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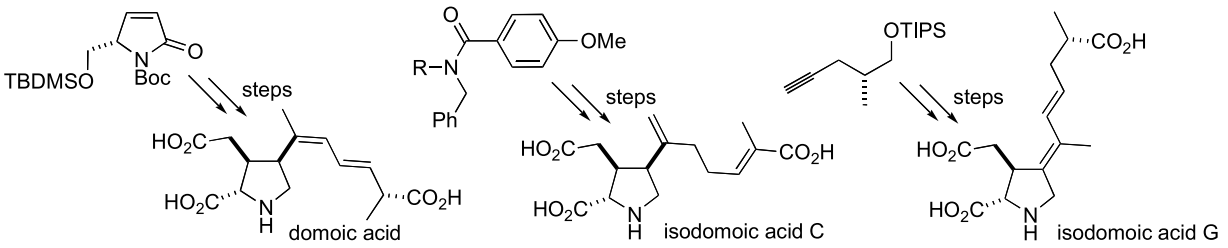

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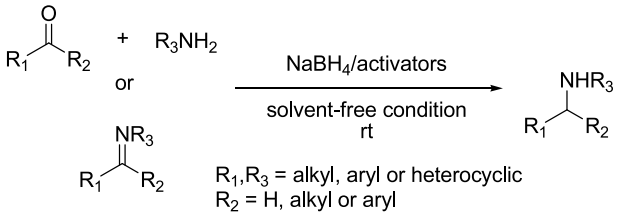
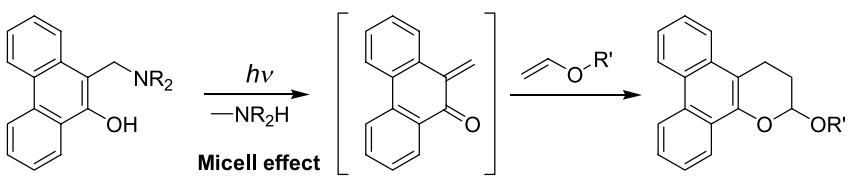
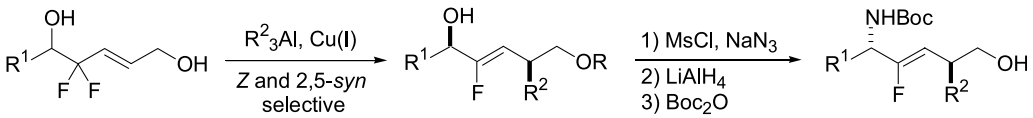
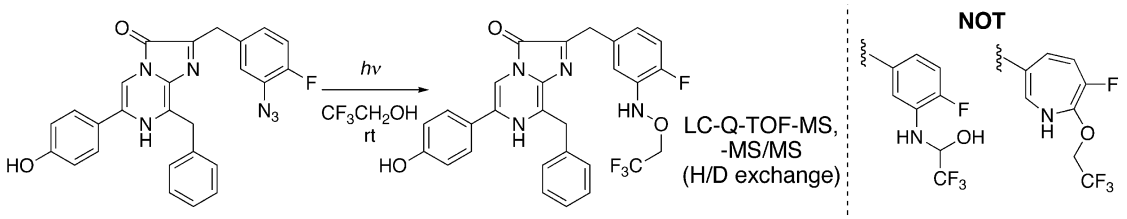
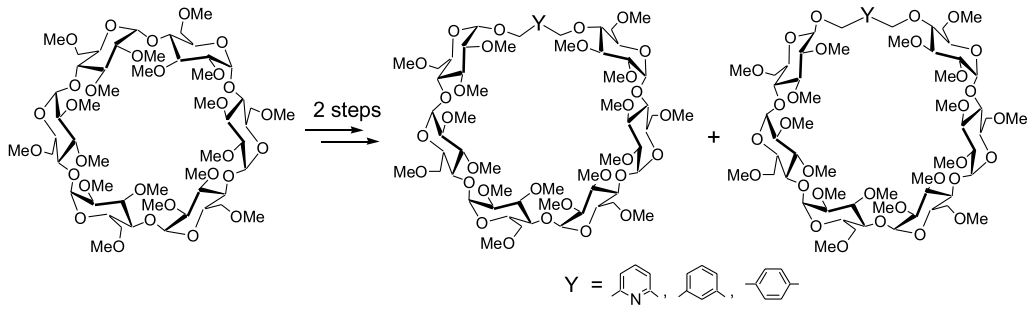
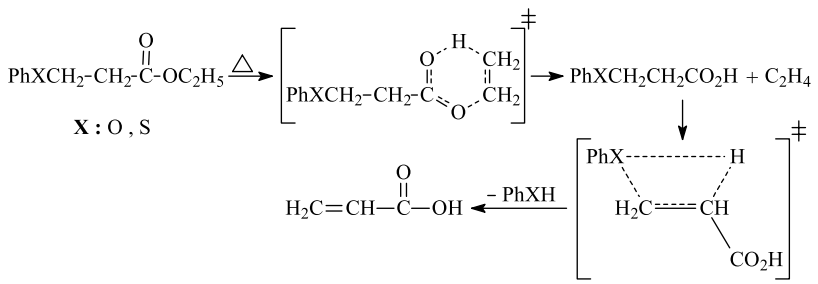




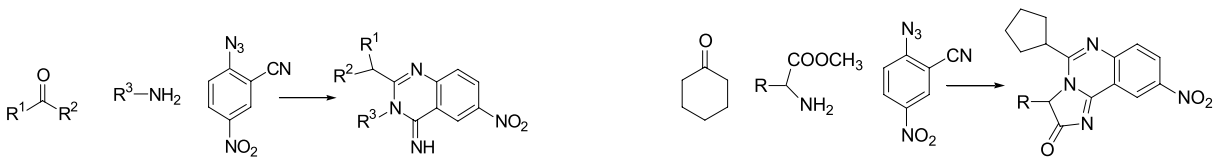


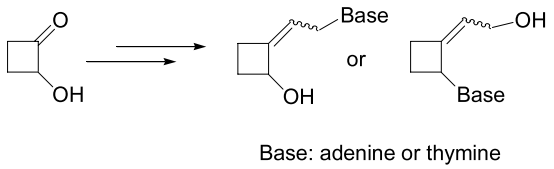


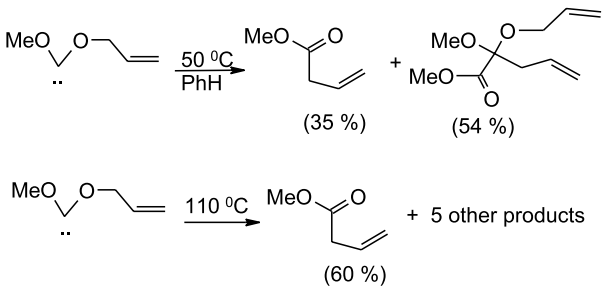


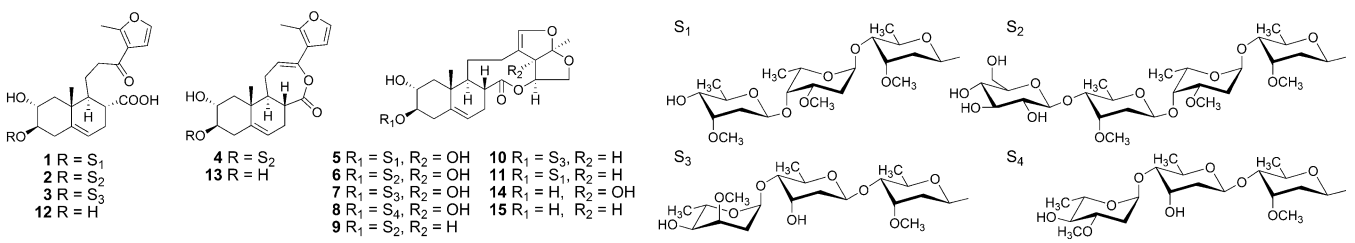


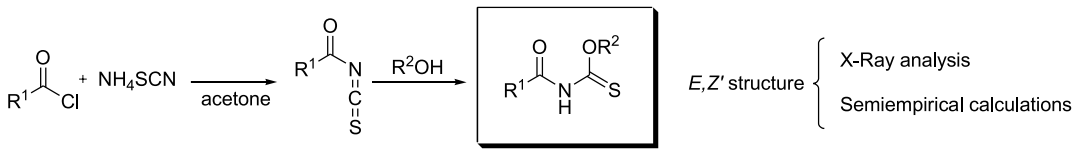
Gas-phase elimination kinetics and product analysis lend support to a thermal retro-Michael reaction pathway involving cyclic transition states.

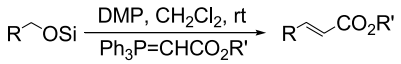


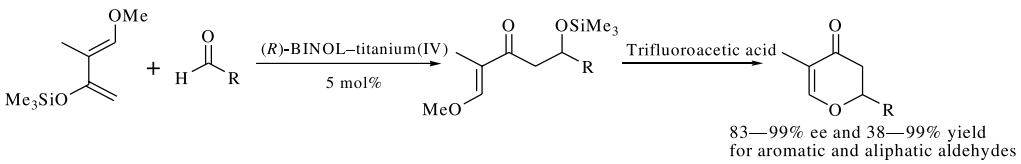


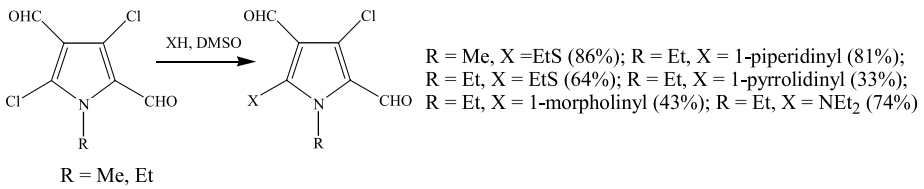


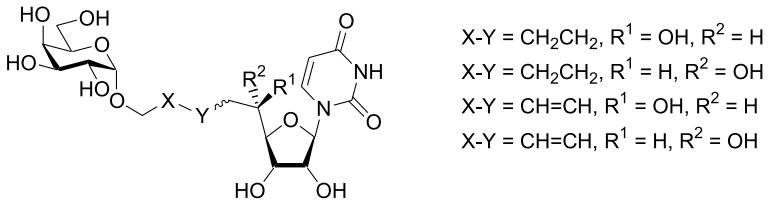


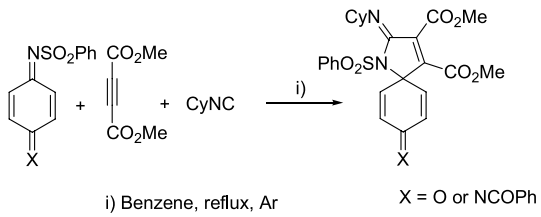


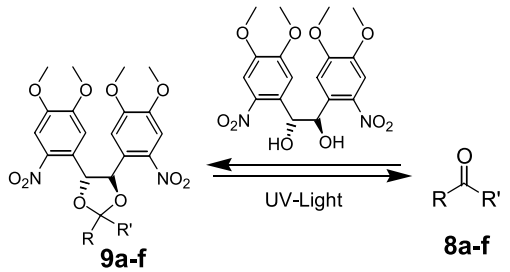


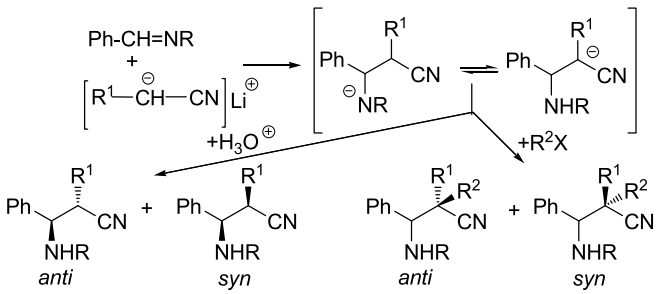


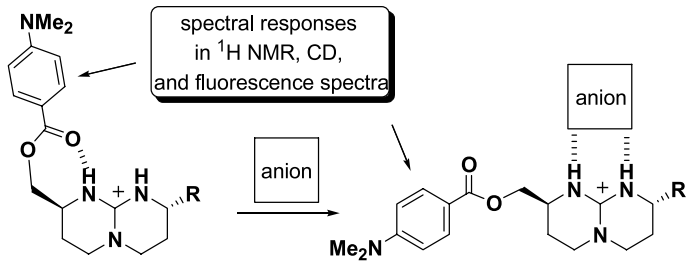


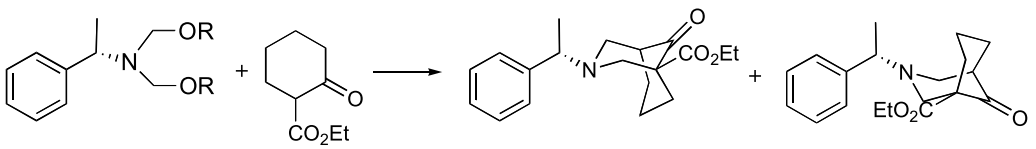


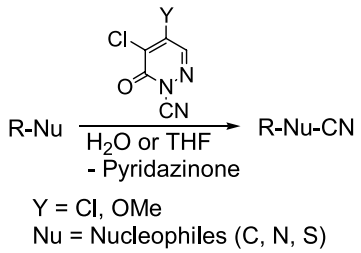


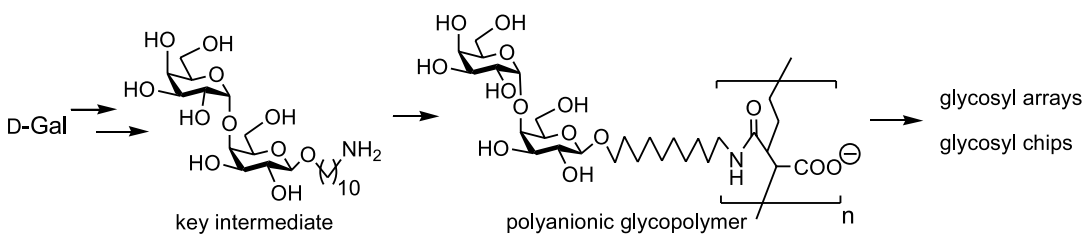


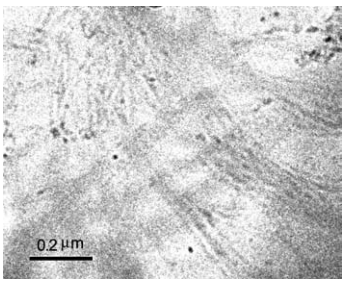


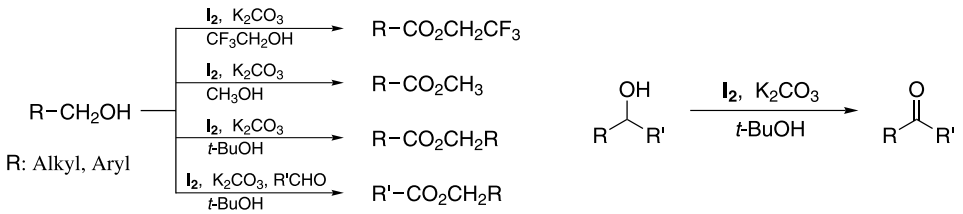


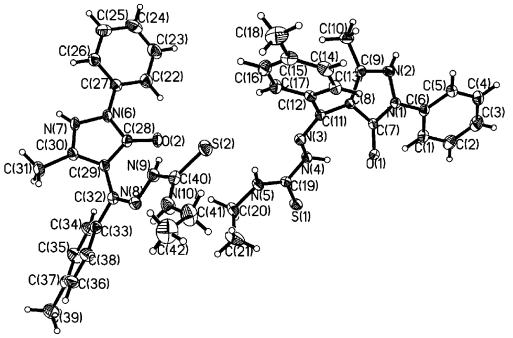




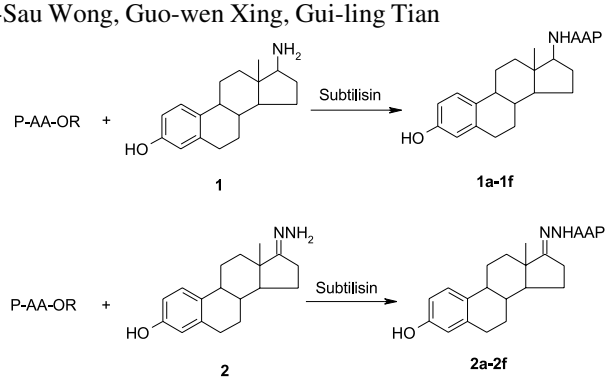


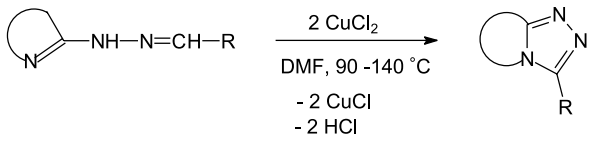






Enzymatic coupling offers noticeable advantages over the chemical method but is limited in terms of its substrate specificity. We extended one of the substrates of subtilisin Calsberg from its natural species to steroidal derivatives. A series of amino acid-steroid conjugates were prepared via the catalysis of subtilisin in DMF and their estrogen-like activities were analyzed.





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Chemistry of domoic acid, isodomoic acids, and their analogues

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Contents

1. Discovery and isolation

Since the isolation of (−)-kainic acid, **1**, from the marine alga *Digenea simplex* in 1953,[1] a number of structurally related compounds have been discovered, including the domoilactones **2a**,**b**,[2] acromelic acids,[3] domoic acid **3** and the isodomoic acids **4a**–**h** (Fig. 1).[4] (−)-Domoic acid carries an octadienoic side chain at C4; the isodomoic acid family is a related group of diene geometrical and regioisomers.

Domoic acid was originally isolated from a red marine alga, *Chondria armata*, in 1958.[5] More than 25 years later, this alga proved also to be a source of isodomoic acids A to D.[6]

In 1989, an HPLC method was developed to allow the rapid, sensitive and unequivocal identification of domoic acid and its known isomers in various shellfish and phytoplankton.[7] Since then, various extraction[8] and screening techniques, such as LC–MS,[9] GC–MS[10] and electrophoresis,[11] have allowed the detection of novel compounds in new areas of the world's waters. Examination of samples of edible mussel *Mytulis edulis* from Canada yielded two new isodomoic acid isomers, E and F, in 1990;[12] re-examination of *Chondria armata* from Kyushu Island, Japan, gave two further isomers, acids G and H.[13]

Much work has been carried out between 1989 and the present time into identifying primary producers of domoic acid and its isomers, other than *Chondria armata*.[14] *Pseudo-nitzschia multiseries* (formally named *Nitzschia pungens* f. *multiseries*) was suggested as the diatom responsible for the contamination of shellfish with domoic acid in 1987, which led to human poisoning in Prince Edward Island, Canada;[15] a cultured sample did indeed produce domoic acid.[16] Other species of *Pseudo-nitzschia* have also been shown to

Keywords: Domoic acid; Isodomoic acids; Marine alga.
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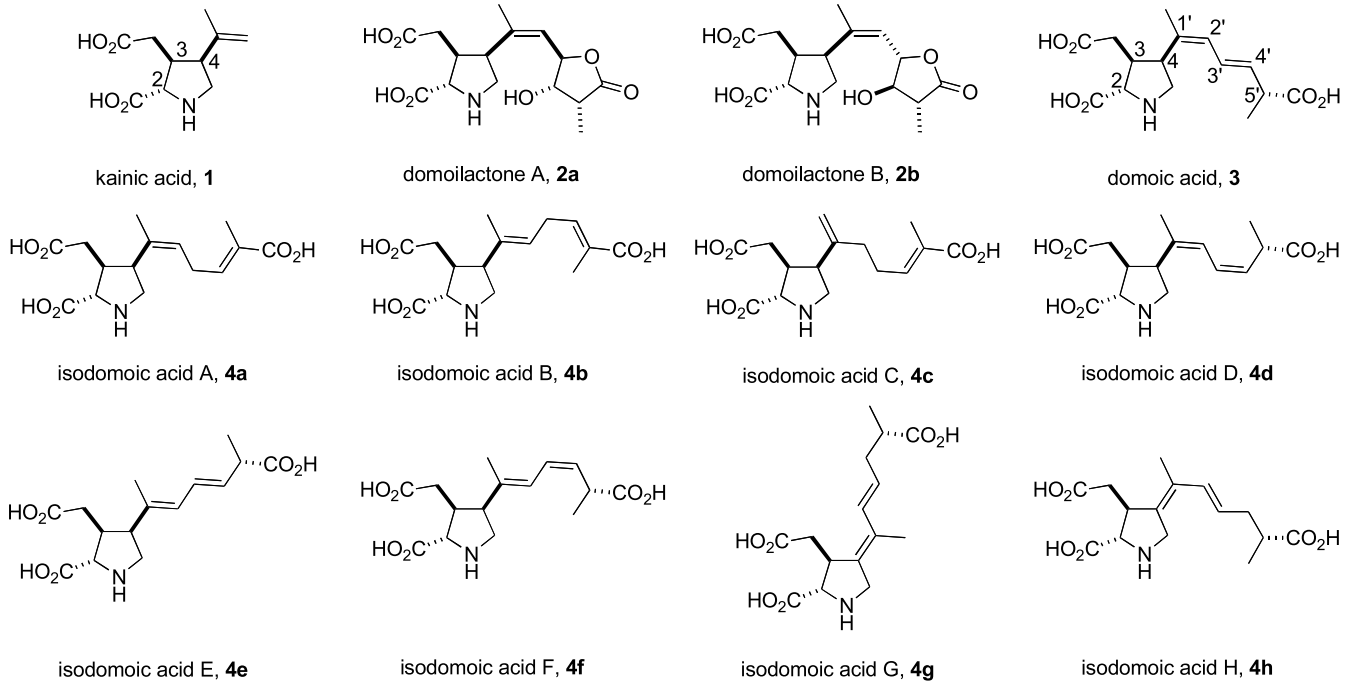


Figure 1. Kainic acid and some members of the domoic/isodomoic acid amino acid family.

produce domoic acid, including *P. calliantha*[14a] (Bay of Fundy, Canada—not *P. pseudodelicatissima*, as reported in 1990[17]), *P. australis*,[18] *P. delicatissima*,[19] *P. multistriata*,[20] *P. pungens*,[19] *P. seriata*,[21] *P. turgidula*[19] and *P. fraudulenta*.[22]

2. Biological and environmental activity[23]

Interest in the study of the kainoids has been aroused by their potent biological effects. Insecticidal[6a,24] and anthelmintic[5,25] (anti-intestinal worm) properties have long been reported and applied therapeutically, but it is their neuroexcitatory properties[26] that have provoked investigation into the kainoid amino acids in recent years.

Neuronal degeneration is a factor that brain diseases such as epilepsy, Huntington's disease and senile dementia have in common. Injection of kainoids gives rise to symptoms that mimic those observed in epilepsy[27] and Huntington's disease,[28] and leads to specific neuronal death in a manner that closely models dementia.[26] Therefore, this family of amino acids offers possibilities for the development of useful tools in the fight against these debilitating diseases.

The mode of kainoid biological action is thought to arise from their structural similarity to glutamic acid (Fig. 2),[29] a mammalian central nervous system neurotransmitter. The potency of neuroexcitation depends on the strength of binding at the kainate receptor, one of three types of the ionotropic (ion channel) class of glutamate receptors.[30] Binding is influenced strongly by C4 stereochemistry,[29b] C4 substituent[29c] and molecular conformation.[29d] The nature of the C4 substituent is particularly critical, with the *Z*-configuration of a C1′ alkene more active than the *E*-configuration, and compounds bearing sp^2 substituents having an activity more than 1000-fold that of a compound with an analogous saturated substituent.[31]

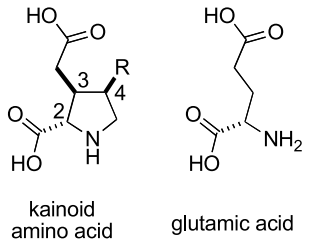


Figure 2. Structural comparison of kainoids with glutamic acid.

Following the 1987 case of shellfish poisoning in Prince Edward Island, Canada, during which three people died and more than 150 were taken ill as a result of eating contaminated blue mussels (*Mytulis edulis*), domoic acid was identified as the causative substance.[32] The contamination resulted from ingestion of toxic domoic acid-producing algae by the shellfish. Manifestation of this 'amnesic shellfish poisoning' has been observed in sea mammals[33] and birds,[34] as well as humans.

The biological mode of action of domoic acid results in neuronal depolarisation; the resultant short-term memory loss is symptomatic of domoic acid poisoning (reported by 25% of those affected by the Canadian amnesic shellfish poisoning outbreak). Other symptoms include dizziness, nausea and vomiting, ultimately leading to coma and brain damage or death in the most severe cases.

Outbreaks of domoic acid poisoning occur when populations of domoic acid-producing organisms 'bloom' to a sufficiently high concentration for levels of the poison to become dangerous to health. This can be a perennial problem in areas of the world's oceans where essential nutrients are brought up from deep water to levels

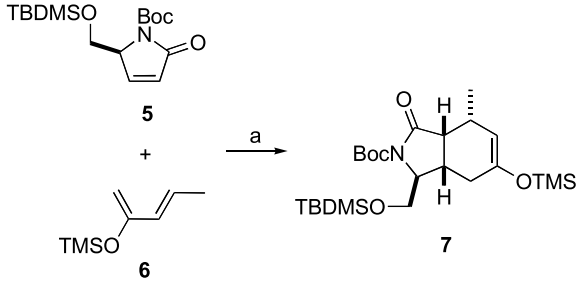
penetrated by light, such as the western coast of North America.

The future threat to human health by shellfish poisoning resulting from domoic acid ingestion should be minimised by the extensive monitoring programmes now in place. These, for example, have led to the detection of domoic acid production by *P. seriata* in Scottish waters[35] and by *P. australis* in Irish waters.[36] Other novel methods of protection employed by the shellfish industry include delaying mussel harvest until after ice-cover in Canada,[37] and the ongoing research into isolation of bacteria that can break down domoic acid.[38]

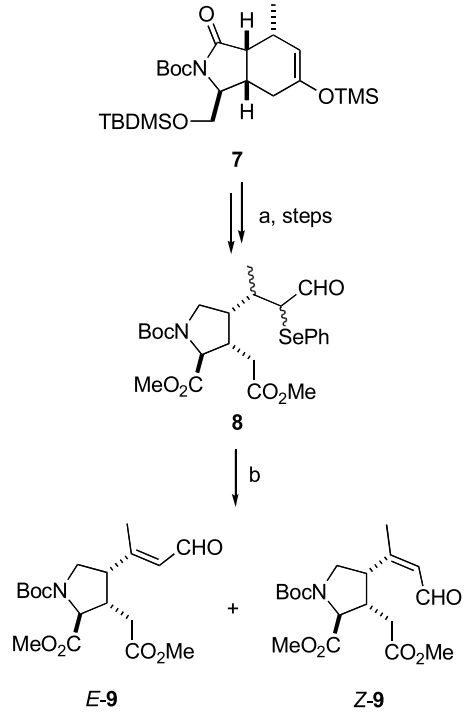
3. Synthesis of members of the domoic acid family

3.1. Domoic acid

Although many syntheses of kainic acid have been reported,[39] domoic acid has been synthesised just once, by Ohfune and Tomita, in 1982,[40] with the aim of proving its absolute stereochemistry. From the advanced intermediate **5**, a Diels–Alder reaction with **6** gave bicyclic compound **7** (Scheme 1).



Scheme 1. Synthesis of domoic acid: key Diels–Alder step. Reagents and conditions: (a) 135 °C, sealed tube, 3 d.



Scheme 2. Synthesis of domoic acid: formation of *E* and *Z* diene precursors. Reagents and conditions: (a) O_3, DCM, −78 °C; DMS, rt, 6 h; (b) see Table 1 below.

Control of stereochemistry was remarkable, with just one stereoisomer of **7** detected; this can be explained by the Woodward–Hoffmann rules,[41] along with second-order effects. The diene approached from the lower face of **5**, as drawn, to avoid the bulky TBDMS group; regiochemistry was controlled by asynchronous diene–dienophile orbital overlap and bond formation, with *para* OTMS and amide carbonyl favoured over *meta*; secondary diene–dienophile interactions promoted *endo* product formation. With the *cis*-fused ring junction controlling the crucial stereochemistry around the pyrrolidinone ring, ozonolysis of **7** opened the six-membered ring, and subsequent steps led to selenide **8** (Scheme 2).

Two methods were developed for the deselenation of **8** (Table 1), giving moderate control of the alkene geometry of **9**.

Table 1. Conditions for controlled deselenation of **8**

Conditions	Total yield of **9** (%)	*E*-**9**:*Z*-**9** ratio
O_3, DCM, −78 °C; Et_3N	33	10:1
NBS, THF, rt, 2 min; NaOAc (aq), 15 min	67	1:2

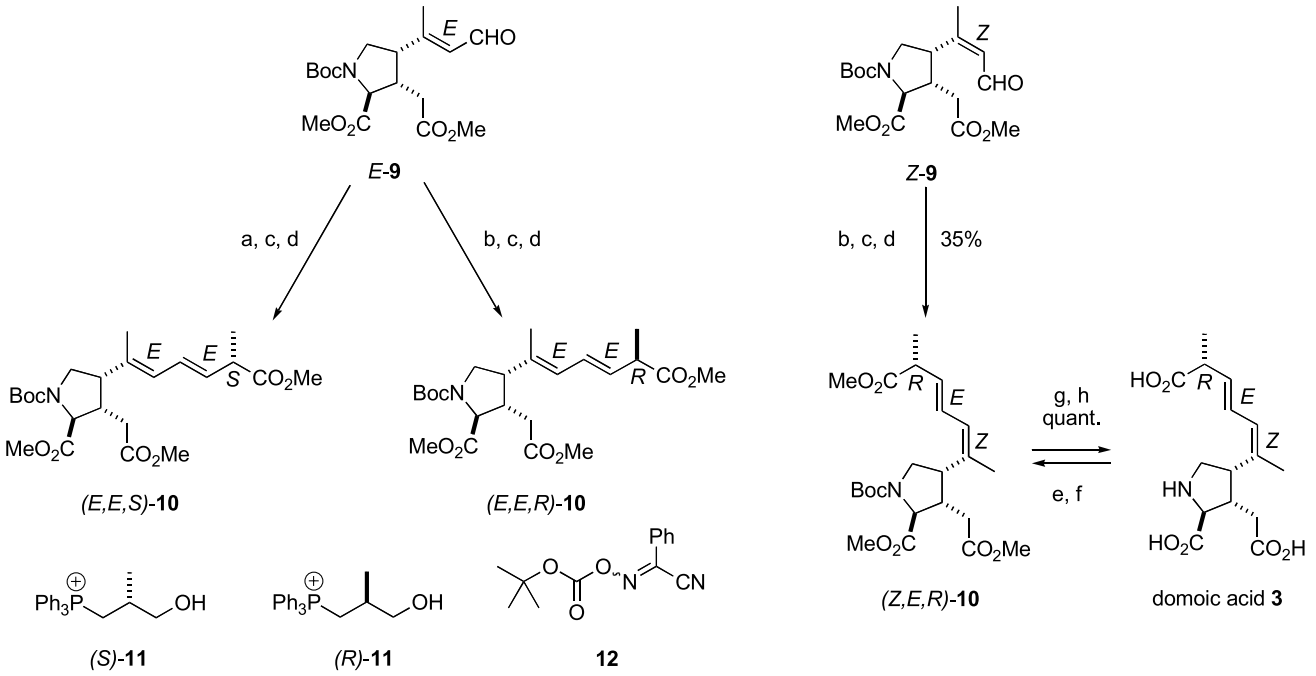
Wittig chemistry was then used to form three derivatives of **9**; derivatisation of an authentic sample of (−)-domoic acid gave (*Z*,*E*,*R*)-**10**, thus proving its absolute stereochemistry (Scheme 3).

3.2. Semisynthesis of isodomoic acids from domoic or kainic acid

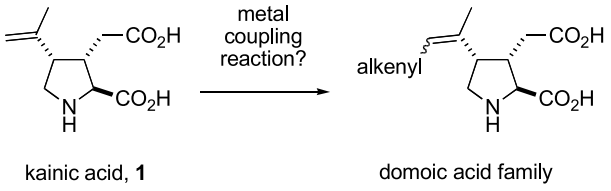
Although scarce in its natural sources, domoic acid is far more abundant than the isodomoic acids. It has been reported to exist at levels of greater than 1% dry weight in the Canadian phytoplankton, *Nitzschia pungens*.[37,42] Work by Wright et al.[12] has demonstrated that, of the mixture of domoic acid isomers obtained by purification of the concentrated extraction mixture of the mussel, *Mytilus edulis*, more than 90% is domoic acid, with isodomoic acids D, E and F at just 5, 2 and 1%, respectively. The relative abundance of the isodomoic acids was improved by photochemical isomerisation, with exposure to ultraviolet light (λ=250 nm) for nine to twelve minutes giving domoic acid, isodomoic acid D, isodomoic acid E and isodomoic acid F in a ratio of 0.28:0.12:0.27:0.13, respectively, per unit of starting material.

Modification of the isopropenyl side chain of kainic acid **1** with a metal coupling reaction also seems a possible route to the domoic acid family of kainoids (Scheme 4), as a semisynthesis from the relatively abundant natural product, or utilising one of the many published syntheses of kainic acid.[39]

Mertes et al. have applied this chemistry to investigate how substitution on the kainic acid isopropenyl group affects

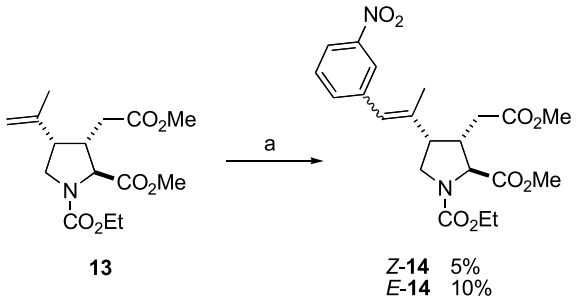


Scheme 3. Routes to isomers of domoic acid. Reagents and conditions: (a) (*S*)-**11**, *n*-BuLi (2 equiv), THF, −78 °C, 2 min; 0 °C, 10 min; (b) (*R*)-**11**, *n*-BuLi (2 equiv), THF, −78 °C, 2 min; 0 °C, 10 min; (c) Jones' reagent, 0 °C, 1 h; (d) CH_2N_2; (e) **12**, Et_3N; (f) CH_2N_2; (g) 2.5% KOH (aq), rt, 24 h; (h) TFA, rt, 15 min; NaOH (1 equiv).



Scheme 4. Isopropenyl modification of kainic acid.

receptor binding.[43] Only an aryl coupling was attempted, giving a mixture of isomers of the domoic analogue **14** in low yield (Scheme 5).



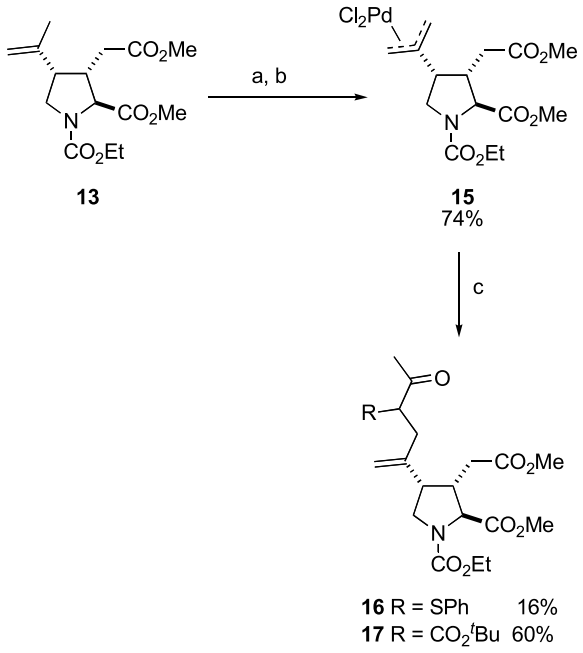
Scheme 5. Arylation of (−)-kainic acid. Reagents and conditions: (a) $Pd(OAc)_2$, 3-nitroaniline, MeCN, 60 °C; *t*-butylnitrite, 60 °C, portion-wise over 3 h; 60 °C, 4 h; 25 °C, overnight; $Pd(OAc)_2$, 3-nitroaniline, *t*-butylnitrite, 60 °C, 2 h; 25 °C, 2 d.

Methyl, rather than methylene, substitution was also reported in this paper, with π-allylpalladium complex **15** proving susceptible to nucleophilic attack (Scheme 6).

No attempts to extend this work to the synthesis of domoic acid or isodomoic acids D, E or F (methylene coupling), or isodomoic acid C (methyl substitution), have since been reported in the literature. The poor yields, lack of stereocontrol and sensitivity to aryl substrate probably mean that this would be an inefficient route to the isodomoic acids.

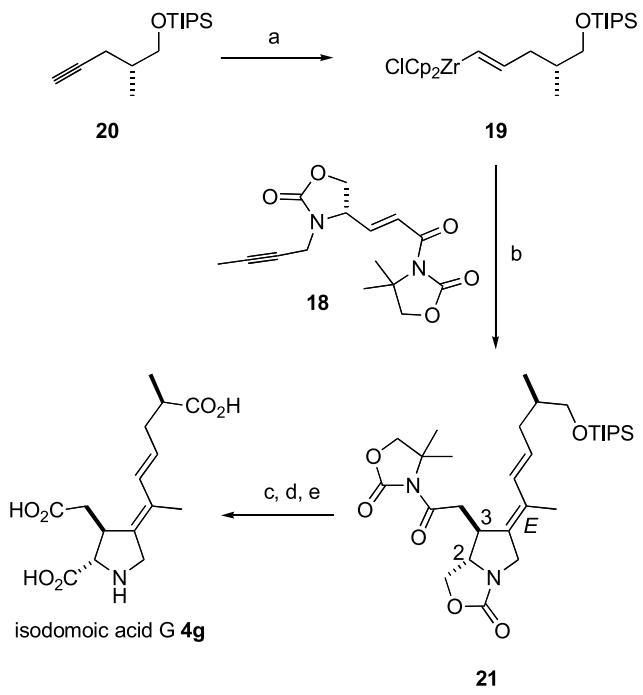
3.3. Isodomoic acid G

A total synthesis of isodomoic acid G has been reported by



Scheme 6. Homologation of (−)-kainic acid. Reagents and conditions: (a) $Pd(OCOCF_3)_2$, EtOAc, rt, overnight; (b) *n*-Bu_4NCl, rt, 24 h; (c) *t*-butylacetoacetate or phenylthioacetone, NaH, THF; **15**, Ph_3P, THF, 25 °C, overnight (**16**) or 38 h (**17**).

Montgomery et al.[44] Isodomoic acids G and H differ from isodomoic acids A to F and domoic acid in that they possess exocyclic alkenes. Therefore, this work was able to utilise a key nickel-catalysed alkyne-alkenylzirconium coupling of **18** with **19** (Scheme 7). This step gave complete control of C2–C3 relative stereochemistry and simultaneously yielded the correct diene geometry.

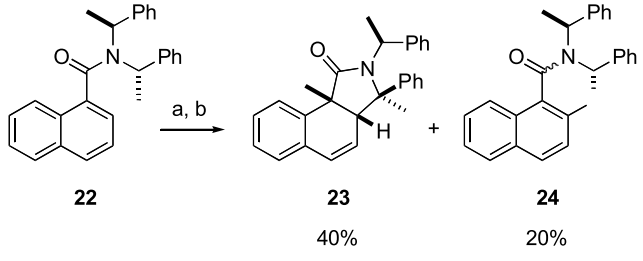


Scheme 7. Synthesis of isodomoic acid G. Reagents and conditions: (a) Cp_2ZrHCl, THF, rt; (b) **18**, $Ni(COD)_2$ (10 mol%), $ZnCl_2$ (20 mol%), THF, 0 °C; (c) MeONa, MeOH; (d) Dess Martin periodinane, CH_2Cl_2 then $NaClO_2$; (e) NaOH, MeOH, H_2O.

Although the stereocontrol demonstrated in this synthesis is remarkable, there is no scope for its extension to other isodomoic acids, except isomer H.

3.4. Isodomoic acids via dearomatising anionic cyclisation reactions

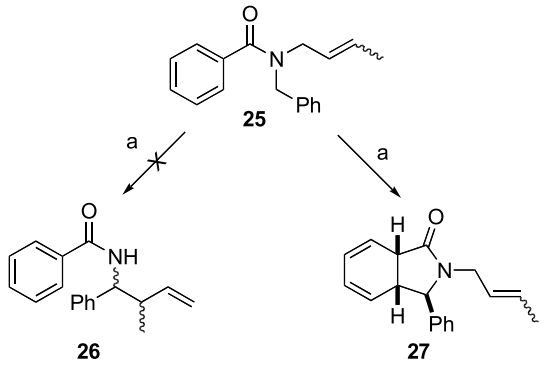
3.4.1. Discovery of the dearomatising anionic cyclisation. Two independent, coincidental observations at the University of Manchester in the mid-1990s prompted research into the previously unreported anionic dearomatising cyclisation of naphthamides and benzamides.[45] Lithiation of **22** gave **23** as the major product, in 40% yield, with



Scheme 8. First dearomatising cyclisation of a lithiated naphthamide. Reagents and conditions: (a) *s*-BuLi, THF, −78 °C; (b) MeI.

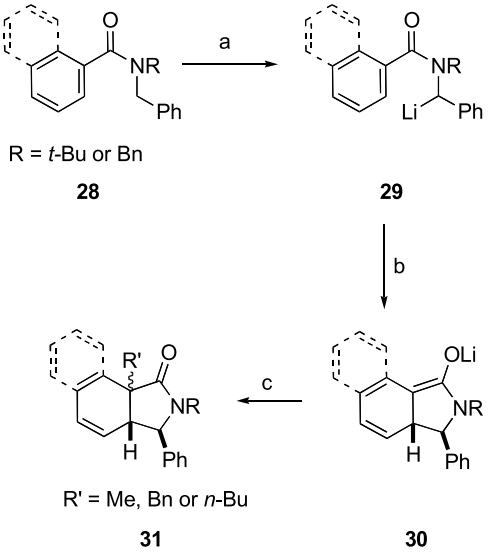
half as much of the expected *ortho*-methylated compound **24** (as a 1:1 mixture of atropisomers) (Scheme 8).[46]

Secondly, the attempted [2,3]-aza Wittig rearrangement of **25** to **26** actually resulted in another cyclised product, **27**, with lost aromaticity (Scheme 9).[47]



Scheme 9. First dearomatising cyclisation of a lithiated benzamide. Reagents and conditions: (a) *t*-BuLi, HMPA, THF, −78 °C.

These two manifestations of the same reaction were subsequently found to be a general reaction of *N*-benzylnaphthamides[48] and *N*-benzylbenzamides[49] (Scheme 10).



Scheme 10. Dearomatising cyclisations of lithiated amides. Reagents: (a) *t*-BuLi; (b) HMPA; (c) MeI, BnBr or *n*-BuBr.

While these reactions are interesting, their true value is tested by their applicability to synthesis. The observation that the dearomatised products possess the relative stereochemistry around the pyrrolidinone ring observed in kainoid natural products (Fig. 3) prompted further investigation of this potential synthetic application.[50]

3.4.2. Development of the cyclisation: synthesis of (±)-kainic acid. Key modifications to the cyclisation conditions and substrates made the reaction more amenable to use in synthesis:

- *t*-Butylbenzylbenzamide cyclised satisfactorily, but E1 elimination of the *t*-butyl group proved almost

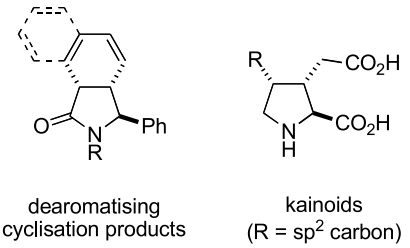
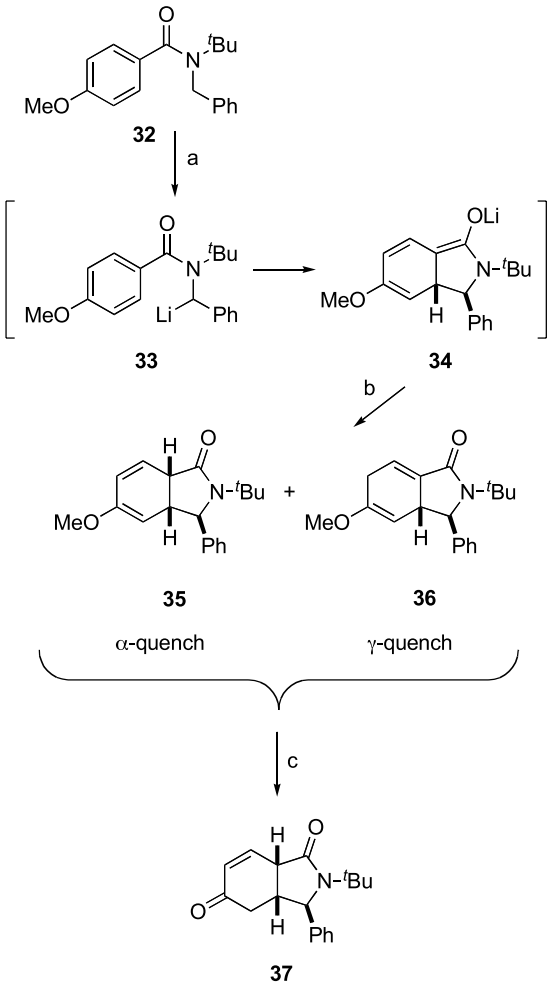


Figure 3. Stereochemical comparison of dearomatised products with kainoid natural products.

impossible;[39e] when compounds without a bulky group on nitrogen were used, for example, *N*,*N*-dibenzylbenzamide, the benzylic organolithium preferred by oxygen–lithium coordination effects must decomplex to cyclise, and poor yields and diastereocontrol result.[51] Replacement of *t*-butyl with the acid-labile[52] cumyl (2-phenylisopropyl) group avoids the removal difficulties, although the cumylamine starting material is somewhat expensive commercially.

- Methoxy functionalisation of the benzamide ring leads to methyl enol ethers, such as **35** and **36**, as the dearomatised products; these can be hydrolysed, without isolation of the enol ether, to give enones, with regioselectivity controlled thermodynamically (Scheme 11).[51]



Scheme 11. Cyclohexenones from anisamides. Reagents: (a) *t*-BuLi, HMPA; (b) H^+; (c) HCl, H_2O–MeOH.

With this groundwork completed, a racemic synthesis of kainic acid was published in 2000 (outlined in Scheme 12).[39e]

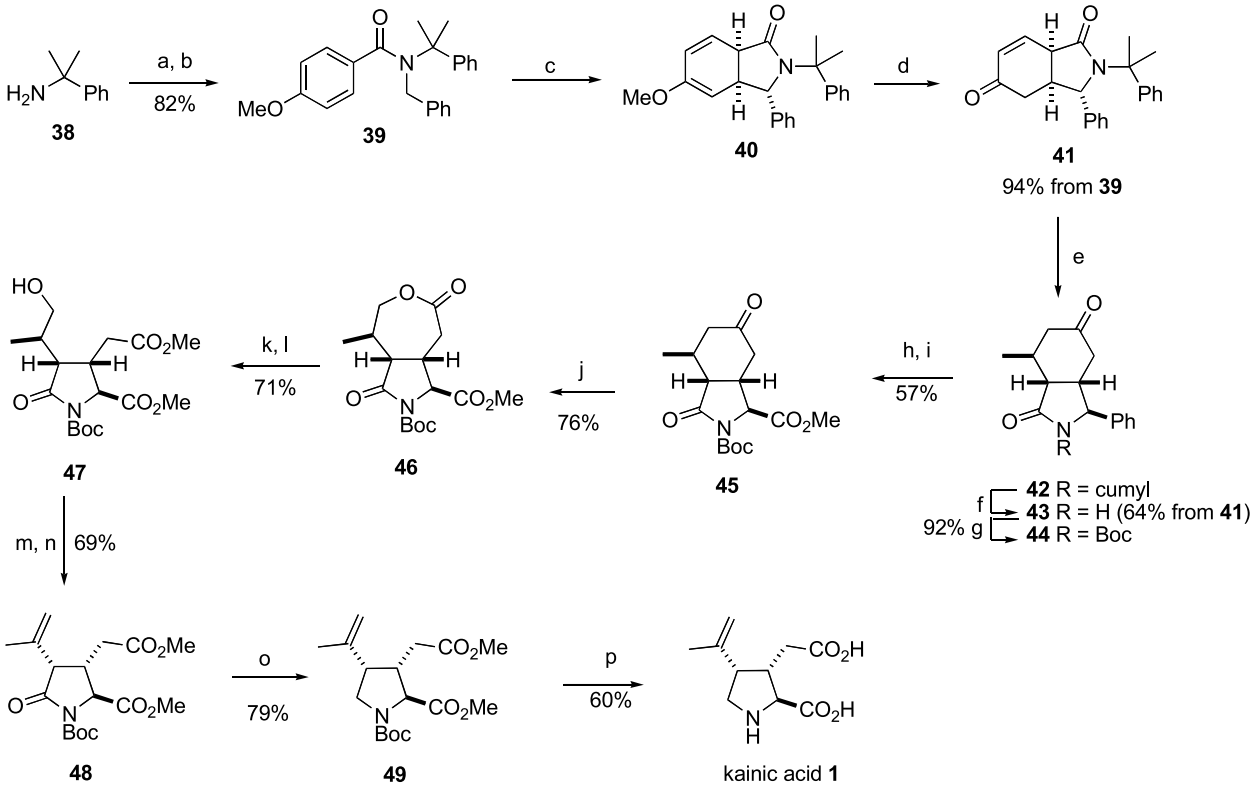
Cumylamine **38** was acylated with *p*-anisoyl chloride, and then alkylated with benzyl bromide. Benzylic lithiation was effected with *t*-BuLi and HMPA, but the cyclisation required 60 h to go to completion because of the bulky cumyl group. In situ enol ether hydrolysis gave the bicyclic enone **41** with correct pyrrolidinone stereochemistry. *Exo*-face conjugate methyl addition followed by acid hydrolysis of the TMS-trapped enolate also cleaved the *N*-cumyl group, which was subsequently reprotected with Boc. The phenyl ring was oxidised with catalytic ruthenium tetroxide, formed in situ from ruthenium trichloride and sodium periodate, the resulting carboxylic acid being esterified with trimethylsilyldiazomethane. Baeyer–Villiger oxidation was surprisingly regioselective; the resultant lactone was cleaved with sodium hydroxide. Elimination of water was via oxidation and elimination of a selenide. Borohydride reduction and acid deprotection gave (±)-kainic acid, which was purified by recrystallisation from methanol.

Subsequent practical developments came with the discoveries that LDA was sufficiently basic to perform the benzylic lithiation (at temperatures above −30 °C), and that raising the temperature to around 0 °C was sufficient to promote cyclisation without the need for carcinogenic HMPA.[51] The milder conditions were also compatible with a wider range of substrates, such as those that are reactive towards *t*-BuLi (e.g., bromides or nitriles), or those containing electron-withdrawing or donating groups.

3.4.3. Asymmetric cyclisation and the synthesis of (−)-kainic acid. Replacement of LDA with the chiral base **50** (Fig. 4) immediately gave good enantiomeric excesses, which were optimised to 75% ee at lower reaction temperatures (facilitated by the higher basicity of **50** than LDA).[39ae] The overall yields were low using **50**, reflecting difficulties removing the hydrochloride salt of **50** from the product. Fortunately, the hydrochloride salt of base **51** was less soluble in organic solvents than that of **50**, facilitating clean extraction and allowing further optimisation of the reaction to give a yield of 88%, with 81% ee.[39ae]

In the full published synthesis of (−)-kainic acid,[39ad] a yield of 52% using base **51** for this key step was obtained after recrystallisation, with an enantiomeric excess of 99%.

3.4.4. Extension of the kainic acid route to the synthesis of (−)-isodomoic acid C. Most members of the isodomoic acid family contain at least one stereochemically defined trisubstituted double bond, a feature which presents an additional synthetic challenge. In Isodomoic acid C, however, one of the two alkenes in the side chain is 1,1-disubstituted, as in kainic acid, and indeed isodomoic acid C can be seen as a homologated version of kainic acid. Its synthesis was therefore approached in a similar way, with the introduction of the side chain as a three-carbon precursor by conjugate cuprate addition to enone **41** (Scheme 13). The need for aryl oxidation to provide the C2 carboxylate precludes the early incorporation of the second double bond.



Scheme 12. Synthesis of kainic acid. Reagents and conditions: (a) *p*-anisoyl chloride, Et_3N, DCM; (b) NaH, DMF, BnBr, rt, 18 h; (c) *t*-BuLi (2 equiv), HMPA (12 equiv), THF, −40 °C, 60 h; satd NH_4Cl soln.; (d) 1 M HCl (aq), THF; (e) Me_2CuLi, TMSCl, THF, −78 °C, 1 h; (f) TFA, reflux, 6 h; (g) Boc_2O, Et_3N, DMAP, DCM; (h) $NaIO_4$, cat. $RuCl_3$, 1:1 acetone–H_2O; (i) Me_3SiCHN_2, PhH, MeOH; (j) *m*-CPBA, DCM; (k) NaOH (2.2 equiv), MeOH, reflux, 2 h; (l) Me_3SiCHN_2, PhH, MeOH; (m) *o*-$NO_2C_6H_4SeCN$, Bu_3P, THF, rt; (n) H_2O_2, py, THF, −40 °C; (o) $NaBH(OMe)_3$ (2 equiv), THF, reflux; (p) 10:1 TFA–H_2O, reflux, 4 h.

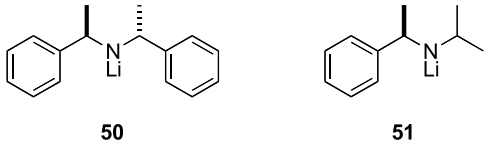


Figure 4. Chiral bases used to promote asymmetric cyclisation.

3.4.5. Studies towards other isodomoic acids: approaches to the trisubstituted alkene. We attempted to extend this approach[39e] to the domoic and isodomoic acids by using sequential nucleophilic and electrophilic addition to the β-methyl enone **64**, aiming to generate the C1′ alkene in a subsequent fragmentation (Scheme 14). The side chain E would be chosen to correspond to the desired member of the isodomoic acid family.

The tin-mediated fragmentation[53] was successful, although initially low-yielding (Scheme 15). Fragmentation of the alcohols **73a–b** indicated that the reaction was stereospecific[53b] with a single geometrical isomer of the alkene **74** generated by the *trans* relationship in **73** (Scheme 16). Both diastereoisomers of **73** gave the same product **74** on treatment with portions of lead tetraacetate, although the reaction proceeded more quickly with **73a**.

The stereochemistry of the resultant double bond was unambiguously assigned as *trans*, from the magnitude of the ^3J coupling observed across the alkene. This is consistent with *anti*-elimination of tin by a radical mechanism,[54] or an analogous ionic elimination by acetate.[53a]

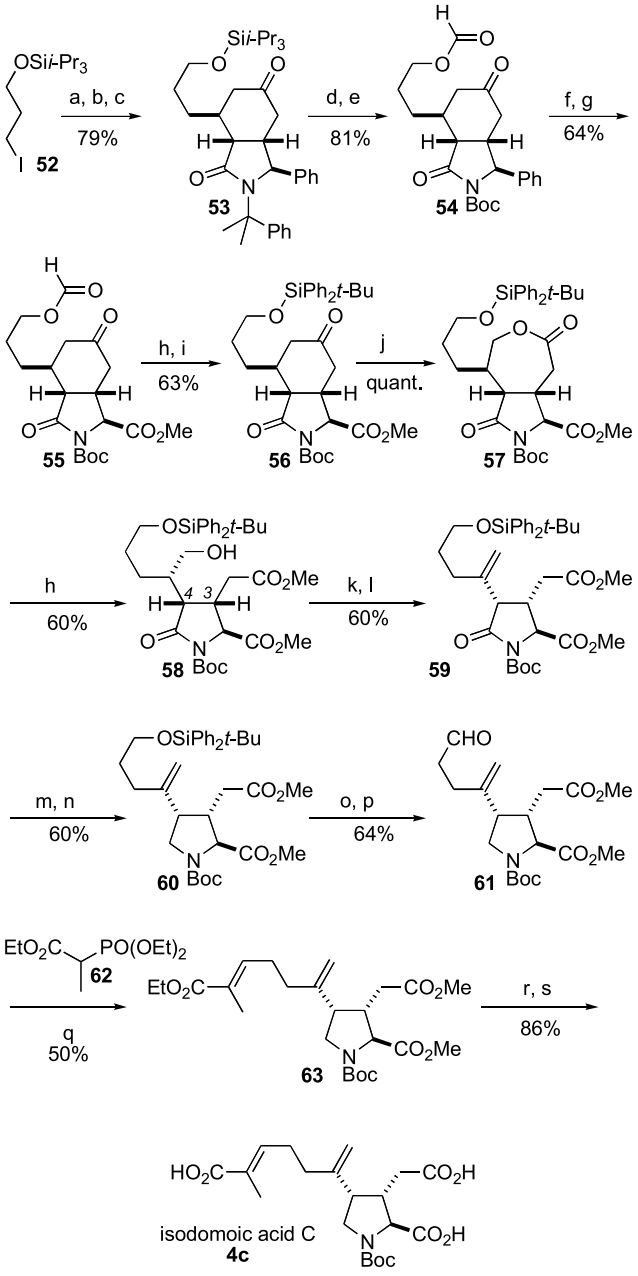
3.5. Domoic acid analogues[55]

Modification of a procedure previously applied to the synthesis of kainic acid[39ai] has allowed Baldwin et al. to synthesise **75** (Fig. 5), a side chain analogue of domoic acid that is structurally similar to isodomoic acid C.

The side chain was introduced early in the synthesis as citral, by reductive amination. Although the commercially available citral **77** was a 95:5 mixture of geometrical isomers, this turned out to be unimportant, as both gave a common product on Co(I)-mediated cyclisation of **78**, the key step in this synthesis (Scheme 17).

Compound **81** could be isolated as a stable compound, but deprotection with aqueous potassium hydroxide and then trifluoroacetic acid gave only a trace of **82**, with **83** as the major product, obtained as the trifluoroacetate salt in 72% yield from **81**.[55]

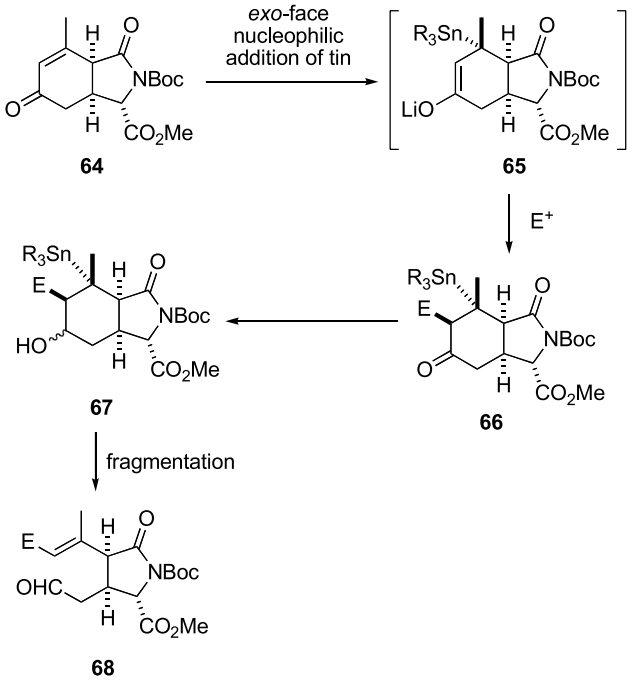
More recent work in this area has focused on 4-arylsulphonyl analogues **84**,[56] and 4-aryl, alkenyl and alkynyl analogues[57] of the acromelic acids **85** (Fig. 6).



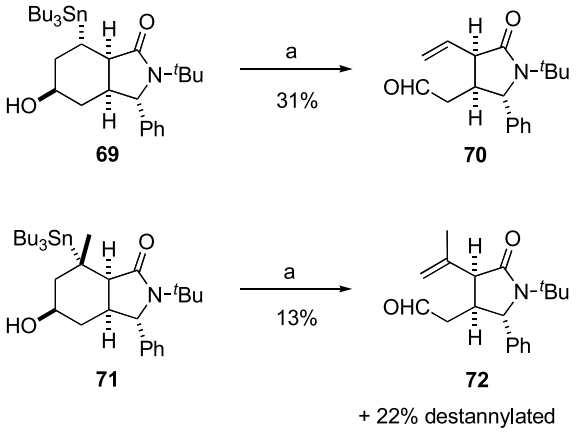
Scheme 13. Synthesis of (−)-isodomoic acid C. Reagents and conditions: (a) *t*-BuLi, −78 °C, Et_2O; (b) MeLi, CuCN, Et_2O, −78 to 25 °C; (c) **41**, −78 °C; (d) HCO_2H, reflux, 30 min; (e) Boc_2O, Et_3N, DMAP, DCM, 25 °C, 18 h; (f) $NaIO_4$, $RuCl_3$, H_2O, MeCN, EtOAc, 18 h; (g) Me_3SiCHN_2, PhMe, MeOH, 20 °C, 5 min; (h) NaOMe, MeOH, −78 °C, 1 h.; (i) *t*-$BuPh_2$SiCl, imid., DCM, 20 °C, 18 h; (j) *m*-CPBA (70%), DCM, 25 °C, 72 h; (k) *o*-$NO_2C_6H_4SeCN$, Bu_3P, THF, 20 °C, 2 h; (l) H_2O_2, py., −40 to 25 °C, 12 h; (m) *i*-Bu_2AlH, PhMe, THF, −78 °C, 1 h; (n) Et_3SiH, $BF_3{:}OEt_2$, −78 °C, 2.5 h; (o) Bu_4NF, THF, 25 °C, 2 h; (p) Dess–Martin, DCM, 25 °C, 30 min; (q) **62**, DBU, LiCl, MeCN, 25 °C, 1 h; (r) LiOH, H_2O, THF, 25 °C, 12 h; (s) CF_3CO_2H, DCM, △, 2 h.

4. Conclusion

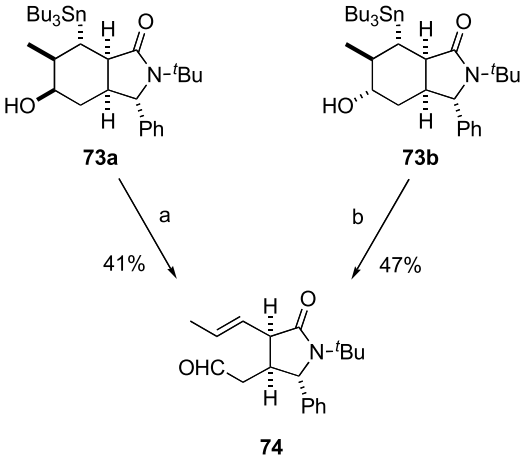
Despite the enormous activity in the last 20 or so years in synthetic studies towards kainic acid, relatively little work has been carried out on related, more complex kainoids. Of the biologically active analogues of kainic acid known as the domoic/isodomoic acid family, only three compounds have so far been synthesised, by very different routes. As the



Scheme 14. Extension of the isodomoic acid C synthesis to trisubstituted alkene-containing domoic acid isomers.



Scheme 15. Kainoid-like pyrrolidines by fragmentation reactions. Reagents and conditions: (a) $Pb(OAc)_4$, C_6H_6, reflux.



Scheme 16. Stereospecificity in the fragmentation. Reagents and conditions: (a) $Pb(OAc)_4$, $CaCO_3$, C_6H_6, reflux, 1.5 h; (b) $Pb(OAc)_4$, $CaCO_3$, C_6H_6, reflux, 3 h.

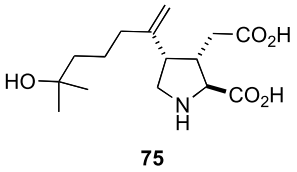


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Figure 5. Baldwin's domoic acid analogue.

important environmental, toxicological and physiological properties of these compounds come under further scrutiny, synthetic work in the area is certain to expand.

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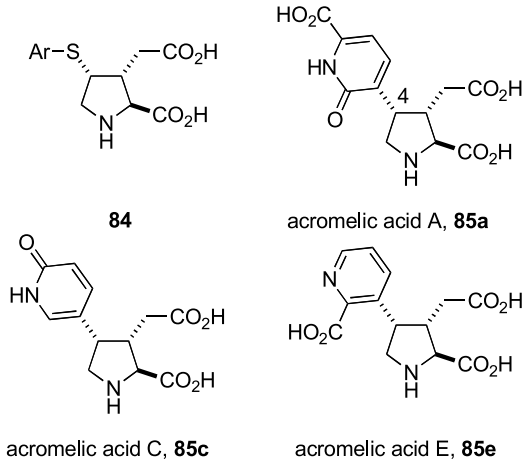
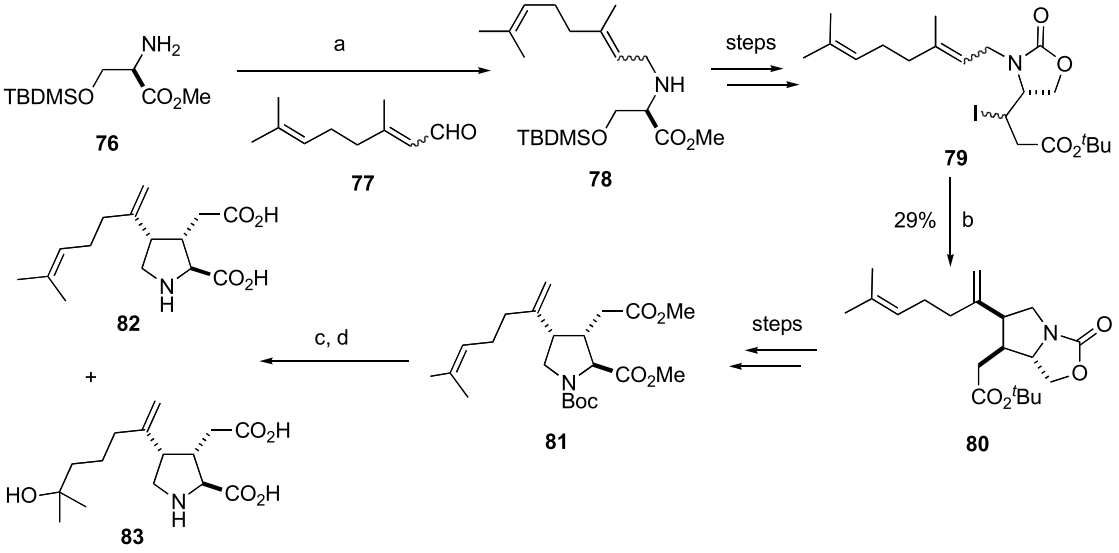


84 acromelic acid A, **85a**

acromelic acid C, **85c** acromelic acid E, **85e**

Figure 6. Current domoic acid analogue targets.



Scheme 17. Cobalt-mediated synthesis of a domoic analogue. (a) NaOH, citral (**77**); (b) Co(I); (c) KOH (aq); (d) TFA.

References and notes

1. Murakami, S.; Takemoto, T.; Shimizu, Z. *J. Pharm. Soc. Jpn.* **1953**, *73*, 1026.
2. Maeda, M.; Kodama, T.; Tanaka, T.; Yoshizumi, H.; Takemoto, T.; Nomoto, K.; Fujita, T. *Tetrahedron Lett.* **1987**, *28*, 633.
3. (a)Acromelic acids A and B: Konno, K.; Shirahama, H.; Matsumoto, T. *Tetrahedron Lett.* **1983**, *24*, 939. (b) Acromelic acid C: Fushiya, S.; Sato, S.; Kanazawa, T.; Kusano, G.; Nozoe, S. *Tetrahedron Lett.* **1990**, *31*, 3901. (c) Acromelic acids D and E: Fushiya, S.; Sato, S.; Kera, Y.; Nozoe, S. *Heterocycles* **1992**, *34*, 1277.
4. For a general review of the kainoid amino acids, see: Parsons, A. F. *Tetrahedron* **1996**, *52*, 4149.
5. (a) Takemoto, T.; Daigo, K. *Chem. Pharm. Bull.* **1958**, *6*, 578. (b) Daigo, K. *J. Pharm. Soc. Jpn.* **1959**, *79*, 350.
6. (a) Isodomoic acids A to C: Maeda, M.; Kodama, T.; Tanaka, T.; Yoshizumi, H.; Takemoto, T.; Nomoto, K.; Fujita, T. *Chem. Pharm. Bull.* **1986**, *34*, 4892. (b) Isodomoic acid D: Maeda, M.; Kodama, T.; Tanaka, T.; Yoshizumi, H.; Takemoto, T.; Nomoto, K.; Fujita, T. *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu* **1985**, *27*, 616.
7. Quilliam, M. A.; Sim, P. G.; McCulloch, A. W.; McInnes, A. G. *Int. J. Env. Anal. Chem.* **1989**, *36*, 139.
8. Vale, P.; Sampayo, M.A.de M. *Harmful Algae* **2002**, *1*, 127.
9. Pineiro, N.; Vaquero, E.; Leao, J. M.; Gago-Martinez, A.; Vazquez, J. A. R. *Chromatographia* **2001**, *53*, 231.
10. Hadley, S. W.; Braun, S. K.; Wekell, M. M. In *Seafood safety, processing and biotechnology*; Shahidi, F., Jones, Y., Kitts, D. D., Eds.; Technomic: Lancaster, 1997; p 25.
11. Zhao, J. Y.; Thibault, P.; Quilliam, M. A. *Electrophoresis* **1997**, *18*, 268.
12. Walter, J. A.; Falk, M.; McInnes, A. G.; Wright, J. L. C. *Can. J. Chem.* **1990**, *68*, 22.
13. Zaman, L.; Arakawa, O.; Shimosu, A.; Onoue, Y.; Nishio, S.; Shida, Y.; Noguchi, T. *Toxicon* **1997**, *35*, 205.
14. For a detailed account of the process of discovery of domoic acid-producing organisms and an explanation of taxonomy, see (a) Lundholm, N.; Moestrup, Ø.; Hasle, G. R.; Hoef-Emden, K. *J. Phycol.* **2003**, *39*, 797. (b) Bates, S. S. *J. Phycol.* **2000**, *36*, 978.

15. Bates, S. S.; Bird, C. J.; De Freitas, A. S. W.; Foxall, R.; Gilgan, M.; Hanic, L. A.; Johnson, G. R.; McCulloch, A. W.; Odense, P. *Can. J. Fish. Aquat. Sci.* **1989**, *46*, 1203.
16. Douglas, D. J.; Bates, S. S. *Can. J. Fish. Aquat. Sci.* **1992**, *49*, 85.
17. Martin, J. L.; Haya, K.; Burridge, L. E.; Wildish, D. J. *Mar. Ecol.: Prog. Ser.* **1990**, *67*, 177.
18. Garrison, D. L.; Conrad, S. M.; Eilers, P. P.; Waldron, E. M. *J. Phycol.* **1992**, *28*, 604.
19. Rhodes, L.; White, D.; Syhre, M.; Atkinson, M. In *Harmful and toxic algal blooms*; Oshima, Y., Fukuyo, Y., Yasumoto, T., Eds.; Intergovernmental Oceanographic Commission, UNESCO: Paris, 1996; p 155.
20. Sarno, D.; Dahlmann, J. *Harmful Algae News* **2000**, *21*, 5.
21. Lundholm, N.; Moestrup, Ø.; Skov, J.; Pocklington, R. *Phycologia* **1994**, *33*, 475.
22. Rhodes, L.; Scholin, C.; Garthwaite, I.; Haywood, A.; Thomas, A. In *Harmful algae*; Reguera, B., Blanco, J., Fernández, M. L., Wyatt, T., Eds.; Intergovernmental Oceanographic Commission, UNESCO: Paris, 1998; p 274.
23. For a detailed review of the role of domoic acid as a marine toxin, see Mos, L. *Environ. Toxicol. Pharmacol.* **2001**, *9*, 79 and references therein.
24. Maeda, M.; Kodama, T.; Tanaka, T.; Ohfune, Y.; Nishimura, K.; Nomoto, K.; Fujita, T. *J. Pestic. Sci.* **1984**, *9*, 27.
25. (a) Watase, H.; Tomiie, Y.; Nitta, I. *Nature (London)* **1958**, *181*, 761. (b) Husinec, S.; Porter, A. E. A.; Roberts, J. S.; Strachan, C. H. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2517.
26. (a) *Kainic acid as a tool in neurobiology*; McGeer, E. G., Olney, J. W., McGeer, P. L., Eds.; Raven: New York, 1978. (b) *Excitatory amino acids*; Simon, R. P., Ed.; Thieme Medical: New York, 1992. (c) *Excitatory amino acids and synaptic transmission*; Wheal, H. V., Thomson, A. M., Eds.; Academic: London, 1991. (d) Watkins, J. C.; Krogsgaard-Larsen, P.; Honoré, T. *Trends Pharmacol. Sci.* **1990**, *11*, 25. (e) Waring, R. H.; Steventon, G. B.; Mitchell, S. C. Molecules of death; Imperial College: London, 2002.
27. Sperk, G. *Prog. Neurobiol. (Oxford)* **1994**, *42*, 1.
28. (a) Coyle, J. T.; Schwarcz, R. *Nature (London)* **1976**, *263*, 244. (b) McGeer, E. G.; McGeer, P. L. *Nature (London)* **1976**, *263*, 517.
29. (a) Johnson, G. A. R.; Curtis, D. R.; Davis, J.; McCulloch, R. M. *Nature (London)* **1974**, *248*, 804. (b) Hansen, J. J.; Krogsgaard-Larsen, P. *Med. Res. Rev.* **1990**, *10*, 55. (c) Ishida, M.; Shinozaki, H. *Br. J. Pharmacol.* **1991**, *104*, 873. (d) Hashimoto, K.; Ohfune, Y.; Shirahama, H. *Tetrahedron Lett.* **1995**, *36*, 6235.
30. (a) Monaghan, D. T.; Bridges, R. J.; Cotman, C. W. *Annu. Rev. Pharmacol. Toxicol.* **1989**, *29*, 365. (b) Young, A. B.; Fagg, G. E. *Trends Pharmacol. Sci.* **1990**, *11*, 126. (c) Sommer, B.; Seeburg, P. H. *Trends Pharmacol. Sci.* **1992**, *13*, 291. (d) Seeberg, P. H. *Trends Neurosci.* **1993**, *16*, 359. (e) Hollmann, M.; Heinemann, S. *Annu. Rev. Neurosci.* **1994**, *17*, 31.
31. Hampson, D. R.; Huang, X.; Wells, J. W.; Walter, J. A.; Wright, J. L. C. *Eur. J. Pharmacol.* **1992**, *218*, 1.
32. (a) Bates, S. S.; Bird, C. J.; De Freitas, A. S. W.; Foxall, R.; Gilgan, M.; Hanic, L. A.; Johnson, G. R.; McCulloch, A. W.; Odense, P. *Can. J. Fish. Aquat. Sci.* **1989**, *46*, 1203. (b) Wright, J. L. C.; Boyd, R. K.; De Freitas, A. S. W.; Falk, M.; Foxall, R. A.; Jamieson, W. D.; Laycock, M. V.; McCulloch, A. W.; McInnes, A. G. *Can. J. Chem.* **1989**, *67*, 481.
33. Scholin, C. A.; Gulland, F.; Doucette, G. J.; Benson, S.; Busman, M.; Chavez, F. P.; Cordar, J.; DeLong, R.; De Vogelaere, A.; Harvey, J.; Haulena, M.; Lefebvre, K.; Lipscomb, T.; Loscutoff, S.; Lowenstine, L. J.; Martin, R. I.; Miller, P. E.; McLellan, W. A.; Moeller, P. D. R.; Powell, C. L.; Rowles, T.; Silvagni, P.; Silver, M.; Spraker, T.; Trainer, V.; Van Dolah, F. M. *Nature* **2000**, *403*, 80.
34. (a) Work, T. M.; Beale, A. M.; Fritz, L.; Quilliam, M. A.; Silver, M.; Buck, K.; Wright, J. L. C. In *Toxic phytoplankton blooms in the sea*; Smayda, T. J., Shimizu, Y., Eds.; Elsevier Science: Amsterdam, 1993; p 643. (b) Sierra-Beltrán, A.; Palafox-Uribe, M.; Grajales-Montiel, J.; Cruz-Villacorta, A.; Ocha, J. L. *Toxicon* **1997**, *35*, 447.
35. Fehling, J.; Green, D. H.; Davidson, K.; Bolch, C. J.; Bates, S. S. *J. Phycol.* **2004**, *40*, 622.
36. Cusack, C. K.; Bates, S. S.; Quilliam, M. A.; Patching, J. W.; Raine, R. *J. Phycol.* **2002**, *38*, 1106.
37. Villac, M. C.; Roelke, D. L.; Villareal, T. A.; Fryxell, G. A. *Hydrobiologia* **1993**, *269–270*, 213.
38. (a) Stewart, J. E.; Marks, L. J.; Gilgan, M. W.; Pfeiffer, E.; Zwicker, B. M. *Can. J. Microbiol.* **1998**, *44*, 456. (b) Smith, E. A.; Grant, F.; Ferguson, C. M. J.; Gallacher, S. *Appl. Environ. Microbiol.* **2001**, *67*, 2345.
39. Racemic syntheses: (a) Anderson, J. C.; Whiting, M. *J. Org. Chem.* **2003**, *68*, 6160. (b) Yoo, S.-E.; Lee, S.-H.; Yi, K.-Y.; Jeong, N. *Tetrahedron Lett.* **1990**, *31*, 6877. (c) Bachi, M. D.; Bar-Ner, N.; Melman, A. *J. Org. Chem.* **1996**, *61*, 7116. (d) Bachi, M. D.; Melman, A. *Synlett* **1996**, 60. (e) Clayden, J.; Tchabanenko, K. *Chem. Commun.* **2000**, 317. Asymmetric syntheses: (f) Hirasawa, H.; Taniguchi, T.; Ogasawara, K. *Tetrahedron Lett.* **2001**, *42*, 7587. (g) Miyata, O.; Ozawa, Y.; Ninomiya, I.; Naito, T. *Tetrahedron* **2000**, *56*, 6199. (h) Cambell, A. D.; Raynham, T. M.; Taylor, R. J. K. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3194. (i) Chevliakov, M. V.; Montgomery, J. *J. Am. Chem. Soc.* **1999**, *121*, 11139. (j) Rubio, A.; Ezquerra, J.; Escribano, A.; Modesto, J.; Remuiñán, M. J.; Vanquero, J. J. *Tetrahedron Lett.* **1998**, *39*, 2171. (k) Cossy, J.; Cases, M.; Pardo, D. G. *Synlett* **1998**, 507. (l) Bachi, M. D.; Melman, A. *J. Org. Chem.* **1997**, *62*, 1896. (m) Cooper, J.; Knight, D. W.; Gallagher, P. T. *J. Chem. Soc., Chem. Commun.* **1987**, 1220. (n) Oppolzer, W.; Thirring, K. *J. Am. Chem. Soc.* **1982**, *104*, 4978. (o) Cooper, J.; Knight, D. W.; Gallagher, P. T. *J. Chem. Soc., Perkin Trans. 1* **1992**, 553. (p) Takano, S.; Iwabuchi, Y.; Ogasawara, K. *J. Chem. Soc., Chem. Commun.* **1988**, 1204. (q) Takano, S.; Sugihara, T.; Satoh, S.; Ogasawara, K. *J. Am. Chem. Soc.* **1988**, *110*, 6467. (r) Yoo, S.-E.; Lee, S.-H.; Jeong, N.; Cho, I. *Tetrahedron Lett.* **1993**, *34*, 3435. (s) Yoo, S.-E.; Lee, S.-H. *J. Org. Chem.* **1994**, *59*, 6968. (t) Takano, S.; Inomata, K.; Ogasawara, K. *J. Chem. Soc., Chem. Commun.* **1992**, 169. (u) Barco, A.; Benetti, S.; Pollini, G. P.; Spalluto, G.; Zanirato, V. *J. Chem. Soc., Chem. Commun.* **1991**, 390. (v) Hatakeyama, S.; Sugawara, K.; Takano, S. *J. Chem. Soc., Chem. Commun.* **1993**, 125. (w) Hanessian, S.; Ninkovic, S. *J. Org. Chem.* **1996**, *61*, 5418. (x) Nakada, Y.; Sugahara, T.; Ogasawara, K. *Tetrahedron Lett.* **1997**, *38*, 857. (y) Miyata, O.; Ozawa, Y.; Ninomiya, I.; Naito, T. *Synlett* **1997**, 275. (z) Monn, J. A.; Valli, M. J. *J. Org. Chem.* **1994**, *59*, 2773. (aa) Trost, B. M.; Rudd, M. T. *Org. Lett.* **2003**, *5*, 1467. (ab) Campbell, A. D.; Raynham, T. M.; Taylor, R. J. K. *Chem. Commun.* **1999**, 245. (ac) Xia, Q.; Ganem, B. *Organic Lett.* **2001**, *3*, 485. (ad) Clayden, J.; Menet, C. J.; Tchabanenko, K. *Tetrahedron* **2002**, *58*, 4727. (ae) Clayden, J.; Menet, C. J.; Mansfield, D. J. *Chem.*

Commun. **2002**, 38. (af) Nakagawa, H.; Sugahara, T.; Ogasawara, K. *Org. Lett.* **2000**, *2*, 3181. (ag) Kawamura, M.; Ogasawara, K. *Heterocycles* **1997**, *44*, 129. (ah) Cossy, J.; Cases, M.; Pardo, D. G. *Tetrahedron* **1999**, *55*, 6153. (ai) Baldwin, J. E.; Moloney, M. G.; Parsons, A. F. *Tetrahedron* **1990**, *46*, 7263.

40. Ohfune, Y.; Tomita, M. *J. Am. Chem. Soc.* **1982**, *104*, 3511.
41. Hoffmann, R.; Woodward, R. B. *The conservation of orbital symmetry*; Academic: New York, 1970.
42. Wright, J. L. C.; Boyd, R. K.; De Freitas, A. S. W.; Falk, M.; Foxall, R. A.; Jamieson, W. D.; Laycock, M. V.; McCulloch, A. W.; McInnes, A. G.; Odense, P.; Pathak, V. P.; Quilliam, M. A.; Ragan, M. A.; Sim, P. G.; Thibault, P.; Walter, J. A.; Gilgan, M.; Richard, D. J. A.; Dewar, D. *Can. J. Chem.* **1989**, *67*, 481.
43. Conway, G. A.; Park, J. S.; Maggiora, L.; Mertes, M. P.; Galton, N.; Michaelis, E. K. *J. Med. Chem.* **1984**, *27*, 52.
44. Yike, N.; Amarasinghe, K. D.; Ksebati, B.; Montgomery, J. *Org. Lett.* **2003**, *5*, 3771.
45. For related observations, see: (a) Breternitz, H.-J.; Schaumann, E.; Adiwidjaja, G. *Tetrahedron Lett.* **1991**, *32*, 1299. (b) Hoell, D.; Lex, J.; Müllen, K. *J. Am. Chem. Soc.* **1986**, *108*, 5983. (c) Padwa, A.; Filipkowski, M. A.; Kline, D. N.; Murphree, S. S.; Yeske, P. E. *J. Org. Chem.* **1993**, *58*, 2061. (d) Crandall, J. K.; Ayers, T. A. *J. Org. Chem.* **2002**, *67*, 2335. (e) Clayden, J.; Kenworthy, M. N. *Synthesis* **2004**, 1721. (f) Clayden, J. *Organolithiums: selectivity for synthesis*; Pergamon: New York, 2002; pp 327–329.
46. Jaeger, S. Ph.D. Thesis, University of Manchester, 1996.
47. Beech, C. Final Year Undergraduate Project, University of Manchester, 1995.
48. Ahmed, A.; Clayden, J.; Rowley, M. *Chem. Commun.* **1998**, 297.
49. Ahmed, A.; Clayden, J.; Yasin, S. A. *Chem. Commun.* **1999**, 231.
50. For a review, see: Clayden, J. Total synthesis of kainoids by dearomatizing cyclization. In Harmata, M., Ed.; Strategies and tactics in organic synthesis; Academic: New York, 2004; Vol. 4, pp 72–96.
51. (a) Clayden, J.; Menet, C. J.; Mansfield, D. *Org. Lett.* **2000**, *26*, 4229. (b) Clayden, J.; Tchabanenko, K.; Yasin, S. A.; Turnbull, M. D. *Synlett* **2001**, 302.
52. Metallinos, C.; Nerdinger, S.; Snieckus, V. *Org. Lett.* **1999**, *1*, 1183.
53. (a) Posner, G. H.; Wang, Q.; Halford, B. A.; Elias, J. S.; Maxwell, J. P. *Tetrahedron Lett.* **2000**, *41*, 9655. (b) Nakatani, K.; Isoe, S. *Tetrahedron Lett.* **1984**, *25*, 5335.
54. Baldwin, J. E.; Adlington, R. M.; Robertson, J. *J. Chem. Soc., Chem. Commun.* **1988**, 1404.
55. Baldwin, J. E.; Moloney, M. G.; Parsons, A. F. *Tetrahedron* **1991**, *47*, 155.
56. (a) Baldwin, J. E.; Pritchard, G. J.; Williamson, D. S. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1927. (b) Baldwin, J. E.; Pritchard, G. J.; Williamson, D. S. *Tetrahedron* **2001**, *57*, 7991.
57. (a) Baldwin, J. E.; Pritchard, G. J.; Fryer, A. M. *J. Org. Chem.* **2001**, *66*, 2588. (b) Baldwin, J. E.; Pritchard, G. J.; Fryer, A. M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 309. (c) Baldwin, J. E.; Spyvee, M. R.; Whitehead, R. C.; Wood, M. E.; Fryer, A. M. *Tetrahedron* **1997**, *53*, 5273. (d) Baldwin, J. E.; Bamford, S. J.; Rudolph, M. P. W.; Wood, M. E.; Fryer, A. M. *Tetrahedron* **1997**, *53*, 5255. (e) Baldwin, J. E.; Bamford, S. J.; Rudolph, M. P. W.; Wood, M. E.; Fryer, A. M. *Tetrahedron* **1997**, *53*, 5233. (f) Baldwin, J. E.; Fryer, A. M.; Spyvee, M. R.; Whitehead, R. C.; Wood, M. E. *Tetrahedron Lett.* **1996**, *37*, 6923.

Biographical sketch

Jonathan Clayden has been Professor of Organic Chemistry at Manchester since 2001 and was previously (since 1994) a lecturer in organic chemistry at the same university. His research interests include synthesis and stereochemistry, with particular emphasis on lithiation methods, on conformational control and atropisomerism, and on the synthesis of kainoid amino acids. He is a co-author of the major Organic Chemistry text published by OUP in 2001 and his book 'Organolithiums: Selectivity for Synthesis' was published in 2002. He was recently awarded the Royal Society of Chemistry's Corday-Morgan Medal and was named Novartis European Young Investigator of the Year 2004.

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Direct and indirect reductive amination of aldehydes and ketones with solid acid-activated sodium borohydride under solvent-free conditions

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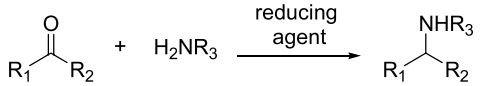
Abstract—A simple and convenient procedure for reductive amination of aldehydes and ketones using sodium borohydride activated by boric acid, *p*-toluenesulfonic acid monohydrate or benzoic acid as reducing agent under solvent-free conditions is described.

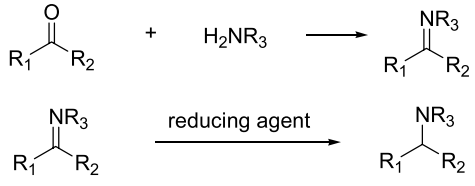
1. Introduction

The transformation of amines from aldehydes and ketones is an important method in organic synthesis because of their versatile utility as intermediates for synthesis of pharmaceuticals[1] and agrochemicals.[2] For the transformation, the two synthetic methods are commonly used. One is the reductive amination, which is termed as a direct reaction. This method allows the conversion of carbonyl functionality to an amine by directly treating a mixture of the carbonyl compound and the amine with suitable reducing agents in a single operation without preformation of an intermediate imine or iminium salt (Scheme 1). The other is a stepwise or indirect reaction, which involves the conversion of amine from the reduction of the imine derivatives isolated in a separate step (Scheme 2). As effective reducing methods for these conversions, catalytic hydrogenation,[3] metal hydride reductions using $NaBH_3CN$,[4a] $LiBH_3CN$,[4b] $(n\text{-}Bu)_4NBH_3CN$,[4c] $NaBH_3CN$–$ZnCl_2$,[4d] $NaBH_3CN$–$Ti(O^iPr)_4$,[4e] $NaBH_3CN$–$Mg(ClO_4)_2$,[4f] $NaBH(OAc)_3$,[5]



R_1, R_3 = alkyl, aryl or heterocyclic
R_2 = H, alkyl, aryl or heterocyclic

Scheme 1.



R_1, R_3 = alkyl, aryl or heterocyclic
R_2 = H, alkyl, aryl or heterocyclic

Scheme 2.

$NaBH_4$–$NiCl_2$,[6a,b] $NaBH_4$–$ZnCl_2$ (nickel boride),[6c] $NaBH_4$–$ZrCl_4$,[6d] $Ti(O^iPr)_4$–$NaBH_4$,[6d] $NaBH_4$–H_2SO_4,[6e] $NaBH_4$–wet clay-microwave,[6f] borohydride exchange resin,[7] $ZnBH_4$,[8a] $ZnBH_4$–$ZnCl_2$,[8b] $ZnBH_4$–SiO_2,[8c] pyridine–borane,[9] picoline–borane,[10] diborane–MeOH,[11] decaborane,[12] Zn–AcOH,[13] polymethylhydrosiloxane (PMHS)–$Ti(O^iPr)_4$,[14a] PMHS–$ZnCl_2$,[14b] PMHS–$BuSn(OCOR)_3$,[14c] Et_3SiH–CF_3CO_2H,[14d] $PhMe_2SiH$–$(C_6F_6)_3$,[14e] Cl_3SiH–DMF,[14f] $PhSiH_3$–Bu_2SnCl_2,[14g] $^nBu_3SnH$–DMF or HMPA,[15a] $^nBu_3SnH$–SiO_2[15b] and $^nBu_2SnIH$ or $^nBu_2$-$SnClH$[15c,d] have been reported. However, most of these reagents may have one drawback or another. For examples, catalytic hydrogenation is incompatible with compounds containing a carbon–carbon double or triple bond and other reducible functional groups such as nitro, cyano and furyl groups.[3] Cyanoborohydride and tin hydride reagents are highly toxic and generate toxic by-products such as HCN, NaCN or organotin compounds[16] upon workup and may result in the contamination of the product with the toxic compounds. Other hydrides such as zinc borohydride,[17] nickel boride[6b,16] and PHMS–$Ti(O^iPr)_4$[18] may be not

Keywords: Reductive amination; Imines; Reductions; Solvent-free reaction; Sodium borohydride.
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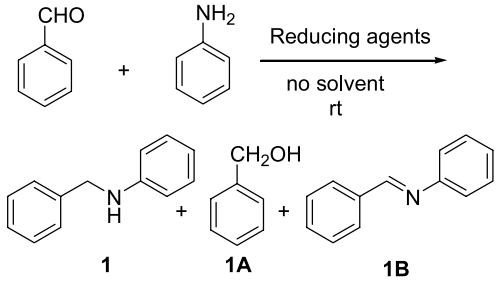
Table 1. Solvent-free reductive amination of benzaldehdye with aniline using boric acid-activated $NaBH_4$ and $NaBH_4$ alone



Method	Time (min)	Ratio of product (%)		
		1	**1A**	**1B**
A	15	35	65	0
B	15	94	6	0
C	15	64	36	0
D	20	3	97	0
E	90	5	10	85

Method A: benzaldehyde was immediately ground with a 1:1:1 mixture of aniline, $NaBH_4$ and boric acid; method B: after benzaldehyde was mixed with aniline for 10 min, the resulting mixture was ground with a 1:1 mixture of $NaBH_4$ and H_3BO_3; method C: the conditions were identical with method B except that the mixing period of benzaldehyde and aniline was 0. 5 min; method D: the conditions were identical with method A except for use of $NaBH_4$ itself; method E: the conditions were identical with method B except for use of $NaBH_4$ itself.

suitable for use of chemoselective reduction of imines having ketone, ester, amide and nitro groups, since these reagents can reduce those functional groups. Sodium borohydride is an inexpensive, safe to handle and environmental friendly reducing agent. Recently, we reported solvent-free chemoselective reduction of aldimines and ketimines including other reducible functional groups, such as ketone, carboxylic acid, ester, nitrile, amide, nitro, furyl and alkenyl groups using boric acid-activated sodium borohydride to the corresponding functionalized amine compounds.[19] This method is not only of interest from ecological point of view,[20] but also proves to be a clean, rapid and very simple procedure for the reduction of imine derivatives. We report here the details, scope, and limitations of direct and indirect process for the reductive amination of aldehydes and ketones using this methodology.

2. Results and discussion

2.1. Direct reductive amination

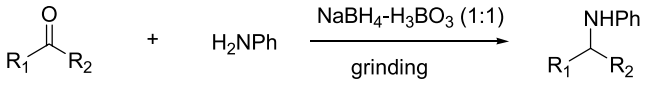
We initially examined a direct solvent-free reductive amination reaction of benzaldehyde with aniline using boric acid-activated sodium borohydride. The reaction was carried out by directly grinding a 1:1:1:1 mixture of benzaldehyde, aniline, sodium borohydride and boric acid with an agate mortar and pestle at room temperature in air until TLC showed complete disappearance of benzaldehyde. As shown in Table 1, it was interestingly found that yields of a product amine, *N*-benzylaniline (**1**) obtained were dependent on a mixing order and period of benzaldehyde and aniline. When benzaldehyde was immediately ground with a 1:1:1 mixture of aniline, boric acid and sodium borohydride (method A) for 15 min, the reaction provided **1** (35%) and benzyl alcohol (**1A**, 65%). In contrast, when benzaldehdye was mixed with aniline for 10 min under solvent-free conditions and the resulting mixture was successively ground with a 1:1 mixture of sodium borohydride and boric acid (method B), the reaction afforded **1** (94%) and **1A** (6%). This reaction gave **1** (64%) and **1A** (36%), even though the mixing period of benzaldehyde and aniline was 0.5 min (method C). On the other hand, the reaction using sodium borohydride itself in the absence of boric acid in method A (method D) gave **1** (3%) and **1A** (97%), whereas the same reaction in method B (method E) provided **1** (5%), **1A** (10%) and 85% of

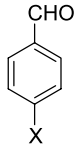
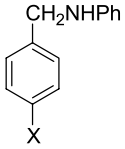
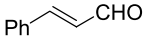
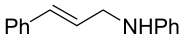
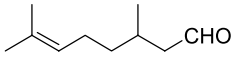
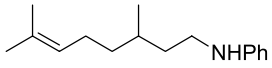
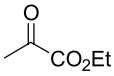
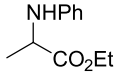
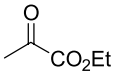
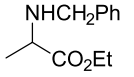
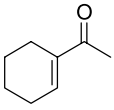
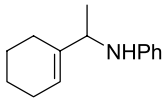
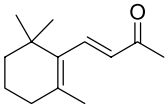
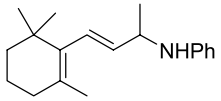
Table 2. Solvent-free reductive amination of aldehyde and ketone with $NaBH_4$ in the presence of activator[a]

Entry no.	Aldehyde/ ketone	Amine	Activator	Time (min)		Product	Yield (%)[b]
1	PhCHO	H_2NPh	H_3BO_3	15		**1**	94
2	PhCHO	H_2NPh	PTSA[c]	15		**1**	92
3	PhCHO	H_2NPh	$PhCO_2H$	15		**1**	93
4	PhCHO	$H_2NC_6H_4OMe$-*p*	H_3BO_3	10	**2**	$PhCH_2NHC_6H_4OMe$-*p*	99
5	PhCHO	H_2NCH_2Ph	PTSA	30	**3**	$PhCH_2NHCH_2Ph$	88
6	PhCHO	H_2NCH_2Ph	H_3BO_3	30		**3**	85
7	PhCHO	$H_2NC_7H_{15}$-*n*	H_3BO_3	20	**4**	*n*-$C_7H_{15}NHCH_2Ph$	93
8	PhCHO	$H_2NC_6H_{11}$-*c*	PTSA	30	**5**	*c*-$C_6H_{11}NHCH_2Ph$	62
9	PhCHO	Morpholine	H_3BO_3	30		$PhCH_2OH$	99
10	*n*-$C_7H_{15}CHO$	H_2NPh	H_3BO_3	20	**6**	*n*-$C_7H_{15}CH_2NHPh$	83
11	*n*-$C_7H_{15}CHO$	H_2NCH_2Ph	$PhCO_2H$	20	**7**	*n*-$C_7H_{15}CH_2NHCH_2Ph$	81
12	*n*-$C_7H_{15}CHO$	$H_2NC_7H_{15}$-*n*	PTSA	30	**8**	*n*-$C_7H_{15}CH_2NHC_7H_{15}$-*n*	70
13	*c*-$C_6H_{11}CHO$	H_2NPh	$PhCO_2H$	20	**9**	*c*-$C_6H_{11}CH_2NHPh$	97
14	*c*-$C_6H_{11}CHO$	$H_2NC_6H_4OMe$-*p*	H_3BO_3	20	**10**	*c*-$C_6H_{11}CH_2NHC_6H_4OMe$-*p*	94
15	*c*-$C_6H_{11}CHO$	H_2NCH_2Ph	PTSA	30	**11**	*c*-$C_6H_{11}CH_2NHCH_2Ph$	77
16	Furfural	H_2NPh	H_3BO_3	10	**12**	*N*-phenylfufrylamine	97
17	Cyclohexanone	H_2NPh	H_3BO_3	10	**13**	*c*-$C_6H_{11}NHPh$	93
18	Cyclohexanone	H_2NCH_2Ph	$PhCO_2H$	20		**5**	62
19	Cyclohexanone	Morpholine	H_3BO_3	20	**14**	*N*-Cyclohexylmorpholine	35[d]
20	Acetophenone	H_2NPh	H_3BO_3	30		PhCH(OH)Me	92

[a] After 1 equiv of aldehydes or ketones was mixed with 1 equiv of amines for 10 min at room temperature under solvent-free conditions, the resulting mixture was ground with a 1:1 mixture of $NaBH_4$ and each activator in an agate mortar and pestle.
[b] Isolated yield after column chromatography.
[c] PTSA = *p*-toluenesulfonic acid monohydrate.
[d] Cyclohexanol was obtained in a major product.

Table 3. Chemoselective reductive amination of functionalized aldehydes and ketones with H_3BO_3-activated $NaBH_4$ under solvent-free conditions[a]

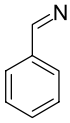
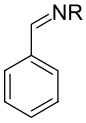
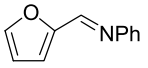
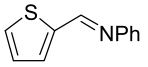
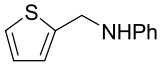
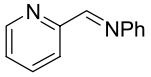
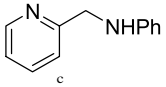
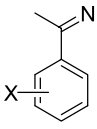
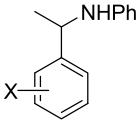
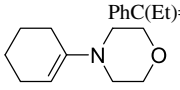
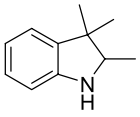
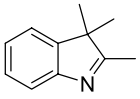


Entry no.			Time (min)				Yield (%)[a]
1		X=COMe	50		**15**	X=COMe	81
2		X=CN	40		**16**	X=CN	99
3		X=CO_2H	40		**17**	X=CO_2H	80
4		X=CO_2Me	30		**18**	X=CO_2Me	82
5		X=NHCOMe	40		**19**	X=NHCOMe	91
6		X=NO_2	50		**20**	X=NO_2	77
7			30	**21**			98
8			20	**22**			92
9			15	**23**			79
10			30	**24**			71
11			20	**25**			88
12			90	**26**			25

[a] See the corresponding footnotes in Table 2.

benzladehyde *N*-phenylimine (**1B**, 85%) even after 90 min. The results indicate that these reactions involve a competitive reduction of benzaldehyde and the imine **1B** formed from the aldehyde and aniline. Indeed, when benzaldehyde was mixed with aniline for 10 min under solvent-free condition at room temperature produced an imine product **1B** in 95% yield, which was rapidly reduced with boric acid-activated sodium borohydride to **1**, but the reduction with sodium borohydride alone was very slow (see Table 4). In these reactions, a mixing order and period of sodium borohydride and boric acid had no discernable effect on the rate of reduction and yield of product amine. To test effectiveness of other solid acids, such as *p*-toluenesulfonic acid monohydrate and benzoic acid, as activator, we compared reductive amination of benzaldehyde with aniline using those acids instead of boric acid under the identical conditions described in method B. As shown in Table 2, the reactions examined were complete within 15 min to give **1** in high yield, showing no significant difference of effectiveness on the role of activators among these acids (entries 1–3). Using the same methodolgy, the reductive aminations of structurally different aldehydes and ketones with various amines were examined. Reductive amination of benzaldehyde with other aromatic and aliphatic primary amines proceeded smoothly to give the corresponding secondary amines (entries 4–8), although the reaction with cyclohexylamine provided somewhat low yield. The reaction with a secondary amine, morpholine, produced only benzyl alcohol without formation of the desired amine (entry 9). The reactions of aliphatic and heterocyclic aldehydes, such as octanal, cyclohexanecarboxaldehyde and furfural, with various primary amines gave the desired secondary amine products in the range of 70–97% yield (entries 10–16). In the cases of ketones, cyclohexanone underwent successfully reductive amination with aniline to give *N*-phenylcyclohexylamine in 93% yield (entry 17), although the reaction with benzylamine and morpholine afforded the desired amines in lower yield (entries 18 and 19). Under the same conditions, however, reductive amination of acetopheone with aniline did not occur. The reaction afforded only reduction product of acetophenone, 1-phenylethanol (entry 20). We next examined chemoselective reductive amination of functionalized aldehydes and ketones bearing other reducible functional groups employing the same methodology using boric acid as activator. As shown in Table 3, aromatic aldehydes having ketone, cyano, carboxylic acid, ester, amide and nitro group underwent reductive amination to give the corresponding *N*-phenyl amines without reduction of any other functional groups in good yields (entries 1–6). In the case of alkenic aldehydes and ketones, such as (*E*)-cinnamaldehyde, citronellal, 1-acetylcyclohexene and β-ionone, all the reductive amination except for β-ionone were successfully achieved in excellent yield (entries 7, 8, and 11). β-Ionone reacted slowly to give the corresponding *N*-phenylamine in a very low yield, showing preferential reduction of carbonyl

Table 4. Solvent-free reduction of imine derivatives with solid acid-activated $NaBH_4$[a]

Entry no.	Imine	Activator	Time (min)	Product	Yield (%)[b]
1		None	180	**1**	26
2		H_3BO_3	10	**1**	99
3		PTSA	10	**1**	99
4		$PhCO_2H$	10	**1**	99
5	 R = *p*-$MeOC_6H_4$	H_3BO_3	10	**2**	99
6	R = *p*-$MeOC_6H_4$	PTSA	10	**2**	99
7	R = $PhCH_2$	H_3BO_3	10	**3**	99
8	R = CMe_3	H_3BO_3	10	**27** $PhCH_2NHMe_3$	99
9	R = CMe_3	$PhCO_2H$	10	**27**	99
10	R = Me	PTSA	10	**28** $PhCH_2NHMe$	99
11	*c*-$C_6H_{11}CH{=}NC_6H_4OMe$-*p*	H_3BO_3	10	**10**	99
12	*c*-$C_6H_{11}CH{=}NCH_2C_6H_5$	PTSA	10	**11**	99
13		H_3BO_3	10	**12**	99
14		$PhCO_2H$	10		99
15		H_3BO_3	10	**29** 	99
16		PTSA	10		99
17		H_3BO_3	10	**30** 	99
18		PTSA	10		99
19	 X = H	None	180	[c]	99
20	X = H	H_3BO_3	20	 **31** X = H	99
21	X = H	$PhCO_2H$	20	**31**	99
22	X = *o*-Me	H_3BO_3	20	**32** X = *o*-Me	99
23	X = *p*-Me	H_3BO_3	20	**33** X = *p*-Me	99
24	X = *p*-MeO	H_3BO_3	20	**34** X = *p*-MeO	99
25	X = *p*-Cl	H_3BO_3	60	**35** X = *p*-Cl	99
26	PhC(Me)=NC_6H_4Cl-*p*	PTSA	30	**36** $PhCH(Me)NHC_6H_4Cl$-*p*	99
27	PhC(Et)=NPh	H_3BO_3	40	**37** PhCH(Et)NHPh	99
28		H_3BO_3	20	**14**	99
29		PTSA	20	**14**	99
30		H_3BO_3	10	**38** 	99
31		PTSA	10		99
32		$PhCO_2H$	15		99

[a] Reactions were carried out by simply grinding a 1:1:1 mixture of imine, $NaBH_4$ and activator with an agate mortar and pestle at room temperature.
[b] Isolated yield.
[c] No reaction.

group (entry 12). The reaction of an α-keto ester, ethyl pyruvate, with aniline and benzylamine provided *N*-phenyl and *N*-benzyl alanine ethyl ester (**23** and **24**, respectively), in good yields (entries 9 and 10).

2.2. Indirect reductive amination (imine reduction)

All the direct solvent-free reductive aminations shown in Tables 1–3 were accompanied with reduction of the starting carbonyls to give the corresponding alcohols as side products. To eliminate such disadvantages, we developed an alternative route by a stepwise (or indirect) reductive amination via solvent-free reduction of preformed aldimine or ketimine. Initially the reduction of **1B** was examined as representative. When a 1:1 mixture of **1B** and sodium borohydride was ground in the absence of activator under solvent-free conditions, the reduction proceeded more slowly to give **1** in 26% yield even after 3 h with recovery of unreacted **1B** in 74% yield (entry 1, Table 4). In contrast, reduction of the imine in the presence of 1 equiv of each of activators using the same methodology was complete within 10 min to give the desired amine in a nearly quantitative yield.[21] Again, significant difference of effectiveness among activators was not observed (entries 2–4).[22] All the reduction of other *N*-aryl and alkyl substituted aromatic (entries 5–10), aliphatic (entries 11 and 12) and heterocyclic (entries 13–18) aldimines using the same methodology gave the corresponding secondary amines within 10 min in quantitative yields. Also, all of the aromatic ketimines (entries 20–27), an enamine (entries 28 and 29), and a cyclic imine (entries 30–32) examined were reduced to the desired amines in excellent yields. The results indicated that this procedure underwent clean reductive amination in these reaction conditions. Isolation of pure products without chromatographic separation in all most cases, high yields and the use of inexpensive reagents requiring no special handling techniques are the notable advantages of this method. However, a ketimines, aetophenone *N*-phenylimine, was not reduced by sodium borohydride alone under the identical conditions (entry 19). As shown in Table 5, this methodology also was very effective for the reduction of various aldimines and ketimines bearing other reducible functional groups, such as ketone, nitrile, carboxylic acid, ester, amide, nitro, and alkenyl groups to amines bearing those functional groups in high yields. Unlike a direct process shown in Tables 1–3, the reduction of imines had no discernable effect by a mixing order and period of reactants on the rate of reduction, yield and the formation of side

Table 5. Solvent-free chemoselective reduction of functionalized imines with solid acid-activated $NaBH_4$[a]

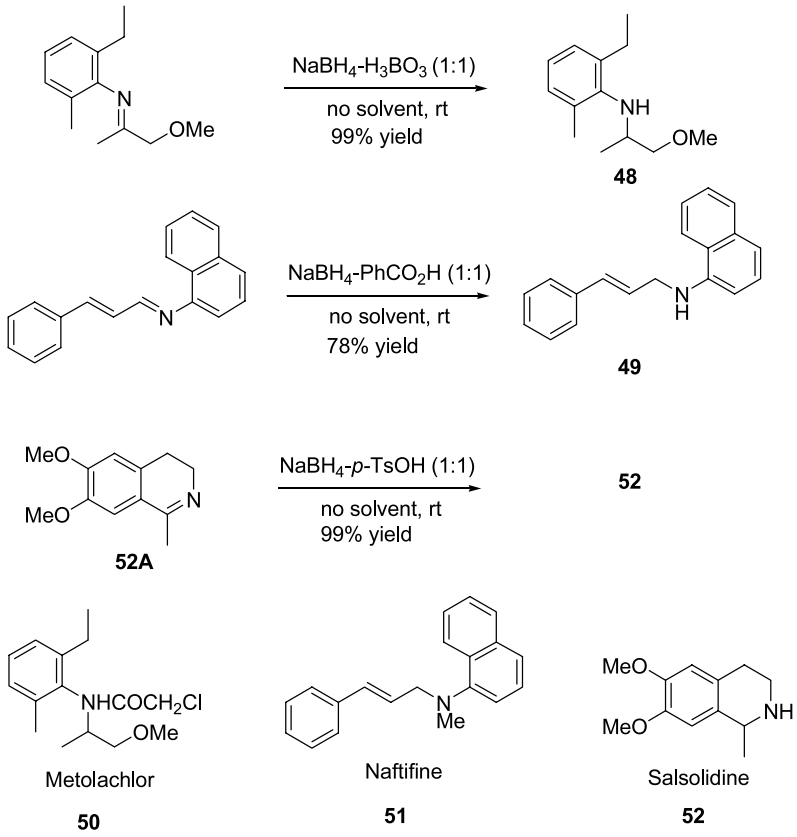
Entry no				Activator	Time (min)		Yield (%)[a]
	R_1	R_2	R_3				
1	*p*-$MeCOC_6H_4$	H	Ph	H_3BO_3	30	**15**	98
2	*p*-$MeCOC_6H_4$	H	Ph	PTSA	40	**15**	98
3	*p*-NCC_6H_4	H	Ph	H_3BO_3	30	**16**	99
4	*p*-NCC_6H_4	H	Me	$PhCO_2H$	20	**16**	97
5	*p*-NCC_6H_4	H	*n*-C_7H_{15}	H_3BO_3	40	**39**	98
6	*p*-NCC_6H_4	H	*n*-C_7H_{15}	$PhCO_2H$	40	**39**	97
7	*p*-NCC_6H_4	H	2-furyl	H_3BO_3	20	**40**	97
8	*p*-NCC_6H_4	H	2-furyl	PTSA	20	**40**	97
9	*p*-$HO_2CC_6H_4$	H	Ph	H_3BO_3	40	**17**	98
10	*p*-$MeO_2CC_6H_4$	H	Ph	H_3BO_3	30	**18**	99
11	*p*-$MeO_2CC_6H_4$	H	Ph	$PhCO_2H$	30	**18**	98
12	*p*-$MeO_2CC_6H_4$	H	Me	H_3BO_3	30	**41**	99
13	*p*-$MeO_2CC_6H_4$	H	Me	PTSA	30	**41**	99
14	*p*-$MeO_2CC_6H_4$	H	CMe_3	H_3BO_3	30	**41**	98
15	*p*-$MeO_2CC_6H_4$	H	CMe_3	PTSA	30	**42**	98
16	*p*-$MeO_2CC_6H_4$	H	CMe_3	$PhCO_2H$	30	**42**	97
17	*p*-$MeCONHC_6H_4$	H	Ph	H_3BO_3	30	**19**	98
18	*p*-$MeCONHC_6H_4$	H	Ph	PTSA	30	**19**	99
19	*p*-$O_2NC_6H_4$	H	Ph	H_3BO_3	40	**20**	99
20	*p*-$O_2NC_6H_4$	H	Ph	PTSA	40	**20**	98
21	*p*-$O_2NC_6H_5$	H	Me	H_3BO_3	30	**43**	99
22	*p*-$O_2NC_6H_5$	H	Me	$PhCO_2H$	30	**43**	97
23	(*E*)-PhCH=CH	H	Ph	H_3BO_3	20	**44**	98
24	(*E*)-PhCH=CH	H	Ph	PTSA	20	**44**	99
25	*p*-NCC_6H_4	Me	Ph	H_3BO_3	40	**45**	98
26	*p*-NCC_6H_4	Me	Ph	PTSA	40	**45**	98
27	*p*-$O_2NC_6H_5$	Me	Ph	H_3BO_3	60	**46**	97
28	*p*-$O_2NC_6H_5$	Me	Ph	$PhCO_2H$	50	**46**	98
29	1-Cyclohexenyl	Me	Ph	H_3BO_3	20	**47**	97
30	1-Cyclohexenyl	Me	Ph	PTSA	20	**47**	97

[a] See the corresponding footnotes in Table 4.

product. Finally, we went on to utilize this methodology to prepare amines **48** and **49**, which can be used as starting materials for synthesis of a herbicide, metolachlor **50** and a topical antifungal agent, naftifine **51**, respectively, and a Salsola alkaloid, salsolidine **52**. As shown in Scheme 3, both of **48** and **52** were successfully obtained from the corresponding imines in nearly quantitative yields, although **49** was obtained in 78% yield through more sluggish reduction. In such solvent-free imine reductions, solid acids used as activator may play a role to form iminium salts, which are easily and selectively reduced to the amines. However, when the reductions of **1B** and 4-acetylbenzaldehyde *N*-phenylimine derivatives with reducing systems generated from grinding a 1:1 mixture of sodium borohydride and activators were carried out, we found that the reductions showed no significant difference in comparison with those given in Tables 4 and 5, respectively. The results suggest that the reduction also occurs selectively in the condition of little chance of the formation of iminium salts by activators. With respect to reducing species of acid-activated sodium borohydride, IR and ^{11}B NMR spectroscopic data of reducing species of $NaBH_4$ activated by solid acids were summarized in Table 6. IR spectra of the reducing agents, reagent A–C, obtained from grinding 1 equiv of $NaBH_4$ with 1 equiv of boric acid, *p*-toluenesulfonic acid and benzoic acid, respectively, showed at 2381 cm^{-1} for reagent A, 2622 cm^{-1} for reagent B and 2562 cm^{-1} for reagent C as medium peaks, which are stretching vibration peaks of B–H bond. Each of ^{1}H decoupled ^{11}B NMR spectra of THF suspension of these reducing agents exhibited at -1.91 ppm for reagent A, $+25.02$ ppm for reagent B and $+23.55$ ppm for reagent C, which were different spectra from those of sodium borohydride itself.[23] Among these, ^{11}B NMR data of reagent B and C indicated the formation of acyl (or sulfonyl)oxyborohydride species [$NaBH_{4-x}(OCOR)_x$ or $NaBH_{4-x}(OSO_2R)_x$].[23] When a 1:1 mixture of $NaBH_4$ and each of activators was ground with excess triethylamine (3 equiv), ^{11}B NMR spectra of THF solution of these reagents showed at -34.40 ± 0.2 ppm indicating the formation of triethylamine–borane complex.[24] This hydride species were stable in air at least for few hours with no loss of hydride activity. Although the structure of this reducing species and the mechanism of this reduction are unclear so far, it appears that a eutectic temperature with melting point lower than the ambient temperature exists in each case. In fact, the reaction mixture became oily or sticky during grinding the mixtures even though they are powder states before grinding. Further studies on the reducing characteristics of these reducing systems for other functional groups are in progress.

3. Conclusion

We have established a direct and indirect reductive amination of aldehydes and ketones using sodium borohydride activated by inorganic and organic solid acid, such



Scheme 3.

Table 6. IR and ^{11}B NMR spectroscopic data of acid-activated $NaBH_4$

Hydride	IR (KBr, cm^{-1})	^{11}B NMR (δ)[a]
$NaBH_4$–H_3BO_3 (1:1, reagent A)	2381 (B–H), 2290	−1.91
$NaBH_4$–PTSA (1:1, reagent B)	2622 (B–H), 1417 (S=O)	+25.02
$NaBH_4$–$PhCO_2H$ (1:1, reagent C)	2562 (B–H), 1682 (C=O)	+23.55

[a] Measured in THF solution of a 1:1 grinding mixture of $NaBH_4$ and the corresponding acid.

as boric acid, *p*-toluenesulfonic acid and benzoic acid, as reducing agents under solvent-free conditions. The use of inexpensive reagents requiring no special handling techniques are the notable advantages of this method. Furthermore, due to compatibility of this reagent system with a variety of otherwise reducible functional groups, this method can provide an easy access to analogues amines bearing functionalized pendant chains.

4. Experimental

4.1. General

The reactions were monitored by TLC using silica gel plates and the products were purified by flash column chromatography on silica gel (Merck; 230–400 mesh). Melting points were uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded at 200 or 300 MHz. The chemical shifts are expressed as δ units with Me_4Si as the internal standard in $CDCl_3$. IR spectra were recorded on an FT-IR spectrophotometer and absorptions are reported in wave numbers (cm^{-1}).

4.2. Materials

Most of organic compounds utilized in this study were commercial products of the highest purity. They were further purified by distillation when necessary. Sodium borohydride, *p*-toluenesulfonic acid monohydrate and benzoic acid were purchased from Aldrich or Lancaster and used without further purification. Aldimines were prepared from stirring a 1:1 mixture of aldehydes and amines at ambient temperature under solvent-free conditions. Ketimines were prepared from reaction of ketones and amines according to the literature procedures.[25,26] 6,7-Dimethoxy-1-methyl-3,4-dihydroisoquinoline (**52A**), which is a starting material of **52** was prepared by known method.[25]

4.3. Direct solvent-free reductive amination of aldehydes and ketones

4.3.1. General procedure. An aldehyde or ketone (5 mmol) was ground with an amine (5 mmol) for 10–15 min in an agate mortar and a pestle at room temperature (ca 25 °C) under solvent-free conditions. To the resulting mixture was

added sodium borohydride (5 mmol) and each boric acid, *p*-toluenesulfonic acid monohydrate, or benzoic acid (5 mmol) and then the mixture was ground under identical conditions until TLC showed complete disappearance of the starting aldehyde. The reaction mixture was quenched with saturated aqueous solution of $NaHCO_3$ (10 ml) and extracted with CH_2Cl_2 or ether (3×10 ml). The combined extract was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude products obtained were further purified by a flash column chromatography on silica-gel (230–400 mesh) using a suitable solvent as eluent. IR, ^1H and ^{13}C NMR spectra of compounds **15**–**21** and **25** obtained from this experiment are identical with those reported in literature.[19]

4.3.2. *N*-Phenylbenzylamine (1). R_f 0.66 (Et_2O/hexane 1:2); oil; 94% yield using boric acid as activator; IR (neat, cm^{-1}) 3384, 3003, 1600, 1498, 1321; ^1H NMR (200 MHz, $CDCl_3$) δ 3.94 (br s, 1H), 4.28 (s, 2H), 6.60 (d, 2H, J=7.63 Hz), 6.70 (t, 1H, J=7.33 Hz), 7.15 (t, 2H, J=7.44 Hz), 7.22–7.34 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 48.9, 113.5, 118.2, 127.9, 128.2, 129.3, 129.9, 140.1, 148.8. Calcd for $C_{13}H_{13}N$: C, 85.21; H, 7.15; N, 7.64. Found: C, 85.24; H, 7.18; N, 7.61.

4.3.3. *N*-(*p*-Methoxyphenyl)benzylamine (2). R_f 0.59 (EtOAc/hexane 1:2); mp 48–49 °C; 98% yield using boric acid as activator; IR (KBr, cm^{-1}) 3385, 2964, 1613, 1510, 1449, 1239, 1031; ^1H NMR (200 MHz, $CDCl_3$) δ 3.59 (br s, 1H), 3.70 (s, 3H), 4.24 (s, 2H), 6.57 (d, 2H, J=8.85 Hz), 6.76 (d, 2H, J=8.85 Hz), 7.21–7.37 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 49.8, 56.4, 114.7, 115.5, 127.8, 128.2, 129.2, 140.4, 143.1, 152.8. Calcd for $C_{14}H_{15}NO$: C, 78.84; H, 7.09; N, 6.57. Found: C, 78.87; H, 7.15; N, 6.58.

4.3.4. Dibenzylamine (3). R_f 0.59 (EtOAc/hexane 1:2); oil; 88% yield using *p*-toluenesulfonic acid as activator; IR (neat, cm^{-1}) 3303, 3025, 2842, 2817, 1494, 1452, 734, 696; ^1H NMR (200 MHz, $CDCl_3$) δ 1.90 (br s, 1H), 3.79 (s, 4H), 7.21–7.34 (m, 10H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 53.6, 127.7, 129.0, 140.7. Calcd for $C_{14}H_{15}N$: C, 85.24; H, 7.66; N, 7.10. Found: C, 85.27; H, 7.65; N, 7.08.

4.3.5. *N*-Benzyl-1-heptylamine (4). R_f 0.68 (EtOAc/hexane 1:2); oil; 88% yield using boric acid as activator; IR (neat, cm^{-1}) 3202, 2955, 2926, 2856, 1455, 1166, 698; ^1H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, 3H, J=6.74 Hz), 1.08–1.26 (m, 6H), 1.53–1.74 (m, 4H), 2.58–2.70 (m, 2H), 3.62 (dd, 1H, J=13.48, 9.08 Hz), 3.76 (br s, 1H). 4.21 (dd, 1H, J=13.48, 3.03 Hz), 7.27–7.40 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.5, 22.9, 26.6, 27.0, 29.1, 31.9, 53.4, 60.1, 128.9, 129.3, 129.6, 134.5. Calcd for $C_{14}H_{23}N$: C, 81.89; H, 11.29; N, 6.82. Found: C, 81.91; H, 11.33; N, 6.91.

4.3.6. *N*-Benzylcyclohexylamine (5). R_f 0.68 (EtOAc/hexane 1:2); oil; 62% yield using boric acid as activator; IR (neat, cm^{-1}) 3197, 2927, 2853, 1447, 1165, 1028, 697; ^1H NMR (300 MHz, $CDCl_3$) δ 0.72–2.05 (m, 10H), 2.71 (m, 1H), 3.40 (br s, 1H), 3.83 (dd, 1H, J=7.98, 13.75 Hz), 4.03 (dd, 1H, J=13.62, 4.26 Hz), 7.23–7.42 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.4, 25.7, 26.9, 30.5, 56.7, 60.0, 128.6, 128.9, 129.3, 135.2. Calcd for $C_{13}H_{19}N$: C, 82.48; H, 10.12; N, 7.40. Found: C, 82.39; H, 10.15; N, 7.40.

4.3.7. *N*-Phenyl-1-octylamine (6). R_f 0.80 (EtOAc/hexane 1:2); oil; 83% yield using boric acid as activator; IR (neat, cm^{-1}) 3405, 2956, 2927, 1603, 1506, 1259, 747, 691; ^1H NMR (300 MHz, $CDCl_3$) δ 0.80–0.91 (m, 3H), 1.20–1.55 (m, 10H), 1.57–1.70 (m, 2H), 3.07 (t, 2H, J=7.15 Hz), 3.60 (br s, 1H), 6.48–6.52 (m, 3H), 7.02–7.30 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.6, 23.1, 27.6, 29.6, 30.0, 32.3, 44.3, 54.2, 112.9, 117.3, 129.4, 148.7. Calcd for $C_{14}H_{23}N$: C, 81.89; H, 11.29; N, 6.82. Found: C, 81.94; H, 11.25; N, 6.79.

4.3.8. *N*-Benzyl-1-octylamine (7). R_f 0.61 (EtOAc/hexane 1:2); oil; 81% yield using benzoic acid as activator; IR (neat, cm^{-1}) 3200, 2955, 2927, 2856, 1455, 1168, 698; ^1H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, 3H, J=7.15 Hz), 1.07–1.45 (m, 10H), 1.49–1.73 (m, 2H), 2.58–2.75 (m, 2H), 3.40 (br s, 1H), 3.62 (dd, 1H, J=13.62, 9.22 Hz), 4.20 (dd, 1H, J=13.75, 3.30 Hz), 7.20–7.42 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.4, 22.9, 26.7, 26.9, 29.1, 31.9, 34.8, 53.6, 60.3, 129.0, 129.3, 129.5, 134.6. Calcd for $C_{15}H_{25}N$: C, 82.13; H, 11.49; N, 6.39. Found: C, 82.18; H, 11.55; N, 6.40.

4.3.9. *N*-(*n*-Heptyl)-1-octylamine (8). R_f 0.62 (EtOAc/hexane 1:2); oil; 70% yield using benzoic acid as activator; IR (neat, cm^{-1}) 3201, 2956, 2927, 2856, 1457, 1378, 1169, 1031, 724; ^1H NMR (300 MHz, $CDCl_3$) δ 0.86–0.90 (m, 6H), 1.03–1.42 (18H), 1.60–1.69 (m, 4H), 2.26–2.42 (m, 2H), 2.64–2.85 (m, 2H), 3.61 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.5, 14.6, 22.9, 23.0, 25.4, 26.9, 27.1, 29.3, 29.7, 31.8, 32.0, 32.3, 34.8, 54.4, 55.7. Calcd for $C_{15}H_{33}N$: C, 79.22; H, 14.63; N, 6.16. Found: C, 79.15; H, 14.65; N, 6.18.

4.3.10. *N*-Phenylcyclohexanemethylamine (9). R_f 0.85 (Et_2O/hexane 1:2); oil; 97% yield using benzoic acid as activator; IR (neat, cm^{-1}) 3418, 3050, 2922, 2850, 1603, 1507, 1448, 1323, 746; ^1H NMR (300 MHz, $CDCl_3$) δ 0.88–1.01 (m, 2H), 1.13–1.29 (m, 3H), 1.54 (m, 1H), 1.64–1.81 (m, 5H), 2.90 (d, 2H, J=6.60 Hz), 3.65 (br s, 1H), 6.54 (d, 2H, J=8.53 Hz), 6.63 (t, 1H, J=7.29 Hz), 7.13 (dd, 2H, J=8.53, 7.43 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.5, 27.1, 31.8, 38.0, 51.1, 112.9, 117.1, 129.5, 148.9. Calcd for $C_{13}H_{19}N$: C, 82.48; H, 10.12; N, 7.40. Found: C, 82.45; H, 10.11; N, 7.43.

4.3.11. *N*-(*p*-Methoxyphenyl)cyclohexanemethylamine (10). R_f 0.77 (Et_2O/hexane 2:1); oil; 94% yield using boric acid as activator; IR (neat, cm^{-1}) 3318, 3025, 2971, 2878, 2789, 1515, 1493, 1045, 738, 698; ^1H NMR (300 MHz, $CDCl_3$) δ 0.92–1.10 (m, 2H), 1.12–1.28 (m, 3H), 1.54 {m, 1H}, 1.65–1.83 (m, 5H), 2.89 (d, 2H, J=6.72 Hz), 3.36 (br s, 1H), 3.78 (s, 3H), 6.55 (d, 2H, J=8.85 Hz), 6.76 (d, 2H, J=8.85 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.6, 27.2, 31.9, 38.2, 52.3, 56.4, 114.5, 115.6, 143.7, 152.5. Calcd for $C_{14}H_{21}NO$: C, 76.67; H, 9.65; N, 6.39. Found: C, 76.69; H, 9.63; N, 6.40.

4.3.12. *N*-Benzylcyclohexanemethylamine (11). R_f 0.70 (EtOAc/hexane 1:2); mp 79–81 °C; 77% yield using *p*-toluenesulfonic acid as activator; IR (KBr, cm^{-1}) 3224, 2926, 2842, 1453, 1416, 1169, 902, 695; ^1H NMR (300 MHz, $CDCl_3$) δ 0.97–1.97 (m, 11H), 2.39–2.57 (m, 2H), 3.55–3.62 (m, 2H), 4.23 (d, 1H, J=10.18 Hz), 7.24–7.42 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.6, 25.9, 26.5, 30.3, 31.3, 34.2, 60.3, 60.9, 129.0, 129.3, 129.5, 134.5.

Calcd for $C_{14}H_{21}N$: C, 82.70; H, 10.41; N, 6.89. Found: C, 82.65; H, 10.36; N, 6.92.

4.3.13. *N*-Phenylfurfurylamine (12). R_f 0.48 (EtOAc/hexane 1:2); oil; 97% yield using boric acid as activator; IR (neat, cm^{-1}) 3323, 3028, 2933, 2842, 2788, 1592, 1452, 1029, 737, 697; ^1H NMR (300 MHz, $CDCl_3$) δ 3.98 (br s, 1H), 4.29 (s, 2H), 6.22 (m, 1H), 6.31 (m, 1H), 6.66 (d, 2H, $J=7.63$ Hz), 6.73 (t, 1H, $J=7.33$ Hz), 7.18 (t, 2H, $J=7.94$ Hz), 7.35 (d, 1H, $J=0.92$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 42.0, 107.6, 111.0, 113.8, 118.7, 129.9, 142.7, 148.3, 153.5. Calcd for $C_{11}H_{11}NO$: C, 76.28; H, 6.40; N, 8.09. Found: C, 76.22; H, 6.44; N, 8.15.

4.3.14. *N*-Phenylcyclohexylamine (13). R_f 0.74 (EtOAc/hexane 1:2); mp 79–81 °C; 93% yield using *p*-toluenesulfonic acid as activator; IR (neat, cm^{-1}) 3401, 2939, 2853, 1602, 1502, 1196, 1058, 785; ^1H NMR (300 MHz, $CDCl_3$) δ 1.07–1.45 (m, 5H), 1.60–1.79 (m, 4H), 2.05 (m, 1H), 3.46 (br s, 1H), 3.62 (m, 1H), 6.57 (d, 2H, $J=7.43$ Hz), 6.64 (t, 1H, $J=7.43$ Hz), 7.14 (dd, 2H, $J=8.53$, 7.43 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.4, 25.4, 26.3, 33.8, 44.5, 52.0, 113.3, 113.4, 117.0, 117.1, 129.5, 147.5. Calcd for $C_{12}H_{17}N$: C, 82.23; H, 9.78; N, 7.99. Found: C, 82.27; H, 9.67; N, 7.97.

4.3.15. *N*-Cyclohexylmorpholine (14). R_f 0.23 (EtOAc/hexane 1:1); oil; 35% yield using boric acid as activator; IR (neat, cm^{-1}) 3460, 2927, 2853, 2808, 1450, 1267, 1117, 1069, 1016, 810; ^1H NMR (300 MHz, $CDCl_3$) δ 1.08–1.30 (m, 5H), 1.63 (d, 1H, $J=10.73$ Hz), 1.78–1.91 (m, 4H), 2.18 (m, 1H), 2.56 (t, 4H, $J=4.68$ Hz), 3.72 (t, 4H, $J=4.68$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.1, 26.6, 29.2, 50.0, 64.1, 67.7. Calcd for $C_{10}H_{19}NO$: C, 70.96; H, 11.31; N, 8.28. Found: C, 70.95; H, 11.30; N, 8.29.

4.3.16. *N*-Phenylcitrolnellylamine (22). R_f 0.68 (EtOAc/hexane 1:4); oil; 92% yield using boric acid as activator; IR (neat, cm^{-1}) 3406, 2961, 2922, 2867, 1604, 1505, 1319, 747, 691; ^1H NMR (300 MHz, $CDCl_3$) δ 0.81–1.81 (m, 5H), 0.93 (d, 3H, $J=5.50$ Hz), 1.60 (s, 3H), 1.68 (s, 3H), 1.83–2.04 (m, 2H), 3.10 (m, 2H), 3.54 (br s, 1H), 5.08 (t, 1H, $J=5.78$ Hz), 6.57 (d, 2H, $J=8.53$ Hz), 6.66 (td, 1H, $J=7.29$, 1.1 Hz), 7.14 (t, 2H, $J=7.43$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.2, 20.1, 25.9, 26.3, 30.8, 37.0, 37.5, 42.3, 112.9, 117.3, 124.9, 129.5, 131.6, 148.7. Calcd for $C_{16}H_{25}N$: C, 83.06; H, 10.89; N, 6.05. Found: C, 83.08; H, 10.90; N, 6.04.

4.3.17. *N*-Phenylalanine ethyl ester (23). R_f 0.65 (EtOAc/hexane 1:2); oil; 79% yield using boric acid as activator; IR (neat, cm^{-1}) 3384, 3016, 2980, 2934, 1740, 1604, 1201, 1051, 784; ^1H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, 3H, $J=7.15$ Hz), 1.47 (d, 3H, $J=6.60$ Hz), 4.06–4.26 (m, 2H), 4.18 (q, 2H, $J=7.15$ Hz), 6.59 (dd, 2H, $J=8.53$, 1.1 Hz), 7.16 (dd, 2H, $J=8.53$, 7.43 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.6, 19.4, 52.3, 61.5, 113.6, 118.5, 129.5, 146.7, 174.8. Calcd for $C_{11}H_{15}N0_2$: C, 68.37; H, 7.82; N, 7.25. Found: C, 68.34; H, 7.83; N, 7.24.

4.3.18. *N*-Benzylalanine ethyl ester (24). R_f 0.65 (EtOAc/hexane 1:1); oil; 71% yield using boric acid as activator; IR (neat, cm^{-1}) 3327, 3028, 3979, 2934, 1733, 1453, 1188, 1152, 1064, 737, 699; ^1H NMR (300 MHz, $CDCl_3$) δ 1.29 (t, 3H, $J=7.01$ Hz), 1.32 (d, 3H, $J=6.88$ Hz), 1.90 (br s, 1H), 3.77 (q, 1H, $J=6.88$ Hz), 3.66 (d, 1H, $J=12.65$ Hz), 3.80 (d, 1H, $J=12.65$ Hz), 4.18 (q, 2H, $J=7.15$ Hz), 7.23–7.33 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.7, 19.6, 52.3, 56.3, 61.1, 127.3, 128.5, 128.6, 139.9, 175.9. Calcd for $C_{12}H_{17}N0_2$: C, 69.54; H, 8.27; N, 6.76. Found: C, 69.59; H, 8.30; N, 6.69.

4.3.19. Compound 26. R_f 0.61 (EtOAc/hexane 1:4); oil; 25% yield using boric acid as activator; IR (neat, cm^{-1}) 3409, 2961, 2926, 2864, 1602, 1504, 1318, 1258, 973, 748, 691; ^1H NMR (300 MHz, $CDCl_3$) δ 0.89 (s, 3H), 0.94 (s, 3H), 1.35 (d, 3H, $J=6.60$ Hz), 1.38–1.44 (m, 2H), 1.47–1.69 (m, 2H), 1.60 (s, 3H), 1.80–1.95 (m, 2H), 3.60 (br s, 1H), 4.00 (quintet, 1H, $J=6.46$ Hz), 5.30 (dd, 1H, $J=15.82$, 6.19 Hz), 6.01 (d, 1H, $J=15.68$ Hz), 6.55–6.67 (m, 3H), 7.10–7.15 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.2, 20.1, 25.9, 26.3, 30.8, 37.0, 37.5, 42.3, 112.9, 117.3, 124.9, 129.5, 131.6, 148.7. Calcd for $C_{19}H_{27}N$: C, 84.70; H, 10.10; N, 5.20. Found: C, 84.68; H, 10.09; N, 5.18.

4.4. Solvent-free reduction of imines with solid acid-activated $NaBH_4$

4.4.1. General procedure. A mixture of imine derivatives (5 mmol), sodium borohydride (5 mmol) and each boric acid, *p*-toluenesulfonic acid monohydrate or benzoic acid (5 mmol) was ground with an agate mortar and pestle at room temperature (ca 25 °C) for 0.5–1.0 h until TLC showed complete disappearance of the starting material. Work-up procedures for isolation of product amines were identical with those described in Section 4.3.1. Among those, compounds **1–3**, **10–12**, **14** and **27–38** were isolated as nearly pure form without chromatographic separation. IR, ^1H and ^{13}C NMR spectra of compounds **39–47** obtained from this experiment are identical with those reported in literature.[19]

4.4.2. *N*-(*tert*-Butyl)benzylamine (27). Oil; 99% yield using boric or benzoic acid as activator; IR (neat, cm^{-1}) 3319, 3027, 2789, 1455, 1358, 1104, 736, 700; ^1H NMR (200 MHz, $CDCl_3$) δ 1.18 (s, 9H), 1.26 (br s, 1H), 3.73 (s, 2H), 7.31–7.34 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 29.8, 47.9, 51.3, 127.4, 129.1, 142.2, 155.5. Calcd for $C_{11}H_{17}N$: C, 80.93; H, 10.50; N, 8.58. Found: C, 80.95; H, 10.49; N, 8.59.

4.4.3. *N*-Methylbenzylamine (28). Oil; 99% yield using *p*-toluenesulfonic acid as activator; IR (neat, cm^{-1}) 3272, 2933, 2842, 2786, 1496, 1360, 1104, 704; ^1H NMR (200 MHz, $CDCl_3$) δ 1.35 (br s, 1H), 2.42 (s, 3H), 3.72 (s, 2H), 7.19–7.31 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 36.6, 56.6, 127.5, 128.7, 128.9, 140.8. Calcd for $C_8H_{11}N$: C, 79.29; H, 9.15; N, 11.56. Found: C, 79.30; H, 9.23; N, 11.51.

4.4.4. *N*-Phenyl-(2′-thiophenemethyl)amine (29). Oil; 99% yield using boric or *p*-toluenesulfonc acid as activator; IR (neat, cm^{-1}) 3281, 3027, 1609, 1515, 1452, 761, 734; ^1H NMR (300 MHz, $CDCl_3$) δ 3.98 (br s, 1H), 4.45 (s, 2H), 6.63 (d, 2H, $J=8.24$ Hz), 6.72 (t, 1H, $J=7.33$ Hz), 6.91–6.98 (m, 2H), 7.13–7.20 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 44.2, 113.8, 118.7, 125.3, 125.7, 127.5, 129.9,

143.6, 148.2. Calcd for $C_{11}H_{11}NS$: C, 69.80; H, 5.86; N, 7.40; S, 16.94. Found: C, 69.84; H, 5.95; N, 7.21; S, 16.82.

4.4.5. *N*-Phenyl-(2′-pyridinemethyl)amine (30). Oil; 99% yield using boric or *p*-toluenesulfonc acid as activator; IR (neat, cm^{-1}) 3416, 3028, 2972, 2843, 2788, 1566, 1542, 1028, 737, 700; ^1H NMR (300 MHz, $CDCl_3$) δ 4.45 (s, 3H), 6.65 (d, 2H, $J=7.63$ Hz), 6.71 (t, 1H, $J=7.33$ Hz), 7.13–7.20 (m, 3H), 7.32 (d, 1H, $J=7.63$ Hz), 7.62 (td, 1H, $J=1.53$, 7.11 Hz), 8.57 (d, 1H, $J=4.27$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 49.8, 1137, 118.2, 122.2, 122.7, 129.8, 137.3, 148.5, 149.7, 159.2. Calcd for $C_{12}H_{12}N_2$: C, 78.23; H, 6.57; N, 15.21. Found: C, 78.27; H, 6.67; N, 15.32.

4.4.6. *N*-Phenyl-1-phenylethylamine (31). Oil; 99% yield using boric acid as activator; IR (neat, cm^{-1}) 3411, 3055, 2926, 1602, 1503; ^1H NMR (300 MHz, $CDCl_3$) δ 1.47 (d, 3H, $J=6.88$ Hz), 3.99 (br s, 1H), 4.44 (q, 1H, $J=6.69$ Hz), 6.46 (d, 2H, $J=7.43$ Hz), 6.61 (t, 1H, $J=7.29$ Hz), 7.05 (dd, 2H, $J=8.53$, 7.43 Hz), 7.17 (m, 1H), 7.2–7.34 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.6, 53.8, 113.6, 117.5, 126.1, 127.2, 128.9, 129.4, 145.5, 147.5. Calcd for $C_{14}H_{15}N$: C, 85.24; H, 7.66; N, 7.10. Found: C, 85.27; H, 7.73; N, 7.08.

4.4.7. *N*-Phenyl-1-(*o*-tolyl)ethylamine (32). Oil; 99% yield using boric acid as activator; IR (neat, cm^{-1}) 3411, 3050, 3016, 2967, 2925, 2867, 1601, 1504, 1318, 749, 691; ^1H NMR (300 MHz, $CDCl_3$) δ 1.45 (d, 3H, $J=6.72$ Hz), 2.42 (s, 3H), 3.97 (br s, 1H), 4.65 (q, 1H, $J=6.61$ Hz), 6.43 (d, 2H, $J=8.24$ Hz), 6.62 (t, 1H, $J=6.72$ Hz), 7.03–7.18 (m, 5H), 7.40 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.6, 23.6, 50.5, 113.7, 117.8, 125.4, 127.3, 129.8, 131.3, 135.2, 143.4, 148.0. Calcd for $C_{15}H_{17}N$: C, 85.26; H, 8.11; N, 6.63. Found: C, 85.27; H, 8.14; N, 6.56.

4.4.8. *N*-Phenyl-1-(*p*-tolyl)ethylamine (33). Oil; 99% yield using boric acid as activator; IR (neat, cm^{-1}) 3407, 3047, 2964, 2919, 2868, 1602, 1505, 1322, 750, 693; ^1H NMR (300 MHz, $CDCl_3$) δ 1.45 (d, 3H, $J=6.60$ Hz), 2.28 (s, 3H), 3.79 (br s, 1H), 4.40 (q, 1H, $J=6.69$ Hz), 6.46 (d, 2H, $J=7.70$ Hz), 6.59 (t, 1H, $J=7.29$ Hz), 7.01–7.11 (m, 4H), 7.20 (d, 2H, $J=7.98$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.6, 25.6, 53.6, 113.6, 117.5, 126.1, 129.4, 129.7, 136.7, 142.5, 147.6. Calcd for $C_{15}H_{17}N$: C, 85.26; H, 8.11; N, 6.63. Found: C, 85.31; H, 8.12; N, 6.67.

4.4.9. *N*-Phenyl-1-(*p*-methoxyphenyl)ethylamine (34). Oil; 99% yield using boric acid as activator; IR (neat, cm^{-1}) 3401, 2962, 2833, 1609, 1504, 1238, 1179, 1035, 831, 751, 695; ^1H NMR (300 MHz, $CDCl_3$) δ 1.46 (d, 3H, $J=6.88$ Hz), 3.74 (s, 3H), 3.82 (s, 1H), 4.41 (q, 1H, $J=6.69$ Hz), 6.48 (dd, 2H, $J=8.67$, 0.93 Hz), 6.61 (t, 1H, $J=7.29$ Hz), 6.82 (d, 2H, $J=8.80$ Hz), 7.06 (dd, 2H, $J=7.43$, 8.53 Hz), 7.24 (d, 2H, $J=8.80$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.5, 53.2, 55.6, 113.5, 114.2, 117.4, 127.1, 129.3, 137.5, 147.5, 158.6. Calcd for $C_{15}H_{17}NO$: C, 79.26; H, 7.54; N, 6.16. Found: C, 79.32; H, 7.62; N, 6.34.

4.4.10. *N*-Phenyl-1-(*p*-chlorophenyl)ethylamine (35). Oil; 99% yield using boric acid as activator; IR (neat, cm^{-1}) 3408, 3050, 2965, 2924, 2866, 1604, 1506, 1322, 1013, 755, 696; ^1H NMR (300 MHz, $CDCl_3$) δ 1.43 (d, 3H, $J=6.88$ Hz), 3.83 (br s, 1H), 4.39 (q, 1H, $J=6.69$ Hz), 6.43 (d, 2H, $J=8.53$ Hz), 6.63 (t, 1H, $J=7.30$ Hz), 7.05 (t, 2H, $J=7.84$ Hz), 7.09–7.30 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.6, 53.4, 113.6, 117.8, 127.5, 129.1, 129.4, 132.6, 144.1, 147.2. Calcd for $C_{14}H_{14}NCl$: C, 72.57; H, 6.09; N, 6.04. Found: C, 72.55; H, 6.05; N, 6.12.

4.4.11. *N*-(*p*-Chlorophenyl)-1-phenylethylamine (36). Mp 59–60 °C; 99% yield using *p*-toluenesulfonic acid as activator; IR (KBr, cm^{-1}) 3432, 2997, 2866, 1596, 1496; ^1H NMR (300 MHz, $CDCl_3$) δ 1.48 (d, 3H, $J=6.88$ Hz), 4.03 (br s, 1H), 4.40 (q, 1H, $J=6.69$ Hz), 6.38 (d, 2H, $J=8.80$ Hz), 6.99 (d, 2H, $J=9.08$ Hz), 7.17–7.36 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.5, 53.9, 114.6, 122.0, 126.0, 127.3, 128.9, 129.1, 144.9, 145.9. Calcd for $C_{14}H_{14}NCl$: C, 72.57; H, 6.09; N, 6.04. Found: C, 72.52; H, 6.12; N, 6.09.

4.4.12. *N*-Phenyl-1-phenylpropylamine (37). Oil; 99% yield using boric acid as activator; IR (neat, cm^{-1}) 3411, 3025, 2963, 2930, 1604, 1509, 1318, 747, 701; ^1H NMR (200 MHz, $CDCl_3$) δ 0.94 (t, 3H, $J=7.33$ Hz), 1.81 (quintet, 2H, $J=7.10$ Hz), 4.04 (br s, 1H), 4.21 (t, 2H, $J=6.72$ Hz), 6.50 (d, 2H, $J=7.63$ Hz), 6.02 (t, 1H, $J=7.33$ Hz), 7.07 (t, 2H, $J=7.94$ Hz), 7.16–7.35 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.4, 32.3, 60.4, 113.9, 117.8, 127.2, 127.5, 129.2, 129.8, 144.6, 148.2. Calcd for $C_{15}H_{17}N$: C, 85.26; H, 8.11; N, 6.63. Found: C, 85.29; H, 8.17; N, 6.72.

4.4.13. 2,3,3-Trimethylindoline (38). Oil; 99% yield using benzoic or *p*-toluenesulfonic acid as activator; IR (neat, cm^{-1}) 3362, 2960, 2861, 1612, 1467, 1378, 1248, 1164, 748; ^1H NMR (300 MHz, $CDCl_3$) δ 1.03 (s, 3H), 1.16 (d, 3H, $J=6.33$ Hz), 1.27 (s, 3H), 3.48 (q, 1H, $J=6.51$ Hz), 3.64 (br s, 1H), 6.58 (d, 1H, $J=7.70$ Hz), 6.71 (m, 1H), 6.95–7.01 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.6, 22.8, 26.6, 43.7, 65.5, 109.6, 119.1, 122.5, 127.4, 139.3, 149.5. Calcd for $C_{11}H_{15}N$: C, 81.94; H, 9.38; N, 8.69. Found: C, 81.82; H, 9.31; N, 8.54.

4.4.14. 1-Methoxy-2-(2′-ethyl-6′-methylanilino)propane (metolachlor base) (48). R_f 0.62 (EtOAc/hexane 1:2); oil; 99% yield using boric acid as activator; IR (neat, cm^{-1}) 3382, 2964, 2929, 2873, 1593, 1462, 1256, 1105, 756; ^1H NMR (300 MHz, $CDCl_3$) δ 1.17 (d, 3H, $J=6.60$ Hz), 1.22 (t, 3H, $J=7.43$ Hz), 2.27 (s, 3H), 2.63 (q, 2H, $J=7.61$ Hz), 3.31 (d, 2H, $J=1.38$ Hz), 3.33 (m, 1H), 3.35 (s, 3H), 6.84 (t, 1H, $J=7.43$ Hz), 6.95–7.01 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.0, 18.9, 19.4, 24.6, 53.2, 59.4, 76.5, 121.9, 126.7, 128.9, 130.0, 135.7, 144.5. Calcd for $C_{13}H_{21}NO$: C, 75.32; H, 10.21; N, 6.76. Found: C, 75.26; H, 10.17; N, 6.80.

4.4.15. *N*-(*trans*-Cinnamyl)-1-naphthylmethylamine (naftifine base) (49). R_f 0.08 (EtOAc/hexane 1:2); 39–40 °C (lit.[28] 39.5–40.5 °C); 78% yield using benzoic acid as activator; IR (KBr, cm^{-1}) 3314, 3057, 3025, 2920, 2817, 1597, 1494, 1447, 967, 779, 692; ^1H NMR (300 MHz, $CDCl_3$) δ 1.88 (br s, 1H), 3.50 (dd, 2H, $J=6.33$, 1.10 Hz), 4.24 (s, 2H), 6.33 (dt, 1H, $J=15.68$, 6.26 Hz), 6.54 (d, 1H, $J=15.95$ Hz), 7.15–7.53 (m, 9H), 7.74 (d, 1H, $J=7.98$ Hz), 7.83 (d, 1H, $J=7.70$ Hz), 8.08 (d, 1H, $J=7.98$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 51.2, 52.1, 123.9, 125.9, 125.7, 126.0, 126.4, 126.5, 126.6, 127.7, 128.1, 128.5, 129.0, 131.9, 132.0, 135.9, 137.3. Calcd for $C_{20}H_{19}N$: C, 87.87; H, 7.01; N, 5.12. Found: C, 87.89; H, 7.02; N, 5.15.

4.4.16. 6,7-Dimethoxy-1-methyl-1,2,3,4-tetrahydroisoquinoline (salsolidine) (52). R_f 0.16 (EtOAc/MeOH 4:1); mp 47–49 °C (lit.[27] 47–49 °C); 99% yield using *p*-toluenesulfonic acid as activator; IR (KBr, cm^{-1}) 3212, 2939, 2380, 2267, 1612, 1513, 1465, 1260, 1167; ^1H NMR (300 MHz, $CDCl_3$) δ 1.72 (d, 3H, $J=6.60$ Hz), 2.94–3.15 (m, 2H), 3.28 (m, 1H), 3.45 (m, 1H), 3.84 (s, 6H), 4.46 (q, 1H, $J=6.41$ Hz), 4.97 (br s, 1H), 6.60 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.3, 25.6, 50.7, 56.2, 108.7, 111.3, 123.7, 125.8, 148.0, 148.3. Calcd for $C_{12}H_{17}NO_2$: C, 69.54; H, 8.27; N, 6.76. Found: C, 69.57; H, 8.25; N, 6.77.

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References and notes

1. Samuelsson, G. *Drugs of Natural Origin*; Swedish Pharmaceutical: Stockholm, 1992; p 214.
2. (a) Sharp, D. B. In *Herbicides: Chemistry, Degradation, and Mode of Action*; Kearney, P. C., Kaufman, D. D., Eds.; Marcel Dekker: New York, 1988; Chapter 7, p 301. (b) Lebaron, H. M.; Mcfarland, J. E.; Simoneaux, B. J. In *Herbicides: Chemistry, Degradation, and Mode of Action*; Kearney, P. C., Kaufman, D. D., Eds.; Marcel Dekker: New York, 1988; Chapter 7, p 335.
3. (a) Rylabder, P. N. *Hydrogenation Methods*; Academic: New York, 1985. (b) Tarasevich, V. A.; Kozlov, N. G. *Russ. Chem. Rev.* **1999**, *68*, 55.
4. (a) Borch, R. F.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897. (b) Borch, R. F.; Durst, H. D. *J. Am. Chem. Soc.* **1969**, *91*, 3996. (c) Hutchins, R. O.; Markowitz, M. *J. Org. Chem.* **1981**, *46*, 3571. (d) Kim, S.; Oh, C. H.; Ko, J. S.; Ahn, K. H.; Kim, Y. J. *J. Org. Chem.* **1985**, *50*, 1927. (e) Mattson, R. J.; Pham, K. M.; Leuck, D. J.; Cowen, K. A. *J. Org. Chem.* **1990**, *55*, 2552. (f) Brussee, J.; van Benthem, R. A. T. M.; Kruse, C. G.; van der Gen, A. *Tetrahedron: Asymmetry* **1990**, *1*, 163.
5. Abdel-Magid, A. F.; Carson, K. G.; Haris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849.
6. (a) Perissamy, M.; Devasagayaraj, A.; Satyanarayana, N.; Narayana, C. *Synth. Commun.* **1989**, *19*, 565. (b) Saxena, I.; Borah, R.; Sarma, J. C. *J. Chem. Soc., Perkin Trans. 1* **2000**, 503. (c) Itsuno, S.; Sakurai, Y.; Ito, K. *Chem. Commun.* **1988**, 995. (d) Bhattacharyya, S. *J. Org. Chem.* **1995**, *60*, 4928. (e) Verardo, G.; Giumanini, A. G.; Strazzolini, P.; Poiana, M. *Synthesis* **1993**, 121. (f) Varma, R. S.; Dahiya, R. *Tetrahedron* **1998**, *54*, 6293.
7. Yoon, N. M.; Kim, E. G.; Son, H. S.; Choi, J. *Synth. Commun.* **1993**, *23*, 1595.
8. (a) Kotsuki, H.; Yoshimura, N.; Kadota, I.; Ushio, Y.; Ochi, M. *Synthesis* **1990**, 401. (b) Bhattacharyya, S.; Chatterjee, A.; Williamson, J. S. *Synth. Commun.* **1997**, *27*, 4265. (c) Ranu, B. C.; Majee, A.; Sarkar, A. *J. Org. Chem.* **1998**, *63*, 370.
9. (a) Bomann, M. D.; Guch, I. C.; DiMare, M. *J. Org. Chem.* **1995**, *60*, 5995. (b) Pelter, A.; Rosser, R. M.; Mills, S. *J. Chem. Soc., Perkin Trans. 1* **1984**, 717.
10. Sato, S.; Sakamoto, T.; Miyazawa, E.; Kikugawa, Y. *Tetrahedron* **2004**, *60*, 7899.
11. Nose, A.; Kudo, T. *Chem. Pharm. Bull.* **1986**, *34*, 4817.
12. Bae, J. W.; Lee, S. H.; Cho, Y. J.; Yoon, C. M. *J. Chem. Soc., Perkin Trans. 1* **2000**, 145.
13. Micćović, I. V.; Ivanović, M. D.; Piatak, D. M.; Bojić, V. D. *Synthesis* **1991**, 1043.
14. (a) Chandrasekhar, S.; Reddy, C. R.; Ahmed, M. *Synlett* **2000**, 1655. (b) Chandrasekhar, S.; Reddy, C. R.; Chandraiah, L. *Synth. Commun.* **1999**, *29*, 3981. (c) Lopez, R. M.; Fu, G. C. *Tetrahedron* **1997**, *53*, 16349. (d) Chen, B.-C.; Sundeen, J. E.; Guo, P.; Bednarz, M. S.; Zhao, R. *Tetrahedron Lett.* **2001**, *42*, 1245. (e) Blackwell, J. M.; Sonmor, E. R.; Scoccitti, T.; Piers, W. E. *Org. Lett.* **2000**, *2*, 3921. (f) Kobayashi, S.; Yasuda, M.; Hachiya, I. *Chem. Lett.* **1996**, 407. (g) Apodaca, R.; Xiao, W. *Org. Lett.* **2001**, *3*, 1745.
15. (a) Suwa, T.; Sugiyama, E.; Shibata, I.; Baba, A. *Synthesis* **2000**, 558 see also p 789. (b) Hiroi, R.; Miyoshi, N.; Wada, M. *Chem. Lett.* **2002**, 274. (c) Suwa, T.; Shibata, I.; Nishino, K.; Baba, A. *Org. Lett.* **1999**, *1*, 1579. (d) Shibata, I.; Moriuchi-Kawakami, T.; Tanizawa, D.; Suwa, T.; Sugiyama, E.; Matsuda, H.; Baba, A. *J. Org. Chem.* **1998**, *63*, 383.
16. Pereyre, M.; Quintard, J.-P.; Rahm, A. *Tin in Organic Synthesis*; Butterworths: London, 1987; p 6.
17. Narasimhan, S.; Balakumar, R. *Aldrichimica Acta* **1998**, *31*, 19.
18. Reding, M. T.; Buchwald, S. L. *J. Org. Chem.* **1995**, *60*, 7884.
19. Cho, B. T.; Kang, S. K. *Synlett* **2004**, 1484. As other reducing agents for solvent-free reductive amination, $NaBH_4$–wet clay-microwave,[6f] picoline–borane[10] and $^nBu_3SnH$–SiO_2[15b] were also reported.
20. Tanaka, K. *Solvent-Free Organic Synthesis*; Wiley-VCH: Weinheim, 2003.
21. When the same reductions of benzaldehyde *N*-phenylimine **1B** were carried out in THF at room temperature, the reductions proceeded very slowly to give **1** in 41% yield (98 h) with $NaBH_4$ itself, 56% yield (98 h) with $NaBH_4$–H_3BO_3, 82% yield (98 h) with $NaBH_4$–PTSA, and 80% yield (98 h) with $NaBH_4$–$PhCO_2H$.
22. For the reduction of **1B**, we also examined effect of other solid acids, such as *p*-nitro (or chloro)benzoic acids and benzene sulfonic acids, as activators. We did not observe any significant difference of effectiveness among those acids including boric acid, PTSA and benzoic acid.
23. IR and ^{11}B NMR spectra of $NaBH_4$ showed at 2610 cm^{-1} (KBr) and -36.28 ppm (in diglyme), respectively. In contrast, IR spectrum of $NaBH(OAc)_3$ purchased from Aldrich Chemical Co. exhibited at 2499 and 1682 cm^{-1} as a stretching vibration bond of B–H and C=O, respectively. ^{11}B NMR spectrum for this reagent showed at $+20.03$ ppm as major peak.
24. ^{11}B NMR spectrum of triethylamine–borane complex purchased from Aldrich Chemical Co. as authentic sample exhibited at -34.40 ppm.
25. Guthrie, R. D.; Burdon, L. G.; Lovell, F. L., Jr. *J. Org. Chem.* **1973**, *38*, 3114.
26. Landor, S. R.; Sonola, O. O.; Tachel, A. R. *J. Chem. Soc., Perkin Trans. 1* **1978**, 605.
27. Battersby, A. R.; Edwards, T. P. *J. Chem. Soc.* **1960**, 1214.
28. Kanno, H.; Taylor, R. J. K. *Tetrahedron Lett.* **2002**, *43*, 7337.

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Generation of quinone methide from aminomethyl(hydroxy)arenes precursors in aqueous solution

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Abstract—*o*-Quinone methides (QMs) are an important reactive intermediate for organic synthetic and biological standpoints of view. Photochemical and thermal transformation of *N*,*N*-dialkyl-9-aminomethyl-10-phenanthrols and their naphthalene analogs, which act as QM precursors, has been studied. These precursors readily reacted with alkyl vinyl ethers to give 2-alkoxydibenzo[*f*,*h*]chroman and 2-alkoxybenzo[*f*]chroman, respectively. Thermal and photochemical generation of QM was accelerated by the presence of water molecule in reaction solvents and by the formation of anionic micelle and vesicle.

1. Introduction

Photochemical reactions in aqueous solution have been received much attention from viewpoints of environmentally benign process. However, the advantages of use of water as reaction solvent are still low, because many reactions in aqueous solution are inefficient than those in organic solvents. Recently, much attention has been paid to the water-enhancing effect on the generation of *o*-quinone methide and the analogs (QMs), which are one of the important reactive intermediates from synthetic and biological standpoints of view.[1] Wan et al., for example, have reported on the photochemical generation of QMs by the elimination of water from *o*-(hydroxymethyl)phenol derivatives.[2] They proposed that an intramolecular proton transfer in the excited state of *o*-hydroxystyrene was accelerated by intervention of water molecule that was named ESIPT (exited state intramolecular proton transfer) mechanism, resulting in more effective reaction in aqueous organic solvent than water-free solvents.[3] Yate[4] and our group[5] have reported the nucleophilic addition to QM via proton transfer in aqueous solution. Also, Nakatani and his co-workers have reported on the photochemical generation of QM from *N*,*N*-dimethyl-6-phenyl-2-(aminomethyl)phenol which is highly accelerated in aqueous solvents.[6] Freccero has reported the photochemical generation of QM using (2-hydroxybenzyl)trimethylammonium salts as more effective precursors in water.[7] In order to develop the efficient generation of QMs in aqueous solution, we will report on solvent and micelle effects on the photochemical and thermal transformations from aminomethyl(hydroxy)arenes (**1**) to QMs.

2. Results and discussion

2.1. Products

N,*N*-Dialkyl-9-aminomethyl-10-phenanthrols (**1a–b**) and *N*,*N*-dialkyl-1-aminomethyl-2-naphthols (**1c–e**) were easily prepared by Mannich reactions of 9-phenanthrol and 2-naphthol with the alkylamines in the presence of formalin, respectively. The thermal reaction of *N*,*N*-dimethyl-9-aminomethyl-10-phenanthrol (**1a**) with ethyl vinyl ether (**2a**) proceeded in aqueous MeOH, MeCN, DMF, and THF solutions at 50 °C to give 2-ethoxydibenzo[*f*,*h*]chroman (**3a**), but did not proceed in water-free MeOH and THF solutions (Table 1). 9,10-Phenanthrenequinone (**4**) was produced as a by-product from the thermal reaction of **1a–b** in MeCN–H_2O and DMF–H_2O.

The photoreactions of **1** with vinyl ethers (**2**) were carried out by irradiating a degassed solution containing **1** and **2** by a high-pressure mercury lamp through a Pyrex filter ($\lambda > 280$ nm) at 20 °C (Scheme 1). The photoreaction of **1a**

Keywords: Quinone methide; Cycloaddition; Micelle effect; Photochemical generation.
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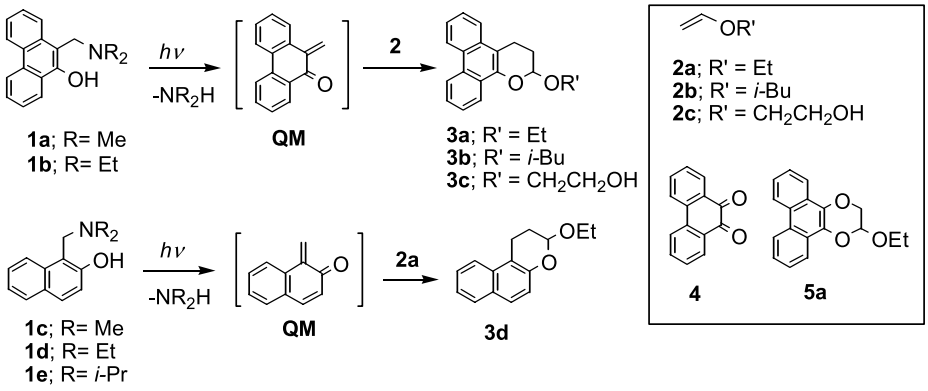
Table 1. Photoreaction of **1** with **2a** in aqueous solution

1	Solvent	Time (h)	Method[a]	3 (Yields/%)[b]
1a	MeCN–H_2O (6:4)	10	A	**3a** (74) [0]
1a	DMF–H_2O (6:4)	10	A	**3a** (30) [0]
1a	MeOH–H_2O (6:4)	10	A	**3a** (25) [0]
1a	THF–H_2O (6:4)	10	A	**3a** (4) [0]
1b	MeCN–H_2O (6:4)	10	A	**3a** (78)
1c	MeCN–H_2O (6:4)	20	A	**3d** (20, 17[c])
1d	MeCN–H_2O (6:4)	20	A	**3d** (30)
1e	MeCN–H_2O (6:4)	20	A	**3d** (43)
1a	MeCN–H_2O (6:4)	10	B	**3a** (50)[d] [0]
1a	MeOH–H_2O (6:4)	10	B	**3a** (84) [0]
1a	DMF–H_2O (6:4)	10	B	**3a** (66)[e] [0]
1a	THF–H_2O (6:4)	10	B	**3a** (70) [0]
1b	MeCN–H_2O (6:4)	10	B	**3a** (72)
1c	MeCN–H_2O (6:4)	10	B	**3d** (15)
1d	MeCN–H_2O (6:4)	10	B	**3d** (44)
1e	MeCN–H_2O (6:4)	10	B	**3d** (58)

[a] Method A: photoreaction at room temperature. Method B: thermal reaction at 50 °C.
[b] The values in the blanket are the yields in the solvents in the absence of water.
[c] The value is the reported yield (Ref. 6).
[d] Accompanied by the formation of 9,10-phenanthrenequinone (**4**) in 32% yield.
[e] Accompanied by the formation of **4** in 6% yield.



Scheme 1. Photoreactions of **1** with vinyl ethers (**2**).

with ethyl vinyl ether (**2a**) in MeCN–H_2O (6:4) gave selectively **3a** in 74% yield without the formation of **4**. However, the photoreaction of **1a** at higher temperature in MeCN–H_2O gave **3a** and 2-ethoxy-4-oxodibenzo[*f*,*h*]-chroman (**5a**) which was formed as a consequence of the photoreaction of **4** with **2a**. As shown in Table 1, the reaction yields were depending on the solvent used. The photoreaction of **1a** with **2a** proceeded more efficiently in aqueous MeCN solution compared with that in MeOH–H_2O and THF–H_2O. It is noteworthy that no reaction occurred entirely in the water-free solvents and even in MeOH regardless of the presence of OH group. Therefore, the presence of water was requisite for the efficient formation of **3** in the photochemical and thermal reactions.

The photochemical and thermal reactions of *N*,*N*-dimethyl-1-aminomethyl-2-naphthol (**1c**) with enamines have been previously reported.[8] However, the yields of the cyclo-adducts were relatively low compared with the case of **1a**. Also, Nakatani et al. have reported that the photoreaction of **1c** with **2a** in MeCN–H_2O (6:4) gave 2-ethoxybenzo[*f*]-chroman (**3d**) but the yield was still low.[6] In order to improve the chemical yields of **3d**, we performed the reaction of *N*,*N*-dialkyl-1-aminomethyl-2-naphthols (**1d–e**) containing more electron-donating alkyl groups on the amino group. The yields were slightly improved in thermal and photochemical reactions.

2.2. Micelle effect

The photocycloaddition of **1a** with **2a** proceeded efficiently in aqueous solution of sodium dodecylsulfate (SDS) that formed a micelle (aggregation number, AN, is 62) in concentrations higher than critical micelle concentration (CMC = 8.1 mM). Figure 1 shows the dependence of the yields of **3a** on the concentration of SDS. In the case of the photochemical reaction, the reaction yields were remarkably enhanced in the presence of SDS of concentrations higher than CMC to reach maximum yield at >9 mM. In the thermal reaction, however, the yield increased gradually, showing no sharp enhancing effect at CMC. It might be due to the disorder of the micelle structure in higher temperature. The formation of **3** occurred effectively in aqueous solution of sodium 1,2-bis(alkyloxycarbonyl)-ethanesulfonate (BES_n: $n=10$ for decyl and 12 for dodecyl groups), which was tend to form a vesicle in aqueous solution.[9,10] Figure 2 shows the dependence of the yields of **3** on the concentration of BES_n. The yields reached to 60%

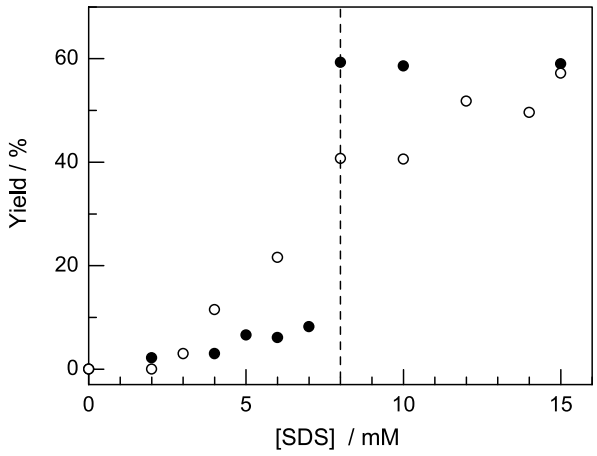


Figure 1. Dependence of the yields on the concentration of SDS in the photochemical (●) and thermal (○) reactions of **1a** with **2a** in aqueous solution.

by the addition of BES_n in concentrations higher than 0.9 mM for BES_{10} and 1.0 mM for BES_{12} to the solution. The advantage of the use of BES_n vesicle is to lower the surfactant concentration than the case of SDS micelle.

However, the reaction in nonionic surfactant such as poly(ethyleneglycol) dodecyl ether (PED; CMC = 0.09 mM, AN = 400) was ineffective (Fig. 3), and the reactions in aqueous solution of cationic surfactant such as hexadecyltrimethylammonium chloride (CTAC; CMC = 1.3 mM, AN = 78) did not occur at all (Table 2). Also, the photoreaction of **1a** with CH_2=CH–OR (**2b**; R = *i*-Bu and **2c**; R = $-CH_2CH_2OH$) in aqueous SDS solution gave the corresponding 2-alkoxydibenzo[*f*,*h*]chroman (**3b**–**c**). But, the photoreaction of **1a** with such other alkenes as 2,3-dimethyl-2-butene and acrylonitrile gave no products.

Although micelle and vesicle operate mainly to dissolve **1** into aqueous solution, the yields were depending on the surfactant used. Anionic surfactants is well known to construct the rigid micelle compared with cationic and nonionic surfactants, because surrounding water molecule

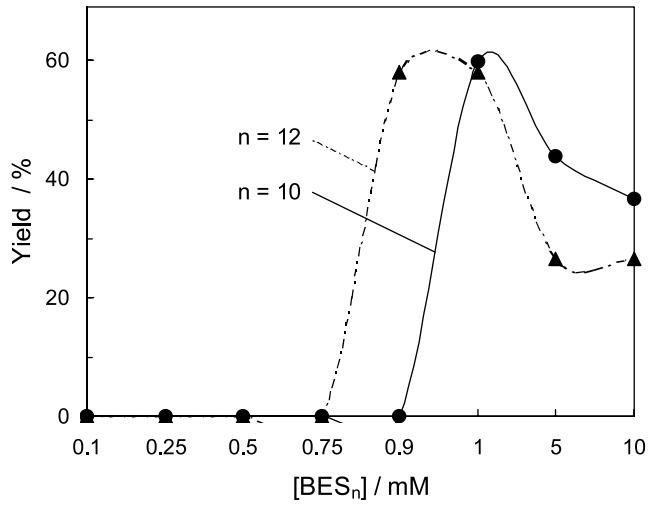


Figure 2. Dependence of the yields on the concentration of BES_n: $n = 10$ (●), $n = 12$ (▲) in the photoreaction of **1a** with **2a** in aqueous solution.

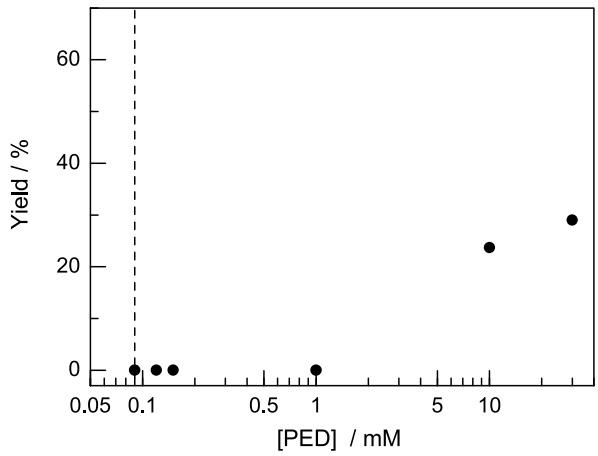


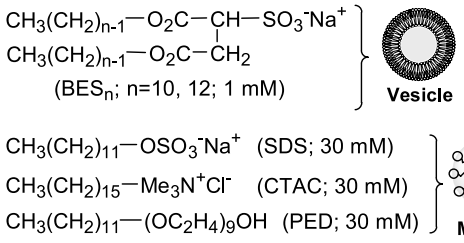
Figure 3. Dependence of the yields on the concentration of PED in the photochemical reactions of **1a** with **2a** in aqueous solution.

can stabilize micelles by the formation of hydrogen bond with the anionic site on the surface of the micelles (Scheme 2). In anionic micelle, therefore, the hydrophilic OH and NR_2 groups of **1** might be fixed on the surface of a

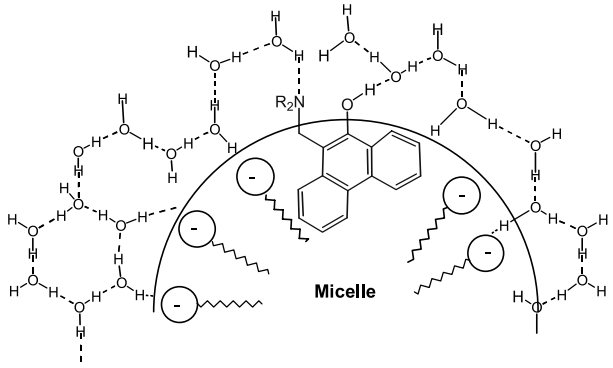
Table 2. Photoreaction of **1a** with **2a**–**c** in aqueous surfactant solution[a]

Solvent/surfactant[b]	**2**	Method[c]	**3** (Yields/%)
H_2O/SDS (30 mM)	**2a**	A	**3a** (61)
H_2O/SDS (30 mM)	**2a**	B	**3a** (72)
H_2O/BES_{12} (1 mM)	**2a**	A	**3a** (58)
H_2O/BES_{12} (1 mM)	**2a**	B	**3a** (41)
H_2O/BES_{10} (1 mM)	**2a**	A	**3a** (58)
H_2O/PED (30 mM)	**2a**	A	**3a** (29)
H_2O/CTAC (30 mM)	**2a**	A	**3a** (0)
H_2O/SDS (30 mM)	**2b**	A	**3b** (29)
H_2O/SDS (30 mM)	**2c**	A	**3c** (43)

[a] Reaction time was 10 h.
[b] The surfactants are as follows:



[c] Method A: photoreaction at room temperature. Method B: thermal reaction at 50 °C



Scheme 2. Schematic representation of micelle surface.

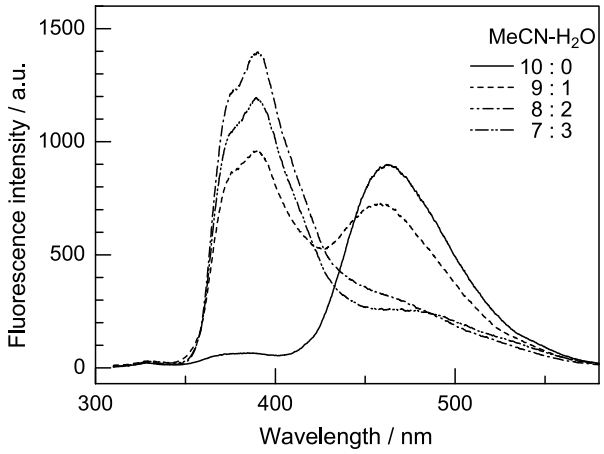


Figure 4. Fluorescence spectra of **1a** in MeCN and MeCN–H_2O (1:1): The excitation at 300 nm, the concentration of **1a** $=1\times10^{-5}$ M.

micelle, while the residual aromatic moiety was oriented toward hydrophobic domain. This causes to induce effective assistance of surrounding water molecule, resulting in the efficient elimination of amine from **1**.

By contrast, the cationic and nonionic surfactants seem unfavorable to form a micelle with a dense of hydrophobic domain, because of little stabilizing effect by surrounding water. Therefore, the hydrophilic OH and NR_2 groups of **1** might arrange randomly in the micelle, resulting that the effect of surrounding water molecule is little. Similar specific enhancing effects of anionic surfactants have already been elucidated on *tetra-O*-acetylriboflavin-photosensitized ring-splitting reaction of pyrimidine cyclobutanes[11] and dehydrogenation of benzyl alcohols[12] in aqueous solution. Thus, the assistance of surrounding water is favorable for the elimination of the amine from **1**.

2.3. Spectroscopic analysis

As has been reported for the formation of QM in the photochemical and thermal reactions of **1c**,[3,6] it is suggested that the QM intermediates, 9-methylene-10-phenanthrone, were formed by the elimination of R_2NH in the cases of the phenanthrene analogs, **1a–b** (Scheme 1). It is well known that the QMs generated from various precursors undergo thermally the cycloaddition reaction with C=C double bonds.[1] Figure 4 shows the fluorescence spectra of **1a** in MeCN and aqueous MeCN. The emission was observed at 460 nm in MeCN. As an increase of the water contents in MeCN, the emission of 460 nm decreased and new emission appeared at 380 nm. It is well known that the phenolic compounds in the excited singlet state are readily converted in the presence of a base to the excited singlet state of the phenolate anion. Therefore, it is safely assigned that the emission at 460 nm comes from the excited singlet states of the zwitter ion (**6**) transformed by the intramolecular proton transfer from the OH to the NH_2 groups of **1a**. Moreover, it is suggested that the emissions at 380 nm come from the excited singlet states of the intermediate (**7**) generated by the protonation on the oxy anion of **6**, since, the emissions at 380 nm observed in the MeCN–H_2O solution was similar to the fluorescence of 9-phenanthrol ($\lambda_{max}=384$ nm). The photochemically and thermally activated **7** induced the Hofmann elimination of R_2NH to give QM intermediate. Similar mechanism has been reported for the photochemical and thermal generation of QM from (2-hydroxybenzyl)-trimethylammonium iodide (Scheme 3).[5]

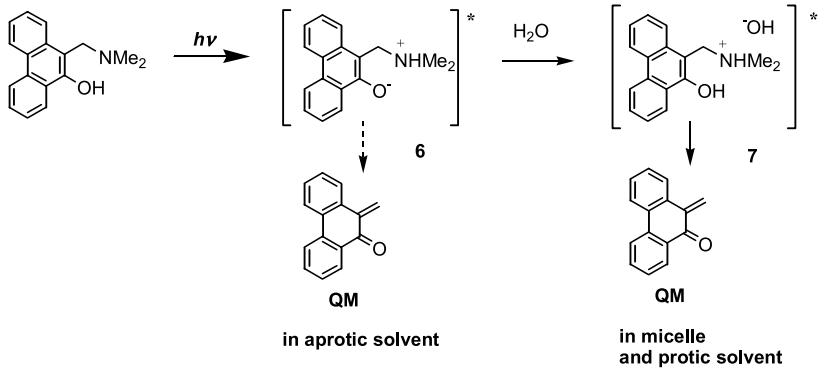
2.4. Participation of water molecule

It has been reported that a water molecule accelerates the intramolecular proton transfer in *o*-hydroxystyrene by ESIPT mechanism. As mentioned above, however, the fluorescence spectra of **1** showed the formation of the zwitter ion (**6**) in MeCN. In contrast to the case of *o*-hydroxystyrene, the proton transfer of **1** proceeds smoothly even in water-free solvents because of the stronger basicity of the amino group than the vinyl group. In the case of **1**, therefore, the enhancing effect of water molecule operates for the elimination step of R_2NH rather than the proton transfer step.

As a conclusion, the recent research of QM intermediate is aimed to the utilization for the bio-molecules involving nucleic acids and peptides.[1] Among a variety of methods to generate QM, therefore, the attention will be focused on the precursor acting in aqueous solution under mild conditions. The photochemical generation of QM from **1** in micelle meets the above requirements.

3. Experimental

9-Bromophenanthrene, 2-naphthol, the amines, and the surfactants were purchased from Wako Chemicals and were used. Commercially available ethyl vinyl ether, isobutyl



Scheme 3. Photochemical generation of *o*-QM.

vinyl ether, and ethylene glycol monovinyl ether were used after distillation to remove the stabilizer (KOH).

^{1}H and ^{13}C NMR spectra were measured on a Bruker AC 250P spectrometer. MS spectra were obtained on a Hitachi M2000A spectrometer. Fluorescence spectra were measured on a Shimadzu RF5300PC fluorometer.

3.1. Preparation of aminomethyl(hydroxy)arenes (1a–e)

Into a flask containing sodium (4.1 g) and pyridine (75 ml) MeOH (75 ml) was added slowly and then CuI (11.4 g) was added into the solution under stirring. Then 9-bromophenanthrene (15.45 g) in pyridine (75 ml) was added into the solution under heating at 80 °C. After heating for 18 h, the reaction was quenched by 10% aqueous HCl solution (75 ml). The extraction with Et_2O of the solution gave the crude 9-methoxyphenanthrene which was purified by column chromatography on SiO_2 and recrystallized from MeOH (yield 86.0%, 10.7 g). The solution of acetic acid (150 ml) of 9-methoxyphenanthrene (7.5 g) was heated at 60 °C and an aqueous solution of HBr (48%, 11.4 ml) was added. After heating at 130 °C for 2 h, the water (150 ml) was added, and the precipitate was obtained by filtration and washing with water to give 9-phenanthrol in 92% yield (6.42 g).

The preparation of **1** was performed by Mannich reaction. Into a reaction mixture of 9-phenanthrol (30 mmol, 5.82 g) and aqueous Me_2NH solution (40%, 40 mmol), a formalin solution (37%, 30 mmol) was slowly added. After stirring at room temperature for 18 h, the solution was extracted with $CHCl_3$. The evaporation of the solvent left the crude *N*,*N*-dimethyl-9-aminomethyl-10-phenanthrol (**1a**) that was subjected to column chromatography on Al_2O_3. Also, *N*,*N*-diethyl-9-aminomethyl-10-phenanthrol (**1b**) and *N*,*N*-dialkyl-1-aminomethyl-2-naphthols (**1c–e**) were prepared according to the method to prepare **1a**.

3.1.1. *N*,*N*-Dimethyl-9-aminomethyl-10-phenanthrol (1a). Yield 76%. ^{1}H NMR δ=2.45 (s, 6H), 4.14 (s, 2H), 7.44–7.53 (m, 3H), 7.83 (d, J=8.1 Hz, 1H), 8.38–8.42 (m, 2H), 8.61–8.66 (m, 2H); ^{13}C NMR δ=44.73, 58.45, 106.34, 121.17, 122.21, 122.67, 122.91, 123.15, 125.81, 126.29, 126.71, 126.80, 130.81, 131.91, 152.79. Exact mass calcd for $C_{17}H_{17}NO$: 251.1310. Found 251.1302.

3.1.2. *N*,*N*-Diethyl-9-aminomethyl-10-phenanthrol (1b). Yield 68%. ^{1}H NMR δ=1.15 (d, J=7.5 Hz, 3H), 2.70 (q, J=7.5 Hz, 4H), 4.23 (s, 2H), 7.40–7.61 (m, 5H), 8.37–8.41 (m, 1H), 8.61–8.66 (m, 2H); ^{13}C NMR δ=11.23, 47.04, 52.87, 106.14, 120.93, 122.10, 122.19, 122.62, 122.69, 123.03, 125.54, 126.45, 126.63, 126.91, 127.07, 130.34, 131.85, 153.05. Exact mass calcd for $C_{19}H_{21}NO$: 279.1623. Found 279.1549.

3.1.3. *N*,*N*-Dimethyl-1-aminomethyl-2-naphthol (1c). Yield 63%. ^{1}H NMR δ=2.36 (s, 6H), 4.04 (s, 2H), 7.06–7.78 (m, 7H); ^{13}C NMR δ=44.57, 57.79, 111.29, 119.15, 120.84, 122.74, 126.19, 128.35, 128.79, 129.10, 132.47, 156.71. Exact mass calcd for $C_{13}H_{15}NO$: 202.1154. Found 202.1165.

3.1.4. *N*,*N*-Diethyl-1-aminomethyl-2-naphthol (1d). Yield 68%. ^{1}H NMR δ=1.12 (d, J=7.2 Hz, 6H), 2.65 (q, J=7.2 Hz, 4H), 4.15 (s, 2H), 7.07 (d, J=8.8 Hz, 1H), 7.18–7.26 (m, 1H), 7.35–7.42 (m, 2H), 7.46–7.76 (m, 3H); ^{13}C NMR δ=11.23, 46.32, 52.21, 111.20, 118.54, 119.32, 120.69, 122.13, 126.16, 128.26, 128.62, 132.53, 157.04. Exact mass. calcd for $C_{15}H_{19}NO$: 229.1467. Found 229.1466.

3.1.5. *N*,*N*-Diisopropyl-1-aminomethyl-2-naphthol (1e). Yield 21%. ^{1}H NMR δ=1.14 (d, J=6.6 Hz, 6H), 3.18 (sept, J=6.6 Hz, 1H), 4.23 (s, 2H), 7.03 (d, J=8.8 Hz, 1H), 7.16–7.25 (m, 1H), 7.35–7.42 (m, 2H), 7.58–7.76 (m, 3H); ^{13}C NMR δ=21.32, 44.65, 51.11, 111.01, 116.64, 120.72, 120.90, 122.16, 126.16, 126.39, 127.71, 129.60, 157.22. Exact mass calcd for $C_{17}H_{23}NO$: 257.1780. Found 257.1788.

3.2. Preparation of BES_n

Decanol (3.3 mmol) was heated with maleic anhydride (1.5 mmol) in the presence of H_2SO_4 (0.1 ml) in benzene (50 ml) at 110 °C on Dean-Stark apparatus. After the neutralization with aqueous $NaHCO_3$ solution, the solution was extracted with benzene. Evaporation of the benzene solution left the crude didecyl maleate that was purified by the recrystallization from hexane. An aqueous solution (50 ml) containing of $NaHSO_3$ (17.5 g) and didecyl maleate (1 mmol) was refluxed for 3 h under bubbling with air. After the neutralization with $NaHCO_3$, the solution was evaporated. The resulting residue was solved into hot methanol, and filtrated. The filtrate was evaporated to give a crude BES_{10} which was purified by recrystallization from hexane.

3.2.1. Didecyl maleate. Yield 78%. ^{1}H NMR ($CDCl_3$) δ=0.88 (t, J=6.9 Hz, 6H), 1.30 (br s, 28H), 1.66 (t, J=7.0 Hz, 4H), 4.17 (t, J=6.8 Hz, 4H), 6.23 (s, 2H).

3.2.2. Sodium 1,2-bis(decylcarbonyl)ethanesulfonate (BES_{10}).[9,10] Yield 16%. ^{1}H NMR (CD_3OD) δ=0.89 (br t, 6H), 1.26–1.31 (m, 28H), 1.63 (br s, 4H), 3.00–3.20 (m, 2H), 4.15–4.20 (m, 5H).

In a similar manner, BES_{12} was prepared using dodecanol instead of decanol.

3.2.3. Didodecyl maleate. Yield 18%. ^{1}H NMR ($CDCl_3$) δ=0.88 (t, J=6.9 Hz, 6H), 1.26 (br s, 28H), 1.60–1.75 (m, 4H), 4.17 (t, J=6.7 Hz, 4H), 6.22 (s, 2H).

3.2.4. Sodium 1,2-bis(dedecylcarbonyl)ethanesulfonate (BES_{12}). Yield 36%. ^{1}H NMR (CD_3OD) δ=0.90 (br t, 6H), 1.30 (br s, 36H), 1.64 (br s, 4H), 3.00–3.20 (m, 2H), 4.15–4.20 (m, 5H).

3.3. General procedure for the reaction of 1 with 2

MeCN–H_2O (6:4), DMF–H_2O (6:4), MeOH–H_2O (6:4), and THF–H_2O (6:4) solutions (100 ml) of **1** (1 mmol) were bubbled with argon gas for 15 min and then **2a** (15 mmol, 1.08 g) was introduced into the solution. Irradiation was performed by an Eikosha high-pressure mercury lamp through a Pyrex filter for 10–20 h at room temperature

(Method A). After the photoreaction, the solvent was evaporated from the photolysate. The crude products were purified by a column chromatography on silica-gel to give **3**. The structure was assigned by ^{1}H NMR and MS spectra. The **2a** was purified by distillation since commercially available **2a** was stabilized by 0.1% NaOH. Under irradiation without the remove of NaOH, **1a** was turned to 9,10-phenanthrenequinone that underwent the formation of the cycloadduct (**5a**) with **2a**.

In the cases of aqueous surfactant solutions, aqueous solution (100 ml) containing **1a** (1 mmol, 251 mg) and the surfactant (SDS 30 mM, 864 mg; PED 30 mM, 1.747 g; CTAC: 30 mM, 960 mg) was bubbled with argon gas for 30 min under cooling with ice and then **2** (15 mmol) was introduced into the solution. Irradiation was performed, as described above (Method A). After the irradiation, the photolysates were extracted with $CHCl_3$. The $CHCl_3$ solution was washed with saturated aqueous NaCl solution to remove the surfactant and was subjected to a column chromatography on silica-gel to give **3a**.

For the case of BES_n, BES_n (0.1–10 mmol, 0.05–5 g for BES_{10}, 0.056–5.6 g for BES_{12}) was solved in MeOH and heated at 40 °C and then cooled to room temperature. After the addition of **1** (0.1 mmol), MeOH was removed by a rotary evaporator to produce a thin film of BES_n and then the film was dried in vacuo. The BES_n film containing **1** was suspended in water (10 ml) and the solution was sonicated at 30 °C for 10 min, giving clear ($\sim$1 mM for BES_n) to slightly turbid ($>$ 5 mM for BES_n) solutions. The obtained solution was bubbled with argon gas for 30 min. After **2a** (1.5 mmol) was introduced into the solution, irradiation was performed. The follow-up process was similar to the method described above.

The thermal reactions of **1** with **2a** were performed for a solution (50 ml) containing **1** (0.2 mmol) and **2a** (3.0 mmol) at bath temperature at 50 °C for 3 h (Method B).

3.3.1. 2-Ethoxydibenzo[*f,h*]chroman (3a). ^{1}H NMR δ= 1.14 (t, J=7.2 Hz, 3H), 2.12–2.26 (m, 4H), 2.90–3.18 (m, 2H), 3.60–3.78 (m, 1H), 3.88–4.00 (m, 1H), 5.41 (t, J= 2.6 Hz, 1H), 7.15–7.60 (m, 4H), 7.73–7.85 (m, 1H), 8.30–8.46 (m, 1H), 8.53–8.59 (m, 2H). ^{13}C NMR δ=15.15, 18.13, 26.56, 63.94. 97.09, 121.79, 122.37, 122.37, 122.72, 124.00, 126.38, 126.38, 126.84, 130.51, 130.82, 131.53, 131.84, 144.80, 152.50. Exact MS calcd for $C_{19}H_{18}O_2$: 278.1307. Found 278.1304.

3.3.2. 2-Isobutoxydibenzo[*f,h*]chroman (3b). ^{1}H NMR δ=0.78 (d, J=6.7 Hz, 3H), 0.83 (d, J=6.7 Hz, 3H), 1.83 (d, J=6.7 Hz, 1H), 2.13–2.31 (m, 2H), 2.99–3.16 (m, 2H), 3.43 (dd, J=9.4, 6.7 Hz, 1H), 3.67 (dd, J=9.5, 6.7 Hz, 1H), 5.45 (dd, J=3.5, 2.6 Hz, 1H) 7.49–7.64 (m, 4H), 7.86–7.90 (m, 1H), 8.29–8.33 (m, 1H), 8.59–8.64 (m, 2H); ^{13}C NMR δ=17.56, 21.03, 28.88, 42.28, 70.80, 109.57, 117.31, 123.38, 123.55, 128.71, 129.92, 131.03, 133.42, 156.74. Exact MS calcd for $C_{21}H_{22}O_2$: 306.1620. Found 306.1584.

3.3.3. 2-(2-Hydroxyethoxy)dibenzo[*f,h*]chroman (3c). ^{1}H NMR δ=1.70 (br s, 1H), 2.20–2.35 (m, 4H), 3.10–3.20 (m, 2H), 3.65–3.74 (m, 1H), 3.80–3.92 (m, 1H), 3.96–4.08 (m, 1H), 5.54 (t, J=3.1 Hz, 1H), 7.15–7.60 (m, 4H), 7.73–7.85 (m, 1H), 8.30–8.46 (m, 1H), 8.53–8.59 (m, 2H); ^{13}C NMR δ=17.96, 26.43, 61.68, 69.96, 97.64, 121.64, 122.41, 122.80, 124.23, 126.53, 126.93. Exact MS calcd for $C_{19}H_{18}O_3$: 294.1283. Found 294.1281.

3.3.4. 2-Ethoxybenzo[*f*]chroman (3d). ^{1}H NMR δ=1.18 (t, J=7.5 Hz, 3H), 2.07–2.21 (m, 2H), 3.05–3.13 (m, 2H), 3.61–3.70 (m, 1H), 3.87–3.96 (m, 1H), 5.31 (t, J=2.5 Hz, 1H), 7.06 (d, J=8.9 Hz, 1H), 7.33 (dd, J=6.8, 1.0 Hz, 1H), 7.48 (dd, J=6.8, 1.3 Hz, 1H), 7.63 (d, J=8.9 Hz, 1H), 7.82 (d, J=5.2 Hz, 1H), 7.85 (d, J=5.2 Hz, 1H); ^{13}C NMR δ= 15.10, 17.43, 26.37, 29.23, 30.85, 63.75, 96.81, 114.37, 119.04, 121.95, 123.27, 126.20, 127.66, 128.33, 129.25. Exact mass calcd for $C_{15}H_{16}O_2$: 228.1150. Found 228.1148.

References and notes

1. Van De Water, R. W.; Petus, T. R. R. *Tetrahedron* **2002**, *58*, 5367.
2. Diao, L.; Yang, C.; Wan, P. *J. Am. Chem. Soc.* **1995**, *117*, 5369.
3. Foster, L. L.; Baker, S.; Brousmiche, D. W.; Wan, P. *J. Photochem. Photobiol. A Chem.* **1999**, *129*, 157.
4. Kalanderopoulos, P.; Yates, K. *J. Am. Chem. Soc.* **1986**, *108*, 6290.
5. Yasuda, M.; Sone, T.; Tanabe, K.; Shima, K. *J. Chem. Soc., Perkin Trans. 1* **1995**, 459.
6. Nakatani, K.; Higashida, N.; Saito, I. *Tetrahedron Lett.* **1997**, *38*, 5005.
7. Modica, E.; Zanaletti, R.; Freddero, M.; Mella, M. *J. Org. Chem.* **2001**, *66*, 41.
8. von Strandtmann, M.; Cohen, M. P.; Shavel, J., Jr. *J. Org. Chem.* **1965**, *30*, 3240.
9. Kunitake, T.; Okahata, Y. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 1877.
10. Ueno, M.; Kishimoto, H.; Kyogoku, Y. *Chem. Lett.* **1977**, 599.
11. Yasuda, M.; Nishinaka, Y.; Nakazono, T.; Hamasuna, T.; Nakamura, N.; Shiragami, T.; Pac, C.; Shima, K. *Photochem. Photobiol.* **1998**, *67*, 192.
12. Yasuda, M.; Nakai, T.; Kawahito, Y.; Shiragami, T. *Bull. Chem. Soc. Jpn.* **2003**, *76*, 601.

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Stereoselective synthesis of (*Z*)-fluoroalkenes directed to peptide isosteres: copper mediated reaction of trialkylaluminum with 4,4-difluoro-5-hydroxyallylic alcohol derivatives

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Abstract—Copper mediated alkyl-transfer reaction of trialkylaluminum (R_3Al) with (*E*)-4,4-difluoro-5-hydroxyallylic alcohol derivative smoothly proceeded to give the corresponding 2-alkylated 4-fluoro-5-hydroxyhomoallylic alcohol derivative with completely *Z* and 2,5-*syn* selective manner. Regio- and stereoselective conversion of the C5-hydroxyl group of the fluoroolefin thus obtained to amino group could be achieved through one-pot mesylation and azidation reaction.

1. Introduction

Fluoroolefin (–CF═CH–) is considered to be an ideal mimic for an amide bond (–CO–NH–) due to the close similarities of the steric and electronic properties.[1] Contrary to these similarities, fluoroolefin should be a non-hydrolyzable bond both chemically and enzymatically, and the lack of rotational freedom of this bond is also a different property from that of amide bond. On the basis of these unique properties, utilization of (*Z*)-fluoroalkene dipeptide or depsipeptide isosteres as non-hydrolyzable and/or conformationally restricted replacements for the parent amide bonds has attracted much attention in the field of medicinal chemistry (Fig. 1).[2–6]

Each of such fluoroalkene-modified dipeptide and depsipeptide isosteres has a set of eight stereoisomers due to two chiral carbon atoms (C2 and C5) and *E*/*Z* configuration of olefin part in the molecule. In Figure 1, a typical dipeptide L-AA_1-L-AA_2 consisting of two L-amino acids, a part of a depsipeptide D-OA_1-L-AA_2 derived from D-oxy acid and L-amino acid, and the corresponding fluoroalkene isosteres are depicted.

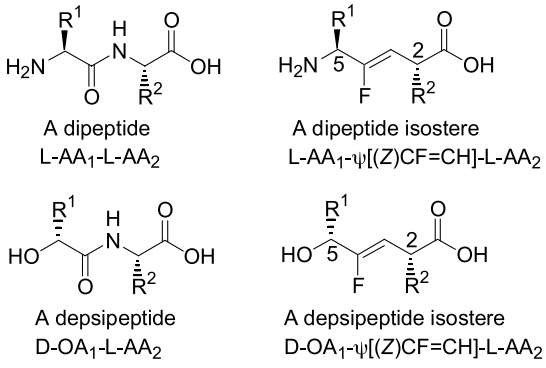

Figure 1. Replacement of amide bond by *Z*-fluoroolefin.

To develop synthetic methods for these fluoroalkene isosteres, stereochemical control of the olefin-configuration (either *Z* or *E*) and the relative stereochemistry of the two chiral centers at C2 and C5 (either *syn* or *anti*) is a major issue to be solved. Furthermore, the use of readily obtainable starting material is also important. So far, extensive efforts to develop highly stereoselective methods for functionalized fluoroolefinic compounds have been reported.[7–15] For example, directed to the preparation of fluoroalkene dipeptide isosteres, Fujii and Otaka et al. recently demonstrated two types of defluorinative reactions using γ,γ-difluoro-α,β-enoate derivatives **1a** as the starting material. That is, the difluoro enoates **1a** having amino group at δ-position could be converted to the dipeptide

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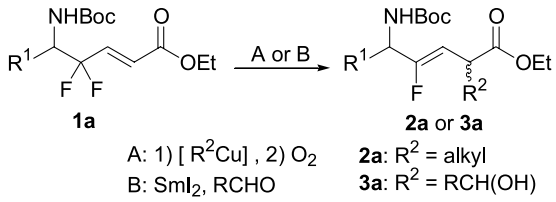


Scheme 1.

isostere form **2a** by utilizing organocopper reagent under reductive–oxidative alkylation conditions (Scheme 1, A).[16] Furthermore, they also reported samarium diiodide (SmI_2)-mediated reduction of **1a** followed by the reaction with carbonyl compounds to give α-substituted fluoroalkenoates **3a** (Scheme 1, B).[17] Although these reactions proceeded with complete *Z*-selectivity, low diastereoselectivity remained as a future subject to be solved.

Independently, we have also reported that using the similar difluoro enoate derivatives **1b–d**, α-alkylated (*Z*)-γ-fluoro-β,γ-enoate **2** can be synthesized upon treating with trialkylaluminum (R_3Al) in the presence of Cu(I) or through lithiocuprate (Me_2CuLi) mediated reductive defluorination followed by regioselective α-alkylation with alkyl halide (Scheme 2, Eqs. 1 and 3).[18] In the lithiocuprate reaction, reductive defluorination product **4** was obtained by quenching with H_2O instead of alkyl halide (Scheme 2, Eq. 2).

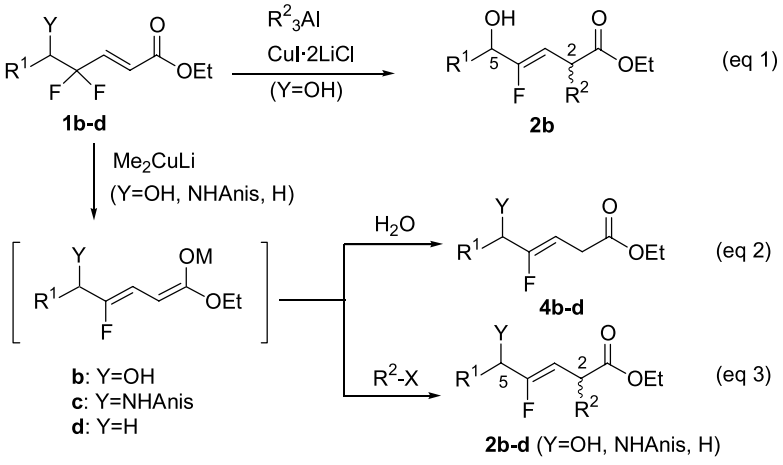
Although these reactions proceeded in excellent *Z*-selectivity, synthetically useful level of diastereo-control could not be realized. Further efforts were made to develop a highly diastereoselective preparation of these compounds. We found that Cu(I)-mediated alkyl-transfer reaction of trialkylaluminum (R_3Al) with (*E*)-4,4-difluoro-5-hydroxyallylic alcohol derivatives *E*-**5** provided the corresponding 2-alkylated 4-fluorohomoallylic alcohol derivatives **6** in completely *Z*- and 2,5-*syn*-selective manner.[19] Furthermore, regio- and stereoselective conversion of the secondary chiral hydroxyl group in the difluoroallylic alcohol **7** into amino functionality could be achieved through one-pot mesylation and azidation protocol (Scheme 3). In this paper, we report these promising results directed to the stereocontrolled preparation of (*Z*)-fluoroalkene depsipeptide or dipeptide isosteres in detail.

2. Results and discussion

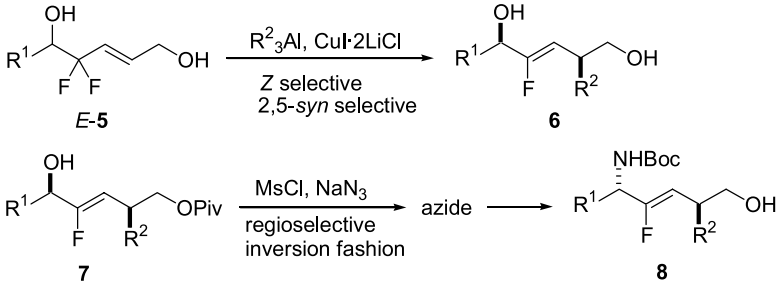
2.1. Reaction with lithiocuprate

First, we examined the reactivity and stereoselectivity in the reaction of (*E*)-4,4-difluoro-5-hydroxyallylic alcohol derivatives **5a**, **5b** with a copper reagent derived from alkyllithium, typically such as Gilman reagent (Table 1).[19]

Contrary to the case of ester derivative **1b** (Y=OH) which provided the reductive defluorination product **4b** rather than expected 2-methylated product **2b** (Y=OH, R^2=Me) as shown in Scheme 2, the reaction of these alcohol derivatives **5a**, **5b** with dialkylcopper lithium R_2CuLi proceeded in SN2′ manner giving rise to 2-alkylated homoallylic alcohol

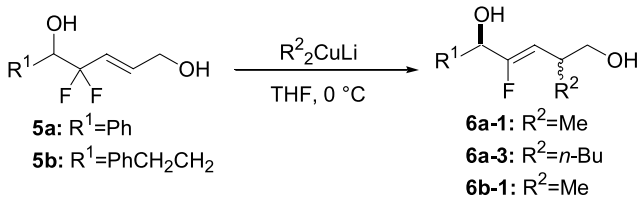


Scheme 2.



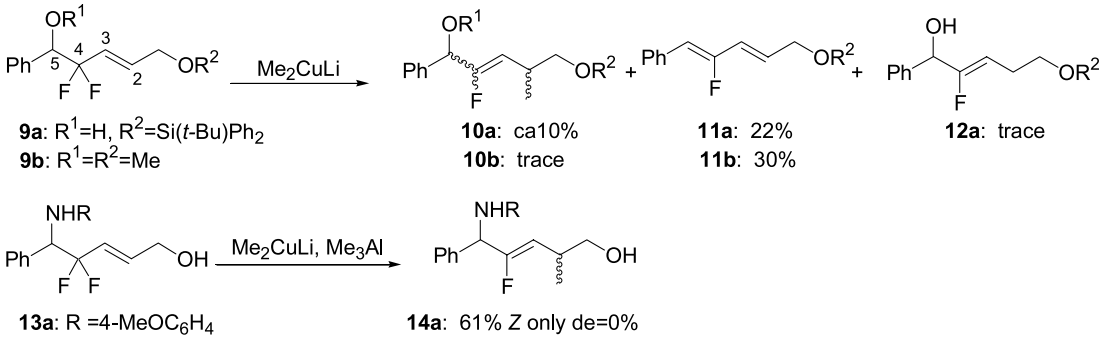
Scheme 3.

Table 1. Reaction of (*E*)-4,4-difluoro-5-hydroxyallylic alcohol with R_2CuLi



Entry	5	Reagent	Additive	R^2	6	Yield (%)[a]	*Z*/*E*[b]	*syn*/*anti*[c]
1	**5a**	Me_2CuLi	—	Me	**6a-1**	90	15	2:1
2		Me_2CuLi	Me_3Al	Me	**6a-1**	88	>95	95:1
3		*n*-Bu_2CuLi	Me_3Al	*n*-Bu	**6a-3**	58	13	1.1:1[d]
4	**5b**	Me_2CuLi	Me_3Al	Me	**6b-1**	90	11	1.1:1[d]

[a] Isolated yield.
[b] Determined by ^1H NMR.
[c] Ratio for *Z* isomer.
[d] Relative stereochemistry was not determined.

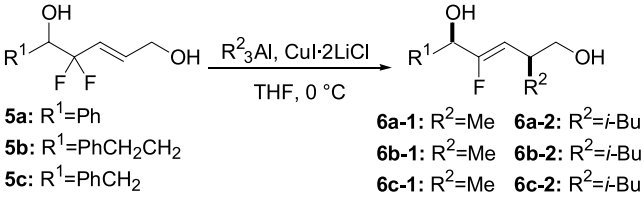


Scheme 4.

derivatives **6a**, **6b**.[20] For example, by treating **5a** with Me_2CuLi (5 equiv) in THF at 0 °C for 2.5 h, 2-methylated homoallylic alcohol **6a-1** was obtained in 90% yield with relatively high *Z* selectivity (*Z*/*E*=15), but with low diastereoselectivity (*syn*/*anti*=2 for *Z* isomer) (entry 1). While limited to the case of phenyl derivative **5a** and Me_2CuLi, a remarkable improvement in diastereroselectivity was observed by the addition of Me_3Al (entry 2). However, the additive effect of Me_3Al on the stereoselectivity was not observed in the reaction of **5a** with *n*-Bu_2CuLi instead of Me_2CuLi or alkyl (β-phenylethyl) derivative **5b** with Me_2CuLi (entries 3 and 4).

Reactions of lithiocuprate with some structure modified substrates were also examined. It was found that both allylic alcohol structure and C5-hydroxyl group may be important

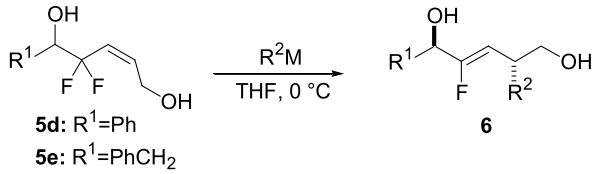
Table 2. Cu(I)-mediated reaction of (*E*)-4,4-difluoro-5-hydroxyallylic alcohol with R_3Al



Entry	5	Reagent	R^2	6	Yield (%)[a]	*Z*/*E*[b]	*syn*/*anti*[c]
1	**5a**	Me_3Al	Me	—	0[d]	—	—
2		Me_3Al, CuI·2LiCl	Me	**6a-1**	77	>95	>95:<1
3		*i*-Bu_3Al, CuI·2LiCl	*i*-Bu	**6a-2**	68	>95	>95:<1
4	**5b**	Me_3Al, CuI·2LiCl	Me	**6b-1**	65	>95	>95:<1
5		*i*-Bu_3Al, CuI·2LiCl	*i*-Bu	**6b-2**	66	>95	>95:<1
6	**5c**	Me_3Al, CuI·2LiCl	Me	**6c-1**	98	>95	>95:<1
7		*i*-Bu_3Al, CuI·2LiCl	*i*-Bu	**6c-2**	78	>95	>95:<1

[a] Isolated yield.
[b] Determined by ^1H NMR.
[c] Ratio for *Z* isomer.
[d] Without CuI·2LiCl, **5a** was recovered in 90%.

Table 3. Alkyl-transfer reaction of (*Z*)-4,4-difluoro-5-hydroxyallylic alcohol



Entry	5	Reagent	Time (h)	R^2	6	Yield (%)[a]	*syn/anti*[b,c]	5 (%)[b,d]
1	**5d**	Me_3Al, CuI·2LiCl	22	Me	**6a-1**	52	1:10.5	41
2		*i*-Bu_3Al, CuI·2LiCl	22	*i*-Bu	**6a-2**	23	1:5.8	60
3		Me_2CuLi	4	Me	**6a-1**	76	1:4.7	—
4	**5e**	*i*-Bu_3Al, CuI·2LiCl	22	*i*-Bu	**6c-2**	32	1:2.9	32
5		Me_2CuLi	4	Me	**6c-1**	89	1:2.0[e]	—

[a] Isolated yield.
[b] Determined by ^1H and ^{19}F NMR.
[c] Ratio for *Z*-isomer is shown.
[d] Recovery of **5**.
[e] *Z*/*E* ratio of **6c-1** was 15:1.

for clean reaction, since the silylation of the primary hydroxyl group (substrate **9a**) or methylation of both hydroxyl groups (substrate **9b**) gave the 2-methylated product **10** as a mixture of *Z*/*E* and *syn*/*anti* isomers along with the formation of the diene compound **11**. In the case of **9a**, reductive defluorination compound **12a** was also detected. With C5-amino substrate **13a**, reaction proceeded in completely non-diastereoselective manner to give the 2-methylated (*Z*)-fluoroalkene compound **14a** in 61% yield (Scheme 4). From these results, a high level of stereocontrol could not be achieved by lithiocuprate reactions.

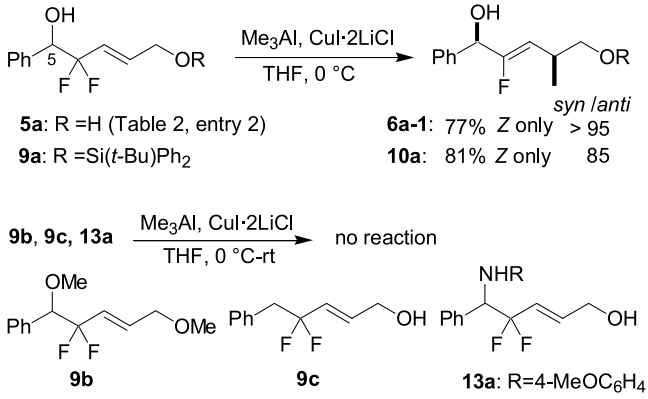
2.2. Reaction with trialkylaluminum in the presence of CuI·2LiCl

Contrary to the low diastereoselectivity in the reaction with lithiocuprate, when the reaction of (*E*)-difluoroallylic alcohols **5a**–**5c** were conducted using a combination of trialkylaluminum (R_3Al, 5–10 equiv) and CuI·2LiCl[21] (2.5 equiv) in THF at 0 °C for 15–22 h, the desired 2-alkylated 4-fluorohomoallylic alcohols **6a**–**6c** were obtained in good to excellent yields (62–98%) with complete *Z*- and 2,5-*syn* selectivity (Table 2, entries 2–7).[19]

In the absence of CuI·2LiCl, no reaction occurred upon treating **5a** with Me_3Al to result in the recovery of **5a** (Table 2, entry 1). Thus, Cu(I) is a crucial additive for the present alkyl-transfer reaction of trialkylaluminum to proceed,[22,23] possibly through the Al–Cu transmetalation and activation of fluorine atom as a leaving group by aluminum–fluorine interaction (see mechanistic discussion).[24,25] As shown in Table 2, with either phenyl or alkyl (phenethyl and benzyl) substituted derivatives, not only methyl but also longer alkyl (*iso*-butyl) aluminum reagent gave the satisfactory results. Thus, we could provide a highly general and stereocontrolled method for 2-alkylated (*Z*)-2,5-*syn*-4-fluoro-5-hydroxy-3-alkenols **6** from *E*-isomer of difluoroallylic alcohols **5**.

Next, the reactivity of *Z*-isomer of difluoroallylic alcohols **5d**, **5e** and their stereochemical outcome were compared with those of *E*-isomers **5a**, **5c**. The results are shown in Table 3. The reaction of *Z*-isomer **5d** under the similar conditions (Me_3Al, CuI·2LiCl, THF, 0 °C, 22 h) proceeded more slowly than that of *E*-isomer **5a** to give the 2-methylated 4-fluorohomoallylic alcohol **6a-1** in moderate yield (52%) along with the recovery of **5d** (41%) (entry 1). The stereochemistry of the product **6a-1** thus obtained indicated that the reaction proceeded in highly *Z*-selective manner (*Z*/*E* = > 95) and in relatively highly *anti*-selective manner (*syn*/*anti* = 1/10.5) opposite to that of *E*-isomer **5a**. Using *i*-Bu_3Al instead of Me_3Al under the similar conditions, the reaction of **5d**, **5e** gave the desired products **6a-2**, **6c-2** in low yields along with the recovery of **5d**, **5e**, respectively (entries 2 and 4). In these cases complete *Z*-selectivity and moderate 2,5-*anti* selectivity were also observed. On the other hand, as shown in entries 3 and 5, the reaction of *Z*-isomers **5d**, **5e** with lithiocuprate Me_2CuLi proceeded smoothly to give **6a-1**, **6c-1** in good yields (76 and 89%, respectively), but with lower diastereoselectivity (*syn*/*anti* = 1/4.7 for **6a-1** and 1/2.0 for **6c-1**). Although the product yield should be improved in the case of *Z*-isomer of difluoroallylic alcohols **5d**, **5e**, the results mentioned above indicated that both *syn*- and *anti*-isomer of 2-alkylated (*Z*)-4-fluoro-5-hydroxyhomoallylic alcohols **6** are stereoselectively constructed by the reaction with R_3Al and CuI·2LiCl.

Next, to see the substrate specificity of the present Cu(I)-mediated alkyl-transfer reaction of trialkylaluminum with



Scheme 5.

4,4-difluoroallylic alcohol derivatives and for mechanistic consideration about the excellent stereoselectivity observed in the reaction with (*E*)-isomer of 5-hydroxyl derivatives **5** (see Table 2), we examined similar reactions using various structure-modified substrates (Scheme 5).

First, the reaction of the substrate **9a** prepared by silylation of the primary hydroxyl group of **5a** was examined to see the effect of allylic alcohol structure on the reactivity. This substrate **9a** showed similar reactivity to that of non-protected substrate **5a** to give the 2-methylated product **10a** in 81% yield with an excellent *Z*-selectivity and high 2,5-*syn* selectivity. On the other hand, etherification of both hydroxyl groups (substrate **9b**) and removal of C5-hydroxyl group (substrate **9c**) resulted in complete recovery of these substrates upon treating with Me_3Al and CuI·2LiCl in THF. Moreover, replacement of C5-hydroxyl group by an amino group such as 4-methoxyanilino derivative **13a** also led to the recovery of the starting material. From these results, it was clearly demonstrated that the free hydroxyl group at 5-position should be essential for the Cu(I)-mediated alkyl-transfer reaction of trialkylaluminum, while the allylic alcohol structure is not always required.

2.3. Mechanism

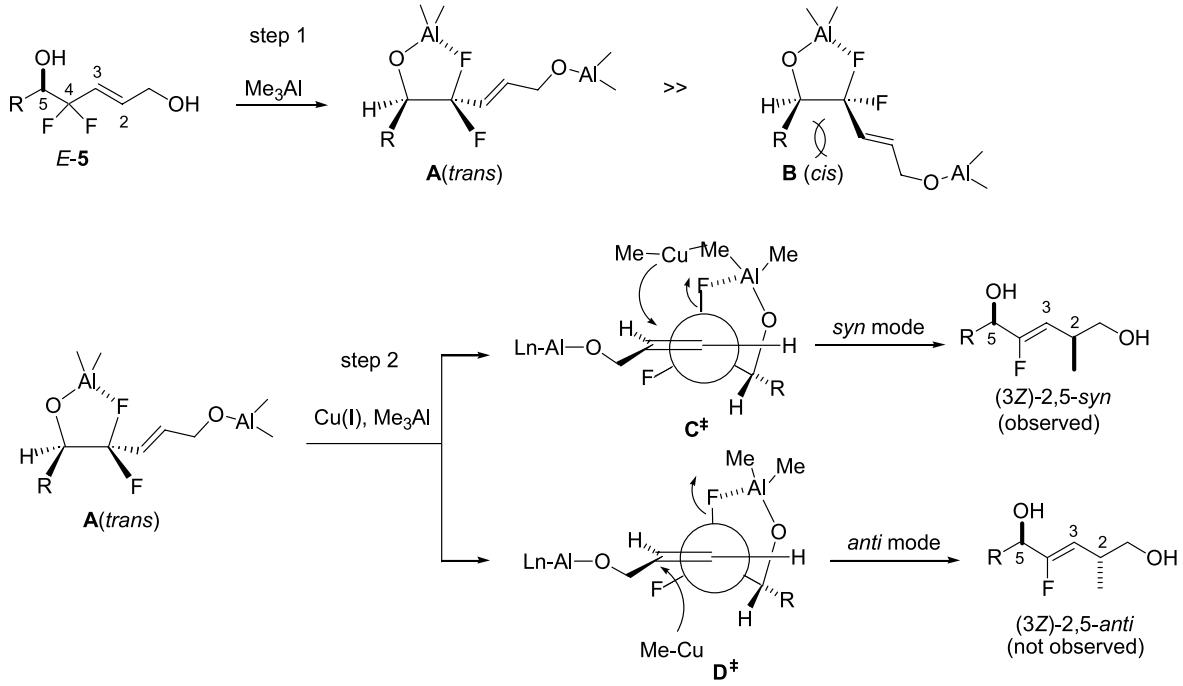
Although mechanistic detail is not clear at this moment, the above mentioned alkyl-transfer reaction may involve Al–Cu transmetalation and activation of fluorine atom as a leaving group through fluorine–aluminum coordination.

As such examples of copper-mediated alkyl-transfer reactions of alkylaluminum were reported conjugate addition with α,β-enones[22] and allylic substitution with allylic phosphates and halides.[23] Similar to these reactions, we believe that the present defluorinative allylic alkylation of difluoroallylic alcohol **5** with trialkylaluminum should involve Al–Cu transmetalation since without Cu(I) no reaction occurred. Furthermore, on the basis of absolutely essential functionality of the C5-hydroxyl group in the substrate **5** as shown in Scheme 5 and relatively strong fluorine–aluminum coordination,[24,25] it would be expected that activation of fluorine atom as a leaving group can be gained by the formation of a five-membered complex **A** easily formed when the substrate **5** is mixed with trialkylaluminum. Due to the steric reason, the complex **A** which is *trans* isomer with respect to the relative stereochemistry between propenyl moiety and R substituent may be more favorable as compared with complex **B**, the corresponding *cis* isomer (Scheme 6, step 1).

Next, we analyzed alkyl-transfer process on the assumption that in the complex form **A** the fluorine atom coordinating to aluminum acts as a leaving group[24] and the reaction proceeds via oxidative addition of aluminocuprate formed by Al–Cu transmetalation.

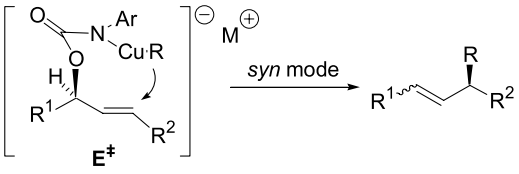
From the accumulated data, the copper-mediated allylic alkylation reactions proceed through either *anti* or *syn* to the leaving group depending on the type of leaving group, nature of organometallics used and sometimes on the additive.[26–30] In our reaction using *E* isomer of 4,4-difluoroallylic alcohol *E*-**5**, when the oxidative addition of methylcopper occurs from *anti* to the leaving fluorine atom as shown in the model $\mathbf{D}^{\ddagger}$, the stereochemistry of the product should be 3*Z* and 2,5-*anti*, although the observed stereochemistry was 2,5-*syn*. Alternatively, if we consider the *syn*-addition of copper to the leaving group through an intramolecular process of the aluminocuprate as shown in the model $\mathbf{C}^{\ddagger}$, the expected stereochemistry of the product is essentially identical with the observed (3*Z*)-2,5-*syn* stereochemistry (Scheme 6, step 2).

This intramolecular process may be similar to the *syn* allylic



Scheme 6. Mechanism for (3*Z*)-2,5-*syn* selectivity with *E*-**5**.

substitution reaction with allylic NH-carbamate derivatives, in which the carbamate anion acts as a ligand onto the alkylcopper species to form the ate complex as shown in the model **E**‡, thereby the alkyl-transfer reaction occurs intramolecularly in *syn* selective manner (Scheme 7).[28] Likewise, in the model **C**‡, methyl group on the aluminum coordinated by the fluorine atom possibly acts as a ligand onto methyl copper to form aluminocuprate ate complex (Scheme 6).



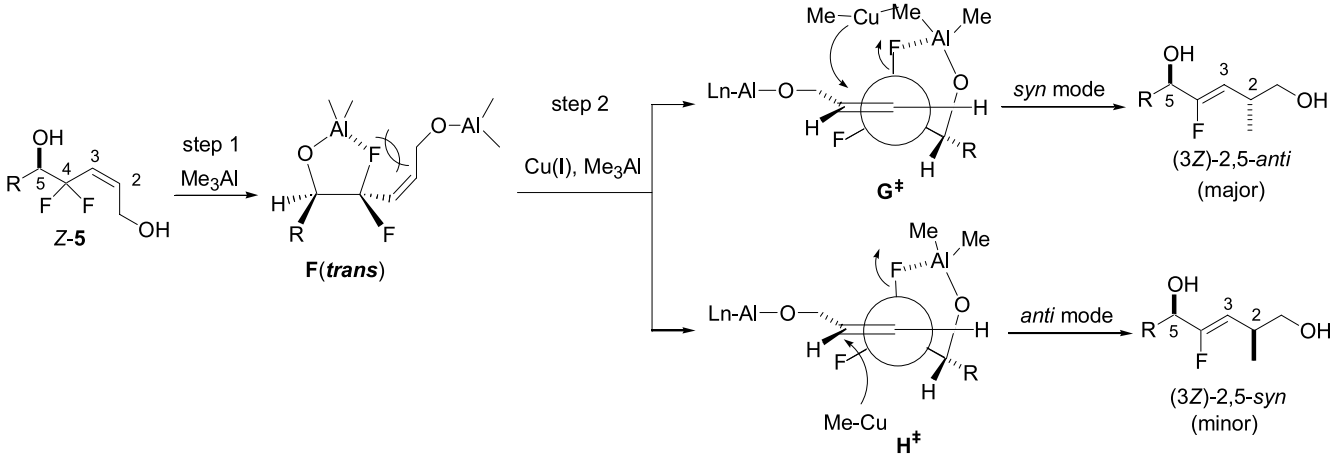
Scheme 7. *syn*-Allylic substitution of NH-carbamate with alkylcuprate.

Contrary to the *E* isomer of the substrate *E*-**5**, in the case of *Z* isomer *Z*-**5**, which showed lower reactivity and moderate 2,5-*anti* selectivity in the product (see Table 3), the five-membered complex form **F** would be more unstable as compared with the complex form **A** due to the unfavorable steric interaction between C1 and C4 (fully substituted carbon atom) in the complex **F**, thereby the reaction of *Z*-**5** showed lower reactivity. Furthermore, as a possible explanation for the moderate 2,5-*anti* selectivity, while the intramolecular alkyl transfer through the model **G**‡ provides the major 2,5-*anti* product, competitive intermolecular oxidative *anti*-addition of methylcopper to the fluorine atom coordinating to aluminum as shown in the model **H**‡ would be a reaction pathway for 2,5-*syn* product (Scheme 8).

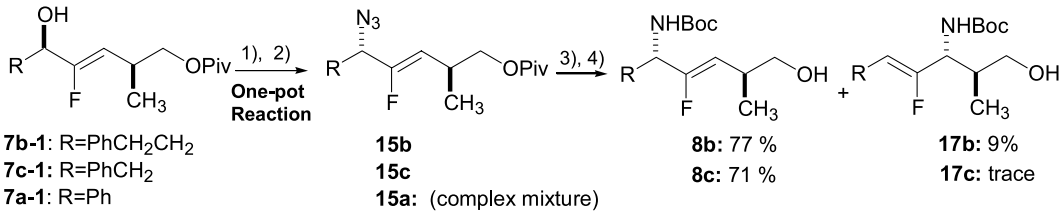
2.4. Conversion of chiral fluoroallylic alcohol to *N*-Boc amino derivative

As mentioned above, towards to the direct production of peptide isostere the present Cu(I)-mediated alkyl-transfer reaction could not be applicable to the substrate having amino group at 5-position (see Scheme 5, **13a**). Therefore, we examined the regio- and stereoselective conversion of C5-hydroxyl group to amino group using the above mentioned fluoroallylic alcohol derivative **6** prepared in completely stereoselective manner (Table 2). In a literature, although the substitution reaction of the primary hydroxyl group of fluoroallylic alcohols by the Mitsunobu reaction (Ph_3P, DEAD, phthalimide) proceeded smoothly,[3,4] those of secondary allylic alcohols seem to be difficult due to the low reactivity.[1e,2a,16a] Similar tendency was also observed in our case. That is, the Mitsunobu reactions of the pivaloyl ester derivative **7b**-**1**, **7c**-**1** were conducted, but these were not fruitful to result in only recovery of the starting materials.

We also examined stepwise procedure involving sulfonylation of the hydroxyl group followed by the subsequent substitution reaction with sodium azide or benzylamine under various reaction conditions. Most of these experiments resulted in a messy mixture due to the instability of these allylic sulfonates. While such a problematic event in allylic substitution reaction is quite common, there have been some successful examples reported so far.[31,32] For example, by one-pot reaction of mesylation of hydroxyl group in the presence of large excess amount of sodium azide, a clean SN2 replacement of an optically pure benzylic hydroxyl group (5,6,7,8-tetrahydroisoquinolin*E*-8-ol or benzhydrol derivative) was reported.[33,34] Similar one-pot operation was found to work very nicely with our fluoroallylic alcohol derivatives **7b**-**1**, **7c**-**1**. Thus, after a mixture of the alcohol **7b**-**1**, dimethylaminopyridine (DMAP, 3 equiv) and sodium azide (20 equiv) suspended in CH_2Cl_2 was treated with methanesulfonyl chloride (3 equiv) for 30 min at room temperature, a clear solution by the addition of DMSO was further stirred for 3 h at the same temperature giving rise to the desired 5-azide derivative **15b** in high yield. Since the rearrangement of the allylic azide forming 3-azide isomer seemed to easily occur, conversion of the azide **15b** to *N*-Boc-protected amino derivative **8b** was carried out immediately after azidation reaction, while we obtained the desired **8b** along with the formation of rearranged isomer **17b** as a by-product. Thus, by treatment of crude **15b** with $LiAlH_4$ followed by with di-*t*-butyl dicarbonate and triethylamine we obtained **8b** and **17b** in 77% and 9% yield, respectively. The 2,5-*anti* stereochemistry was unambiguously confirmed by X-ray analysis of **8b** indicating that the present one-pot azidation reaction proceeded in a complete inversion



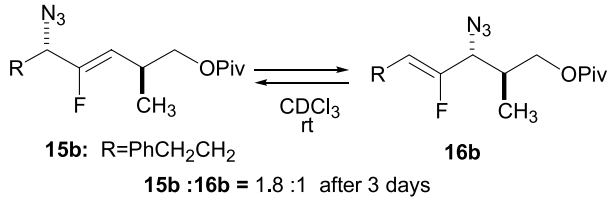
Scheme 8. Mechanism for (3*Z*)-2,5-*anti* selectivity with *Z*-**5**.



Scheme 9. Reagent and conditions: (1) DMAP (3 equiv), NaN_3 (20 equiv), MsCl (3 equiv), CH_2Cl_2, rt, 30 min; (2) DMSO, rt, 3 h; (3) $LiAlH_4$, THF, 1.5 h; (4) $(Boc)_2O$, Et_3N, CH_2Cl_2, rt, 30 min.

fashion. Similar stereoselective conversion from **7c-1** to **8c** was also achieved (Scheme 9). However, under the similar reaction conditions the benzylic alcohol derivative **7a-1** (R=Ph) gave a complex mixture, possibly due to the highly labile nature of the corresponding mesylate.

It should be noted that fluoroallylic azide **15b**, **15c** thus obtained slowly isomerize to 3-azide derivatives.[32,35] Thus, when a solution of 5-azide **15b** in $CDCl_3$ was left at room temperature for 3 days, **15b** changed to a mixture of **15b** and the 3-azide isomer **16b** in a ratio of 1.8:1 (Scheme 10).



Scheme 10.

3. Conclusion

In conclusion, we have established a stereoselective (completely *Z* and 2,5-*syn* selective) synthesis of 5-hydroxylated 2-alkyl-4-fluoro-3-alkene-1-ol derivatives through the defluorinative allylic substitution reaction of difluoroallylic alcohol derivatives with trialkylaluminum and Cu(I). Furthermore, a high yield and complete inversive conversion of C5-hydroxyl group of these stereoselectively synthesized fluoroallylic alcohol derivatives to amino group could be achieved through one-pot preparation of azide compound. The present reaction should provide an efficient method for the preparation of functionalized *Z*-fluoroolefins, which, in particular, are applicable to the preparation of depsipeptide isosteres and dipeptide isosteres.

4. Experimental

4.1. General

Trimethylaluminum (1.0 M in hexane) and triisobutylaluminum (1.0 M in hexane) are available commercially. All reactions were conducted under an argon atmosphere. ^1H and ^{13}C NMR spectra were measured at 400 and 100 MHz in $CDCl_3$, and the chemical shifts are given in ppm using $CHCl_3$ (7.26 ppm) in $CDCl_3$ for ^1H NMR and $CDCl_3$ (77.01 ppm) for ^{13}C NMR as an internal standard, respectively. ^{19}F NMR spectra were measured at 376.5 MHz and the chemical shifts are given in ppm using benzotrifluoride (0 ppm) as an internal standard. Mass spectra and HRMS were recorded by EI or ESI methods. Column chromatography was performed on silica gel (70–230 mesh). Medium-pressure liquid chromatography (MPLC) was performed on a 30 cm×2.2 cm i.d. prepacked column (silica gel, 50 μm) with a UV or RI detector.

4.2. Preparation of 5-substituted (*E*)-4,4-difluoro-2-pentene-1,5-diol (5)

A mixture of ethyl ester of 5-substituted (*E*)-4,4-difluoro-5-hydroxy-2-pentenoic acid[17] (5 mmol) and DIBAL (1 M hexane solution, 15 mmol) in THF was stirred at −70 °C for 30 min. The reaction mixture was quenched with 5% HCl and extracted with AcOEt. The combined organic layer was dried over anhydrous Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica gel column chromatography to give *E*-**5**.

4.2.1. (*E*)-4,4-Difluoro-5-phenyl-2-pentene-1,5-diol (5a). Quant. Colorless oil. IR ($CHCl_3$) ν cm^{-1}; 3622, 3150–3600, 2902, 1683. ^1H NMR (400 MHz, $CDCl_3$) δ; 2.66 (1H, brs), 3.62 (1H, brs), 4.10 (2H, s), 4.84 (1H, t, J=9.6 Hz), 5.73–5.85 (1H, m), 6.07–6.17 (1H, m), 7.26–7.43 (5H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 61.6, 75.8 (t, J=30.5 Hz), 119.7 (t, J=244.3 Hz), 121.5 (t, J=25.4 Hz), 127.7, 128.2, 128.7, 136.2 (d, J=2.7 Hz), 136.6 (t, J=8.2 Hz). ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −41.61 (1F, d, J=247 Hz), −45.09 (1F, dt, J=247, 11 Hz). EI-MS *m/z*; 196 (M^+-H_2O), 177, 107. HRMS; calcd for $C_{11}H_{10}F_2O$ (M^+-H_2O): 196.0700. Found: 196.0712.

4.2.2. (*E*)-4,4-Difluoro-7-phenyl-2-heptene-1,5-diol (5b). 61% yield. Colorless oil. IR ($CHCl_3$) ν cm^{-1}; 3622, 2932, 2866, 1683. ^1H NMR (400 MHz, $CDCl_3$) δ; 1.70–1.84 (1H, m), 1.90–2.04 (1H, m), 2.60–2.74 (1H, m), 2.84–2.96 (1H, m), 3.45 (1H, s), 3.69 (1H, s), 3.74 (1H, m), 4.20 (2H, s), 5.80–5.96 (1H, m), 6.18–6.30 (1H, m), 7.21–7.32 (5H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 31.5, 61.5, 72.7 (t, J=30.2 Hz), 120.5 (t, J=243.3 Hz), 121.6 (t, J=25.6 Hz), 126.0, 128.4, 136.1 (t, J=8.4 Hz), 141.1. ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −42.42 (1F, d, J=248 Hz), −47.98 (1F, d, J=248 Hz). EI-MS *m/z*; 242 (M^+), 224 (M^+-H_2O), 206, 117. HRMS; calcd for $C_{13}H_{16}F_2O_2$ (M^+): 242.1118. Found: 242.1104.

4.2.3. (*E*)-4,4-Difluoro-6-phenyl-2-hexene-1,5-diol (5c). 97% yield. Colorless oil. IR ($CHCl_3$) ν cm^{-1}; 3620, 3460, 2924, 1732, 1682. ^1H NMR (400 MHz, $CDCl_3$) δ; 2.58 (1H, brs), 2.69 (1H, dd, J=14.2, 10.4 Hz), 2.90 (1H, d, J=8.0 Hz), 3.03 (1H, dd, J=14.2, 2.4 Hz), 3.98 (1H, m), 4.23 (2H, s), 5.92–5.96 (1H, m), 6.29–6.34 (1H, m),

7.24–7.36 (5H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 36.44, 61.65, 74.62 (t, J=30.3 Hz), 120.18 (t, J=243.5 Hz), 121.64 (t, J=25.4 Hz), 126.69, 128.53, 129.35, 136.34 (t, J=8.4 Hz), 137.31. ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −42.38 (1F, d, J=249 Hz), −48.46 (1F, dt, J=249, 12 Hz). EI-MS m/z; 228 (M^+), 210 (M^+-H_2O), 190, 121. HRMS; calcd for $C_{12}H_{14}F_2O_2$ (M^+): 228.0962. Found: 228.0954.

4.2.4. (*Z*)-4,4-Difluoro-5-phenyl-2-pentene-1,5-diol (5d). To a suspension of 5% Pd–$BaSO_4$ (183 mg, 0.54 mmol) in MeOH (5 ml) was added dropwise quinoline (183 mg, 1.42 mmol) in MeOH (5 ml) at 0 °C and the mixture was stirred at room temperature for 15 min. To this mixture was added 5-{[*tert*-butyl(diphenyl)silyl]oxy}-2,2-difluoro-1-phenyl-3-pentyn-1-ol[36] (1.971 g, 4.38 mmol) in DMF (5 ml) at room temperature and then under a hydrogen atmosphere the mixture was stirred at room temperature for 4 h. The reaction mixture was filtrated through celite pad with the aid of AcOEt. The combined filtrate was diluted with water and extracted with AcOEt. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by silica gel column chromatography [hexane/AcOEt (5:1)] to give TBDPS-**5d** (1.851 g, 93% yield). After a mixture of TBDPS-**5d** (358 mg, 0.79 mmol) and TBAF (1 M THF solution, 0.5 ml, 0.5 mmol) in THF (5 ml) was stirred at room temperature for 3 h, the reaction mixture was poured into water and extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by silica gel column chromatography [hexane/AcOEt (2:1)] to give **5d** (97 mg, 57% yield) as colorless oil. IR (neat) ν cm^{-1}; 3608, 3448, 3048, 2896. ^{1}H NMR (400 MHz, $CDCl_3$) δ; 2.59 (1H, brs), 3.95–4.10 (2H, br), 4.10–4.20 (1H, br), 4.85 (1H, t, J=9.2 Hz), 5.36–5.49 (1H, m), 5.86–5.95 (1H, m), 7.33–7.40 (5H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 58.6 (d, J=3.2 Hz), 75.5 (t, J=30.3 Hz), 120.7 (t, J=245.4 Hz), 122.0 (t, J=26.5 Hz), 127.7, 128.1, 128.7, 136.0 (d, J=3.6 Hz), 138.3 (t, J=5.1 Hz). ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −37.7 (1F, ddd, J=253, 12, 12 Hz), −38.9 (1F, ddd, J=253, 12, 12 Hz). EI-MS m/z; 196 (M^+-H_2O), 107, 90, 77. HRMS; calcd for $C_{11}H_{10}F_2O$ (M^+-H_2O): 196.0700. Found: 196.0716.

4.2.5. (*Z*)-4,4-Difluoro-6-phenyl-2-hexene-1,5-diol (5e). After a mixture of ethyl (*Z*)-2,2-difluoro-4-phenyl-3-(tetrahydro-2*H*-pyran-2-yloxy)-butanoate (1.90 g, 5.98 mmol) and Red-Al™ (65% toluene solution, 4.3 ml, 7.18 mmol) in THF (15 ml) was stirred at −78 °C for 30 min, the reaction mixture was quenched with 5% HCl. Extractive workup (AcOEt for extraction) followed by concentration in vacuo left an oily aldehyde, which was used for the next step without further purification. Under an argon atmosphere, to a mixture of NaH (60% in oil, 264 mg, 6.58 mmol) and triethyl phosphonoacetate (1.92 g, 5.98 mmol) in DMF (10 ml) at 0 °C was added the aldehyde mentioned above in DMF (5 ml), and the mixture was stirred for 20 min. The reaction mixture was quenched with 5% HCl, extracted with ether. The combined organic layer was washed with $NaHCO_3$aq, brine and dried over anhydrous Na_2SO_4 and then evaporated in vacuo. The residue was purified by silica gel column chromatography [hexane/AcOEt (20:1)] to give a diastereomeric mixture of ethyl (*Z*)-4,4-difluoro-6-phenyl-5-(tetrahydro-2*H*-pyran-2-yloxy)-2-hexenoate (1.25 g, 59% yield), which was separated by MPLC [hexane/AcOEt (5:1)] to give less polar isomer and more polar isomer in the order of elution. Less polar: colorless oil. IR (neat) ν cm^{-1}; 1732. ^{1}H NMR (400 MHz, $CDCl_3$) δ; 1.29 (3H, t, J=7.2 Hz), 1.21–1.33 (1H, m), 1.36–1.49 (1H, m), 1.56–1.73 (3H, m), 2.77–2.85 (1H, m), 2.86 (1H, dd, J=14.2, 9.1 Hz), 2.98–3.05 (1H, m), 3.04 (1H, dd, J=14.2, 4.2 Hz), 4.23 (2H, q, J=7.2 Hz), 4.49–4.59 (1H, m), 4.95 (1H, t, J=2.7 Hz), 5.97 (1H, dt, J=14.6, 12.7 Hz), 6.09 (1H, dt, J=12.7, 1.6 Hz), 7.17–7.23 (1H, m), 7.25–7.32 (4H, m). ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −38.7 (1F, ddd, J=257, 13, 13 Hz), −40.9 (1F, ddd, J=257, 14, 8 Hz). EI-MS m/z; 355 (M^++H), 252. Anal. Calcd for $C_{19}H_{24}F_2O_4$: C, 64.39; H, 6.83. Found: C, 64.53; H, 6.83. more polar: colorless oil. IR (neat) ν cm^{-1}; 1744. ^{1}H NMR (400 MHz, $CDCl_3$) δ; 1.30 (3H, t, J=7.2 Hz), 1.25–1.47 (5H, m), 1.63–1.74 (1H, m), 2.83 (1H, dd, J=14.0, 9.8 Hz), 3.11 (1H, dd, J=14.0, 3.0 Hz), 3.32 (1H, dd, J=11.2, 4.9 Hz), 3.83 (1H, ddd, J=11.5, 5.9, 5.9 Hz), 3.96 (1H, t, J=3.5 Hz), 4.24 (2H, q, J=7.2 Hz), 4.38–4.48 (1H, m), 6.10–6.23 (2H, m), 7.20–7.31 (5H, m). ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −37.2 (1F, ddd, J=258, 9, 9 Hz), −44.3 (1F, ddd, J=258, 13, 13 Hz). EI-MS m/z; 355 (M^++H), 271, 252. HRMS; calcd for $C_{19}H_{24}F_2O_4Na$ (M^++Na): 377.1540. Found: 377.1555. To a solution of the above obtained more polar isomer (1.25 g, 3.52 mmol) in THF (20 ml) was added dropwise DIBAL (0.95 M hexane solution, 15 ml, 14.0 mmol) at −78 °C, and then the mixture was quenched with water after being stirred for 30 min. The organic layer was decanted and the residue was washed with ether twice. The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography [hexane/AcOEt (3:1)] to give (*Z*)-4,4-difluoro-6-phenyl-5-(tetrahydro-2*H*-pyran-2-yloxy)-hexenol (**THP-5e**). After a mixture of **THP-5e** and a catalytic amount of *p*-toluenesulfonic acid in MeOH (10 ml) was stirred at room temperature for 1 h, the reaction mixture was concentrated in vacuo and the residue was purified by silica gel column chromatography [hexane/AcOEt (2:1)] to give **5e** (465 mg, 58% yield) as colorless oil. IR ($CHCl_3$) ν cm^{-1}; 3608, 3440, 3024, 2932, 1954, 1660, 1602. ^{1}H NMR (400 MHz, $CDCl_3$) δ; 2.66 (1H, dd, J=14.0, 10.6 Hz), 2.74 (1H, brs), 3.00 (1H, dd, J=14.0, 1.5 Hz), 3.16 (1H, d, J=2.6 Hz), 3.87–4.00 (1H, m), 4.31 (2H, s), 5.58 (1H, dd, J=15.6, 28.3 Hz), 5.95–6.07 (1H, m), 7.22–7.33 (5H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 36.4, 58.8, 74.5 (t, J=30.2 Hz), 121.0 (t, J=244.6 Hz), 122.3 (t, J=26.6 Hz), 126.8, 128.6, 129.3, 137.2, 138.5 (t, J=5.2 Hz). ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −37.7 (1F, dd, J=256, 2 Hz), −43.0 (1F, dt, J=256, 14 Hz). EI-MS m/z; 229 (M^++H), 210. HRMS; calcd for $C_{12}H_{15}F_2O_2$ (M^++H): 229.1040. Found: 229.1070.

4.3. Preparation of (*E*)-4,4-difluoro-5-phenyl-2-pentenol derivatives

4.3.1. 4,4-Difluoro-5-phenyl-2-pentenol (9c). After a mixture of ethyl 2,2-difluoro-3-phenylpropionate[37] (1.29 g, 7.6 mmol) in THF (10 ml) and DIBAL (11.4 ml, 1 M

hexane solution, 11.4 mmol) was stirred at −78 °C for 30 min, the reaction mixture was quenched by water followed by the similar extractive workup procedure used for **THP-5e** gave an oily aldehyde, which was used for the next step without further purification. To a suspension of NaH (60% oil, 334 mg, 8.36 mmol) in THF (10 ml) was added triethyl phosphonoacetate (1.874 g, 8.36 mmol) in THF (10 ml) at 0 °C, and after 10 min, to this mixture was added the solution of previous aldehyde in THF (10 ml) at 0 °C. The mixture was stirred at 0 °C for 1 h and the reaction mixture was quenched with 5% HCl, extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography [hexane/AcOEt (20:1)] to give a crude mixture, which was further separated by MPLC [hexane/AcOEt (30:1)] to give ethyl (*E*)-4,4-difluoro-5-phenylpent-2-enoate (310 mg, 17% yield) and starting material (240 mg, 15% yield) in the order of elution. Ethyl (*E*)-4,4-difluoro-5-phenylpent-2-enoate: colorless oil. IR (neat) ν cm^{-1}; 3035, 2984, 2929, 1727. ^1H NMR (400 MHz, $CDCl_3$) δ; 1.29 (3H, t, J=7.2 Hz), 3.25 (2H, t, J=16.0 Hz), 4.21 (2H, q, J=7.2 Hz), 6.18 (1H, dt, J=15.6, 11.6 Hz), 6,76 (1H, dt, J=15.6, 2.4 Hz), 7.22–7.38 (5H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ;.13.79, 43.45 (t, J=26.1 Hz), 60.79, 119.15 (t, J=241.4 Hz), 124.97 (t, J=8.5 Hz), 127.34, 128.20, 130.17, 131.37, 138.60 (t, J=27.3 Hz), 164.67. ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −34.61 (2F, m). A mixture of the above ester (51 mg, 0.21 mmol) in THF (5 ml) and DIBAL (1 M hexane solution, 0.84 mmol) was stirred at −78 °C for 1 h. Usual extractive workup mentioned above and purification by silica gel column chromatography [hexane/AcOEt (2:1)] gave **9c** (42 mg, quantitative) as colorless oil. IR (neat) ν cm^{-1}; 3624, 3300–3600, 2932, 1682, 1604. ^1H NMR (400 MHz, $CDCl_3$) δ;1.77–1.93 (1H, brs), 3.20 (2H, t, J=15.7 Hz), 4.08–4.16 (2H, brs), 5.71–5.83 (1H, m), 6.05–6.14 (1H, m), 7.18–7.35 (5H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 44.1 (t, J=27.6 Hz), 61.6, 120.3 (t, J=240.2 Hz), 124.4 (t, J=26,6 Hz), 127.3, 128.3, 130.5, 132.8 (t, J=4.2 Hz), 134.6 (t, J=8.3 Hz). ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −31.77 (2F, td, J=22.5, 15 Hz).

4.3.2. 4,4-Difluoro-5-(4-methoxyphenylamino)-5-phenyl-2-penten-1-ol (13a). Compound **13a** was prepared from 3,3-difluoro-1-(4-methoxyphenyl)-4-phenylazetidin-2-one[38] through the reduction by DIBAL followed by Horner–Emmons reaction. Colorless oil. ^1H NMR (300 MHz, $CDCl_3$) δ; 1.40–1.65 (2H, br), 3.69 (3H, s), 4.17–4.27 (2H, m), 4.62 (1H, t, J=11.0 Hz), 5.80–5.96 (1H, m), 6.16–6.20 (1H, m), 6.50–6.56 (2H, m), 6.64–6.72 (2H, m), 7.24–7.45 (5H, m). ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −40.4 (1F, d, J=244 Hz), −42.0 (1F, dt, J=244 Hz). EI-MS *m/z*; 319 (M^+), 212 (M^+−Anis).

4.4. General procedure for the reaction of difluoroallylic alcohol derivatives 5 with lithium dimethylcuprate in the presence of Me_3Al

4.4.1. (1*R,2*Z*,4*S**)-2-Fluoro-4-methyl-1-phenyl-2-pentene-1,5-diol (*syn*-6a-1).** To a mixture of **5a** (107 mg, 0.5 mmol) and Me_3Al (1 M hexane solution, 5 ml, 5 mmol) in THF (3 ml), was added at −30 °C the Gilman reagent prepared from CuI (476 mg, 2.5 mmol) and MeLi (1.14 M diethyl ether solution, 4.4 ml, 5 mmol) in THF (3 ml). After being stirred at 0 °C for 1.5 h, the reaction mixture was quenched with 5% HCl and filtered through celite pad. The filtrate was extracted with AcOEt and washed with $NaHCO_3$aq and brine. The organic layer was dried over anhydrous Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica gel column chromatography [hexane/AcOEt (1:1)] to give *syn*-**6a-1** (92 mg, 88% yield) as colorless crystals. Mp 102–104 °C. IR ($CHCl_3$) ν cm^{-1}; 3616, 3150–3555, 2968, 2878, 1707. ^1H NMR (400 MHz, $CDCl_3$) δ; 1.01 (3H, d, J=6.9 Hz), 1.68–1.90 (1H, brs), 2.79–2.90 (1H, m), 3.41 (1H, dd, J=10.5, 7.8 Hz), 3.55 (1H, dd, J=10.5, 5.6 Hz), 4.82 (1H, dd, J=37.4, 9.6 Hz), 5.20 (1H, d, J=10.7 Hz), 7.28–7.44 (5H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 16.8, 32.0 (d, J=2.6 Hz), 67.4, 72.5 (d, J=33.1 Hz), 109.5 (d, J=12.6 Hz), 126.7, 128.5 (d, J=22.5 Hz), 139.5, 158.6, 161.1. ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −56.5 (1F, dd, J=37.4, 10.5 Hz). EI-MS *m/z*; 190 (M^+−HF), 162, 147. Anal. Calcd for $C_{12}H_{15}F_2O_2$: C, 68.55; H, 7.19. Found: C, 68.51; H, 7.19.

4.4.2. (2*R,3*Z*,5*R**)-4-Fluoro-2-methyl-6-phenyl-3-hexene-1,5-diol (*anti*-6c-1) and (*E*)-4-fluoro-2-methyl-6-phenyl-3-hexene-1,5-diol (*E*-6c-1).** Compound *anti*-**6c-1** was prepared from **5e** and Gilman reagent. Purification by silica gel column chromatography [hexane/AcOEt (1:1)] gave a mixture of **6c-1** (100 mg, 89% yield, *Z*/*E*=14:1, *syn*/*anti*=1:2 for *Z*-isomer), which was further purified by MPLC [hexane/AcOEt (1:1)] to give *E*-**6c-1**, *syn*-**6c-1**, *anti*-**6c-1** in the order of elution. *anti*-**6c-1**: colorless oil. IR (neat) ν cm^{-1}; 3682, 3604, 3414, 3013, 2962, 2875, 2400, 1708. ^1H NMR (400 MHz, $CDCl_3$) δ; 0.87 (3H, d, J=6.9 Hz), 2.30 (1H, brs), 2.71–2.84 (1H, m), 2.92 (1H, dd, J=13.6, 7.2 Hz), 3.00 (1H, dd, J=13.6, 6.3 Hz), 3.15 (1H, brs), 3.27 (1H, dd, J=10.5, 8.2 Hz), 3.49 (1H, dd, J=10.6, 5.2 Hz), 4.27 (1H, dt, J=17.4, 6.7 Hz), 4.51 (1H, dd, J=37.5, 9.6 Hz), 7.20–7.32 (5H,m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 16.6, 31.8 (d, J=1.7 Hz), 40.1, 67.3, 71.8 (d, J=30.0 Hz), 110.1 (d, J=12.9 Hz), 126.7, 128.4, 129.5, 137.1, 159.2 (d, J=258.6 Hz). ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −62.4 (1F, dd, J=35, 18 Hz). EI-MS *m/z*: 224 (M^+), 207, 204. HRMS; calcd for $C_{13}H_{17}FO_2$ (M^+); 224.1213. Found: 224.1208. *E*-**6c-1**: colorless oil. IR (neat) ν cm^{-1}; 3687, 3599, 3424, 3028, 2965, 2930, 2875, 2401, 1697. ^1H NMR (400 MHz, $CDCl_3$) δ; 0.90 (3H, dd, J=6.7, 0.6 Hz), 2.31–2.48 (2H, m), 2.97 (1H, dd, J=10.3, 8.6 Hz), 3.00 (1H, dd, J=13.3, 6.8 Hz), 3.04 (1H, dd, J=13.3, 7.2 Hz), 3.23 (1H, dd, J=10.3, 4.8 Hz), 4.61 (1H, dt, J=22.7, 7.0 Hz), 4.90 (1H, dd, J=22.5, 10.7 Hz), 7.23–7.34 (5H, m). ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −57.0 (1F, dd, J=23, 23 Hz). EI-MS *m/z*; 225 (M^++H), 207 (M^+−OH), 187. HRMS; calcd for $C_{13}H_{16}FO$ (M^+−OH): 207.1185. Found: 207.1183.

4.5. General procedure for the reaction of difluoroallylic alcohol derivatives 5 with trialkylaluminum in the presence of CuI·2LiCl

4.5.1. (2*R,3*Z*,5*S**)-4-Fluoro-2-methyl-7-phenyl-3-heptene-1,5-diol (*syn*-6b-1).** To a solution of **5b** (121 mg, 0.5 mmol) in THF (3 ml) was added Me_3Al (1 M hexane solution, 2.5 ml, 2.5 mmol) at −30 °C, and then copper

reagent prepared from CuI (468 mg, 2.5 mmol) and LiCl (212 mg, 5 mmol) in THF (3 ml) was added at −30 °C.[21] After being stirred at 0 °C for 20 h, the reaction mixture was quenched with 5% HCl and filtered through celite pad. The filtrate was extracted with AcOEt and washed with $NaHCO_3$aq and brine. The organic layer was dried over anhydrous Na_2SO_4, and then concentrated in vacuo. Purification by silica gel column chromatography [hexane/AcOEt (1:1)] gave a mixture (115 mg) of *syn*-**6b-1** (65% yield) and **5b** (31% recovery), which was further purified by MPLC [hexane/AcOEt (1:1)] to give **5b** and *syn*-**6b-1** in the order of elution. *syn*-**6b-1**: colorless oil. IR ($CHCl_3$) ν cm^{-1}; 3620, 3432, 3032, 2964, 2936, 2876, 1706, 1602. ^1H NMR (400 MHz, $CDCl_3$) δ; 0.99 (3H, d, $J=6.8$ Hz), 1.88–2.06 (2H, m), 2.65–2.87 (3H, m), 3.37 (1H, dd, $J=10.5$, 7.9 Hz), 3.55 (1H, dd, $J=10.5$, 5.4 Hz), 4.09 (1H, ddd, $J=12.8$, 7.6, 5.1 Hz), 4.72 (1H, dd, $J=38.3$, 9.5 Hz), 7.16–7.34 (5H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 16.8, 31.5, 31.8 (d, $J=2.7$ Hz), 35.6, 67.4, 69.4 (d, $J=31.6$ Hz), 108.4 (d, $J=12.8$ Hz), 125.9, 128.4, 128.4, 141.4, 160.9 (d, $J=257.8$ Hz). ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −60.3 (1F, dd, $J=38$, 15 Hz). EI-MS *m/z*; 238(M^+), 220(M^+-H_2O), 201, 190, 91. HRMS; calcd for $C_{14}H^{19}FO_2$ (M^+): 238.1369. Found: 238.1370.

4.5.2. (1*R,2*Z*,4*S**)-2-Fluoro-4-isobutyl-1-phenyl-2-pentene-1,5-diol (*syn*-6a-2).** Compound *syn*-**6a-2** was prepared from **5a** and triisobutylaluminum. Purification by silica gel column chromatography [hexane/AcOEt (1:1)] afforded a mixture (124 mg) of *syn*-**6a-2** (68% yield) and **5a** (30% recovery), which was further purified by MPLC [hexane/AcOEt (1:1)] to give **5a** and *syn*-**6a-2** in the order of elution. *syn*-**6a-2**: colorless crystals. Mp 67–68 °C. IR ($CHCl_3$) ν cm^{-1}; 3616, 3150–3570, 2956, 1706, 1452. ^1H NMR (400 MHz, $CDCl_3$) δ; 0.85 (3H, d, $J=6.7$ Hz), 0.88 (3H, d, $J=6.7$ Hz), 1.13 (2H, dd, $J=7.3$ Hz), 1.54 (1H, qqt, $J=6.7$, 6.7, 6.7 Hz), 2.77–2.89 (1H, m), 3.31 (1H, dd, $J=10.6$, 8.5 Hz), 3.55 (1H, dd, $J=10.6$, 4.7 Hz), 4.68 (1H, dd, $J=37.4$, 10.1 Hz), 5.17 (1H, d, $J=8.6$ Hz), 7.28–7.44 (5H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 21.8, 23.4, 25.7, 35.4, 40.3, 66.4, 72.3 (d, $J=34.4$ Hz), 108.4 (d, $J=12.4$ Hz), 126.8, 128.2, 128.5, 139.7, 161.2 (d, $J=256.7$ Hz). ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −54.4 (1F, d, $J=37$ Hz). EI-MS *m/z*; 204 ($M^+ + H - H_2O - CH_2OH$). HRMS; calcd for $C_{14}H_{17}F$ ($M^+ + H - H_2O - CH_2OH$): 204.1314. Found: 204.1313.

4.5.3. (2*R,3*Z*,5*S**)-4-Fluoro-2-isobutyl-7-phenyl-3-heptene-1,5-diol (*syn*-6b-2).** Compound *syn*-**6b-2** was prepared from **5b** and triisobutylaluminum. Purification by silica gel column chromatography [hexane/AcOEt (1:1)] gave a mixture (127 mg) of *syn*-**6b-2** (66% yield) and **5b** (29% recovery) which was further purified by MPLC [hexane/AcOEt (1:1)] to give *syn*-**6b-2** and **5b** in the order of elution. *syn*-**6b-2**: colorless oil. IR ($CHCl_3$) ν cm^{-1}; 3616, 3416, 3020, 2960, 1706, 1602, 1452. ^1H NMR (400 MHz, $CDCl_3$) δ; 0.89 (3H, d, $J=6.2$ Hz), 0.90 (3H, d, $J=6.3$ Hz), 1.17 (2H, dd, $J=7.3$, 7.3 Hz), 1.50–1.65 (1H, m), 1.87–2.05 (2H, m), 2.71–2.83 (3H, m), 3.36 (1H, dd, $J=10.5$, 8.2 Hz), 3.60 (1H, dd, $J=10.5$, 4.9 Hz), 4.12–4.14 (1H, m), 4.66 (1H, dd, $J=38.3$, 10.1 Hz), 7.18–7.31 (5H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 21.7, 23.5, 25.7, 31.5, 35.4, 35.9, 40.4, 66.6, 69.4 (d, $J=32.5$ Hz), 107.2 (d, $J=12.9$ Hz), 125.9, 128.4, 128.5, 141.5, 161.9 (d, $J=257.2$ Hz). ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −56.8 (1F, dd, $J=38$, 13 Hz). EI-MS *m/z*; 280 (M^+), 243, 232, 156, 91. HRMS; calcd for $C_{17}H_{25}FO_2$ (M^+): 280.1839. Found: 280.1821.

4.5.4. (2*R,3*Z*,5*S**)-4-Fluoro-2-methyl-6-phenyl-3-hexene-1,5-diol (*syn*-6c-1).** Compound *syn*-**6c-1** was prepared from **5c** and trimethylaluminum. Purification by silica gel column chromatography [hexane/AcOEt (1:1)] gave *syn*-**6c-1** (110 mg, 98% yield) as colorless oil. IR ($CHCl_3$) ν cm^{-1}; 3608, 3416, 3024, 2872, 1706. ^1H NMR (400 MHz, $CDCl_3$) δ; 0.93 (3H, d, $J=6.9$ Hz), 2.70–2.83 (1H, m), 2.88 (1H, dd, $J=13.6$, 7.5 Hz), 2.99 (1H, dd, $J=13.6$, 6.0 Hz), 3.24 (1H, dd, $J=10.6$, 7.8 Hz), 3.42 (1H, dd, $J=10.6$, 5.4 Hz), 4.26 (1H, ddd, $J=13.6$, 7.5, 6.0 Hz), 4.56 (1H, dd, $J=38.2$, 9.6 Hz), 7.20–7.32 (5H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 16.6, 31.7 (d, $J=2.7$ Hz), 40.5, 67.1, 71.3 (d, $J=31.9$ Hz), 109.0 (d, $J=12.5$ Hz), 126.6, 128.3, 129.4, 137.1, 159.7 (d, $J=257.9$ Hz). ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −61.0 (1F, dd, $J=38.2$, 13.6 Hz). EI-MS *m/z*; 224 (M^+), 204, 187, 176, 91. HRMS; calcd for $C_{13}H_{17}FO_2$ (M^+): 224.1213. Found: 224.1213.

4.5.5. (2*R,3*Z*,5*S**)-4-Fluoro-2-isobutyl-6-phenyl-3-hexene-1,5-diol (*syn*-6c-2).** Compound *syn*-**6c-2** was prepared from **5c** and triisobutylaluminum. Purification by silica gel column chromatography [hexane/AcOEt (1:1)] gave a mixture (125 mg) of *syn*-**6c-2** (78% yield) and **5c** (19% recovery), which was further purified by MPLC [hexane/AcOEt (1:1)] to give **5c** and *syn*-**6c-2** in the order of elution. *syn*-**6c-2**: colorless solid. Mp 70–72 °C. IR ($CHCl_3$) ν cm^{-1}; 3610, 3412, 2932, 2872, 1707. ^1H NMR (400 MHz, $CDCl_3$) δ; 0.86 (3H, d, $J=6,4$ Hz), 0.86 (3H, d, $J=6,7$ Hz), 1,04–1.16 (2H, m), 1.39–1.54 (1H, m), 1.65 (1H, br), 2.59 (1H, br), 2.71–2.83 (1H, m), 2.92 (1H, dd, $J=13.6$, 7.1 Hz), 3.01 (1H, dd, $J=13.6$, 6.2 Hz), 3.24 (1H, dd, $J=10.6$, 8.0 Hz), 3.47 (1H, dd, $J=10.6$, 4.9 Hz), 4.26–4.37 (1H, m), 4.48 (1H, dd, $J=38.0$, 10.1 Hz), 7.20–7.33 (5H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 21.7, 23.5, 25.5, 35.5 (d, $J=1.5$ Hz), 40.4, 40.6, 66.5, 71.5(d, $J=32.1$ Hz), 108.2 (d, $J=12.5$ Hz), 126.8, 128.5, 129.5, 137.0, 160.4 (d, $J=257.4$ Hz). ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −59.3 (1F, dd, $J=37.7$, 10.5 Hz). EI-MS *m/z*; 267 ($M^+ + H$), 246 ($M^+ - HF$), 218, 91. HRMS; calcd for $C_{16}H_{23}FO_2$ (M^+): 266.1682. Found: 266.1692.

4.5.6. (1*R,2*Z*,4*R**)-2-Fluoro-4-methyl-1-phenyl-2-pentene-1,5-diol (*anti*-6a-1).** Compound *anti*-**6a-1** was prepared from **5d** and trimethylaluminum. Purification by silica gel column chromatography [hexane/AcOEt (1:1)] gave a mixture (104 mg) of *syn*- and *anti*-**6a-1** (52% yield, *syn*/*anti*=1:10.5) and **5d** (41% recovery). This mixture was purified by MPLC [hexane/AcOEt (2:3)] to give **5d**, *syn*-**6a-1** and *anti*-**6a-1** in the order of elution. *anti*-**6a-1**: colorless crystals. Mp 56–58 °C. IR ($CHCl_3$) ν cm^{-1}; 3608, 3396, 3040, 3016, 2968, 2872, 1700. ^1H NMR (400 MHz, $CDCl_3$) δ; 0.96 (3H, d, $J=6.8$ Hz), 2.67–2.88 (1H, m), 3.34 (1H, dd, $J=10.5$, 8.2 Hz), 3.53 (1H, dd, $J=10.5$, 5.3 Hz), 3.95 (1H, brs), 4.80 (1H, dd, $J=36.7$, 9.6 Hz), 5.17 (1H, d, $J=16.7$ Hz), 7.26–7.46 (5H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 16.6, 31.9 (d, $J=2.2$ Hz), 67.3, 72.8 (d, $J=30.4$ Hz), 110.5 (d, $J=12.9$ Hz), 126.6, 128.1, 128.4, 139.3, 159.4 (d, $J=258.9$ Hz). ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −60.1 (1F, dd, $J=37$, 17 Hz). EI-MS *m/z*; 190 ($M^+ - HF$),

162, 150, 147. HRMS; calcd for $C_{12}H_{14}FO_2$ (M^+-HF): 190.0994. Found: 190.1008.

4.5.7. (1*R,2*Z*,4*R**)-2-Fluoro-4-isobutyl-1-phenyl-2-pentene-1,5-diol (*anti*-6a-2).** Compound *anti*-**6a**-**2** was prepared from **5d** and triisobutylaluminum. Purification by silica gel column chromatography [hexane/AcOEt (1:2)] gave a mixture (94 mg) of *syn*- and *anti*-**6a**-**2** (23% yield, *syn*/*anti*=1:5.8) and **5d** (60% recovery), which was further purified by MPLC [hexane/AcOEt (2:3)] to give **5d**, *syn*-**6a**-**2** and *anti*-**6a**-**2** in the order of elution. *anti*-**6a**-**2**: colorless oil. IR ($CHCl_3$) ν cm^{-1}; 3612, 3416, 3024, 2960, 2872, 1700, 1600, 1368. ^1H NMR (400 MHz, $CDCl_3$) δ; 0.85 (3H, d, J=6,6 Hz), 0.89 (3H, d, J=6,6 Hz), 1,13–1.21 (2H, m), 1.49–1.64 (1H, m), 1.85 (1H, brs), 2.76–2.88 (1H, m), 3.30 (1H, brs), 3.39 (1H, dd, J=10.5, 8.1 Hz), 3.60 (1H, dd, J=10.5, 5.0 Hz), 4.76 (1H, dd, J=36.7, 10.1 Hz), 5.22 (1H, d, J=15.5 Hz), 7.26–7.44 (5H, m).^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 21.8, 23.5, 25.7, 35.6, 40.4, 66.5, 72.8 (d, J=31.1 Hz), 109.5 (d, J=12.9 Hz), 126.5, 128.2, 128.5, 139.4, 160.3 (d, J=258.0 Hz). ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −59.6 (1F, d, J=35 Hz). EI-MS *m*/*z*; 232 (M^+-HF), 204, 150, 117, 91. HRMS; calcd for $C_{14}H_{17}F$ ($M^+-OH-CH_2OH$): 204.1314. Found: 204.1311.

4.5.8. (2*R,3*Z*,5*R**)-4-Fluoro-2-isobutyl-6-phenyl-3-hexene-1,5-diol (*anti*-6c-2).** Compound *anti*-**6c**-**2** was prepared from **5e** and triisobutylaluminum. Purification by silica gel column chromatography [hexane/AcOEt (1:1)] gave a mixture (123 mg) of *syn*- and *anti*-**6c**-**2** (32% yield, *syn*/*anti*=1:2.9) and **5e** (32% recovery), which was further purified by MPLC [hexane/AcOEt (1:1)] to give **5e**, *syn*-**6c**-**2** and *anti*-**6c**-**2** in the order of elution. *anti*-**6c**-**2**: colorless solid. Mp 48–49 °C. IR (neat) ν cm^{-1}; 3677, 3602, 3401, 2957, 2871, 1707. ^1H NMR (400 MHz, $CDCl_3$) δ; 0.77 (3H, d, J=6.6 Hz), 0.79 (3H, d, J=6.5 Hz), 0.90–1.06 (2H, m), 1.08–1.22 (1H, m), 2.40 (1H, br), 2.69–2.81 (1H, m), 2.97 (1H, dd, J=13.8, 6.6 Hz), 3.00 (1H, dd, J=13.8, 7.6 Hz), 3.24 (1H, dd, J=10.5, 8.8 Hz), 3.44 (1H, br), 3.53 (1H, dd, J=10.5, 4.7 Hz), 4.31 (1H, dt, J=19.8, 7.0 Hz), 4.38 (1H, dd, J=37.0, 10.2 Hz), 7.19–7.30 (5H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 21.5, 23.6, 25.2, 35.6, 39.8, 40.1, 66.6, 72.3 (d, J=29.5 Hz), 109.9 (d, J=13.3 Hz), 126.6, 128.4, 129.4, 136.9, 159.9 (d, J=258.8 Hz). ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −64.2 (1F, dd, J=37, 19 Hz). EI-MS *m*/*z*; 289 (M^++Na), 229. HRMS; calcd for $C_{16}H_{23}FO_2Na$ (M^++Na); 289.1580. Found: 289.1575.

4.5.9. (*Z*)-5-[(*tert*-Butyldiphenylsilanyl)oxy]-2-fluoro-4-methyl-1-phenyl-2-penten-1-ol (10a). Compound **10a** was prepared from **9a** and Me_3Al in the presence CuI·2LiCl. Purification by silica gel column chromatography [hexane/AcOEt (20:1)] afforded a mixture of *syn*-**10a** (77% yield), *anti*-**10a** (4% yield) and **9a** (4% recovery). **10a** was desilylated with TBAF and the product was identical with **6a**-**1**.

4.5.10. (*E*)-5-[(*tert*-Butyldiphenylsilanyl)oxy]-2,2-difluoro-1-phenyl-3-penten-1-ol (9a). A mixture of **5a** (321 mg, 1.5 mmol), imidazole (112 mg, 1.65 mmol) and *tert*-butyldiphenylchlorosilane (454 mg, 1.65 mmol) in DMF (6 ml) was stirred at room temperature for 3 h. Extractive workup (AcOEt for extraction) followed by silica gel column chromatography [hexane/AcOEt (10:1)] gave **9a** (680 mg, quantitative) as colorless oil. IR ($CHCl_3$) ν cm^{-1}; 3680, 3616, 3028, 1602. ^1H NMR (400 MHz, $CDCl_3$) δ; 1.05 (9H, s), 2.53 (1H, d, J=3.8 Hz), 4.24 (2H,m), 4.93 (1H, td, J=9.4, 3.8 Hz), 5.90–6.03 (1H, m), 6.08–6.16 (1H, m), 7.30–7.50 (11H, m), 7.59–7.68 (4H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 19.2, 26.7, 62.6, 76.1 (t, J=30.0 Hz), 120.2 (t, J=25.0 Hz), 120.2 (t, J=244.1 Hz), 127.6, 127.8, 128.2, 128.6, 129.8, 133.1, 135.4, 136.2, 136.6 (t, J=8.1 Hz). ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −43.3 (1F, d, J=9.4 Hz), −43.5 (1F, d, J=9.4 Hz). EI-MS *m*/*z*; 375 (M^+-Ph), 305, 247. Anal. Calcd for $C_{27}H_{30}F_2O_2Si$: C, 71.65; H, 6.68. Found: C, 71.49; H, 6.70.

4.6. Preparation of the pivalate

4.6.1. (2*S,3*Z*,5*R**)-4-Fluoro-5-hydroxy-2-methyl-7-phenyl-hept-3-enyl-2,2-dimethyl-propionate (7b-1).** After a mixture of *syn*-**6b**-**1** (210 mg, 0.88 mmol), pivaloyl chloride (121 mg, 1.0 mmol) and pyridine (0.20 ml) in CH_2Cl_2 (7 ml) was stirred at room temperature for 4.5 h, usual extractive workup followed by the purification by silica gel column chromatography [hexane/AcOEt (5:1)] gave **7b**-**1** (218 mg, 77% yield) as colorless oil. IR (neat) ν cm^{-1}; 3450, 2970, 1728, 1710. ^1H NMR (400 MHz, $CDCl_3$) δ; 1.05 (3H, t, J=6.9 Hz), 1.20 (9H, s), 1.90–2.05 (2H, m), 2.12–2.28 (1H, brs), 2.66–2.81 (2H, m), 2.94–3.06 (1H, m), 3.92 (1H, dd, J=10.7, 6.6 Hz), 3.96 (1H, dd, J=10.7, 6.1 Hz), 4.02–4.12 (1H, m), 4.71 (1H, dd, J=37.3, 9.5 Hz), 7.17–7.22 (3H, m), 7.25–7.32 (2H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 17.2, 27.1. 28.7 (d, J=3.7 Hz), 31.4, 35.4, 38.8, 68.0, 69.7 (d, J=30.3 Hz), 108.3 (d, J=13.2 Hz), 126.0, 128.4, 141.2, 159.9 (d, J=259.1 Hz), 178.5. ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −60.7 (1F, dd, J=37.0, 2.0 Hz). EI-MS *m*/*z*; 304 (M^+-H_2O), 220. 202. Anal. Calcd for $C_{19}H_{27}FO_3$: C, 70.78; H, 8.44. Found: C, 70.43; H, 8.37.

4.6.2. (2*S,3*Z*,5*R**)-4-Fluoro-5-hydroxy-2-methyl-6-phenyl-hex-3-enyl-2,2-dimethyl-propionate (7c-1).** Compound **7c**-**1** was prepared from *syn*-**6c**-**1**. Purification by silica gel column chromatography [hexane/AcOEt (5:1)] afforded **7c**-**1** (80% yield) as colorless oil. IR (neat) ν cm^{-1}; 3456, 2972, 2929, 2876, 1730, 1712. ^1H- NMR (400 MHz, $CDCl_3$) δ; 1.01 (3H, d, J=6.9 Hz), 1.19 (9H, s), 2.02 (1H, d, J=4.9 Hz), 2.85–3.04 (3H, m), 3.82–3.91 (2H, m), 4.26–4.32 (1H, m), 4.64 (1H, dd, J=37.5, 9.5 Hz), 7.19–7.35 (5H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 17.1, 27.1, 28.7 (d, J=3.8 Hz), 38.8, 40.7, 67.9, 71.2 (d, J=31.4 Hz), 108.3 (d, J=12.6 Hz), 126.8, 128.5, 129.5, 136.8, 159.2 (d, J=258.2 Hz), 178.4. ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −60.0 (1F, dd, J=37, 13 Hz). EI-MS *m*/*z*; 331 (M^++Na), 291, 189. HRMS; calcd for $C_{18}H_{25}O_3FNa$ (M^++Na): 331.1685. Found: 331.1654. Anal. Calcd for $C_{18}H_{25}FO_3$: C, 70.10; H, 8.17. Found: C, 70.06; H, 8.14.

4.7. Preparation of the *N*-Boc derivative 8 through one-pot azidation reaction

4.7.1. (2*S,4*Z*,5*S**)-5-[(*tert*-Butoxycarbonyl)amino]-4-fluoro-2-methyl-7-phenyl-hept-3-en-1-ol (8b) and (2*S**,3*R**,4*Z*)-3-[(*tert*-butoxycarbonyl)amino]-4-fluoro-2-methyl-7-phenyl-hept-4-en-1-ol (17b).** To a mixture of

7b-**1** (260 mg, 0.81 mmol), DMAP (367 mg, 3 mmol) and NaN_3 (1.63 g, 25.0 mmol) in CH_2Cl_2 (10 ml) was added methanesulfonyl chloride (229 mg, 2.0 mmol) at 0 °C. After being stirred at room temperature for 30 min, to the reaction mixture was added DMSO (5 ml), and then the whole was stirred at room temperature for 3 h. The reaction mixture was poured into water and extracted with a mixture of AcOEt and hexane. The aqueous layer was re-extracted with AcOEt, and the combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by short silica gel column chromatography [hexane/AcOEt (5:1)] to give crude **15b** (266 mg, 95% yield). To a suspension of $LiAlH_4$ (76.0 mg, 2.0 mmol) in THF (3 ml) was added **15b** (266 mg) in THF (5 ml) at 0 °C, and then the whole was stirred at room temperature for 90 min. To the reaction mixture was added ice water dropwise at 0 °C and after being stirred for 10 min, the mixture was extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was dissolved in CH_2Cl_2 (2 ml) and to this solution were added Et_3N (0.28 ml, 0.28 mmol) and $(Boc)_2O$ (218 mg, 1.0 mmol) in CH_2Cl_2 (2 ml). After being stirred at room temperature for 30 min, the reaction mixture was poured into water and extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by silica gel column chromatography [hexane/AcOEt (4:1)] to give a mixture of **8b** and **17b** (224 mg, 86% yield, **8b**:**17b**=8.4:1), which was further separated by MPLC [hexane/AcOEt (3:1)]. **8b**: colorless solid. Mp 82–84 °C. IR (neat) ν cm^{-1}; 3400, 3332, 2976, 2931, 1694. ^1H NMR (400 MHz, $CDCl_3$) δ; 0.10 (3H, d, J=6.8 Hz), 1.44 (9H, s), 1.80–2.02 (2H, m), 2.18 (1H, brs), 2.66 (2H, t, J=7.8 Hz), 2.76–2.89 (1H, m), 3.33–3.44 (1H, m), 3.44–3.55 (1H, m), 4.00–4.18 (1H, m), 4.61 (1H, dd, J=37.5, 9.7 Hz), 4.71–4.82 (1H, brd), 7.15–7.22 (3H, m), 7.25–7.31 (2H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 16.7, 28.3, 32.1, 33.6, 52.2 (d, J=30.2 Hz), 67.2, 80.0, 110.4 (d, J=12.0 Hz), 126.1, 128.3, 128.5, 140.9, 155.2, 155.7 (d, J=260.0 Hz). ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −62.8 (1F, dd, J=37.0, 22.0 Hz). EI-MS m/z; 360(M^++Na). Anal. Calcd for $C_{19}H_{28}FNO_3$: C, 67.63; H, 8.36; N, 4.15. Found: C, 67.66; H, 8.33; N, 4.13. **17b**: colorless oil. IR (neat) ν cm^{-1}; 3393, 2976, 2933, 1693, 1499. ^1H NMR (400 MHz, $CDCl_3$) δ; 0.94 (3H, d, J=7.0 Hz), 1.45 (9H, s), 1.71 (1H, br), 2.32–2.50 (2H, m), 2.68 (2H, t, J=7.6 Hz), 2.76 (1H, brs), 3.44 (1H, d, J=11.0 Hz), 3.66 (1H, d, J=10.2 Hz), 4.02 (1H, dt, J=24.3, 9.4 Hz), 4.81 (1H, dt, J=37.7, 7.5 Hz), 5.06 (1H, bd, d, J=9.0 Hz), 7.11–7.19 (3H, m), 7.19–7.29 (3H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 14.4, 25.1(d, J=4.0 Hz), 28.3, 35.3, 37.4, 54.4 (d, J=27.4 Hz), 63.7, 80.3, 107.4 (d, J=14.4 Hz), 126.0, 128.4 (d, J=9.1 Hz), 141.3, 156.3, 156.6 (d, J=256.1 Hz). ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −62.0 (1F, dd, J=38, 24 Hz). EI-MS m/z; 338 (M^++H), 282, 264. HRMS; calcd for $C_{19}H_{29}FNO_3H$ (M^++H); 338.2131. Found: 338.2130.

4.7.2. *tert*-Butyl (1*R,2*Z*,4*R**)-1-benzyl-2-fluoro-5-hydroxy-4-methyl-2-pentenyl carbamate (8c).** Compound **8c** was prepared from **7c**-**1** (308 mg, 1.0 mmol). Purification by silica gel column chromatography [hexane/AcOEt (5:1)] afforded **8c** (235 mg) in 71% yield from **7c**-**1**. **8c**: colorless oil. IR (neat) ν cm^{-1}; 3427, 3334, 2976, 2931, 2872, 1697. ^1H NMR (400 MHz, $CDCl_3$, 50 °C) δ; 0.92 (3H, d, J=6.9 Hz), 1.47 (9H, s), 1.67 (1H, brs), 2.77–2.83 (1H, m), 2.95–3.15 (2H, m), 3.37 (1H, dd, J=10.5, 7.3 Hz), 3.47 (1H, dd, J=10.5, 5.8 Hz), 4.35–4.46 (1H, m), 4.44 (1H, dd, J=37.9, 9.5 Hz), 4.74 (1H, brs), 7.19–7.36 (5H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$, 50 °C) δ; 16.5, 28.3, 32.2 (d, J=2.5 Hz), 38.6, 53.7 (d, J=29.2 Hz), 67.3, 80.0, 110.0 (d, J=12.9 Hz), 126.9, 128.4, 129.4, 136.8, 154.9, 157.4 (d, J=257.7 Hz). ^{19}F NMR (376.5 MHz, $CDCl_3$) δ; −61.0 (1F, dd, J=38.0, 20.0 Hz). ES-MS m/z; 346 (M^++Na). HRMS Calcd for $C_{18}H_{26}FNO_3Na$: 346.1795 (M^++Na). Found: 346.1786.

References and notes

1. (a) Hann, M. M.; Sammes, P. G.; Kennewell, P. D.; Taylor, J. B. *J. Chem. Soc. Perkin Trans. 1* **1982**, 307–314. (b) Abraham, R. J.; Ellison, S. L. R.; Schonholzer, P.; Thomas, W. A. *Tetrahedron* **1986**, *42*, 2101–2110. (c) Cieplak, P.; Kollman, P. A. *J. Comput. Aided Mol. Des.* **1993**, *7*, 291–304. (d) Wipf, P.; Henninger, T. C.; Geib, S. J. *J. Org. Chem.* **1998**, *63*, 6088–6089 and references cited therein. (e) Welch, J. T. In *Biomedical frontiers of fluorine chemistry*; Ojima, I., McCarthy, J. R., Welch, J. T., Eds.; ACS Symposium Series 639; ACS: Washington, DC, 1996. (f) Howard, J. A. K.; Hoy, V. J.; O'Hagan, D.; Smith, G. T. *Tetrahedron* **1996**, *38*, 12613–12622. (g) O'Hagan, D.; Rzepa, H. S. *J. Chem. Soc., Chem. Commun.* **1997**, 645–652.
2. (a) Alemendinger, T.; Furet, P.; Hungerbuehler, E. *Tetrahedron Lett.* **1990**, *31*, 7297–7300. (b) Alemendinger, T.; Felder, E.; Hungerbuehler, E. *Tetrahedron Lett.* **1990**, *31*, 7301–7304.
3. Boros, L. G.; De Corte, B.; Gimi, R. H.; Welch, J. T.; Wu, Y.; Handschumacher, R. E. *Tetrahedron Lett.* **1994**, *35*, 6033–6036.
4. (a) Welch, T.; Lin, J. *Tetrahedron* **1996**, *52*, 291–304. (b) Lin, J.; Toscano, P. J.; Welch, J. T. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 14020–14024. (c) Zhao, K.; Lim, D. S.; Funaki, T.; Welch, J. T. *Bioorg. Med. Chem.* **2003**, *11*, 207–215.
5. Bartlett, P. A.; Otake, A. *J. Org. Chem.* **1995**, *60*, 3107–3111.
6. Examples of trifluoromethyl substituted peptide mimetics, [CH(CF_3)NH] as a replacement for [CONH] bond, see: (a) Volonterio, A.; Bravo, P.; Zanda, M. *Org. Lett.* **2000**, *2*, 1827–1830. (b) Molteni, M.; Volonterio, A.; Zanda, M. *Org. Lett.* **2003**, *5*, 3887–3890 and references cited therein.
7. Machleidt, H.; Wassendorf, R. *Ann.* **1964**, *674*, 1–10.
8. For a review of fluoroalkene syntheses using phosphonium ylides. Burton, D. J.; Yang, Z.-Y.; Qiu, W. *Chem. Rev.* **1996**, *96*, 1641–1715.
9. Kinetic separation of 1-bromo-1-fluoroalkenes using palladium chemistry, see: (a) Xu, J.; Burton, D. J. *Org. Lett.* **2002**, *4*, 831–833. (b) Xu, J.; Burton, D. J. *Tetrahedron Lett.* **2002**, *43*, 4565–4567. See also: (c) Gross, R. S.; Mehdi, S.; McCarthy, J. R. *Tetrahedron Lett.* **1993**, *34*, 7197–7200.
10. Modification of Horner–Emmons type fluoroolefination reactions, see: (a) Sano, S.; Teranishi, R.; Nagao, Y. *Tetrahedron Lett.* **2002**, *43*, 9183–9186. (b) Sano, S.; Yokoyama, K.; Teranishi, R.; Shiro, M.; Nagao, Y. *Tetrahedron Lett.* **2002**, *43*, 281–284. (c) Sano, S.; Saito, K.; Nagao, Y. *Tetrahedron Lett.* **2003**, *44*, 3987–3990.
11. Highly *Z*-selective fluoroolefin synthesis via sulfoxide elimination reaction, see: (a) Allemendinger, T. *Tetrahedron*

1991, *47*, 4905–4914. (b) Nakazato, A.; Kumagai, T.; Sakagami, K.; Yoshikawa, R.; Suzuki, Y.; Chaki, S.; Ito, H.; Taguchi, T.; Nakanishi, S.; Okuyama, S. *J. Med. Chem.* **2000**, *43*, 4893–4909.

12. Hata, H.; Kobayashi, T.; Amii, H.; Uneyama, K.; Welch, J. T. *Tetrahedron Lett.* **2002**, *43*, 6099–6102.
13. 1-Arylsulfonyl-1-fluoroalkenes as a building block, see: (a) McCarthy, J. R.; Matthews, D. P.; Stemerick, D. M.; Huber, E. W.; Bey, P.; Lippert, B. J.; Snyder, R. D.; Sunkara, P. S. *J. Am. Chem. Soc.* **1991**, *113*, 7439–7440. (b) McCarthy, J. R.; Huber, E. W.; Le, T.-B.; Mark Laskovics, F.; Mattews, D. *Tetrahedron* **1996**, *52*, 45–58.
14. Ring-opening reaction of fluorinated cyclopropane derivatives, see: (a) Bessiere, Y.; Savary, D. N.-H.; Schlosser, M. *Helv. Chim. Acta* **1977**, *60*, 1739–1746. (b) Bessiere, Y.; Bessard, Y.; Kotani, T.; Schlosser, M. *Tetrahedron* **1991**, *47*, 4355–4360. (c) Taguchi, T.; Takigawa, T.; Tawara, Y.; Morikawa, T.; Kobayashi, Y. *Tetrahedron Lett.* **1984**, *25*, 5689–5692.
15. Cr(II)-mediated reaction of dibromofluoroacetate with aldehyde, see: Barma, D. K.; Kundu, A.; Zhang, H.; Mioskowski, C.; Falk, J. R. *J. Am Chem. Soc.* **2003**, *125*, 3218–3219.
16. (a) Otaka, A.; Watanabe, H.; Mitsuyama, E.; Yukimasa, A.; Tamamura, H.; Fujii, N. *Tetrahedron Lett.* **2001**, *42*, 285–287. (b) Otaka, A.; Watanabe, H.; Yukimasa, A.; Oishi, S.; Tamamura, H.; Fujii, N. *Tetrahedron Lett.* **2001**, *42*, 5443–5446. (c) Otaka, A.; Yukimasa, A.; Sasaki, Y.; Watanabe, H.; Oishi, S.; Tamamura, H.; Fujii, N. *Chem. Commun.* **2003**, 1834–1835. (d) Otaka, A.; Mitsuyama, E.; Watanabe, H.; Tamamura, H.; Fujii, N. *Chem. Commun.* **2000**, 1081–1082.
17. Otaka, A.; Watanabe, J.; Yukimasa, A.; Sasaki, Y.; Watanabe, H.; Kinoshita, T.; Oishi, S.; Tamamura, H.; Fujii, N. *J. Org. Chem.* **2004**, *69*, 1634–1645.
18. Okada, M.; Nakamura, Y.; Sato, A.; Horikawa, H.; Saito, A.; Taguchi, T. *Chem. Lett.* **2002**, 28–29.
19. Okada, M.; Nakamura, Y.; Saito, A.; Sato, A.; Horikawa, H.; Taguchi, T. *Tetrahedron Lett.* **2002**, *43*, 5845–5848.
20. Yamazaki, A.; Shinohara, N.; Kitazume, T.; Sato, S. *J. Fluorine Chem.* **1997**, *97*, 91–96.
21. Lipshutz, B. H.; Segi, M. *Tetrahedron* **1995**, *51*, 4407–4420.
22. Copper-catalyzed conjugate additions of trialkylaluminium with enones, see: (a) Kabbara, J.; Fleming, S.; Nickisch, K.; Neh, H.; Westermann, J. *Tetrahedron* **1995**, *51*, 743–754. (b) Takemoto, Y.; Kuraoka, S.; Hamaue, N.; Aoe, K.; Hiramatsu, H.; Iwata, C. *Tetrahedron* **1996**, *52*, 14177–14188. (c) Benett, S. M. W.; Brown, S. M.; Muxworthy, J. P.; Woodward, S. *Tetrahedron Lett.* **1999**, *40*, 1767–1770. (d) Pamies, O.; Net, G.; Ruiz, A.; Claver, C.; Woodward, S. *Tetrahedron: Asymmetry* **2000**, *11*, 871–877. (e) Kraus, N.; Hoffmann-Röder, A. *Synthesis* **2001**, 171–196.
23. Copper-catalyzed SN2′ substitution of allylic phosphates and chlorides with trialkylaluminum, see: Fleming, S.; Kabbara, J.; Nickish, K.; Westermann, J.; Mohr, J. *Synlett* **1995**, 183–185.
24. Maruoka demonstrated several specific reactions of fluorinated molecules based on fluorine–aluminum coordination. (a) Ooi, T.; Uraguchi, D.; Kagoshima, N.; Maruoka, K. *Tetrahedron Lett.* **1997**, *38*, 5679–5682. (b) Ooi, T.; Kagoshima, N.; Uraguchi, D.; Maruoka, K. *Tetrahedron Lett.* **1998**, *39*, 7105–7108. (c) Ooi, T.; Kagoshima, N.; Maruoka, K. *J. Am. Chem. Soc.* **1997**, *119*, 5754–5755. (d) Uraguchi, D.; Ooi, T.; Maruoka, K. *J. Synth. Org. Chem. Jpn.* **2000**, *58*, 14–22. (e) Plenio, H. *Chem. Rev.* **1997**, *97*, 3363–3384.
25. Ito, H.; Saito, A.; Taguchi, T. *Tetrahedron: Asymmetry* **1998**, *9*, 1989–1994.
26. (a) Lipshuts, B. H.; Sengupta, S. *Org. React.* **1992**, *41*, 135–631. (b) Nakamura, E.; Mori, S. *Angew. Chem., Int. Ed.* **2000**, *39*, 3750–3771.
27. *anti* mode reaction with allylic esters, halides and phosphates: (a) Goering, H. L.; Singleton, V. D. *J. Am. Chem. Soc.* **1976**, *98*, 7854–7855. (b) Goering, H. L.; Tseng, C. C. *J. Org. Chem.* **1983**, *48*, 3986–3990. (c) Goering, H. L.; Kantner, S. S. *J. Org. Chem.* **1984**, *49*, 422–426. (d) Goering, H. L.; Kantner, S. S.; Seitz, E. P. *J. Org. Chem.* **1985**, *50*, 5495–5499. (e) Goering, H. L.; Kantner, S. S. *J. Org. Chem.* **1981**, *46*, 2144–2148. (f) Tseng, C. C.; Paisley, S. D.; Goering, H. L. *J. Org. Chem.* **1986**, *51*, 2884–2891. (g) Tseng, C. C.; Yen, S.-J.; Goering, H. L. *J. Org. Chem.* **1986**, *51*, 2892–2895. (h) Corey, E. J.; Boaz, N. W. *Tetrahedron Lett.* **1984**, *25*, 3063–3066. (i) Nakamura, E.; Sekiya, K.; Arai, M.; Aoki, S. *J. Am. Chem. Soc.* **1989**, *111*, 3091–3093. (j) Ibuka, T.; Nakao, T.; Nishii, S.; Yamamoto, Y. *J. Am. Chem. Soc.* **1986**, *108*, 7420–7422.
28. *syn* mode reaction, in particular with allylic carbamate derivatives, see: (a) Gallina, C.; Clattini, P. G. *J. Am. Chem. Soc.* **1979**, *101*, 1035–1036. (b) Goering, H. L.; Kantner, S. S.; Tseng, C. C. *J. Org. Chem.* **1983**, *48*, 715–721. (c) Goering, H. L.; Tseng, C. C. *J. Org. Chem.* **1985**, *50*, 1597–1599. (d) Ibuka, T.; Habashita, H.; Otaka, A.; Fujii, N. *J. Org. Chem.* **1991**, *56*, 4370–4382. (e) Smitrovich, J. H.; Woerpel, K. A. *J. Org. Chem.* **2000**, *65*, 1601–1614.
29. Effect of BF_3 on the alkylation of allylic alcohol: Yamamoto, Y.; Yamamoto, S.; Yatagai, H.; Maruyama, K. *J. Am. Chem. Soc.* **1980**, *102*, 2318–2325.
30. SN2′ addition to vinylic epoxide: (a) Marshall, J. A.; Andia, V. H. *J. Org. Chem.* **1987**, *52*, 1106–1113. (b) Saddler, J. C.; Fucks, P. L. *J. Am. Chem. Soc.* **1981**, *103*, 2112–2114.
31. Examples of allylic azide from allylic alcohol by the Mitsunobu reaction, see: (a) Jarvest, R. L.; Berge, J. M.; Brown, P.; Houge-Frydrych, C. S. V.; O'Hanlon, P. J.; McNair, D. J.; Pope, A. J.; Rittenhouse, S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1265–1268. (b) Chandrasekhar, S.; Raza, A.; Takhi, M. *Tetrahedron: Asymmetry* **2002**, *13*, 423–428. (c) Sen, S. E.; Roach, S. L. *Synthesis* **1995**, 756–758.
32. SN2 and SN2′ allylic azidation reaction of allylic mesylate or bromide and rearrangement of allylic azide, see: (a) Hirasawa, H.; Taniguchi, T.; Ogasawara, K. *Tetrahedron Lett.* **2001**, *42*, 7587–7590. (b) Cardillo, G.; Fabbroni, S.; Gentilucci, L.; Perciaccante, R.; Piccinelli, F.; Tolomelli, A. *Org. Lett.* **2005**, *7*, 533–536 and references cited therein.
33. Uenishi, J.; Hamada, M. *Synthesis* **2002**, 625–630.
34. Low temperature preparation of the mesylate of optically pure benzhydrol followed by nucleophilic substitution, see: Bolshan, Y.; Chen, C.-yi.; Chilenski, J. R.; Gosselin, F.; Mathre, D. J.; O'Shea, P. D.; Roy, A.; Tillyer, R. D. *Org. Lett.* **2004**, *6*, 111–114.
35. Kinetic of rearrangement of allylic azides, see: (a) Gragneux, A.; Winstein, S.; Young, W. G. *J. Am. Chem. Soc.* **1960**, *82*, 5956–5957. (b) VanderWerf, C. A.; Heasley, V. L. *J. Org. Chem.* **1966**, *31*, 3534–3539.
36. Kirihara, M.; Takuwa, T.; Takizawa, S.; Momose, T.; Nemoto, H. *Terahedron* **2000**, *56*, 8275–8280.
37. Taguchi, T.; Kitagawa, O.; Morikawa, T.; Nishiwaki, T.; Uehara, H.; Endo, H.; Kobayashi, Y. *Tetrahedron Lett.* **1986**, *27*, 6103–6106.
38. Taguchi, T.; Kitagawa, O.; Suda, Y.; Ohkawa, S.; Hashimoto, A.; Iitaka, Y.; Kobayashi, Y. *Tetrahedron Lett.* **1988**, *29*, 5291–5294.

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Synthesis of azide-fluoro-dehydrocoelenterazine analog as a photoaffinity-labeling probe and photolysis of azide-fluoro-coelenterazine

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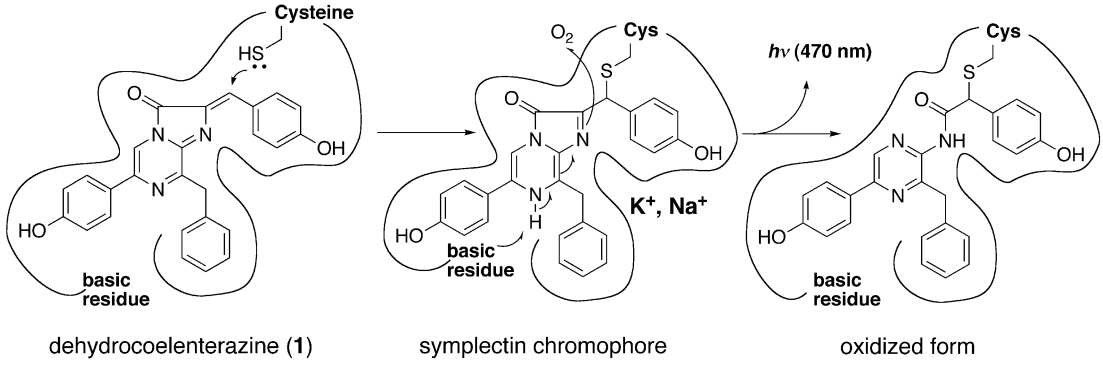
Abstract—A photosensitive azide-fluoro-dehydrocoelenterazine analog (Az-F-DCT) was synthesized, starting from 4-fluorophenylacetic acid, as a photoaffinity-labeling probe in order to analyze symplectin active site. To examine the photo-reactivity of Az-F-DCT, azide-fluoro-coelenterazine analog (Az-F-CT) was used as a potent symplectin chromophore model. Photolysis of Az-F-CT in 2,2,2-trifluoroethanol afforded nitrene intermediate to give an insertion product. The structure of this product was confirmed through spectroscopic analyses particularly by using a proton/deuterium (H/D) exchange experiments with ESI-Q-TOF-MS and -MS/MS measurement.

1. Introduction

Symplectin, the photoprotein of an oceanic luminous squid, TOBIIKA (*Symplectoteuthis oualaniensis*, L.),[1] has a covalently bound chromophore, and its structure has been known to be a conjugate addition product of dehydro-coelenterazine (DCT; **1**) to the protein through an active site cysteine.[2–6] In the presence of mono-valent ions (K^+, Na^+) and molecular oxygen, the chromophore is oxidized at pH 7.8 (optimum) to convert into an oxidized product while emitting a blue light (470 nm) as shown in Scheme 1.

We now focus on the molecular mechanism of symplectin bioluminescent process, especially on the dynamism of symplectin active site surrounding the chromophore. Photoaffinity labeling is the best method for such a research purpose, and has been established with many successful reports.[7,8] Though, there are many photosensitive functional groups such as diazirine and benzophenone,[9] we selected azide group as a photosensitive functional group, since, the introduction of azide into aromatic ring is not so difficult and the size of azide is relatively small to minimize a steric repulsion in the active site. Therefore, we decided to introduce an azide group into DCT.

Azide group always decomposes by light irradiation to give



Scheme 1. Postulated bioluminescent mechanism of symplectin photoprotein.

Keywords: Coelenterazine; Deuteriation MS; Exchangeable protons; Azide-fluoro-phenyl; Photoaffinity probe.
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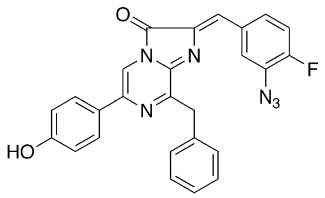
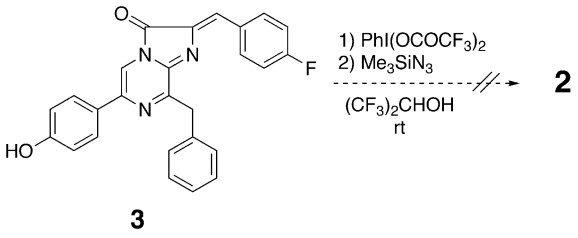


Figure 1. Structure of azide-fluoro-dehydrocoelenterazine analog (**2**).



Scheme 2. Trial for one-pot introduction of azide into 2′-fluoro-DCT analog (**3**) by using Kita's method.

a nitrene intermediate, which after follows a ring expansion to afford an electrophilic azepine intermediate. Actually, Niwa and Ohashi et al. reported the isolation of azepine derivative by photolysis of an azide-coelenteramide analog in MeOH containing Et_2NH.[10] The ring expansion is also useful if some nucleophilic residues exist near the azepine intermediate. However, in order to label the active site of symplectin protein efficiently even if there were no nucleophilic residues, we considered it better to avoid the ring expansion for the successful photoaffinity labeling. Instead of using azepine intermediate, we planned to use nitrene intermediate itself for labeling the symplectin active site. Platz et al. reported that fluorine atom adjacent to nitrene stabilized its singlet state.[11] Furthermore, we have demonstrated that the fluorinated DCT analogs are very active substances for symplectin bioluminescence.[12] Therefore, we planned to synthesize the azide-fluoro-dehydrocoelenterazine analog (Az-F-DCT; **2**) as a photoaffinity-labeling probe to analyze symplectin active site (Fig. 1). In this report, we here, describe the 14-step synthesis of **2** by starting from 4-fluorophenylacetic acid and photolysis of azide-fluoro-coelenterazine analog (Az-F-CT; **15**), which is the final oxidation precursor for **2** having the same oxidation state as the symplectin chromophore, in 2,2,2-trifluoroethanol.

2. Results and discussion

2.1. Synthesis of azide-fluoro-dehydrocoelenterazine analog (Az-F-DCT)

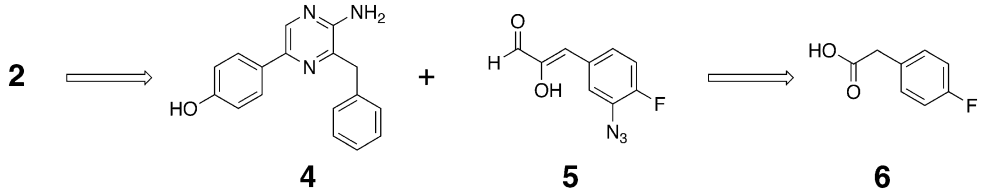
One-pot introduction of azide into DCT is an ideal strategy for the preparation of probe 2. For such a reason, Kita's method for the introduction of azide into an aromatic ring in one-pot is very attractive for us.[13] However, we could not prepare Az-F-DCT (**2**) from 2′-fluoro-DCT analog (**3**) selectively by using Kita's method (Scheme 2).

We then changed the strategy for the introduction of azide into 2′-fluoro-DCT analog (**3**); thus, we planned to prepare the probe 2 through using the conventional synthetic method established by Kishi, Inoue, and Goto et al.[14–17] As shown in Scheme 3, Az-F-DCT (**2**) would be derived from the condensation of coelenteramine (**4**)[15] and 3-(3-azide-4-fluorophenyl)-2-oxopropanal (**5**). The ketoaldehyde (**5**) would be prepared starting from 4-fluorophenylacetic acid (**6**).

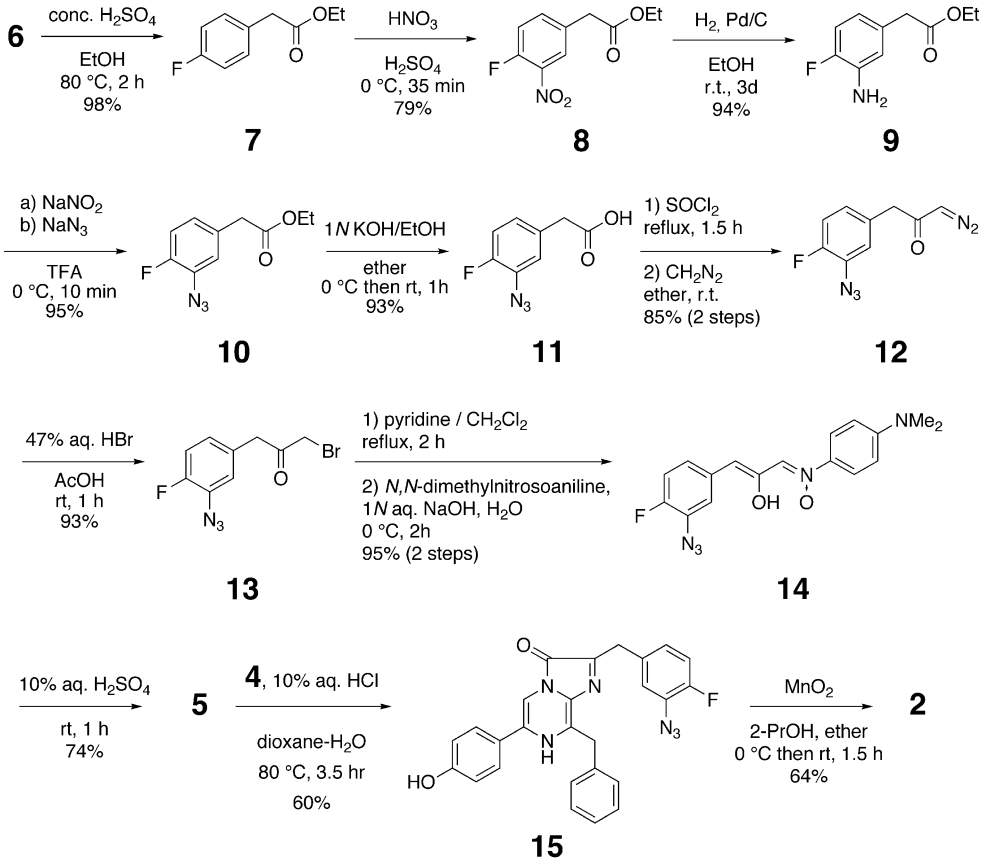
Esterification of 4-fluorophenylacetic acid (**6**) in EtOH afforded the ethyl ester (**7**) in 98% yield, followed by nitration of **7** provided ethyl-4-fluoro-3-nitrophenylacetate (**8**) in 79% yield as shown in Scheme 4 first row. Hydrogenolysis of the nitro group of **8** was facilitated by Pd/C catalyst to give ethyl-(3-amino-4-fluorophenyl)acetate (**9**) and the subsequent azidation[18] afforded ethyl-(3-azide-4-fluorophenyl)acetate (**10**). Saponification of the ester (**10**) afforded (3-azide-4-fluorophenyl)acetic acid (**11**) in 93% yield, which was further, converted to acid chloride. It was further, treated with diazomethane to give homologation productin (**12**) 85% (2 steps) (Scheme 4, second row). Bromination of this diazoketone (**12**) with HBr in acetic acid provided bromoketone (**13**) in 93% yield. After converted to its pyridinium salt, it was condensed with *N*,*N*-dimethyl-nitrosoaniline to give *N*-(*N*,*N*-dimethyl-aminophenyl)-3-(3-azide-4-fluorophenyl)-2-oxopropanimine oxide (**14**: nitrone) in 95% (2 steps) (Scheme 4, third row). Hydrolysis of the nitrone (**14**) in aqueous H_2SO_4 afforded 3-(3-azide-4-fluorophenyl)-2-oxopropanal (**5**) in 74% yield. Condensation of the ketoaldehyde (**5**) with coelenteramine (**4**) in a mixture of H_2O and dioxane in the presence of aqueous HCl provided azide-fluoro-coelenterazine analog (**15**: Az-F-CT) in 60% yield. Finally, oxidation of Az-F-CT (**15**) with manganese(IV) oxide in a mixture of ether and 2-PrOH afforded azide-fluoro-dehydrocoelenterazine analog (**2**: Az-F-DCT) in 64% yield (Scheme 4). Az-F-DCT (**2**) is a powerful probe to analyze symplectin active site by using photoaffinity-labeling, furthermore, Az-F-CT (**15**) will be also useful for the photoaffinity-labeling of aequorin photoprotein, which is the famous substance for jellyfish (*Aequoria aequoria*) bioluminescence.[19]

2.2. Photolysis of Az-F-coelenterazine analog

Although, we synthesized Az-F-DCT (**2**) as described in the previous section, the structure of symplectin chromophore is a reduced form of **2**. Thus, Az-F-CT (**15**) has the same



Scheme 3. Retro-synthesis of **2** from coelenteramine (**4**) and 3-(3-azide-4-fluorophenyl)-2-oxopropanal (**5**), and of **5** from 4-fluorophenylacetic acid (**6**).

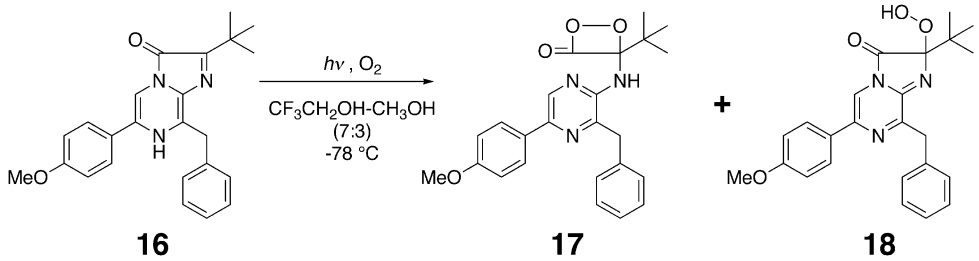


Scheme 4. Synthetic route for Az-F-DCT (**2**) starting from 4-fluorophenylacetic acid (**6**).

oxidation state with symplectin chromophore. To test the photo-reactivity of Az-F-DCT (**2**) in symplectin active site, we examined the photo-reactivity of Az-F-CT (**15**) as a symplectin chromophore model.

During the analysis of chemiluminescent mechanism of coelenterazine analog (**16**), we have reported that photo-oxygenation of the analog (**16**) in 2,2,2-trifluoroethanol (TFE)/methanol (7:3) afforded 2-peroxide (**17**) and dioxetanone (**18**) intermediates effectively as shown in Scheme 5.[20,21]

Since, luminous intermediates (**17**, **18**) were accumulated only when used (TFE) as solvent along with affording high light yield of luminescence,[20,21] we thought trifluoromethyl group might have some stabilization effect on the excited state of the coelenterazine analog (**16**) to give luminous intermediates in high yield. Therefore, we selected TFE as the solvent for light irradiation of **15** to decompose azide with anticipating the efficient production of a nitrene intermediate. The photolysis with a high-pressure mercury lamp was monitored with a nano-LC-Q-TOF-MS with our pre-packed-gradient (PPG) program.[22] The nano-LC chromatogram was shown in Figure 2 monitoring at 254 nm absorption. Az-F-CT (**15**) was almost consumed only remained 4% at 25.0 min after 15 min irradiation, and three new peaks were observed at 20.2, 20.5, 24.6 min in the chromatogram (Fig. 2). Then, these peaks were analyzed with ESI-Q-TOF-MS and -MS/MS measurements. The molecular weight of peak **A** (20.2 min, 33% yield) was *m/z* 441 $(M+H)^+$, which was assigned as reduced amine (**19**)[23] as shown in Figure 3. The peak at 20.5 min (24% yield) was proved to be coelenteramine (**4**) with molecular ion at *m/z* 278 $(M+H)^+$. The peak **B** at 24.6 min was a solvent insertion product with molecular ion at *m/z* 539 $(M+H)^+$. There were three possible structures for **B** as shown in Figure 3; O–H inserted product (**20**), C–H inserted product (**21**), or ring-expanded azepine derivative (**22**).



Scheme 5. Photooxygenation of coelenterazine analog (**16**) afforded 2-peroxide (**17**) and dioxetanone (**18**) intermediates.[20,21]

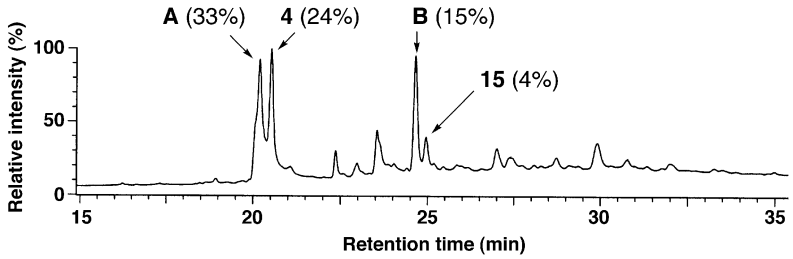


Figure 2. PPG-nano-LC chromatogram of photo-irradiated products of **15** in TFE (detection at UV 254 nm absorption). Values in parentheses show yields estimated by integration of peak areas.

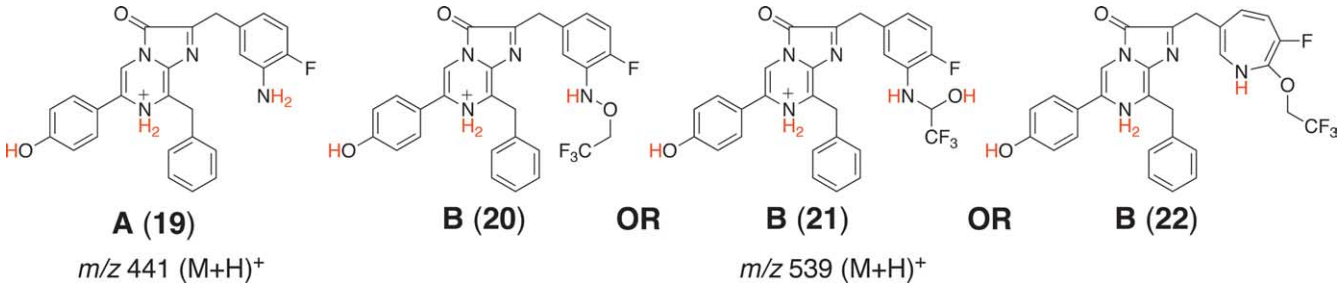


Figure 3. Assignment of the peaks with ESI-Q-TOF-MS analysis, and three possible structures for the solvent insertion product (**B**) (**20**, **21**, or **22**).

For the characterization of structure of peak **B**, proton/deuterium (H/D) exchange experiments were carried out with ESI-Q-TOF-MS and -MS/MS measurement,[24,25] on the basis of different numbers in the estimated exchangeable protons of **20** and **21**. Comparing the respective MS spectrum with both proton and deuterium measurement, the structure of peak **B** would be assigned from the fact of four exchangeable protons (**20**) or five (**21**).

The samples for proton/deuterium (H/D) exchange experiments were prepared as such that photo-irradiated sample of **15** was completely dried in vacuo, then redissolved with

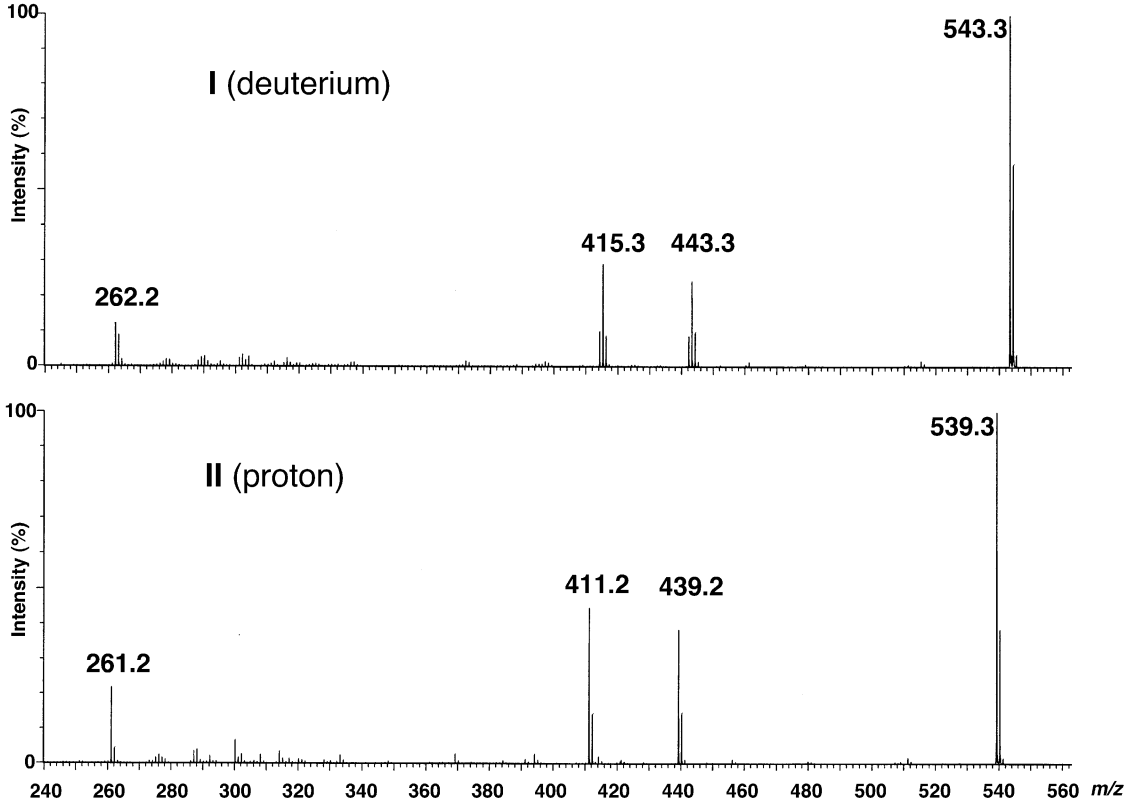


Figure 4. MS/MS spectra of solvent insertion product **B**: (I) MS/MS spectrum of deuteriated **B** precursor *m/z* 543; (II) MS/MS spectrum of natural abundant **B** precursor *m/z* 539.

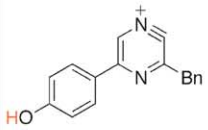
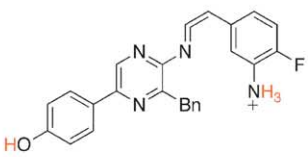
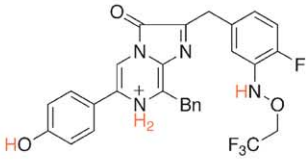
Structure				
m/z in D solvent	262	415	443	543
m/z in H solvent	261	411	439	539
Dn*	1	4	4	4

Figure 5. Assignment of precursor and fragment ions shown in MS/MS spectra (Fig. 4). *Dn: numbers of the exchangeable protons.

99% CH_3OD:1% CH_3COOD for ESI-Q-TOF-MS with deuterium measurement or with 99% CH_3OH:1% CH_3-COOH for MS with proton measurement. The resultant spectra are shown in Figure 4 (I: deuterium measurement, II: proton measurement). Pseudo molecular ion of peak **B** was observed at 539 $(M+H)^+$, which was employed as the precursor ion for MS/MS measurement (sample cone 60 V, collision 15–40 V), and the result is shown in Figure 4II. The product ions from *m/z* 539 precursor are seen at *m/z* 439, 411, and 261 in protonic solvent (Fig. 4II), while those from *m/z* 543 are also varied to appear at *m/z* 443, 415, and 262 in deuterium solvent as shown in Figure 4I.

Figure 5 summarizes the assignment of these fragment structures. For the validation of the above product ions generated during the MS/MS collision process, deuteriation of all the exchangeable protons, as shown in Figure 4I, made mass increase of mass numbers *m/z* 539 to *m/z* 543. The increased mass units (4, 4, 4, and 1) to the product ions also provide strong support for their structures in detail as shown in Figure 5. It has been concluded that the structure of solvent insertion product (**B**) is now concluded to be O–H inserted product (**20**) from the facts that four exchangeable protons of **B** was not for **21** (five exchangeable protons) but **20**.

Concerning the ring-expanded azepine derivative (**22**), from the results of MS and MS/MS measured in proton/deuterium, we excluded the possibility that the solvent adduct was azepine derivative (**22**).[26]

Through these experiments, we demonstrated that photolysis of compound **15** in TFE produced a nitrene intermediate to give the solvent O–H inserted adduct (**20**). We designed Az-F-DCT (**2**) to suppress ring-expansion of nitrene to give azepine intermediate up on photolysis. By using Az-F-CT (**15**) as symplectin chromophore model, no ring expansion was observed during photolysis as we expected.[23] These results enable us to utilize singlet nitrene itself for the photo-affinity labeling of symplectin active site. We also demonstrated that proton/deuterium (H/D) exchange experiments are very powerful method when coupled with LC-Q-TOF-MS and -MS/MS to investigate organic reaction. Photo-affinity labeling of semi-synthetic symplectin by using Az-F-DCT (**2**), and analysis of the photo-labeled site with nano-LC-Q-TOF-MS and -MS/MS equipped with PPG program[22] is now underway in our group.

3. Conclusion

Synthesis of photosensitive azide-fluoro-dehydrocoelenterazine analog (Az-F-DCT; **2**) has been accomplished starting from 4-fluorophenylacetic acid (**6**). Az-F-DCT (**2**) must be a powerful photoaffinity-labeling probe for the analysis of symplectin active site. For the examination of the photoreactivity of Az-F-DCT (**2**), the azide-fluoro-coelenterazine analog (Az-F-CT; **15**) was used as a symplectin chromophore model, due to the fact that chromophore structure in symplectin bound form of Az-F-DCT (**2**) should have the same chromophoric structure as Az-F-CT (**15**). Photoirradiation into a solution of Az-F-CT (**15**) in 2,2,2-trifluoroethanol (TFE) afforded a nitrene intermediate to give solvent O–H insertion product (**20**); this product (**20**) has been characterized from proton/deuterium (H/D) exchange data in ESI-Q-TOF-MS and -MS/MS spectra.

4. Experimental

4.1. General

All melting points were measured on a Yanaco MP-S3 and uncorrected. UV spectra were obtained on a JASCO U-best 50 spectrometer. IR spectra were recorded on a PERKIN ELMER Paragon 1000 FT-IR spectrophotometer. Proton NMR spectra were recorded on a JEOL GSX 270 for 270 MHz, or on a JEOL JNML-500 for 500 MHz, or on a Bruker AMX-600 for 600 MHz. Chemical shifts (δ) are given in parts per million relative to tetramethylsilane (δ 0.00), CD_3OD (δ 3.30), or DMSO-d_6 (δ 2.49) as internal standard. Coupling constants (J) are given in Hz. Proton decoupled carbon NMR spectra were recorded on a JEOL GSX 270 for 67.8 Hz, or on a JEOL JNML-500 for 125.7 Hz, or on a Bruker AMX-600 for 150.9 MHz. Chemical shifts are (δ) given in parts per million relative to $CDCl_3$ (δ 77.0), CD_3OD (δ 49.0), or DMSO-d_6 (δ 45.0) as

internal standard. Coupling constants (J) are given in Hz. Fluorine NMR spectra were recorded on a JEOL A-400 for 376 MHz. Chemical shifts are (δ) given in parts per million relative to 1,1,1-trifluorotoluene (δ 0.00) as external standard. Low-resolution EI MS and FAB MS were measured with a JEOL JMS-700. High-resolution (HR) MS were measured with a JEOL JMS-700. ESI mass spectra were measured with a Q-TOF mass spectrometer (Micromass, Manchester, UK) equipped with a Z-spray type ESI sources. Mr. S. Kitamura in Analytical Laboratory of this school performed the combustion elemental analyses. Fluorescence spectra were measured with a JASCO FP-777 spectrometer. All the solvents were of reagent grade. Analytical thin-layer chromatography (TLC) was conducted on precoated TLC plates: silica gel 60 F-254 [E.Merck (Art 5715) Darmstadt, Germany], layer thickness 0.25 mm. Silica gel column chromatography utilized Silica Gel 60 (spherical) 40–50 mm [KANTO CHEMICAL CO., INC].

4.1.1. Ethyl-(4-fluorophenyl)acetate (7). A solution (6.0 ml) of 4-fluorophenylacetic acid (**6**) (3.06 g, 19.9 mmol) in 6.0 ml of EtOH was refluxed at 80 °C for 2.5 h in the presence of 98% H_2SO_4 (0.84 ml) under Ar atmosphere. The solution was then poured into water at 0 °C, and the resultant solution was extracted with ether. The organic layer was washed with water and brine, and then was passed through a Na_2SO_4–SiO_2–Na_2SO_4 column. Evaporation of the effluent afforded Ethyl-(4-fluorophenyl)-acetate (**1**) in 98%. The product **7** was used for the following reaction without any purification. For the spectroscopic analyses, compound **7** was recrystallized with hexane to afforded a colorless crystalline. Mp 29.0–30.0 °C. IR (KBr) ν_{max} 1736, 1511, 1224 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 1.23 (3H, t, J=7.3 Hz, CH_3), 3.57 (2H, s, ArCH_2), 4.14 (2H, q, J=7.3 Hz, OCH_2), 6.99 (2H, t, J=8.8 Hz, Ar-3H), 7.24 (2H, dd, J=8.8, 5.1 Hz, Ar-2H) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 14.2, 40.5, 60.9, 115.2 (d, $^2J_{C-F}$=22 Hz), 129.7, 130.6 (d, $^3J_{C-F}$=8 Hz), 161.8 (d, $^1J_{C-F}$=244 Hz), 171.2 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ −53.1 ppm. EI-MS m/z 182 (M^+), 154, 109. HRMS (EI) calcd for $C_{10}H_{11}O_2F$: 182.0743, found 182.0719 (M^+). Anal. Calcd for $C_{10}H_{11}O_2F$: C, 65.92; H, 6.09; N, 0.00. Found: C, 65.91; H, 6.14; N, 0.27.

4.1.2. Ethyl-(4-fluoro-3-nitrophenyl)acetate (8). To a solution of **7** (3.54 g, 19.5 mmol) in 14 ml of H_2SO_4 at 0 °C was added 69% HNO_3 (1.4 ml) dropwise over 5 min. After stirring for 35 min at 0 °C, the solution was diluted with AcOEt at 0 °C, then the resultant solution was poured into a cold water. Water layer was extracted with AcOEt. The combined organic layer was washed with water, brine then dried over Na_2SO_4. After removing solvent in vacuo, the residue was purified by silica gel column chromatography (1:2 ether/hexane) to give 3.5 g (79%) of Ethyl-(4-fluoro-3-nitrophenyl)acetate (**8**) as pale yellow oil. IR (KBr) ν_{max} 1737, 1540, 1352, 1254, 1178 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 1.28 (3H, t, J=7.1 Hz, CH_3), 3.68 (2H, s, ArCH_2), 4.19 (2H, q, J=7.1 Hz, OCH_2), 7.26 (1H, dd, J=10.8, 8.6 Hz, Ar-4H), 7.57 (1H, ddd, J=8.6, 4.2, 2.4 Hz, Ar-5H), 8.00 (1H, dd, J=7.9, 2.3 Hz, Ar-2H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1, 39.9, 61.4, 118.4 (d, $^2J_{C-F}$=21 Hz), 126.8, 131.1, 134.7 (d, $^2J_{C-F}$=7 Hz), 136.4 (d, $^3J_{C-F}$=8 Hz), 154.7 (d, $^1J_{C-F}$=263 Hz), 170.1 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ −49.8 ppm. EI-MS m/z 227 (M^+). HRMS (EI) calcd for $C_{10}H_{10}O_4NF$: 227.0594, found 227.0619 (M^+).

4.1.3. Ethyl-(3-amino-4-fluorophenyl)acetate (9). To a solution of **8** (3.5 g, 15.4 mmol) in 150 ml of EtOH was added 10% Pd/C (175 mg) at rt in a round flask. The flask was plugged with hydrogen at atmospheric pressure. After stirred at rt for 2 days, Pd/C (175 mg) was further, added to the mixture. Then the stirring was continued one more day at rt under hydrogen atmosphere. The mixture was filtered through a Celite pad. After removing solvent in vacuo, the residue was purified by silica gel column chromatography (2:3 ether/hexane) to give 2.87 g (94%) of Ethyl-(2-amino-4-fluorophenyl)acetate (**9**) as orange oil. IR (KBr) ν_{max} 3375, 1731, 1633, 1519, 1445, 1303, 1207, 1161 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 1.25 (3H, t, J=7.1 Hz, CH_3), 3.47 (2H, s, ArCH_2), 3.70 (2H, br s, NH_2), 4.14 (2H, q, J=7.1 Hz, OCH_2), 6.58 (1H, ddd, J=8.3, 4.5, 2.0 Hz, Ar-6H), 6.70 (1H, dd, J=8.6, 2.0 Hz, Ar-2H), 6.90 (1H, dd, J=11.0, 8.3 Hz, Ar-5H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1, 40.7, 60.8, 115.1 (d, $^2J_{C-F}$=18 Hz), 117.6 (d, $^3J_{C-F}$=3 Hz), 119.3 (d, $^3J_{C-F}$=6 Hz), 130.3 (d, $^4J_{C-F}$=4 Hz), 134.4 (d, $^2J_{C-F}$=14 Hz), 150.9 (d, $^1J_{C-F}$=236 Hz), 171.6 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ −75.1 ppm. EI-MS m/z 197 (M^+), 124. HRMS (EI) calcd for $C_{10}H_{12}O_2NF$: 197.0852, found 197.0871 (M^+).

4.1.4. Ethyl-(2-azide-4-fluorophenyl)acetate (10). To a solution of **9** (2.87 g, 14.6 mmol) in 30 ml of trifluoroacetic acid (TFA) at 0 °C was added $NaNO_2$ (2.01 g, 29.2 ml) followed by NaN_3 (2.85 g, 43.8 mmol). After stirring for 10 min at 0 °C, the solution was diluted with ether at 0 °C, then the resultant solution was poured into a cold water. Water layer was extracted with ether. The combined organic layer was washed with water, brine then dried over Na_2SO_4. After removing solvent in vacuo, the residue was purified by silica gel column chromatography (1:2 ether/hexane) to give 3.09 g (95%) of Ethyl-(2-azide-4-fluorophenyl)acetate (**10**) as yellow oil. IR (KBr) ν_{max} 2125, 1736, 1515, 1427, 1320, 1227, 1160 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 1.26 (3H, t, J=7.1 Hz, CH_3), 3.56 (2H, s, ArCH_2), 4.16 (2H, q, J=7.1 Hz, OCH_2), 6.97–7.07 (3H, m, Ar-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1, 40.3, 61.1, 116.6 (d, $^2J_{C-F}$=19 Hz), 121.7, 126.6 (d, $^3J_{C-F}$=6 Hz), 127.8 (d, $^2J_{C-F}$=12 Hz), 131.0, 154.0 (d, $^1J_{C-F}$=248 Hz), 171.0 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ −66.0 ppm. EI-MS m/z 223 (M^+). HRMS (EI) calcd for $C_{10}H_{10}O_2N_3F$: 223.0757, found 223.0771 (M^+). Anal. Calcd for $C_{10}H_{10}O_2N_3F$: C, 53.81; H, 4.52; N, 18.83. Found: C, 53.81; H, 4.71; N, 18.53.

4.1.5. 2-Azide-4-fluorophenylacetic acid (11). To a solution of **10** (3.09 g, 13.9 mmol) in 10 ml of ether was added 10 ml of 2 N KOH/EtOH at 0 °C. After stirring at 0 °C for 15 min, the reaction was warmed to rt and stirred for 1 h. Then the solution was cooled to 0 °C and was acidified with 1 N aqueous HCl until pH 1–2. The resultant solution was extracted with AcOEt. The organic layer was washed with water then dried over Na_2SO_4. Concentration in vacuo yielded 2.50 g (93%) of 2-Azide-4-fluorophenyl-acetic acid (**11**). The product **11** was used for the following reaction without any purification. For the spectroscopic

analyses, compound **11** was recrystallized with ether–hexane to afforded an orange crystalline. Mp 48.5–49.5 °C. IR (KBr) ν_{max} 2130, 1718, 1707, 1518, 1239 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 3.60 (2H, s, ArCH_2), 6.97–7.07 (2H, m, Ar-*H*), 7.05 (1H, br t, J=9.6 Hz, Ar-*H*) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 40.0, 116.8 (d, $^2J_{C-F}$=19 Hz), 122.0, 126.7 (d, $^3J_{C-F}$=7 Hz), 128.0 (d, $^2J_{C-F}$=12 Hz), 130.1 (d, $^4J_{C-F}$=4 Hz), 154.2 (d, $^1J_{C-F}$=248 Hz), 177.3 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ −65.3 ppm. EI-MS *m/z* 195 (M^+), 167 (M^+-N_2). HRMS (EI) calcd for $C_8H_6O_2N_3F$: 195.0444, found 195.0449 (M^+). Anal. Calcd for $C_8H_6O_2N_3F$: C, 49.24; H, 3.10; N, 21.53. Found: C, 49.25; H, 3.31; N, 21.35.

4.1.6. (3-Azide-4-fluorophenyl)methyl diazomethyl ketone (12). A solution of **11** (2.50 g, 12.8 mmol) in 8.0 ml of thionylchloride was refluxed 1.5 h. Generating vapors (SO_2 and HCl) were trapped with saturated aqueous $NaHCO_3$. Concentration of the reaction mixture in vacuo equipped a trap cold by liquid N_2 gave a crude oil of acyl chloride of **5**. The acyl chloride was purified with Kugelrohr (145 °C) in vacuo, and was immediately used for the following reaction. To the solution of acyl chloride (2.54 g) of **5** in 60 ml of ether was added a ether solution of diazomethane until acylchloride was perfectly consumed at 0 °C. After removing solvent in vacuo, the residue was purified by silica gel column chromatography (2:1 ether/hexane) to give 2.23 g (85%, two steps) of (3-Azide-4-fluorophenyl)methyl diazomethyl ketone (**12**) as a lemon colored crystalline. Mp 34.8–35.5 °C. IR (KBr) ν_{max} 2092, 1614, 1512, 1426, 1112 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 3.56 (2H, s, ArCH_2), 5.18 (1H, s, C*H*N_2), 6.92–7.10 (3H, m, Ar-*H*) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 46.7, 55.1, 116.9 (d, $^2J_{C-F}$=19 Hz), 121.8 (d, $^3J_{C-H}$=1 Hz), 126.5 (d, $^3J_{C-F}$=7 Hz), 128.1 (d, $^2J_{C-F}$=11 Hz), 131.3, 154.0 (d, $^1J_{C-F}$=248 Hz), 191.2 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ −65.6 ppm. EI-MS *m/z* 219 (M^+), 163 (M^+-2N_2). HRMS (EI) calcd for $C_9H_6ON_5F$: 219.0556, found 219.0540 (M^+). Anal. Calcd for $C_9H_6ON_5F$: C, 49.32; H, 2.76; N, 31.95. Found: C, 49.36; H, 2.67; N, 31.61.

4.1.7. (3-Azide-4-fluorophenyl)methyl bromomethyl ketone (13). To a solution of **12** (2.10 g, 9.59 mmol) in 12 ml of AcOH at 0 °C was added 47% aqueous HBr (2.6 ml, 14.9 mmol) dropwise slowly. The reaction was warmed to rt and stirred for 1 h. Then the solution was poured into water washing with H_2O and ether. The mixture was neutralized with Na_2CO_3 to pH 4, then with $NaHCO_3$ to pH 7. The solution was extracted with ether. The organic layer was washed with water, brine, then dried over Na_2SO_4. Concentration in vacuo afforded crude compound. Recrystallization of the crude compound with ether yielded 2.44 g (93%) of (3-azide-4-fluorophenyl)methyl bromomethyl ketone (**13**) as a pale yellow needle. Mp 74.6–75.6 °C. IR (KBr) ν_{max} 2128, 1736, 1513, 1240 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 3.92 (4H, s, ArCH_2, CH_2Br), 6.90–6.96 (2H, m, Ar-*H*), 7.07 (1H, br t, J=9.5 Hz, Ar-*H*) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 33.4, 45.4, 117.0 (d, $^2J_{C-F}$=28 Hz), 122.0 (d, $^3J_{C-H}$=2 Hz), 126.7 (d, $^3J_{C-F}$=7 Hz), 128.0, 129.9 (d, $^2J_{C-F}$=7 Hz), 154.1 (d, $^1J_{C-F}$=249 Hz), 198.5 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ −65.1 ppm. EI-MS *m/z* 273 (M^+), 271 (M^+), 245 (M^+-N_2), 243 (M^+-N_2). HRMS (EI) calcd for $C_9H_7ON_3F^{79}Br$: 270.9757, found 270.9755 (M^+). Anal. Calcd for $C_9H_7ON_3FBr$: C, 39.73; H, 2.59; N, 15.44. Found: C, 39.84; H, 2.46; N, 15.56.

4.1.8. *N*-(*N*,*N*-Dimethylaminophenyl)-3-(3-azide-4-fluorophenyl)-2-oxopropanimine oxide (14). A solution of **13** (2.44 g, 8.97 mmol) in 30 ml of CH_2Cl_2 was refluxed at 70 °C in the presence of 3.0 ml of pyridine under Ar atmosphere for 2 h. Concentration of the solution in vacuo afforded a pyridinium salt as a foamy solid. To a solution of the pyridinium salt in 90 ml of H_2O at 0 °C was added *N*,*N*-dimetyl-4-nitrosoaniline (1.4 g, 9.42 mmol) followed by 9 ml of 1 N aqueous NaOH. The resultant suspension was warmed to rt and stirred for 2 h at rt with sonicating occasionally. Filtration of the suspension yielded 2.19 g (95%, two steps) of *N*-(*N*,*N*-dimethylaminophenyl)-3-(3-azide-4-fluorophenyl)-2-oxopropanimine oxide (**14**). The product **14** was used for the following reaction without any purification. IR (KBr) ν_{max} 2121, 1605, 1490, 1170 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 3.03 (6H, s, N*Me*), 5.45, (1H, s, ArC*H*), 6.66 (2H, d, J=9.2 Hz, *ortho*-aniline), 7.02 (1H, dd, J=10.6, 8.6 Hz, Ar-*5H*), 7.42 (1H, ddd, J=8.6, 5.0, 2.3 Hz, Ar-*6H*), 7.50 (1H, s, C*H*=NO), 7.57 (2H, d, J=9.2 Hz, *meta*-aniline), 7.62 (1H, dd, J=8.2, 2.3 Hz, Ar-*2H*), 13.77 (1H, br s, O*H*) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 40.3, 110.7, 111.2, 116.5 (d, $^2J_{C-F}$=19 Hz), 120.9, 121.5, 122.3, 126.4 (d, $^2J_{C-F}$=7 Hz), 131.0, 133.3 (d, $^3J_{C-F}$=5 Hz), 134.1, 149.4 (d, $^3J_{C-F}$=2 Hz), 151.4, 153.1 (d, $^1J_{C-F}$=250 Hz) ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ −64.7 ppm. EI-MS *m/z* 341 (M^+), 325 (M^+-16). HRMS (EI) calcd for $C_{17}H_{16}O_2N_5F$: 341.1288, found 341.1238 (M^+).

4.1.9. 3-(3-Azide-4-fluorophenyl)-2-oxopropanal (5). Compound **14** (700 mg, 2.05 mmol) was suspended into 10% aqueous H_2SO_4 (40 ml) at 0 °C. The suspension was warmed to rt and stirred for 1 h with sonicating occasionally. The suspension was extracted with ether. The organic layer was washed with water and brine, and then was passed through a Na_2SO_4–SiO_2–Na_2SO_4 column. Removing solvent in vacuo, yielded 314 mg (74%) of 3-(3-azide-4-fluorophenyl)-2-oxopropanal (**5**) as a dark yellow amorphous. The product **5** was used for the following reaction without any purification. For the spectroscopic analyses, compound **5** was recrystallized with ether–hexane to afforded a pale yellow amorphous. Mp 102.0–104.0 °C (decomposed). IR (KBr) ν_{max} 3329, 2137, 1672, 1650, 1409 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$) δ 6.09 (1H, d J=0.9 Hz, ArC*H*), 6.47 (1H, s, O*H*), 7.12 (1H, dd, J=10.5, 8.4 Hz, Ar-*5H*), 7.51 (1H, ddd, J=8.4, 4.6, 2.1 Hz, Ar-*6H*), 7.66 (1H, dd, J=8.1, 2.1 Hz, Ar-*2H*), 9.25 (1H, d, J=0.9 Hz, CO*H*) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 116.9 (d, $^2J_{C-F}$=20 Hz), 120.2, 122.5, 127.9 (d, $^3J_{C-F}$=7 Hz), 128.2, 130.7, 148.6 (d, $^3J_{C-F}$=3 Hz), 154.7 (d, $^1J_{C-F}$=253 Hz), 187.8 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ −60.8 ppm. EI-MS *m/z* 207 (M^+), 179 (M^+-28), 163 (M^+-N_2–CHO). HRMS (EI) calcd for $C_9H_6O_2N_3F$: 207.0444, found 207.0436 (M^+). Anal. Calcd for $C_9H_6O_2N_3F$: C, 52.18; H, 2.92; N, 20.28. Found: C, 52.17; H, 2.66; N, 20.37.

4.1.10. 8-Benzyl-2-(3-azide-4-fluorophenylmethyl)-6-(4-hydroxyphenyl)-3,7-dihydroimidazo[1,2-*a*]pyrazine-3-one (15) (Az-F-CT). To a degassed solution of

coelenteramine (**4**) (214 mg, 0.77 mmol) and ketoaldehyde (**5**) (223 mg, 1.08 mmol) in 6.0 ml of 20% water/dioxane was added 1.0 ml of 10% aqueous HCl, and was stirred under argon atmosphere at rt for 5 min. Then the reaction was warmed to 80 °C and stirred for 3.5 h. After cooled, the solution was poured into water at 0 °C. The resultant mixture was extracted with AcOEt. The organic layer was washed with water, brine, and dried over anhydrous Na_2SO_4. After removing solvent in vacuo, the residue was purified by silica gel column chromatography (4% MeOH in CH_2Cl_2) to give 215 mg (60%) of 8-benzyl-2-(3-azide-4-fluorophenylmethyl)-6-(4-hydroxyphenyl)-3,7-dihydroimidazo[1,2-*a*]pyrazine-3-one (**15**) as a brown solid. For the spectroscopic analyses, compound **15** was recrystallized with ether–MeOH to afforded a yellow powder. Mp 140–143 °C (decomposed). UV (MeOH) λ_{max} (log ε) 438 (3.97) nm. FL (MeOH) Em. 526.5 nm (Ex. 350 nm). IR (KBr) ν_{max} 3100 (br), 2357, 2349, 2121, 1564, 1548, 1513 cm^{-1}. ^1H NMR (CD_3OD, 600 MHz), δ 4.14 (2H, s, CH_2Ph), 4.41 (2H, s, CH_2Ph), 6.88 (3H, ddd, J=9.6, 8.4, 4.8 Hz, Ar-*H*), 7.07 (1H, dd, J=10.8, 8.4 Hz, F-Ar-*H*), 7.13 (1H, m, Ar-*H*), 7.19 (1H, br d, J=8.4 Hz, Ar-*H*), 7.23 (1H, t, J=7.2 Hz, Ar-*H*), 7.30 (2H, t, J=7.5 Hz, Ar-*H*), 7.38 (2H, d, J=7.8 Hz, Ar-*H*), 7.45 (2H, br s, Ar-*H*) ppm. ^{13}C NMR (CD_3OD, 150 MHz), δ 49.9, 108.0, 116.8, 117.4 (d, $^2J_{C-F}$=20 Hz), 122.5, 127.4 (d, $^3J_{C-F}$=6 Hz), 128.2, 128.7 (d, $^2J_{C-F}$=11 Hz), 129.3, 129.6, 129.7, 137.1, 137.7, 154.8 (d, $^1J_{C-F}$=247 Hz), 160.1 ppm. ^{19}F NMR (376 MHz, CD_3OD) δ −68.9 ppm. FAB-MS (NBA) *m*/*z* 467 (MH^+). HRMS (FAB/NBA) calcd for $C_{26}H_{20}N_6O_2F$: 467.1632, found 467.1663 (MH^+). Anal. Calcd for $C_{26}H_{19}N_6O_2F$: C, 66.95; H, 4.11; N, 18.02. Found: C, 66.95; H, 4.37; N, 17.72.

4.1.11. 8-Benzyl-2-(3-azide-4-fluorobenzylidene)-6-(4-hydroxyphenyl)-2,3-dyhydroimidazo[1,2-*a*]pyrazine-3-one (2) (Az-F-DCT). To a solution of coelenterazine analog (**15**) (70 mg, 0.15 mmol) in a mixture of diethyl ether (175 ml) and EtOH (35 ml) was added 85% manganese(II) oxide (700 mg, 6.83 mmol) at 0 °C under Ar atmosphere. After stirring for 1 h, the reaction was warmed to rt and stirred for 1.5 h. The mixture was filtered through a Celite pad, then was concentrated in vacuo. Recrystallization of the residue with 2-PrOH/ether yielded 45 mg (64%) of 8-benzyl-2-(3-azide-4-fluorobenzylidene)-6-(4-hydroxyphenyl)-2,3-dyhydroimidazo[1,2-*a*]pyrazine-3-one (**2**) as a purple amorphous. Mp 154–156 °C (decomposed). UV (2-PrOH) λ_{max} (log ε) 541 (3.55), 371 (3.93), 281 (4.11) nm. IR (KBr) ν_{max} 2359, 2133, 1721, 1591, 1503 cm^{-1}. ^1H NMR (DMSO-d_6, 600 MHz) δ 4.29 (2H, s, CH_2Ph), 6.81 (2H, d, J=8.8 Hz, Ar-*H*), 7.24 (1H, t, J=7.3 Hz, Ar-*H*), 7.35 (2H, t, J=7.7 Hz, Ar-*H*), 7.50 (3H, dd, J=13.2, 5.4 Hz, Ar-*H*), 7.53 (1H, s, 5-*H*), 7.78 (2H, d, J=8.6 Hz, Ar-*H*), 7.94 (1H, s, Ar-*H*), 8.10–8.12 (1H, m, Ar-*H*), 8.62 (1H, d, J=8.4 Hz, Ar-*H*), 9.66 (1H, br s, O*H*) ppm. ^{13}C NMR (DMSO-d_6, 150 MHz) δ 49.3, 108.0, 116.8, 117.4 ($^2J_{C-F}$=20 Hz), 127.4 ($^3J_{C-F}$=6 Hz), 128.1, 128.7 ($^2J_{C-F}$=11 Hz), 129.3, 129.6, 129.7, 137.1, 137.7, 154.8 ($^1J_{C-F}$=247 Hz), 160.1 ppm. ^{19}F NMR (376 MHz, CD_3OD) δ −58.4 ppm. FAB-MS (NBA) *m*/*z* 465 (MH^+). HRMS (FAB/NBA) calcd for $C_{26}H_{18}N_6O_2F$: 465.1475, found 465.1501 (MH^+). Anal. Calcd for $C_{26}H_{17}N_6O_2F$: C, 67.24; H, 3.69; N, 18.09. Found: C, 67.02; H, 3.80; N, 18.16.

4.1.12. Photolysis of Az-F-CT (15): general procedure. To a solution of Az-F-CT (**15**) (2.0 mM) in 2,2,2-trifluoroethanol in a NMR tubing was irradiated light with a high-pressure mercury lamp (100-W high pressure Hg lamp) for 15 min at rt with Ar bubbling. For nano-LC-Q-TOF-MS measurement, 1 μl of the sample was injected. For proton/deuterium (H/D) exchange measurement, 2 μl of the sample was dried in vacuo, then redissolved with 99% CH_3OD:1% CH_3COOD (200 μl) for ESI-Q-TOF-MS with deuterium measurement or with 99% CH_3OH:1% CH_3COOH for MS (200 μl) with proton measurement. The resultant solutions were injected into ion-source with a mechanical syringe. TFE insertion product **20**. HRMS (FAB/NBA) calcd for $C_{28}H_{23}N_4O_3F_4$: 539.1706, found 539.1710 (MH^+).

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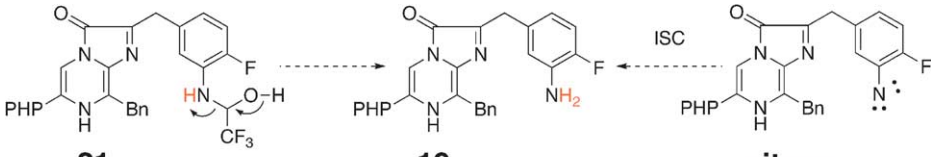
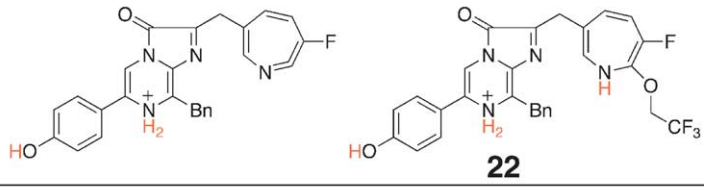
References and notes

1. Tsuji, F. I.; Leisman, G. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 6719–6723.
2. Takahashi, H.; Isobe, M. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2647–2652.
3. Takahashi, H.; Isobe, M. *Chem. Lett.* **1994**, 843–846.
4. Isobe, M.; Kuse, M.; Yasuda, Y.; Takahashi, H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2919–2924.
5. Kuse, M.; Isobe, M. *Tetrahedron* **2000**, *56*, 2629–2639.
6. Fujii, T.; Ahn, J. Y.; Kuse, M.; Mori, H.; Matsuda, T.; Isobe, M. *Biochem. Biophys. Res. Commun.* **2002**, *293*, 874–879.
7. Souto, M. L.; Um, J.; Borhan, B.; Nakanishi, K. *Helv. Chim. Acta* **2000**, *83*, 2617–2628.
8. Dorman, G.; Prestwich, G. D. *Trends Biotechnol.* **2000**, *18*, 64–77.
9. Kurono, M.; Shimomura, A.; Isobe, M. *Tetrahedron* **2004**, *60*, 1773–1780.
10. Zheng, J. L.; Chen, F. Q.; Hirano, T.; Ohmiya, Y.; Maki, S.; Niwa, H.; Ohashi, M. *Bull. Chem. Soc. Jpn.* **2000**, *73*, 465–469.
11. (a) Poe, R.; Scnapp, K.; Young, M. J. T.; Grayzar, J.; Platz, M. S. *J. Am. Chem. Soc.* **1992**, *114*, 5054–5067. (b) Platz, M. S. In *Reactive intermediate chemistry*; Moss, R. A., Platz, M. S., Jones, M., Jr., Eds.; Wiley: New Jersey, 2004; pp 501–559.
12. Isobe, M.; Fujii, T.; Kuse, M.; Miyamoto, K.; Koga, K. *Tetrahedron* **2002**, *58*, 2117–2126.
13. Kita, Y.; Tohma, H.; Inagaki, M.; Hatanaka, K.; Yakura, T. *Tetrahedron* **1991**, *32*, 4321–4324.
14. Kishi, Y.; Tanino, H.; Goto, T. *Tetrahedron Lett.* **1972**, *13*, 2747–2748.
15. Kakoi, H. *Chem. Pharm. Bull.* **2002**, *50*, 301–302.
16. Inoue, S.; Sugiura, S.; Kakoi, H.; Hashizume, K.; Goto, T.; Iio, H. *Chem. Lett.* **1975**, 141–144.
17. Recently, we reported the alternative synthetic route for various 6-substituted coelenteramine analogs: Kuse, M.;

Kondo, N.; Ohyabu, Y.; Isobe, M. *Tetrahedron* **2004**, *60*, 835–840.
18. Pinney, K. G.; Katzenellenbogen, J. A. *J. Org. Chem.* **1991**, *56*, 3125–3133.



19. Head, J.; Inoue, S.; Teranishi, K.; Shimomura, O. *Nature* **2000**, *405*, 372–376.
20. Usami, K.; Isobe, M. *Tetrahedron Lett.* **1995**, *36*, 8613–8616.
21. Usami, K.; Isobe, M. *Tetrahedron* **1996**, *52*, 12061–12090.
22. Kurahashi, T.; Miyazaki, A.; Suwan, S.; Isobe, M. *J. Am. Chem. Soc.* **2001**, *123*, 9268–9278.
23. In deuterium ESI-MS measurement, the molecular weight of **19** (*m*/*z* 441) increased to plus 5 mass units to give molecular ion at *m*/*z* 446. The exchangeable 5 protons strongly support our assignment. We also suppose it possible that **19** might be produced from C–H inserted product (**21**) by hydrolyzed aminal moiety, or be directly produced from nitrene intermediate through intersystem crossing.[11] No ring expansion product to benzazepine type intermediate was observed.[26] This may be responsible for the fluorine atom, which prevents carbon migration to the nitrene due to its electronegativity.



m/z in D solvent		
calculated	442	543
observed	443	543
m/z in H solvent		
calculated	439	539
observed	439	539

24. Kuse, M.; Kanakubo, A.; Suwan, S.; Koga, K.; Isobe, M.; Shimomura, O. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1037–1040.
25. Kanakubo, A.; Isobe, M. *J. Mass Spectrom.* **2004**, *39*, 1260–1267.
26. We considered the possibility for the ring expansion of the nitrene intermediate. If ring expansion happened, the resultant azepine derivative (**22**) also has four exchangeable protons. However, the initial fragmentation product observed at *m*/*z* 439 should have three exchangeable protons. If this is the case, *m*/*z* 442 should be observed in deuterium solvent. Thus, the peak at *m*/*z* 443 in Figure 4I was critical to exclude the possibility of ring expansion product (**22**).

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Synthesis of novel cyclodextrin derivatives by aromatic spacer insertion and their inclusion ability

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Abstract—Novel cyclic host molecules were synthesized by the insertion of three types of aromatic spacers into the skeleton of permethylated α-cyclodextrin. These host molecules formed a 1:1 complex with sodium 3- and 4-nitrobenzenesulfonates (3- and 4-NBS), and sodium 2,4-dinitrobenzenesulfonate (DNBS) in D_2O/CD_3OD (4:1) solution. The type of spacer inserted remarkably affected the inclusion ability of the hosts toward DNBS. The *p*-xylylene-inserted CDs showed greater inclusion ability toward DNBS than permethylated α- and β-CDs.

1. Introduction

Cyclodextrins (CDs) have a hydrophobic cavity into which organic molecules of an appropriate size and shape can be incorporated in aqueous media. This inclusion ability has found applications in many areas.[1] A great deal of effort has been devoted to the modification of the hydroxyl groups on the upper and/or lower rims of CDs, such as capping, the introduction of ionic groups, and dimerization, in order to improve and control their inclusion ability.[2] On the other hand, much less attention has been paid to modifications of the CD ring by the insertion of noncarbohydrate spacers into the CD ring,[3] because such modification processes require considerably more reaction steps than modification of the CD hydroxyl groups. If a new methodology for the facile synthesis of this 'spacer-inserted' CD can be developed, one can easily construct novel types of CD derivatives in which secondary bonding interactions between the incorporated guest and the spacer in the host are possible, and the cavity size and shape can be adjusted to the structure of a given guest by the choice of spacer to be inserted. Recently, we developed a facile synthetic route for novel types of CD derivatives by the insertion of an aromatic dicarbonyl spacer into a permethylated α-CD skeleton.[4] Preliminary complexation studies revealed that the inclusion ability of such CD derivatives was affected by the type of spacer inserted. We report here an extension of this approach to the synthesis of aromatic dimethylene spacer-inserted CDs, in which the spacer is linked through ether linkages to the glucose units of permethylated α-CD. We also determined their inclusion ability towards aromatic guest molecules.

2. Results and discussion

Novel spacer-inserted CDs **3**, **4**, and **5**, in which 2,6-pyridinedimethylene, *m*-xylylene, and *p*-xylylene spacers were inserted into the skeleton of permethylated α-CD **1**, respectively, were synthesized by the cleavage of a single glucosidic bond in **1** and the subsequent cyclization of the resulting acyclic maltohexaose derivative **2** with an aromatic dimethylene diiodide. The synthetic route to **3**, **4**, and **5** is shown in Scheme 1. The selective cleavage of one glucosidic bond in **1** was carried out in 30% aq $HClO_4$ at room temperature to afford the maltohexaose derivative **2** (α-anomer:β-anomer = 55:45), according to a previously reported method.[4] The reaction of **2** with 2,6-bis(iodomethyl)pyridine in the presence of sodium hydride and tetra(*n*-butyl)ammonium iodide (TBAI) gave the desired cyclic product **3**, which was a mixture of α- and β-isomers. The separation of the α- and β-isomers was successfully carried out by silica gel column chromatography using chloroform–methanol (30:1) as an eluent and subsequent reverse phase column chromatography with methanol–water (5:1) as an eluent to give pure **3α** and **3β** in 7 and 12% isolated yields, respectively. Here, cyclization in the absence of TBAI as a phase-transfer catalyst lowered the yields of **3α** and **3β** to 2 and 1%, respectively. In a similar manner to **3**, the reactions of **2** with *m*- and *p*-xylylene diiodides were carried out to afford the corresponding cyclic

Keywords: Host molecule; Cyclodextrin; Spacer insertion; Inclusion ability.
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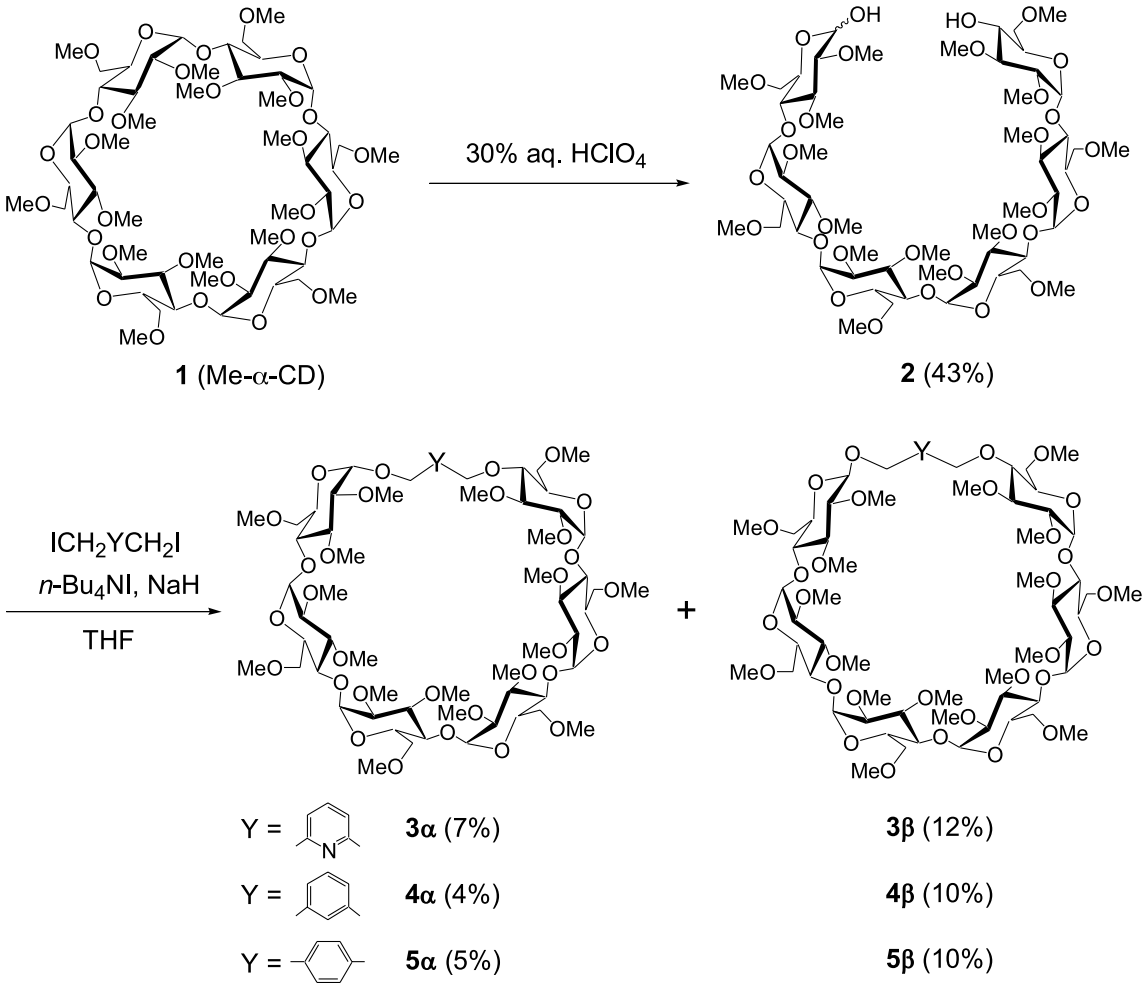

Scheme 1. Synthesis of spacer-inserted cyclodextrins.

host molecules. On the other hand, the reaction of **2** with *o*-xylylene diiodide gave a complex mixture of products from which the desired cyclization products could not be isolated. The structures of the macrocycles **3α**, **3β**, **4α**, **4β**, **5α**, and **5β** were confirmed by their NMR, mass, and IR spectra. Intense $[M+Na]^+$ ion signals were observed in the MALDI-TOF mass spectra of all macrocycles.

Figure 1 shows the ^{1}H NMR signals corresponding to anomeric protons in **3α** and **3β**. The anomeric proton signals for **3α** were separated into five signals in the range of 5.0–5.4 ppm. On the other hand, the anomeric proton signals for **3β** were clearly separated into six signals in the range of 4.2–5.5 ppm. Among the anomeric protons of **3β**, a signal present at 4.24 ppm as a doublet could be assigned to the anomeric proton with a β-D-configuration by the larger coupling constant ($J_{1,2}$=7.7 Hz). In the cases of both *m*-xylylene-inserted CDs **4** and *p*-xylylene-inserted CDs **5**, clear differences in the chemical shift of the anomeric proton signals were also observed between the α- and β-isomers. These results suggest that the ring structures of spacer-inserted CDs are remarkably affected by the configuration at the anomeric center linked to the spacer. CPK-modeling studies showed that the cavities of 2,6-pyridinedimethylene-inserted CDs (**3α**, **3β**) and *m*-xylylene-inserted CDs (**4α**, **4β**) have ellipse-like shapes in the top

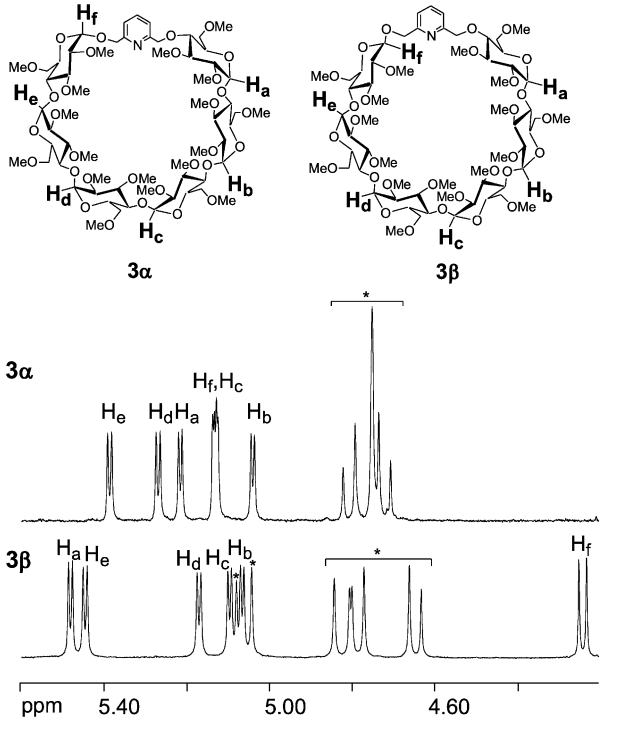

Figure 1. Partial ^{1}H NMR spectra of **3α** and **3β** in $CDCl_3$ at 25 °C. Asterisks indicate the methylene proton signals at the α-position of the pyridine ring.

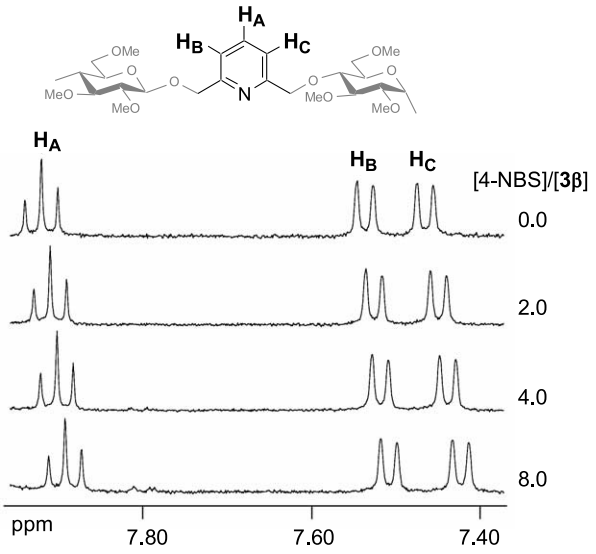

Figure 2. ^{1}H NMR spectral changes observed for host **3β** (4.0×10^{-3} M) in D_2O/CD_3OD (4:1) upon the addition of sodium 4-nitrobenzenesulfonate (4-NBS) at 25 °C.

derivatives and the guest molecules are summarized in Table 1. There was little difference in the inclusion ability towards 4-NBS among the spacer-inserted CDs examined here. On the other hand, a large difference in the inclusion ability towards 3-NBS was observed between hosts **4α** and **4β**, which have a different configuration at the anomeric center. Interestingly, host **4β** showed clear inclusion selectivity for sodium *m*-nitrobenzenesulfonate over the corresponding *p*-isomer, in contrast to the cases of permethylated CDs as well as all other spacer-inserted CDs examined here. These results may indicate that the size and shape of the aromatic part of 3-NBS fit those of the cavity of **4β**. On the other hand, the size of the aromatic part of 4-NBS is too small to cause a difference in the inclusion ability among the spacer-inserted CDs examined here. When 2,4-disubstituted benzenesulfonate, DNBS, whose aromatic part is bulkier than those of 3- and 4-NBS, was used as the guest, the inclusion ability of the spacer-inserted host was remarkably affected by the type of spacer inserted as well as the anomeric configuration in the host. The inclusion ability of 2,6-pyridinedimethylene-inserted CD **3α** (or **3β**) toward DNBS was lower than that of *m*-xylylene-

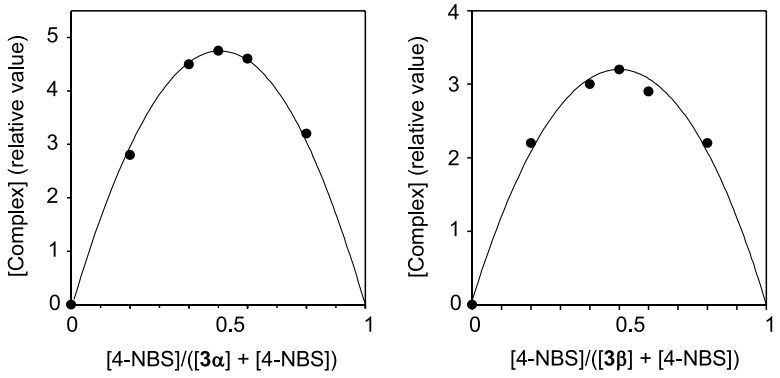

Figure 3. Job plots for **3α**-4-NBS complex (left) and **3β**-4-NBS complex (right).

view, whose long axis/short axis ratio (L/S) is affected by the configuration at the anomeric center linked to the spacer (the ellipsoidal cavities of **3β** and **4β** have a higher L/S than those of **3α** and **4α**, respectively). On the other hand, the cavities of *p*-xylylene-inserted CDs (**5α**, **5β**) have nearly circular shapes with 10–11 Å in diameter.

We determined the stability constants of complexes of these host molecules with sodium 4-nitrobenzenesulfonate (4-NBS), sodium 3-nitrobenzenesulfonate (3-NBS), and sodium 2,4-dinitrobenezesulfonate (DNBS) by the ^{1}H NMR titration method. Due to the poor water solubility of the hosts **4α**, **4β**, **5α**, and **5β**, the titration was performed in a D_2O/CD_3OD (4:1) mixed solvent. The upfield shift of the signals of both the aromatic and carbohydrate parts of these hosts was observed upon the addition of the aromatic guest molecules, indicating that the aromatic parts of the guests were incorporated into the cavities of the hosts. Figure 2 illustrates the upfield shift of the aromatic proton signals of **3β** induced by complexation with 4-NBS. The Job plots indicated that the hosts formed a 1:1 complex with the aromatic guest molecules. As typical examples, Job plots for the **3α**-4-NBS and **3β**-4-NBS complexes are shown in Figure 3. The stability constants of complexes of the CD inserted CD **4α** (or **4β**), though these hosts should have almost the same ring structure. This result can be explained by considering that the pyridine nitrogen atom in the spacer decreases the hydrophobicity of the host cavity. It is noteworthy that the hosts **4β**, **5α**, and **5β** showed higher inclusion ability towards DNBS than any of the permethylated α-, β-, and γ-CDs. CPK-modeling studies indicated

Table 1. Stability constants (M^{-1}) of complexes of cyclodextrin derivatives with aromatic guest molecules[a]

Host	Guest		
	3-NBS[b]	4-NBS[b]	DNBS[b]
3α	13±5	16±5	3±1
3β	14±4	12±4	3±1
4α	9±3	9±2	9±1
4β	25±6	11±2	18±5
5α	8±2	9±2	25±5
5β	12±4	9±3	14±4
Me-α-CD	31±2	85±10	5±1
Me-β-CD	15±5	16±5	7±2
Me-γ-CD	2±1	13±3	7±1

[a] In D_2O/CD_3OD (4/1) at 25 °C.
[b] 3-NBS, sodium 3-nitrobenzenesulfonate; 4-NBS, sodium 4-nitrobenzenesulfonate; DNBS, sodium 2,4-dinitrobenzenesulfonate.

that the host **5α** possesses a cavity of almost the same size and shape as that of permethylated β-CD. This result suggests that the interaction between DNBS and the *p*-xylylene spacer in the host **5α** should contribute to its enhanced inclusion ability.

3. Conclusion

We synthesized novel CD derivatives by the insertion of an aromatic dimethylene spacer into a permethylated α-cyclocyclodextrin skeleton. The type of aromatic spacer inserted remarkably affected the inclusion ability of these hosts towards sodium 2,4-dinitrobenzenesulfonate (DNBS) bearing a moderately bulky aromatic group. The *p*-xylylene-inserted CDs showed higher inclusion ability towards DNBS than any of the permethylated α-, β-, and γ-CDs.

4. Experimental

4.1. General

^{1}H and ^{13}C NMR spectra were recorded with a JEOL JNM-GSX-400 spectrometer. IR spectra were measured on a Horiba FT-710 spectrometer. High-resolution mass spectra were measured on a JEOL JMS-700 mass spectrometer. MALDI-TOF Mass spectra were measured on an Applied Biosystems Voyager RP mass spectrometer. Elemental analyses were measured with a Yanagimoto CHN-Corder. Melting points were measured with a Yanaco MP-S3 apparatus. THF was freshly distilled before use. 2,6-Bis(iodomethyl)pyridine, 1,3-bis(iodomethyl)benzene, and 1,4-bis(iodomethyl)benzene were prepared from the corresponding dibromides via the Finkelstein reaction. The NMR signals of the aromatic and carbohydrate protons of compounds **3α**, **3β**, **4α**, **4β**, **5α**, and **5β** were assigned by 2D NMR spectroscopy (^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY, long-range ^{13}C–^{1}H COSY, TOCSY, and ROESY).[5]

4.1.1. 2,6-Bis(iodomethyl)pyridine. NaI (0.60 g, 4.0 mmol) was added to 2,6-bis(bromomethyl)pyridine (0.106 g, 0.40 mmol) dissolved in acetone (10 mL). This mixture was heated to reflux for 12 h. After the solvent was removed in vacuo, the residue was dispersed in CH_2Cl_2 (150 mL). The resulting precipitate was filtered off, and the solvent was removed in vacuo to yield the desired diiodide (0.135 g, 94%) as a white solid, which was used without further purification in the next step: mp 72–74 °C; ^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.61 (t, J=7.7 Hz, 1H), 7.28 (d, J=7.7 Hz, 2H), 4.50 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.28, 138.01, 121.80, 5.73; HRMS Calcd for $C_7H_7NI_2$: 358.8666. Found: 358.8681. This compound was determined to be >95% pure by ^{1}H NMR spectroscopy.

4.1.2. 1,3-Bis(iodomethyl)benzene. This compound was synthesized in a similar manner to 2,6-bis(iodomethyl)-pyridine. Yield 99%; mp 104–106 °C; ^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.41 (t, 3H), 7.22–7.29 (m, 3H), 4.45 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.97, 129.38, 128.99, 128.35, 4.66; HRMS Calcd for $C_8H_8I_2$: 357.8713. Found: 357.8718. This compound was determined to be >95% pure by ^{1}H NMR spectroscopy.

4.1.3. 1,4-Bis(iodomethyl)benzene. This compound was synthesized in a similar manner to 2,6-bis(iodomethyl)-pyridine. Yield 96%; sp 133 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (s, 4H), 4.40 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.06, 129.26, 4.83; HRMS Calcd for $C_8H_8I_2$: 357.8713. Found: 357.8728. This compound was determined to be >95% pure by ^{1}H NMR spectroscopy.

4.1.4. Maltohexaose derivative (2). This compound was synthesized according to the previously reported method.[4] Permethylated α-CD (**1**) (9.20 g, 7.51 mmol) was dissolved in 30% aq $HClO_4$ (500 mL), and the solution was stirred at room temperature for 4 days. After neutralization with aq NaOH, the mixture was extracted with $CHCl_3$ (150 mL×4). The resultant $CHCl_3$ solution was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (gradient 1:2–1:1 acetone/hexane) to yield the maltohexaose derivative **2** as a white solid (4.01 g, 43%): mp 75–76 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.65 (d, J=3.7 Hz, 1H), 5.52–5.57 (m, 4H), 5.35 (t, J=3.3 Hz, 0.55H), 4.62 (dd, J=5.9, 7.3 Hz, 0.45H), 3.19–3.92 (m, 90H); IR (KBr) 3444, 2929, 1149, 1088 cm^{-1}; MALDI-TOF MS *m/z* 1266 $[M+Na]^+$, 1282 $[M+K]^+$. Anal. Calcd (%) for $C_{54}H_{98}O_{31}\cdot H_2O$: C, 51.42; H, 7.99. Found: C, 51.39; H, 7.62.

4.1.5. 2,6-Pyridinedimethylene-inserted α-cyclodextrin derivatives (3α and 3β). To a suspension of NaH (60% assay, 0.053 g, 1.32 mmol) in THF (20 mL) were added compound **2** (0.410 g, 0.33 mmol) and tetra(*n*-butyl)-ammonium iodide (0.122 g, 0.33 mmol) under an Ar atmosphere, and the mixture was stirred for 1 h at room temperature. 2,6-Bis(iodomethyl)pyridine (0.237 g, 0.66 mmol) was then added. The resulting mixture was stirred at room temperature for 5 h. After the addition of THF (15 mL), the mixture was heated to reflux with stirring for 12 h. Unreacted sodium hydride was deactivated by the addition of methanol into the reaction mixture, and then the solvent was removed in vacuo. The residue was dispersed in $CHCl_3$ (50 mL), and any insoluble solid was filtered off. After the filtrate was concentrated in vacuo, the resulting solid was purified by silica gel column chromatography (gradient 100:0–30:1 chloroform/methanol) and subsequent reverse phase column chromatography (5:1 methanol/H_2O) to yield **3α** and **3β** as white solids. **3α** (yield 7%): mp 88–90 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (t, J=7.7 Hz, 1H), 7.52 (d, J=7.7 Hz, 1H), 7.33 (d, J=7.7 Hz, 1H), 5.39 (d, J=3.7 Hz, 1H), 5.27 (d, J=3.7 Hz, 1H), 5.22 (d, J=3.3 Hz, 1H), 5.14 (d, J=3.7 Hz, 1H), 5.13 (d, J=3.7 Hz, 1H), 5.04 (d, J=3.3 Hz, 1H), 4.81 (d, J=11.4 Hz, 1H), 4.75 (s, 2H), 4.72 (d, J=11.4 Hz, 1H), 3.12–3.89 (m, 90H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.59, 157.23, 137.42, 121.33, 120.64, 98.91, 98.76, 98.60, 98.26, 97.55, 96.82, 83.27, 82.52, 82.43, 82.28, 82.08, 82.04, 81.86, 81.77, 81.61, 80.75, 79.88, 78.75, 78.47, 77.85, 77.25, 77.20, 76.62, 75.93, 75.36, 71.48, 71.40, 71.10, 71.02, 70.98, 70.94, 70.91, 70.77, 70.66, 70.64, 70.35, 69.72, 61.45, 61.43, 61.06, 60.85, 60.74, 60.69, 59.28, 58.97, 58.90, 58.85, 58.82, 58.79, 58.42, 57.94; IR (KBr) 2929, 2839, 1626, 1107, 1039 cm^{-1}; MALDI-TOF MS *m/z* 1347 $[M+H]^+$, 1369 $[M+Na]^+$, 1385 $[M+K]^+$. Anal. Calcd (%) for $C_{61}H_{103}NO_{31}$: C, 54.41; H, 7.71; N, 1.04. Found: C, 54.36; H, 7.50; N, 1.07. **3β** (yield 12%): mp 88–92 °C; ^{1}H NMR

(400 MHz, $CDCl_3$) δ 7.69 (t, J=7.7 Hz, 1H), 7.41 (d, J=7.7 Hz, 1H), 7.19 (d, J=7.7 Hz, 1H), 5.48 (d, J=3.7 Hz, 1H), 5.45 (d, J=3.7 Hz, 1H), 5.17 (d, J=3.7 Hz, 1H), 5.10 (d, J=3.7 Hz, 1H), 5.07 (d, J=3.7 Hz, 1H), 5.06 (d, J=14.7 Hz, 1H), 4.82 (d, J=14.7 Hz, 1H), 4.79 (d, J=11.4 Hz, 1H), 4.65 (d, J=11.4 Hz, 1H), 4.24 (d, J=7.7 Hz, 1H), 3.06–3.91 (m, 90H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.39, 157.30, 137.11, 121.65, 120.74, 101.27, 99.20, 99.18, 98.18, 97.57, 96.95, 86.49, 84.20, 82.69, 82.33, 82.30, 82.22, 82.19, 82.02, 81.85, 81.79, 81.73, 80.84, 80.81, 79.68, 79.54, 76.50, 74.86, 74.16, 73.51, 71.52, 71.43, 71.23, 71.08, 71.06, 71.03, 70.95, 70.85, 70.79, 70.44, 61.73, 61.64, 61.50, 60.69, 60.40, 60.24, 60.02, 59.50, 59.35, 59.29, 59.19, 59.09, 59.04, 59.00, 58.99, 58.50, 58.24, 58.13; IR (KBr) 2933, 2839, 1630, 1101, 1036 cm^{-1}; MALDI-TOF MS m/z 1347 $[M+H]^+$, 1369 $[M+Na]^+$, 1385 $[M+K]^+$. Anal. Calcd (%) for $C_{61}H_{103}NO_{31}$: C, 54.41; H, 7.71; N, 1.04. Found: C, 54.23; H, 7.43; N, 0.98.

4.1.6. *m*-Xylylene-inserted α-cyclodextrin derivatives (4α and 4β). Compounds **4α** and **4β** were synthesized in a similar manner to **3α** and **3β**. **4α** (yield 4%): mp 82–86 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30–7.40 (m, 3H), 7.19–7.20 (m, 1H), 5.51 (d, J=3.7 Hz, 1H), 5.32 (d, J=3.3 Hz, 1H), 5.19 (d, J=3.7 Hz, 1H), 5.09 (d, J=3.9 Hz, 1H), 5.08 (d, J=3.7 Hz, 1H), 5.04 (d, J=3.3 Hz, 1H), 4.92 (d, J=11.7 Hz, 1H), 4.62 (d, J=11.7 Hz, 1H), 4.62 (s, 2H), 3.07–3.91 (m, 90H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.08, 137.43, 128.46, 127.44, 126.92, 126.34, 98.94, 98.73, 98.67, 98.55, 96.68, 96.03, 83.41, 83.36, 82.31, 82.29, 82.16, 82.09, 82.06, 81.89, 81.81, 81.66, 80.56, 80.49, 79.27, 77.68, 77.20, 71.68, 71.28, 71.24, 71.12, 71.03, 70.89, 70.85, 70.80, 70.68, 70.66, 70.64, 70.57, 70.54, 69.47, 61.66, 61.46, 61.15, 60.60, 60.54, 59.30, 59.15, 58.98, 58.94, 58.92, 58.88, 58.58, 58.36, 58.23, 58.01; IR (KBr) 2933, 2821, 1635, 1107, 1039 cm^{-1}; MALDI-TOF MS m/z 1368 $[M+Na]^+$, 1384 $[M+K]^+$. Anal. Calcd (%) for $C_{62}H_{104}O_{31}$: C, 55.35; H, 7.79. Found: C, 55.68; H, 7.47. **4β** (yield 10%): mp 85–86 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (s, 1H), 7.27–7.34 (m, 2H), 7.11–7.15 (m, 1H), 5.50 (d, J=3.7 Hz, 1H), 5.47 (d, J=3.7 Hz, 1H), 5.21 (d, J=3.7 Hz, 1H), 5.08 (d, J=3.7 Hz, 1H), 5.06 (d, J=3.7 Hz, 1H), 4.82 (d, J=11.4 Hz, 1H), 4.77 (s, 2H), 4.63 (d, J=11.4 Hz, 1H), 4.29 (d, J=7.3 Hz, 1H), 3.07–3.95 (m, 90H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.00, 137.68, 128.52, 127.37, 127.17, 126.66, 100.86, 98.96, 98.86, 98.78, 97.66, 96.35, 86.13, 84.08, 83.48, 82.43, 82.27, 82.24, 82.09, 81.94, 81.92, 81.77, 81.65, 81.61, 80.82, 80.70, 79.07, 77.95, 77.20, 74.67, 74.55, 74.09, 73.17, 71.65, 71.24, 71.21, 71.11, 71.05, 71.00, 70.99, 70.98, 70.86, 70.79, 70.63, 70.35, 69.80, 61.71, 61.46, 61.09, 60.11, 60.04, 59.43, 59.32, 59.17, 59.02, 58.89, 58.48, 58.11, 57.93; IR (KBr) 2933, 2829, 1637, 1103, 1039 cm^{-1}; MALDI-TOF MS m/z 1368 $[M+Na]^+$, 1384 $[M+K]^+$. Anal. Calcd (%) for $C_{62}H_{104}O_{31}$: C, 55.35; H, 7.79. Found: C, 55.59; H, 7.50.

4.1.7. *p*-Xylylene-inserted α-cyclodextrin derivatives (5α and 5β). Compounds **5α** and **5β** were synthesized in a similar manner to **3α** and **3β**. **5α** (yield 5%): mp 86–89 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (d, J=8.1 Hz, 2H), 7.31 (d, J=8.1 Hz, 2H), 5.41 (d, J=3.7 Hz, 1H), 5.30 (d, J=3.7 Hz, 1H), 5.21 (d, J=3.7 Hz, 1H), 5.13 (d, J=3.3 Hz, 1H), 5.10 (d, J=3.3 Hz, 1H), 5.06 (d, J=3.3 Hz, 1H), 4.92 (d, J=11.7 Hz, 1H), 4.84 (d, J=11.7 Hz, 1H), 4.57 (d, J=11.7 Hz, 2H), 3.10–3.89 (m, 90H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.46, 137.19, 128.34, 127.10, 99.29, 99.05, 98.41, 98.18, 97.71, 83.27, 83.10, 82.45, 82.35, 82.29, 82.16, 82.05, 81.83, 81.50, 80.96, 79.57, 79.04, 77.71, 75.76, 74.16, 72.45, 71.91, 71.47, 71.21, 71.06, 71.04, 70.98, 70.84, 70.79, 70.68, 70.48, 69.78, 61.46, 61.45, 61.12, 60.94, 60.75, 59.31, 59.24, 58.99, 58.93, 58.91, 58.87, 58.85, 58.30, 57.87; IR (KBr) 2927, 2831, 1647, 1095, 1039 cm^{-1}; MALDI-TOF MS m/z 1368 $[M+Na]^+$, 1384 $[M+K]^+$. Anal. Calcd (%) for $C_{62}H_{104}O_{31}$: C, 55.35; H, 7.79. Found: C, 55.51; H, 7.43. **5β** (yield 10%): mp 91–93 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (d, J=8.1 Hz, 2H), 7.29 (d, J=8.1 Hz, 2H), 5.37 (d, J=3.7 Hz, 1H), 5.31 (d, J=3.7 Hz, 1H), 5.30 (d, J=3.7 Hz, 1H), 5.17 (d, J=3.7 Hz, 1H), 5.08 (d, J=3.3 Hz, 1H), 4.92 (d, J=12.8 Hz, 1H), 4.78 (d, J=10.6 Hz, 1H), 4.62 (d, J=12.8 Hz, 1H), 4.58 (d, J=10.6 Hz, 1H), 4.19 (d, J=7.3 Hz, 1H), 3.06–3.91 (m, 90H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.16, 137.38, 128.63, 127.90, 101.31, 98.67, 98.52, 97.84, 97.33, 86.06, 84.09, 83.36, 82.77, 82.26, 82.05, 81.95, 81.91, 81.83, 81.68, 80.35, 79.46, 77.81, 77.52, 77.20, 75.26, 74.95, 74.15, 71.64, 71.27, 71.10, 71.05, 71.01, 70.96, 70.86, 70.77, 70.65, 70.62, 70.53, 70.40, 61.49, 61.43, 61.07, 60.89, 60.57, 60.43, 60.13, 59.33, 59.28, 59.04, 58.95, 58.88, 58.82, 58.40, 57.98; IR (KBr) 2925, 2821, 1635, 1093, 1039 cm^{-1}; MALDI-TOF MS m/z 1368 $[M+Na]^+$, 1384 $[M+K]^+$. Anal. Calcd (%) for $C_{62}H_{104}O_{31}$: C, 55.35; H, 7.79. Found: C, 55.70; H, 7.59.

4.2. NMR titration and Job plots

The ^{1}H NMR titrations were performed at 25 °C in CD_3OD–D_2O (1:4) with DSS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate) as an external standard. A solution of the host molecule (0.5 mL, 4.0 mM) was titrated in a NMR tube with increasing amounts of guest stock solution (0.5 mL, 80 mM) as follows (in μL): 0, 12.5, 25.0, 37.5, 50.0, 75.0, 100, 150, 200, 250. The titration curves (changes in the chemical shift of the host protons ($\Delta\delta$) against the guest/host ratio) were analyzed by a non-linear least-squares curve fitting method to generate stability constants of the host–guest complexes.

Job plots were carried out by monitoring the changes in the chemical shift of the host protons ($\Delta\delta$) in a series of solutions with varying host/guest ratios but the total concentrations of the host and guest being kept constant (4.0 mM). The relative concentration of the host–guest complex estimated from the $\Delta\delta\cdot$ [host] value was plotted against ([guest]/{[host]+[guest]}).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.tet.2005.04.026

References and notes

1. (a) Wenz, G. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 803–822. (b) Szejtli, J.; Osa, T. *Comprehensive supramolecular chemistry*; Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Vögtle, F., Lehn, J.-M., Eds.; Pergamon: Oxford, 1996; Vol. 3.
2. (a) Croft, A. P.; Bartsch, R. A. *Tetrahedron* **1983**, *39*, 1417–1474. (b) Easton, C. J.; Lincoln, S. F. *Chem. Soc. Rev.* **1996**, *25*, 163–170. (c) Khan, A. R.; Forgo, P.; Stine, K. J.; D'Souza, V. T. *Chem. Rev.* **1998**, *98*, 1977–1996. (d) Breslow, R.; Dong, S. D. *Chem. Rev.* **1998**, *98*, 1997–2011.
3. (a) Bürli, R.; Vasella, A. *Angew. Chem., Int. Ed.* **1997**, *36*, 1852–1853. (b) Morales, J. C.; Zurita, D.; Penadés, S. *J. Org. Chem.* **1998**, *63*, 9212–9222. (c) Hoffmann, B.; Zanini, D.; Ripoche, I.; Bürli, R.; Vasella, A. *Helv. Chim. Acta* **2001**, *84*, 1862–1888. (d) Hoffmann, B.; Bernet, B.; Vasella, A. *Helv. Chim. Acta* **2002**, *85*, 265–287.
4. Kida, T.; Michinobu, T.; Zhang, W.; Nakatsuji, Y.; Ikeda, I. *Chem. Commun.* **2002**, 1596–1597.
5. Schneider, H.-J.; Hacket, F.; Rüdiger, V.; Ikeda, H. *Chem. Rev.* **1998**, *98*, 1755–1785.

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Kinetics and mechanism of thermal gas-phase elimination of β-substituted carboxylic acids

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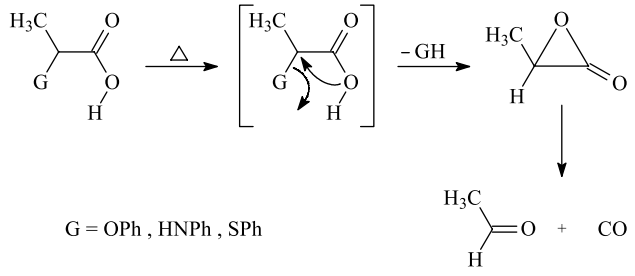
Abstract—3-Phenoxypropanoic acid (**1**), 3-(phenylthio)propanoic acid (**2**), and 4-phenylbutanoic acid (**3**) were pyrolysed between 520 and 682 K. Analysis of the pyrolysates showed the elimination products to be acrylic acid and the corresponding arene. Pyrolysis of ethyl 3-phenoxypropanoate (**4**) and its methyl analogue (**5**), ethyl 3-(phenylthio)propanoate (**6**) and its methyl counterpart (**7**), and 3-phenoxypropane nitrile (**8**) were also investigated between 617 and 737 K. The thermal gas-phase elimination kinetics and product analysis are compatible with a thermal retro-Michael reaction pathway involving a four-membered cyclic transition state.

1. Introduction

A feasible mechanism for the gas-phase pyrolytic reaction of α-substituted carboxylic acids, namely 2-phenoxypropanoic acid and its phenylthio and phenylamino analogues, has been proposed (Scheme 1).[1] Besides, we have recently, reported on the thermal gas-phase elimination of β-aminoarylpropanoic acid.[2] Product analysis indicates the formation of acrylic acid and substituted aniline. Two acceptable mechanistic pathways were suggested to account for the formation of the reaction products (Scheme 2). To discriminate between the two possibilities, the two alternative reaction pathways were subjected to theoretical calculations using the ab initio SCF method, and the computational results favour the cyclic four-membered transition state (Scheme 2: ii; TS-II). In this study, we report on the thermal gas-phase elimination reaction of eight β-substituted propanoic acids and derivatives (Fig. 1).



Scheme 1. Reaction pathway of gas-phase pyrolysis of α-substituted propanoic acid.

2. Results and discussion

2.1. Kinetics and product analysis

The products of pyrolysis were analyzed and spectrometrically characterized. The constituents of the pyrolysates of the β-substituted acids under study (**1**–**3**, **8**) were established to be the corresponding arene fragments (PhOH, PhSH, $PhCH_3$) together with acrylic acid, and for compound **8** the second pyrolytic product was acrylonitrile. The ethyl and methyl esters (**4**–**7**) also gave the corresponding arenes (PhOH, PhSH). However, the ethyl esters (**4**, **6**) gave acrylic acid, while the methyl esters (**5**, **7**) produced methyl acrylate.

The kinetic behaviour of each substrate was investigated, and the electronic effects of substituents at the β-carbon of the propanoic acid and its derivatives studied by comparison of the molecular reactivity of substrates **1**–**8**. These compounds were each well behaved kinetically, and gave reproducible first-order rate constants with strict Arrhenius

Keywords: β-Substituted carboxylic acids; Pyrolysis; Kinetics; Mechanism.
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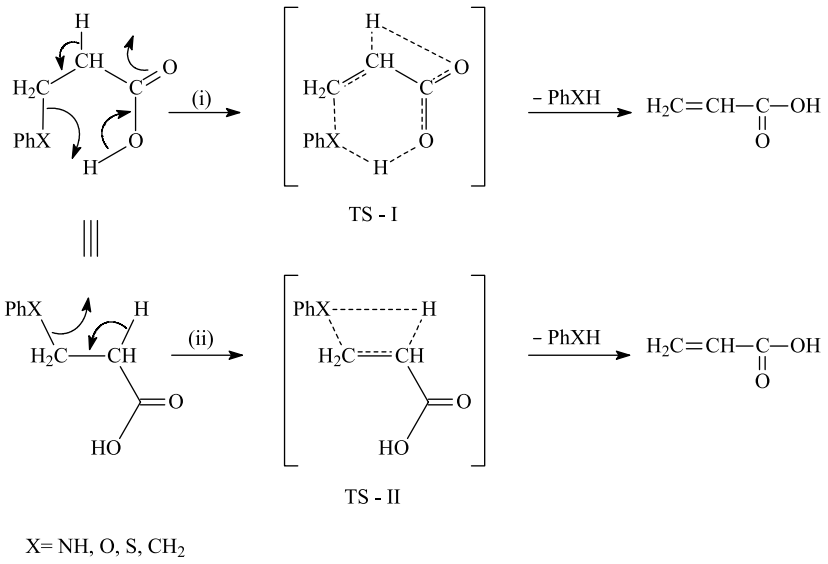
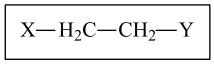


Scheme 2. Gas-phase elimination reaction of β-substituted propanoic acid.



	X	Y	
(**1**)	OPh	COOH	3-Phenoxypropanoic acid
(**2**)	SPh	COOH	3-(Phenylthio)propanoic acid
(**3**)	CH_2Ph	COOH	4-Phenylbutanoic acid
(**4**)	OPh	COOEt	Ethyl 3-phenoxypropanoate
(**5**)	OPh	COOMe	Methyl 3-phenoxypropanoate
(**6**)	SPh	COOEt	Ethyl 3-(phenylthio)propanoate
(**7**)	SPh	COOMe	Methyl 3-(phenylthio)propanoate
(**8**)	OPh	CN	3-Phenoxypropane nitrile

Figure 1. β-Substituted propanoic acid and its derivatives.

plot linearity over 90% of the reaction duration. Since, a sixfold change in the amount of substrate per kinetic run indicated no significant change in rate coefficient, these reactions were deemed to be unimolecular first-order processes. The kinetic data are summarized in Table 1, and the kinetic consequences of changing the β-substituent from $PhCH_2$ to PhO, PhS and PhNH, and of changing Y from acid (CO_2H) function to CN and ester moieties are recorded in Figure 2.

The elimination pathway of acids **1**–**3** involves the transition state (TS) shown in Figure 2. An identical transition state is proposed for compound **8**. Our earlier theoretical ab initio calculations have argued in favour of the reaction pathway for the thermal gas-phase elimination reaction of acid **9**, which involves a four-membered cyclic TS (Scheme 2, pathway (ii)).[2] We have now extended the theoretical investigation to include the three acids **1**–**3** (see Section 2.2). The results for the four acids displayed in Figure 3 clearly demonstrate that the four-membered transition state (TS-I) is energetically more favourable than TS-II (Scheme 2, pathway (i)). The results also illustrate a dramatic difference between TS-I and TS-II for X=S and CH_2 than for X=O and NH. In the transition state of the elimination reaction (TS-I) two σ bonds are being weakened (C–X (bond a) and C–H (bond b) in Fig. 2), while σ and π bonds are being formed. This complex process of bond-breaking and making resulted in an order of molecular reactivities in which acid **3** (X=CH_2) is ca. 19–34 times less reactive than the acids in which X=O, S or NH. This finding correlates well with the relative polarity of the (C–X) bond in these substrates. The effect of substituents on C–H bond-breaking (bond b) and π bond formation (C=C bond) is reflected in a rate-reduction factor of ca. 158 when a carboxylic (CO_2H) acid moiety (**1**) is replaced by the more electron-withdrawing cyano (CN) group (**8**). A rate-reduction factor of ca. 72 is also observed when the acid group is replaced by methyl ester group (**5**), and a larger (227-fold) decrease in rate is obtained on comparing the reactivities of acid **2** and methyl ester **7**.

The proposed mechanisms of the thermal gas-phase elimination reaction of the ethyl and methyl esters are noteworthy (Scheme 3). The ethyl esters (**4**, **6**) react, respectively, ca. 165 and 173 times slower than the corresponding carboxylic acids (**1**, **2**). However, the products of pyrolysis include acrylic acid and no ethyl acrylate. It is, therefore, suggested that these esters first, eliminate ethene and the acid ($PhCH_2CH_2CO_2H$) via a cyclic six-membered TS, and the acid then undergoes rapid secondary decomposition via a faster step to give PhXH and acrylic acid as shown for acid pyrolysis. Ethyl acrylate would have been obtained if the four-membered TS was involved in the pyrolysis of the ethyl esters (**4**, **6**). Pyrolysis of the methyl esters (**5**, **7**), on the other hand, produced methyl acrylate and no acrylic acid. These results seem to substantiate a methyl ester reaction pathway involving a

Table 1. Rate coeffecients and Arrhenius parameters of β-substituted carboxylic acids and their derivatives (**1–8**)

No.	*T*/K	10^4 k/s^{-1}	log *A*/s^{-1}	E_a/kJ mol^{-1}	$k_{600\ K}$/s^{-1}
1	544.7	1.723	12.21±0.18	166.55±1.91	5.13×10^{-3}
	554.7	3.486			
	564.8	6.427			
	574.9	11.53			
	604.7	67.07			
2	520.2	1.526	8.63±0.62	124.23±6.46	6.59×10^{-3}
	532.5	2.434			
	543.8	5.429			
	555.1	10.93			
	568.8	16.47			
3	615.6	3.416	5.29±0.24	103.48±2.97	1.93×10^{-4}
	629.0	4.944			
	643.2	7.321			
	655.6	10.75			
	668. 5	15.91			
	681.9	25.41			
4	616.6	3.934	8.48±0.23	149.26±2.92	3.10×10^{-5}
	650.0	6.128			
	664.6	8.720			
	679.9	15.28			
	720.2	20.49			
5	633.3	2.243	5.26±0.14	108.06±1.80	7.13×10^{-5}
	654.8	4.544			
	666.0	5.840			
	677.1	8.132			
	687.3	11.13			
	698.3	15.18			
	711.0	21.38			
6	634.8	2.345	9.80±0.47	163.33±5.93	3.80×10^{-5}
	645.1	4.011			
	655.1	5.953			
	665.0	9.05			
	675.1	13.79			
	684.9	22.65			
	694.7	36.16			
7	651.5	2.137	6.05±0.30	121.65±3.98	2.90×10^{-5}
	665.6	3.068			
	679.6	4.590			
	693.6	8.314			
	722.0	17.47			
	736.9	27.53			
8	635.2	2.161	10.65±0.37	173.94±4.60	3.25×10^{-5}
	644.6	3.799			
	655.6	6.376			
	665.8	10.16			
	674.6	14.21			
	685.0	25.63			

cyclic four-membered TS analogous to that shown for the acids [Scheme 2, pathway (ii)].

It is of interest to note that the results of the theoretical ab initio calculations show the gas-phase thermodynamic stabilities of the arene fragments to be in the order: $PhNH_2 \gg PhCH_3 \sim PhOH > PhSH$. The results appear to correlate with the expected gas-phase acidities of these compounds and the relative proton affinities of their incipient conjugate bases. The thermodynamic stability of the products of gas-phase elimination is an important contributing factor to both molecular reactivity and reaction pathway.[3]

It is worth mentioning here, that ethyl β-hydroxycarboxylates were reported to decompose thermally in the gas-phase to a mixture of aldehydes and/or ketones and ethyl acetate (Scheme 4).[4] Small amounts of ethene and carboxylic acid were also detected among the products of reaction (Scheme 5). This mechanistic pathway was a subject of a theoretical calculation by Natrio and his group.[5] The free energy profile, evaluated at the MP2/3-31 G(d) level of theory, showed clearly that the formation of ethyl acetate and carbonyl compounds is kinetically more favourable than the formation of ethene and carboxylic acid. The theoretical study concluded that the C=O bond breaking and O–H bond forming can be seen as the driving force of the reaction. A safe conclusion from these studies is that thermal gas-phase elimination reactions of ethyl esters of carboxylic acids proceed by a mechanism involving preferred six-membered cyclic transition states.

2.2. Computational studies

The theoretical studies on the gas-phase thermolysis of the three β-substituted propanoic acids (**1–3**) were carried out using ab initio SCF method. The calculations were undertaken to explore the nature of the reaction mechanism

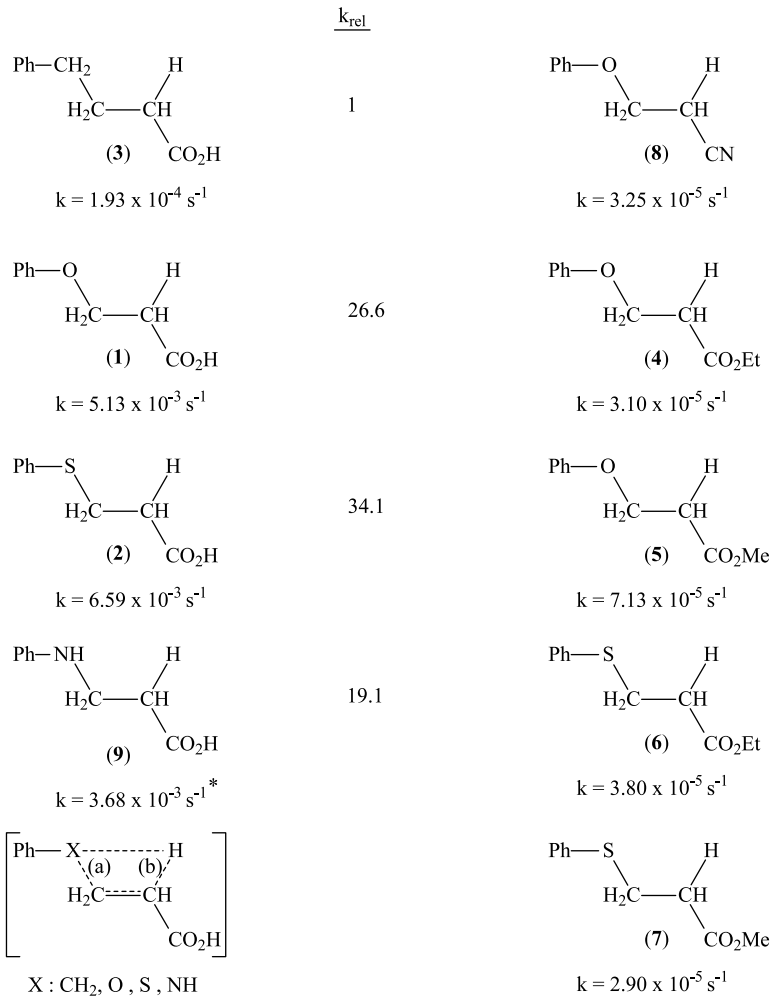


Figure 2. Transition state and relative reactivities at 600 K for the gas-phase pyrolysis of β-substituted propanoic acids (**1**–**3**, **8**, **9**) and esters (**4**–**7**). *Ref. 2.

of the unimolecular thermal decomposition of the three acids. Two alternative reaction pathways for the decomposition process have been evaluated. All the calculations have been performed on the TITAN computational package.[6]

The geometric parameters for the reactants, the transition states (TS), and the products of the two reaction pathways were fully optimized at the HF/6-31G* level of theory to obtain the energy profiles corresponding to the three reactions. A scaling factor[7] of 0.9135 for the zero-point vibrational energies has been used. The structures obtained from the optimization calculations are represented in Figure 4.

Table 2 illustrates the main distances of each optimized structure of the three studied reactions. During the thermolytic process, when the reactant is being transformed into TS-II, the X_1–C_2 and C_3–H_7 distances increase, whereas the C_2–C_3 and H_7–X_1 distances decrease, for X=O, S or CH_2.

The electronic energies, zero-point vibrational energies, enthalpies and entropies, evaluated at the HF/6-31G* level of theory for the reactants, transition states and reaction products involved in the two alternative pathways of the three reactions are collated in Table 3. The free energy profiles obtained at the HF/6-31G* level for the decomposition process of the three β-substituted propanoic acids (**1**–**3**) under study and that of acid (**9**)[2] are presented in Figure 3. The free energy values of the two suggested transition states show that TS-II has an energy barrier lower than TS-I. The calculated activation free energies are 316, 15.21, and 21.07 kJ mol^{-1} for the four-membered ring cyclic transition states (TS-II): X=O, S and CH_2, respectively. The corresponding values based on the cyclic six-membered transition state (TS-I) are, 363, 370 and 490 kJ mol^{-1}, respectively. The present and reported results indicate that the cyclic four-membered transition state (TS-II) is more favoured than the alternative cyclic six-membered transition state.[2]

3. Experimental

3.1. General

Melting points were determined on a Shimadzu-Gallenkamp apparatus and are uncorrected. Elemental analysis was by means of a LECO CHNS-932 Elemental Analyzer. NMR spectra were measured using a Bruker DPX 400 MHz

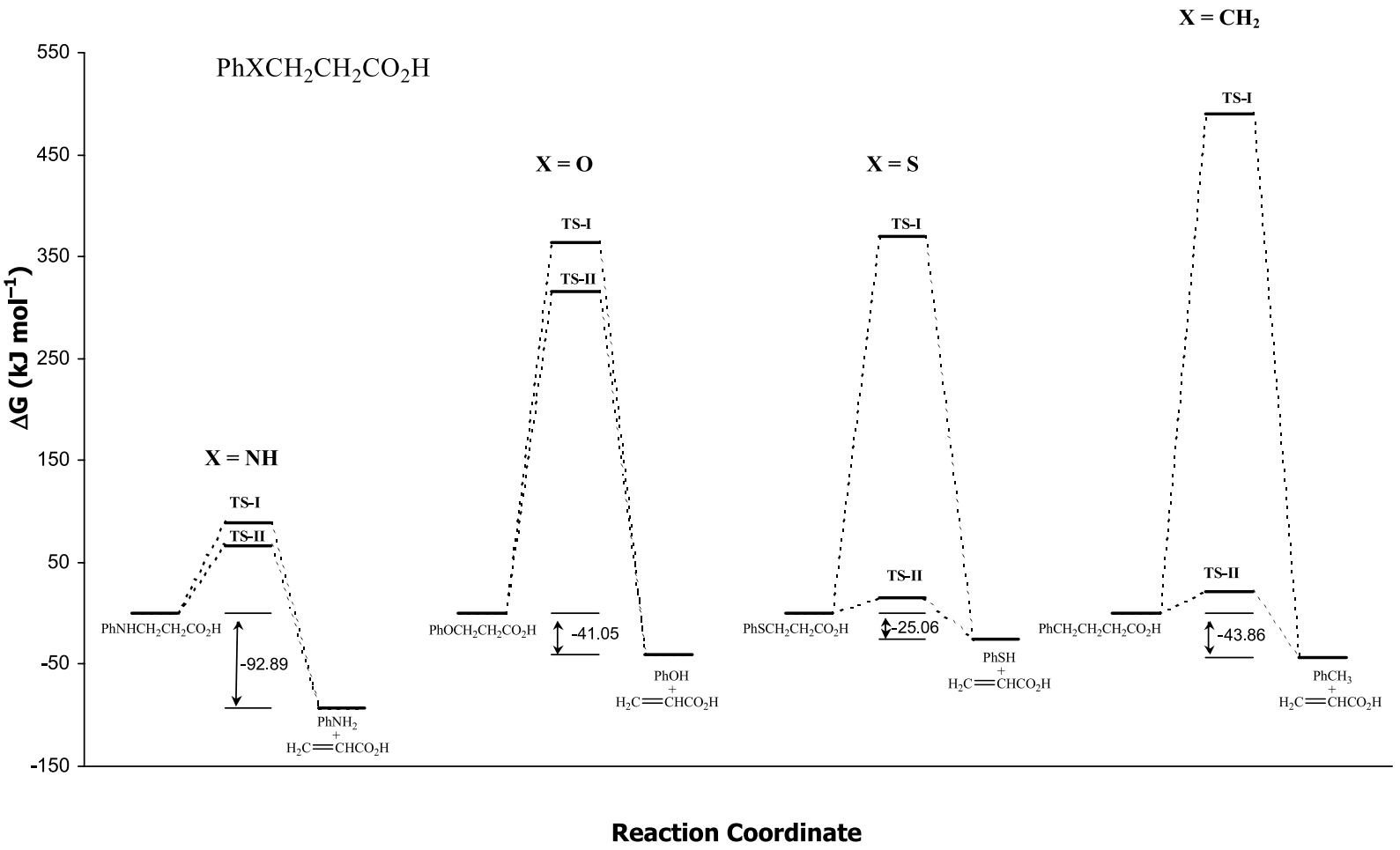
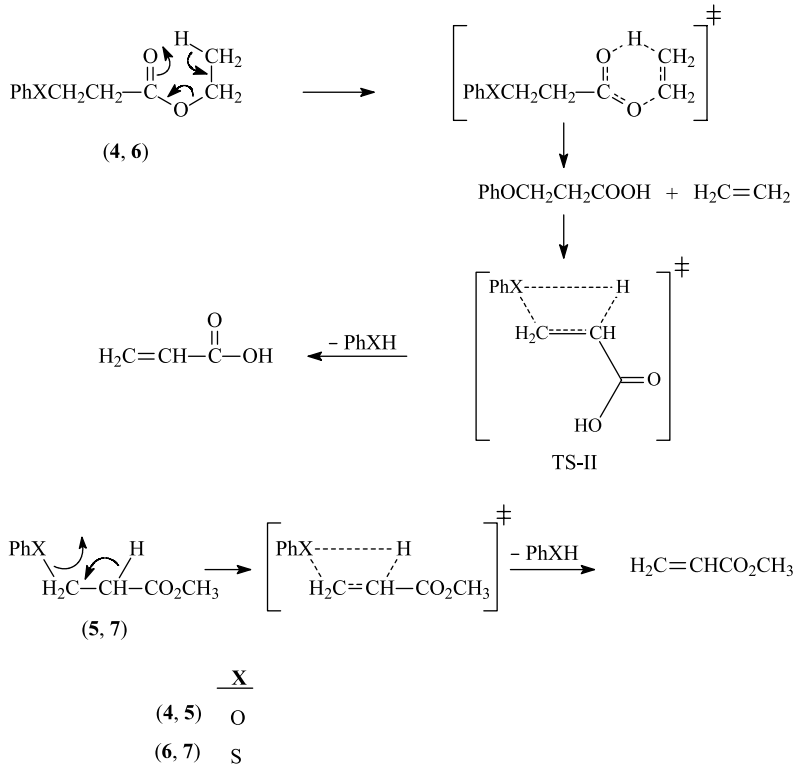
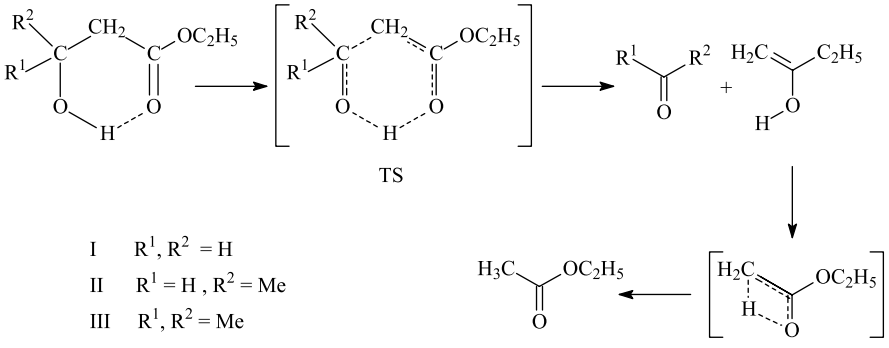


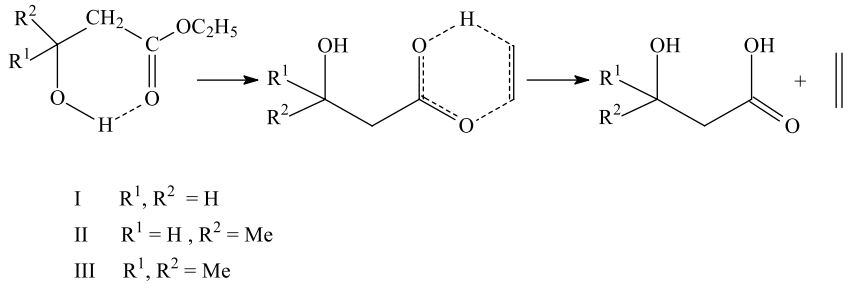
Figure 3. Theoretical ab initio calculations of TS of alternative thermolysis pathways.



Scheme 3. Alternative gas-phase elimination pathway of ethyl and methyl 3-phenoxy- (**4**, **5**) and ethyl and methyl 3-(phenylthio)propanoate (**6**, **7**).



Scheme 4. Mechanism of decomposition of ethyl β-hydroxycarboxylates.[3]



Scheme 5. Side-reaction of decomposition of ethyl β-hydroxycarboxylate.

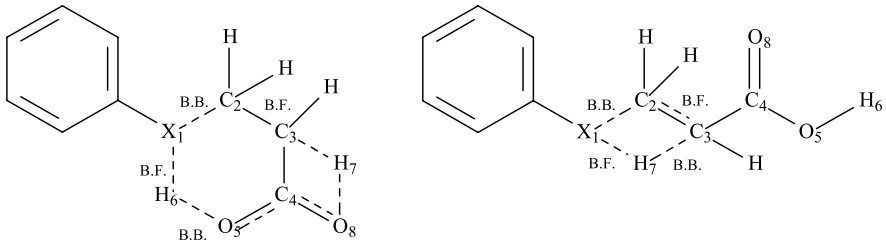


Figure 4. Schematic representation of the optimized structure of TS-I and TS-II.

Table 2. Main distances (Å), in reactants and transition states of the three reactions calculated at the HF/6-31G* level

Species	Distance (Å)			
	X_1–C_2	C_2–C_3	C_3–H_7	H_7–X_1
A: *X*=*O*				
Reactant	1.410	1.512	1.087	2.053
TS-I	1.442	1.500	1.528	3.822
TS-II	1.660	1.453	1.537	1.142
B: *X*=*S*				
Reactant	1.825	1.526	1.088	3.697
TS-I	1.851	1.505	1.519	4.251
TS-II	1.872	1.500	1.522	2.673
C: *X*=*CH*$_2$				
Reactant	1.535	1.526	1.087	2.787
TS-I	1.535	1.507	1.073	2.788
TS-II	1.537	1.505	1.091	2.561

3.2. Synthesis

3.2.1. 3-Phenoxypropanoic acid (1). A mixture of 3-phenoxypropionitrile (14.6 g, 0.1 mol) and HCl (20 mL, 6 M) was heated under reflux for 3 h. The reaction mixture was cooled and the precipitate was collected and crystallized from benzene/petroleum ether (40:60) as colourless crystals: 13.6 g (85%), mp 95–96 °C (lit.[8] mp 95–96 °C). MS: m/z=166 (M^+); IR (KBr): 1695 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$): δ 2.88 (t, J=6.2 Hz, 2H, CH_2CO), 4.28 (t, J=6.2 Hz, 2H, OCH_2), 6.98 (m, 3H, ArH), 7.31 (t, J=7.8 Hz, 2H, ArH); ^{13}C NMR ($CDCl_3$): δ 34.9, 63.5, 115.2, 121.7, 130.1, 158.9, 177.4. Anal. Calcd for $C_9H_{10}O_3$ (166.18): C, 65.06; H, 6.02. Found: C, 65.12; H, 6.03.

Table 3. Total energy, zero-point vibrational energy (ZPE), and thermal correction to enthalpy and entropy, evaluated at HF/6-31G*, for the reactants, transition states and products

Species	Total energy (Hartrees)	ZPE (kcal mol^{-1})	Enthalpy (kcal mol^{-1})	Entropy (cal mol^{-1} K^{-1})
A: *X*=*O*				
Reactant	−571.238336	119.638	125.99	103.074
TS-AI	−571.095924	115.878	122.098	101.65
TS-AII	−571.111045	114.853	121.283	103.345
PhOH	−305.557894	70.511	73.764	73.625
B: *X*=*S*				
Reactant	−893.895435	117.337	124.177	109.38
TS-CI	−893.751866	113.514	120.154	105.849
TS-CII	−893.892301	117.235	123.63	105.29
PhSH	−628.210176	67.040	70.76	78.57
C: *X*=*CH*$_2$				
Reactant	−535.526826	135.393	142.099	106.723
TS-BI	−535.236821	132.783	139.541	106.332
TS-BII	−535.422609	135.448	141.62	102.3
$PhCH_3$	−269.740079	85.944	89.63	79.21
H_2C=CHCOOH	−265.653665	45.866	48.866	70.635

superconducting spectrometer, and FT-IR measurements were from a Perkin Elmer 2000 FT-IR system. Mass spectrometric analysis was carried out on a VG-Autospec-Q high performance tri-sector GC/MS/MS, and the instrument for HPLC was an Agilent 1100 series LC/MSD with an API-ES/APCI ionization mode.

4-Phenylbutanoic acid (**3**) is a commercial sample obtained from Merck.

3.2.2. 3-(Phenylthio)propanoic acid (2). A mixture of ethyl 3-(phenylthio)propanoate (10.5 g, 0.05 mol) and hydrochloric acid (20 mL, 6 M) was heated under reflux for 4 h with vigorous stirring. The reaction mixture was cooled and the precipitate was collected and crystallized from hexane to give white crystals: 7.5 g (82%), mp 59–60 °C (lit.[9] mp 58.5–59.5 °C). MS: m/z=182 (M^+); IR (KBr): 1695 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$): δ 2.70 (t, J=7.3 Hz, 2H, CH_2CO), 3.18 (t, J=7.3 Hz, 2H, SCH_2),

7.2–7.4 (m, 5H, ArH), 12.19 (s, 1H, OH); ^{13}C NMR ($CDCl_3$): δ 29.4, 34.7, 127.4, 129.7, 130.9, 135.4, 178.1. Anal. Calcd for $C_9H_{10}SO_2$ (182.24): C, 59.34; H, 5.49; S, 17.58. Found: C, 59.85; H, 5.58; S, 17.37.

3.2.3. Ethyl 3-phenoxypropanoate (4). 3-Phenoxypropanoic acid (1.66 g, 0.01 mol) was dissolved in 17 mL of absolute ethanol saturated with dry hydrogen chloride. The solution was heated under reflux for 1 h. Normal work up resulted in a colourless oil (lit.[10] bp 170 °C, 40 Torr), yield is 1.74 g (90%). MS: $m/z=194$ (M^+); ^{1}H NMR ($CDCl_3$): δ 1.30 (t, $J=7.1$ Hz, 3H, CH_2CH_3), 2.82 (q, $J=7.1$ Hz, 2H, CH_2CH_2CO), 4.22 (q, $J=7.1$ Hz, 2H, OCH_2CH_3), 4.26 (q, $J=7.1$ Hz, 2H, OCH_2CH_2), 6.95–7.00 (m, 3H, ArH), 7.30–7.32 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$): δ 14.79, 35.25, 61.34, 63.96, 115.24, 121.58, 130.04, 159.09, 171.69. Anal. Calcd for $C_{11}H_{14}O_3$ (194.23): C, 68.04; H, 7.20. Found; C, 68.10; H, 7.09.

3.2.4. Methyl 3-phenoxypropanoate (5). 3-Phenoxypropanoic acid (1.66 g, 0.01 mol) was dissolved in 15 mL dry methanol saturated with dry hydrogen chloride. The mixture was heated under reflux for 1 h. Normal work up resulted in colourless oil (lit.[11] bp 85 °C, 0.4 Torr), yield is 1.5 g (90%). MS: $m/z=180$ ($M^{+}\cdot$); ^{1}H NMR ($CDCl_3$): δ 2.83 (t, $J=6.4$ Hz, 2H, CH_2), 3.75 (s, 3H, CH_3), 4.27 (t, $J=6.4$ Hz, 2H, CH_2), 6.92 (d, $J=8.2$ Hz, 2H, ArH), 6.98 (t, $J=7.8$ Hz, 1H, ArH), 7.30 (t, $J=7.8$ Hz, 2H, ArH). Anal. Calcd for $C_{10}H_{12}O_3$ (180.20): C, 66.65; H, 6.71. Found; C, 66.55; H, 6.63.

3.2.5. Ethyl 3-(phenylthio)propanoate (6). A mixture of thiophenol (11.2 g, 0.1 mol) and THF (25 mL) and triethyl amine (9 g, 0.1 mol) was cooled at 0 °C, and then ethyl acrylate (10 g, 0.1 mol) was added to the mixture. The mixture was stirred and kept on ice for 2 h and then over night at room temperature. The product was extracted with ether (50 mL), washed with aqueous sodium hydroxide (10 mL, 1 M), dried over sodium sulfate and filtered. After removal of the solvent, the product was subjected to vacuum distillation and a residue was obtained as yellow oil (15 g, 71%), (lit.[12] bp 116–117 °C, 3 Torr). MS: $m/z=210$ (M^+); IR (KBr): 1734 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$): δ 1.27 (t, $J=7.1$ Hz, 3H, CH_3), 2.63 (t, $J=7.4$ Hz, 2H, CH_2), 3.18 (t, $J=7.4$ Hz, 2H, CH_2), 4.15 (q, $J=7.2$ Hz, 2H, CH_2), 7.22 (t, $J=6.8$ Hz, 1H, ArH), 7.28 (t, $J=7.2$ Hz, 2H, ArH), 7.38 (d, $J=7.6$ Hz, 2H, ArH); ^{13}C NMR ($CDCl_3$): δ 14.8, 29.6, 35.0, 61.3, 127.1, 129.6, 130.7, 135.8, 172.3. Anal. Calcd for $C_{11}H_{14}O_2S$ (210.29): C, 62.85; H, 6.66; S, 15.23. Found: C, 63.11; H, 6.86; S, 15.38.

3.2.6. Methyl 3-(phenylthio)propanoate (7). Methyl acrylate (20 g) was added to 17 mL thiophenol containing 0.1 g of concentrated HCl. The mixture was kept cold in an ice bath for 16 h. The product was extracted with ether (50 mL), washed with aqueous sodium hydroxide (10 mL, 1 M), dried over anhydrous sodium sulfate and filtered. After removal of the solvent, the product was distilled under vacuum and the residue obtained was yellow oil (16 g, 60%), (lit.[13] bp 113–116 °C, 2 Torr). MS: $m/z=196$ (M^+); ^{1}H NMR ($CDCl_3$): δ 2.61 (t, $J=7.4$ Hz, 2H, CH_2), 3.15 (t, $J=7.4$ Hz, 2H, CH_2), 3.62 (s, 3H, CH_3), 7.18 (t, $J=7.2$ Hz, 1H, ArH), 7.27 (t, $J=7.4$ Hz, 2H, ArH), 7.34 (d, $J=7.4$ Hz, 2H, ArH). Anal. Calcd for $C_{10}H_{12}O_2S$ (196.26): C, 61.20; H, 6.16; S, 16.34. Found: C, 61.12; H, 6.10; S, 16.30.

3.2.7. 3-Phenoxypropionitrile (8). A mixture of phenol (0.25 mol), acrylonitrile (2–4 mol) and triton B (dimethyl benzyl acetylammonium hydroxide) (2–5 mL) was heated under reflux for 20 h. The reaction mixture was diluted with two volumes of solvent (ether or $CHCl_3$) filtered and washed successively with 5% sodium hydroxide, dilute hydrochloric acid followed by water. The solvent was evaporated and the residue was recrystallized from benzene/petroleum ether (40:60) to yield 3-phenoxypropionitrile (70%), mp 59–60 °C (lit.[14] mp 59–60 °C). MS: $m/z=147$ (M^+); ^{1}H NMR ($CDCl_3$): δ 2.85 (t, $J=6.2$ Hz, 2H, CH_2), 4.22 (t, $J=6.2$ Hz, 2H, CH_2), 6.93 (d, $J=7.6$ Hz, 2H, ArH), 7.03 (t, $J=7$ Hz, 1H, ArH), 7.28–7.35 (m, 2H, ArH). Anal. Calcd for C_9H_9NO (147.18): C, 73.45; H, 6.16; N, 9.52. Found: C, 73.00; H, 5.92; N, 9.57.

3.3. Product analysis

Both kinetic (Section 3.4 below) and reaction product analyses were conducted using a Chemical Data System (CDS) custom-made pyrolyzer comprising an insulated aluminium alloy block fitted with a platinum resistance thermocouple connected to a Comark microprocessor thermometer for reactor temperature read-out. The temperature of the reactor is controlled by means of a Eurotherm 093 precision temperature regulator.

Each of the substrates (0.2 g) was introduced in the reaction tube, which is cooled in liquid nitrogen, sealed under vacuum and placed in the pyrolyzer for 900 s at a temperature comparable with that used to achieve complete pyrolysis in the kinetic studies. The contents of the tube were then analysed by NMR and LC/MS, and quantitative estimates were obtained by HPLC. A sample of known mass was subjected to complete pyrolysis. The pyrolysate was dissolved in a known volume of solvent and then injected in the HPLC chromatograph. The percentage yield of each product was estimated by comparing the area under the peak of the product with that of an authentic sample of known concentration. The spectral data of the pyrolysates were compared with reference spectra.

3.4. Kinetic runs and data analysis

A stock solution (7 mL) was prepared by dissolving 6–10 mg of the substrate in acetonitrile as solvent to give a concentration of 1000–2000 ppm. An internal standard was then added, the amount of which was adjusted to give the desired peak area ratio of substrate to standard (2.5:1). The solvent and the internal standard are selected because both are stable under the conditions of pyrolysis, and because they do not react with either substrate or product. The internal standard used in this study is chlorobenzene, 1,3-dichlorobenzene or 1,2,4-trichlorobenzene. Each solution was filtered to ensure that a homogeneous solution is obtained. The weight ratio of the substrate with respect to the internal standard was calculated from the ratio of the substrate peak area to the peak area of the internal standard. The kinetic rate was obtained by tracing the rate of disappearance of the substrate with respect to the internal

standard as follows: An aliquot part (0.2 mL) of each solution containing the substrate and the internal standard is pipetted into the reaction tube, which is then placed in the pyrolyzer for 6 min under non-thermal conditions. A sample is then analyzed using a Waters HPLC probe (pump model 515, UV detector model 2487), or a Metrohm HPLC (pump model 7091C, and SPD 10 AV Shimadzo UV detector) and UV detector at wavelength of 256 nm, and the standardization value (A_0) was then calculated. Several HPLC measurements were obtained with an accuracy of $\geq 2\%$. HPLC columns used for the analysis were Supelco (25 cm length, 4.6 mm ID) ABZ^+, LC-8 and LC-18. The temperature of the pyrolysis (aluminium) block is then raised and held for ca. 900 s to allow approximately 10% pyrolysis to take place at this temperature. This procedure is repeated after each 10–15 °C rise in the temperature of the pyrolyzer until $\geq 90\%$ pyrolysis takes place. The relative ratios of the integration values of the sample and the internal standard (A) at the pyrolysis temperature are then calculated. A minimum of three kinetic runs were carried out at each reaction temperature, following every 10–15 °C rise in the temperature of the pyrolyzer, in order to ensure reproducible values of (A). Treatment of the kinetic data has been detailed elsewhere.[1,15–16]

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References and notes

1. Al-Awadi, N.; Kaul, K.; El-Dusouqui, O. M. E. *J. Phys. Org. Chem.* **2000**, *13*, 499–504.
2. Al-Awadi, S. A.; Abdallah, M. R.; Hasan, M. A.; Al-Awadi, N. A. *Tetrahedron* **2004**, *60*, 3045–3049.
3. Al-Juwaiser, I. A.; Al-Awadi, N. A.; El-Dusouqui, O. M. E. *Can. J. Chem.* **2002**, *80*, 499–503.
4. Rotinov, A.; Chuchani, G.; Machado, R. A.; Rivas, C.; Quijano, J.; Yepes, M. S.; Restrepo, I. *Int. J. Chem. Kinet.* **1992**, *24*, 909–915.
5. Natrio, R.; Quijano, J.; Quijano, J. C.; Gutierrez, L. P.; Suarez, W. A.; Chamorro, E. *J. Phys. Chem. A* **2002**, *106*, 4377–4383.
6. *Titan, Tutorial and User's Guide*; Wavefunction, Inc., 18401 Von Karman Avenue: U.S.A., 1999.
7. Scott, A.; Radom, L. *J. Phys. Chem.* **1996**, *100*, 16502–16513.
8. Lai, S. M. F.; Orchison, J. J. A.; Whiting, D. A. *Tetrahedron* **1989**, *45*, 5895–5906.
9. Ahn, Y.; Cohen, T. *J. Org. Chem.* **1994**, *59*, 3142–3150.
10. Powell, S. G. *J. Am. Chem. Soc.* **1923**, *45*, 2708–2711.
11. Rehberg, C. E.; Dixon, M. B. *J. Am. Chem. Soc.* **1950**, *72*, 2205–2206.
12. Davis, F. A.; Friedman, A. J.; Nadir, U. K. *J. Am. Chem. Soc.* **1978**, *100*, 2844–2852.
13. Hurd, C. D.; Gershbein, L. *J. Am. Chem. Soc.* **1947**, *69*, 2328–2335.
14. Bachmann, G. B.; Levine, H. A. *J. Am. Chem. Soc.* **1948**, *70*, 599–601.
15. Al-Awadi, N. A.; Elnagdi, M. H.; Kaul, K.; Ilingovan, S.; El-Dusouqui, O. M. E. *J. Phys. Org. Chem.* **1999**, *12*, 654–656.
16. Al-Awadi, N. A.; El-Dusouqui, O. M. E.; Kaul, K.; Dib, H. H. *Int. J. Chem. Kinet.* **2000**, *32*, 403–407.

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Single step synthesis of 2,3-dialkyl-6-nitro-quinazolin-4(3*H*)-imines and 3,5-dialkyl-9-nitro-imidazo[1,2-*c*]quinazolin-2(3*H*)-ones

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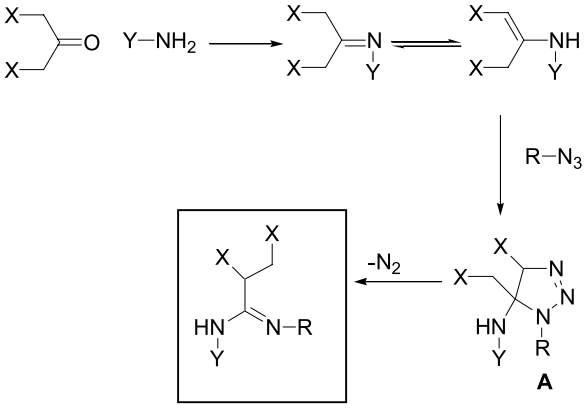
Abstract—A single step synthesis of 2,3-dialkyl-6-nitro-quinazolin-4(3*H*)-imines and 3,5-dialkyl-9-nitro-imidazo-[1,2-*c*]-quinazolin-2(3*H*)-ones from simple carbonyl compounds, primary amines or amino acid methyl esters and 2-azido-5-nitro-benzonitrile was developed. Key intermediates were *N*,*N*′-disubstituted amidines obtained by rearrangement of 4,5-dihydrotriazoles; the new heterocyclic rings were formed by spontaneous intramolecular reaction of the amino and cyano groups which are present in the intermediates.

1. Introduction

Recently, we described a convenient synthesis of *N*,*N*′-disubstituted amidines from tosyl azide, primary amines and ketones.[1] As depicted in Scheme 1, the amidines were easily obtained through a transformation reaction of 4-amino-4,5-dihydrotriazole intermediates **A**. It is well known that compounds **A** readily rearrange into amidines[2] when the **R** group on N-1 is an highly electron-withdrawing substituent (e.g. tosyl and aryl groups having formyl, nitro or cyano substituents). This simple approach to the synthesis of substituted amidines prompted us to investigate the preparation of *N*,*N*′-disubstituted amidines bearing the *ortho*-cyanoaryl group on N-1 and to study the formation of heterocyclic products by intramolecular cyclization processes.



Scheme 1.

Indeed, the formation of a fused azaheterocyclic ring could be expected by virtue of the nucleophilic character of the NH group and as a consequence of the known ability of *N*-aryl amidine intermediates to give intramolecular condensation reactions with suitable ortho groups (Scheme 2).

2. Results and discussion

As a further development of the general synthesis of *N*,*N*′-disubstituted amidines and in analogy with previous results, propanal **1a**, benzylamine **2a** and 2-azido-5-nitro-benzonitrile **3** were reacted in an inert solvent and in the presence of molecular sieves (see Scheme 3). Surprisingly, a single product was obtained, i.e. 3-benzyl-2-ethyl-6-nitro-quinazolin-4(3*H*)-imine **4a**, derived from the direct reaction between the secondary benzylamino group and the cyano group. The structure of **4a** was established by analytical and spectroscopic data. The ^{1}H NMR spectrum of **4a** were in agreement with the proposed structure and IR and ^{13}C NMR spectra ruled out the presence of the cyano group.

Keywords: 2,3-Dialkyl-6-nitro-4(3*H*)-quinazolinimines; 3,5-Dialkyl-9-nitro-imidazo[1,2-*c*]quinazolin-2(3)-ones; *N*,*N*′-disubstituted amidines; Intramolecular cyclization.
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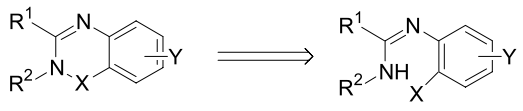
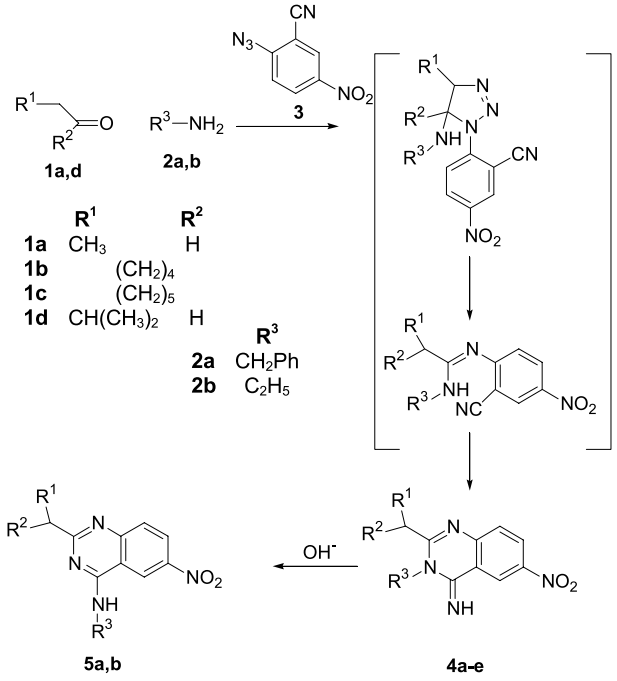


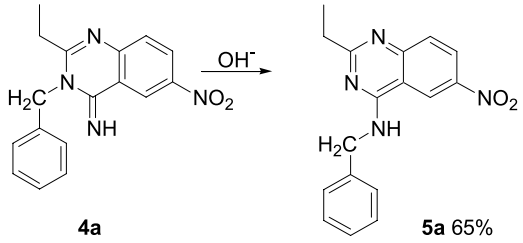
Scheme 2.



	R^1	R^2	R^3	%4	%5
4,5a	CH_3	H	CH_2Ph	78	65
4,5b	$(CH_2)_4$		C_2H_5	88	52
4c	$(CH_2)_4$		CH_2Ph	70	
4d	$(CH_2)_5$		CH_2Ph	68	
4e	$CH(CH_3)_2$	H	CH_2Ph	89	

Scheme 3. Reaction conditions; CH_2Cl_2, room temperature, molecular sieves.

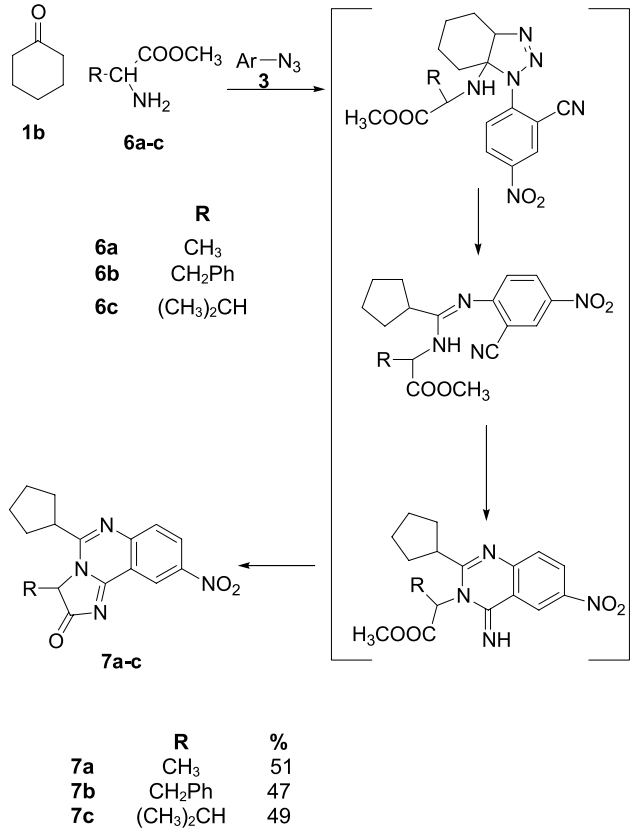
The ^{1}H NMR spectrum of **4a** hydrochloride in DMSO solution showed that the resonance of the NH_2^+ group was split into two signals with considerable difference of frequency (0.9 ppm). The rigid NH_2^+ structure fits this evidence, because the environment of the two protons is very different. In order to confirm this structural hypothesis the reactivity of **4a** was examined. The base catalyzed transposition of the alkyl group of 3-alkyl-quinazoline-4(3*H*)-imines which affords 4-alkylaminoquinazolines is well known as the Dimroth rearrangement.[3] As expected, in basic conditions (1 M sodium hydroxide), iminoquinazoline **4a** yielded 4-alkylaminoquinazoline **5a** (Scheme 4).



Scheme 4.

The ^{1}H NMR spectrum of **5a** hydrochloride showed a single signal associated with the NH_2^+ group and the ^{13}C NMR spectrum was in agreement with the proposed structure. In order to further validate structure **4** we reacted quinazoline-imine **4b** with a base. This second experiment gave rise to the expected Dimroth rearrangement product **5b**. The one-pot method previously described was applied to the synthesis of quinazoline-4(3*H*)imines **4a–e** from carbonyl derivatives **1a–d**, primary amines **2a**,**b** and 2-azido-5-nitrobenzonitrile **3** (Scheme 3).

The use of amino acid esters as primary amines in the above reaction conditions was further investigated. This procedure should allow the amino acid function to be linked onto the quinazoline ring. Reaction of cyclohexanone **1b** and aminoacid methyl esters **6a–c** (D,L-alanine, L-phenylalanine, L-valine methyl esters) with 2-azido-5-nitro-benzonitrile **3** afforded imidazo[1,2-*c*]quinazolin-2(3*H*)-one derivatives **7a–c** in good yields and as single products. Analytical and spectroscopic data ruled out the presence of the methyl ester group and of exchangeable protons whereas signals related to an aromatic group, to the cyclopentyl substituents and those corresponding to the aminoacid backbone were clearly identified. All ^{13}C NMR spectra of **7a–c** were characterized by two signals (quaternary carbons) at low frequencies (185.5–187.2 and 170.3–170.7 δ) associated with a cyclic amide group and with a cyclic N–C=N carbon conjugated with C=O,[4] respectively. These analytical data



	R	%
7a	CH_3	51
7b	CH_2Ph	47
7c	$(CH_3)_2CH$	49

Scheme 5. Reaction conditions: CH_2Cl_2, room temperature, molecular sieves.

allowed assignment of the structure of 3,5-dialkyl-9-nitro-imidazo[1,2-*c*]quinazolin-2(3*H*)-one to compounds **7a–c**. Mass spectra and elementary analysis were in agreement with the proposed structures.

The formation of tricyclic derivatives **7** can be rationalized through the cyclization of 4-iminoquinazoline intermediates which could not be isolated in the present case. The adopted reaction conditions were responsible for the formation of the cyclic imide **7** by condensation of the imino group with the ester function (Scheme 5).

It is necessary to remark that compounds **7** arising from optically pure aminoacids lose optical activity. This is explained by enolization of the intermediate product.

In conclusion, readily available starting materials have been used in one-pot reactions to obtain two different heterocyclic rings in good yield by suitably exploiting the reactivity of *N*,*N*′-disubstituted amidines. This synthetic method is versatile and allows access to several heterocyclic rings with various substituents, depending on the structure of the starting materials.

3. Experimental

Mps were determined by a Büchi 510 (capillary) apparatus. IR spectra were measured with a JASCO IR Report 100 instrument (Nujol; cm^{-1}). NMR spectra were obtained with Bruker Advance 300 and Varian Gemini 200 instruments. *J* values are given in Hz for solutions in $CDCl_3$ if not indicated. Mass spectra were recorded with LCQ Advantage Thermofinnigan equipped with electrospray ionisation (ESI). 2-Azido-5-nitro-benzonitrile is a known compound.[5]

3.1. Synthesis of 3,5-dialkyl-6-nitro-quinazolin-4(3*H*)-imines 4a–c: general procedure

Carbonyl compound **1a**,**d** (10 mmol) and amine **2a**,**b**[6] (10 mmol) were dissolved in CH_2Cl_2 (20 mL). To the solution molecular sieves (4 Å, 5 g) were added. After 30 min, 2-azido-5-nitro-benzonitrile **3** (10 mmol) was added. The solution was stirred at room temperature for 12 h until disappearance of the starting materials (TLC: ethyl acetate–cyclohexane 2:3). The reaction suspension was filtered and evaporated. The crude was chromatographed with ethyl acetate–cyclohexane (2:3).

3.1.1. 3-Benzyl-2-ethyl-6-nitro-quinazolin-4(3*H*)-imine 4a. Yield 2.6 g, 78%. Mp 142–144 °C (yellow crystals from EtOH). IR 3342 (NH), 1629 (C=N), ^{1}H NMR 1.33 (3H, t, J=7.4 Hz, CH_3), 2.75 (2H, q, J=7.4 Hz, CH_2), 5.49 (2H, s, CH_2Ph), 7.22–7.41 (5H, m, Ph), 7.64 (1H, d, J=8.8 Hz, H-8), 8.40 (1H, dd J=8.8, 2.2 Hz, H-7), 8.74 (1H, d, J=2.2 Hz, H-5), 7.40–8.90 (1H, bs, NH), ^{1}H NMR of the hydrochloride (DMSO) 1.35 (3H, t, J=6.9 Hz, CH_3), 4.43 (2H, q, J=6.9 Hz, CH_2), 5.76 (2H, s, CH_2Ph), 7.25–7.44 (5H, m, Ph), 8.02 (1H, d, J=8.7 Hz, H-8), 8.70 (1H, dd, J=1.8, 8.7 Hz, H-7), 9.82 (1H, d, J=1.83 Hz, H-5), 10.30 and 11.17 (2H, 2bs, NH_2), ^{13}C NMR 11.3 (CH_3), 28.4 (CH_2), 47.2 (CH_2), 116.7 (C), 122.8 (CH), 126.6 (CH), 127.4 (CH), 127.6 (CH), 129.3 (CH), 130.0 (CH), 136.1 (C), 145.1 (C), 149.8 (C), 156.8 (C), 162.6 (C). ESI Mz+309.2. Calcd for $C_{17}H_{16}N_4O_2$ (308.33) C, 66.23; H, 5.19; N, 18.18. Found: C, 65.93; H, 5.24; N, 18.03.

3.1.2. 2-Cyclopentyl-3-ethyl-6-nitro-quinazolin-4(3*H*)-imine 4b. Yield 2.5 g, 88%. Mp 164 °C (yellow crystals from EtOH). IR 3345 (NH), 1632 (CN), ^{1}H NMR 1.41 (3H, t, J=6.9 Hz, CH_3), 1.68–2.10 (8H, m, 4CH_2), 3.12–3.24 (1H, m, CH), 4.28 (2H, q, J=6.9 Hz, CH_2), 5.25 (1H, bs, NH), 7.55 (1H, d, J=9.1 Hz, H-8), 8.64 (1H, dd, J=2.5, 9.1 Hz, H-7), 8.70 (1H, d, J=2.5 Hz, H-5), ^{1}H NMR of the hydrochloride (DMSO) 1.36 (3H, t, J=6.9 Hz, CH_3), 1.70–2.12 (8H, m, 4CH_2), 3.14–3.26 (1H, m, CH), 4.44 (2H, q, J=6.9 Hz, CH_2), 7.97 (1H, d, J=9.1 Hz, H-8), 8.70 (1H, dd, J=2.2, 9.1 Hz, H-7), 10.21 and 10.98 (2H, 2bs, NH_2). ^{13}C NMR 13.4 (CH_3), 26.2 (CH_2), 32.6 (CH_2), 40.2 (CH_2), 43.3 (CH), 119.5 (C), 120.8 (CH), 126.8 (CH), 128.9 (CH), 144.9 (C), 149.9 (C), 156.4 (C), 164.4 (C). ESI Mz+287.3. Calcd for $C_{15}H_{18}N_4O_2$ (286.14) C, 62.92; H, 6.34; N, 19.57. Found: C, 62.74; H, 6.50; N, 19.32.

3.1.3. 3-Benzyl-2-cyclopentyl-6-nitro-quinazolin-4(3*H*)-imine 4c. Yield 2.4 g, 70%. Mp 150 °C (yellow crystals from EtOH). IR 3346 (NH), 1629 (C=N), ^{1}H NMR 1.45 (8H, m, 4CH_2), 3.01–3.33 (1H, m, CH), 5.54 (2H, s, CH_2Ph), 7.19–7.40 (5H, m, Ph), 7.61 (1H, d, J=9.1 Hz, H-8), 8.00 (1H, bs, NH), 8.38 (1H, dd, J=2.2, 9.1 Hz, H-7), 8.74 (1H, d, J=2.2 Hz, H-5), ^{1}H NMR of the hydrochloride (DMSO) 1.45–1.93 (8H, m, 4CH_2), 3.25–3.42 (1H, m, CH–C=N), 5.75 (2H, s, CH_2Ph), 7.25–7.44 (5H, m, Ph), 8.02 (1H, d, J=8.7 Hz, H-8), 8.73 (1H, dd, J=1.8, 8.7 Hz, H-7), 9.80 (1H, d, J=1.8 Hz, H-5), 10.3 and 11.17 (2H, 2bs, NH_2), ^{13}C NMR 26.4 (CH_2), 32.7 (CH_2), 43.8 (CH), 48.3 (CH_2), 119.7 (C), 121.2 (CH), 126.1 (CH), 127.2 (CH), 127.9 (CH), 129.2 (CH), 129.4 (CH), 136.5 (C), 145.3 (C), 150.0 (C), 157.0 (C), 165.3 (C). ESI Mz+349.3. Calcd for $C_{20}H_{20}N_4O_2$ (348.16) C, 68.95; H, 5.79; N, 16.08. Found: C, 69.00; H, 5.75; N, 16.09.

3.1.4. 3-Benzyl-2-cyclohexyl-6-nitro-quinazolin-4(3*H*)-imine 4d. Yield 2.5 g, 68%. Mp 146–147 °C (yellow crystals from 2-propanol). IR 3350 (NH), 1630 (CN), ^{1}H NMR 1.52–2.59 (10H, m, 5CH2), 2.62–2.77 (1H, m, CH), 5.48 (1H, s, CH2), 7.17–7.42 (6H, m, Ph and NH), 7.58 (1H, d, J=8.8 Hz, H-8), 8.46 (1H, dd, J=8.8, 1.4 Hz, H-7), 8.70 (1H, d, J=1.4 Hz, H-5), ^{13}C NMR 25.8 (CH_2), 26.2 (CH_2), 31.5 (CH_2), 43.0 (CH), 47.8 (CH_2), 119.6 (C), 121.1 (CH), 126.2 (CH), 127.1 (CH), 127.8 (CH), 129.0 (CH), 136.5 (C), 146.1 (C), 149.9 (C), 156.9 (C), 165.0 (C). ESI M+363.1. Calcd for $C_{21}H_{22}N_4O_2$ C, 69.59; H, 6.12; N, 15.46 (362.43). Found: C, 69.37; H, 6.24; N, 15.27.

3.1.5. 3-Benzyl-2-isobutyl-6-nitro-quinazolin-4(3*H*)-imine 4e. Yield 2.9 g, 89%. Mp 156–155 °C (yellow crystals from 2-propanol). IR 3349 (NH), 1632 (CN), ^{1}H NMR 1.00 (6H, d, J=7.3 Hz, 2CH_3), 2.25–2.63 (1H, m, CH), 2.62 (2H, d, J=7.0 Hz), 5.57 (2H, s, CH_2), 7.16–7.38 (6H, m, Ph and NH), 7.65 (1H, d, J=8.8 Hz, H-8), 8.42 (1H, dd, J=8.8, 2.2 Hz, H-7), 9.0 (1H, d, J=2.2 Hz, H-5), ^{13}C NMR 22.7 (CH_3), 27.2 (CH), 44.3 (CH_2), 48.2 (CH_2), 119.7 (C), 121.1 (CH), 126.1 (CH), 127.1 (CH), 127.8 (CH), 129.1 (CH), 135.9 (C), 145.2 (C), 149.5 (C), 156.5 (C), 160.7 (C).

ESI M+337.0. Calcd for $C_{19}H_{20}N_4O_2$ (336.39) C, 67.84; H, 5.99; N, 16.66. Found: C, 67.68; H, 6.23; N, 16.58.

3.2. Dimroth rearrangement: general procedure

3,5-Dialkyl-6-nitro-quinazolin-4(3*H*)-imines **4a**,**b** (10 mmol) was suspended in 30 mL of 1 M NaOH solution and heated to 80 °C for 24 h. The reaction was monitored by TLC (ethyl acetate–cyclohexane 1:1). The reaction mixture was extract with dichloromethane, dried with Na_2SO_4 and evaporated. The crude reaction was chromatographed with ethyl acetate–cyclohexane (1:1).

3.2.1. 4-Benzylamino-2-ethyl-6-nitro-quinazoline 5a. Yield 2.0 g, 65%. Mp 186 °C (yellow crystals from EtOH). IR 2220 (NH). ^{1}H NMR 1.25 (3H, t, J=8.1 Hz, CH_3), 2.75 (2H, q, J=8.1 Hz, CH_2), 4.80 (2H, d, J=5.4 Hz, CH_2Ph), 7.25–7.44 (5H, m, Ph), 7.75 (1H, d, J=9.1 Hz, H-8), 8.42 (1H, dd, J=9.1, 1.8 Hz, H-7), 9.36 (1H, d, J=1.8 Hz, H-5), 9.42 (1H, t, J=5.4 Hz, NH), ^{13}C NMR (DMSO) 12.0 (CH_3), 32.4 (CH_2), 43.7 (CH_2), 112.2 (C), 120.4 (CH), 126.0 (CH), 126.8 (CH), 127.7 (CH), 128.2 (CH), 128.4 (CH), 138.8 (C), 143.2 (C), 153.4 (C), 160.0 (C), 171.0 s. ESI Mz+309.2. Calcd for $C_{17}H_{16}N_4O_2$ (308.33) C, 66.23; H, 5.19; N, 18.18. Found: C, 65.89; H, 5.27; N, 18.00.

3.2.2. 2-Cyclopentyl-4-ethylamino-6-nitro-quinazoline 5b. Yield 1.4 g, 52%. 188–189 (yellow crystals from EtOH). IR 2220 (NH), ^{1}H NMR 1.40 (3H, t, J=7.4 Hz, CH_3), 1.67–2.19 (8H, m, 4CH_2), 3.27–3.34 (1H, m, CH), 3.71–3.84 (2H, q, J=7.4 Hz, CH_2), 6.08 (1H, bs, NH), 7.84 (1H, d, J=9.2 Hz, H-8), 8.45 (1H, dd, J=9.2, 2.6 Hz, H-7), 8.72 (1H, d, J=2.6 Hz, H-5), ^{13}C NMR (DMSO) 14.6 (CH_3), 26.4 (CH_2), 32.8 (CH_2), 36.3 (CH_2), 49.1 (CH), 113.1 (C), 121.1 (CH), 126.5 (CH), 129.2 (CH), 143.8 (C), 154.2 (C), 160.8 (C), 174.4 (C). ESI Mz+287.3. Calcd for $C_{15}H_{18}N_4O_2$ (286.14) C, 62.92; H, 6.34; N, 19.57. Found C, 62.62; H, 6.59; N, 19.24.

3.3. Synthesis of 3,5-dialkyl-9-nitro-imidazo[1,2-*c*] quinazolin-2(3*H*)-ones 7a–c: general procedure

Amino-acid methyl ester hydrochloride **6a–c** (10 mmol) and TEA (10 mmol) were dissolved in CH_2Cl_2 (20 mL). Then, cyclohexanone (10 mmol) and 7 g of 4 Å molecular sieves were added. After 30 min 2-azido-5-nitro-benzonitrile **3** (10 mmol) was added. The solution was stirred for 24 h until disappearance of the starting material (TLC ethyl acetate–cyclohexane 1:1). After filtration and evaporation the crude reaction product was chromatographed with ethyl acetate–cyclohexane (2:3).

3.3.1. 5-Cyclopentyl-3-methyl-9-nitro-imidazo[1,2-*c*]-quinazolin-2(3*H*)-one 7a. Yield 1.6 g, 51%. Mp 218 °C (yellow crystals from EtOH). IR 1741 (C=O), 1622 (C=N), ^{1}H NMR 1.58–2.37 (11H, m, 4CH_2 and CH_3), 3.08–3.28 (1H, m, CH), 4.60 (1H, q, J=7.3 Hz, CH–N), 7.85 (1H, d, J=9.1 Hz, H-7), 8.63 (1H, dd, J=9.1, 2.5 Hz, H-8), 9.26 (1H, d, J=2.5 Hz, H-10), ^{13}C NMR 18.5 (CH_3), 26.2 (CH_2), 26.4 (CH_2), 33.2 (CH_2), 43.2 (CH), 58.9 (CH), 115.4 (C), 123.9 (CH), 129.2 (CH), 129.8 (CH), 145.7 (C), 151.18 (C), 161.4 (C), 170.3 (C), 187.2 (C). ESI Mz+313.3. Calcd for $C_{16}H_{16}N_4O_3$ (312.32) C, 61.53; H, 5.16; N, 17.94. Found: C, 61.32; H, 5.29; N, 17.49.

3.3.2. 3-Benzyl-5-cyclopentyl-9-nitro-imidazo[1,2-*c*]-quinazolin-2(3*H*)-one 7b. Yield 1.8 g, 47%. Mp 202–203 °C (yellow crystals from EtOH). IR 1622 (C=N), 1741 (C=O), ^{1}H NMR 1.78–2.30 (8H, m, 4CH_2), 3.18–3.37 (1H, m, CH–C=N), 3.58 (2H, d, J=4.4 Hz, CH_2Ph), 4.86 (1H, t, J=4.4 Hz, CH–N), 6.97–7.15 (5H, m, Ph), 7.78 (1H, d, J=9.1 Hz, H-7), 8.53 (1H, dd, J=9.1, 2.5 Hz, H-8), 8.99 (1H, d, J=2.5 Hz, H-10), ^{13}C NMR 26.3 (CH_2), 26.5 (CH_2), 31.9 (CH_2), 34.1 (CH_2), 37.7 (CH_2), 43.5 (CH), 63.6 (CH), 114.9 (C), 123.7 (CH), 128.0 (CH), 128.8 (CH), 129.1 (CH), 129.7 (CH), 132.4 (C), 145.5 (C), 150.9 (C), 161.4 (C), 170.8 (C), 186. 3 (C). ESI Mz+389.4. Calcd for $C_{22}H_{20}N_4O_3$ (388.42) C, 68.03; H, 5.19; N, 14.43. Found: C, 67.79; H, 5.25; N, 14.23.

3.3.3. 5-Cyclopentyl-3-isopropyl-9-nitro-imidazo[1,2-*c*] quinazolin-2(3*H*)-one 7c. Yield 1.6 g, 49%. Mp 176–177 °C (yellow crystals from EtOH). IR 1620 (C=N), 1739 (C=O), ^{1}H NMR 0.79 (3H, d, J=6.9 Hz, CH_3), 1.43 (3H, d, J=6.9 Hz, CH_3), 1.70–2.30 (8H, m, 4CH_2), 2.56–2.65 (1H, m, CH), 3.04–3.24 (1H, m, CH–C=N), 4.52 (1H, d, J=3.6 Hz, CH–N), 7.83 (1H, d, J=9.1 Hz, H-7), 8.60 (1H, dd, J=9.1, 2.5 Hz, H-8), 9.23 (1H, d, J=2.5 Hz, H-10), ^{13}C NMR 15.5 (CH_3), 17.6 (CH_3), 26.6 (CH_2), 26.8 (CH_2), 33.6 (CH_2), 34.3 (CH_2), 31.6 (CH), 43.6 (CH), 67.4 (CH), 115.3 (C), 124.3 (CH), 129.4 (CH), 130.0 (CH), 146.0 (C), 151.4 (C), 161.8 (C), 170.8 (C), 185.5 (C). ESI Mz+341.4. Calcd for $C_{18}H_{20}N_4O_3$ (340.38) C, 63.52; H, 5.92; N, 16.64. Found: C, 63.37; H, 6.04; N, 16.52.

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References and notes

1. Cassani, F.; Celentano, G.; Erba, E.; Pocar, D. *Synthesis* **2004**, 1041–1046.
2. Kadaba, P. K.; Stanovnik, B.; Tišler, M. *Adv. Heteroatom Chem.* **1984**, *37*, 217–349.
3. Brown, D. J.; Ienega, K. *J. Chem. Soc., Perkin Trans. 1* **1975**, 2182–2185.
4. Papadopulos, E. P. *J. Heterocycl. Chem.* **1982**, *18*, 515–518.
5. Nishiyama, K.; Anselme, J. P. *J. Org. Chem.* **1977**, *42*, 2636–2637.
6. In the case of ethylamine hydrochloride, an equivalent amount of TEA was added.

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Synthesis of new nucleoside analogues comprising a methylenecyclobutane unit

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Abstract—Synthesis of eight nucleoside analogues **3–10** with a methylene cyclobutane unit is described. Wittig or Peterson reactions with protected 2-hydroxycyclobutanones **12** and **13** gave *E*- and *Z*-derivatives, respectively. After functional modifications the heterocyclic moieties were introduced via a Mitsunobu reaction either on the lateral chain or on the cycle. When adenine was used in this reaction only the *N*-9 substitution products were obtained. Removal of the protecting groups provided the target products.

1. Introduction

Nucleoside analogues are at the center of current interest because they display a wide range of biological activities especially as antiviral and antitumor agents.[1] Several analogues have been prepared and evaluated in order to obtain compounds with better properties particularly towards enzymatic cleavage. Products with good activity were found among a large number of structures,[2–6] and it is difficult to predict which compounds will be sufficiently active and selective against viral enzymes. Structures of these analogues could be close of nucleosides as the HIV drug 3′-azido-2′,3′-dideoxythymidine (AZT) or very different as the acyclic analogues gancyclovir. Among these products, several compounds with a methylenecyclobutane unit **1** and **2** (Fig. 1) were described by Zemlicka.[5] A moderated effect of **1** against EBV was established but was not separated from cytotoxicity. The methylenecyclobutane system is a rigid linker between the hydroxymethyl group and base residue so that a modification of this system could lead to more active compounds. And thus we examined different geometries in this series, keeping the same distance between the two active parts but with different positions of double bond and cyclobutane.

Figure 1.

In the course of our research program towards nucleoside analogues, we have already synthesized some carbocyclic compounds with double bonds in endocyclic[7a,b,d,e] or exocyclic[7c] position and acyclic dienic[8a] and methylenic[8b] compounds. We then planned to synthesize products related to **1** and **2** but not bearing the base in the vinylic position. We thus selected compounds **3–10** (Fig. 2) as targets and we describe here a short route to these compounds from 2-hydroxycyclobutanone **11**.

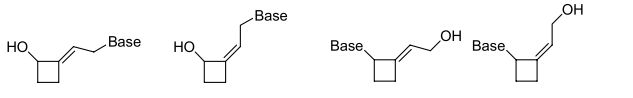

Figure 2.

Keywords: Nucleoside analogues; Methylenecyclobutane; Mistsunobu conditions.
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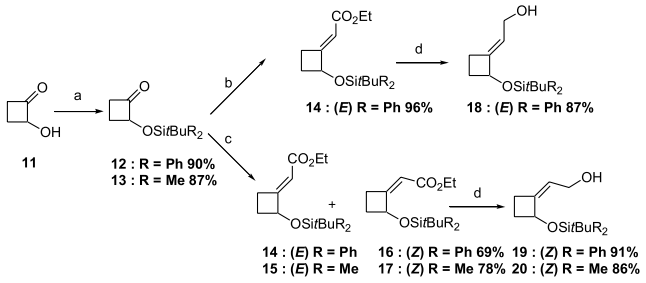
2. Results and discussion

The starting material for the synthesis of *Z*- and *E*-methylenecyclobutane analogues was 2-hydroxycyclobutanone **11**[9] (Scheme 1). This ketol was first protected as silyl ether. The resulting compounds **12** and **13**[10] were treated by two different methods. The first one was the Wittig reaction with ethoxycarbonylmethylene phosphorane, which led to *E*-isomers **14** as sole product. The other one was the Peterson reaction with ethyl trimethylsilylacetate in basic medium, which led to a mixture of *E*- and *Z*-isomers **14** and **16** (ratio 1/12) or **15** and **17** (ratio 1/18).



Scheme 1. Reagents and conditions: (a) tBuR$_2$SiCl, imidazole, DMF, rt, 20 h; (b) $Ph_3P{=}CHCOOEt$, C_6H_6, rt, 72 h; (c) LDA, $(Me)_3SiCH_2COOEt$, THF −78 °C, 1 h; (d) DIBALH, CH_2Cl_2, toluene −60 °C, 2 h.

These compounds were separated by column chromatography. The NOE experiments were used to distinguish the two isomers. Thus, *Z* isomer **14** showed NOE enhancement of the olefinic signal after irradiation of the H_2, similar effect was absent in *E* isomer **16**. Reduction of esters with DIBALH gave, respectively, alcohol **18** from ester **14** and alcohol **20** from ester **17**. With these key compounds in our hands, two different methods were used to obtain the target products either **3**–**6** or **7**–**10** (Fig. 3).

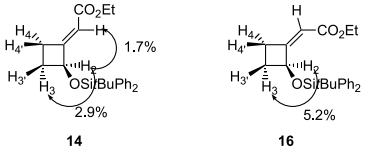
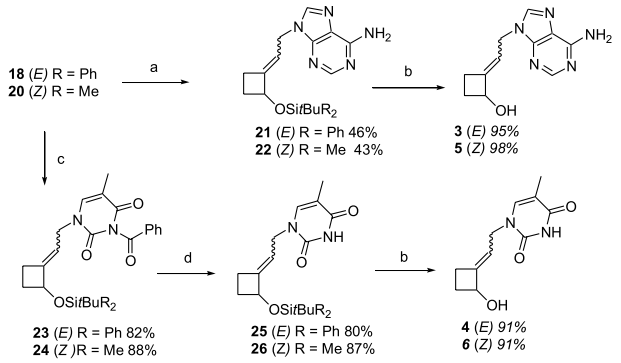


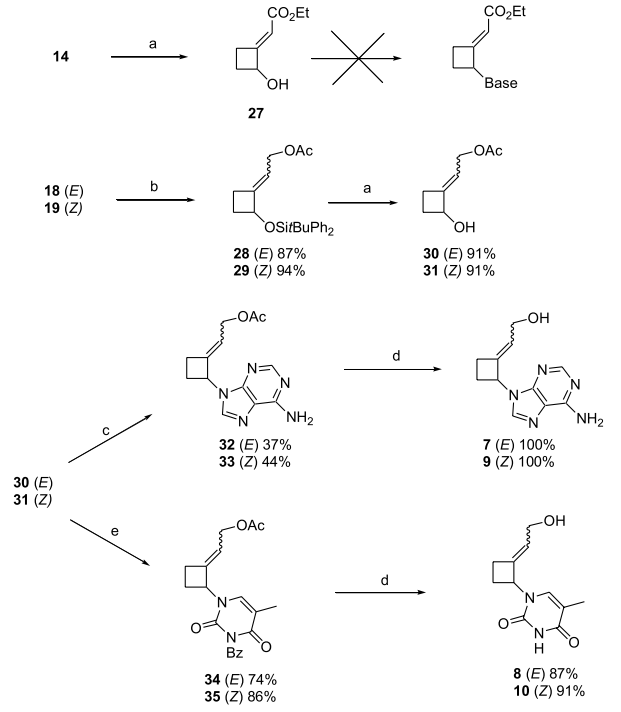
Figure 3. NOE enhancements of **14** and **16**.

To access the series **3**–**6** (Scheme 2) with the nucleic base in allylic position, direct substitutions with protected thymine and free adenine were performed, in Mitsunobu conditions, separately from alcohols **18** (*E*) or **20** (*Z*). The reactions were carried out with triphenylphosphine and DIAD in THF and led to good results. It is worth mentioning that reaction with adenine only led to the *N*-9 substitution products. We could not detect any other isomer by NMR analysis of the crude products. These assignments were proved by $^1H/^{13}C$ HMBC NMR spectra. In the adenine series the target products **3** and **5** were obtained after desilylation with TBAF. For the thymine analogs **4** and **6**, an additional mild treatment with NH_3/MeOH was necessary to remove the benzoyl group. In every case the final product was obtained as single isomer.

For obtaining compounds **7**–**10** (Scheme 3) with base directly linked to the cycle, we first tried to introduce the



Scheme 2. Reagents and conditions: (a) Adenine, DIAD, Ph_3P, THF, rt, 7 days; (b) $(n\text{Bu})_4NF$, THF, rt, 2.5 h; (c) *N*3-benzoyl thymine, DIAD, Ph_3P, THF, rt, 7 days; (d) sat NH_3/MeOH, rt, 48 h.



Scheme 3. Reagents and conditions: (a) $(n\text{Bu})_4NF$, THF, rt, 2.5 h; (b) Ac_2O, pyridine, rt, 14 h; (c) Adenine, DIAD, Ph_3P, THF, rt, 7 days; (d) sat NH_3/MeOH, rt, 15 h; (e) *N*3-benzoyl thymine, DIAD, Ph_3P, THF, rt, 7 days.

nucleic base by substitution in Mitsunobu conditions with alcohol **27** resulting from removal of the silyl group on **14**. Unfortunately, in these conditions, benzoyl thymine led to several products including the expected product in low yield and another one resulting from Michael addition to the conjugated double bond. As to adenine, it did not react. We thought that these difficulties could be avoided by using compounds **30** and **31**. Both of these products were prepared from alcohols **18** and **19** by acetylation providing compounds **28** and **29**, followed by desilylation. These very volatile compounds can only be obtained in good yield if suitable precautions were taken for solvent evaporation stage. The subsequent Mitsunobu reactions provided compounds **32** to **35** and cleavage of acetyl group only (from **32** and **33**) or of acetyl and benzoyl groups (from **34**

and **35**) with NH_3/MeOH gave the target molecules in satisfying overall yields.

Compounds **3** to **10** were tested against HIV-1 and HSV-1, none of them had a significant antiviral activity.

3. Experimental

3.1. General

NMR spectra were recorded at 400 and 100 MHz for ^1H and ^{13}C, respectively. IR spectra were recorded with a FT infrared spectrophotometer. Melting points are uncorrected. Elemental analyses were performed by the service of microanalyses, CNRS ICSN, Gif sur Yvette. High-resolution mass measurements were performed at the CRMPO (Rennes). The column chromatographies were run on silica gel Gerudan SI 60, 230–400 mesh, under 1–2 bars.

3.1.1. 2-{(*tert*-Butyldiphenylsilyl)oxycyclobutanone} 12. To a solution of alcohol **11** (1.50 g, 17.5 mmol) in DMF (4 mL) were added imidazole (1.43 g, 21 mmol) and *tertio*-butylchlorodiphenylsilane (5.4 mL, 21 mmol). The resulting mixture was stirred 20 h at rt. Water (15 mL) was added and the aqueous layer was extracted with diethyl ether. The combinated organic layers were dried and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel to give **12** (5.1 g, 90%) as a colorless oil. IR (film, cm^{-1}): 1793, 1170, 1113, 704. ^1H NMR ($CDCl_3$) δ: 1.08 (9H, s), 1.92 (1H, m), 2.19 (1H, m), 2.61 (2H, m), 4.82 (1H, dtt, $J=8.1$, 1.7 Hz), 7.40 (6H, m), 7.68 (2H, m), 7.77 (2H, m). ^{13}C NMR ($CDCl_3$) δ: 19.3, 22.4, 26.7, 38.4, 82.3, 127.91, 127.93, 130.1, 132.8, 133.4, 135.6, 135.7, 206.6. Anal. Calcd for $C_{20}H_{24}SiO_2$: C, 74.03; H, 7.45. Found C, 73.77; H, 7.54.

3.1.2. 2-{(*tert*-Butyldimethylsilyl)oxycyclobutanone} 13.[10] With the same procedure as above from **11** (1.17 g, 14 mmol), imidazole (1.295 g, 19 mmol) and *tertio*-butylchlorodimethylsilane (1.38 g, 19 mmol) in DMF (3 mL), **13** (2.36 g, 87%) was obtained as a colorless oil. ^1H NMR ($CDCl_3$) δ: 0.10 (3H, s), 0.12 (3H, s), 0.91 (9H, s), 1.86 (1H, m), 2.37 (1H, m), 3.72 (2H, m), 4.88 (1H, m). ^{13}C NMR ($CDCl_3$) δ: −4.7, −4.5, 18.5, 22.5, 25.8, 26.0, 38.5, 82.2, 207.3.

3.1.3. (*E*)-2-{(*tert*-Butyldiphenylsilyl)oxycyclobutylidene}ethyl acetate 14. To a solution of ketone **12** (0.953 g, 2.94 mmol) in benzene (6 mL) was added carbethoxymethylene triphenylphosphorane (1.637 g, 4.70 mmol). The resulting mixture was stirred for 3 days at rt. The solvent was removed under reduced pressure and the residue was diluted with petroleum ether. After filtration the solid was washed with petroleum ether and the filtrate was evaporated under reduced pressure. The resulting oil was purified by column chromatography on silica gel to give **14** (1.109 g, 96%) as a colorless oil. IR (film, cm^{-1}): 1716, 1685, 704. ^1H NMR ($CDCl_3$) δ: 1.07 (9H, s); 1.28 (3H, d, $J=7.4$ Hz), 1.99 (2H, m), 2.40 (1H, m), 2.95 (1H, m), 4.18 (2H, m), 4.75 (1H, m), 5.87 (1H, m), 7.40 (6H, m), 7.65 (3H, m). ^{13}C NMR ($CDCl_3$) δ: 13.9, 18.7, 24.8, 26.3, 29.4, 59.4, 71.4, 109.8, 127.2, 129.4, 132.8, 133.1, 135.1, 166.1, 167.3. Anal. Calcd for $C_{24}H_{30}SiO_3$: C, 73.05; H, 7.66. Found C, 73.49; H, 7.74.

3.1.4. (*Z*)-2-{(*tert*-Butyldiphenylsilyl)oxycyclobutylidene}ethyl acetate 16. To a solution of diisopropylamine (0.21 mL, 1.5 mmol) in THF (0.5 mL) at 0 °C was added a 1.6 M solution of BuLi in hexane (0.98 mL). The resulting mixture was stirred 30 min at 0 °C before cooling at −78 °C. At this temperature ethyl trimethylsilylacetate (0.275 mL, 1.5 mmol) was added. After 45 min a solution of ketone **12** (0.487 g, 1.5 mmol) in THF (2 mL) was added slowly. After 1 h the reaction mixture was allowed to warm to rt, and hydrolyzed with a 3 M solution of HCl (2 mL). The aqueous layer was extracted with dichloromethane and the organic layer was dried and concentrated under reduced pressure. The crude product (mixture *Z*/*E* 12/1) was purified by column chromatography on silica gel to give isomer *Z* **16** (0.377 g, 69%) as a colorless oil. IR (film, cm^{-1}): 1724, 1687, 1190, 1112, 703. ^1H NMR ($CDCl_3$) δ: 1.05 (9H, s), 1.20 (3H, t, $J=6.9$ Hz), 1.82 (2H, m), 2.26 (1H, m), 2.50 (1H, m), 3.96 (1H, dq, $J=14.4$, 6.9 Hz), 4.16 (1H, dq, $J=14.4$, 6.9 Hz), 5.17 (1H, m), 5.63 (1H, m), 7.38 (6H, m), 7.74 (4H, m). ^{13}C NMR($CDCl_3$) δ: 14.3, 19.2, 25.3, 16.9, 59.9, 72.5, 114.0, 127.5, 127.6, 129.5, 129.6, 133.8, 134.4, 135.7, 135.9, 163.5, 165.5. Anal. Calcd for $C_{24}H_{30}SiO_3$: C, 73.05; H, 7.66. Found C, 73.04; H, 7.68.

3.1.5. (*Z*)-2-{(*tert*-Butyldimetylsilyl)oxycyclobutylidene}ethyl acetate 17. With the same procedure as above from **13** (1 g, 5 mmol), (*Z*) ester **17** (1.05 g, 78%) was obtained as a colorless oil. IR (film, cm^{-1}: 1726, 1686, 1192, 1083. ^1H NMR ($CDCl_3$) δ: 0.09 (3H, s), 0.13 (3H, s), 0.87 (9H, s), 1.24 (3H, t, $J=7.5$ Hz), 1.99 (1H, m), 2.35 (1H, m), 2.41 (1H, m), 2.62 (1H, m), 4.07 (1H, m), 4.17 (1H, m), 5.05 (1H, m), 5.61 (1H, m). ^{13}C NMR ($CDCl_3$) δ: −5.3, −4.9, 2.3, 14.4, 18.2, 25.1, 26.1, 29.8, 59.8, 71.5, 114.3, 165.1. Anal. Calcd for $C_{14}H_{26}O_3Si$: C, 62.18; H, 9.69. Found C, 62.41, H, 9.64.

3.1.6. (*E*)-2-{(*tert*-Butyldiphenylsilyl)oxycyclobutylidene}ethanol 18. To a stirred solution of the ester **14** (1.3 g, 3.34 mmol) in dry dichloromethane (185 mL) at −60 °C was added dropwise a 1 M solution of DIBALH in toluene (16.85 mL). The mixture was stirred for 2 h at this temperature. A solution of 10% citric acid (150 mL) was added at −20 °C, the aqueous layer was extracted with toluene (2×50 mL) then the combinated organic layers were dried and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel to afford **18** (1.01 g, 87%) as a colorless oil. IR (film, cm^{-1}): 3378, 1428, 1112, 703. ^1H NMR ($CDCl_3$) δ: 1.07 (s, 9H), 1.60 (1H, br s), 1.99 (3H, m), 2.45 (1H, m) 4.10 (2H, m), 4.70 (1H, m), 5.64 (1H, m), 7.39 (6H, m), 7.67 (4H, m). ^{13}C NMR ($CDCl_3$) δ: 18.7, 21.2, 26.3, 29.8, 59.1, 71.1, 117.3, 127.2, 129.2, 133.5, 135.1, 147.9. Anal. Calcd for $C_{22}H_{28}SiO_2$: C, 74.95; H, 8.01. Found C, 74.64; H, 7.98.

3.1.7. (*Z*)-2-{(*tert*-Butyldiphenylsilyl)oxycyclobutylidene}ethanol 19. With the same procedure as above from ester **16** (0.5 g, 1.27 mmol), **19** (0.405 g, 91%) was obtained as a colorless oil. IR (film, cm^{-1}): 3421, 1428, 1112, 704. ^1H NMR ($CDCl_3$) δ: 1.09 (9H, s), 1.62 (1H, m),

1.76 (1H, m), 2.04 (1H, m), 2.23 (1H, m), 2.94 (1H, br s), 4.20 (1H, dd, $J=13.1$, 4.3 Hz), 4.33 (1H, dd, $J=13.1$, 5.3 Hz), 4.95 (1H, m), 5.46 (1H, m), 7.40 (6H, m), 7.71 (4H, m). ^{13}C NMR ($CDCl_3$) δ: 18.9, 23.0, 26.8, 29.5, 59.5, 72.4, 121.4, 127.6, 127.7, 129.8, 132.9, 133.6, 135.6, 135.7, 145.5. Anal. Calcd for $C_{22}H_{28}SiO_2 \cdot 0.2\ H_2O$: C, 74.19; H, 8.04. Found C, 74.19; H, 8.11.

3.1.8. (*Z*)-2-{(*tert*-Butyldimethylsilyl)oxycyclobutylidene}ethanol 20. With the same procedure as above from ester **17** (1.3 g, 4.82 mmol), **20** (0.94 g, 86%) was obtained as a colorless oil. IR (film, cm^{-1}): 3374, 1254, 1131. ^{1}H NMR ($CDCl_3$) δ: 0.08 (3H, s), 0.10 (3H, s), 0.89 (9H, s), 1.88 (1H, m), 2.20 (2H, m), 2.33 (1H, m), 3.10 (1H, br s), 4.07 (1H, m), 4.15 (1H, m), 4.86 (1H, m), 5.42 (1H, m). ^{13}C NMR ($CDCl_3$) δ: −5.1, −4.7, 2.1, 17.9, 23.1, 25.7, 29.9, 59.7, 71.4, 121.6, 145.4. HRMS Calcd for $C_8H_{15}O_2Si$ [M−*t*Bu] 171.08413. Found 171.0843.

3.1.9. (*E*)-{2-[2-(*tert*-Butyldiphenyl-silanyloxy)-cyclobutylidene]-ethyl}-9*H*-purin-6-ylamine 21. To a solution of alcohol **18** (0.61 g, 1.73 mmol), triphenylphosphine (0.99 g) and adenine (0.495 g) in THF (10 mL), was added for 2.5 h a solution of DIAD (0.55 mL) in THF (10 mL). The mixture was stirred at rt for 1 week. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel to give **21** (0.374 g, 46%) as white needles, mp 174–175 °C. IR (film, cm^{-1}): 3301, 3147, 1670, 1601, 1144, 1109. ^{1}H NMR ($CDCl_3$) δ: 1.05 (9H,s), 1.99 (1H, m), 2.10 (2H, m), 2.44 (1H, m), 4.70 (2H, d, $J=7$ Hz), 4.74 (1H, m), 5.60 (1H, m), 5.81 (2H, br s), 7.36 (6H, m), 7.62 (4H, m), 7.76 (1H,s), 8.36 (1H, s). ^{13}C NMR ($CDCl_3$) δ: 19.1, 21.6, 26.8, 29.9, 41.1, 71.5, 112.1, 119.6, 127.7, 129.8, 133.7, 135.5, 140.0, 150.0, 151.6, 153.0, 155.4. Anal. Calcd for $C_{27}H_{31}N_5SiO$: C, 69.05; H, 6.65, N, 14.91. Found C, 68.90; H, 6.64, N, 14.88.

3.1.10. (*Z*)-{2-[2-(*tert*-Butyldimethyl-silanyloxy)-cyclobutylidene]-ethyl}-9*H*-purin-6-ylamine 22. With the same procedure as above from ester **20** (0.47 g, 2.06 mmol), **22** (0.305 g, 43%) was obtained as white powder, mp 178.6–180 °C (methanol). IR (film, cm^{-1}): 3430, 3293, 3148, 1671, 1604, 1135. ^{1}H NMR ($CDCl_3$) δ: 0.11 (3H, s), 0.12 (3H, s), 0.89 (9H, s), 1.96 (1H, m), 2.26 (2H, m), 2.37 (1H, m), 4.92 (2H, d, $J=7$ Hz), 4.98 (1H, m), 6.20 (2H, br s), 8.01 (1H, s), 8.37 (1H, s). ^{13}C NMR($CDCl_3$) δ: −5.0, −4.5, 17.9, 23.2, 25.7, 29.9, 40.1, 71.4, 115.17, 119.5, 140.9, 149.8, 150.1, 152.8, 155.5. HRMS Calcd for $C_{17}H_{27}N_5OSi$ 345.1985. Found 345.1964.

3.1.11. (*E*)-2-[2-(6-Amino-purin-9-yl)-ethylidene]-cyclobutanol 3. To a solution of protected alcohol **21** (0.32 g, 0.68 mmol) in THF (5.5 mL) was added tetrabutylammonium fluoride (1 M, 1.3 mL) and the mixture was stirred for 2.5 h at rt. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel to give alcohol **3** (0.26 g, 95%). IR (film, cm^{-1}): 3272, 1678, 1605, 1577, 1298. ^{1}H NMR (DMSO-d_6) δ: 1.75 (1H, m), 2.22 (2H, m), 2.55 (1H, m), 4.57 (1H, m), 4.74 (2H, d, $J=7.0$ Hz), 5.47 (1H, m), 5.64 (1H, m), 7.30 (2H, br s), 8.21 (1H, s), 8.23 (1H, s). ^{13}C NMR (DMSO-d_6) δ: 21.2, 29.2, 40.6, 69.9, 112.7, 118.7, 140.5, 149.4, 151.3, 152.5, 156.0. HRMS Calcd for $C_{11}H_{13}ON_5$ 231.1120. Found 231.1133. Anal. Calcd for $C_{11}H_{13}N_5O$: C, 57.13, H, 5.66, N, 30.28. Found C, 56.66, H, 5.52, N, 30.12.

3.1.12. (*Z*)-2-[2-(6-Amino-purin-9-yl)-ethylidene]-cyclobutanol 5. With the same procedure as above from protected alcohol **22** (0.15 g, 0.434 mmol), alcohol **5** was obtained (0.098 g, 98%) as a white powder, mp 196.8–197.5 °C (methanol). IR (film, cm^{-1}): 3427, 3290, 1688, 1614, 1576, 1159. ^{1}H NMR (DMSO-d_6) δ: 1.78 (1H, m), 2.20 (3H, m), 4.80 (1H, m), 4.89 (1H, m), 5.35 (1H, m), 6.09 (1H, d, $J=8$ Hz), 7.16 (2H, br s), 8.10 (1H, s), 8.15 (1H, s). ^{13}C NMR 22.9, 29.3, 39.8, 70.4, 115.3, 119.1, 140.7, 149.2, 150.8, 152.3, 156.2. Anal. Calcd for $C_{11}H_{13}N_5O$: C, 57.13, H, 5.66, N, 30.28. Found C, 57.02, H, 5.71, N, 30.11.

3.1.13. (*E*)-Benzoyl-1-{2-[2-(*tert*-butyldiphenyl-silanyloxy)-cyclobutylidene]-ethyl}-5-methyl-1*H*-pyrimidine-2,4-dione 23. With the same procedure as for **21** from alcohol **18** (0.45 g, 1.27 mmol) and *N*-3-benzoylpyrimidine (0.585 g, 2.55 mmol) **23** was obtained (0.591 g, 82%) as a white powder, mp 68.8–69.7 °C. IR (film, cm^{-1}): 1748, 1699, 1656, 1234, 1110. ^{1}H NMR ($CDCl_3$) δ: 1.07 (9H, s), 1.98 (3H, s), 2.12 (3H, m), 2.50 (1H, m), 4.25 (2H, m), 4.73 (1H, m), 5.40 (1H, m), 7.03 (1H, s), 7.40 (5H, m), 7.48 (4H, m), 7.63 (4H, m), 7.92 (2H, d, $J=7$ Hz). ^{13}C NMR($CDCl_3$) δ: 12.3, 15.1, 18.9, 21.6, 26.6, 29.7, 45.1, 71.3, 100.5, 111.6, 127.5, 128.9, 129.6, 130.3, 131.5, 133.5, 134.8, 135.4, 139.1, 149.7, 152.1, 162.9, 169.0.

3.1.14. (*Z*)-Benzoyl-1-{2-[2-(*tert*-butyldimethyl-silanyloxy)-cyclobutylidene]-ethyl}-5-methyl-1*H*-pyrimidine-2,4-dione 24. With the same procedure as for **23** from alcohol **20** (0.40 g, 1.75 mmol), **24** was obtained (0.678 g, 88%) as a white powder, mp 130–131.2 °C. IR (film, cm^{-1}): 1701, 1658, 1599, 1252. ^{1}H NMR ($CDCl_3$) δ: 0.16 (6H, s), 0.97 (9H, s), 1.97 (3H, s), 1.98 (1H, m), 2.30 (2H, m), 2.43 (1H, m), 4.25 (1H, dd, $J=13.8$, 9.8 Hz), 4.73 (1H, dd, $J=13.8$, 5.3 Hz), 4.96 (1H, m), 5.26 (1H, m), 7.50 (2H, dd, $J=7$, 7 Hz), 7.56 (1H, s), 7.63 (2H, t, $J=7.7$ Hz) 7.94 (2H, d, $J=7$ Hz). ^{13}C NMR ($CDCl_3$) δ −4.7, −4.2, 12.5, 18.2, 23.6, 26.0, 30.4, 44.0, 71.6, 110.5, 115.1, 129.3, 130.6, 132.0, 140.3, 150.2, 151.0, 163.5, 169.6.

3.1.15. (*E*)-1-{2-[2-(*tert*-Butyldiphenyl-silanyloxy)-cyclobutylidene]-ethyl}-5-methyl-1*H*-pyrimidine-2,4-dione 25. A solution of **23** (0.34 g, 0.60 mmol) in methanol saturated with ammonia (9.5 mL) was stirred for 48 h at rt. After removal of the volatile substances, the residue was purified by column chromatography on silica gel to give **25** (0.22 g, 80%) as a white powder, mp 58.8–59.8 °C. IR (film, cm^{-1}): 3170, 1680, 1112, 702. ^{1}H NMR ($CDCl_3$) δ: 1.08 (9H, s), 1.94 (3H, s), 1.98 (1H, m), 2.10 (2H, m), 2.48 (1H, m), 4.25 (2H, m), 4.72 (1H, m), 5.39 (1H, m), 6.96 (1H, s), 7.38 (6H, m), 7.68 (4H, m) 10.05 (1H, br s). ^{13}C NMR ($CDCl_3$) δ: 12.4, 19.1, 21.8, 26.7, 26.8, 29.9, 44.9, 71.5, 110.7, 112.3, 127.6, 127.8, 129.7, 129.8, 133.7, 133.8, 135.5, 139.6, 151.2, 151.5, 164.7. HRMS Calcd for $C_{27}H_{32}N_2O_3Si$ 460.2182. Found 460.2169.

3.1.16. (*Z*)-1-{2-[2-(*tert*-Butyldimethyl-silanyloxy)-cyclobutylidene]-ethyl}-5-methyl-1*H*-pyrimidine-2,4-dione 26. With the same procedure as above from protected alcohol **24** (0.33 g, 0.75 mmol), **26** was obtained (0.219 g,

87%) as a white powder, mp 134.8–135.9 °C (petroleum ether/ether 2/1). IR (film, cm^{-1}): 3464, 3414, 1695, 1681, 1640, 1134. ^1H NMR ($CDCl_3$) δ: 0.12 (6H, s), 0.91 (9H, s), 1.91 (3H, s), 1.92 (1H, m), 2.27 (2H, m), 2.39 (1H, m), 4.19 (1H, dd, $J=14.0$, 10.3 Hz), 4.73 (1H, dd, $J=14.0$, 4.3 Hz), 4.94 (1H, m), 5.25 (1H, m), 7.42 (1H, s), 9.75 (1H, br s). ^{13}C NMR($CDCl_3$) δ: −4.5, 12.3, 18.0, 23.3, 25.8, 30.2, 43.5, 71.4, 110.4, 115.5, 140.3, 150.1, 151.4, 164.7. HRMS Calcd for $C_{17}H_{28}O_3N_2Si$ 336.1869. Found 336.1849.

3.1.17. (*E*)-1-[2-(2-Hydroxy-cyclobutylidene)-ethyl]-5-methyl-1*H*-pyrimidine-2,4-dione 4. With the same procedure as for **3** from protected alcohol **25** (0.17 g, 0.369 mmol), alcohol **4** was obtained (0.074 g, 91%) as a white powder, mp 137.6–138.4 °C (methanol/ether 1/4). IR (film, cm^{-1}): 3406, 1696, 1673, 1117. ^1H NMR (CD_3OD) δ: 1.72 (1H, m), 1.77 (3H, s), 2.19 (2H, m), 2.45 (1H, m), 4.18 (2H, d, $J=7.5$ Hz), 4.49 (1H, m), 5.36 (1H, m), 7.28 (1H, s). ^{13}C NMR (CD_3OD) δ: 14.0, 24.3, 32.1, 48.1, 73.4, 113.1, 115.8, 144.5, 154.2, 154.7, 168.8. Anal. Calcd for $C_{11}H_{14}N_2O$: C, 59.45, H, 6.35, N, 12.61. Found C, 59.01, H, 6.30, N, 12.22. HRMS Calcd for $C_{11}H_{14}O_3N_2Si$ 222.1004. Found 222.0094.

3.1.18. (*Z*)-1-[2-(2-Hydroxy-cyclobutylidene)-ethyl]-5-methyl-1*H*-pyrimidine-2,4-dione 6. With the same procedure as for **3** from protected alcohol **26** (0.16 g, 0.48 mmol), alcohol **6** was obtained (0.096 g, 91%) as a white powder, mp 129–130 °C (methanol/ether 1/4). IR (film, cm^{-1}): 3452, 1680, 1640, 1104. ^1H NMR (CD_3OD) δ: 1.88 (3H, s), 1.90 (1H, m), 2.30 (2H, m), 2.40 (1H, m), 4.39 (1H, dd, $J=14.2$, 8.3 Hz), 4.48 (1H, dd, $J=14.2$, 7.0 Hz), 4.85 (2H, br s), 5.26 (1H, m), 7.50 (1H, s). ^{13}C NMR (CD_3OD) δ: 14.0, 25.7, 31.8, 47.8, 73.5, 113.0, 118.5, 144.5, 144.6, 153.8, 154.7, 168.7. Anal. Calcd for $C_{11}H_{14}N_2O$: C, 59.45, H, 6.35, N, 12.61. Found C, 59.39, H, 6.41, N, 12.69.

3.1.19. (*E*)-Acetic acid 2-[2-(*tert*-butyldiphenyl-silanyl-oxy)-cyclobutylidene]-ethyl ester 28. To a solution of alcohol **18** (0.252 g, 0.71 mmol) in pyridine (10 mL) at 0 °C acetic anhydride (0.3 mL, 3.18 mmol) was added slowly. After 10 min at this temperature the mixture was stirred for one night at rt. Dichloromethane (15 mL) was added and the organic layer was washed with water, the aqueous layer was extracted with dichloromethane (2×20 mL) and the combinated organic layers were dried ($MgSO_4$) then evaporated under reduced pressure. The residue was purified by column chromatography on silica gel to afford **28** (0.245 g, 87%) as a colorless oil. IR (film, cm^{-1}): 1744, 1237, 1117, 704. ^1H NMR ($CDCl_3$) δ: 1.07 (9H, s), 2.03 (3H, m), 2.07 (3H, s), 2.48 (1H, m), 4.51 (2H, m), 4.69 (1H, m), 5.58 (1H, m), 7.39 (6H, m), 7.67 (4H, m). ^{13}C NMR 19.1, 21.0, 21.7, 26.8, 30.0, 61.1, 71.5, 112.8, 127.5, 127.6, 129.6, 133.7, 134.0, 135.5, 150.9, 170.9. Anal. Calcd for $C_{24}H_{30}O_3Si$: C, 73.05, H, 7.66. Found C, 72.96, H, 7.76.

3.1.20. (*Z*)-Acetic acid 2-[2-(*tert*-butyldiphenyl-silanyl-oxy)-cyclobutylidene]-ethyl ester 29. With the same procedure as above from alcohol **19** (1.473 g, 4.18 mmol), pyridine (30 mL) and acetic anhydride (1 mL, 10.6 mmol), **29** (1.548 g, 94%) was obtained as a colorless oil. IR (film, cm^{-1}): 1742, 1233, 1114, 705. ^1H NMR ($CDCl_3$) δ: 1.07 (9H, s), 1.80 (2H, m), 2.06 (3H, s), 2.09 (1H, m), 2.28 (1H, m), 4.80 (2H, dd, $J=1.4$, 0.8 Hz), 4.91 (1H, m), 5.35 (1H, m), 7.38 (6H, m), 7.68 (4H, m). ^{13}C NMR ($CDCl_3$) δ: 18.9, 21.0, 23.2, 26.8, 29.5, 61.0, 72.1, 115.7, 127.5, 127.7, 129.6, 133.4, 134.0, 135.7, 135.8, 149.9, 170.9. Anal. Calcd for $C_{24}H_{30}O_3Si\cdot0.2\ H_2O$: C, 72.39, H, 7.70. Found C, 72.15, H, 7.53.

3.1.21. (*E*)-Acetic acid 2-(2-hydroxy-cyclobutylidene)-ethyl ester 30. With the same procedure as for alcohol **3** from acetate **28** (0.177 g, 0.45 mmol), alcohol **30** was obtained as a colorless oil (0.64 g, 91%). IR (film, cm^{-1}): 3399, 1744, 1251. ^1H NMR ($CDCl_3$) δ: 1.85 (1H, m), 2.06 (3H, s), 2.28 (1H, m), 2.37 (1H, m), 2.57 (1H, m), 4.53 (2H, m), 4.69 (1H, m), 5.60 (1H, m). ^{13}C NMR ($CDCl_3$) δ: 20.9, 21.7, 30.0, 60.9, 70.9, 113.4, 151.4, 171.0. HRMS: calcd for $C_6H_{10}O$ [M−C_2H_2O] 114.06808, found 114.0688.

3.1.22. (*Z*)-Acetic acid 2-(2-hydroxy-cyclobutylidene)-ethyl ester 31. With the same procedure as for alcohol **3** from acetate **29** (1.53 g, 3.88 mmol), alcohol **31** was obtained as a colorless oil (0.549 g, 91%). IR (film, cm^{-1}) 3443, 1735, 1244^1H NMR ($CDCl_3$) δ: 1.83 (1H, m), 2.08 (3H, s), 2.30 (3H, m), 3.92 (1H, dd, $J=8.9$ Hz), 4.39 (1H, m), 4.82 (1H, m), 5.07 (1H, dd, $J=12.3$, 9.8 Hz), 5.26 (1H, m). ^{13}C NMR ($CDCl_3$) δ: 21.2, 23.3, 29.6, 61.3, 71.3, 115.7, 150.7, 171.9. HRMS Calcd for $C_6H_{10}O$ [M− C_2H_4O] 112.05243. Found 112.0515.

3.1.23. (*E*)-Acetic acid 2-[2-(6-amino-purin-9-yl)-cyclobutylidene]-ethyl ester 32. With the same procedure as for **21** from **30** (0.047 g, 0.3 mmol), alcohol **32** was obtained (0.03 g, 37%) as a white powder, mp 155–157 °C (methanol). IR (film, cm^{-1}): 1727, 1675, 1606, 1571, 1240. ^1H NMR ($CDCl_3$) δ: 2.06 (3H, s), 2.49 (1H, m), 2.74 (2H, m), 2.93 (1H, m), 4.56 (2H, m), 5.44 (1H, m), 5.71 (1H, m), 5.93 (2H, br s), 7.98 (1H, s), 8.36 (1H, s). ^{13}C NMR ($CDCl_3$) δ: 20.6, 24.7, 27.7, 53.1, 60.4, 117.5, 119.4, 138.7, 145.1, 149.8, 152.9, 155.7, 170.7. Anal. Calcd for $C_{13}H_{15}N_5O_2$: C, 57.13, H, 5.53, N, 25.63. Found C, 57.14, H, 5.65, N, 25.11.

3.1.24. (*Z*)-Acetic acid 2-[2-(6-amino-purin-9-yl)-cyclobutylidene]-ethyl ester 33. With the same procedure as for **21** from **31** (0.384 g, 2.46 mmol), alcohol **33** was obtained (0.296 g, 44%) as a white powder, mp 155–157 °C (methanol). IR (film, cm^{-1}). ^1H NMR ($CDCl_3$) δ: 1.88 (3H, s), 2.47 (1H, m), 2.74 (2H, m), 2.90 (1H, m), 4.07 (1H, ddd, $J=12.8$, 7.4, 1.0 Hz), 4.21 (1H, ddd, $J=12.8$, 6.8, 1.5 Hz), 5.63 (1H, m), 5.81 (1H, m), 5.85 (1H, br s), 7.98 (1H, s), 8.38 (1H, s). ^{13}C NMR ($CDCl_3$) δ: 20.6, 26.3, 27.3, 53.0, 59.6, 119.7, 119.9, 139.0, 144.4, 149.5, 153.0, 155.9, 170.5. Anal. Calcd for $C_{13}H_{15}N_5O_2$: C, 57.13, H, 5.53, N, 25.63. Found C, 57.16, H, 5.56, N, 25.41.

3.1.25. (*E*)-2-[2-(6-Amino-purin-9-yl)-cyclobutylidene]-ethanol 7. A solution of **32** (0.112 g, 0.41 mmol) in methanol saturated with ammonia (9.5 mL) was stirred for 15 h at rt. After removal of the volatile substances, the residue was purified by column chromatography on silica gel to give **7** (0.095 g, 100%) as a white powder, mp 192 °C. IR (film, cm^{-1}): 3120, 1684, 1614, 1571. ^1H NMR (DMSO-d_6): δ: 2.57 (3H, m), 2.80 (1H, m), 3.90 (2H, m), 4.53 (1H, br s), 5.23 (1H, m), 5.56 (1H, m), 7.18 (2H, s), 8.15 (1H, s), 8.25 (1H, s). ^{13}C NMR (DMSO-d_6): 24.2, 26.0, 52.9, 57.3,

118.7, 122.3, 139.1, 140.9, 149.3, 152.3, 155.9. Anal. Calcd for $C_{11}H_{13}N_5O \cdot 0.1\ H_2O$: C, 56.69, H, 5.71, N, 30.05. Found C, 56.53, H, 5.73, N, 29.67.

3.1.26. (*Z*)-2-[2-(6-Amino-purin-9-yl)-cyclobutylidene]-ethanol 9. With the same procedure as above from acetate **33** (0.29 g, 1.06 mmol), alcohol **9** was obtained (0.245 g, 100%) as a white powder, mp 159.5–160.5 °C (methanol). IR (film, cm^{-1}): 3271, 3120, 1683, 1612, 1573, 1000. ^1H NMR (DMSO-d_6) δ: 2.52 (2H,m), 2.62 (1H, m), 2.77 (1H, m), 3.36 (1H, m), 3.45 (1H, m), 4.39 (1H, m), 5.42 (1H, m), 5.66 (1H, m), 7.17 (1H, s), 8.14 (1H, s), 8.22 (1H,s). ^{13}C NMR (DMSO-d_6): 25.4, 26.0, 52.3, 56.5, 118.7, 125.2, 139.3, 139.5, 149.0, 152.3, 155.9. Anal. Calcd for $C_{11}H_{13}N_5O$: C, 57.13, H, 6.67, N, 30.28. Found C, 56.81, H, 5.59, N, 30.23.

3.1.27. (*E*)-Acetic acid 2-[2-(3-benzoyl-5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-cyclobutylidene]-ethyl ester 34. With the same procedure as for **21** from **31** (0.086 g, 0.55 mmol), alcohol **34** was obtained (0.151 g, 74%) as a white powder, mp 124–125 °C (methanol). IR (film, cm^{-1}): 1751, 1725, 1691, 1655, 1646, 1229. ^1H NMR ($CDCl_3$) δ: 1.97 (3H, s), 2.07 (3H, s), 2.21 (1H, m), 2.51 (1H, m), 2.60 (1H, m), 2.79 (1H, m), 4.51 (1H, ddd, $J=12.7$, 6.1, 1.5 Hz), 4.61 (1H, ddd, $J=12.7$, 7.4, 0.8 Hz), 5.45 (1H, m), 5.68 (1H, m), 7.30 (1H, s), 7.49 (2H, m), 7.64 (1H, m), 7.92 (2H, m). ^{13}C NMR ($CDCl_3$) δ: 12.5, 20.8, 24.2, 26.2, 54.6, 60.4, 111.1, 117.9, 129.0, 130.3, 131.5, 134.9, 136.4, 144.4, 149.6, 162.6, 168.9, 170.7. Anal. Calcd for $C_{20}H_{20}N_2O_5$: C, 65.21, H, 5.47, N, 7.60. Found C, 65.33, H, 5.65, N, 7.41.

3.1.28. (*Z*)-Acetic acid 2-[2-(3-benzoyl-5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-cyclobutylidene]-ethyl ester 35. With the same procedure as for **21** from **31** (0.052 g, 0.33 mmol), alcohol **35** was obtained (0.104 g, 86%) as a white powder, mp 127–130 °C (methanol). IR (film, cm^{-1}): 1732, 1686, 1641, 1598, 1230. ^1H NMR ($CDCl_3$) δ: 1.99 (3H, s), 2.01 (3H, s), 2.21 (1H, m), 2.54 (1H, m), 2.64 (1H, m), 2.71 (1H, m), 4.41 (2H, m), 5.64 (1H, m), 5.77 (1H, m), 7.32 (1H, s), 7.51 (2H, m), 7.65 (1H, m), 7.95 (2H, m). ^{13}C NMR ($CDCl_3$) δ: 12.4, 20.5, 25.5, 25.6, 54.6, 59.6, 111.1, 119.7, 129.0, 130.3, 131.4, 134.8, 136.6, 144.0, 149.3, 162.5, 168.8, 170.3. Anal. Calcd for $C_{20}H_{20}N_2O_5 \cdot 0.2\ H_2O$: C, 64.58, H, 5.53, N, 7.53. Found C, 64.52, H, 5.54, N, 7.81.

3.1.29. (*E*)-1-[2-(2-Hydroxy-ethylidene)-cyclobutyl]-5-methyl-1*H*-pyrimidine-2,4-dione 8. With the same procedure as for **25** from acetate **34** (0.163 g, 0.44 mmol), alcohol **8** was obtained (0.085 g, 87%) as a white powder, mp 133–139 °C (methanol). IR (film, cm^{-1}): 3422, 3022, 1673, 1636, 1265. ^1H NMR (CD_3OD) δ: 1.89 (3H, d, $J=$ 1 Hz), 2.24 (1H, m), 2.41 (1H, m), 2.56 (1H, m), 2.75 (1H, m), 4.06 (2H, m), 5.37 (1H, m), 5.61 (1H, m), 7.57, (1H, q, $J=1$ Hz). ^{13}C NMR(CD_3OD) δ: 12.4, 24.8, 26.8, 56.2, 59.1, 111.8, 123.2, 139.4, 143.4, 152.9, 166.5. Anal. Calcd for $C_{11}H_{14}N_2O_3$: C, 59.45, H, 6.35, N, 12.60. Found C, 59.21, H, 6.44, N, 12.28.

3.1.30. (*Z*)-1-[2-(2-Hydroxy-ethylidene)-cyclobutyl]-5-methyl-1*H*-pyrimidine-2,4-dione 10. With the same procedure as for **25** from acetate **35** (0.118 g, 0.32 mmol), alcohol **10** was obtained (0.065 g, 91%) as a white powder, mp 177 °C (methanol). IR (film, cm^{-1}) 3450, 3165, 1739, 1675, 1641, 1226. ^1H NMR (CD_3OD) δ: 1.89 (3H, d, $J=$ 1 Hz), 2.21 (1H, m), 2.44 (1H, m), 2.59 (1H, m), 2.69 (1H, m), 3.84 (2H, m), 5.54 (1H, m) 5.69 (1H, m), 7.54 (1H, q, $J=1$ Hz). ^{13}C NMR (CD_3OD) δ: 12.4, 26.3, 26.5, 56.1, 58.9, 111.9, 125.8, 139.6, 141.7, 152.5, 166.5. Anal. Calcd for $C_{11}H_{14}N_2O_3$: C, 59.45, H, 6.35, N, 12.60. Found C, 59.36, H, 6.42, N, 12.57.

Acknowledgements

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References and notes

1. For reviews see for instance: Huryn, D. M.; Okabe, M. *Chem. Rev.* **1992**, *92*, 1745–1768. Borthwick, A. D.; Biggadike, K. *Tetrahedron* **1992**, *48*, 571–623. Agrofoglio, L.; Suhas, E.; Farese, A.; Condom, R.; Challand, S. R.; Earl, R. A.; Guedj, R. *Tetrahedron* **1994**, *50*, 10611–10670. Herdewijn, P. *Drug Discov. Today* **1997**, *2*, 235–242. Crimmins, M. T. *Tetrahedron* **1998**, *54*, 9229–9272. De Clercq, E. *Collect. Czech. Chem. Commun.* **1998**, *63*, 449–479. Agrofoglio, L. A.; Challand, S. R. In *Acycllic, carbocyclic and L-nucleosides*; Kluwer Academic: Dordrecht, 1998. Ichikawa, E.; Kato, K. *Synthesis* **2002**, 1–28. De Clercq, E.; Neyts, J. *Rev. Med. Virol.* **2004**, *14*, 289–300.
2. Qiu, Y.-L.; Ksebati, M. B.; Zemlicka, J. *Nucleosides Nucleotides Nucleic Acids* **2000**, *19*, 31–37.
3. Panda, J.; Ghosh, S.; Ghosh, S. *J. Chem. Soc., Perkin Trans. 1* **2001**, 3013–3016.
4. Guan, H.-P.; Ksebati, M. B.; Cheng, Y.-C.; Drach, J. C.; Kern, E. R.; Zemlicka, J. *J. Org. Chem.* **2000**, *65*, 1280–1290.
5. Guan, H.-P.; Ksebati, M. B.; J, C.; Kern, E. R.; Zemlicka, J. *J. Org. Chem.* **2000**, *65*, 5177–5184.
6. Demaison, C.; Hourioux, C.; Roingeard, P.; Agrofoglio, L. A. *Tetrahedron Lett.* **1998**, *39*, 9175–9178.
7. (a) Gourdel-Martin, M.-E.; Huet, F. *J. Org. Chem.* **1997**, *62*, 2166–2172. (b) Gourdel-Martin, M.-E.; Huet, F. *Nucleosides Nucleotides* **1999**, *18*, 645–648. (c) Gauvry, N.; Bhat, L.; Mévellec, L.; Zucco, M.; Huet, F. *Eur. J. Org. Chem.* **2000**, 2717–2722. (d) Gauvry, N.; Huet, F. *J. Org. Chem.* **2001**, *66*, 583–588. (e) Hubert, C.; Alexandre, C.; Aubertin, A.-M.; Huet, F. *Tetrahedron* **2002**, *58*, 3575–3578.
8. (a) Hubert, C.; Alexandre, C.; Aubertin, A.-M.; Huet, F. *Tetrahedron* **2003**, *59*, 3127–3130. (b) Guillarme, S.; Legoupy, S.; Bourgougnon, N.; Aubertin, A.-M.; Huet, F. *Tetrahedron* **2003**, *59*, 9635–9639.
9. Bloomfield, J. J.; Nelke, J. M. In *Organic syntheses*, *Collect. Vol. 6*; Wiley: New York, 1988; p 167.
10. Kazunori, T.; Akio, M. *Tetrahedron Lett.* **1992**, *33*, 4345–4348.

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Reactions of allyloxy(methoxy)carbene in solution. Carbene rearrangement and Claisen rearrangement of the carbene dimer

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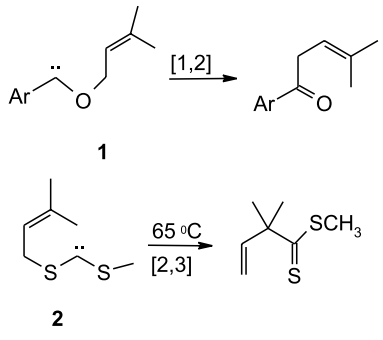
Abstract—Allyloxy(methoxy)carbene, with and without deuterium in the α-position of the allyloxy group, was generated in benzene at 50 and at 110 °C. At the higher temperature, the carbene fragmented to allyl and methoxycarbonyl radicals that subsequently coupled. At the lower temperature, most of the carbene dimerised. The structure of the major product and the distribution of deuterium indicated that the dimer underwent Claisen rearrangement at 50 °C to methyl 2-allyloxy-2-methoxy-4-pentenoate. Facile rearrangement of the dimer was supported by the results of a computation which placed the barrier at about 18 kcal mol^{-1}.

1. Introduction

Both [1,2]- and [2,3]-sigmatropic rearrangement of allylic, heteroatom carbenes, as well as radical-pair formation, are known. In 1977 and 1978, Iwamura and co-workers[1] ascribed rearrangement of aryl(4,4-dimethylallyloxy)carbenes (**1**) to a [1,2] shift, Scheme 1. In 1972, Baldwin and Walker[2] showed that *S*-methyl-(3,3-dimethylallyl)carbene (**2**) undergoes a clean [2,3] sigmatropic rearrangement to a dithiocarboxylic acid methyl ester at 65 °C, Scheme 1. In the same year, Hoffmann and co-workers[3] published a detailed study of the gas phase thermolysis of **3a** and **3b**, at 250 °C, Scheme 2. They concluded that a sequential carbene (**4**)/radical pair (**5**) mechanism best accounted for their results.



Scheme 1.

We had shown[4] that two phenyl-substituted allyloxy-(methoxy)carbenes (e.g., **6**) fragment to radical pairs in benzene at 110 °C, Scheme 3. Carbene **6**, and the isomeric species with Ph at the terminus of the double bond, favored the product from apparent 1,2-migration. Although it was evident that some, but not all, of the product could have come from concerted 1,2-rearrangement, it was unclear whether the product distribution reflected some sigmatropic rearrangement or whether it could also be explained in terms of a concurrent radical-pair mechanism, with a cage effect operating in solution. Very rapid radical-pair coupling, in systems that do not involve a molecule, such as N_2, between the initially-formed radicals, might occur without complete rotational equilibration, favoring re-attachment at the allylic site originally bonded to oxygen. Such coupling would be indistinguishable from concerted [1,2]-migration. [1,2]-Migration is theoretically unfavorable in comparison with [2,3]-sigmatropic rearrangement, but it can eclipse the latter if steric demand is high.[1]

In the gas phase study of Hoffmann and co-workers, a cage effect could not operate and it was pointed out that the high temperature (250 °C) favored the radical-pair mechanism on the grounds of entropy.[3]

We decided to examine the rearrangement of allyloxy-(methoxy)carbene with precursors **7a–d** (Scheme 4), from which **4a** and **4b** can be generated in solution at 110 °C (**7a**,

Keywords: Allyloxy(methoxy)carbene; Carbene dimer; Claisen; Rearrangement.
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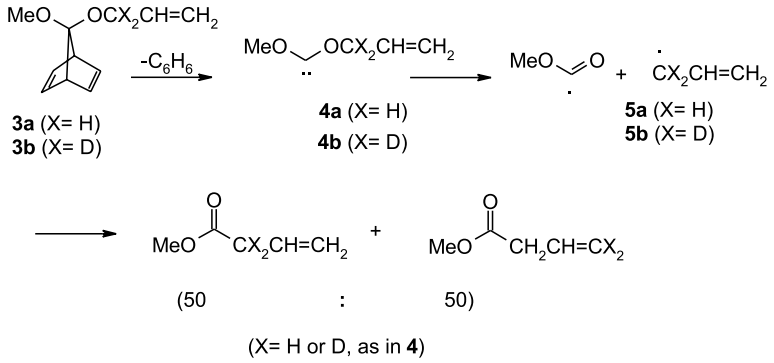
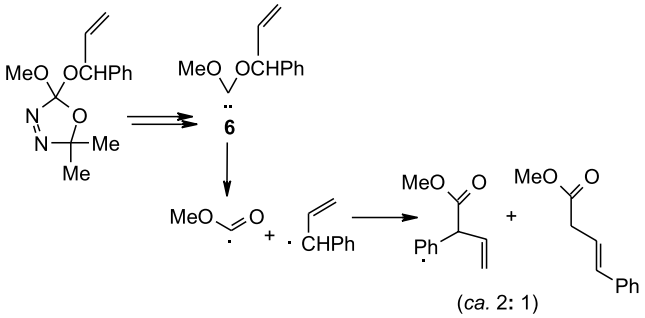
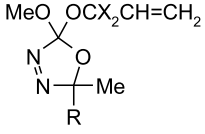


Scheme 2.



Scheme 3.



7a: R= Me, X= H)
b: R= Me, X= D)
c: R= p-MeOC$_6$H$_4$, X = H)
d: R= p-MeOC$_6$H$_4$, X= D)

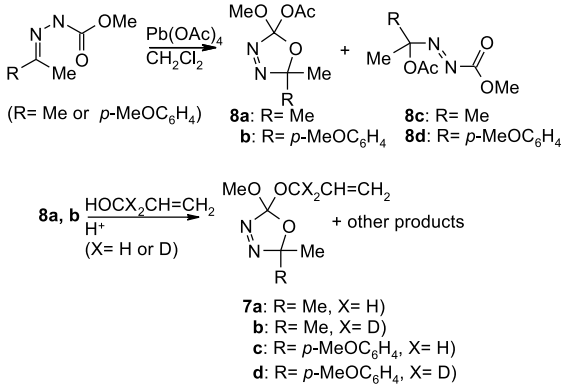
Scheme 4.

7b) or 50 °C (**7c**, **7d**). Sigmatropic rearrangement, as observed by Baldwin and Walker,[2] might well become a competitive (or exclusive) process for **4**, especially at the lower temperature, given that fragmentation should be less likely than was the case with the phenyl-substituted allyloxycarbenes, such as **6**, which were studied previously.[4]

Results of that study, as well as Claisen rearrangement of 1,2-diallyloxy-1,2-dimethoxyethene under relatively mild conditions, are reported.

2. Methods, results, and discussion

2-Allyloxy-2-methoxy-5,5-dimethyl-Δ^3-1,3,4-oxadiazoline (**7a**) and the dideuterio analogue (**7b**), as well as the corresponding 5-methyl-5-(*p*-methoxyphenyl) compounds, **7c** and **7d** were prepared by the route[5] shown in Scheme 5. Compound **7a** was thermolysed at 110 °C, in ordinary



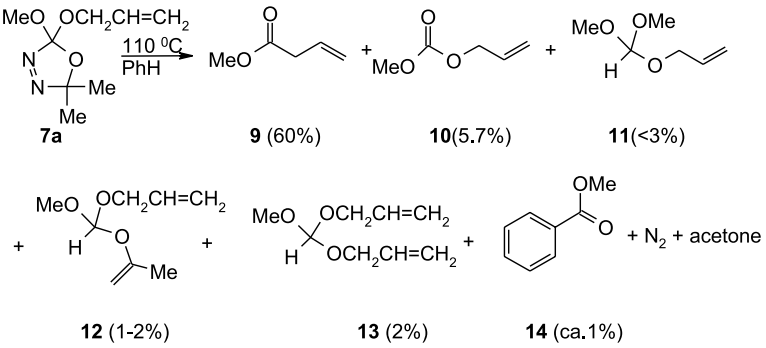
Scheme 5.

benzene and in carefully dried benzene, in a sealed tube to determine the effects of adventitious water. *tert*-Butyl alcohol, or the stable free radical TEMPO, were added in separate experiments to ascertain whether both a carbene and radicals could be trapped. After 24 h, the solutions were analyzed by gas chromatography.

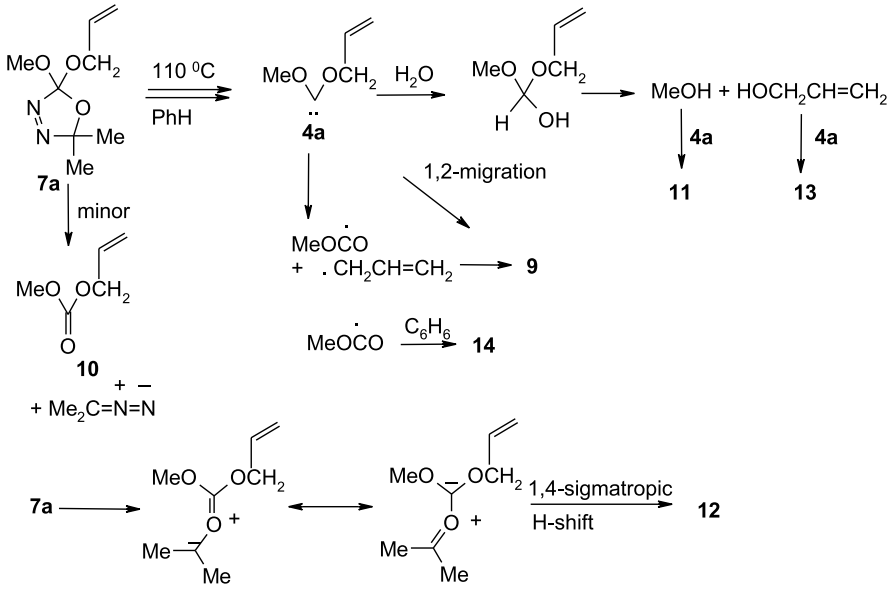
3. Thermolysis of 7a in ordinary benzene

A solution of **7a** in dry benzene at 110 °C for 24 h gave one major product and several minor products noticeable by GC. Thinking that some of those could be caused by adventitious water, we ran the reaction in ordinary benzene which gave, in addition to a solvent peak, six fractions large enough to be collected by GC. Yields from reactions in ordinary benzene are not relevant because such reactions were used only to identify the minor components that were always detectable by GC. Fraction one, not collected, contained benzene and, presumably, acetone and other volatile products. Fractions 2–7 were methyl 3-butenoate (**9**), methyl 2-propenyl carbonate (**10**), allyl dimethyl orthoformate (**11**), allyl methyl 2-propenyl orthoformate (**12**), diallyl methyl orthoformate (**13**) and methyl benzoate (**14**). Yields in Scheme 6 pertain to reaction in dry benzene (distilled from CaH_2); conditions for detecting but not isolating **11** and **13**.

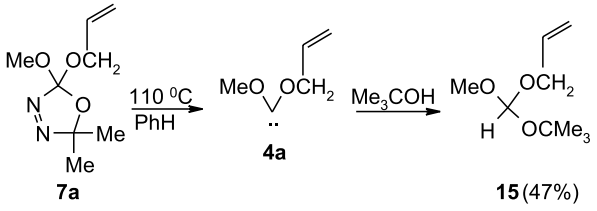
Probable origins of products **9**–**14**, orthoformates **11** and **13** coming from capture of the carbene by adventitious water, are shown in Scheme 7. Formation of carbonates, such as **10**, is a known process in the thermolysis of oxadiazolines



Scheme 6.



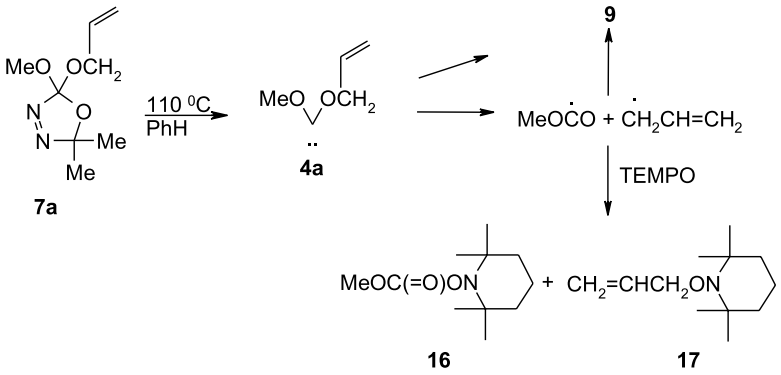
Scheme 7.



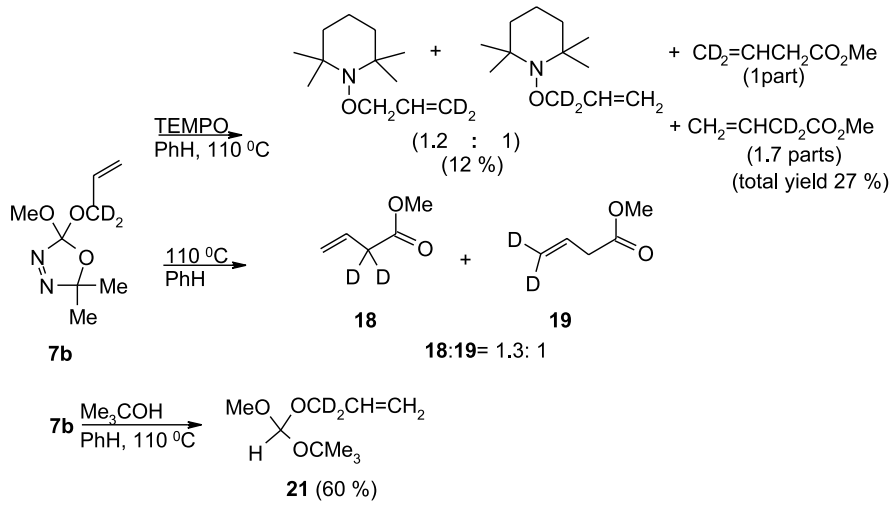
Scheme 8.

analogous to **7a**[6] and the [1–4] sigmatropic shift to afford **12** is a known reaction of carbonyl ylides, particularly in acetoxy analogues.[7,8] Methyl benzoate (**14**) is formed by attack of the methoxycarbonyl radical on benzene.

Thermolysis of **7a** in the presence of *tert*-butyl alcohol gave allyl *tert*-butyl methyl orthoformate (**15**), in support of the intermediacy of carbene **4a**, Scheme 8.



Scheme 9.

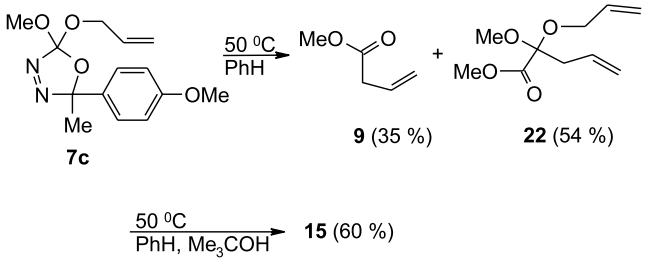


Scheme 10.

Thermolysis of **7a** in the presence of TEMPO and collection by GC afforded the known adducts of the methoxycarbonyl[4b,9] and allyl[10] radicals, **16** and **17**, respectively, Scheme 9. Yields of **16** and **17** were 13 and 18%, respectively. Methyl 3-butenoate (**9**, 17%) was also obtained.

Thermolysis of **7b** at 110 °C gave **18** (1.3 parts) and **19** (1 part), Scheme 10. With added TEMPO, adducts of deuterated allyl and TEMPO (12%, 1 part α- to 1.2 parts γ-deuterated) were obtained as well as deuteriated methyl butenoates (27%, 1.7 parts α- to 1 part γ-deuteriated). Thermolysis of **7b** in the presence of *tert*-butyl alcohol gave **21** (60%), in which all of the deuterium was alpha.

In contrast, at 50 °C, **7c** gave methyl-3-butenoate (**9**) (35%) and **22** (54%), while in the presence of *tert*-butyl alcohol it afforded **15** (60%), Scheme 11. Under the same conditions, **7d** gave **18**, **19**, and **24**, Scheme 12. In the presence of TEMPO, **7c** and **7d** afforded only trace amounts of adducts **16** and **17** (presumably D-labeled from **7d**).

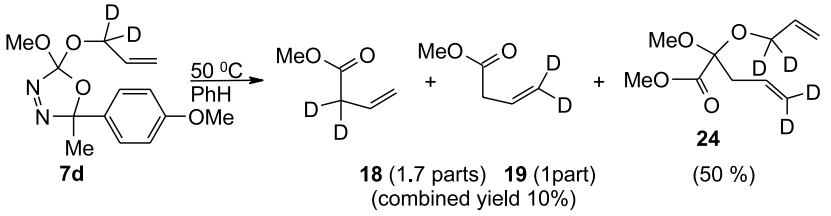


Scheme 11.

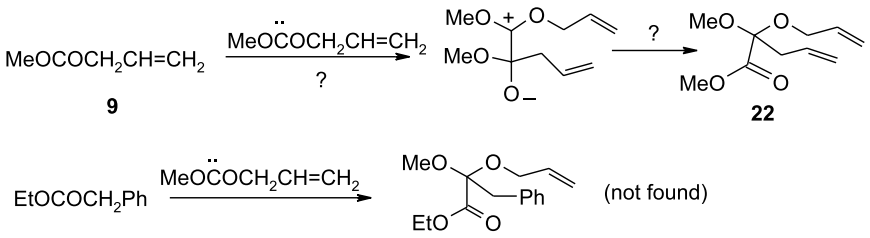
We interpret the high temperature results as follows. First, we can dismiss compounds **10**–**14**, Scheme 6, because they are really minor when dry benzene is used and we know their origins (Scheme 7). Second, we know that allyloxy(methoxy)carbene is formed from **7a**,**b** because that carbene can be captured by *tert*-butyl alcohol. Third, a part of the carbene from **7a** undergoes scission to a radical pair if it is not trapped quickly, as indicated by the finding of methyl benzoate (Scheme 6) and the TEMPO adducts of the methoxycarbonyl and allyl radicals (Scheme 9). The low yields of the TEMPO adducts suggests that methyl 3-butenoate (**9**) arises largely by sigmatropic or 1,2-migration in the carbene. Efficient, in cage radical coupling, relative to radical separation, cannot be ruled out because TEMPO would not trap radicals that are not separated by diffusion.

Thermolysis of **7b** in the presence of TEMPO (Scheme 10, top) gave a 1.7:1 ratio (α- to γ-deuteriated) of ester products rather than the 1:1 ratio observed by Hoffmann et al.[3] in their gas-phase study. This result implies that in concerted processes [1,2]- is favored over [2,3]-rearrangement. The allyl fragments captured by TEMPO are nearly equally deuteriated at the α- and γ-positions, as expected. Those adducts are expected to form in 1:1 ratio and we cannot explain the apparent 1.2:1 ratio obtained from the ^{2}H NMR spectra.

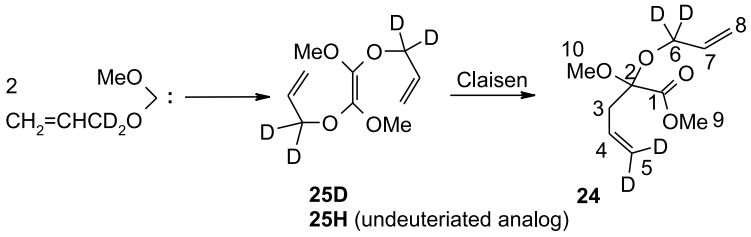
In the absence of TEMPO, where radical processes compete (Scheme 10, center) the ester ratio was down from 1.7:1 to 1.3:1, implying that the portion of ester from radical coupling is formed in a ratio closer to the 1:1 ratio expected from radicals separated by diffusion. If we assume that



Scheme 12.



Scheme 13.



Scheme 14.

TEMPO captures essentially all of the diffusion-separated radicals, then 1.7:1 represents the ratio of the methyl 4-butenoates arising from in-cage coupling, or the net effect of 1,2-migration superimposed on in-cage coupling. A cage effect, favoring coupling of the methoxycarbonyl- and the allylic radical at the allyl site originally bonded to the carbene oxygen, had been observed before,[4] in a system more likely than **7b** to react by the radical pair mechanism. The analysis cannot be more detailed because we do not know what fraction of the radicals is not scavenged by TEMPO but leads to methyl 3-butenoate without ever becoming free. Alternatively, there could be some 1,2-migration in the carbene, in competition with radical-pair formation. What is certain is that there is a radical pair component and that sigmatropic rearrangement, as observed by Baldwin[2] for a thio analogue, can be ruled out as the major mechanism because it would have led primarily to **19**.

At the lower temperature available with **7c**, fragmentation of carbene **4a** to radicals was barely detectable, as indicated by the negligible yields of TEMPO adducts. The carbene was still formed, as indicated by the formation of **9** as well as the trapping with *tert*-butyl alcohol, Scheme 11. We were surprised to find **22**, a strikingly-new product that we had never seen at the higher temperature required to thermolyze **7a**. A simple rationale for compound **22**, based on the precedented ring expansion when strained carbonyl compounds are treated with dimethoxycarbene,[11] is shown in Scheme 13. That result might follow if fragmentation (and rearrangement) of the carbene were slow at 50 °C, permitting it to accumulate sufficiently to attack **9** by addition to carbonyl carbon. Although the mechanism had attractive features, it would be surprising if the nucleophilic carbene **4a** were to add efficiently to the carbonyl group of **9**. This simple explanation lost credence when it was found that inclusion of ester **9** at the outset actually decreased the yield of **22**. Moreover, inclusion of ethyl phenylacetate did not lead to even a trace of the product of carbene attack on that ester, Scheme 13.

These results led us to look for a new mechanism for the formation of **22**. One could arrive at that structure by means of a Claisen rearrangement of the dimer of **4a**, which is an allyl vinyl ether twice over, Scheme 14. The dimer was not observed, but that is not surprising in view of the large substituent effects on rearrangement rates. For example, an alkoxy substituent at C-2 can lead to facile rearrangement at about 35 °C.[12] Coupling of two α-deuteriated carbenes, followed by Claisen rearrangement, would lead to **24** with α-deuterium in the allyloxy group and γ-deuterium in the allyl group, as shown in Scheme 14. That was shown to be the case with the ^{1}H NMR spectrum. Whereas the ^{1}H NMR spectrum of **22** has the α-CH_2 signal of the $OCH_2CH{=}CH_2$ group at 3.96 and 4.06δ, the spectrum of **24** did have any absorption at that position, indicating that the allyloxy group in **24** was $OCD_2CH{=}CH_2$. Moreover, whereas **22** has the terminal vinyl signals of the $CCH_2CH{=}CH_2$ group at 5.12 and 5.14δ, that of **24** did not absorb in that region, indicating that its C-allyl group was $CH_2CH{=}CD_2$. The rest of the signals from **24** were in agreement with that structure, taking into account the effects of deuterium. These results are in keeping with coupling of the carbene to generate a tetraalkoxyethene and sigmatropic Claisen rearrangement of the latter. Coupling of dialkoxycarbenes that do not rearrange fast has been observed before[13] and all that is required in this case is that both concerted rearrangement and fragmentation to radical pairs be slow at 50 °C.

4. Computational studies

The Claisen rearrangement of carbene dimer **25H** to **22** was modeled with Gaussian 03[14a] at the DFT level of theory using the Becke3PW91 functional.[14b,c] The optimized equilibrium geometry of **25H** and transition state **25H-TS** for its rearrangement were obtained at the B3PW91/6-31+G(d) level using the default convergence criteria.

Frequency calculations were performed to characterize **25H** and **25H-TS**. The latter, as expected, exhibited one

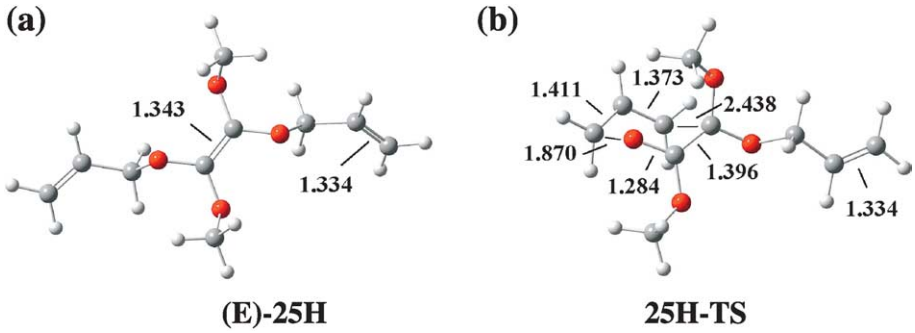


Figure 1. Geometrical structures of (a) (*E*)-1,2-diallyloxy-1,2-dimethoxyethene (**25H**) and (b) transition state (**25H-TS**) for [3,3] Claisen rearrangement of (*E*)-**25H** with selected inter-nuclear distances in angstroms.

Table 1. Thermochemical data for [3,3] Claisen rearrangement of (*E*)-1,2-diallyloxy-1,2-dimethoxyethene at the B3PW91/6-31+G(d) level

Compound	E_{elec}[a]	E_o (E_{elec}+ZPE)[a]	H[a,b] (E+RT)	G[a,c]
25H	−691.213958	−690.964018	−690.945705	−691.012480
25H-TS	−691.184465	−690.936315	−690.918880	−690.981539
Barrier (kcal mol^{-1})	18.50	17.38	16.84	19.41

[a] In hartrees.
[b] Corrected to 298.15 K. $E=E_o+E_{vib}+E_{rot}+E_{trans}$.
[c] Corrected to 298.15 K. $G=H-TS$.

imaginary frequency (−432.0 cm^{-1}). Moreover, animation of the vibration showed that it connected the reactant to the product. The conformations of (*E*)-**25H** and **25H-TS** (the transition state for the [3,3] sigmatropic rearrangement) that were used in calculating the barrier are displayed in Figure 1 and the thermochemical data are listed in Table 1. The Claisen transition state **25H-TS** is chair-like, but it is quite unsymmetrical. The C–C bond (2.438 Å) being formed is significantly longer than the C–O bond (1.870 Å) being broken. That barrier is in the region of 17–18 kcal mol^{-1} and is in good accord with the facile reaction observed experimentally; formation and rearrangement of the dimer in benzene was complete (NMR) at 50 °C in 24 h.

5. Conclusions

The reactions exhibited by allyloxy(methoxy)carbene in solution are very temperature dependent. At 110 °C, a fraction of the carbene fragments to a radical pair that couples to afford methyl 3-butenoate. Alpha deuteriated carbene ($MeOCOCD_2CH{=}CH_2$) rearranges with a preference for methyl 2,2-dideuterio-3-butenoate, suggesting incomplete rotational equilibration (a cage effect) in the radical pair and/or some competition from a 1,2-rearrangment.

At 50 °C, fragmentation to a radical pair is not important (an entropic effect) and it appears that unimolecular reactions are slow enough to permit the carbene to dimerise. A major product (**22**) is one attributed to Claisen rearrangement of the carbene dimer (**25**). The low barrier (17–18 kcal mol^{-1}) computed at the DFT level provides support for the conclusion that **22** is formed via Claisen rearrangement of the carbene dimer **25**.

6. Experimental

6.1. General

Allyl compounds of the type $XCH_2CH{=}CH_{cis}H_{trans}$, in general, have low field ^{1}H NMR spectra in which H_{cis} and H_{trans} appear as doublets. At high field or in expanded-scale low field spectra, those doublets are split further, to show geminal coupling, for example. Many of the spectra below are simplified, to have the appearance of low field spectra although a signal described as a doublet can actually look more complex if it is expanded.

^{1}H NMR spectra were obtained from solutions in C_6D_6, or in $CDCl_3$, with Bruker spectrometers operating at 200 or 600 MHz, unless otherwise indicated, and are referenced to the signal at 7.16 attributed to C_6HD_5 or to $CHCl_3$ at 7.26 ppm. ^{2}H NMR spectra were obtained at 92.1 MHz and are referenced to natural abundance deuterium in the benzene or chloroform solvent. ^{13}C NMR spectra, run at 50.9 or 150.9 MHz, in C_6D_6 or in $CDCl_3$, are referenced to the center line of the solvent triplet at 128.1 or 77.2 ppm, respectively. Mass spectra were run with a Micromass/Waters GCT time of flight instrument. 2,2-Dialkoxyoxadiazolines generally do not afford molecular ions in the mass spectrometer under any conditions and it was not possible to get high resolution data for compounds **7a**–**7d**. By NMR spectroscopy, they were >95% pure.

GC separations were performed with a 6′×4.0 mm (internal) column containing 10% OV 101. The helium flow rate was 40 mL min^{-1} and the temperature program was 60 °C for 3 min, then 5 °C per minute to 200 °C. Infrared spectra were taken with neat samples on a Bruker Tensor 27 FTIR instrument equipped with a Harrick ATR accessory and a ZnSe crystal.

6.1.1. 2-Acetoxy-2-methoxy-5,5-dimethyl-Δ^3-1,3,4-oxadiazoline (8a). This compound has been reported.[16a]

6.1.2. 2-Acetoxy-2-methoxy-5-(4-methoxyphenyl)-5-methyl-Δ^3-1,3,4-oxadiazoline (8b) (single isomer, purified by column chromatography). IR (cm^{-1}): 1747; ^1H NMR (600 MHz, $CDCl_3$) δ: 1.61 (s, 3H), 2.07 (s, 3H), 3.32 (s, 3H), 3.40 (s, 3H), 6.80 (d, $J=7.2$ Hz, 2H), 7.61 (d, $J=7.2$ Hz, 2H); ^{13}C NMR (50.9 MHz, C_6D_6) δ: 20.8, 25.0, 52.6, 54.8, 114.3, 124.5, 127.0, 128.9, 131.5, 160.3, 166.2.

6.1.3. Synthesis of 7a and 7b. To a solution of crude **8a** (7.32 g, ca. 4.7 g of pure **8a**) and 3.7 g of allyl alcohol (3.8 g of 1,1-dideuterioallyl alcohol) in methylene chloride (100 mL) was added *p*-toluenesulfonic acid (ca. 100 mg). The solution was stirred at room temperature for 3 days before it was washed twice with 50 mL of saturated $NaHCO_3$ solution and dried over $MgSO_4$. Evaporation of the solvent and column chromatography on SiO_2 with hexane (9 parts)/ethyl acetate (1 part) gave **7a** (**7b**) as colorless oils in 50–55% yield.

6.1.4. Synthesis of 7c and 7d. To a solution of $Pb(OAc)_4$ (12.4 g, 28 mmol) in methylene chloride (50 mL) was added, slowly at 0 °C, a solution of the methoxycarbonylhydrazone of 4-methoxyacetophenone (7.2 g, 28 mmol) in methylene chloride (30 mL).[15] The mixture was stirred at 0 °C for 7 h before it was washed with saturated $NaHCO_3$ solution and dried over $MgSO_4$. Evaporation of the solvent at 5–10 °C left a crude product that was a mixture of **8b** (ca 37%) and the acyclic isomer[13b,16] **8c**. To a solution of the mixture (6.0 g, 21 mmol) in 50 mL of methylene chloride was added 1.0 g of allyl alcohol, or 1,1-dideuterioallyl alcohol.[17] After 4 h of stirring at room temperature the solution was washed twice with 20 mL of saturated $NaHCO_3$ solution and dried with $MgSO_4$ before the solvent was evaporated at 5–10 °C. Column chromatography on 150 mL of SiO_2, with pentane (85 parts)/diethyl ether (15 parts) gave **7c** (**7d**) as pale yellow oils in about 49% yield. The diastereomers were not separated and the spectra that follow are those of the mixtures. It was possible to see the signals from the major diastereomer easily, but not all of the signals from the minor diastereomer were cleanly resolved. Isomer ratios were estimated from resolved signals.

Compound **7a**. ^1H NMR (200 MHz, C_6D_6) δ: 1.54 (s, 6H), 3.25 (s, 3H), 4.22–4.28 (m, 2H), 4.99 (d, $J=10.4$ Hz, 1H), 5.14 (d, $J=17.2$ Hz, 1H), 5.70– 5.89 (m, 8 lines, 1H); ^{13}C NMR (50.9 MHz, C_6D_6) δ: 23.9, 24.0, 51.7, 65.7, 116.6, 119.1, 134.1, 137.8; ^{13}C NMR (50.9 MHz, $CDCl_3$) δ: 24.0, 24.1, 52.0, 65.7, 117.2, 119.3, 133.4, 137.0. Similar oxadiazolines have absorptions at about 119 (C5) and at 137–138 (C2) ppm in their ^{13}C NMR ($CDCl_3$) spectra.[13c]

Compound **7b**. ^1H NMR (600 MHz, $CDCl_3$) δ: 1.55 (s, 3H), 1.56 (s, 3H), 3.25 (s, 3H), 5.18 (d, $J=10.4$ Hz, 1H), 5.30 (d, $J=17.2$ Hz, 1H), 5.91 (m, 1H); ^2H NMR (92.1 MHz, $CHCl_3$) δ: 4.21 (s), 4.28 (s); ^{13}C NMR (50.9 MHz, $CDCl_3$) δ: 24.1, 52.0, 117.5, 118.9 (C5), 119.3, 133.3, 137.0 (C2) (a signal from OCH_2, which would be expected at ca. 65 ppm, was absent); MS (ESI) (*m/z*): 211.1 $(M+Na)^+$, 187.1 $(M-H)^+$, 157.0 $(M-OMe)^+$.

Compound **7c** (*pale yellow oil*, *major diastereomer*, *ca. 75%*). ^1H NMR (200 MHz, C_6D_6) δ: 1.66 (s, 3H), 3.20 (s, 3H), 3.23 (s, 3H), 4.37 (t, $J=5.2$ Hz, 2H), 4.95 (d, $J=10.4$ Hz, 1H), 5.24 (d, $J=18.0$ Hz, 1H), 5.84 (m, 1H), 6.72 (d, $J=8.8$ Hz, 2H), 7.53 (d, $J=8.8$ Hz, 2H); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ: 26.4, 52.2, 55.2, 65.9, 113.9, 117.3, 121.3, 126.5, 130.5, 133.6, 138.2, 159.8; minor diastereomer (ca. 25%); ^1H NMR (200 MHz, $CDCl_3$) δ: 3.39 (s, 3H), 4.19 (t, $J=6.1$ Hz, 2H), 4.90 (d, $J=10.5$ Hz, 1H), 5.17 (m, 1H), 5.76 (m, 1H); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ: 26.2, 65.8, 117.0, 126.6, 130.3, 130.5, 133.2, 133.3 (4 signals not resolved).

Compound **7d** (*pale yellow oil*, *major diastereomer*, *ca. 72%*). ^1H NMR (600 MHz, $CDCl_3$) δ: 1.79 (s, 3H), 3.37 (s, 3H), 3.81 (s, 3H), 5.22 (d, $J=10.8$ Hz, 1H), 5.35 (d, $J=17.4$ Hz, 1H), 6.91 (d, $J=8.8$ Hz, 2H), 7.49 (d, $J=8.8$ Hz, 2H); ^2H NMR (92.1 MHz, $CHCl_3$) δ : 4.38; ^{13}C NMR (150.9 MHz, $CDCl_3$) δ: 26.4, 52.2, 55.2, 113.9, 117.3, 121.3, 126.5, 130.5, 133.6, 138.2, 159.8; minor diastereomer (ca. 28%): ^1H NMR (600 MHz, $CDCl_3$) δ: 1.69 (s, 3H), 3.60 (s, 3H), 5.13 (d, $J=10.8$ Hz,1H), 5.21 (d, $J=17.4$ Hz, 1H), 5.83 (m, 1H) (2 signals not resolved); ^2H NMR (92.1 MHz, $CHCl_3$) δ: 4.12; ^{13}C NMR (150.9 MHz, $CDCl_3$) δ: 26.2, 67.8, 113.9, 117.0, 126.6, 130.3, 133.2 (5 signals not resolved).

Compound **8d** (*yellow oil*). IR (cm^{-1}) 1765; ^1H NMR (200 MHz, C_6D_6) δ: 1.73 (s, 3H), 2.01 (s, 3H), 3.21 (s, 3H), 3.22 (s, 3H), 6.72 (d, $J=8.8$ Hz, 2H), 7.48 (d, $J=8.8$ Hz, 2H); ^{13}C NMR (150.9 MHz, C_6D_6) δ: 21.9, 24.2, 55.1, 55.4, 102.0, 114.2, 127.3, 130.8, 159.9, 162.0, 168.6.

6.1.5. Thermolysis of 7a in dry benzene. A solution of **7a** (239 mg, 1.5 mmol) in ordinary benzene (1 mL) at 110 °C for 24 h gave six products that were collected directly by GC. Yields were estimated from calibration graphs prepared by injection of benzene solutions of **9**, **10** and **11** and each yield was estimated from 2 or more runs.

6.1.6. Methyl 3-butenoate (9).[18] Yield 60%. ^1H NMR (200 MHz, C_6D_6) δ: 2.77 (d of t, 2H); 3.27 (s, 3H), 4.86–4.98 (m, 2H), 5.78–5.97 (m, 1H). MS (EI) *m/z*: 100 (M^+, 80%), 59 (96%), 41 (100%). The NMR spectrum matched that of an authentic sample of the ester, prepared by treating 3-butenonitrile with methanol.

6.1.7. Methyl-3-butenoate-d2. ^1H NMR (200 MHz, C_6D_6) δ: 2.78 (d, $J=7.0$ Hz, 2H), 3.27 (s, 3H) 5.79–5.93 (m, 1H); ^2H NMR (92.1 MHz, C_6H_6) δ: 4.92; HRMS (CI, NH_3) *m/z*: calcd for $C_5H_7D_2O_2$ $(M+H)^+$, 103.0728 found 103.0724.

6.1.8. Allyl methyl carbonate (10).[19] Colorless liquid. Yield (5.7%). ^1H NMR (600 MHz, C_6D_6) δ: 3.31 (s, 3H); 4.37 (d, $J=6.0$ Hz, 2H); 4.91 (d, $J_{cis}=10.8$ Hz, 1H), 5.08 (d, $J_{trans}=17.4$ Hz, 1H), 5.64 (m, 1H).

6.1.9. Allyl dimethyl orthoformate (11). Colorless liquid. Yield (<3%). ^1H NMR (200 MHz, C_6D_6) δ: 3.14 (s, 6H), 3.99 (d, 2H); 4.96 (s, orthoformyl H, partly superimposed on 5.00 (d, $J=9.5$ Hz, composite 2H), 5.23 (d, $J=17$ Hz, 1H), 5.77 (m, 1H). ^{13}C NMR (150.9 MHz, C_6D_6) δ: 51.0, 65.0, 113.9, 116.1, 134.9; MS *m/z*: calcd for $C_6H_{12}O_3$ 132.0786

found 132.0779. The structure was confirmed with the ^{1}H NMR spectrum of an authentic sample, prepared by reaction of trimethyl orthoformate (32 g, 0.30 mol) with allyl alcohol (8.7 g, 0.15 mol) in the presence of a catalytic amount of *p*-toluenesulfonic acid. The mixture was refluxed for 60 h, before it was washed with aqueous $NaHCO_3$ and extracted with methylene chloride. The allyl dimethyl orthoformate (4 g) was isolated by fractional distillation.

6.1.10. Allyl methyl 2-propenyl orthoformate (12). Colorless liquid. Yield (1–2%). ^{1}H NMR (600 MHz, C_6D_6) δ: 1.72 (s, 3H); 3.18 (s, 3H); 4.04 (br s, 3H, diastereotopic OCH_2 plus 1H of C=CH_2); 4.32 and 4.33 (d, J=3.3 Hz, 1H, other H of C=CH_2); 4.98 (d, J=10.6 Hz, 1H), 5.22 (d, J=17 Hz, 1H), 5.57 (s, 1H), 5.81 (m, 1H). Gradient HSQC and HMBC spectra were in agreement with the assignment. ^{13}C NMR (150.9 MHz, C_6H_6) δ: 20.0, 49.6, 64.0, 86.6, 110.5, 116.6, 133.8, 151.8; HRMS (CI, NH_3) *m/z*: calcd for $C_8H_{13}O_3$ $(M-H)^+$ 157.0865 found 157.0837.

6.1.11. Diallyl methyl orthoformate (13). Colorless liquid. Yield (2%). ^{1}H NMR (200 MHz, C_6D_6) δ: 3.16 (s, 3H); 3.99–4.03 (m, 4H), 5.00 (d, J=10.4 Hz, 2H), 5.10 (s, 1H), 5.24 (d, J=17 Hz, 2H) 5.73–5.89 (m, 2H); ^{13}C NMR (150.9 MHz, C_6D_6) δ: 51.0, 64.3, 112.3, 115.3, 134.1; HRMS (CI, NH_3) *m/z*: calcd for $C_8H_{15}O_3$ $(M+H)^+$ 159.1021 found 159.1021.

6.1.12. Methyl benzoate (14). Colorless liquid. Yield (1%). ^{1}H NMR (200 MHz, C_6D_6) δ: 3.47 (s, 3H), 7.06 (m, partly obscured by solvent signal), 8.09–8.14 (dd, 2H). Its identity was confirmed by running the ^{1}H NMR spectrum of authentic methyl benzoate in C_6D_6.

6.2. Standard procedures for thermolysis of 7a–7e

An oxadiazoline (**7a**,**b**) (1.4–1.5 mmol) in dry benzene (4.8 mL) was heated at 110 °C for 24 h in a sealed tube alone or with either TEMPO or *tert*-BuOH added. The products were separated directly by means of GC. Yields were estimated from calibration graphs and each yield was determined from two or more runs.

6.2.1. Thermolysis of 7a in dry benzene. Thermolysis of **7a** in benzene that was freshly distilled from CaH_2 gave **7** (60%). The other products (Scheme 6) gave only small displacements from the baseline of the GC trace.

6.2.2. Thermolysis of 7a in benzene containing *tert*-butyl alcohol. Thermolysis of **7a** (261 mg, 1.40 mmol) in benzene (5.0 mL) containing 424 mg (5.73 mmol) of *tert*-butyl alcohol gave **15** as a colorless oil, collected by means of GC. Yield 47%. ^{1}H NMR (600 MHz, C_6D_6) δ: 1.15 (s, 9H), 3.22 (s, 3H), 4.08 (m, 2H), 5.03 (d, J=10.2 Hz, 1H), 5.28 (d, J=17.4 Hz, 1H), 5.33 (s, 1H), 5.88 (m, 1H). ^{13}C NMR (150.9 MHz, C_6D_6) δ: 28.8, 49.8, 64.0, 73.8, 109.5, 115.7, 135.4. HRMS (EI) *m/z*: calcd for $C_8H_{15}O_2$ $(M-OMe)^+$ 143.1072, found 143.1072. Carbonate **10** (6.6%) was also obtained.

6.2.3. Thermolysis of 7a in benzene containing TEMPO. Thermolysis of **7a** (254 mg, 1.37 mmol) in dry benzene (5.0 mL) containing TEMPO (451 mg, 2.94 mmol) gave **16** (13%) and **17** (18%) that were separated and collected by GC. Compound **9** was also obtained (15%) as well as carbonate **10** (2.5%).

1-[(Methoxycarbonyl)oxy]-2,2,6,6-tetramethylpiperidine (**16**). This compound gave the ^{1}H NMR spectrum reported in the literature.[4b]

2,2,6,6-Tetramethyl-1-(2-propenyloxypiperidine) (**17**). The ^{1}H NMR spectrum of this compound matched that reported.[10]

6.2.4. Thermolysis of 7b in benzene. A solution of **7b** (243 mg, 1.29 mmol) in 4.4 mL of benzene was heated at 110 °C for 24 h. The workup was the same as those described above for **7a**.

6.2.5. Thermolysis of 7b in benzene containing TEMPO. A solution of **7b** (212 mg, 1.12 mmol) and TEMPO (399 mg, 2.62 mmol) in benzene (3.7 mL) was heated at 110 °C for 24 h. Workup was analogous to that described for **7a**.

6.2.6. Dideuterioallyl adducts of TEMPO. The mixture of α,α- and γ,γ-dideuterioallyl adducts was isolated by GC; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.11 (s, 6H), 1.16 (s, 6H), 1.49 (m, 6H), 4.28 (d, J=5.3 Hz, 0.71H, therefore 1.29D), 5.12 (d, J=10.5 Hz) and 5.27 (d, J=17.2 Hz), composite area 0.90H, therefore 1.1D), 5.88 (m, 1H). The integrations give the isomer ratio (α,α: γ,γ)=1.17:1. MS (ESI) *m/z*: 200.1 $(M+H)^+$.

6.2.7. Thermolysis of 7c. Thermolysis of **7c** at 50 °C and workup as described for **7a** gave methyl 3-butenoate (35%) and compound **22**, 54%. In the presence of dry methyl-3-butenoate (conditions like those for **7a** above) the yield of **22** dropped. Addition of dry ethyl phenylacetate to a benzene solution of **7c** did not produce any benzyl-substituted analogue of **22**.

Methyl 2-allyloxy-2-methoxy-4-pentenoate (**22**). Colorless oil, IR (cm^{-1}) 1735; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 2.67 (dt, J=7.2, 1.2 Hz, 2H, H-3), 3.32 (s, 3H, H-10), 3.77 (s, 3H, H-9), 3.96 (ddt, J=12.6, 6.0, 1.2 Hz, 1H, H-6), 4.06 (ddt, J=12.6, 6.0, 1.2 Hz, 1H, H-6), 5.12 (s, 1H, H-5, *cis*), 5.14 (dd, J=8.4, 1.2 Hz, 1H, H-5, *trans*), 5.18 (dd, J=10.8, 1.2 Hz, 1H, H-8, *cis*), 5.32 (dd, J=17.4, 1.5 Hz, 1H, H-8 *trans*), 5.65–5.71 (m, 1H, H-4), 5.91–5.97 (m, 1H, H-7); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ: 39.1, 50.0, 52.5, 64.0, 102.3, 117.2, 119.3, 130.9, 134.0, 169.3; HRMS (EI) *m/z*: calcd for $C_9H_{13}O_3$ $(M-OMe)^+$ 169.0865, found 169.0855; calcd for $C_7H_{11}O_4$ $(M-CH_2CH=CH_2)^+$ 159.0657, found 159.0640; calcd for $C_8H_{13}O_2$ $(M-MeOCO)^+$ 141.0916, found 141.0902.

6.2.8. Thermolysis of 7d. Thermolysis of **7d** at 50 °C in dry benzene and workup as described for **7a** gave methyl 2-allyloxy-2-methoxy-pentenoate (50%, partially deuteriated, numbering system in Scheme 14).

Partially deuteriated **22** Colorless oil, IR (cm^{-1}) 1735; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 2.66 (m, 2H, H-3), 3.31 (s, 3H,

H-10), 3.77 (s, 3H, H-9), 5.19 (d, $J=10.2$ Hz, 1H, H-8 cis), 5.33 (d, $J=16.8$ Hz, 1H, H-8 trans), 5.67 (bs, 1H, H-4), 5.92 (dd, $J=17.4$, 10.2 Hz, 1H, H-7); H-5 and H-6 signals were not observed, indicating that those sites were deuteriated; ^{13}C NMR (150.9 MHz, $CDCl_3$) δ: 38.9 (C-3), 50.0 (C-9), 52.5 (C-10), 63.6 (quint, $J=23.7$ Hz, C-6), 102.3 (C-2), 117.4 (C-8), 118.7 (quint, $J=23.7$ Hz, C-5), 130.7 (C-4), 133.8 (C-7), 169.3 (C-1); HRMS (EI) *m/z*: calcd for $C_{10}H_{13}D_4O_4$ $(M+H)^+$ 205.1378 found 205.1371.

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References and notes

1. (a) Iwamura, H.; Tukada, H. *Tetrahedron Lett.* **1978**, 3451. (b) Iwamura, H.; Iwai, M.; Kihara, H. *Chem. Lett.* **1977**, 881.
2. Baldwin, J. E.; Walker, J. A. *J. Chem. Soc., Chem. Commun.* **1972**, 354.
3. Hoffmann, R. W.; Hirsch, R.; Fleming, R.; Reetz, M. T. *Chem. Ber.* **1972**, *105*, 3532.
4. (a) Venneri, P. C.; Warkentin, J. *J. Am. Chem. Soc.* **1998**, *120*, 11182. Addendum: *J. Am. Chem. Soc.* **1999**, *121*, 490. (b) Merkley, N.; Venneri, P. C.; Warkentin, J. *Can. J. Chem.* **2001**, *79*, 312.
5. Kassam, K.; Pole, D. L.; El-Saidi, M.; Warkentin, J. *J. Am. Chem. Soc.* **1994**, *116*, 1161.
6. Danheiser, R. L.; Bronson, J. J.; Okano, K. *J. Am. Chem. Soc.* **1985**, *107*, 4579.
7. Majchrzak, M. W.; Warkentin, J. *Can. J. Chem.* **1989**, *67*, 1753.
8. Hitchcock, A. P.; Zweep, S.; Steel, T.; Békhazi, M.; Warkentin, J. *Can. J. Chem.* **1982**, *60*, 2914.
9. Merkley, N.; El-Saidi, M.; Warkentin, J. *Can. J. Chem.* **2000**, *78*, 356.
10. Marque, S.; Le Mercier, P. C.; Tordo, P.; Fischer, H. *Macromolecules* **2000**, *33*, 4403.
11. Venneri, P. C.; Warkentin, J. *Can. J. Chem.* **2000**, *78*, 1194.
12. Ziegler, F. E. *Chem. Rev.* **1988**, *88*, 1423.
13. (a) Couture, P.; El-Saidi, M.; Warkentin, J. *Can. J. Chem.* **1997**, *75*, 326. (b) Ge, C.-S.; Jefferson, E. A.; Moss, R. A. *Tetrahedron Lett.* **1993**, *34*, 7549. (c) El-Saidi, M.; Kassam, K.; Pole, D. L.; Tadey, T.; Warkentin, J. *J. Am. Chem. Soc.* **1992**, *114*, 8751. (d) Moss, R. A.; Wlostowski, M.; Shen, S.; Krogh-Jespersen, K.; Matro, A. *J. Am. Chem. Soc.* **1988**, *110*, 4443. (e) Scheeren, J. W.; Staps, R. J. F. M.; Nivard, R. J. F. *Recl. Trav. Chim. Pays-Bas* **1973**, *92*, 11. (f) Hoffmann, R. W.; Häuser, H. *Tetrahedron Lett.* **1964**, 197.
14. (a) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. Gaussian, Inc.: Pittsburgh, PA, 2003. (b) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1998**, *37*, 785. (c) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.
15. An analogous procedure for the generation of dimethoxy carbene at 40–50 °C has been reported. Zhou, H.; Warkentin, J. *Org. Lett.* **2005**, *7*, 487.
16. (a) Czardybon, W.; Klys, A.; Warkentin, J.; Werstiuk, N. H. *Can. J. Chem.* **2003**, *81*, 1438. (b) Lu, X.; Reid, D. L.; Warkentin, J. *Can. J. Chem.* **2001**, *79*, 319. (c) Er, H.-T.; Pole, D. L.; Warkentin, J. *Can. J. Chem.* **1996**, *74*, 1480. (d) Kassam, K.; Pole, D. L.; El-Saidi, M.; Warkentin *J. Am. Chem. Soc.* **1994**, *116*, 1161.
17. Faller, J. W.; Blankenship, C.; Whitmore, B.; Sena, S. *Inorg. Chem.* **1985**, *24*, 4483.
18. Baird, W. C., Jr.; Hartgerink, R. L.; Surridge, J. H. *J. Org. Chem.* **1985**, *50*, 4601.
19. (a) Shaikh, A. A. G.; Sivaram, S. *Ind. Eng. Chem. Res.* **1992**, *31*, 1167. (b) Woo, E. P.; Cheng, F. C. W. *J. Org. Chem.* **1986**, *51*, 3704.

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Cynanosides A–J, ten novel pregnane glycosides from *Cynanchum atratum*

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Abstract—Four novel 13,14:14,15-diseco-18-nor-pregnane-type steroidal glycosides, cynanosides A–D (**1–4**), and six novel 13,14:14,15-diseco-pregnane-type steroidal glycosides, cynanosides E–J (**5–10**) were isolated from the roots of *Cynanchum atratum*, together with one known compound, cynatratoside F (**11**). Their structures including the absolute stereochemistry were determined on the basis of spectroscopic analysis and chemical evidence, with combination of the modified Mosher method, the exciton chirality method and chemical transformations.

1. Introduction

Pregnanes and their glycosides have established themselves as an important class of biologically active compounds. Pharmacological studies in recent years, have shown that pregnane glycosides possess antitumor,[1] bone resorbing[2] and anti-fungal activities.[3] A number of such compounds have been isolated from the plants, especially the Asclepiadaceae family.[4] *Cynanchum atratum* (Asclepiadaceae) is a perennial herb native to east Asia and its roots, commonly called 'Bai Wei' in Chinese, have been used in traditional medicine for the treatment of hectic fevers, acute urinary infection and abscesses.[5] Previous phytochemical studies on this species have reported the occurrence of 14,15-seco-pregnane type and 13,14:14,15-diseco-pregnane type glycosides.[6] As our current interest in the chemistry of biologically active and structurally unique natural products, we also carried out a chemical investigation on *C. atratum*, which resulted in the isolation of ten new glycosides, cynanosides A–J (**1–10**), together with one known compound cynatratoside F (**11**)[6b] (Fig. 1). Among them, **1–3** and **4**, with two new aglycons, named cynanogenin A (**12**) and cynanogenin B (**13**), have a novel 13,14:14,15-diseco-18-nor-pregnane skeleton, while **5–8** and **9–11**, with two known aglycons, hancopregnane (**14**) and glaucogenin A (**15**), possess a 13,14:14,15-diseco-pregnane skeleton. This paper describes the isolation and structural elucidation including the absolute stereochemistry of the new compounds on the basis of spectroscopic analysis and chemical evidence, with combination of the modified Mosher method, the exciton chirality method and chemical transformations.

2. Results and discussion

Cynanoside A (**1**) was obtained as an amorphous powder. The molecular formula was determined to be $C_{41}H_{62}O_{15}$ by HRFABMS (*m/z* 817.4023 $[M+Na]^+$, calcd for 817.3986). Its IR absorptions at 3423 and 1737 cm^{-1} suggested the presence of hydroxyl and carbonyl groups. The ^{13}C NMR data (Table 1) in combination with analysis of the DEPT and HMQC spectra revealed 41 carbon signals due to six quaternary carbons, 19 methines, eight methylenes and eight methyls, of which 20 were assigned to the aglycon part including two carbonyl carbons at δ 196.0 and 178.9, and the remaining 21 were ascribed to the sugar moiety (including three methoxy groups). The ^1H NMR spectrum of the aglycon moiety showed a set of signals due to a furan ring with 2,3-disubstituents at δ 7.29 (d, $J=2.1$ Hz) and 6.74 (d, $J=2.1$ Hz), one olefinic proton at δ 5.43 (br d, $J=5.1$ Hz), two carbinylic protons at δ 4.03 (ddd, $J=11.7$, 8.8, 4.4 Hz) and 3.63 (ddd, $J=11.5$, 8.8, 5.4 Hz), and two tertiary methyl groups at δ 2.50 (s) and 1.03 (s) (Table 2). The partial structures of the aglycon (C-1 to C-4, C-6 to C-9, C-9 to C-12 and C-15 to C-16) were deduced from the detailed analyses of the DQFCOSY spectrum of **1** (Fig. 2). Interpretation of the HMBC spectral data led to the connectivities of those three partial units and quaternary

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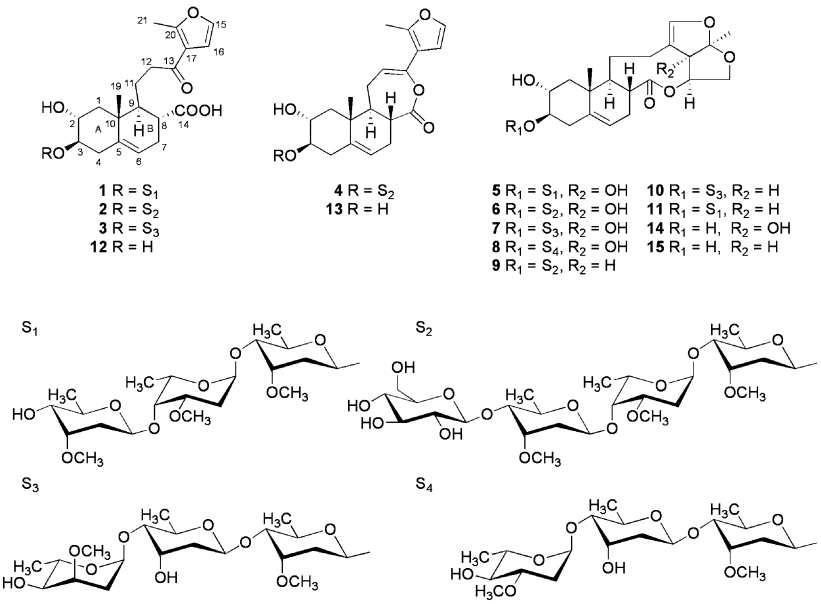


Figure 1. Structures of compounds **1**–**15**.

carbons to construct the whole structure of the aglycon. The HMBC correlations from δ_H 1.82 (H-9) to δ_C 45.0 (C-1), δ_H 5.43 (H-6) to δ_C 37.6 (C-4), as well as from δ_H 1.03 (H-19) to δ_C 45.0 (C-1), 140.0 (C-5) and 48.3 (C-9) indicated the connectivity of the A ring and B ring (Fig. 2). Further evidence of the disubstituted furan ring was provided by the HMBC correlations from δ_H 7.29 (H-15), 6.74 (H-16) and 2.50 (H-21) to δ_C 121.6 (C-17) and 157.9 (C-20), respectively. Although the correlations from δ_H 2.30 (H-11a), 2.05 (H-11b) and 6.74 (H-16) to δ_C 196.0 (C-13) though $^3J_{CH}$ were not observed, the correlations from δ_H 3.24 (H-12a) and 2.97 (H-12b) to δ_C 196.0 (C-13) though $^2J_{CH}$ and the correlation from δ_H 2.50 (H-21) to δ_C 196.0 (C-13) though $^4J_{CH}$ were obviously observed, indicating the furan ring and C-12 was connected through a ketone carbon. In addition, a carboxylic acid unit was attached at C-8 on the basis of the correlation between δ_H 2.79 (H-8) and δ_C 178.9 (C-14). The relative stereochemistry of the aglycon was elucidated by the ^{1}H–^{1}H coupling constants and the NOESY spectrum. The coupling constant between H-2 and H-3 (8.8 Hz) was typical for *trans*-diaxial protons, indicating that both oxygenated substituents were equatorial. Observed 1,3-diaxial NOE correlations for H-2/H-4β, H-2/H-19, H-4β/H-19 and H-1α/H-3 (Fig. 3) further supported the above result and revealed the chair conformation of the A ring and the axial-orientation of H-19. The *trans*-diaxial relationship of H-8 and H-9, namely, the β-orientation of H-8 and α-orientation of H-9, was suggested by the splitting pattern of H-8 (td, J=11.5, 4.9 Hz) and the NOESY correlations for H-8/H-19 and H-1α/H-9. Thus, the aglycon was concluded as 15,20-epoxy-2α,3β-dihydroxy-13,14:14,15-diseco-18-nor-pregna-5,15(16),17(20)-trien-13-one-14-oic acid, which was obtained after acid hydrolysis and named cynanogenin A (**12**) ($[\alpha]_D$ −11.0°). To determine the absolute stereochemistry of **12**, the methyl ester derivative (**16**) was treated with *p*-bromobenzoyl chloride to yield bis-*p*-bromobenzoate (**19**), to which the exciton chirality method was applied (Scheme 1).[7] As the sign of the first Cotton effect [λ_{max} 254 nm ($\Delta\varepsilon$=−31.69)] was negative, while that of the second one [λ_{max} 237 nm ($\Delta\varepsilon$=+19.13)] was positive, the chirality between the two *p*-bromobenzoyloxy groups at C-2 and C-3 was a left-hand screw, indicating 2*R*, 3*R* configurations. The same stereochemical assignment of the aglycon was also confirmed by the modified Mosher method.[8] The methyl ester derivative of **1** (**17**) was converted into its (*R*)- and (*S*)-di-MPTA ester (**22** and **23**) by treatment with (*S*)- and (*R*)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride. As shown in Scheme 1, significant $\Delta\delta$ value ($\delta_S-\delta_R$, ppm) for the protons near the derivatized chiral center C-2 was observed, suggesting that C-2 was of *R* configuration. The type of the sugar units and the sequence of the oligosaccharide chain of **1** were established by a combination of 2D NMR experiments and chemical analysis. The DQFCOSY spectrum showed that three of the sugar units belonged to 2,6-deoxy sugars (systems DS_1, DS_2 and DS_3) because each of them contained four methines, one methylene and a terminal methyl group. Moreover, the location of the methoxy groups was determined by the long-range HMBC correlations (δ_H 3.55/δ_C 77.5 for DS_1, δ_H 3.45/δ_C 74.6 for DS_2 and δ_H 3.47/δ_C 78.9 for DS_3). In the spin systems DS_1 and DS_3, a large coupling constant between H-4 and H-5 (9.6 Hz) disclosed their *trans*-diaxial relationship, while a small coupling constant of 2.8 Hz between H-3 and H-4 indicated a *cis* disposition of those protons. The above evidence suggested the DS_1 and DS_3 sugar units were both cymaropyranose and the β-glycosidic linkages were implied by the J values (9.8, 1.6 Hz) of the anomeric protons. For the remaining spin system, the signals assigned to H-3 at δ 3.83 (ddd, J=12.6, 4.6, 3.0 Hz) and H-4 at δ 4.09 (br d, J=1.8 Hz), as well as NOESY correlation between H-3 and H-5 presented the axial orientation of H-3 and H-5 and the equatorial orientation of H-4. The data allowed the identification of DS_2 sugar as diginopyranose and the α-configuration was inferred from the anomeric proton at δ 5.18 (d, J=3.5 Hz). The connection and the sequence of the sugar chain at C-3 were elucidated by the results of the HMBC experiment, which showed correlations between δ_H

Table 1. ^{13}C NMR data (500 MHz) for **1**–**11** in C_5D_5N

Positions	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	45.0	45.0	45.0	45.0	44.8	44.9	44.8	44.9	44.8	44.8	44.8
2	70.0	70.0	70.0	69.6	70.0	70.0	70.0	70.0	70.0	70.0	70.0
3	85.2	85.2	85.2	85.2	85.4	85.3	85.4	85.4	85.4	85.4	85.4
4	37.6	37.6	37.6	37.3	37.6	37.6	37.6	37.6	37.6	37.6	37.6
5	140.0	140.0	140.0	139.4	139.6	139.7	139.7	139.7	139.8	139.8	139.8
6	120.9	120.9	120.9	121.0	120.9	120.9	120.9	120.9	120.8	120.8	120.8
7	30.5	30.6	30.6	28.3	28.6	28.6	28.6	28.6	28.5	28.5	28.5
8	43.0	43.0	43.0	38.3	40.3	40.3	40.3	40.3	40.3	40.3	40.3
9	48.3	48.3	48.3	55.1	52.7	52.8	52.7	52.7	53.1	53.1	53.1
10	39.5	39.5	39.5	40.1	39.6	39.6	39.6	39.6	39.5	39.5	39.5
11	23.5	23.6	23.6	23.0	30.7	30.7	30.7	30.7	30.1	30.1	30.1
12	42.3	42.4	42.4	110.5	20.8	20.8	20.8	20.8	23.8	23.9	23.8
13	196.0	196.0	196.0	145.3	119.0	119.0	119.0	119.0	114.4	114.4	114.4
14	178.9	179.0	179.0	173.5	175.5	175.5	175.5	175.5	175.4	175.4	175.4
15	140.7	140.7	140.7	141.3	67.2	67.2	67.2	67.2	67.8	67.8	67.8
16	110.7	110.7	110.7	109.7	82.1	82.1	82.1	82.1	75.6	75.6	75.6
17	121.6	121.6	121.6	115.7	92.5	92.5	92.4	92.5	56.2	56.2	56.2
18					144.7	144.7	144.7	144.7	143.9	143.9	143.9
19	19.3	19.3	19.3	20.5	19.0	19.0	19.0	19.0	19.0	19.0	19.0
20	157.9	158.0	157.9	150.0	119.7	119.8	119.8	119.8	118.6	118.6	118.6
21	14.2	14.2	14.2	13.6	20.7	20.7	20.7	20.7	24.8	24.8	24.8
	β-D-cym I	β-D-cym I	β-D-cym	β-D-cym I	β-D-cym I	β-D-cym I	β-D-cym	β-D-cym	β-D-cym I	β-D-cym	β-D-cym I
1	97.4	97.5	97.8	97.5	97.5	97.5	97.9	97.9	97.5	97.9	97.5
2	35.2	35.2	37.0	35.2	35.2	35.2	37.0	37.1	35.2	37.0	35.2
3	77.5	77.5	77.9	77.5	77.5	77.5	77.9	77.9	77.5	77.9	77.5
4	82.1	82.1	82.9	82.1	82.1	82.1	82.9	83.0	82.1	82.9	82.1
5	69.6	69.6	69.4	69.6	69.6	69.6	69.4	69.4	69.6	69.4	69.6
6	18.4	18.3	18.2	18.4	18.4	18.4	18.2	18.2	18.4	18.2	18.4
3-OCH_3	57.4	57.4	59.0	57.4	57.4	57.4	59.0	59.0	57.4	59.0	57.4
	α-L-dgn	α-L-dgn	β-D-dgt	α-L-dgn	α-L-dgn	α-L-dgn	α-D-dgn	α-D-dgt	α-L-dgn	α-D-dgt	α-L-dgn
1	101.1	101.1	100.4	101.1	101.1	101.1	100.4	100.6	101.1	100.5	101.1
2	32.5	32.4	38.5	32.4	32.4	32.4	38.5	39.8	32.4	38.5	32.5
3	74.6	74.6	67.7	74.6	74.6	74.6	67.7	67.7	74.6	67.7	74.6
4	73.9	73.6	80.9	73.7	73.9	73.7	80.8	82.3	73.7	80.8	73.9
5	67.6	67.6	68.9	67.6	67.6	67.6	68.9	68.8	67.6	68.9	67.6
6	17.9	17.9	18.5	17.9	17.9	17.9	18.5	18.6	17.9	18.5	17.9
3-OCH_3	55.4	55.4		55.4	55.4	55.4			55.4		55.4
	β-D-cym II	β-D-cym II	α-L-cym	β-D-cym II	β-D-cym II	β-D-cym II	α-L-cym	α-L-ole	β-D-cym II	α-L-cym	β-D-cym II
1	99.5	99.3	98.5	99.3	99.5	99.3	98.4	100.4	99.3	98.5	99.5
2	35.3	36.2	32.3	36.2	35.3	36.2	32.3	35.8	36.2	32.3	35.4
3	78.9	78.1	76.6	78.2	78.9	78.2	76.6	78.9	78.2	76.6	78.9
4	74.2	83.0	72.7	83.0	74.2	83.0	72.7	76.9	83.0	72.7	74.2
5	71.1	69.6	67.3	69.6	71.1	69.6	67.3	69.6	69.6	67.3	71.1
6	18.8	18.5	18.4	18.5	18.8	18.5	18.4	18.5	18.5	18.4	18.8
3-OCH_3	58.0	58.6	56.8	58.6	58.0	58.6	56.8	57.1	58.6	56.9	58.0
		β-D-glc		β-D-glc		β-D-glc			β-D-glc		
1		106.5		106.5		106.5			106.5		
2		75.4		75.5		75.5			75.5		
3		78.4		78.4		78.4			78.4		
4		71.9		71.9		71.9			71.9		
5		78.4		78.4		78.4			78.4		
6		63.0		63.0		63.0			63.0		

cym, cymaropyranosyl; dgn, diginopyranosyl; dgt, digitoxopyranosyl; ole, oleandropyranosyl; glc, glucopyranosyl.

5.12 (cym II-H-1) and δ_C 73.9 (dgn-C-4), δ_H 5.18 (dgn-H-1) and δ_C 82.1 (cym I-C-4), and δ_H 5.19 (cym I-H-1) and δ_C 85.2 (C-3). 2,6-Deoxy sugars in the glycosides from Asclepiadaceae have been reported to be present as both D- and L-enantiomers.[9] To determine the absolute configurations of 2,6-deoxy sugars in **1**, it was hydrolyzed by 0.05 M HCl (dioxane/H_2O, 1:1) at 60 °C for 1.5 h, and analyzed by HPLC with the detection by using the refractive index (RI) and optical rotation (OR) detectors. The OR detection exhibited a negative peak for the diginose and a positive one for the cymaroses, suggesting L-form of the diginose and D-form of the cymaroses. Based on the above results, the structure of **1** was established as cynanogenin A 3-*O*-β-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-β-D-cymaropyranoside.

Cynanoside B (**2**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{47}H_{72}O_{20}$ by HRFABMS (*m/z* 979.4503 $[M+Na]^+$, calcd for 979.4514). Comparing the ^1H and ^{13}C NMR data of **2** with those of **1**, **2**

Table 2. ^{1}H NMR data (500 MHz) of **1–4** in C_5D_5N

Positions	**1**	**2**	**3**	**4**
1α	1.41 (t, 12.4)	1.41 (t, 12.4)	1.41 (t, 12.4)	1.21 (t, 12.4)
1β	2.61 (dd, 13.0, 4.4)	2.61 (dd, 13.0, 4.4)	2.60 (dd, 13.3, 4.4)	2.41[a]
2	4.03 (ddd, 11.7, 8.8, 4.4)	4.04[a]	4.01 (ddd, 11.7, 8.7, 4.4)	4.05[a]
3	3.63 (ddd, 11.5, 8.8, 5.4)	3.63 (m)	3.62[a]	3.58 (m)
4α	2.53 (dd, 13.9, 5.4)	2.53 (dd, 14.0, 5.5)	2.51 (dd, 13.5, 6.0)	2.49 (dd, 13.7, 5.7)
4β	2.48 (m)	2.48 (m)	2.47 (m)	2.47 (m)
6	5.43 (br d, 5.1)	5.43 (br d, 5.3)	5.43 (br d, 5.1)	5.40 (br d, 5.3)
7	2.69 (m), 2.32 (m)	2.69 (m), 2.33 (m)	2.69 (m), 2.32 (m)	2.77 (m), 2.18 (m)
8	2.79 (td, 11.5, 4.9)	2.80 (td, 11.2, 4.8)	2.79 (td, 11.0, 4.9)	3.15 (td, 11.2, 4.8)
9	1.82[a]	1.82[a]	1.81 (ddd, 11.0, 7.3, 2.0)	2.14 (m)
11	2.30 (m), 2.05 (m)	2.30 (m), 2.05 (m)	2.29 (m), 2.03 (m)	2.57 (m), 2.21 (m)
12a	3.24 (ddd, 16.8, 10.3, 4.8)	3.24 (ddd, 16.8, 10.3, 4.9)	3.24 (ddd, 16.8, 10.5, 5.1)	5.70 (dd, 8.4, 7.1)
12b	2.97 (ddd, 16.8, 9.9, 6.4)	2.97 (ddd, 16.8, 10.1, 6.5)	2.97 (ddd, 16.8, 10.0, 6.6)	
15	7.29 (d, 2.1)	7.29 (d, 2.1)	7.29 (d, 2.1)	7.46 (d, 2.1)
16	6.74 (d, 2.1)	6.74 (d, 2.1)	6.74 (d, 2.1)	6.63 (d, 2.1)
19	1.03 (s)	1.03 (s)	1.02 (s)	1.05 (s)
21	2.50 (s)	2.51 (s)	2.51 (s)	2.41 (s)
	β-D-cym I	β-D-cym I	β-D-cym	β-D-cym I
1	5.19 (dd, 9.8, 1.6)	5.19 (dd, 9.6, 2.0)	5.19 (dd, 9.6, 1.8)	5.19 (dd, 9.6, 1.8)
2a	1.82[a]	1.82[a]	1.87[a]	1.82 (ddd, 13.7, 9.6, 2.3)
2e	2.45[a]	2.48 (ddd, 13.5, 3.5, 2.0)	2.33[a]	2.46[a]
3	3.93 (q like, 2.8)	3.91 (q like, 2.8)	4.08 (q like, 2.8)	3.92 (q like, 2.8)
4	3.45[a]	3.45 (dd, 9.6, 2.8)	3.48 (dd, 9.6, 2.8)	3.45 (dd, 9.6, 2.8)
5	4.26 (dq, 9.6, 6.2)	4.25 (dq, 9.6, 6.2)	4.22 (dq, 9.6, 6.2)	4.25 (dq, 9.6, 6.2)
6	1.31 (d, 6.2)	1.31 (d, 6.2)	1.30 (d, 6.2)	1.32 (d, 6.2)
3-OCH_3	3.55 (s)	3.54 (s)	3.62 (s)	3.55 (s)
	α-L-dgn	α-L-dgn	β-D-dgt	α-L-dgn
1	5.18 (d, 3.5)	5.14 (br s)	5.23 (dd, 9.6, 1.9)	5.14 (br s)
2a	2.34 (td, 12.6, 3.9)	2.33[a]	1.91 (ddd, 13.5, 9.6, 3.0)	2.34 (td, 12.5, 3.5)
2e	2.00 (dd, 12.6, 4.6)	1.99 (dd, 12.4, 4.5)	2.39[a]	2.00 (dd, 12.5, 4.5)
3	3.83 (ddd, 12.6, 4.6, 3.0)	3.80 (ddd, 12.4, 4.5, 2.9)	4.46[a]	3.80 (ddd, 12.5, 4.5, 2.5)
4	4.09 (br d, 1.8)	4.04 (br s)	3.44 (dd, 9.6, 3.0)	4.05 (br d, 2.5)
5	4.24[a]	4.24[a]	4.14 (dq, 9.6, 6.2)	4.23[a]
6	1.47 (d, 6.6)	1.42 (d, 6.6)	1.40 (d, 6.2)	1.42 (d, 6.6)
3-OCH_3	3.45 (s)	3.41 (s)	3.41 (s)	
	β-D-cym II	β-D-cym II	α-L-cym	β-D-cym II
1	5.12 (dd, 9.8, 1.6)	5.15 (dd, 9.6, 2.0)	5.09 (dd, 4.2, 2.5)	5.15 (dd, 9.6, 1.6)
2a	1.86 (ddd, 13.7, 9.8, 2.5)	1.86 (ddd, 13.5, 9.6, 2.5)	1.86[a]	1.87 (ddd, 13.3, 9.6, 2.6)
2e	2.47[a]	2.37 (ddd, 13.5, 3.5, 2.0)	2.40[a]	2.39[a]
3	3.74 (q like, 2.8)	4.09 (q like, 2.8)	3.72 (q like, 3.3)	4.10 (q like, 2.8)
4	3.56[a]	3.70 (dd, 9.8, 2.8)	3.63[a]	3.71 (dd, 9.6, 2.8)
5	4.12 (dq, 9.6, 6.2)	4.28 (dq, 9.8, 6.2)	4.48 (m)	4.28 (dq, 9.6, 6.2)
6	1.53 (d, 6.2)	1.64 (d, 6.2)	1.44 (d, 6.4)	1.65 (d, 6.2)
3-OCH_3	3.47 (s)	3.52 (s)	3.40 (s)	3.52 (s)
		β-D-glc		β-D-glc
1		4.93 (d, 7.8)		4.94 (d, 7.8)
2		4.01 (dd, 8.7. 7.8)		4.02 (dd, 8.7. 7.8)
3		4.24 (t, 9.0)		4.25 (t, 9.0)
4		4.20 (t, 9.0)		4.21 (t, 9.0)
5		3.97 (ddd, 9.0, 5.3, 2.7)		3.97 (ddd, 9.0, 5.3, 2.7)
		4.40 (dd, 11.7, 5.3)		4.40 (dd, 11.7, 5.3)
6		4.55 (dd, 11.7, 2.7)		4.55 (dd, 11.7, 2.7)

cym, cymaropyranosyl; dgn, diginopyranosyl; dgt, digitoxopyranosyl; glc, glucopyranosyl.
[a] Overlapped signals.

had one more glucopyranose unit with the anomeric proton signal resonating at δ 4.93 (d, J=7.8 Hz). Hydrolysis of **2** with naringinase gave the same result that only **1** and D-glucose were detected from the hydrolyzate. The position of the glucopyranosyl group in **2** was suggested to be located at cym II-C-4 from the glycosidation shift observed at cym II-C-3 (−0.8 ppm), cym II-C-4 (+8.8 ppm) and cym II-C-5 (−1.5 ppm). This sugar linkage was also supported by the HMBC correlation from δ_H 4.93 (glc-H-1) to δ_C 83.0 (cym II-C-4). Thus, the structure of **2** was established as cynanogenin A 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-β-D-cymaropyranoside.

Cynanoside C (**3**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{40}H_{60}O_{15}$ by HRFABMS (*m/z* 803.3832 $[M+Na]^+$, calcd for 803.3830). The ^{1}H and ^{13}C NMR spectra indicated that **3** possessed the same aglycon as that of **1**, but differed in the sugar moiety. The anomeric proton signals at δ 5.23 (dd, J=9.6, 1.9 Hz), 5.19 (dd, J=9.6, 1.8 Hz) and 5.09 (dd, J=4.2, 2.5 Hz) in the ^{1}H NMR spectrum revealed that **3** has three sugar units,

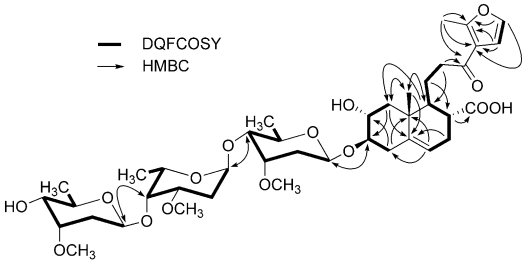


Figure 2. Key DQFCOSY and HMBC correlations of **1**.

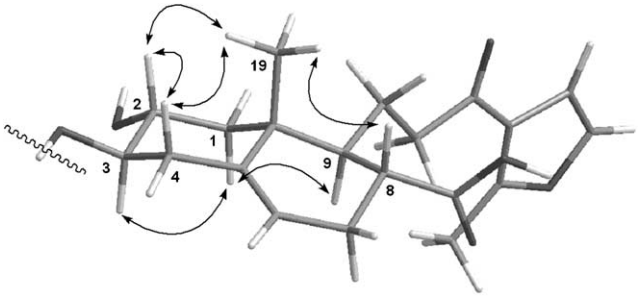
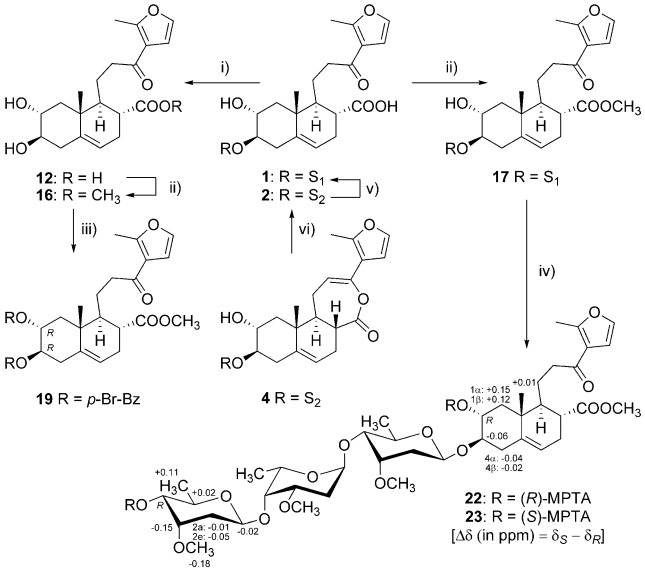


Figure 3. Observed NOESY correlations in the aglycon unit of **1**.



Scheme 1. The structures of S_1 and S_2 are shown as those in Figure 1. (i) 0.05 M HCl (dioxane/H_2O, 1:1), 60 °C, 1.5 h; (ii) CH_2N_2/Et_2O, rt, 1 h; (iii) *p*-bromobenzoyl chloride, *N*,*N*-dimethylamine pyridine, pyridine, rt, 24 h; (iv) (*S*) or (*R*)-MTPA-Cl, pyridine, rt, 4 h; (v) Naringinase, 0.1 M acetate buffer, 40 °C, 24 h; (vi) 0.3% NaOH, acetone, rt, 24 h.

two of which were determined to be α-cymaropyranose and β-cymaropyranose, and the other was elucidated as β-digitoxopyranose by a combination of the DQFCOSY, HMQC experiments and the ^1H-^1H coupling constants. The connection and the sequence of the sugar chain was deduced from observation of the HMBC correlations from δ_H 5.09 (α-cym-H-1) to δ_C 80.9 (dgt-C-4), δ_H 5.23 (dgt-H-1) to δ_C 82.9 (β-cym-C-4), and δ_H 5.19 (β-cym-H-1) to δ_C 85.2 (C-3). Acid hydroysis of **3**, similarly as carried out on **1**, suggested that the digitoxose was D-form and both D- and L-cymaroses occurred in the same molecule since OR of the cymaroses was detected to be zero, namely, no peak of the cymaroses was observed. The absolute configuration of the terminal α-cymarose was elucidated by applying the modified Mosher method after methylation of the carboxyl group at C-14. Treatment of the methyl ester derivative (**18**) with (*S*)- and (*R*)-MPTA chloride gave (*R*)- and (*S*)-MPTA ester (**24** and **25**), respectively. The signals due to the protons at α-cym-C-1, α-cym-C-2, α-cym-C-3 and α-cym-OCH_3 in the (*S*)-MPTA ester (**25**) were observed at lower fields compared with those of (*R*)-MPTA ester (**24**) ($\Delta\delta$: positive), while the signals due to protons at α-cym-C-5 and α-cym-C-6 in **25** were observed at higher field compared with those of **24**. ($\Delta\delta$: negative) (Fig. 4). Consequently, the absolute configuration of α-cym-C-4 was determined as *S*, which indicated the terminal sugar is L-cymarose. Thus, the structure of **3** was established as cynangenin A 3-*O*-α-L-cymaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-cymaropyranoside.

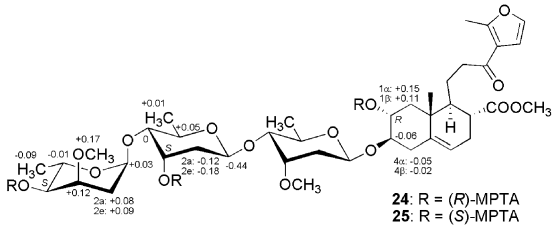


Figure 4. $\Delta\delta$ values ($\delta_S - \delta_R$) for the MPTA ester of **18** in ppm.

Cynanoside D (**4**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{47}H_{70}O_{19}$ by HRFABMS (*m*/*z* 961.4396 $[M+Na]^+$, calcd for 961.4409). The NMR spectral comparison of **4** and **2** revealed that both compounds had the same substitution pattern at C-3 and the difference was only in the signals due to the aglycon, including the absence of a ketone carbonyl carbon at δ 196.0 and an alkyl carbon at δ 42.4 with the appearance of two olefinic carbons at δ 145.3 (C-13) and 110.5 (C-12) in **4**. The assignment of each signal using 2D NMR data led to a tricyclic system through an enol lactone linkage in the structure of **4**. The lactone ring formed between C-13 and C-14 was supported by the HMBC correlations from δ_H 2.57 (H-11a), 2.21 (H-11b) and 2.41 (H-21) to δ_C 145.3 (C-13), as well as δ_H 3.15 (H-8) to δ_C 173.5 (C-14) (Fig. 5). The relative stereochemistry of the aglycon [named cynanogenin B (**13**)] elucidated by NOESY correlations (Fig. 6) was almost the same as that of **2**. Treatment of **4** with 0.3% NaOH/acetone (1:1) afforded **2**, which was confirmed on the basis of HPLC and TLC examination (Scheme 1). Thus, the

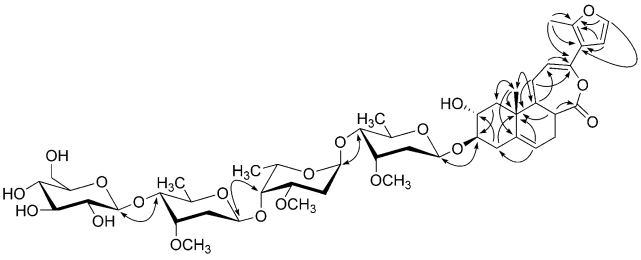


Figure 5. Key HMBC correlations of **4**.

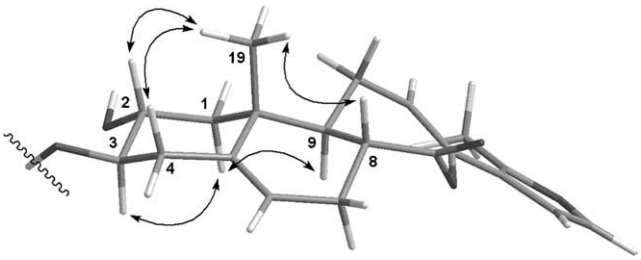


Figure 6. Observed NOESY correlations in the aglycon unit of **4**.

structure of **4** was established as cynanogenin B 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-β-D-cymaropyranoside.

Cynanoside E (**5**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{42}H_{64}O_{16}$ by HRFABMS (*m/z* 847.4111 $[M+Na]^+$, calcd for 847.4092). Of the 42 carbon signals observed in the ^{13}C NMR spectrum (Table 1), 21 were assigned to the aglycon with a pregnane skeleton. A tertiary methyl signal at δ 19.0, and two olefinic

Table 3. ^1H NMR data (500 MHz) for **5–8** in C_5D_5N

Positions	**5**	**6**	**7**	**8**
1α	1.23 (t, 12.4)	1.24 (t, 12.4)	1.22 (t, 12.4)	1.22 (t, 12.4)
1β	2.47[a]	2.47[a]	2.48 (dd, 12.8, 4.6)	2.48[a]
2	4.02[a]	4.03[a]	4.00[a]	4.00[a]
3	3.58[a]	3.59 (ddd, 11.6, 8.9, 5.8)	3.56 (ddd, 11.2, 8.9, 5.7)	3.57[a]
4	2.49[a]	2.50[a]	2.46[a]	2.47[a]
6	5.42 (br d, 5.3)	5.41 (br d, 5.3)	5.42 (br d, 5.1)	5.42 (br d, 5.3)
7	2.68 (m), 2.16 (m)	2.70 (m), 2.16 (m)	2.69 (m), 2.16 (m)	2.69 (m), 2.16 (m)
8	2.54 (td, 11.0, 4.7)	2.54 (td, 10.9, 4.9)	2.53 (td, 11.1, 4.8)	2.54[a]
9	1.38 (t, 11.0)	1.38[a]	1.38[a]	1.38 (t, 10.7)
11	2.23 (m), 1.56 (m)	2.23 (m), 1.57 (m)	2.22 (m), 1.55 (m)	2.22 (m), 1.57 (m)
12	2.47 (m), 1.85 (m)	2.47 (m), 1.84 (m)	2.48 (m), 1.85 (m)	2.47 (m), 1.83 (m)
15a	4.32 (dd, 9.4, 7.3)	4.32 (dd, 9.4, 7.4)	4.32 (dd, 9.2, 7.4)	4.32 (dd, 9.3, 7.4)
15b	4.01 (t, 9.1)	4.01 (t, 9.0)	4.01 (t, 9.1)	4.01 (t, 9.2)
16	6.00 (dd, 8.7, 7.3)	6.01 (dd, 8.8, 7.4)	6.00 (dd, 8.9, 7.4)	6.00 (dd, 8.5, 7.4)
17				
18	6.67 (s)	6.67 (s)	6.67 (s)	6.67 (s)
19	0.94 (s)	0.94 (s)	0.93 (s)	0.92 (s)
21	1.76 (s)	1.77 (s)	1.76 (s)	1.77 (s)
	β-D-cym I	β-D-cym I	β-D-cym	β-D-cym
1	5.18 (dd, 9.6, 1.8)	5.18 (dd, 9.8, 1.8)	5.18 (dd, 9.8, 2.0)	5.20 (dd, 9.8, 2.0)
2a	1.82[a]	1.81 (ddd, 13.7, 9.8, 2.4)	1.87[a]	1.91 (ddd, 13.5, 9.8, 2.5)
2e	2.44[a]	2.47[a]	2.33 (ddd, 13.3, 3.2, 2.0)	2.36 (ddd, 13.5, 3.5, 2.0)
3	3.92 (q like, 2.8)	3.91 (q like, 2.8)	4.07 (q like, 2.8)	4.11 (q like, 2.8)
4	3.45[a]	3.44 (dd, 9.6, 2.8)	3.47 (dd, 9.8, 2.8)	3.49 (dd, 9.6, 2.8)
5	4.26 (dq, 9.7, 6.2)	4.26 (dq, 9.6, 6.2)	4.21 (dq, 9.8, 6.2)	4.23 (dq, 9.6, 6.3)
6	1.32 (d, 6.2)	1.31 (d, 6.2)	1.29 (d, 6.2)	1.30 (d, 6.3)
3-OCH_3	3.55 (s)	3.54 (s)	3.62 (s)	3.65 (s)
	α-L-dgn	α-L-dgn	β-D-dgt	β-D-dgt
1	5.16 (d, 4.3)	5.13 (br d, 2.0)	5.22 (dd, 9.6, 1.7)	5.36 (dd, 9.6, 1.6)
2a	2.34 (td, 12.4, 3.8)	2.33 (td, 12.4, 3.7)	1.91 (ddd, 13.8, 9.6, 2.7)	1.97 (ddd, 13.3, 9.6, 2.5)
2e	1.99 (dd, 12.4, 4.4)	1.99 (dd, 12.4, 4.4)	2.39 (ddd, 13.8, 3.4, 1.7)	2.41[a]
3	3.82 (ddd, 12.4, 4.4, 2.9)	3.80 (ddd, 12.4, 4.4, 2.9)	4.46[a]	4.60 (q like, 2.8)
4	4.08 (br d, 1.9)	4.04 (br d, 2.1)	3.44 (dd, 9.6, 3.0)	3.53[a]
5	4.23[a]	4.23[a]	4.13 (dq, 9.6, 6.2)	4.40 (dq, 9.8, 6.4)
6	1.46 (d, 6.4)	1.41 (d, 6.4)	1.39 (d, 6.2)	1.45 (d, 6.4)
3-OCH_3	3.44 (s)	3.41 (s)		
	β-D-cym II	β-D-cym II	α-L-cym	α-L-ole
1	5.12 (dd, 9.8, 1.9)	5.14 (dd, 9.6, 1.8)	5.09 (dd, 4.1, 2.3)	5.26 (d, 3.7)
2a	1.85 (ddd, 13.4, 9.8, 2.5)	1.87 (ddd, 13.4, 9.6, 2.5)	1.86[a]	1.75 (m)
2e	2.46[a]	2.38 (ddd, 13.4, 3.4, 1.8)	2.40[a]	2.49[a]
3	3.73 (q like, 3.0)	4.10 (q like, 2.8)	3.72 (q like,3.7)	3.84 (ddd, 11.5, 9.0, 4.8)
4	3.56[a]	3.71 (dd, 9.6, 2.8)	3.62[a]	3.54 (t, 9.0)
5	4.11 (dq, 9.6, 6.2)	4.28 (dq, 9.6, 6.2)	4.48 (m)	4.41 (dq, 9.6, 6.2)
6	1.53 (d, 6.2)	1.65 (d, 6.2)	1.44 (d, 6.4)	1.52 (d, 6.2)
3-OCH_3	3.47 (s)	3.52 (s)	3.40 (s)	3.35 (s)
		β-D-glc		
1		4.94 (d, 7.8)		
2		4.02 (dd, 8.8, 7.8)		
3		4.24 (t, 8.9)		
4		4.20 (t, 8.9)		
5		3.97 (ddd, 8.9, 5.3, 2.5)		
		4.40 (dd, 11.7, 5.3)		
6		4.56 (dd, 11.7, 2.5)		

cym, cymaropyranosyl; dgn, diginopyranosyl; dgt, digitoxopyranosyl; ole, oleandropyranosyl; glc, glucopyranosyl.
[a] Overlapped signals.

carbons at δ 139.6 and 120.9, coupled with the information from the ^{1}H NMR data (Table 3) (an angular methyl proton singlet at δ 0.94 and a broad doublet olefinic proton signal at δ 5.42), indicated the aglycon is a Δ5-pregnane. The ^{1}H NMR spectrum of the aglycon moiety also showed signals for the methyl at δ 1.76 (s), one olefinic proton signal at δ 6.67 (s) connected with the trisubstituted double bond, three oxygen-substituted methine protons at δ 6.00 (dd, J=8.7, 7.3 Hz), 4.02 and 3.58, and two oxygen-substituted methylene protons at δ 4.32 (dd, J=9.4, 7.3 Hz) and 4.01 (t, J=9.1 Hz), which exhibited the characteristic feature of 13,14:14,15-diseco-pregnane-type steroidal glycoside.[10] Acid hydrolysis of **5** allowed the aglycon to be identified as hancopregnane (**14**) by comparison of their physical and spectroscopic data.[11] Although the gross structure and the relative stereochemistry of **14** have been established by X-ray analysis, the absolute stereochemistry remains to be defined. The absolute configuration of **14** was elucidated by the exciton chirality method. In the CD spectrum of *p*-bromobenzoate derivative of **14** (**20**), a split CD curve having a negative Cotton effect at 254 nm (Δε=−39.04) and a positive one at 238 nm (Δε=+32.90) was observed, indicating negative chirality between the two chromophores. Therefore, C-2 and C-3 were assigned as both *R* configurations. The assignment was also confirmed by the application of the modified Mosher method for **5** (Fig. 7). The identities of the monosaccharides and the oligosaccharide sequence were determined by extensive analysis of 1D and 2D NMR spectra, as well as the results of acid hydrolysis with the same method as **1**. Since, the glycosidation shifts were observed at C-2 (−2.4 ppm), C-3 (+8.7 ppm) and C-4 (−2.6 ppm), the sugar moiety was linked to the C-3 hydroxyl group of the aglycon. On the basis of all the above evidence, the structure of **5** was established as hancopregnane 3-*O*-β-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-β-D-cymaropyranoside.

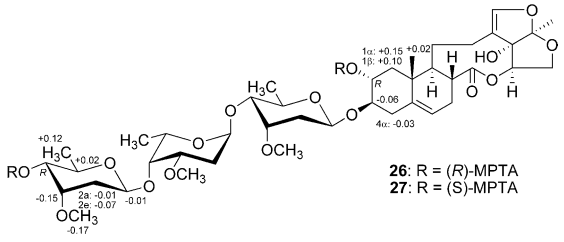


Figure 7. Δδ values ($\delta_S-\delta_R$) for the MPTA ester of **5** in ppm.

Cynanoside F (**6**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{48}H_{74}O_{21}$ by HRFABMS (*m*/*z* 1009.4646 $[M+Na]^+$, calcd for 1009.4626). The overall structure assignment was accomplished by a combination of 1D and 2D NMR spectra. From its ^{1}H and ^{13}C NMR data, it was apparent that **6** possessed the same aglycon as that of **5** and the same sugar structures as those of **2**. Enzymatic hydrolysis of **6** gave D-glucose and a deglucosyl derivative, which was identical as **5**. Thus, the structure of **6** was established as hancopregnane 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-β-D-cymaropyranoside.

Cynanoside G (**7**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{41}H_{62}O_{16}$ by HRFABMS (*m*/*z* 833.3963 $[M+Na]^+$, calcd for 833.3936). On acid hydrolysis, **7** afforded cymarose (a mixture of D- and L-form in the ratio 1:1), D-digitoxose and **14**. From its ^{1}H and ^{13}C NMR (Tables 1 and 3), it was evident that **7** possessed the same sugar moiety at C-3 as that of **3**. Thus, the structure of **7** was established as hancopregnane 3-*O*-α-L-cymaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-cymaropyranoside.

Cynanoside H (**8**) was obtained as an amorphous powder. The HRFABMS of **8** suggested the molecular formula of $C_{41}H_{62}O_{16}$ (*m*/*z* 833.3953 $[M+Na]^+$, calcd for 833.3936), which is the same as that of **7**. Comparison of the NMR spectra of **7** and **8** indicated that **8** differed from **7** only by the replacement of the terminal cymaropyranose by an oleandropyranose, which was deduced from the large coupling constant of H-3 and H-4 (9.0 Hz). The aglycon, D-cymarose and D-digitoxose were identified by comparison with authentic samples after acid hydrolysis, while the absolute configuration of oleandrose was determined by the modified Mosher method due to the lack of an authentic sugar of oleandrose. The (*R*)- and (*S*)-MPTA ester of **8** (**28** and **29**) were synthesized and the values of the chemical shift differences ($\Delta\delta=\delta_S-\delta_R$, ppm) were calculated. As shown in Figure 8, the Δδ values for protons at ole-C-2, ole-C-3 and ole-OCH_3 are positive, while negative Δδ values were observed for protons of ole-C-5 and ole-C-6, suggesting *S* configuration of ole-C-4, namely, L-form of oleandrose. Thus, the structure of **8** was established as hancopregnane 3-*O*-α-L-oleandropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-cymaropyranoside.

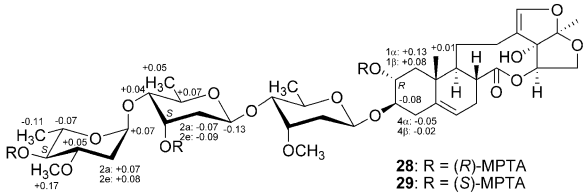


Figure 8. Δδ values ($\delta_S-\delta_R$) for the MPTA ester of **8** in ppm.

Cynanoside I (**9**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{48}H_{74}O_{20}$ by HRFABMS (*m*/*z* 993.4698 $[M+Na]^+$, calcd for 993.4671). The NMR data of **9** (Tables 1 and 4) were superimposable to those of **6**, except for the signals due to the furofuran moiety. The upfield shift of the ring protons and carbons were explainable by the absence of C-17-OH. Enzymatic hydrolysis of **9** gave D-glucose and a known compound (**11**), which was further hydrolyzed with 0.05 M HCl to give D-cymarose, L-diginose and glaucogenin A (**15**).[10b] Since, there was no study on the absolute stereochemistry of **15**, we reported here the determination of the absolute stereochemistry of **15**. The CD spectrum of the *p*-bromobenzoate derivative of **15** (**21**) exhibited a negative Cotton effect at 253 nm (Δε=−35.24) and a positive one at 238 nm (Δε=+29.76), indicating 2*R* and 3*R* configurations. Based on the above results, the structure of **9** was established as glaucogenin A 3-*O*-β-D-glucopyranosyl-

Table 4. ^{1}H NMR data (500 MHz) for **9–11** in C_5D_5N

Positions	**9**	**10**	**11**
1α	1.26 (t, 12.4)	1.25 (t, 12.4)	1.24 (t, 12.4)
1β	2.49[a]	2.49[a]	2.49[a]
2	4.04[a]	4.03 (ddd, 11.7, 8.8, 4.5)	4.01 (ddd, 11.5, 8.7, 4.6)
3	3.62 (ddd, 11.7, 9.0, 5.6)	3.60[a]	3.60 (ddd, 11.4, 8.7, 5.8)
4	2.49[a]	2.49[a]	2.49[a]
6	5.41 (br d, 5.5)	5.43 (br d, 4.0)	5.40 (br d, 5.5)
7	2.67 (m), 2.17 (m)	2.67 (m), 2.16 (m)	2.65 (m), 2.15 (m)
8	2.53[a]	2.51[a]	2.52[a]
9	1.33[a]	1.32[a]	1.32[a]
11	2.12 (m), 1.39 (m)	2.11 (m), 1.41 (m)	2.12 (m), 1.41 (m)
12	2.56 (m), 1.36 (m)	2.55 (m), 1.36 (m)	2.56 (m), 1.37 (m)
15a	4.25 (dd, 8.7, 7.0)	4.25 (dd, 8.6, 7.0)	4.24[a]
15b	3.95 (t, 9.2)	3.96 (dd, 9.6, 8.6)	3.94 (t, 9.6)
16	5.45 (dt, 9.4, 7.6)	5.45 (m)	5.43 (m)
17	3.56[a]	3.55 (d, 8.0)	3.54a
18	6.49 (s)	6.49 (s)	6.47 (s)
19	0.92 (s)	0.91 (s)	0.91 (s)
21	1.55 (s)	1.55 (s)	1.54 (s)
	β-D-cym I	β-D-cym	β-D-cym I
1	5.20 (dd, 9.8, 2.0)	5.21 (dd, 9.7, 2.0)	5.18 (dd, 9.7, 1.6)
2a	1.82 (ddd, 13.7, 9.8, 2.2)	1.88[a]	1.82 (ddd, 13.6 9.7, 2.1)
2e	2.47[a]	2.35[a]	2.44[a]
3	3.91 (q like, 2.8)	4.09 (q like, 2.8)	3.92[a]
4	3.45 (dd, 9.6, 2.8)	3.48 (dd, 9.6, 2.8)	3.44[a]
5	4.27[a]	4.23 (dq, 9.6, 6.2)	4.24[a]
6	1.32 (d, 6.2)	1.30 (d, 6.2)	1.32 (d, 6.2)
3-OCH_3	3.55 (s)	3.62 (s)	3.54 (s)
	α-L-dgn	β-D-dgt	α-L-dgn
1	5.14 (br s)	5.24 (dd, 9.7, 1.9)	5.15 (d, 3.2)
2a	2.34 (td, 12.4, 3.7)	1.92 (ddd, 13.9, 9.7, 2.8)	2.34 (td, 12.6, 3.7)
2e	1.99 (dd, 12.4, 4.4)	2.39[a]	1.99 (dd, 12.6, 4.4)
3	3.80 (ddd, 12.4, 4.4, 3.0)	4.48[a]	3.81 (ddd, 12.6, 4.4, 3.0)
4	4.05 (br s)	3.45 (dd, 9.6, 3.0)	4.07 (br s)
5	4.21[a]	4.15 (dq, 9.6, 6.3)	4.24[a]
6	1.42 (d, 6.4)	1.40 (d, 6.3)	1.45 (d, 6.4)
3-OCH_3	3.41 (s)	3.43 (s)	
	β-D-cym II	α-L-cym	β-D-cym II
1	5.14 (dd, 9.6, 2.0)	5.11 (dd, 4.1, 2.5)	5.12 (dd, 9.5, 1.5)
2a	1.87 (ddd, 13.6, 9.6, 2.2)	1.86[a]	1.84 (ddd, 13.5, 9.5, 2.5)
2e	2.38 (ddd, 13.6, 3.6, 2.0)	2.41[a]	2.46[a]
3	4.10 (q like, 2.8)	3.74 (q like, 3.5)	3.72 (q like, 2.8)
4	3.71 (dd, 9.6, 2.8)	3.62[a]	3.54[a]
5	4.28 (dq, 9.6, 6.4)	4.51 (m)	4.10 (dq, 9.6, 6.2)
6	1.65 (d, 6.4)	1.44 (d, 6.4)	1.53 (d, 6.2)
3-OCH_3	3.52 (s)	3.41 (s)	3.46 (s)
	β-D-glc		
1	4.93 (d, 7.5)		
2	4.01 (dd, 8.7, 7.5)		
3	4.22 (t, 8.7)		
4	4.20 (t, 8.7)		
5	3.97 (ddd, 8.7, 5.2, 2.5)		
	4.40 (dd, 11.7, 5.2)		
6	4.56 (dd, 11.7, 2.5)		

cym, cymaropyranosyl; dgn, diginopyranosyl; dgt, digitoxopyranosyl; glc, glucopyranosyl.
[a] Overlapped signals.

(1→4)-β-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-β-D-cymaropyranoside.

Cynanoside J (**10**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{41}H_{62}O_{15}$ by HRFABMS (*m/z* 817.4023 $[M+Na]^+$, calcd for 817.3986). Comparison of the ^{1}H and ^{13}C NMR spectra of **10** with those of **3** and **9**, suggested that the trisaccharide structure attached to C-3 of the aglycon was identical to that of **3**, while the aglycon was glaucogenin A (**15**) as that of **9**. On acid hydroysis, **10** gave cymarose (a mixture of D- and L-form in the ratio 1:1), D-digitoxose and **15**. Although the specific rotation and the NMR data of **10** were superimposable to those of a previously reported compound, glaucoside C,[10b] the inner cymarose was determined as β-D-cymarose instead of β-L-cymarose. Thus, the structure of **10** was established as glaucogenin A 3-*O*-α-L-cymaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-cymaropyranoside.

The isolated glycosides **1**–**11** were evaluated for their cytotoxic activitiy against HL-60 human promyelocytic leukemia cells.[12] **7**, **10** and **11** showed moderate cytotoxic activity against HL-60 cells with IC_{50} values of 52, 32 and 24 μM, respectively, compared with that of cisplatine used as a positive control (IC_{50} 0.41 μM). **1**–**6**, **8** and **9** were inactive at 100 μM.

3. Experimental

3.1. General

The UV spectra were obtained with a SHIMADZU BIOSPEC-MINI spectrophotometer, whereas the IR spectra were measured with a JASCO FT/IR-300E (by a KBr disk method) spectrometer. Optical rotations were measured with a JASCO DIP-370 digital polarimeter in a 0.5 dm length cell while the CD spectra were recorded on a JASCO J-720W spectropolarimeter. The FABMS and HRFABMS were taken on a JEOL JMS-700 Mstation spectrometer. The ESIMS was taken on an LCQ mass analyzer. The ^1H and ^{13}C NMR spectra were measured with a JEOL ECP-500 spectrometer with TMS as the internal reference, and chemical shifts are expressed in δ (ppm). HPLC separations were carried out with a JASCO PU-2080 HPLC system, equipped with a Shodex RI-101 Differential Refractometer detector and a Senshu Pak RP-C_{18} column (150×20 mm i.d.). For Lobar Normal-phase silica gel chromatography, a JASCO PU-980 HPLC system and a Lichroprep Si 60 column (40–63 μM, Merck) were used. Reversed-phase column chromatography (CC) was accomplished with RP-C_{18} silica gel (100–200 mesh, Chromatorex DM1020T ODS, Fuji Silysia Chemical Ltd). Silica gel CC was carried out using Silica Gel 60N (Kanto Chemical Co., Inc). TLC was conducted in Kieselgel 60 F_{254} plates (E. Merck).

3.2. Isolation of D-cymarose, L-diginose and D-digtoxose

Atratoside B[6c] (100 mg) was heated at 60 °C for 1.5 h with 0.05 M HCl (dioxane/H_2O, 1:1, 2 mL). After dioxane was removed, the solution was extracted with EtOAc (2 mL×3) to remove the aglycon. The aqueous layer was neutralized by passing through an ion-exchange resin (Amberlite MB-3, Organo, Tokyo, Japan) column and concentrated under reduced pressure to give the sugar fraction, which was chromatographed on a silica gel column with $CHCl_3$/MeOH (94:6) to give D-cymarose (**30**, 8 mg) and L-diginose (**31**, 10 mg). Using a similar procedure, D-digitoxose (**32**, 10 mg) was isolated from cynascyroside C (90 mg)[13] by silica gel column with $CHCl_3$/MeOH (9:1).

3.2.1. D-Cymarose (30). $[\alpha]_D$ +18.7° (*c* 0.24, H_2O, 24 °C, 24 h after dissolution). ^1H NMR [(500 MHz, $CDCl_3$); ~18:12:9:7 mixture of α-cymaropyranose, β-cymaropyranose, α-cymarofuranose and β-cymarofuranose]: for α-cymaropyranose, 5.11 (1H, br d, *J*=4.5 Hz, H-1), 3.96 (1H, dq, *J*=9.9, 6.4 Hz, H-5), 3.74 (1H, q like, *J*=2.7 Hz, H-3), 3.54 (3H, s, –OCH_3), 3.24 (1H, m, H-4), 2.32 (1H, ddd, *J*=14.8, 3.5, 1.3 Hz, H-2e), 1.77 (1H, ddd, *J*=14.8, 3.7, 2.6 Hz, H-2a), 1.33 (3H, d, *J*=6.4 Hz, H-6); for β-cymaropyranose, 5.02 (1H, dd, *J*=9.6, 1.8 Hz, H-1), 3.99 (1H, dq, *J*=9.1, 6.4 Hz, H-5), 3.65 (1H, m, H-3), 3.44 (3H, s, –OCH_3), 3.23 (1H, m, H-4), 2.35 (1H, ddd, *J*=14.1, 3.5, 2.2 Hz, H-2e), 1.52 (1H, ddd, *J*=14.1, 9.6, 2.5 Hz, H-2a), 1.30 (3H, d, *J*=6.4 Hz, H-6); for α-cymarofuranose, 5.48 (1H, d, *J*=4.6 Hz, H-1), 3.37 (3H, s, –OCH_3), 1.24 (3H, d, *J*=6.6 Hz, H-6); for β-cymarofuranose, 5.63 (1H, dd, *J*=5.7, 2.2 Hz, H-1), 3.30 (3H, s, –OCH_3), 1.23 (3H, d, *J*=6.4 Hz, H-6). ^{13}C NMR (125 MHz, $CDCl_3$): for α-cymar-cymaropyranose, 91.6 (C-1), 78.3 (C-3), 72.4 (C-4), 64.4 (C-5), 58.2 (–OCH_3), 31.9 (C-2), 18.1 (C-6); for β-cymar-cymaropyranose, 91.9 (C-1), 77.1 (C-3), 72.3 (C-4), 71.0 (C-5), 57.1 (–OCH_3), 34.7 (C-2), 18.2 (C-6); for α-cymarofuranose, 99.1 (C-1), 87.5 (C-3), 80.4 (C-4), 67.4 (C-5), 56.9 (–OCH_3), 39.3 (C-2), 18.7 (C-6); for β-cymarofuranose, 98.7 (C-1), 88.9 (C-3), 79.1 (C-4), 67.7 (C-5), 56.8 (–OCH_3), 40.9 (C-2), 18.1 (C-6).

3.2.2. L-Diginose (31). $[\alpha]_D$ −16.1° (*c* 0.50, H_2O, 24 °C, 24 h after dissolution). ^1H NMR [(500 MHz, $CDCl_3$); ~2:1:1:1 mixture of α-diginopyranose, β-diginopyranose, α-diginofuranose and β-diginofuranose]: for α-diginopyranose, 5.42 (1H, br d, *J*=2.8 Hz, H-1), 4.13 (q, *J*=6.6 Hz, H-5), 3.81 (1H, br d, *J*=1.5 Hz, H-4), 3.71 (1H, ddd, *J*=11.8, 5.2, 3.1 Hz, H-3), 3.41 (1H, s, –OCH_3), 1.94 (1H, dd, *J*=13.0, 5.2 Hz, H-2e), 1.85 (1H, ddd, *J*=13.0, 11.8, 3.5 Hz, H-2a), 1.31 (1H, d, *J*=6.6 Hz, H-6); for β-diginopyranose: 4.73 (1H, br d, *J*=8.6 Hz, H-1; for α-diginofuranose, 5.64 (1H, dd, *J*=5.5, 2.1 Hz, H-1); for β-diginofuranose, 5.49 (1H, m, H-1). ^{13}C NMR (125 MHz, $CDCl_3$): for α-diginopyranose: 92.3 (C-1), 74.2 (C-3), 67.8 (C-4), 65.6 (C-5), 55.6 (–OCH_3), 29.4 (C-2), 16.9 (C-6).

3.2.3. D-Digitoxose (32). $[\alpha]_D$ +50.2° (*c* 0.27, H_2O, 24 °C, 24 h after dissolution). ^1H NMR [(500 MHz, $CDCl_3$); ~7:1 mixture of α-digitoxopyranose and β-digitoxopyranose plus minor amounts of furanose epimers]: for α-digitoxopyranose, 5.25 (1H, d, *J*=3.4 Hz, H-1), 4.08 (1H, d like, *J*=2.9 Hz, H-3), 4.04 (1H, dq, *J*=9.7, 6.2 Hz, H-5), 3.25 (1H, dd, *J*=9.7, 3.1 Hz, H-4), 2.23 (1H, ddd, *J*=14.7, 3.5, 1.3 Hz, H-2e), 1.89 (1H, ddd, *J*=14.7, 3.5, 3.0 Hz, H-2a), 1.33 (3H, d, *J*=6.2 Hz, H-6); for β-digitoxopyranose, 5.18 (1H, dd, *J*=9.8, 2.1 Hz, H-1), 4.14 (1H, d like, *J*=3.2 Hz, H-3), 3.80 (1H, dq, *J*=9.6, 6.2 Hz, H-5), 3.24 (1H, dd, *J*=9.6, 3.2 Hz, H-4), 2.21 (1H, ddd, *J*=14.0, 3.5, 2.2 Hz, H-2e), 1.68 (1H, ddd, *J*=14.0, 9.8, 3.0 Hz, H-2a), 1.32 (3H, d, *J*=6.2 Hz, H-6). ^{13}C NMR (125 MHz, $CDCl_3$): for α-digitoxopyranose, 92.0 (C-1), 74.6, 73.9, 67.6 (C-3, C-4 and C-5), 32.5 (C-2), 17.9 (C-6).

3.3. Plant material

Plant material used in this research was collected from Hebei province in China and identified as *C. atratum* Bge. by Prof. Qishi Sun (Shenyang Pharmaceutical University).

3.4. Extraction and isolation

The roots of *C. atratum* (1.8 kg) were extracted with MeOH (3 L×5). Evaporation of the solvent under reduced pressure from the combined extract gave the MeOH extract (285 g). The extract was then partitioned between EtOAc and H_2O. The EtOAc layer (162 g) was subjected to silica gel CC with a gradient of $CHCl_3$/MeOH to give four fractions (1–4). Fraction 2 (82.9 g) was loaded on a Diaion HP-20 column

and eluted with 50%, 70%, 90% and 100% MeOH. The 90% MeOH fraction (25.6 g) was further separated by chromatography on a silica gel column using $CHCl_3$/MeOH (99:1, 97:3, 95:5 and 9:1) to give five fractions (A–E). Fraction B (6.17 g) was subjected to Sephadex LH-20 CC with MeOH to give three fractions (B_1–B_3). Fraction B_2 (1.72 g) was purified by HPLC (60% CH_3CN) to give **11** (70 mg). Fraction C (3.00 g) was separated by Sephadex LH-20 CC with MeOH to give three fractions (C_1–C_3). The major fraction C_2 (1.77 g) was further purified by HPLC with 45% CH_3CN to give **5** (70 mg) and with 60% CH_3CN to give **10** (3 mg). Fraction D (7.04 g) was chromatographed on a Sephadex LH-20 column with MeOH to give three fractions (D_1–D_3). Fraction D_2 (4.67 g) was fractionated by HPLC (50% CH_3CN) and Lobar Normal-phase silica gel CC [*n*-hexane/$CHCl_3$/EtOH (45:50:5)] to give **1** (98 mg), **3** (123 mg), **7** (45 mg) and **8** (8 mg). Further separation of fraction 3 (19.1 g) was achieved by ODS CC with aqueous MeOH with the ratios of 50%, 80% and 100%. The fraction eluted with 80% MeOH (7.90 g) was purified by HPLC (45% CH_3CN) to give **2** (64 mg), **4** (10 mg), **6** (13 mg) and **9** (95 mg).

3.4.1. Cynanoside A (1). Amorphous powder, $[\alpha]_D$ −26.5° (*c* 0.93, $CHCl_3$, 24 °C). IR (KBr) ν_{max}: 3423, 2926, 1737, 1687, 1065 cm^{-1}. FABMS (positive) *m/z*: 817 $[M+Na]^+$. HRFABMS (positive): observed 817.4023, calcd for $C_{41}H_{62}O_{15}Na$ $[M+Na]^+$, 817.3986. ^1H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N): see Tables 1 and 2.

3.4.2. Cynanoside B (2). Amorphous powder, $[\alpha]_D$ −25.5° (*c* 1.08, MeOH, 24 °C). IR (KBr) ν_{max}: 3423, 2928, 1733, 1686, 1067 cm^{-1}. FABMS (positive) *m/z*: 979 $[M+Na]^+$. HRFABMS (positive): observed 979.4503, calcd for $C_{47}H_{72}O_{20}Na$ $[M+Na]^+$, 979.4514. ^1H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N): see Tables 1 and 2.

3.4.3. Cynanoside C (3). Amorphous powder, $[\alpha]_D$ −44.3° (*c* 0.91, $CHCl_3$, 24 °C). IR (KBr) ν_{max}: 3432, 2930, 1721, 1656, 1058 cm^{-1}. FABMS (positive) *m/z*: 803 $[M+Na]^+$. HRFABMS (positive): observed 803.3832, calcd for $C_{40}H_{60}O_{15}Na$ $[M+Na]^+$, 803.3830. ^1H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N): see Tables 1 and 2.

3.4.4. Cynanside D (4). Amorphous powder, $[\alpha]_D$ −19.8° (*c* 1.00, MeOH, 24 °C). IR (KBr) ν_{max}: 3423, 2926, 1717, 1687, 1068 cm^{-1}. FABMS (positive) *m/z*: 961 $[M+Na]^+$. HRFABMS (positive): observed 961.4396, calcd for $C_{47}H_{70}O_{19}Na$ $[M+Na]^+$, 961.4409. ^1H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N): see Tables 1 and 2.

3.4.5. Cynanoside E (5). Amorphous powder, $[\alpha]_D$ −22.4° (*c* 1.07, $CHCl_3$, 24 °C). IR (KBr) ν_{max}: 3424, 2925, 1738, 1688, 1068 cm^{-1}. FABMS (positive) *m/z*: 847 $[M+Na]^+$. HRFABMS (positive): observed 847.4111, calcd for $C_{42}H_{64}O_{16}Na$ $[M+Na]^+$, 847.4092. ^1H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N): see Tables 1 and 3.

3.4.6. Cynanoside F (6). Amorphous powder, $[\alpha]_D$ −25.3° (*c* 1.06, $CHCl_3$, 24 °C). IR (KBr) ν_{max}: 3450, 2925, 1738, 1687, 1067 cm^{-1}. FABMS (positive) *m/z*: 1009 $[M+Na]^+$. HRFABMS (positive): observed 1009.4646, calcd for $C_{48}H_{74}O_{21}Na$ $[M+Na]^+$, 1009.4626. ^1H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N): see Tables 1 and 3.

3.4.7. Cynanoside G (7). Amorphous powder, $[\alpha]_D$ −40.5° (*c* 1.02, $CHCl_3$, 24 °C). IR (KBr) ν max: 3446, 2932, 1737, 1687, 1090 cm^{-1}. FABMS (positive) *m/z*: 833 $[M+Na]^+$. HRFABMS (positive): observed 833.3963, calcd for $C_{41}H_{62}O_{16}Na$ $[M+Na]^+$, 833.3936. ^1H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N): see Tables 1 and 3.

3.4.8. Cynanoside H (8). Amorphous powder, $[\alpha]_D$ −7.73° (*c* 0.73, $CHCl_3$, 24 °C). IR (KBr) ν_{max}: 3445, 2930, 1721, 1687, 1055 cm^{-1}. FABMS (positive) *m/z*: 833 $[M+Na]^+$. HRFABMS (positive): observed 833.3953, calcd for $C_{41}H_{62}O_{16}Na$ $[M+Na]^+$, 833.3936. ^1H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N): see Tables 1 and 3.

3.4.9. Cynanoside I (9). Amorphous powder, $[\alpha]_D$ −10.5° (*c* 1.10, $CHCl_3$, 24 °C). IR (KBr) ν_{max}: 3423, 2929, 1737, 1687, 1070 cm^{-1}. FABMS (positive) *m/z*: 993 $[M+Na]^+$. HRFABMS (positive): observed 993.4698, calcd for $C_{48}H_{74}O_{20}Na$ $[M+Na]^+$, 993.4671. ^1H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N): see Tables 1 and 4.

3.4.10. Cynanoside J (10). Amorphous powder, $[\alpha]_D$ −11.5° (*c* 0.25, $CHCl_3$, 24 °C). IR (KBr) ν_{max}: 3425, 2926, 1739, 1653, 1058 cm^{-1}. FABMS (positive) *m/z*: 817 $[M+Na]^+$. HRFABMS (positive): observed 817.3977, calcd for $C_{41}H_{62}O_{15}Na$ $[M+Na]^+$, 817.3986. ^1H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N): see Tables 1 and 4.

3.5. Acid hydrolysis of compounds 1, 5 and 11

Each of **1** (10.9 mg), **5** (9.2 mg) and **11** (10.0 mg) was heated at 60 °C for 1.5 h with 0.05 M HCl (dioxane/H_2O, 1:1, 1 mL). After dioxane was removed, the solution was extracted with EtOAc (2 mL×3) and further purified by HPLC to give **12** (2.2 mg) (with 50% MeOH as eluate solvent), **14** (2.9 mg) (55% MeOH) and **15** (2.6 mg) (60% MeOH), respectively. The aqueous layer was neutralized by passing through an ion-exchange resin (Amberlite MB-3, Organo, Tokyo, Japan) column and concentrated under reduced pressure to give the sugar fraction.

3.5.1. Compound 12. $[\alpha]_D$ −11.0° (*c* 0.22, $CHCl_3$, 24 °C). ESIMS (negative) *m/z* 361.5 $[M-H]^-$. ^1H NMR (500 MHz, C_5D_5N): 7.29 (1H, d, *J*=2.1 Hz, H-15), 6.76 (1H, d, *J*=2.1 Hz, H-16), 5.43 (1H, br d, *J*=4.7 Hz, H-6), 4.15 (1H, ddd, *J*=11.9, 9.0, 4.4 Hz, H-2), 3.83 (1H, m, H-3), 3.27 (1H, ddd, *J*=16.8, 10.5, 4.8 Hz, H-12a), 2.97 (1H, ddd, *J*=16.8, 10.1, 6.2 Hz, H-12b), 2.83 (1H, td, *J*=11.3, 5.1 Hz, H-8), 2.68 (1H, m, H-7), 2.67 (2H, m, H-4), 2.63 (1H, dd, *J*=12.8, 4.3 Hz, H-1β), 2.51 (3H, s, H-21), 2.34 (1H, m, H-7), 2.30 (1H, m, H-11), 2.05 (1H, m, H-11),

1.85 (1H, ddd, J=11.3, 7.2, 2.0 Hz, H-9), 1.56 (1H, t, J=12.3 Hz, H-1α), 1.09 (3H, s, H-19). ^{13}C NMR (125 MHz, C_5D_5N): 196.1 (C-13), 179.1 (C-14), 158.0 (C-20), 141.1 (C-15), 140.8 (C-5), 121.6 (C-17), 120.2 (C-6), 110.7 (C-16), 76.7 (C-3), 72.4 (C-2), 48.4 (C-9), 45.8 (C-1), 43.1 (C-8), 42.5 (C-12), 40.6 (C-4), 40.2 (C-10), 30.6 (C-7), 23.7 (C-11), 19.6 (C-19), 14.2 (C-21).

3.5.2. Compound 14. $[\alpha]_D$ +14.6° (c 0.26, MeOH, 24 °C). ESIMS (negative) m/z 391.2 $[M-H]^-$. ^{1}H NMR (500 MHz, C_5D_5N): 6.67 (1H, s, H-18), 6.02 (1H, dd, J=8.8, 7.4 Hz, H-16), 5.42 (1H, br d, J=4.4 Hz, H-6), 4.34 (1H, dd, J=9.3, 7.4 Hz, H-15), 4.15 (1H, ddd, J=11.7, 9.0, 4.4 Hz, H-2), 4.03 (1H, t, J=9.1 Hz, H-15), 3.80 (1H, m, H-3), 2.70 (1H, m, H-7), 2.64 (2H, m, H-4), 2.57 (1H, td, J=10.7, 4.4 Hz, H-8), 2.52 (1H, dd, J=12.9, 4.6 Hz, H-1β), 2.48 (1H, m, H-12), 2.29 (1H, m, H-11), 2.16 (1H, m, H-7), 1.86 (1H, m, H-12), 1.78 (3H, s, H-21), 1.60 (1H, m, H-11), 1.43 (1H, t, J=10.7 Hz, H-9), 1.39 (1H, t, J=12.2 Hz, H-1α), 1.00 (3H, s, H-19). ^{13}C NMR (125 MHz, C_5D_5N): 175.6 (C-14), 144.6 (C-18), 140.7 (C-5), 120.1 (C-6), 119.8 (C-20), 119.1 (C-13), 92.5 (C-17), 82.0 (C-16), 76.7 (C-3), 72.4 (C-2), 67.2 (C-15), 52.9 (C-9), 45.7 (C-1), 40.6 (C-4), 40.4 (C-8), 40.2 (C-10), 30.7 (C-11), 28.5 (C-7), 20.9 (C-12), 20.7 (C-21), 19.3 (C-19).

3.5.3. Compound 15. $[\alpha]_D$ +77.2° (c 0.20, MeOH, 24 °C). ESIMS (positive) m/z 399.2 $[M+Na]^+$. ^{1}H NMR (500 MHz, C_5D_5N): 6.47 (1H, s, H-18), 5.46 (1H, dt, J=9.5, 7.3 Hz, H-16), 5.41 (1H, br d, J=5.0 Hz, H-6), 4.26 (1H, dd, J=8.4, 7.3 Hz, H-15), 4.16 (1H, ddd, J=11.8, 8.8, 4.4 Hz, H-2), 3.97 (1H, dd, J=9.7, 8.6 Hz, H-15), 3.83 (1H, m, H-3), 3.56 (1H, dd, J=8.0, 1.3 Hz, H-17), 2.67 (1H, m, H-7), 2.56–2.65 (4H, m, H-4, H-8 and H-12), 2.51 (1H, dd, J=12.5, 4.6 Hz, H-1β), 2.18 (1H, m, H-7), 2.12 (1H, m, H-11), 1.56 (3H, s, H-21), 1.45 (1H, m, H-11), 1.42 (1H, m, H-12), 1.41 (1H, t, J=12.8 Hz, H-1α), 1.38 (1H, t, J=10.3 Hz, H-9), 0.98 (3H, s, H-19). ^{13}C NMR (125 MHz, C_5D_5N): 175.5 (C-14), 143.8 (C-18), 140.9 (C-5), 120.1 (C-6), 118.6 (C-20), 114.4 (C-13), 76.7 (C-3), 75.6 (C-16), 72.4 (C-2), 67.8 (C-15), 56.2 (C-17), 53.2 (C-9), 45.6 (C-1), 40.6 (C-8), 40.3 (C-10), 40.1 (C-4), 30.1 (C-11), 28.5 (C-7), 24.8 (C-21), 23.9 (C-12), 19.2 (C-19).

3.6. Acid hydrolysis of 3, 7, 8 and 10

A solution of **3**, **7**, **8** and **10** (each 1 mg) in 0.05 M HCl (dioxane/H_2O, 1:1, 200 μL) was heated at 60 °C for 1.5 h. After dioxane was removed, the solution was extracted with EtOAc (1 mL×3). Then, the EtOAc extract was analyzed by HPLC and TLC to identify the aglycon by comparison with the authentic samples of **12**, **14** and **15**. HPLC condition: column, YMC-Pack ODS-A, 150×4.6 mm i.d.; flow rate, 0.8 mL/min; column temperature, 40 °C; t_R, **12** 6.46 min (with 50% MeOH as eluate solvent), **14** 8.04 min (50% MeOH), **15** 6.59 min (60% MeOH); TLC condition: $CHCl_3$/MeOH (9:1), R_f, **12** 0.21; **14** 0.38; **15** 0.48.

Subsequently, the aqueous layer was treated with the same procedure as for **1**, **5** and **11**.

3.7. Enzymatic hydrolysis of 2, 6, and 9

A solution of **2**, **6** and **9** (each 1 mg) in 0.1 M acetate buffer (pH 4.0, 1.0 mL) was treated with naringinase (3 mg, Sigma Chemical Co.), and then the reaction mixture was stirred at 40 °C for 24 h. The reaction mixture was passed through a Sep-Pak C_{18} cartridge using H_2O and MeOH. The MeOH eluate was analyzed by HPLC and TLC to identify the deglucosyl derivative by comparison with the authentic samples. HPLC condition: column, YMC-Pack ODS-A, 150×4.6 mm i.d.; flow rate, 0.8 mL/min; column temperature, 40 °C; t_R, **1** 5.17 min (with 60% MeOH as eluate solvent), **5** 7.25 min (75% MeOH), **11** 7.21 min (80% MeOH). TLC condition: $CHCl_3$/MeOH (95:5), R_f, **1** 0.31; **5** 0.40; **11** 0.42.

The H_2O eluate was concentrated to give a residue of the sugar fraction.

3.8. Methylation of 12, 1 and 3

An excess ethereal solution of diazomethane was added to **12** (1.0 mg) and the mixture was allowed to stand at rt for 1 h. After removal of the solvent by evaporation, the residue was purified by HPLC with 65% MeOH to give **16** (0.9 mg). Through a similar procedure, **17** (4.0 mg) and **18** (3.0 mg) were obtained from **1** (5.2 mg) and **3** (4.1 mg) by HPLC with 80% and 83% MeOH following methylation with diazomethane.

3.8.1. Compound 16. ESIMS (positive) m/z 399.2 $[M+Na]^+$. ^{1}H NMR (500 MHz, C_5D_5N): 7.43 (1H, d, J=2.0 Hz, H-15), 6.81 (1H, d, J=2.0 Hz, H-16), 5.36 (1H, br d, J=4.8 Hz, H-6), 4.13 (1H, ddd, J=11.7, 8.8, 4.4 Hz, H-2), 3.82 (1H, m, H-3), 3.70 (3H, s, C-14-OCH_3), 2.91 (1H, ddd, J=17.0, 10.1, 4.9 Hz, H-12a), 2.83 (1H, ddd, J=17.0, 9.2, 6.6 Hz, H-12b), 2.67 (1H, td, J=11.2, 5.4 Hz, H-8), 2.64 (2H, m, H-4), 2.58 (3H, s, H-21), 2.57 (1H, dd, J=13.0, 4.4 Hz, H-1β), 2.47 (1H, m, H-7), 2.20 (1H, m, H-11), 2.17 (1H, m, H-7), 1.76 (1H, m, H-11), 1.70 (1H, m, H-9), 1.51 (1H, t, J=12.3 Hz, H-1α), 1.03 (3H, s, H-19). ^{13}C NMR (125 MHz, C_5D_5N): 195.7 (C-13), 176.9 (C-14), 158.1 (C-20), 141.1 (C-5), 141.0 (C-15), 121.5 (C-17), 119.7 (C-6), 110.6 (C-16), 76.6 (C-3), 72.2 (C-2), 51.6 (C-14-OCH_3), 48.3 (C-9), 45.6 (C-1), 42.5 (C-8), 42.0 (C-12), 40.5 (C-4), 40.1 (C-10), 30.2 (C-7), 23.2 (C-11), 19.5 (C-19), 14.3 (C-21).

3.8.2. Compound 17. $[\alpha]_D$ −31.9° (c 0.40, $CHCl_3$, 24 °C). ESIMS (positive) m/z 831.5 $[M+Na]^+$. ^{1}H NMR (500 MHz, C_5D_5N): 7.42 (1H, d, J=2.1 Hz, H-15), 6.80 (1H, d, J=2.1 Hz, H-16), 5.36 (1H, br d, J=5.0 Hz, H-6), 5.19 (1H, dd, J=9.6, 1.6 Hz, cym I-H-1), 5.16 (1H, d, J=3.4 Hz, dgn-H-1), 5.12 (1H, dd, J=9.8, 1.7 Hz, cym II-H-1), 4.22–4.28 (2H, m, cym I-H-5 and dgn-H-5), 4.12 (1H, dq, J=9.6, 6.2 Hz, cym II-H-5), 4.09 (1H, br d, J=1.6 Hz, dgn-H-4), 4.01 (1H, ddd, J=11.5, 8.8, 4.3 Hz, H-2), 3.93 (1H, q like, J=2.8 Hz, cym I-H-3), 3.83 (1H, ddd, J=12.1, 4.4, 2.7 Hz, dgn-H-3), 3.74 (1H, q like, J=2.8 Hz, cym II-H-3), 3.69 (3H, s, C-14-OCH_3), 3.62 (1H, ddd, J=11.2, 8.8, 5.5 Hz, H-3), 3.55–3.56 (4H, cym II-H-4 and cym I-OCH_3), 3.47 (3H, s, cym II-OCH_3), 3.45–3.46 (4H, cym I-H-4 and dgn-OCH_3), 2.89 (1H, ddd, J=16.8, 10.1, 5.2 Hz, H-12a),

2.82 (1H, ddd, J=16.8, 9.7, 6.6 Hz, H-12b), 2.64 (1H, td, J=11.4, 5.1 Hz, H-8), 2.58 (3H, s, H-21), 2.55 (1H, dd, J=13.3, 4.8 Hz, H-1β), 2.41–2.52 (5H, H-4, H-7, cym I-H-2 and cym II-H-2), 2.35 (1H, td, J =12.6, 4.7 Hz, dgn-H-2a), 2.17 (1H, m, H-11), 2.16 (1H, m, H-7), 2.01 (1H, dd, J=12.6, 4.6 Hz, dgn-H-2e), 1.86 (1H, ddd, J=13.5, 9.8, 2.5 Hz, cym II-H-2a), 1.82 (1H, ddd, J=13.9, 9.6, 2.0 Hz, cym I-H-2a), 1.74 (1H, m, H-11), 1.69 (1H, m, H-9), 1.54 (3H, d, J=6.2 Hz, cym II-H-6), 1.47 (3H, d, J=6.6 Hz, dgn-H-6), 1.37 (1H, t, J=12.4 Hz, H-1α), 1.31 (3H, d, J=6.2 Hz, cym I-H-6), 0.98 (3H, s, H-19). ^{13}C NMR (125 MHz, C_5D_5N): 195.7 (C-13), 176.8 (C-14), 158.2 (C-20), 141.0 (C-15), 140.1 (C-5), 121.6 (C-17), 120.5 (C-6), 110.7 (C-16), 101.1 (dgn-C-1), 99.5 (cym II-C-1), 97.4 (cym I-C-1), 85.1 (C-3), 82.1 (cym I-C-4), 79.0 (cym II-C-3), 77.5 (cym I-C-3), 74.6 (dgn-C-3), 74.2 (cym II-C-4), 73.9 (dgn-C-4), 71.1 (cym II-C-5), 69.9 (C-2), 69.6 (cym I-C-5), 67.6 (dgn-C-5), 58.0 (cym II-OCH_3), 57.4 (cym I-OCH_3), 55.4 (dgn-OCH_3), 51.6 (C-14-OCH_3), 48.2 (C-9), 44.9 (C-1), 42.4 (C-8), 41.8 (C-12), 39.5 (C-10), 37.6 (C-4), 35.4 (cym II-C-2), 35.2 (cym I-C-2), 32.5 (dgn-C-2), 30.2 (C-7), 23.2 (C-11), 19.2 (C-19), 18.9 (cym II-C-6), 18.4 (cym I-C-6), 17.9 (dgn-C-6), 14.3 (C-21).

3.8.3. Compound 18. $[\alpha]_D$ −55.6° (c 0.30, $CHCl_3$, 24 °C). ESIMS (positive) m/z 817.5 $[M+Na]^+$. HRFABMS (positive): observed 817.4006, calcd for $C_{41}H_{62}O_{15}Na$ $[M+Na]^+$, 817.3987. ^{1}H NMR (500 MHz, C_5D_5N): 7.41 (1H, d, J=2.1 Hz, H-15), 6.80 (1H, d, J=2.1 Hz, H-16), 5.37 (1H, br d, J=5.0 Hz, H-6), 5.23 (1H, dd, J=9.6, 1.8 Hz, dgt-H-1), 5.18 (1H, dd, J=9.7, 1.6 Hz, β-cym-H-1), 5.09 (1H, dd, J=3.9 2.5 Hz, α-cym-H-1), 4.45–4.50 (2H, m, α-cym-H-5 and dgt-H-3), 4.21 (1H, dq, J=9.7, 6.2 Hz, β-cym-H-5), 4.13 (1H, dq, J=9.6, 6.2 Hz, dgt-H-5), 4.07 (1H, q like, J=2.8 Hz, β-cym-H-3), 3.99 (1H, ddd, J=11.7, 8.7, 4.6 Hz, H-2), 3.72 (1H, q like, J=2.6 Hz, α-cym-H-3), 3.69 (3H, s, C-14-OCH_3), 3.62 (1H, m, α-cym-H-4), 3.62 (3H, s, β-cym-OCH_3), 3.59 (1H, ddd, J=11.2, 8.7, 5.5 Hz, H-3), 3.47 (1H, dd, J=9.7, 2.8 Hz, β-cym-H-4), 3.43 (1H, dd, J=9.6, 3.1 Hz, dgt-H-4), 3.40 (3H, s, α-cym-OCH_3), 2.89 (1H, ddd, J=16.8, 9.8, 4.8 Hz, H-12a), 2.82 (1H, ddd, J=16.8, 9.2, 6.8 Hz, H-12b), 2.64 (1H, td, J=11.2, 4.8 Hz, H-8), 2.58 (3H, s, H-21), 2.54 (1H, dd, J=13.1, 4.6 Hz, H-1β), 2.45–2.51 (3H, m, H-4 and H-7), 2.36–2.41 (2H, m, α-cym-H-2e and dgt-H-2e), 2.34 (1H, ddd, J=13.5, 3.4, 2.1 Hz, β-cym-H-2e), 2.17 (1H, m, H-11), 2.16 (1H, m, H-7), 1.91 (1H, ddd, J=13.5, 9.6, 2.7 Hz, dgt-H-2a), 1.84–1.88 (2H, m, β-cym-H-2a and α-cym-H-2a), 1.74 (1H, m, H-11), 1.69 (1H, m, H-9), 1.44 (3H, d, J=6.4 Hz, α-cym-H-6), 1.39 (3H, d, J=6.2 Hz, dgt-H-6), 1.35 (1H, t, J=12.4 Hz, H-1α), 1.29 (3H, d, J=6.2 Hz, β-cym-H-6), 0.96 (3H, s, H-19). ^{13}C NMR (125 MHz, C_5D_5N): 195.7 (C-13), 176.8 (C-14), 158.2 (C-20), 141.0 (C-15), 140.0 (C-5), 121.5 (C-17), 120.4 (C-6), 110.7 (C-16), 100.5 (dgt-C-1), 98.5 (α-cym-C-1), 97.8 (β-cym-C-1), 85.2 (C-3), 82.9 (β-cym-C-4), 80.8 (dgt-C-4), 77.9 (β-cym-C-3), 76.6 (α-cym-C-3), 72.7 (α-cym-C-4), 69.9 (C-2), 69.4 (β-cym-C-5), 68.9 (dgt-C-5), 67.8 (dgt-C-3), 67.3 (α-cym-C-5), 59.0 (β-cym-OCH_3), 56.8 (α-cym-OCH_3), 51.6 (C-14-OCH_3), 48.2 (C-9), 44.8 (C-1), 42.4 (C-8), 41.9 (C-12), 39.5 (C-10), 38.5 (dgt-C-2), 37.6 (C-4), 37.0 (β-cym-C-2), 32.3 (α-cym-C-2), 30.2 (C-7), 23.2 (C-11), 19.2 (C-19), 18.5 (dgn-C-6), 18.4 (α-cym-C-6), 18.2 (β-cym-C-6), 14.3 (C-21).

3.9. *p*-Bromobenzoylation of 16, 14 and 15

To a solution of **16** (0.9 mg), **14** (1.2 mg) and **15** (0.8 mg) in pyridine (100 μL) were added *p*-bromobrnzoyl chloride (5 mg) and *N*,*N*-dimethylamine pyridine (1 mg). The mixture was allowed to stand at rt for 24 h. After evaporation of the solvent, the residue was further purified by HPLC with 97% MeOH to give **19** (0.9 mg), **20** (1.1 mg) and **21** (0.4 mg).

3.9.1. Compound 19. UV (MeOH) λ_{max} nm (log ε): 245 (4.56). CD (MeOH) $\Delta\varepsilon$: −31.69 at 254 nm, +19.13 at 237 nm. ^{1}H NMR (500 MHz, $CDCl_3$): 7.20 (1H, d, J=2.1 Hz, H-15), 6.58 (1H, d, J=2.1 Hz, H-16), 5.51–5.58 (2H, m, H-2 and H-6), 5.14 (1H, ddd, J=11.8, 9.7, 5.5 Hz, H-3), 3.69 (3H, s, C-14-OCH_3), 2.75 (1H, ddd, J=17.2, 10.8, 4.8 Hz, H-12a), 2.54–2.64 (4H, m, H-4, H-8 and H-12b), 2.51 (3H, s, H-21), 2.40 (1H, dd, J=12.7, 4.8 Hz, H-1β), 2.46 (1H, m, H-7), 2.20 (1H, m, H-7), 1.97 (1H, m, H-11), 1.58–1.63 (2H, m, H-9 and H-11), 1.40 (1H, t, J=12.4 Hz, H-1α), 1.23 (3H, s, H-19).

3.9.2. Compound 20. UV (MeOH) λ_{max} nm (log ε): 245 (4.67). CD (MeOH) $\Delta\varepsilon$: −39.04 at 254 nm, +32.90 at 238 nm. ^{1}H NMR (500 MHz, $CDCl_3$): 6.37 (1H, s, H-18), 5.59 (1H, br d, J=5.3 Hz, H-6), 5.51 (1H, ddd, J=11.9, 9.9, 4.6 Hz, H-2), 5.38 (1H, dd, J=10.0, 7.2 Hz, H-16), 5.17 (1H, ddd, J=11.6, 9.9, 5.6 Hz, H-3), 4.19 (1H, dd, J=10.0, 7.2 Hz, H-15), 3.85 (1H, dd, J=9.9, 9.1 Hz, H-15), 2.70 (1H, dd, J=13.7, 5.5 Hz, H-4α), 2.50–2.57 (4H, m, H-4β, H-7, H-8 and H-12), 2.46 (1H, dd, J=12.6, 4.8 Hz, H-1β), 2.11–2.18 (2H, m, H-7 and H-11), 1.48 (3H, s, H-21), 1.36–1.46 (3H, m, H-9, H-11 and H-12), 1.39 (1H, t, J=12.3 Hz, H-1α), 1.15 (3H, s, H-19).

3.9.3. Compound 21. UV (MeOH) λ_{max} nm (log ε): 244 (4.67). CD (MeOH) $\Delta\varepsilon$: −35.24 at 253 nm, +29.76 at 238 nm. ^{1}H NMR (500 MHz, $CDCl_3$): 6.24 (1H, s, H-18), 5.59 (1H, br d, J=5.5 Hz, H-6), 5.51 (1H, ddd, J=11.9, 9.7, 4.7 Hz, H-2), 5.32 (1H, dt, J=9.6, 7.1 Hz, H-16), 5.16 (1H, ddd, J=11.7, 9.7, 5.9 Hz, H-3), 4.17 (1H, dd, J=8.6, 7.1 Hz, H-15), 3.86 (1H, t, J=9.3 Hz, H-15), 3.44 (1H, dd, J=8.6, 1.1 Hz, H-17), 2.69 (1H, dd, J=13.7, 5.9 Hz, H-4α), 2.48–2.58 (4H, m, H-4β, H-7, H-8 and H-12), 2.43 (1H, dd, J=12.8, 4.6 Hz, H-1β), 2.06–2.14 (2H, m, H-7 and H-11), 1.30–1.54 (4H, m, H-1α, H-9, H-11 and H-12), 1.26 (3H, s, H-21), 1.15 (3H, s, H-19).

3.10. Preparation of the (*R*)-MPTA ester and (*S*)-MPTA ester from 17, 18, 5 and 8

(*S*)- and (*R*)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (10 μL each) were added to the solution of **17** (0.9 mg) in pyridine (100 μL). After being stirred at rt for 4 h, the mixture was evaporated to dryness and purified by HPLC with 92% MeOH to give (*R*)-MPTA ester **22** (1.0 mg) and (*S*)-MPTA ester **23** (0.9 mg). Using a similar procedure, (*R*)-MPTA ester [**24** (1.0 mg), **26** (4.9 mg) and **28** (1.2 mg)] and (*S*)-MPTA ester [**25** (1.0 mg), **27** (4.9 mg) and **29** (1.2 mg)] were obtained from **18** (1.1 mg), **5** (5.0 mg) and **8** (1.3 mg), respectively.

3.10.1. Compound 22. ^{1}H NMR (500 MHz, $CDCl_3$): 7.22

(1H, d, J=2.0 Hz, H-15), 6.55 (1H, d, J=2.0 Hz, H-16), 5.44 (1H, br d, J=5.3 Hz, H-6), 5.30 (1H, ddd, J=12.0, 9.6, 4.7 Hz, H-2), 4.98 (1H, d, J=3.7 Hz, dgn-H-1), 4.86 (1H, dd, J=9.4, 1.8 Hz, cym I-H-1), 4.82 (1H, dd, J=9.6, 1.9 Hz, cym II-H-1), 4.72 (1H, dd, J=9.8, 3.2 Hz, cym II-H-4), 3.95–3.99 (3H, m, dgn-H-5, cym II-H-3 and cym II-H-5), 3.86 (2H,m, dgn-H-4 and H-3), 3.78 (1H, dq, J= 9.6, 6.2 Hz, cym I-H-5), 3.69 (1H, m, cym I-H-3), 3.67 (3H, s, C-14-OCH_3), 3.60 (1H, m, dgn-H-3), 3.40 (3H, s, cym I-OCH_3), 3.39 (3H, s, dgn-OCH_3), 3.35 (3H, s, cym II-OCH_3), 3.15 (1H, dd, J=9.6, 2.9 Hz, cym I-H-4), 2.66 (1H, dd, J=17.0, 10.3, 5.1 Hz, H-12a), 2.55 (3H, s, H-21), 2.53–2.56 (3H, m, H-4α, H-8 and H-12b), 2.39 (1H, ddd, J=14.2, 3.4, 1.8 Hz, cym II-H-2e), 2.31 (1H, m, H-7), 2.28 (1H, m, H-4β), 2.18 (1H, dd, J=12.8, 5.1 Hz, H-1β), 2.17 (1H, m, H-7), 2.16 (1H, ddd, J=13.8, 3.5, 2.3 Hz, cym I-H-2e), 2.03 (1H, td, J =12.8, 3.9 Hz, dgn-H-2a), 1.90 (1H, m, H-11), 1.87 (1H, m, cym II-H-2a), 1.83 (1H, m, dgn-H-2e), 1.48–1.53 (3H, m, H-9, H-11 and cym I-H-2a), 1.24 (3H, d, J=6.6 Hz, dgn-H-6), 1.17 (1H, t, J=12.4 Hz, H-1α), 1.12 (3H, s, H-19), 1.12 (3H, d, J=6.2 Hz, cym I-H-6), 1.10 (3H, d, J=6.2 Hz, cym II-H-6).

3.10.2. Compound 23. ^{1}H NMR (500 MHz, $CDCl_3$): 7.22 (1H, d, J=2.0 Hz, H-15), 6.57 (1H, d, J=2.0 Hz, H-16), 5.45 (1H, br d, J=5.0 Hz, H-6), 5.32 (1H, ddd, J=11.9, 9.8, 4.8 Hz, H-2), 4.91 (1H, d, J=3.5 Hz, dgn-H-1), 4.80 (1H, dd, J=9.6, 1.7 Hz, cym II-H-1), 4.76 (1H, dd, J=9.4, 1.6 Hz, cym I-H-1), 4.75 (1H, dd, J=9.7, 3.1 Hz, cym II-H-4), 4.01 (1H, dq, J=9.9, 6.2 Hz, cym II-H-5), 3.93 (1H, q, J=6.5 Hz, dgn-H-5), 3.82–3.85 (2H, m, dgn-H-4 and cym II-H-3), 3.80 (1H, ddd, J=11.4, 9.8, 5.5 Hz, H-3), 3.68 (3H, s, C-14-OCH_3), 3.67 (1H, m, cym I-H-5), 3.60 (1H, m, cym I-H-3), 3.58 (1H, ddd, J=12.4, 4.3, 2.5 Hz, dgn-H-3), 3.39 (3H, s, cym I-OCH_3), 3.38 (3H, s, dgn-OCH_3), 3.17 (3H, s, cym II-OCH_3), 2.96 (1H, dd, J=9.6, 3.0 Hz, cym I-H-4), 2.66 (2H, m, H-12), 2.55 (3H, s, H-21), 2.54 (1H, m, H-8), 2.52 (1H, dd, J=13.7, 5.5 Hz, H-4α), 2.26–2.35 (4H, m, H-1β, H-4β, H-7 and cym II-H-2e), 2.16 (1H, m, H-7), 2.07 (1H, ddd, J=13.8, 3.5, 1.8 Hz, cym I-H-2e), 2.02 (1H, td, J =12.6, 4.6 Hz, dgn-H-2a), 1.97 (1H, m, H-11), 1.86 (1H, m, cym II-H-2a), 1.82 (1H, dd, J=12.6, 4.6 Hz, dgn-H-2e), 1.57 (1H, m, H-11), 1.54 (1H, m, H-9), 1.36 (1H, ddd, J= 13.8, 9.4, 2.1 Hz, cym I-H-2a), 1.32 (1H, t, J=12.6 Hz, H-1α), 1.22 (3H, d, J =6.6 Hz, dgn-H-6), 1.21 (3H, d, J= 6.4 Hz, cym II-H-6), 1.13 (3H, s, H-19), 0.98 (3H, d, J= 6.2 Hz, cym I-H-6).

3.10.3. Compound 24. ^{1}H NMR (500 MHz, $CDCl_3$): 7.21 (1H, d, J=2.1 Hz, H-15), 6.55 (1H, d, J=2.1 Hz, H-16), 5.59 (1H, q like, J=2.8 Hz, dgt-H-3), 5.45 (1H, br d, J= 5.3 Hz, H-6), 5.28 (1H, ddd, J=11.9, 9.7, 4.8 Hz, H-2), 4.86 (br s, α-cym-H-1), 4.85 (dd, J=9.6, 2.2 Hz, β-cym-H-1), 4.78 (1H, dd, J=8.7, 3.2 Hz, α-cym-H-4), 4.70 (1H, dd, J= 9.6, 1.7 Hz, dgt-H-1), 4.26 (1H, dq, J=8.7, 6.4 Hz, α-cym-H-5), 3.80–3.86 (2H, m, dgt-H-5 and H-3), 3.75–3.79 (2H, m, β-cym-H-3 and β-cym-H-5), 3.71 (1H, m, α-cym-H-3), 3.67 (3H, s, C-14-OCH_3), 3.43 (3H, s, β-cym-OCH_3), 3.29 (1H, dd, J=9.5, 2.8 Hz, dgt-H-4), 3.07 (1H, d, J=9.6, 3.0 Hz, β-cym-H-4), 3.07 (3H, s, α-cym-OCH_3), 2.66 (1H, ddd, J=16.9, 10.1, 5.0 Hz, H-12), 2.57 (1H, dd, J=14.0, 5.2 Hz, H-4α), 2.55 (3H, s H-21), 2.54 (1H, m, H-12), 2.52 (1H, m, H-8), 2.23–2.35 (2H, m, H-4β and H-7), 2.18 (1H, dd, J=12.6, 4.8 Hz, H-1β), 2.16 (1H, m, H-7), 2.09–2.14 (2H, m, α-cym-H-2e and dgt-H-2e), 2.06 (1H, ddd, J=13.8, 3.2, 2.2 Hz, β-cym-H-2e), 1.92 (1H, ddd, J=14.2, 9.6, 2.3 Hz, dgt-H-2a), 1.90 (1H, m, H-11), 1.84 (1H, m, α-cym-H-2a), 1.47–1.51 (3H, m, H-9, H-11 and β-cym-H-2a), 1.24 (3H, d, J=6.2 Hz, dgt-H-6), 1.21 (3H, d, J=6.4 Hz, α-cym-H-6), 1.16 (1H, t, J=12.4 Hz, H-1α), 1.12 (3H, s, H-19), 1.04 (3H, d, J=6.1 Hz, β-cym-H-6).

3.10.4. Compound 25. ^{1}H NMR (500 MHz, $CDCl_3$): 7.21 (1H, d, J=2.1 Hz, H-15), 6.57 (1H, d, J=2.1 Hz, H-16), 5.58 (1H, q like, J=2.8 Hz, dgt-H-3), 5.44 (1H, br d, J= 5.3 Hz, H-6), 5.30 (1H, ddd, J=11.9, 9.7, 4.8 Hz, H-2), 4.89 (1H, dd, J=4.7, 2.7 Hz, α-cym-H-1), 4.80 (1H, dd, J=8.7, 3.4 Hz, α-cym-H-4), 4.70 (dd, J=9.6, 1.9 Hz, β-cym-H-1), 4.24–4.28 (2H, m, dgt-H-1 and α-cym-H-5), 3.80–3.86 (2H, m, dgt-H-5 and H-3), 3.83 (1H, m, α-cym-H-3), 3.77 (1H, m, dgt-H-5), 3.79 (1H, ddd, J=11.7, 9.7, 5.0, H-3), 3.68 (3H, s, C-14-OCH_3), 3.58–3.61 (2H, m, β-cym-H-3 and β-cym-H-5), 3.37 (3H, s, β-cym-OCH_3), 3.29 (1H, dd, J= 9.5, 2.8 Hz, dgt-H-4), 3.24 (3H, s, α-cym-OCH_3), 2.60–3.68 (3H, m, β-cym-H-4 and H-12), 2.56 (3H, s H-21), 2.54 (1H, m, H-8), 2.52 (1H, dd, J=13.7, 5.0 Hz, H-4α), 2.34 (1H, m, H-7), 2.29 (1H, dd, J=12.8, 4.8 Hz, H-1β), 2.24 (1H, m, H-4β), 2.20 (1H, m, α-cym-H-2e), 2.14 (1H, m, H-7), 1.89–1.97 (4H, m, H-11, β-cym-H-2e, dgt-H-2e and α-cym-H-2a), 1.80 (1H, ddd, J=14.2, 9.6, 2.3 Hz, dgt-H-2a), 1.49–1.60 (2H, m, H-9 and H-11), 1.31 (1H, t, J=12.6 Hz, H-1α), 1.28 (1H, ddd, J=13.8, 9.6, 2.5 Hz, β-cym-H-2a), 1.25 (3H, d, J=6.2 Hz, dgt-H-6), 1.12 (3H, s, H-19), 1.12 (3H, d, J= 6.2 Hz, α-cym-H-6), 0.76 (3H, d, J=6.2 Hz, β-cym-H-6).

3.10.5. Compound 26. ^{1}H NMR (500 MHz, $CDCl_3$): 6.39 (1H, s, H-18), 5.48 (1H, br d, J=5.1 Hz, H-6), 5.36 (1H, dd, J=10.0, 7.2 Hz, H-16), 5.27 (1H, ddd, J=11.9, 9.6, 4.7 Hz, H-2), 4.96 (1H, d, J=3.4 Hz, dgn-H-1), 4.85 (1H, dd, J= 9.4, 1.6 Hz, cym I-H-1), 4.81 (1H, dd, J=9.6, 1.7 Hz, cym II-H-1), 4.71 (1H, dd, J=9.9, 3.2 Hz, cym II-H-4), 4.16 (1H, dd, J=8.9, 7.2 Hz, H-15a), 3.98–3.99 (3H, m, dgn-H-5, cym II-H-3 and cym II-H-5), 3.84–3.88 (2H, m, H-3 and dgn-H-4), 3.83 (1H, t, J=9.9 Hz, H-15b), 3.77 (1H, dq, J= 9.6, 6.4 Hz, cym I-H-5), 3.68 (1H, q like, J=2.9 Hz, cym I-H-3), 3.59 (1H, m, dgn-H-3), 3.40 (3H, s, cym I-OCH_3), 3.39 (3H, s, dgn-OCH_3), 3.34 (3H, s, cym II-OCH_3), 3.14 (1H, dd, J=9.6, 2.9 Hz, cym I-H-4), 2.55 (1H, dd, J=13.7, 5.4 Hz, H-4α), 2.48, (1H, m, H-12), 2.42–2.46 (2H, m, H-7 and H-8), 2.39 (1H, ddd, J=14.2, 3.8, 1.9 Hz, cym II-H-2e), 2.25 (1H, m, H-4β), 2.16 (1H, dd, J=12.9, 5.1 Hz, H-1β), 2.14 (1H, ddd, J=14.2, 3.5, 2.1 Hz, cym I-H-2e), 2.05–2.08 (2H, m, H-7 and H-11), 2.03 (1H, td, J=12.6, 4.3 Hz, dgn-H-2a), 1.87 (1H, ddd, J=14.2, 9.6, 2.5 Hz, cym II-H-2a), 1.82 (1H, dd, J=12.6, 4.6 Hz, dgn-H-2e), 1.48 (1H, ddd, J=14.2, 9.4, 2.4 Hz, cym I-H-2a), 1.47 (3H, s, H-21), 1.38 (1H, m, H-12), 1.33 (1H, m, H-11), 1.30 (1H, m, H-9), 1.24 (3H, d, J=6.6 Hz, dgn-H-6), 1.15 (1H, t, J=12.4 Hz, H-1α), 1.10 (3H, d, J=6.4 Hz, cym I-H-6), 1.09 (3H, d, J= 6.4 Hz, cym II-H-6), 1.03 (3H, s, H-19).

3.10.6. Compound 27. ^{1}H NMR (500 MHz, $CDCl_3$): 6.40 (1H, s, H-18), 5.49 (1H, br d, J=5.3 Hz, H-6), 5.37 (1H, dd, J=9.9, 7.1 Hz, H-16), 5.30 (1H, ddd, J=11.9, 9.6, 4.6 Hz, H-2), 4.89 (1H, d, J=3.7 Hz, dgn-H-1), 4.80 (1H, dd, J= 9.6, 1.7 Hz, cym II-H-1), 4.74 (1H, dd, J=9.8, 3.0 Hz, cym

II-H-4), 4.74 (1H, dd, J=9.8, 2.1 Hz, cym I-H-4), 4.17 (1H, dd, J=9.2, 7.1 Hz, H-15a), 4.01 (1H, dq, J=9.6, 6.2 Hz, cym II-H-5), 3.93 (1H, q, J=6.6 Hz, dgn-H-5), 3.84 (1H, t, J=9.2 Hz, H-15b), 3.83–3.86 (2H, m, dgn-H-4 and cym II-H-3), 3.80 (1H, ddd, J=11.6, 9.6, 5.2 Hz, H-3), 3.67 (1H, dq, J=9.6, 6.4 Hz, cym I-H-5), 3.60 (1H, m, cym I-H-3), 3.58 (1H, ddd, J=12.6, 4.8, 2.9 Hz, dgn-H-3), 3.39 (3H, s, cym I-OCH_3), 3.38 (3H, s, dgn-OCH_3), 3.17 (3H, s, cym II-OCH_3), 2.92 (1H, dd, J=9.6, 3.0 Hz, cym I-H-4), 2.55 (1H, m, H-12), 2.52 (1H, dd, J=13.9, 5.4 Hz, H-4α), 2.45–2.47 (2H, m, H-7 and H-8), 2.32 (1H, ddd, J=14.3, 3.9, 2.1 Hz, cym II-H-2e), 2.26 (1H, m, H-1β), 2.25 (1H, m, H-4β), 2.05–2.15 (3H, m, H-7, H-11 and cym I-H-2e), 2.01 (1H, td, J=12.6, 3.9 Hz, dgn-H-2a), 1.86 (1H, ddd, J=14.3, 9.6, 2.5 Hz, cym II-H-2a), 1.82 (1H, dd, J=12.6, 4.5 Hz, dgn-H-2e), 1.48 (3H, s, H-21), 1.42 (1H, m, H-12), 1.32–1.37 (3H, m, H-9, H-11 and cym I-H-2a), 1.30 (1H, t, J=12.4 Hz, H-1α), 1.22 (3H, d, J=6.6 Hz, dgn-H-6), 1.21 (3H, d, J=6.2 Hz, cym II-H-6), 1.05 (3H, s, H-19), 0.94 (3H, d, J=6.4 Hz, cym I-H-6).

3.10.7. Compound 28. ^{1}H NMR (500 MHz, $CDCl_3$): 6.40 (1H, s, H-18), 5.52 (1H, q like, 2.8 Hz, dgt-H-3), 5.49 (1H, br d, J=5.2 Hz, H-6), 5.37 (1H, dd, J=9.8, 7.1 Hz, H-16), 5.25 (1H, ddd, J=11.7, 9.4, 4.6 Hz, H-2), 4.98 (br d, J=3.2 Hz, ole-H-1), 4.83 (t, J=9.6 Hz, ole-H-4), 4.83 (1H, m, cym-H-1), 4.54 (1H, dd, J=9.7, 1.9 Hz, dgt-H-1), 4.17 (1H, dd, J=9.0, 7.1 Hz, H-15a), 3.97 (1H, dq, J=9.6, 6.2 Hz, ole-H-5), 3.85 (1H, m, H-3), 3.83 (1H, t, J=9.6 Hz, H-15b), 3.76 (1H, dq, J=9.6, 6.1 Hz, cym-H-5), 3.74 (1H, m, cym-H-3), 3.73 (1H, dq, J=9.7, 6.2 Hz, dgt-H-5), 3.43 (1H, m, ole-3), 3.42 (3H, s, cym-OCH_3), 3.39 (1H, dd, J=9.7, 2.8 Hz, dgt-H-4), 3.09 (3H, s, ole-OCH_3), 3.02 (1H, dd, J=9.6, 2.8 Hz, cym-H-4), 2.57 (1H, dd, J=14.0, 5.3 Hz, H-4α), 2.50 (1H, m, H-12), 2.40–2.46 (2H, m, H-7 and H-8), 2.27 (1H, dd, J=12.4, 5.1 Hz, ole-H-2e), 2.24 (1H, m, H-4β), 2.18 (1H, dd, J=12.7, 5.0 Hz, H-1β), 2.03–2.09 (4H, m, H-7, H-11, cym-H-2e and dgt-H-2e), 1.92 (1H, ddd, J=14.4, 9.7, 2.5 Hz, dgt-H-2a), 1.64 (1H, ddd, J=13.1, 11.7, 3.9 Hz, ole-H-2a), 1.48 (3H, s, H-21), 1.43 (1H, m, cym-H-2a), 1.38 (1H, m, H-12), 1.24–1.30 (2H, m, H-9 and H-11), 1.24 (3H, d, J=6.2 Hz, dgt-H-6), 1.24 (3H, d, J=6.2 Hz, ole-H-6), 1.14 (1H, t, J=12.4 Hz, H-1α), 1.03 (3H, s, H-19), 0.99 (3H, d, J=6.1 Hz, cym-H-6).

3.10.8. Compound 29. ^{1}H NMR (500 MHz, $CDCl_3$): 6.40 (1H, s, H-18), 5.52 (1H, q like, 2.8 Hz, dgt-H-3), 5.49 (1H, br d, J=5.4 Hz, H-6), 5.37 (1H, dd, J=9.9, 7.1 Hz, H-16), 5.29 (1H, ddd, J=11.9, 9.6, 4.5 Hz, H-2), 5.05 (br d, J=3.4 Hz, ole-H-1), 4.86 (t, J=9.7 Hz, ole-H-4), 4.71 (1H, dd, J=9.6, 1.8 Hz, cym-H-1), 4.41 (1H, dd, J=9.6, 1.8 Hz, dgt-H-1), 4.17 (1H, dd, J=9.9, 7.1 Hz, H-15a), 3.90 (1H, dq, J=9.7, 6.2 Hz, ole-H-5), 3.84 (1H, dd, J=9.8, 9.1 Hz, H-15b), 3.80 (1H, dq, J=9.6, 6.2 Hz, dgt-H-5), 3.77 (1H, ddd, J=11.5, 9.6, 5.5 Hz, H-3), 3.59–3.65 (2H, m, cym-H-3 and cym-H-5), 3.48 (1H, m, ole-H-3), 3.43 (1H, dd, J=9.6, 2.8 Hz, dgt-H-4), 3.38 (3H, s, cym-OCH_3), 3.26 (3H, s, ole-OCH_3), 2.73 (1H, dd, J=9.9, 2.9 Hz, cym-H-4), 2.54 (1H, m, H-12), 2.52 (1H, dd, J=13.8, 5.3 Hz, H-4α), 2.45–2.46 (2H, m, H-7 and H-8), 2.35 (1H, dd, J=12.6, 4.7 Hz, ole-H-2e), 2.26 (1H, dd, J=12.9, 4.9 Hz, H-1β), 2.22 (1H, m, H-4β), 2.11 (1H, m, H-11), 2.10 (1H, m, H-7), 1.94–1.97 (2H, m, cym-H-2e and dgt-H-2e), 1.85 (1H, m, dgt-H-2a), 1.71 (1H, ddd, J=13.1, 11.6, 4.0 Hz, ole-H-2a), 1.48 (3H, s, H-21), 1.43 (1H, m, H-12), 1.26–1.33 (4H, m, H-1α, H-9, H-11 and cym-H-2a), 1.29 (3H, d, J=6.2 Hz, dgt-H-6), 1.13 (3H, d, J=6.2 Hz, ole-H-6), 1.04 (3H, s, H-19), 0.75 (3H, d, J=6.4 Hz, cym-H-6).

3.11. Chemical conversion of cynanoside D (4) into cynanoside B (2)

Treatment of **4** (0.5 mg) with 0.3% NaOH (25 μL) and acetone (25 μL) at rt overnight afforded **2**, which was detected on the basis of HPLC analysis [condition: column, CAPCELL PAK C_{18}, 250 × 4.6 mm i.d.; flow rate, 0.8 mL/min; column temperature, 40 °C; t_R, 7.16 min (with 35% CH_3CN as eluate solvent)] and TLC examination [$CHCl_3$/MeOH/H_2O (60:15:1), R_f, 0.30].

3.12. Absolute configuration of sugars in 1–10

The sugar fractions obtained by acid or enzymatic hydrolysis as described above were analyzed by HPLC under the following conditions: column, Shodex SC-1101, 300×8 mm i.d; flow rate, 1 mL/min; column temperature, 80 °C; solvent, H_2O; detection, RI (Shodex RI-101) and OR (Shodex OR-2) detector. Identification of D-cymarose, L-dignose, D-digitoxose and D-glucose in each sugar fraction was carried out by a comparison of the retention times and polarities with those of authentic samples. t_R, (min) 8.00 (D-glucose, positive polarity), 9.19 (D-cymarose, positive polarity), 9.46 (L-diginose, negative polarity) and 9.55 (D-digitoxose, positive polarity). D-cymarose and L-diginose were detected from **1** and **5**. Cymarose (a mixture of D- and L-form in the ratio 1:1) and D-digitoxose were detected from **3**, **7** and **10**. D-Cymarose and D-digitoxose were detected from **8**. D-Glucose was detected from **2**, **6** and **9**.

Acknowledgements

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References and notes

1. (a) Zhang, R. S.; Ye, Y. P.; Shen, Y. M.; Liang, H. L. *Tetrahedron* **2000**, *56*, 3875–3879. (b) Mellado, G. G.; Zubia, E.; Ortega, M. J.; Lopez-Gonzalez, P. J. *Steroids* **2004**, *69*, 291–299. (c) Halaweish, F. T.; Huntimer, E.; Khalil, A. T. *Phytochem. Anal.* **2004**, *15*, 189–194.
2. Yin, J.; Kouda, K.; Tezuka, Y.; Tran, Q. L.; Miyahara, T.; Chen, Y. J.; Kadota, S. *J. Nat. Prod.* **2003**, *66*, 646–650.
3. Liu, H. W.; Xiong, Z.; Li, F.; Qu, G. X.; Kobayashi, H.; Yao, X. S. *Chem. Pharm. Bull.* **2003**, *51*, 1089–1091.
4. Deepak, D.; Srivastav, S.; Khare, A. In Herz, W., Kirby, G. W., Moore, R. E., Strglich, W., Tamm, Ch., Eds.; Progress in the chemistry of organic natural products; Springer: New York, 1997; Vol. 71, pp 169–325.

5. Shanghai Scientific Technological Publishers, Eds.; Zhong Hua Ben Cao; 1985, Vol. 6, pp 330–333.
6. (a) Zhang, Z. X.; Zhou, J.; Hayashi, K.; Mitsuhashi, H. *Chem. Pharm. Bull.* **1985**, *33*, 1507–1514. (b) Zhang, Z. X.; Zhou, J.; Hayashi, K.; Mitsuhashi, H. *Chem. Pharm. Bull.* **1985**, *33*, 4188–4192. (c) Zhang, Z. X.; Zhou, J.; Hayashi, K.; Kaneko, K. *Phytochemistry* **1988**, *27*, 2935–2941. (d) Day, S. H.; Wang, J. P.; Won, S. J.; Lin, C. N. *J. Nat. Prod.* **2001**, *64*, 608–611. (e) Lee, K. Y.; Sung, H.; Kim, Y. C. *Helv. Chim. Acta* **2003**, *86*, 474–483.
7. Harada, N.; Ohashi, M.; Nakanishi, K. *J. Am. Chem. Soc.* **1968**, *90*, 7349–7351.
8. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
9. (a) Deepak, D.; Khare, M. P.; Khare, A. *Phytochemistry* **1985**, *24*, 3015–3017. (b) Tsukamoto, S.; Hayashi, K.; Mitsuhashi, H. *Chem. Pharm. Bull.* **1985**, *33*, 2294–2304. (c) Tsukamoto, S.; Hayashi, K.; Kaneko, K.; Mitsuhashi, H. i.; Snyckers, F. O.; Fourie, T. G. *Chem. Pharm. Bull.* **1986**, *34*, 1337–1339. (d) Abe, F.; Hirokawa, M.; Yamauchi, T.; Honda, K.; Hayashi, N.; Nishida, R. *Chem. Pharm. Bull.* **1999**, *47*, 1384–1387.
10. (a) Nakagawa, T.; Hayashi, K.; Mitsuhashi, H. *Chem. Pharm. Bull.* **1983**, *31*, 2244–2253. (b) Nakagawa, T.; Hayashi, K.; Wada, K.; Mitsuhashi, H. *Tetrahedron* **1983**, *39*, 607–612. (c) Qiu, S. X.; Zhang, Z. X.; Yong, L.; Zhou, J. *Planta Med.* **1991**, *57*, 454–456. (d) Wang, L. Q.; Shen, Y. M.; Xu, X.; Wei, Y. Q.; Zhou, J. *Steroids* **2004**, *69*, 319–324.
11. Konda, Y.; Toda, Y.; Takayanagi, H.; Ogura, H.; Harigaya, Y.; Lou, H. X.; Li, X.; Onda, M. *J. Nat. Prod.* **1992**, *55*, 1118–1123.
12. Sargent, J. M.; Taylor, C. G. *Br. J. Cancer* **1989**, *60*, 206–211.
13. Yeo, H.; Kim, K. W.; Kim, J.; Choi, Y. H. *Phytochemistry* **1998**, *49*, 1129–1133.

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Synthesis of new acyl, furoyl, and benzoylthiocarbamates as polydentate systems. Structural study of isopropyl *N*-(2-furoyl)thiocarbamate

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Abstract—Synthesis of new acylthiocarbamates has been carried out. To establish the preferential conformation and to explain the behaviour chemically, the structure of isopropyl *N*-(2-furoyl)thiocarbamate **3m** has been determined by single-crystal X-ray analysis. The most stable conformation *E*,*Z*′ established by X-ray analysis was corroborated by semiempirical theoretical calculations.

1. Introduction

Some organic sulfur compounds such as thiocarbamates show important biological activity.[1] Most notably, acylthiocarbamates are used as biosensors,[2] elastase inhibitors,[3] and they can exhibit antineoplasic and antiinflammatory or antiarthritic[1] effects. Also molecular modelling studies of related acylthiocarbamates has been previously carried out due to they are potent non-nucleoside HIV-1 reverse transcriptase inhibitors.[4]

In addition, acylthiocarbamates have also been employed as starting compounds to obtain different heterocyclic compounds such as aminothiazoles, thietanes, aminotetrazoles, thiadiazoles, or thiadiazolines.[5] Some of these compounds are important since they are intermediates en route to variety of drugs.[6]

Thiocarbamates can react with weak bases, such as sodium or potassium carbonate, generating the corresponding anion, which can be represented through four plausible conformations as shown in Figure 1.[7] Molecular mechanics calculations onto *N*-acylthiocarbamate predict that *E*,*Z*′ is the most stable conformation.[8] Likewise, Schroeder et al. established, by using nuclear magnetic resonance, that *E*,*Z*′ was the most stable conformation in *N*-benzoyl-*O*-alkylthiocarbamates.[7] X-ray and semiempirical calculations would complete the structural determination of acylthiocarbamates in the literature and would be useful to understand the behaviour of these compounds versus electrophiles.

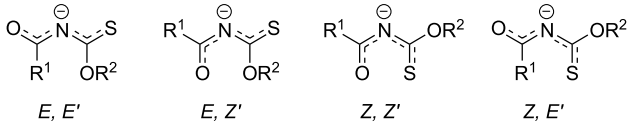
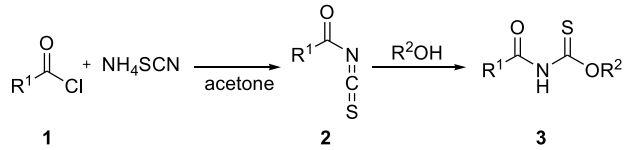


Figure 1.

Previously, we reported the synthesis of 1-benzoyl-3-alkylureas, using microwaves, through transamidation reactions,[9] and the alkylation of benzoyl and furoylthioureas as polydentate systems.[10] Here, we describe the synthesis of new acyl, furoyl and benzoylthiocarbamates and we have determined the structure of isopropyl *N*-(2-furoyl)thiocarbamate **3m** by single-crystal X-ray analysis. We have also confirmed that *E*,*Z*′ is the most stable conformation using the X-ray data and semiempirical theoretical calculations.



Scheme 1.

Keywords: Thiocarbamates; Acylthiocarbamates; Structural studies; Semiempirical methods.
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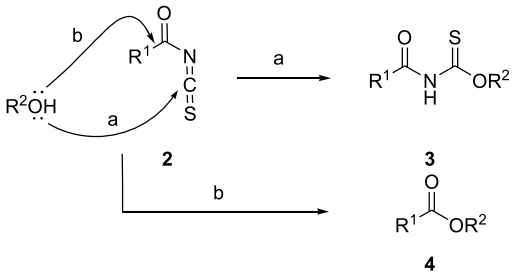
Table 1. Synthesis of compounds **3**

Entry	3	R^1	R^2	Yield (%)
1	**3a**	*t*-Bu	Me	68
2	**3b**	*t*-Bu	Et	67
3	**3c**	*t*-Bu	*i*-Pr	70
4	**3d**	Ph	*n*-C_5H_{11}	74
5	**3e**	Ph	*s*-C_5H_{11}	78
6	**3f**	Ph	*i*-C_5H_{11}	76
7	**3g**	Ph	*n*-$C_{18}H_{37}$	72
8	**3h**	Ph	Bn	81
9	**3i**	*p*NO_2–C_6H_4	*i*-Pr	71
10	**3j**	*p*F–C_6H_4	*i*-Pr	74
11	**3k**	Furyl	Me	72
12	**3l**	Furyl	Et	74
13	**3m**	Furyl	*i*-Pr	73
14	**3n**	Furyl	Bn	78
15	**3o**	2-(2-Furyl)vinyl	Me	78
16	**3p**	2-(2-Furyl)vinyl	Et	78
17	**3q**	2-(2-Furyl)vinyl	*i*-Pr	78

2. Results and discussion

The thiocarbamates were prepared by using acid chlorides **1** and ammonium thiocyanate in acetone. The intermediate isothiocyanate **2** reacts in situ with the corresponding alcohol affording the thiocarbamate **3** (Scheme 1 and Table 1).

Although there are two electrophilic carbon centres in **2**, the major product obtained is **3**, a consequence of the alcohol addition to the isocyanate electrophilic carbon (Scheme 2).



Scheme 2.

AM1, and PM3 semiempirical calculations predict a slightly negative charge value (−0.035, and −0.00075, respectively) on the carbon in the thiocarbamoyl group in products **2** (R^1=Ph). However, steric effects on the carbonyl group lead to products **3** in high yields instead of compounds **4**. In the case of net charges values on *C*=O, AM1 and PM3 methods predict 0.418 and 0.408, respectively.

Synthesis of acylthiocarbamates seems to be general: a variety of aliphatic and aromatic alcohols were shown to be applicable. In general, slightly higher yields were obtained from aromatic, or conjugated alcohols **3d**–**q** in comparison to aliphatic alcohols **3a**–**c**. This result could be explained taking into account that aliphatic acylisothiocyanates are more reactive than conjugated or aromatic equivalents: the decomposition of these products afforded the corresponding acid. Scheme 2 illustrates the two possible mechanisms of the reaction of alcohols with isothiocyanates **2**. When the aliphatic chain in alcohols is branched, better yields were observed in the reaction to afford **3**. When alcohols with smaller aliphatic chain were used, products **3** were obtained in lower yields due to the generation of **4**. This fact could be explained assuming steric effects, as stated before.

To study the structure of acylthiocarbamates, isopropyl *N*-(2-furoyl)thiocarbamate (**3m**) was used as compound model. Thus, X-ray diffraction and semiempirical methods were employed to reveal the most stable conformation of **3m** (Fig. 1).

X-ray analysis of **3m** shows that fragments S1=C6–N1–C5=O2 are in a nearly planar alignment with a torsion angle S1–C6–N1–C5=−177(2)° and C6–N1–C5–O2=−3(4)° (Fig. 2).

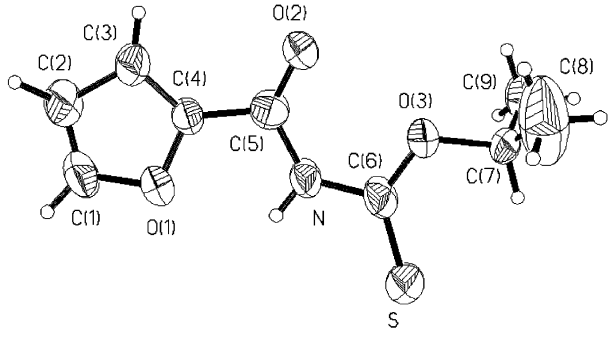


Figure 2. X-ray structure of compound **3m** showing the numbering scheme.

Asymmetry in angles C3–C4–C5 and O1–C4–C5 [131.8(5) and 118.9(4)°, respectively] could be due to the repulsion in the C3–H3···O2 system [C3·O2=3.006(6), H3···O2=2.96 Å] and the attraction in the NH···O1 system [N···O1=2.654(6) Å, H···O1=2.19 Å]. Taking into account that distances in C5–N1 and C6–N1 are 1.389(6) and 1.377(6) Å, respectively, it is possible to assume an electronic delocalization in that part of the molecule. In addition, C6–O3 bond distance [1.300(7) Å] indicates a partial double bond and a π-type conjugation in the whole system, this observation is in agreement with the previously reported results.[7]

In order to predict the minimum energy conformation, and relative stability of the possible conformers for **3m**, the

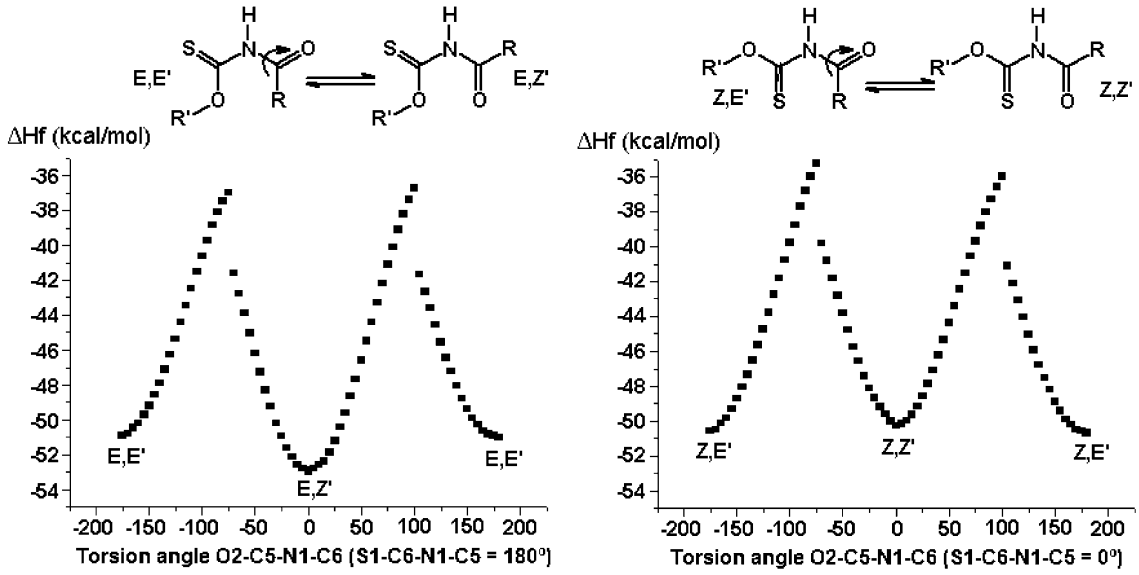


Figure 3. Heat of formation versus torsion angle O2–C5–N1–C6 (S1–C6–N1–C5 = 180 and 0°, respectively) for compound **3m**.

semiempirical PM3 method was used. The strategy was to study the heat of formation against the torsion angle related to the interconversion of these conformations.[11] Figure 3 represents the variation of the torsion angle that corresponds to the interconversion *E*,*E*′ *E*,*Z*′ and *Z*,*E*′ *Z*,*Z*′, respectively.

In the first case (Fig. 3a), the torsion angle O2–C5–N1–C6 was rotated every 5° from −180° (*E*,*E*′) to 180° (*E*,*E*′) passing through 0° (*E*,*Z*′), the torsion angle S1–C6–N1–C5 being fixed at 180°.

In the second case (Fig. 3b), the torsion angle O2–C5–N1–C6 was again rotated every 5° from −180° (*Z*,*E*′) to 180° (*Z*,*E*′) passing through 0° (*Z*,*Z*′), and the torsion angle S1–C6–N1–C5 was fixed at 0°.

In this sense, Figure 3 shows that the four conformers are consistent with the minimum energy in the curve and are close in energy (heat of formation around −50 kcal/mol). A complete optimisation onto the geometry corresponding to the energetic minimum was carried out and

Table 2. Bond distances, valence angles, and torsion angles for compound **3m** (the numbering scheme is shown in Figure 2). Bond distances are given in Å and angles in degrees (standard deviations in parenthesis)

3m	PM3	X-ray	**3m**	PM3	X-ray
Bond distances					
C1–C2	1.375	1.317(9)	C6–O3	1.344	1.300(7)
C1–O1	1.370	1.382(6)	C6–N1	1.404	1.377(6)
C2–C3	1.437	1.411(8)	C6–S1	1.654	1.635(6)
C3–C4	1.379	1.343(7)	C7–C9	1.523	1.46(3)
C4–O1	1.393	1.362(7)	C7–O3	1.449	1.486(8)
C4–C5	1.476	1.466(7)	C7–C8	1.523	1.50(4)
C5–O2	1.215	1.198(6)	N1–H1	1.001	0.8600
C5–N1	1.445	1.389(6)			
Valence angles					
C2–C1–O1	110.8	111.1(6)	O3–C6–S1	131.5	127.8(5)
C1–C2–C3	106.4	106.1(6)	N1–C6–S1	120.9	119.8(4)
C4–C3–C2	106.4	107.5(5)	C9–C7–O3	107.1	108.3(15)
C3–C4–O1	109.8	109.6(5)	C9–C7–C8	111.5	112.3(7)
C3–C4–C5	129.6	131.9(5)	O3–C7–C8	107.1	103.0(2)
O1–C4–C5	120.6	118.3(5)	C4–O1–C1	106.6	105.3(5)
O2–C5–N1	121.0	124.3(5)	C6–O3–C7	118.3	120.3(4)
O2–C5–C4	122.4	122.4(5)	C6–N1–C5	129.9	131.7(4)
N1–C5–C4	116.6	113.0(5)	C6–N1–H1	112.8	114.2
O3–C6–N1	107.5	111.5(5)	C5–N1–H1	117.1	114.2
Torsion angles					
O1–C1–C2–C3	0.0	6(4)	C2–C1–O1–C4	−0.1	−7(4)
C1–C2–C3–C4	0.1	−3(3)	N1–C6–O3–C7	180.0	−173(2)
C2–C3–C4–O1	−0.2	−1(3)	S1–C6–O3–C7	1.0	−4(5)
C2–C3–C4–C5	−179.3	−177(3)	C9–C7–O3–C6	120.0	129(2)
C3–C4–C5–O2	8.8	4(5)	C8–C7–O3–C6	−120.3	−112(3)
O1–C4–C5–O2	−170.2	−170(3)	O3–C6–N1–C5	7.7	−7(4)
C3–C4–C5–N1	−170.1	178(3)	S1–C6–N1–C5	−173.3	−177(2)
O1–C4–C5–N1	0.8	3(3)	O2–C5–N1–C6	7.2	−3(4)
C3–C4–O1–C1	0.2	5(4)	C4–C5–N1–C6	−173.7	−177(3)
C5–C4–O1–C1	179.4	−179(2)			

$E,E' = -51.3$ kcal/mol; $E,Z' = -54.4$ kcal/mol; $Z,E' = -51.2$ kcal/mol and $Z,Z' = -51.9$ kcal/mol heats of formation were found. This lead us to state that E,Z' conformation is the most stable. This result is in agreement with the experimental data from X-ray analysis, in which the torsion angles are S1–C6–N1–C5 = −177(2) and O2–C5–N1–C6 = −3(4)°. This also explains that the carbonyl and thiocarbonyl groups are in opposite positions, the molecule being in a E,Z' conformation (Fig. 2).

Table 2 shows selected data of bond distances, bond angles and torsion angles, obtained from X-ray diffraction studies and values from the semiempirical method PM3 for **3m**. Both methods showed a satisfactory correspondence. These results confirmed that the semiempirical method PM3, reports a reliable geometry for the propose systems.

3. Conclusion

In conclusion, a synthesis of new acythiocarbamates has been carried out. X-ray analysis and semiempirical theoretical calculations, established that the most stable conformation in acylthiocarbamates as E,Z'.

4. Experimental

4.1. General experimental

Reactions which required an inert atmosphere were conducted under dry nitrogen, and the glassware was oven dried (120 °C). All reagents were purchased from Aldrich or Merck and were used without further purification. Silica gel for flash chromatography was purchased from Scharlau or Merck (200–450 mesh), and compounds were visualized (UV light, 254 nm) on analytical thin layer chromatograms (TLC) and using benzene/methanol (9/1) as eluent. ^{1}H NMR spectra were recorded on a Bruker AC spectrometer at 250 MHz. ^{13}C NMR spectra and DEPT experiments were determined at 62 MHz. Chemical shifts are given in ppm relative to tetramethylsilane (TMS), which was used as an internal standard, and coupling constants J are reported in Hz. Melting points (mp) were determined on an Electrothermal C14500 apparatus and are uncorrected. IR spectra (ν_{max}/cm^{-1}) were recorded on a Bruker IRS48 instrument using KBr disc. MS (electronic impact) spectra were measured at 70 eV. Only the most important IR absorptions (cm^{-1}) and the molecular ions and/or base peaks in MS are given. Microanalyses were performed by the Servicio de Microanálisis of Universidad Complutense de Madrid.

4.2. Synthesis of acylthiocarbamates 3

The corresponding acyl chlorides (0.02 mol) were dissolved in dry acetone. To that solution, 0.02 mol of thiocyanate in acetone was added slowly. The mixture was stirred until a precipitate of ammonium chloride appeared. The precipitate indicated the formation of the corresponding organic isothiocyanate. So, to 0.02 mol of the generated isothiocyanate was slowly added the corresponding alcohol dissolved in acetone. The mixture was stirred for between 3 and 8 h. The progress of the reaction was monitored by TLC (using benzene/methanol (9/1) as eluent). When the reaction was completed the product was poured into 100 mL of cold water. The solid acylthiocarbamates were filtered out. Purification of compounds **3** were performed by recrystallization using acetone–H_2O as solvent.

4.2.1. Methyl *N*-pivaloylthiocarbamate 3a. 68% Yield; mp 69–70 °C; ν_{max}/cm^{-1} 3282 (NH), 2966 (C–H), 1714 (C=O), 1525 (NH), 1278 (C=S); δ_H (d_6-DMSO, 250 MHz), 8.72 (br s, 1H), 4.09 (s, 3H), 1.22 (s, 9H); δ_C (d_6-DMSO, 62 MHz), 190.7 (CS), 173.6 (CO), 59.5 (OCH_3), 40.3 (C), 27.1 (CH_3).

4.2.2. Ethyl *N*-pivaloylthiocarbamate 3b. 67% Yield; mp 90–92 °C; ν_{max}/cm^{-1} 3314 (NH), 2983 (CH), 1709 (C=O), 1518 (NH), 1272 (C=S); δ_H (d_6-DMSO, 250 MHz), 8.67 (br s, 1H), 4.58 (q, J=6 Hz, 2H), 1.41 (t, J=6 Hz, 3H), 1.22 (s, 9H); δ_C (d_6-DMSO, 62 MHz), 189.9 (CS), 173.5 (CO), 69.5 (CH_2), 40.3 (C), 27.1 [$(CH_3)_3C$], 13.8 (CH_3).

4.2.3. *Iso*propyl *N*-pivaloylthiocarbamate 3c. 70% Yield; mp 76–78 °C; ν_{max}/cm^{-1} 3207 (NH), 2980 (CH), 1720 (C=O), 1523 (NH), 1290 (C=S); δ_H (d_6-DMSO, 250 MHz), 8.57 (br s, 1H), 5.54 (hept, J=6.5 Hz, 1H), 1.42 (d, J=6.5 Hz, 6H), 1.24 (s, 9H); δ_C (d_6-DMSO, 62 MHz), 189.1 (CS), 173.3 (CO), 77.6 [$CH(CH_3)_2$], 40.2 [$C(CH_3)_3$], 27.1 [$C(CH_3)_3$], 21.2 (CH_3).

4.2.4. *n*-Pentyl *N*-benzoylthiocarbamate 3d. 74% Yield; mp 69–71 °C; ν_{max}/cm^{-1} 3208 (NH), 3063 (CH), 2956 (CH), 1696 (C=O), 1601 (C=C), 1300 (C=S), 1462 (CH_3); δ_H ($CDCl_3$, 250 MHz), 9.32 (br s, 1H), 7.90–7.40 (m, 5H), 4.60 (t, J=6.2 Hz, 2H), 1.85–1.75 (m, 2H), 1.40–1.35 (m, 4H), 0.95 (t, J=6.2 Hz, 3H); δ_C ($CDCl_3$, 62 MHz) 189.7 (CS), 162.8 (CO), 133.1 (C8), 133.0 (C5), 128.9 (C6 and C10), 127.8 (C7 and C9), 73.7 (OCH_2), 27.9 ($CH_2)_2CH_2O$, 22.3 (CH_2CH_3), 13.9 (CH_3); MS (EI): m/z (%), 251 (5), 181 (25), 121 (18), 105 (100), 77 (72), 51 (34); elemental analysis calcd (%) for $C_{13}H_{17}NO_2S$: C, 62.12; H, 6.82; N, 5.57. Found: C, 62.04; H, 6.96; N, 5.41.

4.2.5. 2-Pentyl *N*-benzoylthiocarbamate 3e. 78% Yield; mp 64–65 °C; δ_H ($CDCl_3$, 250 MHz), 9.28 (br s, 1H), 7.90–7.40 (m, 5H), 5.60–5.55 (m, 1H), 1.80–1.60 (m, 2H), 1.45–1.40 (m, 4H), 0.90–0.85 (m, 3H); δ_C ($CDCl_3$, 62 MHz), 189.6 (CS), 162.8 (CO), 133.1 (C8), 133.0 (C5), 128.9 (C6 and C10), 127.8 (C7 and C9), 81.1 (OCH_2), 37.5 (OCH_2CH_2), 19.1 [$CH(CH_3)_2$], 18.4 (CH_3), 13.9 (CH_3).

4.2.6. *Iso*pentyl *N*-benzoylthiocarbamate de 3f. 76% Yield; mp 77–78 °C; ν_{max}/cm^{-1} 3263 (NH), 3059 (CH), 2958 (CH), 1700 (C=O), 1601 (C=C), 1300 (C=S), 1456 (CH_3); δ_H ($CDCl_3$, 250 MHz), 9.22 (br s, 1H), 7.90–7.35 (m, 5H), 4.60 (t, J=6.2 Hz, 2H), 2.00–1.60 (m, 3H), 0.95 (d, J=6.4 Hz, 6H); δ_C ($CDCl_3$, 62 MHz), 189.7 (CS), 162.7 (CO), 133.1 (C8), 133.0 (C5), 129.0 (C6 and C10), 127.8 (C7 and C9), 72.3 (OCH_2), 36.8 (OCH_2CH_2), 24.9 (CH), 22.4 (CH_3); MS (EI): m/z (%), 251 (2), 181 (14), 121 (9), 105 (100), 77 (63), 51 (14); elemental analysis calcd (%) for $C_{13}H_{17}NO_2S$: C, 62.12; H, 6.82; N, 5.57. Found: C, 62.19; H, 6.88; N, 5.43.

4.2.7. Octadecyl *N*-benzoylthiocarbamate 3g. 72% Yield; mp 68–69 °C; ν_{max}/cm^{-1} 3256 (NH), 3021 (CH), 2951 (CH), 1704 (C=O), 1604 (C=C), 1300 (N–C=S); δ_H ($CDCl_3$, 250 MHz), 9.18 (br s, 1H), 7.90–7.41 (m, 5H), 4.60–4.55 (m, 2H), 1.80–1.75 (m, 2H), 1.55–1.0 (m, 30H), 0.90–0.85 (m, 3H); δ_C ($CDCl_3$, 62 MHz), 189.7 (CS), 162.7 (CO), 133.1 (C5 and C8), 129.0 (C6 and C10), 127.7 (C7 and C9), 73.8 (OCH_2), 31.9 ($CH_2(CH_2)_{14}$, 31.9 ($CH_2(CH_2)_{14}$ 22.7 [$CH_2(CH_2)_{14}CH_2$], 14.1 (CH_3); elemental analysis calcd (%) for $C_{26}H_{43}NO_2S$: C, 72.01; H, 9.99; N, 3.23. Found: C, 72.19; H, 9.85; N, 3.34.

4.2.8. Benzyl *N*-benzoylthiocarbamate 3h. 81% Yield; mp 104–106 °C; ν_{max}/cm^{-1}: 3314 (NH), 3073 (CH), 2968 (CH), 1693 (C=O), 1601 (C=C), 1269 (C=S); δ_H ($CDCl_3$, 250 MHz), 9.24 (br s, 1H), 7.83–7.25 (m, 10H), 5.62 (s, 2H); δ_C ($CDCl_3$, 62 MHz), 188.9 (CS), 162.8 (CO), 137.4 (C5 and C8), 131.1 (1C), 128.3 (1C), 127.3 (1C), 127.1 (1C) (aromatic), 129.0 (C6 and C10), 127.7 (C7 and C9), 74.2 (CH_2); elemental analysis calcd (%) for $C_{15}H_{13}NO_2S$: C, 66.40; H, 4.83; N, 5.16. Found: C, 66.29; H, 4.99; N, 5.27.

4.2.9. *Iso*propyl *N*-(4-nitrobenzoyl)thiocarbamate 3i. 71% Yield; mp 101–103 °C; ν_{max}/cm^{-1} 3209 (NH), 3120 (=CH), 3060 (CH), 2976 (CH), 1698 (C=O), 1601 (C=C), 1550 (NO_2), 1280 (C=S); δ_H ($CDCl_3$, 250 MHz), 9.45 (br s, 1H), 8.30 (d, $J=8.1$ Hz, 2H), 8.04 (d, $J=8.1$ Hz, 2H), 5.65 (hept, $J=6.2$ Hz, 1H), 1.40 (d, $J=6.2$ Hz, 6H); δ_C ($CDCl_3$, 62 MHz), 187.7 (CS), 161.7 (CO), 150.0 (C8), 138.7 (C5), 128.9 (C6, C10), 123.8 (C7, C9), 77.9 [$CH(CH_3)_2$], 21.0 (CH_3); MS (EI): *m*/*z* (%), 268 (<1), 226 (3), 166 (5), 150 (100), 122 (7); elemental analysis calcd (%) for $C_{11}H_{12}N_2O_4S$: C, 49.25; H, 4.51; N, 10.44. Found: C, 49.45; H, 4.72; N, 10.50.

4.2.10. *Iso*propyl *N*-(4-fluorobenzoyl)thiocarbamate 3j. 74% Yield; mp 111–112 °C; ν_{max}/cm^{-1} 3256 (NH), 3080 (=CH), 3100 (C–H), 2974 (C–H), 1696 (C=O), 1604 (C=C), 1287 (C=S); δ_H ($CDCl_3$, 250 MHz), 9.22 (br s, 1H), 7.87 (q, $J=5.2$ Hz, 2H), 7.16 (t, $J=8.1$ Hz, 2H), 5.63 (hept, $J=6.1$ Hz, 1H), 1.43 (d, $J=6.1$ Hz, 6H); δ_C ($CDCl_3$, 62 MHz), 188.6 (CS), 167.6 (CO), 167.6, 163.6 (C8), 130.7 (C5), 130.4, 129.3 (C6, C10), 116.2, 115.9 (C7, C9), 77.9 OCH, 21.2 (CH_3); MS (EI): *m*/*z* (%), 241 (3), 199 (15), 139 (22), 123 (100), 95 (46), 75 (19); elemental analysis calcd (%) for $C_{11}H_{12}FNO_2S$: C, 54.76; H, 5.01; N, 5.81. Found: C, 54.88; H, 5.12; N, 5.76.

4.2.11. Methyl *N*-(2-furoyl)thiocarbamate 3k. 72% Yield; mp 97–100 °C; ν_{max}/cm^{-1} 3416 (NH), 3120, (=CH), 2970 (CH), 1712 (C=O), 1581 (C=C), 1298 (C=S), 1025 (C–O–C); δ_H ($CDCl_3$, 250 MHz), 9.46 (br s, 1H), 7.55–7.47 (m, 1H), 7.31–7.26 (m, 1H); 6.58–6.54 (m, 1H), 4.17 (s, 3H); δ_C ($CDCl_3$, 62 MHz), 189.4 (CS), 152.3 (CO), 145.9 (C2), 145.7 (C5), 118.3 (C3), 113.3 (C4), 59.4 (CH_3); elemental analysis calcd (%) for $C_7H_7NO_3S$: C, 45.40; H, 3.81; N, 7.56. Found: C, 45.88; H, 3.92; N, 7.60.

4.2.12. Ethyl *N*-(2-furoyl)thiocarbamate 3l. 74%; mp 98–99 °C; ν_{max}/cm^{-1} 3411 (NH), 3122 (=CH), 2970 (CH); 1712 (C=O), 1583 (C=C), 1310 (C=S), 1025 (C–O–C); δ_H ($CDCl_3$, 250 MHz), 9.49 (br s, 1H), 7.54–7.53 (m, 1H), 7.31–7.27 (m, 1H), 6.58–6.55 (m, 1H), 4.60 (q, $J=7.1$ Hz, 2H), 1.50 (t, $J=7.1$ Hz, 3H); δ_C ($CDCl_3$, 62 MHz), 188.1 (CS), 152.3 (CO), 145.7 (C2), 145.5 (C5), 117.8 (C3), 112.6 (C4), 68.7 (CH_2), 13.4 (CH_3); MS (EI): *m*/*z* (%), 199 (2), 171 (1), 111 (1), 95 (100), 67 (6), 54 (2); elemental analysis calcd (%) for $C_8H_9NO_3S$: C, 48.23; H, 4.55; N, 7.03. Found: C, 48.58; H, 4.92; N, 7.30.

4.2.13. *Iso*propyl *N*-(2-furoyl)thiocarbamate 3m. 73% Yield; mp 76–78 °C; ν_{max}/cm^{-1} 3415 (N–H), 3132 (=CH), 2988 (CH), 1715 (C=O), 1584 (C=C), 1298 (C=S), 1013 (C–O–C); δ_H ($CDCl_3$, 250 MHz), 9.27 (br s, 1H), 7.51–7.50 (m, 1H), 7.27–7.25 (m, 1H), 6.53–6.52 (m, 1H), 5.55 (hept, $J=6.2$ Hz, 1H), 1.39 (d, $J=6.3$ Hz, 6H,); δ_C ($CDCl_3$, 62 MHz), 187.7 (CS), 152.3 (CO), 146.1 (C2), 145.5 (C5), 118.1 (C3), 113.3 (C4), 77.65 [$CH(CH_3)_2$], 21.3. (CH_3); MS (EI): *m*/*z* (%), 213 (6), 171 (23), 111 (40), 109 (1), 95 (100), 67(4), 55 (16); elemental analysis calcd (%) for $C_9H_{11}NO_3S$: C, 50.69; H, 5.20; N, 6.57. Found: C, 50.88; H, 5.72; N, 6.69.

4.2.14. Benzyl *N*-(2-furoyl)thiocarbamate 3n. 78% Yield; mp 114–116 °C; ν_{max}/cm^{-1} 3418 (NH), 3130 (=CH), 2990 (CH), 1718 (C=O), 1609 (C=C), 1290 (C=S), 1012 (C–O–C); δ_H ($CDCl_3$, 250 MHz), 9.39 (s, 1H), 7.50–7.49 (m, 1H), 7.26–7.25 (m, 1H), 6.65–6.60 (m, 1H), 5.63 (s, 2H), 7.46–7.30 (m, 5H); δ_C ($CDCl_3$, 62 MHz), 184.5 (CS), 154.7 (CO), 149.9 (C2), 142.0 (C5), 137.4 (1C), 128.3 (1C), 127.3 (1C), 127.1 (1C), (Ph), 119.7 (C3), 109.5 (C4), 71.9 (CH_2); MS (EI): *m*/*z* (%), 261 (<1), 171 (2), 111 (2), 95 (100), 67 (8); elemental analysis calcd (%) for $C_{13}H_{11}NO_3S$: C, 59.76; H, 4.24; N, 5.36. Found: C, 59.88; H, 4.92; N, 5.60.

4.2.15. Methyl *N*-[3-(2-furylacryloyl)]thiocarbamate 3o. 53% Yield; mp 117–118 °C; ν_{max}/cm^{-1} 3254 (NH), 3118 (=CH), 2980, (C–H), 1713 (C=O), 1626 (C=C), 1278 (C=S), 1023 (C–O–C); δ_H ($CDCl_3$, 250 MHz), 11.83 (br s, 1H), 7.84 (d, $J=1.7$ Hz, 1H), 7.46 (d, $J=15.4$ Hz, 1H), 6.92 (d, $J=3.4$ Hz, 1H), 6.69 (d, $J=15.4$ Hz, 1H), 6.65 (dd, $J=3.4$, 1.7 Hz, 1H), 4.02 (s, 3H); δ_C ($CDCl_3$, 62 MHz), 189.8 (CS), 162.5 (CO), 151.0 (C2), 145.4 (C5), 132.5 (C6), 116.5 (C3), 116.2 (C7), 112.7 (C4), 59.4 (CH_3); elemental analysis calcd (%) for $C_9H_9NO_3S$: C, 51.17; H, 4.29; N, 6.63. Found: C, 51.48; H, 4.12; N, 6.30.

4.2.16. Ethyl *N*-[3-(2-furyl)acryloyl]thiocarbamate 3p. 78% Yield; mp 114–116 °C; ν_{max}/cm^{-1} 3250 (NH), 3030 (=CH), 2982 (CH), 1716 (C=O), 1630 (C=C), 1336 (CH_3), 1275 (C=S); δ_H ($CDCl_3$, 250 MHz), 11.76 (br s, 1H), 7.83 (d, $J=1.6$ Hz, 1H), 7.50 (d, $J=15.4$ Hz, 1H), 6.92 (d, $J=3.4$ Hz, 1H), 6.68 (d, $J=15.4$ Hz, 1H), 6.65 (dd, $J=3.4$, 1.6 Hz, 1H), 4.50 (q, $J=7.1$ Hz, 2H), 1.31 (t, $J=7.1$ Hz, 3H); δ_C ($CDCl_3$, 62 MHz), 188.7 (CS), 161.9 (CO), 150.4 (C2), 145.7 (C5), 130.1 (C6), 117.3 (C3), 116.2 (C7), 112.6 (C4), 67.1 (CH_2), 13.4 (CH_3); elemental analysis calcd (%) for $C_{10}H_{11}NO_3S$: C, 53.32; H, 4.92; N, 6.22. Found: C, 53.58; H, 4.82; N, 6.32.

4.2.17. *Iso*propyl *N*-[3-(2-furyl)acryloyl]thiocarbamate 3q. 62% Yield; mp 70–72 °C; ν_{max}/cm^{-1} 3251 (NH), 3120 (=CH), 2987 (CH), 1718 (C=O), 1630 (C=C), 1280 (C=S), 1020 (C–O–C); δ_H ($CDCl_3$, 250 MHz), 11.76 (br s, 1H), 7.83 (d, $J=1.6$ Hz, 1H), 7.43 (d, $J=15.4$ Hz, 1H), 6.92

(d, $J=3.4$ Hz, 1H), 6.69 (d, $J=15.4$ Hz, 1H), 6.65 (dd, $J=3.4$, 1.6 Hz, 1H), 5.54 (hept, $J=6.1$ Hz, 1H), 1.37 (d, $J=6.1$ Hz, 6H); δ_C ($CDCl_3$, 62 MHz), 188.0 (CS), 161.9 (CO), 150.4 (C5), 145.6 (C2), 130.1 (C7), 117.4 (C6), 116.2 (C4), 112.6 (C3), 77.6 [O*C*H(CH_3)], 21.7 (CH_3); MS (EI): *m*/*z* (%), 239 (<1), 197 (2), 137 (3), 121 (100); elemental analysis calcd (%) for $C_{11}H_{13}NO_3S$: C, 55.21; H, 5.48; N, 5.85. Found: C, 55.48; H, 5.62; N, 5.60.

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References and notes

1. Trivedi, B. K. USA Patent, 8810816, 1988; *Chem. Abstr.* **1988**, *108*, 167428d.
2. Watcher, R. USA Patent, 901053, 1990; *Chem. Abstr.* **1989**, *111*, 128868a.
3. Digenis, G. A.; Rodis, N. P. USA Patent, 5539123, 1996; *Chem. Abstr.* **1996**, *125*, 167993n.
4. Ranise, A.; Spallarossa, A.; Schenone, S.; Bruno, O.; Bondavalli, F.; Vargiu, L.; Marceddu, T.; Mura, M.; La Colla, P.; Pani, A. *J. Med. Chem.* **2003**, *46*, 768.
5. Quante, J. M.; Hoke, R. A.; Mize, P. D.; Woodard, D. L.; Willner, O. L. USA Patent, 5516902, 1996; *Chem. Abstr.* **1996**, *125*, 50759z.
6. Shigeru, M.; Shigeru, M. Eur. Patent, 2394794, 1976; *Chem. Abstr.* **1976**, *85*, 95400x.
7. Schroeder, U.; Beyer, L.; Dietze, F.; Richter, R.; Schmidt, S.; Hoyer, E. *J. Prakt. Chem.* **1995**, *337*, 184.
8. Filleux-Blanchard, M. L.; Durand-Couturier, A. *Bull. Soc. Chim. Fr.* **1972**, 4710.
9. Márquez, H.; Pérez, E. R.; Plutín, A. M.; Morales, M.; Loupy, A. *Tetrahedron Lett.* **2000**, *41*, 1753.
10. Plutín, A. M.; Márquez, H.; Ochoa, E.; Morales, M.; Sosa, M.; Morán, L.; Rodríguez, Y.; Suárez, M.; Martín, N.; Seoane, C. *Tetrahedron* **2000**, *56*, 1533.
11. Furyl substituent was assumed in the same orientation as that found in the crystal and determinated by X-ray diffraction.

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DMP-mediated one-pot oxidative olefination of silyl ethers

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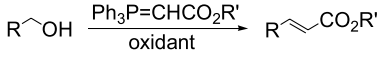
Abstract—Silyl ethers of arylic, allylic, propargylic and unactivated alcohols could be deprotected and oxidized with Dess–Martin periodinane, and the resulting aldehydes could be directly converted to the corresponding α,β-unsaturated esters in one pot with stabilized phosphoranes. Good selectivities were achieved upon a variety of protecting groups of alcohol by using this method. Other advantages of the protocol included simplicity of operations and high efficiency, as well as good to excellent yields.

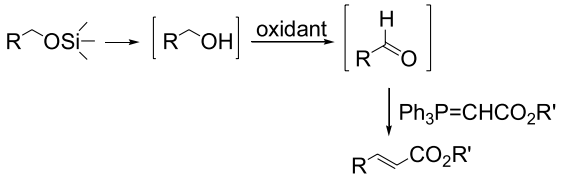
1. Introduction

One-pot methods have broadly been applied in organic synthesis. Syntheses of α,β-unsaturated esters using one-pot methodologies have been attracted attentions due to their widely potential applications in organic chemistry. For instance, one-pot oxidative olefination of alcohol using active manganese dioxide as an oxidant in the presence of Wittig reagents has been studied extensively (Scheme 1).[1–4] In those procedures, the resulting aldehydes by the oxidation of alcohols can be trapped in situ immediately by Wittig reagents to afford the corresponding olefins. Exploration of other one-pot transformations of various substrates to α,β-unsaturated esters still keeps attractive today. In principle, all the precursors, which can be converted to the aldehydes, are able to apply in the methods to generate α,β-unsaturated esters in one pot.



Scheme 1.

In this paper, we report a new one-pot transformation of silyl ethers to α,β-unsaturated esters (Scheme 2). In our initial plan, deprotection of silyl ethers, oxidation of the resulting alcohols and subsequent Wittig reaction to trap the in situ generated aldehydes were combined into a one-step operation. Such a transformation is particularly advantageous because it avoids the isolations of intermediate alcohols and aldehydes, reduces the volatility and toxicity,



Scheme 2.

and evades liability to oligomerisation, facile hydration, acerial oxidation, polymerization[5] or isomerization.[6]

2. Results and discussion

Initially, the reactions were performed under the conditions similar to the reported cases using active manganese dioxide.[1–4] However, no reactions occurred when the silyl ethers were treated under the same conditions. Obviously, active manganese dioxide is not able to remove silyl ether protecting groups in the first step. To accomplish this one-pot transformation, it is crucial to choose an appropriate reagent, which can deprotect silyl ethers and immediately oxidize the resulting alcohols to the corresponding aldehydes. Many reagents are potentially suitable to meet these requirements in the literature.[7] The oxidants that can oxidatively deprotect silyl ethers would be preferred to this transformation. However, these oxidants may encounter the difficulties due to their sensitivities to the Wittig reagents under strong acidic and strong oxidative conditions. With these considerations, Dess–Martin periodinane (DMP), a mild oxidant used for the deprotection–oxidation reaction of silyl ethers,[8] was examined for this purpose.

To our delight, Dess–Martin periodinane was proved to be

Keywords: Silyl ether; Unsaturated ester; Wittig reaction; Dess–Martin periodinane; One-pot method.
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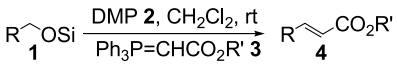
efficient in the procedure without any additives. Treatment of a mixture of silyl ethers **1** and ylide **3** in dichloromethane with DMP **2** at room temperature afforded α,β-unsaturated esters **4**, which were predominantly *trans*-isomers as expected (Scheme 3). The results were summarized in Table 1.



Scheme 3.

As shown in Table 1, the one-pot sequential deprotection–oxidation–Wittig reaction of more active silyl ethers, such as arylic, vinylic and alkynylic silyl ethers, generated α,β-unsaturated esters in 84–94% yields (entries 1–4 in Table 1). The *trans*-vinylic silyl ether was converted to 4-*trans*, 2-*trans*-unsaturated ester (entry 3 in Table 1); while the *cis*-vinylic silyl ether gave 4-*cis*, 2-*trans*-unsaturated ester under the same conditions (entry 4 in Table 1). This indicated the configuration of the *cis*-vinylic silyl ether was remained without any isomerization. However, it was found by us that the stepwise deprotection–oxidation of the *cis*-allylic silyl ether and followed by Wittig reaction predominately gave the (4*E*,2*E*)-isomer under the same conditions. This was easily explained by rapid isomerization of the resulted *cis*-enals.[6] Extension of the methodology to disilyl ethers derived from (*Z*)-1,4-buten-diol and 1,4-butynyl-diol was also successful, affording the corresponding double and symmetrical adducts (entries 5 and 6 in Table 1). Pre-existing *Z*-alkene geometry of silyl ether derived from (2*Z*)-1,4-buten-diol was preserved into its product as compared with the case using (2*Z*)-1,4-buten-diol as the substrate.[2a,4d]

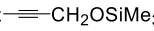
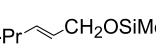
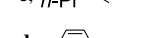
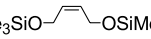
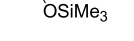
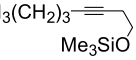
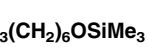
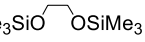
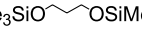
Unactivated silyl ethers, such as alkylic examples, were also proved to be successful (entries 7–9 in Table 1). The examined deprotection–oxidation–Wittig reactions of Me_3Si ethers of 1,2- and 1,3-diols both gave the expected products (entries 10 and 11 in Table 1). In contrast, the sequential oxidation–Wittig reactions of 1,2- and 1,3-diols were reported to give the cleavage products.[2a,9]

Both of THP and $^tBuMe_2Si$ were examined as protection groups of hydroxyls to replace Me_3Si group. The cases with $^tBuMe_2Si$ ethers underwent the same reaction as those with TMS ethers (entry 12 in Table 1); however, the derivatives protected with THP could not proceed the same reaction (entries 12 and 13 in Table 1).

Further investigations showed that the arylic, vinylic, alkynylic and alkylic TMS and/or TBDMS ethers, etc., could be deprotected using 1 equiv of acetic acid, however, the derivatives with THP group could not deprotected under the same conditions.

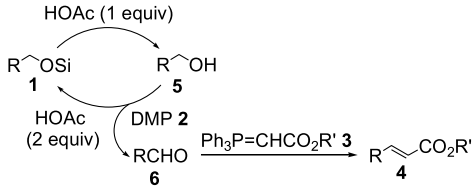
High-valent iodine-mediated cleavage of the oxygen–silyl linkage has been reported. For example, silyl protecting groups can be cleaved by sodium periodate[10] and *o*-iodoxybenzoic acid (IBX).[11] The former reaction went to completion when $NaIO_4$ was used in 1.1–2.5 mol ratio. The latter reaction was performed when IBX was used in 0.5–1.5 mol ratio. It is noteworthy that these reactions are not catalytic. Accordingly, the reaction would require

Table 1. DMP-mediated one-pot oxidative olefination of silyl ethers

Entry	Silyl ether **1**	Product **4**	Reaction time, yield, (selectivity)[a]
1	**a**, **$PhCH_2OSiMe_3$**	**a**, 	20 h, 94%, (*E*:*Z*=20:1)
2	**b**, 	**b**, 	2.5 d, 87%, (*E*:*Z*=9:1)
3	**c**, 	**c**,	2.5 d, 90%, (2*E*,4*E*:2*Z*,4*E*=10.5:1)
4	**d**, 	**d**,	3 d, 84%, (2*E*,4*Z*:2*Z*,4*Z*=8:1)
5	**e**, 	**e**,	3 d, 65%, (2*E*,4*Z*,6*E*:2*Z*,4*Z*,6*E*=3.6:1)
6	**f**,	**f**,	2 d, 78%, (2*E*,6*E*:2*Z*,6*E*=5:1)
7	**g**, 	**g**,	3 d, 48%, (2*E*,5*E*:2*Z*,5*E*=4:1)
8	**h**, 	**h**,	75 h, 60%, (*E*:*Z*=5.5:1)
9	**i**, **$CH_3(CH_2)_6OSiMe_3$**	**i**, 	3 d, 70%, (*E*:*Z*=4.5:1)
10	**j**, 	**j**,	3 d, 60%, (2*E*,4*E*:2*Z*,4*E*=5:1)
11	**k**,	**k**,	3 d, 40%, (2*E*,5*E*:2*Z*,5*E*=3.3:1)
12	**l**, 	**l**,	3 d, 62%, (*E*:*Z*=7:1)
13	**m**, 	**m**,	3 d, 65%, (*E*:*Z*=6:1)

[a] The ratios were determined by ^1H NMR.

2 equiv of DMP, at least 1.5 equiv to carry out this one-pot transformation. However, the experiments showed that only 1 equiv of DMP was enough to finish the above sequential reactions. This suggested that the desilylation process was probably not mediated by DMP except the starting stage. A reasonable explanation was that Dess–Martin periodinane (DMP) **2** or trace amount of acetic acid in available commercial source started the deprotection of silyl ethers **1** to form the corresponding alcohols **5**. The resulting alcohols **5** could be oxidized by Dess–Martin periodinane **2** to generate the corresponding aldehydes **6** and 2 equiv of acetic acid.[12] The aldehydes **6** were trapped immediately in situ with Wittig reagent **3** to give the α,β-unsaturated esters **4** (Scheme 4). With those newly produced acetic acid, the cycle of deprotection of silyl ethers **1** maintained. Therefore, the reaction did not require additional DMP for desilylation once it started.



Scheme 4.

The generation rates of aldehydes **6** may be controlled by deprotection of silyl ethers **1**. The resulting aldehydes **6** were trapped before they were isomerized and/or decomposed. Therefore, the pre-existing *Z*-alkene geometry was retained (entry 5 in Table 1). In addition, the hydroxyl groups protected with THP remained as the protected form in the products because the corresponding deprotections could not occur under such conditions (entries 12 and 13 in Table 1). The actual mechanism is still unclear.

In summary, a new one-pot oxidative olefination of silyl ethers using DMP was investigated for the first time. This protocol proved to be a useful and efficient method to directly convert the silyl ethers to corresponding α,β-unsaturated esters, and presented a significant expansion of the exiting methods.[2–4] The geometry of olefins retained during the reaction and the problems like oxidative cleavage of diols were not observed.[2a,9] Potential uses of this methodology in the multi-step synthesis of complex compounds and natural products are under investigation in our laboratory.

3. Experimental

IR spectra were recorded on a Nicolet 370 FT-IR. ^{1}H NMR spectra were performed on Varian YH 300 in $CDCl_3$ solution using tetramethylsilane as an internal standard. Dichloromethane was distilled before use from calcium hydride. Other solvents were distilled prior to use. Organic extracts were concentrated using a rotary evaporator at below 50 °C. Melting points were uncorrected. Silyl ethers,[13] Wittig reagents[14] and Dess–Martin periodinane (DMP)[12] were prepared according to the known procedures. In addition, DMP was obtained from commercial source.

3.1. General procedure

Silyl ether **1d** (0.317 g, 2 mmol) and methyl triphenylphosphoranylideneacetate (0.868 g, 2.6 mmol) were dissolved in anhydrous CH_2Cl_2 (30 mL). Dess–Martin periodinane (1.70 g, 4 mmol) was added in 3–5 portions over 16–24 h to the reaction mixture. After stirring for 64 h at room temperature, Et_2O (30 mL) and saturated aqueous $NaHCO_3$ (20 mL) were added. After stirring for another 10 min, the mixture was filtered; the organic layer was separated and concentrated under reduced pressure. The residue was purified by chromatography on silica gel by eluting with 15:1 petroleum ether–Et_2O to give methyl (2*E*,4*Z*)-2,4-heptadienoate **4d** (0.235 g, 84%) as a colourless oil.[4d] δ_H ($CDCl_3$, 300 MHz) 0.83 (t, J=7.1 Hz, 3H), 2.09–2.19 (m, 2H), 3.73 (s, 3H), 5.77 (d, J=15.6 Hz, 1H), 6.03–6.16 (m, 2H), 7.21–7.30 (m, 1H). ν_{max} (KBr) 2953, 2924, 2854, 1719, 1666, 1638, 1463, 1377, 1274, 1178 cm^{-1}.

3.1.1. Ethyl (2*E*)-3-phenylpropenoic acid 4a. A colourless oil[3] was obtained in 94% yield. δ_H ($CDCl_3$, 300 MHz) 1.34 (t, J=7.2 Hz, 3H), 4.27 (q, J=7.2 Hz, 2H), 6.44 (d, J=15.9 Hz, 1H), 7.32–7.60 (m, 5H), 7.69 (d, J=15.9 Hz, 1H). ν_{max} (KBr) 3029, 2925, 2854, 1716, 1663, 1578, 1496, 1449, 1367, 1270, 1175, 1040 cm^{-1}.

3.1.2. Methyl (2*E*)-2-hepten-4-ynoate 4b. A colourless oil[15] was obtained in 87% yield. δ_H ($CDCl_3$, 300 MHz) 0.86 (t, J=7.3 Hz, 3H), 2.39 (dq, J=7.3, 2.1 Hz, 2H), 3.76 (s, 3H), 6.15 (d, J=15.9 Hz, 1H), 6.77 (dt, J=15.9, 2.1 Hz, 1H). ν_{max} (KBr) 2953, 2924, 2854, 2217, 1730, 1666, 1625, 1462, 1377, 1274, 1159, 962, 700 cm^{-1}.

3.1.3. Methyl (2*E*,4*E*)-2,4-octadienoate 4c. A colourless oil[16] was obtained in 90% yield. δ_H ($CDCl_3$, 300 MHz) 0.92 (t, J=7.2 Hz, 3H), 1.40–1.52 (m, 2H), 2.12–2.18 (m, 2H), 3.74 (s, 3H), 5.79 (d, J=15.6 Hz, 1H), 6.12–6.23 (m, 2H), 7.23–7.31 (m, 1H). ν_{max} (KBr) 2953, 2925, 2854, 1725, 1665, 1646, 1462, 1377, 1275, 1141, 999, 701 cm^{-1}.

3.1.4. Dimethyl (2*E*,4*Z*,6*E*)-2,4,6-octatrienedioate 4e. White crystals[4d] were obtained in 65% yield, mp 112–116 °C. δ_H ($CDCl_3$, 300 MHz) 3.79 (s, 6H), 6.02 (d, J=15.3 Hz, 2H), 6.35–6.45 (m, 2H), 7.77–7.85 (m, 2H). ν_{max} (KBr) 2919, 2850, 1708, 1624, 1464, 1367, 1318, 1270, 1164, 1025, 982, 727 cm^{-1}.

3.1.5. Dimethyl (2*E*,6*E*)-2,6-octadien-4-ynedioate 4f. White crystals[4d] were obtained in 78% yield, mp 106–110 °C. δ_H ($CDCl_3$, 300 MHz) 3.76 (s, 6H), 6.29 (dd, J=16.8, 2.0 Hz, 2H), 6.87 (dd, J=16.8, 2.0 Hz, 2 Hz). ν_{max} (KBr) 2924, 2853, 2200, 1715, 1610, 1467, 1317, 1273, 1167, 978 cm^{-1}.

3.1.6. Methyl (2*E*,5*Z*)-2,5-octadienoate 4g. Yellow crystals[4d] were obtained in 48% yield, mp 86–90 °C. δ_H ($CDCl_3$, 300 MHz) 1.07 (t, J=7.5 Hz, 3H), 1.89–2.02 (m, 2H), 2.61–2.68 (m, 2H), 3.79 (s, 3H), 6.25 (d, J=15.0 Hz, 1H), 6.46 (d, J=15.0 Hz, 1H), 7.18–7.34 (m, 2H). ν_{max} (KBr) 2922, 2853, 1704, 1600, 1463, 1377, 1283, 1159, 973, 722 cm^{-1}.

3.1.7. Ethyl (2*E*)-2-decen-5-ynoate 4h. A colourless oil[17]

was obtained in 60% yield. δ_H ($CDCl_3$, 300 MHz) 0.96 (t, $J=7.3$ Hz, 3H), 1.09–1.14 (m, 4H), 1.29 (t, $J=7.2$ Hz, 3H), 2.33 (t, $J=7.5$ Hz, 2H), 4.03–4.13 (m, 2H), 4.20 (q, $J=7.2$ Hz, 2H), 5.82 (d, $J=15.6$ Hz, 1H), 6.97 (dt, $J=15.6$, 7.1 Hz, 1H). ν_{max} (KBr) 2927, 2855, 2350, 1726, 1662, 1462, 1377, 1275, 1176, 941, 702 cm^{-1}.

3.1.8. Methyl (2*E*)-2-nonenoate 4i. A colourless oil[18] was obtained in 70% yield. δ_H ($CDCl_3$, 300 MHz) 0.88 (t, $J=7.1$ Hz, 3H), 1.29–1.45 (m, 8H), 2.16–2.23 (m, 2H), 3.73 (s, 3H), 5.82 (d, $J=15.9$ Hz, 1H), 6.98 (dt, $J=15.9$, 7.2 Hz, 1H). ν_{max} (KBr) 2955, 2928, 2857, 1724, 1656, 1600, 1466, 1379, 1266, 1169, 978 cm^{-1}.

3.1.9. Diethyl (2*E*,4*E*)-2,4-hexadienoate 4j. White crystals were obtained in 60% yield, mp 57–59 °C (lit.[2a] 57–59 °C). δ_H ($CDCl_3$, 300 MHz) 1.30 (t, $J=7.1$ Hz, 6H), 4.23 (q, $J=7.1$ Hz, 4H), 6.21 (dd, $J=11.6$, 3.0 Hz, 2H), 7.33 (dd, $J=11.6$, 3.0 Hz, 2H). ν_{max} (KBr) 2924, 2853, 1670, 1609, 1462, 1246, 1160, 1032, 863, 728 cm^{-1}.

3.1.10. Diethyl (2*E*,5*E*)-2,5-heptadienedioate 4k. A yellow oil[19] was obtained in 40% yield. δ_H ($CDCl_3$, 300 MHz) 1.29 (t, $J=7.2$ Hz, 6H), 2.44–2.50 (m, 2H), 4.18 (q, $J=7.2$ Hz, 4H), 5.92 (d, $J=15.8$ Hz, 2H), 6.97 (dt, $J=15.8$, 7.2 Hz, 2H). ν_{max} (KBr) 2981, 2937, 1721, 1655, 1467, 1369, 1271, 1167, 1046, 979, 711 cm^{-1}.

3.1.11. Ethyl (2*E*)-4-[(tetrahydro-2H-pyran-2-yl)oxy]-2-butenoate 4l or 4m. A yellow oil[20] was obtained in 62 and 65% yields, respectively. δ_H ($CDCl_3$, 300 MHz) 1.29 (t, $J=7.2$ Hz, 3H), 1.50–1.83 (m, 6H), 2.48–2.54 (m, 2H), 3.46–3.53 (m, 2H), 3.81–3.88 (m, 2H), 4.20 (q, $J=7.2$ Hz, 2H), 4.59 (t, $J=3.8$ Hz, 1H), 5.90 (d, $J=15.8$ Hz, 1H), 6.99 (dt, $J=15.8$, 7.0 Hz, 1H). ν_{max} (KBr) 2925, 2855, 1725, 1655, 1464, 1367, 1298, 1262, 1176, 1036, 980, 870 cm^{-1}.

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References and notes

1. Ireland, R. E.; Norbeck, D. W. *J. Org. Chem.* **1985**, *50*, 2198–2200.
2. (a) Barrett, A. G. M.; Hamprecht, D.; Ohkubo, M. *J. Org. Chem.* **1997**, *62*, 9376–9378. (b) Crich, D.; Mo, X. *Synlett* **1999**, 67–68. (c) Huang, C. C. *J. Labelled Compd. Radiopharm.* **1987**, *24*, 675–681.
3. Shuto, S.; Niizuma, S.; Matsuda, A. *J. Org. Chem.* **1998**, *63*, 4489–4493.
4. (a) Reid, M.; Rowe, D. J.; Taylor, R. J. K. *Chem. Commun.* **2003**, 2284–2285. (b) Blackburn, L.; Wei, X.; Taylor, R. J. K. *Chem. Commun.* **1999**, 1337–1338. (c) Runcie, K. A.; Taylor, R. J. K. *Chem. Commun.* **2002**, 974–975. (d) Wei, X.; Taylor, R. J. K. *Tetrahedron Lett.* **1998**, *39*, 3815–3818. (e) Wei, X.; Taylor, R. J. K. *J. Org. Chem.* **2000**, *65*, 616–620.
5. Darkins, P.; McCarthy, N.; Mckervey, M. A.; Ye, T. *Chem. Commun.* **1993**, 1222–1223.
6. (a) Thomas, D. A.; Warburton, W. K. *J. Chem. Soc.* **1965**, 2988. (b) Xiao, W. Y.; Prestwich, G. D. *Synth. Commun.* **1990**, *20*, 3125.
7. (a) Chandrasekhar, S.; Mohanty, P. K.; Takhi, M. *J. Org. Chem.* **1997**, *62*, 2628–2629. (b) Chang, S.; Lee, N. H.; Jacobsen, E. N. *J. Org. Chem.* **1993**, *58*, 6939–6941. (c) Astles, P. C.; Thomas, E. J. *J. Chem. Soc. Perkin Trans. 1* **1997**, 845–856. (d) Marshall, J. A.; DeHoff, B. S.; Cleary, D. G. *J. Org. Chem.* **1986**, *51*, 1735–1741. (e) Nicolaou, K. C.; Seitz, S. P.; Pavia, M. R.; Petasis, N. A. *J. Org. Chem.* **1979**, *44*, 4011–4013. (f) Piva, O.; Amougay, A.; Pete, J.-P. *Tetrahedron Lett.* **1991**, *32*, 3993.
8. (a) Oskooie, S. H. A.; Heravi, M. M.; Sarmad, N.; Saednia, A.; Ghassemzadeh, M. *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.* **2003**, *42B*, 2890–2891. (b) Oskooie, S. H. A.; Khalipoor, M.; Saednia, A.; Sarmad, N.; Heravi, M. M. *Phosphorus, Sulfur Silicon Relat. Elem.* **2000**, *166*, 197–200.
9. Outram, H. S.; Raw, S. A.; Taylor, R. J. K. *Tetrahedron Lett.* **2002**, *43*, 6185–6187.
10. Wang, M.; Li, C.; Yin, D.; Liang, X.-T. *Tetrahedron Lett.* **2002**, *43*, 8727–8729.
11. Wu, Y.; Huang, J.-H.; Shen, X.; Hu, Q.; Tang, C.-J.; Li, L. *Org. Lett.* **2002**, *4*, 2141–2144.
12. Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155–4156.
13. Denney, D. B.; Ross, S. T. *J. Org. Chem.* **1962**, *27*, 998–1000.
14. Morita, T.; Yoshida, S.; Okamoto, Y.; Sakurai, H. *Synthesis* **1979**, 379.
15. Fürstner, A.; Dierkes, T.; Thiel, O. R.; Blanda, G. *Chem. Eur. J.* **2001**, *7*, 5286–5289.
16. Ma, D.; Lu, X. *Tetrahedron Lett.* **1989**, *30*, 843–844.
17. Ma, S.; Zhang, A. *J. Org. Chem.* **2002**, *67*, 2287–2294.
18. Rathke, M. W.; Nowak, M. *J. Org. Chem.* **1985**, *50*, 2624–2626.
19. Pettrovich, J. P.; Anderson, J. D.; Baizer, M. M. *J. Org. Chem.* **1966**, *31*, 3897–3903.
20. Harinish, V.; Morera, E.; Ortar, G. *J. Org. Chem.* **1985**, *50*, 1990–1992.

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Highly enantioselective hetero-Diels–Alder reaction between *trans*-1-methoxy-2-methyl-3-trimethylsiloxybuta-1,3-diene and aldehydes catalyzed by (*R*)-BINOL–Ti(IV) complex

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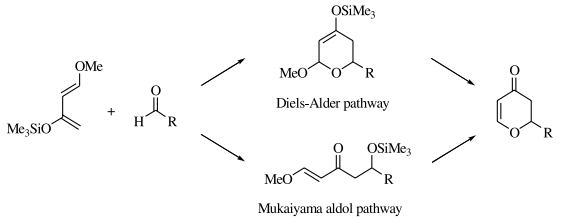
Abstract—An efficient enantioselective approach to 2,5-disubstituted dihydropyrones was developed. Some easily accessible inexpensive diol ligand metal complexes were employed, and $[(R)\text{-BINOL}]_2\text{–Ti(O}i\text{Pr)}_4$ complex was found to be the most effective catalyst (up to 99% yield and 99% ee in the presence of 5 mol% catalyst) for the hetero-Diels–Alder reaction between *trans*-1-methoxy-2-methyl-3-trimethylsiloxybuta-1,3-diene (**1**) and aldehydes. The potential and generality of this catalyst were evaluated by a variety of aldehydes including aromatic, heteroaromatic, α,β-unsaturated and aliphatic aldehydes. Based on the isolated intermediate from the reaction of benzaldehyde being confirmed by ^{1}H, ^{13}C NMR and HRMS data, the mechanism was proposed as a Mukaiyama aldol pathway.

1. Introduction

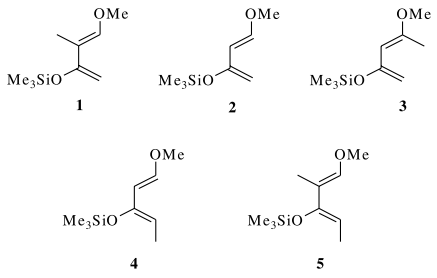
Recent advance of asymmetric reactions in synthetic organic chemistry has been well documented and excellent stereoselective reactions have been successfully established.[1] Among these reactions, asymmetric hetero-Diels–Alder (HDA) reaction allows the direct formation of carbon–carbon bond and carbon–oxygen bond with up to two stereogenic centers in one convergent step from simple achiral precursors. In this context, it has attracted much attention over the past two decades. Following the development of chiral Lewis acids[2] and organocatalysts,[2b,3] considerable progress on this reaction has been achieved.



Scheme 1. Two possible pathways for the HDA reaction of Danishefsky's diene.

When Lewis acid catalyzed HDA reaction is considered, two mechanistic pathways have generally been taken into account, namely that a given Lewis acid will catalyze this reaction by either a traditional Diels–Alder type cycloaddition or a Mukaiyama aldol pathway (Scheme 1). In the course of mechanism investigation, identification of reaction intermediate[4] and semi-empirical calculation[5] are two practical approaches.

Dihydropyrones are highly versatile synthetic intermediates for the preparation of biologically important compounds,[4j,6] including carbohydrates,[6a–i] antibiotics,[6k] and toxines.[4j,6l,m] Asymmetric HDA reaction of activated diene, such as Danishefsky's diene and its derivatives (Scheme 2),[7] is a synthetically powerful approach to these heterocycles. Enantioselective HDA reaction between diene **1** and benzaldehyde using chiral auxiliary-chiral catalyst



Scheme 2. Danishefsky's diene and its derivatives.

Keywords: Asymmetric catalysis; Dihydropyrone; Hetero-Diels–Alder reaction; Titanium (IV) complex.
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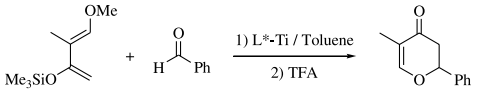
combination strategy (84% ee) was firstly reported by Danishefsky and co-workers.[6j] Recently, we reported our preliminary efforts on the asymmetric HDA reaction of diene **1** catalyzed by (*R*)-BINOL–Ti(O*i*Pr)$_4$ (2:1 ratio) complex,[8] which provided an efficient and enantioselective approach to 2,5-disubstituted dihydropyrones. The present paper, describes our studies on catalyst optimization, substrate generality and mechanistic investigation for this reaction.

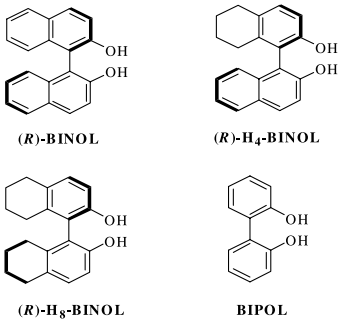
2. Results and discussion

2.1. Optimization of the catalysts

During the course of catalyst optimization, how to make the chiral ligand, metallic ion, substrate, etc. into a perfect match is a key point for a highly enantioselective reaction. To achieve a perfect chiral match, some concepts in asymmetric catalysis must be taken into account, such as 'asymmetric amplification',[9a] 'chiral poisoning',[9b] 'asymmetric activation',[9c,9d] 'ligand-accelerated catalysis',[9e] 'chiral environment amplification',[9f] and 'asymmetric autocatalysis'.[9g] Based on the principle of these concepts, which make the combination of two different ligands with a metallic ion into a practicable approach for increasing the activity and stereospeciality of a target reaction theoretically, some homo- and hetero-combinations of easily accessible diol ligands with titanium(IV) isopropoxide were tested as catalysts for the HDA reaction of *trans*-1-methoxy-2-methyl-3-trimethylsiloxybuta-1,3-diene (**1**) with benzaldehyde (Eq. 1) (Scheme 3).



1 **6a** **7a** (1)



Scheme 3. Diols used as ligands for the catalysts survey.

According to the results shown in Table 1, the yield and enantiomeric excess of dihydropyrone **7a** were greatly affected by the catalyst used. (*R*)-BINOL/Ti^{IV}/(*R*)-BINOL, (*R*)-BINOL/Ti^{IV}/(*R*)-H$_4$-BINOL, (*R*)-BINOL/Ti^{IV}/(*R*)-H$_8$-BINOL and (*R*)-H$_4$-BINOL/Ti^{IV}/(*R*)-H$_4$-BINOL promoted this reaction smoothly to give product **7a** in reasonable yields with high enantioselectivities (Table 1, entries 1–3, and 5). However, (*R*)-H$_4$-BINOL/Ti^{IV}/(*R*)-H$_8$-BINOL complex, an exceptionally efficient catalyst for solvent-free enantioselective HDA reaction of Danishefsky's diene,[10] showed lower catalytic activity to give dihydropyrone **7a** with 38% yield and 79% ee (Table 1, entry 6). And chiral (*R*)-H$_8$-BINOL/Ti^{IV}/(*R*)-H$_8$-BINOL complex also revealed lower catalytic activity in this reaction (Table 1, entry 8). These results showed that a much larger dihedral angle value of axial biaryl groups ((*R*)-H$_8$-BINOL > (*R*)-H$_4$-BINOL > (*R*)-BINOL) on titanium (IV) complex was disadvantageous for the activity of catalyst in this system. It was obviously different from what had been observed in titanium (IV) complexes catalyzed HDA reaction of Danishefsky's diene, in which the reactivity and enantioselectivity of catalyst increased with the dihedral angle value.[11b]

Table 1. Asymmetric hetero-Diels–Alder reaction of diene **1** with benzaldehyde catalyzed by chiral diol titanium(IV) complexes[a]

Entry	Catalyst[b]	Yield (%)[c]	ee (%)[d]
1	(*R*)-BINOL/Ti^{IV}/(*R*)-BINOL	99	87
2	(*R*)-BINOL/Ti^{IV}/(*R*)-H$_4$-BINOL	99	88
3	(*R*)-BINOL/Ti^{IV}/(*R*)-H$_8$-BINOL	89	87
4	(*R*)-BINOL/Ti^{IV}/BIPOL	49	78
5	(*R*)-H$_4$-BINOL/Ti^{IV}/(*R*)-H$_4$-BINOL	99	89
6	(*R*)-H$_4$-BINOL/Ti^{IV}/(*R*)-H$_8$-BINOL	38	79
7	(*R*)-H$_4$-BINOL/Ti^{IV}/BIPOL	43	24
8	(*R*)-H$_8$-BINOL/Ti^{IV}/(*R*)-H$_8$-BINOL	25	71
9	(*R*)-H$_8$-BINOL/Ti^{IV}/BIPOL	37	45

[a] All reactions were carried out at 0 °C in toluene using 10 mol% catalyst, in the presence of 120 mg 4 Å MS, over 48 h, concentration of benzaldehyde = 0.25 M.
[b] The chiral catalyst was generated by combining a diol ligand with Ti(O*i*Pr)$_4$ and an alternative diol ligand (1:1:1) in parallel style.
[c] Isolated yield.
[d] The ee values were determined by HPLC using Chiralcel OJ column.

Then, other (*R*)-BINOL–metal complexes were screened in the HDA reaction of diene **1** with benzaldehyde. As shown in Table 2, among these catalysts, (*R*)-BINOL–Al^{III} complexes (Table 2, entries 5–7), and (*R*)-BINOL–Yb^{III} complex (Table 2, entry 8) could promote this reaction slowly, but gave racemic products. (*R*)-BINOL–Zr^{IV} complexes (Table 2, entries 3 and 4) revealed moderate reactivity and lower enantioselectivity. Only (*R*)-BINOL–Ti^{IV} complexes (Table 2, entries 1 and 2) catalyzed the reaction with high enantioselectivities. However, (*R*)-BINOL–TiCl$_4$ complex was much more sluggish than (*R*)-BINOL–Ti(O*i*Pr)$_4$ (Table 2, entry 2 vs 1).

Table 2. Effects of Lewis acids on asymmetric hetero-Diels–Alder reaction of diene **1** with benzaldehyde[a]

Entry	Metal compound[b]	Yield (%)[c]	ee (%)[d]
1	Ti(O*i*Pr)$_4$	99	87
2	TiCl$_4$	29	82
3	Zr(O*t*Bu)$_4$	87	40
4	ZrCl$_4$	77	9
5	AlEt$_3$	16	<5
6	AlEt$_2$Cl	18	<5
7	Al(O*i*Pr)$_3$	20	0
8	Yb(OTf)$_3$	16	0

[a] All reactions were carried out at 0 °C in toluene using 10 mol% catalyst, in the presence of 120 mg 4 Å MS, over 48 h, concentration of benzaldehyde = 0.25 M.
[b] (*R*)-BINOL was used as chiral ligand, and the ratio of (*R*)-BINOL to metal compound was 2:1.
[c] Isolated yield.
[d] The ee values were determined by HPLC using Chiralcel OJ column.

To obtain higher enantioselectivity for dihydropyrone **7a**, the molar ratio of (*R*)-BINOL to Ti(O*i*Pr)$_4$ was surveyed. As shown in Table 3 (entries 1–4), the enantioselectivity was indeed varied with the change of molar ratio of (*R*)-BINOL to Ti(O*i*Pr)$_4$. When the molar ratio was 1:1, this reaction was carried out smoothly with moderate enantioselectivity (Table 3, entry 1). When the molar ratio increased to 1.1:1 and 1.5:1, enantioselectivities were increased to 82% (Table 3, entries 2 and 3). Catalyst, which was generated using 2:1 molar ratio promoted this reaction with optimal enantioselectivity (87% ee) and yield (99%) (Table 3, entry 4). These results were similar with Keck's report on HDA reaction of Danishefsky's diene.[4e] Considering hexacoordinated titanium atom, the actual catalyst should be [(*R*)-BINOL]$_2$–Ti(O*i*Pr)$_4$ complex.

Table 3. Effects of the ligand/metal ratio and the catalyst loading on the enantioselectivity in this catalysis[a]

Entry	(*R*)-BINOL/Ti(O*i*Pr)$_4$ (mol%)	Yield (%)[b]	ee (%)[c]
1	10:10	93	77
2	11:10	87	82
3	15:10	99	82
4	20:10	99	87
5	10:5	97	90
6	2:1	26	69

[a] All reactions were carried out at 0 °C in toluene, in the presence of 120 mg 4 Å MS, over 48 h, concentration of benzaldehyde = 0.25 M.
[b] Isolated yield.
[c] The ee values were determined by HPLC using Chiralcel OJ column.

In terms of chirality economy, the effect of catalyst loading was also studied. When the catalyst loading was reduced from 10 mol% to 5 mol%, the enantioselectivity increased to 90% ee (Table 3, entry 4 vs 5). However, when this reaction was carried out using 1 mol% catalyst loading, the catalyst efficiency was decreased significantly (Table 3, entry 6). Then, 5 mol% catalyst loading was chosen as the optimal one to examine other parameters.

The studies showed the reactivity and enantioselectivity of this catalyst were dependent on temperature (Table 4, entries 1–3). Under mild condition (0 °C), dihydropyrone **7a** was obtained with excellent isolated yield and enantiomeric excess (Table 4, entry 1). When reaction temperature decreased to −20 °C, the yield and enantioselectivity were slightly affected (Table 4, entry 2). However, when the reaction was performed at 25 °C, dihydropyrone **7a** was obtained only in 60% yield with 66% ee (Table 4, entry 3). Furthermore, solvent effect study revealed that THF afforded product **7a** in much higher enantiomeric excess (99% ee, Table 4, entry 7) than other solvents (Table 4, entries 1, 4–7).

Table 4. Effects of the temperature and the solvent on the enantioselectivity in this catalysis[a]

Entry	Temperature (°C)	Solvent	Yield (%)[b]	ee (%)[c]
1	0	Toluene	97	90
2	−20	Toluene	92	91
3	25	Toluene	60	66
4	0	CH_2Cl_2	69	92
5	0	Et_2O	99	89
6	0	*t*-BuOMe	98	81
7	0	THF	86	99

[a] All reactions were promoted by 5 mol% [(*R*)-BINOL]$_2$–Ti(O*i*Pr)$_4$ complex, in the presence of 120 mg 4 Å MS, over 48 h, concentration of benzaldehyde = 0.25 M.
[b] Isolated yield.
[c] The ee values were determined by HPLC using Chiralcel OJ column.

2.2. Substrate generality

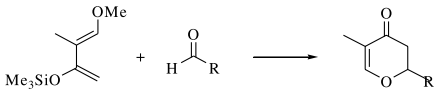
Encouraged by the result obtained from benzaldehyde, a variety of aldehydes were investigated under optimal conditions. Excellent enantioselectivities of 83–99% ee and moderate to high isolated yields were obtained for aromatic, heteroaromatic, α,β-unsaturated and aliphatic aldehydes. As shown in Table 5, 2-chlorobenzaldehyde afforded the corresponding adduct with 90% ee (Table 5, entry 2), while 3-chlorobenzaldehyde (Table 5, entry 3) and 4-chlorobenzaldehyde (Table 5, entry 4) gave the dihydropyrones with higher enantioselectivities. Moreover, 2,6-dichlorobenzaldehyde gave the dihydropyrone **7f** with much lower enantioselectivity (Table 5, entry 6). Similarly, the enantiomeric excess of dihydropyrone **7h** (91% ee, Table 5, entry 8) was lower than that of **7i** (98% ee, Table 5, entry 9) and **7j** (96% ee, Table 5, entry 10). Relatively, 3- and 4-substituents on benzaldehyde had slight effect on enantioselectivity (Table 5, entries 3 vs 4, 9 vs 10, and 11 vs 12). These steric effects were consistent with Chan's and our reports on alkylation of aromatic aldehydes[11] and HDA reaction of aldehydes,[12] respectively.

On the other hand, the electronic effect of aldehydes on enantioselectivity was not so obvious as compared with the steric effect. When 3- and 4-substituents on aromatic ring were electron-withdrawing groups (Table 5, entries 3, 4, 7, 9, and 10), the ee values of products (>95% ee) were distinct higher than those that were electron-donating groups (Table 5, entries 11–13), except for 4-cyanobenzaldehyde (only 92% ee, Table 5, entry 17). In addition, 4-fluorobenzaldehyde gave the product with somewhat lower ee value than 4-chlorobenzaldehyde (Table 5, entry 4 vs 16). This effect may arise from the fact that the resonance electron-donating property (+ M effect) of fluorine is stronger than chlorine. Furthermore, unsubstituted aromatic and heteroaromatic aldehydes, such as benzaldehyde (Table 5, entry 1), 1-naphthaldehyde (Table 5, entry 14), 2-naphthaldehyde (Table 5, entry 15), 2-pyridinecarboxaldehyde (Table 5, entry 18), 3-pyridinecarboxaldehyde (Table 5, entry 19) and 2-furaldehyde (Table 5, entry 20), afforded the corresponding dihydropyrones with excellent enantioselectivities.

2.3. Reaction mechanism

When Lewis acid catalyzed HDA reactions are considered, two mechanistic pathways have generally been taken into account. A Mukaiyama aldol pathway has been identified in the highly enantioselective HDA reaction of Danishefsky's diene with aldehydes developed by Corey,[4d] Keck,[4e] and Kobayashi,[4j] whereas a traditional Diels–Alder pathway was reported by Danishefsky,[4a] Jacbosen,[4f] Molander,[4g] and Ding.[4i] To survey the possible reaction pathway of this [(*R*)-BINOL]$_2$–Ti(O*i*Pr)$_4$ catalyst, the reaction between diene **1** and benzaldehyde was performed under optimal conditions. Intermediate of this reaction could be isolated

Table 5. Asymmetric synthesis of 2,5-disubstituted dihydropyrones catalyzed by [(*R*)-BINOL]$_2$–Ti(O*i*Pr)$_4$ complex[a]

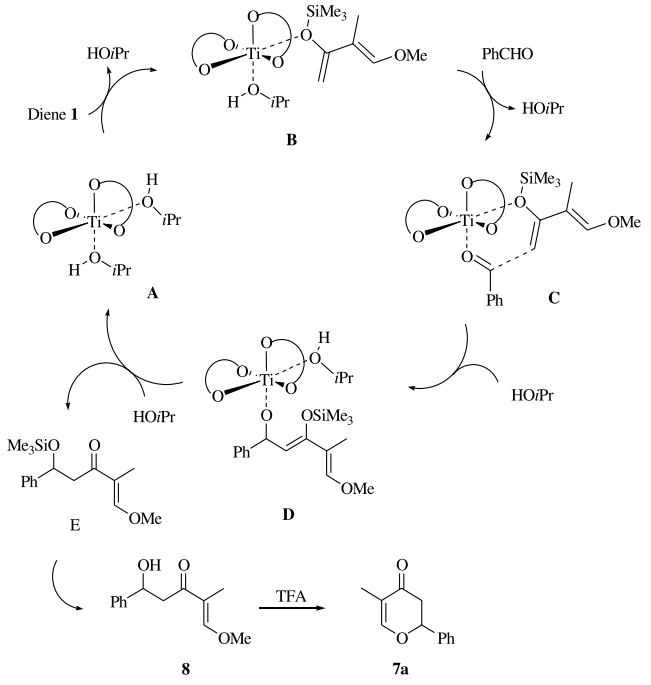


1 **6a-x** **7a-x**

Entry	Aldehyde **6a–x**	Dihydropyrone	Yield (%)[b]	ee (%)
1	Benzaldehyde	**7a**	86	99[c]
2	2-Chlorobenzaldehyde	**7b**	99	90[c]
3	3-Chlorobenzaldehyde	**7c**	82	98[d]
4	4-Chlorobenzaldehyde	**7d**	66	96[e]
5	2,4-Dichlorobenzaldehyde	**7e**	70	94[c]
6	2,6-Dichlorobenzaldehyde	**7f**	63	83[c]
7	3,4-Dichlorobenzaldehyde	**7g**	87	99[d]
8	2-Nitrobenzaldehyde	**7h**	99	91[f]
9	3-Nitrobenzaldehyde	**7i**	85	98[d]
10	4-Nitrobenzaldehyde	**7j**	80	96[d]
11	3-Methoxybenzaldehyde	**7k**	99	91[c]
12	4-Methoxybenzaldehyde	**7l**	84	90[c]
13	3,4-Methylenedioxybenzaldehyde	**7m**	38	90[c]
14	1-Naphthaldehyde	**7n**	40	97[d]
15	2-Naphthaldehyde	**7o**	69	96[d]
16	4-Fluorobenzaldehyde	**7p**	73	94[e]
17	4-Cyanobenzaldehyde	**7q**	95	92[d]
18	2-Pyridinecarboxaldehyde	**7r**	99	98[c]
19	3-Pyridinecarboxaldehyde	**7s**	98	96[d]
20	2-Furaldehyde	**7t**	99	97[c]
21	(*E*)-3-Phenyl-2-propenal	**7u**	47	85[c]
22	*n*-Hexanal	**7v**	41	87[f]

[a] All reactions were carried out at 0 °C in THF using 5 mol% [(*R*)-BINOL]$_2$–Ti(O*i*Pr)$_4$ complex, in the presence of 120 mg 4 Å MS, over 48 h, concentration of aldehyde **6** = 0.25 M.
[b] Isolated yield.
[c] Determined by HPLC using Chiralcel OJ column.
[d] Determined by HPLC using Chiralcel OD column.
[e] Determined by HPLC using Chiralcel OB-H column.
[f] Determined by HPLC using Chiralpak AD-H column.

using triethylamine-treated silica gel column chromatography before workup by TFA. This isolated product was confirmed as Mukaiyama aldol condensation adduct **8** (Scheme 4) by ^{1}H, ^{13}C NMR and HRMS analysis.[13] In addition, we have found the optimal molar ratio of (*R*)-BINOL to Ti(O*i*Pr)$_4$ was 2:1, and presumed the catalyst in this system was a [(*R*)-BINOL]$_2$–Ti(O*i*Pr)$_4$ complex, during the course of catalyst optimization. Futher efforts, on disclosing the absolute structure of this organometallic complex by X-ray and NMR analysis was unsuccessful because of its moisture sensitivity.



Scheme 4. The proposed mechanism of [(*R*)-BINOL]$_2$–Ti(O*i*Pr)$_4$ catalyzed hetero-Diels–Alder reaction of diene **1** with benzaldehyde.

In this context, the proposed mechanism was illustrated in Scheme 4. After diene **1** was added to the solution of catalyst prepared in situ, the active catalytic species **B** was produced. Immediately, benzaldehyde was coordinated with titanium (IV) through lone pair of electrons on carbonyl oxygen (state **C**), followed by a Mukaiyama aldol condensation to afford complex **D**. Then isopropyl alcohol was transferred from reaction solution to titanium (IV), and initial complex **A** and Mukaiyama aldol condensation adduct **E** were released. Finally, 5-methyl-2-phenyl-2,3-dihydro-4*H*-pyran-4-one (**7a**) was produced from **8** by the treatment of trifluoroacetic acid.

Furthermore, to insight into the importance of isopropyl alcohol in this catalyst cycle, control experiments were performed under optimal conditions. When Ti(O*i*Pr)$_2$Cl$_2$ and TiCl$_4$ were used to generate the catalyst, the yield and enantiomeric excess of dihydropyrone **7a** were sacrificed regularly (Table 6). This was probably due to the fact that chloride ion was much smaller than isoproxide considering van de Waals radius, and more electronegative as well. In

Table 6. Control experiments[a]

Entry	Metal compound	Yield (%)[b]	ee (%)[c]
1	$Ti(OiPr)_4$	86	99
2	$Ti(OiPr)_2Cl_2$	62	87
3	$TiCl_4$	26	82

[a] All reactions were carried out at 0 °C in THF, in the presence of 120 mg 4 Å MS, over 48 h, (*R*)-BINOL:metal compound = 10:5 mol%, concentration of benzaldehyde = 0.25 M.
[b] Isolated yield.
[c] The ee values were determined by HPLC using Chiralcel OJ column.

this catalysis, isopropyl alcohol should be of advantage for the procedure of coordinate and decoordinate between substrates and catalyst, which determined the yield and enantiospecificity of product **7a**.

3. Conclusions

In conclusion, a highly enantioselective HDA reaction between *trans*-1-methoxy-2-methyl-3-trimethylsiloxybuta-1,3-diene (**1**) and aldehydes has been documented, which provides a potential tactics for the conformation of highly optical active 2,5-disubstituted dihydropyrones. Under mild conditions, a variety of aldehydes including aromatic, heteroaromatic, α,β-unsaturated and aliphatic aldehydes reacted smoothly with diene **1** to afford corresponding dihydropyrones in highly enantiomeric excesses (up to 99% ee). Based on the experimental results, the reaction mechanism was proposed as a Mukaiyama aldol pathway. Meanwhile, since optical active BINOL can be easily available in large scale, this (*R*)-BINOL–Ti^{IV} complex catalyzed HDA reaction is expected to have excellent potential for practical applications.

4. Experimental

4.1. General methods

All reactions were carried out using anhydrous solvents and under nitrogen in over-dried tubes. Toluene, Et_2O, *t*BuOMe and THF were dried and distilled from sodium/benzophenone under nitrogen prior to use. CH_2Cl_2 was dried over powdered CaH_2 and distilled under nitrogen prior to use. $Ti(OiPr)_4$ (from Acros) was distilled and diluted to 1 M in toluene, stored under nitrogen. HG/T2354-92 silica gel (Qingdao Haiyang Chemical Co., Ltd.) was used for flash chromatography (FC). Melting points (mp) were measured on electrothermal digital melting point apparatus and uncorrected. Enantiomeric excesses (ee) were determined by HPLC using corresponding commercial chiral column as stated in the experimental procedures at 23 °C with UV detection at 254 nm. Optical rotations were measured on Perkin-Elmer-341. ^{1}H NMR spectra were recorded in $CDCl_3$ on Inova-400 (400 MHz) or Bruker Avance 600 (600 MHz) and were reported in ppm using TMS ($\delta=0$) or residual $CDCl_3$ ($\delta=7.26$) as the reference. ^{13}C NMR spectra were recorded in $CDCl_3$ on Inova-400 (100 MHz) or Bruker Avance 600 (150 MHz) and were reported in ppm relative to the central $CDCl_3$ resonance ($\delta=77.00$). HRMS were recorded on BRUKER-APEX-2 (SIMS).

4.2. Materials

trans-1-Methoxy-2-methyl-3-trimethylsiloxybuta-1,3-diene (**1**) was prepared according to the reported procedure.[14] The ligands (*R*)-H_8-BINOL[15] and (*R*)-H_4-BINOL[16] were prepared as described in the literature. (*R*)-BINOL and BIPOL were purchased from Aldrich and Sigma, respectively. 4 Å MS powder was purchased from Aldrich and used as received without further activation. All aldehydes were purchased from Acros, Aldrich or Fluka, and used directly without further purification.

4.3. Typical procedure for the asymmetric synthesis of 2,5-disubstituted dihydropyrones

4.3.1. 5-Methyl-2-phenyl-2,3-dihydro-4*H*-pyran-4-one (7a). A mixture of (*R*)-BINOL (7.2 mg, 0.025 mmol), $Ti(OiPr)_4$ (1 M in toluene, 12.5 μL, 0.0125 mmol) and finely powdered 4 Å MS (120 mg) in THF (0.5 mL) was heated at 35 °C for 1 h. After the brown mixture was cooled to −18 °C, benzaldehyde (26 μL, 0.25 mmol), diene **1** (84 μL) and THF (0.5 mL) were added via syringe successively. The reaction was allowed to stir at 0 °C for 48 h, then it was removed from the bath and treated with TFA (100 μL). After stirring 12 h at ambient temperature, the reaction was quenched with saturated $NaHCO_3$ (5 mL), filtered through a plug of Celite and extracted with Et_2O (10×5 mL). The organic layer was dried over Na_2SO_4 and concentrated to give the crude product. Purified by FC on silica gel (petroleum/Et_2O 9:1) to afford the title compound as white crystals (mp = 46–48 °C) in 86% yield with 99% ee determined by HPLC analysis using a Chiralcel OJ column (hexane/2-propanol 90:10, 1.0 mL min^{-1}, t_r (minor) = 15.400 min, t_r (major) = 21.150 min). $[\alpha]_D^{25}$ −95.9 (*c* 0.20, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta=7.46$–7.40 (m, 5H, Ph-H), 7.39 (s, 1H, =CH), 5.41 (dd, ^3J(H,H) = 14.6, 3.2 Hz, 1H, Ph-CHO), 2.94–2.88 (m, 1H, CH_AH_B), 2.73–2.69 (m, 1H, CH_AH_B), 1.76 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): $\delta=192.59$, 159.49, 138.27, 128.80, 128.78, 126.05, 114.14, 80.99, 43.21, 10.51; HRMS (ESI): Calcd for $C_{12}H_{13}O_2$ [M+H$^+$] 189.0910, found 189.0911.

4.3.2. 5-Methyl-2-(2-chlorophenyl)-2,3-dihydro-4*H*-pyran-4-one (7b). Purified by FC on silica gel (petroleum/Et_2O 5:1) to afford the title compound as light yellow crystals (mp = 42–44 °C) in 99% yield with 90% ee determined by HPLC analysis using a Chiralcel OJ column (hexane/2-propanol 90:10, 1.0 mL min^{-1}, t_r (major) = 7.700 min, t_r (minor) = 11.800 min). $[\alpha]_D^{25}$ +114.9 (*c* 0.19, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.61$–7.59 (m, 1H, Ph-H), 7.40 (s, 1H, =CH), 7.38–7.28 (m, 3H, Ph-H), 5.77 (dd, ^3J(H,H) = 14.4, 3.2 Hz, 1H, Ph-CHO), 2.85–2.80 (m, 1H, CH_AH_B) 2.72–2.64 (m, 1H, CH_AH_B), 1.75 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=192.18$, 159.36, 136.20, 131.58, 129.69, 129.60, 127.34, 127.06, 114.32, 77.92, 41.94, 10.53; HRMS (ESI): Calcd for $C_{12}H_{12}ClO_2$ [M+H$^+$] 223.0520, found 223.0518.

4.3.3. 5-Methyl-2-(3-chlorophenyl)-2,3-dihydro-4*H*-pyran-4-one (7c). Purified by FC on silica gel (petroleum/Et_2O 5:1) to afford the title compound as an oil in 82% yield with 98% ee determined by HPLC analysis using a Chiralcel OD column (hexane/2-propanol 90:10, 1.0 mL min^{-1}, t_r

(minor)=8.100 min, t_r (major)=10.708 min). $[\alpha]_D^{25}$ +23.7 (*c* 0.79, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$): δ=7.42 (s, 1H, =CH), 7.36–7.26 (m, 4H, Ph-H), 5.36 (dd, ^3J(H,H)=14.5, 3.3 Hz, 1H, Ph-CHO), 2.86–2.81 (m, 1H, CH_AH_B), 2.71–2.68 (m, 1H, CH_AH_B), 1.74 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ=191.94, 159.11, 140.33, 134.74, 130.10, 128.84, 126.21, 124.04, 114.37, 80.05, 43.14, 10.47; HRMS (ESI): Calcd for $C_{12}H_{12}ClO_2$ [M+H^+] 223.0520, found 223.0522.

4.3.4. 5-Methyl-2-(4-chlorophenyl)-2,3-dihydro-4*H*-pyran-4-one (7d). Purified by FC on silica gel (petroleum/Et_2O 5:1) to afford the title compound as a white solid (mp=46–48 °C) in 66% yield with 96% ee determined by HPLC analysis using a Chiralcel OB-H column (hexane/2-propanol 80:20, 1.0 mL min^{-1}, t_r (major)=10.117 min, t_r (minor)=17.850 min). $[\alpha]_D^{25}$ −72.8 (*c* 0.21, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ=7.40–7.32 (m, 5H, Ph-H and =CH), 5.36 (dd, ^3J(H,H)=14.4, 3.6 Hz, 1H, Ph-CHO), 2.87–2.79 (m, 1H, CH_AH_B), 2.69–2.64 (m, 1H, CH_AH_B), 1.73 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ=192.19, 159.24, 136.72, 134.54, 128.96, 127.35, 114.27, 80.13, 43.10, 10.48; HRMS (ESI): Calcd for $C_{12}H_{12}ClO_2$ [M+H^+] 223.0520, found 223.0522.

4.3.5. 5-Methyl-2-(2,4-dichlorophenyl)-2,3-dihydro-4*H*-pyran-4-one (7e). Purified by FC on silica gel (petroleum/Et_2O 4:1) to afford the title compound as a white solid (mp=80–82 °C) in 70% yield with 94% ee determined by HPLC analysis using a Chiralcel OJ column (hexane/2-propanol 99:1, 1.0 mL min^{-1}, t_r (major)=12.533 min, t_r (minor)=14.633 min). $[\alpha]_D^{25}$ +52.4 (*c* 0.58, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$): δ=7.56–7.54 (m, 1H, Ph-H), 7.43–7.42 (m, 1H, Ph-H), 7.39 (s, 1H, =CH), 7.36–7.34 (m, 1H, Ph-H), 5.72 (dd, ^3J(H,H)=14.6, 3.3 Hz, 1H, Ph-CHO) 2.83–2.80 (m, 1H, CH_AH_B), 2.67–2.62 (m, 1H, CH_AH_B), 1.76 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ=191.74, 159.11, 134.96, 134.92, 132.32, 129.57, 128.12, 127.77, 114.60, 77.53, 41.92, 10.51; HRMS (ESI): Calcd for $C_{12}H_{11}Cl_2O_2$ [M+H^+] 257.0131, found 257.0139.

4.3.6. 5-Methyl-2-(2,6-dichlorophenyl)-2,3-dihydro-4*H*-pyran-4-one (7f). Purified by FC on silica gel (petroleum/Et_2O 8:1) to afford the title compound as an oil in 63% yield with 83% ee determined by HPLC analysis using a Chiralcel OJ column (hexane/2-propanol 90:10, 1.0 mL min^{-1}, t_r (minor)=7.497 min, t_r (major)=8.425 min). $[\alpha]_D^{25}$ +13.0 (*c* 0.38, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$): δ=7.39–7.37 (m, 3H, =CH and Ph-H), 7.29–7.25 (m, 1H, Ph-H), 6.20 (dd, ^3J(H,H)=15.8, 4.1 Hz, 1H, Ph-CHO), 3.57–3.52 (m, 1H, CH_AH_B), 2.53–2.49 (m, 1H, CH_AH_B), 1.78 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ=191.74, 159.22, 135.12, 132.10, 130.31, 129.60, 113.84, 77.37, 38.32, 10.72; HRMS (ESI): Calcd for $C_{12}H_{11}Cl_2O_2$ [M+H^+] 257.0131, found 257.0138.

4.3.7. 5-Methyl-2-(3,4-dichlorophenyl)-2,3-dihydro-4*H*-pyran-4-one (7g). Purified by FC on silica gel (petroleum/Et_2O 5:1) to afford the title compound as white crystals (mp=96–97 °C) in 88% yield with 99% ee determined by HPLC analysis using a Chiralcel OD column (hexane/2-propanol 90:10, 1.0 mL min^{-1}, t_r (minor)=9.642 min, t_r (major)=14.031 min). $[\alpha]_D^{25}$ +27.8 (*c* 0.19, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$): δ=7.54–7.50 (m, 2H, Ph-H), 7.37 (s, 1H, =CH), 7.25–7.23 (m, 1H, Ph-H), 5.36 (dd, ^3J(H,H)=14.4, 3.4 Hz, 1H, Ph-CHO), 2.85–2.80 (m, 1H, CH_AH_B), 2.72–2.68 (m, 1H, CH_AH_B), 1.76 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ=191.62, 158.90, 138.51, 133.08, 132.82, 130.80, 128.08, 125.17, 114.56, 79.42, 43.07, 10.44; HRMS (ESI): Calcd for $C_{12}H_{11}Cl_2O_2$ [M+H^+] 257.0131, found 257.0138.

4.3.8. 5-Methyl-2-(2-nitrophenyl)-2,3-dihydro-4*H*-pyran-4-one (7h). Purified by FC on silica gel (petroleum/Et_2O 5:1) to afford the title compound as a yellow solid (mp=74–75 °C) in 99% yield with 91% ee determined by HPLC analysis using a Chirapak AD-H column (hexane/2-propanol 95:5, 1.0 mL min^{-1}, t_r (major)=17.417 min, t_r (minor)=23.350 min). $[\alpha]_D^{25}$ −38.9 (*c* 0.42, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ=8.05–8.03 (m, 1H, Ph-H), 7.86–7.84 (m, 1H, Ph-H), 7.76–7.72 (m, 1H, Ph-H), 7.57–7.52 (m, 1H, Ph-H), 7.36 (s, 1H, =CH), 6.00 (dd, ^3J(H,H)=14.0, 3.2 Hz, 1H, Ph-CHO), 3.01–2.96 (m, 1H, CH_AH_B), 2.78–2.70 (m, 1H, CH_AH_B), 1.75 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ=191.33, 158.80, 147.29, 134.21, 133.94, 129.35, 128.10, 124.87, 114.80, 42.95, 29.69, 10.48; HRMS (ESI): Calcd for $C_{12}H_{12}NO_4$ [M+H^+] 234.0761, found 234.0768.

4.3.9. 5-Methyl-2-(3-nitrophenyl)-2,3-dihydro-4*H*-pyran-4-one (7i). Purified by FC on silica gel (petroleum/Et_2O 4:1) to afford the title compound as white crystals (mp=75–76 °C) in 85% yield with 98% ee determined by HPLC analysis using a Chiralcel OD column (hexane/2-propanol 90:10, 1.0 mL min^{-1}, t_r (minor)=18.626 min, t_r (major)=29.123 min). $[\alpha]_D^{25}$ −52.2 (*c* 0.20, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$): δ=8.34 (s, 1H, Ph-H), 8.27–8.26 (m, 1H, Ph-H), 7.74–7.73 (m, 1H, Ph-H), 7.65–7.62 (m, 1H, Ph-H), 7.41 (s, 1H, =CH), 5.52 (dd, ^3J(H,H)=14.3, 3.6 Hz, 1H, Ph-CHO), 2.90–2.85 (m, 1H, CH_AH_B), 2.80–2.76 (m, 1H, CH_AH_B), 1.78 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ=191.34, 158.80, 148.55, 140.50, 131.77, 129.91, 123.58, 121.05, 114.77, 79.48, 43.14, 10.45; HRMS (ESI): Calcd for $C_{12}H_{12}NO_4$ [M+H^+] 234.0761, found 234.0758.

4.3.10. 5-Methyl-2-(4-nitrophenyl)-2,3-dihydro-4*H*-pyran-4-one (7j). Purified by FC on silica gel (petroleum/Et_2O 4:1) to afford the title compound as yellow crystals (mp=102–104 °C) in 80% yield with 96% ee determined by HPLC analysis using a Chiralcel OD column (hexane/2-propanol 90:10, 1.0 mL min^{-1}, t_r (minor)=22.136 min, t_r (major)=36.407 min). $[\alpha]_D^{25}$ −50.7 (*c* 0.22, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$): δ=8.31 (d, ^3J(H,H)=8.6 Hz, 2H, Ph-H), 7.61 (d, ^3J(H,H)=8.7 Hz, 2H, Ph-H), 7.41 (s, 1H, =CH), 5.52 (dd, ^3J(H,H)=14.1, 3.8 Hz, 1H, Ph-CHO), 2.86–2.81 (m, 1H, CH_AH_B), 2.78–2.75 (m, 1H, CH_AH_B), 1.77 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ=191.25, 158.75, 147.99, 145.31, 126.64, 124.08, 114.81, 79.61, 43.19, 10.44; HRMS (ESI): Calcd for $C_{12}H_{12}NO_4$ [M+H^+] 234.0761, found 234.0767.

4.3.11. 5-Methyl-2-(3-methoxyphenyl)-2,3-dihydro-4*H*-pyran-4-one (7k). Purified by FC on silica gel (petroleum/Et_2O 5:1) to afford the title compound as an oil in 99% yield with 91% ee determined by HPLC analysis using a

Chiralcel OJ column (hexane/2-propanol 90:10, 1.0 mL min^{-1}, t_r (minor)=20.617 min, t_r (major)=25.750 min). $[\alpha]_D^{25}$ −22.4 (*c* 0.45, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ=7.36–7.31 (m, 2H, =CH and Ph-H), 6.97–6.89 (m, 3H, Ph-H), 5.35 (dd, ^3J(H,H)=14.8, 3.2 Hz, 1H, Ph-CHO), 3.83 (s, 3H, OCH_3), 2.91–2.83 (m, 1H, CH_AH_B), 2.70–2.65 (m, 1H, CH_AH_B), 1.74 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ=192.63, 159.81, 159.45, 139.75, 129.86, 118.14, 114.09, 114.04, 111.66, 80.79, 55.26, 43.20, 10.51; HRMS (ESI): Calcd for $C_{13}H_{15}O_3$ [M+H$^+$] 219.1016, found 219.1019.

4.3.12. 5-Methyl-2-(4-methoxyphenyl)-2,3-dihydro-4*H*-pyran-4-one (7l). Purified by FC on silica gel (petroleum/Et_2O 4:1) to afford the title compound as an oil in 84% yield with 90% ee determined by HPLC analysis using a Chiralcel OJ column (hexane/2-propanol 90:10, 1.0 mL min^{-1}, t_r (minor)=29.542 min, t_r (major)=36.242 min). $[\alpha]_D^{25}$ −53.3 (*c* 0.12, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$): δ=7.35–7.33 (m, 3H, =CH and Ph-H), 6.95–6.94 (m, 2H, Ph-H), 5.33 (dd, ^3J(H,H)=14.6, 3.3 Hz, 1H, Ph-CHO), 3.84 (s, 3H, OCH_3), 2.94–2.88 (m, 1H, CH_AH_B), 2.67–2.64 (m, 1H, CH_AH_B), 1.74 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ=192.86, 159.99, 159.63, 130.29, 127.66, 114.15, 113.99, 80.78, 55.35, 42.99, 10.51; HRMS (ESI): Calcd for $C_{13}H_{15}O_3$ [M+H$^+$] 219.1016, found 219.1023.

4.3.13. 5-Methyl-2-(3,4-methylenedioxyphenyl)-2,3-dihydro-4*H*-pyran-4-one (7m). Purified by FC on silica gel (petroleum/Et_2O 3:1) to afford the title compound as a yellow solid (mp=100–102 °C) in 38% yield with 90% ee determined by HPLC analysis using a Chiralcel OJ column (hexane/2-propanol 90:10, 1.0 mL min^{-1}, t_r (minor)=28.442 min, t_r (major)=32.183 min). $[\alpha]_D^{25}$ −103.5 (*c* 0.35, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ=7.33 (s, 1H, =CH), 6.90–6.81 (m, 3H, Ph-H), 5.99 (s, 2H, OCH_2O), 5.28 (dd, ^3J(H,H)=14.4, 3.2 Hz, 1H, Ph-CHO), 2.90–2.82 (m, 1H, CH_AH_B), 2.65–2.60 (m, 1H, CH_AH_B), 1.73 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ=192.65, 159.46, 147.93, 147.90, 131.92, 119.94, 113.99, 108.30, 106.64, 101.27, 80.82, 43.10, 10.46; HRMS (ESI): Calcd for $C_{13}H_{13}O_4$ [M+H$^+$] 233.0808, found 233.0813.

4.3.14. 5-Methyl-2-(naphthalen-1-yl)-2,3-dihydro-4*H*-pyran-4-one (7n). Purified by FC on silica gel (petroleum/Et_2O 5:1) to afford the title compound as a white solid (mp=99–100 °C) in 40% yield with 97% ee determined by HPLC analysis using a Chiralcel OD column (hexane/2-propanol 90:10, 1.0 mL min^{-1}, t_r (major)=19.505 min, t_r (minor)=24.740 min). $[\alpha]_D^{25}$ −37.7 (*c* 0.11, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$): δ=8.00–7.98 (m, 1H, napth-H), 7.93–7.88 (m, 2H, napth-H), 7.68–7.66 (m, 1H, napth-H), 7.58–7.52 (m, 3H, napth-H), 7.47 (s, 1H, =CH), 6.14 (dd, ^3J(H,H)=14.5, 3.2 Hz, 1H, napth-CHO), 3.10–3.04 (m, 1H, CH_AH_B), 2.92–2.88 (m, 1H, CH_AH_B), 1.80 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ=192.83, 159.72, 133.84, 133.71, 130.13, 129.40, 129.10, 126.70, 126.58, 126.00, 125.32, 123.80, 122.70, 78.37, 42.58, 10.60; HRMS (ESI): Calcd for $C_{16}H_{15}O_2$ [M+H$^+$] 239.1067, found 239.1066.

4.3.15. 5-Methyl-2-(naphthalen-2-yl)-2,3-dihydro-4*H*-pyran-4-one (7o). Purified by FC on silica gel (petroleum/Et_2O 5:1) to afford the title compound as a white solid (mp=110–112 °C) in 69% yield with 96% ee determined by HPLC analysis using a Chiralcel OD column (hexane/2-propanol 90:10, 1.0 mL min^{-1}, t_r (minor)=16.455 min, t_r (major)=29.510 min). $[\alpha]_D^{25}$ −83.3 (*c* 0.20, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$): δ=7.92–7.87 (m, 4H, napth-H), 7.54–7.50 (m, 3H, napth-H), 7.42 (s, 1H, =CH), 5.56 (dd, ^3J(H,H)=14.5, 3.2 Hz, 1H, napth-CHO), 3.02–2.97 (m, 1H, CH_AH_B), 2.80–2.76 (m, 1H, CH_AH_B), 1.77 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ=192.49, 159.46, 135.62, 133.37, 133.13, 128.74, 128.14, 127.76, 126.57, 125.28, 123.55, 114.24, 81.09, 43.24, 10.54; HRMS (ESI): Calcd for $C_{16}H_{15}O_2$ [M+H$^+$] 239.1067, found 239.1073.

4.3.16. 5-Methyl-2-(4-fluorophenyl)-2,3-dihydro-4*H*-pyran-4-one (7p). Purified by FC on silica gel (petroleum/Et_2O 4:1) to afford the title compound as a light yellow solid (mp=55–57 °C) in 73% yield with 94% ee determined by HPLC analysis using a Chiralcel OB-H column (hexane/2-propanol 80:20, 1.0 mL min^{-1}, t_r (major)=9.850 min, t_r (minor)=19.020 min). $[\alpha]_D^{25}$ +26.2 (*c* 0.49, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$): δ=7.32–7.30 (m, 2H, Ph-H), 7.28 (s, 1H, =CH), 7.05–7.02 (m, 2H, Ph-H), 5.29 (dd, ^3J(H,H)=14.6, 3.3 Hz, 1H, Ph-CHO), 2.82–2.76 (m, 1H, CH_AH_B), 2.61–2.58 (m, 1H, CH_AH_B), 1.67 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ=192.41 162.78 (d, ^1J(C,F)=247 Hz), 159.37, 134.05, 127.93 (d, ^3J(C,F)=8.4 Hz), 115.75 (d, ^2J(C,F)=21.4 Hz), 114.22, 80.27, 43.17, 10.52; HRMS (ESI): Calcd for $C_{12}H_{12}FO_2$ [M+H$^+$] 207.0816, found 207.0823.

4.3.17. 5-Methyl-2-(4-cyanophenyl)-2,3-dihydro-4*H*-pyran-4-one (7q). Purified by FC on silica gel (petroleum/Et_2O 3:1) to afford the title compound as white crystals (mp=140–144 °C) in 95% yield with 92% ee determined by HPLC analysis using a Chiralcel OD column (hexane/2-propanol 90:10, 1.0 mL min^{-1}, t_r (minor)=19.975 min, t_r (major)=27.342 min). $[\alpha]_D^{25}$ −49.1 (*c* 0.22, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ=7.73–7.51 (m, 4H, Ph-H), 7.38 (s, 1H, =CH), 5.45 (dd, ^3J(H,H)=14.0, 4.0 Hz, 1H, Ph-CHO), 2.84–2.69 (m, 2H, CH_AH_B), 1.74 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ=191.53, 158.89, 143.37, 132.64, 126.45, 118.34, 114.66, 112.51, 79.76, 43.07, 29.68, 10.48; HRMS (ESI): calcd for $C_{13}H_{12}NO_2$ [M+H$^+$] 214.0863, found 214.0857.

4.3.18. 5-Methyl-2-(pyridin-2-yl)-2,3-dihydro-4*H*-pyran-4-one (7r). Purified by FC on silica gel (petroleum/Et_2O 2:1) to afford the title compound as a light brown solid (mp=67–69 °C) in 99% yield with 98% ee determined by HPLC analysis using a Chiralcel OJ column (hexane/2-propanol 90:10, 1.0 mL min^{-1}, t_r (minor)=13.825 min, t_r (major)=20.783 min). $[\alpha]_D^{25}$ −107.7 (*c* 0.14, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ=8.62–8.61 (m, 1H, Py-H), 7.79–7.75 (m, 1H, Py-H), 7.50–7.48 (m, 1H, Py-H), 7.37 (s, 1H, =CH), 7.31–7.29 (m, 1H, Py-H), 5.49 (m, ^3J(H,H)=13.2, 4.0 Hz, 1H, Py-CHO), 3.01–2.86 (m, 2H, CH_AH_B), 1.74 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ=192.38, 158.71, 157.13, 149.45, 137.04, 123.40, 120.68, 114.50, 81.09, 41.43, 10.54; HRMS (ESI): Calcd for $C_{11}H_{12}NO_2$ [M+H$^+$] 190.0863, found 190.0859.

4.3.19. 5-Methyl-2-(pyridin-3-yl)-2,3-dihydro-4*H*-pyran-4-one (7s). Purified by FC on silica gel (petroleum/Et_2O

1:1) to afford the title compound as a light brown liquid in 98% yield with 96% ee determined by HPLC analysis using a Chiralcel OD column (hexane/2-propanol 80:20, 1.0 mL min^{-1}, t_r (minor)=14.308 min, t_r (major)=23.875 min). $[\alpha]_D^{25}$ −60.1 (*c* 0.56, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ=8.67–8.63 (m, 2H, Py-H), 7.76–7.73 (m, 1H, Py-H), 7.39–7.36 (m, 2H, Py-H and =CH), 5.44 (dd, ^3J(H,H)=14.8, 3.2 Hz, 1H, Py-CHO), 2.92–2.85 (m, 1H, CH_AH_B), 2.74–2.69 (m, 1H, CH_AH_B), 1.75 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ=191.70, 159.10, 150.07, 147.67, 133.81, 133.57, 123.57, 114.50, 78.61, 42.80, 10.45; HRMS (ESI): Calcd for $C_{11}H_{12}NO_2$ [M+H^+] 190.0863, found 190.0869.

4.3.20. 5-Methyl-2-(furan-2-yl)-2,3-dihydro-4*H*-pyran-4-one (7t). Purified by FC on silica gel (petroleum/Et_2O 5:1) to afford the title compound as yellow crystals (mp=49–51 °C) in 99% yield with 97% ee determined by HPLC analysis using a Chiralcel OJ column (hexane/2-propanol 90:10, 1.0 mL min^{-1}, t_r (minor)=11.533 min, t_r (major)=21.417 min). $[\alpha]_D^{25}$ −302.3 (*c* 0.34, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ=7.47 (s, 1H, Fur-H), 7.25 (s, 1H, =CH), 6.42–6.40 (m, 2H, Fur-H), 5.42 (dd, ^3J(H,H)=13.2, 3.6 Hz, 1H, Fur-CHO), 3.11–3.04 (m, 1H, CH_AH_B), 2.76–2.71 (m, 1H, CH_AH_B), 1.71 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ=191.82, 158.71, 150.48, 143.46, 114.22, 110.51, 109.34, 73.53, 39.38, 10.51; HRMS (ESI): Calcd for $C_{10}H_{11}O_3$ [M+H^+] 179.0703, found 179.0703.

4.3.21. 5-Methyl-2-styryl-2,3-dihydro-4*H*-pyran-4-one (7u). Purified by FC on silica gel (petroleum/Et_2O 5:1) to afford the title compound as white crystals (mp=75–77 °C) in 47% yield with 85% ee determined by HPLC analysis using a Chiralcel OJ column (hexane/2-propanol 90:10, 1.0 mL min^{-1}, t_r (minor)=22.725 min, t_r (major)=28.142 min). $[\alpha]_D^{25}$ −128.3 (*c* 0.12, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ=7.42–7.26 (m, 6H, Ph-H and =CH), 6.70 (d, ^3J(H,H)=16 Hz, 1H, Ph-CH=C), 6.30 (dd, ^3J(H,H)=16, 6.4 Hz, 1H, Ph-C=CH), 5.02 (m, 1H, Styryl-CHO), 2.76–2.60 (m, 2H, CH_AH_B), 1.71 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ=192.43, 159.30, 135.63, 133.38, 128.67, 128.42, 126.71, 125.45, 114.02, 79.56, 41.72, 10.55; HRMS (ESI): Calcd for $C_{14}H_{15}O_2$ [M+H^+] 215.1067, found 215.1064.

4.3.22. 5-Methyl-2-pentyl-2,3-dihydro-4*H*-pyran-4-one (7v). Purified by FC on silica gel (petroleum/Et_2O 9:1) to afford the title compound as an oil in 41% yield with 87% ee determined by HPLC analysis using a Chiralpak AD-H column (hexane/2-propanol 99.5:0.5, 1.0 mL min^{-1}, t_r (major)=14.110 min, t_r (minor)=19.627 min). $[\alpha]_D^{25}$ −93.4 (*c* 0.33, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$): δ=7.24 (s, 1H, =CH), 4.36–4.31 (m, 1H, Pentyl-CHO), 2.51–2.47 (m, 1H, CH_AH_B), 2.45–2.42 (m, 1H, CH_AH_B), 1.81–1.25 (m, 14H, 4×CH_2, 2×CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ=193.29, 159.76, 113.57, 79.46, 41.67, 34.48, 31.50, 24.46, 22.50, 13.96, 10.48; HRMS (ESI): Calcd for $C_{11}H_{19}O_2$ [M+H^+] 183.1380, found 183.1380.

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References and notes

1. (a) Kagan, H. B.; Comprehensive Organic Chemistry; Pergamon: Oxford, 1992; Vol. 8. (b) Noyori, R. Asymmetric catalysis in organic synthesis; Wiley: New York, 1994. (c) Jacobsen, E. N., Pfaltz, A., Yamamoto, H., Eds.; Comprehensive Asymmetric Catalysis; Springer: Berlin, 1999; Vol. 1–3. (d) Ojima, I., Ed. 2nd ed.; Catalysis Asymmetric Synthesis; Wiley: New York, 2000. (e) Lin, G.-Q.; Li, Y.-M.; Chan, A. S. C. Principles and Applications of Asymmetric Synthesis; Wiley: New York, 2001.
2. (a) Jørgensen, K. A. *Angew. Chem. Int. Ed.* **2000**, *39*, 3558–3588 and references therein. (b) Jørgensen, K. A. *Eur. J. Org. Chem.* **2004**, 2093–2102 and references therein. (c) Fan, Q.; Lin, L.-L.; Liu, J.; Huang, Y.-Z.; Feng, X.-M.; Zhang, G.-L. *Org. Lett.* **2004**, *6*, 2185–2188.
3. (a) Juhl, K.; Jørgensen, K. A. *Angew. Chem. Int. Ed.* **2003**, *42*, 1498–1501. (b) Huang, Y.; Unni, A. K.; Thadani, A. N.; Rawal, V. H. *Nature* **2003**, *424*, 146. (c) Du, H.-F.; Zhao, D.-B.; Ding, K.-L. *Chem. Eur. J.* **2004**, *10*, 5964–5970.
4. (a) Larson, E. R.; Danishefsky, S. *J. Am. Chem. Soc.* **1982**, *104*, 6458–6460. (b) Larson, E. R.; Danishefsky, S. *Tetrahedron Lett.* **1982**, *23*, 1975–1978. (c) Danishefsky, S. J.; Larson, E.; Askin, D.; Kato, N. *J. Am. Chem. Soc.* **1985**, *107*, 1246–1255. (d) Corey, E. J.; Cywin, C. L.; Roper, T. D. *Tetrahedron Lett.* **1992**, *33*, 6907–6910. (e) Keck, G. E.; Li, X.-Y.; Krishnamurthy, D. *J. Org. Chem.* **1995**, *60*, 5998–5999. (f) Schaus, S. E.; Brånalt, J.; Jacobsen, E. N. *J. Org. Chem.* **1998**, *63*, 403–405. (g) Molander, G. A.; Rzasa, R. M. *J. Org. Chem.* **2000**, *65*, 1215–1217. (h) Baldoli, C.; Maiorana, S.; Licandro, E.; Zinzalla, G.; Lanfranchi, M.; Tiripicchio, A. *Tetrahedron: Asymmetry* **2001**, *12*, 2159–2167. (i) Yuan, Y.; Long, J.; Sun, J.; Ding, K.-L. *Chem. Eur. J.* **2002**, *8*, 5033–5042. (j) Yamashita, Y.; Saito, S.; Ishitani, H.; Kobayashi, S. *J. Am. Chem. Soc.* **2003**, *125*, 3793–3798.
5. (a) McCarrick, M. A.; Wu, Y.-D.; Houk, K. N. *J. Am. Chem. Soc.* **1992**, *114*, 1499–1500. (b) McCarrick, M. A.; Wu, Y.-D.; Houk, K. N. *J. Org. Chem.* **1993**, *58*, 3330–3343. (c) Roberson, M.; Jepsen, A. S.; Jørgensen, K. A. *Tetrahedron* **2001**, *57*, 907–913. (d) Ujaque, G.; Lee, P. S.; Houk, K. N.; Hentemann, M. F.; Danishefsky, S. J. *Chem. Eur. J.* **2002**, *8*, 3423–3430.
6. For example, see: (a) Danishefsky, S.; Larson, E. R.; Askin, D. *J. Am. Chem. Soc.* **1982**, *104*, 6457–6458. (b) Danishefsky, S.; Bednarski, M. *Tetrahedron Lett.* **1985**, *26*, 3411–3412. (c) Danishefsky, S. J.; Pearson, W. H.; Segmuller, B. E. *J. Am. Chem. Soc.* **1985**, *107*, 1280–1285. (d) Danishefsky, S. J.; DeNinno, M. P. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 15–23. (e) Tietze, L. F.; Schneider, C.; Montenbruck, A. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 980–982. (f) Hu, Y.-J.;

Huang, X.-D.; Yao, Z.-J.; Wu, Y.-L. *J. Org. Chem.* **1998**, *63*, 2456–2461. (g) Bednarski, M.; Danishefsky, S. *J. Am. Chem. Soc.* **1986**, *108*, 7060–7067. (h) Zhuang, W.; Thorhauge, J.; Jørgensen, K. A. *Chem. Commun.* **2000**, 459–460. (i) Audrain, H.; Thorhauge, J.; Hazell, R. G.; Jørgensen, K. A. *J. Org. Chem.* **2000**, *65*, 4487–4497. (j) Bednarski, M.; Danishefsky, S. *J. Am. Chem. Soc.* **1983**, *105*, 6968–6969. (k) Danishefsky, S. J.; Selnick, H. G.; Zelle, R. E.; DeNinno, M. P. *J. Am. Chem. Soc.* **1988**, *110*, 4368–4378. (l) Rainier, J. D.; Allwein, S. P.; Cox, J. M. *Org. Lett.* **2000**, *2*, 231–234. (m) Cox, J. M.; Rainier, J. D. *Org. Lett.* **2001**, *3*, 2919–2922. (n) Williams, D. R.; Heidebrecht, R. W., Jr. *J. Am. Chem. Soc.* **2003**, *125*, 1843–1850.

7. (a) Danishefsky, S.; Kitahara, T. *J. Am. Chem. Soc.* **1974**, *96*, 7807–7808. (b) Danishefsky, S.; Yan, C.-F.; Singh, R. K.; Gammill, R. B.; McCurry, P. M., Jr.; Fritsch, N.; Clardy, J. *J. Am. Chem. Soc.* **1979**, *101*, 7001–7008.
8. Fu, Z.-Y.; Gao, B.; Yu, Z.-P.; Yu, L.; Huang, Y.-Z.; Feng, X.-M.; Zhang, G.-L. *Synlett* **2004**, 1772–1775.
9. For examples of reviews on relative concepts in asymmetric synthesis, see: (a) Girard, C.; Kagan, H. B. *Angew. Chem. Int. Ed.* **1998**, *37*, 2922–2959. (b) Faller, J. W.; Lavoie, A. R.; Parr, J. *Chem. Rev.* **2003**, *103*, 3345–3368. (c) Mikami, K.; Terada, M.; Korenaga, T.; Matsumoto, Y.; Ueki, M.; Angelaud, R. *Angew. Chem. Int. Ed.* **2000**, *39*, 3532–3556. (d) Mikami, K.; Yamanaka, M. *Chem. Rev.* **2003**, *103*, 3369–3400. (e) Berrisford, D. J.; Bolm, C.; Sharpless, K. B. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1059–1070. (f) Walsh, P. J. *Acc. Chem. Res.* **2003**, *36*, 739–749. (g) Soai, K.; Shibata, T.; Sato, I. *Acc. Chem. Res.* **2000**, *33*, 382–390.
10. Long, J.; Hu, J.-Y.; Shen, X.-Q.; Ji, B.-M.; Ding, K.-L. *J. Am. Chem. Soc.* **2002**, *124*, 10–11.
11. (a) Wang, B.; Feng, X.-M.; Cui, X.; Liu, H.; Jiang, Y.-Z. *Chem. Commun.* **2000**, 1605–1606. (b) Wang, B.; Feng, X.-M.; Huang, Y.-Z.; Liu, H.; Cui, X.; Jiang, Y.-Z. *J. Org. Chem.* **2002**, *67*, 2175–2182. (c) Huang, Y.-Z.; Feng, X.-M.; Wang, B.; Zhang, G.-L.; Jiang, Y.-Z. *Synlett* **2002**, 2122–2124.
12. (a) Chan, A. S. C.; Zhang, F.-Y.; Yip, C.-W. *J. Am. Chem. Soc.* **1997**, *119*, 4080–4081. (b) Zhang, F.-Y.; Yip, C.-W.; Cao, R.; Chan, A. S. C. *Tetrahedron: Asymmetry* **1997**, *8*, 585–589. (c) Zhang, F.-Y.; Chan, A. S. C. *Tetrahedron: Asymmetry* **1997**, *8*, 3651–3655.
13. (a) ^{1}H, ^{13}C NMR and HRMS data of compound **8**: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.41$–7.20 (m, 6H, Ph-H and =CH), 5.20–5.17 (m, 1H, Ph-CHO), 3.97–3.96 (m, 1H, OH), 3.84 (s, 3H, OCH_3), 2.94–2.83 (m, 2H, CH_AH_B), 1.72 (s, 3H, CH_3); ^{1}H NMR (400 MHz, $CDCl_3+D_2O$): $\delta=7.45$–7.31 (m, 6H, Ph-H and =CH), 5.23 (dd, $^{3}J(H,H)=8.8$, 3.2 Hz, 1H, Ph-CHO), 3.90 (s, 3H, OCH_3), 3.00–2.88 (m, 2H, CH_AH_B), 1.77 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): $\delta=199.72$, 160.93, 143.25, 128.48, 127.53, 125.72, 117.45, 70.40, 61.60, 45.89, 8.06; HRMS (ESI): Calcd for $C_{26}H_{32}O_6Na$ $[2M+Na^+]$ 463.2091, found 463.2087.
14. (a) Clive, D. L. J.; Bergstra, R. J. *J. Org. Chem.* **1991**, *56*, 4976–4977 and references therein. (b) Miyashita, M.; Yamasaki, T.; Shiratani, T.; Hatakeyama, S.; Miyazawa, M.; Irie, H. *Chem. Commun.* **1997**, 1787–1788.
15. Guo, H.; Ding, K.-L. *Tetrahedron Lett.* **2000**, *41*, 10061–10064.
16. Shen, X.-Q.; Guo, H.; Ding, K.-L. *Tetrahedron: Asymmetry* **2000**, *11*, 4321–4327.

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Regioselectivity in the reactions of polyfunctionalised pyrroles with nucleophiles

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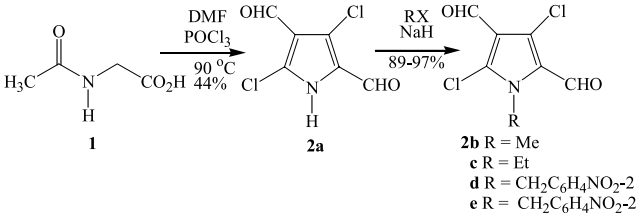
Abstract—The polyfunctionalised pyrrole, 3,5-dichloro-1*H*-pyrrole-2,4-dicarbaldehyde reacts with secondary amines by condensation with the 2-carbaldehyde to give methylene-substituted pyrroles, while its *N*-alkyl derivatives undergo substitution of the 5-chloro group to give 5-substituted pyrroles.

1. Introduction

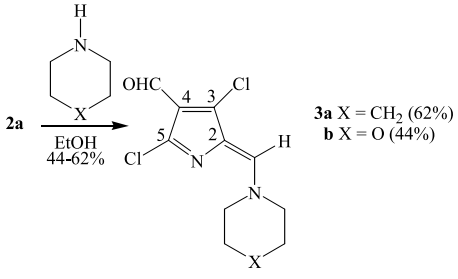
As part of a study involving the generation and reaction of Vilsmeier reagents, we have isolated the polyfunctional pyrrrole **2a** from the reaction of *N*-acetylglycine **1** and *N*,*N*-dimethylformamide in $POCl_3$, Scheme 1, via the method of Balasundaram et al.[1] This pyrrole **2a** has a range of electrophilic centres, and thus, a range of potential adducts from its reaction with nucleophiles. Of particular interest are the differences in the reactivity of the C-2 and C-4 aldehyde groups due to the potential for hydrogen bond formation by the C-2 aldehyde group, and the differences in the electrophilicity of C-3 and C-5 due to the inductive effect of the nitrogen on C-5, and the fact that C-3 is the β-carbon of an enamine. We wish to report here the first study of the reactions of this polyfunctionalised pyrrole **2a** and some alkylated derivatives with ethanethiol and a range of amines.



Scheme 1.

2. Results and discussion

The reaction of 1 equiv of pyrrole **2a** with 5 equiv of morpholine or piperidine, in ethanol at room temperature, afforded crystalline products after purification, which were assigned as 3,5-dichloro-2-(1′-piperidinylmethylene)-2*H*-pyrrole-4-carbaldehyde **3a** and 3,5-dichloro-2-(4′-morpholinylmethylene)-2*H*-pyrrole-4-carbaldehyde **3b**, Scheme 2. The ^{1}H NMR spectrum of **3a** revealed that the adduct was formed from a 1:1 molar ratio of the reactants, with one of the aldehyde group signals disappearing, and a new alkenic signal appearing at $\delta_H = 7.53$ ppm. In the IR spectrum of pyrrole **3a** there was a strong absorption at 1624 cm^{-1}, characteristic of a C=C bond conjugated with an amine group (enamine), and a peak for the C=O of an aldehyde at 1673 cm^{-1}.



Scheme 2.

The pyrrole regioisomer formed was identified with the aid of a heteronuclear multiple bond correlation (HMBC) spectrum. In this spectrum, only two carbons (C-2 and C-3) will give crosspeak signals to the hydrogen of the

Keywords: Vilsmeier; Pyrroles; Nucleophilic substitution; Condensation; HMBC.
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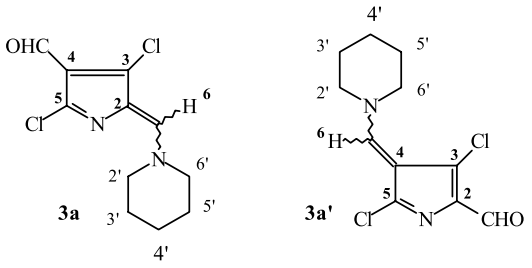
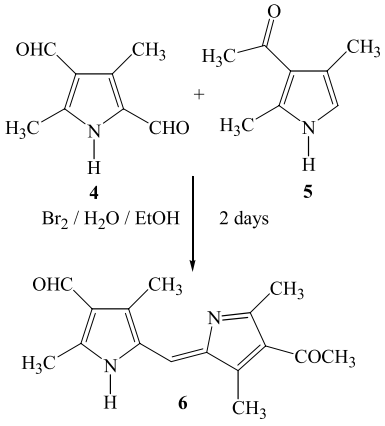


Figure 1.

aldehyde in **3a′**, whereas in isomer **3a** three carbon atoms (C-3, C-4, and C-5) would give crosspeaks, Figure 1. The same logic can be applied to the enamine hydrogen atom (H-6), but in this case four signals [C-2, C-3 and two carbons of the piperidine (C-2′ and C-6′)] would be expected to give crosspeaks for **3a** and for **3a′** five signals would be expected (C-3, C-4, C-5, C-2′, and C-6′). The HMBC spectrum supports structure **3a** as the product.

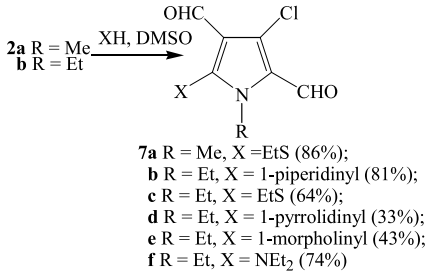
Because of the conjugation of the lone pair of electrons on the nitrogen atom in the piperidinyl group with the rest of the molecule in pyrrole **3a**, the rotation of the amine function about the N–C6 single bond is restricted and this gives rise to a difference in the chemical shifts of the H-7 and H-11 methylenes in the proton spectrum (two clearly distinguished triplets at $\delta_H=3.71$ and 4.68 ppm). These methylenes can be assigned with the aid of NOE experiments, showing which of the two CH_2 groups are closest to the alkene proton, H-6, in space. The irradiation of the alkene proton signal gave an increase in the intensity of the peak at δ 3.71 of 4.5%, indicating that the peak at δ 3.71 belongs to H-6′ and the signal at δ 4.68 to H-2′. The lack of protons on the pyrrole ring precludes the assignment of the stereochemistry of the alkene bond.

In a similar manner, a pyrrole-3,5-dicarbaldehyde **4** has been shown to react with 3-acetyl-2,4-dimethylpyrrole **5** to give a 5-pyrrolylidenemethylpyrrole-3-carbaldehyde **6**, Scheme 3.[2]



Scheme 3.

The presence of the labile proton of the N–H group in pyrrole **2a** made it impossible to achieve nucleophilic substitution of the chloro groups by secondary amines. In order to overcome this problem it was necessary to alkylate the pyrrole **2a** in dry DMF using methyl iodide, ethyl bromide, 2-nitrobenzyl bromide or 4-methoxybenzyl chloride and sodium hydride as the base, to give the pyrroles **2b–e** in good to excellent yields, Scheme 1. The *N*-methyl **2b** and *N*-ethyl **2c** derivatives were chosen for further investigation in nucleophilic substitutions with thioethanol, piperidine, pyrrolidine, morpholine and diethylamine in DMSO at room temperature. All of these reactions gave the products **7** of the substitution at the pyrrole 5-position, Scheme 4.



Scheme 4.

The ^{1}H NMR spectrum of 3-chloro-5-diethylamino-1-ethyl-1*H*-pyrrole-2,4-dicarbaldehyde **7f** indicated the presence of two aldehyde groups and the signals for two different ethyl groups in a ratio of 2:1. The triplet at $\delta_H=1.01$ and the quartet at $\delta_H=3.19$ ppm belong to the *N*,*N*-diethylamino group and the triplet at $\delta_H=1.29$ and the quartet at $\delta_H=4.36$ ppm belong to the ethyl group in the 1-position of the pyrrole ring. Once again, the regioisomer formed was assigned with the use of an HMBC spectrum, Figure 2.

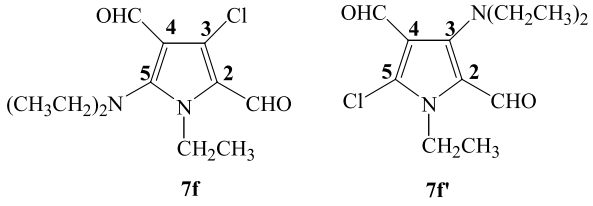


Figure 2.

In this case, in isomer **7f** the carbon in the 5-position would give two crosspeaks—to the protons of the ethyl groups of the N^1-ethyl and 5-diethylamino substituents—whereas in isomer **7f′** common crosspeaks would not be expected for the ethyl groups. In the HMBC experiment the carbon at $\delta_C=149.9$ ppm gave crosspeaks to the protons of the CH_2 groups of the both sets of ethyl groups indicating that the product is pyrrole **7f**. The quartet at $\delta_H=4.36$ ppm also shows a crosspeak to the ^{13}C quaternary signal at $\delta_C=122.95$ ppm, indicating that this is the signal for C-2. This greater electrophilicity of C-5 compared to C-3 correlates well with the chemical shifts of the protons attached to those carbons in the ^{1}H NMR spectrum of 1-methyl-1*H*-pyrrole-2,4-dicarboxaldehyde **8**, with peaks at $\delta_H=7.47$ and 7.34 ppm corresponding to H-5 and H-3, respectively, Figure 3.[3]

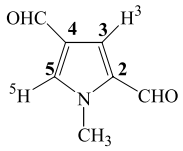


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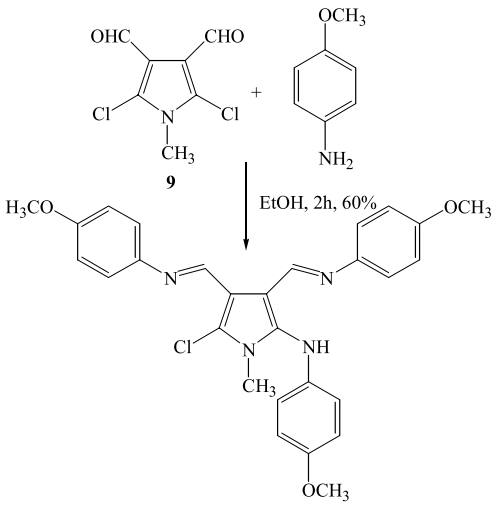
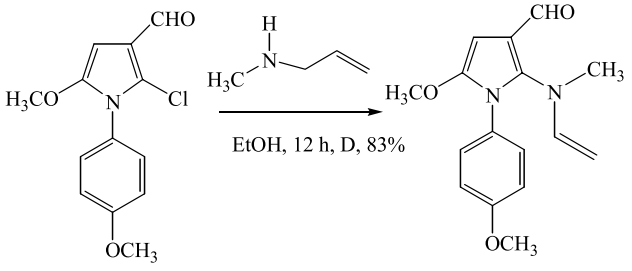
Figure 3.

In the reaction of the *N*-methyl-2,5-dichloropyrrole-3,4-dicarbaldehyde **9** with 4-methoxyaniline, Scheme 5,[4] substitution of one of the chloro groups can be accompanied by imine formation with both aldehyde groups while the reaction of *N*-methylallylamine with the 2-chloropyrrole-3-carbaldehyde **10** led only to the substitution of the chloro group, Scheme 6.[5]



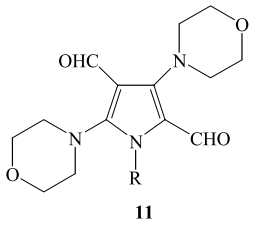
Scheme 5.



Scheme 6.

If the reaction of pyrrole **2c** with an excess of morpholine was performed at 78 °C for 70 h, the di-substituted pyrrole **11**, Figure 4, formed in 21% yield, along with the mono-substituted derivative **7e**.

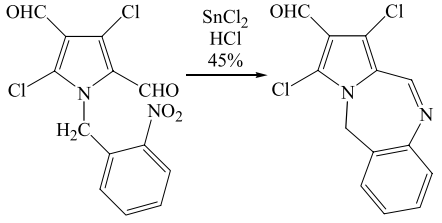
The substitution of the remaining 3-chloro group in pyrrole **7e** is more difficult than in the initial pyrrole **2c** because of the electron-rich character of the carbon at the 3-position and the fact that the lone pair of electrons on the nitrogen atom of the morpholinyl group also contributes to the overall electron density of the pyrrole ring.



11

Figure 4.

Having two active functions on the carbons adjacent to the nitrogen in pyrrole **2d** (an aldehyde group at the 2-position and the chloro group at the 5-position) it was interesting to see which would be more reactive with a nucleophilic reagent in an intramolecular reaction. In order to equalise the chances of either substitution, it was decided to use a primary amine as the nucleophile, and this was prepared in situ by the reduction of the nitro group in pyrrole **2d**. Analysis of the ^{1}H NMR spectrum of the product revealed an absence of the signal for the proton of one of the aldehyde groups and the presence of a signal at δ 8.52, which was assigned to the imine group in the pyrrolo-[2,1-*c*]benzo[*f*]diazepine **12**, Scheme 7, indicating that the amino group reacts via condensation with the aldehyde group rather than via substitution of the chloro group.



2d 12

Scheme 7.

In conclusion, the reaction of the polyfunctional pyrroles **2** with a range of nucleophiles leads to the formation of a range of selectively functionalized pyrroles—pyrrole **2a** reacts with secondary amines by condensation with the 2-carbaldehyde to give methylene-substituted pyrroles, while its *N*-alkyl derivatives **2b**,**c** undergo substitution of the 5-chloro group to give 5-substituted pyrroles **7a–f**.

3. Experimental

3.1. General

Infra-red spectra were obtained on a Unicam Research Series 2000 FTIR; liquid samples were examined as thin films or nujol mulls on sodium chloride plates, and solid samples as potassium bromide (KBr) discs. Low resolution electrospray (ESI) spectra were obtained on a Bruker Esquire 3000+ ion trap mass spectrometer. High resolution electrospray spectra were obtained on a Bruker APEX II FT-MS (ICR) and high resolution electron impact (EI) spectra on a Finnigan MAT 900 XLT at the EPSRC National Mass Spectrometry Service Centre, University of Swansea. ^{1}H NMR spectra were acquired on a Bruker

AVANCE 300 at 300 MHz, or a Bruker AVANCE 500 at 500 MHz. Spectra are recorded as δ values for solutions in $CDCl_3$ unless otherwise stated, and coupling constants (J) are quoted in Hz. ^{13}C NMR spectra were obtained on the Bruker AVANCE 300 (75 MHz) or 500 (125 MHz). Melting points were determined on an Electrothermal 9100, a Gallenkamp melting point apparatus, or a Reichert hot stage microscope.

Fluka silica gel $60F_{254}$ plates were used for tlc analysis and the components in the developed chromatogram were detected by their quenching of fluorescence under UV light. Fluka $60F_{254}$ silica gel in a glass column was used for flash column chromatography. The samples were applied as liquids or pre-adsorbed onto a small amount of silica.

3.2. Materials

3.2.1. 3,5-Dichloro-1*H*-pyrrole-2,4-dicarbaldehyde 2a. *N*-Acetylglycine **1** (0.5 g, 4.27 mmol) was dissolved in dry DMF (5 mL) and cooled to 0 °C in an ice bath and $POCl_3$ (1.46 mL, 15.66 mmol) was added dropwise over 30 min. The resulting mixture was stirred for 1 h at room temperature then 4 h at 90 °C. The contents of the flask were then poured, with stirring, onto a mixture of crushed ice (50 mL), sodium acetate (1.46 g) and water (5 mL). The product was extracted with ether (5×20 mL) and dried over anhydrous $MgSO_4$. After removal of the solvent under reduced pressure, the crude product was purified by column chromatography on silica, eluting with ethyl acetate to give a red solid (0.36 g, 44%), mp 147.5–148.5 °C (lit.[1] 170 °C); IR (KBr) 3095 (NH), 1675 (C=O), 1649 (C=O), and 1536 (C=C) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) $\delta=9.63$ (1H, s, CHO), 9.84 (1H, s, CHO), 12.85 (1H, br s, NH); ^{13}C NMR (75 MHz, DMSO-d_6) $\delta=117.7$ (quat.), 123.7 (quat.), 128.5 (quat.), 130.5 (quat.), 177.4 (CHO), 183.0 (CHO); HRMS (EI): Found: $[M-H]^+$, 189.9457. Calcd for $C_6H_2NO_2{}^{35}Cl_2$: $[M-H]^+=189.9457$.

3.2.2. 3,5-Dichloro-1-methyl-1*H*-pyrrole-2,4-dicarbaldehyde 2b. A solution of pyrrole **2a** (1.0 g, 5.21 mmol) in dry DMF (12 mL) was added dropwise to a stirred suspension of NaH (60% in oil; 0.27 g, 6.77 mmol) in dry DMF (10 mL) and stirred for 15 min at room temperature. Methyl iodide (1.31 mL, 20.84 mmol) was added dropwise to the resulting solution containing the salt of the pyrrole. The reaction mixture was left to stir for a further 2 h at room temperature, quenched with water (20 mL), extracted with diethyl ether (3×20 mL) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography on silica, eluting with light petroleum (60–80 °C)/diethyl ether (1:2) to give **2b** as a pinkish solid (0.95 g, 89%), mp 108.5–109.5 °C; IR (KBr) 1668 (C=O) cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) $\delta=3.99$ (3H, s, NCH_3), 9.83 (1H, s, 2-C*H*O), 9.99 (1H, s, 4-C*H*O); ^{13}C NMR (75 MHz; $CDCl_3$) $\delta=33.7$ (CH_3, NCH_3), 117.3 (quat., C-4), 127.0 (quat., C-2 or C-3), 127.5 (quat., C-2 or C-3), 135.5 (quat., C-5), 178.1 (CH, 2-*C*HO), 182.7 (CH, 4-*C*HO); HRMS (ESI): Found: MNa^+, 227.9596. Calcd for $C_7H_5{}^{35}Cl_2NNaO_2$: $MNa^+=227.9590$.

3.2.3. 3,5-Dichloro-1-ethyl-1*H*-pyrrole-2,4-dicarbaldehyde 2c. This pyrrole was prepared as described above, using 3,5-dichloro-1*H*-pyrrole-2,4-dicarbaldehyde **2a** (0.09 g, 0.47 mmol), NaH (60% in oil, 0.24 g, 6.10 mmol) and ethyl bromide (1.43 mL, 18.76 mmol) to give **2c** as a red solid (0.100 g, 97%), mp 68.0–69.0 °C; IR (KBr) 1667 (C=O) cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) $\delta=1.28$ (3H, t, $J=7.1$ Hz, CH_3CH$_2$), 4.44 (2H, q, $J=7.1$ Hz, CH$_3$CH_2), 9.73 (1H, s, 2-CHO), 9.89 (1H, s, 4-CHO); ^{13}C NMR (75 MHz; $CDCl_3$) $\delta=15.5$ (CH_3, *C*H$_3$CH$_2$), 42.1 (CH_2, CH$_3$*C*H$_2$), 117.3 (quat., C-3), 126.3 (quat., C-4 or C-5), 127.8 (quat., C-4 or C-5), 131.6 (quat., C-2), 177.8 (CH, 2-CHO), 182.7 (CH, 4-CHO); HRMS (ESI): Found: MH^+, 219.9924. Calcd for $C_8H_8{}^{35}Cl_2NO_2$: MH^+, 219.9927.

3.2.4. 3,5-Dichloro-1-(2′-nitrobenzyl)-1*H*-pyrrole-2,4-dicarbaldehyde 2d. Pyrrole **2a** (0.405 g, 2.109 mmol) was dissolved in dry DMF (10 mL) and sodium hydride (60% in oil; 0.101 g, 2.531 mmol) was added in small portions. After all the sodium hydride had been added, 2-nitrobenzyl bromide (0.547 g, 2.531 mmol) was added to the solution of the pyrrole sodium salt. The resulting mixture was stirred at 80 °C for 7 days, quenched with water (10 mL), extracted with diethyl ether (3×10 mL) and the organic phase dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica, eluting with light petroleum (60–80 °C)/diethyl ether (30:70) to give **2d** as a red solid (0.671 g, 97%), mp 167–168 °C; IR (KBr) 1658 (C=O), 1518 and 1346 (NO_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) $\delta=5.96$ (2H, s, H-6), 6.74 (1H, d, $J=7.7$ Hz, H-6′), 7.60 (1H, t, $J=7.7$ Hz, H-4′), 7.69 (1H, t, $J=7.7$ Hz, H-5′), 8.21 (1H, d, $J=7.7$ Hz, H-3′), 9.68 (1H, s, 2-CHO), 9.94 (1H, s, 4-CHO); ^{13}C NMR (75 MHz, $CDCl_3$) $\delta=47.4$ (CH_2, C-6), 117.7 (quat., C-4), 126.2 (quat., C-5), 126.2 (CH, C-3′), 127.1 (quat., C-1′), 127.5 (CH, C-6′), 129.9 (CH, C-4′), 131.8 (quat., C-2), 133.2 (quat., C-3), 135.7 (CH, C-5′), 147.8 (quat., C-2′), 178.8 (CH, 2-CHO), 183.7 (CH, 4-CHO); HRMS (ESI): Found: MH^+, 326.9935. Calcd for $C_{13}H_9{}^{35}Cl_2N_2O_4$: $MH^+=326.9934$.

3.2.5. 3,5-Dichloro-1-(4′-methoxybenzyl)-1*H*-pyrrole-2,4-dicarbaldehyde 2e. Prepared as described for **2d** to give **2e** as an oil (0.158 g, 97%); IR (KBr) 1671 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) $\delta=3.79$ (3H, s, OCH_3), 5.65 (2H, s, CH_2N), 6.85 (2H, d, $J=8.7$ Hz, H-3′ and H-5′), 7.19 (2H, d, $J=8.7$ Hz, H-2′ and H-6′), 9.82 (1H, s, 2-CHO), 10.02 (1H, s, 4-CHO); ^{13}C NMR (75 MHz, $CDCl_3$) $\delta=48.9$ (CH_2, C-6), 55.6 (CH_3, OCH_3), 114.7 (2×CH, C-3′ and C-5′), 116.4 (quat., C-4), 117.7 (quat., C-3), 126.6 (quat.), 127.4 (quat.), 128.0 (quat.), 129.3 (2×CH, C-2′ and C-6′), 160.1 (quat., C-4′), 177.7 (CH, 2-CHO), 182.4 (CH, 4-CHO); HRMS (ESI): Found: MNa^+, 334.0010. Calcd for $C_{14}H_{11}{}^{35}Cl_2NNaO_3$: $MNa^+=334.0008$.

3.2.6. 3,5-Dichloro-2-(1′-piperidinylmethylene)-2*H*-pyrrole-4-carbaldehyde 3a. Pyrrole **2a** (0.125 g, 0.652 mmol) was dissolved in ethanol (2 mL) and piperidine (0.32 mL, 3.255 mmol) was added. The reaction mixture was stirred at room temperature for 7 h, quenched with brine (5 mL), extracted with CH_2Cl_2 (3×5 mL) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica, eluting with CH_2Cl_2/EtOAc (95:5) to give **3a** as a brown solid (0.104 g, 62%), mp 100–101 °C; IR (KBr) 1673

(C=O), 1624 (enamine C=C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ=1.79–1.92 (6H, br s, H-3′, H-4′ and 5′), 3.71 (2H, t, J=5.8 Hz, H-6′), 4.68 (2H, t, J=5.9 Hz, H-2′), 7.53 (1H, s, H-6), 9.90 (1H, s, CHO); ^{13}C NMR (75 MHz; $CDCl_3$) δ=23.8 (CH_2), 26.3 (CH_2) and 27.7 (CH_2), 50.6 (CH_2, C-2′), 59.3 (CH_2, C-6′), 120.6 (quat., C-4), 126.8 (quat., C-2), 135.8 (quat., C-3), 143.9 (quat., C-5), 146.7 (CH, C-6), 183.9 (CHO); HRMS (ESI): Found: MH^+, 259.0404. Calcd for $C_{11}H_{13}{}^{35}Cl_2N_2O$: MH^+=259.0399.

3.2.7. 3,5-Dichloro-2-(4′-morpholinylmethylene)-2*H*-pyrrole-4-carbaldehyde 3b. Prepared as described for **3a** above. Column chromatography on silica, eluting with $CHCl_3$/EtOAc (1:2) gave **3b** as a solid (0.067 g, 44%), mp 160.0–161.0 °C; IR (KBr) 1664 (C=O), 1624 (enamine C=C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ=3.77–3.81 (2H, m, H-6′), 3.89–3.92 (2H, m, H-3′ or H-5′), 3.94–3.98 (2H, m, H-3′ or H-5′), 4.79–4.82 (2H, m, H-2′), 7.52 (1H, s, 6-H), 9.89 (1H, s, 4-CHO); ^{13}C NMR (75 MHz, $CDCl_3$) δ= 50.1 (CH_2, C-2′), 57.8 (CH_2, C-6′), 67.0 (CH_2, C-3′ or C-5′), 67.5 (CH, C-3′ or C-5′), 121.0 (quat., C-4), 127.0 (quat., C-2), 137.0 (quat., C-3), 144.7 (quat., C-5), 146.4 (CH, C-6), 183.9 (CHO); HRMS (ESI): Found: MH^+, 261.0195. Calcd for $C_{10}H_{11}{}^{35}Cl_2N_2O_2$: MH^+=261.0192.

3.2.8. 3-Chloro-5-ethylsulfanyl-1-methyl-1*H*-pyrrole-2,4-dicarbaldehyde 7a. Thioethanol (0.09 mL, 1.213 mmol) was added to a mixture of pyrrole **2b** (0.1 g, 0.454 mmol) and triethylamine (0.17 mL, 1.213 mmol) in DMSO (2 mL). The reaction mixture was left to stand at room temperature for 3 days and then poured into water (10 mL). The product was extracted from aqueous solution with diethyl ether (3×5 mL). The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica, eluting with DCM to give a solid **7a** (0.097 g, 86%), mp 39–40 °C; IR (KBr) 1674 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ=1.22 (3H, t, J=7.3 Hz, CH_3CH$_2$), 2.90 (2H, q, J=7.3 Hz, CH$_3$CH_2), 4.04 (3H, s, NCH_3), 9.86 (1H, s, 2-CHO), 10.09 (1H, s, 4-CHO); ^{13}C NMR (75 MHz, $CDCl_3$) 15.4 (CH$_3$, CH$_3$CH$_2$), 31.5 (CH$_2$, CH$_3$$CH_2$), 34.3 (CH$_3$, NCH$_3$), 123.8 (quat., C-4), 127.5 (quat., C-3), 129.2 (quat., C-2), 140.3 (quat., C-5), 178.9 (CH, 2-CHO), 185.1 (CH, C4-CHO); HRMS (ESI): Found: MH^+, 232.0196. Calcd for $C_9H_{11}{}^{35}ClNO_2S$: MH^+=232.0194.

3.2.9. 3-Chloro-1-ethyl-5-(1′-piperidinyl)-1*H*-pyrrole-2,4-dicarbaldehyde 7b. Pyrrole **2c** (0.1 g, 0.454 mmol) was dissolved in DMSO (2 mL) and piperidine (0.12 mL, 1.146 mmol) was added. The mixture was left to stand at room temperature for 17 days then poured into water (10 mL) and extracted with diethyl ether (3×5 mL). The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica, eluting with petroleum ether (60–80 °C)/diethyl ether (1:1) to give **7b** as an oil (0.099 g, 81%); IR (KBr) 1664 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ=1.30 (3H, t, J=7.0 Hz, CH_3CH$_2$), 1.66 (6H, br s, H-3′, H-4′ and H-5′), 3.09 (4H, br s, H-2′ and H-6′), 4.34 (2H, q, J=7.0 Hz, CH$_3$CH_2), 9.77 (1H, s, 2-CHO), 9.92 (1H, s, 4-CHO); ^{13}C NMR (75 MHz, $CDCl_3$) δ=16.4 (CH$_3$, CH$_3$CH$_2$), 24.0 (CH$_2$, C-4′), 26.6 (2×CH$_2$, C-3′ and C-5′), 40.4 (CH$_2$, CH$_3$$CH_2$), 51.6 (2×CH$_2$, C-2′ and C-6′), 115.0 (quat., C-4), 122.7 (quat., C-2), 130.3 (quat., C-3), 151.1 (quat., C-5), 177.7 (CH, 2-CHO), 183.5 (CH, 4-CHO); HRMS (ESI): Found: MH^+, 269.1046. Calcd for $C_{13}H_{18}{}^{35}ClN_2O_2$: MH^+, 269.1051.

3.2.10. 3-Chloro-1-ethyl-5-ethylsulfanyl-1*H*-pyrrole-2,4-dicarbaldehyde 7c. Thioethanol (0.04 mL, 0.545 mmol) was added to a solution of the pyrrole **2c** (0.100 g, 0.454 mmol) and triethylamine (0.06 mL, 0.0454 mmol) in DMSO (2 mL). The mixture was left to stand at room temperature for 22 days, then diluted with water (10 mL), extracted with ether (10 mL), dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica, eluting with petroleum ether (60–80 °C)/diethyl ether (50:50) to give **7c** as a solid (0.071 g, 64%), mp 68.5–69.5 °C; IR (KBr) 1674 (C=O), 1601 (C=C) cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ=1.26 (3H, t, J=7.4 Hz, CH_3CH$_2$S), 1.33 (3H, t, J=7.1 Hz, CH_3CH$_2$N), 2.98 (2H, q, J=7.4 Hz, CH$_3$CH_2S), 4.64 (2H, q, J=7.1 Hz, CH$_3$CH_2N), 9.87 (1H, s, 2-CHO), 10.16 (1H, s, 4-CHO); ^{13}C NMR (75 MHz; $CDCl_3$) δ=15.4 (CH$_3$, CH$_3$CH$_2$S), 16.7 (CH$_3$, CH$_3$CH$_2$N), 32.0 (CH$_2$, CH$_3$$CH_2$S), 42.1 (CH$_2$, CH$_3$$CH_2$N), 123.5 (quat.), 128.0 (quat.), 128.6 (quat.), 139.3 (quat.), 178.3 (CHO), 184.8 (CHO); HRMS (ESI): Found: MH^+, 246.0354. Calcd for $C_{10}H_{13}{}^{35}ClNO_2S$: MH^+=246.0350.

3.2.11. 3-Chloro-1-ethyl-5-(1′-pyrrolidinyl)-2,4-dicarbaldehyde 7d. Pyrrole **2c** (0.1 g, 0.454 mmol) was dissolved in DMSO (2 mL) and pyrrolidine (0.1 mL, 1.146 mmol) was added. The mixture was left to stand at room temperature for 19 h then poured into water (10 mL) and extracted with $CHCl_3$ (3×5 mL). The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica, eluting with petroleum ether (60–80 °C)/ethyl acetate (30:60) to give **7d** as a colorless oil (0.038 g, 33%); IR (KBr) 1664 (C=O) cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ=1.23 (3H, t, J=7.1 Hz, CH_3CH$_2$), 1.93–2.03 (4H, m, H-3′ and H-4′), 3.24–3.29 (4H, m, H-2′ and H-5′), 4.26 (2H, q, J=7.1 Hz, CH$_3$CH_2), 9.65 (1H, s, 2-CHO), 10.16 (1H, s, 4-CHO); ^{13}C NMR (75 MHz; $CDCl_3$) δ=16.3 (CH$_3$, CH$_3$CH$_2$), 26.6 (2×CH$_2$, C-3′ and C-4′), 40.7 (2×CH$_2$, CH$_2$N), 52.5 (CH$_2$, C-2′ and C-5′), 113.0 (quat., C-4), 122.5 (quat., C-2), 127.7 (quat., C-3), 149.4 (quat., C-5), 177.1 (CH, 2-CHO), 183.1 (CH, 4-CHO); HRMS (ESI): Found: MH^+, 255.0897. Calcd for $C_{12}H_{16}{}^{35}ClN_2O_2$: MH^+, 255.0895.

3.2.12. 3-Chloro-1-ethyl-5-(morpholin-4-yl)-1*H*-pyrrole-2,4-dicarbaldehyde 7e and 1-ethyl-3,5-bis(morpholin-4-yl)-1*H*-pyrrole-2,4-dicarbaldehyde 11. Morpholine (0.2 mL, 2.182 mmol) was added to a solution of pyrrole **2c** (0.096 g, 0.436 mmol) in ethanol (5 mL). The resulting mixture was refluxed for 50 h then poured into water (10 mL) and extracted with diethyl ether (3×5 mL). The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica, eluting with petroleum ether (60–80 °C)/diethyl ether (1:1) to give **7e** and **11**.

3.2.12.1. 3-Chloro-1-ethyl-5-(morpholin-4-yl)-1*H*-pyrrole-2,4-dicarbaldehyde 7e. Solid (0.050 g, 43%), mp 70–71 °C; IR (KBr) 1669 (C=O) cm^{-1}; ^1H NMR (500 MHz; $CDCl_3$) δ=1.31 (3H, t, J=7.1 Hz, CH_3CH$_2$),

3.16 (4H, s, H-2′ and 6′), 3.84 (4H, br s, H-3′ and H-5′), 4.42 (2H, q, $J=7.1$ Hz, CH_2N), 9.82 (1H, s, 2-CHO), 9.96 (1H, s, 4-CHO); ^{13}C NMR (75 MHz; $CDCl_3$) 16.6 (CH_3, C-7), 40.3 (CH_2, C-6), 50.2 (CH_2, C-10(13)), 67.4 (CH_2, C-11(12)), 115.5 (quat., C-4), 122.8 (quat., C-2), 130.1 (quat., C-3), 148.8 (quat., C-5), 177.8 (CH, C-8), 183.5 (CH, C-9); HRMS (ESI): Found: MNa^+ 293.0669. Calcd for $C_{12}H_{15}{}^{35}ClN_2O_3Na$: MNa^+, 293.0663.

3.2.12.2. 1-Ethyl-3,5-bis-(morpholin-4-yl)-1*H*-pyrrole-2,4-dicarbaldehyde 11. Oil (0.026 g, 21%); IR (KBr) 1668 (C=O); ^{1}H NMR (300 MHz; $CDCl_3$) $\delta=1.23$ (3H, t, $J=7.0$ Hz, CH_3CH$_2$), 3.06 (4H, t, $J=4.4$ Hz, 2×CH$_2$), 3.22 (4H, t, $J=4.4$ Hz, 2×CH$_2$), 3.78–3.75 (8H, m, 4×CH$_2$), 4.29 (2H, q, $J=7.0$ Hz, 6-H), 9.77 (1H, s, CHO), 9.93 (1H, s, CHO); ^{13} C NMR (75 MHz; $CDCl_3$) 16.6 (CH_3), 40.6 (CH_2), 51.3 (CH_2, C-2′ or C-6′), 54.7 (CH_2, C-2′ or C-6′), 67.7 (CH_2, C-3′ or C-5′), 67.9 (CH_2, C-3′ or C-5′), 113.8 (quat., C-4), 120.7 (quat., C-2), 150.8 (quat., C-3 or C-5), 151.1 (quat., C-5 or C-3), 178.4 (CH, C-8), 183.6 (CH, C-9); HRMS (ESI): Found: MH^+, 322.1764. Calcd for $C_{16}H_{24}N_3O_4$: $MH^+=322.1761$.

3.2.13. 3-Chloro-5-diethylamino-1-ethyl-1*H*-pyrrole-2,4-dicarbaldehyde 7f. Pyrrole **2c** (0.1 g, 0.454 mmol) was dissolved in DMSO (2 mL) and diethylamine (0.12 mL, 1.146 mmol) was added. The mixture was left to stand at room temperature for 17 days then poured into water (10 mL) and extracted with diethyl ether (3×5 mL). The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica, eluting with petroleum ether (60–80 °C)/diethyl ether (1:1) to give **7f** as an oil (0.063 g, 74%); IR (KBr) 1668 (C=O) cm^{-1}; ^{1}H NMR (300 MHz; $CDCl_3$) $\delta=1.01$ (6H, t, $J=7.2$ Hz, 2×CH$_3$), 1.29 (3H, t, $J=7.0$ Hz, CH_3CH$_2$N^1), 3.18 (4H, q, $J=7.2$ Hz, 2×CH$_2$), 4.36 (2H, q, $J=7.0$ Hz, CH_2N^1), 9.79 (1H, s, 2-CHO), 9.92 (1H, s, 4-CHO); ^{13}C NMR (75 MHz; $CDCl_3$) $\delta=14.3$ (2×CH$_3$), 16.2 (CH$_3$, *C*H$_3$CH$_2$N^1), 40.2 (CH$_2$, CH$_3$*C*H$_2$N^1), 47.9 (2×CH$_2$), 116.0 (quat., C-4), 122.9 (quat., C-2), 130.3 (quat., C-3), 149.9 (quat., C-5), 177.6 (CH, 2-CHO), 183.5 (CH, 4-CHO); HRMS (ESI): Found: MH^+, 257.1054. Calcd for $C_{12}H_{18}{}^{35}ClN_2O_2$: $MH^+=257.1051$.

3.2.14. 1,3-Dichloro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepine-2-carbaldehyde 12. Concd HCl (0.23 mL) and $SnCl_2$ (0.263 g, 1.164 mmol) were added to a solution of pyrrole **2d** (0.127 g, 0.388 mmol) in ethanol (5 mL). The resulting mixture was stirred at 50 °C for 4 h. Approximately 50% of the ethanol was evaporated under reduced pressure and the residue was neutralized with a satd aq. solution of $NaHCO_3$ (10 mL), extracted with ethyl acetate (3×10 mL), dried over $MgSO_4$ and purified by column chromatography on silica, eluting with EtOAc to give **12** as a pink solid (0.049 g, 45%), mp 125.0–126.0 °C; IR (KBr) 1676 (C=O) cm^{-1}; ^{1}H NMR (300 MHz; $CDCl_3$) $\delta=5.05$ (2H, s, H-5), 7.29–7.53 (4H, m, H-6, H-7, H-8 and H-9), 8.52 (1H, s, H-11), 9.94 (1H, s, CHO); ^{13}C NMR (75 MHz; $CDCl_3$) $\delta=48.1$ (CH_2, C-5), 117.7 (quat., C-2), 118.1 (quat., C-1), 125.5 (quat., C-3), 126.0 (quat., C-9a), 126.4 (quat., C-5a), 128.3 (CH), 129.1 (CH), 129.3 (CH), 130.6 (CH), 146.9 (CH, C-11), 147.6 (quat., C-11a), 183.2 (CHO); HRMS (ESI): Found: MH^+, 279.0085. Calcd for $C_{13}H_9{}^{35}Cl_2N_2O$: $MH^+=279.0086$.

References and notes

1. Balasundaram, B.; Venugopal, M.; Perumal, P. T. *Tetrahedron Lett.* **1993**, *34*, 4249–4252.
2. Fischer, H.; Zeile, K. *Liebigs Ann. Chem.* **1930**, *483*, 251–271.
3. Chadwick, D. J. *J. Chem. Soc., Chem. Commun.* **1974**, 790–791.
4. Panfilova, E. A.; Kvitko, I. Ya.; El'tsov, A. V. *Khim. Geterosikl. Soedin.* **1981**, *17*, 1489–1493.
5. Abd El-Nabi, H. A. *Tetrahedron* **2000**, *56*, 3013–3020.

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Synthesis of galactose-linked uridine derivatives with simple linkers as potential galactosyltransferase inhibitors☆

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Abstract—Galactose-linked uridine derivatives without charge or dipole contributions in the linker were designed and synthesized via cross metathesis (CM). This strategy would provide a ready access to a range of hybrid compounds linking uridine and galactose derivatives.

1. Introduction

Oligosaccharides on cell surfaces and other glycoconjugates are responsible for several intercellular and intracellular events, including intercellular adhesion in inflammation, tumor metastasis, bacterial or viral infection, or activation of the innate immune system.[1–5] Since diseases such as rheumatoid arthritis, for example, are caused by malfunction of glycoconjugate biosynthesis, the challenge to modulate glycoconjugate biosynthesis is compelling and is being actively undertaken. Glycosyltransferases are key enzymes responsible for oligosaccharide biosynthesis and for catalysis of the sugar-transfer reaction in a stereo- and regiospecific manner. Each individual glycosyltransferase is responsible for each glycosylation step in oligosaccharide biosynthesis. Thus, inhibition of glycosyltransferases leads to the modulation of oligosaccharide biosynthesis, providing us with an opportunity to study their biological functions and to develop new therapeutic agents. Generally, a nucleoside diphosphate sugar (an NDP-sugar) is utilized as the sugar donor and the sugar moiety found in an NDP-sugar is transferred to the hydroxyl group of the acceptor molecule such as a growing oligosaccharide, a protein, or a lipid. Extensive efforts have been devoted to developing inhibitors for these glycosyltransferases with donor analogues.[9–20] However, only limited success has been achieved.[9–11] The diphosphate linkage found in an NDP-sugar is prone to hydrolysis, and its inherent negative charge is disfavored in terms of cell permeability. Some glycosyltransferases are known to require a divalent metal for their activity and form a salt bridge with the NDP-sugar through the metal in their active site called a DXD motif. Thus, modification of a diphosphate found in an NDP-sugar has been conducted primarily with respect to its ability to interact with the divalent metal ion in the acceptor analogue. In addition to the charge or dipole interaction mentioned above, the relative position and orientation of nucleoside and sugar moiety within the enzyme upon binding are likewise important concerns. Here we describe in an initial study the design and synthesis of galactose-linked uridine derivatives without charge or dipole contributions in the linker, which would be useful for the development of novel NDP-sugar analogues. Bovine β1,4-galactosyltransferase (β4GalTI) was chosen as the target enzyme because it is the most well-studied enzyme among the glycosyltransferases, and because X-ray crystal structure analysis data are available.[6–8] β4GalT catalyzes the transfer of galactose from UDP-galactose (Fig. 1, **1**) to the 4-hydroxyl group of *N*-acetylglucosamine found in oligosaccharides. We designed two sets of galactose-linked uridine derivatives, one of which possesses the simple alkane linker (Fig. 1, **2**), the other the alkene (Fig. 1, **3**), with the same number of atoms between the 5′-position of uridine and the anomeric position of galactose. Each set of compounds also consists of diastereomers at the 5′-position of uridine. With the introduction of an olefin and a hydroxyl group, a systematic structure-activity relationship could be conducted in terms of the distance between the uridine and galactose moiety and the relative orientation toward each other. Compounds **3** are also versatile intermediates because a variety of functional groups can be introduced at the olefinic position, which corresponds to the diphosphate in the natural substrate.

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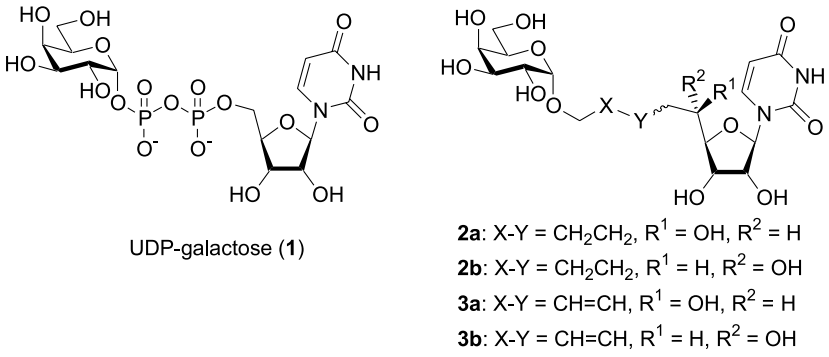
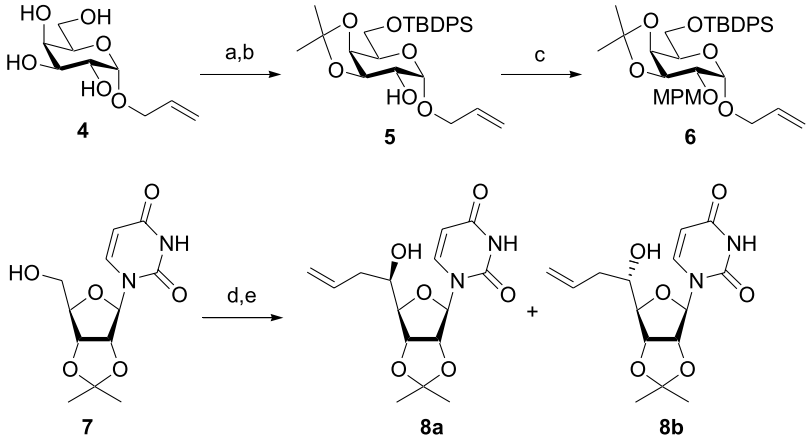


Figure 1. Structures of UDP-Gal (**1**) and designed compounds (**2**, **3**).



Scheme 1. Preparation of **6** and **8**. Reagents and conditions: (a) TBDPSCl, imidazole, DMF, room temperature; (b) 2,2-dimethoxypropane, TsOH, acetone, room temperature (90%, two steps); (c) MPMCl, NaH, DMF, room temperature (87%); (d) IBX, MeCN, 80 °C; (e) allylmagnesium bromide, THF, −78 °C (89%, two steps).

Table 1

Entry	Catalyst	Solvent	Temp. (°C)	Yield (%)		
				9a	**10**	**6**[a] (recovered)
1	**A**	CH_2Cl_2	40	trace	trace	-
2	**B**	CH_2Cl_2	40	35	38	43
3	**B**	toluene	110	15	54	40
4[b]	**B**	CH_2Cl_2	40	89	6	62

[a] Yield was based on the sugar derivative **6**.
[b] The reaction was carried out using 5 equiv of **6**.

2. Results and discussion

Our strategy for the synthesis of compounds **2** and **3** consisted of connecting the suitably protected allyl galactoside and the 5′-*C*-allyluridine derivative via olefin cross metathesis (CM).[21–29]

The CM precursors were prepared as shown in Scheme 1. Allyl α-galactopyranoside **4**[30] was sequentially protected with TBDPS, isopropylidene, and MPM groups to give **6**. 2′,3′-*O*-Isopropylideneuridine (**7**) was oxidized with IBX[31] to give the 5′-aldehyde, which was allylated upon treatment with allylmagnesium bromide to provide the 5′-*C*-allyl uridine[32] **8** in 89% yield (**8a/8b**=5/1) for two steps. Each diastereomer was separated by HPLC to give the pure material.

With these compounds in hand, we next examined the key cross metathesis, and the results are summarized in Table 1 and Scheme 2. When **6** (1 equiv) and **8a** (1 equiv) in a 0.1 M CH_2Cl_2 solution was treated with catalyst **B**[33,34] (Fig. 2) at reflux for 12 h, the desired **9a** was obtained in 35% yield (*E*/*Z*=5/1) as well as the homodimer of the uridine derivative **10** (Fig. 3) in 38% yield (entry 2). The use of catalyst **A**[35,36] gave only a trace amount of **9a** and was not effective for CM, as reported (entry 1). A trace amount of the homodimer of the allyl galactoside **11** was observed in the reaction mixture. Changing the solvent to toluene and the reaction temperature decreased the yield of **9a** (entry 3).

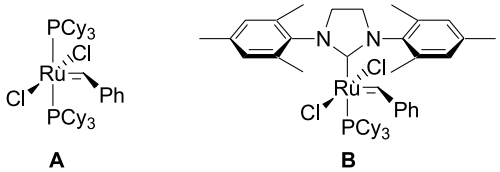
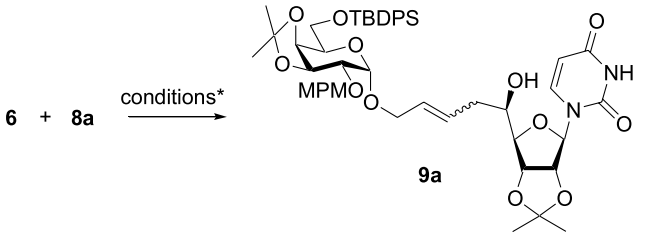


Figure 2. Structure of ruthenium catalysts.

It is known that the ruthenium carbene complex coordinates with the neighboring oxygen, and this coordination affects the selectivity and reactivity.[21] It is suggested that an initial [2+2] cycloaddition of the ruthenium carbene in the catalytic process might selectively proceed with **8a** rather than **6** via coordination with the 5′-hydroxyl group in **8a**. This result prompted us to use an excess of **6** to increase the ratio of the desired cross metathesis product **9a** to the homodimer **10**. Thus, when **6** (1 equiv) and **8a** (5 equiv) in 0.1 M CH_2Cl_2 solution was treated with catalyst **B** at reflux for 12 h, the yield of the desired **9a** was increased to 89% with the decreased amount of homodimer **10** (6% yield, entry 4). A limited amount of dimerized product **11** was also obtained under these conditions and the unreacted **6** was recovered and recycled. The CM with the diastereomer **8b** gave similar results to provide **9b**.

Deprotection of **9a** and **9b** by a two-step sequence afforded the target compounds **3a** and **3b**, respectively. Hydrogenation of **3a** and **3b** gave **2a** and **2b**, respectively (Scheme 3).



Scheme 2. Cross metathesis of **6** and **8a**.

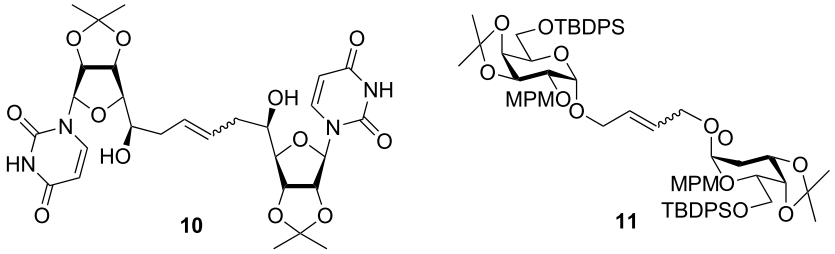
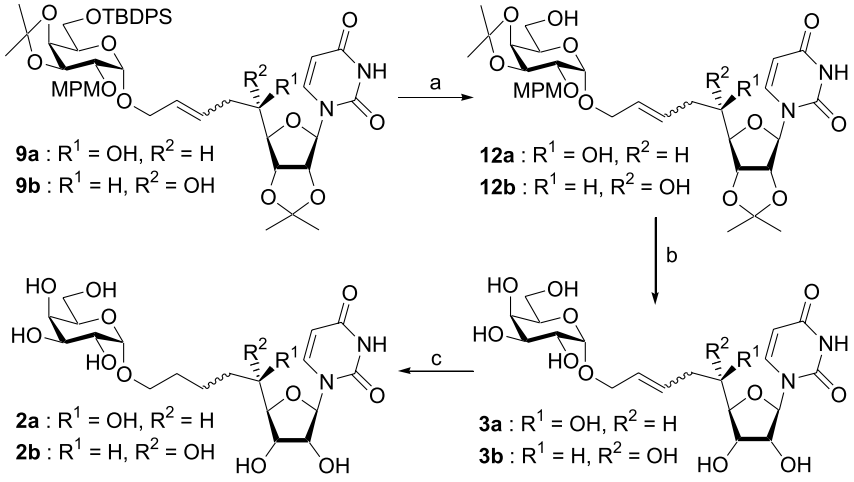


Figure 3. Structures of compound **10** and **11**.



Scheme 3. Synthesis of compound **2** and **3**. Reagents and conditions: (a) TBAF, THF, room temperature (91% for **12a**, 93% for **12b**); (b) 80% aq. TFA, room temperature (quant. for **3a** and **3c**); (c) H_2, $Pd(OH)_2$/C, MeOH, room temperature (quant. for **2a** and **2c**).

All the synthesized target compounds **2a**, **2b**, **3a** and **3b** were tested as inhibitors of bovine β1,4-galactosyltransferase I, according to the previously reported procedure[18,19] using UDP-[^{3}H]Gal as the glycosyl donor. Compounds **2a** and **2b** were tested as a geometric mixture because the geometrical isomers were inseparable. However, none of them exhibited inhibitory activities at concentrations up to 1 mM. Compounds **3** possess neutral and hydrophobic liker between uridine and galactose moieties. Thus, these results suggest that charge and/or dipole interaction with DXD motif found in β4GalTI is important for binding. However, compounds **3** also versatile intermediates because a variety of functional groups can be introduced at the olefinic position, which can interact with DXD motif.

3. Conclusion

Galactose-linked uridine derivatives without charge or dipole contributions in the linker were designed and synthesized via CM. This strategy would provide a ready access to a range of hybrid compounds linking uridine and galactose derivatives. Further transformation of the olefin found in compounds **3** could provide additional functionality, and these studies are in progress.

4. Experimental

4.1. General

NMR spectra were obtained on a JEOL EX270, JEOL

GX270, JEOL AL400 or Bruker ARX-500 and were reported in parts per million (δ) relative to tetramethylsilane (0.00 ppm) as an internal standard otherwise noted. Coupling constant (J) was reported in herz (Hz). Abbreviations of multiplicity were as follows; s: singlet, d; doublet, t: triplet, q: quartet, m: multiplet, br: broad. Data were presented as follows; chemical shift (multiplicity, integration, coupling constant). Assignment was based on ^{1}H–^{1}H COSY, HMBC and HMQC NMR spectra. Optical rotations were recorded on JASCO DIP-370 digital polarimeter or JASCO P-1030 polarimeter. FAB-MS were obtained on a JEOL JMS-HX101 or JEOL JMS-700TZ. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60F_{254} plates. Normal-phase column chromatography was performed on Merck silica gel 5715 or Kanto Chemical silica gel 60N (neutral). Flash column chromatography was performed on Merck silica gel 60. Reverse-phase column chromatography was performed on Waters Preparative C18 125 Å (55–105 μm). HPLC systems were Shimadzu LC-8A, Shimadzu SPD-6A, Shimadzu C-R6A. Dichloromethane and acetonitorile were distilled from P_2O_5 and then CaH_2. Methanol was distilled from sodium metal or directly used HPLC grade solvent from Kanto Chemical Co., Inc. Toluene was distilled from sodium metal/benzophenone ketyl. *N*,*N*-Dimethylformamide and dimethylsulfoxide were distilled from CaH_2 under reduced pressure or purchased dehydrated solvent from Kishida Chemical Co., Ltd. Tetrahydrofuran was purchased dehydrated stabilizer free solvent from Kanto Chemical Co., Inc. Bovine β1,4-galactosyltransferase I (β4GalTI) was purchased from TOYOBO Co. Ltd. Uridine diphosphate-[^{3}H]-galactose was purchased from American Radiolabeled Chemical Inc. Ovalbmin was purchased from nacalai tesque. Scintillation count was performed on PACKARD 1600 TR and Aloka LSC-120.

4.1.1. Allyl 6-*O*-(*tert*-butyldiphenylsilyl)-3,4-*O*-isopropylidene-α-D-galactopyranoside (5). To a solution of **4**[30] (5.00 mmol, 1.10 g) in DMF (40 ml), imidazole (12.0 mmol, 817 mg) and TBDPSCl (6.0 mmol, 1.56 ml) were added and the mixture was stirred for 12 h. The reaction was quenched with MeOH, and the solvent was removed in vacuo. The residue was partitioned between AcOEt (300 ml) and H_2O (100 ml) and the organic layer was washed with H_2O (100 ml×2) and brine (70 ml), dried over Na_2SO_4, filtered and concentrated. To the residue in acetone (100 ml), 2,2-dimethoxypropane (50 mmol, 6.1 ml) and TsOH (0.5 mmol, 86 mg) were added and the mixture was stirred for 12 h. The reaction mixture was neutralized with saturated aqueous $NaHCO_3$ and the solvent was removed in vacuo. The residue was partitioned between AcOEt (200 ml) and H_2O (100 ml) and the organic layer was washed with H_2O (100 ml) and brine (70 ml), dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (SiO_2, 7/1-6/1-5/1; hexane/AcOEt) to give **5** (4.50 mmol, 2.24 g, 90% for two steps) as a colorless syrup. $[\alpha]_D^{20}$ +62.6 (*c* 1.00, $CDCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (m, 4H), 7.38 (m, 6H), 5.90 (m, 1H), 5.29 (ddd, 1H, J=1.7, 17.2 Hz), 5.21 (ddd, 1H, J=1.7, 10.4 Hz), 4.89 (d, 1H, J=3.9 Hz), 4.26 (m, 3H), 4.11 (ddd, 1H, J=2.3, 6.5, 6.6 Hz), 4.03 (ddd, 1H, J=6.3, 12.8 Hz), 3.94 (m, 2H), 3.80 (m, 1H), 2.30 (d, 1H, J=7.1 Hz, exchangeable with D_2O), 1.48, 1.34 (each s, each 3H), 1.07 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.6, 133.6, 133.4, 133.3, 129.6, 127.6, 117.7, 109.3, 96.4, 76.2, 72.9, 69.7, 68.8, 68.4, 63.0, 27.8, 26.8, 26.0, 19.3; FAB-LRMS *m*/*z* 499 (MH^+); FAB-HRMS calcd for $C_{28}H_{39}O_6Si$ 499.2516, found 499.2520 (MH).

4.1.2. Allyl 6-*O*-(*tert*-butyldiphenylsilyl)-3,4-*O*-isopropylidene-2-*O*-(4-methoxybenzyl)-α-D-galactopyranoside (6). To a solution of **5** (4.56 mmol, 2.27 g) in DMF (40 ml), NaH (5.47 mmol, 131 mg) was added and the mixture was stirred for 1 h. After 1 h stirring, MPMCl (5.47 mmol, 742 μl) was added and the mixture was stirred for further 10 h. The reaction was quenched with MeOH and the solvent was removed in vacuo. The residue was diluted with AcOEt (300 ml), and the organic layer was washed with H_2O (100 ml×4) and brine (70 ml), dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by column chromatography (SiO_2, 10/1-8/1-7/1; hexane/AcOEt) to give **6** (3.97 mmol, 2.45 g, 87%) as a colorless syrup. $[\alpha]_D^{19}$ +52.3 (*c* 1.44, $CDCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 (m, 4H), 7.41 (m, 6H), 7.27 (m, 2H), 6.85 (m, 2H), 5.91 (m, 1H), 5.30 (dd, 1H, J=1.4, 17.3 Hz), 5.20 (dd, 1H, J=1.4, 10.6 Hz), 4.76 (d, 1H, J=3.5 Hz), 4.72 (d, 1H, J=12.3 Hz), 4.62 (d, 1H, J=12.3 Hz), 4.33 (m, 1H), 4.25 (dd, 1H, J=2.4, 5.4 Hz), 4.14 (dd, 1H, J=5.1, 13.0 Hz), 4.08 (m, 1H), 3.96 (dd, 1H, J=13.0, 6.4 Hz), 3.90 (m, 1H), 3.83 (m, 1H), 3.79 (s, 3H), 3.49 (dd, 1H, J=3.6, 7.9 Hz), 1.38, 1.35 (each s, each 3H), 1.05 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.1, 135.5, 133.7, 133.5, 133.3, 130.4, 129.7, 129.4, 127.6, 117.8, 113.7, 108.9, 95.8, 76.1, 76.0, 73.3, 71.9, 68.2, 68.0, 63.0, 55.3, 28.3, 26.8, 26.4, 19.3; FAB-LRMS *m*/*z* 619 (MH^+); FAB-HRMS calcd for $C_{36}H_{47}O_7Si$ 619.3091, found 619.3088 (MH^+).

4.1.3. 1-(2′,3′-*O*-Isopropylidene-6′,7′,8′-trideoxy-α-L-*talo*-oct-7′-enofranosyl)uracil (8a) and 1-(2′,3′-*O*-isopropylidene-6′,7′,8′-trideoxy-β-D-*allo*-oct-7′-enofranosyl)-uracil (8b). To a solution of **7** (4.32 mmol, 1.23 g) in MeCN (40 ml), IBX (12.9 mmol, 3.60 g) was added and the mixture was refluxed for 1.5 h. The reaction mixture was cooled to 0 °C and the precipitate was filtered off. The filtrate was concentrated and the residue was coevaporated with toluene (5 ml×2). A solution of the residue in THF (40 ml) was cooled to −78 °C. To the solution, allylmagnesium bromide (1.0 M solution in Et_2O; 13.0 mmol, 13.0 ml) was added dropwise over 10 min and the mixture was stirred for further 10 min. The reaction was quenched with saturated aqueous NH_4Cl and the precipitate was filtered off. The filtrate was diluted with AcOEt (200 ml) and the organic layers were washed with H_2O (70 ml) and brine (70 ml). The aqueous layer was extracted with $CHCl_3$ (70 ml) and combined organic layers were dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by column chromatography (SiO_2, 0–2% ethanol in $CHCl_3$) to give **8** (3.84 mmol, 1.24 g, 89% for two steps; diastereomixture of 5′*R*/*S*-isomer) as a white foam. Separation of 5′-stereoisomer was accomplished by HPLC (YMC-PACK SIL-06; 1/3; hexane/AcOEt). Physical data for 5′*R*-isomer **8a** were identical with the properties of the previously reported.[32] For 5′*R*-isomer **8a**; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.43 (br s, 1H, exchangeable with D_2O), 7.42 (d, 1H, J=8.0 Hz), 5.84 (m, 1H), 5.72 (d, 1H, J=8.0 Hz), 5.62 (d, 1H, J=2.7 Hz), 5.20 (m, 2H), 4.97 (m,

2H), 4.13 (m, 1H), 3.94 (m, 1H), 2.93 (br s, 1H, exchangeable with D_2O), 2.32 (m, 2H), 1.57, 1.37 (each s, each 3H). For 5′*S*-isomer **8b**; $[\alpha]_D^{21}$ −7.15 (*c* 0.91, $CDCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.09 (br s, 1H, exchangeable with D_2O), 7.44 (d, 1H, J=8.3 Hz), 5.83 (m, 1H), 5.73 (d, 1H, J=8.3 Hz), 5.67 (d, 1H, J=3.4 Hz), 5.19 (d, 1H, J=5.8 Hz), 5.15 (s, 1H), 4.96 (dd, 1H, J=3.4, 6.9 Hz), 4.92 (dd, 1H, J=3.4, 6.9 Hz), 4.15 (dd, 1H, J=3.0, 3.4 Hz), 3.83 (m, 1H), 2.65 (d, 1H, J=6.4 Hz, exchangeable with D_2O), 2.36 (m, 2H), 1.55, 1.36 (each s, each 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.3, 142.5, 133.8, 118.8, 114.5, 102.8, 95.0, 87.4, 83.2, 81.3, 71.1, 38.5, 27.4, 25.3; FAB-LRMS *m/z* 325 (MH^+); FAB-HRMS calcd for $C_{15}H_{21}N_2O_6$ 325.1400, found 325.1399 (MH^+).

4.1.4. 1-{9′-*O*-[6″-*O*-(*tert*-Butyldiphenylsilyl)-3″,4″-*O*-isopropylidene-2″-*O*-(4-methoxybenzyl)-α-D-galactopyranosyl]-2′,3′-*O*-isopropylidene-6′,7′,8′-trideoxy-α-L-*talo*-non-7′-enofranosyl}uracil (9a). To a solution of **8a** (59 μmol, 19 mg) and **6** (295 μmol, 182 mg) in CH_2Cl_2 (2 ml), Grubbs second catalyst **B** (10 mol%, 6.0 mg) was added and the mixture was refluxed for 12 h. The solvent was removed in vacuo, and the residue was purified by column chromatography (SiO_2, 0–2% ethanol in $CHCl_3$) to give **9a** (52 μmol, 48 mg, 89%) as a brown foam. ^1H NMR (500 MHz, $CDCl_3$) for *E* geometrical isomer δ 8.13 (br s, 1H, exchangeable with D_2O), 7.67 (m, 3H), 7.41 (m, 7H), 7.36 (m, 3H), 6.85 (m, 2H), 5.71 (m, 3H), 5.58 (d, 1H, J=2.7 Hz), 4.97 (m, 2H), 4.76 (d, 1H, J=3.6 Hz), 4.73 (d, 1H, J=12.1 Hz), 4.61 (d, 1H, J=12.1 Hz), 4.32 (m, 1H), 4.26 (m, 1H), 4.12 (m, 1H), 4.07 (m, 2H), 3.91 (m, 3H), 3.86 (m, 1H), 3.79 (s, 3H), 3.49 (m, 1H), 2.90 (br s, 1H, exchangeable with D_2O), 2.29 (m, 2H), 1.53, 1.37, 1.34, 1.31 (each s, each 3H), 1.05 (s, 9H); FAB-LRMS *m/z* 915 (MH^+); FAB-HRMS calcd for $C_{49}H_{63}N_2O_{13}Si$ 915.4099, found 915.4095 (MH^+).

4.1.5. 1-{9′-*O*-[6″-*O*-(*tert*-Butyldiphenylsilyl)-3″,4″-*O*-isopropylidene-2″-*O*-(4-methoxybenzyl)-α-D-galactopyranosyl]-2′,3′-*O*-isopropylidene-6′,7′,8′-trideoxy-β-D-*allo*-non-7′-enofranosyl}uracil (9b). Compound **9b** (0.14 mmol, 125 mg, 80%) was obtained as a pale brown foam from **8a** (0.17 mmol, 54 mg) and **6** (0.85 mmol, 556 mg) as described above for the synthesis of **9b**, after purification by flash column chromatography (SiO_2, 3/1-2/1-1/1-1/2; hexane/AcOEt). ^1H NMR (500 MHz, $CDCl_3$) for *E* geometrical isomer δ 8.27 (br s, 1H, exchangeable with D_2O), 7.68 (m, 3H), 7.41 (m, 7H), 7.28 (m, 3H), 6.86 (m, 2H), 5.71 (m, 3H), 5.60 (d, 1H, J=3.3 Hz), 4.95 (m, 1H), 4.89 (m, 1H), 4.76 (d, 1H, J=3.3 Hz), 4.71 (d, 1H, J=12.2 Hz), 4.60 (d, 1H, J=12.2 Hz), 4.31 (m, 1H), 4.26 (m, 1H), 4.12 (m, 3H), 4.07 (m, 1H), 3.92 (m, 2H), 3.84 (m, 1H), 3.81 (s, 3H), 3.49 (dd, 1H, J=3.6, 7.9 Hz), 2.87 (d, 1H, J=6.0 Hz, exchangeable with D_2O), 2.33 (m, 2H), 1.56, 1.40, 1.37, 1.35 (each s, each 3H), 1.05 (s, 9H); FAB-LRMS *m/z* 915 (MH^+); FAB-HRMS calcd for $C_{49}H_{63}N_2O_{13}Si$ 915.4099, found 915.4103 (MH^+).

4.1.6. 1-{9′-*O*-[3″,4″-*O*-Isopropylidene-2″-*O*-(4-methoxybenzyl)-α-D-galactopyranosyl]-2′,3′-*O*-isopropylidene-6′,7′,8′-trideoxy-α-L-*talo*-non-7′-enofranosyl}uracil (12a). To a solution of **9a** (140 μmol, 125 mg) in THF (2 ml), TBAF (1 M solution in THF; 210 μmol, 210 μl) was added and stirred for 8 h. The solvent was removed in vacuo, and the residue was purified by flash column chromatography (SiO_2, 0–1–2–3–4% ethanol in $CHCl_3$) to give **12a** (127 μmol, 86 mg, 91%) as a colorless glass. ^1H NMR (500 MHz, $CDCl_3$) for *E* geometrical isomer δ 8.21 (br s, 1H, exchangeable with D_2O), 7.38 (d, 1H, J=8.0 Hz), 7.27 (m, 2H), 6.87 (m, 2H), 5.74 (m, 3H), 5.58 (d, 1H, J=1.5 Hz), 4.97 (m, 2H), 4.83 (d, 1H, J=3.4 Hz), 4.74 (d, 1H, J=12.2 Hz), 4.62 (d, 1H, J=12.2 Hz), 4.35 (dd, 1H, J=5.5, 7.7 Hz), 4.21 (dd, 1H, J=2.7, 5.5 Hz), 4.06 (m, 4H), 3.91 (m, 2H), 3.80 (s, 3H), 3.49 (m, 2H), 3.13 (d, 1H, J=2.5 Hz, exchangeable with D_2O), 2.31 (m, 2H), 1.57, 1.41, 1.36, 1.34 (each s, each 3H); FAB-LRMS *m/z* 677 (MH^+); FAB-HRMS calcd for $C_{33}H_{45}N_2O_{13}$ 677.2922, found 677.2918 (MH^+).

4.1.7. 1-{9′-*O*-[3″,4″-*O*-Isopropylidene-2″-*O*-(4-methoxybenzyl)-α-D-galactopyranosyl]-2′,3′-*O*-isopropylidene-6′,7′,8′-trideoxy-β-D-*allo*-non-7′-enofranosyl}uracil (12b). Compound **12b** (0.10 mmol, 68 mg, 93%) was obtained as a colorless glass from **9b** (0.11 mmol, 100 mg) as described above for the synthesis of **12a**, after purification by flash column chromatography (SiO_2, 0–1–2–3–4% ethanol in $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) for *E* geometrical isomer δ 7.40 (d, 1H, J=8.1 Hz), 7.29 (d, 2H, J=8.5 Hz), 6.87 (d, 2H, J=8.5 Hz), 5.72 (m, 3H), 5.61 (d, 1H, J=3.0 Hz), 4.97 (m, 1H), 4.91 (m, 1H), 4.86 (d, 1H, J=3.5 Hz), 4.72 (d, 1H, J=12.1 Hz), 4.62 (d, 1H, J=12.1 Hz), 4.34 (dd, 1H, J=5.5, 7.7 Hz), 4.19 (dd, 1H, J=2.5, 5.5 Hz), 4.13 (m, 2H), 4.09 (m, 1H), 3.90 (m, 1H), 3.82 (m, 2H), 3.80 (s, 3H), 3.49 (dd, 1H, J=3.6, 8.0 Hz), 3.24 (br s, 1H, exchangeable with D_2O), 1.57, 1.39, 1.35, 1.33 (each s, each 3H); FAB-LRMS *m/z* 677 (MH^+); FAB-HRMS calcd for $C_{33}H_{45}N_2O_{13}$ 677.2922, found 677.2930 (MH^+).

4.1.8. 1-(9′-*O*-α-D-Galactopyranosyl-6′,7′,8′-trideoxy-α-L-*talo*-non-7′-enofranosyl)uracil (3a). 12a (30 μmol, 20 mg) was dissolved in 80% aqueous TFA (200 μl) and the mixture was stirred for 5 min. The solvent was removed in vacuo and the residue was coevaporated with EtOH (1 ml×6). The resulting residue was purified by column chromatography (C18, 5–10–15% MeOH in H_2O) to give **3a** (30 μmol, 14 mg, quant.) as a colorless glass. ^1H NMR (500 MHz, DMSO-d_6, D_2O) for *E* geometrical isomer δ 7.82 (d, 1H, J=8.0 Hz), 5.89 (m, 1H), 5.87 (m, 1H), 5.75 (m, 2H), 4.97 (d, 1H, J=2.3 Hz), 4.18 (m, 2H), 4.20 (dd, 1H, J=5.4, 12.1 Hz), 4.05 (m, 2H), 3.92 (m, 3H), 3.78 (m, 2H), 3.71 (m, 2H), 2.42 (m, 1H), 2.29 (m, 2H); FAB-LRMS *m/z* 477 (MH^+); FAB-HRMS calcd for $C_{19}H_{29}N_2O_{12}$ 477.1720, found 477.1731 (MH^+).

4.1.9. 1-(9′-*O*-α-D-Galactopyranosyl-6′,7′,8′-trideoxy-β-D-*allo*-non-7′-enofranosyl)uracil (3b). Compound **3b** (74 μmol, 35 mg, quant.) was obtained as a colorless glass from **12b** (74 μmol, 50 mg) as described above for the synthesis of **3a**, after purification by column chromatography (C18, 5–10% methanol in H_2O). ^1H NMR (400 MHz, DMSO-d_6, D_2O) δ 7.97 (d, 1H, J=7.8 Hz), 5.77 (d, 1H, J=5.4 Hz), 5.62 (d, 1H, J=5.62 Hz), 4.62 (d, 1H, J=3.4 Hz), 4.00 (m, 3H), 3.53 (m, 7H), 3.44 (m, 2H), 1.48 (m, 6H); FAB-LRMS *m/z* 477 (MH^+); FAB-HRMS calcd for $C_{19}H_{29}N_2O_{12}$ 477.1720, found 477.1735 (MH^+).

4.1.10. 1-(9′-*O*-α-D-Galactopyranosyl-6′,7′,8′-trideoxy-α-L-*talo*-nonofranosyl)uracil (2a). To a solution of **3a** (15 μmol, 7.0 mg) in MeOH (2 ml), palladium hydroxide on carbon (wet. 10%; 5 mg) was added and the mixture was vigorously stirred under H_2 atmosphere for 10 min. The catalyst was filtered off through Celite pad and the filtrate was concentrated. The resulting residue was purified by column chromatography (C18, 5–10–15% methanol in H_2O) to give **2a** (15 μmol, 7.0 mg, quant.) as a colorless glass. $[\alpha]_D^{21}$ +21.6 (*c* 1.09, H_2O); ^{1}H NMR (500 MHz, DMSO-d_6, D_2O) δ 7.82 (d, 1H, J=8.1 Hz), 5.77 (d, 1H, J=6.6 Hz), 5.62 (d, 1H, J=8.1 Hz), 4.61 (d, 1H, J=3.4 Hz), 3.68 (m, 2H), 3.57 (m, 4H), 3.53 (m, 3H), 3.42 (m, 2H), 1.47 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6, D_2O) δ 163.2, 151.0, 140.9, 101.9, 98.8, 87.8, 86.7, 73.3, 71.1, 70.2, 69.6, 69.0, 68.8, 68.4, 66.9, 60.6, 32.8, 29.1, 22.1; FAB-LRMS *m/z* 479 (MH^+); FAB-HRMS calcd for $C_{19}H_{31}N_2O_{12}$ 479.1877, found 479.1868 (MH^+).

4.1.11. 1-(9′-*O*-α-D-Galactopyranosyl-6′,7′,8′-trideoxy-β-D-*allo*-nonofranosyl)uracil (2b). Compound **2b** (10 μmol, 7.0 mg, quant.) was obtained as a colorless glass from **3b** (10 μmol, 7.0 mg) as described above for the synthesis of **2a**, after purification by column chromatography (C18, 5–10% methanol in H_2O). $[\alpha]_D^{21}$ +22.2 (*c* 1.02, H_2O); ^{1}H NMR (400 MHz, DMSO-d_6, D_2O) δ 7.97 (d, 1H, J=7.8 Hz), 5.77 (d, 1H, J=5.4 Hz), 5.63 (d, 1H, J=7.8 Hz), 4.62 (d, 1H, J=3.4 Hz), 4.01 (m, 2H), 3.97 (m, 1H), 3.76 (m, 1H), 3.69 (br s, 1H), 3.50 (m, 7H), 1.46 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6, D_2O) δ 140.5, 101.7, 98.8, 87.6, 86.6, 73.7, 71.1, 70.7, 69.6, 69.3, 68.9, 68.4, 66.9, 60.6, 33.3, 29.1, 22.2; FAB-LRMS *m/z* 479 (MH^+); FAB-HRMS calcd for $C_{19}H_{31}N_2O_{12}$ 479.1877, found 479.1875 (MH^+); FAB-HRMS calcd for $C_{19}H_{31}N_2O_{12}$ 479.1877, found 479.1878 (MH^+).

4.2. β1,4-Galactosyltransferase assay

Assays were performed in a total volume of 100 μl. The assay medium contained ovalbmin (1 mg/ml), 0.1 M sodium cacodylate buffer, 10 mM $MnCl_2$, 2 mM AMP, inhibitor (varying conc.), 1 μCi of UDP-[^{3}H]-galactose, and β1,4-galactosyltransferase (4 mU). The enzyme assay was incubated for 3 h at 37 °C and the reaction stopped with 1 ml of 10% TCA. The precipitate was washed with 10% TCA and once with ethanol/ether (2/1). The precipitate was dissolved in 200 μl of 2 N NaOH and the solution was counted in 2 ml of scintillation fluid by using β-scintillation counter.

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References and notes

1. Varki, A. *Glycobiology* **1993**, *3*, 97.
2. Dwek, R. K. *Chem. Rev.* **1993**, *96*, 683.
3. Kornfield, S.; Kornfield, R. *Annu. Rev. Biochem.* **1985**, *54*, 631.
4. Paulson, J. C.; Colley, K. *J. Biol. Chem.* **1989**, *264*, 17615.
5. Roseman, S. *J. Biol. Chem.* **2001**, *276*, 41527.
6. Berger, E. G.; Rohrer, J. *Biochimica* **2003**, *85*, 261.
7. Gastinel, L. N.; Cambillau, C.; Bourne, Y. *EMBO J.* **1999**, *18*, 491.
8. Ramakrishnan, B.; Balaji, P. V.; Qasba, P. K. *J. Mol. Biol.* **2002**, *318*, 491.
9. Lee, L. V.; Mitchell, L. M.; Huang, S.-J.; Fokin, V. V.; Sharpless, K. B.; Wong, C.-H. *J. Am. Chem. Soc.* **2003**, *125*, 9588.
10. Kim, Y. J.; Ichikawa, M.; Ichikawa, Y. *J. Am. Chem. Soc.* **1999**, *121*, 5829.
11. Hang, H. C.; Chong, Y.; Ten Hagen, K. G.; Tian, E.; Winans, K. A.; Tabak, L. A.; Bertozzi, C. R. *Chem. Biol.* **2004**, *11*, 337.
12. Takayama, S.; Chung, S. J.; Igarashi, Y.; Ichikawa, Y.; Sepp, A.; Lechler, R. I.; Wu, J.; Hayashi, T.; Sizdak, G.; Wong, C.-H. *Bioorg. Med. Chem. Lett.* **1999**, *7*, 401.
13. Takayama, S.; Chung, S. J.; Igarashi, Y.; Ichikawa, Y.; Wang, R.; Steensma, D. H.; Takaoka, Y.; Yun, J. W.; Kajimoto, T.; Wong, C.-H. *Bioorg. Med. Chem.* **1997**, *5*, 661.
14. Behr, J.-B.; Gourlain, T.; Helomi, A.; Guillerm, G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1713.
15. Bhattacharya, A. K.; Stolz, F.; Kurzeck, J.; Ruger, W.; Schmidt, R. R. *Bioorg. Med. Chem.* **2002**, *10*, 1129.
16. Broxterman, H. J. G.; van der Mavel, G. A.; van Boom, J. M. *Tetrahedron Lett.* **1988**, *29*, 4893.
17. Grugier, J.; Xie, J.; Duarte, I.; Valery, J.-M. *Tetrahedron Lett.* **1988**, *29*, 4893.
18. Camarasa, M.-J.; F.-Resa, P.; G.-Lopez, M.-T.; De las Heras, F. G.; M.-Castrillon, P. P.; Alarcon, B.; Carrasco, L. *J. Org. Chem.* **2000**, *65*, 979.
19. Vaghefi, M. M.; Bernacki, R. J.; Dalley, N. K.; Wilson, B. E.; Robins, R. K. *J. Med. Chem.* **1987**, *30*, 1383.
20. Vaghefi, M. M.; Bernacki, R. J.; Hennen, W. J.; Robins, R. K. *J. Med. Chem.* **1987**, *30*, 1391.
21. For recent review on cross metathesis see: Connon, S. J.; Blechert, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 1900.
22. Roy, R.; Das, S. K.; Dominique, R.; Trono, M. C.; H.-Mateo, F.; S.-Gonzalez, F. *Pure Appl. Chem.* **1999**, *71*, 565.
23. Dominique, R.; Liu, B.; Das, S. K.; Roy, R. *Synthesis* **2000**, 862.
24. Dondoni, A.; Giovannini, P. P.; Marra, A. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2380.
25. Furstner, A. *Angew. Chem., Int. Ed.* **2000**, *39*, 3012.
26. Roy, R.; Das, S. K. *Chem. Commun.* **2000**, 519.
27. Hoveyda, A. H.; Schrock, R. R. *Chem. Eur. J.* **2001**, *7*, 945.
28. Batoux, N.; B.-Zerrouki, R.; Bressolier, P.; Granet, R.; Laumont, G.; Aubertin, A.-M.; Krausz, P. *Tetrahedron Lett.* **2001**, *42*, 1491.
29. Lera, M. L.; Hayes, C. J. *Org. Lett.* **2001**, *3*, 2765.
30. Lee, R. T.; Lee, Y. C. *Carbohydr. Res.* **1974**, *37*, 193.
31. More, J. D.; Finny, N. S. *Org. Lett.* **2002**, *4*, 3001.
32. Hannesian, S.; Kloss, J.; Sugawara, T. *J. Am. Chem. Soc.* **1986**, *108*, 2758.
33. Huang, J.; Schanz, H.-J.; Stevens, E. D.; Nolan, S. P. *Organometallics* **1999**, *18*, 5375.
34. Scholl, M.; Trnka, T. M.; Morgan, J. P.; Grubbs, R. H. *Tetrahedron Lett.* **1999**, *40*, 2247.
35. Schwab, P.; France, M. B.; Ziller, J. W.; Grubs, R. H. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2039.
36. Schwab, P.; Grubs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 100.

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The three component reaction involving isocyanides, dimethyl acetylenedicarboxylate and quinoneimides: a facile synthesis of spirofused γ-iminolactams

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Abstract—The three component reaction of the zwitterion generated from dimethyl acetylenedicarboxylate and isocyanides with various quinoneimides is described. The reaction afforded the corresponding γ-spiroiminolactams in good yields.

1. Introduction

Quinonoid compounds have assumed great importance, since they find application as pharmaceuticals, hormones, and pigments besides playing very crucial roles in photosynthesis and electron transport chains in a number of biological processes. They are also versatile building blocks in organic synthesis by virtue of their multiple reactivity profiles.[1] Our own studies have uncovered some novel reaction pathways of *o*-quinones, especially in the area of cycloadditions.[2] In contrast, however, the reactivity of quinoneimides,[3] the aza analogs of quinones, has received much less attention.[4] The available reports on the chemistry of *p*-quinoneimides are essentially focussed on the addition of weak nucleophiles,[5] whereas the chemistry of *o*-quinoneimides has been mainly concerned with their participation in Diels–Alder reaction with alkenes and fulvenes.[6] Very recently, Parker and Mindt have reported the formation of quinolines and indoles from enolalizable *p*-quinoneimides.[7]

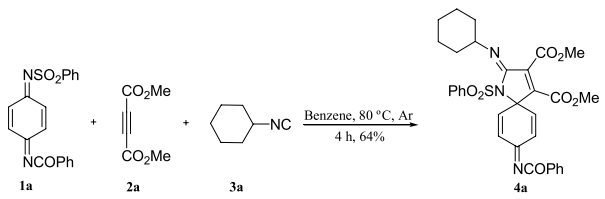
As a part of our ongoing project in the area of cycloaddition reactions of quinoneimides[8] as well as the chemistry of zwitterions, generated by the addition of various nucleophilic species to dimethyl acetylenedicarboxylate (DMAD),[9] we undertook a detailed investigation of the reactivity of zwitterion generated from the latter and alkyl isocyanides to various quinoneimides. Expect for the two examples on the addition of this zwitterionic species to *p*-quinonediimides reported by us,[10] there has been no systematic investigation in this area. In particular there are no reports on their addition to *p*-quinonemonoimides and *o*-quinonediimides. For our present studies *p*-quinonediimides, *p*-quinonemonoimides and *o*-quinonediimides were used as the electrophiles for trapping the zwitterion. The results of the investigations carried out on such systems are presented in this paper.

2. Results and discussion

2.1. Reaction with *p*-quinonediimides

In an initial experiment, we treated the *p*-quinonediimide **1a** and DMAD **2a** with stoichiometric amount of cyclohexyl isocyanide **3a** in refluxing benzene under an atmosphere of argon for 4 h. A facile reaction occurred and the reaction mixture on silica gel column chromatography afforded the γ-iminolactam **4a** as a pale yellow crystalline solid in 64% yield (Scheme 1).

The product **4a** was characterized on the basis of spectroscopic data. The IR spectrum showed strong absorption due



Scheme 1.

Keywords: Multicomponent reactions; Isocyanide; Quinoneimide; Lactam; Dimethyl acetylenedicarboxylate.
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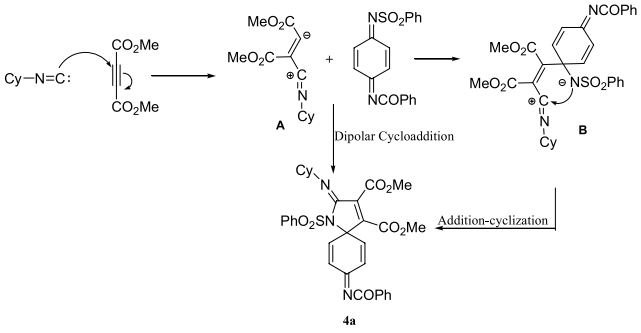
to ester carbonyl at 1744 cm^{-1} and the carbonyl absorption of the benzoyl group was seen at 1667 cm^{-1}. The characteristic bands due to sulfonyl absorption were seen at 1331 and 1175 cm^{-1}. In the ^1H NMR spectrum, the two carbomethoxy groups were discernible as singlets at δ 3.86 and 3.75. The tertiary cyclohexyl proton displayed a multiplet at δ 3.17; other protons were discernible as multiplets between δ 1.72 and 1.11. The four olefinic protons on the cyclohexadiene ring resonated as two separate doublets, one centered at δ 6.63 (J=9.7 Hz, 2H) and the other centered at δ 6.46 (J=9.7 Hz, 2H). In the ^{13}C NMR spectrum, the peak at δ 179.9 was assigned to the carbonyl resonance due to the benzimide functionality. The ester carbonyls were visible at δ 163.8. The resonance due to the imino carbon was observed at δ 155.2. The spirocarbon signal appeared at δ 66.7 and the methoxy carbons were observed at δ 53.2 and 53.0. The signal at δ 59.5 was attributed to the carbon atom on the cyclohexyl ring adjacent to the nitrogen. All other signals were also in good agreement with the proposed structure.

The following rationalization may be invoked for the formation of the product. Conceivably the starting point of the reaction is the formation of a 1:1 zwitterionic species **A** between cyclohexyl isocyanide and DMAD, which adds to the quinonediimide, in a chemoselective manner to the C=N of the sulfonimide leading to the dipolar intermediate **B**. Cyclization of the latter then completes the reaction sequence leading to the iminolactam **4a**. Although less likely, an alternate mechanism involving the 1,3-dipolar cycloaddition reaction of the zwitterionic intermediate **A**, to the C=N bond of the quinoneimide as shown in Scheme 2, cannot be excluded.



Scheme 2.

Similar reactivity was observed with other *p*-quinonediimides, which underwent a facile reaction with the zwitterions formed from DMAD and isocyanides to afford the corresponding iminolactam derivatives. The results obtained are summarized in Table 1.

From Table 1, it is clear that the reaction is general with respect to quinoneimide and isocyanide components. In all the cases, products were isolated by column chromatography and were characterized on the basis of spectroscopic data. The regiochemistry of the product **4f** was established by comparing its ^{13}C NMR data with those of the starting quinoneimide **1d**. Attempts were made to intercept both the imide functionalities of the *p*-quinoneimides, but the results were not promising.

2.2. Reaction with *p*-quinonemonoimides

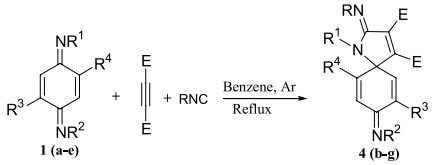
p-Quinonemonoimides offer multiple electrophilic sites,[11] and therefore it was of interest to examine the reactivity profile of the zwitterion towards *p*-quinonemonoimides. Thus, when 3-methoxy-*p*-quinonemonobenzenesulfonimide **5a** was treated with the in situ generated zwitterion, from DMAD and cyclohexyl isocyanide in refluxing benzene, the iminolactam derivative **6a** was obtained in 76% yield. Analogous reactions were observed with other *p*-quinonemonoimides, DMAD and isocyanides. The results are summarized in Table 2.

The structure of the adduct **6a**, which sheds light on the chemoselectivity of the reaction, was established by spectroscopic methods. In the IR spectrum, the characteristic dienone carbonyl absorption was observed at 1662 cm^{-1}. In the ^{13}C NMR spectrum, the characteristic resonance signal due to the dienone carbonyl appeared at δ 188.4. All the other peaks were in agreement with the proposed structure.

2.3. Reaction with *o*-quinonediimides

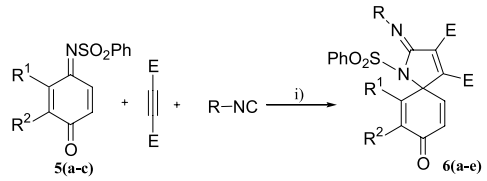
In view of the results obtained by the addition of the zwitterionic intermediate generated from DMAD and isocyanides to both *p*-quinonemono- and di-imides, a logical extension of the work was to explore the reactivity of *o*-quinonediimides towards the zwitterionic intermediate. *o*-Quinonediimides can, in principle, exhibit four different reactivity modes. But in the studies carried out so far, they

Table 1. Reaction of *p*-quinoediimides and acetylenes with isocyanides



Entry	Quinonemonoimides	Acetylene	Isocyanide	Time (h)	Product (%)
1	R^1=SO_2Ph, R^2=COPh, R^3=R^4=H, **1a**	E=CO_2Me	^{t}BuNC	4	**4b** (69)
2	R^1=R^2=SO_2Ph, R^3=R^4=CH_3, **1b**	E=COPh	CyNC	12	**4c** (61)
3	R^1=R^2=SO_2Ph, R^3=R^4=CH_3, **1b**	E=CO_2Me	^{t}BuNC	6	**4d** (82)
4	R^1=R^2=Ts, R^3=R^4=H, **1c**	E=CO_2Me	^{t}BuNC	6	**4e** (62)
5	R^1=R^2=Ts, R^3=Cl, R^4=H, **1d**	E=CO_2Me	^{t}BuNC	6	**4f** (62)
6	R^1=R^2=COPh, R^3=R^4=H, **1e**	E=CO_2Me	CyNC	6	**4g** (57)

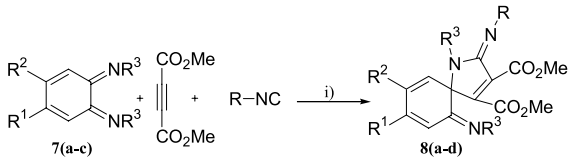
Table 2. Reaction of *p*-quinonemonoimides and acetylenes with isocyanides



Entry	*p*-Quinonemonoimides	Acetylene	Isocyanide	Product (%)
1	R^1=OMe, R^2=H, **5a**	E=CO_2Me	CyNC	**6a** (64)
2	R^1=H, R^2=OMe, **5b**	E=CO_2Me	CyNC	**6b** (69)
3	R^1=H, R^2=OMe, **5b**	E=CO_2Me	^{t}BuNC	**6c** (72)
4	R^1=R^2=H, **5c**	E=CO_2Me	CyNC	**6d** (66)
5	R^1=OMe, R^2=H, **5b**	E=COPh	^{t}BuNC	**6e** (56)

(i) Benzene, 80 °C, Ar, 6 h

Table 3. Reaction of *o*-quinonediimides and DMAD with isocyanides



Entry	*o*-Quinonemonoimides	Isocyanide	Product (%)
1	R^1=R^2=Me, R^3=SO_2Ph, **7a**	^{t}BuNC	**8a** (64)
2	R^1=R^2=Me, R^3=SO_2Ph, **7a**	CyNC	**8b** (48)
3	R^1=R^2=H, R^3=COPh, **7b**	CyNC	**8c** (52)
4	R^1=R^2=Me, R^3=COPh, **7c**	CyNC	**8d** (53)

have manifested only two different reactivity modes viz. as carbodiene or as heterodiene.[4a] In a pilot experiment, we observed that a mixture of 4,5-dimethyl-o-benzoquinonedisulfonimide **7a**, and DMAD at 80 °C, when treated with cyclohexyl isocyanide afforded the iminolactam derivative **8a** in 64% yield. As usual, the product was separated by silica gel column chromatography and characterized on the basis of spectroscopic methods. Analogous reactions were observed with other substituted *o*-quinonediimides and the results are presented in Table 3.

In the IR spectrum of **8a**, the absorption band due to the ester carbonyls was seen at 1727 cm^{-1}. The peaks at 1336 and 1161 cm^{-1} were attributed to sulfonyl absorptions. In the ^{1}H NMR spectrum of **8a**, protons of the *tert*-butyl group exhibited a sharp singlet at δ 1.29. The two methyl groups on the cyclohexadiene ring were observed as two separate singlets at δ 2.01 and 1.46, while the two carbomethoxy groups were observed at δ 3.95 and 3.47. The two protons of the cyclohexadiene ring gave two singlets at δ 7.43 and 5.85. In ^{13}C the NMR spectrum, the carbomethoxy carbonyl resonances were discernible at δ 173.3 and 168.5. The peak at δ 61.2 was attributed to the spiro carbon. The signal due to the methyl carbons of the *tert*-butyl group was seen at δ 30.1. All the other peaks were in good agreement with the assigned structure.

3. Conclusion

In summary, our studies have shown that the interception of the zwitterionic intermediate, generated by the addition of isocyanides to DMAD, using quinoneimides offers a convenient method for the synthesis of spiroiminolactams. The presence of transformable functionalities in these products makes them potentially valuable from the vantage point of further synthetic manipulations.

4. Experimental

4.1. General

All reactions were carried out in oven-dried glasswares under an atmosphere of argon. Progress of reaction was monitored by thin layer chromatography, while purification was effected using silica gel column chromatography. NMR spectra were recorded at 300 (^{1}H) and 75 (^{13}C) MHz, respectively, on a Brücker DPX-300 MHz spectrometer. Chemical shifts (δ) were reported relative to TMS (^{1}H) and $CDCl_3$ (^{13}C) as the internal standards. Coupling constant (J) is reported in Hertz (Hz). IR spectra were recorded in Bomem MB Series FT-IR spectrophotometer. Melting points were recorded on a Buchi melting point apparatus and are uncorrected. All the quinoneimides were prepared according to the literature procedure.[12–14]Commercial grade solvents were distilled prior to use.

4.1.1. Dimethyl 2-(cyclohexylimino)-1-(phenylsulfonyl)-8-[(benzoyl)imino]-1-azaspiro[4.5]deca-3,6,9-triene-3,4-dicarboxylate 4a: Typical procedure and spectral data. A mixture of *p*-quinoneimide **1a** (70 mg, 0.2 mmol) and DMAD (32 mg, 0.22 mmol) in dry benzene (4 mL) was purged with argon. To this mixture, cyclohexyl isocyanide

(24 mg, 0.22 mmol) was added and the refluxing was continued at 80 °C for 4 h. The solvent was removed under vacuum and the residue was purified by chromatography on silica gel using 80:20 hexane/ethylacetate as eluent gave the iminolactam **4a** as a pale yellow crystalline solid (77 mg, 64%). The products were recrystallized from dichloromethane–hexane solvent system.

Pale yellow crystalline solid, mp: 158–159 °C, IR (KBr) ν_{max}: 2934, 2856, 1744, 1667, 1600, 1450, 1331, 1268, 1175, 1087, 1020, 731 cm^{-1}. ^1H NMR: δ 1.11–1.72 (m, 10H), 3.17 (m, 1H), 3.75 (s, 3H), 3.86 (s, 3H), 6.46 (d, 2H, $J=9.7$ Hz), 6.63 (d, 2H, $J=9.7$ Hz), 7.44–7.49 (m, 4H), 7.57–7.58 (m, 2H), 7.99–8.03 (m, 4H). ^{13}C NMR: δ 24.5, 25.4, 29.7, 34.6, 53.0, 53.2, 59.5, 66.7, 127.4, 128.0, 128.1, 128.6, 129.5, 129.7, 131.6, 132.0, 133.0, 133.3, 133.4, 134.0, 138.6, 139.2, 143.0, 143.6, 155.2, 163.8, 179.9.

HRMS (EI) calcd for $C_{31}H_{31}N_3O_7S$: 601.1883. Found: 601.1869.

4.1.2. Dimethyl 2-(*tert*-butylimino)-1-(phenylsulfonyl)-8-[(benzoyl)imino]-1-azaspiro[4.5]deca-3,6,9-triene-3,4-dicarboxylate 4b. Pale yellow crystalline solid, mp: 144–146 °C, IR (KBr) ν_{max}: 2960, 2365, 1739, 1677, 1600, 1450, 1315, 1268, 1087, 1015, 803 cm^{-1}. ^1H NMR: δ 1.11 (s, 9H), 3.72 (s, 3H), 3.82 (s, 3H), 6.55 (d, 2H, $J=9.7$ Hz), 6.64 (d, 2H, $J=9.7$ Hz), 7.23–7.50 (m, 4H), 7.54–7.58 (m, 2H), 7.93–8.00 (m, 4H). ^{13}C NMR: δ 31.0, 52.9, 53.1, 56.1, 64.9, 127.3, 127.4, 128.2, 128.6, 128.8, 128.9, 129.7, 131.6, 133.0, 133.1, 133.2, 133.3, 134.9, 140.0, 140.1, 143.4, 155.3, 163.8, 179.9.

HRMS (EI) calcd for $C_{30}H_{29}N_3O_7S$: 575.1726. Found: 575.1724.

4.1.3. 3,4-Bisbenzoyl-2-(cyclohexylimino)-1-(phenylsulfonyl)-8-[(phenylsulfonyl)imino]-1-azaspiro[4.5]deca-3,6,9-triene 4c. Pale yellow crystalline solid; mp: 112–114 °C, IR (KBr) ν_{max}: 2924, 2851, 1667, 1543, 1450, 1336, 1258, 1165, 1093, 752, 725, 689 cm^{-1}. ^1H NMR: δ 1.32–1.16 (m, 10H), 1.82 (s, 3H), 2.28 (s, 3H), 2.97 (m, 1H), 6.64 (s, 1H), 6.77 (s, 1H), 7.55–7.31 (m, 12H), 7.62 (d, 4H, $J=8.0$ Hz), 8.05 (d, 2H, $J=8.9$ Hz), 8.11 (d, 2H, $J=8.0$ Hz). ^{13}C NMR: δ 17.0, 19.6, 24.3, 25.3, 29.8, 34.5, 60.9, 71.2, 123.2, 127.1, 128.1, 128.5, 128.7, 128.9, 129.1, 129.3, 129.7, 129.8, 129.9, 132.4, 133.7, 134.3, 134.9, 137.6, 139.2, 139.5, 145.9, 154.5, 164.1, 189.7.

Mass spectrometric analysis (FAB) calcd for $C_{43}H_{39}N_3O_6S_2+H$: 758.23. Found: 758.24.

4.1.4. Dimethyl 2-(*tert*-butyllimino)-6,9-dimethyl-1-(phenylsulfonyl)-8-[(phenylsulfonyl)imino]-1-azaspiro-[4.5]deca-3,6,9-triene-3,4-dicarboxylate 4d. Pale yellow crystalline solid, mp: 85–88 °C, IR (KBr) ν_{max}: 2960, 2365, 1739, 1677, 1600, 1450, 1315, 1268, 1087, 1015, 803 cm^{-1}. ^1H NMR: δ 1.20 (s, 9H), 1.87 (s, 3H), 2.08 (s, 3H), 3.68 (s, 3H), 3.82 (s, 3H), 6.41 (s, 1H), 7.40–7.61 (m, 7H), 7.96–8.12 (m, 4H). ^{13}C NMR: δ 17.3, 18.9, 31.2, 53.0, 53.1, 56.2, 67.7, 123.7, 127.0, 128.1, 128.8, 129.2, 132.5, 133.3, 138.0, 138.4, 139.3, 139.4, 139.7, 142.0, 153.8, 163.6, 165.4.

Anal. Calcd for $C_{31}H_{33}N_3O_8S_2$: C, 58.20; H, 5.20; N, 6.57; S, 10.02. Found: C, 58.01; H, 5.62; N, 6.61; S, 9.73.

4.1.5. Dimethyl 2-(*tert*-butylimino)-1-[(4-methylphenyl) sulfonyl]-8-{[(4-methylphenyl)sulfonyl]imino}-1-azaspiro[4.5]deca-3,6,9-triene-3,4- dicarboxylate 4e. Pale yellow crystalline solid, mp: 170–172 °C, IR (KBr) ν_{max}: 2975, 1739, 1667, 1548, 1315, 1274, 1170, 1082, 1015, 870, 770, 668 cm^{-1}. ^1H NMR: δ 1.12 (s, 9H), 2.45 (s, 6H), 3.63 (s, 3H), 3.75 (s, 3H), 6.54 (d, 1H, $J=8.1$ Hz), 6.74 (d, 2H, $J=8.3$ Hz), 7.26–7.29 (m, 4H), 7.34 (d, 1H, $J=8.1$ Hz), 7.81–7.90 (m, 4H). ^{13}C NMR: δ 21.6, 21.7, 31.1, 53.1, 53.2, 56.2, 64.9, 125.0, 127.4, 128.9, 129.2, 129.5, 131.3, 136.2, 138.2, 138.6, 136.8, 141.7, 142.5, 143.7, 144.2, 163.4, 163.7.

Anal. Calcd for $C_{31}H_{33}N_3O_8S_2$: C, 58.20; H, 5.20; N, 6.57; S, 10.02. Found: C, 58.07; H, 5.57; N, 6.63; S, 9.7.

4.1.6. Dimethyl 2-(*tert*-butylimino)-7-chloro-1-[(4-methylphenyl)sulfonyl]-8-{[(4-methylphenyl)sulfonyl]-imino}-1-azaspiro[4.5]deca-3,6,9-triene-3,4-dicarboxylae 4f. Pale yellow crystalline solid; mp: 140–142 °C, IR (KBr) ν_{max}: 2924, 1734, 1672, 1563, 1439, 1320, 1279, 1160, 1082, 1025, 870, 808 cm^{-1}. ^1H NMR: δ 1.19 (s, 9H), 2.40 (s, 3H), 2.45 (s, 3H), 3.72 (s, 3H), 3.83 (s, 3H), 6.73 (d, 2H, $J=9.9$ Hz), 7.26–7.35 (m, 4H), 7.81–7.87 (m, 4H), 6.85 (s, 1H). ^{13}C NMR: δ 21.6, 21.7, 31.2, 53.0, 53.2, 56.4, 65.6, 124.3, 124.4, 127.3, 127.4, 128.7, 128.9, 129.2, 129.4, 129.5, 129.9, 131.0, 136.5, 139.4, 141.3, 142.5, 142.8,143.8, 144.5, 162.5, 184.2.

HRMS (EI) calcd for $C_{31}H_{32}ClN_3O_8S_2$: 673.1319. Found: 673.1320.

4.1.7. Dimethyl 2-(cyclohexylimono)-1-(benzoyl)-8-[(benzoyl)imino]-1-azaspiro-[4.5]deca-3,6,9-triene-3,4-dicarboxylate 4g. Pale yellow semi solid, IR (neat) ν_{max}: 2934, 2856, 1744, 1667, 1600, 1450, 1331, 1268, 1175, 1087, 1020, 731 cm^{-1}. ^1H NMR: δ 1.26–1.63 (m, 10H), 3.25 (m, 1H), 3.76 (s, 3H), 3.83 (s, 3H), 6.52 (d, 2H, $J=9.8$ Hz), 6.66 (d, 2H, $J=9.8$ Hz), 7.16–7.66 (m, 10H). ^{13}C NMR: δ 24.5, 26.6, 29.3, 37.8, 52.7, 53.1, 59.6, 65.8, 120.4, 125.2, 126.9, 127.4, 127.8, 128.2, 128.4, 128.6, 128.8, 129.2, 129.3, 130.0, 130.3, 130.5, 131.3, 134.4, 144.3, 162.3, 164.9, 167.9, 176.7.

HRMS (EI) calcd for $C_{33}H_{31}N_3O_6$: 565.2213. Found: 565.2208.

4.1.8. Dimethyl 2-(cyclohexylimino)-6-methoxy-8-oxo-1-(phenylsulfonyl)-1-azaspiro[4.5]deca-3,6,9-triene-3,4-dicarboxylate 6a. Pale yellow crystalline solid, mp: 186–188 °C, IR (KBr) ν_{max}: 2918, 2846, 1732, 1662, 1605, 1444, 1408, 1382, 1222, 1087 cm^{-1}. ^1H NMR: δ 1.73–1.17 (m, 10H), 3.16–3.23 (m, 1H), 3.43 (s, 3H), 3.63 (s, 3H), 3.74 (s, 3H), 5.78 (s, 1H), 6.41 (d, 1H, $J=9.8$ Hz), 6.49 (d, 1H, $J=9.8$ Hz), 7.44–7.49 (m, 2H), 7.56–7.61 (m, 1H), 8.00 (d, 2H, $J=7.8$ Hz). ^{13}C NMR: δ 19.8, 24.4, 32.9, 34.6, 52.8, 53.1, 56.2, 59.4, 67.0, 104.6, 116.9, 126.0, 127.9, 129.3, 131.1, 133.3, 134.6, 138.9, 139.2, 139.3, 143.9, 152.9, 163.5, 168.2, 188.4.

HRMS (EI) calcd for $C_{26}H_{28}N_2O_8S$: 528.1566. Found: 528.1562.

4.1.9. Dimethyl 2-(cyclohexylimino)-7-methoxy-8-oxo-1-(phenylsulfonyl)-1-azaspiro[4.5]deca-3,6,9-triene-3,4-dicarboxylate 6b. Pale yellow crystalline solid, mp: 138–140 °C, IR (KBr) ν_{max}: 2929, 2851, 1765, 1734, 1662, 1605, 1548, 1439, 1356, 1294, 1232, 1155, 1087, 1020, 989, 793 cm^{-1}. ^1H NMR: δ 1.19–1.73(m, 10H), 3.41–3.54 (m, 1H), 3.67 (s, 3H), 3.78 (s, 3H), 3.93 (s, 3H), 5.67 (s, 1H), 6.29 (d, 1H, $J=9.7$ Hz), 6.38 (d, 1H, $J=9.7$ Hz), 7.54–7.71 (m, 3H), 8.00 (d, 2H, $J=7.1$ Hz). ^{13}C NMR: δ 24.7, 25.7, 29.6, 33.3, 52.9, 53.2, 56.4, 57.1, 97.7, 103.5, 127.2, 128.8, 130.8, 131.7, 132.7, 136.9, 137.8, 153.6, 165.6, 167.7, 185.9.

HRMS (EI) calcd for $C_{26}H_{28}N_2O_8S$: 528.1566. Found: 528.1593.

4.1.10. Dimethyl 2-(*tert*-butylimino)-7-methoxy-8-oxo-1-(phenylsulfonyl)-1-azaspiro[4.5]deca-3,6,9-triene-3,4-dicarboxylate 6c. Pale yellow crystalline solid; mp: 176–178 °C, IR (KBr) ν_{max}: 2970, 1775, 1739, 1662, 1548, 1439, 1356, 1222, 1165, 1031, 984, 798 cm^{-1}. ^1H NMR: δ1.27 (s, 9H), 3.72 (s, 3H), 3.79 (s, 3H), 3.91 (s, 3H), 5.65 (s, 1H), 6.61 (d, 1H, $J=8.6$ Hz), 6.79 (d, 1H, $J=8.6$ Hz), 7.54–7.60 (m, 3H), 7.99 (d, 2H, $J=8.2$ Hz). ^{13}C NMR: δ 29.9, 52.5, 52.8, 56.2, 56.8, 82.7, 103.2, 126.9, 128.4, 128.6, 128.7, 130.5, 131.4, 132.5, 136.9, 137.7, 139.4, 140.7, 159.5, 165.4, 167.5, 185.6.

HRMS (EI) calcd for $C_{24}H_{26}N_2O_8S$: 502.1410. Found: 502.1426.

4.1.11. Dimethyl 2-(cyclohexylimino)-8-oxo-1-(phenylsulfonyl)-1-azaspiro[4.5]deca-3,6,9-triene-3,4-dicarboxylate 6d. Pale yellow semi solid, IR (neat) ν_{max}: 2924, 1763, 1734, 1672, 1563, 1439, 1350, 1289, 1160, 1082, 1025, 870, 808 cm^{-1}. ^1H NMR: δ 1.26–1.77 (m, 10H), 3.13 (m, 1H), 3.87 (s, 3H), 3.93 (s, 3H), 6.65 (d, 2H, $J=7.9$ Hz), 6.86 (d, 2H, $J=7.9$ Hz), 7.91 (d, 2H, $J=6.9$ Hz), 7.67–7.56 (m, 3H). ^{13}C NMR: δ 24.5, 25.5, 29.7, 33.8, 51.9, 52.1, 59.6, 99.2, 115.9, 125.8, 126.4, 127.3, 128.8, 129.0, 132.5, 132.6, 133.8, 144.7, 159.1, 161.5, 165.0, 179.5.

HRMS (EI) calcd for $C_{25}H_{26}N_2O_7S$: 498.1461. Found: 498.1460.

4.1.12. 3,4-Bisbenzoyl 2-(*tert*-butyliminoimino)-7-methoxy-8-oxo-1-(phenylsulfonyl)-1-azaspiro[4.5]deca-3,6,9-triene 6e. Pale yellow crystalline solid, mp: 126–128 °C, IR (KBr) ν_{max}: 2924, 1758, 1734, 1672, 1563, 1439, 1320, 1279, 1160, 1082, 1025, 870, 808 cm^{-1}. ^1H NMR: δ 1.28 (s, 9H), 3.98 (s, 3H), 5.74 (s, 1H), 6.35 (d, 1H, $J=8.4$ Hz), 6.51 (d, 1H, $J=8.4$ Hz), 7.34–7.29 (m, 4H), 7.51–7.62 (m, 7H), 7.99 (d, 4H, $J=6.8$ Hz). ^{13}C NMR: δ 29.7, 55.4, 57.0, 83.8, 111.4, 127.2, 127.5, 128.5, 128.6, 128.7, 128.8, 129.3, 129.4, 131.5, 132.7, 134.1, 134.3, 135.8, 136.1, 137.3, 148.0, 165.4, 168.2, 188.6, 189.1.

HRMS (EI) calcd for $C_{34}H_{30}N_2O_6S$: 594.1825. Found: 594.1823.

4.1.13. Dimethyl 2-(*tert*-butylimino)-7,8-dimethyl-1-(phenylsulfonyl)-10-[(phenylsulfonyl)imino]-1-azaspiro-[4.5]deca-3,6,8-triene-3,4-dicarboxylate 8a. Pale yellow crystalline solid, mp: 144–145 °C, IR (KBr) ν_{max}: 2969, 1727, 1660, 1516, 1449, 1336, 1269, 1161, 1094, 1017, 908, 687 cm^{-1}. ^1H NMR: δ1.29 (s, 9H), 1.46 (s, 3H), 2.01 (s, 3H), 3.47 (s, 3H), 3.95 (s, 3H), 5.85 (s, 1H), 7.43 (s, 1H), 7.46–7.52 (m, 6H), 7.57 (d, 2H, $J=8.3$ Hz), 8.05 (d, 2H, $J=7.4$ Hz). ^{13}C NMR: δ 18.5, 20.0, 30.1, 51.6, 53.6, 61.2, 62.7, 111.6, 114.0, 118.0, 121.0, 127.1, 127.5, 127.8, 128.2, 128.4, 128.6, 128.7, 128.8, 128.9, 129.9, 132.6, 132.7, 133.1, 136.9, 138.5, 138.8, 139.9, 168.5, 173.3.

Mass spectrometric analysis (FAB) calcd for $C_{31}H_{33}N_3O_8S_2+H$: 640.17. Found: 640.62.

4.1.14. Dimethyl 2-(cyclohexylimino)-7,8-dimethyl-1-(phenylsulfonyl)-10-[(phenylsulfonyl)imino]-1-azaspiro-[4.5]deca-3,6,8-triene-3,4-dicarboxylate 8b. Pale yellow crystalline solid; mp: 148–150 °C, IR (KBr) ν_{max}: 2929, 2846, 1729, 1667, 1600, 1450, 1331, 1268, 1175, 1087, 1020, 731 cm^{-1}. ^1H NMR: δ 1.71–1.22 (m, 10H), 2.03 (s, 3H), 2.17 (m, 1H), 2.21 (s, 3H), 3.67 (s, 3H), 4.07 (s, 3H), 4.89 (s, 1H), 6.50 (s, 1H), 7.31–7.79 (m,10H). ^{13}C NMR: δ 18.9, 20.1, 24.8, 25.5, 32.8, 51.6, 53.7, 59.6, 72.9, 111.6, 120.8, 127.2, 127.5, 128.5, 128.9, 129.2, 131.3, 132.5, 132.6, 133.0, 133.5, 134.3, 140.2, 145.7, 163.5.

Mass spectrometric analysis (FAB) calcd for $C_{33}H_{35}N_3O_8S_2+H$: 666.19. Found: 666.26.

4.1.15. Dimethyl 2-(cyclohexylimino)-1-benzoyl-10-[(benzoyl)imino]-1-azaspiro[4.5] deca-3,6,8-triene-3,4-dicarboxylate 8c. Pale yellow crystalline solid, mp: 120–122 °C, IR (KBr) ν_{max}: 2928, 1737, 1676, 1563, 1439, 1279, 1160, 1082, 1025, 870, 808 cm^{-1}. ^1H NMR: δ 0.98–1.67 (m, 10H), 2.02 (m, 1H), 3.65 (s, 3H), 3.98 (s, 3H), 6.95 (d, 1H, $J=7.3$ Hz), 7.30–7.38 (m, 6H), 7.39–7.57 (m, 4H), 8.04 (d, 2H, $J=7.7$ Hz), 8.71 (d, 2H, $J=8.4$ Hz). ^{13}C NMR: δ 23.8, 29.7, 32.9, 51.9, 53.2, 62.1, 70.1, 113.7, 124.4, 127.5, 127.7, 127.9, 128.3, 128.4, 128.7, 129.2, 129.9, 130.3, 131.8, 132.2, 132.9, 134.3, 145.6, 164.7, 166.8.

Mass spectrometric analysis (FAB) calcd for $C_{33}H_{31}N_3O_6+H$: 567.22. Found: 567.86.

4.1.16. Dimethyl 2-(cyclohexylimino)-1-benzoyl-10-[(benzoyl)imino]-7,8-dimethyl-1-azaspiro[4.5]deca-3,6,8-triene-3,4-dicarboxylate 8d. Pale yellow crystalline solid, mp: 88–90 °C, IR (KBr) ν_{max}: 2924, 2866, 1746, 1674, 1605, 1528, 1445, 1255, 1014, 776 cm^{-1}. ^1H NMR: δ1.26–1.76 (m, 10H), 2.15 (s, 3H), 2.35 (s, 3H), 2.37 (m, 1H), 3.72 (s, 3H), 3.83 (s, 3H), 6.37 (s, 1H), 7.13 (s, 1H), 7.54–7.31(m, 4H), 7.98–7.74 (m, 4H), 8.34 (d, 2H, $J=8.8$ Hz). ^{13}C NMR: δ 15.9, 16.6, 26.4, 29.6, 30.7, 51.9, 53.0, 63.2, 122.3, 126.3, 127.0, 127.4, 127.9, 128.2, 128.3, 128.4, 128.5, 128.8, 129.9, 130.1, 131.8, 132.1, 133.0, 139.0, 156.3, 165.1, 169.6, 174.4.

Mass spectrometric analysis (FAB) calcd for $C_{35}H_{35}N_3O_6+H$: 594.25. Found: 594.43.

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References and notes

1. Patai, S., Rappoport, Z., Eds.; The Chemistry of Quinonoid Compounds; Wiley: New York, 1988; Vol. 2; Parts 1 and 2.
2. (a) Nair, V.; Kumar, S. *Synlett* **1996**, 1035. (b) Nair, V.; Kumar, S. *J. Chem. Soc., Chem. Commun.* **1994**, 1341. (c) Nair, V.; Radhakrishnan, K. V.; Sheela, K. C.; Rath, N. P. *Tetrahedron* **1999**, *55*, 14199. (d) Nair, V.; Mathew, B. *Tetrahedron Lett.* **2000**, *41*, 6919.
3. Adams, R.; Reifschneider, W. *Bull. Chim. Soc. Fr.* **1958**, 23 and references cited therein.
4. (a) Friedrichsen, W.; Bottcher, A. *Heterocycles* **1981**, 425. (b) Heine, H. W.; Barchiesi, B. J.; Williams, E. A. *J. Org. Chem.* **1984**, *49*, 2560. (c) Fujita, S. *J. Chem. Soc., Chem. Commun.* **1981**, 425. (d) Holmes, T. J., Jr.; Lawton, R. G. *J. Org. Chem.* **1983**, *48*, 3146.
5. (a) Boger, D. L.; Coleman, R. S. *J. Am. Chem. Soc.* **1988**, *110*, 4796. (b) Boger, D. L.; Zarrinmayeh, H. *J. Org. Chem.* **1990**, *55*, 1379. (c) Engler, T. A.; Lynch, K. O., Jr.; Chai, W.; Meduna, S. P. *Tetrahedron Lett.* **1995**, *36*, 2713. (d) Engler, T. A.; Chai, W.; Latessa, K. *J. Org. Chem.* **1996**, *61*, 9297.
6. (a) Friedrichsen, W.; Oeser, H. G. *Liebigs Ann. Chem.* **1978**, 1139. (b) Friedrichsen, W.; Oeser, H. G. *Liebigs Ann. Chem.* **1978**, 1146. (c) Friedrichsen, W.; Oeser, H. G. *Liebigs Ann. Chem.* **1978**, 1161.
7. Parker, K. A.; Mindt, T. L. *Org. Lett.* **2002**, *4*, 4265.
8. (a) Nair, V.; Rajesh, C.; Dhanya, R.; Rath, N. P. *Tetrahedron Lett.* **2002**, *43*, 5349. (b) Nair, V.; Rajesh, C.; Dhanya, R.; Rath, N. P. *Org. Lett.* **2002**, *4*, 953. (c) Nair, V.; Rajesh, C.; Dhanya, R.; Vinod, A. U. *Tetrahedron Lett.* **2001**, *42*, 2045. (d) Nair, V.; Dhanya, R.; Rajesh, C.; Bhadbhade, M. M.; Manoj, K. *Org. Lett.* **2004**, *6*, 4743.
9. (a) Nair, V.; Rajesh, C.; Vinod, A. U.; Bindu, S.; Sreekanth, A. R.; Mathen, J. S.; Balagopal, L. *Acc. Chem. Res* **2003**, *36*, 899 and the references cited therein. (b) Nair, V.; Remadevi, B.; Vidya, N.; Menon, R. S.; Abhilash, N.; Rath, N. P. *Tetrahedron Lett.* **2004**, *45*, 3203. (c) Nair, V.; Bindu, S.; Sreekumar, V.; Rath, N. P. *Org. Lett.* **2003**, *5*, 665. (d) Nair, V.; Menon, R. S.; Beneesh, P. B.; Sreekumar, V.; Bindu, S. *Org. Lett.* **2004**, *6*, 767.
10. Nair, V.; Vinod, A. U.; Rajesh, C. *J. Org. Chem.* **2001**, *62*, 4427.
11. Engler, T. A.; Wanner, J. *Tetrahedron Lett.* **1997**, *38*, 6135.
12. For the preparation of *p*-quinonediimides see (a) Adams, R.; Anderson, J. L. *J. Am. Chem. Soc.* **1950**, *72*, 5154. (b) Adams, R.; Nagarkatti, A. S. *J. Am. Chem. Soc.* **1950**, *72*, 460.
13. For the preparation of *p*-quinonemonoimides see (a) Adams, R.; Looker, J. H. *J. Am. Chem. Soc.* **1951**, *73*, 1146. (b) Engler, T. A.; Chai, W.; LaTessa, K. O. *J. Org. Chem.* **1996**, *61*, 9297. (c) Engler, T. A.; Scheibe, C. M. *J. Org. Chem.* **1998**, *63*, 6247.
14. For the preparation of *o*-quinonediimides see (a) Adams, R.; Winnick, C. N. *J. Am. Chem. Soc.* **1951**, *73*, 5687. (b) Adams, R.; Way, J. W. *J. Am. Chem. Soc.* **1954**, *76*, 2763.

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Bis(4,5-dimethoxy-2-nitrophenyl)ethylene glycol: a new and efficient photolabile protecting group for aldehydes and ketones

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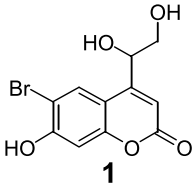
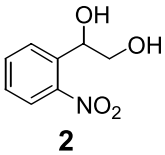
Abstract—Synthesis of a new photolabile protecting group, bis(4,5-dimethoxy-2-nitrophenyl)ethylene glycol (**4**) from 4,5-dimethoxy-2-nitrobenzyl alcohol in three steps in good yields is described. The acetals and ketals of **4** are stable against acidic and basic reaction conditions and are cleaved smoothly on irradiation at 350 and 400 nm with regeneration of carbonyl compounds in high yields and efficiency.

1. Introduction

The use of photolabile molecules as protecting groups has received considerable attention in recent years, as it offers a mild method to deprotect without the requirement of any reagent.[1] It also provides a greater specificity in the presence of other protecting groups and can be smoothly handled in variable acidic, basic and other very sensitive reaction conditions.[2] Although, many photo-removable protecting groups are known for carboxylic acids,[3] amines,[4] amides,[5] carbamates,[6] phenols,[7] alcohols[8] and phosphates,[9,10] surprisingly, less attention has been paid to develop photo-removable groups for aldehydes and ketones, though, they are most commonly used in organic synthesis.[1a] Earlier 6-bromo-4-(1,2-dihydroxyethyl)-7-hydroxy coumarin **1** (Bhc-diol),[11] 2-nitrophenyl ethylene glycol **2**[12] and bis(2-nitrophenyl)ethane diol **3**[13] were developed as photolabile protecting molecules for carbonyl compounds and found to have certain drawbacks.



1



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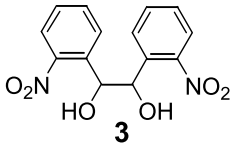

3

Bhc-diol is not a suitable substrate due to the more sensitive coumarin moiety and diol **2** takes several hours for photochemical deprotection under UV-light at longer wavelength (350 nm). We therefore, undertook the design of an efficient protecting group in terms of stability and ease of deprotection. We chose the 4,5-dimethoxy-2-nitrobenzyl group as it exhibited absorbance at longer wavelength than the 2-nitrobenzyl group. We describe herein, the synthesis of the new photolabile protecting group bis(4,5-dimethoxy-2-nitrophenyl)ethylene glycol **4** from 4,5-dimethoxy-2-nitrobenzyl alcohol in three steps in good yields. The acetals and ketals of **4** have been found to be stable under all normal reaction conditions and were efficiently deprotected at 350 and 400 nm with liberation of carbonyl compounds in high yields.

2. Results and discussion

4,5-Dimethoxy-2-nitrobenzyl alcohol (**5**),[17] was converted to 4,5-dimethoxy-2-nitro benzyl chloride (**6**) by reaction with PCl_5 and treated further, with KOH in DMSO–ethanol for 45 h to afford stilbene derivative **7** as pale yellow crystals. Stillbene **7** was dihydroxylated with a catalytic amount of OsO_4/NMO to obtain the diol **4** as a crystalline pale yellow solid, mp 154–157 °C (Scheme 1).

In order to study the efficiency of the new photosensitive protecting group, **4** was reacted with various aldehydes **8a–b** and ketones **8c–f** (entries i–vi, Table 1) by refluxing in benzene containing a catalytic amount of pyridinium *p*-toluenesulphonate (PPTS) to obtain the corresponding acetals **9a–b** and ketals **9c–f**, respectively, (Scheme 1). The products **9a–f** were purified by silica gel column chromatography and characterized by IR, NMR and mass spectra. It is worth mentioning the absence of double bond isomerization in enone substrates (entries iii, vi and viii).

Keywords: Protecting groups; Cage compounds; Photolysis; Radical ions.
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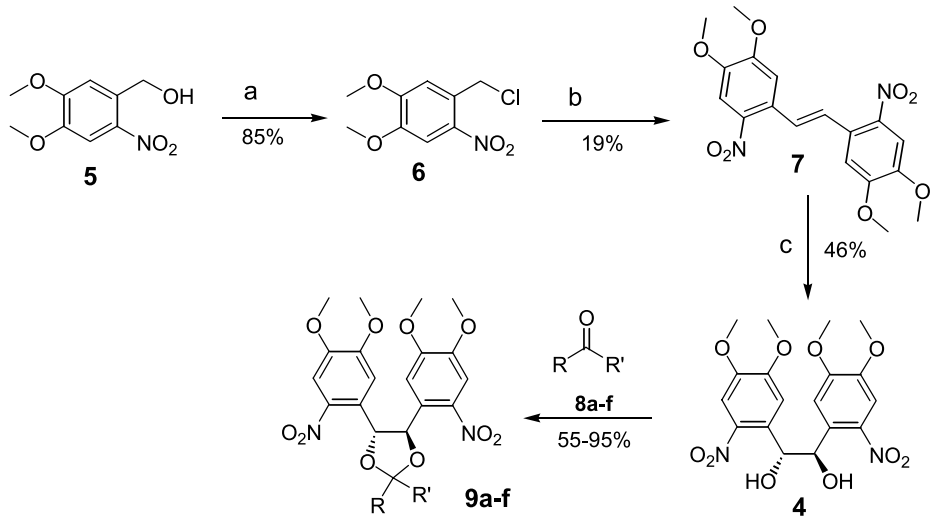


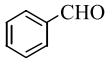
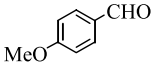
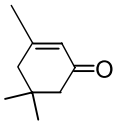
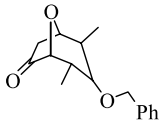
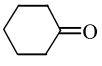
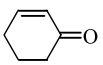
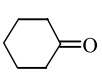
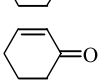
Scheme 1. (a) $PCl_5/CHCl_3$; (b) KOH/DMSO–ethanol; (c) OsO_4/NMO; (d) PPTS, benzene.

The use of 4 Å molecular sieves was essential to avoid deconjucation during the formation of the ketal.

The acetals **9a–b** and ketals **9c–f** were exposed to UV-light at 350 and 400 nm for 2–10 h to regenerate the carbonyl compounds **8a–f**, respectively, in good yields (Table 1). In a typical deprotection experiment 0.1 mmol solution of **9e** in acetonitrile in a Pyrex vessel was irradiated at 350 nm using Rayonet apparatus until TLC showed the disappearance of the starting material. Chromatography of the residue on silica gel afforded cyclohexanone **8e** in 92% yield. Examination of the UV–visible spectra revealed that dimethoxy diol **4** and its acetals **9a–b** and ketals **9c–f** showed very good absorbance at 400 nm (Fig. 1). Accordingly, on irradiation of **9e** at 400 nm for 10 h in a Pyrex vessel, 75% of cyclohexanone **8e** was regenerated (Table 1).

The stability of the compounds was tested in a broad variety of chemical conditions and for commonly used reagents to assess the general applicability of the new protecting group (Table 2). Here, it is worth mentioning that the acetals **9a–b** and ketals **9c–f** were found to be highly stable in acidic and basic reaction media. They did not show any significant decomposition in polar solvents such as DMSO and alcohols. We noticed that **9e** did not undergo reduction with $LiAlH_4$ (5 equiv) even after 48 h at rt in solvents such

Table 1. Protection and deprotection of aldehydes and ketones with diols **3** and **4**

Entry	Diol	Substrate (**8**)	Ketal	Yield (%)	Photolysis	
					350 nm	400 nm
i	**4**	(a) 	**9a**	77	80	58
ii	**4**	(b) 	**9b**	95	82	62
iii	**4**	(c) 	**9c**	55	68	—
iv	**4**	(d) 	**9d**	65	74	—
v	**4**	(e) 	**9e**	90	92	75
vi	**4**	(f) 	**9f**	73	81	—
vii	**3**	(e) 	**10a**	90	89	50
viii	**3**	(f) 	**10b**	80	78	—

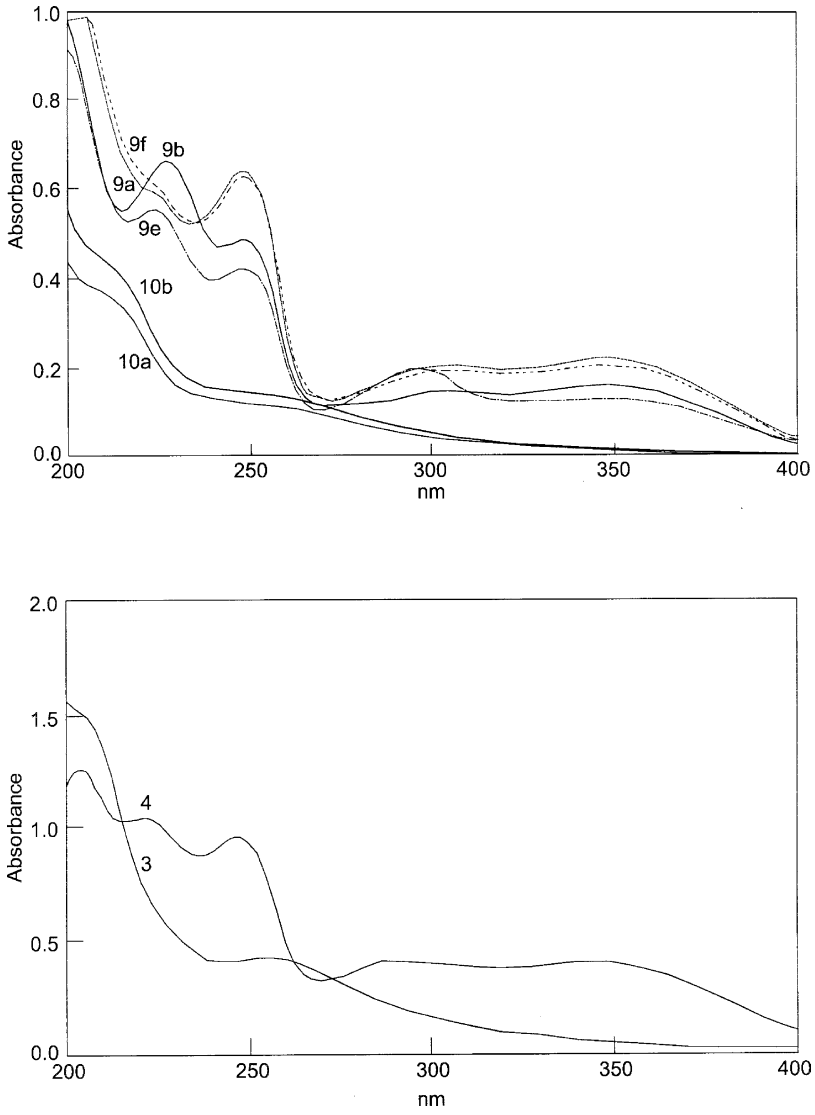
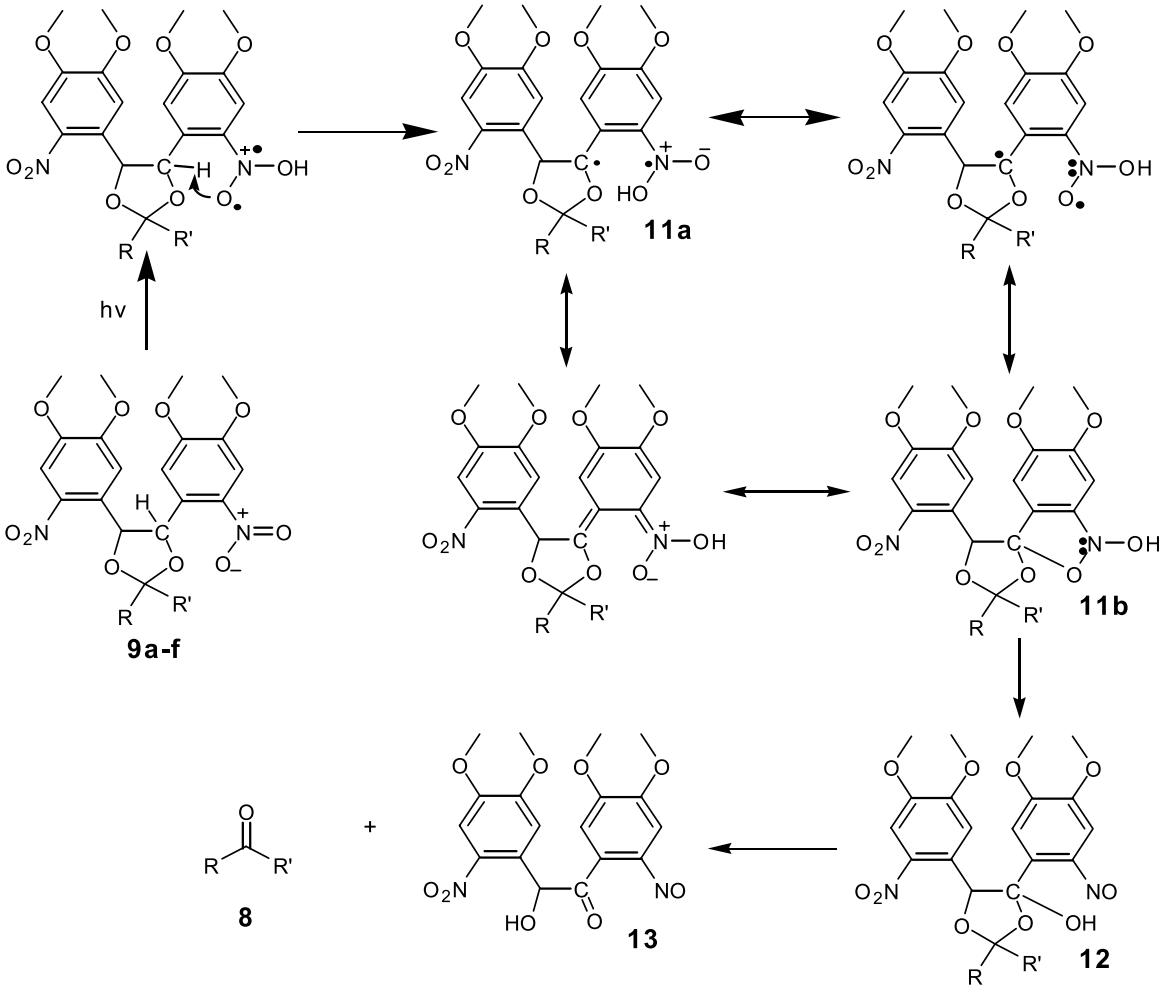


Figure 1. UV–visible spectra of diols **3**, **4**; acetals **9a**, **9b** and ketals **9e**, **9f**, **10a** and **10b**.

Table 2. Stability of ketal **8c** in acidic, basic, oxidation and reduction conditions

S. No	Solvent	Reagent	Reaction conditions[a]		Ketal **8c**[b] (%)	Cyclohexanone
			Temp (°C)	Time (h)		
a	THF	aq HCl (5%)	rt	48	100	0
b	THF	aq H_2SO_4 (10%)	rt	78	99	Trace
c	Dioxane	2 N NaOH	rt	24	100	0
d	Dioxane	2 N NaOH	Reflux	24	89	8
e	THF	NaH	rt	24	98	Trace
f	DMSO	NaH	rt	24	95	Trace
g	DMSO	NaH	100	10	65	Trace
h	CH_3CN	NaH	rt	20	100	0
i	Ether	NaH	rt	24	100	0
j	Ether	$LiAlH_4$	rt	48	98	Trace
k	THF	$LiAlH_4$	rt	48	96	Trace
l	DME	NaH	rt	24	100	0
m	*t*-Butanol	Potassium *t*-butoxide	rt	24	80	Trace
n	Methanol	NaBH4	rt	24	100	0
o	CH_3CN	DDQ	rt	18	85	Trace
p	THF	TBAF	rt	20	95	Trace
q	CH_3CN	CAN	rt	24	85	Trace
r	TFA	TFA	rt	1	58	40
s	Methanol	$NaBH_4 + CeCl_3 \cdot 7H_2O$	rt	24	98	Trace
t	Methanol	Pd/C, H_2	rt	24	0	0

[a] 0.04 mmol of ketal in appropriate solvent was stirred with 5 equiv of reagent in the dark.
[b] Yields are determined by ^{1}H NMR analysis of crude reaction mixture after workup.



Scheme 2. Proposed mechanism for the photodeprotection of ketal **9a–f**.

as ethyl ether and THF (entries j and k, Table 2). Hydrogenation of **9e** with $Pd/C/H_2$ resulted in the formation of the corresponding aniline and deprotection of acetal was not observed. Under aqueous acidic conditions, ketal **9e** showed high stability, whereas in neat TFA at rt, the respective carbonyl compound was isolated in 1 h (entry r, Table 2).

Although, the exact mechanism of the photochemical deprotection is not yet fully understood, it is probably analogous to that described for uncaging of caged calcium,[14] caged neurotransmitters[15] caged peptides[16] and protected diols[12] (Scheme 2). The key step involves intramolecular abstraction of benzylic hydrogen in a singlet or a triplet state to give **11a** or **11b**, its rearrangement to hemiacetal **12** and collapse of the latter afford the free carbonyl compound **8** and nitroso compound **13**. The sensitivity of **13** to irradiated light prevented its isolation and it could not therefore, be characterized.

In conclusion, we have synthesized a new photolabile protecting group bis(4,5-dimethoxy-2-nitrophenyl)ethylene glycol **4** for aldehydes and ketones and demonstrated the stability of its ketals in various reaction conditions. The ketals were efficiently deprotected both at 350 and 400 nm to regenerate carbonyl compounds in good yields.

3. Experimental

3.1. General

Melting points were recorded on Buchi 535 melting point apparatus and are uncorrected. All the reactions were monitored by thin layer chromatography performed on precoated silica gel $60F_{254}$ plates (Merck). Compounds were visualized with UV-light at 254 and 365 nm, iodine and heating plates after dipping in 2% phosphomolybdic acid in 15% aq H_2SO_4 solution. Column chromatography was carried out using silica gel 60–120 mesh purchased from ACME Chemical Company, Bombay. All solvents used were purified and dried according to the standard procedures. IR spectra were recorded on Perkin-Elmer 683 or 1310 FT-IR spectrometer with KBr pellets. NMR spectra were recorded on Varian Unity-400 MHz and BRUKER AMX 300 MHz spectrometers using tetramethylsilane as an internal standard. ^{13}C NMR was recorded on Varian Unity 100 MHz using $CDCl_3$ as internal standard. Mass spectra were recorded on a VG Micromass 7070H and Finnigan Mat 1020B mass spectrometers operating at 70 eV. UV-spectra were recorded on a Perkin-Elmer UV/Vis spectrophotometer. Benzaldehyde, *p*-methoxybenzaldehyde, cyclohexanone, 2-cyclohexenone, 3,5,5-trimethyl-2-cyclohexenone were purchased from SD-Fine chemicals,

Bombay. Bis(2-nitrophenyl)ethanediol **3** and its cyclohexanone ketal **10a** and cyclohexanone ketal **10b** were synthesized according to the literature procedure.[13]

3.1.1. 4,5-Dimethoxy-2-nitrobenzyl chloride 6. 4,5-Dimethoxy-2-nitrobenzyl alcohol **5** (3.0 g, 14.0 mmol) in chloroform (80 ml) was treated with PCl_5 (3.2 g, 15.4 mmol) at rt for 30 min. The reaction was quenched with addition of water (80 ml), organic layer was separated, dried over sodium sulfate and evaporated under reduced pressure to give a solid residue, which was passed over silica gel column (eluted with ethyl acetate/hexane 1:9) to isolate the titled compound **6** (2.72 g, 85%) as a pale yellow solid, mp 65–66 °C. IR (ν_{max}, cm^{-1}) 3030, 2985, 1425, 1505, 1425, 1285, 1100, 675. ^1H NMR (400 MHz, $CDCl_3$) δ: 3.94 (s, 3H), 4.04 (s, 3H), 5.00 (s, 2H), 7.14 (s, 1H), 7.55 (s, 1H). MS (*m/z*, %) 231 (M+, 10), 183 (100), 153 (8), 79 (28). Analysis found: C, 46.84; H, 4.44; Cl, 15.22; N, 6.12. Calcd for $C_9H_{10}ClNO_4$: C, 46.67; H, 4.35; Cl, 15.35; N, 6.05.

3.1.2. *trans*-2,2′-Dinitro-3,3′,4,4′-tetramethoxystilbene 7. To a solution of 4,5-dimethoxy-2-nitrobenzyl chloride **6** (2.0 g, 8.6 mmol) in DMSO (1 ml) and ethanol (3 ml) was added KOH (1.54 g, 26.8 mmol) in ethanol (14 ml) dropwise slowly and stirred for 45 h at rt. The solid precipitate was filtered and washed with ethanol (10 ml) and redissolved in hot ethyl acetate. The insoluble portion was filtered and filtrate was cooled to give the title compound **7** (0.300 g, 19%) as yellow solid, mp 245–248 °C. IR (ν_{max}, cm^{-1}) 2950, 1650, 1500, 1250, 1200, 820, 740. ^1H NMR (300 MHz, $CDCl_3$) δ: 3.95 (s, 6H), 4.60 (s, 6H), 7.14 (s, 2H), 7.59 (s, 2H), 7.65 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 153.5, 148.8, 145.2, 128.8, 128.1, 110.0, 107.9, 56.4. MS (EI, *m/z*, %): 390 (M+, 28), 358 (10), 211 (18), 194 (18), 179 (24), 164 (70), 152 (38), 136 (100), 125 (14), 108 (24), 79 (20). Analysis found: C, 55.12; H, 4.58; N, 7.19. Calcd for $C_{18}H_{18}N_2O_8$: C, 55.39; H, 4.65; N, 7.18.

3.1.3. Bis(4,5-dimethoxy-2-nitrophenyl)ethylene glycol 4. To a mixture of *trans*-2,2′dinitro-3,3′,4,4′-tetramethoxystilbene **7** (150 mg, 0.38 mmol) in dichloromethane (4 ml) and water (1 ml) and *N*-methyl morpholine oxide (NMO) (50 mg, 0.5 mmol) in water (0.5 ml), OsO_4 (0.1 ml, 0.02 mmol, 4% in water) was added. The reaction mixture was vigorously stirred at rt. After 48 h, reaction mixture was quenched with $Na_2S_2O_4$ (0.45 g in 4.5 ml water) and stirred for an additional 24 h. The dichloromethane layer was separated and the aqueous phase was extracted with ethyl acetate (2×5 ml). The combined organic layers were dried over sodium sulfate and evaporated under vacuum. The residue obtained was chromatographed over silica gel (eluted with hexane/ethyl acetate, 1:1) to isolate diol **4** (50 mg, 46%) as a pale yellow solid, mp 154–157 °C. IR (ν_{max}, cm^{-1}): 3320, 2985, 2895, 1420, 1340, 1280, 100, 1080, 810, 780. ^1H NMR (300 MHz, $CDCl_3$) δ: 3.99 (s, 6H), 4.00 (s, 6H), 5.69 (s, 2H), 7.19 (s, 2H), 7.31 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 153.4, 148.7, 141.3, 129.8, 111.4, 108.4, 72.3 and 56.4. MS (ESI−ve, $CHCl_3$): 459 (M+Cl−), 884 (2M+Cl−). Analysis found: C, 50.88; H, 4.62; N, 6.71. Calcd for $C_{18}H_{20}N_2O_{10}$: C, 50.95; H, 4.75; N, 6.60.

3.2. General procedure for preparation of acetals 9a–b and ketals 9c–f

A solution of bis(4,5-dimethoxy-2-nitrophenyl)ethylene glycol **4** (1.0 mmol), carbonyl compounds **8a–f** (1.0 mmol), pyridinium *p*-toluenesulfonate (0.1 mmol) in dry benzene (10 ml) was taken in a flask equipped with Dean-Stark water separator (protected from day light) and was heated to reflux. Progress of the reaction was monitored by TLC After completion of reaction, benzene was evaporated under vacuum to obtain residue, which was dissolved in ethyl acetate (10 ml). The organic phase was washed with saturated $NaHCO_3$, brine, dried over sodium sulfate, filtered and evaporated to obtain a residue, which was purified by silica gel column chromatography (eluted with hexane:ethyl acetate) to isolate the acetals and ketals as crystalline solids.

3.2.1. Benzaldehyde acetal 9a. Yield: 77%, pale yellow solid, mp 117 °C. IR (ν_{max}, cm^{-1}): 2923, 2852, 1582, 1516, 1459, 1269, 1064, 869, 752. ^1H NMR (300 MHz, $CDCl_3$) δ: 3.70 (s, 3H), 3.92 (s, 3H), 3.98 (s, 3H), 4.98 (s, 3H), 5.90 (d, J=5 Hz, 1H), 6.00 (d, J=5 Hz, 1H), 6.39 (s, 1H), 7.10 (s, 1H), 7.35 (s, 1H), 7.44 (m, 3H), 7.51 (s, 1H), 7.54 (s, 1H), 7.60 (d, J=7.6 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 153.7, 153.5, 148.7, 148.6, 141.3, 140.6, 137.7, 129.5, 128.6, 110.1, 109.4, 108.3, 107.7, 104.5, 81.0, 80.5, 56.5, 56.4, 56.3, 56.1. MS (ESI−ve, $CHCl_3$): 547 (M+ Cl−), 1059 (2M+Cl−). Analysis found: C, 58.32; H, 4.68; N, 5.48. Calcd for $C_{25}H_{24}N_2O_{10}$: C, 58.59; H, 4.72; N, 5.47.

3.2.2. *p*-Anisaldehyde acetal 9b. Yield: 95%; yellow solid, mp 167 °C. IR (ν_{max}, cm^{-1}): 2933, 2880, 1600, 1520, 1456, 1380, 1200, 1140, 1115, 799. ^1H NMR (300 MHz, $CDCl_3$) δ: 3.76 (s, 3H), 3.85 (s, 3H), 3.95 (s, 3H), 3.98 (s, 3H), 4.09 (s, 3H), 5.90 (d, J=7.8 Hz, 1H), 5.98 (d, J=7.8 Hz, 1H), 6.30 (s, 1H), 6.98 (d, J=10.5 Hz, 2H), 7.18 (s, 1H), 7.35 (s, 1H), 7.52–7.54 (d, J=10.5 Hz, 2H; s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 160.6, 153.6, 153.4, 148.5, 148.4, 141.0, 140.5, 129.5, 129.4, 128.9, 127.7, 114.0, 109.9, 109.2, 108.1, 107.5, 104.5, 80.8, 80.4, 56.4, 56.2, 56.1, 55.3. MS (ESI−ve, $CHCl_3$): 577 (M+Cl−), 1119 (2M+Cl−). Analysis found: C, 57.78; H, 4.78; N, 5.12. Calcd for $C_{26}H_{26}N_2O_{11}$: C, 57.56; H, 4.83; N, 5.16.

3.2.3. 3,5,5-Trimethyl-2-cyclohexenone ketal 9c. Yield: 55%; yellow solid, mp 152–155 °C. IR (ν_{max}, cm^{-1}): 2925, 2858, 2359, 1667, 1585, 1458, 1274, 1171, 1080, 798, 760. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.09 (s, 6H), 1.29 (s, 3H), 1.80 (s, 2H), 2.00 (s, 2H), 3.91 (s, 6H), 4.09 (s, 6H), 5.51–5.60 (m, 3H), 7.32 (s, 1H), 7.34 (s, 1H), 7.38 (s, 2H). MS (ESI−ve, $CHCl_3$): 579 (M+C1−), 1123 (2M+C1−). Analysis found: C, 59.68; H, 5.88; N, 5.12. Calcd for $C_{27}H_{32}N_2O_{10}$: C, 59.55; H, 5.92; N, 5.14.

3.2.4. Ketal 9d. Yield: 65%, 61–64 °C. IR (ν_{max}, cm^{-1}): 2924, 2855, 2360, 1514, 1458, 1266, 1215, 1083, 868, 802. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.95 (d, J=6.8 Hz, 3H), 1.08 (d, J=6.8 Hz, 3H), 2.20–2.40 (m, 3H), 2.75 (m, 1H), 3.58 (t, J=3.4 Hz, 1H), 3.65 (d, J=3.6 Hz, 1H), 4.31 (m, 1H), 3.71 (s, 3H), 3.96 (s, 3H), 4.10 (s, 3H), 4.55 (ABq, J= 10.8 Hz, 2H), 5.82 (d, J=8.7 Hz, 1H), 6.01 (d, J=8.7 Hz,

1H), 7.12–7.62 (m, 9H). Analysis found: C, 61.51; H, 5.78; N, 4.26. Calcd for $C_{24}H_{28}N_2O_{10}$: C, 61.25; H, 5.75; N, 4.20.

3.2.5. Cyclohexanone ketal 9e. Yield: 90%; pale yellow solid, mp 197–200 °C. IR (ν_{max}, cm^{-1}): 2930, 1520, 1350, 1290, 1210, 1130, 1090, 790. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.50 (m, 2H), 1.75 (m, 4H), 1.92 (m, 4H), 3.90 (s, 6H), 4.02 (s, 6H), 5.60 (s, 2H), 7.39 (s, 2H), 7.40 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 153.5, 148.5, 141.3, 127.8, 109.8, 107.6, 79.3, 56.3, 56.2, 36.8, 25.5, 23.7. MS (ESI− ve, $CHCl_3$): 539 (M+Cl−), 1043 (2M+Cl−). Analysis found: C, 57.39; H, 5.58; N, 5.62. Calcd for $C_{24}H_{28}N_2O_{10}$: C, 57.14; H, 5.59; N, 5.55.

3.2.6. Cyclohexenone ketal 9f. Yield: 73%; yellow solid, mp 140–142 °C. IR (ν_{max}, cm^{-1}): 2924, 2855, 1584, 1515, 1464, 1386, 1332, 1216, 1117, 1071, 1026, 873. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.80–1.90 (m, 2H), 2.19 (m, 4H), 3.90 (s, 6H), 4.08 (s, 6H), 5.61 (d, J=7.5 Hz, 1H), 5.72 (d, J=7.5 Hz, 1H), 5.89 (d, J=9.3 Hz, 1H), 6.11 (d, J=9.2 Hz, 1H), 7.39 (s, 2H), 7.40 (s, 2H). MS (ESI− ve, $CHCl_3$): 537 (M+Cl−), 1040 (2M+Cl−). Analysis found: C, 57.56; H, 5.08; N, 5.11. Calcd for $C_{24}H_{26}N_2O_{10}$: C, 57.37; H, 5.22; N, 5.01.

3.3. General procedure for photolytic deprotection of acetals and ketals 9a–f

A solution of acetals **9a**–**b** or ketals **9c**–**f** (0.1 mmol) in acetonitrile (10 ml) in a Pyrex vessel was degassed by bubbling dry nitrogen gas and irradiated at 350 and 450 nm separately with stirring in Rayonet apparatus equipped with monochromatic lamps. Progress of deprotection was monitored by thin layer chromatography (hexane/ethyl acetate 4:1). Samples were drawn intermittently and yields were determined by GC and proton NMR spectroscopy (Table 1). The reddish brown solution so obtained was evaporated to a residue and purified by flash column chromatography over silica gel to recover carbonyl compounds **8a**–**e**. The irradiation at 400 nm was carried out for 10 h and analogously analyzed by thin layer chromatography and NMR spectroscopy. The carbonyl compounds **8a**–**f** recovered were characterized by comparison with the authentic samples.

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References and notes

1. (a) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 3rd ed.; Wiley: New York, 1999. (b) Pillai, V. N. R. *Synthesis* **1980**, 1–26. (c) Pillai, V. N. R. *Org. Photochem.* **1987**, *9*, 225–323. (d) Dorman, G.; Prestwich, G. D. *Trends Biotechnol.* **2000**, *18*, 64–72.
2. (a) Bochet, C. G. *Synlett* **2002**, 2268–2274. *J. Chem. Soc., Perkin Trans. 1* **2002**, 125–142. (b) Blank, A.; Bochet, C. G. *J. Org. Chem.* **2002**, *67*, 5567–5577. (c) Kessler, M.; Glatthar, R.; Giese, B.; Bochet, C. G. *Org. Lett.* **2003**, *5*, 1179–1181.
3. (a) Bochet, C. G. *Tetrahedron Lett.* **2000**, *41*, 6341. (b) Schaper, K.; Mobarekah, S. A. M.; Grewer, C. *Eur. J. Org. Chem.* **2002**, 1037–1046.
4. Peng, L.; Wirz, J.; Goeldner, M. *Angew. Chem., Int. Ed.* **1997**, *36*, 398–400. *Tetrahedron Lett.* **1997**, *38*, 2961–2964.
5. (a) Salerrno, C. P.; Resat, M.; Magde, D.; Kraut, J. *J. Am. Chem. Soc.* **1997**, *119*, 3403–3404. (b) Cohen, B. E.; Stoddard, B. L.; Koshland, D. E., Jr. *Biochemistry* **1997**, *36*, 9035–9044. (c) Helgen, C.; Bochet, C. G. *Synlett* **2001**, 1968–1970.
6. (a) Helgen, C.; Bochet, C. G. *J. Org. Chem.* **2003**, *68*, 2483–2486. (b) Milburn, T.; Matsubara, N.; Billington, A. P.; Udgaonkar, J. B.; Walker, J. W.; Carpenter, B. K.; Webb, W. W.; Marque, J.; Denk, W.; McCray, J. A.; Hess, G. P. *Biochemistry* **1989**, *28*, 49–55.
7. Walker, J. W.; Martin, H.; Schmitt, F. R.; Barsotti, R. J. *Biochemistry* **1993**, *32*, 1338–1345.
8. (a) Loudwig, S.; Goeldner, M. *Tetrahedron Lett.* **2001**, *42*, 7957–7959. (b) Corrie, J. E. T. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2161–2166.
9. (a) Dinkel, C.; Wichmann, O.; Schultz, C. *Tetrahedron Lett.* **2003**, *44*, 1153–1155. (b) Dinkel, C.; Schultz, C. *Tetrahedron Lett.* **2003**, *44*, 1157–1159. (c) Baldwin, J. E.; McConnanghie, A. W.; Moloney, M. G.; Pratt, A. J.; Shin, S. B. *Tetrahedron* **1990**, *46*, 6879–6884.
10. Li, W.-h.; Llopis, J.; Whitney, M.; Zlokarnik, G.; Tsien, R. Y. *Nature* **1998**, *392*, 936–941.
11. (a) Lin, W.; Lawrence, D. S. *J. Org. Chem.* **2002**, *67*, 2723–2726. (b) Lu, M.; Fedoryank, O. D.; Moister, B. R.; Dore, T. M. *Org. Lett.* **2003**, *5*, 2119–2122.
12. (a) Herbert, J.; Gravel, D. *Can. J. Chem.* **1974**, *52*, 187–189. (b) Gravel, D.; Herbert, J.; Thorava, D. *Can. J. Chem.* **1983**, *61*, 400–410. (c) Aurell, M. J.; Boix, C.; Ceita, M. L.; Ciopis, C.; Tortajada, A.; Mesters, R. *J. Chem. Res., Synop.* **1995**, 452–454.
13. Blanc, A.; Bochet, C. G. *J. Org. Chem.* **2003**, *68*, 1138–1141.
14. Ellis-Davies, G. C. R. *Methods Enzymol.* **2003**, *360*, 226–248.
15. (a) Gee, K. R.; Carpenter, B. K.; Hess, G. P. *Methods Enzymol.* **1998**, *291*, 30–56. (b) Hess, G. P. *Biophys. Chem.* **2003**, *100*, 493–501.
16. (a) Lawrence, D. S. *Acc. Chem. Res.* **2003**, *36*, 401–408. (b) Bosques, C. J.; Imperialli, B. *J. Am. Chem. Soc.* **2003**, *125*, 7530–7531.
17. (a) Vogel, A. I. *Textbook of Practical Organic Chemistry*, 5th edition, Longman: London, **1989**, 987–988. (b) Zehavi, U.; Amit, B.; Patchonik, A. *J. Org. Chem.* **1972**, *37*, 2281–2285. (c) Buck, J. S. In *Organic Syntheses Collect. Vol. II*; Wiley: New York, 1977; pp 619–621.

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Diastereoselectivity in addition of nitrile-stabilized carbanions to Schiff bases and in subsequent alkylation reactions

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Abstract—The stereochemical course in the addition of lithiated benzylcyanide and propionitrile to aromatic Schiff bases, as well as of the subsequent alkylation reaction has been investigated. The stereochemical ratios in the condensation reaction are proved to result from the intermediacy of a prochiral carbanionic intermediate, produced by a fast proton shift in the initially formed azanion. Subsequent one-pot alkylation reaction with a variety of electrophiles leads in high to moderate yields to diastereoselective formation of a second carbon–carbon bond at the same carbon center. Diastereoselectivity in alkylation, which varies from outstanding to high and poor is rationalized in terms of open-chain, product-like or reactant-like transition state models.

1. Introduction

Due to their specific properties, nitrile-stabilized carbanions are valuable intermediates in organic synthesis.[1] Being powerful nucleophiles, they are widely used for C–C bond formation in aldol[2] and alkylation reactions.[1] The small steric requirement of the cyano group is an important characteristic which makes them suitable intermediates for the creation of quaternary carbon center(s) that are present in a number of biologically active natural products.[3]

Several years ago, we reported that diastereoselectivity in the Michael addition of phenylacetonitrile to cinnamic acid *N*,*N*-dimethylamide is determined by kinetic asymmetric protonation of a prochiral intermediate with an adjacent chiral center, resulting from intramolecular proton transfer.[4] Later, we developed an alternative method for the direct preparation of analogous intermediates by conjugate addition of acetic acid to α-phenylcinnamic acid derivatives[5] and investigated their behavior in trapping reactions with electrophiles other than protons.[6] The diastereoselectivity of alkylation was found to depend on the type of metal intermediate and its γ-substitution patterns and to be consistent with a rigidly chelated transition state model.

With these results in mind and based on our interest in diastereoselective C–C bond formation[7] we decided to explore the stereochemical course in the addition of nitrile-stabilized carbanions to azomethinic compounds. It is worth noting that despite the considerable research focused on aldol and Michael condensations of nitrile carbanions[2] to the best of our knowledge very little attention has been paid to the use of imines as electrophilic partners.[8]

Another reason to undertake this investigation was the presumption that, as in our previous study,[4] isomerization of the initially formed azanions to nitrile-stabilized carbanions would give access to intermediates which could be subjected to alkylation reactions. This useful one-pot synthetic procedure was expected to furnish relatively complex amino-nitrile molecules with two adjacent chiral centers, one of which was quaternary. The diastereoselectivity of alkylation was also of considerable interest.

Herein, we report our study on the stereochemical course of condensation of nitrile carbanions with aromatic imines and of the subsequent alkylation reaction. The origin of the stereochemical outcome in both reactions is discussed.

2. Results and discussion

The addition of lithiated nitriles to azomethinic compounds was carried out in THF (concentration = 0.33 M) in the temperature range of −78 to 22 °C. (Scheme 1, pathway A). The results obtained are summarized in Table 1.

The reaction between phenylacetonitrile and benzylideneaniline (example **5**) has been previously studied in the

Keywords: Tandem reactions; Aldol addition; Schiff bases; Alkylation; Diastereoselectivity; Nitrile-stabilized carbanions.
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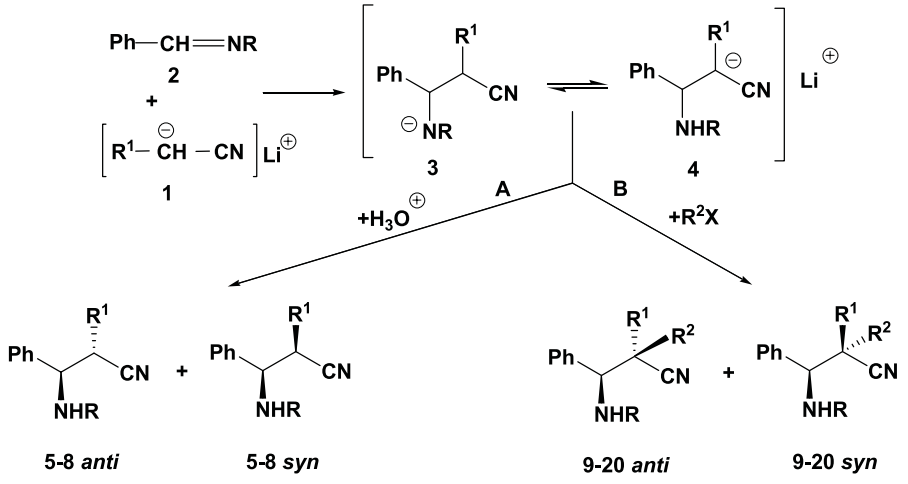


	R	R^1
5	Ph	Ph
6	β-naphthyl	Ph
7	Ph	α–naphthyl
8	Ph	CH_3

	R	R^1	R^2
9	Ph	Ph	CH_3
10	Ph	Ph	C_2H_5
11	Ph	Ph	$CH_2CH{=}CH_2$
12	Ph	Ph	$CH_2C_6H_5$
13	Ph	Ph	$CH_2CO_2CH_3$
14	β-naphthyl	Ph	CH_3
15	β-naphthyl	Ph	C_2H_5
16	β-naphthyl	Ph	$CH_2CH{=}CH_2$
17	β-naphthyl	Ph	$CH_2C_6H_5$
18	β-naphthyl	Ph	$CH_2CO_2CH_3$
19	Ph	CH_3	$CH_2CH{=}CH_2$
20	Ph	CH_3	$CH_2C_6H_5$

Scheme 1.

Table 1. Yields and distereoselectivity in addition of lithiated nitriles to aromatic imines

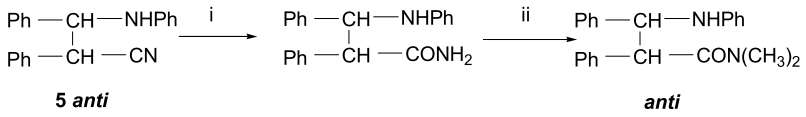
Compound	R	R^1	Temp (°C)	Time	Yield (%)	*anti*/*syn*
5[a]	Ph	Ph	−78	10 s→60 min	45 (85)	46:54
	Ph	Ph	22	60 s→60 min	70 (80)	47:53
	Ph	Ph	22	24 h	53	45:55
6	β-naphthyl	Ph	−78	10 s→60 min	52 (82)	46:54
	β-naphthyl	Ph	22	60 s→60 min	75 (87)	46:54
7	Ph	α-naphthyl	−78→22	60 s→24 h	—	—
8	Ph	CH_3	−78	10 s	62	40:60
	Ph	CH_3	−78	15 min	82	60:40
	Ph	CH_3	22	60 s→60 min	72 (0)	60:40

[a] The use of sodium reagent does not influence the reaction parameters.

presence of aluminium chloride[8a] and lithium amide.[8b] In both cases only one isomer was isolated, but its relative configuration was not determined and the stereochemical course of the reaction not elucidated, due to the lack of a precise method of analysis before the advent of NMR spectroscopy.

The relative configurations of **5** (*anti*) and (*syn*) were assigned by chemical correlation with the diastereoisomeric *N*,*N*-dimethyl-2,3-diphenyl-3-(phenylamino)-propanamides of known stereochemistry[9] as shown in Scheme 2.

The relative stereochemistry of diastereoisomeric pairs **6**



Scheme 2. Reagents and conditions: (i) concd H_2SO_4, −15 °C, 4 days (71%); (ii) NaH, MeI, THF, rt, 2 h (94%).

and **8** were attributed using the difference of 0.16 ppm between the ^{1}H NMR chemical shifts of the H-2 proton in the diastereoisomeric pair **5** of known configuration. Thus, *anti* configuration was assigned to the isomers with the H-2 proton located upfield and *syn* configuration to the isomers with a downfield location of the same proton.

The diastereoisomeric ratios reported in Table 1 were determined by integration of the appropriate signals in the ^{1}H NMR spectra of the crude reaction products.

With the exception of example **7**, the reactions occured in high yields. When the lithium reagent of benzylcyanide was used (examples **5** and **6**) the diastereoselectivity observed was uniformly low with a slight predominance of the *syn* isomer (*anti*/*syn*=40:60). No detectable effect of temperature or reaction time on the diastereoisomeric excess was found. The change of lithium for a sodium counterion in the case of **5** does not affect the diastereofacial selectivity. With propionitrile (example **8**) the diastereoisomeric ratio at −78 °C changes with time from 40:60 in favor of the *syn* isomer (10 s) to 60:40 in favor of the *anti* isomer (15 min). At 22 °C, prolongation of the reaction time causes a significant decrease in the reaction yields. This process is especially dramatic in the case of **8** where after 60 min the reaction adduct disappears completely, resulting in a complex unresolved mixture.

The lithiated α-naphthylacetonitrile failed to react with benzylideneaniline. This fact is due, in our opinion, to the decreased nucleophilicity of the reagent (better delocalization of the negative charge) compared to the lithiated benzylcyanide. This observation is in agreement with our former study on the reactivity of *N*,*N*-dialkylamides to aromatic imines, where electronic factors were found to be of great importance.[9a]

To understand the origin of the observed diastereoselectivity, we first had to ascertain whether carbanion **4** participates in the stereochemistry forming processes. The formation of **4** seems rather probable if we take into account our previous results[4] and data existing in the literature. Thus, Binev et al. have reported that isomerization of 3-amino-2-butennitrile obtained by base-catalyzed dimerizaion of acetonitrile takes place via delocalized product-like anionic intermediate rather than via the protonated form.[10] A main argument for anion formation was also found in our preliminary trapping experiments with alkyl halides which resulted in isolation of C-2 alkylated products.

To evaluate quantitatively the formation of carbanion **4** we performed D_2O quenching experiments of the reaction mixtures obtained at different temperatures and reaction times. Thus, in the case of propionitrile up to 20% of deuterium was found incorporated at the C-2 position both after 10 s and 60 min at −78 °C. Therefore, we assume that the equilibrium of **3** to **4** in this intermediate is very fast. When benzylcyanide is used, the formation of carbanion **4** must be more important due to its higher H-2 acidity and higher stability (delocalization of negative charge on the phenyl ring) compared to the example with propionitrile Unfortunately, in this case D_2O quenching experiments are not applicable because of overlapping of the signals for H-2, H-3 and NH-protons in the ^{1}H NMR spectra of the diastereoisomeric mixtures.

We then focused our attention on the elucidation of the reaction reversibility. The change of the diastereoisomeric ratio from *anti*/*syn*=40:60 to *anti*/*syn*=60:40 observed with propionitrile within 15 min at −78 °C is an indication that reversible condensation is involved in the formation of the stereochemical results. According to some generalizations made for aldol type reactions[11] the rate of *syn–anti* equilibration increases with decreasing basicity of the enolate moiety. Hence, the lithium metallate of benzylcyanide must equilibrate faster than that of propionitrile. To check the reversibility in this case, we subjected pure **5** (*syn*) to the reaction conditions at −78 °C and found a ratio *anti*/*syn*=40:60 within 2 min. The presence of starting reactants (^{1}H NMR, TLC) indicates that epimerization and reversible condensation are involved in the mechanism of isomerization.

Based on the above considerations we conclude that the stereochemical results in addition of nitrile-stabilized carbanions to Schiff bases are due to participation of carbanion **4**. Thus, although reversible condensation seems rather improbable for very short reaction time (10 s) at −78 °C, the isomer ratios cannot be considered as kinetically controlled in the general sense of an aldol type reaction. Because of the fast **3**–**4** isomerization in the anionic intermediate, the observed diastereoselection will result from superposition between the kinetically controlled *anti*/*syn* ratio from the aldol reaction step, represented by azanion **3**, and that determined by kinetic protonation of the prochiral intermediate **4**. Due to the lack of data for the precise kinetic control of the adduct's configuration, relation of the stereochemical results to any transition state hypothesis seems unhelpful.

By analogy, the stereochemical results at longer reaction times will be obtained from superposition of *anti*/*syn* ratio due to equilibration through azanion **3** and the ratio obtained from the protonation of the carbanion **4**.

The low diastereoselectivity, observed in the condensation of nitriles to aromatic imines is similar to the one discussed in the literature for nitrile aldol reactions.[2h–j] Several years ago Carlier et al. reported the first case of *anti* selective addition of phenylacetonitrile to benzaldehyde at a concentration range below 0.1 M in THF, where lithiated benzylcyanide exists as monomeric tight ion pairs.[12] *Anti*

selectivity is in agreement with a cyclic-6-membered transition state model, while at higher concentrations coordination is hampered, due to the involvement of the lithium reagent in dimeric aggregates.

In an attempt to elucidate the influence of the aggregation state of the lithiated benzyl cyanide on diastereoselectivity, we examined the condensation at a concentration of 0.05 M at −78 °C in the case of **5**. Under these conditions, however, the change in the stereochemical outcome in favour of the *anti* isomer is negligible (*anti*/*syn*=53:47) accompanied by a much lower yield (12%).

Further, we directed our efforts to a thorough study of the subsequent alkylation reaction (Scheme 1, pathway B). As discussed above, isomerization in the anionic intermediate affords prochiral nitrile carbanions with an adjacent stereogenic center, which could be subjected to an alkylation procedure in a one-pot, tandem addition–alkylation reaction. It is worth noting that this finding is a contribution to the methods of generating nitrile anions reported in the literature,[1] which allows creation of two carbon–carbon bonds at the same carbon center and construction of relatively complex molecules, starting from simple materials.

The alkylation of the prochiral intermediates **4** was performed with a variety of electrophiles, including methyl, ethyl, and allyl iodides, benzyl bromide and methyl-2-bromoacetate. To enhance reactivity and to inhibit adduct decomposition, we conducted the reaction at −15 °C for 4 h. The results obtained are collected in Table 2.

The alkylation reaction occurs smoothly, in high to low yields, exceeding substantially the primary concentration of the carbanionic species **4**. Undoubtedly, the driving force of this conversion is the irreversible transformation of **4** to alkylated products. The diastereoisomeric ratios reported in Table 2, are taken from the ^{1}H NMR spectra of the crude reaction mixtures.

To be sure that the observed alkylation diastereoselectivity is not a result of a reversible condensation, we examined the stereochemical course in addition of lithiated α-methylbenzylcyanide to benzilidenaniline, which leads directly to the synthesis of **9**. In all the investigated temperature range of −15 to 22 °C the reaction is non-selective (*anti*/*syn*= 40:60 vs *anti*/*syn* >95 in the tandem procedure). The reaction yield is very low (about 10%), evidently due to steric hindrance in the carbanionic species. This result emphasizes the synthetic utility of our sequential one-pot procedure, where both high yields and high diastereoselectivity were achieved.

A detailed NMR spectroscopy study allows determination of the relative stereochemistry of **9**, obtained as a single isomer (see Fig. 1). Full assignment of the ^{1}H and ^{13}C NMR signals has been carried out. Of particular importance was the discrimination of the individual signals belonging to the three different phenyl rings in the molecule. Both proton and carbon signals have similar chemical shifts, some of them heavily overlapped and of second order. HSQC spectra without decoupling[13] proved to be very useful for assignment of the individual signals. The chemical shifts of the *ortho* protons are 7.50, 7.42 and 6.36 ppm for rings **A**, **B** and **C**, indicating an antiperiplanar position of rings **A** and **B**. One large (7.2 Hz) and two small (1.9 and 3.0 Hz) values for the vicinal proton–carbon couplings around the C2–C3 bond reveal that in $CDCl_3$ solution a single predominant conformer of this penta-substituted ethane is present. As expected, the two smallest substituents-proton H-3 and the cyano group occupy antiperiplanar positions. The close proximity between the CH_3-group and the *ortho* protons of the two phenyl rings **A** and **B**, deduced from the NOESY spectrum, establish unambiguously an *anti* configuration for compound **9**. In the alternative *syn* configuration the

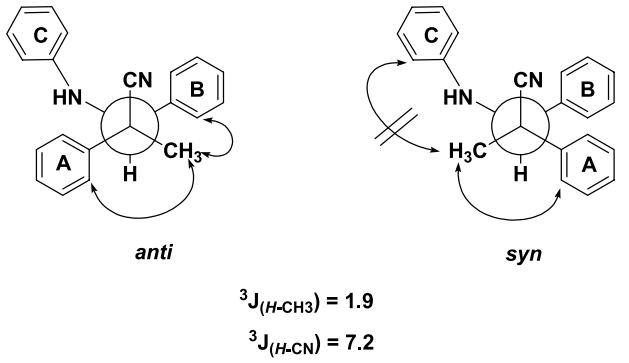


Figure 1. Conformation proof of **9**. $^3J_{CH}$ constants determine predominant conformations for *syn* and *anti* isomers. The arrows indicate important experimental NOEs.

Table 2. Yields and diastereoselectivity by tandem addition–alkylation reactions

Compound	R	R^1	R^{2}X	Yield (%)	*anti*/*syn*
9	Ph	Ph	CH_3I	81	>95[a]
10	Ph	Ph	CH_3CH_2I	75	>95
11	Ph	Ph	$CH_2CH{=}CH_2I$	83	>95
12	Ph	Ph	$C_6H_5CH_2Br$	81	84:16
13[b]	Ph	Ph	$CH_3O_2CCH_2Br$	43[c]	73:27
14	β-naphthyl	Ph	CH_3I	83	>95
15	β-naphthyl	Ph	CH_3CH_2I	72	>95
16	β-naphthyl	Ph	$CH_2CH{=}CH_2I$	77	>95
17	β-naphthyl	Ph	$C_6H_5CH_2Br$	75	83:17
18[b]	β-naphthyl	Ph	$CH_3O_2CCH_2Br$	57	89:11
19	Ph	CH_3	$CH_2CH{=}CH_2I$	51	53:47
20	Ph	CH_3	$C_6H_5CH_2Br$	33	51:49

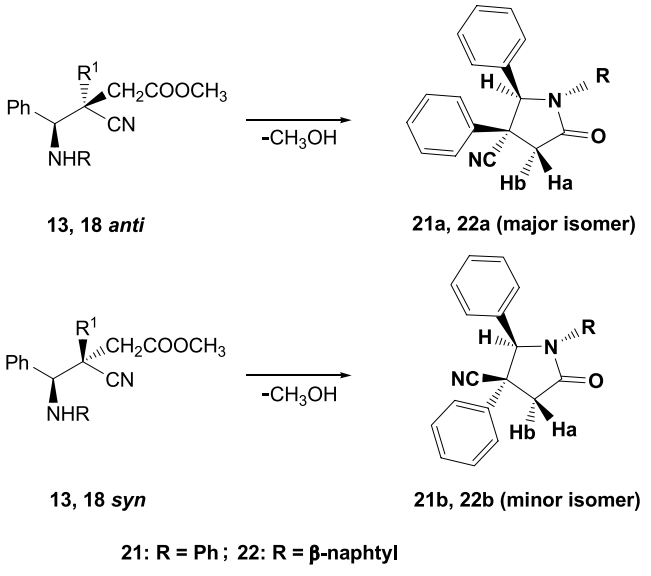
[a] The presence of HMPT does not affect the reaction diastereoselectivity.
[b] Alkylation reaction has been carried out at −70 °C, 5 h.
[c] High % of non-alkylated products has been recovered.

CH_3-group should be close to the *ortho* protons of the phenyl rings **A** and **C**, as shown in Figure 1.

The same *anti* configuration has been established for compounds **10**–**18** by comparison of the ^{1}H NMR spectra. Predominance of the conformers with *anti* disposition of proton H-3 and the cyano group has been proved by selective 1D DEPT spectra for both isomers of **19**. This facilitated configuration elucidation by the use of NOESY-spectra. All aliphatic protons, located in *gauche* position to the carbon-substituted phenyl rings are shielded by 0.3–0.6 ppm, that has been used to distinguish **20** *syn* and **20** *anti*.

When methyl-2-bromoacetate was applied (cases **13** and **18**) the products of alkylation were isolated from the reaction performed at −70 °C for 5 h. At higher temperature (−15 °C) the initially formed aminoesters undergo elimination of methanol, thus furnishing in good yields diastereoisomeric 5-oxo-1,2,3-trisubstituted-pyrrolidine-3-carbonitriles **21** and **22** (Scheme 3).

The cyclization reaction should occur stereospecifically, as *anti* isomers produce derivatives with *cis* relationship of the phenyl groups at the stereogenic centers and *syn* isomers-products with the opposite, *trans* relationship.



Scheme 3.

The NOESY experiments, carried out on the pure major isomer **21a** as well as on a mixture enriched with the minor isomer **21b** proved unambiguously a *cis* location of the phenyl groups in the former product and their *trans* location in the second one. Hence, isomer **21a** derives from the *anti*-configurated precursor while isomer **21b** originates from the *syn*-alkylated one. The good coincidence in the diastereoisomeric ratios of the alkylated adducts and the derived cyclic products evidences that *anti–syn* isomerization does not occur under the reaction conditions. Some characteristic NOESY correlations observed in compound **21** are presented in Figure 2.

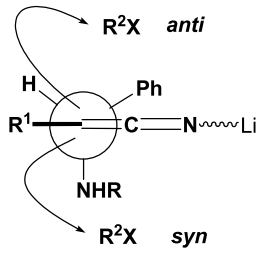


Figure 3. Transition structure for the alkylation reaction.

The relative configurations in the diastereoisomeric pair **22** were assigned using the observed similarities between the H-2 chemical shifts with **21** of known configuration.

The diastereoselectivity in alkylation varies from outstanding to high and poor. Analysis of the data, presented in Table 2 implies two general trends: (1) dependence of the diastereofacial selectivity on the substituent R^1, attached to the prochiral center of the nitrile anion and (2) dependence on the electrophile used.

When R^1 is a phenyl group, alkylation occurs with complete to excellent *anti* diastereoselection. Thus, with methyl, ethyl, and allyl iodides (cases **9**–**11** and **14**–**16**) only a single isomer is apparent in the ^{1}H NMR spectra of the crude reaction products, while with benzyl bromide and methyl-2-bromoacetate the diastereoselectivity level decreases (examples **12**, **13**, **17**, **18**). Alternatively, when R^1 is a methyl group, the diastereoselectivity is low regardless of the alkylating agent applied.

To rationalize the results obtained, we first examined the influence of highly ionizing conditions (excess of HMPT as additive) on the stereochemistry of methylation in the case

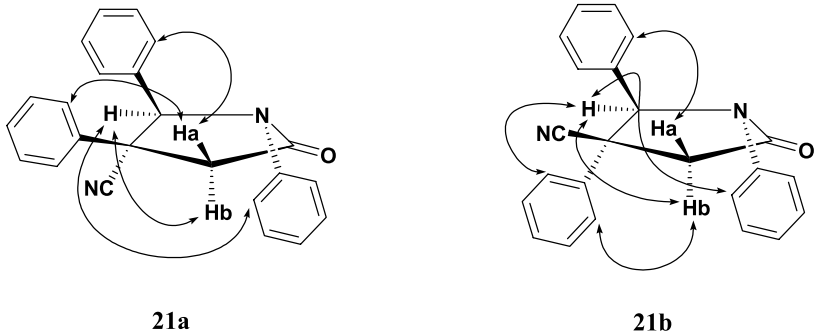


Figure 2. Configurations of **21a** and **21b**. The arrows indicate the important effects in NOE experiments.

of **9**. The lack of variation in the stereochemical ratio compared to pure THF is evidence that the alkylation reaction occurs through an open-chain rather than chelated transition state.

The diastereoselectivity course of electrophilic attack on a trigonal carbon adjacent to a stereogenic center in open-chain systems was predicted by Houk's theoretical model,[14] which has been later confirmed by numerous experimental data.[15]

To interpret the alkylation diastereoselectivity we consider the open-chain transition state model, depicted in Figure 3. An analogous transition state has been previously proposed by Fleming as an alternative to Houk's model for methylation of nitrile derived carbanions.[15c] This transition conformation must be strongly favored, due to the lack of allylic 1,3 strain because of the small steric volume of the cyano group.

As noted above, diastereoselectivity in alkylation dramatically depends on the substituent R^1. This effect can be referred to the electronic structure of the nitrile anions. According to the literature, anions derived from α-aromatic nitriles are 'softer' nucleophiles (with more delocalized negative charge) compared to alkane nitrile anions.[16] Consequently, their reactions will proceed through later, product-like transition states. This difference seems to be of crucial importance in stereoselective nitrile anion alkylations.

Thus, with benzylcyanide derived carbanions (cases **9**–**18**) the transition state would be expected to be product-like. In these cases the electrophilic attack will take place from the less sterically shielded π-face of the carbanionic species, thus furnishing *anti*-alkylated products with an outstanding level of diastereofacial selectivity. The inferior diastereoselectivity with benzyl bromide and methyl-2-bromoacetate (cases **12**, **13**, **17**, **18**) could be explained with their higher reactivity and hence relatively earlier transition state that makes possible a small proportion of *syn* attack. In the case of propionitrile derived anion (cases **19** and **20**) the transition state is supposed to be reactant-like, thus diminishing the influence of the steric control elements. Electrophilic attacks from both π-faces of the carbanionic intermediate in these cases results in strong decrease of diastereofacial selectivity.

3. Conclusion

The stereochemical course in addition of lithiated benzylcyanide and propionitrile to aromatic Schiff bases has been investigated and the origin of the stereochemical results has been discussed. Carbanionic intermediates with an adjacent stereogenic center, formed as a result of fast isomerization of the initially formed azanions, has been proved to be involved in the formation of the stereochemical ratios. Based on this isomerization, a convenient one-pot tandem addition–alkylation reaction, which allows formation of two carbon–carbon bonds at the same stereogenic center has been developed. It has been demonstrated, that appropriate selection of the starting reagents results in an outstanding level of alkylation diastereoselectivity. Stereochemical results have been interpreted on the assumption of open-chain, reactant-like or product-like transition state hypothesis.

The paper contributes to elucidation of the strereochemical course in condensation of nitrile-stabilized carbanions with azomethinic compounds–a poorly investigated area. The developed tandem addition—alkylation procedure has definite synthetic utility, giving ready access to relatively complex molecules with two chiral centers, in some of cases with outstanding level of diastereoselection.

4. Experimental

All reactions involving lithium derivatives were carried out under dry argon atmosphere using oven-dried flasks, equipped with a rubber septum for introduction of the reagents by a syringe. THF was distilled under argon from $LiAlH_4$ prior to use. HMPT was distilled on CaH_2 and kept over molecular sieves (13×). LDA was prepared before use from *n*-BuLi (1.6 M in hexane, Fluka) and diisopropylamine. Other starting materials were obtained from commercial suppliers and were used without further purification.

Flash column chromatography was performed using silica gel (Merck, 0.040–0.063 mm). Analytical TLC was carried out on Merck silica gel plates 60F-254. Melting points were determined using a Kofler apparatus and uncorrected values were presented. IR spectra were recorded on a Bruker FTR-113 V spectrometer using KBr pallets and only partial data are reported. Mass spectra were done on Hewlett Packard 5973 mass spectrometer. ^1H and ^{13}C NMR spectra were recorded at room temperature on a Bruker DRX-250 spectrometer operating at 250.13 MHz for ^1H and 62.89 MHz for ^{13}C. Chemical shifts are reported in δ with TMS as internal standard, coupling constants are given in Hz. The *anti*/*syn* ratios in all cases studied were determined by integration of appropriate multiplets in the ^1H NMR spectra of the crude reaction products for protons having sufficiently different chemical shifts. Assignment of the NMR signals has been achieved on the base of the multiplicities of the signals and the values of the vicinal coupling constants. Where necessary for the elucidation of the stereochemistry (compounds **9**, **19** and **21**), full assignment of the proton and carbon signals has been done by the aid of 2D HSQC with and without decoupling and NOESY spectra. Selective 1D and 2D DEPT spectra have been used for check of the through-bond carbon–proton connectivities and estimation or measurement of the values of the carbon–proton coupling constants.[17]

4.1. Condensation reaction. General experimental procedure

To a stirred solution of BuLi (0.69 mL, 1.1 mmol, 1.6 M in hexane) in THF (1 mL) diisopropylamine (0.15 mL, 1.1 mmol) was added at room temperature. Then the reaction mixture was cooled to −78 °C and the corresponding nitrile (1 mmol) dissolved in dry THF (1 mL) was introduced drop-wise via syringe. After 30 min the reaction

mixture was allowed to reach the desired temperature (if necessary) and the corresponding azometinic compound (1 mmol) dissolved in dry THF (1 mL) was added. Stirring was continued for the required reaction time and then the reaction was quenched with saturated aqueous NH_4Cl solution (3 mL). The THF was removed under reduced pressure and the residue was extracted with methilene-chloride (2×5 mL). The combined organic layers were dried ($MgSO_4$), filtered and the solvent was removed in vacuum. The reaction yields given in Table 1 concern diastereoisomeric *anti*/*syn* mixtures and were determined after purification of the crude products by flash chromatography.

4.1.1. 2,3-Diphenyl-3-phenylamino-propionitrile 5 (*anti*). Following the general experimental procedure from the reaction mixture obtained at −40 °C for 30 min the title compound **5** (*anti*) (72 mg, 24%) was isolated after preparative thin-layer chromatography separation procedure (Kieselgel 60 PF_{254}, Et_2O/hexane=1/5, twofold elution) and subsequent recrystallisation (EtOH). White crystals, mp 140–142 °C; [Found: C, 84.70; H, 6.25; N, 9.01. $C_{21}H_{18}N_2$ requires C, 84.53; H, 6.08; N, 9.39]; MS (EI, 70 eV) *m*/*z* 298 (M^+); R_f (Et_2O/hexane=1:2) 0.29; ν_{max} (KBr) 3413 (NH), 2245 (CN) cm^{-1}; δ_H (250 MHz, $CDCl_3$) 4.26 (d, 1H, *J*=5.0 Hz, H-2), 4.32 (d, 1H, *J*=7.0 Hz, NH), 4.82 (dd, 1H, *J*=7.0, 5.0 Hz, H-3), 6.49–7.32 (m, 15H, 3×C_6H_5); δ_C (62.9 MHz, $CDCl_3$, DEPT) 45.3 (C-2), 61.2 (C-3), 118.8 (CN), 114.1, 118.7, 126.8, 128.3, 128.4, 128.6, 128.7, 128.9, 129.2, (C_{arom}), 132.2, 138.5, 145.0 (C_{quat}).

4.1.2. 2,3-Diphenyl-3-phenylamino-propionitrile 5 (*syn*). From the described above separation procedure the title compound was isolated as a white solid. Recrystallisation (EtOH) afforded **5** (*syn*) (83 mg, 28%) as white crystals, mp 110–112 °C; [Found: C, 84.48; H, 5.93; N, 9.21. $C_{21}H_{18}N_2$ requires C, 84.53; H, 6.08; N, 9.39]; MS (EI, 70 eV) *m*/*z* 298 (M^+); R_f (Et_2O/hexane=1:2) 0.31; ν_{max} (KBr) 3407 (NH), 2248 (CN) cm^{-1}; δ_H (250 MHz, $CDCl_3$) 4.35 (d, 1H, *J*=7.2 Hz, NH), 4.42 (d, 1H, *J*=5.4 Hz, H-2), 4.77 (dd, 1H, *J*=7.2, 5.4 Hz, H-3), 6.57–7.30 (m, 15H, 3×C_6H_5); δ_C (62.9 MHz, $CDCl_3$, DEPT) 44.8 (C-2), 61.3 (C-3), 118.6 (CN), 114.0, 118.8, 127.3, 128.3, 128.4, 128.6, 128.8, 129.3 (C_{arom}), 131.9, 137.2, 145.6 (C_{quat}).

4.1.3. 3-(Naphthalen-2-ylamino)-2,3-diphenyl-propionitrile-6 (*anti*). Following the general experimental procedure from the reaction mixture obtained at −40 °C for 30 min the title compound **6** (*anti*) (107 mg, 31%) was isolated after preparative thin-layer chromatography (Kieselgel 60 PF_{254}, Et_2O/hexane=1/4, twofold eluation). Viscous oil; [Found: C, 85.70; H, 5.42; N, 7.82. $C_{25}H_{20}N_2$ requires C, 86.18; H, 5.79; N, 8.04]; MS (EI, 70 eV) *m*/*z* 348 (M^+); R_f (Et_2O/hexane=1:2) 0.30; ν_{max} (KBr) 3383 (NH), 2244 (CN) cm^{-1}; δ_H (250 MHz, $CDCl_3$) 4.37 (d, 1H, *J*=4.8 Hz, H-2), 4.51 (br d, 1H, NH), 5.01 (d, 1H, *J*=4.8 Hz, H-3), 6.66–7.70 (m, 17H, Ar*H*); δ_C (62.9 MHz, $CDCl_3$, DEPT) 44.9 (C-2), 61.1 (C-3), 118.7 (CN), 106.8, 118.1, 122.6, 126.1, 126.4, 126.9, 127.5, 128.3, 128.4, 128.6, 128.8, 128.9, 129.1 (C_{arom}), 127.9, 132.1, 134.6, 138.2, 143.4 (C_{quat}).

4.1.4. 3-(Naphthalen-2-ylamino)-2,3-diphenyl-propionitrile-6 (*syn*). The title compound **6** (*syn*) was isolated from the above described separation procedure as a viscous oil (92 mg, 26%). Recrystallisation (Et_2O) afforded **6** (*syn*) as white crystals, mp 139–141 °C; [Found: C, 85.93; H, 5.56; N, 7.90. $C_{25}H_{20}N_2$ requires C, 86.18; H, 5.79; N, 8.04]; MS (EI, 70 eV) *m*/*z* 348 (M^+); R_f (Et_2O/hexane=1:2) 0.36; ν_{max} (KBr), 3379 (NH), 2243 (CN) cm^{-1}; δ_H (250 MHz, $CDCl_3$) 4.53 (d, 2H, *J*=5.3 Hz, H-2+ NH), 4.92 (d, 1H, *J*=5.3 Hz, H-3), 6.75–7.33 (m, 17H, Ar*H*); δ_C (62.9 MHz, $CDCl_3$, DEPT) 44.6 (C-2), 61.3 (C-3), 118.7 (CN), 106.7, 118.2, 122.7, 126.1, 126.4, 127.4, 127.6, 128.3, 128.5, 128.7, 128.9, 129.2 (C_{arom}), 128.0, 131.8, 134.6, 137.0, 143.0 (C_{quat}).

4.1.5. 2-Methyl-3-phenyl-3-(phenylamino)-propanenitriles 8 (*anti*) and 8 (*syn*). Following the general experimental procedure from the synthesis conducted at −40 °C for 30 min the titled compounds (184 mg, 78%) were isolated by column chromatography (silica gel, Et_2O/hexane=1:2) as diastereoisomeric mixture. Colorless oil; [Found: C, 81.53; H, 6.56; N, 11.60. $C_{16}H_{16}N_2$ requires C, 81.32; H, 6.82; N, 11.85]; MS (EI, 70 eV) *m*/*z* 236 (M^+); R_f (Et_2O/hexane=1:2) 0.26; ν_{max} (KBr), 3377 (NH), 2241 (CN) cm^{-1}; ^1H NMR chemical shifts for **8** (*anti*) and **8** (*syn*) are taken from the spectrum of the diastereoisomeric mixture purified as described above.

Compound **8** (*anti*). δ_H (250 MHz, $CDCl_3$) 1.43 (d, 3H, *J*=7.2 Hz, CH_3), 3.08 (m, 1H, H-2), 4.42 (br d, 1H, NH), 4.55 (dd, 1H, *J*=7.2, 4.8 Hz, H-3), 6.58–7.42 (m, 10H, 2×C_6H_5).

Compound **8** (*syn*). δ_H (250 MHz, $CDCl_3$) 1.29 (d, 3H, *J*=7.2 Hz, CH_3), 3.27 (m, 1H, H-2), 4.42 (br d, 1H, NH), 4.53 (dd, 1H, *J*=7.2, 4.9 Hz, H-3), 6.58–7.42 (m, 10H, 2×C_6H_5).

4.2. Proof of the relative configuration of 5 (*anti*)

The relative configuration of **5** (*anti*) was determined by the chemical correlation presented in Scheme 2. Thus, the higher melting isomer of **5** (mp 140–142 °C) (298 mg, 1 mmol) was dissolved in concd H_2SO_4 (4.5 mL). The reaction mixture was then kept at −15 °C for four days. The hydrolysis at higher temperature (0 °C or room temperature) is accompanied by isomerization of the staring product (TLC analysis). Further, the reaction mixture was poured into ice-cold water, neutralized with solid Na_2CO_3 (pH 7) and extracted with methylenechloride (4×8 mL). The combined organic layer was dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. Flash chromatography of the crude product (silica gel, Et_2O) afforded 2,3-diphenyl-3-(phenylamino)-propanamide (224 mg, 71%) as a white solid.

To a stirred suspension of sodium hydride (50 mg, 2.1 mmol) in dry THF (3 mL) under argon atmosphere the solution of the isolated amide (224 mg, 0.7 mmol) in dry THF (10 mL) was introduced via syringe at room temperature. The reaction was kept at stirring for 30 min and CH_3I (298 mg, 0.19 mL, 2.1 mmol) was added. After 2 h the reaction was quenched with saturated aqueous NH_4Cl solution (4 mL). Standard work-up procedure follows, including THF removal under vacuum, extraction with

methylenechloride (3×5 mL), drying and evaporation of the solvent. The pure *N*,*N*-dimethyl-2,3-diphenyl-3-(phenylamino)-propanamide (229 mg, 94%) was isolated by flash chromatography (silica gel, Et_2O/hexane=1:1) as a white solid, mp 183–185 °C. The ^1H NMR spectrum of the isolated product is identical with that of previously obtained *anti N*,*N*-dimethyl-2,3-diphenyl-3-(phenylamino)-propanamide.[9]

4.3. General procedure for the alkylation reaction

To a solution of the desired metal intermediate prepared from 1 mmol starting products at −40 °C for 30 min as described in Section 4.1 a solution of the corresponding alkyl halide (1.5 mmol) dissolved in THF (1 mL) was introduced via syringe. Then the reaction mixture was warmed to −15 °C and was kept at this temperature for 4 h. When methyl-2-bromoacetate was used (cases **13** and **18**), to avoid the subsequent cyclization reaction the alkylation was performed at −70 °C for 5 h. After addition of saturated aqueous NH_4Cl solution (4 mL), THF was distilled in vacuum and the residue was extracted with methylenechloride (3×5 mL). The combined organic solution was dried ($MgSO_4$), the solvent was removed under reduced pressure and the product was further purified as described below. Yields, given in Table 2 are determined at the stage of flash chromatography purification.

4.3.1. 2-Methyl-2,3-diphenyl-3-phenylamino-propionitrile 9 (*anti*). Following the general experimental procedure, from the metal intermediate of **5** prepared from 1 mmol starting products and CH_3I (213 mg, 1.5 mmol) the title compound (255 mg, 81%) was isolated after flash chromatography (silica gel, Et_2O/hexane=1:4) as a white solid. Recrystallization (EtOH) afforded **9** (*anti*) (130 mg, 42%) as white crystals, mp 116–118 °C; [Found: C, 84.69; H, 6.25; N, 8.62. $C_{22}H_{20}N_2$ requires C, 84.58; H, 6.45; N, 8.97]; MS (EI, 70 eV) *m*/*z* 312 (M^+); R_f (Et_2O/hexane=1:4) 0.36; ν_{max} (KBr) 3365 (NH), 2243 (CN) cm^{-1}; δ_H (250 MHz, $CDCl_3$) 1.61 (s, 3H, CH_3), 4.13 (d, 1H, *J*=5.8 Hz, NH), 4.60 (d, 1H, *J*=5.8 Hz, H-3), 6.35–7.5 (m, 15H, 3×C_6H_5); δ_C (62.9 MHz, $CDCl_3$, DEPT) 25.5 (*C*H_3), 48.6 (C-2), 65.4 (C-3), 121.4 (CN), 114.0 (*ortho*-ring C), 118.3 (*para*-ring C), 126.1 (*ortho*-ring A), 128.3 (*ortho*-ring B), 128.37 (*meta*-ring A), 128.39 (*para*-ring A), 128.42 (*para*-ring B), 128.9 (*meta*-ring C), 129.0 (*meta*-ring B), 137.7, 137.9 (*ipso*-ring A, B), 146.2 (*ipso*-ring C).

4.3.2. 2-Phenyl-2-(phenyl-phenylamino-methyl)-butyronitrile 10 (*anti*). Analogously, starting from the metal intermediate of **5** prepared from 1 mmol starting products and C_2H_5I (232 mg, 1.5 mmol) the title compound (244 mg, 75%) was isolated after purification of the crude reaction product by flash chromatography (silica gel, Et_2O/hexane=1:7) as a viscous oil. Recrystallization (hexane) afforded **10** (*anti*) (107 mg, 33%) as white crystals, mp 53–55 °C; [Found: C, 84.38; H, 6.28; N, 8.82. $C_{23}H_{22}N_2$ requires C, 84.58; H, 6.45; N, 8.97]; MS (EI, 70 eV) *m*/*z* 326 (M^+); R_f (Et_2O/hexane=1:7) 0.31; ν_{max} (KBr) 3393 (NH), 2235 (CN) cm^{-1}; δ_H (250 MHz, $CDCl_3$) 0.78 (t, 3H, *J*=7.3 Hz, H-4), 1.85 (m, 1H, H_a-3), 2.04 (m, 1H, H_b-3), 4.09 (d, 1H, *J*=5.4 Hz, NH), 4.62 (d, *J*=5.4 Hz, C*H*–C_6H_5), 6.32–7.68 (m, 15H, 3×C_6H_5); δ_C (62.9 MHz, $CDCl_3$, DEPT) 9.4 (C-4), 30.8 (C-3), 55.4 (C-2), 65.5 (*C*H–C_6H_5), 120.2 (CN), 114.0, 118.3, 126.7, 128.5, 128.9, 129.1 (C_{arom}), 135.6, 138.1, 146.3 (C_{quat}).

4.3.3. 2-Phenyl-2-(phenyl-phenylamino-methyl)-pent-4-enenitrile 11 (*anti*). According to the general experimental procedure, from the metal intermediate of **5** prepared from 1 mmol starting products and allyl iodide (252 mg, 1.5 mmol) the title compound (280 mg, 83%) was isolated after flash chromatography (silica gel, Et_2O/hexane=1:7) as a white solid. Recrystallization (EtOH) afforded **11** (*anti*) (179 mg, 53%) as white crystals, mp 139–141 °C; [Found: C, 85.38; H, 6.75; N, 8.34. $C_{22}H_{20}N_2$ requires C, 85.17; H, 6.55; N, 8.28]; MS (EI, 70 eV) *m*/*z* 338 (M^+); R_f (Et_2O/hexane=1:7) 0.33; ν_{max} (KBr) 3383 (NH), 2241 (CN) cm^{-1}; δ_H (250 MHz, $CDCl_3$) 2.64 (ddt, 1H, *J*=14.0, 7.5, 1.0 Hz, H_a-3), 2.84 (ddt, 1H, *J*=14.0, 6.6, 1.1 Hz, H_b-3), 4.14 (d, 1H, *J*=6.5 Hz, NH), 4.75 (d, 1H, *J*=6.5 Hz, C*H*–C_6H_5), 5.06 (d, 1H, *J*=16.1 Hz, H_{trans}-5), 5.08 (d, 1H, *J*=11.2 Hz, H_{cis}-5), 5.47–5.57 (m, 1H, H-4), 6.41–7.51 (m, 15H, 3×C_6H_5); δ_C (62.9 MHz, $CDCl_3$, DEPT, HMQC) 41.5 (C-3), 54.1 (C-2), 64.6 (*C*H–C_6H_5), 118.4 (CN), 120.4 (C-5), 131.1 (C-4), 114.1, 114.2, 120.1, 127.0, 128.4, 128.5, 128.6, 128.9, 129.0 (C_{arom}), 135.1, 137.6, 146.1 (C_{quat}).

4.3.4. 2-Benzyl-2,3-diphenyl-3-phenylamino-propionitrile 12 (*anti*). From the metal intermediate of **5** obtained from 1 mmol starting products and benzyl bromide (256 mg, 1.5 mmol) the title compound (314 mg, 81%) was isolated after purification of the crude reaction product by flash chromatography (silica gel, Et_2O/hexane=1:7) as a white solid. Recrystallization (EtOH) yielded **12** (*anti*) (197 mg, 51%) as white crystals, mp 164–166 °C; [Found: C, 86.28; H, 6.08; N, 7.02. $C_{28}H_{24}N_2$ requires C, 86.56; H, 6.23; N 7.33]; MS (EI, 70 eV) *m*/*z* 388 (M^+); R_f (Et_2O/hexane=1:7) 0.29; ν_{max} (KBr) 33783 (NH), 2239 (CN) cm^{-1}; δ_H (250 MHz, $CDCl_3$) 3.15 (d, 1H, *J*=13.3 Hz, H_a-3), 3.22 (d, 1H, *J*=13.3 Hz, H_b-3), 4.10 (d, 1H, *J*=6.0 Hz, NH), 4.86 (d, 1H, *J*=6.0 Hz, C*H*–C_6H_5), 6.35–7.56 (m, 20H, 4×C_6H_5); δ_C (62.9 MHz, $CDCl_3$, DEPT) 43.6 (C-3), 55.8 (C-2), 64.0 (*C*H- C_6H_5), 119.8 (CN), 114.1, 118.4, 127.2, 127.9, 128.5, 128.6, 128.7, 128.9, 129.0, 130.3 (C_{arom}), 134.3, 135.0, 137.7, 146.0, (C_{quat}).

4.3.5. Methyl-3-cyano-3,4-diphenyl-4-(phenylamino)-butanoate 13 (*anti*). From the metal intermediate of **5** prepared from 1 mmol starting products and methyl-2-bromoacetate (230 mg, 1.5 mmol) at −70 °C for 5 h the title compound **13** (159 mg, 43%) was isolated by flash chromatography (silica gel, Et_2O/hexane=1:3) as a diastereoisomeric mixture (*anti*/*syn*=73/27). Significant quantity of unreacted **5** (64 mg, 22%) has been recovered. Following recrystallization (Et_2O/hexane) afforded **13** (*anti*) (76 mg, 20%) as white crystals, mp 164–166 °C; [Found: C, 78.01; H, 6.15; N, 7.34. $C_{24}H_{22}N_2O_2$ requires C, 77.81; H, 5.99; N, 5.56]; MS (EI, 70 eV) *m*/*z* 370 (M^+); R_f (Et_2O/hexane=1:2) 0.41; ν_{max} (KBr) 3403 (NH), 2250 (CN), 1743 ($COOCH_3$) cm^{-1}; δ_H (250 MHz, $CDCl_3$) 2.89 (d, 1H, *J*=14.4 Hz, H_a-2), 3.14 (d, 1H, *J*=14.4 Hz, H_b-2), 3.53 (s, 3H, $COOCH_3$), 4.13 (br d, 1H, NH), 4.92 (s, br d, 1H,H-4), 6.42–7.51 (m,15H, 3×C_6H_5); δ_C (62.9 MHz, $CDCl_3$, DEPT) 41.5 (C-2), 50.6 (C-3), 52.0 (COO*C*H_3), 64.2 (C-4), 119.9 (*C*N), 114.2, 118.8, 126.7, 128.4, 128.5,

128.6, 128.7, 128.9 (C_{arom}), 134.8, 137.1, 146.0 (C_{quat}), 168.9 (CO).

4.3.6. Methyl-3-cyano-3,4-diphenyl-4-(phenylamino)-butanoate 13 (*syn*). R_f (Et_2O/hexane = 1:2) 0.41; The ^1H NMR chemical shifts are taken from the spectrum of diastereoisomeric mixture, obtained by the above described flash column chromatography purification procedure. δ_H (250 MHz, $CDCl_3$) 3.27 (d, 1H, J = 16.6 Hz, H_a-2), 3.57 (s, 3H, $COOCH_3$), 3.66 (d, 1H, J = 16.6 Hz, H_b-2), 4.65 (d, 1H, J = 10.1 Hz, NH), 4.82 (d, 1H, J = 10.1 Hz, H-4).

4.3.7. 2-Methyl-3-(naphthalen-2-ylamino)-2,3-diphenyl-propionitrile 14 (*anti*). Following the general experimental procedure, from the metal intermediate of **6** prepared from 1 mmol starting products and CH_3I (213 mg, 1.5 mmol) the title compound (300 mg, 83%) was isolated after flash chromatography (silica gel, Et_2O/hexane = 1:4) as a white solid. Recrystallization (EtOH) afforded **14** (*anti*) (170 mg, 47%) as white crystals, mp 148–150 °C; [Found: C, 85.84; H, 5.84; N 7.51. $C_{26}H_{22}N_2$ requires C, 86.15; H 6.12; N 7.73]; MS (EI, 70 eV) m/z 362 (M^+); R_f (Et_2O/hexane = 1:5) 0.20; ν_{max} (KBr) 3397 (NH), 2237 (CN) cm^{-1}; δ_H (250 MHz, $CDCl_3$) 1.67 (s, 3H, CH_3), 4.30 (s, br d, 1H, NH), 4.74 (s, 1H, H-3), 6.41–7.58 (m, 17H, Ar*H*); δ_C (62.9 MHz, $CDCl_3$, DEPT) 25.6 (CH_3), 48.6 (C-2), 65.3 (C-3), 121.4 (CN), 106.7, 118.1, 122.4, 126.0, 126.1, 127.4, 128.3, 128.5, 128.7, 129.1 (C_{arom}), 127.7, 134.4, 137.5, 137.9, 143.7 (C_{quat}).

4.3.8. 2-[(Naphthalen-2-ylamino)-phenyl-methyl]-2-phenylbutyronitrile 15 (*anti*). According to the general experimental procedure, from the metal intermediate of **6** prepared from 1 mmol starting products and C_2H_5I (232 mg, 1.5 mmol) the title compound (270 mg, 72%) was isolated after flash chromatography (silica gel, Et_2O/hexane = 1:4) as a viscous oil. Recrystallization (EtOH) afforded **15** (*anti*) (124 mg, 33%) as white crystals, mp 150–152 °C; [Found: C, 85.97; H, 6.22; N, 7.28. $C_{27}H_{24}N_2$ requires C, 86.13; H 6.43; N 7.44]; MS (EI, 70 eV) m/z 376 (M^+); R_f (Et_2O/hexane = 1:5) 0.24; ν_{max} (KBr) 3391 (NH), 2235 (CN) cm^{-1}; δ_H (250 MHz, $CDCl_3$) 0.79 (t, 3H, J = 7.3 Hz, H-4), 1.88 (dq, 1H, J = 14.6, 7.3 Hz, 3-H_a), 2.07 (dq, 1H, J = 14.6, 7.3 Hz, 3-H_b), 4.26 (d, 1H, J = 5.6 Hz, NH), 4.75 (d, 1H, J = 5.6 Hz, C*H*–C_6H_5), 6.49–7.56 (m, 17H, Ar*H*); δ_C (62.9 MHz, $CDCl_3$, DEPT) 9.4 (C-4), 30.8 (C-3), 55.4 (C-2), 65.5 (*C*H–C_6H_5), 120.2 (CN), 106.7, 118.1, 122.4, 122.5, 126.0, 126.2, 127.0, 127.4, 128.5, 128.6, 128.7, 129.0 (C_{arom}), 127.7, 134.5, 135.7, 137.9, 143.9 (C_{quat}).

4.3.9. 2-[(Naphthalen-2-ylamino)-phenyl-methyl]-2-phenyl-pent-4-enenitrile 16 (*anti*). Analogously, from the metal intermediate of **6** prepared from 1 mmol starting products and allyl iodide (252 mg, 1.5 mmol) the title compound (298 mg, 77%) was isolated after flash chromatography (silica gel, Et_2O/hexane = 1:4) as a viscous oil. Recrystallization (EtOH) afforded **16** (*anti*) (120 mg, 31%) as white crystals, mp 154–156 °C; [Found: C, 86.27; H, 6.45; N, 7.08. $C_{28}H_{24}N_2$ requires C, 86.56; H 6.23; N 7.21]; MS (EI, 70 eV) m/z 388 (M^+); R_f (Et_2O/hexane = 1:5) 0.22; ν_{max} (KBr) 3405 (NH), 2234 (CN) cm^{-1}; δ_H (250 MHz, $CDCl_3$) 2.61 (dd, 1H, J = 13.9, 6.6 Hz, H_a-3), 2.81 (dd, 1H, J = 13.9, 6.6 Hz, H_b-3), 4.26 (d, 1H, J = 5.9 Hz, NH), 4.82 (d, 1H, J = 5.9 Hz, C*H*–C_6H_5), 5.01 (ddt, 1H, J = 16.9, 1.5 Hz, H-5$_{trans}$), 5.02 (ddt, 1H, J = 11.7, 1.5 Hz, H-5$_{cis}$), 5.45 (m, 1H, H-4), 6.52–7.49 (m, 17H, Ar*H*); δ_C (62.9 MHz, $CDCl_3$, DEPT) 41.6 (C-3), 54.2 (C-2), 64.6 (*C*H–C_6H_5), 120.1 (CN), 120.5 (C-5), 131.1 (C-4), 106.8, 118.1, 122.5, 126.0, 126.2, 127.0.127.4, 128.4, 128.5, 128.6, 128.7, 129.0 (C_{arom}), 127.7, 134.5, 135.1, 137.4, 143.7 (C_{quat}).

4.3.10. 2-Benzyl-3-(naphthalen-2-ylamino)-2,3-diphenyl-propionitrile 17 (*anti*). Using the general experimental procedure, from the metal intermediate of **6** prepared from 1 mmol starting products and benzyl bromide (256 mg, 1.5 mmol) the title compound (328 mg, 75%) was isolated after flash chromatography (silica gel, Et_2O/hexane = 1:4) as a white solid. Recrystallization (EtOH) yielded **17** (*anti*) (170 mg, 39%) as white crystals, mp 224–225 °C; [Found: C, 87.35; H, 5.73; N, 6.54. $C_{32}H_{26}N_2$ requires C, 87.64; H 5.98; N 6.39]; MS (EI, 70 eV) m/z 438 (M^+); R_f (Et_2O/hexane = 1:5) 0.22; ν_{max} (KBr) 3409 (NH), 2239 (CN) cm^{-1}; δ_H (250 MHz, DMSO) 3.18 (d, 1H, J = 13.2 Hz, CH_aH$_b$–C_6H_5), 3.27 (d, 1H, J = 13.2 Hz, CH$_a$$H_b$–$C_6H_5$), 4.28 (d, 1H, J = 6.4 Hz, NH), 4.99 (d, 1H, J = 6.4 Hz, H-3), 6.5–7.56 (m, 22H, Ar*H*); δ_C (62.9 MHz, DMSO, DEPT) 43.1 (*C*H$_2$–C_6H_5), 55.5 (C-2), 63.1 (C-3), 120.2 (CN), 104.7, 118.7, 121.6, 125.5, 126.0, 126.1, 126.9, 127.3, 127.8, 128.2, 128.3, 129.1, 130.1 (C_{arom}), 127.8, 134.6, 135.2, 136.2, 139.1, 145.0 (C_{quat}).

4.3.11. Methyl-3-cyano-4-(naphtalen-2-ylamino)-3,4-diphenylbutanoate 18 (*anti*). Using the general experimental procedure the title compound **18** was obtained from the metal intermediate of **6** prepared from 1 mmol starting products and methyl-2-bromoacetate (230 mg, 1.5 mmol) at −70 °C for 5 h. Flash chromatography (silica gel, Et_2O/hexane = 1:2) afforded **18** (239 mg, 57%) as diastereoisomeric mixture (*anti*/*syn* = 89/11). Unreacted **6** (84 mg, 25%) has been recovered. Two recrystallizations (Et_2O) yielded **18** (*anti*) (96 mg, 23%) as white crystals, mp 170–172 °C; [Found: C, 79.71; H, 5.49; N, 6.39. $C_{28}H_{24}N_2O_2$ requires C, 79.98; H, 5.75; N, 6.66]; MS (EI, 70 eV) m/z 420 (M^+); R_f (Et_2O/hexane = 1:1) 0.34; ν_{max} (KBr) 3410 (NH), 2238 (CN), 1739 ($COOCH_3$) cm^{-1}; δ_H (250 MHz, $CDCl_3$) 3.03 (d, 1H, J = 16.2 Hz, H_a-2), 3.18 (d, 1H, J = 16.2 Hz, H_b-2), 3.54 (s, 3H, $COOCH_3$), 3.83 (br d, 1H, NH), 5.08 (s, 1H, H-4), 6.56–7.56 (m, 17H, Ar*H*); δ_C (62.9 MHz, $CDCl_3$, DEPT) 41.5 (C-2), 50.6 (C-3), 52.0 ($COOCH_3$), 64.1 (C-4), 119.9 (*C*N), 107.0, 118.1, 122.6, 126.0, 126.3, 126.7, 127.4, 128.4, 128.6, 128.8, 129.0 (C_{arom}), 127.9, 134.5, 134.8, 136.9, 143.5 (C_{quat}), 168.9 (C=O).

4.3.12. Methyl-3-cyano-4-(naphtalen-2-ylamino)-3,4-diphenylbutanoate 18 (*syn*). R_f (Et_2O/hexane = 1:1) 0.34; The title compound has not been isolated. ^1H NMR chemical shifts are taken from the spectrum of the diastereoisomeric mixture obtained as described above. δ_H (250 MHz, $CDCl_3$) 3.58 (s, 3H, $COOCH_3$), 4.95 (s, 1H, H-4).

4.3.13. 2-Methyl-2-(phenylamino-methyl)-pent-4-enenitrile 19 (*anti*). Using the general experimental procedure, from the metal intermediate of **8** prepared from 1 mmol starting products and allyl iodide (252 mg, 1.5 mmol) the title compound **19** (*anti*) (74 mg, 27%) was isolated after

preparative thin-layer chromatography (Kieselgel 60 PF_{254}, Et_2O/hexane = 1:3, twofold eluation) as a viscous colorless oil; [Found: C, 82.38; H, 7.03; N, 9.94. $C_{19}H_{20}N_2$ requires C, 82.57; H, 7.29; N 10.14]; MS (EI, 70 eV) *m/z* 276 (M^+); R_f (Et_2O/hexane = 1:2) 0.43; ν_{max} (KBr) 3386 (NH), 2233 (CN) cm^{-1}; δ_H (250 MHz, $DMSO_{d6}$) 1.52 (s, 3H, CH_3), 2.09 (dd, 1H, J = 13.8, 8.1 Hz, H_a-3), 2.30 (dd, 1H, J = 13.8, 6.5 Hz, H_b-3), 4.31 (s, 1H, C*H*–C_6H_5), 4.42 (br d, 1H, NH), 5.14 (dq, 1H, J = 16.9, 1.5 Hz, H_{trans}-5), 5.22 (dt, 1H, J = 10.3, 1.5 Hz, H_{cis}-5), 5.84 (m, 1H, H-4), 6.56–7.41 (m, 10H, 2×C_6H_5); δ_C (62.9 MHz, DMSO, DEPT) 22.4 (*C*H_3), 41.2 (C-3), 42.8 (C-2), 63.5 (*C*H–C_6H_5), 120.5 (C-5), 122.3 (CN), 131.5 (C-4), 113.9, 127.8, 128.3, 128.6, 129.2, 131.5 (C_{arom}), 138.0, 146.2 (C_{quat}).

4.3.14. 2-Methyl-2-(phenylamino-methyl)-pent-4-enenitrile 19 (*syn*). From the above described separation procedure the title compound **19** (*syn*) (66 mg, 24%) was isolated as a viscous oil. [Found: C, 82.70; H, 7.43; N, 10.33. $C_{19}H_{20}N_2$ requires C, 82.57; H, 7.29; N 10.14]; MS (EI, 70 eV) *m/z* 276 (M^+); R_f (Et_2O/hexane = 1:2) 0.54; ν_{max} (KBr) 3386 (NH), 2234 (CN) cm^{-1}; δ_H (250 MHz, $CDCl_3$) 1.15 (s, 3H, CH_3), 2.45 (dd, 1H, J = 13.7, 8.2 Hz, H_a-3), 2.85 (dd, 1H, J = 13.7, 6.5 Hz, H_b-3), 4.26 (d, 1H, J = 11.9 Hz, C*H*–C_6H_5), 4.49 (s, br d, 1H, NH), 5.23 (ddt, 1H, J = 15.3, 1.4 Hz, H_{trans}-5), 5.28 (ddt, 1H, J = 10.5, 1.4 Hz, H_{cis}-5), 5.91 (m, 1H, 4-H), 6.56–7.49 (m, 10H, 2×C_6H_5); δ_C (62.9 MHz, $CDCl_3$, DEPT) 21.5 (CH_3), 42.0 (C-3), 42.6 (C-2), 62.6 (*C*H–C_6H_5), 120.8 (C-5), 122.3 (CN), 131.4 (C-4), 113.9, 118.3, 127.7, 128.2, 128.5, 129.1 C_{arom}, 138.1, 146.2 (C_{quat}).

4.3.15. 2-Benzyl-2-methyl-3-phenyl-3-phenylamino-propionitrile 20 (*anti*). Using the general experimental procedure, from the metal intermediate of **8** prepared from 1 mmol starting products and benzyl bromide (256 mg, 1.5 mmol) the title compound (55 mg, 17%) was isolated after preparative thin-layer chromatography (Kieselgel 60 PF_{254}, Et_2O/hexane = 1:2) as a white solid. Recrystallization (EtOH) afforded **20** (*anti*) (36 mg, 11%) as white crystals, mp 184–186 °C; [Found: C, 84.30; H, 6.58; N 8.32. $C_{23}H_{22}N_2$ requires C, 84.63; H, 6.79; N, 8.58]; MS (EI, 70 eV) *m/z* 326 (M^+); R_f (Et_2O/hexane = 1:2) 0.40; ν_{max} (KBr) 3379 (NH), 2241 (CN) cm^{-1}; δ_H (250 MHz, THF_{d8}) 1.37 (s, 3H, CH_3), 2.73 (d, 1H, J = 13.2 Hz, C$H_a$$H_b$–$C_6H_5$), 2.97 (d, 1H, J = 13.2 Hz, $CH_a$$H_b$–$C_6H_5$), 4.48 (d, 1H, J = 9.0 Hz, H-3), 5.46 (d, 1H, J = 9.0 Hz, NH), 6.55–7.31 (m, 15H, 3×C_6H_5); δ_C (62.9 MHz, THF_{d8}, DEPT) 21.4 (CH_3), 43.3 (*C*H_2–C_6H_5), 44.8 (C-2), 63.4 (C-3), 122.8 (CN), 114.3, 118.1, 127.8, 128.5, 128.8, 129.1, 129.5, 131.1 (C_{arom}), 136.5, 139.6, 147.8 (C_{quat}).

4.3.16. 2-Benzyl-2-methyl-3-phenyl-3-phenylamino-propionitrile 20 (*syn*). From the above described separation procedure the title compound (52 mg, 16%) was isolated as a viscous oil. Recrystallization (Et_2O) afforded **20** (*syn*) as white crystals, mp 129–131 °C; [Found: C, 84.48; H, 6.68; N, 8.40. $C_{23}H_{22}N_2$ requires C, 84.63; H, 6.79; N, 8.58]; MS (EI, 70 eV) *m/z* 326 (M^+); R_f (Et_2O/hexane = 1:2) 0.52; ν_{max} (KBr) 3426 (NH), 2232 (CN) cm^{-1}; δ_H (250 MHz, $CDCl_3$) 1.06 (s, 3H, CH_3), 3.12 (d, 1H, J = 13.3 Hz, C$H_a$$H_b$–$C_6H_5$), 3.38 (d, 1H, J = 13.3 Hz, $CH_a$$H_b$–$C_6H_5$), 4.24 (d, 1H, J = 9.2 Hz, H-3), 4.63 (d, 1H, J = 9.2 Hz, NH), 6.59–7.53 (m, 15H, 3×C_6H_5); δ_C (62.9 MHz, $CDCl_3$, DEPT) 21.7 (CH_3), 43.7 (*C*H_2–C_6H_5), 62.4 (C-3), 122.4 (CN), 114.1, 118.5, 127.5, 127.9, 128.3, 128.5, 128.6, 129.2, 130.7 (C_{arom}), 134.7, 138.1, 146.0 (C_{quat}).

4.3.17. 5-Oxo-1,2,3-triphenyl-pyrolidine-3-carbonitrile 21a. Using the general experimental procedure, from the metal intermediate of **5** prepared from 1 mmol starting products and methyl-2-bromoacetate (230 mg, 1.5 mmol) the titled compound **21** (239 mg, 70%) was isolated as a diastereoisomeric mixture (**21a/21b** = 70/30) after flash chromatography (silica gel, Et_2O/hexane = 1:1). Subsequent recrystallization (Et_2O) yielded **21a** (98 mg, 29%) as white crystals, mp 141–143 °C; [Found: C, 81.48; H, 5.22; N, 8.03. $C_{23}H_{18}N_2O$ requires C, 81.63; H, 5.36; N, 8.28]; MS (EI, 70 eV) *m/z* 338 (M^+); R_f (Et_2O/hexane = 1:1) 0.33; ν_{max} (KBr) 2244 (CN) 1708 (CO) cm^{-1}; δ_H (250 MHz, $CDCl_3$) 3.30 (d, 1H, J = 16.8 Hz, H_a-4), 3.5 (d, 1H, J = 16.8 Hz, H_b-4), 5.68 (s, 1H, H-2), 6.73–7.36 (m, 15H, 3×C_6H_5); δ_C (62.9 MHz, $CDCl_3$, DEPT) 40.3 (C-4), 47.5 (C-3), 72.4 (C-2), 122.1 (CN), 122.6, 125.9, 126.9, 127.2, 128.4, 128.5, 128.6, 128.7, 128.8 (C_{arom}), 132.2, 132.9, 137.0 (C_{quat}), 170.4 (C=O).

4.3.18. 5-Oxo-1,2,3-triphenyl-pyrolidine-3-carbonitrile 21b. The product has not been isolated. ^1H NMR chemical shifts are taken from the spectrum of diastereoisomeric mixture, isolated as described above. R_f (Et_2O/hexane = 1:1) 0.33; δ_H (250 MHz, $CDCl_3$) 3.31 (d, 1H, J = 17.3 Hz, H_a-4), 3.53 (d, 1H, J = 17.3 Hz, H_b-4), 5.31 (s, 1H, H-2).

4.3.19. 1-Naphthalen-2-yl-5-oxo-2,3-diphenyl-pyrolidine-3-carbonitrile 22a. According to the general experimental procedure from the metal intermediate of **6** (1 mmol) and methyl-2-bromoacetate (230 mg, 1.5 mmol) after flash chromatography (silica gel, Et_2O/hexane = 1:1) the title compound **22** (263 mg, 68%) was isolated as diastereoisomeric mixture (**22a/22b** = 71/29). Further recrystallization (Et_2O) yielded **22a** (100 mg, 26%) as white crystals, mp 104–106 °C; [Found: C, 83.22; H, 5.08; N, 7.02. $C_{27}H_{20}N_2O$ requires C, 83.48; H, 5.19; N, 7.21]; MS (EI, 70 eV) *m/z* 388 (M^+); R_f (Et_2O/hexane = 1:1) 0.35; ν_{max} (KBr) 2240 (CN) 1706 (CO) cm^{-1}; δ_H (250 MHz, $CDCl_3$) 3.35 (d, 1H, J = 16.9 Hz, H_a-4), 3.57 (d, 1H, J = 16.9 Hz, H_b-4), 5.82 (s, 1H, H-2), 6.78–7.77 (m, 17H, Ar*H*); δ_C (62.9 MHz, $CDCl_3$) 40.5 (C-4), 47.5 (C-3), 72.8 (C-2), 122.1 (CN), 120.9, 121.4, 125.9, 126.5, 127.0, 127.3, 127.5, 127.8, 128.4, 128.6, 128.8 (C_{arom}), 131.3, 132.3, 132.9, 133.1, 134.6 (C_{quat}), 170.6 (C=O).

4.3.20. 1-Naphthalen-2-yl-5-oxo-2,3-diphenyl-pyrolidine-3-carbonitrile 22b. The minor isomer **22b** has not been isolated. Chemical shifts are taken from the ^1H NMR spectrum of diastereoisomeric mixture, isolated as described above. R_f (Et_2O/hexane = 1:1) 0.35; δ_H (250 MHz, $CDCl_3$) 3.36 (d, 1H, J = 17.4 Hz, H_a-4), 3.6 (d, 1H, J = 17.4 Hz, H_b-4), 5.44 (s, 1H, H-2).

References and notes

1. (a) Arseniyadis, S.; Kyler, K. S.; Watt, D. S. *Org. React.* **1984**,

31. (b) Fleming, F. F.; Shook, B. C. *Tetrahedron* **2002**, *58*, 1–23.
2. (a) Baradel, A. M.; Dreux, J.; Longeray, R. *Bull. Soc. Chim. Fr.* **1970**, 255–258. (b) Kyriakakou, G.; Roux-Schmitt, M. C.; Seyden-Penne, J. *Tetrahedron* **1975**, *31*, 1883–1888. (c) Sauvetre, R.; Roux-Schmitt, M. C.; Seyden-Penne, J. *Tetrahedron* **1978**, *34*, 2135–2140. (d) El-Bouz, M.; Roux-Schmitt, M. C.; Wartsky, L. *J. Chem. Soc., Chem. Commun.* **1979**, 779–780. (e) Roux-Schmitt, M. C.; Wartsky, L.; Seyden-Penne, J. *Tetrahedron* **1981**, *37*, 1927–1934. (f) Cossentini, M.; Seyden-Penne, J. *Synth. Commun.* **1985**, *15*, 689–696. (g) Cossentini, M.; Strzalko, T.; Seyden-Penne, J. *Bull. Soc. Chim. Fr.* **1987**, 531–534. (h) Dobrev, A.; Ivanov, C.; Lattes, A.; Bon, M. *Bull. Soc. Chim. Fr.* **1987**, 1084–1088. (i) Kasatkin, A. N.; Bictimirov, R. Kh.; Tolstikov, G. A.; Nikonenko, A. G. *J. Org. Chem. USSR* **1990**, *26*, 1037–1045. (j) Hamana, H.; Sugasawa, T. *Chem. Lett.* **1982**, 1401–1404. (k) Kauffmann, T.; Kieper, H.; Pieper, H. *Chem. Ber.* **1992**, *125*, 899–905.
3. Fuji, K. *Chem. Rev.* **1993**, *93*, 2037–2066.
4. Viteva, L.; Stefanovsky, Y. *Tetrahedron Lett.* **1989**, *30*, 4565–4568.
5. Viteva, L.; Gorrichon, L. *Mh. Chem.* **1995**, *126*, 1001–1009.
6. Viteva, L.; Gospodova, Tz.; Stefanovsky, Y.; Angelova, S.; Gorrichon, L. *Tetrahedron* **2002**, *58*, 3371–3378.
7. (a) Stefanovsky, Y.; Viteva, L. *Mh. Chem.* **1980**, *111*, 1287–1298. (b) Stefanovsky, Y.; Viteva, L. *Mh. Chem.* **1981**, *112*, 125–128. (c) Stefanovsky, Y.; Viteva, L. *Mh. Chem.* **1982**, *113*, 181–190. (d) Stefanovsky, Y.; Gospodova, Tz.; Viteva, L. *Tetrahedron* **1986**, *42*, 5355–5362. (e) Viteva, L.; Stefanovsky, Y. *Tetrahedron Lett.* **1989**, *30*, 4565–4568. (f) Viteva, L.; Stefanovsky, Y. *J. Chem. Res. (S)* **1990**, 232–233. (g) Viteva, L.; Stefanovsky, Y. *Tetrahedron Lett.* **1990**, *31*, 5649–5652. (h) Viteva, L. Z.; Gospodova, Tz. S.; Stefanovsky, Y. N. *Tetrahedron* **1994**, *50*, 7193–7202.
8. (a) Mollov, N. M.; Petrova, P. Ch. *Commun. Inst. Org. Chem. Bulg. Acad. Sci.* **1964**, 127–138. (b) Ivanov, C.; Dryanska, V. *C. R. Acad. Bulg. Sci.* **1969**, *22*, 423–426. (c) Dryanska, V.; Tasheva, D. *Synth. Commun.* **1992**, *22*, 63–71.
9. (a) Viteva, L. Z.; Gospodova, Tz. S.; Stefanovsky, Y. N. *J. Chem. Res. (S)* **2000**, 133–135. (b) Stefanovsky, Y. N.; Kurtev, B. J. *C. R. Acad. Bulg. Sci.* **1969**, *22*, 53–55.
10. Novkova, S. I.; Georgieva, M. K.; Binev, Y. I.; Petkov, Ch. Ts.; Binev, I. G. *J. Mol. Struct. (Teochem.)* **2003**, *636*, 167–183.
11. Heathcock, C. H. The aldol addition reaction. In Morrison, J. D., Ed.; Asymmetric Synthesis; Academic: New York, 1984; Vol. 3, pp 111–212.
12. (a) Garlier, P. R.; Lo, K. M. *J. Org. Chem.* **1994**, *59*, 4053–4055. (b) Garlier, P. R.; Lo, K. M.; Lo, M. M.-C.; Williams, I. D. *Org. Chem.* **1995**, *60*, 7511–7517.
13. Simova, S. *Magn. Res. Chem.* **1998**, *36*, 505–510.
14. (a) Paddon-Row, M. N.; Rondon, N. G.; Houk, K. N. *J. Am. Chem. Soc.* **1982**, *104*, 7162–7166. (b) Houk, K. N.; Rondon, N. G.; Wu, Y.-D.; Metzand, M. N.; Paddon-Row, M. N. *Tetrahedron* **1984**, *40*, 2257–2274.
15. (a) Fleming, I.; Lewis, J. J. *J. Chem. Soc., Perkin Trans. 1* **1992**, 3257–3266. (b) Fleming, I.; Lewis, J. J. *J. Chem. Soc., Perkin Trans. 1* **1992**, 3267–3276. (c) Crump, R. A. N. C.; Fleming, I.; Hill, J. H. M.; Parker, D.; Reddy, N. L.; Waterson, D. *J. Chem. Soc., Perkin Trans. 1* **1992**, 3277–3294. (d) Fleming, I.; Kilbrun, J. D. *J. Chem. Soc., Perkin Trans. 1* **1992**, 3295–3302. (e) Fleming, I. *J. Chem. Soc., Perkin Trans. 1* **1992**, 3303–3308. (f) Alexakis, A.; Trevitt, G. P.; Bernardinelli, G. *J. Am. Chem. Soc.* **2001**, *123*, 4358–4359.
16. (a) Klopman, G. *J. Am. Chem. Soc.* **1968**, *90*, 223–234. (b) Binev, I. G.; Tsenov, J. A.; Velcheva, E. A.; Juchnovsky, I. N. *J. Mol. Struct.* **1995**, *344*, 205–215. (c) Albagnac, G.; Brun, B.; Calas, B.; Giral, L. *Bull. Soc. Chim. Fr.* **1974**, 1469–1473. (d) Deschamps, B.; Roux-Schmitt, M. C.; Wartsky, L. *Tetrahedron Lett.* **1979**, *20*, 1337–1380. (e) McMahon, T. B.; Kebarle, P. *J. Am. Chem. Soc.* **1976**, *98*, 3399–3406.
17. Parella, T.; Sanchez-Ferrando, F.; Virgili, A. *Magn. Res. Chem.* **1994**, *32*, 343–347.

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Versatility of an intramolecularly hydrogen-bonded 4-(*N*,*N*-dimethylamino)benzoate group as a signaling subunit for anion recognition

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Abstract—The versatility of the 4-(*N*,*N*-dimethylamino)benzoate (DMAB) group embedded in host **1** as a signaling subunit for anion recognition was elucidated in terms of ^{1}H NMR, CD, and fluorescence studies. Host **1** showed 1:1 complexation with monovalent anions and stepwise 1:1 and 2:1 (host **1**: anion) complexation with divalent phosphate anions. The binding constants between host **1** and anions were determined by means of ^{1}H NMR titrations in CD_3CN (HPO_4^{2-}: log $K_{1:1}=6.2$, log $K_{2:1}=4.9$; $H_2P_2O_7^{2-}$: log $K_{1:1}=4.4$, log $K_{2:1}=1.8$; AMP^{2-}: log $K_{1:1}>7$, log $K_{2:1}>5$) and the affinity of host **1** toward divalent anions, HPO_4^{2-}, $H_2P_2O_7^{2-}$, and AMP^{2-}, is stronger than that toward monovalent anions, NO_3^-, BF_4^-, ClO_4^-, HSO_4^-, and PF_6^-. The CD exciton chirality studies of host **1** with divalent anions, HPO_4^{2-} and AMP^{2-}, revealed that the two DMAB groups in the 2:1 complexes were arranged with negative chirality (counterclockwise). The dual fluorescence behavior of the DMAB group demonstrated not only the complexation stoichiometry but also the role(s) of the lipophilic countercation such as tetrabutylammonium and/or the hydrophilic residue in AMP during anion recognition.

1. Introduction

The design of artificial receptors (hosts) for recognition of anionic species is a subject of current interest due to their importance in a wide area ranging from chemical, biological, and environmental fields.[1] Central to the designing of the receptors with high complexation ability as well as excellent selectivity in anion recognition has been the development of new binding principles.[2] In order to estimate the complexation behavior of receptors toward anions, signaling of the anion coordination process is of great importance. In this sense, separation of the signaling subunit and the binding subunit in a receptor would lead to more intelligent and sophisticated systems to obtain clear binding information (Fig. 1).[3] Chromophores such as nitrophenyl,[4] anthraquinone,[5] azo dye derivatives,[6] porphyrins,[7] and sapphyrins[8] have been applied for this purpose as signaling subunits to show absorption changes in

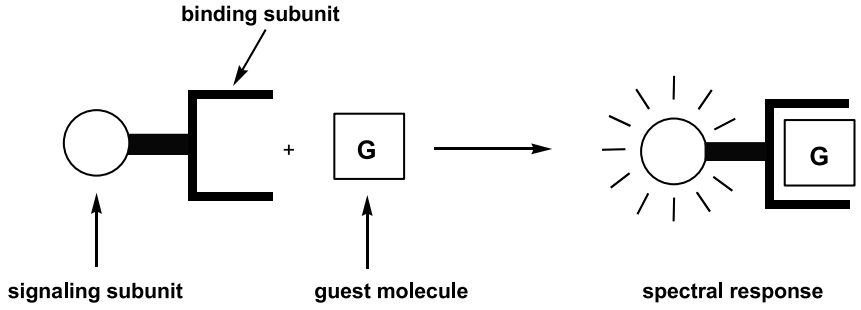

Figure 1. Detection of guest molecule by the binding subunit-signaling subunit strategy.

Keywords: Intramolecular hydrogen bonding; 4-(*N*,*N*-Dimethylamino)benzoate group; Chiral guanidinium ion; Anion recognition; NMR titration; CD titration; Dual fluorescence titration.
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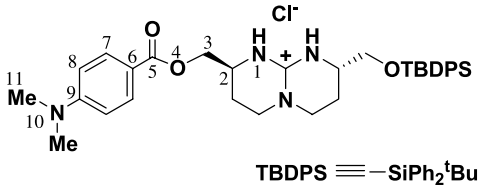
electronic absorption spectra or direct color changes in the visible region. Fluorophores, the properties of which are generally more sensitive than the absorption properties of chromophores, for example, polycyclic aromatic compounds[9] and transition metal complexes with bipyridine derivatives[10] have been used in many sensing applications as signaling subunits. Electrochemical properties such as the redox potential of metallocenes have also been utilized for detection of complexation.[11]

In the previous research, we designed a new type of simple host molecule **1** (Fig. 2) having a 4-(*N*,*N*-dimethylamino)-benzoate (DMAB) group as a signaling subunit linked to a chiral bicyclic guanidinium ion moiety as a binding subunit for anions through both of the dual-hydrogen-bonding and ion-pairing interactions.[12,13,14] By using host **1**, we demonstrated that the DMAB group which is embedded in host **1** was an alternative excellent signaling subunit in complexation with sulfate anion. Electronic absorption spectra of **1** revealed that strong intramolecular hydrogen bonding was present between the carbonyl oxygen atom of the DMAB group and one of the N–H groups of the guanidinium ion moiety even in a polar solvent such as acetonitrile. The stepwise 1:1 and 2:1 (host **1**: sulfate anion) complexation constants were determined by ^{1}H NMR titration. The CD exciton chirality method allowed us to determine the chiral sense of the two DMAB groups in the 2:1 complex as negative. The dual fluorescence behavior of the DMAB group was employed for elucidation of the role of the countercation upon complexation of host **1** with the sulfate anion.



1

Figure 2. The structure of host **1**.

We report, herein, the scope and limitations of the signaling subunit, 4-(*N*,*N*-dimethylamino)benzoate, upon complexation with a variety of anions possessing monovalent or divalent negative charge with trigonal planar, tetrahedral, dual tetrahedral, or octahedral geometry (Fig. 3). Versatility of the signaling group in anion recognition is also discussed by means of ^{1}H NMR, CD, and fluorescence spectra.

2. Results and discussion

2.1. ^{1}H NMR spectral titrations

The ^{1}H NMR spectral titration method is a useful and powerful technique to investigate complexation behavior between hosts and guests, especially in the case that hosts have no chromophore or fluorophore.[15] When bis-tetrabutylammonium sulfate $(TBA)_2SO_4$ was added to host **1**, clear chemical shift changes of aromatic protons (H7 and H8) and *N*-methyl proton (H11) of the signaling subunit in host **1** were observed by ^{1}H NMR in acetonitrile.[13] The observation of the clear chemical shift changes is quite interesting, since these protons (H7, H8, and H11) are quite distant from the NH group of the binding site and the existence of three single bonds between N1 and O4 would make it difficult to transmit any electronic effect from the NH groups in the binding subunit to the DMAB chromophore through the bonds. The clear chemical shift changes observed are, therefore, rationalized by fission of the intramolecular hydrogen bonding (Scheme 1), the existence of which was indicated by electronic absorption spectra of host **1**.[13] When a guest anion binds to the guanidinium ion moiety of host **1** with 'covered' structure, the intramolecular hydrogen bonding will be broken to release a free carbonyl group with an 'open' structure, which would influence the electronic and/or steric environment on the DMAB group. In order to make it clear whether the chemical shift changes are a common aspect in anion binding or not, we selected a variety of anions potentially able to bind to the guanidinium ion moiety. Monovalent anions such as nitrate (NO_3^-, trigonal planar), tetrafluoroborate (BF_4^-, tetrahedral), perchlorate (ClO_4^-, tetrahedral), hydrogensulfate (HSO_4^-, tetrahedral), dihydrogenphosphate ($H_2PO_4^-$, tetrahedral), and hexafluorophosphate (PF_6^-, hexagonal) and divalent anions such as hydrogenphosphate (HPO_4^{2-}, tetrahedral), dihydrogenpyrophosphate ($H_2P_2O_7^{2-}$, dual tetrahedral), and adenosine 5′-monophospate (AMP^{2-}, tetrahedral) which is a biologically important derivative of hydrogenphosphate were selected to elucidate the sensitivity of the chromophore for complexation of **1** with anions having different charges and different structures.

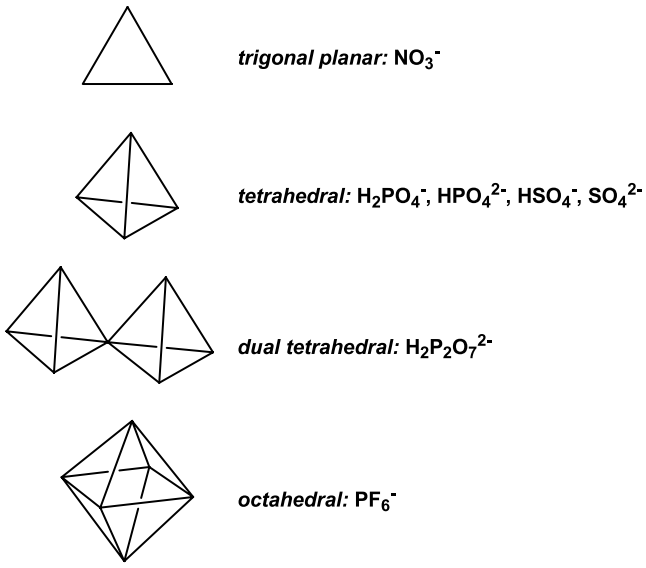
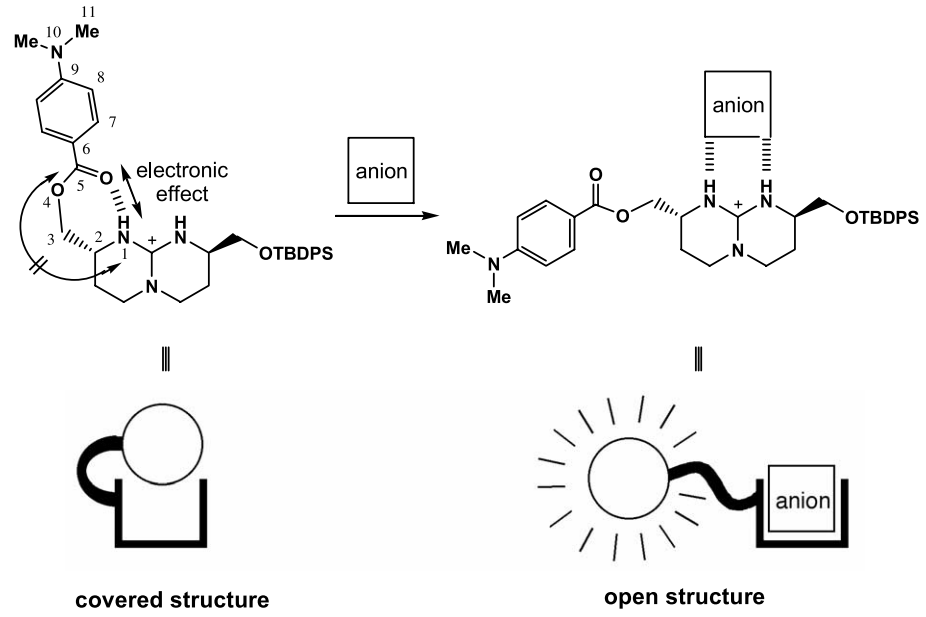


Figure 3. The geometry of a variety of anions.

The complexation behavior of host **1** with divalent anions was studied by the ^{1}H NMR titration method in CD_3CN at 25 °C. The aromatic protons (H7 and H8) and *N*-methyl proton (H11) were monitored during the titrations. As can be seen in Figure 4, when host **1** was titrated with HPO_4^{2-}, a representative anion having a tetrahedral array of four oxygen atoms with divalent negative charge, similar titration curves were obtained as compared with those for



Scheme 1.

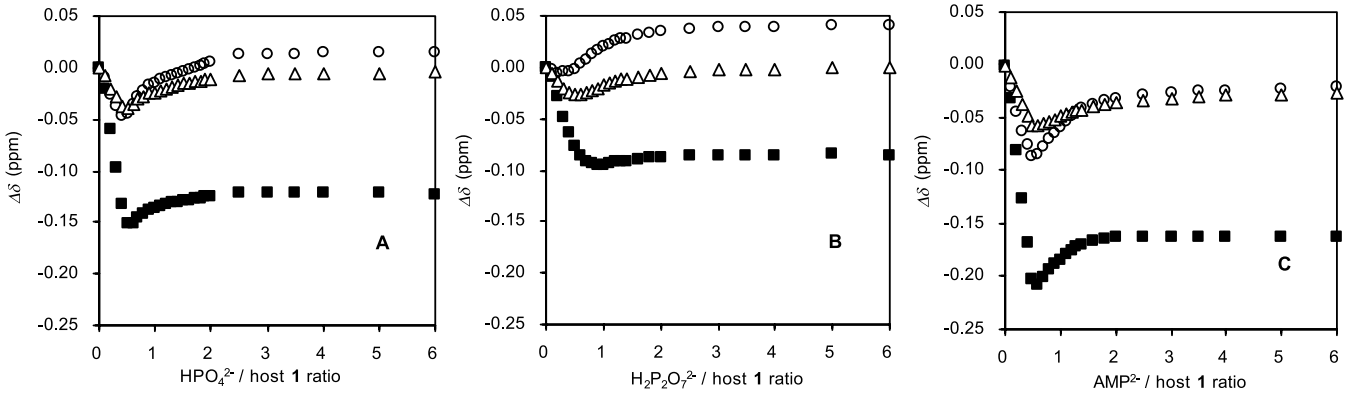


Figure 4. Chemical shift changes of aromatic (■: H7 and ○: H8) and *N*-methyl (△: H11) protons of host **1** upon ^{1}H NMR titrations with A: $(TBA)_2HPO_4$, B: $(TBA)_2H_2P_2O_7$, and C: $(TBA)_2AMP$ in CD_3CN.

SO_4^{2-}. All of the H7, H8, and H11 signals, namely, shifted to high field first almost proportionally to the amount of added HPO_4^{2-} until the HPO_4^{2-}/**1** ratio reached 0.5, and then they moved in the opposite direction to low field until the ratio reached almost 2, and finally leveled off thereafter. Similar titration results were obtained in the cases of structurally related anions $H_2P_2O_7^{2-}$ and AMP^{2-}, both of which have the phosphate moiety as a partial structure, irrespective of a dual tetrahedral array of seven oxygen atoms and a large volume of an organic residue on one of the oxygen atoms of the phosphate anion, respectively. The characteristic titration curves indicate that 2:1 complexes at the beginning and then 1:1 complexes were formed by continuous addition of the divalent anions in these cases.

The complexation behavior of host **1** toward monovalent anions was also studied using ^{1}H NMR titrations in CD_3CN at 25 °C by monitoring the chemical shift changes of H7, H8, and H11 protons. Results obtained for the H7 proton with ClO_4^-, one of the most representative anions which have a tetrahedral array of four oxygen atoms with monovalent negative charge, are shown in Figure 5, while almost no change was observed in H8 and H11 protons. The observed simple and moderate upfield shift suggests that weak 1:1 complexation occurred between host **1** and ClO_4^-. Similar results were obtained in the case of other monovalent anions such as NO_3^-, BF_4^-, HSO_4^-, and PF_6^-.

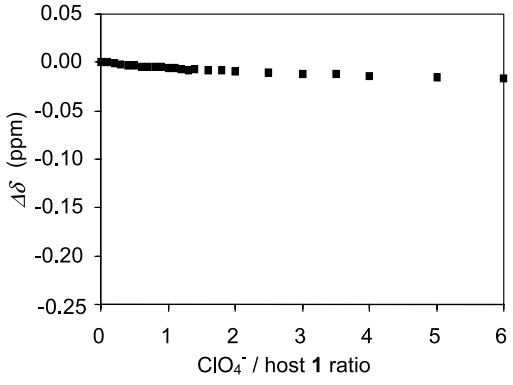
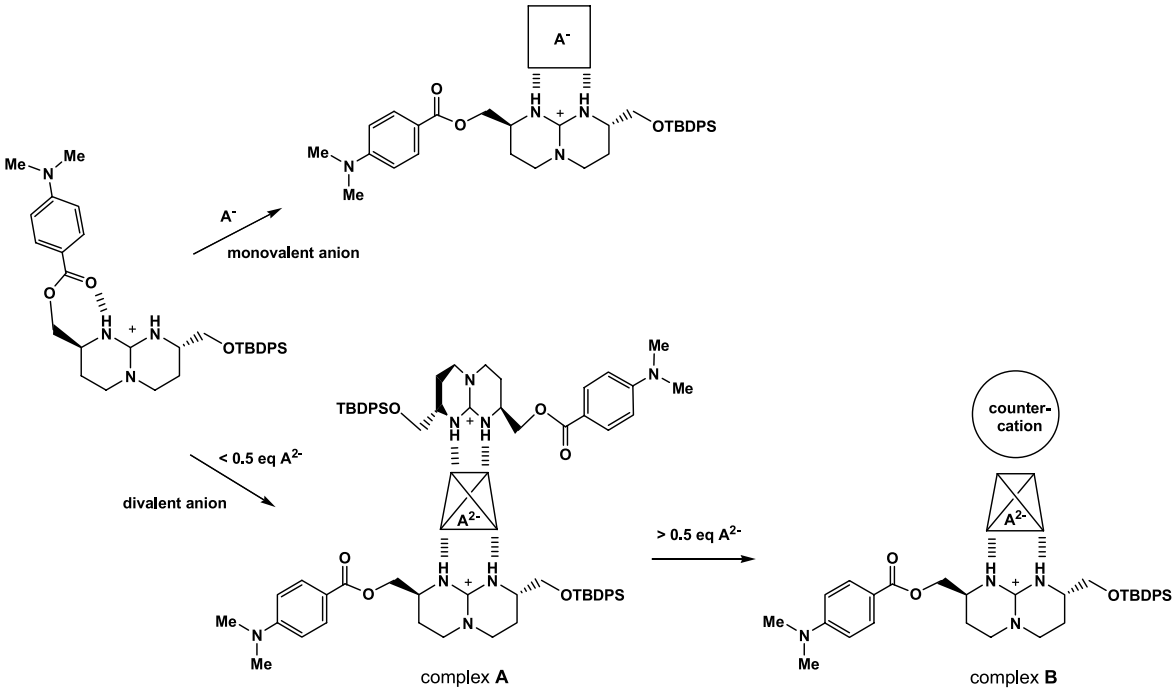


Figure 5. Chemical shift changes of H7 of host **1** upon ^{1}H NMR titration with $(TBA)ClO_4$ in CD_3CN.



Scheme 2.

2.2. Binding constants and anion selectivity of host 1

The binding constant $K_{1:1}$ for the 1:1 complexation (Scheme 2) is defined by (Eq. 1)

$$\mathbf{1}^+ + \mathrm{A}^- \overset{K_{1:1}}{\rightleftharpoons} \mathbf{1}^+ \cdot \mathrm{A}^-$$

$$K_{1:1} = [\mathbf{1}^+ \cdot \mathrm{A}^-]/([\mathbf{1}^+][\mathrm{A}^-]) \quad (1)$$

where A^- denotes a monovalent anion. In the case of a divalent anion, on the other hand, not only 1:1 complex (**B**) but also 2:1 complex (**A**) will be given by addition of the anion as illustrated in Scheme 2. The stepwise binding constants $K_{1:1}$ and $K_{2:1}$ for the 1:1 and 2:1 complexes are defined by (Eqs. 2 and 3), respectively

$$\mathbf{1}^+ + \mathrm{A}^{2-} \overset{K_{1:1}}{\rightleftharpoons} \mathbf{1}^+ \cdot \mathrm{A}^{2-}$$

$$K1:1 = [\mathbf{1}^+ \cdot \mathrm{A}^{2-}]/([\mathbf{1}^+][\mathrm{A}^{2-}]) \quad (2)$$

$$\mathbf{1}^+ \cdot \mathrm{A}^{2-} + \mathbf{1}^+ \overset{K_{2:1}}{\rightleftharpoons} (\mathbf{1}^+)_2 \cdot \mathrm{A}^{2-}$$

$$K_{2:1} = [(\mathbf{1}^+)_2 \cdot \mathrm{A}^{2-}]/([\mathbf{1}^+ \cdot \mathrm{A}^{2-}][\mathbf{1}^+]) \quad (3)$$

where A^{2-} denotes a divalent anion. The titration data obtained with the H7 proton of **1** were submitted to the non-linear least square curve-fitting[15a,16] to calculate $K_{1:1}$ and $K_{2:1}$ values, because the H7 proton exhibited the largest chemical shift change among three protons upon titration. The calculation results are summarized in Table 1.

The tetrahedral divalent anion HPO_4^{2-} exhibited quite large binding affinities ($\log K_{1:1}=6.2$ and $\log K_{2:1}=4.9$) and these binding constants are almost identical with those for SO_4^{2-}. With respect to $H_2P_2O_7^{2-}$ having divalent negative charge with dual tetrahedral arrays of seven oxygen atoms, the binding constants of $\log K_{1:1}$ (4.4) and $\log K_{2:1}$ (1.8) are relatively large. Unfortunately, binding constants, $K_{1:1}$ and $K_{2:1}$, for AMP^{2-} were too large to be calculated accurately. On the other hand, the binding constants for monovalent anions NO_3^- (trigonal planar), BF_4^- (tetrahedral), ClO_4^- (tetrahedral), HSO_4^- (tetrahedral), and PF_6^- (octahedral) turned out to be small ($\log K_{1:1}<2$). Interestingly, $H_2PO_4^-$ (tetrahedral) showed relatively strong binding affinity ($\log K_{1:1}=4.4$).

Indeed, anions must compete with and then overcome the inherent strong internal hydrogen bonding existing between one of the NH groups of the guanidinium moiety and the carbonyl oxygen atom of the DMAB group to give complexes. Geometric matching between the binding site

Table 1. Binding constants of host **1** with anions in CD_3CN

Anion	Binding constants ($\log M^{-1}$)	
	$\log K_{1:1}$	$\log K_{2:1}$
HPO_4^{2-}	6.2	4.9
$H_2P_2O_7^{2-}$	4.4	1.8
AMP^{2-}	>7[a]	>5[a]
SO_4^{2-}	6.2[b]	4.7[b]
NO_3^-	<2[c]	—
BF_4^-	<2[c]	—
ClO_4^-	<2[c]	—
$H_2PO_4^-$	4.4[d]	—
HSO_4^-	<2[c]	—
PF_6^-	<2[c]	—

[a] Binding constants $K_{1:1}$ and $K_{2:1}$ are too large to be accurately determined.
[b] Taken from Ref. 13.
[c] Binding constant $K_{1:1}$ is not determined accurately because chemical shift changes were so small (< 0.03 ppm) and clear endpoint of titration was not obtained.
[d] Estimated by CD titration. Ref. 17.

Table 2. Calculated interatomic distances between oxygen atoms or fluorine atoms, charges on oxygen or fluorine atoms, and heat of formation

Anion	O···O or F···F distances (Å)	Charges on O or F atom (au)	ΔH_f (kcal mol^{-1})
HPO_4^{2-}	2.56×2, 2.55, 2.54×2, 2.41	−1.29, −1.27×2, −0.85	−205
SO_4^{2-}	2.39×6	−1.20×4	−133
NO_3^-	2.12×3	−0.57×3	−89
BF_4^-	2.12×6	−0.32×4	−397
ClO_4^-	2.89×6	−0.50×4	166
$H_2PO_4^-$	2.66, 2.56×2, 2.47, 2.44×2	−1.17×2, −0.82×2	−316
HSO_4^-	2.44×2, 2.37×2, 2.36×2	−1.11, −1.08×2, −0.82	−234
PF_6^-	2.22×15	−0.65×6	−504

of host **1** and anions as well as charge densities on O or F atoms of guest anions must play a crucial role in complexation in this regard, because host **1** binds the anions through both dual-hydrogen-bonding and ion-pairing interactions.[12] The geometric matching can be estimated by comparison of the interatomic distance of two NH groups of host **1** and those of O atoms or F atoms of the anions. Thus,

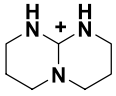


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Figure 6. The simplified model structure of host **1**.

semiempirical AM1 calculations[18] of anions were performed in order to determine the interatomic distances of O atoms or F atoms and charges on the anions (Table 2). The interatomic distance of and charges on hydrogen atoms of cyclic guanidinium ion **2** (Fig. 6), a simplified model compound for host **1**, were also calculated to give the values of 2.37 Å and +0.273 au, respectively.

Several interesting trends can be gleaned from the data of Tables 1 and 2. Host **1** showed much higher complexation affinities to the divalent negatively charged anions, HPO_4^{2-}, $H_2P_2O_7^{2-}$, and AMP^{2-}, than those to the monovalent negatively charged anions, NO_3^-, BF_4^-, ClO_4^-, HSO_4^-, and PF_6^-. The higher affinities of host **1** to the divalent anions, HPO_4^{2-} and SO_4^{2-}, should be attributable to the large negative charges on the O atoms (−1.29 to −0.85 au: HPO_4^{2-} and SO_4^{2-}). The distances of the O atoms in SO_4^{2-} (2.39 Å) are almost equal to that of the H atoms of the guanidinium NH groups (2.37 Å) in **2**. Similarly, the corresponding distances of HPO_4^{2-} are almost equal to or only a little bit longer (2.56–2.41 Å) than that of the calculated distance of the guanidinium NH groups (2.37 Å). By contrast with the divalent anions, monovalent anions, NO_3^-, BF_4^-, ClO_4^-, and PF_6^-, exhibiting very low affinity toward host **1**, have smaller negative charges on their O or F atoms (−0.65 to −0.32 au) as well as a little bit shorter (2.22–2.12 Å: NO_3^-, BF_4^-, and PF_6^-) or quite longer (2.89 Å: ClO_4^-) interatomic distances between the O atoms or F atoms than that of the NH groups in **2** (2.37 Å). The low affinity of host **1** to the anions is most probably due to small charges on the O or F atoms of the anions and the geometric disagreement between the binding site of host **1** and guest anions. The exceptional higher affinity of $H_2PO_4^-$ is presumably due to the significantly large charge of the O atoms (−1.17 to −0.82 au) among monovalent anions. On the contrary, HSO_4^- has comparable negative charge on the O atoms (−1.11 to −0.82 au) to those of HPO_4^{2-} and SO_4^{2-} (−1.29 to −0.85 au) and interatomic distances between the O atoms (2.44–2.36 Å) are almost equal to the NH distance of the guanidinium ion (2.37 Å), while the binding affinity resulted in unexpectedly low value.[19]

In conclusion, the DMAB signaling subunit gives us quantitative information on complexation of host **1** with anions in terms of ^{1}H NMR titrations. Host **1** showed 1:1 complexation with NO_3^-, BF_4^-, ClO_4^-, $H_2PO_4^-$, HSO_4^-, and PF_6^-, and successive 1:1 and 2:1 complexation with HPO_4^{2-}, $H_2P_2O_7^{2-}$, and AMP^{2-}. It is worth noting that the unsymmetric structure of HPO_4^{2-} and the existence of an extraordinarily large organic residue of AMP^{2-} did not influence their complexation behavior with host **1**. The complexation ability of host **1** toward HPO_4^{2-}, $H_2P_2O_7^{2-}$, and AMP^{2-} having a tetrahedral array of oxygen atoms with divalent negative charge turned out to be quite high as compared with those with monovalent anions such as NO_3^-, BF_4^-, ClO_4^-, HSO_4^-, and PF_6^-, except for $H_2PO_4^-$.

2.3. CD spectral titrations.[20]

The exciton chirality method is a simple and practical approach for establishing absolute configurations and conformations of organic compounds in solution on a microscale.[21] Although the magnitude of CD couplets tends to decrease with increasing distance between two chromophores, the 2:1 complex of host **1** with SO_4^{2-}, whose C3/C3′ distance was estimated to be ca. 8 Å by CPK molecular model examination, exhibited a clear bisignate CD

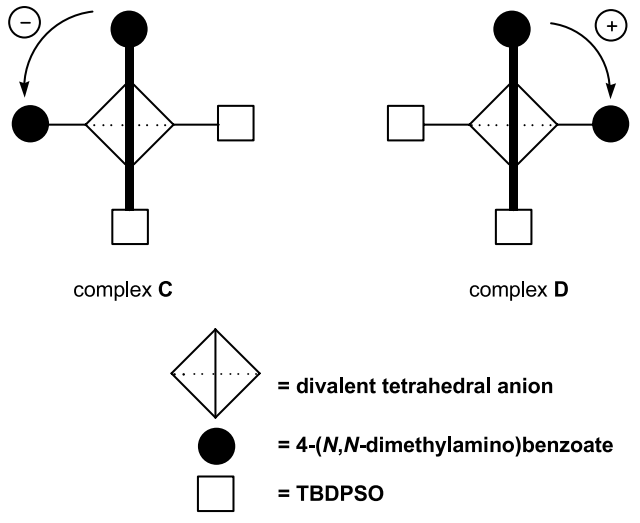

Figure 7. Schematic expressions of the 2:1 complex.

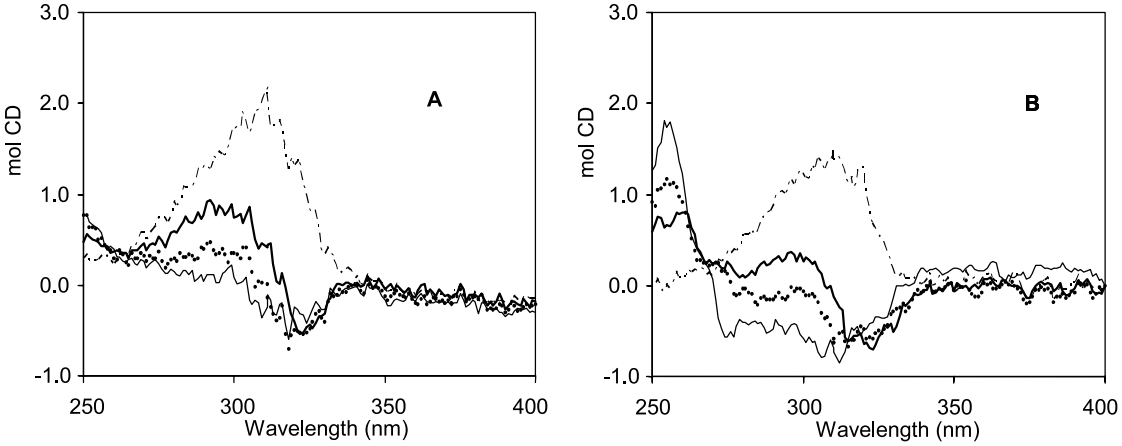

Figure 8. CD spectra of acetonitrile solution of **1** in the absence of (– – – –) and in the presence of 0.5 (——), 1 (- - - - - -), and 3 (—) equivalents of A: $(TBA)_2HPO_4$ and B: $(TBA)_2AMP$.

spectrum with the first negative and second positive Cotton effect peaks.[13] The observation of the exciton coupling peaks clearly indicates that the spatial array of the two DMAB chromophores in the 2:1 complex should be a counterclockwise arrangement (complex **C**) rather than a clockwise arrangement (complex **D**) (Fig. 7). In order to clarify whether the favorable formation of complex **C** is an intrinsic aspect in complexation of host **1** with divalent anions possessing a tetrahedral array of four oxygen atoms, we carried out titrations of host **1** with several anions using CD spectra.

The titration experiments of host **1** by HPO_4^{2-} and AMP^{2-}, which are divalent anions with a tetrahedral array of four oxygen atoms, exhibited similar results to SO_4^{2-} (Fig. 8). In the case of HPO_4^{2-}, for example, simple positive Cotton effect peaks were monitored in the absence of anion and, then, the profile of CD spectrum varied to negative first and positive second Cotton effect peaks upon addition of a half-amount of anion. Finally, a negative Cotton effect peak appeared on addition of an excess amount of HPO_4^{2-}. The observed weak but clear negative first and positive second Cotton effect peaks indicate that two DMAB groups in the 2:1 complex are arranged counterclockwise. Similar results were given in the case of AMP^{2-}, regardless of the sterically bulky organic residue on the oxygen atom of the phosphate moiety. Therefore, the formation of complex **C** is certainly favorable in 2:1 complexation of host **1** with both HPO_4^{2-} and AMP^{2-}.

In contrast, addition of a half-amount of $H_2P_2O_7^{2-}$, having a 'dimer' structure of HPO_4^{2-}, to host **1** resulted in simple decrease of CD intensity (Fig. 9) rather than exciton coupling peaks, though host **1** tends to give the 2:1 complex with the anion as demonstrated by ^1H NMR titration. The different titration profiles of HPO_4^{2-} and $H_2P_2O_7^{2-}$ could be explained by the flexibility of the 2:1 complexes. Since the 2:1 complex **3** (Fig. 10) of host **1** with $H_2P_2O_7^{2-}$ has P–O–P single bonds leading to a number of conformers by rotation, the relative position of the two DMAB groups in the complex would not be fixed well, while the 2:1 complex of **1** with HPO_4^{2-} has no such single bond in the corresponding complex. Further addition of an excess amount of $H_2P_2O_7^{2-}$ finally led to a negative Cotton effect peak. Similarly, addition of monovalent anion $H_2PO_4^-$ led to a simple decrease of CD intensity and finally an almost flat line. The simple change in Cotton effect peaks on addition of $H_2P_2O_7^{2-}$ or $H_2PO_4^-$ to host **1** could be ascribed to some conformational changes around the DMAB groups caused by anion coordination, which would drive the DMAB

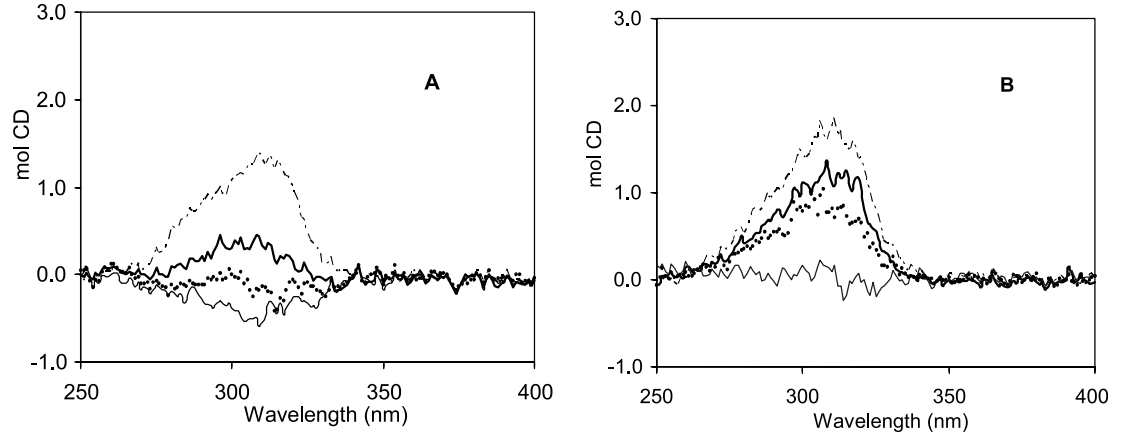

Figure 9. CD spectra of acetonitrile solution of **1** in the absence of (– – – –) and in the presence of 0.5 (——), 1 (- - - - - -), and 3 (—) equivalents of A: $(TBA)_2H_2P_2O_7$ and B: $(TBA)H_2PO_4$.

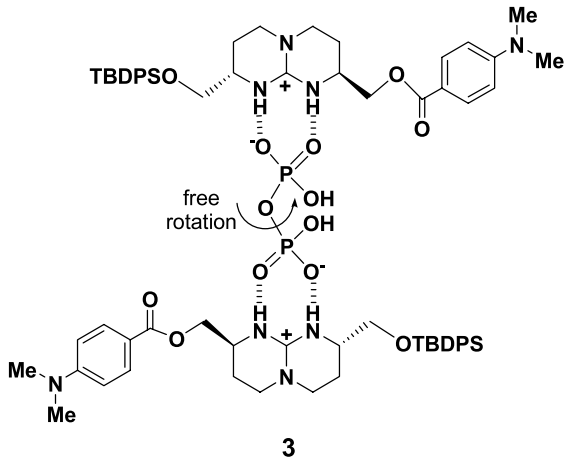

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Figure 10. The postulated structure of the 2:1 complex of host **1** with $(TBA)_2H_2P_2O_7$.

chromophores from the original position to a different sector of the chiral field created by the chiral guanidinium skeleton. In the case of ClO_4^-, having quite weak complexation ability as shown by ^1H NMR, no change in CD profiles was observed on addition of even an excess amount of ClO_4^-, as expected.

As a result, we succeeded in getting complexation information for **1** with anions through the DMAB group on the CD spectra. Anions having strong affinity for host **1** gave the characteristic intensity changes in CD profiles during titrations. Divalent anions HPO_4^{2-} and AMP^{2-} exhibited clear intensity changes with exciton coupling peaks. In addition, the observation of the bisignate Cotton effect peaks in the 2:1 complexes indicates that the two DMAB groups in the corresponding 2:1 complexes are arranged with negative chirality (counterclockwise) rather than positive chirality (clockwise). Divalent anion $H_2P_2O_7^{2-}$ and monovalent anion $H_2PO_4^-$ showed simple changes in CD intensities. Monovalent anion ClO_4^- with weak coordination ability exhibited no change. Thus, combination of the DMAB signaling subunit and the chiral guanidinium binding subunit makes it possible to obtain detailed information on the complexation process of **1** with anions using the CD spectroscopy.

2.4. Fluorescence titrations

Dual fluorescence behavior of the DMAB group originating from the simultaneous emissions of the locally excited (LE) state and intramolecular charge transfer (TICT) state at different wavelengths is known to be highly susceptible to the media polarity.[22] The dual fluorescence phenomena of closely related 4-(*N*,*N*-dimethylamino)benzamide derivatives have been applied as a signaling principle in anion receptors.[23] In the previous paper, we demonstrated that the DMAB group in host **1** also showed two fluorescence peaks at 346 and 491 nm in acetonitrile upon excitation at 280 nm, which are attributable to the LE and TICT emissions, respectively.[13] Upon addition of up to a half-amount of SO_4^{2-}, the LE intensity first drops quickly, then rapidly increases up to addition of twice amount. In contrast, the TICT band showed a simple increase until a half-amount addition of SO_4^{2-} but no appreciable increase thereafter. These observations were ascribed to the formation of two complexes, first the 2:1 then the 1:1 complex. The feasibility of rotation to give a TICT state from the LE state is suppressed by the inherent internal hydrogen bonding in host **1**.[13] When anion binds to host **1**, the internal hydrogen bonding is cleaved to lead to easy rotation giving a TICT state from the LE state with increasing intensity of TICT emission concomitant with the decreasing intensity of LE emission in the 2:1 complex. Moreover, active participation of lipophilic countercation (TBA) with the DMAB chromophore, which decreases polarity of the microenvironment around the DMAB group, leads to increase of the fluorescence intensity in the 1:1 complex.

In order to investigate the influence of anions on the unique fluorescence behavior of the DMAB group in host **1**, titrations of **1** with a variety of anions were performed on fluorescence spectra. As can be seen in Figure 11, the titration curves obtained with HPO_4^{2-}, $H_2P_2O_7^{2-}$, and AMP^{2-} were very close to those with SO_4^{2-}. The characteristic titration curves of the LE bands observed in these anions illustrate that the 2:1 complexation occurred on addition of up to 0.5 equiv of the anions and further addition of anions would replace the 2:1 complexes with the 1:1 complexes concomitant with lipophilic TBA cation (Scheme 2). Addition of an excess amount of AMP^{2-} resulted in a decrease of both LE and TICT intensities,

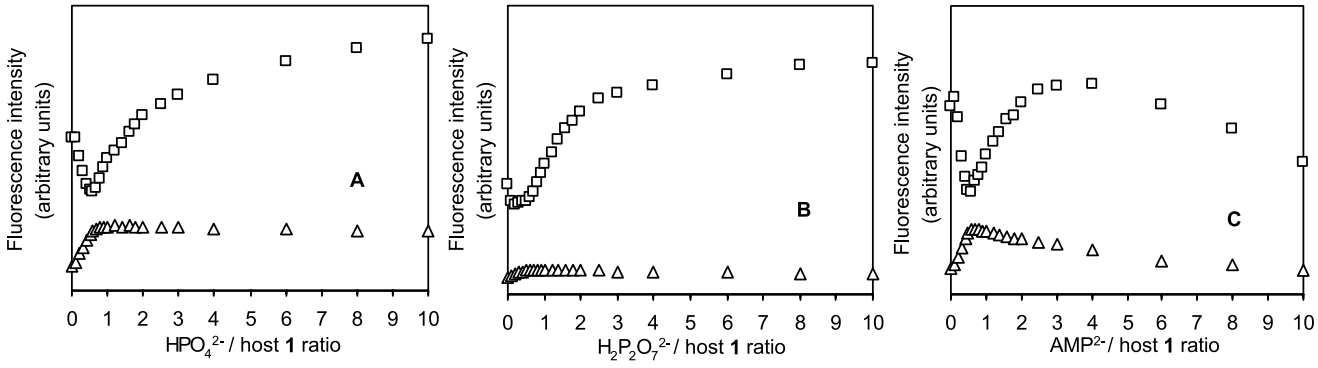

Figure 11. Fluorescence intensity changes in the LE band at 348 nm (□) and those in the TICT band at 491 nm (△) upon titration of host **1** with A: $(TBA)_2HPO_4$, B: $(TBA)_2H_2P_2O_7$, and C: $(TBA)_2AMP$ in acetonitrile, excitation wavelength: 280 nm.

which can be ascribed to the increased polarity of the microenvironment around the DMAB group on addition of a large amount of quite hydrophilic AMP^{2-} salt.

In the case of ClO_4^-, however, titration curves were quite simple (Fig. 12). LE intensity became gradually stronger with increasing amount of added (TBA)ClO_4, while almost no change was observed in TICT intensity. The appreciable differences in coordination affinities between divalent anions, HPO_4^{2-}, $H_2P_2O_7^{2-}$, and AMP^{2-}, and monovalent anion, ClO_4^-, should be the reason for the differences in the observed titration curves. Fission of the intrinsic internal hydrogen bonding between one of the NH groups of the guanidinium moiety and the ester carbonyl oxygen in host **1** would increase the feasibility of rotation leading to a TICT state from the LE state.[13] However, weak (or almost no) coordination of ClO_4^- should have no influence on the internal hydrogen bonding and therefore leads to almost no change in TICT intensity. The simple increase in LE emission is most likely due to the active participation of lipophilic TBA countercation to the DMAB group of **1**, as discussed in the previous paper.[13] Furthermore, quite similar results to ClO_4^- were obtained in the titrations of **1** with NO_3^-, BF_4^-, HSO_4^-, and PF_6^-, all of which have low affinities for **1** as indicated by ^{1}H NMR titrations. The profiles of the LE and TICT intensity changes by addition of $H_2PO_4^-$ were quite different from those of the divalent anions and the other monovalent anions.[24]

In summary, the fluorescence probe of the DMAB group in **1** successfully showed distinctive fluorescence responses to the anions. Divalent anions with a tetrahedral structure exhibited substantial decreases of LE emissions first up to a half-amount addition of anions and then gradual increases on further addition of the anions, while TICT emissions showed simple increases up to a half-amount addition of anions. With respect to the monovalent anions with weak coordination ability, the corresponding LE emissions exhibited a simple increase on gradual addition of the anions, though there was no change in TICT bands.

3. Conclusion

The reported novel host **1**[13] with a chiral bicyclic guanidinium ion moiety as a binding subunit linked to a DMAB group as a signaling subunit was applied for anion binding. In the host, the binding site is first covered with its own DMAB signaling subunit via the internal hydrogen bonding to give a 'covered' structure. Anions with stronger affinity than the DMAB group cleave the hydrogen binding and drive out the DMAB group to give an 'open' structure, which leads to the spectral changes of host **1** on ^{1}H NMR, CD, and fluorescence. The rational design of the above signaling principle gave us not only quantitative information about binding constants but also detailed understanding of the complexation behavior such as stoichiometry of the complexes, chiral sense of the 2:1 complexes, and participation of a countercation and a hydrophilic group in the coordination process. The intramolecularly hydrogen-bonded DMAB group would be of use in designing new artificial receptors for anion recognition as a versatile signaling subunit.

4. Experimental

4.1. General

^{1}H NMR spectra were obtained on a Varian UNITY INOVA 400 NMR spectrometer. CD spectra were obtained on a JASCO J-820 spectropolarimeter. Fluorescence spectra were recorded by a Fluorolog JOBIN YVON-SPEX spectrophotometer. Elemental analyses were performed on an Elementar Vario EL III. pH Measurements were done on a HORIBA F-22 pH meter. Spectroscopic grade acetonitrile from Dojindo Laboratories was used without further purification for all spectrophotometric measurements. Tetrabutylammonium nitrate, tetrabutylammonium dihydrogenphosphate, tetrabutylammonium tetrafluoroborate, tetrabutylammonium perchlorate, tetrabutylammonium hydrogensulfate, tetrabutylammonium hexanfluorophosphate, and tetrabutylammonium hydroxide were purchased from Aldrich and used without further purification.

4.1.1. Preparation of bis(tetrabutylammonium) hydrogenphosphate, bis(tetrabutylammonium) dihydrogenpyrophosphate, and bis(tetrabutylammonium) adenosine 5′-monophosphate. A potentiometric titration method in

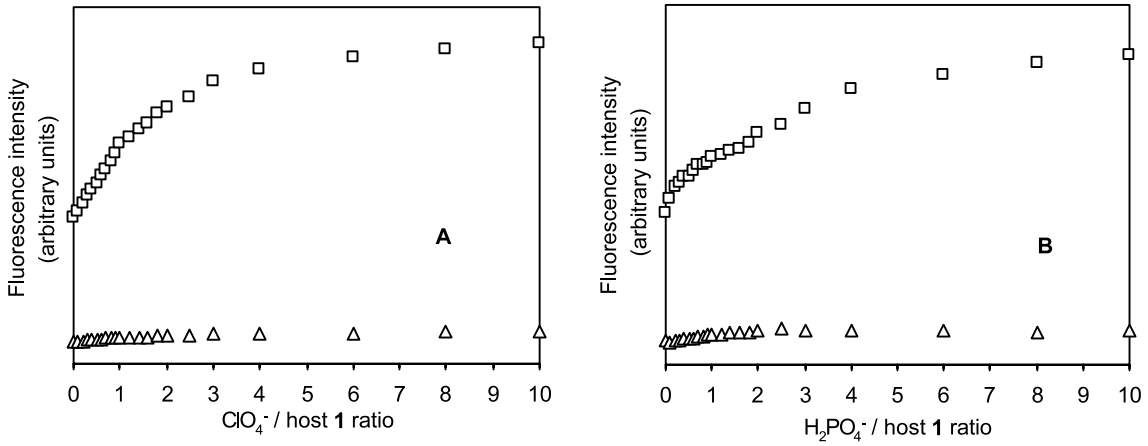

Figure 12. Fluorescence intensity changes in the LE band at 348 nm (□) and those in the TICT band at 491 nm (△) upon titration of host **1** with A: (TBA)ClO_4 and B: (TBA)H_2PO_4 in acetonitrile, excitation wavelength: 280 nm.

water was applied to prepare bis(tetrabutylammonium) hydrogenphosphate, bis(tetrabutylammonium) dihydrogenpyrophosphate, and bis(tetrabutylammonium) adenosine 5′-monophosphate. An acid solution (0.1 mol/L solution of phosphoric acid, pyrophosphoric acid, or adenosine 5′-monophosphoric acid in water) was titrated by 0.1 mol/L water solution of tetrabutylammonium hydroxide. The endpoint of the titration was monitored by pH meter. The resulting solution containing tetrabutylammonium salt was lyophilized to give pure salt. Bis(tetrabutylammonium) hydrogenphosphate·$5H_2O$ (white solid, hygroscopic). ^{1}H NMR (400 MHz, CD_3CN) δ 3.16–3.09 (m, 16H), 1.67–1.56 (m, 16H), 1.44–1.29 (m, 16H), 0.97 (t, J=7.4 Hz, 24H). Anal. Calcd for $C_{32}H_{83}N_2O_9P$: C, 57.28; H, 12.47; N, 4.18. Found: C, 57.35; H, 12.65; N, 4.23. Bis(tetrabutylammonium) pyrophosphate·$3H_2O$ (white solid, hygroscopic). ^{1}H NMR (400 MHz, CD_3CN) δ 3.16–3.09 (m, 16H), 1.68–1.56 (m, 16H), 1.44–1.28 (m, 16H), 0.97 (t, J=7.4 Hz, 24H). Anal. Calcd for $C_{32}H_{80}N_2O_{10}P_2$: C, 53.76; H, 11.28; N, 3.92. Found: C, 53.61; H, 11.41; N, 3.84. Bis(tetrabutylammonium) 5′-adenosine monophosphate·$3H_2O$ (white solid, hygroscopic). ^{1}H NMR (400 MHz, D_2O) δ 8.44 (s, 1H, adenine CH), 8.07 (s, 1H, adenine CH), 5.95 and 5.94 (d, J=3.0 Hz, 1H, O–CH–N), 4.66–4.52 (m, 1H, C*H*–OH), 4.35–4.30 (dd, J=4.2 Hz, 1H, O–C*H*–CH_2), 4.18 (br, 1H, C*H*–OH), 3.84–3.77 (t, J=3.8 Hz, 2H, CH_2O), 3.08–2.92 (m, 16H), 1.52–1.40 (m, 16H), 1.24–1.10 (m, 16H), 0.76 (t, J=7.2 Hz, 24H). Anal. Calcd for $C_{42}H_{90}N_7O_{10}P$: C, 57.05; H, 10.26; N, 11.09. Found: C, 57.39; H, 10.61; N, 10.76.

4.2. Spectral titrations of host 1 by anions

^{1}H NMR, CD, and fluorescence spectral titrations were performed by a similar procedure described before except for the concentration used.[13] ^{1}H NMR titrations in CD_3CN: a 2.00×10^{-3} M solution of host **1** and a 1.20×10^{-2} M solution of anions as tetrabutylammonium salts were used. CD titrations in CH_3CN: HPO_4^{2-}, $H_2PO_4^-$, and ClO_4^-: a 4.50×10^{-5} M solution of host **1** and a 2.70×10^{-3} M solution of anions as tetrabutylammonium salts were used, $H_2P_2O_7^{2-}$ and AMP^{2-}: a 3.65×10^{-5} M solution of host **1** and a 2.19×10^{-3} M solution of anions as tetrabutylammonium salts were used. $(TBA)_2$AMP itself has strong absorption around 270 nm in the CD spectra, which interferes with the signal of host **1** in CD titration. To cancel the influence of the AMP signal, the following procedures were performed. CD spectra of $(TBA)_2$AMP were recorded, then the intensity of $(TBA)_2$AMP was subtracted from each titration result in each experiment. Fluorescence titrations of host **1** in CH_3CN: a 4.05×10^{-5} M solution of host **1** and a 2.43×10^{-3} M solution of anions as tetrabutylammonium salts were used.

4.4. Semiempirical molecular orbital calculation

AM1 semiempirical calculations were performed using CAChe WorkSystem Pro Version 6.01 software.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.tet.2005.04.006

These data comprise of ^{1}H NMR titrations with tetrabutylammonium nitrate, tetrabutylammonium tetrafluoroborate, tetrabutylammonium hydrogensulfate, and tetrabutylammonium hexafluorophosphate, fluorescence titrations with tetrabutylammonium nitrate, tetrabutylammonium tetrafluoroborate, tetrabutyl-ammonium hydrogensulfate.

References and notes

1. (a) *Supramolecular chemistry of anions*; Bianchi, A., Bowman-James, K., Garcia-España, E., Eds.; Wiley-VCH: New York, 1997. (b) Steed, J. W.; Atwood, J. L. *Supramolecular chemistry*; Wiley: West Sussex, 2000.
2. For reviews, see: (a) Schmidtchen, F. P.; Berger, M. *Chem. Rev.* **1997**, *97*, 1609–1646. (b) Gale, P. A. *Coord. Chem. Rev.* **2000**, *199*, 181–233. (c) Gale, P. A. *Coord. Chem. Rev.* **2001**, *213*, 79–128.
3. For reviews, see: (a) Beer, P. D.; Gale, P. A. *Angew. Chem., Int. Ed.* **2001**, *40*, 486–516. (b) Martínez-Máñez, R.; Sancenón, F. *Chem. Rev.* **2003**, *103*, 4419–4476.
4. (a) Nishizawa, S.; Kato, R.; Hayashita, T.; Teramae, N. *Anal. Sci.* **1998**, *14*, 595–597. (b) Hayashita, T.; Onodera, T.; Kato, R.; Nishizawa, S.; Teramae, N. *Chem. Commun.* **2000**, 755–756. (c) Miyaji, H.; Sato, W.; Sessler, J. L.; Lynch, V. M. *Tetrahedron Lett.* **2000**, *41*, 1369–1373. (d) Kato, R.; Nishizawa, S.; Hayashita, T.; Teramae, N. *Tetrahedron Lett.* **2001**, *42*, 5053–5056. (e) Piatek, P.; Jurczak, J. *Chem. Commun.* **2002**, 2450–2451. (f) Miyaji, H.; Collinson, S. R.; Prokeš, I.; Tucker, J. H. R. *Chem. Commun.* **2003**, 64–65.
5. (a) Miyaji, H.; Sato, W.; Sessler, J. L. *Angew. Chem., Int. Ed.* **2000**, *39*, 1777–1778. (b) Miyaji, H.; Sessler, J. L. *Angew. Chem., Int. Ed.* **2001**, *40*, 154–157. (c) Jiménez, D.; Martínez-Máñez, R.; Sancenón, F.; Soto, J. *Tetrahedron Lett.* **2002**, *43*, 2823–2825.
6. (a) Lee, D. H.; Lee, K. H.; Hong, J.-I. *Org. Lett.* **2001**, *3*, 5–8. (b) Lee, D. H.; Lee, H.-Y.; Lee, K. H.; Hong, J.-I. *Chem. Commun.* **2001**, 1188–1189. (c) Lee, K. H.; Lee, H.-Y.; Lee, D. H.; Hong, J.-I. *Tetrahedron Lett.* **2001**, *42*, 5447–5449. (d) Lee, C.; Lee, D. H.; Hong, J.-I. *Tetrahedron Lett.* **2001**, *42*, 8665–8668. (e) Sancenón, F.; Martínez-Máñez, R.; Soto, J. *Angew. Chem., Int. Ed.* **2002**, *41*, 1416–1419. (f) Lee, D. H.; Im, J. H.; Son, S. U.; Chung, Y. K.; Hong, J.-I. *J. Am. Chem. Soc.* **2003**, *125*, 7752–7753.
7. (a) Beer, P. D.; Drew, M. G. B.; Hesek, D.; Jagessar, R. *J. Chem. Soc., Chem. Commun.* **1995**, 1187–1190. (b) Starnes, S. B.; Arungundram, S.; Saundars, C. H. *Tetrahedron Lett.* **2002**, *43*, 7785–7788. (c) Kim, Y.-H.; Hong, J.-I. *Chem. Commun.* **2002**, 512–513.

8. For a review, see: Sessler, J. L.; Davis, J. M. *Acc. Chem. Res.* **2001**, *34*, 989–997.
9. (a) Vance, D. H.; Czarnik, A. W. *J. Am. Chem. Soc.* **1994**, *116*, 9397–9398. (b) Nishizawa, S.; Kaneda, H.; Uchida, T.; Teramae, N. *J. Chem. Soc., Perkin Trans. 2* **1998**, 2325–2328. (c) Kubo, Y.; Tsukahara, M.; Ishihara, S.; Tokita, S. *Chem. Commun.* **2000**, 653–654. (d) Yoshida, H.; Saigo, K.; Hiratani, K. *Chem. Lett.* **2000**, 116–117. (e) Hennrich, G.; Sonnenschein, H.; Resch-Genger, U. *Tetrahedron Lett.* **2001**, *42*, 2805–2808. (f) Cabell, L. A.; Best, M. D.; Lavigne, J. J.; Schneider, S. E.; Perreault, D. M.; Monahan, M. K.; Anslyn, E. V. *J. Chem. Soc., Perkin Trans. 2* **2001**, 315–323. (g) Fabbrizzi, L.; Licchelli, M.; Mancin, F.; Pizzeghello, M.; Rabaioli, G.; Taglietti, A.; Tecilla, P.; Tonellato, U. *Chem. Eur. J.* **2002**, *8*, 94–101. (h) Kubo, Y.; Ishihara, S.; Tsukahara, M.; Tokita, S. *J. Chem. Soc., Perkin Trans. 2* **2002**, 1455–1460. (i) Kim, S. K.; Yoon, J. *Chem. Commun.* **2002**, 770–771. (j) Kwon, J. Y.; Jang, Y. J.; Kim, S. K.; Lee, K.-H.; Kim, J. S.; Yoon, J. *J. Org. Chem.* **2004**, *69*, 5155–5157.
10. (a) Beer, P. D.; Szemes, F.; Balzani, V.; Salà, C. M.; Drew, M. G. B.; Dent, S. W.; Maestri, M. *J. Am. Chem. Soc.* **1997**, *119*, 11864–11875. (b) Deetz, M. J.; Smith, B. D. *Tetrahedron Lett.* **1998**, *39*, 6841–6844. (c) Watanabe, S.; Onogawa, O.; Komatsu, Y.; Yoshida, K. *J. Am. Chem. Soc.* **1998**, *120*, 229–230. (d) Beer, P. D.; Timoshenko, V.; Maestri, M.; Passaniti, P.; Balzani, V. *Chem. Commun.* **1999**, 1755–1756. (e) Watanabe, S.; Higashi, N.; Kobayashi, M.; Hamanaka, K.; Takata, Y.; Yoshida, K. *Tetrahedron Let.* **2000**, *41*, 4583–4586. (f) Padilla-Tosta, M. E.; Lloris, J. M.; Martínez--Máñez, R.; Pardo, T.; Soto, J.; Benito, A.; Marcos, M. D. *Inorg. Chem. Commun.* **2000**, *3*, 45–48.
11. For a review, see: Beer, P. D. *Acc. Chem. Res.* **1998**, *31*, 71–80.
12. X-ray structure analyses on guanidinium ion complexes with anions were reported: (a) Chertanova, L.; Pascard, C. *Acta Crystallogr.* **1996**, *B52*, 677–684. (b) Haj-Zaroubi, M.; Mitzel, N. W.; Schmidtchen, F. P. *Angew. Chem., Int. Ed.* **2002**, *41*, 104–107.
13. Kobiro, K.; Inoue, Y. *J. Am. Chem. Soc.* **2003**, *125*, 421–427.
14. Schug, K. A.; Lindner, W. *Chem. Rev.* **2005**, *105*, 67–114.
15. (a) Connors, K. A. *Binding constants. The measurement of molecular complex stability*; Wiley-Interscience: New York, 1987; pp 189–215. (b) Wilcox, C. S. In *Frontiers in supramolecular organic chemistry and photochemistry*; Schneider, H.-J., Dürr, H., Eds.; VCH: Weinheim, 1991; pp 123–143.
16. Hynes, M. J. *J. Chem. Soc., Dalton Trans.* **1993**, 311–312.
17. Precipitation occurred during ^{1}H NMR titration of **1** with $H_2PO_4^-$. The binding constant of $K_{1:1}$ was calculated, instead, by the use of titration data on CD shown in Figure 9 B.
18. Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902–3903.
19. Low affinity of HSO_4^- as compared with $H_2PO_4^-$ toward anion receptors is also reported in the literature.[6d,22b].
20. The UV–vis spectral titration method is a simple and convenient method to study interactions between hosts and guests. We applied UV–vis spectral titration technique to investigate complexation behavior of host **1** toward anions in CH_3CN, while the spectral change was too small to discuss in detail. Host **1** is instead CD active, because the DMAB group is connected to the chiral guanidinium ion moiety. Thus, we utilized CD spectra instead of UV–vis spetra to get complexation information through the DMAB group.
21. (a) Berova, N.; Nakanishi, K.; Woody, R. W. *Circular dichromism, principle and applications*, 2nd ed.; Wiley-VCH: New York, 2000. (b) Lightner, D. A.; Gurst, J. E. *Organic conformational analysis and stereochemistry from circular dichromism spectroscopy*; Wiley-VCH: New York, 2000.
22. (a) Cowley, D. J.; Healy, P. J. *Proc. R. Ir. Acad. Soc.* **1977**, *B77B*, 397. (b) Grabowski, Z. R.; Rotkiewicz, K.; Siemiarczuk, A.; Cowley, D. J.; Baumann, W. *Nouv. J. Chim.* **1979**, *3*, 443. (c) Rettig, W. *Angew. Chem., Int. Ed.* **1986**, *25*, 971. (d) Hayashi, R.; Tazuke, S.; Frank, C. W. *Macromolecules* **1987**, *20*, 983–988. (e) Tazuke, S.; Guo, R. K.; Hayashi, R. *Macromolecules* **1989**, *22*, 729–734. (f) Tazuke, S.; Guo, R. K.; Ikeda, T.; Ikeda, T. *Macromolecules* **1990**, *23*, 1208–1211. (g) Paczkowski, J.; Neckers, D. C. *Macromolecules* **1991**, *24*, 3013–3016. (h) Paczkowshi, J.; Neckers, D. C. *J. Polym. Sci., Polym. Chem.* **1993**, *31*, 841. (i) Rettig, W. In *Topics in current chemistry*; Mattay, J., Ed.; Springer: Berlin, 1994; Vol. 169, pp 253–299.
23. (a) Wu, F.-Y.; Jiang, Y.-B. *Chem. Phys. Lett.* **2002**, *355*, 438–444. (b) Wu, F.-Y.; Li, Z.; Wen, Z.-C.; Zhou, N.; Zhao, Y.-F.; Jiang, Y.-B. *Org. Lett.* **2002**, *4*, 3203–3205.
24. The LE intensity exhibited an intricate increase with increasing amount of $H_2PO_4^-$, while the TICT intensity increased slightly till the anion/host **1** ratio reached about 1 and, then, became almost flat. Relatively strong complexation of $H_2PO_4^-$ would lead to decrease of the LE intensity with increase of the TICT intensity. Judging from the increment of the TICT intensity, the decrease of the LE intensity seems to be very small. The expected slight decrease of the LE intensity could overlap with the large increase of the LE intensity which is caused by the active participation of the lipophilic TBA to show the observed intricate increase of the LE profile.

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Use of bis-(aminol) ethers derived from *N*-(*S*)-(−)-α-methylbenzylamine in reactions with resorcinarenes and double Mannich reactions

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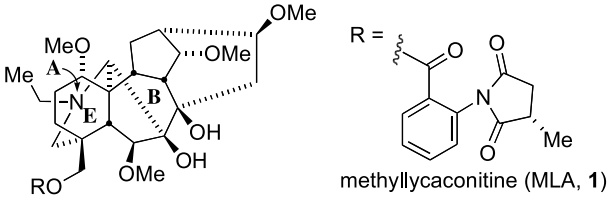
Abstract—The synthesis of some chiral bis-(aminol)ethers are described. Reaction of a solution of the resorcin[4]arene derived from propanal with *N*,*N*-bis(methoxymethyl)-*N*-(*S*)-(−)-α-methylbenzylamine in toluene at 85 °C initially afforded a 1:1 mixture of two diastereoisomeric tetrakis(benzoxazines). Further, heating of this mixture under reflux in ethanol for 24 h afforded the crystalline (α*S*),(*S*)-diastereoisomer in 77% yield. *N*,*N*-bis(ethoxymethyl)-*N*-(*S*)-(−)-α-methylbenzylamine and *N*,*N*-bis(ethoxymethyl)-*N*-(*R*)-(+)-α-methylbenzylamine were reacted with β keto esters to afford a 1:1 mixture of the diastereoisomeric double Mannich adducts. Two of the double Mannich adducts were converted into tricyclic ABE analogues of the alkaloid methyllycaconitine **1**.

1. Introduction

Bis-(aminol) ethers derived from primary amines are efficient *bis*-aminoalkylating agents used for the synthesis of tertiary amines upon reaction with a nucleophilic source. Our synthetic work in this area has focused on their use for the efficient preparation of secondary and tertiary amines using aromatic nucleophiles[1] and for the construction of the azabicyclo[3.3.1]nonane ring system present in the alkaloid methyllycaconitine **1**[2] via use of ethyl 2-oxocyclohexane1-carboxylate as the nucleophile in a double Mannich



methyllycaconitine (MLA, **1**)

reaction.[3] We herein report our studies on the use of chiral bis-(aminol) ether *N*,*N*-bis(methoxymethyl)-*N*-(*S*)-(−)-α-methylbenzylamine **2** as a bis-aminoalkylating agent in an effort to exert diastereocontrol in reactions to prepare tetrakis(benzoxazines) and the double Mannich reaction.

2. Results and discussion

Some time ago we[4] and others[5,6] showed that the reactions of formaldehyde and either (*R*)-(+)-α-methylbenzylamine or (*S*)-(−)-α-methylbenzylamine with a number of resorcin-[4]arenes in ethanol led to the formation of crystalline tetrakis(benzoxazines) with high diastereoselectivity and in high yield, also that the ^{1}H and ^{13}C NMR spectroscopic data for the two sets of diastereoisomers were identical and their optical rotation values were of opposite signs. These data established that the two sets of diastereoisomers were enantiomers. Further, we also showed that the bis-(aminol) ethers derived from either (*R*)-(+)-α-methylbenzylamine or (*S*)-(−)-α-methylbenzylamine together with methanol and paraformaldehyde gave the same diastereoisomeric tetrakis(benzoxazines) in high yield in ethanol.[7] The diastereoisomerization of the tetrakis(benzoxazines) in, for example, carefully purified and acid-free deuteriochloroform, must proceed via a series of iminium ions that are generated intramolecularly as a result of hydrogen bonding between the residual phenolic hydroxyl groups and the adjacent benzoxazine oxygen atoms and led to an approximately 2:1

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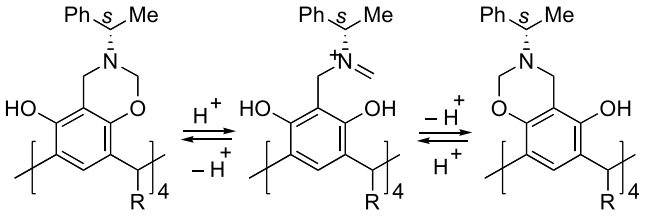


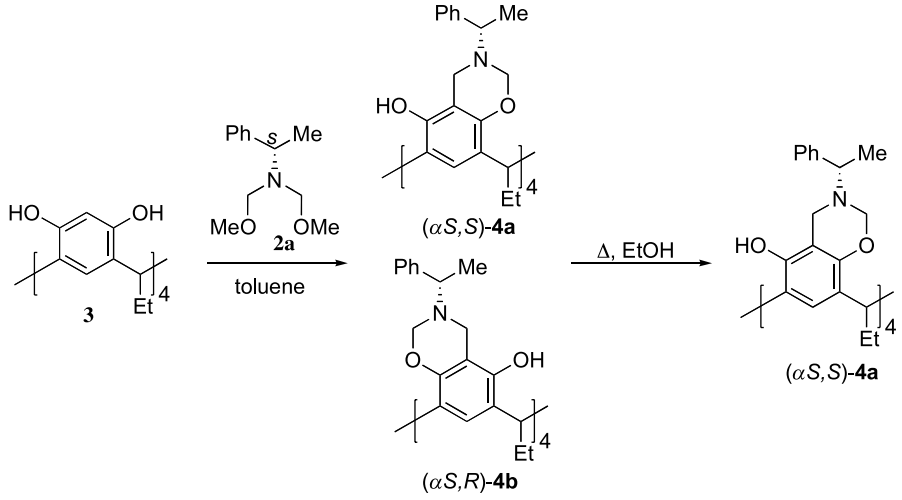
Scheme 1.

mixture of the previously isolated product together with its diastereoisomer (Scheme 1).[4]

We presumed that the reason for the diastereoselectivity in ethanol was related to the isolated products being the most thermodynamically stable stereoisomers as a result of intramolecular hydrogen bonding and gearing of the phenyl groups that are evident in the X-ray structure that we had obtained.[4] Böhmer[8] carried out an experiment of the dodecanal derived resorcin[4]arene with formaldehyde and (*R*)-(+)-α-methylbenzylamine in ethanol in which an aliquot was taken and quenched just as precipitation had started, that reaction established that the initial reaction involved the random formation of benzoxazines in equilibrium and that the observed diastereoselectivity resulted from the lower solubility of one of the two diastereoisomers. We had also carried out experiments that were designed to establish the reason for the preferred isolation of a single diastereoisomer in related reactions.[9]

We now present details of our experiments using *N*,*N*-bis(methoxymethyl)-*N*-(*S*)-(−)-α-methylbenzylamine **2a** (Scheme 2). When a solution of the resorcin[4]arene **3** derived from propanal was heated in toluene at 85 °C with *N*,*N*-bis(methoxymethyl)-*N*-(*S*)-(−)-α-methylbenzylamine **2a** the reaction mixture remained homogeneous and a ^{1}H NMR spectrum of an aliquot taken after a few hours showed that a complex mixture of products was present. The solvent was removed after 24 h and a portion of the residue was examined by ^{1}H NMR spectroscopy. The spectrum showed that the product was made up of an approximately 1:1 mixture of two diastereoisomers **4a**, **4b** related to those that had been observed in the diastereoisomerization reactions reported earlier,[4] together with a small amount of other regioisomers. The AB quartets for the methylene group(s) between the oxygen and nitrogen atoms are the diagnostic resonances and were observed for one diastereoisomer at δ_H 5.16 and 4.96 and for the second diastereoisomer at δ_H 5.02 and 4.74 ppm. Ethanol was then added to the oily mixture of products and the mixture was heated under reflux for a further, 24 h, after which time the cooled solution gave a crystalline product in 77% yield which was shown to be the (α*S*),(*S*)-diastereoisomer **4a** δ_H 5.16 and 4.96 for the O–CH_2–N AB quartet, by comparison with data from related compounds. These results establish that the tetrakis-(3,4-dihydro-2*H*−1,3-benzoxazines) are initially formed as a random mixture of regio- and diastereo-isomers and that isomerization under equilibrium reaction conditions leads eventually to the two thermodynamically favoured diastereoisomers, one of which crystallizes from ethanolic solution. We were interested to establish whether the equilibrium position for the two major diastereoisomers was solvent dependent, particularly in view of the observed ratios in deuteriochloroform (2:1) and toluene (1:1). A sample of the 1:1 mixture of diastereoisomers, obtained from the reaction carried out in toluene, was dissolved in [$^2H_4$]methanol and its ^{1}H NMR spectrum was taken after ca. 1.5 h. The ^{1}H NMR spectrum showed that the diastereoisomer that crystallized from ethanol was the major isomer present in solution, the second diastereoisomer was only present in a trace amount. These results support the view that, in solution, the position of the equilibrium between the two major diastereoisomers is dependent on the polarity of the solvent and that the isolation of a single diastereoisomer from an ethanolic solution results from a solvent–solute interaction that results in the equilibrium favouring its preferential formation both as a result of the hydrogen bonding between the residual phenolic hydroxyl groups and the adjacent benzoxazine oxygen atoms and the gearing of the phenyl residues, as observed previously in the X-ray crystal structures.

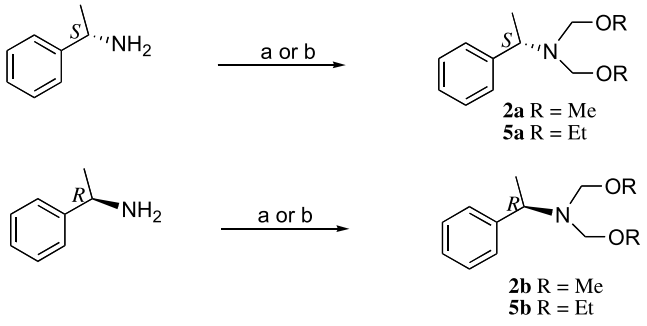
In light of the ability of *N*,*N*-bis(methoxymethyl)-*N*-(*S*)-(−)-α-methylbenzylamine **2a** to influence diastereocontrol in the above reaction with resorcinarene **3**, it was decided to investigate the ability of chiral bis-(aminol) ether **5** to exert



Scheme 2.

diastereocontrol in double Mannich reactions with cyclic β-keto esters. Use of ethyl 2-oxocyclohexane-1-carboxylate **6** in this latter reaction provides an efficient entry to the AE bicyclic ring system present in methyllycaconitine **1**, an alkaloid that binds with high affinity to the α7 nicotinic acetylcholine receptor.[10] A number of approaches to the synthesis of small molecule analogues of methyllycaconitine have been reported, including the synthesis of E,[11] AE[12] and AEF[13] ring systems, some of which display significant biological activity.[11,14] The present investigation provided an opportunity to construct the AE azabicyclo[3.3.1]nonane ring system in enantiopure form through implementation of an asymmetric variant of the key double Mannich reaction we had previously developed using bis-(aminol) ethers derived from achiral amines.

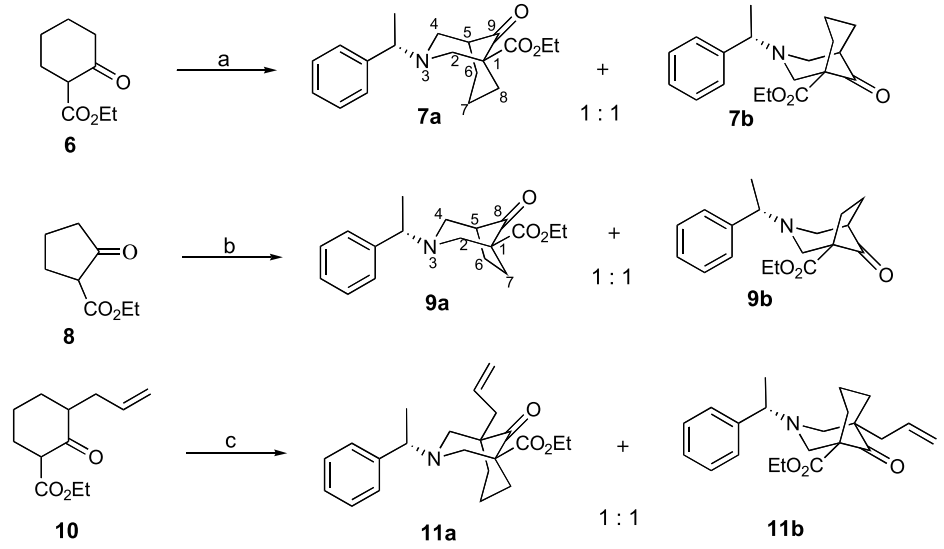
Chiral bis-(aminol)ethers **2a**, **2b** and **5a**, **5b** were prepared by reaction of (*S*)-(−)-α-methylbenzylamine or (*R*)-(+)-α-methylbenzylamine with potassium carbonate (1.0 equiv) and paraformaldehyde (2.0 equiv) in either methanol or ethanol for 48 h followed by purification by vacuum distillation (Scheme 3). The double Mannich reaction of chiral bis-(aminol) ether **5a** with keto ester **6** was carried out in an analogous manner to the racemic work.[3] Thus, a mixture of β-keto ester **6** and bis-(aminol)ether **5a** (2.0 equiv) in acetonitrile was treated with trichloromethylsilane (2.0 equiv) with stirring for 20 h (Scheme 4). It was envisaged that the reaction would result in the formation of a mixture of two diastereomeric bicyclic ketones **7a**, **7b** which would then be separated by flash chromatography and further, elaborated to provide the azabicyclo[3.3.1]nonane ring system in enantiopure AE analogue of MLA after removal of the chiral amine group. Disappointingly the two diastereomeric double Mannich products **7a**, **7b** only appeared as a single spot upon analysis by TLC using several solvent systems. Numerous attempts to separate the diastereomers by flash chromatography and HPLC were fruitless. The double Mannich product albeit as a 1:1 mixture of diastereomers **7a**, **7b** was afforded as a colourless oil in 76% yield.



Scheme 3. Reagents, conditions and yields: (a) K_2CO_3, CH_2O, MeOH, 48 h, **2a**, 65%; **2b**, 66%. (b) K_2CO_3, CH_2O, EtOH, 48 h, **5a**, 40%; **5b**, 22%.

A similar reaction of the five membered β-keto ester **8** with bis-(aminol) ether **5a** afforded an inseparable 1:1 mixture of the diastereoisomeric adducts **9a**, **9b** in 74% yield. It was next, decided to investigate the use of allylated keto ester **10** in the double Mannich reaction with bis-(aminol) ether **2a** hoping that the presence of an allyl side chain would afford separable diastereomeric adducts **11a**, **11b**. As it transpired the adducts **11a**, **11b** were afforded in 77% yield also as an inseparable 1:1 mixture. Similar yields were obtained for the analogous reaction of the enantiomeric chiral bis-(aminol) ether **5b** with β-keto esters **6**, **8** and **10**.

The structures of the adducts **7a**, **7b** were supported by a molecular ion at *m/z* 315.1830 (M^+) in the high resolution mass spectrum, corresponding to the molecular formula $C_{19}H_{25}NO_3$. The ^1H NMR spectrum of a basic 3-azabicyclo[3.3.1]nonane ring system is complex yet distinct due to the non-equivalence of the methylene protons in the bicyclic ring system. The ^1H NMR spectrum of bicyclic ketones **7a**, **7b** was further, complicated due to the doubling of signals resulting from the presence of a 1:1 diastereomeric mixture of products. Using 2D COSY and heteronuclear ^1H-^{13}C correlated NMR spectra it was possible to completely assign all protons and carbons relating to either diasteromeric adduct **7a** or **7b**. Using data obtained through structural studies performed by Arias-Pérez



Scheme 4. Reagents, conditions and yields: (a) **5a**, $SiCl_3Me$, acetonitrile, 20 h, **7a**:**7b** (1:1), 76%; (b) **5a**, $SiCl_3Me$, acetonitrile, 20 h, **9a**:**9b** (1:1), 74%; (c) **5a**, $SiCl_3Me$, acetonitrile, 20 h, **11a**:**11b** (1:1), 77%.

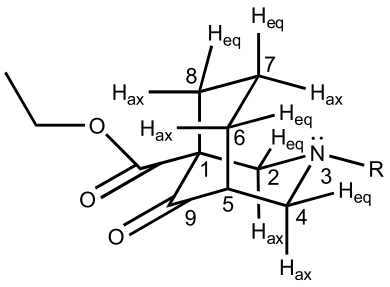


Figure 1. Conformation of the 3-azabicyclo[3.3.1]nonane-1-carboxylate ring system.

et al[15] it was possible to assign the protons on the bicyclic ring to axial or equatorial positions (Fig. 1).

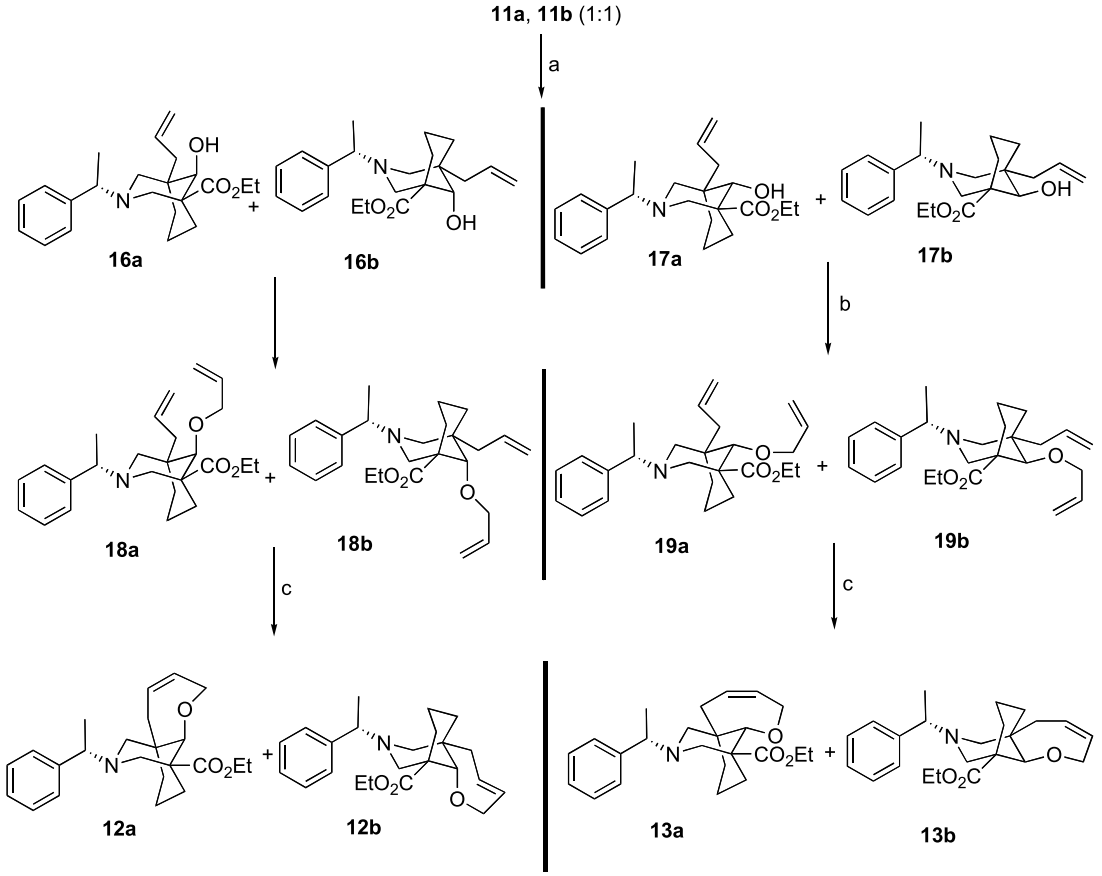
The axial proton attached to C-7 at δ_H 2.83–3.00 resonates further, downfield than the equatorial proton positioned on the same carbon that resonates at δ_H 1.51–1.69, due to the intense deshielding effect by the nitrogen lone pair that lies in close proximity to the axial proton.[15] A doublet of doublets at δ_H 2.88 (J=1.6, 11.7 Hz) was assigned to 2-H_{ax} and a multiplet at δ_H 2.98–3.02 was assigned to 4-H_{eq} and 2-H_{ax}*. The deshielded doublet of doublets at δ_H 3.10 (J= 2.3, 11.7 Hz) was assigned to 2-H_{eq}, while the doublet of doublets of doublets at δ_H 3.30 (J=2.3, 2.3, 11.0 Hz) was assigned to 4-H_{eq}*. The doublet of doublets at δ_H 3.36 (J= 2.7, 11.3 Hz) was assigned to the more deshielded 2-H_{eq}*.

The axial protons of 2-H_{ax} and 4-H_{ax} are more shielded than the equatorial protons 2-H_{eq} and 4-H_{eq} due to delocalisation of the nitrogen lone pair electrons into the *trans*-coplanar C–H bond[15] (Fig. 1). The assignment of the 4-H and 2-H protons is usually derived from the four-bond coupling constants; however, the presence of a diastereomeric mixture in this case complicated the assignment.

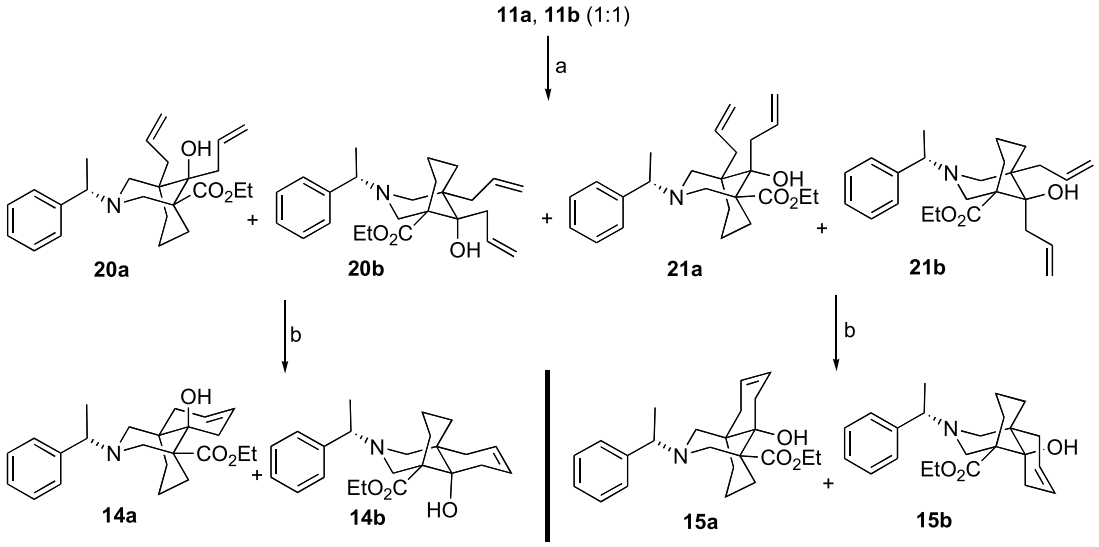
Given that the two diastereomers of the double Mannich adduct **11a**, **11b** were also not separable by flash chromatography, it was decided to pursue the synthesis of the oxygen containing tricyclic analogues of methyllycaconitine **12a/12b** and **13a/13b** (Scheme 5) and the all carbon tricyclic analogues of methyllycaconitine **14a/14b**, **15a/15b** (Scheme 6) with the hope that separation of the individual diastereoisomers would be possible at a later stage in the synthesis.

The next, step in the synthesis of the ABE tricyclic analogues **12a/12b** and **13a/13b** involved reduction of the allyl substituted bicyclic ketones **11a**, **11b** using sodium borohydride in aqueous THF. After careful purification by flash chromatography the major alcohols **16a**, **16b**, were afforded in 39% yield as an inseparable 1:1 mixture of the two diastereomers together with a 1:1 mixture of the minor alcohols **17a**, **17b** isolated in 32% yield.

The ^{1}H NMR spectra of the major **16a**, **16b** and minor alcohols **17a**, **17b** displayed a broad singlet at δ_H 3.61



Scheme 5. Reagents, conditions and yields: (a) $NaBH_4$, 1:1 THF–H_2O, 2 h, **16a**:**16b** (1:1), 39%; **17a**:**17b** (1:1), 32%; (b) NaH, 0 °C to room temperature, THF, 30 min, allyl bromide, THF, 48 h, **18a**:**8b** (1:1), 68%; **19a**:**19b** (1:1), 67%; (c) $Cl_2(PCy_3)_3Ru$=CHPh (5 mol %), CH_2Cl_2, 48 h, **12a**:**12b** (1:1), 95%; **13a**:**13b** (1:1), 95%.



Scheme 6. Reagents, conditions and yields: (a) allyl magnesium bromide, 0 °C to room temperature, 30 min, THF, **20a**:**20b**: **21a**:**21b** (1:1:1:1), 74%; (b) $Cl_2(PCy_3)_3Ru{=}CHPh$ (10 mol %), CH_2Cl_2, 48 h, **14a**:**14b** (1:1), 41%; **15a**:**15b** (1:1), 36%.

(major) and δ_H 2.72 (minor), assigned to the hydroxyl protons. Doublets at δ_H 3.10 (major) and δ_H 3.69 (minor) were assigned to the methine C*H*OH proton. The ^{13}C NMR data was used to assign the stereochemistry of the 9-OH group. The γ-gauche effect establishes that the alcohols with a *syn* hydroxyl group experiences an upfield shift of the neighbouring bicyclic ring carbons in the ^{13}C NMR spectrum.[16] Using this analysis the stereochemistry of alcohols **16a**, **16b** was established to be (1*R**, 5*R**, 9*R**) and hence the stereochemistry of alcohols **17a**, **17b** was established to be (1*R**, 5*R**, 9*S**).

The next, step in the synthesis of tricyclic methyllycaconitine analogues **12a**, **12b** required the allylation of alcohols **16a**, **16b** by treatment with sodium hydride and allyl bromide to afford the allyl ethers **18a**, **18b** in 67% yield as a 1:1 mixture of diastereomers. Allylation of the 1:1 mixture of the minor alcohols **17a**, **17b** in a similar fashion afforded the desired allyl ethers **19a**, **19b**, in 68% yield.

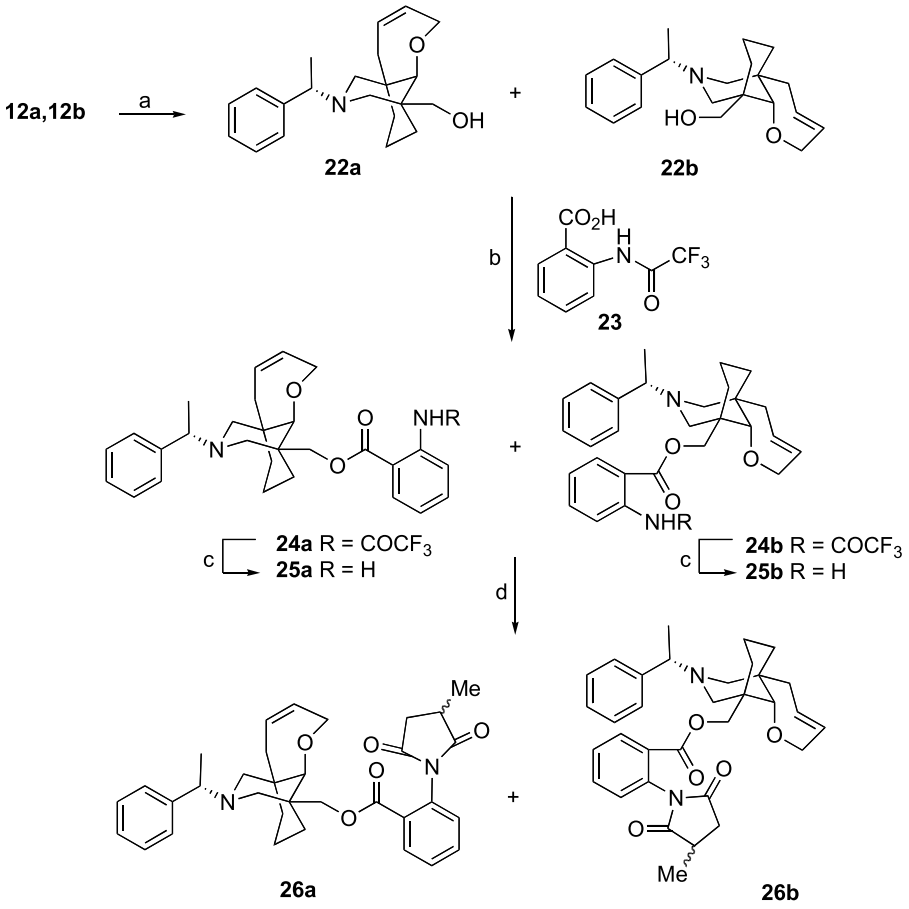
With the necessary diene functionality installed in the AE bicyclic dienes **18a**, **18b** and **19a**, **19b**, subsequent ring closing metathesis using Grubbs' first, generation catalyst was undertaken. Dienes **18a**, **18b** (1:1 mixture) were treated with 5 mol % Grubbs' catalyst in dichloromethane at room temperature for 22 h. Work-up and chromatography afforded the tricyclic ethers **12a**, **12b** in 95% yield as a 1:1 mixture of diastereomers. Subsequent treatment of dienes **19a**, **19b** with 5 mol % Grubbs' catalyst afforded tricyclic ethers **13a**, **13b** in 95% yield also as a 1:1 mixture of diastereomers.

It was hoped that the diastereomeric adducts of the azabicyclic ring system could be separated during one of the steps in the conversion of the allyl keto esters **11a**, **11b** to the tricyclic compounds **12a**, **12b** and **13a**, **13b**. Disappointingly, no separation of the individual isomers by flash chromatography was possible in any of the synthetic steps undertaken. Attempts to separate the diastereomeric mixture of tricyclic ethers **12a**, **12b** by HPLC were also unsuccessful. At this stage it was therefore, decided to investigate the synthesis of the six-membered all carbon tricyclic analogues of methyllycaconitine **14a**, **14b** and **15a**, **15b** as outlined in Scheme 6.

The first, step involved treatment of bicyclic ketones **11a**, **11b** with allyl magnesium bromide in THF to afford an inseparable 1:1:1:1 mixture of the four possible isomeric alcohols **20a**, **20b** and **21a**, **21b** in 74% yield. Ring closing metathesis of the 1:1:1:1 mixture of isomeric dienes **20a/20b**, **21a/21b** was next, carried out by treatment with 10 mol % Grubbs' catalyst under reflux in dichloromethane for 24 h to afford the ABE tricyclic carbon analogues of methyllycaconitine **14a**, **14b** and **15a**, **15b**. After work-up of the reaction mixture careful separation by flash chromatography afforded the tricyclic ABE analogues **14a**, **14b** and **15a**, **15b** in 41% and 36% yield respectively, both as a 1:1 mixture of diastereomers.

It was next, decided to continue with the attachment of the methylsuccinimido anthranilate ester pharmacophore to the ether containing ABE analogues **12a**, **12b** with the hope that later separation of the individual diastereomers would be possible when further, functionality had been added to the molecule (Scheme 7).

Reduction of the ethyl esters **12a**, **12b** was performed by treatment with lithium aluminium hydride in THF affording alcohols **22a**, **22b** in 85% yield. The next, step involved the attachment of the pharmacophore, using *N*-(trifluoroacetyl)-anthranilic acid **23** following a well established protocol.[3] Alcohols **22a**, **22b** were treated with *N*-(trifluoroacetyl)-anthranilic acid **23**, DMAP and DCC in acetonitrile to afford trifluoroacetyl anthranilate esters **24a**, **24b** that were subsequently treated with sodium borohydride to afford anthranilates **25a**, **25b**. Finally fusion with methylsuccinimide afforded the substituted methyllycaconitine analogues **26a**, **26b**. Disappointingly the individual diastereomers were not able to be separated during any of the steps in the further, conversion of esters **12a**, **12b** to methylsuccinimido anthranilate esters **26a**, **26b**.



Scheme 7. Reagents, conditions and yields: (a) $LiAlH_4$, THF, 24 h, **22a**:**22b** (1:1), 86%; (b) **23**, DCC, DMAP, CH_3CN, **24a**:**24b** (1:1), 53%; (c) $NaBH_4$, EtOH, **25a**: **25b** (1:1), 82%; (d) methylsuccinic anhydride, 125 °C, **26a**:**26b** (1:1), 55%.

In summary, the synthesis of four analogues of the alkaloid methyllycaconitine albeit as a 1:1 mixture of diastereomers was successfully completed. Tricyclic ABE analogues **12a**, **12b** and **13a**, **13b** contained a seven membered ether B ring whereas tricyclic analogues **14a**, **14b** and **15a**, **15b** contained a six membered all carbon B ring. The AE azabicyclo[3.3.1]nonane ring system was constructed using a key double Mannich reaction starting from a chiral bis(aminol)ether. The attempted asymmetric variant of the double Mannich reaction was the first, example of the use of a chiral bis(aminol)ether as an electrophile in a reaction with a cyclic β-keto ester, however, disappointingly no diastereomeric excess was observed.

3. Experimental

3.1. General details

All reactions were conducted in flame-dried or oven-dried glassware under a dry nitrogen atmosphere unless otherwise noted. Tetrahydrofuran was dried over sodium/benzophenone and distilled prior to use. Flash chromatography was performed using Merck Kieselgel 60 (230–400 mesh) with the indicated solvents. Thin layer chromatography (TLC) was carried out on precoated silica plates (Merck Kieselgel $60F_{254}$) and compounds were visualized by UV fluorescence or by staining with vanillin in methanolic sulfuric acid and heating. Infrared spectra were recorded with a Perkin–Elmer 1600 series Fourier-transform infrared spectrometer as thin films between sodium chloride plates. Absorption maxima are expressed in wavenumbers (cm^{-1}) with the following abbreviations: s=strong, m=medium, w=weak and br=broad. ^1H and ^{13}C NMR spectra were obtained using Bruker AC 200B, AM 400, and DPX400 spectrometers and a JEOL EX400 spectrometer. All chemical shifts are given in parts per million (ppm) downfield from tetramethylsilane as internal standard (^1H) or relative to $CDCl_3$ (^{13}C) and *J* values are given in Hz. ^1H NMR data are tabulated as s, singlet; d, doublet; t, triplet; q, quartet: m, multiplet, br, broad. High resolution mass spectra were recorded using a VG70-SE spectrometer operating at nominal accelerating voltage of 70 eV. Chemical ionisation (CI) mass spectra were obtained with ammonia as the reagent gas.

3.2. Standard procedure for the formation of *N*,*N*-bis(alkoxymethyl)amines

Dry potassium carbonate (1 mol equiv) was added to a mixture of dry amine (1 mol equiv) and anhydrous ethanol or methanol (4 mol equiv). Dry paraformaldehyde (2 mol equiv) was added and the resulting mixture stirred for 48 h. The suspension was filtered to remove all solids. Excess ethanol or methanol was removed by distillation at ambient pressure. The crude product was purified by vacuum

distillation to afford the *N*,*N*-bis(alkoxymethyl)amine as a colourless oil.

3.2.1. *N*,*N*-Bis(methoxymethyl)-*N*-(*R*)-α-methylbenzylamine 2b. The reaction was carried out according to the standard procedure using dry (*R*)-α-methylbenzylamine (5.32 mL, 41.3 mmol), anhydrous methanol (6.7 mL, 165 mmol), potassium carbonate (5.70 g, 41.26 mmol) and paraformaldehyde (2.47 g, 82.5 mmol). The crude product was purified by Kugelrohr distillation to afford the title compound **2b** (5.69 g, 66%) as a colourless oil; $[\alpha]_D+4.5$ (*c* 1.32, $CHCl_3$); (Found: M^+, 209.1412, $C_{12}H_{19}NO_2$ requires 209.1416); ν_{max} (neat)/cm^{-1} 3509 (C=H), 2924, 1492 (C=C, Ar), 1382 (C=H), 1066 (C=O, ether); δ_H (400 MHz, $CDCl_3$), 1.54 (3H, d, $J=6.7$ Hz, CH_3), 3.08 (6H, s, OCH_3) 4.20 (2H, d, $J=9.8$ Hz, NCH_2O), 4.28 (2H, d, $J=9.8$ Hz, NCH_2O), 4.29 (1H, q, $J=6.7$ Hz, CHN), 7.24–7.28 (5H, m, PhH); δ_C (100 MHz, $CDCl_3$), 20.3 (CH_3, CH*C*H$_3$), 54.7 (OCH_3), 57.7 (CH, Ph*C*H), 84.6 (NCH_2O), 126.6 (CH, C-4), 127.5 (CH, C-3, C-5), 128.3 (CH, C-2, C-6), 144.2 (quat., C-1); *m*/*z* (EI, %) 209 (M^+, 1).

3.2.2. *N*,*N*-Bis(ethoxymethyl)-*N*-(*S*)-α-methylbenzylamine 5a. The reaction was carried out according to the standard procedure using dry (*S*)-α-methylbenzylamine (15.0 g, 123.8 mmol), anhydrous ethanol (27.9 mL, 22.8 g, 495.1 mmol), potassium carbonate (17.1 g, 123.8 mmol) and paraformaldehyde (7.4 g, 247.6 mmol). The crude product was purified by vacuum distillation to afford the title compound **5a** (11.7 g, 40%) as a colourless oil (bp 95–105 °C at 0.75 mmHg); $[\alpha]_D^{22}-15.8$ (*c* 2.08, $CHCl_3$); (Found: M^+, 237.1727, $C_{14}H_{23}NO_2$ requires 237.1729); ν_{max} (NaCl)/cm^{-1} 2973 (C=H), 1453 (C=C, Ar), 1376 (C=H), 1072 (C–O, ether); δ_H (400 MHz, $CDCl_3$) 1.15 (6H, t, $J=7.0$ Hz, 2×CH_2CH_3), 1.48 (3H, q, 6.9 Hz, CHCH_3), 3.34–3.40 (4H, m, 2×OCH_2CH$_3$), 4.25 (1H, q, $J=7.0$ Hz, CH*N*), 4.29 (4H, s, 2×NCH_2O), 7.24–7.38 (5H, m, PhH); δ_C (100 MHz, $CDCl_3$) 15.2 (2×CH_3, CH_2CH_3), 20.2 (CH_3, CH*C*H$_3$), 57.0 (CH, Ph*C*H), 62.3 (2×CH_2, O*C*H$_2$CH$_3$), 82.3 (NCH_2O), 126.8 (CH, C-4), 127.5 (CH, C-3, C-5), 128.1 (CH, C-2, C-6), 144.5 (quat., C-1); *m*/*z* (EI, %) 237 (M^+, 1), 192 (10), 118 (11), 105 (100), 91 (11), 79 (12), 77 (12).

3.2.3. Synthesis of tetrakis-(3,4-dihydro-2*H*-1,3-benzoxazine) 4a. A solution of *C*-ethylcalix[4]resorcinarene **3** (500 mg, 0.83 mmol) and *N*,*N*-bis(methoxymethyl)-*N*-(*S*)-α-methylbenzylamine **2a** (710 mg, 3.4 mmol) in toluene (20 mL) was heated at 85 °C for 24 h. After this time the reaction mixture was allowed to cool to room temperature and the solvent removed under reduced pressure to give a pale brown oil. A sample was submitted for analysis by ^1H NMR spectroscopy and showed the presence of resonances at δ_H 5.16 and 4.96 and at δ_H 5.02 and 4.74 ppm in a 1:1 ratio. Ethanol (20 mL) was added to the mixture which was then heated under reflux for a further, 24 h. After this time the reaction mixture was cooled using an ice-bath and the resulting off-white precipatate was collected by filtration and washed with cold anhydrous ethanol to give the tetrakis(3-[1*S*)-1-phenylethyl]-3,4-dihydro-2H-1,3-benzoxazine) derivative **4a** (720 mg, 77%); $[\alpha]_D^{22}$ −102.2 (*c* 1.19, $CHCl_3$); (Found: C=76.86, H=7.16, N=4.77% $C_{76}H_{84}N_4O_8$ requires C=77.26, H=7.17, N=4.74%); ν_{max} (thinfilm) 3355, 3034, 2962, 2928, 2870, 1601, 1468, 885; δ_H (400 MHz, $CDCl_3$) 0.95 (12H, t, $J=6.8$ Hz, –CH_2CH_3), 1.33 (12H, d, $J=6.4$ Hz, ArCHCH_3), 2.24 (8H, m, CHCH_2CH$_3$), 3.75 (4H, d, $J_{AB}=17.6$ Hz, NCH_A-H_BAr), 3.83 (4H, q, $J=6.0$ Hz, C*H*CH$_3$), 4.01 (4H, d, $J_{AB}=17.6$ Hz, NCH_AH_BAr), 4.14 (4H, t, $J=7.6$ Hz, C*H*CH$_2$), 4.96 (4H, d, $J_{AB}=10.0$ Hz, NCH_AH_BO), 5.16 (4H, d, $J_{AB}=10.0$ Hz, NCH_AH_BO), 6.96–7.21 (24H, m, ArH) and 7.71 (4H, s, ArOH); δ_C (100 MHz, $CDCl_3$) 12.73 (CH_3), 21.47 (CH_3), 26.76 (CH), 34.85 (CH_2), 44.58 (CH), 58.06 (CH_2), 80.93 (CH_2), 108.98 (C), 121.00 (CH), 123.38 (C), 124.14 (C), 127.04 (CH), 127.07 (CH), 128.25 (CH), 144.55 (C), 148.84 (C) 149.70 (C).

3.3. General procedure for the double mannich reaction

To a mixture of β-ketoester (1 mol equiv) and *N*,*N*-bis(ethoxymethyl)amine (2 mol equiv) in acetonitrile (2 mL) was added trichloromethylsilane (2 mol equiv). The mixture was stirred under an atmosphere of nitrogen at room temperature for 20 h. The reaction was quenched by the addition of sat. $NaHCO_3$ (20 mL) and the aqueous layer extracted with ethyl acetate (3×20 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. The crude product was purified by flash chromatography to afford the double Mannich adduct.

3.3.1. Ethyl (1*R,5*R**)-3-((*S*)-1-methylbenzyl)-9-oxo-3-azabicyclo[3.3.1]nonane-1-carboxylate 7a, 7b.** The reaction was carried out according to the standard procedure using ethyl 2-oxo-cyclohexane-1-carboxylate **6** (100 mg, 0.59 mmol), *N*,*N*-bis(ethoxymethyl)-(*S*)-α-methylbenzylamine **5a** (280 mg, 1.17 mmol) and trichloromethylsilane (140 mL, 1.17 mmol). Use of 19:1 hexane–ethyl acetate as eluent for flash chromatography afforded the title compounds **7a**, **7b** (141 mg, 76%) as a colourless oil and as a 1:1 mixture of diastereomers; (Found: M^+, 315.1830, $C_{19}H_{25}NO_3$ requires 315.1834); ν_{max} (NaCl)/cm^{-1} 1732 (C=O, ester), 1716 (C=O, ketone), 1453 (C=C, Ar), 1257 (*tert*-N, amine); δ_H (300 MHz, $CDCl_3$) 1.22–1.33 (3H, m, OCH_2CH_3 and OCH_2CH_3*), 1.39–1.42 (3H, m, CHCH_3Ph and CHCH_3Ph*), 1.51–1.69 (1H, m, 7-H_{eq}, 7-H_{eq}*), 2.01–2.14 (1H, m, 6-H_{ax}, 6-H_{eq}), 2.15–2.26 (1.5H, m, 6-H_{ax}*, 6-H_{eq}*, 8-H_{eq}), 2.27–2.32 (0.5H, m, 8-H_{eq}*), 2.34–2.41 (0.5H, m, 5-H), 2.44–2.60 (2H, m, 8-H_{ax}, 8-H_{ax}*, 5-H*, 4-H_{ax}), 2.64 (0.5H, dd, $J=2.3$, 11.0 Hz, 4-H_{ax}*), 2.83–3.00 (1H, m, 7-H_{ax},7-H_{ax}*), 2.88 (0.5H, dd, $J=1.6$, 11.7 Hz, 2-H_{ax}), 2.98–3.03 (1H, m, 4-H_{eq}, 2-H_{ax}*), 3.10 (0.5H, dd, $J=2.3$, 11.7 Hz, 2-H_{eq}), 3.30 (0.5H, ddd, $J=2.3$, 2.3, 11.0 Hz, 4-H_{eq}*), 3.36 (0.5H, dd, $J=2.7$, 11.3 Hz, 2-H_{eq}*), 3.42 (1H, q, $J=6.7$ Hz, C*H*CH$_3$Ph), 4.10–4.20 (1H, m, OCH_2CH$_3$), 4.18–4.25 (1H, m, OCH_2CH$_3$*), 7.23–7.34 (5H, m, Ar-H, Ar-H*); δ_C (75 MHz, $CDCl_3$), 14.0 (CH_3, OCH_2CH_3), 14.1 (CH_3, OCH_2CH_3*), 19.7 (CH_3, CH*C*H$_3$-Ph), 19.9 (CH_3, CH*C*H$_3$Ph*), 20.6 (CH_2, C-7), 20.7 (CH_2, C-7*), 33.7 (CH_2, C-6), 34.1 (CH_2, C-6*), 36.3 (CH_2, C-8), 36.7 (CH_2, C-8*), 47.1 (2×CH, C-5, C-5*), 56.9 (CH_2, C-4), 58.3 (CH_2, C-4*), 58.8 (CH_2, C-2), 60.0 (CH_2, C-2*), 61.0 (CH_2, O*C*H$_2$CH$_3$), 61.1 (CH_2, O*C*H$_2$CH$_3$*), 64.2 (2×CH, *C*HCH$_3$Ph, *C*HCH$_3$Ph*), 127.1, 127.2, 127.3, 128.4, 128.5 (10×CH, Ar, Ar*), 143.8 (quat., Ar), 143.9 (quat., Ar*), 171.1 (quat., OC=O), 171.2 (quat., OC=O*), 212.7

(quat., C-9), 212.8 (quat., C-9*); *m/z* (EI, %) 315 (M^+,10), 300 (22), 298 (27), 272 (21), 105 (100), 79 (13), 44 (22).

3.3.2. Ethyl (1*R,5*R**)-3-((*S*)-1-methylbenzyl)-9-oxo-3-azabicyclo[3.2.1]octane-1-carboxylate 9a, 9b.** The reaction was carried out according to the standard procedure using ethyl 2-oxo-cyclopentane-1-carboxylate **8** (100 mg, 0.64 mmol), *N*,*N*-bis(ethoxymethyl)-(*S*)-α-methylbenzylamine **5a** (300 mg, 1.28 mmol) and trichloromethylsilane (150 μL, 1.28 mmol). Use of 19:1 hexane–ethyl acetate as eluent for flash chromatography afforded the title compounds **9a**, **9b** (131 mg, 74%) as a colourless oil and as a 1:1 mixture of diastereomers; (Found: M^+, 301.1679, $C_{18}H_{23}NO_3$ requires 301.1678); ν_{max} (NaCl)/cm^{-1} 1759 (C=O, ester), 1726 (C=O, ketone), 1453 (C=C, Ar), 1262 (*tert*-N, amine); δ_H (400 MHz, $CDCl_3$) 1.22–1.28 (3H, m, OCH_2CH_3 and OCH_2CH_3*), 1.37–1.41 (3H, m, $CHCH_3Ph$ and $CHCH_3Ph$*), 1.88–2.01 (2H, m, 7-H_A, 7-H_A*, 7-H_B, 7-H_B*), 2.23–2.31 (1.5H, m, 5-H, 6-H_B, 6-H_B*), 2.33–2.42 (1.5H, m, 5-H*, 6-H_A, 6-H_A*), 2.51 (0.5H, d, J=11.0 Hz, 4-H_{ax}), 2.58 (0.5H, d, J=11.0 Hz, 4-H_{ax}*), 2.74–2.79 (1H, m, 2-H_A, 2-H_{ax}*), 2.99 (0.5H, ddd, J=3.5, 3.5, 11.0 Hz, 4-H_{eq}), 3.01 (0.5H, ddd, J=2.8, 2.8, 11.0 Hz, 4-H_{eq}*), 3.08 (0.5H, dd, J=2.8, 11.0 Hz, 2-H_{eq}), 3.21 (0.5H, dd, J=2.8, 11.0 Hz, 2-H_{eq}*), 3.68 (1H, dq, J=2.7, 11.0 Hz, C*H*CH_3-Ph), 4.15–4.22 (2H, m, OCH_2CH_3, OCH_2CH_3*), 7.23–7.36 (5H, m, Ar-H, Ar-H*); δ_C (100 MHz, $CDCl_3$), 14.2 (2×CH_3, OCH_2CH_3*), 18.7 (CH_3, $CHCH_3Ph$), 19.1 (CH_3, $CHCH_3Ph$*), 21.7 (2×CH_2, C-7, C-7*), 27.4 (CH_2, C-6), 27.5 (CH_2, C-6*), 46.6 (2×CH, C-5, C-5*), 57.3 (CH_2, C-4), 58.8 (CH_2, C-2), 59.6 (CH_2, C-4*), 60.6 (CH_2, C-2*), 60.9 (CH_2, OCH_2CH_3), 61.9 (CH, $CHCH_3Ph$), 62.2 (CH, $CHCH_3Ph$*), 127.1, 127.2, 127.3, 128.3, 128.4 (10×CH, Ar, Ar*), 143.5 (quat., Ar), 143.6 (quat., Ar*), 170.2 (quat., OC=O), 170.4 (quat., OC=O*), 213.5 (quat., C-8, C-8*); *m/z* (EI, %) 301 (M^+, 12), 286 (17), 105 (100), 79 (13), 77 (12), 57 (15), 44 (30).

3.3.3. Ethyl (1*R,5*R**)-3-((*S*)-1-methylbenzyl)-9-oxo-5-(2′-propenyl)-3-azabicyclo[3.3.1]nonane-1-carboxylate 11a, 11b.** The reaction was carried out according to the standard procedure using ethyl 2-oxo-3-(2′-propenyl)cyclohexane-1-carboxylate **10**[17] (100 mg, 0.51 mmol), *N*,*N*-bis(ethoxymethyl)-(*S*)-α-methylbenzylamine **5a** (240 mg, 1.02 mmol), and trichloromethylsilane (110 μL, 1.02 mmol). Use of 19:1 hexane–ethyl acetate as eluent for flash chromatography afforded the title compounds **11a**, **11b** (0.14 g, 77%) as a colourless oil and as a 1:1 mixture of diastereomers; (Found: M^+, 355.2148, $C_{22}H_{29}NO_3$ requires 355.2147); ν_{max} (NaCl)/cm^{-1} 1732 (C=O, ester), 1714 (C=O, ketone), 1639 (C=C), 1454 (C=C, Ar), 1259 (*tert*-N, amine); δ_H (300 MHz, $CDCl_3$) 1.26 (3H, m, OCH_2CH_3 and OCH_2CH_3*), 1.39 (3H, dq, J=1.9, 6.7 Hz, $CHCH_3Ph$ and $CHCH_3Ph$*), 1.48–1.62 (1H, m, 7-H_{eq}, 7-H_{eq}*), 1.71–1.89 (1H, m, 6-H_{eq}, 6-H_{eq}*), 1.95–2.07 (1H, m, 6-H_{ax}, 6-H_{ax}*), 2.09–2.20 (3H, m, 1′-CH_2, 1′-CH_2*, 8-H_{eq}, 8-H_{eq}*), 2.22–2.30 (0.5H, dd, J=1.6, 11.0 Hz, 4-H_{ax}), 2.35 (0.5H, dd, J=1.4, 11.0 Hz, 4-H_{ax}*), 2.44–2.58 (1H, m, 8-H_{ax}, 8-H_{ax}*), 2.84–2.92 (1H, m, 2-H_{ax}, 4-H_{eq}), 2.92–3.02 (1H, m, 7-H_{ax}, 7-H_{ax}*), 2.99–3.04 (0.5H, m, 2-H_{ax}*), 3.08 (0.5H, dd, J=2.5, 11.0 Hz, 2-H_{eq}), 3.18 (0.5H, dd, J=2.5, 11.0 Hz, 4-H_{eq}*), 3.28 (0.5H, dd, J=2.4, 11.0 Hz, 2-H_{eq}*), 3.38 (0.5H, q, J=6.7 Hz, C*H*CH_3Ph), 3.47 (0.5H, q, J=6.7 Hz, C*H*CH_3Ph*), 4.17 (2H, m, OCH_2CH_3, OCH_2CH_3*), 4.95–4.98 (1H, m, 3′-H_B, 3′-H_B*), 5.03–5.06 (1H, m, 3′-H_A, 3′-H_A*), 5.62–5.86 (1H, m, 2′-H, 2′-H*), 7.23–7.34 (5H, m, Ar-H, Ar-H*); δ_C (75 MHz, $CDCl_3$), 14.0 (CH_3, OCH_2CH_3), 14.1 (CH_3, OCH_2CH_3*), 18.9 (CH_3, $CHCH_3Ph$), 20.1 (CH_3, $CHCH_3Ph$*), 20.5 (2×CH_2, C-7, C-7*), 36.3 (CH_2, C-8), 36.6 (CH_2, C-8*), 38.7 (CH_2, C-6), 39.1 (quat., C-5), 39.3 (CH_2, C-1′), 39.4 (CH_2, C-1′*), 48.8 (quat., C-1), 48.9 (quat., C-1*), 58.6 (CH_2, C-2), 59.0 (CH_2, C-2*), 60.9 (CH_2, OCH_2CH_3), 61.0 (CH_2, OCH_2CH_3*), 61.7 (CH_2, C-4), 62.8 (CH_2, C-4*), 63.9 (CH, $CHCH_3Ph$), 64.3 (CH, $CHCH_3Ph$*), 117.8 (CH_2, C-3′), 118.0 (CH_2, C-3′*), 127.0, 127.2, 127.3, 128.3, 128.4 (10×CH, Ar, Ar*), 133.7 (2×CH, C-2′, C-2′*), 143.4 (quat., Ar), 143.7 (quat., Ar*), 171.1 (quat., OC=O), 171.3 (quat., OC=O*), 212.7 (2×quat., C-9, C-9*); *m/z* (EI, %) 355 (M^+, 6), 340 (33), 312 (14), 209 (12), 105 (100), 79 (14), 42 (16).

3.3.4. Ethyl (1*R,5*R**,9*R**)-9-hydroxy-3-((*S*)-1-methylbenzyl)-5-(2′-propenyl)-3-azabicyclo[3.3.1]nonane-1-carboxylate 16a,16b and ethyl (1*R**, 5*R**, 9*S**)-9-hydroxy-3-((*S*)-1-methylbenzyl)-5-(2′-propenyl)-3-azabicyclo[3.3.1]nonane-1-carboxylate 17a, 17b.** To a solution of sodium borohydride (43 mg, 1.125 mmol) in THF (7 mL) and distilled water (14 mL) at 0 °C was added dropwise a solution of ketones **11a**, **11b** (0.80 g, 2.25 mmol) in THF (7 mL). The reaction mixture was stirred for 2 h then quenched with water (14 mL) and the volatile solvents were removed in vacuo. The residual aqueous layer was then extracted with ethyl acetate (3×25 mL). The combined organic extracts were washed with brine (50 mL), dried ($MgSO_4$) and concentrated in vacuo. The crude product was purified by flash chromatography using 32:1 hexane–ethyl acetate as eluent to afford: (i) *alcohols* **16a**, **16b** (0.31 g, 39%) as a colourless oil and as a 1:1 mixture of diastereomers; (Found: M^+, 357.2298, $C_{22}H_{31}NO_3$ requires 357.2304); ν_{max} (NaCl)/cm^{-1} 3535 (O=H), 1710 (C=O, ester), 1638 (C=C), 1452 (C=C, Ar), 1259 (*tert*-N, amine); δ_H (400 MHz, $CDCl_3$) 1.26 (3H, t, J=7.1 Hz, OCH_2CH_3 and OCH_2CH_3*), 1.30–1.33 (3H, m, $CHCH_3Ph$ and $CHCH_3Ph$*), 1.51–1.59 (1H, m, 7-H_{eq}, 7-H_{eq}*), 1.63–1.71 (1H, m, 6-H_{eq}, 6-H_{eq}*), 1.78–1.85 (1H, m, 6-H_{ax}, 6-H_{ax}*), 1.86 (0.5H, dd, J=1.8, 11.4 Hz, 4-H_{ax}), 1.96–2.13 (3.5H, m, 1′-CH_2, 1′-CH_2*, 4-H_{ax}*, 8-H_{eq}, 8-H_{eq}*), 2.20 (0.5H, dd, J=1.8, 11.4 Hz, 2-H_{ax}), 2.58 (0.5H, dd, J=1.8, 11.4 Hz, 4-H_{eq}), 2.78–2.86 (1H, m, 7-H_{ax}, 7-H_{ax}*), 2.89 (0.5H, dd, J=1.8, 11.4 Hz, 2-H_{ax}*), 2.96 (0.5H, dd, J=1.8, 11.4 Hz, 2-H_{eq}), 3.03 (0.5H, d, J=7.3 Hz, 9-H), 3.10 (0.5H, d, J=7.3 Hz, 9-H*), 3.17–3.22 (1.5H, m, C*H*CH_3Ph, C*H*CH_3Ph*, 4-H_{eq}*), 3.29 (0.5H, dd, J=1.8, 11.0 Hz, 2-H_{eq}*), 3.61 (1H, br s, O–H), 4.01–4.19 (2H, m, OCH_2CH_3, OCH_2CH_3*), 4.92–4.96 (1H, m, 3′-H_B, 3′-H_B*), 5.02–5.06 (1H, m, 3′-H_A, 3′-H_A*), 5.68–5.88 (1H, m, 2′-H, 2′-H*), 7.21–7.33 (5H, m, Ar-H, Ar-H*); δ_C (100 MHz, $CDCl_3$), 14.0 (2×CH_3, OCH_2CH_3, OCH_2CH_3*), 20.1 (2×CH_3, $CHCH_3Ph$, $CHCH_3Ph$*), 20.9 (CH_2, C-7), 21.0 (CH_2, C-7*), 26.8 (CH_2, C-8), 27.2 (CH_2, C-8*), 29.5 (CH_2, C-6), 37.5 (quat., C-5), 37.6 (quat., C-5*), 42.5 (CH_2, C-1′), 42.9 (CH_2, C-1′*), 48.0 (quat., C-1), 57.7 (CH_2, C-2), 59.3 (CH_2, C-2*), 60.3 (CH_2, OCH_2CH_3), 60.7 (CH_2, C-4), 60.8 (CH_2, C-4*), 65.1 (CH, $CHCH_3Ph$), 74.0 (CH, C-9), 74.4 (CH, C-9*), 117.3 (CH_2, C-3′), 117.4 (CH_2,

C-3′*), 126.7, 127.3, 128.3 (10×CH, Ar, Ar*), 134.3 (CH, C-2′), 134.4 (CH, C-2′*), 144.2 (quat., Ar), 144.3 (quat., Ar*), 176.7 (quat., OC=O), 176.9 (quat., OC=O*); *m/z* (EI, %) 357 (M^+, 10), 342 (46), 315 (15), 242 (28), 105 (100), 91 (23), 79 (18).

(ii) *Alcohols* **17a**, **17b** (0.25 g, 32%) as a colourless oil and as a 1:1 mixture of diastereomers; (Found: M^+, 357.2298, $C_{22}H_{31}NO_3$ requires 357.2304); ν_{max} (NaCl)/cm^{-1} 3536 (O=H), 1710 (C=O, ester), 1638 (C=C), 1452 (C=C), 1259 (*tert*-N, amine); δ_H (400 MHz, $CDCl_3$) 1.26 (3H, t, J= 7.1 Hz, OCH_2CH_3 and OCH_2CH_3*), 1.30–1.33 (3H, m, $CHCH_3Ph$ and $CHCH_3Ph$*), 1.41–1.61 (2H, m, 6-H_{eq}, 6-H_{eq}*, 7-H_{eq}, 7-H_{eq}*), 1.73–1.91 (1H, m, 6-H_{ax}, 6-H_{ax}*), 1.96–2.07 (3.5H, m, 1′-CH_2, 1′-CH_2*, 4-H_{ax}, 8-H_{eq}, 8-H_{eq}*), 2.11–2.31 (1.5H, m, 4-H_{ax}*, 8-H_{ax}, 8-H_{ax}*), 2.62 (0.5H, dd, J=1.8, 11.0 Hz, 2-H_{ax}), 2.72 (1H, br s, OH), 2.74–2.87 (2H, m, 2-H_{ax}, 4-H_{eq}, 7-H_{ax}, 7-H_{ax}*), 2.89 (0.5H, dd, J=1.8, 11.4 Hz, 2-H_{eq}), 2.96 (0.5H, dd, J=1.8, 11.4 Hz, 4-H_{eq}*), 3.19–3.31 (1.5H, m, $CHCH_3Ph$, $CHCH_3$-Ph*, 2-H_{eq}*), 3.69 (1H, d, J=7.5 Hz, 9-H), 4.01–4.18 (2H, m, OCH_2CH_3, OCH_2CH_3*), 4.96–5.01 (1H, m, 3′-H_B, 3′-H_B*), 5.02–5.07 (1H, m, 3′-H_A, 3′-H_A*), 5.76–5.86 (1H, m, 2′-H, 2′-H*), 7.21–7.33 (5H, m, Ar-H, Ar-H*); δ_C (100 MHz, $CDCl_3$), 14.1 (CH_3, OCH_2CH_3) 14.2 (CH_3, OCH_2CH_3*), 20.1 (CH_3, $CHCH_3Ph$), 20.2 (CH_3, $CHCH_3$-Ph*), 20.8 (CH_2, C-7), 20.9 (CH_2, C-7*), 34.3 (CH_2, C-8), 34.7 (CH_2, C-8*), 34.9 (CH_2, C-6), 35.2 (CH_2, C-6*), 38.1 (quat., C-5), 41.6 (CH_2, C-1′), 42.0 (CH_2, C-1′*), 48.9 (quat., C-1), 51.6 (CH_2, C-2), 54.1 (CH_2, C-2*), 60.6 (CH_2, OCH_2CH_3), 60.7 (CH_2, C-4), 65.1 (CH, $CHCH_3Ph$), 65.2 (CH, $CHCH_3Ph$*), 74.0 (CH, C-9), 74.4 (CH, C-9*), 117.6 (CH_2, C-3′), 117.7 (CH_2, C-3′*), 126.6, 126.8, 127.3, 127.4, 128.3 (10×CH, Ar, Ar*), 134.2 (CH, C-2′), 134.3 (CH, C-2′*), 145.1 (quat., Ar), 176.5 (quat., OC=O); *m/z* (EI, %) 357 (M^+, 11), 342 (100), 252 (33), 242 (27), 105 (90), 91 (16), 79 (14).

3.3.5. Ethyl (1*R,5*R**,9*R**)-3-((*S*)-1-methylbenzyl)-5-(2′-propenyl)-9-(2′-propenyloxy)-3-azabicyclo[3.3.1]-nonane-1-carboxylate 18a, 18b.** To a suspension of sodium hydride (78 mg, 3.25 mmol), in dry THF (15 mL) at 0 °C was added a solution of alcohols **16a**, **16b** (290 mg, 0.81 mmol) in dry THF (1.5 mL) and the mixture was stirred for 30 mins. Allyl bromide (393 mg, 280 mL, 3.25 mmol) was then added and the reaction mixture stirred for 48 h at room temperature. The reaction was quenched by the addition of distilled water (15 mL) and the volatile solvents were removed in vacuo. The residual aqueous layer was then extracted with ethyl acetate (2×20 mL). The combined organic extracts were washed with brine (30 mL), dried ($MgSO_4$) and concentrated in vacuo. The crude product was purified by flash chromatography using 19:1 hexane–ethyl acetate as eluent to afford the title compounds **18a**, **18b** (220 mg, 67%) as a colourless oil and as a 1:1 mixture of diastereomers; (Found: M^+, 397.2603, $C_{25}H_{35}NO_3$ requires 397.2617); ν_{max} (NaCl)/cm^{-1} 2924 (C=H), 1731 (C=O, ester), 1638 (C=C), 1452 (C=C, Ar), 1258 (*tert*-N, amine); δ_H (400 MHz, $CDCl_3$) 1.22–1.28 (3H, m, OCH_2CH_3, OCH_2CH_3*), 1.30–1.33 (3H, m, $CHCH_3Ph$ and $CHCH_3Ph$*), 1.51–1.57 (1H, m, 6-H_{eq}, 6-H_{eq}*), 1.61–1.72 (2H, m, 7-H_{eq}, 7-H_{eq}*, 8-H_{eq}, 8-H_{eq}*), 1.79–1.84 (1H, m, 6-H_{ax}, 6-H_{ax}*), 1.90–2.02 (2H, m, 4-H_{ax}, 4-H_{ax}*, 8-H_{ax}, 8-H_{ax}*), 2.09–2.21 (2H, m, 1′-CH_2, 1′-CH_2*), 2.19 (0.5H, dd, J=1.8, 11.0 Hz, 2-H_{ax}), 2.39 (0.5H, dd, J=1.8, 11.0 Hz, 4-H_{eq}), 2.62 (0.5H, dd, J=1.8, 11.0 Hz, 2-H_{ax}*), 2.73 (0.5H, dd, J=1.8, 11.0 Hz, 2-H_{eq}), 2.69–2.83 (1H, m, 7-H_{ax}, 7-H_{ax}*), 2.99 (0.5H, dd, J=1.8, 11.0 Hz, 4-H_{eq}*), 3.04 (0.5H, dd, J=1.8, 11.0 Hz, 2-H_{eq}*), 3.11 (0.5H, q, J=6.7 Hz, $CHCH_3Ph$), 3.21 (0.5H, q, J= 6.7 Hz, $CHCH_3Ph$*), 3.43 (1H, br s, 9-H), 3.94–4.14 (4H, m, OCH_2CH_3, OCH_2CH_3*, 1″-CH_2, 1″-CH_2*), 4.89–4.94 (1H, m, 3′-H_B, 3′-H_B*), 4.98–5.08 (2H, m, 3″-H_B, 3″-H_B*, 3′-H_A, 3′-H_A*), 5.19–5.24 (1H, m, 3″-H_A, 3″-H_A*), 5.62–5.72 (0.5H, m, 2′-H), 5.78–5.87 (1.5H, m, 2′-H*, 2″-H, 2″-H*), 7.21–7.30 (5H, m, Ar-H, Ar-H*); δ_C (100 MHz, $CDCl_3$), 14.1 (CH_3, OCH_2CH_3), 14.2 (CH_3, OCH_2CH_3*), 19.6 (2×CH_3, $CHCH_3Ph$, $CHCH_3Ph$*), 20.7 (CH_2, C-7), 21.0 (CH_2, C-7*), 26.2 (CH_2, C-8), 26.6 (CH_2, C-8*), 30.0 (CH_2, C-6), 30.1 (CH_2, C-6*), 39.0 (quat., C-5), 39.1 (quat., C-5*), 43.0 (CH_2, C-1′), 43.3 (CH_2, C-1′*), 48.0 (quat., C-1), 58.5 (2×CH_2, C-2, C-2*), 59.8 (CH_2 C-4), 59.9 (CH_2, C-4*), 60.5 (CH_2, OCH_2CH_3), 60.6 (CH_2, OCH_2CH_3*), 64.9 (CH, $CHCH_3Ph$), 65.5 (CH, $CHCH_3Ph$*), 73.7 (CH_2, C-1″), 84.3 (CH, C-9), 84.7 (CH, C-9*), 115.2 (CH_2, C-3″), 117.2 (CH_2, C-3′), 117.4 (CH_2, C-3′*), 126.7, 127.2, 127.3, 128.2, 128.3 (10×CH, Ar, Ar*), 134.1 (CH, C-2′), 134.2 (CH, C-2′*), 135.4 (CH, C-2″), 135.5 (CH, C-2″*), 144.3 (quat., Ar), 144.7 (quat., Ar*), 175.6 (quat., OC=O), 175.8 (quat., OC=O*); *m/z* (EI, %) 397 (M^+, 7) 382 (50), 356 (18), 206 (16), 105 (100), 91 (15), 41 (15).

3.3.6. Ethyl (1*R,5*R**,9*S**)-3-((*S*)-1-methylbenzyl)-5-(2′-propenyl)-9-(2′-propenyloxy)-3-azabicyclo[3.3.1]-nonane-1-carboxylate 19a, 19b.** To a suspension of sodium hydride (33 mg, 1.38 mmol) in dry THF (9 mL) at 0 °C was added a solution of alco hols **17a**, **17b** (123 mg, 0.34 mmol) in dry THF (1.0 mL) and the mixture was stirred for 30 mins. Allyl bromide (167 mg, 120 mL, 1.38 mmol) was then added and the reaction mixture stirred for 48 h at room temperature. The reaction was quenched by the addition of distilled water (10 mL) and the volatile solvents were removed in vacuo. The residual aqueous layer was extracted with ethyl acetate (2×15 mL). The combined organic extracts were washed with brine (20 mL), dried ($MgSO_4$) and concentrated in vacuo. The crude product was purified by flash chromatography (19:1 hexane–ethyl acetate) to afford the title compounds **19a**, **19b** (90 mg, 68%) as a colourless oil and as a 1:1 mixture of diastereomers; (Found: M^+, 397.2606, $C_{25}H_{35}NO_3$ requires 397.2617); ν_{max} (NaCl)/cm^{-1} 2924 (C=H), 1731 (C=O, ester), 1639 (C=C), 1452 (C=C, Ar), 1258 (*tert*-N, amine);*)*; δ_H (400 MHz, $CDCl_3$) 1.22–1.28 (3H, m, OCH_2CH_3, OCH_2CH_3*), 1.30–1.33 (3H, m, $CHCH_3Ph$ and $CHCH_3Ph$*), 1.46–1.63 (2H, m, 6-H_{eq}, 6-H_{eq}*, 7-H_{eq}, 7-H_{eq}*), 1.62–1.83 (2H, m, 8-H_{eq}, 8-H_{eq}*, 6-H_{ax}, 6-H_{ax}*), 1.81–2.23 (3H, m, 1′-CH_2, 1-CH_2*, 8-H_{ax}, 8-H_{ax}*), 2.69–2.91 (1H, m, 7-H_{ax}, 7-H_{ax}*), 2.58–2.82 (2H, m, 2-H_{ax}, 2-H_{eq}, 2-H_{ax}*, 4-H_{eq}), 3.01 (0.5H, dd, J=1.8, 11.0 Hz, 4-H_{eq}*), 3.08–3.31 (1.5H, m, $CHCH_3Ph$, $CHCH_3$-Ph*, 2-H_{eq}*), 3.42–3.44 (1H, m, 9-H, 9-H*), 3.97–4.13 (4H, m, OCH_2CH_3, OCH_2CH_3*, 1″-CH_2, 1″-CH_2*), 4.85–4.97 (1H, m, 3′-H_B, 3′-H_B*), 4.98–5.08 (2H, m, 3″-H_B, 3″-H_B*, 3′-H_A, 3′-H_A*), 5.16–5.26 (1H, m, 3″-H_A, 3″-H_A*), 5.62–5.75 (0.5H, m, 2′-H), 5.77–5.91 (1.5H, m, 2″-H, 2″-H*, 2′-H*), 7.21–7.30 (5H, m, Ar-H, Ar-H*); δ_C (100 MHz,

$CDCl_3$), 14.1 (2×CH_3, OCH_2CH_3, OCH_2CH_3*), 20.3 (CH_3, $CHCH_3Ph$), 20.5 (CH_3, $CHCH_3Ph$*), 20.9 (CH_2, C-7), 26.4 (CH_2, C-8), 30.0 (CH_2, C-6), 39.4 (quat., C-5), 41.7 (CH_2, C-1′), 42.2 (CH_2, C-1′*), 48.9 (quat., C-1), 50.6 (CH_2, C-2) 52.4 (CH_2, C-2*), 59.8 (CH_2, C-4), 59.9 (CH_2, C-4*), 60.2 (CH_2, OCH_2CH_3), 65.6 (CH, $CHCH_3Ph$), 65.7 (CH, $CHCH_3Ph$*), 73.7 (CH_2, C-1″), 73.8 (CH_2, C-1″*), 83.2 (CH, C-9), 83.5 (CH, C-9*), 114.8 (CH_2, C-3″), 114.9 (CH_2, C-3″*), 117.4 (CH_2, C-3′), 117.6 (CH_2, C-3′*), 126.4, 126.5, 127.2, 127.3, 128.2, 128.3 (10×CH, Ar, Ar*), 134.2 (CH, C-2′), 135.4 (2×CH, C-2″, C-2″*), 145.9 (2×quat., Ar, Ar*), 175.7 (quat., OC=O), 175.9 (quat., OC=O*); *m/z* (EI, %) 397 (M^+, 3) 382 (33), 156 (35), 141 (51), 105 (84), 71 (61), 57 (100).

3.3.7. Ethyl (1*R,7*R**,8*R**)-10-((*S*)-1-methylbenzyl)-6-oxa-10-azatricyclo[6.3.3.0]tetradec-3-ene-8-carboxylate 12a, 12b.** To a solution of bis(tricyclohexylphosphine)-benzylidene ruthenium(IV) dichloride (7 mg, 0.009 mmol) in dry dichloromethane (2.5 mL) was added dropwise a solution of dienes **18a**, **18b** (70 mg, 0.176 mmol) in dry dichloromethane (1 mL). The mixture was stirred for 22 h, concentrated in vacuo and the crude product purified by flash chromatography using 19:1 hexane–ethyl acetate as eluent to afford the title compounds **12a**, **12b** (62 mg, 95%) as a colourless oil and as a 1:1 mixture of diastereomers; (Found: M^+, 369.2301, $C_{23}H_{31}NO_3$ requires 369.2304); ν_{max} (NaCl)/cm^{-1} 2974 (C=H), 1734 (C=O, ester), 1654 (C=C), 1451 (C=C, Ar), 1255 (*tert*-N, amine); δ_H (400 MHz, $CDCl_3$) 1.17–1.20 (3H, m, OCH_2CH_3, OCH_2CH_3*), 1.23–1.32 (5H, m, 12-H_{eq}, 12-H_{eq}*, 14-H_{eq}, 14-H_{eq}*, $CHCH_3Ph$, $CHCH_3Ph$*), 1.53–1.60 (1H, m, 13-H_{eq}, 13-H_{eq}*), 1.68–1.84 (1.5H, m, 2-H_B, 11-H_{ax}, 11-H_{ax}*), 1.87–2.16 (4H, m, 2-H_A, 2-H_A*, 2-H_B*, 9-H_{ax}, 12-H_{ax}, 12-H_{ax}*, 14-H_{ax}, 14-H_{ax}*), 2.25 (0.5H, dd, J=1.8, 11.0 Hz, 11-H_{ax}), 2.42 (0.5H, dd, J=1.8, 11.0 Hz, 9-H_{ax}), 2.53 (0.5H, dd, J=1.8, 11.0 Hz, 9-H_{eq}), 2.75 (0.5H, dd, J=1.8, 11.0 Hz, 11-H_{eq}), 2.77–2.91 (1H, m, 13-H_{ax}, 13-H_{ax}*), 2.89 (0.5H, dd, J=1.8, 11.0 Hz, 9-H_{eq}), 3.09–3.17 (1H, m, $CHCH_3Ph$, $CHCH_3Ph$*), 3.61 (1H, br s, 7-H, 7-H*), 3.96–4.23 (4H, m, 5-CH_2, 5-CH_2*, OCH_2CH_3, OCH_2CH_3*), 5.61–5.89 (2H, m, 3-H, 3-H*, 4-H, 4-H*), 7.21–7.32 (5H, m, Ar-H, Ar-H*); δ_C (100 MHz, $CDCl_3$), 14.2 (2×CH_3, OCH_2CH_3, OCH_2CH_3*), 20.5 (CH_3, $CHCH_3Ph$), 20.6 (CH_2, C-13), 20.7 (CH_2, C-13*), 26.0 (CH_2, C-14), 26.4 (CH_2, C-14*), 28.8 (CH_2, C-12), 29.2 (CH_2, C-12*), 37.0 (quat., C-1), 37.1 (quat., C-1*), 40.0 (CH_2, C-2), 40.3 (CH_2, C-2*), 49.0 (quat., C-8), 49.1 (quat., C-8*), 58.8 (CH_2, C-9), 59.8 (CH_2, C-9*), 60.2 (CH_2, C-11), 60.3 (CH_2, C-11*), 62.2 (CH_2, OCH_2CH_3), 65.1 (CH, $CHCH_3Ph$), 65.3 (CH, $CHCH_3Ph$*), 68.4 (CH_2, C-5), 88.5 (CH, C-7), 88.6 (CH, C-7*), 126.8, 127.2, 128.3 (10×CH, Ar, Ar*), 130.2 (CH, C-3), 130.3 (CH, C-3*), 130.7 (CH, C-4), 130.8 (CH, C-4*), 144.4 (quat., Ar), 144.7 (quat., Ar*), 175.1 (quat., OC=O), 175.3 (quat., OC=O*); *m/z* (EI, %) 369 (M^+, 20), 354 (59), 264 (25), 134 (28), 105 (100), 91 (26), 44 (28).

3.3.8. Ethyl (1*R,7*S**,8*R**)-10-((*S*)-1-methylbenzyl)-6-oxa-10-azatricyclo[6.3.3.0]tetradec-3-ene-8-carboxylate 13a, 13b.** To a solution of bis(tricyclohexylphosphine)-benzylidene ruthenium(IV) dichloride (6 mg, 0.007 mmol) in dry dichloromethane (2.5 mL) was added dropwise a solution of dienes **19a**, **19b** (60 mg, 0.151 mmol) in dry dichloromethane (1 mL). The mixture was stirred for 22 h, concentrated in vacuo and the crude product purified by flash chromatography using 19:1 hexane–ethyl acetate as eluent to afford the title compounds **13a**, **13b** (53 mg, 95%) as a colourless oil and as a 1:1 mixture of diastereomers; (Found: M^+, 369.2297, $C_{23}H_{31}NO_3$ requires 369.2304); ν_{max} (NaCl)/cm^{-1} 2975 (C=H), 1731 (C=O, ester), 1652 (C=C), 1452 (C=C, Ar), 1257 (*tert*-N, amine); δ_H (400 MHz, $CDCl_3$) 1.17–1.21 (3H, m, OCH_2CH_3, OCH_2CH_3*), 1.17–1.34 (5H, m, 12-H_{eq}, 12-H_{eq}*, 14-H_{eq}, 14-H_{eq}*, $CHCH_3Ph$, $CHCH_3Ph$*), 1.41–1.57 (1H, m, 13-H_{eq}, 13-H_{eq}*), 1.81–2.16 (4H, m, 2-H_A, 2-H_B, 9-H_{ax}, 11-H_{ax}, 12-H_{ax}, 12-H_{ax}*,14-H_{ax}, 14-H_{ax}*), 1.61–1.77 (1.5H, m, 2-H_B*, 9-H_{ax}*, 11-H_{ax}*), 2.24–2.33 (0.5H, m, 2-H_A*), 2.50–2.53 (0.5H, m, 9-H_{eq}), 2.54–2.56 (0.5H, m, 11-H_{eq}), 2.72–2.74 (0.5H, m, 9-H_{eq}*), 2.75–2.77 (0.5H, m, 11_{eq}*), 2.83–3.01 (1H, m, 13-H_{ax}, 13-H_{ax}*), 3.13–3.18 (1H, m, $CHCH_3Ph$, $CHCH_3Ph$*), 3.69 (1H, s, 7-H, 7-H*), 3.96–4.26 (4H, m, 5-CH_2, 5-CH_2*, OCH_2CH_3, OCH_2CH_3*), 5.69–5.75 (2H, m, 3-H, 3-H*, 4-H, 4-H*), 7.19–7.32 (5H, m, Ar-H, Ar-H*); δ_C (100 MHz, $CDCl_3$), 14.2 (CH_3, OCH_2CH_3) 14.3 (CH_3, OCH_2CH_3*), 20.6 (CH_3, $CHCH_3Ph$), 20.7 (CH_3, $CHCH_3Ph$*), 21.2 (CH_2, C-13), 21.3 (CH_2, C-13*), 26.0 (CH_2, C-14), 29.2 (CH_2, C-12), 38.2 (quat., C-1), 39.2 (CH_2, C-2), 39.5 (CH_2, C-2*), 48.8 (quat., C-8), 49.1 (quat., C-8*), 59.8 (CH_2, C-9), 60.0 (CH_2, C-9*), 60.2 (CH_2, C-11), 60.4 (CH_2, C-11*), 62.2 (CH_2, OCH_2CH_3), 65.1 (CH, $CHCH_3$-Ph), 65.3 (CH, $CHCH_3Ph$*), 68.3 (CH_2, C-5), 68.8 (CH_2, C-5*), 85.5 (CH, C-7), 85.6 (CH, C-7*), 126.4, 126.8, 127.3, 127.4, 128.2, 128.3 (10×CH, Ar, Ar*), 129.1 (CH, C-3), 130.3 (CH, C-4), 130.5 (CH, C-4*), 145.6 (quat., Ar), 146.2 (quat., Ar*), 175.1 (quat., OC=O), 175.4 (quat., OC=O*); *m/z* (EI, %) 369 (M^+, 19), 354 (76), 264 (32), 105 (100), 91 (35), 79 (25), 41 (29).

3.3.9. Ethyl (1*R,5*R**,9*R**)-9-hydroxy-3-((*S*)-1-methylbenzyl)-5-(2′-propenyl)-9-(2′-propenyl)-3-azabicyclo-[3.3.1]nonane-1-carboxylate 20a, 20b and ethyl (1*R**, 5*R**, 9*S**)-9-hydroxy-3-((*S*)-1-methylbenzyl)-5-(2′-propenyl)-9-(2′-propenyl)-3-azabicyclo[3.3.1]nonane-1-carboxylate 21a, 21b.** To a solution of ketones **11a**, **11b** (330 mg, 0.928 mmol) in THF (10 mL) at 0 °C was added dropwise allyl magnesium bromide (1.4 mL, 1 M in diethyl ether, 1.4 mmol) under an atmosphere of nitrogen. The reaction was stirred for 1 h then warmed to room temperature and stirred for a further, 48 h. The reaction was quenched by the addition of sat. NH_4Cl (10 mL) and the residual aqueous layer was extracted with diethyl ether (1× 20 mL, 2×10 mL). The aqueous mixture was further, extracted with diethyl ether (2×10 mL) and the combined organic extracts washed with brine (20 mL), dried ($MgSO_4$) and concentrated in vacuo. The crude product was purified by flash chromatography using 19:1 hexane–ethyl acetate as eluent to afford the title compounds **20a/20b**, **21a/21b** (265 mg, 74%) as a colourless oil and as a 1:1:1:1 mixture of four diastereomers; (Found: M^+, 397.2621, $C_{25}H_{35}NO_3$ requires 397.2617); ν_{max} (NaCl)/cm^{-1} 3463 (O=H), 2984 (C=H), 1741 (C=O, ester), 1639 (C=C), 1447 (C=C, Ar), 1373 (*tert*-N, amine), 1241 (*tert*-N, amine); δ_H (400 MHz, $CDCl_3$) 1.18–1.23 (3H, m, $CHCH_3Ph$, $CHCH_3Ph$*, $CHCH_3Ph$*′, $CHCH_3Ph$*″), 1.27–1.34 (1.5H, m, $CHCH_3Ph$, $CHCH_3Ph$*), 1.34–1.41 (1.5H, m, $CHCH_3Ph$*′, $CHCH_3Ph$*″), 1.43–2.99 (11.5H, m, 1′-H_A,

1′-H_B, 1′-H_A*, 1′-H_B*, 1″-H_A, 1″-H_B, 1″-H_A*, 1″-H_B*, 2-H_{ax}, 2-H_{ax}*, 2-H_{eq}, 2-H_{eq}*, 2-H_{eq}*′, 2-H_{eq}*″, 4-H_{eq}, 4-H_{eq}*, 4-H_{eq}*′, 4-H_{eq}*″, 4-H_{ax}, 4-H_{ax}*, 4-H_{ax}*′, 4-H_{ax}*″,6-H_{eq}, 6-H_{eq}*, 6-H_{eq}*′, 6-H_{eq}*″, 6-H_{ax}, 6-H_{ax}*, 6-H_{ax}*′,6-H_{ax}*″, 7-H_{eq}, 7-H_{eq}*, 7-H_{eq}*′, 7-H_{eq}*″, 7-H_{ax}, 7-H_{ax}*, 7-H_{ax}*′, 7-H_{ax}*″, 8-H_{eq}, 8-H_{eq}*, 8-H_{eq}*′, 8-H_{eq}*″, 8-H_{ax}, 8-H_{ax}*, 8-H_{ax}*′, 8-H_{ax}*″), 3.12–3.36 (1.5H, m, 2-H_{ax}*′, 2-H_{ax}*″, C*H*CH_3Ph, C*H*CH_3Ph*,C*H*CH_3Ph*′, C*H*CH_3Ph*″), 3.97–4.14 (2.5H, m, OCH_2CH_3, OCH_2CH_3*, OCH_2CH_3*′, OCH_2CH_3*″, OH, OH*), 4.54 (0.25, s, OH*′), 4.59 (0.25, s, OH*″), 4.87–5.07 (4H, m, 3′-H_A, 3′-H_A*, 3′-H_A*′, 3′-H_A*″, 3′-H_B, 3′-H_B*, 3′-H_B*′, 3′-H_B*″, 3″-H_A, 3″-H_A*, 3″-H_A*′, 3″-H_A*″, 3″-H_B, 3″-H_B*, 3″-H_B*′, 3″-H_B*″), 5.65–5.91 (2H, m, 2′-H_A, 2′-H_A*, 2′-H_A*′, 2′-H_A*″, 2″-H_B, 2″-H_B*, 2″-H_B*′, 2″-H_B*″); δ_C (100 MHz, $CDCl_3$), 13.6 (CH_3, OCH_2*C*H_3), 13.8 (CH_3, OCH_2*C*H_3*), 13.9 (CH_3, OCH_2*C*H_3*′), 14.1 (CH_3, OCH_2*C*H_3*″), 18.8, 19.7, 20.0, 20.1, 20.2, 20.4, 21.2 (4×CH_3, CH*C*H_3Ph, CH*C*H_3Ph*, CH*C*H_3Ph*′, CH*C*H_3Ph*″ and 4×CH_2, C-7, C-7*, C-7*′, C-7*″), 29.5, 29.8, 30.5, 30.7, 31.8, 32.4, 32.6, 32.8 (8×CH_2, C-6, C-6*, C-6*′, C-6*″, C-8, C-8*, C-8*′, C-8*″), 37.8, 37.9, 38.0 (4×CH_2, C-1″, C-1″*, C-1″*′, C-1″*″), 39.0, 39.1, 39.4, 39.5 (4×CH_2, C-1′, C-1′*, C-1′*′, C-1′*″), 50.3, 50.6, 50.7 (4×quat., C-1, C-1*, C-1*′, C-1*″), 53.1, 54.0, 55.1, 55.3, 56.6, 57.1, 57.2 (8×CH_2, C-2, C-2*, C-2*′, C-2*″, C-4, C-4*, C-4*′, C-4*″), 60.6, 60.7, 60.9, 61.0 (4×CH_2, O*C*H_2CH$_3$, O*C*H_2CH$_3$*, O*C*H_2CH$_3$*′, O*C*H_2CH$_3$*″), 64.8, 65.1, 65.2, 65.8 (4×CH, *C*HCH$_3$Ph, *C*HCH$_3$Ph*, *C*HCH$_3$Ph*′, *C*HCH$_3$Ph*″), 74.0, 74.1, 74.4, 74.5 (4× quat., C-9, C-9*, C-9*′, C-9*″), 116.7, 116.8, 117.0, 117.2 (8×CH_2, C-3′, C-3′*, C-3′*′, C-3′*″, C-3″, C-3″*, C-3″*′, C-3″*″), 126.6, 126.7, 127.2, 127.3, 128.1, 128.2, 128.3 (10×CH, Ar, Ar*, Ar*′, Ar*″), 134.6, 134.7, 134.8, 134.9, 135.0, 135.1, 135.2 (8×CH, C-2′, C-2′*, C-2′*′, C-2′*″, C-2″, C-2″*, C-2″*′, C-2″*″), 144.2, 144.3, 144.8, 145.6 (4×quat., Ar, Ar*, Ar*′, Ar*″), 177.0, 177.2, 177.4 (4× OC=O, OC=O*, OC=O*′, OC=O*″); *m/z* (EI, %) 397 (M^+, 13), 382 (36), 292 (18), 206 (9), 105 (100), 79 (13), 41 (13).

3.3.10. Ethyl (1*R, 6*R**, 7*R**)-6-hydroxy-9-((*S*)-1-methylbenzyl)-9-azatricyclo[5.3.3.0^{1,6}]tridec-3-ene-7-carboxylate 14a, 14b and ethyl (1*R**, 6*S**, 7*R**)-6-Hydroxy-9-((*S*)-1-methylbenzyl)-9-azatricyclo[5.3.3.0^{1,6}]tridec-3-ene-7-carboxylate 15a, 15b.** To a solution of bis(tricyclohexylphosphine)benzylidene ruthenium(IV) dichloride (46 mg, 0.06 mmol) in dry dichloromethane (7 mL) was added dropwise a solution of dienes **20a/20b**, **21a/21b** (0.22 g, 0.6 mmol) in dry dichloromethane (3 mL). The mixture was heated under reflux for 48 h, concentrated in vacuo and the crude product purified by flash chromatography using 19:1 hexane–ethyl acetate as eluent to afford:

(i) *alkenes* **14a**, **14b** (91 mg, 41%) as a colourless oil and as a 1:1 mixture of diastereomers; (Found: M^+, 369.2305, $C_{23}H_{31}NO_3$ requires 369.2304); ν_{max} (NaCl)/cm^{-1} 3518 (O–H), 2976 (C–H), 1731 (C=O, ester), 1698 (C=C), 1453 (C=C,Ar), 1261 (*tert*-N, amine); δ_H (400 MHz, $CDCl_3$) 1.22–1.45 (7H, m, 11-H_{eq}, 11-H_{eq}*, CHC*H*$_3$Ph, CHC*H*$_3$Ph*, OCH$_2$C*H*$_3$, OCH$_2$C*H*$_3$*), 1.57–1.64 (1.5H, m, 12-H_{eq}, 12-H_{eq}*, 5-H_B), 1.73–1.78 (0.5H, m, 5-H_B*), 1.82–2.19 (3.5H, m, 2-H_A, 2-H_B, 2-H_A*, 2-H_B*, 5-H_A, 13-H_{eq}, 13-H_{eq}*), 2.23 (0.5H, dd, *J*=1.8, 11.0 Hz, 10-H_{ax}), 2.36 (0.5H, d, *J*=11.0 Hz, 5-H_A*), 2.47 (0.5H, dd, *J*=1.8, 11.0 Hz, 10-H_{eq}), 2.52–2.68 (2.5H, m, 8-H_{ax}, 8-H_{ax}*, 10-H_{ax}*, 13-H_{ax}, 13-H_{ax}*), 2.72 (0.5H, dd, *J*=1.8, 11.0 Hz, 8-H_{eq}), 2.94–3.06 (2H, m, 12-H_{ax}, 12-H_{ax}*, 8-H_{eq}*, 10-H_{eq}*), 3.21 (0.5H, q, *J*=6.7 Hz, C*H*CH$_3$Ph) 3.33 (0.5H, q, *J*=6.7 Hz, C*H*CH$_3$Ph*), 3.41 (0.5H, d, *J*=11.0 Hz, OH), 3.67 (0.5H, d, *J*=11.0 Hz, OH*), 4.13 (1H, q, *J*=7.1 Hz, OC*H*$_2$CH$_3$), 4.23 (1H, q, *J*=7.1 Hz, OC*H*$_2$CH$_3$*), 5.53–5.69 (2H, m, 3-H, 4-H), 7.25–7.36 (5H, m, Ar-H, Ar-H*); δ_C (100 MHz, $CDCl_3$), 14.1 (CH_3, OCH_2C*H*$_3$), 14.2 (CH_3, OCH_2C*H*$_3$*), 19.4 (CH_2, C-12), 20.9 (CH_3, CHC*H*$_3$Ph), 20.1 (CH_3, CHC*H*$_3$Ph*), 31.4 (CH_2, C-2), 31.8 (CH_2, C-13), 33.9 (CH_2, C-11), 34.2 (CH_2, C-11*), 35.0 (CH_2, C-5), 35.4 (CH_2, C-5*), 49.6 (quat., C-7), 49.7 (quat., C-7*), 54.8 (CH_2, C-8), 56.0 (CH_2, C-8*), 57.4 (CH_2, C-10), 59.4 (CH_2, C-10*), 60.7 (CH_2, O*C*H$_2$CH$_3$), 60.8 (CH_2, O*C*H$_2$CH$_3$*), 65.1 (CH, *C*HCH$_3$Ph), 65.7 (CH, *C*HCH$_3$Ph*), 71.3 (quat., C-6), 71.4 (quat., C-6*), 121.7 (CH, C-4), 121.9 (CH, C-4*), 125.4 (CH, C-3), 125.5 (CH, C-3*), 126.7, 127.2, 127.3, 128.2, 128.3 (10×CH, Ar, Ar*), 144.7 (quat., Ar), 145.3 (quat., Ar*), 176.4 (quat., OC=O), 176.6 (quat., OC=O*); *m/z* (EI, %) 369 (M^+, 37), 354 (100), 296 (14), 264 (26), 105 (81), 91 (15), 79 (13).

(ii) *Alkenes* **15a**, **15b** (80 mg, 36%) as a colourless oil and as a 1:1 mixture of diastereomers; (Found: M^+, 369.2297, $C_{23}H_{31}NO_3$ requires 369.2304); ν_{max} (NaCl)/cm^{-1}3507 (O–H), 2911 (C–H), 1731 (C=O),1698 (C=C), 1452 (C=C, Ar), 1262 (*tert*-N, amine); δ_H (400 MHz, $CDCl_3$) 1.21–1.49 (7.5H, m, 5-H_B, 11-H_{eq}, 11-H_{eq}*, CHC*H*$_3$Ph, CHC*H*$_3$Ph*, OCH$_2$C*H*$_3$, OCH$_2$C*H*$_3$*), 1.51–1.63 (1.5H, m, 12-H_{eq}, 12-H_{eq}*, 5-H_B*), 1.72–2.24 (6H, m, 2-H_A, 2-H_B, 2-H_A*, 2-H_B*, 5-H_A, 10-H_{ax}, 10-H_{ax}*, 13-H_{eq}, 13-H_{eq}*, 11-H_{ax}, 11-H_{ax}*), 2.28 (0.5H, d, *J*=11.0 Hz, 5-H_A*), 2.52 (0.5H, dd, *J*=1.8, 11.0 Hz, 10-H_{eq}), 2.58–2.65 (1.5H, m, 10-H_{ax}*, 13-H_{ax}, 13-H_{ax}*), 2.96 (0.5H, dd, *J*=1.8, 11.0 Hz, 10-H_{eq}*), 2.94–3.11 (3H, m, 8-H_{ax}, 8-H_{ax}*, 8-H_{eq}, 8-H_{eq}*, 12-H_{ax}, 12-H_{ax}*), 3.24–3.31 (1H, m, C*H*CH$_3$Ph, C*H*CH$_3$Ph*), 3.63–3.68 (1H, m, OC*H*$_2$CH$_3$), 4.19–4.24 (1H, m, OC*H*$_2$CH$_3$*), 5.57–5.70 (2H, m, 3-H, 3-H*, 4-H, 4-H*), 7.24–7.37 (5H, m, Ar-H, Ar-H*); δ_C (100 MHz, $CDCl_3$), 14.1 (CH_3, OCH_2C*H*$_3$), 14.2 (CH_3, OCH_2C*H*$_3$*), 19.4 (CH_2, C-12), 20.0 (CH_2, C-12*), 20.7 (CH_3, CHC*H*$_3$Ph), 20.9 (CH_3, CHC*H*$_3$Ph*), 31.2 (CH_2, C-2), 31.6 (CH_2, C-2*), 31.7 (CH_2, C-13), 31.8 (CH_2, C-13*), 33.9 (CH_2, C-11), 34.0 (CH_2, C-11*), 34.2 (CH_2, C-5), 34.5 (CH_2, C-5*), 50.3 (quat., C-7), 50.4 (quat., C-7*), 54.5 (CH_2, C-8), 54.8 (CH_2, C-8*), 56.1 (CH_2, C-10), 59.0 (CH_2, C-10*), 60.8 (CH_2, O*C*H$_2$CH$_3$), 60.9 (CH_2, O*C*H$_2$CH$_3$*), 65.1 (CH, *C*HCH$_3$Ph), 65.5 (CH, *C*HCH$_3$Ph*), 71.4 (quat., C-6), 71.5 (quat., C-6*), 122.6 (CH, C-4), 122.7 (CH, C-4*), 125.4 (CH, C-3), 125.5 (CH, C-3*), 126.6, 126.7, 127.2, 127.3, 128.3 (10×CH, Ar, Ar*), 145.5 (quat., Ar), 145.6 (quat., Ar*), 175.9 (quat., OC=O), 176.2 (quat., OC=O*); *m/z* (EI, %) 369 (M^+, 40), 354 (100), 292 (11), 164 (62), 105 (73), 91 (14), 44 (20).

3.3.11. (1*R,7*S**,8*R**)-10-((*S*)-1-Methylbenzyl)-6-oxa-10-azatricyclo[6.3.3.0]tetradec-3-en-8-yl)methanol 22a, 22b.** To a slurry of lithium aluminium hydride (17 mg, 0.44 mmol) in dry THF (5 mL) was slowly added a solution

of esters **12a**, **12b** (33 mg, 0.088 mmol) in dry THF (2 mL) and the mixture stirred under an atmosphere of nitrogen for 24 h. The reaction was quenched on the addition of $Na_2SO_4 \cdot 10H_2O$ and stirring was continued for 1 h. The mixture was filtered through Celite® and the filtrate concentrated in vacuo. The residue was dissolved in ethyl acetate (50 mL) washed with sat. $NaHCO_3$ (20 mL), brine (20 mL) then dried ($MgSO_4$) and concentrated in vacuo. The crude product was purified by flash chromatography using 19:1 hexane–ethyl acetate as eluent to afford the title compounds **22a**, **22b** (25 mg, 85%) as a colourless oil and as a 1:1 mixture of diastereomers; (Found: M^+, 327.2199, $C_{21}H_{29}NO_2$ requires 327.2198); ν_{max} (NaCl)/cm^{-1} 3429 (O–H), 2921 (C–H), 1451 (C=C, Ar), 1105 (C=O, ether), 1255 (*tert*-N, amine); δ_H (400 MHz, $CDCl_3$) 0.82–1.23 (1H, m, 12-H_{eq}, 14-H_{eq}) 1.26–1.33 (4H, m, 12-H_{eq}*, 14-H_{eq}*, CHCH_3Ph, CHCH_3Ph*), 1.54–1.59 (1H, m, 13-H_{eq}, 13-H_{eq}*), 1.64–1.71 (1H, m, 2-H_B, 11-H_{ax}), 1.76 (0.5H, dd, J= 1.8, 11.0 Hz, 9-H_{ax}), 1.85–2.01 (4H, m, 2-H_A*, 2-H_B*, 9-H_{ax}*, 11-H_{ax}*, 12-H_{ax}, 12-H_{ax}*, 14-H_{ax}, 14-H_{ax}*), 2.10–2.17 (0.5H, m, 2-H_A), 2.44 (0.5H, dd, J=1.8, 11.0 Hz, 9-H_{eq}), 2.49 (0.5H, dd, J=1.8, 11.0 Hz, 11-H_{eq}), 2.68–2.93 (2H, m, 9-H_{eq}*, 11-H_{eq}*, 13-H_{ax}, 13-H_{ax}*), 3.06–3.11 (1H, m, CHCH$_3$Ph, CHCH$_3$Ph*), 3.12–3.19 (0.5H, m, CH_AH$_B$OH), 3.27–3.28 (1H, m, 7-H, 7-H*), 3.27–3.34 (0.5H, m, CH$_A$$H_B$OH), 3.32 (0.5H, d, J=11.0 Hz, CH_AH$_B$OH*), 3.48 (0.5H, d, J=11.0 Hz, CH$_A$$H_B$OH*), 4.00–4.07 (1H, m, 5-H_B), 4.23–4.30 (1H, m, 5-H_A), 5.69–5.93 (2H, m, 3-H, 4-H), 7.22–7.30 (5H, m, Ar-H, Ar-H*); δ_C (100 MHz, $CDCl_3$) 20.5 (CH$_3$, CHCH$_3$Ph), 20.7 (CH$_2$, C-13), 20.8 (CH$_2$, C-13*), 26.6 (CH$_2$, C-14), 27.0 (CH$_2$, C-14*), 29.1 (CH$_2$, C-12), 29.5 (CH$_2$, C-12*), 36.9 (quat., C-1), 37.0 (quat., C-1*), 39.7 (quat., C-8), 39.9 (CH$_2$, C-2), 40.0 (CH$_2$, C-2*), 58.6 (CH$_2$, C-9), 59.5 (CH$_2$, C-9*), 62.4 (CH$_2$, C-11), 63.7 (CH$_2$, C-11*), 65.4 (CH, CHCH$_3$Ph), 65.6 (CH, CHCH$_3$Ph*), 67.9 (CH$_2$, C-5), 70.8 (CH$_2$, CH$_2$OH), 71.0 (CH$_2$, CH$_2$OH*), 92.8 (CH, C-7), 92.9 (CH, C-7*), 126.7, 127.2, 127.3, 128.3 (10×CH, Ar, Ar*), 129.7 (CH, C-3), 129.8 (CH, C-3*), 132.1 (CH, C-4), 132.3 (CH, C-4*), 144.6 (quat., Ar), 145.0 (quat., Ar*); *m/z* (EI, %) 327 (M^+, 34), 312 (78), 222 (23), 134 (39), 105 (100), 91 (23), 44 (25).

3.3.12. (1*S*,7*R*,8*S*)-(10-((1*S*)-1-Phenylethyl)-6-oxa-10-azatricyclo[6.3.3.0^{1,7}]tetra-dec-3-en-8-yl)methyl 2-(*N*-trifluoroacetyl)aminobenzoate 24a and (1*R*,7*S*,8*R*)-(10-((1*S*)-1-phenylethyl)-6-oxa-10-azatricyclo[6.3.3.0^{1,7}]-tetradec-3-en-8-yl)methyl 2-(*N*-trifluoroacetyl)amino-benzoate 24b. To a mixture of alcohols **22a**, **22b** (32 mg, 0.098 mmol), *N* (trifluoroacetyl)anthranilic acid **23**[18] (69 mg, 0.294 mmol) and 4 (dimethylamino)pyridine (6 mg, 0.049 mmol) in acetonitrile (10 mL) was added 1,3-dicyclohexyl-carbodiimide (61 mg, 0.294 mmol), and the reaction mixture was stirred for 20 h at 40 °C. The reaction mixture was cooled, filtered, and the filtrate evaporated to dryness. The residue was purified by flash chromatography (10% EtOAc in hexane) to afford the title compounds **24a**, **24b** (28 mg, 53%) as a colourless oil and as a 1:1 mixture of diasteromers; (Found: M^+, 542.2391, $C_{30}H_{33}F_3N_2O_4$ requires 542.2392); δ_H (400 MHz, $CDCl_3$) 1.08–1.17 (0.5H, m, 14-H_{ax}*), 1.23–1.43 (4H, m, 12-H_{ax}*, 14-H_{ax}, NCH(CH_3)Ph, NCH(CH_3)Ph*), 1.48–1.77 (3.5H, m, 2-H_b*, 9H_{ax}, 12-H_{ax}, 12-H_{eq}, 12-H_{eq}*, 13H_{eq}, 13H_{eq}*), 1.88–2.28 (4H, m, 2-H_a, 2-H_a*, 2-H_b, 9H_{ax}*, 11H_{ax}, 11H_{ax}*, 14H_{eq}, 14H_{eq}*), 2.56 (0.5H, d, $^2J_{gem}$=12.8 Hz, 9H_{eq}), 2.80–2.98 (2H, m, 9H_{eq}*, 11-H_{eq}, 13H_{ax}, 13H_{ax}*), 3.07–3.19 (2.5H, m, 7H, 7H*, 11-H_{eq}*, NCH(CH$_3$)Ph, NCH(CH$_3$)-Ph*), 3.86–4.28 (m, 4H, 5H_a, 5H_a*, 5-H_b, 5-H_b*, CH$_2$O, CH$_2$O*), 5.67 5.96 (2H, m, 3-H, 3-H*, 4-H, 4-H*), 7.13–7.31 (6H, m, 4′-H, 4′-H*, H(Ph), H*(Ph*)), 7.59–7.72 (1.5H, m, 3′-H*, 5′-H, 5′ H*), 8.05 (0.5H, dd, $^3J_{3',4'}$= 8.0 Hz, $^4J_{3',5'}$=1.5 Hz, 3′-H), 8.64 (0.5H, d, $^3J_{5'*,6'*}$= 8.4 Hz 6′-H*), 8.69 (0.5H, d, $^3J_{5',6'}$=8.4 Hz 6′-H), 12.33 (0.5H, s, NH*), 12.40 (0.5H, s, NH); δ_C (75 MHz, $CDCl_3$) 20.4 (NCH(CH_3)Ph*), 20.6 (C-13, C 13*), 20.7 (NCH(CH_3)Ph), 27.5 (C 12*), 27.8 (C 12), 29.3 (C-14*), 29.7 (C-14), 36.7 (C-1*), 36.9 (C-1), 39.9 (C-8, C-8*), 40.0 (C-2*), 40.2 (C-2), 58.6 (C-11*), 59.8 (C-11), 62.7 (C-9*), 63.7 (C-9), 65.4 (NCH(CH$_3$)Ph*), 65.5 (NCH(CH$_3$)Ph), 68.4 (C-5, C-5*), 71.0 (CH$_2$O*), 71.3 (CH$_2$O), 88.2 (C 7*), 88.6 (C 7), 116.4 (C-1′*), 116.5 (C-1′), 120.7 (C 6′*), 120.8 (C 6′), 124.7 (C-4′*), 124.8 (C-4′), 126.7 (C-4*), 126.8 (C-4), 127.3 (C-2, C-2*, C 6, C 6*), 128.3 (C 3, C 3*, C-5, C-5*), 130.2 (C-3*), 130.3 (C-3), 130.7 (C-3′, C 3′*), 131.2 (C-4*), 131.3 (C-4), 134.7 (C 5′*), 134.9 (C 5′), 139.1 (C-2′*), 139.2 (C-2′), 144.6 (C-1*), 144.8 (C-1), 168.0 (C=O* (ester)), 168.2 (C=O (ester)).

3.3.13. (1*S*,7*R*,8*S*)-(10-((1*S*)-1-phenylethyl)-6-oxa-10-azatricyclo[6.3.3.0^{1,7}]-tetradec-3-en-8-yl)methyl 2-amino-benzoate 25a and (1*R*,7*S*,8*R*)-(10-((1*S*)-1-phenylethyl)-6-oxa-10-azatricyclo[6.3.3.0^{1,7}]tetradec-3-en-8-yl)methyl 2-aminobenzoate 25b. To a mixture of trifluoroaceates **24a**, **24b** (24 mg, 0.044 mmol) in dry ethanol (5 mL) was added sodium borohydride (7 mg, 0.176 mmol) and the reaction mixture was stirred for 20 h at room temperature. The reaction was quenched with water (5 mL) and the volatiles were removed at reduced pressure. The residue was dissolved in ethyl acetate (30 mL), washed with aq. $NaHCO_3$ (10 mL) and brine (10 mL), dried ($MgSO_4$) and concentrated in vacuo. The crude product was purified by flash chromatography (10% EtOAc in hexane) to afford the title compounds **25a**,**25b** (16 mg, 82%) as a colourless oil and as a 1:1 mixture of diastereomers; (Found: M^+, 446.2573, $C_{28}H_{34}N_2O_3$ requires 446.2569); δ_H (400 MHz, $CDCl_3$) 1.07–1.16 (0.5H, m,14-H_{ax}*), 1.22–1.44 (4H, m, 12-H_{ax}*, 14-H_{ax}, NCH(CH_3)Ph, NCH(CH_3)Ph*), 1.47–1.76 (3.5H, m, 2-H_b*, 9H_{ax}, 12H_{ax}, 12-H_{eq}, 12-H_{eq}*, 13H_{eq}, 13H_{eq}*), 1.87 2.17 (3.5H, m, 2-H_a, 2H_a*, 2-H_b, 9H_{ax}*, 11H_{ax}, 14H_{eq}, 14H_{eq}*), 2.25 (0.5H, dd, $^2J_{gem}$=10.7 Hz, $^4J_{11ax*,12ax*}$=1.8 Hz, 11H_{ax}*), 2.53 (0.5H, d, $^2J_{gem}$= 11.4 Hz, 9H_{eq}), 2.78–2.95 (2H, m, 9H_{eq}*, 11-H_{eq}, 13H_{ax}, 13H_{ax}*), 3.05–3.17 (1.5H, m, 11-H_{eq}*, NCH(CH$_3$)Ph, NCH(CH$_3$)Ph*), 3.21 (0.5H, s, 7H*), 3.22 (0.5H, s, 7H), 3.86–4.27 (4H, m, 5H_a, 5H_a*, 5-H_b, 5-H_b*, CH$_2$O, CH$_2$O*), 5.62 5.91 (4H, m, 3-H, 3-H*, 4-H, 4-H*, NH$_2$, NH$_2$*), 6.56–6.69 (2H, m, 3′-H, 3′ H*, 5′-H, 5′ H*), 7.20–7.34 (6H, m, 4′-H, 4′-H*, H(Ph), H*(Ph*)), 7.56 (0.5H, dd, $^3J_{5'*,6'*}$= 8.0 Hz, $^4J_{4'*,6'*}$=1.4 Hz, 6′-H*), 7.84 (0.5H, dd, $^3J_{5',6'}$= 8.3 Hz, $^4J_{4',6'}$=1.5 Hz, 6′-H); δ_C (75 MHz, $CDCl_3$) 20.5 (NCH(CH_3)Ph*), 20.7 (C-13, C 13*), 20.8 (NCH(CH_3)Ph), 27.6 (C 12*), 27.9 (C 12), 29.5 (C-14*), 29.9 (C-14), 36.7 (C-1*), 36.9 (C-1), 39.8, 39.9, 40.0, 40.3 (C-2, C-2*, C-8, C-8*), 58.8 (C 11*), 59.8 (C-11), 62.6 (C-9*), 64.0 (C-9), 65.4 (NCH(CH$_3$)Ph*), 65.6 (NCH(CH$_3$)Ph), 68.4 (C-5, C-5*), 69.2 (CH$_2$O*), 69.4 (CH$_2$O), 88.1 (C 7*), 88.4 (C 7),

111.1 (C-1′, C-1′*), 116.2 (C-3′*), 116.3 (C-3′), 116.6 (C 5′*), 116.7 (C 5′), 126.7 (C-4, C-4*), 127.3 (C-2*, C-6*), 127.4 (C-2, C-6), 128.3 (C 3, C 3*, C-5, C-5*), 130.2 (C-3*), 130.4 (C-3), 131.0 (C 6′, C 6′*), 131.1 (C-4*), 131.2 (C-4), 133.9 (C-4′*), 134.0 (C-4′), 144.9 (C-1*), 145.1 (C-1), 150.4 (C-2′*), 150.5 (C-2′), 167.9 (C═O* (ester)), 168.1 (C═O (ester)).

3.3.14. (1*S*,7*R*,8*S*)-(10-((1*S*)-1-Phenylethyl)-6-oxa-10-azatricyclo[6.3.3.0^{1,7}]-tetradec-3-en-8-yl)methyl 2-(3-methyl-2,5-dioxopyrrolidin-1-yl)benzoate 26a and (1*R*,7*S*,8*R*)-(10-((1*S*)-1-phenylethyl)-6-oxa-10-azatricyclo-[6.3.3.0^{1,7}]tetra-dec-3-en-8-yl)-methyl 2-(3-methyl-2,5-dioxopyrrolidin-1-yl)benzoate 26b. A mixture of amines **25a**, **25b** (14 mg, 0.031 mmol) and methylsuccinic anhydride (11 mg, 0.093 mmol) was heated at 125 °C for 4 h. The reaction mixture was dissolved in warm ethyl acetate (20 mL), washed with aq. $NaHCO_3$ (10 mL) and brine (10 mL), dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography (100% $CHCl_3$) to afford the title compounds **26a**, **26b** (9 mg, 55%) as a colourless oil and as a 1:1 mixture of diastereomers; (Found: M^+, 542.2776, $C_{33}H_{38}N_2O_5$ requires 542.2781); δ_H (400 MHz, $CDCl_3$) 1.06–1.14 (0.5H, m, 12-H_{ax}*), 1.40–1.62 (4.5H, m, 12H_{ax}, 14-H_{ax}, 14H_{ax}*, NCH(CH_3)Ph, NCH(CH_3)Ph*), 1.55–1.62 (4H, m, 13H_{eq}, 13H_{eq}*, 3″-CH_3, 3″-CH_3*), 1.60–1.74 (1.5H, m, 9H_{ax}, 12-H_{eq}, 12-H_{eq}*), 1.85–2.23 (4.5H, m, 2-H_a, 2H_a*, 2-H_b, 2-H_b*, 9H_{ax}*, 11H_{ax}, 11H_{ax}*, 14H_{eq}, 14H_{eq}*), 2.45–2.62 (1.5H, m, 9H_{eq}, 4″ H_b, 4″-H_b*), 2.76 2.93 (2H, m, 9H_{eq}*, 11-H_{eq}, 13H_{ax}, 13H_{ax}*), 3.00–3.18 (4.5H, m, 3″ H, 3″-H*, 4″-H_a, 4″-H_a*, 7H, 7H*, 11-H_{eq}*, NC*H*(CH_3)Ph, NC*H*(CH_3)Ph*), 3.87 4.26 (4H, m, 5H_a, 5H_a*, 5-H_b, 5-H_b*, CH_2O, CH_2O*), 5.63 5.92 (2H, m, 3-H, 3-H*, 4-H, 4-H*), 7.21–7.36 (6H, m, 3′-H, 3′ H*, H(Ph), H(Ph*)), 7.44 (0.5H, ddd, $^3J_{4'*,5'*}$= 7.7 Hz, $^3J_{5'*,6'*}$=7.7 Hz, $^4J_{3'*,5'*}$=1.1 Hz, 5′ H*), 7.54 (0.5H, ddd, $^3J_{4',5'}$=7.7 Hz, $^3J_{5',6'}$=7.7 Hz, $^4J_{3',5'}$=1.1 Hz, 5′-H), 7.61–7.70 (1H, m, 4′-H, 4′-H*), 7.75 (0.5H, d, $^3J_{5'*,6'*}$=7.9 Hz, 6′-H*), 8.08 (0.5H, d, $^3J_{5',6'}$=7.8 Hz, 6′-H,); δ_C (75 MHz, $CDCl_3$) 16.3 (3″ CH_3*), 16.5 (3″-CH_3), 20.6 (C-13, C-13*, NCH(*C*H_3)Ph, NCH(*C*H_3)Ph*), 27.5 (C-12*), 27.8 (C-12), 29.4 (C-14*), 29.8 (C-14), 35.2 (C-3″*), 35.4 (C-3″), 36.6 (C-1*), 36.8 (C-1), 37.0 (C-4″, C-4″*), 39.8, 39.9, 40.3 (C-2, C-2*, C-8, C-8*), 58.6 (C-11*), 59.7 (C-11), 62.6 (C-9*), 63.8 (C-9), 65.4 (N*C*H(CH_3)Ph*), 65.5 (N*C*H(CH_3)Ph), 68.4 (C-5, C-5*), 70.1 (CH_2O, CH_2O*), 88.0 (C 7*), 88.4 (C 7), 126.6 (C-4*), 126.7 (C-4), 127.3 (C-2*, C-6*, C-1′, C-1′*), 127.4 (C 2, C-6), 128.3 (C 3, C 3*, C-5, C-5*), 129.4 (C 5′*), 129.5 (C 5′), 129.9 (C-3′*), 130.0 (C-3′), 130.3 (C-3, C-3*), 130.4 (C-4, C-4*), 131.2 (C 6′*), 131.4 (C 6′), 132.9 (C-2′*), 133.0 (C-2′), 133.3 (C-4′*), 133.4 (C-4′), 144.9 (C-1, C-1*), 164.0 (C═O, C═O* (ester)), 176.1 (C 5″, C 5″*), 180.0 (C-2″, C-2″*).

References and notes

1. (a) Earle, M. J.; Fairhurst, R. A.; Heaney, H.; Papageorgiou, G.; Wilkins, R. F. *Tetrahedron Lett.* **1990**, *31*, 4229. (b) Cooper, M. S.; Earle, M. J.; Fairhurst, R. A.; Heaney, H.; Papageorgiou, G.; Wilkins, R. F. *Synlett* **1990**, 617. (c) Heaney, H.; Papageorgiou, G.; Wilkins, R. F. *Tetrahedron* **1995**, *51*, 10737.
2. (a) Manske, R. H. *Can. J. Res.* **1938**, *16B*, 57. (b) Pelletier, S. W.; Joshi, B. S. In Pelletier, S. W., Ed.; Alkaloids: Chemical and Biological Perspectives; Springer: New York, 1991; 7, p 297.
3. Brocke, C.; Brimble, M. A.; Lin, D. S.; McLeod, M. D. *Synlett* **2004**, 2359.
4. El Gihani, M. T.; Heaney, H.; Slawin, A. M. Z. *Tetrahedron Lett.* **1995**, *36*, 4905.
5. Iwanek, W.; Mattay, J. *Liebigs Ann. Chem.* **1995**, 1463.
6. Arnecke, R.; Böhmer, V.; Friebe, S.; Gebauer, S.; Krauss, G. J.; Thondorf, I.; Vogt, W. *Tetrahedron Lett.* **1995**, *36*, 6221.
7. Page, P. C. B.; Heaney, H.; Sampler, E. P. *J. Am. Chem. Soc.* **1999**, *121*, 6751.
8. Schmidt, C.; Paulus, E. F.; Böhmer, V.; Vogt, W. *New J. Chem.* **2001**, *25*, 374.
9. Sampler, E. P. PhD Thesis, Loughborough University, 2001.
10. Davies, A. R. L.; Hardick, D. J.; Blagbrough, I. S.; Potter, B. V. L.; Wolstenholme, A. J.; Wonnacott, S. *Neuropharmacology* **1999**, *38*, 679.
11. (a) Bergmeier, S. C.; Lapinsky, D. J.; Free, R. B.; McKay, D. B. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2263. (b) Doisy, X.; Blagbrough, I. S.; Wonnacott, S.; Potter, B. V. L. *Pharm. Pharmacol. Commun.* **1998**, *4*, 313.
12. Trigg, W. J.; Hardick, D. J.; Grangier, G.; Wonnacott, S.; Lewis, T.; Rowan, M. G.; Potter, B. V. L.; Blagbrough, I. S. *ACS Symp. Ser.* **1998**, *686*, 194.
13. (a) Baillie, L. C.; Bearder, J. R.; Li, W.-S.; Sherringham, J. A.; Whiting, D. A. *J. Chem. Soc., Perkin Trans. 1* **1998**, 4047. (b) Kraus, G. A.; Dneprovskaia, E. *Tetrahedron Lett.* **1998**, *39*, 2451.
14. (a) Davies, A. R. L.; Hardick, D. J.; Blagbrough, I. S.; Potter, B. V. L.; Wolstenholme, A. J.; Wonnacott, S. *Biochem. Soc. Trans.* **1997**, *25*, 545S. (b) Bryant, D. L.; Free, R. B.; Thomasy, S. M.; Lapinsky, D. J.; Ismail, K. A.; McKay, S. B.; Bergmeier, S. C.; McKay, D. B. *Neurosci. Res.* **2002**, *42*, 57.
15. Arias-Pérez, M. S.; Alejo, A.; Gálvez, E.; Pérez, S. M.; Santos, M. J. *J. Mol. Struct.* **1995**, *349*, 169.
16. Jeyaraman, R.; Jawaharsingh, C. B.; Avila, S.; Eliel, E. L.; Ganapathy, K.; Manoharan, M.; Morris-Natschke, S. *J. Heterocycl. Chem.* **1982**, *19*, 449.
17. Gravel, D.; Labelle, M. *Can. J. Chem.* **1985**, *63*, 1874.
18. Schepartz, A.; Breslow, R. *J. Am. Chem. Soc.* **1987**, *109*, 1814.

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2-Cyanopyridazin-3(2*H*)-ones: effective and chemoselective electrophilic cyanating agents

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Abstract—2-Cyanopyridazin-3(2*H*)-ones are novel, effective, selective and electrophilic cyanating agents. A variety of amino, thiol and carbon nucleophiles are chemoselectively *N*-, *S*- or *C*-cyanated in excellent yield using 2-cyanopyridanzin-3(2*H*)-ones in water or tetrahydrofuran.

1. Introduction

The introduction of a cyano group via carbon–carbon, carbon–nitrogen and carbon–sulfur forming reactions is a fundamental process in organic synthesis. Compounds containing the *N*-, *S*-, or *C*-cyano functional group are also found among many pharmaceuticals and their intermediates.[1,2] There are a limited number of reagents that can serve as a cyano cation (CN^+) equivalent or synthon, inter alia, tosyl cyanide,[3,4] 2-chlorobenzylthiocyanate,[5] cyanogen chloride,[6] 1-cyanobenzotriazole,[7,8] and 1-cyanoimidazole.[9] However, problems with poor solubility, lack of reactivity, cost, stability, corrosiveness, toxicity, complicated preparation, and/or availability have prompted the continuing search for a more effective, mild, and convenient class of electrophilic cyanating agents. Since, pyridazin-3(2*H*)-ones **1** readily form stable anions,[10] are good leaving groups, and have been used as a novel synthetic auxiliary.[11–14] Our attention focused upon their utility as electrophilic cyanating agents. Herein, we report the first synthesis and the application of the hitherto unknown 2-cyanopyridazin-3(2*H*)-ones **2** as electrophilic cyanating reagents toward various amines, sulfur, and carbon nucleophilies.

2. Results and discussion

A variety of 2-cyanopyridazin-3(2*H*)-ones **2** were readily prepared by treating the corresponding pyridazin-3(2*H*)-ones **1** with cyanogen bromide and triethylamine in tetrahydrofuran at room temperature (Table 1). Yields were good to excellent even in the presence of halide, phenolic, azide, and heteroatom substituents (Scheme 1).

Table 1. Preparation of 2-cyanopyridazin-3(2*H*)-ones (**2**)[a]

Entry	**2**	Time (h)	Yield (%)[b]
1	**2a**	1.2	92
2	**2b**	1.4	88
3	**2c**	1.0	85
4	**2d**	1.0	80
5	**2e**	1.2	80
6	**2f**	1.5	83
7	**2g**	1.3	85

[a] Compound **1** (1 equiv), BrCN (1 equiv) and Et_3N (1 equiv), THF, at room temperature.
[b] Isolated yield.

Initially, the efficacy of **2a**–**g** for *N*-cyanation was evaluated using *N*-methylbenzylamine (**3a**) in water at 15–17 °C (Table 2, entries 1–7). Among seven *N*-cyanopyridazin-3(2*H*)-ones, **2a** and **2b** were the best *N*-cyanating agents. Consequently, **2a** and **2b** were further, studied in a variety of representative organic solvents (entries 8–17). Exclusive

Keywords: *N*-, *S*- *or C*-cyanation; Electrophilic cyanating agent; 2-Cyanopyridazin-3(2*H*)-ones.
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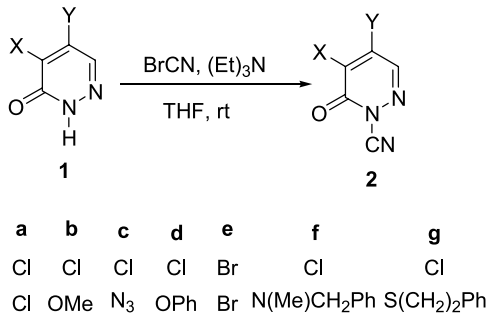


1,2	a	b	c	d	e	f	g
X	Cl	Cl	Cl	Cl	Br	Cl	Cl
Y	Cl	OMe	N_3	OPh	Br	$N(Me)CH_2Ph$	$S(CH_2)_2Ph$

Scheme 1.

N-cyanation in good to excellent yields was obtained in acetonitrile, methanol, and toluene.

Cyanation of various nitrogen and sulfur nucleophiles **3b–h** with **2a–b** in water under neutral condition gave the corresponding *N*- or *S*-cyano derivatives **4b–h** in good to excellent yields (Table 3, entries 1–14).

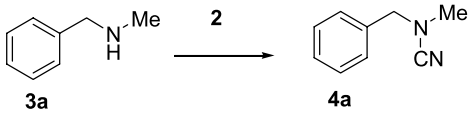
We also investigated the chemoselectivity in the cyanation of bifunctional nucleophiles such as 4-aminophenol, 4-aminobenzenethiol and 4-mercaptophenol. Firstly, compounds **3i–k** was treated with **2a–b** in water to give both 5-substituted-pyridazin-3(2*H*)-ones and cyanated **4i–k**. To enhance the chemoselectivity of the cyanation for bifunctional nucleophiles, we found that the best reaction condition requires the presence of 1 equiv of zinc chloride from preliminary experiments. Treatment of bifunctional nucleophiles **3i–k** with **2a**,**b** (entries 15–20) in the presence of zinc chloride in water chemoselectively afforded *N*- or *S*-cyano derivatives **4i–k** in good yields. In order to expand the applications of the α-cyanation for 1,3-dicarbonyl compounds, we examined the cyanation of **3l** and **3m**. Otherwise the cyanations of *N*- or *S*-nucleophile, the reactions of 1,3-dicarbonyl compounds in water, methanol, acetonitrile and toluene suffer from low yield, low selectivity, and long reaction time. Therefore, we chose tetrahydrofuran as solvent for the α-cyanation of 1,3-dicarbonyl compounds. Reaction of **2b** with β-diketone **3l** in tetrahydrofuran mediated by $ZnCl_2$ or NaH (entries 21 and 22, respectively) gave rise to deacetylated α-cyano ketone **4l** in excellent yields. A similar deacetylation has been reported.[15] On the other hand, deacetylation was not observed for **3m** under identical conditions. Reaction of **3m** with **2b** (2 equiv) in the presence of $ZnCl_2$ or NaH (entries 23 and 24, respectively) gave the corresponding α,α-dicyano derivative **4m** in excellent yields, whereas the use of just 1 equiv of **2b** under the same conditions afforded **4m** in 46–48% yields. In contrast to the behavior of **2b**, compound **2a** was not capable of cyanating the carbon nucleophiles and yielded 5-substituted-pyridazin-3(2*H*)-ones instead of **4l** or **4m**. Enhancement of the reactivity of cyano group at N-2 position for the cyanation of *N*-, *S*- or *C*-nucleophiles with **2** may be due to the chelation of **2** with zinc chloride (Fig. 1).

The different product selectivity between substrate **3l** and **3m** may be due to the different tautomerization of two substrates under our condition. In order to occur an intermolecular pyridazinone-mediated deacylation like Katritzky's mechanism,[15] the terminal acetyl group of **3l** and **3m** must be present as a keto-form. Under our reaction system, the structure (I) of **3l** may be more favorable than the structure (II), whereas the structure (IV) of **3m** may be more favorable than the structure (III) (Scheme 2).

3. Conclusion

In conclusion, 2-cyanopyridazin-3(2*H*)-ones such as **2a** and **2b** are novel and stable electrophilic cyanating agents. The methodology presented here, is an efficient, chemoselective, mild and/or eco-friendly procedure for cyanation of nitrogen, sulfur and carbon nucleophiles. The solvents

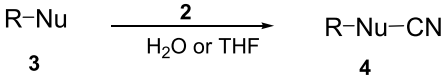
Table 2. Preparation of *N*-cyano-*N*-methylbenzylamine (**4a**) using **2** in various solvents

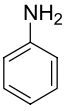
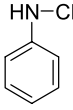
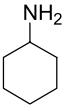
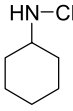
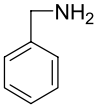
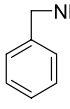
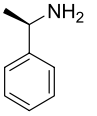
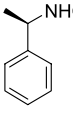
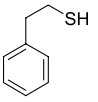
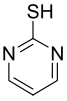
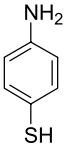
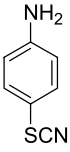
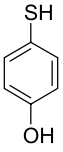
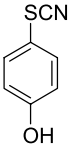
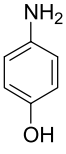
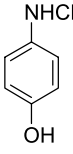
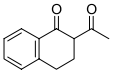
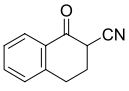
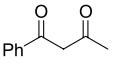
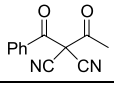


Entry	2	Solvent	Temp (°C)	Time (h)	**4a** (%)[a]
1	**2a**	H_2O	15–17	0.5	95
2	**2b**	H_2O	15–17	0.5	96
3	**2c**	H_2O	15–17	1	88
4	**2d**	H_2O	15–17	1	83
5	**2e**	H_2O	15–17	1.2	82
6	**2f**	H_2O	15–17	1.5	85
7	**2g**	H_2O	15–17	1.3	84
8	**2a**	CH_2Cl_2	Reflux	22	52
9	**2b**	CH_2Cl_2	Reflux	28	41
10	**2a**	THF	Reflux	5	—[b]
11	**2b**	THF	Reflux	20	75
12	**2a**	CH_3CN	Reflux	9	88
13	**2b**	CH_3CN	Reflux	14	94
14	**2a**	MeOH	Reflux	6	91
15	**2b**	MeOH	Reflux	4	89
16	**2a**	$C_6H_5CH_3$	Reflux	2	92
17	**2b**	$C_6H_5CH_3$	Reflux	2	97

[a] Isolated yield. Compound **1** was isolated in good to excellent yields except for entries 1–4 and could be recycled.
[b] 5-(*N*-Methyl-*N*-benzylamino)-4-chloropyridazin-3(2*H*)-one was obtained in 80% yield.

Table 3. Cyanation of the substrates (**3**) with **2a–b** in water or THF



Entry	2	Substrate (3)		Temp (°C)	Solvent	Time (h)	4 (%)[a]	
1	**2a**	**3b**		15–17	H_2O	1.5		**4b** (91)
2	**2b**			15–17	H_2O	0.5		**4b** (92)
3	**2a**	**3c**		15–17	H_2O	1.0		**4c** (89)
4	**2b**			15–17	H_2O	0.5		**4c** (93)
5	**2a**	**3d**		15–17	H_2O	1.2		**4d** (84)
6	**2b**			15–17	H_2O	0.5		**4d** (86)
7	**2a**	**3e**	*n*-Bu–NH_2	15–17	H_2O	1.0	*n*-Bu–NHCN	**4e** (82)
8	**2b**			15–17	H_2O	0.8		**4e** (80)
9	**2a**	**3f**		15–17	H_2O	0.8		**4f** (88)
10	**2b**			15–17	H_2O	0.8		**4f** (98)
11	**2a**	**3g**		15–17	H_2O	0.5		**4g** (82)
12	**2b**			15–17	H_2O	0.8		**4g** (78)
13	**2a**	**3h**		15–17	H_2O	1.5		**4h** (85)
14	**2b**			15–17	H_2O	1.2		**4h** (81)
15	**2a**	**3i**		15–17	H_2O	1.5		**4i** (78)[b]
16	**2b**			15–17	H_2O	1.5		**4i** (85)[b]
17	**2a**	**3j**		15–17	H_2O	1.0		**4j** (82)[b]
18	**2b**			15–17	H_2O	2.1		**4j** (85)[b]
19	**2a**	**3k**		15–17	H_2O	1.2		**4k** (86)[b]
20	**2b**			15–17	H_2O	2.0		**4k** (85)[b]
21	**2b**	**3l**		Reflux	THF	24		**4l** (93)[c]
22	**2b**			7–8	THF	9		**4l** (94)[d]
23	**2b**	**3m**		Reflux	THF	14		**4m** (97)[e]
24	**2b**			7–8	THF	5		**4m** (95)[f]

[a] Isolated yield. Compound **1** was isolated in good to excellent yield except for entry 12 and could be recycled.
[b] Using $ZnCl_2$ (30 mol%) as the catalyst.
[c] Using $ZnCl_2$ (1 equiv).
[d] Using NaH (60%, 1.2 equiv).
[e] Reaction conditions: **2b** (2 equiv), $ZnCl_2$ (1 equiv).
[f] Reaction conditions: **2b** (2 equiv), NaH (60%, 2.4 equiv).

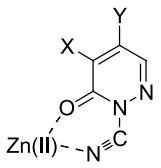
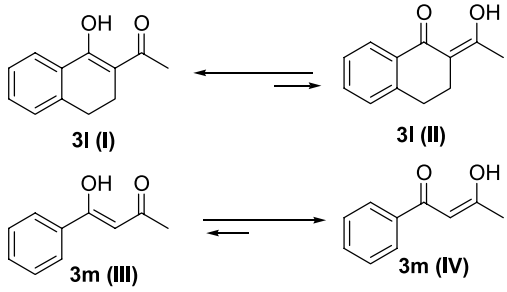


Figure 1. Chleation of **2** with Zn(II).



Scheme 2.

also affect the reactivity and the selectivity of these systems. Our investigation of the reactions of these new compounds is continuing and the results will be reported in due course.

4. Experimental

4.1. General

Melting points were determined with a capillary apparatus and uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a 300 MHz spectrometer with chemical shift values reported in units (ppm) relative to an internal standard (TMS). IR spectra were obtained on a IR spectrophotometer. Elemental analyses were performed with a Perkin Elmer 240C. Open-bed chromatography was carried out on silica gel (70–230 mesh, Merck) using gravity flow. The column was packed as slurries with the elution solvent. 4,5-Dichloropyridazin-3(2*H*)-one (**1a**) and 4,5-dibromopyridazin-3(2*H*)-one (**1e**) were prepared by the literature method.[16] 4-Chloro-5-substituted-pyridazin-3(2*H*)-ones **1b–d**, **1f** and **1g** were prepared from **1a** by the reported method.[17]

4.2. Synthesis of 2-cyanopyridazin-3(2*H*)-ones 2

Triethylamine (0.6 mL, 4.36 mmol) was added slowly to a stirred solution of **1** (1.1 g, 4.36 mmol) in THF (40 mL) at room temperature. After stirring 5 min, cyanogen bromide (4.36 mmol) was added. The reaction mixture was stirred at room temperature until **1** disappeared. After evaporating the solvent under reduced pressure, the resulting residue was applied to the top of an open-bed silica gel column (3.0× 5 cm). The column was eluted with methylene chloride. Fractions containing **2** were combined and evaporated under reduced pressure to give **2**.

4.2.1. 2-Cyano-4,5-dichloropyridazin-3(2*H*)-one (2a). Yield 92%. Mp 104–105 °C. R_f=0.56 (methylene chloride). IR (KBr) 3100, 3050, 2245, 1700, 1600, 1580, 1360, 1260, 1180, 1160, 960 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.00 (s, 1H) ppm; ^{13}C NMR ($CDCl_3$) δ 104.9, 134.9, 138.3, 141.2, 154.4 ppm. Elemental analysis calcd for $C_5HCl_2N_3O$: C, 34.52; H, 0.58; N, 24.15; found: C, 34.77; H, 0.53; N, 24.07.

4.2.2. 2-Cyano-4-chloro-5-methoxypyridazin-3(2*H*)-one (2b). Yield 88%. Mp 105–106 °C. R_f=0.61 (methylene chloride). IR (KBr) 3100, 3030, 2980, 2250, 1690, 1600, 1460, 1400, 1300, 1260, 1120, 960 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 4.24 (s, 3H), 8.19 (s, 1H) ppm; ^{13}C NMR ($CDCl_3$) δ 59.1, 105.6, 115.8, 133.8, 155.4, 156.9 ppm. Elemental analysis calcd for $C_6H_4ClN_3O_2$: C, 38.83; H, 2.17; N, 22.64; found: C, 38.87; H, 2.13; N, 23.46.

4.2.3. 2-Cyano-4-chloro-5-azidopyridazin-3(2*H*)-one (2c). Yield 85%. R_f=0.46 (methylene chloride). Mp 114–115 °C. R_f=0.67. IR (KBr) 3100, 3070, 2250, 2160, 2120, 1700, 1600, 1520, 1380, 1310, 1230, 1130, 1000, 860, 730, 700 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.74 (s, 1H) ppm; ^{13}C NMR ($CDCl_3$) δ 105.2, 122.0, 136.3, 140.5, 155.1 ppm. Elemental analysis calcd for C_5HClN_6O: C, 30.55; H, 0.51; N, 42.76; found: C, 30.77; H, 0.53; N, 42.37.

4.2.4. 2-Cyano-4-chloro-5-phenoxypyridazin-3(2*H*)-one (2d). Yield 80%. R_f=0.42 (methylene chloride). Mp 115–116 °C. IR (KBr) 3070, 2250, 1720, 1620, 1590, 1540, 1490, 1380, 1280, 1220, 1150, 860, 820, 780, 730, 690 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.12–7.17 (m, 2H), 7.34–7.41 (m, 1H), 7.47–7.55 (m, 2H), 7.65 (s, 1H) ppm; ^{13}C NMR ($CDCl_3$) δ 105.4, 118.8, 119.9, 127.2, 130.9, 135.3, 152.7, 153.7, 156.8 ppm. Elemental analysis calcd for $C_{11}H_6ClN_3O_2$: C, 53.35; H, 2.44; N, 16.97; found: C, 53.57; H, 2.33, N, 16.96.

4.2.5. 2-Cyano-4,5-dibromopyridazin-3(2*H*)-one (2e). Yield 80%. R_f=0.62 (methylene chloride). Mp 84–85 °C. R_f=0.67. IR (KBr) 3080, 3040, 2950, 2260, 1680, 1590, 1510, 1280, 1220, 1110, 960, 860, 720 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.98 (s, 1H) ppm; ^{13}C NMR ($CDCl_3$) δ 137.4, 138.2, 141.1, 142.4, 154.4 ppm. Elemental analysis calcd for $C_5HBr_2N_3O$: C, 21.53; H, 0.36; N, 15.07; found: C, 21.71; H, 0.33; N, 15.15.

4.2.6. 2-Cyano-4-chloro-5-(benzylmethylamino)pyridazin-3(2*H*)-one (2f). Yield 83%. R_f=0.49 (methylene chloride). Mp 105–106 °C. IR (KBr) 3050, 2980, 2950, 2260, 1690, 1625, 1560, 1500, 1460, 1420, 1360, 1310, 1260, 1240, 1200, 1140, 1100, 1060, 1030, 980, 900, 750, 730 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 3.18 (s, 3H), 4.87 (s, 2H), 7.30 (dd, 3H, J=7.12 Hz, 7.39), 7.39 (dd, 2H, J=7.43 Hz, 7.51), 8.27 (s, 1H) ppm; ^{13}C NMR (DMSO-d_6) δ 41.1, 56.8, 104.6, 107.6, 127.4, 127.9, 129.1, 136.9, 138.9, 147.5, 156.8 ppm. Elemental analysis calcd for $C_{13}H_{11}ClN_4O$: C, 56.84; H, 4.04; N, 20.40; found: C, 56.87; H, 4.13; N, 20.51.

4.2.7. 2-Cyano-4-chloro-5-(phenethylsulfanyl)pyridazin-3(2*H*)-one (2g). Yield 85%. R_f=0.51 (methylene chloride). Mp 141–143 °C. IR (KBr) 3080, 3000, 2950, 2270, 1700, 1590, 1500, 1460, 1440, 1380, 1310, 1290, 1230, 1120, 960, 860, 780, 740 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.07 (t, 2H, J=7.34 Hz), 3.38 (t, 2H, J=7.34 Hz), 7.20–7.37 (m, 5H), 7.64 (s, 1H) ppm; ^{13}C NMR ($CDCl_3$) δ 33.5, 35.7, 127.6, 128.7, 129.0, 136.9, 137.6, 143.9, 145.2, 152.3, 159.2 ppm. Elemental analysis calcd for $C_{13}H_{10}ClN_3OS$: C, 53.52; H, 3.45; N, 14.40; found: C, 53.63; H, 3.42; N, 14.50.

4.3. Typical cyanation of nucleophiles in water

Nucleophiles (1.2 equiv) was added slowly to a stirred solution of **2** (200 mg, 1.05 mmol) in water (20 mL) at 15–17 °C. The reaction mixture was stirred at room temperature until **2** disappeared. The product was extracted with CH_2Cl_2 (30 mL×2). The organic layer was dried over anhydrous magnesium sulfate and then the solvent evaporated under reduced pressure, the resulting residue was applied to the top of an open-bed silica gel column (3.0× 7 cm). The column was eluted with methylene chloride or methylene chloride: *n*-hexane=2:1 (v/v); methylene chloride/ether=10:1 (v/v). Fractions containing **4** were combined and evaporated under reduced pressure to give **4** in 78–98% yield.

4.4. Typical cyanation of 1,3-dicarbonyl compounds in presence of base or acid

NaH or $ZnCl_2$ (1.1 equiv) was added slowly to a stirred solution of active methylene (1.08 mmol) in dry-THF (20 mL) at 7–8 °C (for NaH) and at reflux temperature (for $ZnCl_2$). After stirring 5 min, **2** (200 mg, 1.08 mmol) was added. The reaction mixture was refluxed until carbanion or **2** disappeared. After evaporating the solvent under reduced pressure, the resulting residue was applied to the top of an open-bed silica gel column (3.0×10 cm). The column was eluted with methylene chloride: ether= 7:1 (v/v). Fractions containing **4l** or **4m** were combined and evaporated under reduced pressure to give **4l** or **4m** in 93–97% yield.

4.4.1. *N*-Cyano-*N*-methylbenzylamine (4a). Oil (colorless). R_f=0.70 (methylene chloride/*n*-hexane=2:1 (v/v)). IR (KBr) 3050, 2950, 2240, 1500, 1460, 1380, 1230, 1160, 1040, 740, 700 cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.75 (s, 3H), 4.13 (s, 2H), 7.34 (m, 5H) ppm. ^{13}C NMR ($CDCl_3$) δ 37.7, 57.1, 118.8, 128.3, 128.5, 128.8, 134.3 ppm. Elemental analysis calcd for $C_9H_{10}N_2$: C, 73.94; H, 6.89; N, 19.16; found C, 73.98; H, 6.84; N, 19.21.

4.4.2. *N*-Cyanoaniline (4b). Oil (colorless). R_f=0.43 (methylene chloride/ether=10:1 (v/v)). IR (KBr) 3190, 3100, 3000, 2930, 2230, 1600, 1500, 1440, 1300, 1250, 750, 690, 490 cm^{-1}. ^1H NMR ($CDCl_3$) δ 6.19 (s, NH, D_2O exchangable), 6.99 (d, 2H, J=8.65 Hz), 7.05 (m, 1H), 7.31 (m, 2H) ppm. ^{13}C NMR ($CDCl_3$) δ 111.7, 115.5, 123.5, 129.7, 137.4 ppm. Elemental analysis calcd for $C_7H_6N_2$: C, 71.17; H, 5.12; N, 23.71; found C, 71.21; H, 5.15; N, 23.73.

4.4.3. *N*-Cyanocyclohexylamine (4c). Oil (colorless). R_f= 0.36 (methylene chloride). IR (KBr) 3200, 2940, 2860, 2230, 1450, 1370, 1260, 1170, 1140, 950, 900 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.98–1.20 (m, 10H), 3.09 (m, 1H), 4.14 (s, NH, D_2O exchangable) ppm. ^{13}C NMR ($CDCl_3$) δ 24.3, 25.1, 32.6, 54.4, 115.7 ppm. Elemental analysis calcd for $C_7H_{12}N_2$: C, 67.70; H, 9.74; N, 22.56; found C, 67.75; H, 9.75; N, 22.53.

4.4.4. *N*-Cyanobenzylamine (4d). Oil (colorless). R_f=0.32 (methylene chloride). IR (KBr) 3260, 3080, 3030, 2220, 1500, 1460, 1440, 1340, 1310, 1220, 1160, 750, 700 cm^{-1}. ^1H NMR ($CDCl_3$) δ 4.14 (d, 2H, J=5.57 Hz), 4.41 (bs, NH, D_2O exchangable), 7.28–7.36 (m, 5H) ppm. ^{13}C NMR ($CDCl_3$) δ 50.1, 116.3, 127.8, 128.4, 128.9, 136.3 ppm. Elemental analysis calcd for $C_8H_8N_2$: C, 72.70; H, 6.10; N, 21.20; found C, 72.81; H, 6.15; N, 21.19.

4.4.5. *N*-Cyano-*n*-butylamine (4e). Oil (colorless). R_f= 0.48 (methylene chloride). IR (KBr) 3220, 2970, 2950, 2880, 2220, 1460, 1380, 1360, 1170 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.92–1.00 (t, 3H, J=3.36 Hz), 1.34–1.47 (m, 2H), 1.62–1.73 (m, 2H), 2.16 (m, 2H), 3.92 (bs, NH, D_2O exchangable) ppm. ^{13}C NMR ($CDCl_3$) δ 13.8, 20.2, 28.8, 44.4, 147.2 ppm. Elemental analysis calcd for $C_5H_{10}N_2$: C, 61.19; H, 10.27; N, 28.54; found C, 61.22; H, 10.28; N, 28.49.

4.4.6. (*R*)-*N*-Cyano-α-methylbenzylamine (4f). Oil (colorless). R_f=0.29 (methylene chloride). IR (KBr) 3210, 3000, 2920, 2230, 1500, 1460, 1380, 1210, 1160, 760, 700 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.53 (d, 3H, J=6.80 Hz), 4.31 (bs, NH, D_2O exchangable), 4.32–4.41 (m, 1H), 7.25–7.40 (m, 5H) ppm. ^{13}C NMR ($CDCl_3$) δ 22.0, 55.6, 115.3, 126.2, 128.3, 128.9, 141.5 ppm. Elemental analysis calcd for $C_9H_{10}N_2$: C, 73.94; H, 6.89; N, 19.16; found C, 73.88; H, 6.91; N, 19.19.

4.4.7. 2-Phenylethyl thiocyanate (4g). Oil (colorless). R_f= 0.50 (methylene chloride). IR (KBr) 3080, 3050, 2950, 2880, 2180, 1610, 1500, 1460, 1330, 1290, 1240, 1080, 1040, 760, 710 cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.11 (m, 2H), 3.16 (m, 2H), 7.21 (d, 2H, J=7.00 Hz), 7.27 (dd, 1H, J= 7.50 Hz), 7.33 (dd, 2H, J=7.00, 7.50 Hz) ppm. ^{13}C NMR ($CDCl_3$) δ 34.0, 34.9, 110.8, 126.1, 127.5, 127.7, 136.5 ppm. Elemental analysis calcd for C_9H_9NS: C, 66.22; H, 5.56; N, 8.58; found C, 66.24; H, 5.60; N, 8.56.

4.4.8. 2-Thiocyanatopyrimidine (4h). Mp 112–113 °C (colorless). R_f=0.61 (methylene chloride). IR (KBr) 3100, 3000, 2180, 1570, 1390, 1280, 1180, 820, 770, 740, 700, 630 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.33 (dd, 1H, J=4.88 Hz), 8.72 (d, 2H, J=4.88 Hz) ppm. ^{13}C NMR ($CDCl_3$) δ 107.3, 119.7, 158.9, 164.2 ppm. Elemental analysis calcd for $C_5H_3N_3S$: C, 43.78; H, 2.20; N, 30.64; found C, 43.91; H, 2.30; N, 29.99.

4.4.9. 4-Thiocyanatoaniline (4i). Mp 55–56 °C (colorless). R_f=0.23 (methylene chloride). IR (KBr) 3430, 3350, 3250, 3060, 2970, 2930, 2150, 1640, 1600, 1500, 1440, 1300, 1180, 1080, 820, 670, 520 cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.97 (bs, NH_2, D_2O exchangeable), 6.64–6.68 (dd, 2H, J= 6.80 Hz), 7.32–7.36 (dd, 2H, J=6.86 Hz) ppm. ^{13}C NMR ($CDCl_3$) δ 109.6, 112.3, 116.1, 134.4, 148.9 ppm. Elemental analysis calcd for $C_7H_6N_2S$: C, 55.97; H, 4.03; N, 18.65; found C, 56.01; H, 4.06; N, 18.56.

4.4.10. 4-Thiocyanatophenol (4j). Mp 62–63 °C (colorless). R_f=0.38 (Ethyl acetate/*n*-hexane=1:3 (v/v)). IR (KBr) 3400, 2960, 2890, 2180, 1620, 1600, 1500, 1440, 1370, 1280, 1240, 1180, 840 cm^{-1}. ^1H NMR ($CDCl_3$) δ 6.00 (bs, OH, D_2O exchangeable), 6.88 (dd, 2H, J= 6.50 Hz), 7.44 (dd, 2H, J=6.50 Hz) ppm. ^{13}C NMR ($CDCl_3$) δ 112.1, 113.4, 117.5, 134.2, 158.0 ppm. Elemental analysis calcd for C_7H_5NOS: C, 55.61; H, 3.33; N, 9.26; found C, 55.68; H, 3.36; N, 9.20.

4.4.11. *N*-Cyano-4-hydroxyaniline (4k). Mp 218–219 °C (colorless). R_f=0.41 (ethyl acetate/*n*-hexane=1:2 (v/v)). IR (KBr) 3200, 2990, 2930, 2230, 1620, 1510, 1440, 1360, 1280, 1260, 1220, 1110, 810, 620, 500 cm^{-1}. ^1H NMR (DMSO-d_6) δ 6.77 (m, 4H), 9.17 (bs, NH, D_2O exchangable), 9.62 (bs, OH, D_2O exchangeable) ppm. ^{13}C NMR (DMSO-d_6) δ 111.9, 115.1, 115.2, 128.8, 151.8 ppm. Elemental analysis calcd for $C_7H_6N_2O$: C, 62.68; H, 4.51; N, 20.88; found C, 62.60; H, 4.66; N, 20.90.

4.4.12. 2-Cyano-3,4-dihydro-1(2*H*)-naphthalenone (4l). Mp 75–77 °C (colorless). R_f=0.76 (methylene chloride/*n*-hexane=2:1 (v/v)). IR (KBr) 3070, 2950, 2880, 2250, 1700, 1600, 1460, 1440, 1360, 1310, 1230, 1200, 920, 750 cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.43–2.63 (m, 2H), 3.02–3.21 (m, 2H), 3.72–3.78 (dd, 1H, J=4.60, 4.61 Hz), 7.34–7.40 (dd, 1H, J=7.74, 7.44 Hz), 7.52–7.59 (m, 1H), 8.05–8.09 (dd, 1H, J=7.86, 7.89 Hz) ppm. ^{13}C NMR ($CDCl_3$) δ 27.7, 27.8, 40.8, 116.7, 127.5, 128.4, 129.0, 130.5, 134.8, 142.9, 187.8 ppm. Elemental analysis calcd for $C_{11}H_9NO$: C, 77.17; H, 5.30; N, 8.18; found C, 77.22; H, 5.32; N, 8.09.

4.4.13. 2,2-Dicyano-1-benzoylacetone (4m). Mp 68–69 °C (colorless). R_f=0.42 (methylene chloride). IR (KBr) 3070, 2930, 2220, 1735, 1690, 1600, 1550, 1440, 1400, 1360, 1290, 1180, 1020, 990, 860 cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.54 (s, 3H), 7.48–7.54 (m, 2H), 7.59–7.65 (m, 1H), 8.01–8.05 (m, 2H) ppm. ^{13}C NMR ($CDCl_3$) δ 25.6, 88.2, 117.7, 128.6, 128.7, 128.8, 133.1, 133.8, 190.0, 200.2 ppm. Elemental analysis calcd for $C_{12}H_8N_2O_2$: C, 67.92; H, 3.80; N, 13.20; found C, 67.88; H, 3.78; N, 13.16.

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References and notes

1. Gilman, A. G.; Goodman, L. S.; Rall, T. W.; Murad, F. *Goodman and Gilman's the pharmacological basis of therapeutics*, 7th ed.; Pergamon: New York, 1990; p 899.
2. Hu, L. Y.; Guo, J.; Mager, S. S.; Fischer, J. B.; Burke-Howie, K. J.; Durant, G. J. *J. Med. Chem.* **1997**, *40*, 4281–4289.
3. Kahne, D.; Collum, D. B. *Tetrahedron Lett.* **1981**, *22*, 5011–5014.
4. Miyashita, A.; Nakasaki, I.; Kawano, A.; Suzuki, Y.; Iwamoto, K.-I.; Higashino, T. *Heterocycles* **1997**, *45*, 745–755.
5. Davis, W. A.; Cava, M. P. *J. Org. Chem.* **1983**, *48*, 2774–2775.
6. Wheland, R. C.; Martin, E. L. *J. Org. Chem.* **1975**, *40*, 3101–3109.
7. Hughes, T. V.; Cava, M. P. *J. Org. Chem.* **1999**, *64*, 313–315.
8. Hughes, T. V.; Hammond, S. D.; Cava, M. P. *J. Org. Chem.* **1998**, *63*, 401–402.
9. Wu, Y.-Q.; Limburg, D. C.; Wilkinson, D. E.; Hamilton, G. S. *Org. Lett.* **2000**, *2*, 795–797.
10. Kim, S. K.; Cho, S. D.; Kweon, D. H.; Yoon, Y. J.; Kim, J. H.; Heo, J. N. *J. Heterocycl. Chem.* **1997**, *34*, 209–214.
11. Kang, Y. J.; Chung, H. A.; Kim, J. J.; Yoon, Y. J. *Synthesis* **2002**, 733–738.
12. Kim, J. J.; Park, Y. D.; Lee, W. S.; Cho, S. D.; Yoon, Y. J. *Synthesis* **2003**, 1517–1520.
13. Park, Y. D.; Kim, H. K.; Kim, J. J.; Cho, S. D.; Kim, S. K.; Shiro, M.; Yoon, Y. J. *J. Org. Chem.* **2003**, *68*, 9113–9115.
14. Kim, J. J.; Park, Y. D.; Kweon, D. H.; Kang, Y. J.; Kim, H. K.; Lee, S. G.; Cho, S. D.; Lee, W. S.; Yoon, Y. J. *Bull. Korean Chem. Soc.* **2004**, *25*, 501–505.
15. Katritzky, A. R.; Wang, Z.; Wang, M.; Wilkerson, C. R.; Hall, C. D.; Akhmedov, N. G. *J. Org. Chem.* **2004**, *69*(20), 6617–6622.
16. Dury, K. *Angew. Chem., Int. Ed. Engl.* **1965**, *4*, 292–300.
17. Chung, H.-A.; Kweon, D. H.; Kang, Y. J.; Park, J. W.; Yoon, Y. J. *J. Heterocycl. Chem.* **1999**, *36*, 905–910.

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Synthesis of polyanionic glycopolymers for the facile assembly of glycosyl arrays

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Abstract—Polyanionic glycopolymers were synthesized aiming at establishing a simple process for assembling glycosyl arrays. The synthetic glycopolymers carry the key carbohydrate epitopes of α-D-galactobioside (Gb_2), β-lactoside, and α-D-mannopyranoside, each of which serves as a ligand of bacterial toxins and adhesion proteins. The Gb_2 epitope, prepared from penta-*O*-acetyl-D-galactopyranose, was coupled with poly(ethylene-*alt*-maleic anhydride) in a polymer reaction to afford a Gb_2-embedded glycopolymer having also carboxylate (COO^-) polyanions at the side chain. The polyanionic glycopolymer was then applied to a preparation of sugar-coated gold electrodes, which involves an alternating layer-by-layer adsorption based on electrostatic interactions. The presence of the Gb_2-coat on the surface was evidenced by Fourier transform infrared reflection absorption spectroscopy. The Gb_2-coated glyco-chip was stable in 10 mM HEPES buffer containing 150 mM NaCl aq. Other glycopolymers carrying the β-lactoside and α-D-mannopyranoside epitopes were applied to the same assembling process. The derived glycosyl arrays will be useful for detecting Shiga toxins, other pathogenic toxins and viruses when applied as glyco-chips for surface plasmon resonance or quartz crystal microbalance technique.

1. Introduction

The cell-surface oligosaccharides bound to glycoproteins and glycolipids are associated with various biological roles on cell surfaces like cell-adhesion, signal transduction and regulation as well as bacterial and viral infections.[1] Therefore, much attention is paid to the species-specific interactions involved in carbohydrate–protein and carbohydrate–carbohydrate bindings. These interactions are analyzed with artificial models assisted by different techniques of quartz crystal microbalance (QCM), surface plasmon resonance (SPR), Au-nanoparticles, and other biological assays.[2] For all of these analyses, the fabrication of glycosyl arrays, chips, or nanoparticles is of crucial significance.[3] For such purpose, simple and convenient immobilization of carbohydrates to these sensor substrates is indispensable. Conventional immobilization processes apply the natural affinity of biotin and streptavidin and the formation of the self-assembled monolayers between the thiolated compounds and gold surfaces.[4]

Recently, Houseman et al. proposed a method for sugar immobilization, which is based on the Diels–Alder coupling reaction on gold surface between benzoquinone and glycosyl cyclopentadiene.[3a] Wong et al. proposed 1,3-dipolar cycloaddition between azido sugars and alkynes.[3b,e] Park et al. attached sugars onto substrate surfaces by Michel addition between maleimides and thiols.[3f] In these studies, monomeric carbohydrate molecules are routinely utilized. However, with an aim to establish a simple process for assembling glycosyl arrays, the utility of glycopolymers has not yet been fully explored,[5] albeit glycomaterials consisting of the glycopolymers can be generally expected to have a multivalent and cluster effect.[6] This effect can potentially enhance binding interactions with the receptor proteins. We describe herein the synthesis and convenient use of polyanionic glycopolymers for a facile immobilization of carbohydrates to Au-substrates. In this paper, an alternating layer-by-layer adsorption technique was applied, which is clearly distinct from the previous immobilization methods.

Keywords: Glycopolymers; Glycosyl arrays; Carbohydrate epitopes; Layer-by-layer; Polyanions.
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As part of our ongoing project to assemble a library of various sulfo sugars,[7] we are interested in the artificial glycopolymers carrying Gb_3 and Gb_2 in clusters, which are able to adsorb the pathogenic *Escherichia coli* O-157 bacteria and their produced Shiga toxins.[8] In our preceding study, we applied the Gb_3-glycopolymers[8] as well as alkyl Gb_2-monolayers[9] in our QCM sensors targeting the Shiga toxins.

In the present study, we synthesized various polyanionic glycopolymers involving galactobioside (Gb_2) and other biologically important sugar epitopes for the purpose of simple, rapid and practical immobilization. These glycopolymers can be applicable to the fabrication of glycosyl arrays and glyco-chips. Our approach has an intriguing feature in that the polymer has two different functional groups, carbohydrates like Gb_2 as a toxin–ligand and an anionic species like COO^-. The anions distributed at the sugar-embedded polymer chain can tether the cationic counterpart surfaces coated with, for instance, quaternary ammonium ions through electrostatic interactions as depicted in Figure 1.

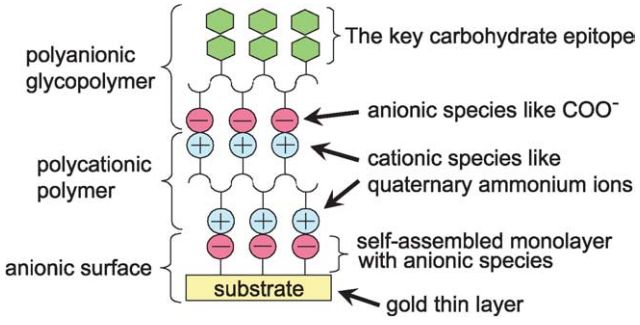


Figure 1. Schematic figure of alternating layer-by-layer membranes onto the substrate surface.

2. Results and discussion

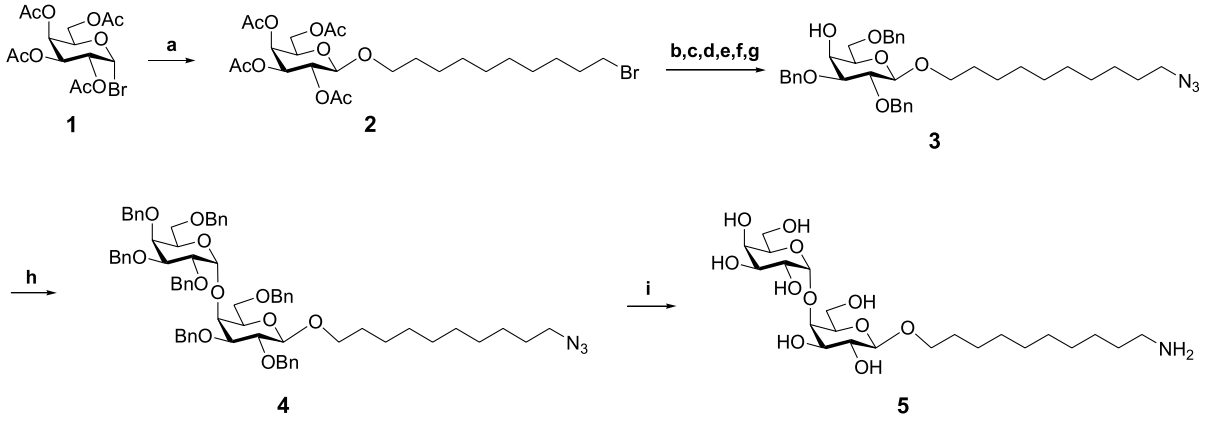
2.1. Synthesis of Gb_2-embedded polyanionic glycopolymers for the detection of *E. coli* O-157 Shiga toxins

Synthesis of the Gb_3-carrying glycopolymers has already been accomplished in the literature and found to show notable activity to block the Shiga toxins-host cell infections.[8] Most of the synthetic studies have focused on the molecular assembly to construct the glycosyl clusters as glycopolymers, glycodendrimers and starfish models.[10] Moreover, substituted polyacrylate-based neoglycoconjugates are reported as versatile chemical tools for biochemical and medicinal applications.[11] In the present approach, we synthesized novel polyanionic glycopolymers to fabricate glycosyl arrays based on the alternating layer-by-layer adsorption method.

For the aimed glycopolymers, the key intermediate **5** was synthesized from the known 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **1** in a manner similar to our previously reported way (Scheme 1).[12] 10-Bromodecyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside **2** carrying a hydrophobic spacer at the reducing terminal was derived from commercially available **1** in 72% yield. The anomeric configuration in **2** was confirmed by ^1H and ^{13}C NMR spectroscopies. The doublet signal of H-1 with $J=8.0$ Hz appears at δ 4.45 ppm in its ^1H NMR spectrum, showing the β-coupling. The peak at δ 101.2 ppm (C-1) in ^{13}C NMR also supports this result.

Compound **2** was then converted to 10-azidodecyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside **3** possessing a reaction point at O-4 in the following way: (1) azide substitution of the terminal bromide by treatment of NaN_3 at 80 °C, (2) de-*O*-acetylation with NaOMe-MeOH, (3) 3,4-*O*-isopropylidenation by treatment with acetone and 2,2-dimethoxypropane in the presence of camphorsulfonic acid (CSA), (4) 2,6-di-*O*-benzylation with BnBr and NaH in DMF, (5) de-*O*-isopropylidenation by treatment of CSA, and (6) selective 3-*O*-benzylation using Bu_2SnO, BnBr and Bu_4NBr (56% in 6 steps).

Stereoselective glycosylation of **3** and 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride[13] was carried out in the presence of $AgClO_4$ as activator in diethylether to obtain α1-4 globobioside **4** and β1-4 disaccharide (α/β=ca. 10:1), which was isolated by silica gel column chromatography, respectively. The ^{13}C NMR signal at δ 100.4 ppm (C-1′) of



Scheme 1. Synthesis of a key intermediate Gb_2 derivative with an amino group at the terminal. Reagents and conditions: (a) 10-bromo-1-decanol, Ag_2CO_3, CH_2Cl_2, MS4A (72%); (b) NaN_3, DMF, 80 °C (99%); (c) NaOMe, MeOH; (d) 2,2-dimethoxypropane, acetone, camphorsulfonic acid (CSA) (73%, two steps); (e) BnBr, NaH (quant.); (f) MeOH, CSA (84%); (g) Bu_2SnO, toluene, reflux, then BnBr, Bu_4NBr (0.5 equiv), 93%; (h) 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride, $AgClO_4$, Et_2O, MS4A, 0 °C–rt, 22 h (α, 67%; β, 7%); (i) $Pd(OH)_2$, H_2, MeOH–AcOEt (2:5, v/v) (85%).

disaccharide **4** shows to be the α-linkage of the newly formed glycosidic bond. COSY and HMQC analyses also confirmed the α1-4 linkage. The FAB-MS spectrum also supports the disaccharide ($[M+Na]^+$ 1176.8). Compound **4** was then subjected to catalytic hydrogenolysis with H_2 and $Pd(OH)_2$ to give the fully deprotected **5** carrying an amino group at the terminal (for **5**; δ 4.97 ppm (d, $J=2.9$ Hz, H-1′) and δ 4.26 ppm (d, $J=7.3$ Hz, H-1)), which gave similar results to the data reported for Galα1-4Gal octadecyl glycoside.[9]

Coupling of 2.0 mol equiv of the key monomeric epitope **5** (0.02 mmol) with a reactive polymer, poly(ethylene-*alt*-maleic anhydride) ($M_w=100{,}000$–$500{,}000$) (0.01 mmol) at 70 °C was performed to produce the Gb_2-embedded anionic polymer **6** containing 43% of the Gb_2 sugar moiety after being dialyzed (M_w 2000 cut-off) in water for 3 days, further purified with a gel-permeation chromatography and lyophilized (Scheme 2). In its IR spectrum, two characteristic signals appeared at 1643 and 1566 cm^{-1}, showing the presence of amide (NHCO) groups in the polymer.

A particularly notable point is that the terminal amino group selectively reacted with an anhydride moiety in the polymer, almost alternately, to generate an amide group and a free carboxylic acid in a ratio of 1:1. Thus, sugar contents do not exceed the 50% value theoretically. The carboxylate anions (COO^-) can effectively serve as the binding sites with the counterpart cationic surfaces or the cationic polymers through electrostatic interactions. The characteristic polymer **6** carrying both Gb_2 carbohydrate and carboxylate anions is advantageous for the immobilization onto sensor surfaces rather than random or block polymers, because the polyanionic glycopolymer **6** distributes carboxylate anions alternately to give almost homogeneous clusters in the polymer chain, while random or block polymerization gave heterogeneous clusters on which carboxylate anions tend to be irregularly distributed in the polymers.

To determine the precise sugar content for the Gb_2 moiety in polymer **6**, the phenol-H_2SO_4 method[14] was carried out. This method is a reliable and sensitive analysis to determine total sugar (Gb_2) amounts after complete hydrolysis of **6**. In a similar approach shown in Scheme 2, various different Gb_2-containing anionic polymers depending on the feed ratio of the key module segment **5** were synthesized, and each sugar content was determined as summarized in Table 1. These polymers can be useful for the fabrication of sensor chips according to varying sugar density.

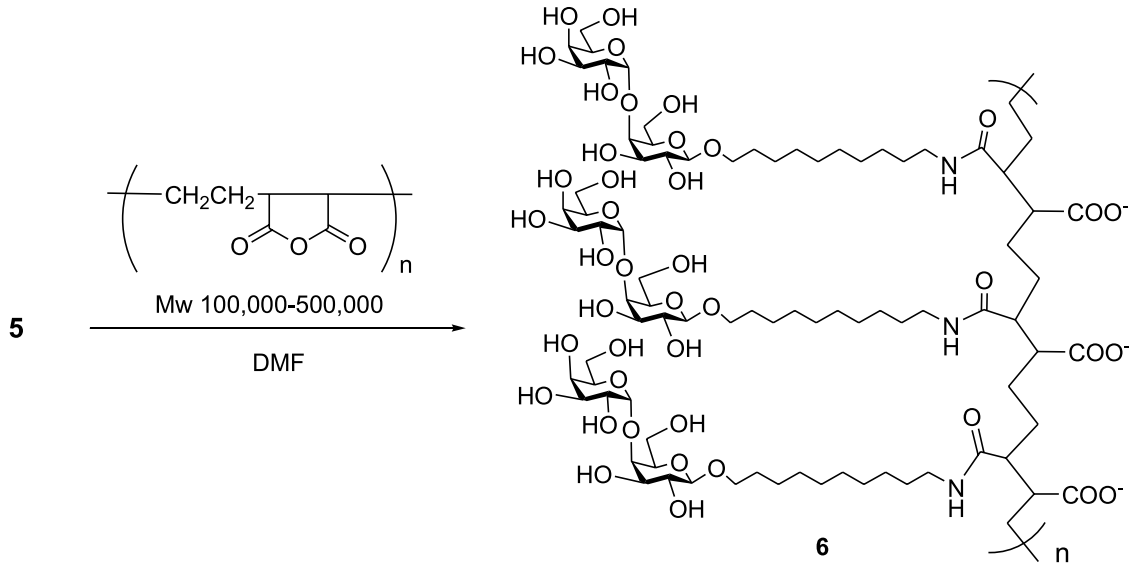
Table 1. Reaction conditions for preparing polyanionic glycopolymer **6** with various Gb_2 contents

Monomer **5** (mol equiv)[a]	Reaction time (h)	Temperature (°C)	Sugar content of **6** (%)[b]	M_w[c]
0.1	35	rt	3	4.0×10^5
1.0	60	rt	13	5.0×10^5
2.0	45	rt	25	6.7×10^5
2.0	32	70	43	7.8×10^5

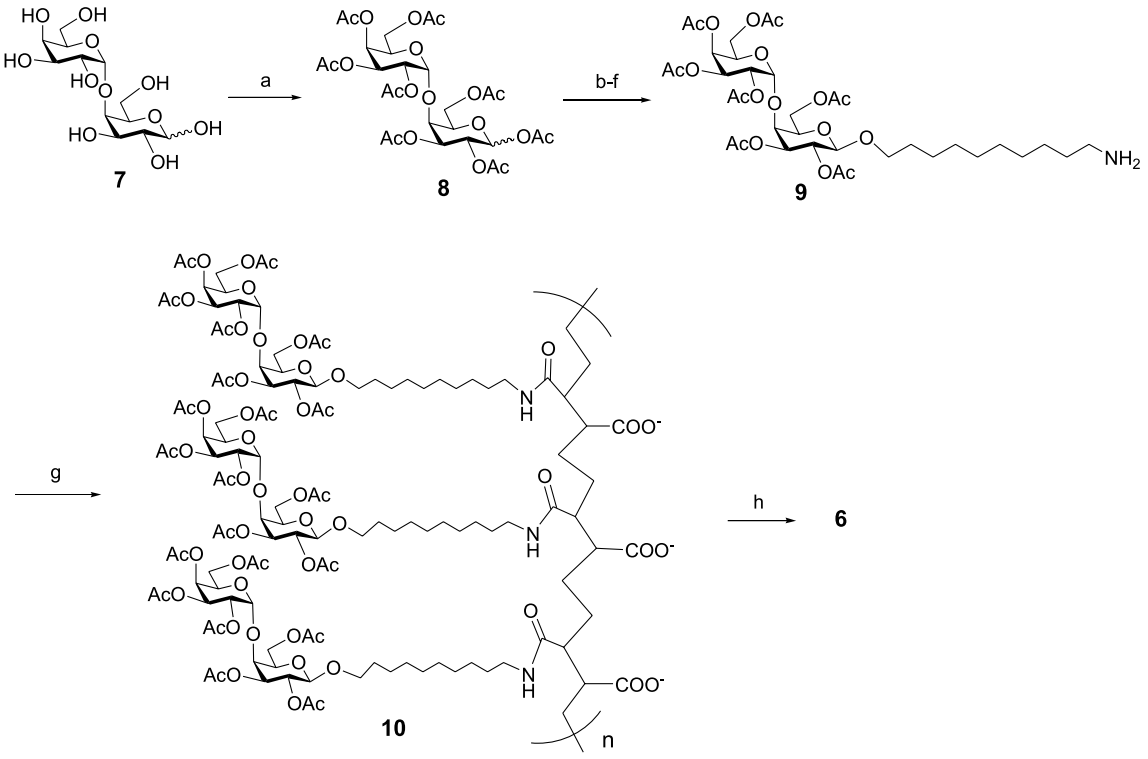
[a] mol equiv to poly(ethylene-*alt*-maleic anhydride).
[b] Determined by the H_2SO_4-phenol method.
[c] Determined by the static light scattering method.

The structure of **6** was further confirmed by an alternative synthesis shown in Scheme 3. Another key carbohydrate intermediate **9** was obtained from commercially available **7** in 6 steps (67% overall yield). Then, compound **9** was reacted with poly(ethylene-*alt*-maleic anhydride) to produce the fully protected Gb_2-embedded polymer **10**. In the ^1H NMR spectrum, broad signals appeared at near δ 7.4 ppm in DMF-d_7, indicating the presence of the amide groups. Deprotection of **10** was achieved in DMF containing excess amount of 1 M NaOH aq to give polyanionic glycopolymer **6**. In the ^1H NMR spectrum, none of the Ac groups was observed. The ^1H NMR of **6** in Scheme 3 is well-consistent with the data for **6** synthesized in Scheme 2. This strategy completely eliminates the possibility of the ester formation by a side reaction between hydroxyl groups like O-6 in the Gb_2 moiety and the active anhydride in the polymer.

When using another polymer, poly(isobutylene-*alt*-maleic



Scheme 2. Synthesis of Gb_2-embedded anionic polymer **6** in the polymer reaction.



Scheme 3. Synthesis of another key module Gb_2 derivative **9** and polyanionic glycopolymer **6**. Reagents and conditions: (a) Ac_2O, DMAP, pyridine (98%); (b) hydrazine–AcOH (93%); (c) Cl_3CCN, DBU (90%; α only); (d) 10-bromo-1-decanol, trimethylsilyltriflate, 0 °C (90%); (e) NaN_3, DMF, 80 °C (99%); (f) $Pd(OH)_2$, H_2, MeOH (91%); (g) poly(ethylene-*alt*-maleic anhydride), DMAP, DMF, then H_2O; (h) 1 M NaOH aq, DMF.

anhydride) (M_w = 60,000), the polymer reaction similar to **6** hardly proceeded. This large difference probably is ascribed to the steric hindrance for the isobutyl group neighboring on the reaction site in the polymer chain.

2.2. Synthesis of other sugar-embedded anionic polymers

One advantage of glycosyl arrays is the potential of concurrent analysis for various viruses, bacteria and other carbohydrate receptor proteins. To this end, several sugar-embedded polymers are required. As model compounds, we newly synthesized two polyanionic polymers carrying β-lactosyl and α-D-mannosyl residues, respectively. These sugar epitopes ubiquitously exist on the cell surfaces.

In this section, we chose the *p*-nitrophenyl (*p*NP) group at the aglycon because the *p*NP group is readily linked to the polymer chains, after transformation to a *p*-aminophenyl (*p*AP) group by chemical reduction.[8,15]

Commercially available *p*NP lactoside **11** was reduced in the presence of Pd under H_2 atmosphere to obtain *p*AP lactoside **12**, quantitatively. Compound **12** was then reacted with poly(ethylene-*alt*-maleic anhydride) in a similar manner to polymer **6** to give **13** carrying lactoside residues. In a similar approach, the mannose-embedded anionic polymer **16** was produced from the corresponding *p*NP α-D-mannoside **14** in Scheme 4. This characterization was carried out as stated above, summarized in Table 2.

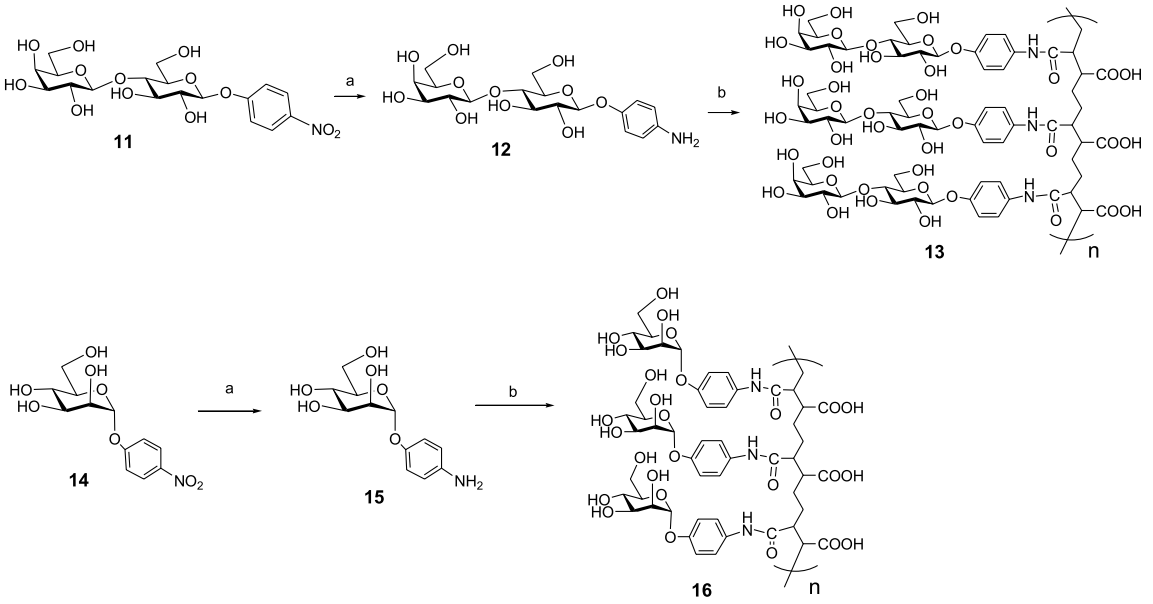
2.3. Alternating layer-by-layer formation of sugar-embedded polyanionic glycopolymer 6 and its confirmation by the FTIR-RAS

The alternating layer-by-layer adsorption technique has provided a convenient way to fabricate thin-layer films onto solid surfaces or polymer-support,[16] and is applied to the study of molecular imprinting and chemical filters.[17] We applied this technique to prepare the glycosyl surfaces.

Mercaptopropionic acid was adsorbed onto a gold surface by a self-assembled monolayer technique[18] to fabricate the surface A in Scheme 5. On this surface A, quaternary ammonium cationic polymer, [poly(diallyldimethylammonium chloride); M_w 400,000–500,000] was adsorbed in 10 mM HEPES buffer (pH 7.4) for 10 min to obtain surface B. Finally, Gb_2-embedded polyanionic polymer **6** was adsorbed by electrostatic interactions to form a three-layer structure (surface C) as illustrated in Scheme 5.

The surface C was analyzed by Fourier transform infrared reflection absorption spectroscopy (FTIR-RAS). Two well-defined adsorptions were observed at 1650 and 1570 cm^{-1}, showing amide groups (Fig. 2). Apparently, this result supports the presence of the Gb_2-sugar epitope on the surface C.

Subsequently, we examined the stability of the Gb_2-containing polyanionic glycopolymer **6** on the surface C. The FTIR-RAS analysis shows that the corresponding amide peaks are nearly completely retained even when

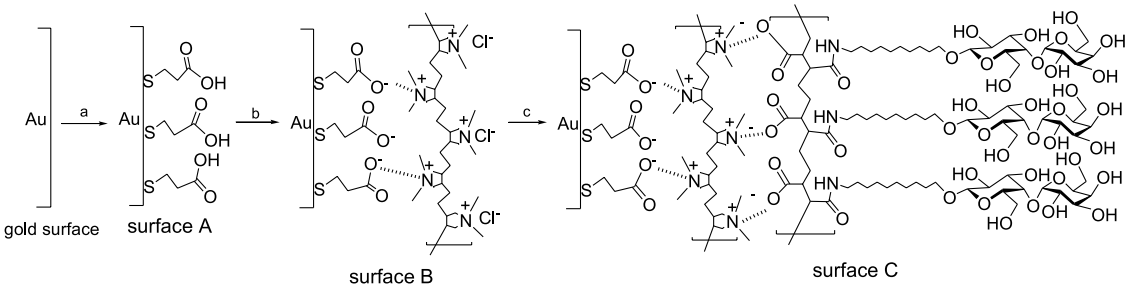


Scheme 4. Synthesis of other sugar-embedded polymers. Reagents and conditions: (a) $Pd(OH)_2$, H_2, H_2O (99% for **12**, 99% for **15**); (b) poly(ethylene-*alt*-maleic anhydride), DMF.

Table 2. Reaction conditions for preparing polyanionic glycopolymer **13** and **16**.

Polymer	Monomer (mol equiv)[a]	Reaction time (h)	Temperature °C	Sugar content (%)[b]	M_w[c]
13	**12** (2.0)	50	rt	40	7.2×10^5
16	**15** (3.6)	22	rt	37	6.0×10^5

[a] mol equiv to poly(ethylene-*alt*-maleic anhydride).
[b] Determined by the H_2SO_4-phenol method.
[c] Determined by the static light scattering method.



Scheme 5. Formation of layer-by-layer adsorption. Reagents and conditions: (a) 3-mercaptopropionic acid in ethanol; (b) poly(diallyldimethylammonium chloride) in 10 mM HEPES buffer (pH 7.4); (c) **6** in the same condition as (b).

washed with 50 mM HCl aq and also exposed to 10 mM HEPES buffer containing 150 mM NaCl aq for at least 24 h (data are not shown), and are stable during the SPR measurements. Those results show that the Gb_2-coated surface C can be usefully applied for toxin detection.

Other surfaces coated with polymers **13** and **16** were also prepared in a similar manner to **6**. More detailed information for surface characterization by the FTIR-RAS, the X-ray photoelectron spectroscopy (XPS) analysis and thickness of each layer by ellipsometry are under study and will be reported in a separate paper.

3. Conclusion

In conclusion, we have synthesized novel polyanionic glycopolymers for the facile fabrication of glycosyl arrays by applying an alternating layer-by-layer method. The present polyanionic glycopolymers are useful to assemble glycosyl arrays or glycosyl chips, for instance, targeting *E. coli* O-157 Shiga toxins. This approach will be a highly practical and promising way to detect toxins, viruses, bacteria and other receptor proteins with the SPR and a quartz crystal microbalance (QCM) method, and the details will be reported in due course.

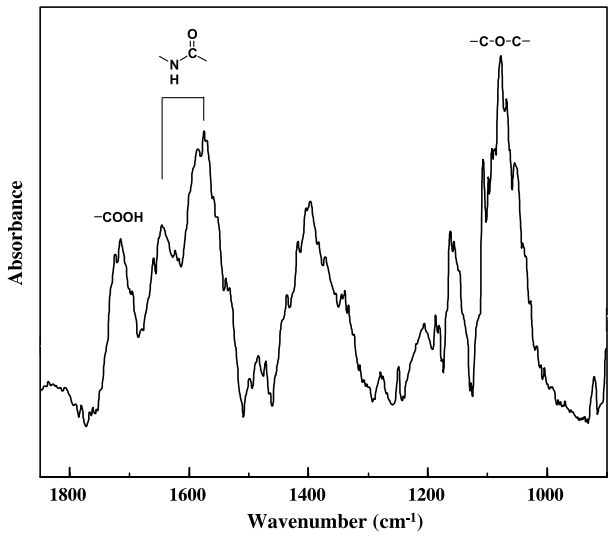


Figure 2. FTIR-RAS spectrum of surface C in Scheme 5.

4. Experimental

4.1. General methods

Globobiose was purchased from Toronto Research Chemicals (Canada). *p*NP β-lactoside and *p*NP α-D-mannopyranoside was purchased from Sigma. 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide, poly(ethylene-*alt*-maleic anhydride) (M_w=100,000–500,000) and poly(diallyldimethylammonium chloride) (M_w 400,000–500,000) were purchased from Aldrich. Reactions were monitored by thin-layer chromatography (TLC) on Silica Gel 60 F_{254} (E. Merck), which were visualized by UV light and by spraying with 20% H_2SO_4 in EtOH followed by charring at 180 °C. Column chromatography was performed on Silica Gel 60 N (Cica Reagent). NAP™-10 Column (Sephadex G-25) was purchased from Amersham Pharmacia Biotech, Ultrafree-MC filter from Millipore and they were used for a gel permeation chromatography. Optical rotations were measured with JASCO DIP-1000 digital polarimeter at ambient temperature, using a 10-cm micro cell. ^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA 400 or a JEOL LA-600 spectrometer for solutions in $CDCl_3$, D_2O or DMF-d_7. Chemical shifts are given in ppm and referenced to internal *tert*-butyl alcohol (δ_H 1.23 in D_2O or δ_C 31.2 in D_2O), TMS (δ_H 0.00 in $CDCl_3$ and DMF-d_7), or $CDCl_3$ (δ_C 77.7 in $CDCl_3$). All data are assumed to be first order with apparent doublet and triplets reported as d and t, respectively. Resonances that appear broad are designated b. A digital resolution is ca. 0.4 Hz. FAB mass spectra (FAB-MS) were recorded using a JEOL DX 303 mass spectrometer, and high-resolution mass spectra (HR-MS) were recorded using a Hitachi M 80 mass spectrometer or Mariner Biospectroscopy Workstation ESI-TOF MS. Elemental analyses were performed with a Carlo Elba EA-1108 or Perkin-Elmer EA-2400 instrument. Average molecular weights (M_w) of each glycopolymer were determined by static light scattering using Zetasizer Nano ZS instrument (Malvern Instruments Ltd, Worcestershire, UK), and poly[ethylene-*alt*-maleic acid] was used as the standard polymer (M_w 300,000). Fourier transform infrared reflection absorption spectroscopy (FTIR-RAS) measurements were made using a Digilab FTS-7000 spectrometer equipped with a Harrick Scientific reflection accessory and a liquid-N_2-cooled MCT detector.

4.1.1. Preparation of layer-by-layer substrates by the alternating adsorption. Gold substrates were cleanly washed with a freshly prepared 'piranha solution', H_2SO_4–H_2O_2 (3/1, v/v) to completely remove any organic adsorption, and they were rinsed with milli-Q water several times. The gold substrates were then immersed into ethanolic solution containing mercaptopropionic acid (1 mM). After 16 h, the substrates were rinsed extensively with absolute ethanol and dried under nitrogen atmosphere to give surface A in Scheme 5. On this surface A, quaternary ammonium cationic polymer, [poly(diallyldimethylammonium chloride); M_w 400,000–500,000] (100 μg/mL) was adsorbed in 10 mM HEPES buffer (pH 7.4) for 10 min to obtain surface B. Finally, Gb_2-embedded polyanionic polymer **6** (100 μg/mL) was adsorbed in 10 mM HEPES buffer (pH 7.4) for 10 min to form a three-layer structure (surface C).

4.1.2. FTIR-RAS measurements. FTIR-RAS measurements were carried out using refractor top plate under nitrogen atmosphere.

4.1.3. 10-Bromodecyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside 2. A solution of 10-bromo-1-decanol (123 mg, 0.52 mmol) and Ag_2CO_3 (137.9 mg, 0.5 mmol) in dry CH_2Cl_2 (2 mL) containing freshly activated MS-4 Å (200 mg) was stirred for 1 h at 25 °C under N_2. To the reaction mixture, 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **1** (206 mg, 0.50 mmol) in dry CH_2Cl_2 (3 mL) was slowly added during 40 min. The reaction mixture was stirred at 25 °C for 20 h and then filtered through a Celite pad. The organic layer was washed with satd aq $NaHCO_3$, and water, respectively. The organic layer was dried ($MgSO_4$), filtered and concentrated. Column chromatography of the product on silica gel with 4:6 EtOAc–hexane afforded compound **2** (209 mg, 72%). $[\alpha]_D$ −9.9° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.39 (dd, H-4, J= 3.2, 0.8 Hz), 5.20 (dd, H-2, J=8.0, 10.4 Hz), 5.02 (dd, H-3, J=3.2, 10.4 Hz), 4.45 (d, H-1, J=8.0 Hz), 4.19 (dd, H-6, J=6.4, 11.2 Hz), 4.13 (dd, H-6′, J=7.2, 11.2 Hz), 3.91–3.85 (m, H-5 and –(CH_2)–, 2H), 3.49–3.44 (m, –(CH_2)–, 1H), 3.41 (t, –CH_2–Br, J=6.8 Hz, 2H), 2.149 (Ac), 2.051 (Ac), 2.049 (Ac), 1.986 (Ac), 1.88–1.81 (m, –(CH_2)–, 2H), 1.62–1.24 (m, –(CH_2)–, 14H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 170.2, 170.1, 169.2, 101.2, 70.8, 70.4, 70.1, 68.8, 67.0, 61.2, 33.9, 32.7, 29.30, 29.27, 29.22, 29.1, 28.6, 28.0, 25.6, 20.64, 20.55, 20.47; IR (liquid film): 2931, 2857, 1749, 1648, 1434, 1369, 1223, 1175, 1138, 1079, 1057, 957, 901, 840, 737, 645, 597 cm^{-1}; HRMS calcd for $C_{24}H_{39}BrO_{10}Na$ $[M+Na]^+$ 589.1625, found 589.1635.

4.1.4. 10-Azidodecyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside 3.

4.1.4.1. Synthesis of 10-azidodecyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside. 10-Bromodecyl glycoside **2** (1.46 g, 2.58 mmol) and NaN_3 (300 mg, 4.61 mmol) was dissolved in DMF (20 mL). The reaction mixture was stirred at 80 °C for 4 h. The organic layer was diluted with EtOAc, washed with brine solution, dried ($MgSO_4$), filtered and

concentrated. Column chromatography of the product on silica gel with 4:6 EtOAc–hexane gave 10-azidodecyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (1.35 g, 99%). $[\alpha]_D$ −12.9° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.39 (d, H-4, *J*=3.2 Hz), 5.20 (dd, H-2, *J*=8.0, 10.4 Hz), 5.02 (dd, H-3, *J*=3.2, 10.4 Hz), 4.45 (d, H-1, *J*=8.0 Hz), 4.19 (dd, H-6, *J*=6.4, 11.2 Hz), 4.13 (dd, H-6′, *J*=7.2, 11.2 Hz), 3.91–3.85 (m, H-5 and –(CH_2)–, 2H), 3.49–3.44 (m, –(CH_2)–, 1H), 3.26 (t, –CH_2–N_3, *J*=6.8 Hz, 2H), 2.147, 2.051, 2.049 and 1.986 (4×Ac), 1.64–1.24 (m, –(CH_2)–, 16H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 170.2, 170.1, 169.2, 101.2, 70.8, 70.4, 70.1, 68.8, 67.0, 61.2, 51.3, 29.3, 29.2, 29.1, 29.0, 28.7, 26.6, 25.7, 20.62, 20.55, 20.47; IR (liquid film): 2932, 2858, 2098, 1756, 1456, 1435, 1370, 1224, 1175, 1135, 1080, 1058, 957, 902, 737, 597 cm^{-1}. Anal. Calcd for $C_{24}H_{39}O_{10}N_3$: C, 54.43; H, 7.42; N, 7.93. Found C, 54.33; H, 7.54; N, 7.90.

4.1.4.2. Synthesis of 10-azidodecyl 3,4-*O*-isopropylidene-β-D-galactopyranoside. Treatment of the above compound (708 mg, 1.34 mmol) with 0.1 M NaOMe for 3 h followed by neutralization with Dowex 50 WXH^+ resin afforded deacetylated product, 10-azidodecyl β-D-galactopyranoside. To a solution of crude 10-azidodecyl β-D-galactopyranoside in dry acetone (20 mL), 2,2-dimethoxypropane (0.86 mL, 7 mmol) and camphorsulfonic acid (255 mg, 1.1 mmol) were added. The reaction mixture was stirred at 25 °C for 2.5 h and then neutralized with Et_3N. The reaction mixture was then diluted with EtOAc. The organic layer was washed with water, dried ($MgSO_4$), filtered and concentrated. Column chromatography on silica gel with EtOAc–hexane (8:2) gave 10-azidodecyl 3,4-*O*-isopropylidene-β-D-galactopyranoside (392 mg, 73%); $[\alpha]_D$ +11.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.18 (d, H-1, *J*=8.4 Hz), 3.25 (t, –CH_2–N_3, *J*=6.8 Hz), 1.52 and 1.35 (2×Me of isopropylidene); ^{13}C NMR (100 MHz, $CDCl_3$): δ 110.9 (Me_2C), 102.9 (C-1), 79.5, 74.5, 74.2, 74.1, 70.7, 62.9, 52.0 (–CH_2–N_3), 30.2, 29.97, 29.95, 29.93, 29.7, 29.4, 28.7 (Me_2C), 27.3, 26.9 (Me_2C), 26.5; IR (liquid film): 3313, 2986, 2933, 2857, 2095, 1456, 1381, 1372, 1241, 1218, 1161, 1144, 1096, 1077, 1036, 964, 904, 891, 873, 808, 738 cm^{-1}; HRMS calcd for $C_{19}H_{35}N_3O_6Na$ [M+Na]$^+$ 424.2424, found 424.2424.

4.1.4.3. Synthesis of 10-azidodecyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranoside. A mixture of isopropylidene compound synthesized in Section 4.1.4.2 (338 mg, 0.84 mmol) and NaH (52 mg, 2.2 mmol) in dry DMF (20 mL) was stirred at 25 °C for 30 min and benzyl bromide (0.26 mL, 2.2 mmol) was added. The reaction mixture was further stirred at 25 °C for 2 h. The reaction mixture was then diluted with EtOAc. The organic layer was washed with water, dried ($MgSO_4$), filtered and concentrated. Column chromatography of the product on silica gel with 4:6 EtOAc–hexane gave 10-azidodecyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (530 mg, quant.); $[\alpha]_D$ +18.7° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz,$CDCl_3$): δ 7.42–7.22 (m, 10H, aromatic), 4.85 (d, CH_2–C_6H_5, 11.6 Hz), 4.78 (d, CH_2–C_6H_5, *J*=11.6 Hz), 4.64 (d, CH_2–C_6H_5, *J*=11.6 Hz), 4.55 (d, CH_2–C_6H_5, *J*=11.6 Hz), 4.3 (d, H-1, *J*=8.0 Hz), 4.16–3.88 (m, H-3, H-4, H-5, –(CH_2)–), 3.82–3.74 (m, H-6 and H-6′), 3.54–3.46 (m, –(CH_2)–, 1H), 3.41–3.35 (m, H-2, 1H), 3.23 (t, –CH_2–N_3, *J*=7.2 Hz, 2H), 1.34 and 1.32 (s, 2×Me), 1.69–1.24 (m, –(CH_2)–, 16H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.3, 138.2, 128.3, 128.1, 127.5, 127.4, 109.8 (Me_2C), 102.9 (C-1), 79.6, 79.0, 73.8, 73.5, 72.2, 69.8, 69.5, 51.4 (–CH_2–N_3), 29.6, 29.4, 29.32, 29.30, 29.1, 28.7, 27.7, 26.6, 26.3, 26.0; IR (liquid film): 3031, 2986, 2931, 2857, 2095, 1497, 1455, 1371, 1243, 1219, 1163, 1097, 1080, 1045, 1029, 871, 806, 736, 697 cm^{-1}; HRMS calcd for $C_{33}H_{47}N_3O_6Na$ [M+Na]$^+$ 604.3363, found 604.3350.

4.1.4.4. Synthesis of 10-azidodecyl 2,6-di-*O*-benzyl-β-D-galactopyranoside. A mixture of the above di-*O*-benzyl compound (508 mg, 0.84 mmol) and camphorsulfonic acid (139 mg, 0.6 mmol) in dry MeOH (10 mL) was stirred at 25 °C for 2 h. The reaction mixture was then neutralized with Et_3N. The reaction mixture was then diluted with EtOAc. The organic layer was washed with water, dried ($MgSO_4$), filtered and concentrated. Column chromatography on silica gel with EtOAc–hexane (4:6) gave 10-azidodecyl 2,6-di-*O*-benzyl-β-D-galactopyranoside (387 mg, 84%); $[\alpha]_D$ +5.2° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38–7.25 (m, aromatics, 10H), 4.97 (d, CH_2–C_6H_5, *J*=11.6 Hz), 4.67 (d, CH_2–C_6H_5, *J*=11.6 Hz), 4.59 (s, CH_2–C_6H_5, 2H), 4.36 (d, H-1, *J*=7.6 Hz), 4.00–3.92 (m, –(CH_2)– and H-4, 2H), 3.82–3.71 (m, H-6 and H-6′, 2H), 3.63–3.46 (m, H-3, H-5, H-2 and –(CH_2)–, 4H), 3.24 (t, –CH_2–N_3, *J*=7.2 Hz, 2H), 1.71–1.22 (m, –(CH_2)–, 16H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 139.1, 138.6, 129.1, 129.0, 128.7, 128.4, 128.33, 128.30, 104.3 (C-1), 79.7, 75.1, 74.2, 73.9, 73.8, 70.5, 70.0, 69.6, 52.0 (–CH_2–N_3), 30.3, 30.03, 29.98, 29.96, 29.7, 29.4, 27.3, 26.7; IR (liquid film): 3401, 3064, 3034, 2929, 2856, 2096, 1454, 1368, 1288, 1260, 1077, 736, 697 cm^{-1}. Anal. Calcd for $C_{30}H_{43}O_6N_3$: C, 66.52; H, 8.00; N, 7.76. Found C, 66.47; H, 8.13; N, 7.57.

4.1.4.5. Synthesis of 3. To a solution of the above de-*O*-isopropylidenated compound (157 mg, 0.29 mmol) in toluene (10 mL), Bu_2SnO (79.7 mg, 0.32 mmol) was added and the mixture was refluxed with azeotropic removal of water for 6 h. The reaction mixture was allowed to 25 °C. Benzyl bromide (0.1 mL, 0.87 mmol) and Bu_4NBr (48 mg, 0.15 mmol) were added and stirred at 25 °C for 3 h. The reaction mixture was then diluted with EtOAc. The organic layer was washed with water, dried ($MgSO_4$), filtered and concentrated. Column chromatography of the product on silica gel with 4:6 EtOAc–hexane gave pure **3** (171 mg, 93%); $[\alpha]_D$ −7.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39–7.25 (m, aromatics, 15H), 4.92 (d, CH_2–C_6H_5, *J*=10.8 Hz), 4.72 (d, CH_2–C_6H_5, *J*=10.8 Hz), 4.72 (s, CH_2–C_6H_5, 2H), 4.59 (s, CH_2–C_6H_5, 2H), 4.35 (d, H-1, *J*=7.6 Hz), 4.02 (br s, H-4), 3.98–3.91 (m, –CH_2O–, 1H), 3.80 (dd, H-6, *J*=6.0, 10.0 Hz), 3.72 (dd, H-6′, *J*=6.0, 10.0 Hz), 3.63 (dd, H-2, *J*=8.0, 9.2 Hz), 3.55 (br t, H-5, *J*=6.0 Hz), 3.53–3.47 (m, –(CH_2)–, 1H), 3.49 (dd, H-3, *J*=3.2, 9.2 Hz), 3.24 (t, –CH_2–N_3, *J*=7.2 Hz, 2H), 1.71–1.22 (m, –(CH_2)–, 16H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 139.3, 138.6, 138.5, 129.03, 129.01, 128.9, 128.7, 128.41, 128.36, 128.32, 128.2, 104.2 (C-1), 81.0, 79.4, 75.6, 74.1, 73.6, 72.8, 70.4, 69.7, 67.4, 52.0 (–CH_2–N_3), 30.3, 30.0, 30.0, 29.7, 29.4, 27.3, 26.7; IR (liquid film): 3536, 3065, 3031, 2929, 2857, 2095, 1454, 1366, 1300, 1264, 1210, 1159, 1096, 1077, 1029, 917, 736, 697 cm^{-1}. Anal. Calcd for

$C_{37}H_{49}O_6N_3$: C, 70.34; H, 7.82; N, 6.65. Found C, 70.60; H, 7.82; N, 6.47.

4.1.5. 10-Azidodecyl *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranoside 4. A solution of compound **3** (116 mg, 0.18 mmol) and tetra-*O*-benzyl-α-D-galactopyranosyl chloride[13] (203 mg, 0.36 mmol) in dry diethyl ether (20 mL) containing freshly activated 4 Å MS (1 g) was stirred under N_2 for 1 h and cooled at 0 °C, $AgClO_4$ (112 mg, 0.54 mmol) was then added. The reaction temperature was gradually elevated to 25 °C, and stirring was continued in the dark for 22 h at the temperature. The reaction mixture was diluted with EtOAc and then filtered through a Celite pad, washed with satd aq $NaHCO_3$, water, respectively. The organic layer was dried ($MgSO_4$), filtered and concentrated. Column chromatography of the product on silica gel with 2:8 EtOAc–hexane afforded disaccharide **4** (134 mg, 67%) and β1-4 isomer (14 mg, 7%). Compound **4**; $[\alpha]_D$ +47.5° (*c* 0.71, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.37–7.16 (m, aromatics, 35H), 5.03 (br s, H-1′), 4.92–4.87 (m, C*H*$_2$–C_6H_5, 3H), 4.80–4.76 (m, C*H*$_2$–C_6H_5, 4H), 4.68 (d, C*H*$_2$–C_6H_5, *J*=11.7 Hz, 1H), 4.55 (d, C*H*$_2$–C_6H_5, *J*=11.4 Hz, 1H), 4.54 (d, C*H*$_2$–C_6H_5, *J*=12.8 Hz, 1H), 4.46–4.42 (m, H-5′, 1H), 4.31 (d, H-1, *J*=7.7 Hz), 4.26 (d, C*H*$_2$-C_6H_5, *J*=12.0 Hz, 1H), 4.22 (d, C*H*$_2$–C_6H_5, *J*=12.0 Hz, 1H), 4.15 (d, C*H*$_2$–C_6H_5, *J*=12.0 Hz), 4.13 (d, C*H*$_2$–C_6H_5, *J*=12.0 Hz), 4.10 (br, H-2′, H-3′, H-4′, 3H), 4.01 (bd, H-4, *J*=2.9 Hz, 1H), 3.98–3.90 (m, H-6a and –OC*H*$_2$–, 2H), 3.66 (dd, H-2, *J*=7.7, 9.9 Hz), 3.56–3.46 (m, H-6′a, H-6b, H-5 and –OC*H*$_2$–, 4H), 3.38 (dd, H-3, *J*=2.9, 9.9 Hz), 3.26–3.22 (m, H-6′b and –C*H*$_2$–N_3, 3H), 1.71–1.26 (m, –(C*H*$_2$)–, 16H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.9, 138.8, 138.7, 138.6, 138.1, 138.0, 128.3, 128.21, 128.16. 128.06, 128.0, 127.8, 127.6, 127.5, 127.4, 127.3, 103.9 (C-1), 100.4 (C-1′), 80.8, 78.9, 76.5, 75.0, 74.8, 74.7, 74.6, 73.7, 73.5, 73.1, 73.0, 72.3, 72.2, 70.1, 69.2, 68.0, 67.9, 51.4 (–C*H*$_2$–N_3), 29.7, 29.4, 29.3, 29.1, 28.8, 26.6, 26.1; IR (liquid film): 3062, 3030, 2929, 2858, 2095, 1497, 1454, 1365, 1269, 1208, 1097, 1056, 1028, 735, 697 cm^{-1}. Anal. Calcd for $C_{71}H_{83}O_{11}N_3$: C, 73.87; H, 7.25; N, 3.64. Found C, 74.05; H, 7.18; N, 3.74; FAB-MS(pos): $[M+Na]^+$ 1176.8. β**1–4** isomer; $[\alpha]_D$ +12° (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47–7.18 (m, aromatics, 35H), 5.12 (d, C*H*$_2$–C_6H_5, *J*=10.8 Hz, 1H), 4.99 (d, C*H*$_2$–C_6H_5, *J*=12.0 Hz, 1H), 4.91 (d, H-1′, *J*=7.6 Hz), 4.81–4.69 (m, C*H*$_2$–C_6H_5, 6H), 4.59–4.49 (m, C*H*$_2$–C_6H_5, 3H), 4.42 (d, C*H*$_2$–C_6H_5, *J*=10.8 Hz, 1H), 4.35 (d, H-1, *J*=8.0 Hz), 4.34 (s, C*H*$_2$–C_6H_5, 2H), 4.24 (br d, H-4′, *J*=2.4 Hz), 3.98–3.93 (m, –O–C*H*$_2$–(CH$_2$)–, 1H), 3.86 (br d, H-4, *J*=2.8 Hz), 3.84–3.67 (m, H-2, H-2′, H-6′a and H-6′b, 4H), 3.56–3.42 (m, H-3, H-3′, H-5, H-5′, H-6a, H-6b and –O–C*H*$_2$–(CH$_2$)–, 7H), 3.23 (t, –C*H*$_2$–N_3, 2H), 1.67–1.25 (m, –(C*H*$_2$)–, 16H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.8, 137.9, 137.7, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9. 127.8, 127.7, 127.6, 127.5, 127.3, 103.9 (C-1), 102.8 (C-1′), 82.0, 81.9, 80.0, 79.9, 75.2, 74.9, 74.5, 74.2, 73.8, 73.5, 73.4, 73.2, 72.7, 70.7, 70.0, 69.9, 68.7, 51.5 (–C*H*$_2$–N_3), 29.8, 29.5, 29.4, 29.3, 28.8, 26.7, 26.2.

4.1.6. 10-Aminodecyl *O*-(α-D-galactopyranosyl)-(1→4)-β-D-galactopyranoside 5. A solution of **4** (49 mg, 0.042 mmol) and a catalytic amount of $Pd(OH)_2$ in EtOAc–MeOH (2:5, 7 mL) was hydrogenated at room temperature under atmospheric pressure for 4 h. The reaction mixture was filtered through a Celite pad and concentrated to give fully deprotected **5** (18 mg, 85%). $[\alpha]_D$ +64.6° (*c* 0.5, CH_3OH); ^{1}H NMR (600 MHz, CD_3OD): δ 4.97 (d, H-1′, *J*=2.9 Hz), 4.29 (t, H-5′, *J*=6.2 Hz), 4.26 (d, H-1, *J*=7.3 Hz), 3.99 (d, H-4, *J*=2.9 Hz), 3.92 (br s, H-4′), 3.87 (dt, –C*H*$_2$O–, *J*=7.0, 9.5 Hz, 1H), 3.83 (dd, H-6a, *J*=7.7, 11.0 Hz), 3.79–3.75 (m, H-2′, H-3′), 3.75–3.70 (m, H-6b, H-6′a), 3.68 (dd, H-6′b, *J*=5.5, 11.0 Hz), 3.60 (t, H-5, *J*=8.4 Hz), 3.56 (dt, –C*H*$_2$O–, *J*=6.6, 9.5 Hz, 1H), 3.53 (dd, H-3, *J*=2.9, 9.9 Hz), 3.46 (dd, H-2, *J*=7.3, 9.9 Hz), 2.90 (t, –C*H*$_2$–NH_2, *J*=7.3 Hz), 1.65–1.28 (m, –(CH$_2$)$_8$–, 16H); ^{13}C NMR (100 MHz, CD_3OD): δ 106.0 (C-1), 103.3 (C-1′), 79.7, 76.9, 75.5, 73.7, 73.4, 72.2, 71.9, 71.6, 63.5, 61.7, 41.7 (–C*H*$_2$–NH_2), 31.7, 31.4, 31.3, 31.2, 31.0, 29.4, 28.3, 27.9; IR (liquid film): 3349, 2930, 2856, 1615, 1376, 1150, 1075, 807 cm^{-1}; HRMS calcd for $C_{22}H_{44}NO_{11}$ $[M+H]^+$ 498.2914, found 498.292.

4.1.7. Polyanionic glycopolymer 6. The reaction conditions are summarized in Tables 1 and 2. A typical procedure is as follows. Sugar monomer, **5** (10 mg, 0.02 mmol) and poly(ethylene-*alt*-maleic anhydride) (1.3 mg, 0.01 mmol as maleic anhydride unit) were dissolved in dry DMF (1 mL), and stirred at 70 °C for 32 h. The reaction mixture was concentrated, the precipitate was dissolved in water (20 mL), dialyzed for 3 days in water (M_w 2000 cut-off) and further purified with NAP™-10 Column (1.3×2.6 cm) to afford the polyanionic glycopolymer **6** (4.1 mg). The sugar content was determined by H_2SO_4-phenol method to be ca. 43%. $[\alpha]_D$ −74.0° (*c* 0.2, H_2O); ^{1}H NMR (400 MHz, D_2O): δ 5.02 (br, H-1′), 4.40 (br, H-1 and H-5′, 2H), 1.7–1.1 (br, –(CH$_2$)–); IR (KBr): 3400, 2930, 2857, 1713, 1643, 1566, 1465, 1406, 1226, 1151, 1075 cm^{-1}.

4.1.8. *O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→4)-1,2,3,6-tetra-*O*-acetyl-α/β-D-galactopyranoside 8. *O*-(α-D-galactopyranosyl)-(1→4)-α/β-D-galactopyranoside **7** (50 mg, 0.15 mmol) was dissolved in dry pyridine (4 mL), and acetic anhydride (340 μL, 3.6 mmol) and *N*,*N*-dimethylaminopyridine (8.9 mg, 0.073 mmol) was added. The reaction mixture was stirred at 40 °C for 20 h. The mixture was then diluted with EtOAc, and washed with satd aq $NaHCO_3$, water, respectively. The organic layer was dried ($MgSO_4$), filtered and concentrated. Column chromatography of the product on silica gel with 6:4 EtOAc–hexane afforded peracetylated disaccharide **8** (97.3 mg, 98%) of α/β=ca. 3:2; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.38 (d, $H_α$-1, *J*=3.6 Hz), 5.71 (d, $H_β$-1, *J*=8.0 Hz), 5.59 (dd, $H_β$-4′, *J*=1.2, 3.2 Hz), 5.57 (dd, $H_α$-4′, *J*=1.6, 3.2 Hz), 5.32 (dd, $H_β$-2, *J*=8.0, 10.4 Hz), 5.01 (d, $H_α$-1′, *J*=3.6 Hz), 5.00 (d, $H_β$-1′, *J*=3.6 Hz), 4.87 (dd, $H_β$-3, *J*=2.8, 10.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 99.5, 99.1, 92.0, 89.8; IR (KBr): 2934, 1752, 1650, 1436, 1373, 1231, 1134, 1069, 1015, 938, 905 cm^{-1}. Anal. Calcd for $C_{28}H_{38}O_{19}$: C, 49.56; H, 5.64. Found C, 49.68; H, 5.52.

4.1.9. 10-Aminodecyl *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranoside 9.

4.1.9.1. Synthesis of *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-α/β-D-galactopyranose. The disaccharide **8** (103 mg, 0.15 mmol)

was treated with hydrazine acetate (27 mg, 0.30 mmol) in DMF (4 mL) at 0 °C for 4 h. The mixture was then diluted with $CHCl_3$, and washed with brine. The organic layer was dried ($MgSO_4$), filtered and concentrated. Column chromatography of the product on silica gel with 8:2 EtOAc–hexane gave *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-α/β-D-galactopyranoside (88.5 mg, 93%). Selective ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.51 (d, H_α−1, J=3.2 Hz), 5.12 (dd, H_β-2, J=8.0, 10.8 Hz), 5.03 (d, H_β-1′, J=3.6 Hz), 5.00 (d, H_α-1′, J=4.0 Hz), 4.91 (dd, H_β-3, J=2.4, 10.8 Hz), 4.70 (br d, H_β-1, J=7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 98.9 (C-1′), 95.5 (C_β-1), 90.4 (C_α-1); IR (KBr): 3469, 2973, 1751, 1660, 1437, 1374, 1235, 1157, 1131, 1070, 979, 908, 758, 600 cm^{-1}; HRMS calcd for $C_{26}H_{36}O_{18}Na$ $[M+Na]^+$ 659.1800, found 659.1776.

4.1.9.2. Synthesis of *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate. To a mixture of the above selectively deacetylated compound (87 mg, 0.14 mmol) and trichloroacetonitrile (400 μL, 4.0 mmol), 1,8-diazabicyclo [5.4.0]undec-7-ene (30 μL, 0.20 mmol) was added at 0 °C and stirred for 2 h. The reaction mixture was then subjected to a silica gel column chromatography with 4:6 EtOAc–hexane to afford *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate (95 mg, 90%). $[\alpha]_D$ +148.6° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.68 (s, NH), 6.61 (d, H-1, 3.6 Hz), 5.57 (dd, H-4′, J=1.2, 3.2 Hz), 5.42 (dd, H-2, J=3.6, 11.2 Hz), 5.38 (dd, H-3′, J=3.2, 11.2 Hz), 5.31 (dd, H-3, J=2.8, 11.2 Hz), 5.24 (dd, H-2′, J=3.6, 11.2 Hz), 5.03 (d, H-1′, 3.2 Hz), 4.55–4.51 (m, H-5′), 4.37–4.31 (m, H-5 and H-6′), 4.28 (br d, H-4, J=2.4 Hz), 4.16–4.08 (m, H-6a, H-6b and H-6′), 2.15 (Ac), 2.12 (2×Ac), 2.04 (Ac), 2.03 (2×Ac), 1.99 (Ac); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.3, 170.1, 169.8, 169.7, 160.8 (C=NH), 109.7 (C-1), 98.9 (C-1′), 93.6 (CCl_3), 70.6, 69.4, 68.2, 67.7, 67.3, 67.1, 66.7, 61.8, 60.7, 20.9, 20.7, 20.6, 20.5, 20.4; IR (liquid film): 3320, 2977, 1748, 1677, 1434, 1372, 1225, 1134, 1068, 971, 904, 837, 797, 752, 644 cm^{-1}.

4.1.9.3. Synthesis of 10-bromodecyl *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranoside. A solution of the above trichloroacetimidate compound (61 mg, 0.077 mmol) and 10-bromo-1-decanol (182 mg, 0.76 mmol) in dry CH_2Cl_2 (2 mL) containing freshly activated 4 Å-MS (400 mg) was stirred for 1 h at 25 °C under N_2. The reaction mixture was then cooled to 0 °C and Me_3SiOTf (9 μL, 0.05 mmol) was added. The mixture was stirred at 0 °C for 2.5 h and then triethylamine was added. The mixture was filtered through a Celite pad, then diluted with $CHCl_3$ and washed with satd aq $NaHCO_3$, water, respectively. The organic layer was dried ($MgSO_4$), filtered and concentrated. Column chromatography of the product on silica gel with 4:6 EtOAc–hexane afforded 10-bromodecyl glycoside (59 mg, 90%); $[\alpha]_D$ +60.3° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.56 (dd, H-4′, J=1.2, 3.2 Hz), 5.39 (dd, H-3′, J=3.6, 11.2 Hz), 5.21–5.15 (m, H-2′ and H-2), 5.00 (d, H-1′, J=3.6 Hz), 4.81 (dd, H-3, J=2.8, 10.8 Hz), 4.55–4.52 (m, H-5′), 4.48–4.44 (m, H-6 and H-1), 4.20–4.07 (m, H-6′a, H-6′b and H-6), 4.05 (br d, H-4, J=2.4 Hz), 3.91–3.85 (m, –O–CH_2–(CH$_2$)–, 1H), 3.77 (br t, H-5, J=6.8 Hz), 3.49–3.43 (m, –O–CH_2–(CH$_2$)–, 1H), 3.41 (t, –CH_2–Br, J=6.8 Hz, 2H), 2.13 (Ac), 2.10 (Ac), 2.08 (Ac), 2.07 (Ac), 2.04 (2×Ac), 1.98 (Ac), 1.89–1.81 (m, –CH_2–CH$_2$Br, 2H), 1.61–1.54 (m, –OCH$_2$–CH_2–, 2H), 1.44–1.26 (m, –(CH$_2$)–, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.4, 170.3, 170.2, 169.9, 169.5, 168.9, 101.1 (C-1), 99.2 (C-1′), 72.6, 71.7, 69.9, 68.7, 68.4, 67.7, 67.2, 66.9, 61.8, 60.4, 33.9, 32.6, 29.3, 29.3, 29.2, 29.1, 28.6, 28.0, 25.7, 20.8, 20.6, 20.55, 20.53, 20.48; IR (liquid film): 2931, 2856, 1750, 1434, 1371, 1225, 1180, 1132, 1070, 903, 600 cm^{-1}. Anal. Calcd for $C_{36}H_{55}O_{18}Br$: C, 50.53; H, 6.48. Found C, 50.33; H, 6.35.

4.1.9.4. Synthesis of 10-azidodecyl *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranoside. A solution of the 10-bromodecyl glycoside (59 mg, 0.069 mmol) and NaN_3 (8 mg, 0.12 mmol) in dry DMF (4 mL) was stirred at 80 °C for 5.5 h. The reaction mixture was then processed in the same way described for the Section 4.1.4.1 to give 10-azidodecyl *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranoside (55 mg, 99%). $[\alpha]_D$ +70.4° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.56 (dd, H-4′, J=1.2, 3.2 Hz), 5.39 (dd, H-3′, J=3.2, 10.8 Hz), 5.21–5.15 (m, H-2′ and H-2), 5.00 (d, H-1′, J=3.6 Hz), 4.81 (dd, H-3, J=2.4, 11.2 Hz), 4.55–4.52 (m, H-5′), 4.48–4.44 (m, H-6 and H-1), 4.20–4.07 (m, H-6′a, H-6′b and H-6), 4.05 (br d, H-4, J=2.0 Hz), 3.91–3.85 (m, –O–CH_2–(CH$_2$)–, 1H), 3.77 (br t, H-5, J=6.8 Hz), 3.49–3.43 (m, –O–CH_2–(CH$_2$)–, 1H), 3.26 (t, –CH_2–N$_3$, J=6.8 Hz), 2.13 (Ac), 2.10 (Ac), 2.08 (Ac), 2.07 (Ac), 2.04 (2×Ac), 1.98 (Ac), 1.63–1.54 (m, –(CH$_2$)–, 4H), 1.38–1.28 (m, –(CH$_2$)–, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.4, 170.3, 170.2, 169.9, 169.5, 168.9, 101.0 (C-1), 99.2 (C-1′), 72.6, 71.7, 69.8, 68.6, 68.4, 67.7, 67.2, 66.9, 61.8, 60.3, 51.3 (–CH$_2$N$_3$), 29.3, 29.2, 29.1, 28.9, 28.6, 26.5, 25.6, 20.8, 20.6, 20.51, 20.48, 20.43; IR (liquid film): 2933, 2857, 2098, 1751, 1371, 1225, 1133, 1067, 903, 668 cm^{-1}. Anal. Calcd for $C_{36}H_{55}O_{18}N_3$: C, 52.87; H, 6.78; N, 5.14. Found: C, 52.59; H, 6.42; N, 4.77.

4.1.9.5. Synthesis of 9. A mixture of the above azide compound (55 mg, 0.068 mmol) and a catalytic amount of $Pd(OH)_2$ (ca. 5 mg) was stirred in MeOH (5 mL) at room temperature under hydrogen atmosphere for 3 h. The reaction mixture was then processed in the same way described for compound **5** to give **9** (49 mg, 91%). $[\alpha]_D$ +70.4° (*c* 1.0, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD): δ 5.54 (dd, H-4′, J=1.2, 3.2 Hz), 5.40 (dd, H-3′, J=3.2, 11.2 Hz), 5.22–5.14 (m, H-2′ and H-2), 5.06 (d, H-1′, 4.0 Hz), 5.01 (dd, H-3, J=2.4, 10.8 Hz), 4.63 (d, H-1, J=8.0 Hz), 4.58–4.54 (m, H-5′), 4.43 (dd, H-6, J=7.2, 11.2 Hz), 4.21–4.11 (m, H-4, H-6′a, H-6′b and H-6), 4.00–3.96 (m, H-5), 3.90–3.85 (m, –O–CH_2–(CH$_2$)–, 1H), 3.57–3.51 (m, –O–CH_2–(CH$_2$)–, 1H), 2.92 (t, –CH_2–N$_3$, J=7.6 Hz, 2H), 2.13 (Ac), 2.11 (Ac), 2.08 (Ac), 2.05 (Ac), 2.04 (Ac), 2.02 (Ac), 1.96 (Ac), 1.69–1.33 (m, –(CH$_2$)–, 16H); ^{13}C NMR (100 MHz, CD_3OD): δ 172.2, 172.0, 171.9, 171.6, 171.3, 102.3, 100.4, 78.5, 73.8, 73.5, 71.0, 70.6, 69.6, 69.5, 69.0, 68.4, 64.1, 62.0, 40.8, 30.6, 30.5, 30.4, 30.3, 30.2, 28.5, 27.4, 27.0, 21.0, 20.81, 20.76, 20.7, 20.6, 20.5; IR (KBr): 3458, 2935, 2859, 1753, 1631, 1435, 1373, 1230, 1134, 1072, 904, 601 cm^{-1};

HRMS calcd for $C_{36}H_{58}NO_{18}$ $[M+H]^+$ 792.3654, found 792.3629.

4.1.10. Synthesis of acetylated glycopolymer 10. A solution of **9** (33.1 mg, 0.043 mmol) and poly(ethylene-*alt*-maleic anhydride) (2.5 mg, 0.02 mmol as maleic anhydride unit) were dissolved in dry DMF (1 mL), and was stirred at room temperature for 45 h. A few drops of water were added, and the mixture was concentrated and then purified with Ultrafree-MC filter (DMF as an eluent) to afford the acetylated glycopolymer **10** (9.2 mg). $[\alpha]_D$ −38.9° (*c* 0.61, DMF); ^{1}H NMR (400 MHz, DMF-d_7): δ 7.4 (br, N*H*CO), 5.58 (br, H-4′), 5.35 (br, H-3′), 5.25 (br dd, J=3.4, 11.0 Hz, H-2′), 5.2–5.1 (br, H-2, H-1′, H-3), 4.77 (br d, J=6.8 Hz, H-1), 4.62 (br, H-5′), 3.85 (br, –OC*H*$_2$CH$_2$–), 2.17, 2.16, 2.12, 2.09, 2.07, 2.04, 1.99 (7×br s, Ac).

4.1.11. Conversion of acetylated glycopolymer 10 to fully deprotected 6. The above polymer **10** (3 mg) was deacetylated with 1 M NaOH aq. (3 mL) in DMF (1 mL) at room temperature for 3 h. The reaction mixture was then processed in the same way described for the Section 4.1.7 to afford polyanionic glycopolymer **6** (1.3 mg).

4.1.12. Synthesis of glycopolymer 13. A solution of commercially available *p*NP lactoside **11** (683.2 mg, 1.47 mmol) and a catalytic amount of $Pd(OH)_2$ (ca. 5 mg) in H_2O (200 mL) was hydrogenated at room temperature under atmospheric pressure for 15 h. The reaction mixture was filtered through a Celite pad and concentrated to give **12** (636.0 mg, 99%). Then, monomer **12** (86.7 mg, 0.2 mmol) and poly(ethylene-*alt*-maleic anhydride) (12.8 mg, 0.1 mmol as maleic anhydride unit) were dissolved in dry DMF (3 mL), and the mixture was stirred at room temperature for 50 h. The reaction mixture was then processed in the same way described for glycopolymer **6** to give **13** (87 mg). The sugar content was determined by the H_2SO_4-phenol method to be ca. 40%. $[\alpha]_D$ +87° (*c* 0.2, H_2O); ^{1}H NMR (400 MHz, D_2O): δ 7.2 (br, aromatics), 5.12 (br, H-1), 4.48 (br, H-1′), 3.9–3.5 (br, 12H), 2.7 (br, –C*H*(COO)–C*H*(CONH)–), 1.6 (br, –(CH$_2$)–); IR (KBr): 3339, 2903, 1703, 1657, 1549, 1510, 1429, 1373, 1318, 1235, 1163, 1061, 897 cm^{-1}.

4.1.13. Synthesis of glycopolymer 16. A solution of commercially available *p*NP α-D-mannopyranoside **14** (1 g, 3.3 mmol) and a catalytic amount of $Pd(OH)_2$ (ca. 5 mg) in H_2O (200 mL) was hydrogenated at room temperature under atmospheric pressure for 15 h. The reaction mixture was filtered through a Celite pad and concentrated to give **15** (890 mg, 99%). Then, monomer **15** (100 mg, 0.36 mmol) and poly(ethylene-*alt*-maleic anhydride) (12.8 mg, 0.1 mmol as maleic anhydride unit) were dissolved in dry DMF (3 mL), and the mixture was stirred at room temperature for 22 h. The reaction mixture was then processed in the same way described for glycopolymer **6** to give **16** (32 mg). The sugar content was determined by the H_2SO_4-phenol method to be ca. 37%. $[\alpha]_D$ +47° (*c* 0.2, H_2O); ^{1}H NMR (400 MHz, D_2O): δ 7.12 (br, aromatics), 5.52 (br, H-1), 4.0–3.5 (br, H-2, H-3, H-4, H-5, H-6 and H-6′), 2.6 (br, –C*H*(COO)–C*H*(CONH)–), 1.6 (br, –(CH$_2$)–); IR (KBr): 3439, 2932, 1707, 1648, 1548, 1510, 1454, 1410, 1228, 1123, 1065, 1010, 975, 831 cm^{-1}.

Acknowledgements

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References and notes

1. (a) Esko, J. D. Microbial carbohydrate-binding proteins. In *Essentials of glycobiology*; Varki, A., Cummings, R., Esko, J., Freeze, H., Hart, G., Marth, J., Eds.; Cold Spring Harbor Laboratory: Spring Harbor, NY, 1999; pp 429–440. (b) Angata, T.; Varki, A. *Chem. Rev.* **2002**, *102*, 439–470. (c) Smith, A. E.; Helenius, A. *Science* **2004**, *304*, 237–242. (d) Karlsson, K. *Annu. Rev. Biochem* **1989**, *58*, 309–350. (e) Hakomori, S. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 225–232.
2. (a) Rojo, J.; Morales, J. C.; Penadés, S. *Top. Curr. Chem.* **2002**, *218*, 45–92. (b) Marx, K. *Biomacromolecules* **2003**, *4*, 1099–1120. (c) Matsuura, K.; Oda, R.; Kitakouji, H.; Kiso, M.; Kitajima, K.; Kobayashi, K. *Biomacromolecules* **2004**, *5*, 937–941. (d) Barrientos, Á.; Fuente, J. M.; Rojas, T. C.; Fernández, A.; Penadés, S. *Chem. Eur. J.* **2003**, *9*, 1909–1921. (e) Otsuka, H.; Akiyama, Y.; Nagasaki, Y.; Kataoka, K. *J. Am. Chem. Soc.* **2001**, *123*, 8226–8230. (f) Matsuura, K.; Kitakouji, H.; Sawada, N.; Ishida, H.; Kiso, M.; Kitajima, K.; Kobayashi, K. *J. Am. Chem. Soc.* **2000**, *122*, 7406–7407. (g) Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555–578.
3. (a) Houseman, B. T.; Mrksich, M. *Chem. Biol.* **2002**, *9*, 443–454. (b) Fazio, F.; Bryan, M. C.; Blixt, O.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.* **2002**, *124*, 14397–14402. (c) Love, K. R.; Seeberger, P. H. *Angew. Chem., Int. Ed.* **2002**, *41*, 3583–3586. (d) Flitsch, S. L.; Ulijn, R. V. *Nature* **2003**, *421*, 219–220. (e) Fazio, F.; Bryan, M. C.; Lee, H.-K.; Chang, A.; Wong, C.-H. *Tetrahedron Lett.* **2004**, *45*, 2689–2692. (f) Park, S.; Shin, I. *Angew. Chem., Int. Ed.* **2002**, *41*, 3180–3182.
4. (a) Wilchek, M.; Bayer, E. A. *Anal. Biochem.* **1988**, *171*, 1–32. (b) Ulman, A. *Chem. Rev.* **1996**, *96*, 1533–1554.
5. Some chemical and hydrophobic adsorption techniques of artificial polymers onto plate surfaces were reported. See the following references; (a) Suzuki, N.; Quesenberry, M. S.; Wang, J. K.; Lee, R. T.; Kobayashi, K.; Lee, Y. C. *Anal. Biochem.* **1997**, *247*, 412–416. (b) Matsuura, K.; Tsuchida, A.; Okahata, Y.; Akaike, T.; Kobayashi, K. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 2973–2977.
6. Mammen, M.; Choi, S.-K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2754–2794.
7. (a) Uzawa, H.; Nishida, Y.; Sasaki, K.; Minoura, N.; Kobayashi, K. *ChemBioChem.* **2003**, *4*, 640–647. (b) Uzawa, H.; Zeng, X.; Minoura, N. *Chem. Commun.* **2003**, 100–101. (c) Uzawa, H.; Toba, T.; Nishida, Y.; Kobayashi, K.; Minoura, N.; Hiratani, K. *Chem. Commun.* **1998**, 2311–2312. (d) Uzawa, H.; Nishida, Y.; Sasaki, K.; Nagatsuka, T.; Hiramatsu, H.; Kobayashi, K. *Carbohydr. Res.* **2004**, *339*, 1597–1602.
8. (a) Nishida, Y.; Dohi, H.; Uzawa, H.; Kobayashi, K. *Tetrahedron Lett.* **1998**, *39*, 8681–8684. (b) Dohi, H.; Nishida, Y.; Mizuno, M.; Shinkai, M.; Kobayashi, T.; Takeda, T.; Uzawa, H.; Kobayashi, K. *Bioorg. Med. Chem.* **1999**, *7*, 2053–2062. (c) Dohi, H.; Nishida, Y.; Furuta, Y.; Uzawa, H.; Yokoyama, S.-I.; Ito, S.; Mori, H.; Kobayashi, K. *Org. Lett.* **2002**, *4*, 355–357.

9. Uzawa, H.; Kamiya, S.; Minoura, N.; Dohi, H.; Nishida, Y.; Taguchi, K.; Yokoyama, S.; Mori, H.; Shimizu, T.; Kobayashi, K. *Biomacromolecules* **2002**, *3*, 411–414.
10. (a) Matsuoka, K.; Terabatake, M.; Esumi, Y.; Terunuma, D.; Kuzuhara, H. *Tetrahedron Lett.* **1999**, *40*, 7839–7842. (b) Kitov, P. I.; Sadowska, J. M.; Mulvey, G.; Armstrong, G. D.; Ling, H.; Pannu, N. S.; Read, R. J.; Bundle, D. R. *Nature* **2000**, *403*, 669–672. (c) Lundquist, J. J.; Debenham, S. D.; Toone, E. J. *J. Org. Chem.* **2000**, *65*, 8245–8250.
11. Bovin, N. V.; Gabius, H.-J. *Chem. Soc. Rev.* **1995**, *24*, 413–421.
12. Choudhury, I.; Minoura, N.; Uzawa, H. *Carbohydr. Res.* **2003**, *338*, 1265–1270.
13. Austin, P. W.; Hardy, F. E.; Buchanan, J. G.; Baddiley, J. *J. Chem. Soc.* **1965**, 1419–1424.
14. Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. *Anal. Chem.* **1956**, *28*, 350–356.
15. (a) Nishida, Y.; Uzawa, H.; Toba, T.; Sasaki, K.; Kondo, H.; Kobayashi, K. *Biomacromolecules* **2000**, *1*, 68–74. (b) Sasaki, K.; Nishida, Y.; Tsurumi, T.; Uzawa, H.; Kondo, H.; Kobayashi, K. *Angew. Chem., Int. Ed.* **2002**, *41*, 4463–4467.
16. (a) Decher, G. *Science* **1997**, *277*, 1232–1237. (b) Decher, G.; Lvov, Y.; Schmitt, J. *Thin Solid Films* **1994**, *244*, 772–777.
17. (a) Kanekiyo, Y.; Inoue, K.; Ono, Y.; Sano, M.; Shinkai, S.; Reinhoudt, D. N. *J. Chem. Soc., Perkin Trans. 2* **1999**, 2719–2722. (b) Onda, M.; Lvov, Y.; Ariga, K.; Kunitake, T. *Biotechnol. Bioeng.* **1996**, *51*, 163–167.
18. Nuzzo, R. G.; Allara, D. L. *J. Am. Chem. Soc.* **1983**, *105*, 4481–4483.

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Supramolecular parallel β-sheet and amyloid-like fibril forming peptides using δ-aminovaleric acid residue

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Abstract—Four terminally blocked tripeptides containing δ-aminovaleric acid residue self-assemble to form supramolecular β-sheet structures as are revealed from their FT-IR data. Single crystal X-ray diffraction studies of two representative peptides also show that they form parallel β-sheet structures. Self-aggregation of these β-sheet forming peptides leads to the formation of fibrillar structures, as is evident from scanning electron microscopic (SEM) and transmission electron microscopic (TEM) images. These peptide fibrils bind to a physiological dye, Congo red and exhibit a typical green-gold birefringence under polarized light, showing close resemblance to neurodegenerative disease causing amyloid fibrils.

1. Introduction

Supramolecular β-sheet forming peptides are important for their numerous potential uses in material[1] and biological[2] sciences. Self-aggregating β-sheet forming peptides sometimes provide a fibrous network to form gels.[3] Under suitable conditions, they can also provide suitable molecular scaffolds for growing neurons[2a] and cartilage.[2b]

Many fatal neurodegenerative diseases like Alzheimer's disease,[4] Parkinson's disease,[5] and prion related diseases[6] are believed to occur due to protein misfolding and subsequent protein/protein fragment aggregation to form β-sheet rich amyloid fibrils. This fact has motivated extensive research on the general mechanism of β-sheet formation and the subsequent aggregation to form fibrillar quaternary structures. Recent evidence suggests that not only disease-related proteins, but also other non-disease related proteins can be induced to form aggregated β-sheet rich amyloid fibrils under appropriate conditions.[7] Moreover, some very recent results have established that not the matured fibrils but the intermediates, that is the protofibrils, are potent neurotoxic agents for Alzheimer's disease[8] and even for prion related diseases.[9] Thorough knowledge of β-sheet aggregation is thus, important to understand the mechanism of fibrillogenesis in order to design therapeutic agents against amyloidoses. Due to non-crystallinity and extremely poor solubility of real amyloidogenic sequences, there are no significant studies that clearly demonstrate the mechanism of β-sheet formation and its aggregation at atomic resolution using single crystal X-ray diffraction studies. So, it is worthwhile to design supramolecular β-sheet forming model peptides that can form amyloid-like fibrils and are capable of forming single crystals.

It is still a controversial issue whether amyloid fibril formation proceeds preferentially through the self-assembly of parallel or antiparallel β-sheets. Recent studies have suggested that Aβ10-35 give rise to parallel[10a] β-strand whereas Aβ16-22 forms antiparallel β-strands.[10b] In our previous study, we have demonstrated that short peptides composed of non-coded amino acids can self-assemble to form amyloid-like fibril forming supramolecular parallel β-sheet structure in crystals.[11]

δ-Ava (δ-aminovaleric acid) has been used for conformational interest to design new foldamers. Previous reports described that an oligopeptide with a centrally located δ-Ava residue forms a helical conformation in solution[12a] and an octapeptide with a δ-Ava residue at the central position can form a β-hairpin structure in solution.[12b] Very recently, Baldauf et al. have demonstrated all possible helix types in oligomers of δ-amino acids (δ-peptides) and their stabilities employing various methods of ab initio MO theory.[13] However, in this paper, we report the use of the δ-aminovaleric acid residue (δ-Ava) in model terminally

Keywords: δ-Aminovaleric acid; Supramolecular parallel β-sheet; Amyloid-like fibril.
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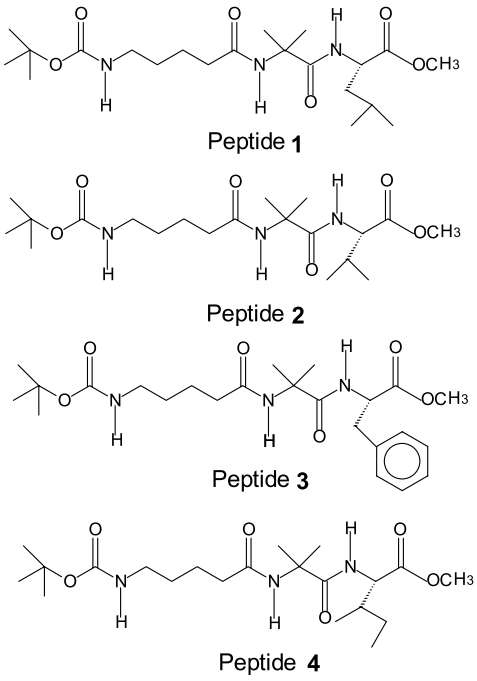

Figure 1. Schematic representation of peptides **1**, **2**, **3** and **4**.

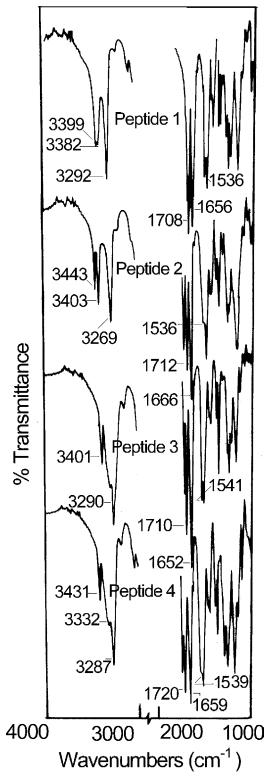

Figure 2. FT-IR spectra at the region 3000–4000 cm^{-1} (a) and 1000–2000 cm^{-1} (b) of peptides **1**, **2**, **3** and **4** in solid state.

blocked tripeptides to design and construct supramolecular β-sheet structures in the solid state and subsequent self-assembly of these β-sheets leads to the formation of amyloid-like fibrils. The schematic presentation of all terminally protected tripeptides are shown in Figure 1. To the best of our knowledge this is the first, crystallographic study of oligopeptides containing a δ-Ava residue.

2. Results and discussion

In our previous results we have demonstrated that the combination of conformationally flexible amino acid Acp (ε-aminocaproic acid) and conformationally constrained Aib (α-aminoisobutyric acid) provides overall extended structures[14] whereas by contrast a combination of conformationally flexible γ-Abu (γ-aminobutyric acid) and Aib gives a turn structure.[15] In this paper, a series of terminally blocked tripeptides containing conformationally conflicting amino acid residues (δ-aminovaleric acid and α-aminoisobutyric acid) have been designed and synthesized. The δ-aminovaleric acid residue has been used to exploit its CO and NH groups as hydrogen bonding functionalities and the centrally located tetramethylene unit provides sufficient flexibility to the peptide backbone that might help these peptides to adopt an extended backbone conformation. The conformationally restricted α-aminoisobutyric acid (Aib) residue has been used in each of the peptides to decrease conformational heterogeneity and to increase the crystallinity of the corresponding peptides. The third residues for peptides **1** and **2** are leucine (Leu) and valine (Val), respectively, having hydrophobic side chains that might help these peptides to aggregate also using van der Waals interactions. Similarly, for peptides **3** and **4** we have used Phe and Ile, respectively. Out of the four reported peptides, we obtained suitable single crystals for peptides **1** and **2** and obtained their structures. All peptides were studied using FT-IR, NMR, scanning electron microscopy, transmission electron microscopy and optical microscopy.

2.1. Solid-state FT-IR study

Preliminary information on the conformational preferences of all peptides were obtained from solid-state FT-IR studies (Fig. 2). In solid state (KBr matrix), intense bands at 3275–3375 cm^{-1} have been observed for all reported peptides indicating the presence of strongly hydrogen bonded NH groups. Important IR data of all these reported peptides are listed in Table 1. The bands corresponding to NH stretching appear over 3430 cm^{-1} suggesting the occurrence of free NH groups for peptide **2** and peptide **4**.[16] The absence of a band attributable to free NH (over 3430 cm^{-1}) indicates

Table 1. Infrared (IR) absorption frequencies (cm^{-1}) for all reported peptides in solid state (on KBr pellet)

Peptide	CO stretch (cm^{-1})	NH bend (cm^{-1})	NH stretch (cm^{-1})
Boc-δ-Ava-Aib-Leu-OMe **1**	1656 (s)	1536 (m)	3399 (m), 3382 (m), 3292 (s)
Boc-δ-Ava-Aib-Val-OMe **2**	1666 (s)	1536 (m)	3443 (m), 3403 (m), 3269 (s)
Boc-δ-Ava-Aib-Phe-OMe **3**	1652 (s)	1541 (m)	3401 (w), 3290 (s)
Boc-δ-Ava-Aib-Ile-OMe **4**	1659 (s)	1539 (m)	3431 (w), 3332 (m), 3287 (s)

s = strong, w = weak, m = medium.

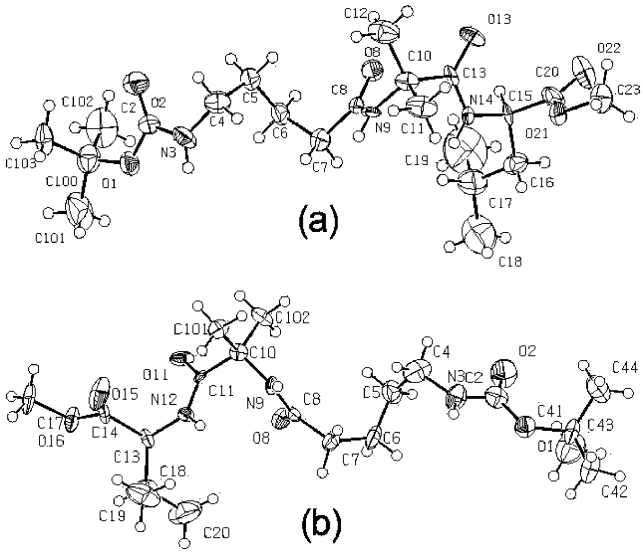

Figure 3. The ORTEP diagram of (a) peptide **1** and (b) peptide **2** with the atomic numbering scheme. Ellipsoids at 20% probability.

that all NHs are involved in intermolecular hydrogen bonding for peptides **1** and **3**.[16] The CO stretching band at around 1650–1660 cm^{-1} (amide I) and the NH bending peak near 1535 cm^{-1} (amide II) suggest the presence of intermolecularly hydrogen bonded supramolecular β-sheet-like conformations[16] for all peptides in the solid state. So, from solid-state FT-IR data it can be concluded that all the peptides share a common structural feature, the intermolecularly hydrogen-bonded sheet.

2.2. Single crystal X-ray diffraction study

These preliminary conformational data were further, supported by single crystal X-ray diffraction studies. Single crystals of peptide**1** and peptide **2** were grown from methanol–water solution. The molecular conformations of the peptides **1** and **2** in the crystals are illustrated in Figure 3. This reveals that the reported peptides are unable to form any kind of intramolecularly hydrogen bonded (N–H···O or N–H···N) turn structures despite the fact that there is a centrally positioned helicogenic Aib[17] residue for both peptides and the ϕ and ψ values of the majority of the constituent amino acid residues fall within the helical region of the Ramachandran map (Table 2 for peptide **1** and Table 3 for peptide **2**). Self-assembly of each individual monomer leads to the formation of parallel β-sheet columns along the crystallographic *a* axis (Fig. 4). For peptide **1** the parallel β-sheet column is stabilized by three intermolecular hydrogen bonds (N3–H3···O8, N9–H9···O13 and N14–H14···O22) (Table 2) exploiting all its hydrogen bonding functionalities. However, only two intermolecular hydrogen bonds (N3–H3···O8 and N9–H9···O11) are present in peptide **2** connecting individual peptide molecules to form the supramolecular parallel β-sheet column (Table 3). In peptide **2**, one N–H and one carbonyl group do not form any type of hydrogen bond. These data confirm our initial insight from the solid-state FT-IR data, that both peptides **1** and **2** possess an intermolecularly hydrogen-bonded β-sheet structure, although peptide **2** has one free NH while in peptide **1** all NHs are engaged in hydrogen-bonding. Parallel β-sheet columns self-assemble along the crystallographic *b* axis for peptide **1** (Fig. 5a) and along the

Table 2. Characteristics of peptide **1** (Boc-δ-Ava-Aib-Leu-OMe)

(a) Selected torsional angles[a] (°) of peptide **1**

Residue	ϕ	ψ	ω	θ_1	θ_2	θ_3
δ-Ava(1)	−67.14	135.37	169.55	−55.06	−69.7	−60.79
Aib(2)	−49.12	−44.92	179.26	—	—	—
Leu(3)	62.74	36.43	176.95	—	—	—

(b) Intermolecular hydrogen bonding parameters of peptide **1**

D–H···A	H···A (Å)	D···A (Å)	D–H···A (°)
N3–H3···O8[b]	2.25	3.023(10)	150
N9–H9···O13[b]	2.04	2.904(8)	179
N14–H14···O22[b]	2.53	3.358(9)	161

[a] The torsion angles for rotation about the bonds of peptide backbone: ϕ, ψ, ω. Torsions in the main chain in the N-terminal δ-Ava residue about C^{α}-C^{β}, C^{β}–C^{γ} and C^{γ}–C^{δ} θ_3 to θ_1, respectively.
[b] Symmetry element $-1+x, y, z$.

Table 3. Characteristics of peptide **2** (Boc-δ-Ava-Aib-Val-OMe)

(a) Selected torsional angles[a] (°) of peptide **2**

Residue	ϕ	ψ	ω	θ_1	θ_2	θ_3
δ-Ava(1)	80.09	−129.31	−175.43	59.27	76.26	57.75
Aib(2)	56.73	40.79	170.98	—	—	—
Val(3)	−73.76	150.88	173.46	—	—	—

(b) Intermolecular hydrogen bonding parameters of peptide **2**

D–H···A	H···A (Å)	D···A (Å)	D–H…A (°)
N3–H3···O8[b]	2.31	3.028(8)	142
N9–H9···O11[b]	2.12	2.915(6)	153

[a] The torsion angles for rotation about the bonds of peptide backbone: ϕ, ψ, ω. Torsions in the main chain in the N-terminal δ-Ava residue about C^{α}–C^{β}, C^{β}–C^{γ} and C^{γ}–C^{δ} θ_3 to θ_1, respectively.
[b] Symmetry element $-1+x, y, z$.

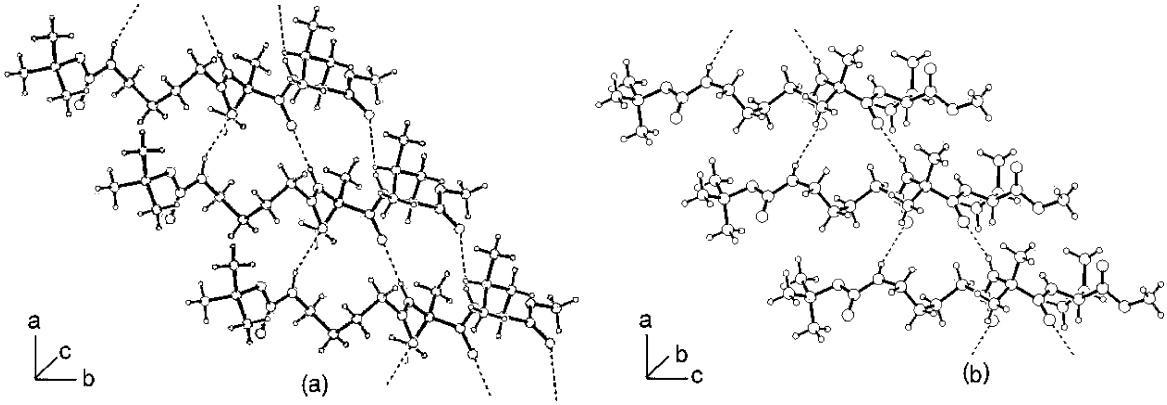

Figure 4. (a) Packing diagram of peptide **1** along *a* projection and (b) Packing diagram of peptide **2** along *a* projection illustrating intermolecular hydrogen bonding in solid state and the formation continuous β-sheet columns. Hydrogen bonds are shown as dotted lines.

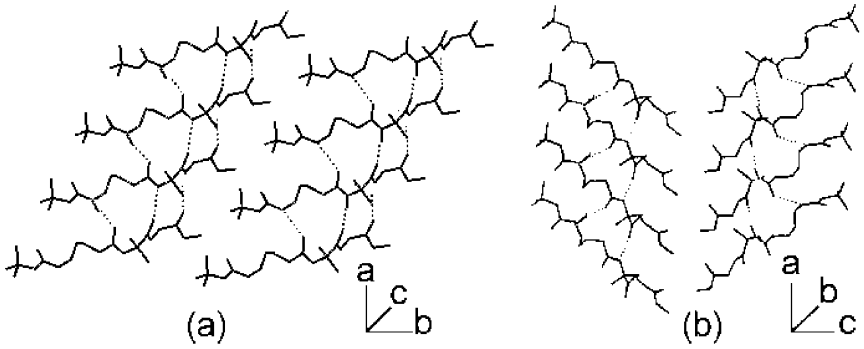

Figure 5. (a) Packing of peptide **1** along crystallographic *b* direction and (b) packing of peptide **2** along crystallographic *c* direction forming β-sheet layer structures. Hydrogen bonds are shown as dotted lines.

crystallographic *c* axis for peptide **2** (Fig. 5b) to form two-dimensional β-sheet layers. Each β-sheet layer is then regularly stacked along crystallographic *c* axis for peptide **1** (Fig. 6) and along crystallographic *b* axis for peptide **2** (Fig. 7) using van der Waals' interactions forming complex quaternary β-sheet structures. Crystal data for peptides **1** and **2** are listed in Table 4.

Except the ψ_1 for peptide **1** and ψ_1 and ψ_3 for peptide **2**, all ϕ, ψ values of these peptides **1** and **2** fall within the helical region of the Ramachandran plot (Tables 2 and 3). However, neither of these peptide backbones adopts turn or turn-like structure. No intramolecular hydrogen bond has been observed. Instead, these peptides self-aggregate to form intermolecularly hydrogen-bonded supramolecular β-sheet structures. It is also noteworthy that C–C bonds along the polymethylene units of the δ-Ava residue adopt gauche conformations. However, the overall molecular conformations of peptides **1** and **2** are flat without any noticeable bend or turn.

2.3. Scanning electron microscopic study

The morphological studies of all peptides were carried out using a scanning electron microscope (SEM). The scanning electron microscopic (SEM) images of peptides **1**, **2**, **3** and **4** (Figs. 8–11, respectively) of the dried fibrous material grown from methanol–water clearly demonstrate that the aggregates in the solid state are bunches of long small filaments, resembling amyloid fibrils.[18]

2.4. Transmission electron microscopic study

Transmission electron microscopy of all the peptides at high magnification provides information concerning their detailed structure although only a two dimensional

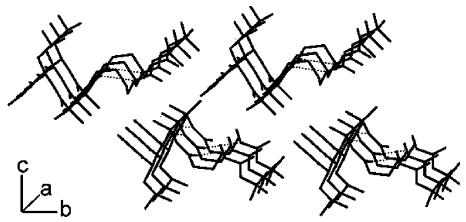

Figure 6. Higher order packing of peptide **1** along crystallographic *c* axis and forming quaternary β-sheet structures. Hydrogen bonds are shown as dotted lines.

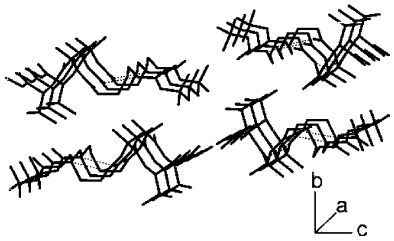

Figure 7. Higher order packing of peptide **2** along crystallographic *b* axis and forming quaternary β-sheet structures. Hydrogen bonds are shown as dotted lines.

Table 4. Crystallographic data for peptide **1** and **2**

	Peptide **1**	Peptide **2**
Formula	$C_{21}H_{39}N_3O_6$	$C_{20}H_{37}N_3O_6$
Formula weight	429.55	415.53
Crystallizing solvent	Methanol–water	Methanol–water
Crystal system	Orthorhombic	Orthorhombic
Temperature (K)	293	293
Space group	*P*212121	*P*212121
a (Å)	6.100 (8)	6.143 (8)
b (Å)	14.325 (16)	13.156 (15)
c (Å)	29.94 (3)	29.50 (3)
U (Å^3)	2616 (5)	2384 (5)
Z	4	4
*D*calcd (g cm^{-3})	1.091	1.158
λ (Å)	0.71073	0.71073
*R*1 ($I>2\sigma(I)$)	0.1050	0.0958
*wR*2 ($I>2\sigma(I)$)	0.2643	0.2041

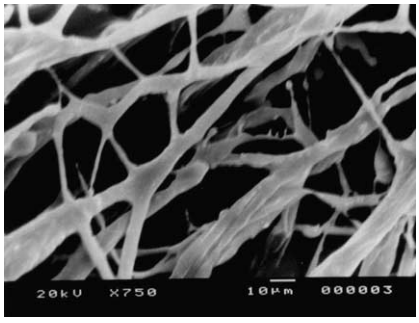

Figure 8. SEM image of dried material of peptide **1** obtained from methanol–water solution by slow evaporation.

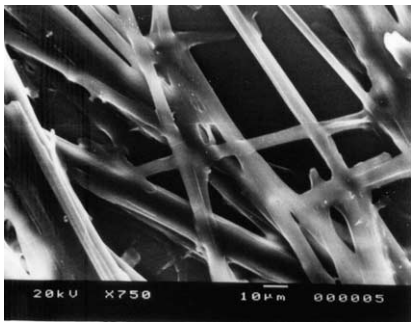

Figure 9. Typical SEM image of dried fibrous material of peptide **2** obtained from methanol–water solution by slow evaporation.

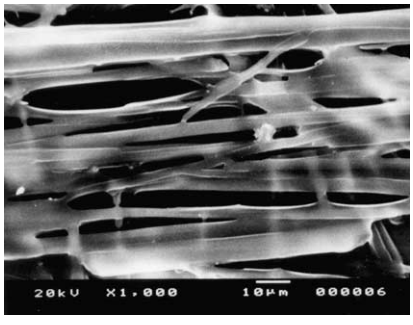

Figure 10. Typical SEM image of dried material of peptide **3** showing amyloid-like fibrillar morphology obtained from methanol–water solution by slow evaporation.

projection of the specimen could be imaged. All the peptides exhibit fibrillar morphology under the TEM. The representative TEM image of peptide **3** (Fig. 12) reveals that the peptide exists as a bunch of long unbranched filaments having diameter ~20–40 nm.

2.5. Congo red binding study

Air-dried drops of the solution of all these peptides were stained with a physiological dye Congo red and under cross polarizers these peptide fibrils exhibit green birefringence, characteristic feature of amyloid fibrils when investigated

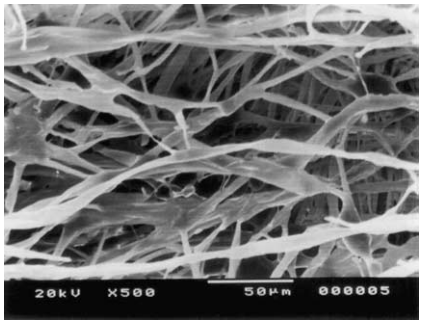

Figure 11. Typical SEM image of dried material of peptide **4** showing amyloid-like fibrillar morphology obtained from methanol–water solution by slow evaporation.

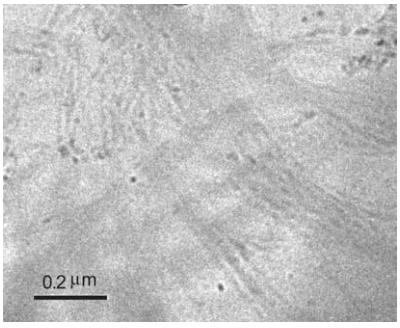

Figure 12. Transmission electron micrograph of peptide **3** showing amyloid-like morphology. The sample was prepared on a carbon coated copper grid by slow evaporation of methanol–water solution of the peptide **3**.

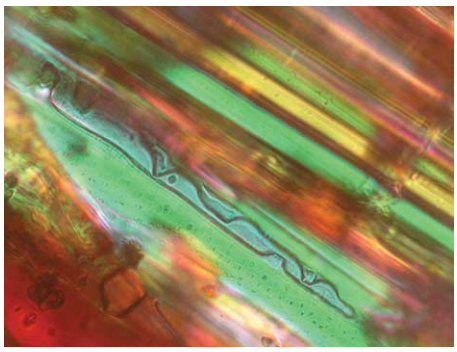

Figure 13. Congo red stained peptide **1** fibrils observed through crossed polarizers showing green-gold birefringence a characteristic feature of amyloid fibrils.

microscopically.[19] Figure 13 is a representative picture of peptide **1** stained with Congo red, and exhibits distinct green-gold birefringence under polarized light.

3. Conclusion

Solid-state FT-IR data of all peptides (peptides **1**, **2**, **3** and **4**) reveal that, all reported peptides self-associate to form intermolecularly hydrogen bonded supramolecular β-sheet structure. Crystal structures of two peptides (peptide **1** and peptide **2**) not only support the preliminary informational feature obtained from the solid-state FT-IR data but also suggest that these two peptides self-aggregate in a parallel orientation. Upon further self-assembly, these beta sheet structures produce peptide fibrils. All reported peptides show their morphological similarity with amyloidogenic proteins or protein fragments. They also bind to the physiological dye Congo red and exhibit a typical green-gold birefringence under polarized light like amyloid fibrils. Previously, it has been shown that fibril formation of Alzheimer's-associated Aβ peptide fragment proceeds via parallel β-sheet formation.[12a,20a] Some very recent results also demonstrate that fragments of Prion proteins[20b,c] and even Tau filament fragments[20d] also self-aggregate via parallel β-sheet formation. So, this study of the amyloid-like fibril forming model peptide with parallel β-sheet structure at atomic resolution may assist the scientific community studying amyloid diseases in investigating the pathway(s) and self-assembly mechanism during amyloid fibril formation. Moreover, insertion of δ-Ava residues into the peptide backbone not only helps to form hydrogen-bonded supramolecular β-sheet structures, but also provides proteolytic resistance as δ-Ava is homomorphous with the Gly-Gly segment of any peptide.

4. Experimental

4.1. Synthesis of peptides

All peptides were synthesized by conventional solution phase methods by using racemization free fragment condensation strategy.[21] The Boc group was used for N-terminal protection and the C-terminus was protected as a methyl ester. Deprotections were performed using the saponification method. Couplings were mediated by dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC/HOBt). All the intermediates were characterized by ^{1}H NMR (300 MHz) and thin layer chromatography (TLC) on silica gel and used without further purification. The final products were purified by column chromatography using silica (100–200 mesh size) gel as stationary phase and ethyl acetate/ethyl acetate–toluene mixture as eluent. The purified final compounds were fully characterized by 300 MHz ^{1}H NMR spectroscopy, mass spectrometry and elemental analysis.

4.1.1. Boc-δ-Ava-OH 5. A solution of δ-aminovaleric acid (3.51 g, 30 mmol) in a mixture of dioxane (60 mL), water (30 mL) and 1 M NaOH (60 mL) was stirred and cooled in an ice-water bath. Di-*tert*-butylpyrocarbonate (7.2 g, 33 mmol) was added and stirring was continued at room temperature for 6 h. Then the solution was concentrated in vacuo to about 30–40 mL, cooled in an ice water bath, covered with a layer of ethyl acetate (about 50 mL) and acidified with a dilute solution of $KHSO_4$ to pH 2–3 (Congo red). The aqueous phase was extracted with ethyl acetate and this operation was done repeatedly. The ethyl acetate extracts were pooled, washed with water and dried over anhydrous Na_2SO_4 and evaporated concentrated in vacuo. The pure material **5** was obtained as a waxy solid. Yield=5.6 g (25.8 mmol, 86%); δ_H (300 MHz, CD_3SOCD_3) 11.76 (–COOH, 1H, br), 6.54 (δ-Ava(1) NH, 1H, t, J=9 Hz), 2.64–2.70 (δ-Ava(1) C^{δ}Hs, 2H, m), 1.94–1.99 (δ-Ava(1) C^{α}Hs, 2H, m), 1.21–1.26 (δ-Ava(1) C^{β} and C^{γ} Hs, 4H, m), 1.14 (Boc-CH_3, 9H, s); mass spectral (ESI) data $(M+Na)^+$=240.1, M_{calcd}=217; (found: C, 55.35; H, 8.72; N, 6.42 $C_{10}H_{19}N_1O_4$ (217) requires C, 55.30; H, 8.76; N, 6.45%).

4.1.2. Boc-δ-Ava(1)-Aib(2)-OMe 6. Boc-δ-Ava-OH (5.4 g, 25 mmol) was dissolved in dichloromethane (DCM) (50 mL) in an ice-water bath. H-Aib-OMe was isolated from the corresponding methyl ester hydrochloride (7.67 g, 50 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration to 15 mL and this was added to the reaction mixture, followed immediately by dicyclohexylcarbodiimide (DCC) (5.15 g, 25 mmol). The reaction mixture was allowed to come to room temperature and stirred for 48 h. DCM was evaporated, the residue was dissolved in ethyl acetate (60 mL), and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 M HCl (3×50 mL), brine (2×50 mL), then 1 M sodium carbonate (3×50 mL) and brine (2×50 mL) and dried over anhydrous sodium sulfate, and evaporated in vacuo to yield Boc-δ-Ava-Aib-OMe **6** as a white solid. Yield=6.68 g (21.14 mmol, 84.5%); mp 104–106 °C; δ_H (300 MHz, $CDCl_3$) 6.24 (Aib(2) NH, 1H, s), 4.72 (δ-Ava(1) NH, 1H, t, J=9.6 Hz), 3.73 (–OCH_3, 3H, s), 3.14–3.10 (δ-Ava(1) C^{δ}Hs, 2H, m), 2.17–2.22 (δ-Ava(1) C^{α}Hs, 2H, m), 1.60–1.71 (δ-Ava(1) C^{β} and C^{γ}Hs, 4H, m), 1.53 (Aib(2) C^{β}Hs, 6H, s), 1.44 (Boc-CH_3s, 9H, s); mass spectral (ESI) data $(M+Na)^+$=339.2, M_{calcd}=316; (found: C, 56.93; H, 8.82; N, 8.92 $C_{15}H_{28}N_2O_5$ (316) requires C, 56.96; H, 8.86; N, 8.86%).

4.1.3. Boc-δ-Ava(1)-Aib(2)-OH 7. To a sample of **6** (6.64 g, 21 mmol), MeOH (75 mL) and 2 M NaOH (30 mL) were added and the progress of saponification

was monitored by thin layer chromatography (TLC). The reaction mixture was stirred. After 10 h. methanol was removed in vacuo, the residue was dissolved in water (50 mL), and washed with diethyl ether (2×50 mL). Then the pH of the aqueous layer was adjusted to 2 using 1 M HCl and it was extracted with ethyl acetate (3×50 mL). The extracts were pooled, dried over anhydrous sodium sulfate, and evaporated in vacuo to yield compound **7** as a white solid. Yield=6.08 g (20.13 mmol, 95.86%); mp 122–124 °C; δ_H (300 MHz, CD_3SOCD_3) 12.18 (–COOH, 1H, br), 7.89 (Aib(2) NH, 1H, s), 6.71 (δ-Ava(1) NH, 1H, t, J= 8.7 Hz), 3.89–3.97 (δ-Ava(1) C^{δ}Hs, 2H, m), 1.52–1.61 (δ-Ava(1) C^{α}Hs, 2H, m), 1.28–1.35 (δ-Ava(1) C^{β} and C^{γ}Hs, 4H, m), 1.13 (Boc-CH_3, 9H, s), 0.81 (Aib (2) C^{β}Hs, 6H, s); mass spectral (ESI) data $(M+Na)^+$=325.2, M_{calcd}=302; (found: C, 55.65; H, 8.58; N, 9.31. $C_{14}H_{26}N_2O_5$ (302) requires C, 55.63; H, 8.61; N, 9.27%).

4.1.4. Boc-δ-Ava(1)-Aib(2)-Leu(3)-OMe 1. Boc-δ-Ava-Aib-OH **7** (1.51 g, 5 mmol) was dissolved in DMF (10 mL) in an ice-water bath. H-Leu-OMe was isolated from methyl ester hydrochloride (1.82 g, 10 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration to 7 mL and it was added to the reaction mixture, followed immediately by dicyclohexylcarbodiimide (DCC) (1.03 g, 5 mmol) and HOBt (0.68 g, 5 mmol). The reaction mixture was allowed to come to room temperature and stirred for 72 h. The residue was taken in ethyl acetate (30 mL), and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 M HCl (3×30 mL), brine (2×30 mL), then 1 M sodium carbonate (3×30 mL) and brine (2×30 mL) and dried over anhydrous sodium sulfate and evaporated in vacuo to yield **1** (1.59 g) in form of white solid. Purification was done by silica gel column (100–200 mesh) using ethyl acetate as eluent.

Yield=1.59 g (3.7 mmol, 74%); mp 114–116 °C; δ_H (300 MHz, $CDCl_3$) 7.07 (Leu(3) NH, 1H, d, J=7.5 Hz), 6.18 (Aib(2) NH, 1H, s), 4.76 (δ-Ava(1) NH, 1H, t, J= 7.15 Hz), 4.60–4.53 (Leu(3) C^{α}H, 1H, m), 3.73 (-OCH_3, 3H, s), 3.14–3.12 (δ-Ava(1) C^{δ}Hs, 2H, m), 2.25–2.20 (δ-Ava(1) C^{α} Hs, 2H, m), 1.86–1.82 (Leu(3) C^{β}Hs, 2H, m), 1.68–1.62 (δ-Ava(1) C^{β} Hs and C^{γ}Hs, 4H, m), 1.58 (Aib(2) C^{β}Hs, 3H, s), 1.56 (Aib(2) C^{β}Hs, 3H, s), 1.54–1.49 (Leu(3) C^{γ}H 1H, m), 1.44 (Boc-CH_3, 9H, s), 0.96–0.91 (Leu(3) C^{δ}Hs, 6H, m); mass spectral (ESI) data $(M+H)^+$=430.4, M_{calcd}= 429; $[\alpha]_D^{24.9}$ +6.39 (c 2.12, $CHCl_3$); (found: C, 58.78; H, 9.07; N, 9.83 $C_{21}H_{39}N_3O_6$ (429) requires C, 58.74; H, 9.1; N, 9.79%).

4.1.5. Boc-δ-Ava(1)-Aib(2)-Val(3)-OMe 2. A sample of **7** (1.51 g, 5 mmol) in DMF (10 mL) was cooled in an ice water bath. H-Val-OMe was isolated from of the corresponding methyl ester hydrochloride (1.68 g, 10 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration to 7 mL and this was added to the reaction mixture, followed immediately by DCC (1.03 g, 5 mmol) and HOBt (0.68 g, 5 mmol). The reaction mixture was stirred for 3 days. The residue was taken in ethyl acetate (50 mL) and DCU was filtered off. The organic layer was washed with 2 M HCl (3×50 mL), brine (2×50 mL), 1 M sodium carbonate (3×50 mL), brine (2×50 mL), dried over anhydrous sodium sulfate and evaporated in vacuo to yield white solid of **2**. Purification was done by silica gel column using ethyl acetate as eluent. Yield=1.5 g (3.6 mmol, 72%); mp 110–112 °C; δ_H (300 MHz, $CDCl_3$) 7.13 (Val(3) NH, 1H, d, J=8.1 Hz), 6.10 (Aib(2) NH, 1H, s), 4.70 (δ-Ava(1) NH, 1H, br), 4.48–4.52 (Val(3) C^{α}H, 1H, m), 3.73 (–OCH_3, 1H, s), 3.15–3.11 (δ-Ava(1) C^{δ}Hs, 2H, m), 2.25–2.21 (δ-Ava(1) C^{α}Hs, 2H, m), 2.18–2.16 (Val C^{β}H, 1H, m), 1.63–1.71 (δ-Ava(1) C^{β} and C^{γ}Hs, 4H, m and 1H, m), 1.43 (Boc-CH_3, 9H, s), 1.25 (Aib(2) C^{β}Hs, 6H, s), 0.96–0.88 (Val(3) C^{γ}Hs, 6H, m); Mass spectral (ESI) data $(M+H)^+$=416.4, M_{calcd}=415; $[\alpha]_D^{25}$ +17.4 (c 2.16, $CHCl_3$); (found: C, 57.87; H, 8.95; N, 10.07; $C_{20}H_{37}N_3O_6$ (415) requires C, 57.83; H, 8.92; N, 10.12%).

4.1.6. Boc-δ-Ava(1)-Aib(2)-Phe(3)-OMe 3. Boc-δ-Ava(1)-Aib(2)-OH (1.51 g, 5 mmol) in DMF (10 mL) was cooled in an ice-water bath and H-Phe-OMe was isolated from the corresponding methyl ester hydrochloride (2.16 g, 10 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration to 7 mL and it was added to the reaction mixture, followed immediately by DCC (1.03 g, 5 mmol) and of HOBt (0.68 g, 5 mmol). The reaction mixture was stirred for three days. The residue was taken in ethyl acetate (60 mL) and the DCU was filtered off. The organic layer was washed with 2 M HCl (3× 50 mL), brine (2×50 mL), 1 M sodium carbonate (3× 50 mL), brine (2×50 mL), dried over anhydrous sodium sulfate and evaporated in vacuo to yield **3** as white solid. Purification was done by silica gel column (100–200 mesh) using ethyl acetate as eluent. Yield=1.67 g (3.6 mmol, 72%); mp 117–119 °C; δ_H (300 MHz, $CDCl_3$) 7.11–7.33 (Ph ring protons of Phe(3), 5H, m), 6.88 (Phe(3) NH, 1H, d, J=7.26 Hz), 6.05 (Aib(2) NH, 1H, s), 4.86–4.80 (Phe(3) C^{α}H, 1H, m), 4.70 (δ-Ava(1) NH, 1H, m), 3.73 (–OCH_3, 3H, s), 3.21–3.06 (Phe(3) C^{β}Hs, 2H, m and δ-Ava(1) C^{δ}Hs, 2H, m), 2.20–2.09 (δ-Ava(1) C^{α}Hs, 2H, m), 1.66–1.59 (δ-Ava(1) C^{β} and C^{γ}Hs, 4H, m), 1.49 (Aib(2) C^{β} Hs, 6H, s), 1.43 (Boc-CH_3, 9H, s); mass spectral (ESI) data $(M+Na)^+$=486.2, M_{calcd}=463; $[\alpha]_D^{25.1}$ +36.91 (c 2.25, $CHCl_3$); (found: C, 62.27; H, 8.12; N, 9.02. $C_{24}H_{37}N_3O_6$ (463) requires C, 62.20; H, 7.99; N, 9.07%).

4.1.7. Boc-δ-Ava(1)-Aib(2)-Ile(3)-OMe 4. Boc-δ-Ava-Aib-OH (1.51 g, 5 mmol) was dissolved in DMF (10 mL) in an ice-water bath. H-Ile-OMe was isolated from methyl ester hydrochloride (1.82 g, 10 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration to 7 mL and it was added to the reaction mixture, followed immediately by dicyclohexylcarbodiimide (DCC) (1.03 g, 5 mmol) and HOBt (0.68 g, 5 mmol). The reaction mixture was allowed to come to room temperature and stirred for 72 h. The residue was taken in ethyl acetate (30 mL), and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 M HCl (3×30 mL), brine (2×30 mL), then 1 M sodium carbonate (3×30 mL) and brine (2× 30 mL) and dried over anhydrous sodium sulfate and evaporated in vacuo to yield **4** as white solid. Purification was done by silica gel column (100–200 mesh) using ethyl acetate as eluent. Yield =1.58 g (3.68 mmol, 73.6 %); mp 108–110 °C; δ_H (300 MHz, $CDCl_3$) 7.18 (Ile(3) NH, 1H, d, J=8.1 Hz), 6.25 (Aib(2) NH, 1H, s), 4.77 (δ-Ava(1) NH, 1H, m), 4.62–4.51 (Ile(3) C^{α}H, 1H, m), 3.73 (–OCH_3, 3H, s), 3.19–3.06 (δ-Ava(1) C^{δ}Hs, 2H, m), 2.29–2.21 (δ-Ava(1)

C^{α}Hs, 2H, m), 1.97–1.88 (Ile C^{β}Hs,1H, m), 1.70–1.62 (δ-Ava(1) C^{β} and C^{γ}Hs, 4H, m), 1.58 (Aib(2) C^{β}Hs, 3H, s), 1.55 (Aib(2) C^{β}Hs, 3H, s), 1.44 (Boc-CH_3, 9H, s), 1.24–1.11 (Ile(3) C^{γ}Hs, 2H, m), 0.99–0.90 (Ile(3) C^{γ} and C^{δ}Hs, 6H, m); Mass spectral (ESI) data $(M+Na)^+ = 452.2$, $M_{calcd} = 429$; $[\alpha]_D^{25.3} +21.35$ (*c* 2.04, $CHCl_3$); (found: C, 58.68; H, 9.13; N, 9.76. $C_{21}H_{39}N_3O_6$ (429) requires C, 58.74; H, 9.1; N, 9.79).

4.2. NMR experiments

All NMR studies were carried out on a Brüker DPX 300 MHz spectrometer at 300 K. Peptide concentrations were in the range 1–10 mM in $CDCl_3$ and CD_3SOCD_3.

4.3. FT-IR spectroscopy

The FT-IR spectra were taken using Shimadzu (Japan) model FT-IR spectrophotometer. The solid-state FT-IR measurements were performed using the KBr disk technique.

4.4. Scanning electron microscopic study

Morphologies of all reported tripeptides were investigated using optical microscopy and scanning electron microscopy (SEM). For the SEM study, fibrous materials (slowly grown from methanol–water mixtures) were dried and gold coated. Then the micrographs were taken in a SEM apparatus (Jeol Scanning Microscope-JSM-5200).

4.5. Transmission electron microscopic study

The morphologies of the reported compounds were investigated using transmission electron microscopy (TEM). The transmission electron microscopic studies of all the peptides were carried out using a small amount of the solution of the corresponding compounds on carbon-coated copper grids (200 mesh) by slow evaporation and allowed to dry in vacuum at 30 °C for two days. Images were taken at an accelerating voltage of 200 kV. TEM was performed using a JEM-2010 electron microscope.

4.6. Congo red binding study

An alkaline saturated Congo red solution was prepared. The peptide fibrils were stained by alkaline Congo red solution (80% methanol/ 20% glass distilled water containing 10 μL of 1% NaOH) for 2 min and then the excess stain (Congo red) was removed by rinsing the stained fibril with 80% methanol/20% glass distilled water solution for several times. The stained fibrils were dried in vacuum at room temperature for 24 h, then visualized at 100× or 500× magnification and birefringence was observed between crossed polarizers.

4.7. Single crystal X-ray diffraction study

For peptides**1** and **2**, single crystals were obtained from methanol–water solution by slow evaporation. Crystal data for both peptides were collected on a Marresearch Image Plate with Mo Kα radiation. The crystals were positioned at 70 mm from the Image Plate. Hundred frames were measured at 2° intervals with a counting time of 2 min. Data analyses were carried out with the XDS program.[22] The structures were solved using direct methods with the Shelx86 program.[23] Non-hydrogen atoms were refined with anisotropic thermal parameters. The hydrogen atoms bonded to carbon were included in geometric positions and given thermal parameters equivalent to 1.2 times those of the atom to which they were attached. The structures were refined on F^2 using Shelxl.[24] Crystallographic data have been deposited at the Cambridge Crystallographic Data Center references CCDC 258126, 258127 for peptides **1** and **2**.

4.8. Mass spectrometry

Mass spectra were recorded on a Hewlett Packard Series 1100MSD mass spectrometer by positive mode electrospray ionization.

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References and notes

1. (a) Aggeli, A.; Boden, N.; Cheng, Y.; Findlay, J. B. C.; Knowles, P. F.; Kovatchev, P.; Turnbull, P. J. H. *Biochemistry* **1996**, *35*, 16213–16221. (b) Scheibel, T.; Parthasarathy, R.; Sawicki, G.; Lin, X.-M.; Jaeger, H.; Lindquist, S. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 4527–4532.
2. (a) Holmes, T. C.; Lacalle, S. D.; Su, X.; Liu, G.; Rich, A.; Zhang, S. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6728–6733. (b) Kisiday, J. D.; Jin, M.; Kurz, B.; Huang, H.; Semino, C. E.; Zhang, S.; Grodzinsky, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 9996–10001. (c) Wang, W.; Hect, M. H. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 2760–2765.
3. Aggeli, A.; Bell, M.; Boden, N.; Keen, J. N.; Knowles, P. F.; Mcleish, T. C. B.; Pitkeathly, M.; Radford, S. E. *Nature* **1997**, *386*, 259–262.
4. (a) Taubes, G. *Science* **1996**, *271*, 1493–1495. (b) Lansbury, P. T., Jr. *Acc. Chem. Res.* **1996**, *29*, 317–321. (c) Rochet, J. C.; Lansbury, P. T., Jr. *Curr. Opin. Struct. Biol.* **2000**, *10*, 60–68. (d) Walsh, D. M.; Lomakin, A.; Benedek, G. B.; Condron, M. M.; Teplow, D. B. *J. Biol. Chem.* **1997**, *272*, 22364–22372. (e) Walsh, D. M.; Hartley, D. M.; Kusumoto, Y.; Fezoui, Y.; Condron, M. M.; Lomakin, A.; Benedek, G. B.; Selkoe, D. J.; Teplow, D. B. *J. Biol. Chem.* **1999**, *274*, 25945–25952.
5. (a) Goedert, M.; Spillntini, M. G.; Davies, S. W. *Curr. Opin. Neurobiol.* **1998**, *8*, 619–632. (b) Baba, M.; Nakajo, S.; Tu, P. H.; Tomita, T.; Nakaya, K.; Lee, V. M.-Y.; Trojanowski, J. Q.; Iwatsubo, T. *Am. J. Pathol.* **1998**, *52*, 879–884. (c) Spillantini, M. G.; Crowther, R. A.; Jakes, R.; Hasegawa, M.; Goedert, M. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6469–6473.
6. (a) Pan, K. M.; Baldwin, M.; Nguyen, J.; Gasset, M.; Serban, A.; Groth, D.; Mehlhorn, I.; Huang, Z.; Fletterick, R. J.;

Cohen, F. E. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10962–10966. (b) Prusiner, S. B. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 13363–13383.
7. (a) Dobson, C. M. *Trends Biochem. Sci.* **1999**, *24*, 329–332. (b) Fändrich, M.; Fletcher, M. A.; Dobson, C. M. *Nature* **2001**, *410*, 165–166.
8. (a) Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353–356. (b) Klein, W. L.; Krafft, G. A.; Finch, C. E. *Trends Neurosci.* **2001**, *24*, 219–224. (c) Walsh, D. M.; Hartley, D. M.; Kusumoto, Y.; Fezoui, Y.; Condron, M. M.; Lomakin, A.; Benek, G. B.; Selkoe, D. J.; Teplow, D. B. *J. Biol. Chem.* **1999**, *274*, 25945–25952.
9. DeMaco, M. L.; Daggett, V. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 2293.
10. (a) Hou, L.; Zagorski, M. G. *Biophys. J.* **2004**, *86*, 1–2. (b) Gordon, D. J.; Balbach, J. J.; Tycko, R.; Meredith, S. C. *Biophys. J.* **2004**, *86*, 428–434.
11. Maji, S. K.; Drew, M. G. B.; Banerjee, A. *J. Chem. Soc., Chem. Commun.* **2001**, 1946–1947.
12. (a) Banerjee, A.; Pramanik, A.; Bhattacharya, S.; Balaram, P. *Biopolymers* **1996**, *39*, 769–777. (b) Shankaramma, S. C.; Singh, S. H.; Sathyamurthy, A.; Balaram, P. *J. Am. Chem. Soc.* **1999**, *121*, 5360–5363.
13. Baldauf, C.; Gunther, R.; Hoffman, H.-J. *J. Org. Chem.* **2004**, *69*, 6214–6220.
14. Banerjee, A.; Maji, S. K.; Drew, M. G. B.; Haldar, D.; Das, A. K.; Banerjee, A. *Tetrahedron* **2004**, *60*, 5935–5944.
15. Maji, S. K.; Banerjee, R.; Velmurugan, D.; Razak, A.; Fun, H. K.; Banerjee, A. *J. Org. Chem.* **2002**, *67*, 633–639.
16. (a) Toniolo, C.; Palumbo, M. *Biopolymers* **1977**, *16*, 219–224. (b) Mutter, M.; Maser, F.; Altman, K.-H.; Toniolo, C.; Bonora, G. M. *Biopolymers* **1985**, *24*, 1057–1074. (c) Chirgadze, Y. N.; Nevskaya, N. A. *Biopolymers* **1976**, *15*, 627–636. (d) Baron, M. H.; De Loze, C.; Toniolo, C.; Fasman, G. D. *Biopolymers* **1979**, *18*, 411–424. (e) Narita, M.; Ishikawa, K.; Sugasawa, H.; Doi, M. *Bull. Chem. Soc. Jpn.* **1985**, *158*, 1731–1737. (f) Moretto, V.; Crisma, M.; Bonora, G. M.; Toniolo, C.; Balaram, H.; Balaram, P. *Macromolecules* **1989**, *22*, 2939–2944.
17. Karle, I. L.; Balaram, P. *Biochemistry* **1990**, *29*, 4756–6747.
18. (a) Sipe, J. D.; Cohen, A. S. *J. Struct. Biol.* **2000**, *130*, 88–98. (b) Goldsbury, C. S.; Wirtz, S.; Müller, S. A.; Sunderji, S.; Wicki, P.; Aebi, U.; Frey, P. *J. Struct. Biol.* **2000**, *130*, 217–231.
19. (a) Kim, Y. S.; Randolph, T. W.; Manning, M. C.; Stevens, F. J.; Carpenter, J. F. *J. Biol. Chem.* **2003**, *278*, 10842–10850. (b) Taylor, D. L.; Allen, R. D.; Benditt, E. P. *J. Histochem. Cytochem.* **1974**, *22*, 1105–1112. (c) Lim, A.; Makhov, A. M.; Bond, J.; Tnouye, H.; Conmors, L. H.; Griffith, J. D.; Erickson, W. B.; Kirschner, D. A.; Costello, C. E. *J. Struct. Biol.* **2000**, *130*, 363–370. (d) Azriel, R.; Gazit, E. *J. Biol. Chem.* **2001**, *226*, 34156–34161.
20. (a) Benzinger, T. L. S.; Gregory, D. M.; Burkoth, T. S.; MillerAuer, H.; Lynn, D. G.; Botto, R. E.; Meredith, S. C. *Proc. Natl. Acad. Sci U.S.A.* **1998**, *95*, 13407–13412. (b) Kajava, A. V.; Baxa, U.; Wickner, R. B.; Steven, A. C. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 7885–7890. (c) Kuwata, K.; Matumoto, T.; Cheng, H.; Nagayama, K.; James, T. L.; Roder, H. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 14790–14795. (d) Margittai, M.; Langen, R. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 10278–10283.
21. Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*; Springer: New York, 1984; pp 1–282.
22. Kabsch, W. *J. Appl. Cryst.* **1988**, *21*, 916.
23. Sheldrick, G. M. *Acta Crystallogr. Sect. A* **1990**, *46*, 467.
24. Sheldrick, G. M. *Program for Crystal Structure Refinement*; University of Göttingen: Germany, 1993.

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Facile oxidative conversion of alcohols to esters using molecular iodine

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Abstract—A simple, efficient, and high-yield procedure for the oxidative conversion of alcohols to various types of esters and ketones, with molecular iodine and potassium carbonate was successfully carried out.

1. Introduction

1.1. Oxidation of alcohols

Oxidation of alcohols with less toxic reagents is very important in organic synthesis. Therefore, it has been intensively studied and numerous reagents and methods have been developed.[1]

Oxidation with molecular halogens and related reagents is attractive due to the simple operation and low cost. To date, halogen-mediated oxidation of alcohols to aldehydes, esters, and ketones with aqueous NaOCl,[2] $Ca(OCl)_2$/AcOH,[3a,b] *t*-BuOCl/Py,[3c] Cl_2-dimethyl sulfide,[4a,b,e] Cl_2-dimethyl sulfoxide,[4c] Cl_2-pyridine,[4d] Cl_2-HMPT or Br_2-HMPT,[4f,g] Br_2/KBr,[5a] Br_2 and HOBr,[5b] $NaBrO_2$,[5c] $NaBrO_3$,[5d] $PhCH_2N^+Me_3Br_3^-$,[5e] NaBr/CH_3CO_3H,[5f] PhIO/KBr,[5g] and $PyH^+Br_3^-$[5h] has been well studied. However, these methods still have several drawbacks such as strong oxidative conditions, or acidic or basic conditions.

Direct oxidative condensation of primary alcohols to the corresponding esters, where both the acid and the alcohol portion of the esters are derived from the alcohols, is interesting and useful.[6] Therefore, extensive study has been carried out as follows: oxidative condensation with metal catalysts such as ruthenium or palladium; $Ru_3(CO)_{12}$,[7a] $RuH_2(Ph_3P)_4$,[7b,c] $PdCl_2$,[7d,e] $Pd(OAc)_2$,[7f] $PhCH_2N^+Me_3Br_4MoO^-$,[7g] under heating conditions, with chromic acids such as $Na_2Cr_2O_7/H_2SO_4$,[8a] PCC/Al_2O_3,[8b] and with oxoammonium salt.[9] However, these reactions have drawbacks from a practical point of view, that is, the reaction requires high temperature, yield of the esters is not high, or the alcohols used are limited.

1.2. Oxidation of aldehydes

It is often required to transform aldehydes directly into esters during various stages in organic synthesis and natural product synthesis.[10] The oxidation of aldehydes to carboxylic acids or esters is one of the most frequently encountered reactions in organic chemistry. Such a process has been accomplished in a variety of ways. Two-step methods include the oxidation of hemiacetals,[11] acetals,[12] cyanohydrins,[13] etc. Conversion of aldehydes to the methyl esters or acids by NaOCl,[14a] *t*-BuOCl,[14b] and $Ca(OCl)_2$[14c] is effective; however, activated aromatic aldehydes gave lower yield of the products, because of the ring chlorination. Generally, one-pot or one-step methods reported require the use of heavy-metal oxidants such as $KMnO_4$,[15] PDC,[16] or the very expensive silver,[17] rhodium,[18] ruthenium,[19] or vanadium[20] catalysts. Oxidation using $NaClO_2/H_2O_2$[21] is the most common method. NIS-mediated oxidation,[22] electrochemical oxidation,[23] as well as very recently using cat. V_2O_5/H_2O_2,[24a] cat. $QHSO_4/H_2O_2$,[24b] cat. $S{\cdot}SnO_2/H_2O_2$,[24c] and the conversion of aldehydes into glycol monoesters with $Al_2O_3/MeSO_3H$,[25] also have been reported.

Most of the reported methods are useful for oxidation of aldehydes into the corresponding esters. However, conversion of aldehydes with a stoichiometrical amount of

Keywords: Molecular iodine; Oxidation; Alcohol; Aldehyde; 2,2,2-Trifluoroethyl ester; Methyl ester; Condensed ester; Ketone.
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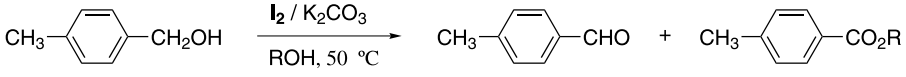
Table 1. Oxidation of *p*-methylbenzyl alcohol with I_2/K_2CO_3 in protic polar solvents



1f **2f** **3f (R = CH_2CF_3)** **4f (R = CH_3)**

Entry	I_2/K_2CO_3 (equiv)	ROH (pK_a)	Time (h)	Yields (%)		
				If	**2f**	**Xf**
1	5/5	$(CH_3)_3COH$ (18.0)	19	0	87	7 (R=H)
2	5/5	CH_3CH_2OH (15.9)	14	90	10	0
3	5/5	CH_3OH (16.0)	24	30	4	66 (**4f**)
4	5/5	H_2O (15.7)	21	0	47	53 (R=H)
5	3/3	CF_3CH_2OH (12.4)	18	0	16	83 (**3f**)
6	5/0	CF_3CH_2OH	18	100	0	0
7	5/5	CF_3CH_2OH	14	0	6	85 (**3f**)
8	5/5	CF_3CH_2OH	18[a]	0	5	91 (**3f**)
9	5/5	$(CF_3)_2CHOH$ (9.2)	14	76	24	0

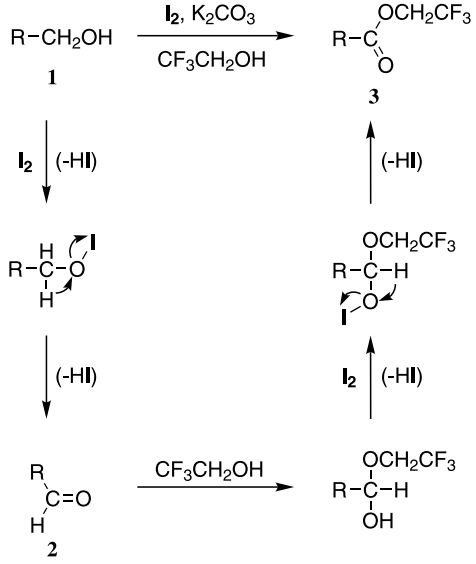
[a] Half amount each of I_2 and K_2CO_3 was added at first, and the rest was added later.

alcohols into esters with molecular iodine has been never studied.

1.3. Oxidation with molecular iodine

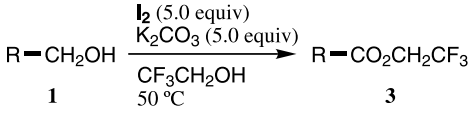
Molecular iodine is a mild, cheap and easily available oxidizing reagent, and, moreover, it is useful because of its solid form and it is less toxic than molecular bromine or chlorine. Previously, simple and effective conversion of aldehydes to the corresponding methyl esters,[26a,b] carboxylic acids,[26c] and nitriles[26d] in the presence of molecular iodine in methanol, in water/acetonitrile, and in ammonia water, respectively, was reported. The Lieben iodoform reaction, which is the reaction of methyl ketones with molecular iodine in aqueous basic solution, is known for the detection of the methyl ketone group.[27]

Here, as a part of our basic study of molecular iodine for organic synthesis,[28] we would like to report a useful oxidation of alcohols to the corresponding esters and ketones by a simple procedure using molecular iodine and potassium carbonate in protic polar solvents (i.e., 2,2,2-trifluoroethanol, methanol, and *t*-butyl alcohol). To the best of our knowledge, reports on the direct oxidative esterification of primary alcohols with molecular iodine in such alcoholic solvents have not yet appeared,[29] while the

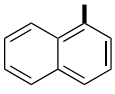


Scheme 1. Plausible reaction pathway for 2,2,2-trifluoroethyl ester.

Table 2. Oxidation of primary alcohols to 2,2,2-trifluoroethyl esters with I_2/K_2CO_3 in CF_3CH_2OH

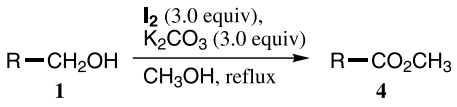


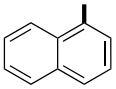
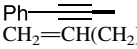
1 **3**

Entry	R–	Time (h)	Yield (%)
1	3-$O_2NC_6H_4$–	1	80 (**3a**)
2	3,4-$Cl_2C_6H_3$–	2[a]	91 (**3b**)
3	4-$CF_3C_6H_4$–	4	87 (**3c**)
4	4-ClC_6H_4–	5[a]	93 (**3d**)
5		5[b]	78
6	Ph–	5[a]	91 (**3e**)
7	4-$CH_3C_6H_4$–	18[a]	91 (**3f**)
8		18[b]	83
9	4-$CH_3OC_6H_4$–	24[a]	85 (**3g**)
10		24[b]	63
11	2,5-$(CH_3)_2C_6H_3$–	24[a]	85 (**3h**)
12	3,4,5-$(CH_3O)_3C_6H_2$–	16	93 (**3i**)
13		14	95 (**3j**)
14		1	78 (**3k**)
15		24[a]	67 (**3l**)
16	$CH_3(CH_2)_{11}$–	84	60 (**3III**)
17		15[b]	91 (**3VI**)
18		5[b]	100 (**3X**)

[a] Half amount each of I_2 and K_2CO_3 was added at first, and the rest was added later.
[b] Molar ratio of I_2/K_2CO_3 is 3.0/3.0.

Table 3. Oxidation of primary alcohols to methyl esters with I_2/K_2CO_3 in CH_3OH

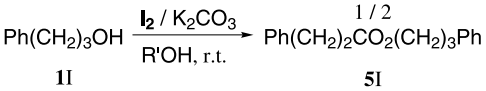


Entry	R–	Time (h)	Yield[a] (%)
1	3-$O_2NC_6H_4$–	20	86
2	4-ClC_6H_4–	21	93
3	Ph–	14	86
4	4-$CH_3C_6H_4$–	15	87
5	4-$CH_3OC_6H_4$–	28	74
6	3,4,5-$(CH_3O)_3C_6H_2$–	19	77
7		30	82
8		24	82
9		39	91
10	$PhCH_2CH_2$–	40[b]	76
11	$CH_3(CH_2)_6$–	5	73
12	$CH_3(CH_2)_{11}$–	7	70
13	4-$CH_3OC_6H_4(CH_2)_2$–	80[b]	82
14		23	70
15		8	90
16		27	76
17	CH_2=$CH(CH_2)_8$–	20[c]	75

[a] Method A. Alcohol (1 mmol) in MeOH (0.5 ml).
[b] Method B. Alcohol (2 mmol) in MeOH (0.6 ml).
[c] After the reaction, zinc powder was added to regenerate the olefinic group.

electrochemical conversion of alcohols to the corresponding ketones or esters using potassium iodide via the formation of iodonium ion species generated by electroxidation,[30] and oxidative conversion of secondary alcohols into α-iodoketones with NaI, H_2O_2, and acid,[31a] and oxidation of alcohols using IPy_2BF_4/I_2[31b] were reported.

Table 4. Oxidative condensation of 3-phenylpropanol with I_2/K_2CO_3 in protic polar solvents



Entry	I_2/K_2CO_3 (equiv)	R′OH	Time (h)	Yields (%)
1	1.2/5	CH_3OH	21	0 (26,[a] 70[b])
2	1.2/5	CH_3CH_2OH	21	0 (8,[c] 89[b])
3	1.2/5	$(CH_3)_2CHOH$	21	8 (92[b])
4	1.2/5	CF_3CH_2OH	21	34 (50[b])
5	1.2/5	$(CH_3)_3COH$	21	96
6	1.2/1.2	$(CH_3)_3COH$	66	42 (58[b])
7	1.2/2.4	$(CH_3)_3COH$	66	84 (16[b])
8	1.2/3.6	$(CH_3)_3COH$	66	93 (7[b])
9	3.0/3.0	$(CH_3)_3COH$	45	92 (7[b])

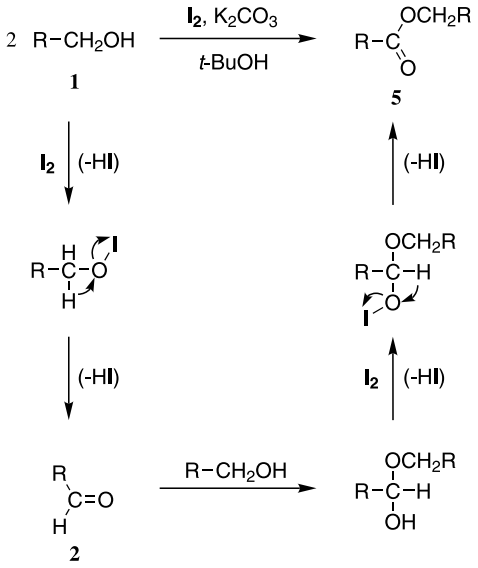
[a] Yield of methyl 3-phenylpropanoate.
[b] Yield of recovered 3-phenylpropanol.
[c] Yield of ethyl 3-phenylpropanoate.

2. Results and discussion

2.1. Preparation of 2,2,2-trifluoroethyl esters

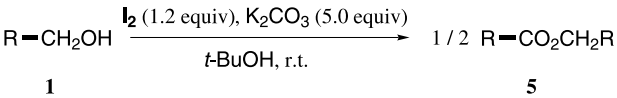
At first, direct oxidative conversion of alcohols to the esters with molecular iodine in the presence of potassium carbonate was carried out. Table 1 shows the effect of a protic polar solvent (5 ml for 1 mmol of alcohol), that is, *t*-butyl alcohol, ethanol, methanol, water, and 2,2,2-trifluoroethanol in the oxidative conversion of *p*-methylbenzyl alcohol to the corresponding esters or acid (with water). It indicates 2,2,2-trifluoroethanol is the best solvent among them, to provide the corresponding 2,2,2-trifluoroethyl ester in high yield (entry 8). Thus, less acidic solvents such as water, methanol, ethanol, *t*-butyl alcohol gave the ester or acid products in low yield under the present conditions. Since it is known that the pK_a value of 2,2,2-trifluoroethanol is ca. 12.4,[32] the result suggests that the acidic polar solvent promotes the present reaction. Probably, 2,2,2-trifluoroethanol activates molecular iodine as an oxidant through a hydrogen bond between its OH proton and molecular iodine. Recently, it has been known that 2,2,2-trifluoroethanol sometimes promotes the reactions; therefore, synthetic use of 2,2,2-trifluoroethanol as a solvent is interesting.[33] For example, 2,2,2-trifluoroethanol activates hydrogen peroxide for the oxidation of thiols to disulfides,[33a] epoxidation of olefins,[33b–d] Mn(III)/Cu(II)-mediated oxidative radical cyclization of α-(methylthio) acetamides,[33e] Et_3N-induced β-cleavage reaction,[33f] etc. The addition of a small amount of methanesulfonic acid to the methanol or ethanol solvent was not effective. The present reaction requires potassium carbonate as a base (entry 6). Though 1,1,1,3,3,3-hexafluoro-2-propanol (pK_a 9.2) is more acidic than 2,2,2-trifluoroethanol, the yield was much reduced due to the poor solubility of molecular iodine (entry 9).

In the present oxidative conversion of alcohols in 2,2,2-trifluoroethanol with molecular iodine and potassium carbonate, it was found that the solvent, 2,2,2-trifluoroethanol, was not oxidized at all by the NMR measurement of the reaction mixture. Here, exactly, aldehyde is formed at

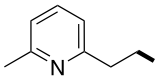


Scheme 2. Plausible reaction pathway for condensed ester.

Table 5. Oxidative condensation of primary alcohols with I_2/K_2CO_3 in *t*-BuOH



1 **5**

Entry	R–	Time (h)	Yields (%)	Entry	R–	Time (h)	Yields (%)
1	3-$O_2NC_6H_4$–	16	95 (**5a**)	10	4-$CH_3OC_6H_4(CH_2)_2$–	27	98 (**5IV**)
2	3,4-$Cl_2C_6H_3$–	48	99 (**5b**)	11	$PhOCH_2$–	42[a]	78 (**5V**)
3	4-$CF_3C_6H_4$–	48	99 (**5c**)	12		27	93 (**5VI**)
4		19	99 (**5k**)	13		19	88 (5VII)
5	4-$CHDOC_6H_4$–	31	3 (97[b])	14		33	76 (**5VIII**)
6	$Ph(CH_2)_2$–	21	96 (**5I**)	15		50	72 (**5IX**)
7		48[c]	97	16	$Br(CH_2)_5$–	42	94[d]
8	$CH_3(CH_2)_6$–	33	97 (**5II**)	17		26	32 (41[b])
9	$CH_3(CH_2)_{11}$–	41	98 (**5III**)				

[a] I_2 (2.4 equiv) was used.
[b] Yield of aldehyde.
[c] Reaction was carried out with 3 g (22 mmol) of alcohol.
[d] Bromine atom was partly substituted by iodine atom (ca. 12%).

first, and, therefore, it can be obtained in high yield when the reaction is carried out at room temperature. For example, the treatment of *p*-methoxybenzyl alcohol, 2,4,6-trimethylbenzyl alcohol, and α-thienylmethanol with molecular iodine (2.5 equiv) and potassium carbonate (2.5 equiv) in 2,2,2-trifluoroethanol at room temperature for 19, 83, and 70 h provided the corresponding aldehydes in 99, 85, and 87% yields together with trace amounts of the corresponding 2,2,2-trifluoroethyl esters, respectively. Though TEMPO-mediated oxidation of alcohols to aldehydes with molecular iodine was reported recently,[34] aldehydes can be formed by the reaction of alcohols with molecular iodine alone in 2,2,2-trifluoroethanol. Once aldehydes are formed, then oxidative conversion to 2,2,2-trifluoroethyl esters proceeds smoothly via the hemiacetals, as shown in Scheme 1.

Based on these results, a variety of primary alcohols were directly oxidized to the corresponding 2,2,2-trifluoroethyl esters in good yields, as shown in Table 2. Benzylic alcohols (entries 1–15), aliphatic primary alcohols (entries 16 and 17), and neopentyl-typed 1-adamantanemethanol (entry 18) could be oxidized to the corresponding 2,2,2-trifluoroethyl esters in good to moderate yields. In benzylic alcohols, formation of the esters with high yields requires 5 equiv each of molecular iodine and potassium carbonate, while in aliphatic primary alcohols, 3 equiv each was required. The present reaction is clean and the esters are obtained in quite good yields from the simple primary alcohols.

2.2. Preparation of methyl esters

When methanol was used as a solvent, instead of 2,2,2-trifluoroethanol, under the same conditions with molecular iodine and potassium carbonate, the formation of methyl esters was observed. However, the yields were not high. When a mixture of alcohol with I_2 and K_2CO_3 in a small amount of methanol was refluxed, the corresponding methyl esters were obtained in high yields, as shown in Table 3. In the present oxidative conversion of alcohols to methyl esters with methanol solvent, methanol is partly oxidized competitively. Therefore, the amount of solvent was quite reduced, and conditions were concentrated (0.3–0.5 ml for 1 mmol of alcohol) to provide the corresponding esters in the best yield.

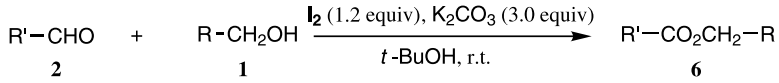
2.3. Preparation of oxidative condensed esters

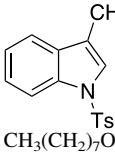
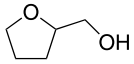
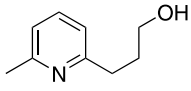
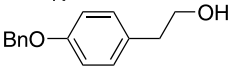
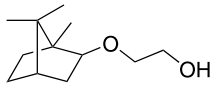
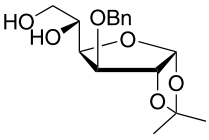
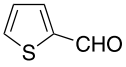
3-Phenylpropanol was treated with molecular iodine and potassium carbonate in various alcoholic solvents (0.5 ml for 1 mmol alcohol), such as methanol, ethanol, isopropyl alcohol, 2,2,2-trifluoroethanol, and *t*-butyl alcohol, at room temperature, and the results are shown in Table 4. Thus, the results indicate that *t*-butyl alcohol is the best solvent, with 1.2 equiv of molecular iodine and 5.0 equiv of potassium carbonate, to provide 3-phenylpropyl 3-phenylpropanoate in high yields (entries 5–8).

The reaction probably proceeds as follows: oxidation of alcohol to aldehyde by molecular iodine, formation of hemiacetal by the reaction of the formed aldehyde and alcohol, and then, oxidation of the hemiacetal to an ester by molecular iodine, as shown in Scheme 2.

Based on these results, various benzylic alcohols (entries 1–4) and primary alcohols (entries 6–16) could be directly converted to the corresponding oxidatively condensed esters in high yields in *t*-butyl alcohol at room temperature, except for *p*-methoxybenzyl alcohol which has an electron-donating group (entry 5), and 1-adamantanemethanol which is a neopentyl-type alcohol (entry 17), as shown in Table 5. When the reaction was carried out with 3 g

Table 6. Stoichiometrically oxidative condensation of aldehydes and alcohols with I_2/K_2CO_3 in *t*-BuOH



Entry	R′-CHO (1.0 equiv)	R-CH_2OH (1.05 equiv)	Time (h)	Yields (%)
1	$PhCH_2CH_2CHO$	4-$ClC_6H_4CH_2OH$	22	90 (**6d**)
2	$PhCH_2CH_2CHO$	4-$CH_3C_6H_4CH_2OH$	22	87 (**6f**)
3	$PhCH_2CH_2CHO$		22[a]	61 (**6m**)
4	$PhCH_2CH_2CHO$	$CH_3(CH_2)_7OH$	22	91 (**6II**)
5	$PhCH_2CH_2CHO$	$CH_3(CH_2)_{12}OH$	24	84 (**6III**)
6	$PhCH_2CH_2CHO$	$PhO(CH_2)_2OH$	22	89 (**6V**)
7	$PhCH_2CH_2CHO$		22	91 (**6VIII**)
8	$PhCH_2CH_2CHO$		22	76 (**6IX**)
9	$PhCH_2CH_2CHO$		22	**80** (**6XI**)
10	$PhCH_2CH_2CHO$		25	84 (**6XII**)
11	$PhCH_2CH_2CHO$		23	63[b] (**6XIII**)
12	$CH_3(CH_2)_3CHO$	$CH_3(CH_2)_7OH$	22	87 (**6II′**)
13	$PhCH(CH_3)CHO$	$CH_3(CH_2)_7OH$	22	62
14	PhCHO	$CH_3(CH_2)_7OH$	22	47 (46[c])
15		$CH_3(CH_2)_7OH$	22	20 (45[c])

[a] Aldehyde/alcohol = 1.5/1.0 (equiv), and reaction temperature was 45 °C.
[b] Yield of mono ester.
[c] Yield of octyl octanoate (**5II**).

(22 mmol) of 3-phenylpropanol, instead of 1 mmol scale, 3-phenylpropyl 3-phenylpropanoate was obtained again in high yield (entry 7). Thus, this reaction can be carried out easily for large-scale preparation of oxidatively condensed esters from primary alcohols.

2.4. Stoichiometrically oxidative condensation of aldehydes and alcohols to esters

At first, esterification of 3-phenylpropanal with various alcohols was carried out using iodine and potassium carbonate in *t*-butyl alcohol at room temperature (entries 1–11). Next, some of the aldehydes with 1-octanol were examined (entries 12–15). The results are summarized in Table 6, and indicate that the oxidative esterification of aldehydes bearing a primary alkyl group, with primary alcohols proceeded smoothly to provide the corresponding esters in high yields. The reactions of aldehydes bearing a primary alkyl group, with secondary alcohols, or aldehydes bearing a secondary alkyl group, with alcohols, gave the corresponding esters in moderate to low yields. Thus the present oxidative condensation of aldehydes with alcohols is rather sensitive to steric hindrance and electronic effect, since the formation of hemiacetal is the key step.

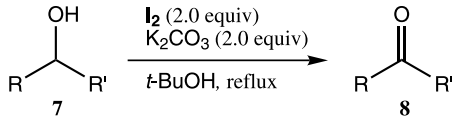
2.5. Preparation of ketones

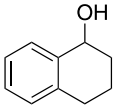
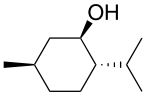
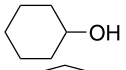
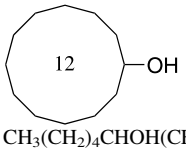
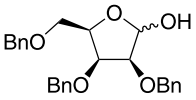
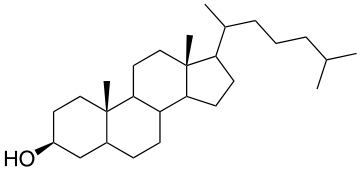
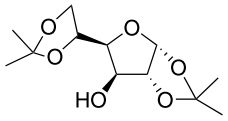
Benzylic (entries 1–5) and aliphatic (entries 6–12) alcohols could be also oxidized in *t*-butyl alcohol with molecular iodine and potassium carbonate under the refluxing conditions smoothly, to the corresponding ketones in high yields, as shown in Table 7. 2,3,5-Tri-*O*-benzyl-D-ribofuranose and cholestanol were oxidized to 2,3,5-tri-*O*-benzyl-D-ribofuranone and cholestanone in good yields, respectively (entries 11 and 12). Generally, purine-like *C*-nucleosides such as formycin A and formycine B were synthesized from 2,3,5-tri-*O*-benzyl-D-ribofuranone.[35] However, diacetone-D-glucose was not oxidized at all, even for long reaction time. Probably, molecular iodine cannot approach the hydroxyl group, due to the steric hindrance (entry 13).

3. Conclusion

The present method is a simple, efficient, and high-yield procedure for the oxidative conversion of primary alcohols to the corresponding esters, condensed esters, and secondary alcohols to the corresponding ketones, with molecular

Table 7. Oxidation of secondary alcohols to ketones with I_2/K_2CO_3 in *t*-BuOH



Entry	Secondary alcohol	Time (h)	Yield (%)
1	$PhCHOHC_2H_5$	8	99
2	$4\text{-}ClC_6H_4CHOHCH_3$	6	86
3	$4\text{-}CH_3C_6H_4CHOHCH_3$	5	99
4	$PhCHOH(CH_2)_7CH_3$	6	99
5		7	99
6		16	99
7		4	78
8		6	99
9	$CH_3(CH_2)_4CHOH(CH_2)_4CH_3$	5	99
10	$Ph(CH_2)_3CHOHCH_3$	6	99
11		8	79
12		40	90
13		20	0 (88[a])

[a] Yield of starting alcohol.

iodine and potassium carbonate in 2,2,2-trifluoroethanol, methanol, and *t*-butyl alcohol. The advantages of the present reactions are the operational simplicity, elimination of the use of toxic oxidants or solvents, generality of the reactions, and high yield of the products.

4. Experimental

4.1. General

^1H NMR and ^{13}C NMR spectra were obtained with JEOL-JNM-LA-400, JEOL-JNM-LA-400s, and JEOL-JNM-LA-500 spectrometers. Chemical shifts are expressed in ppm downfield from tetramethylsilane (TMS) in δ units. IR spectra were measured with JASCO FT/IR-200 and FT/IR-4100 spectrometers. Mass spectra were recorded on JEOL-HX-110 and JEOL-JMS-ATII15 spectrometers. Melting points were determined on Yamato melting points apparatus Model MP-21. Silica Gel 60 (Kanto Kagaku Co.) was used for column chromatography and Wakogel B-5F was used for preparative TLC.

Most of the organic chemical substrates are commercially available. All the compounds gave satisfactory spectroscopic data, most methyl esters and ketones were identified with commercially available authentic materials.[36]

4.2. Typical procedure for oxidative conversion of primary alcohols to 2,2,2-trifluoroethyl esters

To a solution of benzyl alcohol (1 mmol) in CF_3CH_2OH (5 ml) were added I_2 (2.5 mmol) and K_2CO_3 (2.5 mmol) under an argon atmosphere. The mixture obtained was stirred for 2 h at 50 °C, then I_2 (2.5 mmol) and K_2CO_3 (2.5 mmol) were added to the mixture again. After 3 h at the same temperature, the mixture was quenched with satd aq Na_2SO_3 (3–5 ml) at 0 °C, and was extracted with Et_2O three times. The organic layer was washed with brine and dried over Na_2SO_4 to provide 2,2,2-trifluoroethyl benzoate in 91% yield in an almost pure state. If necessary, the product was purified by flash column chromatography on silica gel (hexane–EtOAc=4:1) to give pure 2,2,2-trifluoroethyl benzoate as an oil.

4.2.1. 2,2,2-Trifluoroethyl 3-nitrobenzoate (3a). Colorless solid; mp 46–47 °C; IR (KBr): 1750, 1300, 1250, 1170, 1130 cm^{-1}; ^1H NMR ($CDCl_3$) δ=4.78 (2H, q, J=8.4 Hz), 7.73 (1H, t, J=8.1 Hz), 8.42 (1H, dt, J=8.1, 1.2 Hz), 8.49 (2H, ddd, J=8.1, 2.2, 1.2 Hz), 8.91 (1H, t, J=2.2 Hz); HRMS (FAB): obsd M+H=250.0320, calcd for $C_9H_7F_3NO_4$ M+H=250.0327.

4.2.2. 2,2,2-Trifluoroethyl 3,4-dichlorobenzoate (3b). Colorless solid; mp 32–33 °C; IR (KBr): 1740, 1300, 1265, 1235, 1170, 1110 cm^{-1}; ^1H NMR ($CDCl_3$) δ=4.71 (2H, q, J=8.4 Hz), 7.57 (1H, d, J=8.4 Hz), 7.90 (1H, dd, J=8.4, 2.0 Hz), 8.15 (1H, d, J=2.0 Hz); HRMS (EI): obsd M+=271.9624, calcd for $C_9H_5Cl_2F_3O_2$ M+=271.9619.

4.2.3. 2,2,2-Trifluoroethyl 4-trifluoromethylbenzoate (3c). Oil; IR (neat): 1745, 1165, 1130, 1100, 1065 cm^{-1}; ^1H NMR ($CDCl_3$) δ=4.74 (2H, q, J=8.3 Hz), 7.75 (2H, d, J=8.1 Hz), 8.20 (2H, d, J=8.1 Hz); HRMS (EI): obsd M+=272.0293, calcd for $C_{10}H_6F_6O_2$ M+=272.0298.

4.2.4. 2,2,2-Trifluoroethyl 4-chlorobenzoate (3d). Oil; IR (neat): 1740, 1295, 1255, 1170, 1105 cm^{-1}; ^1H NMR ($CDCl_3$) δ=4.70 (2H, q, J=8.4 Hz), 7.46 (2H, d, J=8.8 Hz), 8.02 (2H, d, J=8.8 Hz); HRMS (EI): obsd M+=238.0005, calcd for $C_9H_6ClF_3O_2$ M+=238.0008.

4.2.5. 2,2,2-Trifluoroethyl benzoate (3e). Oil; bp 84–86 °C/19 mm Hg (lit.[37] bp 77 °C/13 mm Hg); IR (neat): 1740, 1295, 1255, 1170, 1115 cm^{-1}; ^1H NMR ($CDCl_3$) δ=4.70 (2H, q, J=8.4 Hz), 7.48 (2H, t, J=7.7 Hz), 7.62 (1H, tt, J=7.7, 1.4 Hz), 8.09 (2H, dt, J=7.7, 1.4 Hz); HRMS (EI): obsd M+=204.0413, calcd for $C_9H_7F_3O_2$ M+=204.0398.

4.2.6. 2,2,2-Trifluoroethyl *p*-toluate (3f). Oil; IR (neat): 1740, 1295, 1260, 1170, 1110 cm^{-1}; ^1H NMR ($CDCl_3$) δ=2.43 (3H, s), 4.68 (2H, q, J=8.4 Hz), 7.27 (2H, d,

J=8.1 Hz), 7.97 (2H, d, J=8.1 Hz); HRMS (EI): obsd M+=218.0561, calcd for $C_{10}H_9F_3O_2$ M+=218.0555.

4.2.7. 2,2,2-Trifluoroethyl 4-methoxybenzoate (3g). Oil; IR (neat): 1735, 1255, 1165 cm^{-1}; ^1H NMR ($CDCl_3$) δ=3.88 (3H, s), 4.67 (2H, q, J=8.4 Hz), 6.95 (2H, d, J=9.0 Hz), 8.03 (2H, d, J=9.0 Hz); HRMS (EI): obsd M+=234.0494, calcd for $C_{10}H_9F_3O_3$ M+=234.0504.

4.2.8. 2,2,2-Trifluoroethyl 2,5-dimethylbenzoate (3h). Oil; ^1H NMR ($CDCl_3$) δ=2.36 (3H, s), 2.56 (3H, s), 4.67 (2H, q, J=8.4 Hz), 7.16 (1H, d, J=8.0 Hz), 7.26 (1H, d, J=8.0 Hz), 7.77 (1H, s); HRMS (EI): obsd M+=232.0708, calcd for $C_{11}H_{11}F_3O_2$ M+=232.0711.

4.2.9. 2,2,2-Trifluoroethyl 3,4,5-trimethoxybenzoate (3i). Colorless solid; mp 67–68 °C; IR (KBr): 1735, 1160, 1130 cm^{-1}; ^1H NMR ($CDCl_3$) δ=3.92 (6H, s), 3.93 (3H, s), 4.70 (2H, q, J=8.4 Hz), 7.32 (2H, s); HRMS (FAB): obsd M+=294.0698, calcd for $C_{12}H_{13}F_3O_5$ M+=294.0715.

4.2.10. 2,2,2-Trifluoroethyl 1-naphthoate (3j). Colorless solid; mp 28–29 °C; IR (KBr): 1720, 1160, 1125 cm^{-1}; ^1H NMR ($CDCl_3$) δ=4.79 (2H, q, J=8.4 Hz), 7.55 (2H, m), 7.66 (1H, td, J=7.8, 1.4 Hz), 7.91 (1H, d, J=7.8 Hz), 8.09 (1H, dd, J=7.8, 1.4 Hz), 8.29 (1H, d, J=7.8 Hz), 8.92 (1H, d, J=7.8 Hz); HRMS (FAB): obsd M+=254.0549, calcd for $C_{13}H_9F_3O_2$ M+=254.0555.

4.2.11. 2,2,2-Trifluoroethyl nicotinate (3k). Oil; IR (neat): 1745, 1300, 1260, 1180, 1120 cm^{-1}; ^1H NMR ($CDCl_3$) δ= 4.75 (2H, q, J=8.3 Hz), 7.45 (1H, dd, J=8.0, 4.9 Hz), 8.34 (1H, dt, J=8.0, 1.9 Hz), 8.85 (1H, dd, J=4.9, 1.9 Hz), 9.28 (1H, d, J=1.9 Hz); HRMS (EI): obsd M+=205.0348, calcd for $C_8H_6F_3NO_2$ M+=205.0351.

4.2.12. 2,2,2-Trifluoroethyl thiophene-2-carboxylate (3l). Oil; IR (neat): 1725, 1250, 1160, 1095 cm^{-1}; ^1H NMR ($CDCl_3$) δ=4.68 (2H, q, J=8.4 Hz), 7.15 (1H, dd, J=5.0, 3.9 Hz), 7.66 (1H, dd, J=5.0, 1.2 Hz), 7.89 (1H, dd, J=3.9, 1.2 Hz); HRMS (EI): obsd M+=209.9967, calcd for $C_7H_5F_3O_2S$ M+=209.9962.

4.2.13. 2,2,2-Trifluoroethyl tridecanoate (3III). Oil; ^1H NMR ($CDCl_3$) δ=0.88 (3H, t, J=6.9 Hz), 1.22–1.32 (18H, m), 1.61 (2H, q, J=7.5 Hz), 2.41 (2H, t, J=7.5 Hz), 4.46 (2H, q, J=8.4 Hz); HRMS (EI): obsd M+=296.1957, calcd for $C_{15}H_{27}F_3O_2$ M+=296.1963.

4.2.14. 2,2,2-Trifluoroethyl cyclohexanecarboxylate (3VI). Oil; ^1H NMR ($CDCl_3$) δ=1.28 (3H, m), 1.47 (2H, m), 1.65 (1H, m), 1.75 (2H, m), 1.94 (2H, m), 2.42 (1H, tt, J=11.1, 3.6 Hz), 4.47 (2H, q, J=8.5 Hz); HRMS (EI): obsd M+=210.0860, calcd for $C_9H_{13}F_3O_2$ M+=210.0868.

4.2.15. 2,2,2-Trifluoroethyl adamantanecarboxylate (3X). Oil; bp 90–100 °C/1 mm Hg; IR (neat): 1745, 1160, 1080 cm^{-1}; ^1H NMR ($CDCl_3$) δ=1.73 (6H, m), 1.93 (6H, m), 2.04 (3H, m), 4.45 (2H, q, J=8.4 Hz); HRMS (EI): obsd M+=262.1178, calcd for $C_{13}H_{17}F_3O_2$ M+=262.1181.

4.3. Typical procedure for oxidative conversion of primary alcohols to methyl esters

To a solution of 4-chlorobenzyl alcohol (1 mmol) in CH_3OH (0.5 ml) were added I_2 (3 mmol) and K_2CO_3 (3 mmol) under an argon atmosphere. The mixture obtained was stirred at 70 °C. After 21 h at the same temperature, the mixture was quenched with satd aq Na_2SO_3 (3–5 ml) at 0 °C, and was extracted with Et_2O three times. The organic layer was washed with brine and dried over Na_2SO_4 to provide methyl 4-chlorobenzoate in 93% yield in an almost pure state. If necessary, the product was purified by flash column chromatography on silica gel (hexane–EtOAc= 8:1) to give pure methyl 4-chlorobenzoate as a colorless solid. Mp 43 °C (lit.[36] mp 43 °C). ^1H NMR ($CDCl_3$): δ=3.92 (3H, s), 7.42 (2H, d, J=8.8 Hz), 7.98 (2H, d, J=8.8 Hz).

4.4. Typical procedure for oxidative condensation of primary alcohols to esters

To a solution of 3-phenylpropanol (1 mmol) in *t*-butyl alcohol (0.5 ml) were added I_2 (1.2 mmol) and K_2CO_3 (5.0 mmol) under an argon atmosphere. The obtained mixture was stirred at room temperature, until the iodine color almost disappeared. After 21 h at the same temperature, the mixture was quenched by the addition of water (20 ml), Et_2O (5 ml), and satd aq Na_2SO_3 (0.5 ml) at 0 °C. Then the mixture was extracted with Et_2O three times. The organic layer was washed with brine and dried over Na_2SO_4 to provide 3-phenylpropyl 3-phenylpropanoate in 96% yield in an almost pure state. If necessary, the product was purified by flash column chromatography on silica gel (hexane–EtOAc=10:1) to give pure 3-phenylpropyl 3-phenylpropanoate as a colorless oil.

4.4.1. 3-Nitrobenzyl 3-nitrobenzoate (5a). Colorless solid; mp 146–147 °C; IR (KBr): 1720, 1280, 1265, 1140 cm^{-1}; ^1H NMR ($CDCl_3$) δ=5.52 (2H, s), 7.62 (1H, t, J=8.1 Hz), 7.70 (1H, t, J=8.1 Hz), 7.82 (1H, m), 8.24 (1H, m), 8.34 (1H, m), 8.41 (1H, m), 8.45 (1H, m), 8.89 (1H, m); ^{13}C NMR ($CDCl_3$) δ=66.1 (s), 123.2 (t), 123.3 (t), 123.6 (t), 124.7 (t), 127.8 (t), 129.8 (t), 131.3 (q), 134.3 (t), 135.4 (t), 137.3 (q), 148.3 (q), 148.4 (q), 164.1 (q); HRMS (EI): obsd M+=302.0541, calcd for $C_{14}H_{10}N_2O_6$ M+=302.0539.

4.4.2. 3,4-Dichlorobenzyl 3,4-dichlorobenzoate (5b). Colorless solid; mp 90–91 °C; IR (KBr): 1725, 1295, 1270, 1245, 1110 cm^{-1}; ^1H NMR ($CDCl_3$) δ=5.30 (2H, s), 7.27 (1H, dd, J=8.3, 2.0 Hz), 7.47 (1H, d, J=8.3 Hz), 7.53 (2H, m), 7.88 (1H, dd, J=8.3, 2.0 Hz), 8.12 (1H, d, J=2.0 Hz); ^{13}C NMR ($CDCl_3$) δ=65.7 (s), 127.6 (t), 128.7 (t), 129.5 (q), 130.3 (t), 130.6 (t), 130.7 (t), 131.6 (t), 132.8 (q), 132.9 (q), 133.1 (q), 135.6 (q), 138.0 (q), 164.3 (q); HRMS (EI): obsd M+=347.9285, calcd for $C_{14}H_8Cl_4O_2$ M+=347.9278.

4.4.3. 4-(Trifluoromethyl)benzyl 4-(trifluoromethyl)-benzoate (5c). Colorless solid; mp 57–58 °C (lit.[38] mp 61–62 °C); IR (KBr): 1720, 1325, 1270, 1105, 1065 cm^{-1}; ^1H NMR ($CDCl_3$) δ=5.45 (2H, s), 7.57 (2H, d, J=8.2 Hz), 7.66 (2H, d, J=8.0 Hz), 7.72 (2H, d, J=8.2 Hz), 8.19 (2H, d, J=8.0 Hz); ^{13}C NMR ($CDCl_3$) δ=66.2 (s), 125.5 (t),

125.7 (t), 128.3 (t), 130.1 (t), 130.6 (q), 133.0 (q), 134.7 (q), 139.5 (q), 165.0 (q); HRMS (FAB): obsd M+H= 349.0661, calcd for $C_{16}H_{11}O_2F_6$ M+H=349.0663.

4.4.4. 3-Pyridinylmethyl nicotinate (5k). Oil; IR (neat): 1720, 1275, 1110 cm^{-1}; ^1H NMR ($CDCl_3$) δ=5.42 (2H, s), 7.35 (1H, ddd, J=7.9, 4.9, 0.8 Hz), 7.41 (1H, ddd, J=7.9, 4.9, 0.8 Hz), 7.81 (1H, dt, J=7.9, 2.0 Hz), 8.32 (1H, dt, J= 7.9, 2.0 Hz), 8.62 (1H, dd, J=4.9, 1.5 Hz), 8.74 (1H, d, J= 1.5 Hz), 8.80 (1H, dd, J=4.9, 2.0 Hz), 9.25 (1H, dd, J=2.0, 0.8 Hz); ^{13}C NMR ($CDCl_3$) δ=64.5 (s), 123.3 (t), 123.5 (t), 125.6 (q), 131.1 (q), 136.1 (t), 137.1 (t), 149.7 (t), 149.8 (t), 149.9 (t), 150.9 (t), 153.7 (t), 164.9 (q); HRMS (FAB): obsd M+H=215.0814, calcd for $C_{12}H_{11}N_2O_2$ M+H= 215.0821.

4.4.5. 3-Phenylpropyl 3-phenylpropanoate (5I). Oil; bp 210 °C/1 mm Hg (lit.[8b] bp 146–150 °C/0.8 mm Hg); IR (neat): 1730, 1160, 1030 cm^{-1}; ^1H NMR ($CDCl_3$) δ= 1.93 (2H, m), 2.63 (2H, t, J=7.7 Hz), 2.64 (2H, t, J= 7.7 Hz), 2.95 (2H, t, J=7.7 Hz), 4.09 (2H, t, J=6.5 Hz), 7.18 (6H, m) 7.28 (4H, m); ^{13}C NMR ($CDCl_3$) δ=30.2 (s), 31.0 (s), 32.1 (s), 35.9 (s), 63.8 (s), 126.0 (t), 126.3 (t), 128.3 (t), 128.4 (t), 128.5 (t), 140.5 (q), 141.2 (q), 173.0 (q); HRMS (FAB): obsd M+H=269.1552, calcd for $C_{18}H_{21}O_2$ M+H=269.1542.

4.4.6. Octyl octanoate (5II). Oil; IR (neat): 1735, 1460, 1165 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.88 (3H, t, J=7.0 Hz), 0.88 (3H, t, J=7.0 Hz), 1.25–1.40 (18H, m), 1.62 (4H, t, J=7.5 Hz), 2.29 (2H, t, J=7.5 Hz), 4.06 (2H, t, J=6.7 Hz); ^{13}C NMR ($CDCl_3$) δ=14.0 (p), 22.6 (s), 25.0 (s), 25.9 (s), 28.6 (s), 28.9 (s), 29.1 (s), 29.2 (s), 31.6 (s), 31.7 (s), 34.4 (s), 64.3 (s), 173.9 (q).

4.4.7. Tridecyl tridecanoate (5III). Colorless solid; mp 35–36 °C; IR (KBr): 1735, 1370, 1230, 1205, 1185 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.88 (6H, t, J=6.9 Hz), 1.25–1.38 (38H, m), 1.63 (4H, m), 2.29 (2H, t, J=7.6 Hz), 4.05 (2H, t, J=6.7 Hz); ^{13}C NMR ($CDCl_3$) δ=14.1 (p), 22.7 (s), 25.0 (s), 26.0 (s), 28.7 (s), 29.2 (s), 29.3 (s), 29.5 (s), 29.6 (s), 29.7 (s), 31.9 (s), 34.4 (s), 64.4 (s), 174.0 (q); HRMS (FAB): obsd M+H=397.4037, calcd for $C_{26}H_{53}O_2$ M+H= 397.4046.

4.4.8. 3-(4′-Methoxyphenyl)propyl 3-(4′-methoxyphenyl) propanoate (5IV). Colorless solid; mp 38–39 °C; IR (KBr): 1730, 1510, 1245, 1030 cm^{-1}; ^1H NMR ($CDCl_3$) δ=1.89 (2H, tt, J=7.9, 6.5 Hz), 2.58 (2H, t, J=7.9 Hz), 2.60 (2H, t, J=7.9 Hz), 2.89 (2H, t, J=7.9 Hz), 3.77 (3H, s), 3.78 (3H, s), 4.07 (2H, t, J=6.5 Hz), 6.83 (4H, dd, J=7.8, 2.1 Hz), 7.06 (2H, d, J=8.7 Hz), 7.13 (2H, d, J=8.7 Hz); ^{13}C NMR ($CDCl_3$) δ=30.1 (s), 30.3 (s), 31.1 (s), 36.1 (s), 55.2 (p), 63.7 (s), 113.7 (t), 113.8 (t), 129.2 (t), 132.5 (q), 133.2 (q), 157.8 (q), 158.0 (q), 173.0 (q); HRMS (FAB): obsd M+ H=328.1671, calcd for $C_{20}H_{24}O_4$ M+H=328.1675.

4.4.9. 2-Phenoxyethyl 2-phenoxyacetate (5V). Colorless solid; mp 82–83 °C (lit.[39] mp 83–84 °C); IR (KBr): 1740, 1365, 1215 cm^{-1}; ^1H NMR ($CDCl_3$) δ=4.19 (2H, t, J= 4.7 Hz), 4.57 (2H, t, J=4.7 Hz), 4.67 (2H, s), 6.90 (4H, m), 6.98 (2H, m), 7.28 (4H, m); ^{13}C NMR ($CDCl_3$) δ=63.5 (s), 65.2 (s), 65.6 (s), 114.6 (t), 114.7 (t), 121.3 (t), 121.8 (t), 129.5 (t), 157.7 (q), 158.3 (q), 168.9 (q); HRMS (FAB): obsd M+ =272.1059, calcd for $C_{16}H_{16}O_4$ M+ =272.1049.

4.4.10. Cyclohexylmethyl cyclohexanecarboxylate (5VI). Oil; IR (neat): 1730, 1170, 1130 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.97 (2H, m), 1.12–1.32 (6H, m), 1.44 (2H, m), 1.57–1.78 (9H, m), 1.90 (2H, m), 2.29 (1H, tt, J=11.3, 3.6 Hz), 3.87 (2H, d, J=6.4 Hz); ^{13}C NMR ($CDCl_3$) δ=25.5 (s), 25.7 (s), 25.8 (s), 26.4 (s), 29.1 (s), 29.7 (s), 37.2 (t), 43.3 (t), 69.2 (s), 176.2 (q); HRMS (FAB): obsd M+H=225.1858, calcd for $C_{14}H_{25}O_2$ M+H=225.1855.

4.4.11. Cyclopentylmethyl cyclopentanecarboxylate (5VII). Oil; IR (neat): 1730, 1180, 1155 cm^{-1}; ^1H NMR ($CDCl_3$) δ=1.25 (2H, m), 1.50–1.64 (6H, m), 1.65–1.84 (6H, m), 1.88 (2H, m), 2.20 (1H, septet, J=7.5 Hz), 2.73 (1H, quint, J=7.9 Hz), 3.96 (2H, d, J=7.5 Hz); ^{13}C NMR ($CDCl_3$) δ=25.3 (s), 25.8 (s), 29.3 (s), 30.0 (s), 38.6 (t), 44.0 (t), 68.2 (s), 176.9 (q); HRMS (FAB): obsd M+H= 197.1531, calcd for $C_{12}H_{21}O_2$ M+H=197.1542.

4.4.12. (Tetrahydrofuran-2-yl)methyltetrahydrofuran-2-carboxylate (5VIII). Oil; IR (neat): 1745, 1200, 1175, 1080 cm^{-1}; ^1H NMR ($CDCl_3$) δ=1.62 (1H, m), 1.80–2.10 (6H, m), 2.26 (1H, m), 3.80 (1H, m), 3.90 (2H, m), 4.00–4.18 (3H, m), 4.22 (1H, m), 4.51 (1H, m); ^{13}C NMR ($CDCl_3$) δ=25.2 (s), 25.6 (s), 25.6 (s), 27.9 (s), 30.2 (s), 66.6 (s), 68.4 (s), 69.3 (s), 76.3 (t), 76.4 (t), 76.6 (t), 173.4 (q); HRMS (FAB): obsd M+H=201.1145, calcd for $C_{10}H_{17}O_4$ M+H=201.1127.

4.4.13. 3-(6′-Methylpyridin-2′-yl)propyl 3-(6′-methylpyridin-2′-yl)propanoate (5IX). Oil; IR (neat): 1730, 1455, 1160 cm^{-1}; ^1H NMR ($CDCl_3$) δ=2.04 (2H, tt, J= 7.6, 6.5 Hz), 2.51 (3H, s), 2.52 (3H, s), 2.78 (4H, tt, J=7.6, 3.1 Hz), 3.07 (2H, t, J=7.6 Hz), 4.11 (2H, t, J=6.5 Hz), 6.91 (1H, d, J=7.6 Hz), 6.97 (3H, m), 7.47 (2H, t, J= 7.6 Hz); ^{13}C NMR ($CDCl_3$) δ=24.5 (p), 28.7 (s), 33.0 (s), 33.8 (s), 34.6 (s), 63.9 (s), 119.6 (t), 119.7 (t), 120.7 (t), 120.8 (t), 136.6 (t), 157.9 (q), 159.4 (q), 160.3 (q), 173.1 (q); HRMS (EI): obsd M+ =298.1678, calcd for $C_{18}H_{22}N_2O_2$ M+ =298.1681.

4.5. Typical procedure for stoichiometrically oxidative condensation of aldehydes with primary alcohols to esters

To a solution of 3-phenylpropanal (1 mmol) and 1-octanol (1.05 mmol) in *t*-butyl alcohol (0.5 ml) were added I_2 (1.2 mmol) and K_2CO_3 (3.0 mmol) under an argon atmosphere. The obtained mixture was stirred at room temperature, until the iodine color almost disappeared. After 22 h at the same temperature, the mixture was quenched by the addition of water (20 ml), Et_2O (5 ml), and satd aq Na_2SO_3 (0.5 ml) at 0 °C. Then the mixture was extracted with Et_2O three times. The organic layer was washed with brine and dried over Na_2SO_4 to provide octyl 3-phenylpropanoate in 91% yield. If necessary, the product was purified by flash column chromatography on silica gel (hexane–EtOAc=10:1) to give pure octyl 3-phenylpropanoate as a colorless oil.

4.5.1. 4-Chlorobenzyl 3-phenylpropanoate (6d). Oil; IR (neat): 1735, 1495, 1145, 1090 cm^{-1}; ^1H NMR ($CDCl_3$)

δ=2.68 (2H, t, J=7.8 Hz), 2.96 (2H, t, J=7.8 Hz), 5.06 (2H, s), 7.19 (5H, m), 7.28 (4H, m); ^{13}C NMR ($CDCl_3$) δ= 30.9 (s), 35.8 (s), 65.4 (s), 126.3 (t), 128.3 (t), 128.5 (t), 128.7 (t), 129.5 (t), 134.1 (q), 134.4 (q), 140.3 (q), 172.6 (q); HRMS (FAB): obsd M+H=275.0839, calcd for $C_{16}H_{16}O_2Cl$ M+H=275.0839.

4.5.2. 4-Methylbenzyl 3-phenylpropanoate (6f). Oil; IR (neat): 1730, 1150 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ=2.34 (3H, s), 2.66 (2H, t, J=7.8 Hz), 2.95 (2H, t, J=7.8 Hz), 5.06 (2H, s), 7.13–7.22 (7H, m), 7.26 (2H, m); ^{13}C NMR ($CDCl_3$) δ=21.1 (p), 30.9 (s), 35.9 (s), 66.2 (s), 126.2 (t), 128.3 (t), 128.4 (t), 128.5 (t), 129.2 (t), 132.9 (q), 138.0 (q), 140.4 (q), 172.7 (q); HRMS (FAB): obsd M+H=255.1392, calcd for $C_{17}H_{19}O_2$ M+H=255.1385.

4.5.3. (*N*-Tosyl-indol-3-yl)methyl 3-phenylpropanoate (6m). Oil; ^{1}H NMR ($CDCl_3$) δ=2.34 (3H, s), 2.65 (2H, t, J=7.8 Hz), 2.94 (2H, t, J=7.8 Hz), 5.22 (2H, s), 7.12–7.27 (7H, m), 7.34 (1H, t, 7.6 Hz), 7.47 (1H, d, 7.6 Hz), 7.60 (1H, s), 7.78 (2H, d, 7.6 Hz), 7.97 (1H, d, 7.6 Hz); ^{13}C NMR ($CDCl_3$) δ=21.5 (p), 30.8 (s), 35.7 (s), 57.7 (s), 113.6 (t), 117.2 (q), 119.7 (t), 123.4 (t), 125.0 (t), 125.6 (t), 126.2 (t), 126.9 (t), 128.2 (t), 128.4 (t), 129.4 (q), 129.9 (t), 135.0 (q), 135.1 (q), 140.2 (q), 145.1 (q), 172.7 (q); HRMS (FAB): obsd M+ =433.1339, calcd for $C_{25}H_{23}NO_4S$ M+ = 433.1348.

4.5.4. Octyl 3-phenylpropanoate (6II). Oil; IR (neat): 1735, 1160 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ=0.88 (3H, t, J= 7.0 Hz), 1.22–1.38 (10H, m), 1.59 (2H, m), 2.63 (2H, t, J= 7.9 Hz), 2.95 (2H, t, J=7.9 Hz), 4.06 (2H, t, J=6.8 Hz), 7.20 (3H, m), 7.29 (2H, m); ^{13}C NMR ($CDCl_3$) δ=14.1 (p), 22.6 (s), 25.9 (s), 28.6 (s), 29.1 (s), 29.2 (s), 31.0 (s), 31.8 (s), 35.9 (s), 64.6 (s), 126.2 (t), 128.3 (t), 128.4 (t), 140.5 (q), 173.0 (q); HRMS (FAB): obsd M+H=263.2017, calcd for $C_{17}H_{27}O_2$ M+H=263.2011.

4.5.5. Tridecyl 3-phenylpropanoate (6III). Oil; IR (neat): 1735, 1455, 1160 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ=0.88 (3H, t, J=7.0 Hz), 1.22–1.38 (20H, m), 1.63 (2H, m), 2.62 (2H, t, J=7.9 Hz), 2.95 (2H, t, J=7.9 Hz), 4.06 (2H, t, J=6.7 Hz), 7.20 (3H, m), 7.28 (2H, m); ^{13}C NMR ($CDCl_3$) δ=14.2 (p), 22.8 (s), 26.0 (s), 28.7 (s), 29.3 (s), 29.4 (s), 29.6 (s), 29.7 (s), 29.8 (s), 31.1 (s), 32.0 (s), 36.0 (s), 64.7 (s), 126.3 (t), 128.4 (t), 128.5 (t), 140.7 (q), 173.1 (q).

4.5.6. 2-Phenoxyethyl 3-phenylpropanoate (6V). Colorless solid; mp 52–53 °C; IR (KBr): 1730, 1240, 1160 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ=2.67 (2H, t, J=7.8 Hz), 2.95 (2H, t, J=7.8 Hz), 4.12 (2H, t, J=4.7 Hz), 4.42 (2H, t, J=4.7 Hz), 6.90 (2H, d, J=7.5 Hz), 6.96 (1H, t, J=7.5 Hz) 7.19 (3H, m), 7.26 (4H, m); ^{13}C NMR ($CDCl_3$) δ=30.8 (s), 35.7 (s), 62.8 (s), 65.8 (s), 114.6 (t), 121.1 (t), 126.2 (t), 128.3 (t), 128.5 (t), 129.5 (t), 140.3 (q), 158.4 (q), 172.8 (q); HRMS (FAB): obsd M+H=271.1324, calcd for $C_{17}H_{19}O_3$ M+ H=271.1334.

4.5.7. (Tetrahydrofuran-2-yl)methyl 3-phenyl-propanoate (6VIII). Oil; IR (neat): 1730, 1160, 1075 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ=1.55 (2H, m), 1.90 (3H, m), 2.68 (2H, t, J=8.0 Hz), 2.96 (2H, t, J=8.0 Hz), 3.79 (1H, m), 3.87 (1H, m), 4.01 (1H, m), 4.09 (1H, m), 4.16 (1H, m), 7.23 (3H, m), 7.28 (2H, m); ^{13}C NMR ($CDCl_3$) δ=25.6 (s), 27.9 (s), 30.9 (s), 35.7 (s), 66.5 (s), 68.4 (s), 76.4 (t), 126.2 (t), 128.2 (t), 128.4 (t), 140.4 (q), 172.8 (q); HRMS (FAB): obsd M+H= 235.1314, calcd for $C_{14}H_{19}O_3$ M+H=235.1334.

4.5.8. 3-(6′-Methylpyridin-2′-yl)propyl 3-phenyl-propanoate (6IX). Oil; IR (neat): 1730, 1455, 1155 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ=2.04 (2H, m), 2.52 (3H, s), 2.63 (2H, t, J=7.7 Hz), 2.78 (2H, t, J=7.7 Hz), 2.95 (2H, t, J=7.7 Hz), 4.12 (2H, t, J=6.6 Hz), 6.90 (1H, d, J=7.6 Hz), 6.96 (1H, d, J=7.6 Hz), 7.20 (3H, m), 7.28 (2H, m), 7.47 (1H, t, J= 7.6 Hz); ^{13}C NMR ($CDCl_3$) δ=24.5 (p), 28.7 (s), 30.9 (s), 34.7 (s), 35.8 (s), 64.0 (s), 119.6 (t), 120.7 (t), 126.2 (t), 128.3 (t), 128.5 (t), 136.6 (t), 140.5 (q), 157.9 (q), 160.3 (q), 172.9 (q); HRMS (FAB): obsd M+H=284.1634, calcd for $C_{18}H_{22}NO_2$ M+H=284.1651.

4.5.9. 4-(Benzyloxy)phenethyl 3-phenylpropanoate (6XI). Colorless solid; mp 61–62 °C; IR (KBr): 1725, 1240, 1170 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ=2.60 (2H, t, J= 7.8 Hz), 2.83 (2H, t, J=7.0 Hz), 2.91 (2H, t, J=7.8 Hz), 4.23 (2H, t, J=7.0 Hz), 5.02 (2H, s), 6.89 (2H, d, J= 8.7 Hz), 7.08 (2H, d, J=8.7 Hz), 7.17 (3H, m), 7.28 (3H, m), 7.38 (4H, m); ^{13}C NMR ($CDCl_3$) δ=30.9 (s), 34.2 (s), 35.9 (s), 65.2 (s), 70.0 (s), 114.8 (t), 126.2 (t), 127.5 (t), 127.9 (t), 128.3 (t), 128.5 (t), 128.6 (t), 129.9 (t), 130.1 (q), 137.1 (q), 140.5 (q), 157.5 (q), 172.8 (q); HRMS (FAB): obsd M+ =360.1721, calcd for $C_{24}H_{24}O_3$ M+ =360.1725.

4.5.10. 2-(1′,7′,7′-Trimethyl-bicyclo[2,2,1] hept-2′-yloxy)ethyl 3-phenylpropanoate (6XII). Oil; IR (neat): 1735, 1165, 1120 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ=0.80 (3H, s), 0.87 (3H, s), 0.96 (3H, s), 0.97 (2H, m), 1.45–1.75 (5H, m), 2.63 (2H, t, J=7.8 Hz), 2.95 (2H, t, J=7.8 Hz), 3.20 (1H, dd, J=7.5, 3.5 Hz), 3.47 (1H, m), 3.58 (1H, m), 4.17 (2H, m), 7.20 (3H, m), 7.28 (2H, m); ^{13}C NMR ($CDCl_3$) δ=11.7 (p), 20.1 (p), 20.2 (p), 27.3 (s), 30.9 (s), 34.4 (s), 35.9 (s), 38.5 (s), 45.0 (t), 46.4 (q), 49.2 (q), 63.8 (s), 66.9 (s), 87.4 (t), 126.2 (t), 128.3 (t), 128.5 (t), 140.6 (q), 172.8 (q); HRMS (FAB): obsd M+ =330.2214, calcd for $C_{21}H_{30}O_3$ M+ = 330.2195.

4.5.11. (*R*)-2-((3a*R*,5*R*,6*R*,6a*S*)-6-(Benzyloxy)-tetrahydro-2,2-dimethylfuro[2,3-*d*][1,3]dioxol-5-yl)-2-hydroxyethyl 3-phenylpropanoate (6XIII). Oil; IR (neat): 1735, 1215, 1165, 1070, 1025 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ= 1.30 (3H, s), 1.46 (3H, s), 2.46 (1H, broad), 2.65 (2H, t, J= 7.8 Hz), 2.94 (2H, t, J=7.8 Hz), 4.09 (2H, m), 4.14 (2H, m), 4.38 (1H, dd, J=14.4, 5.5 Hz), 4.55 (1H, d, J=11.9 Hz), 4.60 (1H, d, J=3.9 Hz), 4.69 (1H, d, J=11.9 Hz), 5.91 (1H, d, J=3.9 Hz), 7.18 (3H, t, J=3.9 Hz), 7.25 (2H, m), 7.33 (5H, m); ^{13}C NMR ($CDCl_3$) δ=26.2 (p), 26.7 (p), 30.8 (s), 35.6 (s), 66.5 (s), 67.4 (t), 72.0 (s), 79.3 (t), 81.6 (t), 82.0 (t), 105.1 (t), 111.7 (q), 126.2 (t), 127.7 (t), 128.1 (t), 128.4 (t), 128.6 (t), 137.1 (q), 140.2 (q), 173.0 (q); HRMS (FAB): obsd M+H=443.2065, calcd for $C_{25}H_{31}O_7$ M+H= 443.2070.

4.5.12. Octyl pentanoate (6II′). Oil; IR (neat): 1735, 1170 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ=0.88 (3H, t, J=7.0 Hz), 0.92 (3H, t, J=7.4 Hz), 1.22–1.38 (12H, m), 1.61 (4H, m), 2.30 (2H, t, J=7.4 Hz), 4.06 (2H, t, J=6.7 Hz); ^{13}C NMR ($CDCl_3$) δ=13.7 (p), 14.0 (p), 22.3 (s), 22.6 (s), 25.9 (s),

27.1 (s), 28.6 (s), 29.2 (s), 31.8 (s), 34.1 (s), 64.4 (s), 174.0 (q); HRMS (EI): obsd M+ =214.1940, calcd for $C_{13}H_{26}O_2$ M+ =214.1933.

4.6. Typical procedure for oxidation of secondary alcohols to ketones

To a solution of (−)-menthol (1 mmol) in *t*-butyl alcohol (1 ml) were added I_2 (2 mmol) and K_2CO_3 (2 mmol) under an argon atmosphere. The mixture obtained was stirred at 90 °C. After 16 h at the same temperature, the mixture was quenched with satd aq Na_2SO_3 (3–5 ml) at 0 °C, and was extracted with Et_2O three times. The organic layer was washed with brine and dried over Na_2SO_4 to provide (−)-menthone in 99% yield in an almost pure state. If necessary, the product was purified by flash column chromatography on silica gel (hexane–EtOAc=4:1) to give pure (−)-menthone as an oil.[36] ^{1}H NMR ($CDCl_3$) δ=0.85 (3H, d, J= 6.8 Hz), 0.92 (3H, d, J=6.8 Hz), 1.01 (3H, d, J=6.1 Hz), 1.28–1.45 (2H, m), 1.79–2.20 (6H, m), 2.35 (1H, m).

4.6.1. 2,3,5-Tri-*O*-benzyl-D-ribofuranone. Oil; ^{1}H NMR ($CDCl_3$) δ=3.52 (1H, dd, J=11.1, 2.7 Hz), 3.62 (1H, dd, J=11.1, 2.7 Hz), 4.08 (1H, dd, J=5.6, 1.7 Hz), 4.41 (3H, m), 4.53 (2H, m), 4.67 (1H, d, J=11.9 Hz), 4.72 (1H, d, J= 11.9 Hz), 4.91 (1H, d, J=11.9 Hz), 7.18 (2H, m), 7.25–7.37 (13H, m); ^{13}C NMR ($CDCl_3$) δ=68.6 (s), 72.2 (s), 72.6 (s), 73.4 (s), 73.6 (t), 75.2 (t), 81.6 (t), 127.4 (t), 127.8 (t), 127.9 (t), 128.0 (t), 128.1 (t), 128.3 (t), 128.4 (t), 136.8 (q), 137.0 (q), 137.1 (q), 173.7 (q).

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References and notes

1. (a) Larock, R. C. Comprehensive Organic Transformation; VCH: New York, 1989. (b) Hudlicky, M. Oxidations in Organic Chemistry In *ACS Monograph Series*; American Chemical Society: Washington, DC, 1990. (c) Sheldon, R. A.; Kochi, J. K. Metal-Catalyzed Oxidations of Organic Compounds; Academic: New York, 1984.
2. (a) Meyers, C. Y. *J. Org. Chem.* **1961**, *26*, 1046. (b) Lee, G. A.; Freedman, H. H. *Tetrahedron Lett.* **1976**, 1641. (c) Regan, S. L. *J. Org. Chem.* **1977**, *42*, 875. (d) Stevens, R. V.; Chapman, K. T.; Weller, H. N. *J. Org. Chem.* **1980**, *45*, 2030.
3. (a) Nwaukwa, S. O.; Keehn, P. M. *Tetrahedron Lett.* **1982**, *23*, 35. (b) McDonald, C. E.; Nice, L. E.; Shaw, A. W.; Nestor, N. B. *Tetrahedron Lett.* **1993**, *34*, 2741. (c) Milovanović, J. N.; Vasojević, M.; Gojković, S. *J. Chem. Soc., Perkin Trans. 2* **1991**, 1231.
4. (a) Corey, E. J.; Kim, C. U. *J. Am. Chem. Soc.* **1972**, *94*, 7586. (b) Corey, E. J.; Kim, C. U. *J. Org. Chem.* **1973**, *38*, 1233. (c) Corey, E. J.; Kim, C. U. *Tetrahedron Lett.* **1973**, 919. (d) Wicha, J.; Zarecki, A. *Tetrahedron Lett.* **1974**, 3059. (e) Crosby, G. A.; Weinshenker, N. M.; Uh, H. S. *J. Am. Chem. Soc.* **1975**, *97*, 2232. (f) Neirabeyeh, M. A.; Ziegler, J. C.; Gross, B. *Synthesis* **1976**, 811. (g) Neirabeyeh, M. A.; Pujol, M. D. *Tetrahedron Lett.* **1990**, *31*, 2273.
5. (a) Farkas, L.; Schächter, O. *J. Am. Chem. Soc.* **1949**, *71*, 2827. (b) Perlmutter-Hayman, B.; Weissmann, Y. *J. Am. Chem. Soc.* **1969**, *91*, 668. (c) Kageyama, T.; Kawahara, S.; Kitamura, K.; Ueno, Y.; Okawara, M. *Chem. Lett.* **1983**, 1097. (d) Kajigaeshi, S.; Nakagawa, T.; Nagasaki, N.; Yamasaki, H.; Fujisaki, S. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 747. (e) Kajigaeshi, S.; Kawamukai, H.; Fujisaki, S. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 2585. (f) Morimoto, T.; Hirano, M.; Hamaguchi, T.; Shimoyama, M.; Zhuang, X. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 703. (g) Tohma, H.; Maegawa, T.; Kita, Y. *Synlett* **2003**, 723. (h) Sayama, S.; Onami, T. *Synlett* **2004**, 2739.
6. Otera, J. *Esterification*; Wiley: New York, 2003.
7. (a) Blum, Y.; Reshef, D.; Shvo, Y. *Tetrahedron Lett.* **1981**, *22*, 1541. (b) Murahashi, S. I.; Ito, K.; Naota, T.; Maeda, Y. *Tetrahedron Lett.* **1981**, *22*, 5327. (c) Murahashi, S. I.; Naota, T.; Ito, K.; Maeda, Y.; Taki, H. *J. Org. Chem.* **1987**, *52*, 4319. (d) Nagashima, H.; Tsuji, J. *Chem. Lett.* **1981**, 1171. (e) Nagashima, H.; Sato, K.; Tsuji, J. *Tetrahedron* **1985**, *41*, 5645. (f) Tamaru, Y.; Yamada, Y.; Inoue, K.; Yamamoto, Y.; Yoshida, Z. *J. Org. Chem.* **1983**, *48*, 1286. (g) Masuyama, Y.; Takahashi, M.; Kurusu, Y. *Tetrahedron Lett.* **1984**, *25*, 4417.
8. (a) Robertson, R. G. In *Organic Syntheses*, *Collect. Vol. 1* 1941 p 138. (b) Bhar, S.; Chaudhuri, S. K. *Tetrahedron* **2003**, *59*, 3493.
9. Merbouh, N.; Bobbitt, J. M.; Brückner, C. *J. Org. Chem.* **2004**, *69*, 5116.
10. (a) Sakuragi, H.; Tokumaru, K. *Chem. Lett.* **1974**, 475. (b) Ogawa, T.; Matsui, M. *J. Am. Chem. Soc.* **1976**, *98*, 1629. (c) Sundararaman, P.; Walker, E. C.; Djerassi, C. *Tetrahedron Lett.* **1978**, 1627. (d) Chiba, T.; Okimoto, M.; Nagai, H.; Takata, Y. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 335. (e) Garegg, P. J.; Olsson, L.; Oscarson, S. *J. Org. Chem.* **1995**, *60*, 2200.
11. Craig, J. C.; Horning, E. C. *J. Org. Chem.* **1960**, *25*, 2098.
12. Marvell, E. N.; Joncich, M. J. *J. Am. Chem. Soc.* **1951**, *73*, 973.
13. Corey, E. J.; Gilman, N. W.; Ganem, B. E. *J. Am. Chem. Soc.* **1968**, *90*, 5616.
14. (a) Stevens, R. V.; Chapman, K. T.; Stubbs, C. A.; Tam, W. W.; Albizati, K. F. *Tetrahedron Lett.* **1982**, *23*, 4647. (b) Wilson, S. R.; Tofigh, S.; Misra, R. N. *J. Org. Chem.* **1982**, *47*, 1360. (c) Nwaukwa, S. O.; Keehn, P. M. *Tetrahedron Lett.* **1982**, *23*, 3131.
15. Abiko, A.; Roberts, J. C.; Takemasa, T.; Masamune, S. *Tetrahedron Lett.* **1986**, *27*, 4537.
16. O'Connor, B.; Just, G. *Tetrahedron Lett.* **1987**, *28*, 3235.
17. Thomason, S. C.; Kubler, D. G. *J. Chem. Educ.* **1968**, *45*, 546.
18. Grigg, R.; Mitchell, T. R. B.; Sutthivaiyakit, S. *Tetrahedron* **1981**, *37*, 4313.
19. Rajendran, S.; Trivedi, D. C. *Synthesis* **1995**, 153.
20. Gopinath, R.; Barkakaty, B.; Talukdar, B.; Patel, B. K. *J. Org. Chem.* **2003**, *68*, 2944.
21. Dalcanale, E.; Montanari, F. *J. Org. Chem.* **1986**, *51*, 567.
22. McDonald, C.; Holcomb, H.; Kennedy, K.; Kirkpatrick, E.; Leathers, T.; Vanemon, P. *J. Org. Chem.* **1989**, *54*, 1213.
23. Okimoto, M.; Chiba, T. *J. Org. Chem.* **1988**, *53*, 218.
24. (a) Gopinath, R.; Patel, B. K. *Org. Lett.* **2000**, *2*, 577. (b) Sato, K.; Hyodo, M.; Takagi, J.; Aoki, M.; Noyori, R. *Tetrahedron Lett.* **2000**, *41*, 1439. (c) Qian, G.; Zhao, R.; Ji, D.; Lu, G.; Qi, Y.; Suo, J. *Chem. Lett.* **2004**, *33*, 834.
25. Sharghi, H.; Sarvari, M. H. *J. Org. Chem.* **2003**, *68*, 4096.
26. (a) Inch, T. D.; Ley, R. V.; Rich, P. *J. Chem. Soc. C* **1968**,

1693. (b) Eliel, E. L.; Clawson, L.; Knox, D. E. *J. Org. Chem.* **1985**, *50*, 2707. (c) Yamada, S.; Morizono, D.; Yamamoto, K. *Tetrahedron Lett.* **1992**, *33*, 4329. (d) Talukdar, S.; Hsu, J. L.; Chou, T. C.; Fang, J. M. *Tetrahedron Lett.* **2001**, *42*, 1103.
27. Lieben, A. *Ann.* **1870**, *7*, 218.
28. (a) Togo, H.; Katohgi, M. *Synlett* **2000**, 565. (b) Togo, H.; Harada, Y.; Yokoyama, M. *J. Org. Chem.* **2000**, *65*, 926. (c) Katohgi, M.; Togo, H. *Tetrahedron* **2001**, *57*, 7481.
29. As a preliminary report: Mori, N.; Togo, H. *Synlett* **2004**, 880.
30. Shono, T.; Matsumura, Y.; Hayashi, J.; Mizoguchi, M. *Tetrahedron Lett.* **1979**, 165.
31. (a) Barluenga, J.; Marco-Arias, M.; González-Bobes, F.; Ballesteros, A.; González, J. M. *Chem. Commun.* **2004**, 2616. (b) Barluenga, J.; González-Bobes, F.; Murguía, M. C.; Ananthoju, S. R.; González, J. M. *Chem. Eur. J.* **2004**, *10*, 4206.
32. (a) Detar, D. F. *J. Am. Chem. Soc.* **1982**, *104*, 7205. (b) Bégué, J. P.; Bonnet-Delpon, D.; Crousse, B. *Synlett* **2004**, 18.
33. (a) Kesavan, V.; Bonnet-Delpon, D.; Bégué, J. P. *Synthesis* **2000**, 223. (b) Neimann, K.; Neumann, R. *Org. Lett.* **2000**, *2*, 2861. (c) van Vliet, M. C. A.; Arends, I. W. C. E.; Sheldon, R. A. *Synlett* **2001**, 248. (d) de Visser, S. P.; Kaneti, J.; Neumann, R.; Shaik, S. *J. Org. Chem.* **2003**, *68*, 2903. (e) Chikaoka, S.; Toyao, A.; Ogasawara, M.; Tamura, O.; Ishibashi, H. *J. Org. Chem.* **2003**, *68*, 312. (f) Harmata, M.; Lee, D. R. *J. Am. Chem. Soc.* **2002**, *124*, 14328. (g) Abe, M.; Adam, W.; Minamoto, T.; Ino, Y.; Nojima, M. *J. Org. Chem.* **2003**, *68*, 1796. (h) Grainger, R. S.; Owoare, R. B.; Tisselli, P.; Steed, J. W. *J. Org. Chem.* **2003**, *68*, 7899. (i) Ben-Daniel, R.; de Visser, S. P.; Shaik, S.; Neumann, R. *J. Am. Chem. Soc.* **2003**, *125*, 12116. (j) Iskra, J.; Bonnet-Delpon, D.; Bégué, J. P. *Tetrahedron Lett.* **2003**, *44*, 6309.
34. Miller, R. A.; Hoerrner, R. S. *Org. Lett.* **2003**, *5*, 285.
35. Kourafalos, V. N.; Marakos, P.; Pouli, N.; Townsend, L. B. *J. Org. Chem.* **2003**, *68*, 6466.
36. On line data from Sigma-Aldrich products. http://www.sigmaaldrich.com/Brands/Aldrich.html
37. Kubota, T.; Miyashita, S.; Kitazume, T.; Ishikawa, N. *J. Org. Chem.* **1980**, *45*, 5052.
38. Halton, B.; Boese, R.; Dixon, G. M. *Eur. J. Org. Chem.* **2003**, 4507.
39. Liu, H. J.; Chan, W. H.; Lee, S. P. *Tetrahedron Lett.* **1978**, 4461.

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Studies on solid-state proton transfer along hydrogen bond of pyrazolone-ring

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Abstract—Evidence from the time-dependent UV–vis reflection spectra studies indicates the compound 1-phenyl-3-methyl-4-(4-methylbenzal)-5-pyrazolone 4-ethylthiosemicarbazone (PM4MBP-ETSC) undergoes a solid-state photochromism. The reaction rate constant was studied by the first-order kinetics curves. X-ray single crystal structural analysis shows that the pyrazolone-ring stabilizes in the keto form. The conclusion can be made that its photochromism in crystalline is associated with a photoinduced proton transfer reaction (inter- and intra-molecular hydrogen transfer) along hydrogen bond leading to a colored tautomer as the compound crystallizes in H-bonded supramolecular configuration.

1. Introduction

Photochromism in organic systems is a phenomenon entailing the reversible change of a selected chemical species from one molecular conformation to another. The transformation is accompanied by a readily discernible change in the optical absorption spectrum between two states having distinguishable absorption spectra. Structural transformation is induced in at least one direction by light energy.[1] Recently, photochromic materials have gained much attention and they now constitute an active research area because they are responsible for a variety of biological phenomena and in their potential applications in the areas of linear and non-linear optics.[2,3] Although, a great number of photochromic compounds have been reported, compounds which undergo photochromism in crystalline phase are rare. The crystalline photochromic materials show a fatigue resistant character and have promising potential for optoelectronic devices.[4]

Hydrogen bonds play a key role in a large number of chemical and biological processes. These interactions contribute significantly to the structural feature and the stability of molecules.[5] Information on hydrogen bonds can be very useful to understand various molecular properties and to control the structures of a vast multitude of biomolecules, host–guest complexes and supramolecular assemblies.[6] The special features of such hydrogen bonds include hydrogen transfer of interactions between acid–base centers of the molecule, hydrogen bridge bonding effects, diminishing of the acid–base interactions and, as a consequence of this, the red shift of ν_{OH} band and decreasing intensity.[7] The presence of electron-rich centers (=N, =O) and hydrogen atoms covalently bonded to electronegative atoms provide the possibility of forming different type of intermolecular hydrogen bonds, O···H–O and N···H–O. The amide N–H···O=C hydrogen bond is of the paramount importance to molecular structure in biological and chemical systems.[8]

Renewed interest on the subject has recently arisen due to the discovery of proton transfer reactions along hydrogen bonds which not only occur in solution but also proceed in the solid-state.[9] As proton transfer in these systems causes a change in their optical properties, therefore, these molecules are candidates for optical switches and storage devices. There have been a few studies of proton transfer reaction.[10,11] When the proton donor and proton acceptor sites are sufficiently close, they are often linked by hydrogen bonds which enable direct tunneling of the proton.

In this paper, we describe a photochromic compound, 1-phenyl-3-methyl-4-(4-methylbenzal)-5-pyrazolone 4-ethylthiosemicarbazone (PM4MBP-ETSC), which undergoes a solid-state photochromism. With the aim of gaining a deeper insight into the structural aspects responsible for the observed phenomenon, a crystallographic analysis of the title compound has been carried out. Its photochromism is

Keywords: Thiosemicarbazone; Photochromism; Crystal structure; Proton transfer.
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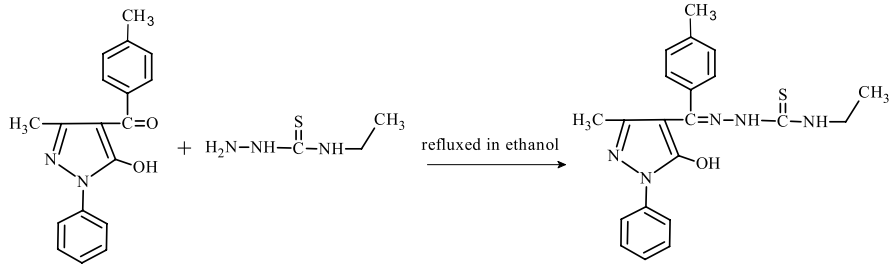

Scheme 1. Reaction equation for PM4MBP-ETSC (yield: 82%).

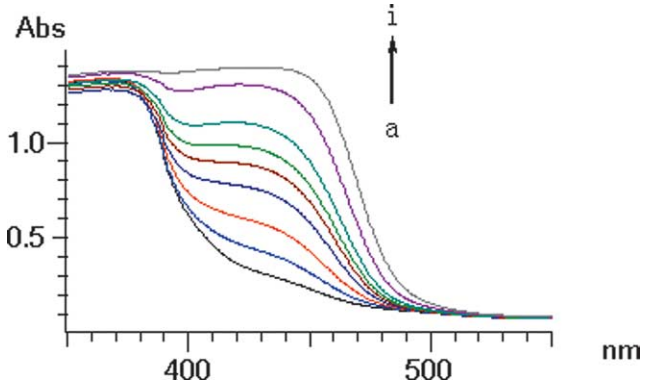

Figure 1. UV–vis reflection spectral changes of PM4MBP-ETSC in the solid-state at room temperature. From *a–i*, each irradiation time (min) is: 0, 3, 6, 9, 12, 15, 20, 36, ∞.

due to proton transfer through the hydrogen bonds, meanwhile the molecule changes from enol form to the keto form (Scheme 2). The photochromic behavior of the compound was characterized by UV–vis reflection spectra. The objective of this paper is to gain information on the solid-state proton transfer reaction from reflection spectral experiment and crystal analysis.

2. Experimental

2.1. Materials

1-Phenyl-3-methyl-5-pyrazolone (PMP), 4-methylbenzoylchloride and all the other reagents are analytical pure and used without further purification, 4-ethylthiosemicarbazide (ETSC) was obtained from Aldrich.

2.2. Instrumentation

Melting point was measured with a TECH XT-5 melting point apparatus and was uncorrected. The elemental analyses were determined on Perkin–Elmer-2400 Elemental analyzer. IR spectrum was recorded from KBr pellet on a BRUKER EQUINOX-55 Spectrometer. ^{1}H NMR (DMSO-d_6) was carried out on an INOVA-400 ^{1}H NMR Spectrometer. The mass spectrum was collected on an HP 1100 LC/MS mass Spectrometer with an ionization energy of 120 eV. UV–vis reflection spectra were monitored on Hitachi UV-3010 Spectrometer. Irradiation of microcrystalline sample of the compound was carried out using a pressed pellet under 365 nm light.

X-ray diffraction data were collected at 296 K on a Siemens *P*4 Four-circle diffractermeter using graphite

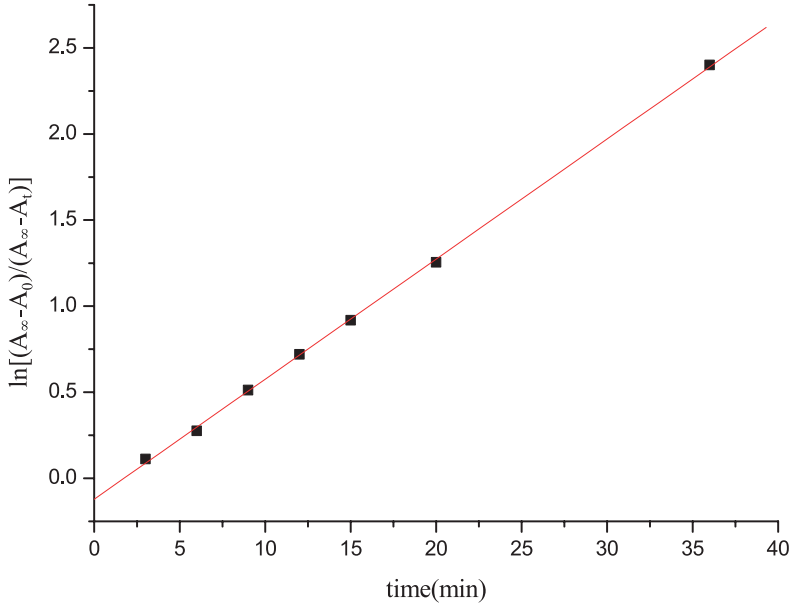

Figure 2. First-order kinetic plot of photoisomerization reaction of PM4MBP-ETSC induced by 365 nm light at room temperature.

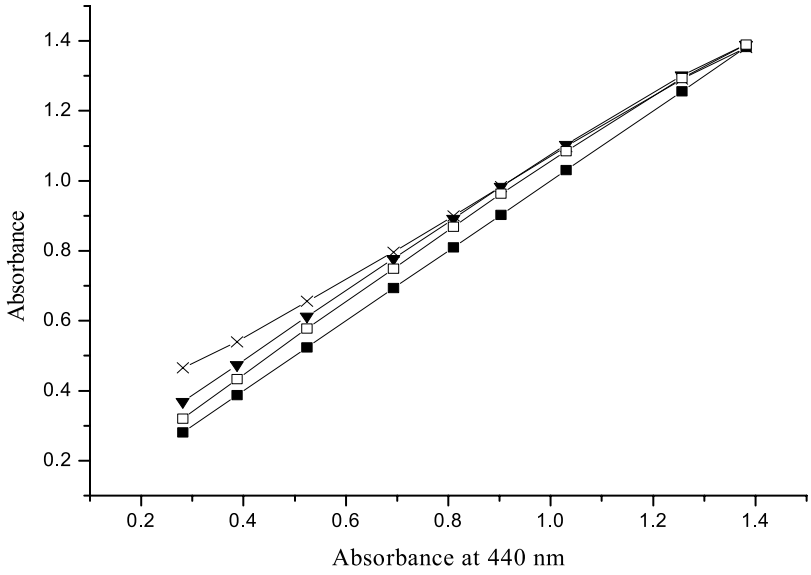

Figure 3. E-diagram of PM4MBP-ETSC. The reflection at 440 nm (■) is plotted against those at 410 nm (×), 420 nm (▲) and 430 nm (□).

monochromatic $M_o K_\alpha$ radiation ($\lambda=0.71073$ Å). The molecular structure was solved by direct method and refined by full-matrix least squares on F^2. Empirical absorption was applied. The intensity was measured by ω-scans. The diffraction data were collected from reflections in the range of 1.44–25.25°. Hydrogen atoms were found from their calculated positions and refined isotropically. All calculations and drawings were performed by the SHELXTL-97 crystallographic software package of molecular structure. Crystallographic data (excluding structure factors) for the structure has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC: 256179. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

2.3. Preparation and characterization

The preparation of 1-phenyl-3-methyl-4-(4-methylbenzoyl)-5-pyrazolone (PM4MBP) was according to literature procedure.[12]

Compound 1-phenyl-3-methyl-4-(4-methylbenzal)-5-pyrazolone 4-ethylthiosemicarbazone (PM4MBP-ETSC) was synthesized by direct condensation of PM4MBP with ETSC in ethanol followed by repeated recrystallization from the same solvent. Reaction equation is shown in Scheme 1. Yield: 82%, mp 216–218 °C. Elemental analyses for $C_{21}H_{23}N_5SO$: found (%): C, 63.69; H, 6.19; N, 18.07; calculated (%): C, 64.10; H, 5.89; N, 17.80. IR (ν/cm^{-1}) (after irradiation): 3247 (N–H), 1631 (C=O), 1610 (C=N), 1550, 1488 (phenyl), 1408, 1308 (pyrazolone-ring), 1212, 839 (C=S). ^{1}H NMR (DMSO-d_6) (δ/ppm): 9.65 (1H, Pz-NH), 8.55 (1H, NH–C=S), 7.23–7.97 (m, 10H, 2Ph+NH), 3.61 (s, 3H, Pz-CH_3), 2.50–2.36 (m, 2H, N–CH_2CH_3), 1.18 (s, 3H, Ph-CH_3), 1.14 (m, 3H, N–CH_2CH_3). MS (ESI^-): $m/z=392.1$ (M−1, 100), 305.1 (M−87, 90).

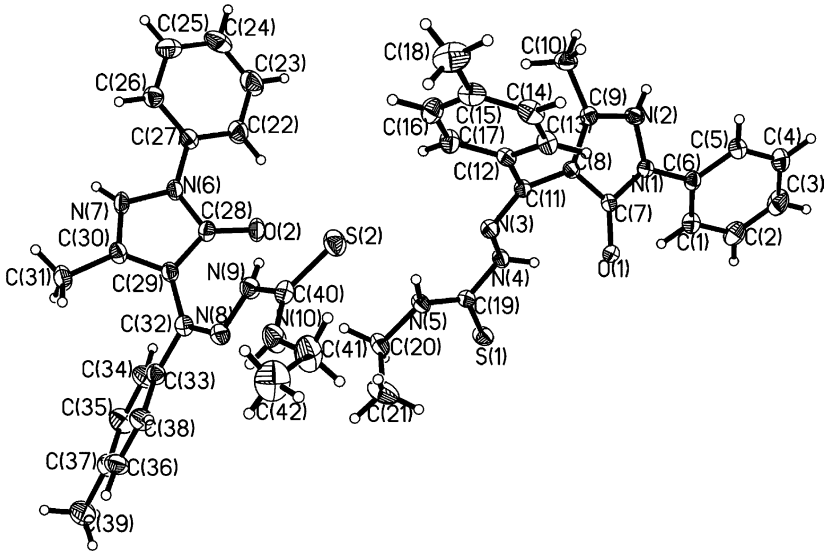

Figure 4. Crystal structure of PM4MBP-ETSC (solvent: methanol and diochloromethane).

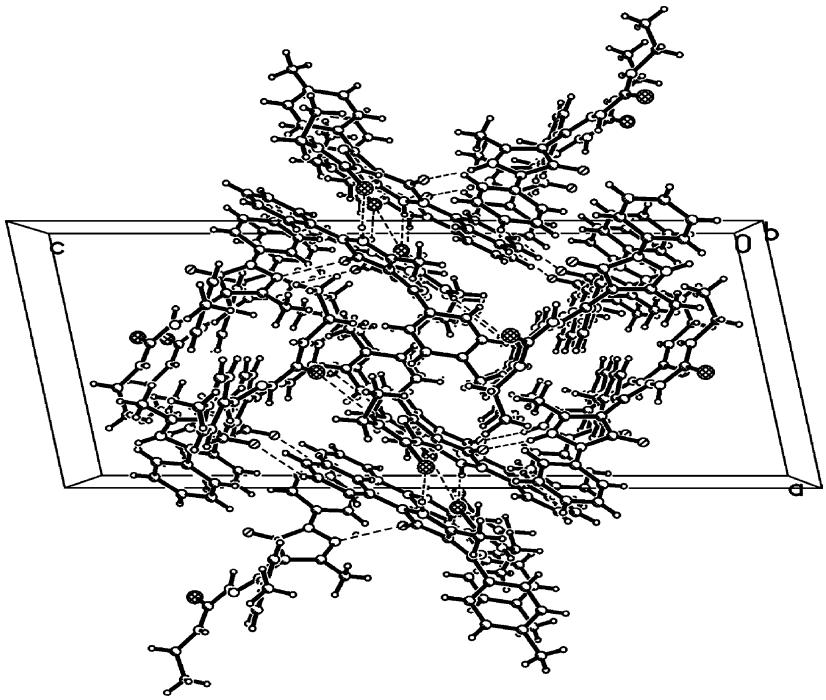

Figure 5. Crystal packing of PM4MBP-ETSC.

3. Results and discussion

3.1. Photochromic properties in solid-state and kinetics of the reaction

Irradiation of the crystalline sample of PM4MBP-ETSC was carried out using a thin pressed pellet. The results obtained are as follows: the white PM4MBP-ETSC at room temperature changes to yellow after irradiation. Thus, the strongly white crystalline PM4MBP-ETSC is photochromic. The UV–vis reflection spectra after different irradiation times are shown in Figure 1. Upon UV light irradiation, a broad reflection band appeared at around 350–500 nm and continuously increased depending on the irradiation time, which identifies the title compound exhibits photochromic properties in the solid-state.

Table 1. Crystal data and structure refinement for PM4MBP-ETSC

Empirical formula	$C_{21}H_{23}N_5OS$
Formula weight	393.50
Temperature (K)	296(2)
Wavelength (Å)	0.71073
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimensions	$a=12.729(2)$ Å $\alpha=90°$ $b=11.438(2)$ Å $\beta=100.06(2)°$ $c=28.641(5)$ Å $\gamma=90°$
Volume (Å^3), Z	4106.0(12), 8
Density (calculated) (g·cm^{-3})	1.273
Absorption coefficient (mm^{-1})	0.179
$F(000)$	1664
Crystal size (mm)	$0.60\times0.48\times0.36$
Limiting indices	$0\le h\le15$, $0\le k\le13$, $-34\le l\le33$
Reflections collected	8501
Independent reflections	7437 [$R_{int}=0.0165$]
Theta range for data collection (°)	1.44–25.25
Absorption correction	Empirical
Max. and min. transmission	0.9809 and 0.9238
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	7437/6/539
Goodness-of-fit on F^2	0.842
Final R indices [$I>2\sigma(I)$]	$R_1=0.0412$, $\omega R_2=0.0859$
R indices (all data)	$R_1=0.0837$, $\omega R_2=0.0947$
Extinction coefficient	0.0026(2)
Largest diff. peak and hole (e·Å^{-3})	0.240 and −0.231

The absorption was monitored at a $\lambda=430$ nm (at which the maximum change in optical density occurred) as a function of time to obtain good first-order kinetics rate. The kinetic curve (shown in Fig. 2) is plotted according to the Eq. 1[13,14]

$$kt = \ln[(A_\infty - A_0)/(A_\infty - A_t)] \quad (1)$$

where k is the first-order rate constant. A_0, A_∞ and A_t are the observed reflection data measured at the beginning, at the end and at time t of the reaction, respectively. From Figure 2, it can be seen that it represents a good linear fit and the photochromic reaction process follows the first-order kinetics. The pseudo-first-order rate constant is obtained as $k_1=1.16\times10^{-3}$ s^{-1}. Spectra measured at any time after UV irradiation is proportional to each other in the visible region, indicating that only one species is formed. The absorbance diagram (E-diagram) of the system is plotted (Fig. 3).[15] The reflection at $\lambda=440$ nm is plotted against those at 410, 420 and 430 nm during the coloration of the compound. The straight lines in the E-diagram also indicate that the reaction is a uniform one. In other words, only one linear independent step is contained in the coloration process.[16]

3.2. Crystallographic explanation and mechanism of photochromism

By slow evaporating the mix solution of methanol and dichloromethane at room temperature without avoiding of

Table 2. Selected bond lengths (Å) and angles (°) for PM4MBP-ETSC

Molecule A		Molecule B	
Bond lengths			
S(1)–C(19)	1.694(2)	S(2)–C(40)	1.664(3)
O(1)–C(7)	1.265(2)	O(2)–C(28)	1.240(2)
N(1)–N(2)	1.377(2)	N(6)–N(7)	1.378(2)
N(1)–C(7)	1.372(3)	N(6)–C(28)	1.397(3)
N(2)–C(9)	1.344(3)	N(7)–C(30)	1.331(3)
N(3)–C(11)	1.290(2)	N(8)–C(32)	1.294(3)
N(3)–N(4)	1.383(2)	N(8)–N(9)	1.392(3)
N(4)–C(19)	1.360(3)	N(9)–C(40)	1.362(3)
N(5)–C(19)	1.321(3)	N(10)–C(40)	1.330(3)
C(8)–C(11)	1.474(3)	C(29)–C(32)	1.477(3)
C(11)–C(12)	1.482(3)	C(32)–C(33)	1.481(3)
Bond angles			
C(11)–N(3)–N(4)	118.9(2)	C(32)–N(8)–N(9)	116.9(2)
C(19)–N(4)–N(3)	117.7(2)	C(40)–N(9)–N(8)	119.5(2)
N(5)–C(19)–N(4)	116.8(2)	N(10)–C(40)–N(9)	114.6(2)
N(4)–C(19)–S(1)	117.9(2)	N(9)–C(40)–S(2)	120.5(2)
N(5)–C(19)–S(1)	125.2(2)	N(10)–C(40)–S(2)	124.9(2)
N(2)–N(1)–C(7)	108.3(2)	N(7)–N(6)–C(28)	108.2(2)
C(9)–N(2)–N(1)	109.3(2)	C(30)–N(7)–N(6)	109.6(2)
N(1)–C(7)–C(8)	106.9(2)	N(6)–C(28)–C(29)	105.4(2)
N(2)–C(9)–C(8)	109.0(2)	N(7)–C(30)–C(29)	109.3(2)
C(9)–C(8)–C(7)	106.4(2)	C(30)–C(29)–C(28)	107.3(2)
N(3)–C(11)–C(8)	127.4(2)	N(8)–C(32)–C(29)	125.3(2)
C(8)–C(11)–C(12)	118.9(2)	C(29)–C(32)–C(33)	118.8(2)
N(3)–C(11)–C(8)	127.4(2)	N(8)–C(32)–C(29)	125.3(2)
C(8)–C(11)–C(12)	118.9(2)	C(29)–C(32)–C(33)	118.8(2)
N(3)–C(11)–C(12)	113.7(2)	N(8)–C(32)–C(33)	115.9(2)

H-bonds: N2 H2N S1 0.869(9) 2.390(10) 3.256(2) 175(2) 3_756. N4 H4N O1 0.863(9) 1.916(12) 2.732(2) 157(2). N5 H5N S2 0.859(10) 2.835(16) 3.551(2) 141.9(19). N7 H7N O1 0.870(9) 1.847(10) 2.710(2) 171(2) 4_566. N9 H9N O2 0.875(10) 1.963(14) 2.781(3) 155(2).

the sunlight, we obtained the yellowish transparent crystal of PM4MBP-ETSC. The molecular structure, atomic labeling and possible configuration of the compound are shown in Figures 4 and 5. The crystal data and structure refinement details are given in Table 1. Selected bond lengths and bond angles are compiled in Table 2.

The crystal structure of PM4MBP-ETSC is composed of two crystallographically independent molecules (marking them as Molecule A and Molecule B), stabilized by intermolecular hydrogen bonds N(5)-H···S(2) (3.551 Å). A dimer formed and displayed in an asymmetric way (Fig. 4). X-ray crystallographic study reveals that the pyrazolone-ring is the 'NH' form (the keto tautomer is favoured over the enol form for this compound) [17,18] according to the distances of C(9)–N(2) (1.344 Å) and C(30)–N(7) (1.331 Å). The bond distances shown in Table 2 indicate that C(19)–S(1) (1.694 Å) and C(40)–S(2) (1.664 Å) distances are in between that of C–S single bond (1.82 Å) and that of C═S double bond (1.56 Å).[19] Like the C═S bond, all the other bonds lengths have some double bond and some single bond properties (show a bond equalization), which identifies that in this compound, there are a big electron delocalization around pyrazolone-ring and thiosemicarbazide moiety. Although, the bond lengths of Molecules A and B have some variations, the corresponding angles differ little.

The compound is non-planar and corresponding dihedral angles of different moieties about Molecules A and B are shown in Table 3. From Table 3, we can see the corresponding dihedral angles of Molecules A and B differ greatly. It was concluded that the significant differences are related to the manner of molecular packing in the crystal, which may be attributed to the different hydrogen bond requirement about the two molecules and make the flat arrangement unfavorable. Another factor acting against planarity is the steric repulsion due to the presence of 4-acyl substitution group of pyrazolone-ring.

For these two independent molecules, crystal structure reveals an unusual hydrogen bonding configuration. The asymmetric distribution of the two molecules in the unit cell results in the variety hydrogen bonding modes and would be expected to increase the strength of intermolecular hydrogen bond, so do the different C═S and C═O bond lengths correspondingly. Structural analysis indicates the existence of both N(4)–H···O(1) (2.732 Å, 157°) and N(9)–H···O(2) (2.781 Å, 155°) intramolecular hydrogen bonds at the same position of the two molecules, but one has the N(2)–H···S′ (3.256 Å, 175°) intermolecular hydrogen bond while another has the N(7)–H···O″ (2.710 Å, 171°) bond (′ and ″ represent the different molecules). These distances are significantly shorter than the sum of Vander Waals' radii for corresponding atom.[20] The assemblies of this general type tend to adopt a structure that maximises as an infinite three-dimensional configuration like a swirl (Fig. 5). The preference of PM4MBP-ETSC form has been explained by several factors, including better linearity of hydrogen bonds and the stabilizing influence of stacking interactions.

From the structural analysis above, it is concluded that, after irradiation, the white product was converted into the yellowish products, which is the keto form. So certain reaction must have taken place during the irradiation process. The color change of the compound may be due to the configuration transformation between the two isomers, as in this compound, the enol and keto form of the pyrazolone-ring. That's to say, a proton transfer reaction between the two tautomers must take place.

Table 3. Dihedral angles (°) for PM4MBP-ETSC

Molecule A: plane (mean deviation from plane)	I	II	III
I C(7)C(8)C(9)N(2)N(1) (0.0135 Å)			
II C(1)C(2)C(3)C(4)C(5)C(6) (0.0075 Å)	31.6		
III C(12)C(13)C(14)C(15)C(16)C(17) (0.0186 Å)	111.5	142.7	
IV N(4)N(3)C(19)N(5) (0.0288 Å)	35.3	14.9	138.4
Molecule B: plane (mean deviation from plane)	I′	II′	III′
I′ C(28)C(29)C(30)N(6)N(7) (0.0179 Å)			
II′ C(22)C(23)C(24)C(25)C(26)C(27) (0.0097 Å)	23.7		
III′ C(33)C(34)C(35)C(36)C(37)C(38) (0.011 Å)	107.0	83.9	
IV′ N(8)N(9)C(40)N(10) (0.0572 Å)	124.7	101.8	18.0

Excited-state intra- and inter-molecular proton transfer reactions have received considerable attention both experimentally and theoretically. Molecules, which contain two vicinal (proton donating and proton accepting) groups, are likely to have excited state proton transfer reaction. This process involves transfer of hydroxyl proton from the donor group to an acceptor such as carbonyl oxygen or nitrogen atom in the excited state. This may occur when increasing the acidity of the hydroxyl group and basicity of nitrogen or oxygen atom on ring in the excited state.[21] There have been many studies of proton transfer in the solid-state and in solution state. *N*-salicylideneanilines are an interesting class of compounds undergoing intramolecular proton transfer in the solid-state.[22,23] In studies of solid-state photochemistry, it has been recognized that the topochemical reactions among neighboring molecules and atoms dictate the outcome of photochemical excitation. It seems that the crystal structure determines the photochromic behaviour, rather than the molecule as such. The molecular packing manner in the lattice determines the proton transfer modes.[24] In the planar molecules of PM4MBP-ETSC, the lone pair electrons of the nitrogen does not overlap with the π electrons of the pyrazolone-ring whereas in the non-planar structure such overlap is possible and consequently the basicity of the nitrogen and hence, the strength of the N–H bond is greater in the non-planar. It is clear that excited state proton transfer can take place from the hydroxyl moiety of one molecule to the pyrazolone-ring N atom of the H-bonded partner (due to the nature of the crystal structure). The subsequent loss of hydroxide ion will necessarily be assisted by the partner as well. To explain, one can suggest that intermolecular proton transfer along with intramolecular proton transfer takes place in PM4MBP-ETSC. The increasing electron density caused by electron delocalization of intermolecular hydrogen bonds and the linear arrangement of the intermolecular hydrogen bonds led to a strengthened intermolecular H-bond and facilitating the excited state proton transfer.[25]

By comparing the intermolecular hydrogen bond lengths and angles with that of the intramolecular hydrogen bond of Schiff bases,[22,23,26] which have excited state intramolecular proton transfer through hydrogen bond, we found that in our compound, hydrogen bond length is shorter than the corresponding sum of Vander Waals' radii but slightly longer than Schiff bases' hydrogen bond length, and the bond angles are inclined to 180°. This enables the direct tunneling of the proton with lower energy barrier. Because of the N(5)–H···S(2) hydrogen bond effect, the thione-form of the compound tautomerized to thiol-form (I⇔II(II′), there is a little difference between II and II′ because of the different hydrogen bonds mode) (Scheme 2). For one molecule, by intermolecular proton transfer from SH to N′(2) along with intramolecular proton transfer from pyrazolone-ring-OH to N(4), II transformed to form III. For another molecule, only by intermolecular protons transfer from SH to N″(5) and pyrazolone-ring-OH to pyrazolone-ring N‴(7) (′, ″, ‴ represent different molecules), form II′ tautomerized to form III. The different hydrogen bond mode determines the different proton transfer modes, but the final compound is the same. Scheme 2 gives the corresponding tautomerization reaction of the two modes.

Acknowledgements

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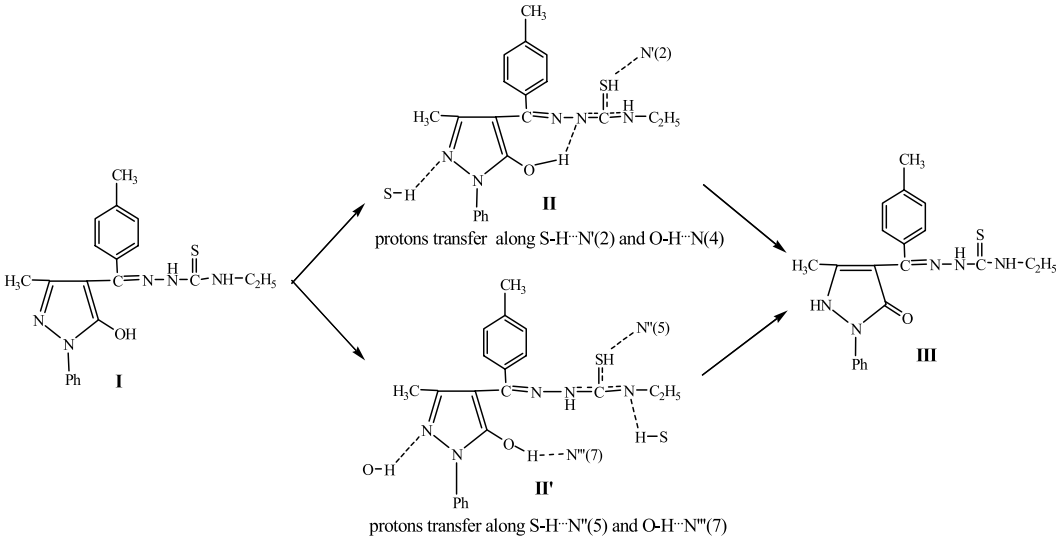
References and notes

1. Sun, X. D.; Fan, M. G.; Meng, S. J.; Knobbe, E. T. *J. Photochem. Photobiol., A: Chem.* **1997**, *102*, 213–216.
2. Kim, S. H.; Suh, H. J.; Cui, J. Z.; Gal, Y. S.; Jin, S. H.; Koh, K. *Dyes and Pigments* **2002**, *53*, 251–256.
3. Ziane, O.; Casalegno, R.; Corval, A. *Chem. Phys.* **1999**, *250*, 199–206.



Scheme 2. Tautomerization reaction of two different modes about the two crystallographically molecules in the crystal unit cell (′, ″, ‴ represent different molecules).

4. Irie, M.; Lifka, T.; Kobatake, S.; Kato, N. *J. Am. Chem. Soc.* **2000**, *122*, 4871–4876.
5. Kato, T.; Kihara, H.; Ujiie, S.; Uryu, T.; Fréchet, J. M. J. *Macromolecules* **1996**, *29*, 8734–8739.
6. Cubero, E.; Orozco, M.; Hobza, P.; Luque, F. J. *J. Phys. Chem. A* **1999**, *103*, 6394–6401.
7. Lipkowski, P.; Koll, A.; Karpfen, A.; Wolschann, P. *Chem. Phys. Lett.* **2002**, *360*, 256–263.
8. Georgieva, I.; Binev, D.; Trendafilova, N.; Bauer, G. *Chem. Phys.* **2003**, *286*, 205–217.
9. Alarcón, S. H.; Olivieri, A. C.; Labadie, G. R.; Cravero, R. M.; Sierra, M. G. *Tetrahedron* **1995**, *51*, 4619–4626.
10. Herbich, J.; Kijak, M.; Luboradzki, R.; Gil, M.; Zielińska, A.; Hu, Y. Z.; Thummel, R. P.; Waluk, J. *J. Photochem. Photobiol., A: Chem.* **2002**, *154*, 61–68.
11. Ledesma, G. N.; Ibanñez, G. A.; Escandar, G. M.; Olivieri, A. C. *J. Mol. Struct.* **1997**, *415*, 115–121.
12. Jensen, B. S. *Acta Chem. Scand.* **1959**, *13*, 1668–1670.
13. Bénard, S.; Yu, P. *Chem. Commun.* **2000**, 65–66.
14. Tang, X. C.; Jia, D. Z.; Liang, K.; Zhang, X. G.; Xia, X.; Zhou, Z. Y. *J. Photochem. Photobiol., A: Chem.* **2000**, *134*, 23–29.
15. Li, Y. T.; Zhou, J. W.; Wang, Y. Q.; Zhang, F. S.; Song, X. Q. *J. Photochem. Photobiol., A: Chem.* **1998**, *113*, 65–72.
16. Zheng, X. J.; Zhuang, J. Y.; Jin, L. P.; Wang, Z. M.; Yan, C. H.; Zhu, L. G.; Mei, Y. H. *J. Mol. Struct.* **2001**, *595*, 201–208.
17. Holzer, W.; Claramunt, R. M.; Torralba, M. P.; Guggi, D.; Brehmer, T. H. *J. Org. Chem.* **2003**, *68*, 7943–7950.
18. Kataeva, O. N.; Gubaidullin, A. T.; Litvinor, I. A.; Lodochnikova, O. A.; Islamor, L. R.; Movchan, A. I.; Chmutova, G. A. *J. Mol. Struct.* **2002**, *610*, 175–179.
19. Jouad, E. M.; Allain, M.; Khan, M. A.; Bouet, G. M. *J. Mol. Struct.* **2002**, *604*, 205–208.
20. Grabowski, S. J. *J. Phys. Chem. A* **2001**, *105*, 10739–10746.
21. Mehata, M. S.; Joshi, H. C.; Tripathi, H. B. *Chem. Phys. Lett.* **2002**, *359*, 314–320.
22. Kobayashi, T.; Sekikawa, T.; Inabe, T. *J. Lumin.* **1997**, *72*, 508–510.
23. Űnver, H.; Kabak, M.; Zengin, D. M.; Durlu, T. N. *J. Chem. Crystallogr.* **2001**, *31*, 203–209.
24. Hadjoudis, E.; Dziembowska, T.; Rozwadowski, Z. *J. Photochem. Photobiol., A: Chem.* **1999**, *128*, 97–99.
25. Denisov, G. S.; Golubev, N. S.; Schreiber, V. M.; Shajakhmedov, S. S.; Shurukhina, A. V. *J. Mol. Struct.* **1997**, *436*, 153–160.
26. Kabak, M. *J. Mol. Struct.* **2003**, *655*, 135–139.

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Enzymatic synthesis and bioactivity of estradiol derivative conjugates with different amino acids

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Abstract—A series of *N*-protected amino acid-estradiol derivative conjugates have been synthesized by coupling of 17β-aminoestra-1,3,5 (10)-trien-3-ol (**1**) or 17β-hydrazonoestra-1,3,5 (10)-trien-3-ol (**2**) with different amino acids via the catalysis of subtilisin Carlsberg in organic solvent. Various factors, including the structure of amino acid residue, different *N*-protecting groups of amino acids, different esters of carboxyl group and water content of the reaction media that influence the efficiency of enzymatic reactions were systematically studied. In vitro biological activity studies revealed that the binding interactions between estradiol derivative conjugates and estrogen receptor can be affected by the properties of the conjugated amino acid, but the effects of the change in binding properties did not result in changes in biological activities in both MCF-7 and HeLa cell lines.

1. Introduction

Amino acid-steroidal conjugates include such a class of compounds in, which the amino acids are linked with steroids through amide or ester bonds. This class of conjugates covers a spectrum of important molecules in nature, such as bufetoxin, a 3-arginyl derived steroid product isolated from the Chinese hoptoad; cholyl glycine and cholyl taurine, which exist in the bile of animals and contains a glycyl or a taurinyl group at 17 position of steroid; polymastiamide A,[1] a tyrosine conjugated steroid analog isolated from the Norwegian marine sponge *polymastia boletiformis* as well as a starfish steroid in which a 24-carboxylic acid functionality is linked via an amide bond to D-cysteinolic acid.[2] These compounds have been found to play a diversity of critical roles in a large number of organisms. Introduction of the amino acid or peptide to the steroid backbone offers a combination of a hydrophilic functional moiety as well as a hydrophobic carrier in a same molecule and therefore represents as an important class of molecules for drug development. A wide variety of steroid derivatives conjugated with amino acids or peptides have been prepared with the purposes to enhance the oral antiarrhythmic activity,[3] to help delivering the prodrugs to specific target tissues,[4] as well as to achieve the 'permissive action'.[5]

Previous studies on steroidal derivatives have indicated that an unblocked 3-oxygenated group that should maintain as a carbonyl or a hydroxyl group is required to exhibit high binding affinities with their receptors.[6,7] As a consequence, we focused our interests on the modification of the other functional groups on steroid matrix rather than the 3 position. In the previous paper, a series of 17β-amino or 17β-hydrazono steroidal derivatives have been synthesized using a variety of chemical coupling reagents and the relative binding affinities of the deprotected derivatives to the estrogen receptor were examined by competitive radioligand binding assay.[8] Recently, we focus our attention on enzymatic reactions in organic media owing to its noticeable advantages over the chemical method, such as enzymatic coupling usually can be performed under mild conditions, displays high regio- and stereoselectivity, requires minimal protection and is generally racemization free. Moreover, most organic compounds are soluble in organic solvents and enzymes are stable in organic media,[9]

Keywords: Conjugate; Enzymatic synthesis; Chemoselectivity; Steroids; Estrogen receptor.
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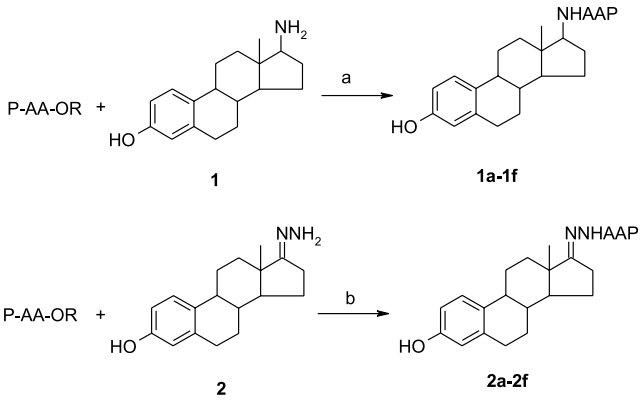
therefore, the formation of peptide bond is favorable over the hydrolysis of the product in organic media.[10–12] A large number of bioactive peptides have been synthesized via the catalysis of different proteases in organic media and the factors that influence the enzymatic peptide bond formation have been investigated by us and others.[12–15] However, at present, proteases are limited in the synthetic application in terms of their substrate specificity. In order to extend the synthetic applications of proteases in organic media, a number of approaches such as medium-engineering, substrate engineering, enzyme engineering have been developed.[16–19] In an earlier study, we have extended the nucleophilic substrates of proteases to amino alcohols and a series of *N*-protected peptide alcohols have been generated with the catalysis of subtilisin Carlsberg or α-chymotrypsin.[15] In this paper, we present our exploration of utilizing two steroid derivatives, 17 β-aminoestra-1, 3, 5 (10)-trien-3-ol **1** or 17 β-hydrazonoestra-1, 3, 5 (10)-trien-3-ol **2**, as the nucleophilic substrates of subtilisin Carlsberg to synthesize a series of *N*-protected amino acid-estradiol conjugates in an organic solvent. Various factors, which influence the catalytic efficiency were studied and the optimum reaction conditions were systematically investigated. Our results demonstrate that steroid derivatives **1** and **2** can be applied as nucleophilic substrates for subtilisin in organic media under certain reaction condition. The synthetic strategy was briefly reported previously by us.[20]

Incorporation of amino acids with 17β-amino or 17β-hydrazono estra-1, 3, 5 (10)-trien-3-ol (**1** and **2**) resulted in a series of molecules, which is analogous to estradiol, an endogenous estrogen hormone. Previous efforts to develop novel anti-estrogenic agents have resulted in several estradiol derivatives, which contain the modifications on 7α or 11β position, such as ICI 164387, ICI 182780, RU 39411, RU 58668[21] whereas no systematic studies on the 17 β-derivatives were examined so far. Thus, it is interesting to measure the effects of the synthesized compounds on the estrogen receptor (ER) positive cells with regard to their agonistic or antagonistic activities. The MCF-7 and HeLa cell lines[22a,b] were chosen in this paper to analyze the estrogen like activities of these synthetic analogues as well as to determine their abilities to activate ER dependent gene transcription. In addition, the binding affinities of selected synthesized compounds towards estrogen receptor were determined by competitive receptor binding assay.

2. Results and discussion

In order to generate a nucleophile moiety at the 17 position of steroid backbone, we started with estrone and derived its 17-carbonyl group to amine (compound **1**) or hydrazono (compound **2**) as described previously.[8] Because these two compounds are not the natural neucleophilic substrates of proteases, subtilisin Carlsberg, which has been shown to display flexible specificity for its P'_1 residue was utilized and the kinetic approach, which is usually faster than thermodynamic one[10] was employed in our study. The reaction was carried out in DMF in the presence of certain amount of essential water[23] as demonstrated in Scheme 1. Various factors, which affect the efficiency of enzymatic



Scheme 1. Schematic representative of the enzymatic coupling reaction between a variety of *N*-protected amino acid esters and **1** or **2** carried out in DMF in the presence of certain amount of essential water. (a), (b) Subtilisin Carlsberg, DMF, 8% water (v/v); P=Z, Boc or Fmoc; R=Me, Et, *t*Bu, Ph, Bzl, Cam, or CH_2CF_3; AA=Ala, Leu, Phe, Asp(OBzl), Asp, or Lys(*Z*).

reactions (P, AA, R etc.) were also shown in this scheme and they were examined subsequently.

2.1. *N*-Protected amino acid trifluoroethyl ester is the best acyl donor among various esters

Since the kinetic approach requires the use of amino acid esters as the acyl donor substrates, we first analyzed the influence of different esters in the acyl donor component for their feasibility to be coupled with substrate **1** or **2** under the catalysis of subtilisin Carlsberg. As shown in Table 1, among the various esters we analyzed, the trifluoroethyl ester of *Z*-protected alanine acts as the best acyl donor. The *t*-butyl ester, phenyl ester, benzyl ester and Cam(Carboxamidomethyl)ester also displays activation effects, yet not as good as trifluoroethyl ester, whereas methyl or ethyl ester as acyl donor substrate does not yield any detectable product. This result was expected because a strong electron withdrawing group, trifluoroethyl, causes electron deficiency on the carbonyl group. As a result, the free energy required to form the acyl donor-enzyme intermediate, which is believed as the prerequisite for the subsequent aminolysis, is diminished so that the enzymatic coupling becomes achievable.[24] The IR spectrum of the carbonyl group in the trifluoroethyl esters, which shows a shift to high move number (1771 cm^{-1}) compared with the carbonyl group in an unactivated ester (1723–1747 cm^{-1})

Table 1. Coupling of compound **1** or **2** with different *Z*-Ala-OR catalyzed by subtilisin Carlsberg in DMF

Acyl donor	Nucleophile	Product	Yield (%)
Z-Ala-OMe (**6a**)	**1**	**1a**	0
Z-Ala-OEt (**6b**)	**1**	**1a**	0
Z-Ala-OBu^t (**6c**)	**1**	**1a**	Trace
Z-Ala-OPh (**6d**)	**1**	**1a**	18.7
Z-Ala-OBzl (**6e**)	**1**	**1a**	Trace
Z-Ala-OCam (**6f**)	**1**	**1a**	32.2
Z-Ala-OCH_2CF_3 (**6g**)	**1**	**1a**	35.0
Z-Ala-OMe (**6a**)	**2**	**2a**	0
Z-Ala-OEt (**6b**)	**2**	**2a**	0
Z-Ala-OBu^t (**6c**)	**2**	**2a**	22.3
Z-Ala-OPh (**6d**)	**2**	**2a**	21.5
Z-Ala-OBzl (**6e**)	**2**	**2a**	Trace
Z-Ala-OCam (**6f**)	**2**	**2a**	36.9
Z-Ala-OCH_2CF_3 (**6g**)	**2**	**2a**	56.0

Table 2. Effect of different protecting groups on the yields of coupling products

Entry	Acyl donor	Nucleophile	Product	Yield (%)
1	BocAlaOCH$_2$CF$_3$ (**5b**)	**1**	**1b**	77.0
2	Z-AlaOCH$_2$CF$_3$ (**6g**)	**1**	**1a**	35.0
3	FmocAlaOCH$_2$CF$_3$ (**7a**)	**1**	**1c**	0
4	BocAlaOCH$_2$CF$_3$ (**5b**)	**2**	**2b**	87.0
5	Z-AlaOCH$_2$CF$_3$ (**6g**)	**2**	**2a**	56.0
6	FmocAlaOCH$_2$CF$_3$ (**7a**)	**2**	**2c**	40.0

also supports the proposal. However, it is noticeable that such activation effect of trifluoroethyl esters is not sufficient to provide the free energy required to form the amide bond without the catalysis of subtilisin. For an instance, we carried out the control reaction, that is, the reaction between compound **2** and Boc-Ala-OCH$_2$CH$_3$ (**5b**) without enzyme added, the result was negative and we did not get the desired product (**2b**). Our previous studies demonstrated that the methyl or ethyl esters of corresponding amino acids or peptides acted as attainable substrates for proteases catalyzed native peptide bond formation in organic media,[14,25] but they are not acceptable in the current study when non-natural substrates of proteases act as acyl acceptors. This result implied that under special reaction conditions, subtilisin could accommodate steroid derivative as its acyl acceptor substrate to catalyze the non-peptidyl amide bond formation in organic solvent, however, to compensate for the unfavorable effects of the weak nucleophilic substrates, strong electron withdrawing ester is required in acyl donor substrate.

2.2. Boc- is more favorable than *Z*- or Fmoc- moiety as the *N*-protecting group in the acyl donor substrate in the subtilisin catalyzed non-typical peptide bond formation

To avoid the unexpected coupling between amine and carboxyl groups in a same molecule, which results in oligomerization, the amine group in the amino acid ester usually is blocked by selective protecting groups during the coupling procedure. We then investigated the effect of diverse *N*-protecting groups on the enzymatic reactions. As revealed in Table 2, Boc is more favorable as the amino protecting group for the acyl donor than *Z* or Fmoc both in the presence of compound **1** and **2** as an acyl acceptor. This observation is consistent with the suggestion that the S_2 binding pocket of subtilisin prefers to a small, hydrophobic moiety.[26] Notably, under the circumstance when a poor hydrophilic component **1** (see discussion below) acted as an acyl acceptor and an unfavorable *N*-protecting group was placed in the acyl donor (Fmoc), the capability of subtilisin to catalyze the aminolysis was depleted or if any, was undetectable (see entry 3 in Table 2).

2.3. Compound 2 is a better acyl acceptor than Compound 1 in the subtilisin catalyzed non-peptidyl amide bond formation

It was traditionally believed that proteases act on the amide bond formed by natural amino acids, hydrolysis in physiological condition and aminolysis in organic solvent. However, we have shown that under particular condition, subtilisin is able to accommodate non amino acid component, steroid derivatives, **1** or **2** as its acyl acceptor substrates. As demonstrated both in Tables 1 and 2, **2** behaves as a preferable nucleophile than **1** in the enzymatic reactions we studied. This result is consistent with the proposal that despite that reaction rate is determined mainly by the specificity of the enzyme toward the acyl donor, a specific binding of the nucleophile to the S′ subsite of the protease is crucial for high yields.[12] The preference of the compound **2** as an acyl acceptor probably is the consequence of both electronic and stereo effect of hydrozono group. The electron accumulation on the nucleophilic moiety in **2** due to the conjugating effect between two nitrogen atoms and double bond of N═C in hydrazono group obviously favors nucleophilic attack. On the other hand, the nucleophilic moiety of amino group in **2** is not directly attached to the bulk steroid backbone thereby is more accessible to the acyl donor-enzyme complex in the process of aminolysis compared with the amino group in **1**.

2.4. The optimum reaction condition

It was believed that although most of the proteases are stable in non-aqueous media, certain amount of water (essential water) is required to maintain their catalytic conformation.[9] On the other hand, however, excess of water in the system will cause the undesired hydrolysis of the product. Hence, there must be an optimum water content range within which high yield of the coupling product could be achieved. We subsequently examined the optimum water content of the reaction using the condensation of Boc-Ala-OCH$_2$CF$_3$ (**5b**) and **1** as model reaction and found that the optimum water content is 8% (V/V) (Fig. 1). As expected, without water or low water content in the system led to a complete depletion of the catalytic activity of subtilisin whereas when the water content of the system is higher than 8%, the yield of the coupling product declined again due to the side effect of

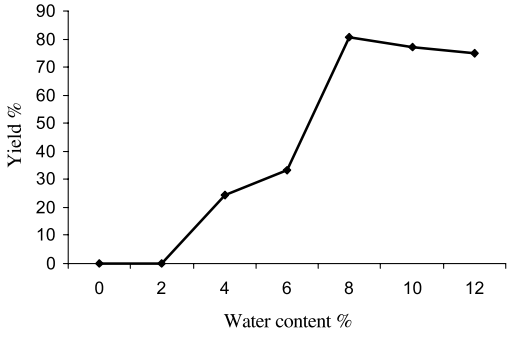


Figure 1. Effect of water content on the enzymatic reaction. Using the coupling between **1** (0.2 mmol) and **5b** (0.2 mmol) as the model reaction, 0.0, 0.06, 0.12, 0.18, 0.24, 0.30 and 0.36 mL phosphate buffer (KH_2PO_4-$\sim Na_2HPO_4$, 50 mmol/L, pH 7.73) were added to certain amount of anhydrous DMF to the total volume of 3 mL. The reaction was carried out at 40 °C oil bath for 48 h and the product was purified by column chromatography or preparative TLC.

hydrolysis (Fig. 1). We also examined the effect of molar ratio between two reactive components on the yield of coupling product using the same model reaction and found that the molar ratio of acyl donor versus acyl acceptor of 2:1 resulted in highest yield (Table 3).

Table 3. Effect of molar ratio of acyl donor versus acyl acceptor on the yield of the coupling product

Boc-Ala-OCH_2CF_3: compound **1**	1:2	1:1	2:1
Product	**1b**	**1b**	**1b**
Yield (%)	83.4	77.0	88.0

2.5. Characterization of the S_1 binding pocket of subtilisin under the reaction conditions studied

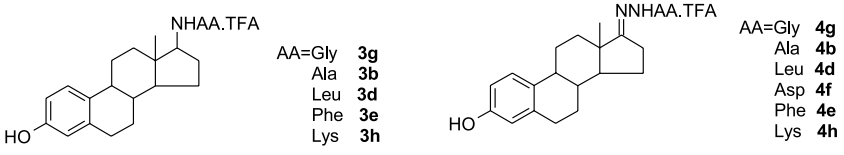
To study whether the non-native acyl acceptor affect the substrate selectivity and regioselectivity of subtilisin in its S_1 position in the reactions studied, we synthesized a series of amino acid trifluoroethyl esters, which bear a variety of different side chains as the acyl donors to be reacted with **1** or **2**. As listed in Table 4, among the various acyl donor substrates, the one contains alanine residue, which bears a small, alkyl side chain in the P_1 position resulted in highest yield (entries 1 and 7). Longer alkyl residue of the leucyl side chain (entries 2 and 8) or bulk aromatic residue of the phenylalaninyl side chain (entries 3 and 9) in this position led to poor yield of the corresponding product. Particularly, when the side chain of aspartic acid was blocked by benzyl group, subtilisin was incapable to accept it as an acyl donor substrate. However, when the side chain was deblocked, the corresponding α-carboxyl active ester becomes to be recognized by the enzyme. A moderate coupling yield was obtained when **1** acts as the acyl donor and a significant improvement of the yield was achieved when **2** acts as the acyl donor, which is in agreement with the proposal that **2** acted as a better acyl acceptor than **1**. It is noteworthy that only α-carboxyl group rather than β-carboxyl group in Asp residue was selected to be condensed with the nucleophiles indicating that the regioselectivity of subtilisin was maintained under the conditions we studied. In the case of using Boc-Lys(*Z*)-OCH_2CF_3 as an acyl donor, which bears a bulk moiety in its P_1 position, no coupling product was detected even in the presence of a favorable acyl acceptor substrate **2**. Therefore, we speculate that the S_1 binding pocket of subtilisin favors a small, acidic moiety rather than a bulk, basic one under the reaction conditions studied.

2.6. In vitro estrogen like activity of the synthetic amino acid-steroid conjugates

Since the amino acid-steroid conjugates we synthesized are analogs of estradiol, an endogenous hormone, we removed the artificial *N*-protecting groups on amino acid moieties (Scheme 2) and measured their abilities to stimulate cell proliferation in ER positive human breast cancer (MCF-7) cell line. As revealed in Figure 2, 10^{-9} M of all synthetic conjugates, except for the lysine conjugates (**3h** and **4h**), significantly increased [^{3}H]-methylthymidine incorporation in MCF-7 cells ($p<0.05$). To determine whether the up-regulation of thymidine incorporation by the analogues is mediated via the ER, cells were incubated with different analogues in the presence or absence of the estrogen antagonist ICI 182780. ICI 182780 (10^{-6} M) completely blocked the increase in [^{3}H]-methylthymidine incorporation in MCF-7 by all synthetic conjugates and 17-β-estradiol, suggesting that their effects on MCF-7 cell proliferation are mediated through ER. Several conjugates with amino acids of different hydrophilicity (Leu, Phe, Lys) were chosen to test for their abilities to induce ER dependent activation of luciferase gene transcription in HeLa cells. Figure 3 indicated that all chosen synthetic 17β-derivatives of estradiol increased ERE-dependent luciferase activities and that these activities can be blocked by co-treatment with ICI 182780. However, it appears that the hydrophilicity of amino acid in these conjugates did not affect

Table 4. Coupling of various of acyl donors bearing different amino acid residues at P_1 position with **1** or **2** under the catalysis of subtilisin Carlsberg

Entry	Acyl donor	Acyl acceptor	Product	Yield (%)
1	Boc-Ala-OCH_2CF_3 (**5b**)	**1**	**1b**	88.0
2	Boc-Leu-OCH_2CF_3 (**5d**)	**1**	**1d**	32.3
3	Boc-Phe-OCH_2CF_3 (**5e**)	**1**	**1e**	22.3
4	Boc-Asp(OBzl)-OCH_2CF_3 (**5f–1**)	**1**	—	—
5	Boc-Asp-OCH_2CF_3 (**5f**)	**1**	**1f**	40.0
6	Boc-Lys(*Z*)-OCH_2CF_3 (**5h**)	**1**	—	—
7	Boc-Ala-OCH_2CF_3 (**5b**)	**2**	**2b**	89.0
8	Boc-Leu-OCH_2CF_3 (**5d**)	**2**	**2d**	49.0
9	Boc-Phe-OCH_2CF_3 (**5e**)	**2**	**2e**	32.6
10	Boc-Asp(OBzl)-OCH_2CF_3 (**5f–1**)	**2**	—	—
11	Boc-Asp-OCH_2CF_3 (**5f**)	**2**	**2f**	80.0
12	Boc-Lys(*Z*)-OCH_2CF_3 (**5h**)	**2**	—	—



Scheme 2. Structures of a series of amino acid-steroid conjugates for bioactivity assay.

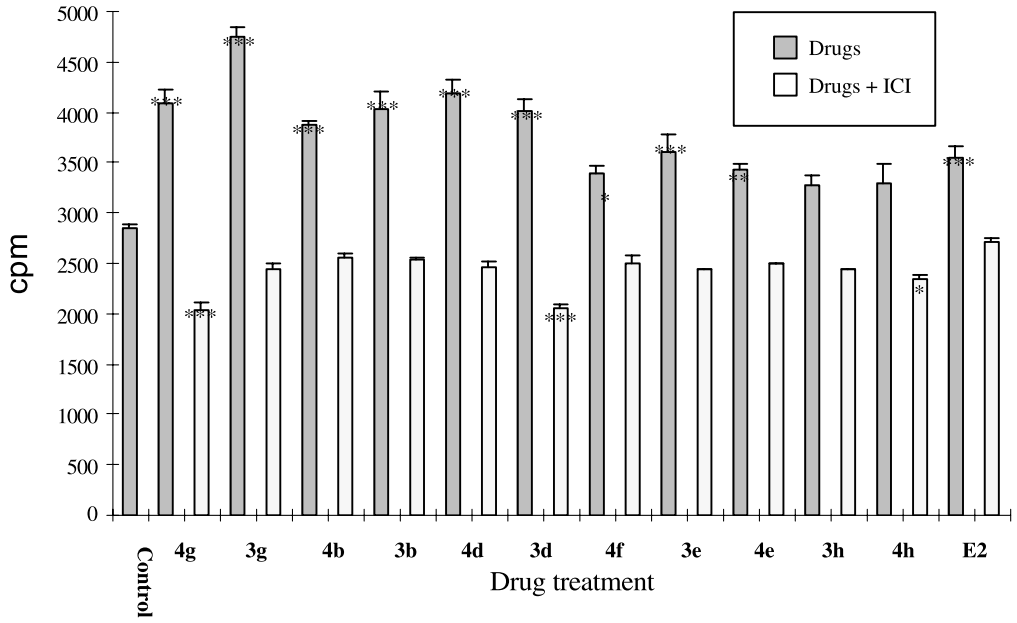


Figure 2. HeLa cell-based ER-luciferase reporter gene system was treated with 10^{-9} M estradiol and different estradiol analogues with or without 10^{-6} M pure estrogen-receptor antagonist. Arbitrary amount of light emitted was measured after substrate luciferin was added. Data represented mean ± SEM, for $n=3$. Mean difference was compared by one-way ANOVA and followed by Tukey's Post Test. The effect of each individual E_2 and its analogues treatment was significantly blocked by ICI 182780 ($^{\#}p<0.001$); $^{*}p<0.001$ versus control.

their abilities to stimulate ERE-dependent luciferase activities.

2.7. Difference in binding affinities of selected synthetic amino acid-steroid conjugates towards estrogen receptor

We then determined the binding affinities of several conjugates towards human estrogen receptor alpha using competitive binding assay. As shown in Figure 4, increasing concentration of estradiol or its derivative conjugates displaced the binding of radiolabel estradiol. The calculated EC50 for 17-estradiol, **3g**, **3d** and **3e** are 4 nM, 5.8 nM, 1.7 μM and 13.9 nM, respectively. The order of affinity for ER is: $E_2 > \mathbf{3g} > \mathbf{3e} \gg \mathbf{3d}$. These results are in agreement with our previous studies[8] that E_2 has a much stronger affinity toward ER than **3d** does. These results also indicated that estradiol derivative conjugated with Leu has a much lower affinity for ER than conjugates with Gly or Phe. Thus, the degree of bulkiness of the amino acid in estradiol

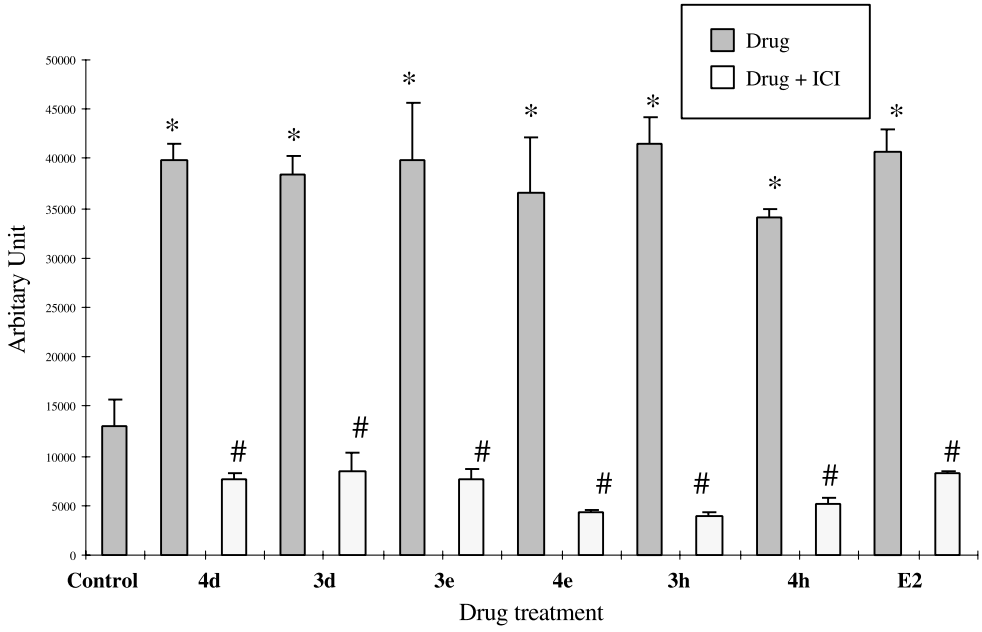


Figure 3. HeLa cell-based ER-luciferase reporter gene system was treated with 10^{-9} M estradiol and different estradiol analogues with or without 10^{-6} M pure estrogen-receptor antagonist. Arbitrary amount of light emitted was measured after substrate luciferin was added. Data represented mean ± SEM, for $n=3$. Mean difference was compared by one-way ANOVA and followed by Tukey's Post Test. The effect of each individual E_2 and its analogues treatment was significantly blocked by ICI 182780 ($^{\#}p<0.001$); $^{*}p<0.001$ versus control.

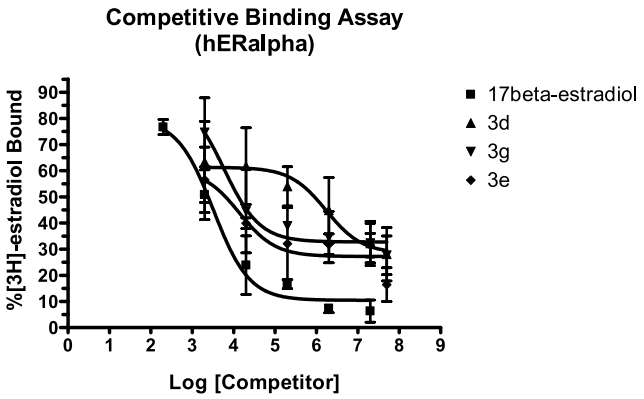


Figure 4. Competitive ER binding assay. Receptor binding assays were carried out as described in methodology. The percent [3H]-estradiol bound was calculated based on the equation: 100%(TB-NSB/TB). The data are the mean of triplicate determinations, and the experiment was repeated twice. The displacement of [3H]-estradiol increases as concentration of non-radiolabeled estradiol or conjugated estradiol derivative analog increases.

derivative conjugates might affect the binding interactions with ER.

Taken together, these results seem to indicate that even though these compounds have different affinities for ER, they have similar abilities to activate human breast cancer cell proliferation as well as ERE-dependent transcription of luciferase gene in HeLa cells. One explanation for this could be the high sensitivity of both MCF-7 cell as well as luciferase gene construct in HeLa cell to activated estrogen receptor. In other word, even though their abilities to bind ER are weaker than E_2, all the compounds demonstrate sufficient binding to ER for subsequent activation of MCF-7 cell growth and ERE-dependent gene transcription.

3. Conclusion

In this paper, we have successfully extended the application of subtilisin Carlsberg to the catalysis of non-peptidyl amide bond formation. 17 β-aminoestra-1,3,5 (10)-trien-3-ol **1** or 17 β-hydrazonoestra-1, 3, 5 (10)-trien-3-ol **2** are compatible nucleophilic substrates of subtilisin at P'_1 position in DMF with 8% water (V/V). *N*-protected amino acid trifluoroethyl ester is the best acyl donor among various esters, and Boc- is more favorable than *Z*- or Fmoc- moiety as the *N*-protecting group in the acyl donor substrate. Compound **2** is a better acyl acceptor than Compound **1** in the subtilisin catalyzed non-peptidyl amide bond formation. Probing the selectivity of the S_1 binding pocket of subtilisin indicates that this subsite prefers to a small, acidic moiety rather than a bulk, basic one under the reaction conditions in this study. Most importantly, when Boc-Asp-OCH_2CF_3 (**5f**) as an acyl donor in which the side chain β-carboxyl group was not protected, only α-carboxyl group rather than β-carboxy group was selected to be reacted with the nucleophiles indicating that the high regioselectivity of the enzymatic synthesis in organic media was maintained. However, the substrate selectivity of the enzyme on its S_1 position has led to moderate yields in the cases of those amino acids, which bear bulk side chains, partially limited its extensive application in routine organic synthesis. This problem may be solved by modern molecular biology tools. Our results provide helpful information for expanding the proteases catalyzed amide bond formation to routine organic synthesis in the future. The in vitro bioactivity assay indicates that all of the synthesized steroid-amino acid conjugates possess ER-dependent biological activities in human MCF-7 cell lines. The competitive binding assay indicated that the binding affinities of the conjugates for ER were indeed influenced by the structure of the conjugate amino acids. However, such hydrophobicity differences did not alter the abilities of these conjugates to induce ERE-dependent gene transcription in HeLa cells.

4. Experimental

4.1. General

Subtilisin Carlsberg, (EC 3.4.21.14, specific activity 20.3 U/mg powder) was purchased from Shanghai Dong-Feng Biochemical Technology Company. DCC, HOBt, DMAP and trifluoroethanol were supplied by Aldrich Chemical Company. All the amino acids were obtained from Nova Biochemical Company. The estrone used in this paper, was a generous gift from Prof. Guang-Dian Han in Institute of Materia Medica, Chinese Academy of Medical Sciences. Melting points were recorded by Yanaco micro melting point apparatus given without correction; Molecular masses were determined with VG-ZAB-HS, Bruker APEX™II; ^1H was recorded with Bruker ARX400 (200 and 400 MHz); Elemental analysis were performed with Carlo Erba 1106, Hezaeus CHN-Rapid, ST-02 or Elementar Vario EL (Germany); Optical rotation was analyzed by Perkin–Elmer 241 MC or 341 LC. Standard abbreviations for amino acids and protecting groups are according to the suggestion of IUPAC-IUB joint Commission on Biochemistry Nomenclature, *Eur. J. Biochem.* **1984**, *138*, 937.

4.2. Synthesis of 17β-aminoestra-1,3,5(10)-trien-3-ol (1) and 17β-hydrazonoestra-1,3,5(10)-trien-3-ol (2)

These two starting materials were prepared virtually as described in literature.[8] 17β-aminoestra-1, 3, 5 (10)-trien-3-ol (**1**), as white crystals, 52.0%, mp 235–237 °C; 17β-hydrazonoestra-1, 3, 5 (10)-trien-3-ol (**2**). As white needle crystals, 81.0%, mp 252–254 °C.

4.3. General procedure of synthesis of *N*-protected amino acid trifluoroethyl esters[27]

DCC (5 mmol) and HOBt (2.5 mmol) were added to an ice cooled solution of the *N*-protected amino acid (5 mmol) and trifluoroethanol (5 mmol) in anhydrous DMF or CH_2Cl_2 (10 mL) followed by addition of DMAP (0.5 mmol) and Et_3N drop by drop till the pH becomes 8–9. The mixture was stirred overnight at room temperature and the DMF or CH_2Cl_2 was removed in vacuo. The residue was dissolved in EtOAc (100 mL); washed with 5% citric acid, 5% $NaHCO_3$ and saturated NaCl; and dried over $MgSO_4$. The solvent was removed and the residue was recrystallized from EtOAc/hexane.

4.3.1. *Z*-Ala-OMe (6a), *Z*-Ala-OEt (6b). These two compounds were synthesized as described in literature.[28] *Z*-Ala-OMe (**6a**). As white crystals, yield 70.0%, mp 44–46 °C, $[\alpha]_D^{22}=-34.5$ ($c=0.5$ in MeOH). [Lit.[28], mp 46–47 °C, $[\alpha]_D^{20}=-36.5$ ($c=1.0$ in MeOH)]; *Z*-Ala-OEt (**6b**). As light yellow crystals, yield 83.0%, mp 25–27 °C, $[\alpha]_D^{22}=-30.2$ ($c=1.0$ in MeOH); IR ν_{max} (cm^{-1}) 3343, 3034, 1724, 1455, 1530. [Lit.[28], oil, $[\alpha]_D^{20}=-38.4$ ($c=1.0$ in MeOH)].

4.3.2. *Z*-Ala-OBu^t (6c). As colorless oil, yield 71.0%, $[\alpha]_D^{22}=-33.2$ ($c=1.2$ in MeOH); IR ν_{max} (cm^{-1}) 3341, 3066, 3034, 1722, 1455, 1527. [Lit.[29], colorless oil, 58%, $[\alpha]_D^{20}=-22.9$ ($c=1.2$ in EtOH).

4.3.3. *Z*-Ala-OPh (6d). As white crystals, yield 42.0%, mp 94.5–95.5 °C, $[\alpha]_D^{22}=-18.9$ ($c=2.1$ in $CHCl_3$); IR ν_{max} (cm^{-1}) 3356, 3062, 1690, 1700, 1771, 1485, 1527. [Lit.[30], mp 94–95 °C, $[\alpha]_D^{25}=-18.8$ ($c=2.0$ in $CHCl_3$)].

4.3.4. *Z*-Ala-OBzl (6e). As white crystals, yield 83.0%, mp 34–36 °C, $[\alpha]_D^{22}=-28.5$ ($c=1.0$ in MeOH); IR ν_{max} (cm^{-1}) 3339, 3064, 1692, 1718, 1747, 1454, 1527. [Lit.[31], mp 38–39 °C, $[\alpha]_D^{25}=-31.4$ ($c=1.0$ in MeOH); IR ν_{max} (cm^{-1}) 3338, 1747, 1688].

4.3.5. *Z*-Ala-OCam (6f). As white crystals, yield 84.0%, mp 65–68 °C, $[\alpha]_D^{22}=-15.0$ ($c=2.0$ in DMF); IR ν_{max} (cm^{-1}) 3351, 3202, 3065, 3034, 1691, 1737, 1751, 1771, 1455, 1536. [Lit.[32], 80%, mp 60–64 °C, $[\alpha]_D^{22}=-13.0$ ($c=2.1$ in DMF)].

4.3.6. *Z*-Ala-OCH_2CF_3 (6g). As white crystals, yield 63.9%, mp 43–46 °C, $[\alpha]_D^{22}=-23.9$ ($c=1.0$ in MeOH); MS (EI) $m/z=305.2[M]^+$. Anal. Calcd for $C_{13}H_{14}NO_4F_3$%: C, 51.15; H, 4.62; N, 4.59. Found %: C, 51.55; H, 4.65; N, 4.72; IR ν_{max} (cm^{-1}) 3333, 3038, 1689, 1756, 1774, 1454, 1536.

4.3.7. Boc-Ala-OCH_2CF_3 (5b).[27] As white crystals, yield 69.0%, mp 106–108 °C, $[\alpha]_D^{22}=-17.5$ ($c=0.5$ in MeOH).

4.3.8. Fmoc-Ala-OCH_2CF_3 (7a). As white crystals, yield 87.0%, mp 120–121 °C, $[\alpha]_D^{22}=-21.1$ ($c=0.5$ in MeOH). Anal. Calcd for $C_{20}H_{18}NO_4F_3$%: C, 61.07; H, 4.61; N, 3.56. Found: C, 61.24; H, 4.52; N, 3.37.

4.3.9. Boc-Leu-OCH_2CF_3 (5d). As colorless oil, yield 75.0%; MS (FAB) $m/z=314.3$ $[M+H]^+$, $[\alpha]_D^{22}=-25.4$ ($c=0.9$ in DMF).[33]

4.3.10. Boc-Phe-OCH_2CF_3 (5e). As white crystals, yield 87.0%, mp 75–77 °C, $[\alpha]_D^{22}=-11.8$ ($c=1.1$ in MeOH); MS (FAB) $m/z=348.4[M+H]^+$. Anal. Calcd for $C_{16}H_{20}O_4NF_3$%: C, 55.33; H, 5.80; N, 4.03. Found: C, 55.69; H, 5.85; N, 3.94. δ_H ($CDCl_3$, 200 MHz); 1.41 (s, 9H, $3CH_3$), 3.10 (m, 2H, CH_2), 4.28–4.80 (m 3H, CH, CH_2O), 4.91 (d, $J=8.0$ Hz, 1H, CONH), 7.07–7.41 (m, 5H, Ar-H); IR ν_{max} (cm^{-1}) 3350, 3055, 1692, 1768, 1452, 1540.

4.3.11. Boc-Asp(OBzl)-OCH_2CF_3 (5f–1). As white crystals, yield 90.0%, mp 59–61 °C, $[\alpha]_D^{22}=-14.3$ ($c=1.0$ in MeOH); MS (FAB) $m/z=406.2$ $[M+H]^+$. Anal. Calcd for $C_{18}H_{22}O_6NF_3$%: C, 53.33; H, 5.47; N, 3.46. Found: C, 53.15; H, 5.64; N, 3.37. δ_H ($CDCl_3$, 200 MHz); 1.45 (s, 9H, $3CH_3$), 2.80–3.21 (m, 2H, CH_2), 4.39–4.71 (m, 3H, CH, CH_2O), 5.13 (s, 2H, CH_2O), 5.48 (d, $J=8.2$ Hz, 1H, CONH), 7.26–7.39 (m, 5H, Ar-H); IR ν_{max} (cm^{-1}) 3360, 3050, 1700, 1780, 1450, 1542.

4.3.12. Boc-Lys(*Z*)OCH_2CF_3 (5h). As white crystals, yield 85.0%, mp 85–86 °C, $[\alpha]_D^{22}=-13.5$ ($c=1.0$ in MeOH); MS (FAB) $m/z=463.4$ $[M+H]^+$. Anal. Calcd for $C_{21}H_{29}O_6N_2F_3$%: C, 54.54; H, 6.32; N, 6.06. Found: C, 54.82; H, 6.36; N, 5.93. δ_H ($CDCl_3$, 200 MHz); 1.43 (s, 9H, $3CH_3$), 1.50–1.79 (m, 6H, $3CH_2$), 3.15–3.21 (m, 2H, CH_2), 4.34–4.69 (m, 3H, CH, CH_2O), 4.68–4.81 (m, 2H, 2CONH), 5.10 (s, 2H, CH_2O), 7.26–7.45 (m, 5H, Ar-H); IR ν_{max} (cm^{-1}) 3360, 3050, 1695, 1778, 1450, 1550.

4.3.13. Boc-Asp-OCH_2CF_3 (5f). The standard hydrogenization procedure was followed to generate **5f** from compound **5f–1**. As white crystals, yield 84.0%, mp 120–121 °C, $[\alpha]_D^{22}=-19.7$ ($c=1.0$ in EtOH); MS (FAB) $m/z=316.2$ $[M+H]^+$.

4.4. General procedure of enzymatic condensation between various *N*-protected amino acid trifluoroethyl esters and 1 or 2

To a mixture of P-AA-OCH_2CF_3 (0.2 mmol) and compound **1** or **2** (0.2 mmol) in anhydrous DMF (2.7 mL), 0.3 mL phosphate buffer ($KH_2PO_4 \sim Na_2HPO_4$, 50 mmol/L, pH 7.73) and subtilisin Carlsberg (4 mg) were added. The mixture was stirred 48 h at 40 °C oil bath and DMF was subsequently removed in vacuo. The residue was dissolved in EtOAc (60 mL), washed with 5% citric acid, 5% $NaHCO_3$ and saturated NaCl and dried over $MgSO_4$. The solvent was removed and the residue was recrystallized from appropriate solvents or purified by column chromatography or preparative TLC.

4.4.1. 17β-(*N*-*t*-Butyloxycarbonyl-L-alanyl)aminoestra-1, 3, 5 (10)-trien-3-ol (1b). As white powder, mp 129–131 °C, $[\alpha]_D^{19}=-31.0$ ($c=0.5$ in EtOH); MS (FAB) $m/z=443.5$ $[M+H]^+$. Anal. Calcd for $C_{26}H_{38}O_4N_2$%: C, 70.56; H, 8.65; N, 6.33. Found: C, 70.76; H, 8.86; N, 6.12.

4.4.2. 17β-(*N*-*t*-Butyloxycarbonyl-L-alanyl)hydrazonoestra-1, 3, 5 (10)-trien-3-ol (2b). As white powder, mp 174–176 °C, $[\alpha]_D^{19}=+120.5$ ($c=0.4$ in DMF); MS (FAB) $m/z=456.6[M+H]^+$. Anal. Calcd for $C_{26}H_{37}O_4N_3$%: C, 68.54; H, 8.19; N, 9.22. Found: C, 68.71; H, 8.38; N, 8.91.

4.4.3. 17β-(*N*-Benzyloxycarbonyl-L-alanyl)aminoestra-1, 3, 5 (10)-trien-3-ol (1a). As white powder,[8] mp 108–110 °C, $[\alpha]_D^{19}=-16.7$ ($c=0.5$ in EtOH); MS (FAB) $m/z=477.6$ $[M+H]^+$.

4.4.4. 17β-(*N*-Benzyloxycarbonyl-L-alanyl)hydrazonoestra-1, 3, 5 (10)-trien-3-ol (2a). As white powder,[8] mp 173–176 °C, $[\alpha]_D^{19}=+56.8$ ($c=1.0$ in DMF); MS (FAB) $m/z=490.6$ $[M+H]^+$.

4.4.5. 17β-(*N*-9-Fluorenylmethyloxycarbonyl-L-alanyl)-hydrazonoestra-1, 3, 5 (10)-trien-3-ol (2c). As white

powder, mp 147–150 °C, $[\alpha]_D^{19}=+95.2$ ($c=0.3$ in EtOH); MS (FAB) $m/z=578.6$ $[M+H]^+$. Anal. Calcd for $C_{36}H_{39}O_4N_3\cdot 1/2H_2O$ %: C, 73.70; H, 6.87; N, 7.16. Found: C, 74.10; H, 6.93; N, 6.78.

4.4.6. 17β-(*N*-*t*-Butyloxycarbonyl-L-leucinyl)aminoestra-1, 3, 5 (10)-trien-3-ol (1d). As white powder,[8] mp 221–223 °C, $[\alpha]_D^{20}=-32.6$ ($c=0.2$ in EtOH); MS (FAB) $m/z=485.6$ $[M+H]^+$.

4.4.7. 17β-(*N*-*t*-Butyloxycarbonyl-L-leucinyl)hydrazonoestra-1, 3, 5 (10)-trien-3-ol (2d). As white powder,[8] mp 129–131 °C, $[\alpha]_D^{20}=+57.8$ ($c=0.8$ in DMF); MS (FAB) $m/z=498.5$ $[M+H]^+$.

4.4.8. 17β-(*N*-*t*-Butyloxycarbonyl-L-phenylalanyl)-aminoestra-1, 3, 5 (10)-trien-3-ol (1e). As white powder, mp 128–130 °C, $[\alpha]_D^{20}=+21.1$ ($c=0.4$ in EtOH); MS (FAB) $m/z=519.6$ $[M+H]^+$. Anal. Calcd for $C_{32}H_{42}O_4N_2\cdot H_2O$ %: C, 71.61; H, 8.26; N, 5.22. Found: C, 71.40; H, 8.02; N 5.00.

4.4.9. 17β-(*N*-*t*-Butyloxycarbonyl-L-phenylalanyl)-hydrazonoestra-1, 3, 5 (10)-trien-3-ol (2e). As white powder, mp 128–130 °C, $[\alpha]_D^{20}=+76.2$ ($c=0.9$ in EtOH); MS (FAB) $m/z=532.6$ $[M+H]^+$. Anal. Calcd for $C_{32}H_{41}O_4N_3$: C, 72.29; H, 7.77; N, 7.90. Found: C, 72.24; H, 7.73; N, 7.88.

4.4.10. 17β-(*N*-*t*-Butyloxycarbonyl-L-aspartyl)amino-estra-1, 3, 5 (10)-trien-3-ol (1f). As white powder, mp 162–164 °C, $[\alpha]_D^{20}=-8.9$ ($c=0.9$ in EtOH); MS (FAB) $m/z=487.6$ $[M+H]^+$. Anal. Calcd for $C_{27}H_{38}O_6N_2\cdot H_2O$ %: C, 64.27; H, 7.99; N, 5.55. Found: C, 64.57; H, 7.77; N 5.32.

4.4.11. 17β-(*N*-*t*-Butyloxycarbonyl-L-aspartyl)hydrazono-estra-1, 3, 5 (10)-trien-3-ol (2f). As white powder, mp 205–208 °C (dec), $[\alpha]_D^{20}=+74.7$ ($c=1.0$ in DMF); MS (FAB) $m/z=500.6$ $[M+H]^+$. Anal. Calcd for $C_{27}H_{37}O_6N_3$%: C, 64.91; H, 7.46; N, 8.41. Found: C, 65.13; H, 7.69; N, 8.44.

4.5. General procedure of deprotection of Boc by 50% trifluoroacetic acid

The Boc-protected amino acid-steroidal conjugates (1 mmol) were dissolved in 6 mL DCM: TFA (1:1), several drops of anisole were added to quench the cations generated. The mixture was stirred at room temperature and the completion of the reaction was detected by TLC. The DCM and TFA were removed in vacuo followed by the addition of anhydrous ether to obtain precipitates of products.

4.6. In vitro binding affinity assay

4.6.1. Culture of estrogen receptor positive and negative human breast cancer cell lines (MCF7 and MDA-MB-231). MCF7 and MDA-MB-231 cells were routinely cultured in Dulbecco's Modified Eagle Medium (DMEM) with 5% fetal bovine serum (FBS) at a confluency below 70.[22] Culture medium was shifted to phenol-red free DMEM supplemented with 1% charcoal-stripped fetal bovine serum (FBS) for five days before the cells were seeded in a 96-well microtitre plate at a density of 2×10^4 cells per well. Cells were then treated with 10^{-9} M estrogen or its analogues amino acid estrogens with or without estrogen receptor antagonist ICI 182, 780 at 10^{-6} M (Tocris, UK). Drug treatments continued for three days with the medium changed everyday.

4.6.2. [^{3}H]-Methylthymidine incorporation assay. After drug treatment, cells were washed with serum-free medium once before adding serum-free medium containing 1 mCi/mL [^{3}H]-methylthymidine (Amersham Pharmacia Biotech, UK). Cells were returned to incubator for 2 h before they were chilled at 4 °C for 15 min. Cells were then washed with ice-cold phosphate-buffered saline (PBS) twice. 200 μL ice-cold 0.75 M trichloroacetic acid (TCA) per well was added and incubated at 4 °C further for 15 min. TCA was then removed and 200 μL fresh TCA per well was added. Plates were baked at 75 °C for 2 h. TCA from each well was transferred into scintillation vial and its radioactivity was measured by a scintillation counter.[22a]

4.6.3. Luciferase assay. HeLa cell based human estrogen-receptor (ER)-luciferase reporter cell line (a gift from Dr. H. Gronemeyer, Strasbourg, France)[22b] was used to determine the degree of activation of ER induced by estrogen and peptidyl estrogen. Cells were cultured in phenol-red free DMEM with 5% charcoal-stripped FBS for five days before they were seeded at a density of 3×10^5 cells per well in a 96-well plate. Different drug treatments, as indicated in the result section, were used to treat cells for 24 h before they were washed with PBS and lysed in lysis buffer (Promega). Cells were frozen at −80 °C and were thawed to room temperature before the substrate luciferin (Promega) was added to the cell lysate. The amount of light produced, as a direct correlation of the degree of ER activation, was measured by TR717 microplate luminometer (Applied Biosystems, USA).

4.6.4. ER binding assay. *Escherichia. coli* TOPP-3 cells (Stratagene, La Jolla, CA) harboring the desired expression plasmid pAF9His were grown in LB medium and induced by IPTG.[34] The cell lysate was used for the binding assay. Total [3H]-estradiol binding (TB) to the hERα was determined at different concentrations of non-radiolabeled ligand in the presence of 10 nM [3H]-estradiol. Radioactive counts were also measured in the presence of a 2000-fold molar excess of non-radiolabeled estradiol attempting displacement of [3H]-estradiol from hERα (NSB).

4.6.5. Statistical analysis. Data are reported as the mean ± standard error of mean (SEM). Significance of differences between group means was determined by analysis of variance (ANOVA) when more than two groups were compared. Group means differing by P values of 0.05 and less are considered statistically significant.

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Huang for the synthesis of the precursors of **4g**, **3g**, **3h** and **4h** by chemical method.

References and notes

1. Kong, F.; Andersen, R. J. *J. Org. Chem.* **1993**, *58*, 6924–6927.
2. De Riccardis, F.; Minal, L.; Riccio, R. *Tetrahedron Lett.* **1993**, *34*, 4381–4384.
3. Mokotoff, M.; Zhao, M.; Richard, J.; Marshall, R. J.; Winslow, E.; Wong, L. K.; Liao, Q.-J. *Steroids* **1990**, *55*, 399–404.
4. Lapatsanls, L. *J. Chem. Eng. Data* **1980**, *25*, 287–289.
5. Wang, C.; Zhao, M.; Yang, J.; Peng, S. Q. *Steroids* **2001**, *66*, 811–815.
6. Tang, Z. M.; Chen, M. Y.; Zhang, Q. *Acta Pharmacologica Sinica* **1983**, *4*, 186–191.
7. Jordan, V. C. *Pharmacol. Rev.* **1984**, *36*, 245–276.
8. Ye, Y.-H.; Huang, Y.-S.; Wang, Z.-Q.; Chen, S.-M.; Tian, Y. *Steroids* **1993**, *58*, 35–39.
9. Zaks, A.; Klibanov, A. M. *Science* **1984**, *224*, 1249–1251.
10. Wong, C.-H.; Wang, K. T. *Experitia* **1991**, *47*, 1123–1129.
11. Yan, A. X.; Tian, G. L.; Ye, Y. H. *Chin. J. Chem. Prog.* **2001**, *13*, 203–208. (*Chem. Abstr.* **2001**, *134*, 237763).
12. Bordusa, F. *Chem. Rev.* **2002**, *102*, 4817–4867.
13. Xing, G. W.; Tian, G. L.; Ye, Y. H. *J. Peptide Res.* **1998**, *52*, 300–304.
14. Ye, Y. H.; Tian, G. L.; Xing, G. W.; Dai, D. C.; Chen, G.; Li, C. X. *Tetrahedron* **1998**, *54*, 12585–12596.
15. Liu, P.; Tian, G. L.; Lee, K. S.; Wong, M. S.; Ye, Y. H. *Tetrahedron Lett.* **2002**, *43*, 2423–2425.
16. Fernandez, M. M.; Margot, A. O.; Falender, C. A.; Blanch, H. W.; Clark, D. S. *Enzyme Microb. Technol.* **1995**, *17*, 964.
17. Khumtaveeporn, K.; Ullmann, A.; Matsumoto, K.; Davis, B. G.; Jones, J. B. *Tetrahedron: Asymmetry* **2001**, *12*, 249–261.
18. Witte, K.; Seitz, O.; Wong, C.-H. *J. Am. Chem. Soc.* **1998**, *120*, 1979–1982.
19. Liu, P.; Tian, G. L.; Lo, W. H.; Lee, K. S.; Ye, Y. H. *Prep. Biochem. Biotechnol.* **2002**, *32*, 29–37.
20. Yan, A. X.; Xing, G. W.; Ye, Y. H.; Tian, G. L.; Wong, M. S.; Lee, K. S. *Tetrahedron Lett.* **2000**, *41*, 5379–5381.
21. William, H. C.; Jordan, V. C. In *Molecular, Cellular, and Systemic Mechanisms of Antiestrogen Action*; Vedeckis, W. V., Ed.; Hormones and Cancer; Birkhauser: Boston, 1996; pp 307–355.
22. (a) Chan, R. Y. K.; Dong, Al.; Guo, D.; Wong, M. S. *Regul. J. Clin. Endo. Metab.* **2002**, *87*, 3691–3695. (b) Chen, W. F.; Huang, M. H.; Tsang, C. H.; Yang, M. S.; Wong, M. S. *Biochim. Biophys. Acta* **2003**, *1638*, 187–196.
23. Zaks, A.; Klibanov, A. M. *J. Biol. Chem.* **1988**, *263*, 8017–8021.
24. Miyazawa, T.; Tanaka, K.; Ensatsu, E.; Yanagihara, R.; Yamada, T. *Tetrahedron Lett.* **1998**, *39*, 997–1000.
25. Xing, G. W.; Liu, D. J.; Ye, Y. H.; Ma, J. M. *Tetrahedron Lett.* **1999**, *40*, 1971–1974.
26. Matsubara, H.; Hagihara, B.; Nakai, M.; Komaki, T.; Yonetani, T.; Okunuki, K. *J. Biochem.* **1958**, *45*, 251–258.
27. Liu, P.; Yan, A. X.; Ye, Y. H. *Huaxue Tongbao* **2001**, *No. 12*, 777–780.
28. Yamada, T.; Isono, N.; Inui, A.; Miyazawa, T.; Kuwata, S.; Watanabe, H. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 1897–1898.
29. Strazzolini, P.; Scuccato, M.; Giumanini, A. G. *Tetrahedron* **2000**, *56*, 3625–3633.
30. Barton, M. A.; Singh, B.; Fraga, E. *J. Am. Chem. Soc.* **1977**, *99*, 8491–8498.
31. Miyazawa, T.; Tanaka, K.; Ensatsu, E.; Yanagihara, R.; Yamada, T. *J. Chem. Soc., Perkin Trans. 1* **2001**, 87–94.
32. Martinex, J.; Laur, J.; Castro, B. *Tetrahedron Lett.* **1983**, *24*, 5219–5222.
33. Park, O. J.; Jeon, G. J.; Yang, J. W. *Enzyme Microb. Technol.* **1999**, *25*, 455–462.
34. Nygaard, F. B.; Harlow, K. W. *Protein Expr. Purif.* **2001**, *21*, 500–509.

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A convenient new synthesis of fused 1,2,4-triazoles: the oxidation of heterocyclic hydrazones using copper dichloride

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Abstract—A series of 1,2,4-triazoles have been prepared by oxidative intramolecular cyclization of heterocyclic hydrazones with copper dichloride. General applicability of this simple transformation was confirmed by the synthesis of moderate to high yields of 1,2,4-triazolo[4,3-*a*]pyridines, 1,2,4-triazolo[4,3-*a*]pyrimidines, 1,2,4-triazolo[4,3-*b*]pyridazines, 1,2,4-triazolo[4,3-*a*]phthalazines, and 1,2,4-triazolo[4,3-*a*]quinoxalines. A 1,2,4-triazolo[4,3-*e*]purine-6,8(*7H*)-dione was obtained in a lower yield.

1. Introduction

Triazoles are an important class of heterocyclic compounds. In particular, fused 1,2,4-triazoles **1**–**5** (Fig. 1) express antifungal,[1] bactericidal,[1,2] anxiolytic,[3,4] anticonvulsant[5] or herbicidal[6] activities or can act as antidepressants.[7] Therefore, versatile and widely applicable methods for the synthesis of **1**–**5** are of considerable interest. Most methods for the preparation of **1**–**5** are based on heterocyclic hydrazones or hydrazides as precursors. However, these methods have some restrictions as regards their applicability and the use of toxic reagents like lead tetraacetate,[8,9] bromine[9,10] or phosphorus oxychloride.[8] In order to overcome these limitations, the oxidant chloramine T[11] and (diacetoxy)iodobenzene[12,13] as well as an electrochemical method[14] have been introduced.

Recently, we have shown that heterocyclic substituted imines undergo copper-catalyzed oxidation, thus forming imidazo[1,5-*a*]pyridines, imidazo[1,5-*a*]imidazoles, and imidazo[1,5-*a*]isochinolines using the nonhazardous, less toxic, and inexpensive reagent copper dichloride.[15,16]

As part of our ongoing studies dealing with copper(II) in synthesis, we now describe a novel copper-mediated oxidative heterocyclization of hydrazones yielding the corresponding 1,2,4-triazoles (Scheme 1).

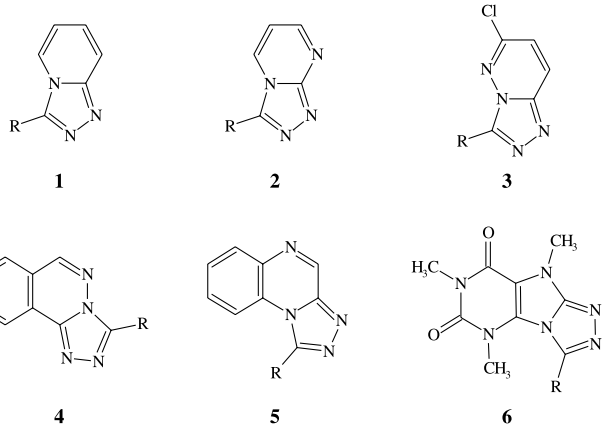
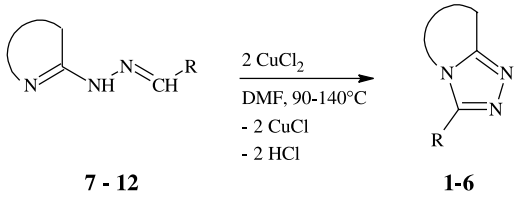


Figure 1.



Scheme 1.

2. Results and discussion

The synthesis of 1,2,4-triazolo derivatives from hydrazones has a remarkably wide scope of application. Hydrazones of aromatic and aliphatic aldehydes **7**–**11** (Fig. 2) with both electron-withdrawing and electron-donating substituents

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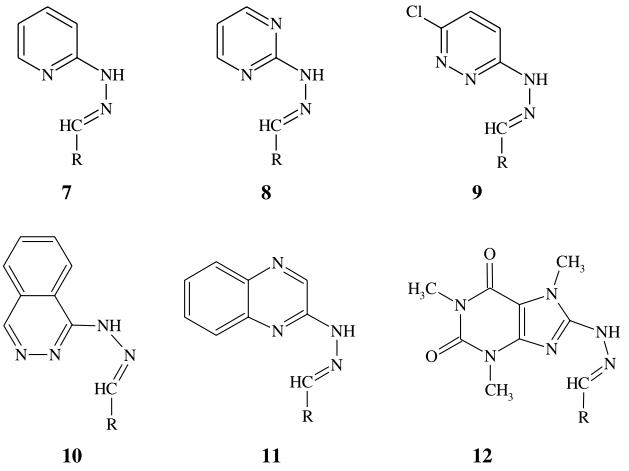

Figure 2.

were oxidized to give the corresponding 1,2,4-triazolo[4,3-*a*]pyridines **1**, 1,2,4-triazolo[4,3-*a*]pyrimidines **2**, 1,2,4-triazolo[4,3-*b*]pyridazines **3**, 1,2,4-triazolo[4,3-*a*]phthalazines **4**, and 1,2,4-triazolo[4,3-*a*]quinoxalines **5** in high yields (Table 1). Heterocyclization of **12a** was achieved in the same way, but the yield of the 1,2,4-triazolo[4,3-*e*]purine-6,8(*7H*)-dione **6a** was rather poor.

The required hydrazones **7**–**12** were obtained by treating the corresponding hydrazino heterocycles with aldehydes.

Heterocyclization was carried out in absolute DMF under argon. After the hydrazone had been dissolved, a solution of two equivalents of copper dichloride was added and then, the mixture was heated. The reaction had been completed when its initially brown color turned to yellow due to reduction of the copper(II) ions to copper(I). Isolation of the 1,2,4-triazolo compounds **1**–**5** was easily carried out. The solvent was distilled off in vacuum, and the residue was treated with an aqueous solution of ammonia in order to remove the copper ions as a water-soluble complex. Then, the precipitated crude products were filtered off and pure 1,2,4-triazolo compounds **1**–**5** were obtained by recrystallization. Only 1,2,4-triazolo[4,3-*e*]purine-6,8(*7H*)-dione **6a** had to be separated from impurities by column chromatography.

Triazoles **1a**,[17] **1b**,[17] **1d**[14], **2a**,[18,19] **3a**[20], and **5b**[21] have already been described. Their melting points agree with those reported in the literature except for **1d** and **2a**. Nevertheless, spectroscopic data of these compounds confirm the structure proposed and are listed in the experimental section. The conditions applied for the synthesis of each compound are listed in Table 1.

Comparison of the reactivities of the tested hydrazones indicates that the cyclization is not strongly affected by the substituent R. The reactivity to copper dichloride remains unchanged when the phenyl group in the hydrazones is replaced by an *n*-alkyl residue. In addition, hydrazones of donor-substituted benzaldehydes (R = 2,3-dimethoxy-phenyl and 4-hydroxy-phenyl) and those bearing an acceptor substituent (R = 2-chloro-phenyl and 4-nitro-phenyl) yielded triazoles under similar conditions. Functionalities of the substrate are maintained, even when substituents susceptible to oxidation, e.g. a hydroxy group, are considered. On the other hand, the influence of the nature of the heterocyclic moiety is more significant. If the –NH–N=CH group is attached to a six-membered nitrogen-containing ring, the triazoles are obtained in a smooth reaction. The lower selectivity in the oxidation of **12** indicates that a five-membered ring is less favorable. However, the difficulties of the heterocyclization of **12** could also be caused by the sterically demanding effect of the methyl group at position 5 or by a stabilized intermediate of the radical-based reaction. Investigations concerning mechanistical studies are still in progress.

Unlike heterocyclic aldimines,[15] the hydrazones **7**–**12** could not be oxidized by atmospheric oxygen in the presence of catalytic amounts of copper(II)chloride. Copper(I)complexes of substituted hydrazones are not able to coordinate oxygen in order to form copper(II)species and are likely to decompose. Thus, reactive intermediates for a copper-catalyzed oxidative cyclization are not generated.

3. Conclusion

In conclusion, we have developed a convenient and simple method for the preparation of a wide variety of 1,2,4-triazolo compounds by oxidation of heterocyclic substituted hydrazones using copper dichloride as oxidation agent. Besides, the presence of several functionalities in the substrate is tolerated and does not influence the yield of the resulting 1,2,4-triazole. Our current studies are directed to

Table 1. Triazolo (**1**–**6**) compounds from the reaction of heterocyclic hydrazones (**7**–**12**) with copper dichloride

Hydrazone	R	Time (h)	Temp. (°C)	Triazole	Yield (%)	mp (°C)
7a	4-Chloro-phenyl	1.0	90	**1a**	60	198–199
7b	2-Chloro-phenyl	1.0	90	**1b**	57	130–132
7c	4-Hydroxy-phenyl	1.0	90	**1c**	71	249–250
7d	4-Nitro-phenyl	1.0	100	**1d**	59	312–314
8a	4-Chloro-phenyl	1.0	110	**2a**	63	244–245
8b	3,4-Dimethoxy-phenyl	1.0	110	**2b**	43	207–209
9a	4-Chloro-phenyl	1.0	130	**3a**	72	197–198
9b	3,4-Dimethoxy-phenyl	1.5	120	**3b**	62	235–237
10a	2-Chloro-phenyl	0.5	140	**4a**	74	214–216
11a	4-Chloro-phenyl	1.0	100	**5a**	72	192–194
11b	*n*-Propyl	0.75	100	**5b**	61	149–153
11c	3,4-Dimethoxy-phenyl	0.5	90	**5c**	84	262–263
12a	*n*-Propyl	0.75	100	**6a**	15	205–208 (dec.)

extend the scope of the method to cover additional heterocyclic systems.

4. Experimental

4.1. General

Melting points were measured using the Büchi melting point apparatus B-545 and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AC 250 spectrometer at 250 and 63 MHz, respectively. Mass spectra were recorded on a Hewlett Packard 1100 MSD using the electrospray ionization (ES) technique and on a Micromass GCT spectrometer using an electron impact source (EI). Elemental analysis was made by a Vario EL III from Elementar Analysensysteme GmbH.

The references of the hydrazones **7a**, **7b**, **7c**, **7d**, **8a**, **9a** and **12a** which have already been described in the literature are noted in the experimental description of the corresponding triazolo compounds. Preparation and experimental data of **8b**, **9b**, **10a**, **11a**, **11b** and **11c** are described in Section 4.2.

4.2. General procedure for the preparation of hydrazones 8b, 9b, 10a, 11a, 11b, 11c

The hydrazine (25 mmol) was dissolved in a sufficient amount of boiling ethanol and the aldehyd (25 mmol), dissolved in 20 ml ethanol, was added dropwise. After that, the solution was stirred and heated under reflux for 20 min. The formed hydrazone was filtered from the cooled solution and used in the next reaction without any purification.

4.2.1. 3,4-Dimethoxybenzaldehyde-pyrimidin-2-yl-hydrazone 8b. ^{1}H NMR (DMSO-D_6) δ 11.19, 8.49, 8.13, 7.33, 7.16, 7.04, 6.85, 3.56, 3.84; MS (EI) $m/z=258$ (M^+).

4.2.2. 3,4-Dimethoxybenzaldehyde-(5-chloropyridin-2-yl)-hydrazone 9b. ^{1}H NMR (DMSO-D_6) δ 11.66, 8.11, 7.71, 7.41, 7.20, 7.04, 3.89, 3.85; MS (EI) $m/z=292$ (M^+).

4.2.3. 2-Chlorobenzaldehyde-phthalazin-1-ylhydrazone 10a. ^{1}H NMR (DMSO-D_6) δ 12.26, 8.74, 8.65, 8.33, 8.15, 7.73, 7.43; MS (EI) $m/z=282$ (M^+-H).

4.2.4. 4-Chlorobenzaldehyde-quinoxalin-2-ylhydrazone 11a. ^{1}H NMR (DMSO-D_6) δ 9.12, 8.76, 8.33, 7.94, 7.36, 7.60; MS (EI) $m/z=282$ (M^+).

4.2.5. Butanal-quinoxalin-2-ylhydrazone 11b. ^{1}H NMR (DMSO-D_6) δ 11.16, 8.86, 7.83, 7.58, 7.41, 2.19, 1.46, 0.86; MS (EI) $m/z=214$ (M^+).

4.2.6. 3,4-Dimethoxybenzaldehyde-quinoxalin-2-yl-hydrazone 11c. ^{1}H NMR (DMSO-D_6) δ 11.59, 9.12, 8.05, 7.88, 7.66, 7.41, 7.18, 7.00; MS (EI) $m/z=308$ (M^+).

4.2.7. 3-(4-Chlorophenyl)-1,2,4-triazolo[4,3-*a*]pyridine 1a. 1.85 g (8.0 mmol) **7a**[17] and 2.15 g (16.0 mmol) $CuCl_2$ were dissolved each in 25 ml absolute DMF Both solutions were combined and the mixture was stirred under argon at 50 °C for 20 min and then at 90 °C for 1 h. After cooling to ambient temperature, the solution was concentrated in vacuum and 70 ml 10% ammonia solution were added to the residue. After stirring for 20 min at 40 °C in the presence of air, the precipitated solid was filtered off and suspended again in 70 ml 10% ammonia solution. Then, the solid was filtered off, washed with water, and dried. The mixed ammonia solutions were extracted with ethyl acetate twice. The solid was boiled in 100 ml ethyl acetate. An insoluble impurity was removed by filtration of the hot mixture. All organic solutions were combined and the solvent was distilled off in vacuum. Then, the crude product was dissolved in 100 ml boiling ethanol, 50 ml water were added and a small amount of a dark impurity was removed by filtration of the hot solution. Pure **1a** crystallized upon slow cooling of the filtrate; yield 60%; mp 198–199 °C (lit. 192 °C[17]).

4.2.8. 3-(2-Chlorophenyl)-1,2,4-triazolo[4,3-*a*]pyridine 1b. 2.31 g (10 mmol) **7b**[17] and 2.69 g (20 mmol) $CuCl_2$ were dissolved at 50 °C each in 30 ml absolute DMF Both solutions were combined and the mixture was stirred under argon at 50 °C for 20 min and afterwards at 90 °C for 1 h. After cooling to ambient temperature, the solution was concentrated in vacuum to approximately 10 ml. Then, 100 ml of 10% ammonia solution and 100 ml of ethyl acetate were added and the mixture was stirred at 40 °C for 30 min in the presence of air. The organic layer was separated and the ammoniacal solution was extracted with ethyl acetate twice. All organic solutions were combined, extracted with water, and dried over sodium sulfate. Then, the solvent was removed. The viscous oil obtained was dissolved in 60 ml of boiling toluene/*n*-hexane (1/1). A small amount of impurity remained undissolved and was removed by filtration of the hot solution. Pure **1b** crystallized upon slow cooling of the solution down to −20 °C; yield 57%, mp 130–132 °C (lit. 132 °C[17]).

4.2.9. 3-(4-Hydroxyphenyl)-1,2,4-triazolo[4,3-*a*]pyridine 1c. A solution of 1.62 g (12.0 mmol) $CuCl_2$ in 20 ml absolute DMF was added to 1.28 g (6.0 mmol) **7c**[22] which was dissolved in 10 ml absolute DMF and heated to 50 °C. After stirring for 1 h at 90 °C, the solvent was removed in vacuum and the residue treated for 30 min with 30 ml warm 10% ammonia solution and 5 g NaCl. The formed precipitate was filtered out, whereas the aqueous solution was extracted with ethyl acetate. The solvent was distilled off and the solid obtained was combined with the former one. Recrystallization from ethanol gave pure **1c** as white crystals; yield 71%; mp 249–250 °C; ^{1}H NMR (DMSO-D_6) δ 10.04, 8.46, 7.79, 7.70, 7.36, 6.99; ^{13}C NMR (DMSO-D_6) δ 159.45, 130.16, 127.96, 124.25, 117.57, 116.49, 116.06, 114.56; MS (EI) $m/z=211$ (M^+); calcd. (%) for $C_{12}H_9N_3O$ (211.22) C 68.24, H 4.29, N 19.89; found (%) C 68.00, H 4.40, N 20.32.

4.2.10. 3-(4-Nitrophenyl)-1,2,4-triazolo[4,3-*a*]pyridine 1d. 1.35 g (10.0 mmol) $CuCl_2$ dissolved in 20 ml absolute DMF were added to a heated (50 °C) suspension of 1.21 g (5.0 mmol) **7d**[22] in 10 ml absolute DMF The solution was stirred for 1 h at 100 °C and concentrated after cooling to room temperature. Then, a solution of 30 ml 10% ammonia and 10 g NaCl was added, and the mixture was stirred at 45 °C for 15 min in the presence of air. A

precipitate was formed, filtered out, dissolved in boiling acetone and filtered again. Ocher-colored **1d** crystallized upon cooling of the solution; yield 59%; mp 312–314 °C (lit. 296–298 °C[14]), ^{1}H NMR (DMSO-D_6) δ 8.73, 8.43, 8.24, 7.93, 7.50, 7.12; ^{13}C NMR (DMSO-D_6) δ 148.18, 148.09, 129.39, 128.97, 125.54, 123,54, 116.09; MS (EI) $m/z=240$ (M^+); calcd. (%) for $C_{12}H_8N_4O_2$ (240.22) C 60.00, H 3.36, N 23.32; found (%) C 59.41, H 3.38, N 24.05.

4.2.11. 3-(4-Chlorophenyl)-1,2,4-triazolo[4,3-*a*]pyrimidine 2a. 1.51 g (6.5 mmol) **8a**[19] were dissolved in 25 ml absolute DMF at 50 °C and a solution of 1.75 g (13 mmol) $CuCl_2$ in 25 ml warm absolute DMF was added. The reaction mixture was stirred under argon at 50 °C for 20 min and then heated at 110 °C for 1 h. After cooling to approximately 30 °C, the mixture was concentrated to 5 ml in vacuum. Then, a mixture of 100 ml water, 50 ml concentrated ammonia solution, and 10 g NaCl was added. After stirring for 20 min at 40 °C in the presence of air the precipitate was filtered off. Treatment with diluted ammonia solution was repeated, the precipitate was filtered off, washed with water, and dried. The crude product was first recrystallized from ethanol/water (200 ml, 70/30) and then from ethyl acetate; yield 63%; mp 244–245 °C (lit. 284–286 °C,[18] 278–280 °C[19]), ^{1}H NMR (DMSO-D_6) δ 9.36, 8.87, 8.21, 7.60, 7.34; ^{13}C NMR (DMSO-D_6) δ 164.20, 156.06, 137.65, 135.91, 129.67, 129.54, 129.10, 111.55; MS (EI) $m/z=230$ (M^+); calcd. (%) for $C_{11}H7N_4Cl$ (230.66) C 57.27, H 3.06, N 24.29; found (%) C 57.27, H 3.00, N 24.54.

4.2.12. 3-(3,4-Dimethoxyphenyl)-1,2,4-triazolo[4,3-*a*]-pyrimidine 2b. 2b was prepared from **8b** in analogy to the synthesis of **2a**; yield 43%; mp 207–209 °C; ^{1}H NMR ($CDCl_3$) δ 8.82–8.72, 7.92–7.84, 7.80, 7.03–6.96, 6.93–6.85, 3.94, 3.88; ^{13}C NMR ($CDCl_3$) δ 154.46, 151.69, 149.53, 135.61, 123.33, 121.07, 111.49, 110.65, 110.10, 56.33, 56.34; MS (EI) $m/z=256$ (M^+); calcd (%) for $C_{13}H_{12}N_4O_2$ (256.27) C 60.93, H 4.72, N 21.86; found (%) C 60.80, H 4.46, N 21.86.

4.2.13. 6-Chloro-3-(4-chlorophenyl)-1,2,4-triazolo[4,3-*b*]pyridazine 3a. A solution of 2.02 g (15 mmol) $CuCl_2$ in 30 ml warm absolute DMF was added to a suspension of 1.53 g (7.22 mmol) **9a**[20] in 40 ml absolute DMF. The reaction mixture was stirred at 50 °C for 20 min and then heated at 130 °C for 1 h under argon. After cooling to ambient temperature, the mixture was concentrated in vacuum. The residue obtained was stirred with a mixture of 150 ml 10% ammonia solution and 20 g NaCl for 20 min at 40 °C in the presence of air. The solid precipitated from the deep blue solution was filtered off. Treatment with diluted ammonia solution was repeated, the crude product was filtered off and dried on air. The crude product was refluxed in 150 ml ethyl acetate, with a small amount of impurity remaining undissolved. Then, it was filtered from the hot solution and the solvent was removed in vacuum. The pure compound was obtained by recrystallization from ethanol; yield 72%; mp 197–198 °C (lit. 193–194 °C[20]).

4.2.14. 6-Chloro-3-(3,4-dimethoxyphenyl)-1,2,4-triazolo[4,3-*b*]pyridazine 3b. Both 2.18 g (7.4 mmol) **9b** and 2.02 g (15 mmol) $CuCl_2$ were dissolved in 35 ml warm absolute DMF. The solutions were mixed at 50 °C. The mixture was stirred at 60 °C for 20 min and then heated at 120 °C for 1.5 h under argon. After cooling to approximately 30 °C, the volume of the mixture was reduced to 7 ml in vacuum. A solution of 100 ml water, 50 ml concentrated ammonia, and 20 g NaCl was added and the mixture was stirred for 20 min at 40 °C in the presence of air. The precipitate was filtered off and treated again with 70 ml of 10% ammonia solution in the same manner. The solid obtained was washed with water and dried on air. It was dissolved in 200 ml of boiling xylene and a small amount of an impurity was separated by filtration of the hot solution. The filtrate was cooled down slowly, with **3b** being obtained in the form of small crystals; yield 62%; mp 235–237 °C; ^{1}H NMR ($CDCl_3$) δ 8.12, 8.07, 7.94, 7.07, 6.97, 6.94, 3.93, 3.89; ^{13}C NMR ($CDCl_3$) δ 151.44, 149.52, 127.00, 121.08, 118.71, 111.38, 110.96, 56.44, 56.40; MS (EI) $m/z=290$ (M^+); calcd. (%) for $C_{13}H_{11}ClN_4O_2$ (290.71) C 53.71, H 3.81, N 19.27; found (%) C 53.58, H 3.64, N 19.46.

4.2.15. 3-(2-Chlorophenyl)-1,2,4-triazolo[3,4-*a*]phthalazine 4a. 1.61 g (12 mmol) $CuCl_2$ dissolved in 20 ml DMF were added to a suspension of 1.70 g (6 mmol) **10a** in 20 ml DMF at 70 °C The mixture was stirred at 80 °C for 20 min and then heated at 140 °C for 30 min under argon. After cooling, the yellow-brown solution was concentrated in vacuum. A solution of 100 ml water, 50 ml concentrated ammonia solution, and 20 g NaCl was added. This mixture was stirred for 20 min at 40 °C in air and a solid precipitated. It was filtered off and suspended again in 70 ml diluted ammonia solution. After stirring in air (15 min), the solid was separated by filtration, washed with water, and dried. The crude product was dissolved in 80 ml of boiling ethanol and the hot ethanolic solution was filtered. After addition of water (40 ml), the solution was cooled down slowly and **4a** was obtained as brass-colored crystals. The product was isolated by filtration. The filtrate was heated again and treated with a solution of 5 g NaCl in 50 ml water. A brown impurity precipitated. It was removed by filtration of the boiling solution. The filtrate was cooled down slowly and a second fraction of the product was obtained; yield 74%; mp 214–216 °C; ^{1}H NMR (DMSO-D_6) δ 9.07, 8.60–8.56, 8.25–8.21, 8.13–8.06, 7.99–7.92, 7.77–7.54; ^{13}C NMR (DMSO-D6) δ 148.49, 134.39, 133.63, 132.82, 132.20, 131.21, 129.71, 128.99, 127.25, 125.61, 123.00, 122.50, 122.12; MS (EI) $m/z=280$ (M^+); calcd. (%) for $C_{15}H_9ClN_4$ (280.72) C 64.18, H 3.23, N 19.96; found (%) C 63.98, H 3.07, N 20.01.

4.2.16. 1-(4-Chlorophenyl)-1,2,4-triazolo[4,3-*a*]quinoxaline 5a. 1.27g (4.5 mmol) **11a** were dissolved in 20 ml absolute DMF at 50 °C and a warm solution of 1.21 g (9 mmol) $CuCl_2$ was added The mixture was stirred at 50 °C for 20 min and then heated at 100 °C for 1 h under argon. After the reaction mixture had been cooled to approximately 30 °C, it was concentrated to 5 ml in vacuum. Then, a solution of 100 ml water, 50 ml concentrated ammonia, and 20 g NaCl was added and the mixture was stirred at 40 °C for 20 min in the presence of air. After cooling to room temperature, the precipitated solid was filtered off and treated again with diluted ammonia solution (70 ml, 15 min). The solid obtained was washed with water and dissolved in 60 ml of boiling ethanol. Small amounts of a

brown precipitate were filtrated from the solution and the filtrate was cooled down slowly. The product was collected by filtration. After the volume of the filtrate had been reduced to 25 ml, a second fraction of **5a** was obtained; yield 72%; mp 192–194 °C; ^{1}H NMR ($CDCl_3$) δ 9.27–9.20, 8.09–8.05, 7.65–7.50, 7.42–7.34; ^{13}C NMR ($CDCl_3$) δ 144.09, 137.90, 137.09, 131.69, 129.97, 129.88, 128.27, 126.46, 126.96, 116.26; MS (ES) m/z=281 ($M+H^+$); calcd. (%) for $C_{15}H_9ClN_4$ (280.72) C 64.18, H 3.23, N 19.96; found (%) C 64.03, H 3.11, N 19.90.

4.2.17. 1-Propyl-1,2,4-triazolo[4,3-*a*]quinoxaline 5b. A warm solution of 1.34 g (10 mmol) $CuCl_2$ in 25 ml absolute DMF and a solution of 1.07 g (5 mmol) **11b** in 20 ml absolute DMF were mixed and stirred for 20 min at 50 °C. Then, the reaction mixture was heated at 100 °C for 45 min under argon. After the solution had been cooled to 30 °C, it was concentrated to 5 ml in vacuum. Then, a solution of 80 ml water, 40 ml concentrated ammonia solution, and 20 g NaCl was added and the mixture was stirred for 20 min at 40 °C in the presence of air. A solid substance separated, which was collected by filtration. The solid was treated again with diluted ammonia solution (10%, 50 ml, 15 min). The crude product was washed with water and then dissolved in a boiling mixture of 80 ml water and 20 ml ethanol. Small amounts of a dark impurity were removed by filtration of the boiling solution and the filtrate was cooled down slowly. **5a** crystallized as large needles. A second fraction of the product was obtained after adding NaCl to the filtrate; yield 61%; mp 149–153 °C (lit. 150 °C[21]).

4.2.18. 1-(3,4-Dimethoxyphenyl)-1,2,4-triazolo[4,3-*a*]quinoxaline 5c. 1.23 g (4 mmol) **11c** and 1.08 g (8 mmol) $CuCl_2$ were dissolved each in 25 ml warm absolute DMF Both solutions were mixed. The brown reaction mixture was stirred at 50 °C for 20 min and heated at 90 °C for 30 min under argon. While cooling, a pale yellow solid precipitated. After the main part of the solvent had been removed in vacuum, 50 ml water, 50 ml concentrated ammonia solution, and 20 g NaCl were added to the residue. The mixture was stirred for 20 min at 40 °C in the presence of air, cooled to room temperature, and the precipitated substance was collected by filtration. It was suspended again in diluted ammonia solution (50 ml). After stirring in air (15 min), the crude product was collected by filtration, washed with water, and dried. Then, it was dissolved in 200 ml of boiling xylene. Small amounts of a dark substance had been filtered from the boiling mixture and the solution was cooled down slowly, whereby the product was obtained as sand-colored crystals; yield 84%; mp 262–263 °C; ^{1}H NMR (DMSO-D_6) δ 9.32, 8.10, 7.66–7.53, 7.37–7.35, 3.95, 3.82; ^{13}C NMR ($CDCl_3$) δ 151.97, 150.13, 149.51, 144.91, 144.23, 136.83, 130.78, 127.86, 129.66, 126.67, 123.67, 121.04, 116.45, 115.10, 113.62, 56.84, 56.66; MS (EI) m/z=306 (M^+); calcd. (%) for $C_{17}H_{14}N_4O_2$ (306.11) C 66.66, H 4.61, N 18.29; found (%) C 66.41, H 4.55, N 18.49.

4.2.19. 5,7,9-Trimethyl-3-propyl-5,9-dihydro-6*H*-1,2,4-triazolo[4,3-*e*]purine-6,8(7*H*)-dione 6a. 1.21 g (9 mmol) $CuCl_2$ were dissolved in 25 ml absolute DMF, while warming gently This solution was added to a solution of 1.25 g (4.5 mmol) **12a**[23] in 25 ml of absolute DMF under argon at 50 °C. The reaction mixture was stirred at 50 °C for 20 min and then heated at 100 °C for 45 min under argon. After cooling of the clear yellow-brown solution, its volume was reduced to approximately 5 m. Then, 150 ml of an ammonia solution (10%) containing 20 g NaCl was added. The mixture was stirred for 30 min at room temperature, the precipitated solid was filtered off, and treated again with a diluted ammonia solution (50 ml). The ammoniacal filtrates were extracted with ethyl acetate. The solid was refluxed with 150 ml ethyl acetate. Small amounts of a substance remained unsolved and were removed by filtration from the boiling mixture. All organic solutions were mixed and dried over Na_2SO_4. After the solvent had been removed in vacuum, a brown solid was obtained. **6a** was isolated by flash chromatography on alumina (eluent: chloroform). The crude product was dissolved in 20ml hot ethyl acetate, the boiling mixture was filtrated, and 15 ml hot *n*-hexane were added. While slowly cooling, **6a** was obtained as sand-colored crystals; yield 15%; mp 205–208 °C (dec.); ^{1}H NMR ($CDCl_3$) δ 3.89, 3.77, 3.05–2.90, 2.00–1.80, 1.08–0.98; ^{13}C NMR ($CDCl_3$) δ 155.90, 150.19, 128.39, 110.61, 34.31, 32.05, 31.02, 29.03, 21.06, 14.12; MS (EI) m/z=276 (M^+); calcd. (%) for $C_{12}H_{16}N_6O_2$ (276.30) C 52.16, H 5.84, N 30.42; found (%) C 51.98, H 5.76, N 30.55.

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References and notes

1. El-Hawash, S. A.; Habib, N. S.; Fanaki, N. H. *Pharmazie* **1999**, *54*, 808–813.
2. Brown, D. J.; Iwai, Y. *Aust. J. Chem.* **1979**, *32*, 2727–2733.
3. Tarzia, G.; Ocelli, E.; Toja, E.; Barone, D.; Corsico, N.; Gallico, L.; Luzzani, F. *J. Med. Chem.* **1988**, *31*, 1115–1123.
4. Trust, R. I.; Albright, J.D. U.S. Patent 4 242 515, 1980.
5. Tarzia, G.; Ocelli, E.; Barone, D. *Il Farmaco* **1989**, *44*, 3–16.
6. Peignier, R., Chêne, A., Cantregril, R., Mortier, J. Eur. Patent 441718. ; *Chem. Abstr.* **1991** *115* 208000. Cantegril, R., Chêne, A., Mortier, J., Peignier, R. Eur. Patent 483027.; *Chem. Abstr.* **1992** *117* 131214.
7. Sarges, S.; Howard, H. R.; Browne, R. G.; Lebel, L. A.; Seymour, P. A.; Koe, B. K. *J. Med. Chem.* **1990**, *33*, 2240–2254.
8. Bower, J. D.; Doyle, F. P. *J. Chem. Soc.* **1957**, 727–732.
9. Pollak, A.; Tišler, M. *Tetrahedron* **1966**, *22*, 2073–2079.
10. Gibson, M. S. *Tetrahedron* **1963**, *19*, 1587–1589.
11. Bourgeois, P.; Cantegril, R.; Chêne, A.; Gelin, J.; Mortier, J.; Moyroud, J. *Synth. Comm.* **1993**, *23*, 3195–3199.
12. Sadana, A. K.; Mirza, Y.; Aneja, K. R.; Prakash, O. *Eur. J. Med. Chem.* **2003**, *38*, 533–536.
13. Kumar, D.; Kondapalli, V. G.; Chandra, S.; Harmeet, D.; Vajja, S. R.; Rajender, S. *Green Chem.* **2004**, *6*, 156–157.
14. Crljenak, S.; Tabaković, I.; Jeremić, D.; Gaon, I. *Acta Chem. Scand.* **1983**, *B37*, 527–535.
15. (a) Bluhm, M. E.; Ciesielski, M.; Görls, H.; Döring, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 2962–2965. (b) Bluhm, M. E.;

Ciesielski, M.; Görls, H.; Döring, M. *Angew. Chem.* **2002**, *114*, 3104–3107.
16. Bluhm, M. E.; Ciesielski, M.; Görls, W.; Walter, O.; Döring, M. *Inorg. Chem.* **2003**, 8878–8885.
17. Naqui, S.; Srinivasan, V. R. *Indian J. Chem.* **1965**, *3*, 162–164.
18. Tsujikawa, T.; Tatsuta, M. *Chem. Pharm. Bull.* **1977**, *25*, 3137–3146.
19. Brown, D. J.; Namagatsu, T. *Aust. J. Chem.* **1977**, *30*, 2515–2524.
20. Pollak, A.; Tisler, M. *Tetrahedron* **1966**, *22*, 2073–2079.
21. Shiho, D.; Tagami, S. J. *Am. Chem. Soc.* **1960**, *82*, 4044–4054.
22. Todeschini, A. R.; Miranda, A.L.P de; Silva, K. C. da; Parrini, S. C.; Barreiro, E. J. *Eur. J. Med. Chem. Chim. Ther.* **1998**, *33*, 189–200.
23. Klosa *J. Chem. Ber.* **1955**, *93*, 211–217.