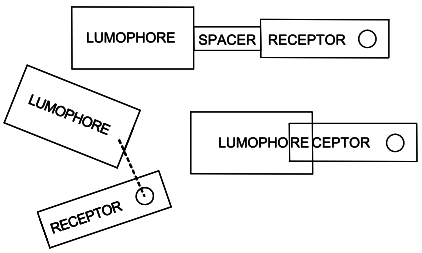
ELSEVIER

Tetrahedron

Tetrahedron Vol. 61, No. 36, 2005

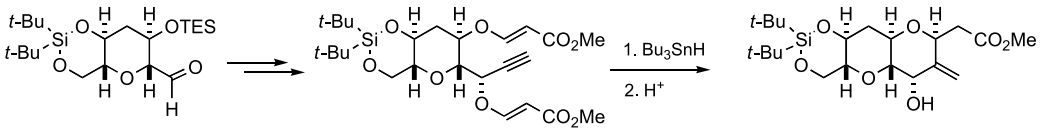
Contents

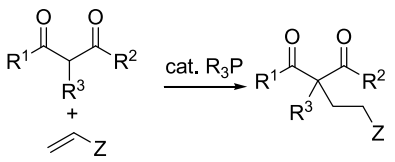
REPORT



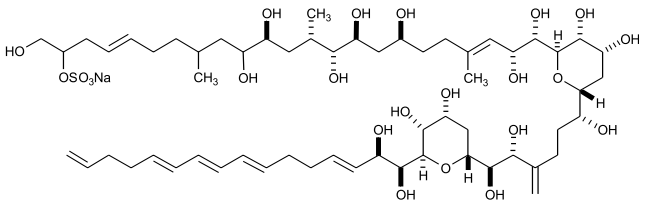
The ideas and experimental results within 350 references are marshalled to illustrate the design bases and application potential of molecular luminescent sensing and switching devices that have appeared since the turn of the century. All of these molecules possess lumophores and receptors connected or associated in some way.

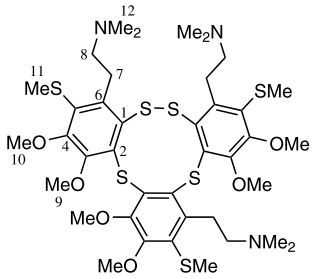
ARTICLES



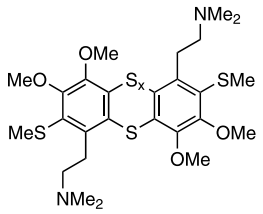


General method as shown by the vast selection of reagents

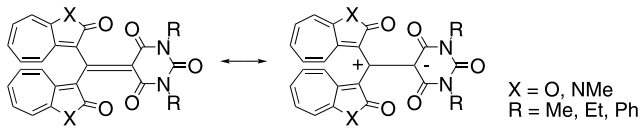




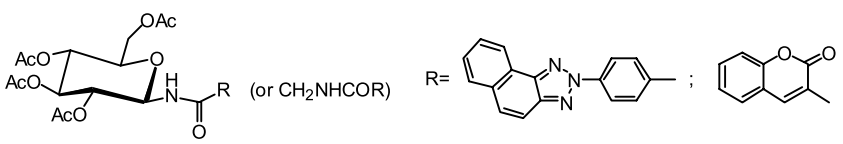
Lissoclibadin 1 (**1**)

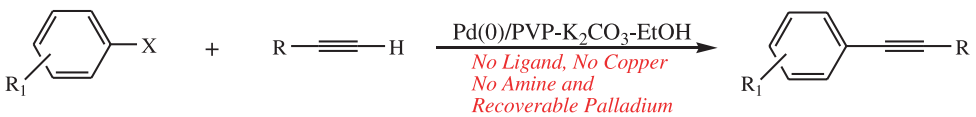


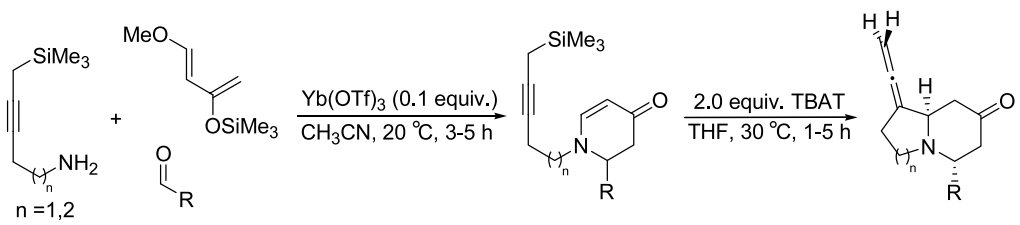
Lissoclibadin 2 (**2**): x = 2
Lissoclibadin 3 (**3**): x = 1

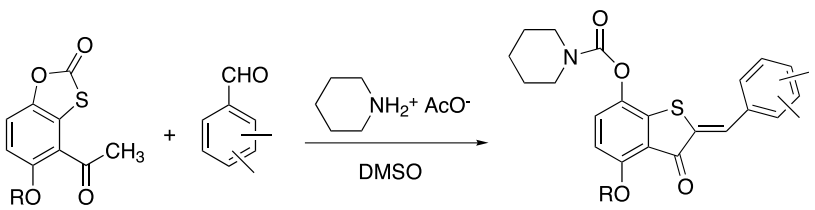


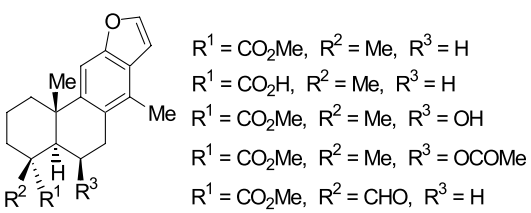
X = O, NMe
R = Me, Et, Ph

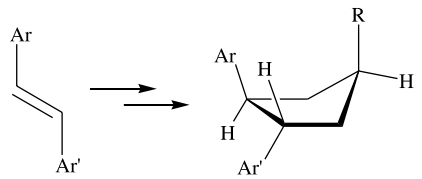




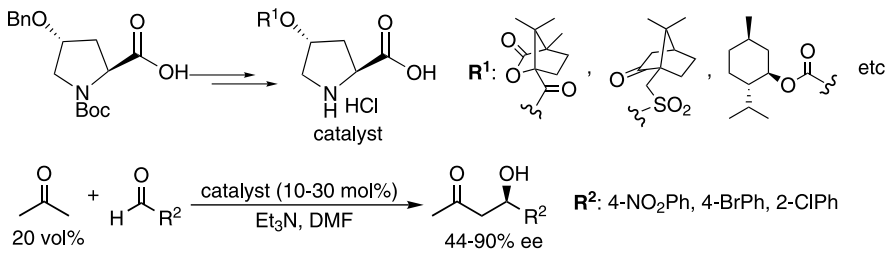


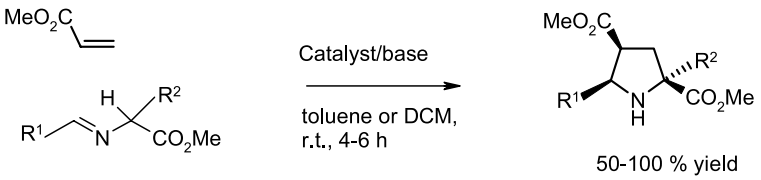




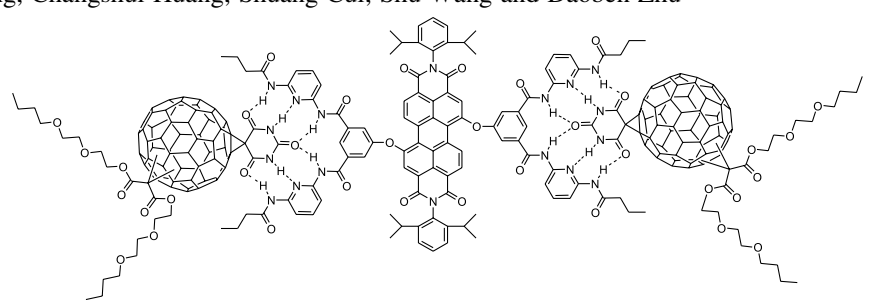


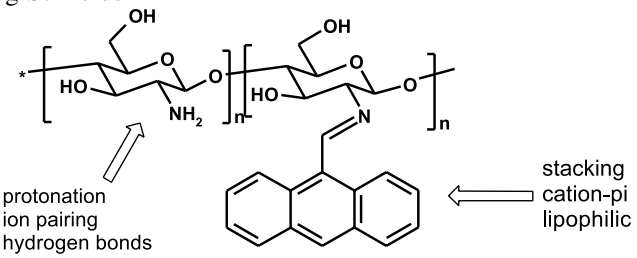
Ar and/or Ar' = C_6H_5, 2-$C_{10}H_7$, 2- or 4-Pyridyl, 2-$(CH_3O)C_6H_4$, 3,5-$(CH_3O)_2C_6H_3$; R = H, OH



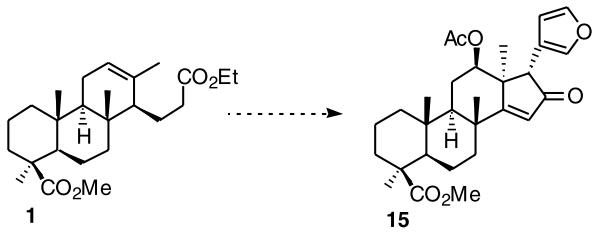


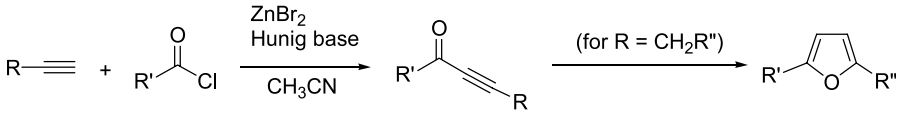
Catalysts: Ag_2O, Ag zeolite, $NiCl_2$, CuI. Base: DBU, NEt_3

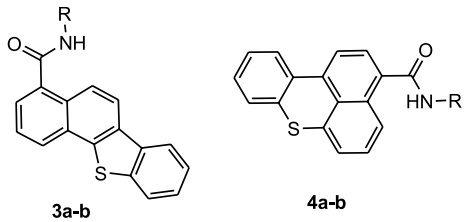


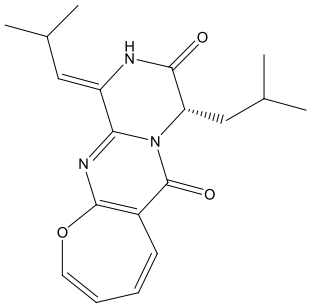


Chitosan with a covalently attached anthrylunit leads by stacking interactions with charged hetaryls such as benzimidazole to reversible volume expansions by up to 65%. Aminoacid esters trigger an increase selective towards the lipophilicity of the residues.

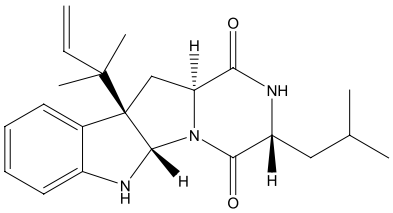




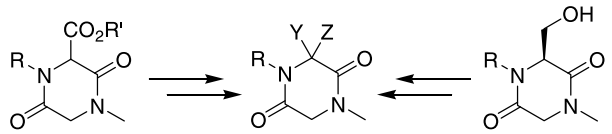




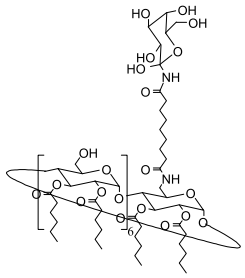
Janoxepin

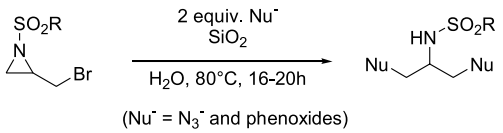


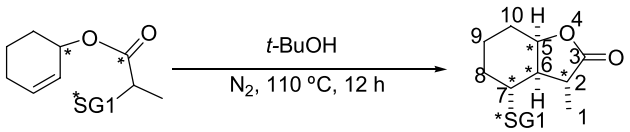
Brevicompanine B



Alkoxycarbonyl- and hydroxymethyl-piperazine-2,5-diones are versatile precursors for the α-functionalisation of piperazine-2,5-diones.







OTHER CONTENTS

*Corresponding author
ⓘ+ Supplementary data available via ScienceDirect

COVER

ELSEVIER

ISSN 0040-4020

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
ELSEVIER
Tetrahedron 61 (2005) 8551–8588

Tetrahedron report number 731

Luminescent sensors and switches in the early 21st century

John F. Callan,[a,b,*] A. Prasanna de Silva[a,*] and David C. Magri[a,*]

[a]*School of Chemistry, Queen's University, Belfast BT9 5AG, Northern Ireland, UK*
[b]*School of Pharmacy, Robert Gordon University, Aberdeen AB10 1FR, Scotland, UK*

Accepted 18 May 2005

Available online 27 June 2005

Contents

Keywords: Luminescence; Fluorescence; Sensors; Switches; Logic gates; Photoinduced electron transfer; Internal charge transfer; Energy transfer.
* Corresponding authors. Tel.: +44 1224 262280; fax: +44 1224 262500 (J.F.C.); Tel.: +44 2890 974422; fax: +44 2890 974890 (A.P.de S.; D.C.M.); e-mail addresses: j.callan@qub.ac.uk; j.callan@rgu.ac.uk; a.desilva@qub.ac.uk; d.magri@qub.ac.uk

doi:10.1016/j.tet.2005.05.043

1. Introduction

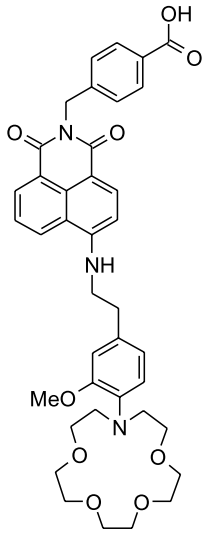
Chemically induced switching of luminescence remains one of the more dramatic visual phenomena to be found in a laboratory. The past sliver of this century provides enough evidence that this phenomenon continues to entertain scientists in laboratories around the world in good numbers. It is also natural that such a humanly comprehensible signal would be put to good use in quantitating chemical species of various kinds, that is, luminescence sensors serve real-life needs in the wider world. Organic chemistry remains at the centre of this overall enterprise, since design, synthesis and characterization of mostly carbon-based molecules precedes the demonstration of luminescence switching. Our observation is that organic chemists as a community, especially those at the higher end of molecular synthesis, have largely remained aloof from luminescent sensors and switches. Our aim in this review is to appeal to this skills-base and to suggest that luminescent sensors and switches can be a fitting creative output for hard-core organic chemists. After all, even molecules as small as aspirin had important enough social ramifications to inspire organic chemists.

To keep this review within reasonable bounds, we will only track the trends of the past 5 years. Even then, we will only illustrate trends with those discoveries that caught our eye. Older literature will be cited only in an effort to note the foundations of these fresher discoveries. Readers wishing more background have a string of older reports to consult.[1–24]

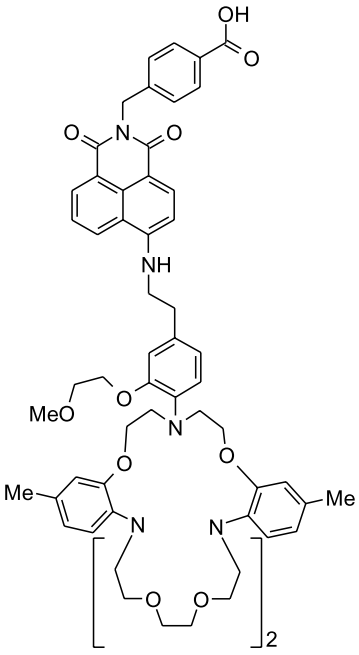
1.1. Real-life case: from design to deployment

A victim of a car crash is lying in the street. Someone dials the emergency number. The ambulance arrives within minutes and the paramedics take a quick blood sample from the victim while preparing him for transfer to the hospital. Every minute counts. In 30 seconds, the patient's blood electrolyte levels are known thanks to a small portable instrument. These are telephoned through to the hospital. A blood bag is balanced with electrolytes to match the patient and is ready for use as soon as the ambulance has fought its way through the city traffic to the accident and emergency centre of the hospital.

Those blood electrolyte levels are measured quickly and reliably with a family of fluorescent PET sensors.[1,3,5] The sensor molecules **1**[25] for, say, Na^+ are covalently immobilised on an aminocellulose fibre substrate. K^+ is tackled similarly with **2**.[26] The whole blood is filtered through the cellulose fibre mat before encountering the sensor molecules, so that only the relatively colourless serum makes the crossing. The Na^+ in the serum equilibriates with the sensor molecules so that the appropriate fraction of the sensor molecule population becomes Na^+-bound. A flash from an inexpensive blue light-emitting diode excites all the sensor molecules. The Na^+-bound fraction is strongly fluorescent and replies with a green-yellow flash. The Na^+-free fraction remains virtually silent. The intensity of the green-yellow light flash is measured by an inexpensive photodiode and easily converted into a blood Na^+ level.



1



2

The luminescent PET sensor principle[1,3,5] was particularly attractive for the present application because of its simplicity. A lumophore is attached to a receptor via a spacer so that the terminal units act in a modular fashion, that is, their properties are largely preserved. Except for one thing: the luminescence of the lumophore is switched 'off' due to presence of the receptor. Arrival of the analyte which is selected by the receptor causes the luminescence to be switched back 'on' again. The 'off–on' nature of the analyte-induced switching and the modular nature of the system are two manifestations of simplicity. Equally simple is the design, that is, the choice of the modules. How do we choose the receptor from the wealth of ideas and examples available in the literature?

A quick glance at a medical textbook[27] tells us the normal levels of extracellular Na^+ and K^+ in whole blood are 140 and 4 mM, respectively, and so the accurate measurement of the Na^+ level requires matching of the Na^+ binding constant of the chosen receptor to the reciprocal of the

normal concentration as well as ensuring adequate selectivity with respect to the other constituents. The selectivity of binding is the province of coordination and supramolecular chemists. Elegant solutions are available for this situation following the developments in macrocyclic chemistry.[28]

Crown ethers have a well-deserved reputation as Na^+ binders.[29] Adequate selectivity against K^+ can be obtained by choosing a 15-crown-5 derivative.[28] Selectivity against H^+ can be obtained with all-oxygen crowns, but an *N*-phenyl monoazacrown would work just as well at physiological pH values and have extra advantages. First, they are more electroactive due to the lower oxidation potential of the anilinic motif (0.8 V vs saturated calomel electrode).[30] Electroactivity is needed, since these sensors rely on photoinduced electron transfer,[31–36] and in fact, this is the origin of the PET acronym. Such a transfer requires an oxidation, say, of the receptor and a corresponding reduction of the lumophore under the influence of photoexcitation. This transfer is responsible for draining the energy deposited in the lumophore upon excitation and, hence, the lack of luminescence in the switched 'off' state of the sensor. Second, the *N*-phenyl motif can be outfitted with a 2-methoxy substituent which swings into action when Na^+ is captured by the crown in order to increase the number of oxygen atoms in the coordination sphere. In effect, the 2-methoxy substituent serves to cap the Na^+ corralled by the crown ether. This capping requires a rotation about the aromatic C–N bond such that the nitrogen electron pair is deconjugated from the benzene ring π-system and so the oxidation potential of the Na^+-bound *N*-phenylazacrown is naturally raised. Of course, the very presence of the Na^+ raises the oxidation potential of the aniline motif due to straightforward electrostatics.[37,38] This double boost of the oxidation potential is the reason why the photoinduced electron transfer becomes energetically impossible in the Na^+-bound sensor. The excitation is therefore unused by PET and returned as luminescence emission—a clear switching 'on'.

2. PET (photoinduced electron transfer) systems

2.1. Brief theory

Photoinduced electron transfer has had whole books devoted to it,[31,32,34–36] and luminescence switching with it has also been reviewed.[1,3,5] It was also discussed above concerning the case study. Nevertheless, the basic approach can be summarised in a few sentences. A lumo/fluorophore is connected to a receptor via a spacer, which can be virtual or even absent. Known redox potentials[30] can be used to choose components so that the receptor will transfer an electron to/from the excited lumo/fluorophore. This means emission is absent at this stage. Binding of a target species will now change the redox potential of the receptor so that the electron transfer is no longer energetically feasible, and the excited state energy of the lumo/fluorophore is therefore dumped as a humanly visible emission. This is the target-induced emission enhancement. Distinguishing features of this method are its quantitative design and the predictability of many observable parameters. The 'on/off' emission intensity ratios can be easily useful at values of 2, can be visually dramatic in the dark at values of 10, and can be much larger than 100 when instrumental errors take over the numbers. Values clearly less than 1 can also be useful, and can be designed into the systems.

2.2. Proton targets

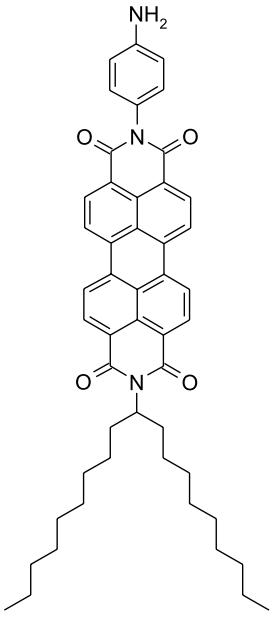
This survey begins with a special example which is based on a common structural format. The imide nitrogen creates a node in the molecular orbital system, and so Zang et al.'s compound **3**[39] is clearly of the 'fluorophore-spacer-receptor' format. Indeed, PET from the anilinic unit to the perylenetetracarboximide leads to negligible fluorescence. Protonation of the anilinic unit gives a strong fluorescence enhancement (FE). Other binders of the anilinic nitrogen such as $ZnCl_2$ also give a similar effect. The earliest PET sensors by Morawetz in 1976 (not far from Zang et al.'s premises in New York) also found this similar action between H^+ and Zn^{2+}.[40] However, what distinguishes the current study is the observation of the switching properties at the single-molecule level by confocal scanning microscopy. The time trajectory of fluorescence intensity is similar to that seen in the one previous case of a fluorescent ICT sensor (see below) for pH.[41]

The 'fluorophore-spacer-receptor' format is also clearly recognisable in Fahrni et al.'s compound **4**,[42] which switches on its emission when the aniline unit picks up H^+. The structure and function of **4** is largely subsumed within **5**[43] and **6**[44] designed to respond principally to Ca^{2+} and Na^+, respectively. Both **5** and **6** have been tested for their response to H^+ during selectivity studies. Indeed, strong fluorescence switching 'on' is seen. PET from the aniline unit to the pyrazoline fluorophore carrying electron-withdrawing groups like CN was first appreciated by Pragst and co-workers.[45]

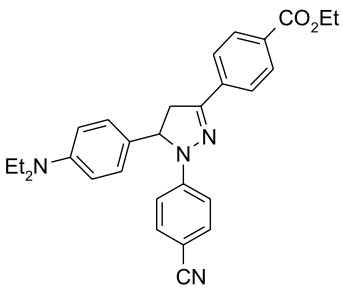
While the interactions of H^+ with naphthylmethylpolyamines were studied during the formative phase of PET sensors, Pina et al.[46] cleverly analyse the PET rates extracted from **7** and its relatives as a function of the electron-transfer distance. The former is derived from fluorescence lifetime measurements, whereas the latter is obtained from NMR data. This work contributes to the understanding of the distance dependence of PET,[47] and so molecules like **7** enjoy a new life in a new field. Clear isotope effects indicate a proton shift prior to PET or a coupled proton–electron movement.[48]

Tian et al.'s copolymer **8**[49] includes the 'fluorophore-spacer-receptor' system where H^+ binding to the tertiary aliphatic amine inhibits PET and switches on the emission of the naphthalimide unit.[50–52] This phenomenon is cleverly combined with photogenerated acids, from precursors such as triphenylsulfonium salts, to achieve 'light writing' of fluorescent images.

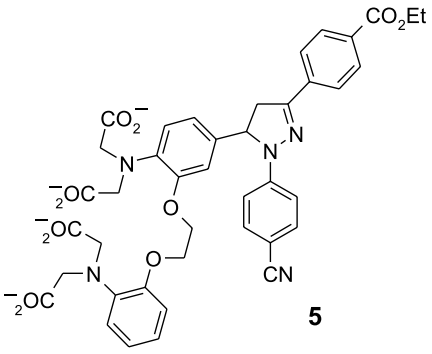
Overall, acid–base interactions remain the simplest, most convenient and most defined testing ground for sensor designers.



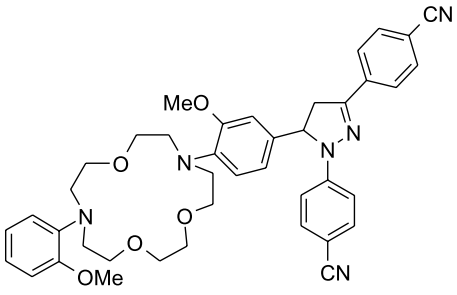
3



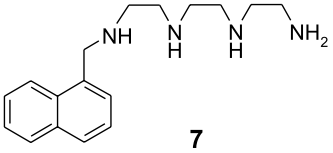
4



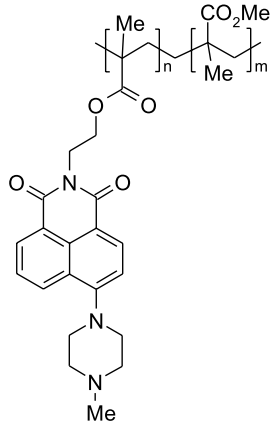
5



6



7



8

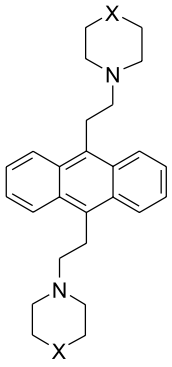
2.3. Alkali and alkaline earth cation targets

The opening cases of Na^+ and K^+ blood analysers[25,26] belong here. A related case for Na^+ employing the same receptor is also available from Gunnlaugsson et al.[53] What follows shows how much this sub-field has grown.

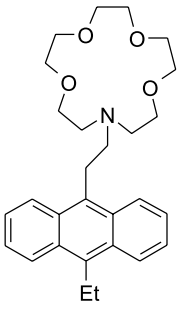
The amine donor, which is two methylene groups away from the anthracene fluorophore, gives away the PET sensor design of **9–11**.[54,55] If one methylene group each were shaved off **11** we would arrive at a classical PET sensor for H^+.[56] Both **9** and **10** form all three possible structures $[ML]^{2+}$, $[M_2L]^{4+}$ and $[ML']^{2+}$(sandwich) for the alkaline earth metal ions but only $[ML]^+$ and $[M_2L]^{2+}$ for the alkali metals. Fluorescence would be switched 'on' only when both tertiary nitrogen lone electron pairs are blocked by the metal ions or H^+. The secondary nitrogen in **9** allows it to better chelate metal ions than **10** to an extent that results in higher stabilities of the complexes formed. While the binding data can be useful, the poor selectivities reduce their usefulness as sensors. Whereas these sensors operate in acetonitrile, derivatives operating in water would be generally more useful.

Another study from the Lincoln laboratory[57] compares PET sensors with one or two crowns, as we did before for very similar structures concerning H^+.[58] The mono-azacoronand **12** has a moderate fluorescence quantum yield of 0.25 in acetonitrile. An Li^+-induced FE of 3 is found, with larger alkali cations performing less well. The stability constants obey a similar trend. The bis-azacoronand **13** exhibits poor quantum yields of less than 0.07, even when only one of the receptors is occupied by an alkali metal, due to PET readily

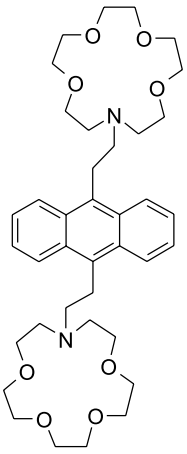
occurring from the uncomplexed receptor. The lower fluorescence efficiency is due to the increased reducibility of the fluorophore when **13** is mono-complexed. However, when alkali metals occupy both receptor sites the quantum yields are as high as 0.73.



9; X = NH
10; X = S
11; X = O

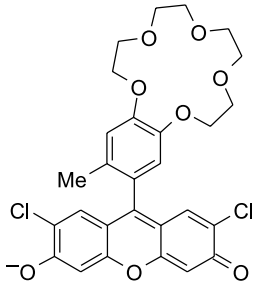


12

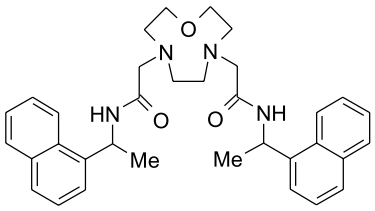


13

Kenmoku et al.[38] found an Na^+-induced FE of 5 for **14** with a small binding constant of 2.5 under simulated physiological conditions. This is clearly a virtually spaced PET system[3] aided by the steric effect of the strategically placed methyl group. Nice thermodynamic and quantum calculational arguments are offered to support PET in this case. A very similar system was unveiled a few years ago by Kollmannsberger et al.[59] Older cases of benzocrown ether-based PET sensors for Na^+ are known.[60] As an aside, a nice electrochemical study of benzocrown ether is included and shows the long-expected[60] Na^+-induced anodic shift.



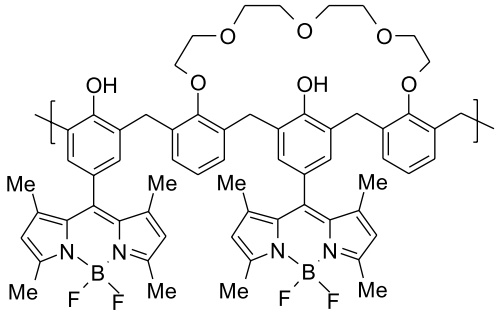
14



15

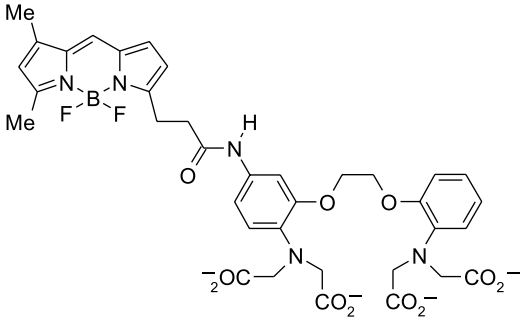
Sensing Li^+ in water via fluorescence remains a tough nut to crack due to the unavailability of suitably selective and strong receptors. Nevertheless, Gunnlaugsson et al.[61] make progress by developing **15** for use in acetonitrile. Compound **15** has a 'fluorophore-spacer-receptor-spacer-fluorophore' format. It produces an FE of 9 and a final quantum yield of 0.11. The relatively high pK_a of the diazacrown ether (7.2 in water) is a weak point, however.

Compound **16**[62] contains the boron-dipyrromethine fluorophore, which is building up a considerable usage in sensor studies[63–65] as well as in general biochemical research.[66] It is found to be selective for Ca^{2+} over other cations in methanol solution. However, the fact that the fluorescence quantum yield decreases rapidly as the amount of water is increased to even 20% limits **16**'s general usefulness. The fluorescence of **16** switches 'off' in the presence of Ca^{2+} because of the increased oxidizability of the phenolate part of the calixcrown receptor. Ca^{2+} inclusion in the calixcrown allows H^+ ejection from the proximal phenol to produce the phenolate. This is reminiscent of older boron-dipyrromethine -based PET systems responding to H^+ via their phenolate receptor.[67] It is notable, however, that **16** requires a particular pH range to see 'on–off' switching action, as found in the pioneering (non-fluorescent) case.[68]



16

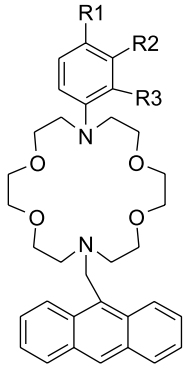
More examples using the boron-dipyrromethine fluorophore are available in the work by Gee et al.,[69] who connect Tsien's Ca^{2+}-receptor BAPTA[70] via a relatively long spacer to produce a family of PET sensors like **17**. Acetoxymethyl esters of these are cell permeable as expected and give order-of-magnitude FE in the cell environment.



17

As might be expected, there are more crown ether- and cryptand-based sensors to discuss in this section. For instance, a large Ca^{2+}-induced FE is seen for **18**[71] compared to both **19** and **20**, and **21** also shows a moderate increase in fluorescence with Ca^{2+}, but is not as selective against Mg^{2+} as **18**. Changing the electronic properties of the *N*-aryl group thus affects the selectivity of the molecules—ion selection is not just a size-match scenario. It is clear that the electron richness of the aromatic amine moiety is in the order: **21** > **18** > **19** = **20**. The aptitude of **21** for Mg^{2+} seems to be due to the electron-rich phenylene-diamine unit binding strongly with charge-dense Mg^{2+}, which has been noted in a previous report from the Pearson group.[72,73] NMR experiments suggest that Mg^{2+} is too small to fit snugly into the crown cavity and resides towards the aliphatic amine side. This can be rationalised since the

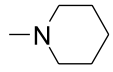
hard Mg^{2+} avoids the softer aniline side. The large effect on NMR chemical shifts near the anthracene, when compared to the small FE for Mg^{2+} on **18**, **19** and **20**, suggests the ion probably moves towards the aniline side of the molecule during the anthracene's excited state lifetime. Related cation decoordination is known in ICT sensors which develop a substantial cationic charge at the business end of the receptor.[74,75] The small amount of electronic coupling between the anthracene and the amine across the methylene spacer[76] can create a small amount of ICT character which, in turn, can serve the current purpose.



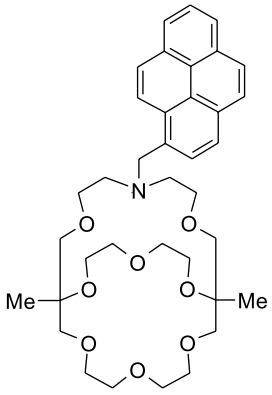
18; R1, R2, R3 = H

19; R1 = Cl; R2, R3 = H

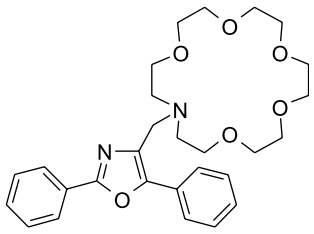
20; R1, R3 = H; R2 = Cl

21; R2, R3 = H; R1 = 

Most school chemical laboratory experiences include the precipitation of white $BaSO_4$ from its water-soluble components. From then on, however, a chemical test for Ba^{2+} is hard to come by, so much so that developing optical sensors for Ba^{2+} is an important research goal for many. The report of a fluorescent sensor for Ba^{2+} in aqueous solution by Nakahara et al.[77] is therefore quite an event. The 'fluorophore-spacer-receptor' system **22** in water shows poor binding to Ba^{2+} and thus poor FE. Water molecules offer strong hydration and thus reduce the complexing abilities of the ion. Adding the non-ionic detergent Triton X-100 to the solution, such that the critical micelle concentration is exceeded, allows the sensor to reside in a less polar micellar location[78] and therefore offers stronger binding of the metal ion which cancels the PET process and switches fluorescence 'on'. The rigidity of the cryptand then asserts itself by giving very good selectivity for Ba^{2+} over other alkali and alkaline earth cations. Of course, the dielectric effect of the micellar environment which repels ions to some extent[79] is a mitigating factor. The significant proton sensitivity of **22** also could bear improvement, with the current experiments being conducted at pH 10. In spite of this, **22** is a real advance on a previously intransigent target. Interestingly, the successful micelle is the neutral one, as also found by Bhattacharya and Gulyani (see below).[80]



22



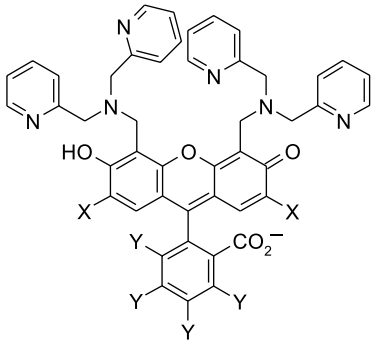
23

It is a particular pleasure to see how far PET sensors and switches can go in the hands of inventive scientists. The case under discussion does not deal with fluorescence at all, but rather with scintillation counting. Both processes end up with light emission from the lowest electronically excited state. The two processes have very different starting points, however. Of course, fluorescence requires the optical pumping of the molecule, which is a sensor or a switch in the context of this review. On the other hand, scintillation begins with ionising radiation (α, β or γ) hitting the scintillation solvent, usually an aromatic such as toluene. The electron–hole pairs created therein can recombine to give rise to high-energy excited states of the solvent. The energy proceeds to run downhill by transferring from the solvent to the scintillant solute. 2,5-Diphenyloxazole is a common example of the latter. Once the lowest electronic excited state is reached, we can apply PET principles as usual. So PET will deactivate the excited state of the scintillant **23**[81] non-radiatively. When Clapham and Sutherland's compound **23** captures K^+ within the azacrown cavity, PET is blocked in the time-honoured way.[82] So, in the presence of ionising radiation, **23** exhibits an increase in the amount of scintillation upon addition of K^+. H^+ does the same thing.[56] The generalisation is that 'scintillant-spacer-receptor' systems stand ready to bring radiochemical techniques into the general sensing arena.

2.4. Transition metal ion targets

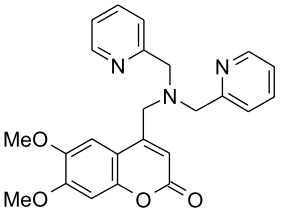
2.4.1. Zn^{2+}. Though hard-core inorganic chemists would debate whether Zn^{2+} should be considered as a transition metal ion, we consider it here for organisational simplicity. We also start Section 2.4 with it because Zn^{2+} has been a particular triumph of PET sensor design all the way to practical use in physiology laboratories for neurochemical investigations. Of course, the success can also be attributed to the closed-shell nature of Zn^{2+}, which simplifies design greatly regarding the photophysics. Another contributor to the success is the availability of rather selective receptors. For instance, the dipicolylamine unit has been a recent favourite with designers.[83–86] Nevertheless, the tertiary nitrogens are protonated at physiological pH causing high background fluorescence. The pK_a values of the tertiary nitrogens are now tuned by Chang et al.[83] by changing groups X and Y on the fluorophore unit of **24** to the more

electronegative halogens in different combinations. This has the extra benefit, known previously,[87] of moving the inherent pH sensitivity of the fluorophore away from the physiological region. Though not directly attached, electronegative X groups influence the amine pK_a value, since the methylene spacers transmit field/inductive interactions quite well. Of course, the fluorescein fluorophore allows excitation and emission in the visible region to reduce the possibility of tissue damage and also avoids autofluorescence. The observation of a Zn^{2+}-induced FE of 6 for one derivative of **24** (X=F, Y=H) shows the success of this combinatorial approach to structure optimisation. Compound **24** is clearly a 'fluorophore-spacer-receptor' system operating PET from the tertiary amine to the fluorophore until Zn^{2+} blocks it. It permits imaging high levels of neuronal Zn^{2+} which have been associated with Alzheimer's disease and other neurological disorders. Interestingly, **24** can be directly loaded into neurons by passive diffusion without the usual membrane-permeant acetoxymethyl ester derivatives.[87] Also notable is the Zn^{2+}-binding contribution of the phenolate group of the fluorophore which also brings a modicum of ICT character to the sensory mechanism. Another contribution of the phenolate is best discussed in Section 2.6.

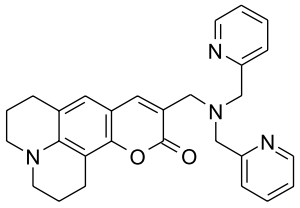


24

The dipicolylamine unit also raises its head in **25**[88] and **26**,[88] where the latter is an interesting ratiometric sensor for Zn^{2+}. The value of ratiometric systems in intracellular studies[87] and the inability of most PET systems in this aspect have been clear for some time. ICT systems naturally produce ratiometric systems, even though their overall track record in producing practically useful sensors for a variety of targets is not as good with PET systems, partly because of the predictive nature of the latter. Compound **25** is a straightforward PET system of the 'fluorophore-spacer-receptor' type, which produces the fluorescence switching 'on' with Zn^{2+}, as predicted, while **26** behaves differently, since the participation of the lactone oxygen of coumarin in complexing the Zn^{2+}, directly perturbs the chromophore and causes a red shift of 21 nm, allowing for ratiometric detection. This is the ICT contribution to this otherwise PET system. Increasing amounts of water in the methanol solvent reduce the shift, suggesting water is competing with the lactone oxygen for co-ordination to the metal centre. Unfortunately, this does not augur well for biological studies. Also, **26** does not show a significant FE, and is switched 'on' in the absence of Zn^{2+} in its free base form. This is because the fluorophore in **26** is an aminocoumarin with a higher reduction potential than the dimethoxycoumarin in **25**. Therefore the variation in the position of the chelate on the fluorophore is not the only change in going from **25** to **26**. Older examples of regiochemical effects on fluorescent PET systems are available,[50,51,89] along with commentaries.[89,90]

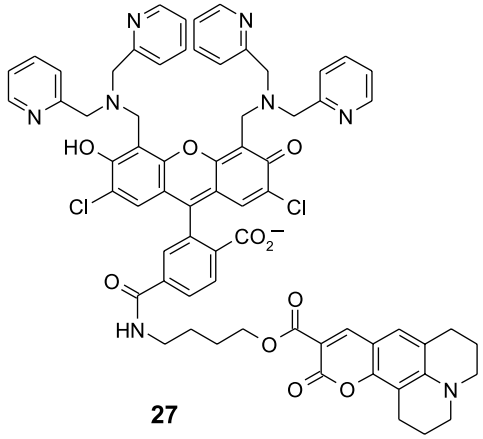


25



26

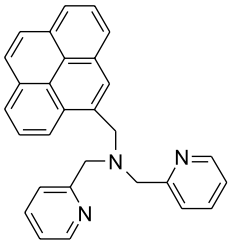
Woodroofe and Lippard[91] tackle the problem of building ratiometric systems out of tried-and-tested PET systems for intracellular use.[87] They build on their previous successes by coupling their Zn^{2+}-sensitive dipicolylaminomethylfluorescein moiety to a Zn^{2+}-insensitive coumarin unit to produce **27**. Upon entry into the cell, nonspecific esterases cleave the ester linkage to yield two fluorescent molecules, observable at separately distinct wavelengths. Upon binding Zn^{2+}, the green fluorescein signal undergoes enhancement, which can be quantified against the blue coumarin signal as the reference. This general approach of using two unconnected fluorophores to feed two colour channels[92] is very useful, though as the authors indicate, co-localisation of the two fluorophores must be ensured, at least microscopically. Approaches to build unimolecular ratiometric sensors out of polymeric[93] and PET[94] systems in the past have depended on long chains or carefully chosen fluorophore pairs, respectively, to minimise energy transfer between them.



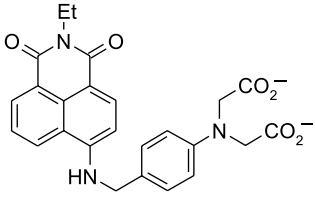
27

Bhattacharya and Gulyani's compound **28**[80] displays weak monomer and excimer emission due to aggregation in water. The anthracenyl model[84,95] does not show excimers, partly because of the shorter excited-state lifetimes, besides differences in aqueous solubility. Incidentally, the latter was described earlier as a PET sensor by de Silva for Zn^{2+} in acetonitrile.[84] The Zn^{2+} sensor action of **28** is almost non-existent, probably because the hydrated Zn^{2+} ions cannot access most of the sensor molecules locked within

the aggregate. However, **28** can be molecularly dispersed in membranes such as vesicles and micelles.[78] Then, no excimer emission is observed and the monomer emission is enhanced. The bis(pyridylmethyl)amine receptor peeps out into the water since the hydrophobic pyrene unit is held within the membrane. Normal PET sensor service is resumed with Zn^{2+}-induced FE, except that membrane-bounded Zn^{2+} is being sensed rather than bulk Zn^{2+}. Previous PET sensors which targeted membrane-bounded H^+ showed similar effects.[79] The intrinsic pH sensitivity of the bis(pyridylmethyl)amine group shows up in the anthracenyl version as a fluorescent 'off–on–off' action[84,96] and **28** itself does not display any major protonic interference against Zn^{2+} sensing if the bulk water phase is buffered at pH 7. The dielectric effect of the micellar surface region also disfavours protons to some extent.[79] However, the negative surface charge of sodium dodecyl-sulfate (SDS) micelles produces higher H^+ densities there with respect to the bulk solution. pH buffering is complex in the micelle-bounded regions. This can rationalise the lower Zn^{2+}-induced FE in SDS. On the other hand, positively charged cetyltrimethylammonium bromide micelles show no FE at all, due to the repulsion of Zn^{2+} away from the micellar surface region where **28** resides and so the neutral Triton X-100 micelles emerge as the best performers.[77]



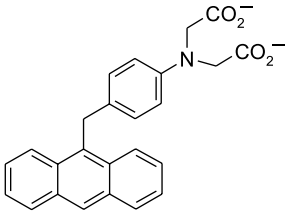
28



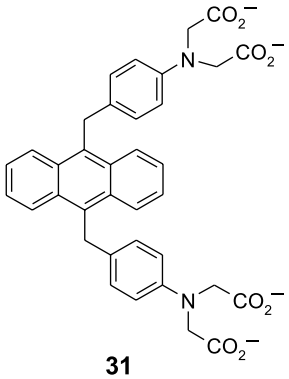
29

Another 'off–on' fluorescent PET sensor for Zn^{2+} in water, Gunnlaugsson et al.'s compound **29**,[97] has the appeal of perhaps being the simplest receptor. Nevertheless, an excellent FE of 50 is found with a final quantum yield of 0.21—a very respectable value. The sensor is pH independent in the physiological pH range, though it is switched on by 100-fold when the pH drops below 5.

Designed receptors for Zn^{2+} are frequently dogged by some response towards Cd^{2+} and so it is natural that the receptor used in **29** resurfaces in Gunnlaugsson et al.'s **30** and **31**.[98,99] The latter **31** acts as a typical 'off–on' fluorescent PET sensor for Zn^{2+}, but titration of **30** with Zn^{2+} results in the formation of an exciplex band at 468 nm. Hence, these cases would have been equally at home in Section 5.2. The exciplex could arise from interaction of a receptor-bound Zn^{2+} or the Zn^{2+}-bound receptor and the anthracene moiety. Cd^{2+} produces exciplex emission at 500 nm for both **30** and **31** with slightly stronger binding than for **29**.



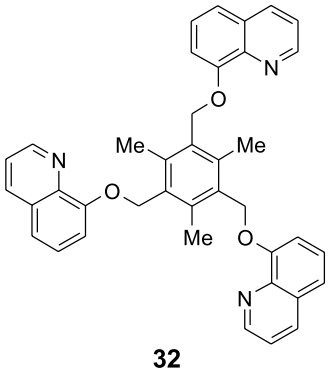
30



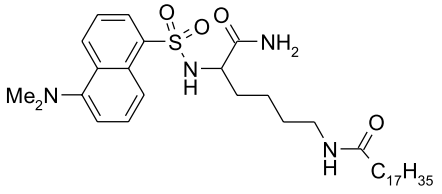
31

2.4.2. Cu^{2+}. The best performer according to the Irving–Williams series,[17] Cu^{2+}, is expected to yield more easily than most metals to sensor designers' efforts. Indeed, several nice receptors are available for incorporation into sensor and switch molecules. For instance, tripodal structural motifs continue to be popular in sensor design.[100–103] The fluorescence of **32**[100] is quenched strongly by Cu^{2+} by usual PET/EET mechanisms, in acetonitrile:water (4:1). Selectivity against Hg^{2+} is not great, but several other late- or post-transition metals are successfully rejected. Though 8-hydroxyquinoline is a versatile receptor[104,105] (see below), its etherification within **32** should remove some of its versatility.

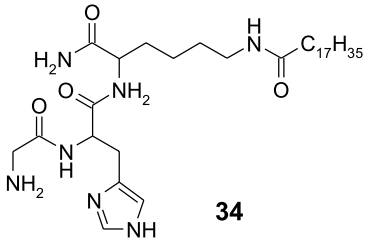
The majority of sensors and switches discussed within these pages are molecules swimming free in solution. Many applications prefer these molecules to be anchored on a surface. An example of this was discussed in the case study in Section 1.1.[25,26] It has also been our experience that people are convinced more readily by a solid object they can hold firmly in their hands rather than by a liquid swilling in a vessel. Leblanc's team[106] prepared monolayers of lipoidal peptides by the Langmuir method and deposited the films by the Langmuir–Blodgett techniques. Fluorophore **33**, receptor **34** and a 'fluorophore-spacer-receptor' system **35** are used, as well as mixed monolayers of **33** and **34**. The lipid-free version of **35** is known to be a selective sensor for Cu^{2+},[107] so the success of films of **35** is not unexpected. Both these cases, as well as other polyamines derivatised with the same fluorophore,[102] show fluorescence switching 'off'. Cu^{2+}-induced deprotonation of the sulfonamide contribute to this phenomenon, besides the usual PET/EET effects caused by the open-shell ion. Fluorescence quenching with Cu^{2+} is also seen in the mixed monolayer, indicating that intramolecularity is not necessary in such pseudo-intramolecular situations. Multilayers of pure **33** (inner) and **34** (outer), respectively, with or without intervening layers of a simple lipid, would provide a nice counterpoint to these studies. Micelle-assembled sensors for Cu^{2+} are not dissimilar.[108–111] Brasola et al.'s silica-based case[112] is formally similar, too.



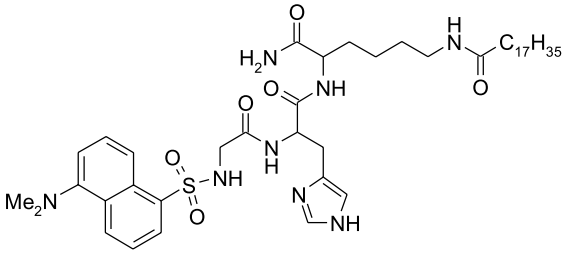
32



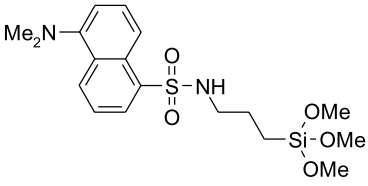
33



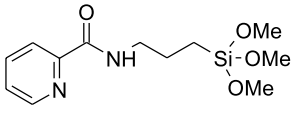
34



35



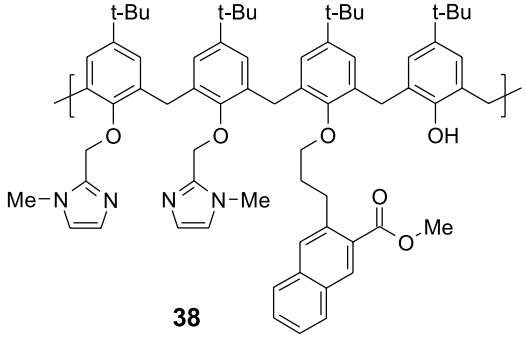
36



37

The alkoxysilane side chains of fluorophore **36** and receptor **37** allow grafting onto silica nanoparticles, as described by Brasola et al.[112] Foundational material is available concerning fluorophores anchored on such nanoparticles.[113] Latex particle-based sensors are also similarly valuable.[114] By varying the fluorophore-receptor ratio the sensitivity can be tuned. This effect is not seen in solution for **36** and **37**, suggesting the pseudo-intramolecular arrangement of fluorophore and receptor on the solid is critical. The pH effect on the sensor action towards Cu^{2+} is usual for such metal–ligand interactions, as is the selectivity against Ni^{2+}. The fluorescence quenching caused by Cu^{2+} is due to the PET/EET processes commonly seen during fluorophore-transition metal interactions.[115] Related systems grafted onto macroscopic quartz surfaces also form a valuable sensor configuration.[116]

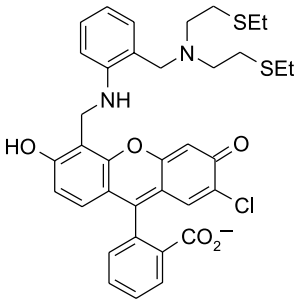
Calixarene **38**[117] is interesting because of its mix of functionalities, but its fluorescent sensory behaviour is not unusual. Compound **38** itself has a moderate fluorescence. Cu^{2+} switches fluorescence off due to PET/EET, whereas closed-shell Zn^{2+} increases fluorescence somewhat. H^+ also causes a small fluorescence increase. Acidification of the complex **38** Cu^{2+} ejects the metal, and hence emission is switched on. Addition of base also switches on fluorescence to some extent because of the change of the Cu^{2+} species into a mixed hydroxophenoxy species with attenuated PET activity, before eventual ejection as $Cu(OH)_2$.



38

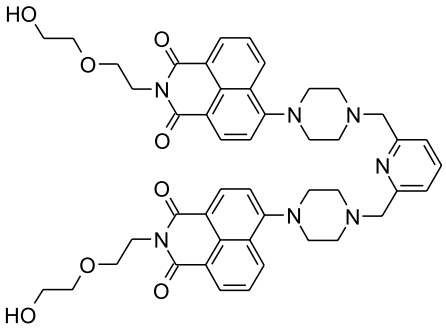
2.4.3. Hg^{2+}. The notoriety of Hg^{2+} as a toxic metal in the environment has naturally brought it to the attention of PET sensor designers. We are delighted how quickly solutions have been attained, from not one but several, teams. One is **39**, due to Nolan and Lippard,[118] which shows fluorescence switching 'on' with H^+ in water with a pK_a of 7.1. This rather high pK_a value can arise by protonation of the aliphatic amine. Protonated aminomethyl groups are very electron withdrawing[119] and so the oxidation potential of the aniline unit is raised sufficiently to stop PET. So **39** will be partially switched 'on' under simulated physiological conditions. Now, Hg^{2+} binds strongly to the NS_2 donor atom set and, by proximity, to the aniline nitrogen, too. So fluorescence is strongly switched 'on', which means that the

Hg^{2+}-induced FE is not huge, but useful. Though Hg^{2+} is normally sufficiently redox-active to spoil 'off–on' sensing schemes, S-coordination tamps this propensity down to manageable proportions.[120]



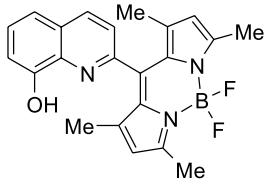
39

Another excellent example (**40**) is from Qian's team.[121] This also functions in neutral aqueous solution, but is built from a 2,6-bis(aminomethyl)pyridine receptor. Two aminonaphthalimide fluorophores are also incorporated, apparently for synthetic convenience, within the classic PET system. The two hydroxyethyloxyethyl groups assist with solubility. A red shift of 8 nm is observed upon addition of Hg^{2+}, possibly due to some intramolecular excimer formation. The selectivity for Hg^{2+} is very good. Hg^{2+} produces a FE of 17, whereas no other cation displayed a value greater than 3. Even 0.1 nM Hg^{2+} could be detected. The amine groups within **40** have a low enough pK_a value (5.2) to minimise protonic interference at pH 7.



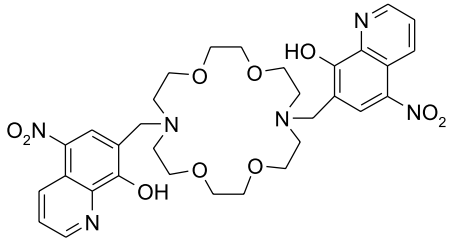
40

8-Hydroxyquinoline is a classical fluorescent sensor e.g. for Mg^{2+},[122] of the ICT type and so it is interesting to see it being employed as a receptor rather than as a fluorophore within **41**.[105] The fluorophore role is assigned to a boron dipyrromethine unit, though the situation is unlikely to be so clear-cut given the relative excited state energies of the two components. The two components are joined via a virtual spacer, given the steric forcing of a near-orthogonality of the two π-electron systems. Such systems are ripe for TICT[123] or PET behaviour.[3] Compound **41** is selective for Hg^{2+} in an aqueous dioxane solvent with fluorescence switching 'off' upon ion binding. However, 40% switching 'off' also happens with Cu^{2+}. The 30 nm red shift in the UV–vis absorption spectrum for Hg^{2+} indicates the ICT contributions to the emissive excited state.



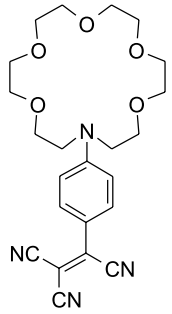
41

The origin[124] of the excellent 'off–on' Hg^{2+}-sensing ability of **42**[125] (though in mixed aqueous solution) is now analysed further by Bronson et al.[124] X-ray crystallography of the complex, **42**·Hg^{2+}, clearly shows that the metal is only bound by the pair of hydroxyquinolines and not by the diazacrown. So the latter serves two important roles: (a) as a scaffold for the ligating groups and (b) as an intramolecular base to pick up the protons from the phenol upon metal binding. PET from the amines would be inhibited in this way and strong fluorescence of the complex, **42**·Hg^{2+}, would be tolerated.

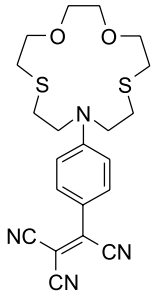


42

2.4.4. Distinguishing between Hg^{2+}, Pb^{2+} and Fe^{2+}. Martínez-Máñez's work[126] with **43** and **44** is distinguished by applying three techniques to examine their heavy metal coordination chemistry—UV–vis spectrometry (as seen in classical analytical colour reagents[104]) and cyclic voltammetry (as found by Beer[37]), in addition to fluorescence spectrometry which is our main aim in this review. Sensor behaviour can result from any, some or all of these three methods. For **43**, a blue shift is observed in the absorption spectrum on addition of Pb^{2+} only; an FE is also observed with closed-shell ion Pb^{2+}, but the emission is quenched by open-shell ions Cu^{2+} and Fe^{3+} by PET/EET effects. Though **43** appears structurally to be an ICT fluorophore, such systems are known to have TICT excited states[123] due to twisting about anilinic C–N or aromatic C–vinyl C bonds.[127] TICT phenomena have similar criteria as for PET and so the FE with Pb^{2+} is not surprising. Anodic shifts are observed during cyclic voltammetry for Hg^{2+}, Pb^{2+} and Fe^{3+}, due to the cationic destabilization of the sensor radical cation. These results show that one molecule **43** can selectively detect three different ions via three separate outputs. While this work involves multiple targets, it is not considered along with logic systems (Section 2.11), since the targets are not presented as sets to the molecular device.

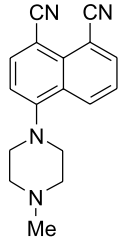


43



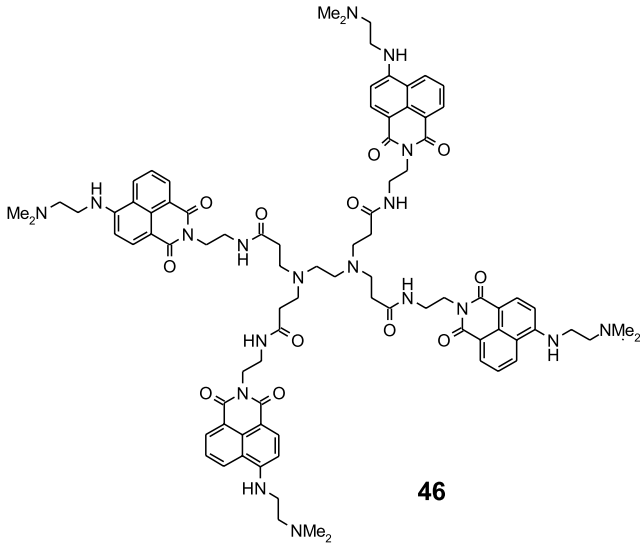
44

2.4.5. Detecting various transition metal ions/protons. Xiao and Qian's **45**[128] is just one example of a set of sensors which possess amine receptors spaced from several fluorophores, each of which have substantial ICT character due to their donor–acceptor nature. Those with the cyclic piperazine substituent, rather than acyclic groups, show twisting about the aromatic C–N bond owing to a *peri*-effect. Thus, the propensity for TICT state formation is increased.[123] The strategy of the design is to assist PET by the electric field of the TICT state, as has been found for the less dipolar ICT states.[50] Large FE values (>100) are seen with Fe^{3+} and Cr^{3+} and significant enhancements are caused by Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Pb^{2+}, but not with Hg^{2+} and Zn^{2+}.

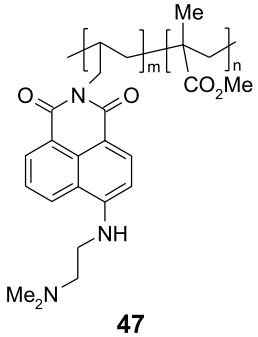


45

However, the rather basic amino receptor is likely to be protonated by the acidic hydration shells of these transition metal salts in (moist) organic solvents and caution must be exercised about the interpretation of the FE.[129] The same caution is probably necessary concerning the rather basic polyamidoamine (PAMAM) dendrimer **46**,[130] which contains the 4-dimethylaminoethylamino-1,8-naphthalimide 'fluorophore-spacer-receptor' system. FE values of 3–10 are found for **46** in DMF in the presence of various transition metals, notably Zn^{2+}, Ni^{2+}, Fe^{2+}, and Pb^{2+}. The authors suggest that the dimethylamino groups of the dendrimer serve to complex the metals. Certainly, the pseudo-chelating nature of these groups within the dendrimer must not be ignored. The basic sensing unit which invoked caution in the dendrimer **46**[130] is included in the copolymer **47**,[131] which is also tested with various transition metals in DMF. The latter study also finds that low-polarity solvents switch on the fluorescence. We reported this polarity effect in 2001 for a similar aminoethylaminonaphthalimide monomer.[132] Such polarity effects are also seen in related PET systems with hydrocarbon fluorophores, since photoinduced charge separation in neutral molecules is the main cause.[133]

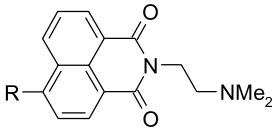


46



47

Compounds **48**[134] are another set where the problem of amine protonation by the acidic hydration shell of the highly charged metal ion rears its head. As the substituents becomes more electron deficient within **48**, Zn^{2+}- or Cr^{3+}-induced FE in acetonitrile solution increases substantially. The same order of FE values is seen when H^+ is added to **48** in mixed aqueous media.[50,135,136] The fact that the kinetically inert Cr^{3+} cannot exchange an amine ligand from **48** into its coordination shell during the conditions of a typical fluorescence spectroscopic experiment is an added complication.[137]



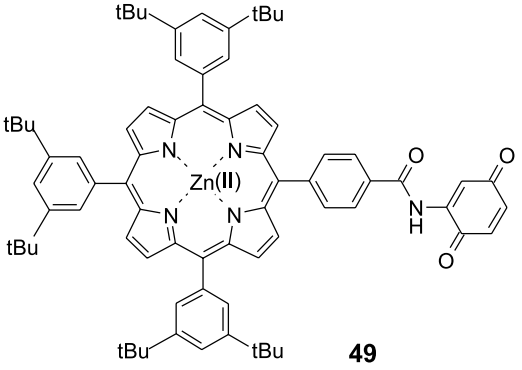
R = NH_2, OCH_3, Cl, H

48

2.5. Other metal ion targets

Compound **49**[138] is a porphyrin-Zn(II)-quinone dyad. PET occurs in benzonitrile as expected from the corresponding redox potentials and the fluorescence is switched 'off' initially. PET is confirmed by observation of the ESR spectrum of the zinc porphyrin radical cation and the quinone radical anion. Y^{3+} produces a large FE in the

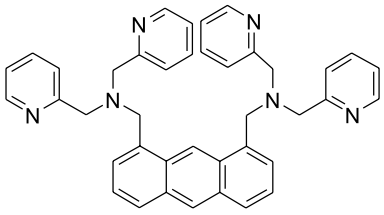
porphyrin. Interestingly, the isomer with the linker amide reversed shows no such switching. The quite selective binding (and fluorescence switching) of Y^{3+} originates in the correct size-fit to the two carbonyls of the dyad. However, this makes the ΔG_{PET} (-1.7 eV) even more negative than in the Y^{3+}-free case (-0.9 eV). So how does the Y^{3+}-induced FE arise? Okamoto and Fukuzumi[138] attack this question by fitting measured PET rates to the famed Marcus equation.[139] They find that the barrier to PET (due to the reorganisation energy) is raised with increasing driving force which slows its rate, allowing fluorescence to compete—a case of entering the Marcus inverted region.[139]



49

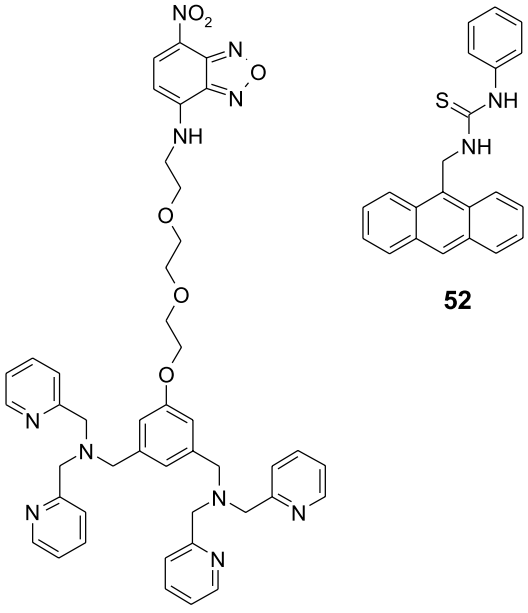
2.6. Anion targets

In the presence of Zn^{2+}, the ability of **50** to monitor the biochemically vital phosphorylation reactions of peptides in water[140] is quite rare and is likely to be very useful. Though the choice of dipicolylamine as a zinc receptor within **50** is not surprising (Section 2.4.1), the availability of two receptors is crucial for its success. After the first Zn^{2+} has bound to one receptor, it is much harder to bind a second metal ion into the second receptor due to electrostatic repulsion. Binding of an anion to the first Zn^{2+} is a common behaviour of such dipicolylamine complexes with incomplete coordination shells. Importantly, Hamachi and colleagues choose this anion as a phosphorylated peptide sequence. This anion binding to the first Zn^{2+} facilitates the binding of a second Zn^{2+}, by cutting down the aforementioned electrostatic repulsion. Now both PET channels from the benzylic nitrogens to the anthracene fluorophore are blocked, and a large FE is seen. The preliminary paper[141] did not carry this mechanism, however. As has been observed for a long time, blocking one PET channel out of two is not sufficient to obtain a good FE.[142–144] Crucially, the complex, **50**$\cdot 2Zn^{2+}$, can discriminate between phosphorylated and non-phosphorylated peptide sequences since the former possess the all-important phosphate unit. Also, the fluorescence response is shown to be sequence dependent—the more negative the phosphorylated peptide sequence then the greater the fluorescence response. Naturally, dephosphorylation reactions can also be monitored using the complex, **50**$\cdot 2Zn^{2+}$, since it is based on reversible interactions. The complex, **50**$\cdot 2Zn^{2+}$, has been cleverly applied by Koulov et al.[145] as a sensor for the anionic phospholipid phosphatidylserine on the outer leaflet of cell membranes which in turn signals that the cell has proceeded to the apoptotic phase. Given the apocalyptic possibilities of the latter, Koulov et al.'s application promises to be a particularly powerful one.



50

Lakshmi et al.[146] now extend the excitation wavelength of these sensors to 470 nm with **51**, so that it is compatible with the specifications of the current generation of flow cytometers. Indeed, **51** works well by being selective for 5% incorporation of phosphatidylserine in vesicle surfaces, but not for related compounds in homogeneous solution. Two other cases like this which only succeed in membrane media are covered in this report.[77,80] Here, the fluorescence enhancement is at least partly due to **51** being bound to the anionic vesicle surface of lower polarity than bulk water. The ICT fluorophore is well known for fluorescence switching 'on' in non-polar solvents.[147,148] The probability of PET involvement is lowered further when we consider the length of the spacer in **51**. The Hamachi et al.[140] work has an important relevance to several of the dipicolylamine-based Zn^{2+} sensors discussed in Section 2.4.1. For instance, Lippard's cases[83,91] use a fluorescein fluorophore which has phenolate groups next to the dipicolylamine units. This provides the negative charge which tamps down the positive charge density caused by the first Zn^{2+}, so that the second Zn^{2+} can arrive at relatively low concentrations. Hamachi's case has no such anionic centre and, hence, depends on the phosphate group of the peptide to provide it.

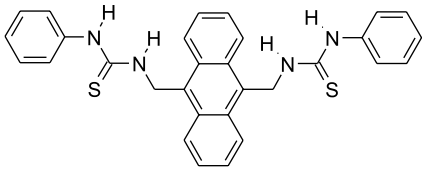


52

51

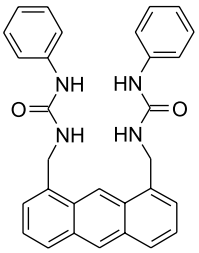
Gunnlaugsson et al.'s compound **52**[149,150] contains a thiourea receptor, a methylene spacer and anthracene as

the fluorophore. The distal end of the thiourea has been altered to modulate its binding strength towards a given anion. The fluorescence in DMSO is switched 'off' in the presence of AcO^-, $H_2PO_4^-$ and F^- by 70, 50 and 90% of the maximum quantum yield of 0.10. The work is smoothly extended with **53**[151] carrying two thiourea binding sites for improved selectivity towards bidentate anions, e.g. $P_2O_7^{2-}$ and malonate. The plan, its implementation and the results carry on the tradition of bisazacrown ether-[142] and bisboronate[144]-based PET sensors. A related PET-based ditopic sensor for $P_2O_7^{2-}$ itself[143] is also available in the literature.

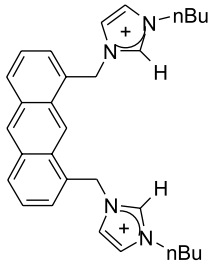


53

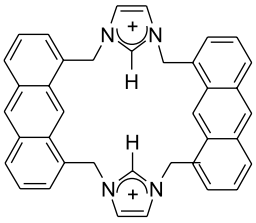
In a clutch of papers, Yoon, Kim and their co-workers examine the sensory consequences of locating a pair of hydrogen bond acidic sites near/on hydrocarbon fluorophores. They succeed with F^- targets in most cases, and not very well with $H_2PO_4^-$ in others, but almost always in aprotic solvent mixtures. The hydrogen bond acidic sites are either imidazolium or urea groups. Their first effort which we consider is sensor **54**[152] in aprotic solvent mixtures, which shows significant F^-/Cl^- selectivity by chelating F^- between its urea groups. Such chelation can stretch H–N bonds to the point of deprotonation. The consequent increase in electron density of the ureas would allow them to engage in PET with the excited anthracene and so a substantial decrease ($\times 20$) in the emission intensity is the result. Then, they proceed to sensor **55**[153] which is set up with the imidazolium groups to bind with anions through a tweezer-like conformation. Fluorescence quenching is seen with $H_2PO_4^-$, though F^- is a significant interferent. The anthracene moiety in the host acts not on only as a fluorophore, but also as the origin of binding selectivity. Such a dual role of anthracene in fluorescent-sensing contexts has a decade of precedence,[142,144] one of which is from Yoon and Czarnik.[143] The binding can be assigned to $(C–H)^+\cdots X^-$ hydrogen bonding, which is clearly indicated by NMR downfield shifts. It is hard to see how PET can be the mechanism of sensory action, in spite of the structurally obvious 'fluorophore-spacer-receptor' nature of **55**. On the other hand, the bound $H_2PO_4^-$ will present its O–H bonds as hydrogen bond donors to the anthracene π-system in its excited state causing the observed fluorescence quenching.[154] Interestingly, F^- does not show consistent quenching, even though its calculated binding strength is the best of all. Compound **56**,[155] which is the bifluorophoric and macrocyclic version of **55**,[153] mitigates the F^- interference of the binding of $H_2PO_4^-$ somewhat, but there is still much room for improvement. The reliance on aprotic solvents also has not been shaken off. On the positive side, no excimer complications are seen. Closely related cases without spacers from this laboratory[156] and another[157] will be considered below.



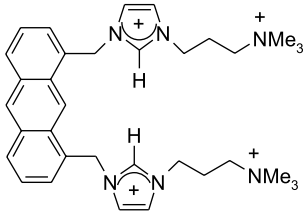
54



55



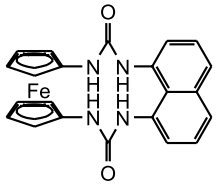
56



57

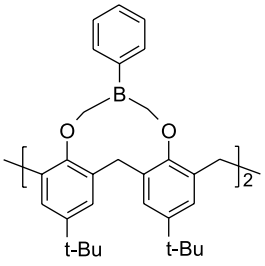
Yoon and Kim's saga with anthrylmethylimidazolium systems targeting anions continues with a welcome foray into neutral aqueous media.[158] Now, the macrocyclic **56**[155] is abandoned in favour of a more highly charged version of **55**,[153] that is, **57**.[158] The fluorescence of **57** is quenched by a factor of 6 by guanosine triphosphate (GTP), whereas several other anions including all three adenosine phosphates only show weak enhancements. The authors attribute this behaviour to an enhanced fluorescence rate due to the larger dipole component of GTP normal to the anthracene plane in a theoretically optimised structure of the complex. Unfortunately, no X-ray crystallographic structure is available. However, chemical enhancements of radiative rates of any substantial significance are very rare in our experience,[10] except for metal plasmon-enhanced fluorescence.[159] Anyhow, this can be checked by fluorescence lifetime measurements on **57** with and without GTP. Another rationale for the observation is the well-known oxidisability of guanine, c.f. the other nucleic acid bases leading to PET from GTP to the electron-deficient anthracene fluorophore. Additional quenching can arise from a hydrogen bond from the bound GTP to the anthracene π-cloud. Such hydrogen bonds from carboxylic acids are known to quench the fluorescence of anthracene derivatives.[154]

Macrocyclic sensor **58**[160] exhibits a naphthalene-like emission spectrum with a FE of 12-fold in the presence of 2 equiv of F^-. The stoichiometry is confirmed by NMR studies. Cyclic voltammetry shows a 190 mV cathodic shift in the oxidation potential of the ferrocene moiety upon addition of F^-. $H_2PO_4^-$ is a poorer performer, whereas other anions such as Cl^-, Br^-, and $H_2SO_4^-$ showed no evidence for complexation from any of the three techniques used in this wide-ranging study. However, fluorescence quantum yields cannot usually be expected to be high in systems containing fluorophores and redox centres.



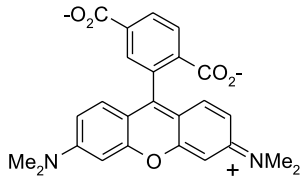
58

Boronic acids have played a valuable part in the growth of the field of luminescent sensors, especially regarding their applicability in aqueous media,[161,162] something the hydrogen-bonding systems still struggle with.[152,153,155,156,158] Therefore, it is a pleasure to welcome a new boron compound to the field. Bis(bora)calixarene **59**[163] shows a decrease in fluorescence upon encountering F^-.

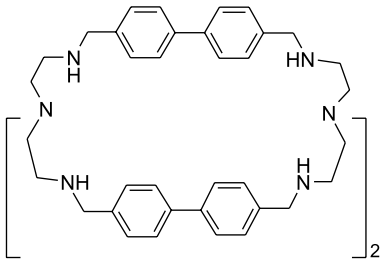


59

Being an important neurotransmitter, glutamate is an important target for sensor designers. Now Bonizzoni et al.[164] achieve this goal with an indicator displacement assay. The fragility of assembled systems means that they are yet to be successfully operated in multi-compartmental systems prevalent in biology. Nevertheless, the present achievement deserves applause for its elegant design. Fluorophore **60** binds partly electrostatically to the complex, $\mathbf{61}\cdot 2Cu^{2+}$, and is switched 'off' completely due to quenching by the neighbouring Cu^{2+} centres by EET/PET. Glutamate displaces **60** into bulk water, so its fluorescence switches back 'on' with a huge FE and an apparent association constant of 10^7. Other neurotransmitters are almost silent, or in the case of glycine, respond with much lower association constants (2×10^3). However, glutarate binds with a constant of 10^7. Overall, this is a nice solution to a tough problem, at least for use in cuvet- or well-based assays. This is just one example where Fabbrizzi and his team have helped to popularise the indicator displacement method, which is currently championed by Anslyn and co-workers,[101] especially where transition metal sites are involved. Such competitive displacement assays have had a short, but fruitful, history in the luminescence field, following the radiochemical archetype.[165] Various receptors such as cyclodextrins,[166,167] calixarenes,[168,169] crown ethers[170] and tripodal systems[101] have been co-opted into it during this time.



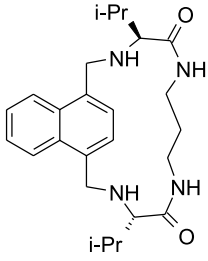
60



61

2.7. Other organic targets

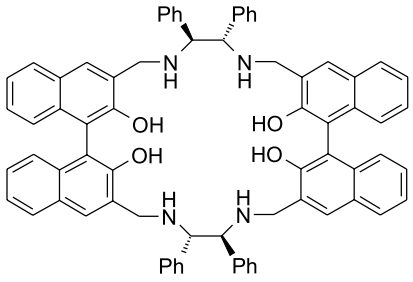
2.7.1. Schemes using reversible interactions. Compound **62**[171] in acetonitrile displays a broad emission band centred at 390 nm, red-shifted by 60 nm from the typical naphthalenic emission. This is an intramolecular exciplex[172] produced by interaction of the naphthalene excited state and the proximal amines. Some related classical systems show this behaviour,[76,173] while others do not,[133] since exciplex formation and PET can be quite finely balanced. Protonation of amine groups restores naphthalenic fluorescence,[56,174] while removing the exciplex emission.[174] Even though **62** can be seen as a ratiometric sensor for H^+ in organic solvents, the need for such targets is not great. It also displays various FE values with different *N*-protected amino acids, with L-phenylalanine showing the largest FE, perhaps due to the π–π stacking between phenyl units of the guest and naphthalene moiety of **62**. Such results are likely to be dominated by protonation effects rather than more complex supramolecular interactions. The small degree of enantioselective recognition of *N*-protected amino acids found with **62** illustrates this problem. However, such studies are interesting because of the challenge.[175]



62

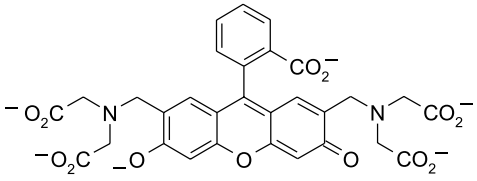
Bisbinaphthol macrocycles **63**[176] with chiral subunits display enantioselective recognition of amino acid derivatives in mixed dimethoxyethane solution. The latter solvent component is required to aid dissolution. The binaphthol

fluorophore is clearly spaced from the electron-donor amines, which occupy the rim of the macrocycle. Hence, **63** has the hallmarks of a PET system. Nevertheless, it also has phenolic groups, which are receptor elements integral to the fluorophore in the ICT manner. In particular, (+)-**63** showed a FE of 5.7 on addition of D-*N*-benzyloxycarbonyl-phenylglycine, but virtually no enhancement for the L-analogue. Conversely, (−)-**63** showed enhancement for the L-amino acid derivative, but not for the D-form. The general discussion concerning the work of Galindo et al.[171] also applies here.



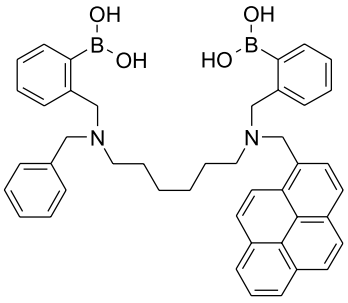
63

As its name implies, calcein (**64**) is a classical fluorescent sensor, which switches 'on' with Ca^{2+} in water.[177] Suitably alkaline solutions are needed for this action. PET underlies this behaviour, since the methylene groups can play a spacer role, though the coordination of the phenolic oxygens introduces a modicum of ICT character. Compound **64** itself is fluorescent at neutral pH when the amine groups pick up H^+. When this species coordinates with the open-shell ion, Cu^{2+}, the resulting complex is non-fluorescent.[177] In the presence of amino acids, Cu^{2+} is pulled away from the sensor, which recovers its fluorescence. Thus, a high-throughput screen for proteases becomes possible.[178] The screen is not suitable at acidic pH because of lowered fluorescence quantum yield of the fluorescein fluorophore, as well as the lowered coordinating ability to Cu^{2+}. Care is also probably needed concerning the free Cu^{2+} in solution to avoid interference with the protease.



64

James' laboratory continues its expertise in sugar sensing by developing a series of bis(aminomethylphenylboronic acid) systems carrying various fluorophores such as **65**.[179] These are typical PET systems for glucose and its relatives.[161,162] However, the binding selectivity is controlled by the solvation and/or sterics of the fluorophore. Some of the earliest PET systems for protons also displayed this kind of perturbation of the receptor properties by the fluorophore.[56,58] Compound **65** was most selective towards glucose, probably due to the largest hydrophobic π-surface displayed by pyrene. As the π-surface is reduced, the selectivity among monosaccharides switches from glucose to galactose. Unfortunately, the FE values do not show much selectivity with respect to the monosaccharides, at least in MeOH solution.



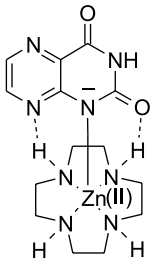
65

Fluorimetric detection of thiols, and those amino acids/peptides that contain thiols, e.g. cysteine, in neutral water remains an active area[180] because most of the older detection systems rely on covalent bond formation. For instance, several fluorescent PET systems employ electrophiles such as malemides[181,182] to react with thiols. No reversible chemistry is involved and there is no contribution from supramolecular or coordination interactions in these cases. Therefore, true sensors for thiols (with reversible action) are still needed. Compound **66**[180] is therefore an interesting entrant. Kimura's prior work has established the usefulness of Zn^{2+}-cyclen for binding imide equivalents in water.[183] The fluorescence of such structures has also been reported by De Cola, Koenig and colleagues,[184] whereas the imide itself is switched 'off'. The mechanism probably has to do with low-lying $n\pi^*$ excited states in the imide, which are destabilised upon Zn^{2+} binding. The fluorescence of **66** and its quenching upon thiol addition are, therefore, not surprising. This is due to competition by thiols against the imide for the Zn^{2+} site. Competition assays are popular,[101,164] but, in these cases, an intrinsically fluorescent dye is used to compete with the analyte, and the dye-receptor complex is switched 'off'. Compound **66** is opposite in its fluorescence behaviour. It is effective at measuring levels of the tripeptide, glutathione. However, the fact that aspartic and glutamic acids cause a reduction in fluorescence is a warning that Zn^{2+} complexes of this type can also bind carboxylates at the axial position[185] to cloud the selectivity profile of **66**.

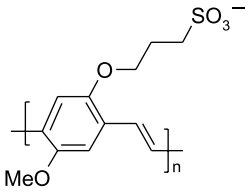
The fluorescence of polyanionic **67** is switched 'off' by **68**. The distinguishing feature is its huge efficiency, brought about by electrostatic concentration of **68** in the neighbourhood of **67** and subsequent PET, which, again, is amplified by the extensive exciton length. This is superquenching, as named by Whitten et al.[186] 'Quencher-tether-ligand' (QTL) system **69** has an effect similar to **68**. Now, the fluorescence of **67** can be switched back 'on' by the protein, Avidin. Avidin is well known to tightly bind to the biotin ligand within **69** and sterically displaces the polymeric **67** and so this is also an indicator displacement assay.[101,164–170]

Yang et al.[187] combine components from several previous ditopic PET sensors[188–190] within **70**, which selectively switches on in response to glucarate, but, as in a previous

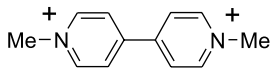
case,[188] the guanidinium unit does not play a major role in the switching action. However, it does improve the binding selectivity.



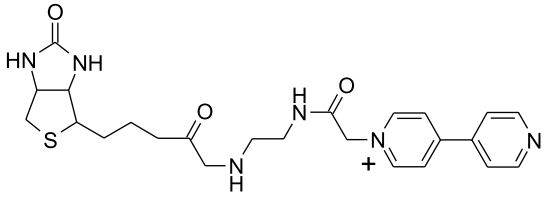
66



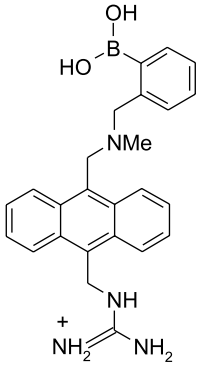
67



68

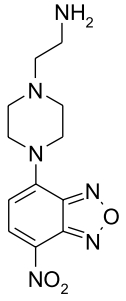


69

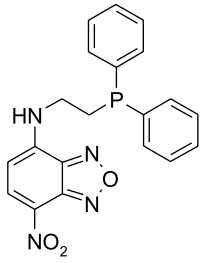


70

2.7.2. Schemes using irreversible interactions. PET switching is robust enough to deal with targets which are covalently bound into the switch molecule upon first encounter. Therefore, fluorescent derivatisation reagents can benefit from the same design principles used for sensors and switches. Imai's team apply PET principles to design a reagent **71** for carboxylic acids.[191] It contains a primary amine, which readily reacts with carboxylic acids to form an amide bond under a defined protocol. The products are subjected to capillary electrophoresis with laser fluorescence detection in acidic aqueous acetonitrile. The acidic medium ensures the protonation of the aliphatic tertiary amine and stops PET. Older examples targetted isocyanates, for instance.[192] Amine **71** contains a member of the family of 4,7-disubstituted benzofurazan fluorophores favoured by many workers. Indeed, the derivatives with a nitro group and chlorine or fluorine have been used for years as fluorescent reagents for amines,[193] and the sulfonamide with fluorine as a selective fluorogenic reagent for thiols.[148] The reagent function is aided by the fact that halide derivatives in the 4-position readily undergo nucleophilic aromatic substitution with nucleophiles such as amines and thiols. Several sensor designers have also succumbed to the pull of the benzofurazans.[129,194–199] However, their photophysics could do with more clarity.[147] Uchiyama et al. now take up the challenge,[148] by studying benzofurazans with various substituents on the 4- and 7-positions with a range of methods including time-resolved thermal lensing. They find that replacement of 4-halide by amines removes an intersystem crossing channel caused by a triplet $n\pi^*$ state. The major deactivation pathway turns out to be internal conversion, but its nature still remains veiled.



71

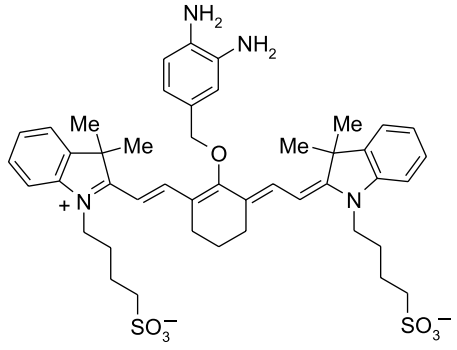


72

Fluorescent PET reagents, though not recognised as such, have existed for longer than sensors. For instance, cases which undergo a large fluorescence increase in the presence of thiols have been known since 1973.[182] An early instance of isocyanate detection is also available.[192] Deliberately designed cases for thiols appeared in 1998.[181] Now, Imai's laboratory comes up with a good reagent for hydroperoxides[200] building on older work by Akasaka et al.[201] Free **72** has a very low quantum yield of fluorescence in solvents such as acetonitrile or methanol, because of PET from the diphenylphosphine unit to the benzofurazan fluorophore across the dimethylene spacer. Upon addition of micromolar quantities of *t*-butyl hydroperoxide or cumyl hydroperoxide, the reagent reacts quantitatively at 50 °C, resulting in the oxidation of the phosphorus atom, and thus preventing PET as observed by FE values of 30. As is usual with PET reagents, it is not easy to reverse the fluorogenic

reaction. The same laboratory has unveiled another reagent for peracids based on sulfur instead of phosphorus,[202] but this does not react with simple hydroperoxides.

Following his[16] and Plater's[203] success with PET reagents for NO which are excited and emit in the visible region, Nagano and his group now tackle those which can function in the near-infrared.[204] It has long been appreciated that near-infrared radiation can penetrate deeper into tissue. The cyanine fluorophore within **73**[204] engages in PET from the *o*-phenylenediamine unit. NO reacts as usual with the diamine reactive site within **73** to yield the corresponding benzotriazole.[16] A nice FE of 12 is seen. We must not forget that, essentially, the fluorophore and the aniline-based Tsien receptor for Ca^{2+} were successfully used by Ozmen and Akkaya previously in a PET sensing context,[205] so that the success of **73** is to be expected. Nevertheless, the ability to apply NO reagents deep in tissue is laudable.

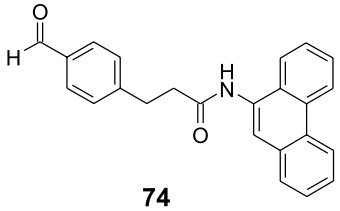


73

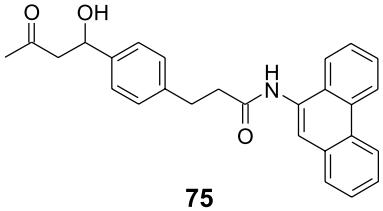
2.8. Reaction targets

The availability of luminescent reagents which bind covalently with target molecules can be viewed from a slightly different perspective as a way of monitoring a particular type of reaction. The latter approach will thus provide a new avenue of application for old luminescent switches.

PET systems based on the 'fluorophore-spacer-receptor' format have been previously used to track reactions at carbon-based functional groups by fluorescence switching 'on'. For instance, Michael additions of thiols to enones were handled in this way.[181] Virtually spaced PET systems are even older.[182] Now, Tanaka et al.[206] show that poorly fluorescent aldehyde **74** undergoes the aldol reaction with acetone in aqueous buffer (pH 7) with 1% cosolvents to give **75**, which is strongly emissive. Though an EET mechanism is assigned in this paper, we feel this is unlikely for several reasons. The lowest excited singlet state of benzaldehyde is higher in energy than that of phenanthrene, so the latter cannot be an EET donor.[207] Benzaldehyde also has a small extinction coefficient for excitation to its lowest excited singlet, which also disfavours EET to it. The emission of the amidophenanthrene unit (>350 nm) does not overlap well with the benzaldehyde absorption band (ca. 330 nm)—another requirement for EET. On the other hand, PET fits the bill very well due to the electron-rich amidophenanthrene and the electron-poor benzaldehyde. In fact, we estimate ΔG_{PET} to be −0.6 eV from available redox potentials,[30] which is a very favourable value. Of course, aldol formation removes the electron-withdrawing aldehyde from the benzene ring. Thus, the benzene ring is no longer sufficiently electron deficient to allow PET. We have seen a similar PET switching 'on' when benzoic acid with its electron-withdrawing carboxylic acid unit is changed to benzoate.[208]



74

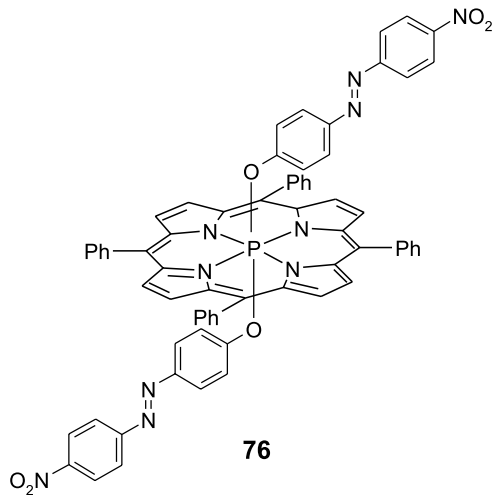


75

2.9. Physical property targets

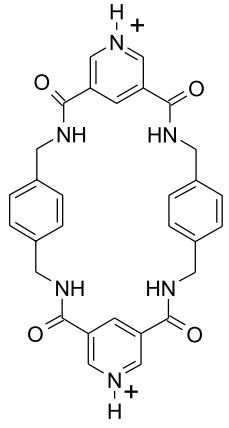
All of the previous discussion has concerned interactions between a designed molecule with a chemical species which switches a fluorescence signal 'off' or 'on'. The latter effects can also be achieved by taking advantage of the weak interactions offered by solvation, for instance. A case of this type was mentioned in passing concerning Grabchev et al.'s compound **47**.[131] However, chemical effects can also arise from non-chemical entities such as light. Photochemistry can thus give rise to fluorescence signals. Indeed, Zweig developed this general approach over three decades ago.[209] It is notable that light dose, rather than light intensity, is the real input variable.

The photochemistry of the azobenzene and the tetraphenylporphyrin units in **76**[210] can be individually addressed. Irradiation at 345 nm achieves the classical *trans* to *cis* azobenzene transformation. The fluorescence (excited at 565 nm) of the *cis* form is less than that of the corresponding *trans* form, which, in turn, is less than that of a simple porphyrin control. The photoswitching function of the molecules survives many irradiationl/dark relaxation cycles, and is probably due to the different distance of the *cis* or *trans* form of the axial azoarene from the porphyrin, giving different PET rates. PET is clearly thermodynamically feasible, but no decomposition is seen from this pathway. Different donor–acceptor orbital overlap in the two states may also contribute.

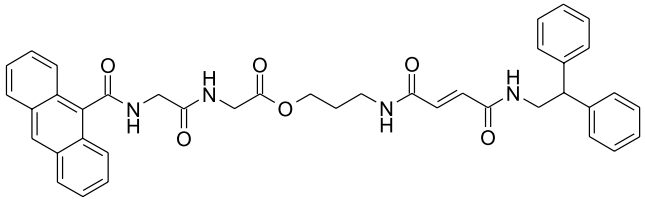


76

Perez et al.[211] designed the 'bead on a string' rotaxane **77**–**78** such that the bead sits on the *trans* fumaric diamide station due to a hydrogen-bond array of high strength in dichloromethane solution. Then, the anthracenamide fluorophore is remote from the electron-acceptor pyridinium units, so that PET is absent and the fluorescence is switched 'on'. However, light irradiation input at 312 nm isomerises the *trans* fumaric diamide into the *cis* maleic diamide. The hydrogen-bond array is disrupted in this new station geometry and so the bead moves across to the glycylglycine station, which allows hydrogen-bond array with intermediate strength. Now, PET is strong at this small distance of separation between the PET pair and the fluorescence is switched 'off'. The 'on–off' intensity ratio is 85. This constitutes a lovely example of light writing with fluorescence readout somewhat related to Tian's case using photogenerated acid.[49]



77



78

An equally beautiful example of a rotaxane based on a cyclodextrin bead and an azobenzene station with two different naphthalimides as stoppers is also available from Tian's laboratory.[212] This shows switching 'on' and 'off' in two different colours upon irradiation to cause the *cis–trans* isomerisation in either direction. The same group has also demonstrated light-induced fluorescence switching by substituting rotaxane-type machinery with phthalocyanine–dithienylethene photochromics.[213]

2.10. Nanoparticle targets

Even though metal nanoparticles are considered to be efficient quenchers of fluorescence, Thomas and Kamat[214] managed to overcome this with **79**, the fluorescence of which is switched 'on' with gold nanoparticles capped with quaternary ammonium ions. This is attributed to the blocking of PET from the amine to the pyrene fluorophore by coordination to the gold surface. Even cases of voltage-induced fluorescence switching 'on' under electrochemical conditions for similarly functionalised nanoparticles are now known.[214] The nanoparticle effect on fluorescence is more complicated with the discovery of metal-enhanced fluorescence, which is thought to be surface plasmon-induced acceleration of the fluorescence radiative rate.[159]

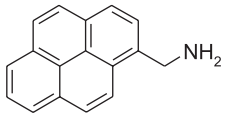
The story so far is a clear illustration of the vitality and value of PET sensors in the hands of designers in far-flung parts of the world. A big reason for this popularity is the quantitatively predictive nature of PET designs.[3]

2.11. Multiple targets

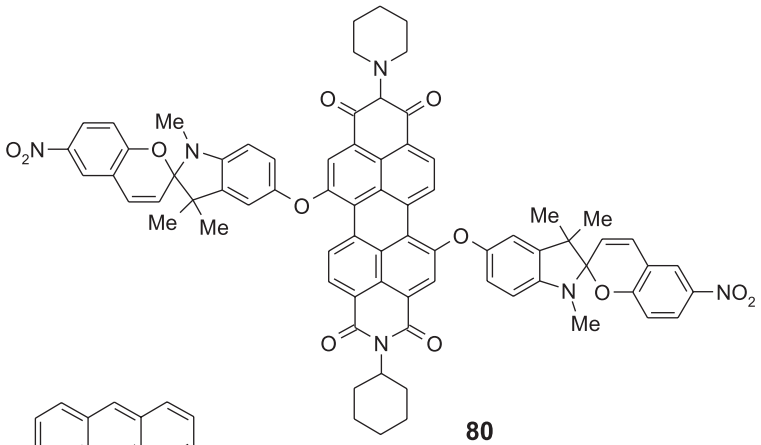
The past decade has started to provide examples of how molecules can begin to achieve[23,215–219] at least some of the computational functions developed in silicon technology.[220] The recognition[221] that two-way communication with molecular logic devices can be achieved with chemical species and light signals has allowed movement down this road. The use of PET systems based on the 'fluorophore-spacer$_1$-receptor$_1$-spacer$_2$-receptor$_2$'[221] and 'receptor$_1$-spacer$_1$-fluorophore-spacer$_2$-receptor$_2$'[119] format allowed AND logic gates with fluorescence output to be demonstrated. More recent AND gates of this type concern Na^+ and various phosphate species,[222] K^+ and F^-,[223] and Na^+ and transition metal ions.[224] Schneider's group even interpret gel swelling in terms of AND logic.[225] It is perhaps worth reiterating that AND logic is not confined to a computer. It can be seen at work in human cooperation, for instance.

Connecting two photoactive switches opens up the danger of EET destroying the photoactivity of the higher-energy excited state. For instance, azobenzene–porphyrin conjugates do not show significant *cis–trans* isomerisation of the azobenzene unit.[226] Guo et al.[227] have managed to overcome this situation with **80**, where the usual spiropyran photochromic ring opening to merocyanine[228] can be achieved by uv irradiation, in spite of the presence of the perylenediimide. The merocyanine can complex with Fe^{3+},[229] moving its absorption spectrum to higher energies and stopping EET acceptance from the perylenediimide fluorophore. The aniline moiety of the spiropyran can bind

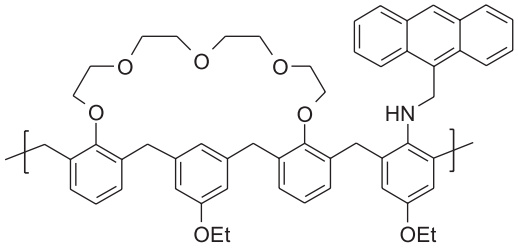
to H^+ and stop its PET donor action towards the perylenediimide fluorophore. Indeed, the fluorescence is switched 'on' only if UV light, Fe^{3+} and H^+ are all supplied in THF. This constitutes a three-input AND logic gate. Interestingly, the authors do not report irreversibilities arising from any Fe^{3+}-induced oxidation of the aniline moieties. Fe^{3+}-induced oxidative electroconversion of related compounds with similar oxidation potentials is known.[45]



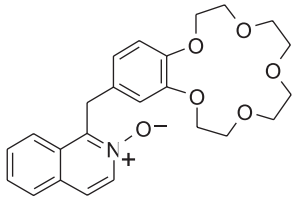
79



80



81



82

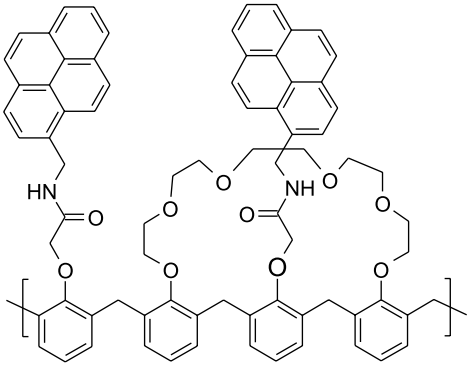
Though AND logic is perhaps the easiest to appreciate, it is only one of many gate types.[220] For instance, INH logic has one input holding a veto over the other. A nice example, **81**,[230] has its fluorescence switched 'on' by H^+, but only as long as K^+ is absent. The 1,3-dialternate calixcrown binds K^+ via ion-dipole and cation-π interactions.[231] The bound K^+ electrostatically ejects H^+ from the anilinium moiety (if it had been previously protonated), but is apparently incapable of sufficient direct interaction with the aniline nitrogen lone pair and so PET is re-established from the aniline to the anthracene.

Though several INH logic gates are known,[230,232–236] **82**[235] is special because PET produces an observable charge transfer (CT) emission when the *N*-oxide attaches onto an H^+. In all cases discussed in this report, PET causes only the loss of the characteristic emission of the fluorophore from a locally excited (LE) state. However, in the present instance, the PET is arrested by attaching K^+ to the benzocrown ether and so the CT emission subsides as well. K^+ is therefore the disabling input of this INH gate. The fact that **82**'s emission can be observed from the CT or LE states, or even at intermediate wavelengths, is notable and that it can receive H^+ or Zn^{2+} at the *N*-oxide, besides receiving K^+ or Ba^{2+} at the crown, is an added distinction. Such flexibility in inputs and outputs smoothly leads to reconfigurability of the logic function in several ways—some of these even simultaneously. Such achievement of reconfigurable or even superposed logic has only a short history.[236–239] From another viewpoint, **82** combines PET with ICT processes (since the *N*-oxide is integrated into the fluorophore). Such combined switching mechanisms are rare, too.[129]

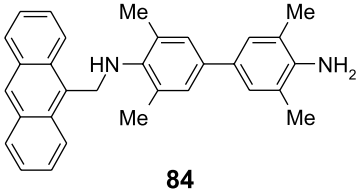
Now, we move to other logic types. Compound **83**[240] is composed of a calix[4]arene-18-crown-6 receptor and two pyrene fluorophores linked to it via two amide linkages. In its free form in acetonitrile, normal pyrene monomer emission is seen. Upon Pb^{2+} binding to the crown and the carbonyl oxygens, fluorescence is switched 'off' due to PET from pyrene to the electron-deficient amide. Also, addition of acid to the free **83** causes quenching of fluorescence due to PET from pyrene to the protonated amide. Addition of base, however, removes the amidic proton, resulting in PET from this electron-rich moiety to pyrene. The availability of three inputs allows logic functions such as NOR and XNOR to be demonstrated, though stoichiometric amounts of acid and base need to be maintained.[241] Several earlier examples of molecular NOR[232,242] and XNOR[243] logic are available. An original molecular logic function obtained by Lee et al.[240] is a nice disabled XNOR, which is perhaps best represented as an XNOR gate (driven by acid and base inputs) feeding a two-input AND gate, the other input of which is the output from a NOT gate driven by Pb^{2+} input. The disabling input is therefore Pb^{2+}. Such disabling lines have only been demonstrated within INH gates before.[230,232–235,244]

Mohr has developed the idea of fluoro/chromoreactands,[245] where a reversible reaction leads to a change in the fluorescence or absorption spectrum of a sensor. Many of

his cases function best in a polymer membrane reminiscent of ion-selective electrodes, when the analyte is extracted out of bulk water solution. Yang et al.'s compound **84**[246] is an anthracenylmethylbenzidine. The anthracene is clearly a fluorophore spaced from the π-electron system of the benzidine which contains integrated amines. The latter is also a fluorophore emitting in the same region as the anthracene, though it needs excitation at shorter wavelengths. Both amines can act as receptors for H^+ and the distal amine can act as a reactive site for the analyte aldehyde. Both receptors inhibit PET to anthracene when protonated, and the distal amine inhibits PET to anthracene upon forming a Schiff base in the presence of aldehydes. Compound **84** is particularly interesting because it mixes a protonation and a more complex reaction. The full logic ramifications of cases like it are yet to be explored.

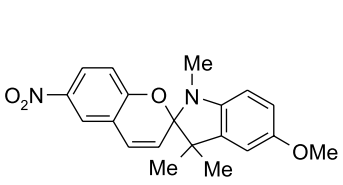


83

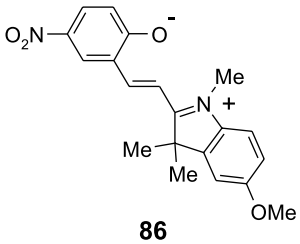


84

Raymo and Giordani[247] used non-luminescent dyes to show how the output of one molecular switch could be fed as input into another—via protons. Guo et al.[248] now demonstrate a case involving fluorescence. Compound **79** is a simple PET pH sensor[56] the fluorescence of which is switched 'on' when protonated. As noted before, the spiropyran photochromic **85** converts into the merocyanine **86** via UV irradiation. The increased basicity of **86** leads to the acceptance of a proton from **79**·H^+, thus switching 'off' fluorescence. Irradiation with visible light converts the cation **86**· H^+ back into the spiropyran **85** and releases the proton, allowing it to join **79** again and switch fluorescence back 'on'. At least formally, two inputs of UV and visible light doses are involved.



85



86

Older textbooks concerning computing show how two diodes can be wired to produce AND logic activity.[249] Two nanosheet diodes built from polymer Langmuir–Blodgett films are now operated at different wavelengths to produce an AND logic gate.[250] One nanosheet is constructed with a phenanthrene- and an electron-acceptor-based (dinitrobenzene) acrylate–acrylamide copolymer film and the other with an anthracene- and an electron-donor-based (dimethylamine) acrylate–acrylamide copolymer film. The input signals are the excitation wavelengths for the two chromophores, phenanthrene and anthracene, each of which can be selectively excited. When the phenanthrene layer is excited at 300 nm, PET occurs to the dinitrobenzene-containing film, and a photocurrent of 70 pA, similar to the dark current, is recorded. When the anthracene layer is excited at 380 nm, ET occurs from the dimethylamino layer to the anthracene film, again with a low photocurrent of 70 pA. However, when both chromophore-containing polymers are excited simultaneously, charge transport occurs from the phenanthrene to the dinitrobenzene to the dimethylamino to the anthracene layer with a high photocurrent of 190 pA. Thus, AND logic is demonstrated with two optical inputs and an electrical output. We note that the first experimental molecular AND gates[221] also used two PET processes, but with a single acceptor which was responsible for the fluorescence output. The two inputs were also chemical, rather than optical. Yet older proposals for AND gates, which never reached the primary literature,[251] did discuss two optical inputs and an optical output. Matsui et al. now extend this nanosheet approach to include XOR logic as well.[252] Even though these nanosheets are molecule-based, it is clear that logic behaviour at the level of a single molecule is not attainable by this approach. Of course, single-molecule logic is not essential for many imagined applications.

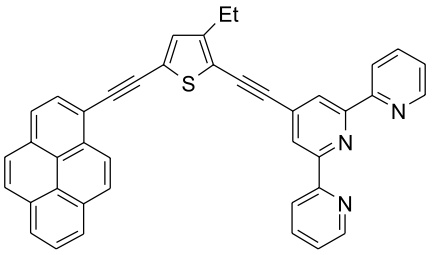
To conclude this general section, we are delighted to note the widespread success of PET systems whether they are chasing single or several targets. Luminescent sensors and logic gates of various descriptions arise naturally from this general mechanistic approach.

3. ICT (internal charge transfer) systems

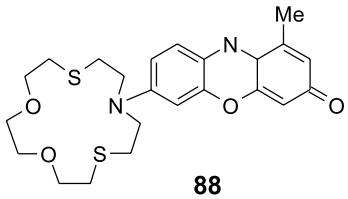
3.1. Brief theory

In contrast with PET systems, a lumo/fluorophore can be directly integrated with a receptor[3] so that their orbitals overlap to such an extent that the seam does not show. Then, one terminal tends to be electron rich and the other electron poor. In such 'push–pull' cases, excitation leads to a serious redistribution of electron density, so that a substantial dipole

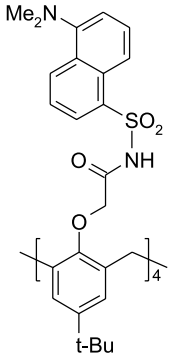
is created. Binding of a target species, especially a charged one, into the receptor naturally causes an interaction with this excited state dipole.[18] This interaction energy shows up in the emission signature, that is, a repulsive interaction is seen as a blue shift. Even if the emission does not show this, the excitation spectrum of the emission will betray this effect.[74,75] Spectral shifts allow analysis by ratioing intensities at two separated wavelengths—a trick much used in intracellular sensing.[87] PET systems do not give such shifts, which can be a blessing (in terms of simplicity) or a curse (in terms of being tricky to use in undefined environments). We note that many, many fluorescent pH indicators that have been discovered over the years[253] are now seen to be ICT systems.



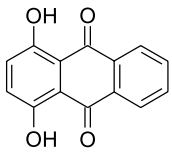
87



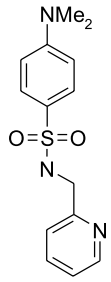
88



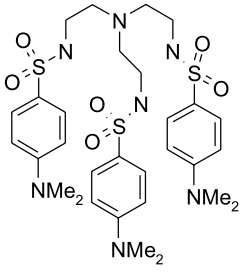
89



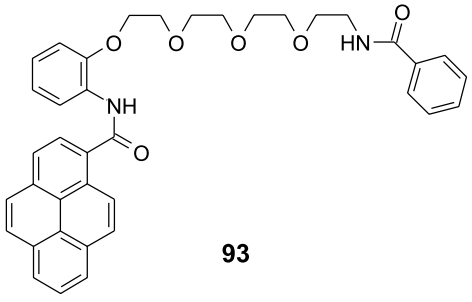
90



91



92



93

3.2. Metal ion targets

The structure **87**[254] suggests an ICT sensor with a π-electron system spreading over a pyrene moiety, a terpyridine (tpy) receptor and a 2,5-diethynylated thiophene connector. Zn^{2+} binding is visualised not only by a switching 'off' of the fluorescence in acetonitrile, but also by shifts in the UV–visible spectrum and in the cyclic voltammogram. The latter point suggests that the ICT will be so extensive in the Zn^{2+}-bound state as to border on complete electron transfer. A little later, Jimenez et al.[126] analyse the patterns emerging from these three analytical techniques for various analytes. Of course, the terpyridine receptor is quite promiscuous and this shows up in the quenching of fluorescence of **87** with many ions, as well as H^+. The Zn^{2+}-tpy interaction has also been exploited for sensing by the groups of Sauvage and Barigeletti.[255]

The water solubility of the phenoxazinone fluorophore is a plus point of the sensor **88**,[256] which is selective for 10^{-7} M Hg^{2+}. The ICT nature of the sensor is clear from the colour change from pink to yellow on coordination with Hg^{2+}. The emission spectra also show a small blue-shift, besides a degree of quenching. This allows ratiometry in absorption, as well as emission channels. Rurack's previous experience with Hg^{2+} sensors would have aided this work.[120]

By combining an ICT fluorophore with a calixarene receptor, Métivier et al.[257] found a useful Pb^{2+}-induced blue shift (52 nm) for **89** in 40% aqueous acetonitrile at pH 5.2. The latter pH value is crucial since protonation equilibria concerning the N–H group control fluorescence

to a high degree. Good selectivities versus Cu^{2+}, Zn^{2+}, Cd^{2+} and Hg^{2+} are notable.

Br^- (in excess) is the agent which reacts with Pb^{2+} in acetonitrile to produce a nice fluorescence switching 'on'. As Dutta and Perkovic[258] perceptively note, the analyte is more responsible for the emission than the agent. However, the emission arises from a state delocalised over both atom types with considerable charge transfer.[259] Also, the formation of the emissive cluster $Pb_4Br_{11}^{3-}$ does not use organic sensors, as in the rest of this report. Early Russian work concerning the fluorescent detection of Pb^{2+} with concentrated HBr must not be forgotten,[260] even though $Pb_4Br_{11}^{3-}$ does not survive water.

Quinizarin (**90**) is a member of the classical hydroxyanthraquinone family of fluorescent sensors. Quinizarin itself was used to detect boron and beryllium.[261] Quinizarin-2-sulfonic acid was similarly employed with regard to Be^{2+} and Al^{3+}.[262] All of these can now be rationalised as being ICT fluorophores with intramolecular hydrogen bonds as their de-excitation channels. Analyte-induced FE arises when these offending hydrogen bonds are replaced with dative bonds to the analyte. It is interesting that such sensors still hold the attention of 21st century researchers. Ga^{3+} or In^{3+} binds to **90** in alcoholic solutions and an FE of about 7 is observed, though Al^{3+} produce even larger values.[263]

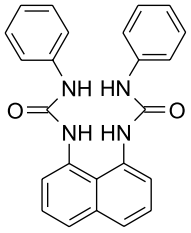
The fluorophore component of the sensors **91**[264] and **92**[264] has dual fluorescence, originating from a locally excited (LE) state and a TICT state, as found in the archetypal dimethylaminobenzonitrile.[123] In acetonitrile:water (1:1), The TICT emission of **91** blue shifts and disappears upon alkalinisation. Acidification gives a red shift and disappearance of **92**'s TICT band, whereas both bands of **91** are quenched. Given its dominant position in the Irving–Williams series,[17,137] it is no surprise that Cu^{2+} is the only transition metal to affect fluorescence. The quenching (by a factor of 2) of **91** seen in the 2:1 (ligand:metal) complex is due to the usual PET/EET, as well as sulfonamide deprotonation. In the case of **92**, only the TICT band is quenched for the 1:1 complex. On the other hand, d^{10} Zn^{2+} greatly enhances the LE band. Sensor **92** shares several features with the prior work by Corradini et al.[102]

Morozumi et al.'s compound **93**[265] is not a simple ICT system, but is best viewed as an extreme case based on TICT.[123] Such systems have the same thermodynamic criteria as PET and behave like the latter cases but with virtual spacers.[3,266] Compound **93** shows an FE of 37 with a broad spectrum, suggesting some residual ICT character when confronted with Ca^{2+} in acetonitrile. Ca^{2+} binds to the podand and both carbonyl oxygens to prevent the twisting necessary for the population of a TICT state. Sr^{2+} is less effective, whereas Ba^{2+} and Mg^{2+} show nothing at all.

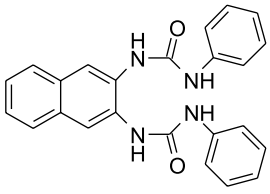
Ca^{2+}-induced colour changes in non-fluorescent ICT sensors are worthy of discussion, since they display aspects of quantum computing. This is a startling conclusion[235] because any sensory molecule which shows a colour change, e.g. a simple pH indicator, would do the same. Nevertheless, observations of such target-induced transmittance changes at four clearly chosen wavelengths are seen to exhibit the four different logic types for a single input–single output device, i.e. PASS 0, PASS 1, YES and NOT. The fact that the four wavelengths can be simultaneously observed then leads us to conclude that four different computational operations can be run concurrently. Here is a trick barred to electronics devices because colour is meaningless to electrons in wires.

3.3. Anion targets

The interaction of F^- with the urea units of Cho et al.'s compound **94**[156] in acetonitrile:DMSO (9:1, v/v) shows a broad peak at 445 nm in the emission spectrum. However, with other halide ions, the emission remains at 379 nm (as expected for such a naphthalene derivative) with only small intensity changes. The stronger hydrogen-bond basicity of F^- stretches both the naphthyl N–H bonds enough to distort the π-electron cloud of this ICT system, which can be produced from the PET system **54**[152] by deleting the two methylene spacers and the central benzene ring. The F^- selectivity of **94** in aprotic solvent mixtures features again in work from another group,[157] but now only an FE (of 3) is claimed. The different observations in the two reports can be assigned to the change of the acetonitrile:DMSO ratio from 9:1 to 4:6 in the present work. Rigidification by F^- complexation may contribute to the FE. Other halide ions only show a slight fluorescence quenching. In contrast, quenching for **95**[157] was observed with all halide anions, including F^-. The 2,3-diaminonaphthalene unit is sterically incapable of accommodating a F^- between them.

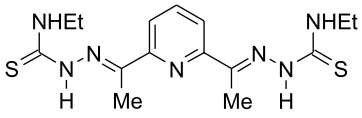


94



95

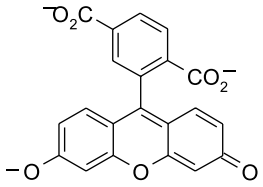
Though no fluorescent effects are involved, we note an interesting study concerning **96**.[267] Since it also involves F^--induced stretching/breaking of H–N bonds to increase π-delocalization as seen in Ref. 156. A yellow colour formation is visible evidence of this and the stoichiometry is found to be 1:1.



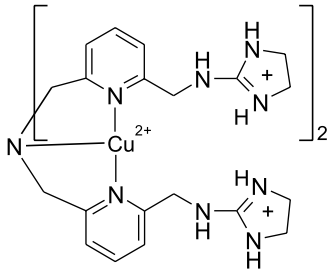
96

Though the fluorescence of **97** could have been exploited, Zhang and Anslyn[268] use its ICT chromophore properties to develop a nice indicator displacement assay for *O*-phosphoserine. An orange-to-yellow colour change is observed as **98**-bound **97** is released into bulk water when the stronger-binding *O*-phosphoserine arrives at **98**.

Naturally, the absorption spectrum of **97** is affected when it is held close to the Cu^{2+} centre and the guanidinium groups. The Anslyn laboratory has given the indicator displacement method a new lease of life.[101]



97

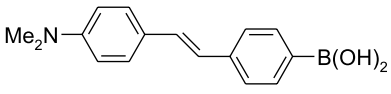


98

In closing this short section, we note that many anion-receptor interactions depend on hydrogen bonds.[269,270] Then, new anion sensory systems based on ICT systems will benefit from the fact that fluorescence can be strongly quenched when the excited state energy is drained via strong hydrogen-bond arrays by vibrational loss.[271]

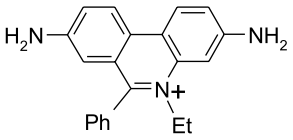
3.4. Other organic targets

We exploited the electron-withdrawing nature of the carboxylic acid group to design fluorescent switches based on PET.[208] The boronic acid group is now similarly used by DiCesare and Lakowicz[272] with **99** and its ICT nature. The fluorescence spectra show blue shifts (45 nm) upon basification with a pK_a value of 9.1. A similar blue shift is found, but with a pK_a of 6.6, in the presence of fructose. Yoon and Czarnik[273] found a similar sugar-induced pK_a shift for anthrylboronic acid with much less ICT character. Thus, **99** allows saccharides to be measured by ratioing intensities at two chosen wavelengths.

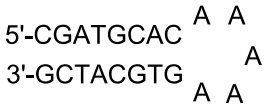


99

Compound **100** is a venerable fluorescence 'off–on' switch for ds-DNA[274] occurring by intercalation. Its ICT excited state deposits considerable positive charge on the NH_2 groups leading to strong hydrogen bonding to water with subsequent de-excitation by vibrational loss. The intercalation offers protection from this loss channel. It is not surprising, then, that the DNA-type short hairpin **101** will do the same with **100**.[275,276] Now, any other potential intercaland will compete with **100** for the binding site(s) on **101**, and the fluorescence of **100** will decrease, owing to its displacement into bulk water. The magnitude of this decrease is analysable to yield the binding affinity of the intercaland. Older examples of fluorescent/coloured indicator displacement assays exist.[164–170]



100

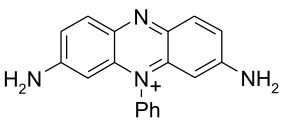


101

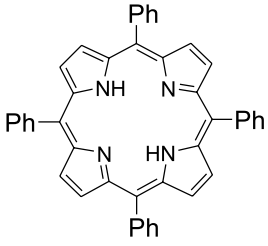
We can draw together the examples discussed in these sections concerning ICT systems in one sentence. Modern sensor and switch researchers appear to spend less effort on ICT designs than PET, but the former continues be a very fruitful mechanism, too.

3.5. Multiple targets

Komura et al.'s experiment[277] involves the common dye **102** which is normally monoprotonated in aqueous media and is fluorescent. This is switched off upon diprotonation at high acidities as the ICT nature is lessened. Compound **102** is held in a polyanionic Nafion® film on an ITO (indium tin oxide) electrode. Upon reduction, **102** switches 'off' its fluorescence because the exciting wavelength is no longer absorbed. Fluorescence is observed when **102** is in an oxidised state, and only one of the amine groups is protonated. Thus, electrochemical (oxidation) and chemical (H^+) inputs combine in AND logical fashion to produce fluorescence output.[221]



102



103

Though not principally a fluorescence study, the report by Langford and Yann[278] about **103** is interesting for several reasons. First, it is the first molecular demonstration of the primeval mathematical operation of subtraction. Second, it achieves this within one compound. Third, the compound itself can be bought from almost any chemical supply house. The interest in this work is sharpened by the fact that the first numerical operations performed by a small molecule (or two) had only been accomplished five short years ago.[279] This was the case of addition. Similar half-adders (which can only count up to 2) were soon demonstrated within one compound.[280,281] One of these was also commercially available,[280] but required laser methods for the numeracy demonstration. The numerical function of **103**, however, is exposed with simple UV–visible spectrometry and fluorescence spectrometry. The value of both these techniques in this general pursuit had been revealed earlier.[221,236,279,280,282] Compound **103** also requires the small binary numbers to be input in chemical form. The trick of using stoichiometric amounts of acid and base as inputs which can annihilate each other for building XOR logic gates had previously been achieved by Langford, along with Balzani, Stoddart

and Credi.[241] Half-subtractors are built from INHIBIT and XOR logic gates in silicon technology.[220]

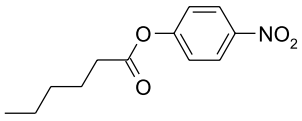
The free base porphyrin **103** can form either dicationic or dianionic species on addition of H^+ or $tBuO^-$, respectively, in DMF solvent. In its free base form, **103** absorbs strongly at 417 nm. Addition of acid or base leads to red shifting of about 15 nm. This behaviour is not unlike a pH indicator with an ICT excited state.[283] Monitoring transmittance (*T*) at 417 nm, a 'difference' output can be observed (XOR logic). Monitoring fluorescence emission (*F*) yields INHIBIT logic or the 'borrow' output (Table 1). The free base displays a weak emission band at 395 nm. Notably, this is not the normal fluorescence of porphyrins such as **103**.[284] Deprotonation causes a red shift to 440 nm and protonation a red shift to 405 nm. Monitoring emission at 440 nm thus yields the Borrow output. The origin of the anomalous fluorescence would deserve further examination, especially because one of the emissions is anti-Stokes in nature—a further anomaly.

Table 1. Truth table for half-subtractor **103**

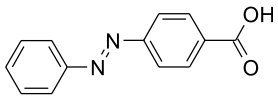
Input		Output	
Acid	Base	F	T
0	0	0	0
0	1	1	1
1	0	0	1
1	1	0	0

A non-fluorescent, but interesting, approach by Sivan et al.[285] to AND logic uses an enzyme (α-chymotrypsin), as it hydrolyses **104** to the coloured phenolate product. The integration of the reactive group of **104** with the chromophore gives it an ICT designation in the current context. Judicious replacement of **104** would easily give a fluorescent product in order to conform to the remit of this report. The hydrolysis implies that there is an aspect of irreversibility here, but the conceptual value is not diminished. A lysine unit on the gateway to the active site of the enzyme is modified by derivatisation with **105** to form an amide. Irradiation at 334 nm converts the *trans* form of the derivatised **105** into its *cis* version which blocks the gateway. Thus, the substrate **104** and the inhibitor **106** are rendered unemployed, since the enzyme is disabled. Irradiation at 420 nm restores the *trans* form of **105** amide, opens the gateway for **104** and **106** to reach the active site and, so, if the inhibitor is reduced to a non-inhibitory version, **104** can be processed (output high). Reduction of **106** is achieved photochemically (436 nm) in the absence of air. The corresponding oxidation back to **106** can be accomplished by simple aeration and, so, the reduction of **106** and the 420 nm radiation to give the *trans* **105** amide are the two inputs (high states) of the AND gate. Some cross talk is unavoidable at such close wavelengths of photochemical irradiation.

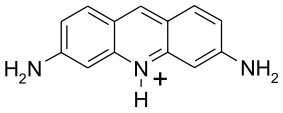
The distinguishing feature of ICT systems is the integration of receptors with fluoro/chromophores.[3] Appreciating the presence of a receptor integrated within a photochromic such as a spiropyran, first seen by Tamaki[286] and also by Buncel,[229] was developed by Raymo and Giordani into a nice approach towards molecular logic systems containing small-scale integration.[282] Guo et al. apply this idea by using Fe^{3+} as an input which is also an oxidising agent to demonstrate half-adder action in **85**.[281] This differs from our older example,[279] since we needed separate molecules for AND and XOR gates. Remacle et al. also showed half-adder action within the simple fluorophores **107** and **108**.[280] A case by Guo et al.[227] involving PET was discussed earlier in this report.



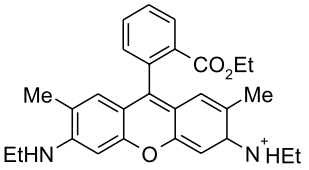
104



105

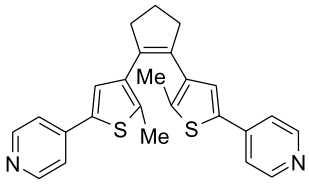


106



107

108



109

Deliberately building-in a receptor to a photochromic is another way to tackle related logic problems, first done by Inouye with spiropyrans[287] and later by Diederich for dihydroazulenes.[288] Earlier cases by Inouye et al. without logic interpretations are also available.[289] Now, Tian et al.[290] modify Irie's celebrated dithienylethene photochromics[291] with pyridyl groups, so as to benefit from a Zn^{2+} input. An ICT behaviour can be assigned, owing to the 'fluorophore-receptor' integration. Compound **109** allows small-scale integration, especially when different emission and absorption wavelengths are observed[238] to achieve superposed logic configurations.[236]

We conclude this general section as follows: though the number of modern ICT sensor and switch systems may not be huge, some of these are among the most imaginative.

4. EET (electronic energy transfer) systems

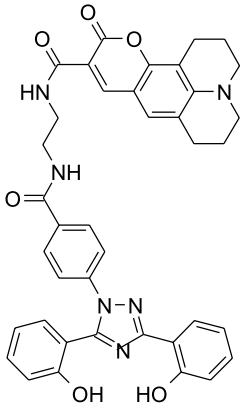
4.1. Brief theory[14,18,292,293]

The argument starts with the fact that energy runs downhill and so an excited state of high energy will donate its excitation to a lower-energy excited state. Since each excited state can emit its own signature, we have here the conditions for a two-colour sensory system. The intensities of the two wavelengths can be ratioed. Singlet energy

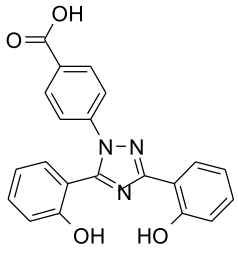
transfer is our concern in most, though not all, cases in this report. Its efficiency is crucially dependent on the distance of separation of the two states concerned. At biorelevant distances of, say, 0.5 nm or larger, dipoles of the two states can interact. This is the Forster resonance energy transfer (FRET). At smaller distances, orbital overlap between the two states dominates. Either way, the amount of overlap between the absorption spectrum of the acceptor and the emission spectrum of the donor controls the efficiency of EET.

4.2. Various targets

Al^{3+} is one of those ions for which fluorescent sensors are available from classical analytical chemistry.[294] A weakness, however, is that careful pH adjustment is needed for the success of many of these phenolic compounds. Nevertheless, this weakness is quite hard to avoid. Phenols **110**[295] and **111**[295] are likely to need pH adjustment, too. The importance of this work arises from the use of EET in **110** to increase a FE value, which is inherently low with **111**, by optically pumping the latter fluorophore. The classical mechanism of FE in these ICT-type phenols, including **111**, is that Al^{3+} replaces the phenolic hydrogens which vibrationally couple the excited state to water, leading to fluorescence loss.[271] The emission of **111**·Al^{3+}, however, overlaps well with the absorbance of the aminocoumarin unit. Direct excitation of the aminocoumarin shows no Al^{3+}-induced FE, confirming that the aminocoumarin is not involved in the binding process. How does the FE increase take place? The beginning of an explanation may be that, in the Al^{3+}-free state, the phenolic unit has a very small excited state lifetime owing to the vibrational coupling. Hence, the EET process is very inefficient and gives very little emission from the aminocoumarin. On the other hand, the Al^{3+}-bound state has a much longer lifetime for the phenolic unit, which allows excellent EET to the aminocoumarin. We also note that the exact type of EET at these short distances is not as clear-cut as that found in biological systems with their larger size scales. Dexter-type electron exchange comes into its own at chemically relevant distances, whereas the Forster-type dipole resonance interaction (FRET) revels at biologically relevant scales.[292]

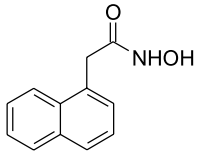


110



111

Hydroxamates are under-used receptors in luminescent sensor research, with the notable exception of Shanzer's work.[236] Now, De Costa and Jayasinghe's 'fluorophore-spacer-receptor' system **112**[296] switches 'off' its naphthalene-like fluorescence upon binding Fe^{3+} in 50% aqueous methanol at pH 3. There is also an appearance of a CT absorption band at longer wavelengths. The latter is the probable sink for the EET process, though, a contribution from PET to this redox-active ion is probable, too.

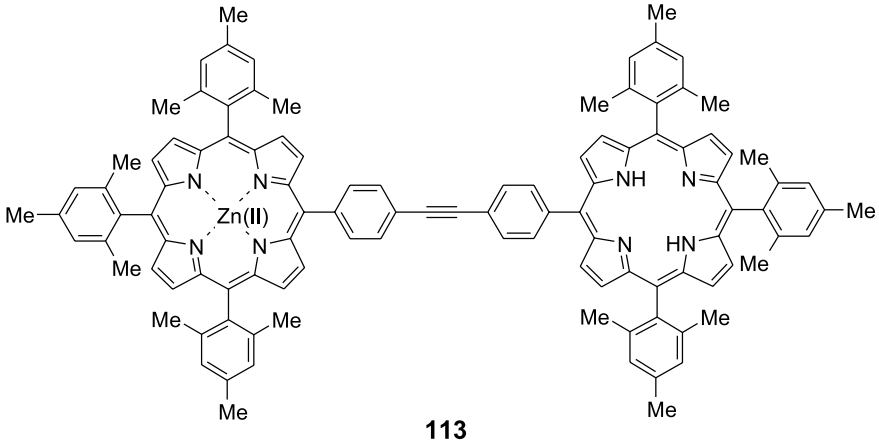


112

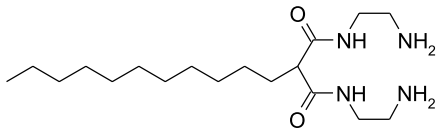
Wagner, Johnson and Lindsey's compound **113**[297] is cleverly exploited by Otsuki et al.[298] as an EET switch. Axial coordination of phenylazopyridine (PAP) to the Zn(II) centre provides an internal PET channel for the porphyrin-Zn(II) unit, which is much faster than EET to the free base porphyrin unit of **113**. A previous example of PET/EET competition within a switch context is known,[94] and so the fluorescence signature of the latter is perhaps 10-fold lower in intensity. Addition of a stronger axial ligand like dimethylaminopyridine (DMAP) displaces the offending PAP into bulk dichloromethane solution and the fluorescence switches back 'on'. Addition of dichloroacetic acid protonates just the DMAP, so that the PAP can sneak back into the Zn(II) site to switch fluorescence 'off' again.

The assembly[109,110]of the hydrophobic pyrene fluorophore and the receptor **114** within Triton X-100 micelles in water is quenched by Cu^{2+} via EET, as found in the case of the corresponding 'fluorophore-spacer-receptor' system **115**.[115] Unsurprisingly, the micelle structure is not perturbed by the small mole fraction of the fluorophore and receptors included in it. The convenience of the assembly approach is clear, since synthetic procedures are simplified or even avoided. The only price to be paid is in terms of the fragility of these multicomponent systems, especially in multicompartmental host environments. Above all, we must not forget the closely related studies of enhanced quenching of stilbene in sodium dodecylsulfate micelles by Cu^{2+}.[111]

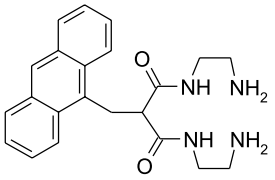
The fluorescence of **116** is strongly switched 'off' by **117** if it is held close by. The usual mechanism is EET, even though the distortions seen in the UV–vis spectra suggests CT, or even PET, components. If **116** and **117** grace the termini of a DNA-type hairpin (with a short stem and a large loop), the fluorescence of **116** can be switched back 'on' by straightening out the stem-loop by hybridisation to a long complementary DNA single strand. The fluorescence switching 'on' seen here is nothing extraordinary from a sensory/switching viewpoint, though DNA's obvious glamour is probably behind the catchy descriptor, 'molecular beacon'.[299,300] Many clever applications are available already.[300]



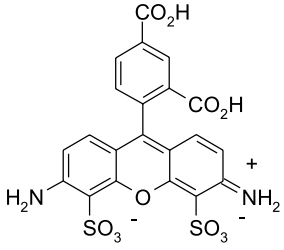
113



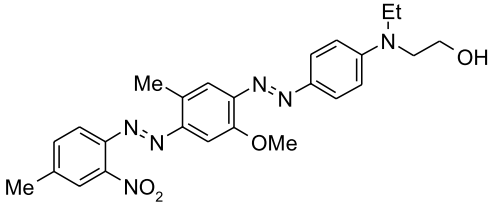
114



115



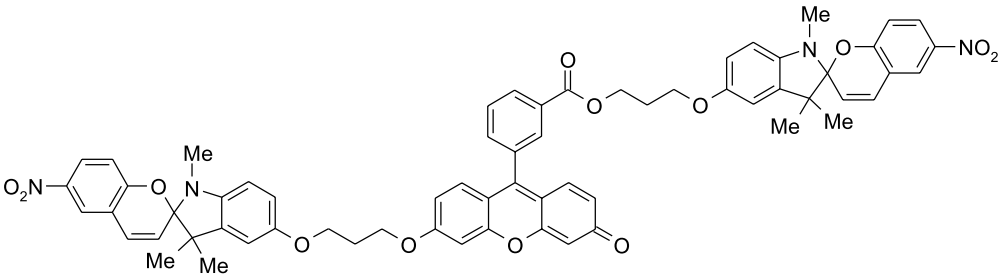
116



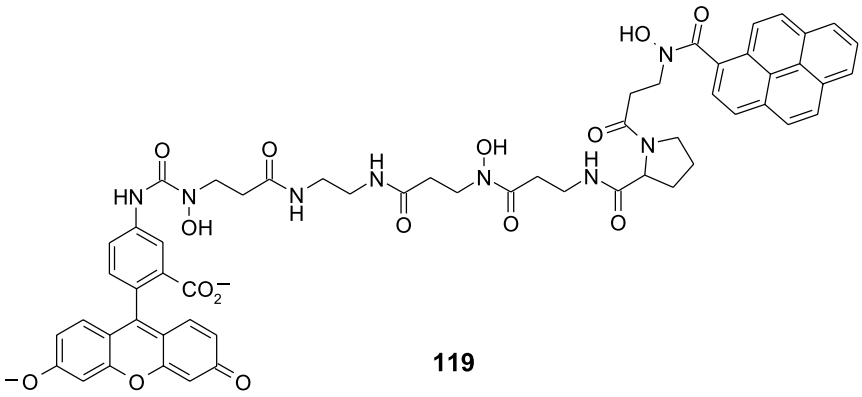
117

Raymo and Giordani have recently made valuable contributions to the field of molecular logic devices[215–219] by considering the chemical response of photochromics in addition to their photoeffects.[282,217] However, the time scales of the state interconversions need to be borne in mind, so that we are clear whether a given discussion concerns data processing or storage, especially since photochromics were treated in a data-processing context from their inception.[228] Since this review is restricted to luminescent systems, we will only consider cases where emission processes hold an important key. Raymo and Giordani[238] report cases involving intermolecular EET and fluorescence output. Upon irradiation with UV light (at 365 nm), the colourless spiropyran passes to the coloured merocyanine. This gives different degrees of overlap between the fluorescence emission spectrum and the absorption spectra of the two photochromic states. The EET mechanism here should be the emission–reabsorption mechanism, owing to the large distance of separation between the energy donor and acceptor. Different light-transmittance patterns can therefore be achieved with different fluorophores. Even though the same binary patterns (logic descriptors) can be generated by using different wavelengths of a spectrophotometer, the molecular communication ideas are imaginative.

Guo et al.[301] use the connected fluorophore-spiropyran system **118**, so that intramolecular EET can confront the fluorophore excited state with the two states of the photochromic. Spiropyran **118** is complicated, since the fluorophore is a fluorescein mono-ether,[302] rather than a simple fluorescein. Further, the environments the two spiropyrans are not equivalent. Nevertheless, the merocyanine absorption band overlaps well with the emission band of the fluorescein fluorophore, but the protonated merocyanine does not. Of course, the spiropyran with its rather high-energy absorption will not overlap either and so EET would be strong for the merocyanine only. The emission spectrum of **118** in THF when excited at 430 nm is the typical fluorescein band at 550 nm. Upon irradiation with UV light, the spiropyran isomerises to the merocyanine, which reduces the fluorescein intensity due to EET. Addition of an acid (to produce the protonated merocyanine) or irradiation with visible light (to regenerate the spiropyran) re-establishes the normal intensity, as neither of these forms absorb at the emission wavelength of fluorescein. The fact that the two inputs, H^+ and visible light can produce the same fluorescence effect in some situations will probably limit the logic capabilities of **118**.



118



119

Shanzer's group exploit their experience of siderophore-based fluorescent sensors for Fe^{3+} to develop the first arithmetic processor **119**,[237] which is capable of both elementary binary addition and subtraction.[303] Monomolecular processors described previously could do each of these operations before, but not both.[278,280,281] Bimolecular systems have an even older vintage[279] and only decimal numbers 0, 1 and 2 are recognised. As we have seen, addition needs AND logic for the carry digit and XOR for the sum digit. Subtraction needs INH logic for the borrow digit and XOR for the difference digit. By using a small menu of input chemicals (acetic acid, acetate as base and EDTA as complexant), several 2-input logic gates can be configured. These inputs interact with hydroxamate, carboxylate and phenolate, as well as Fe^{3+} sites. The variety of logic configurations is magnified further, since blue (pyrene) or green (fluorescein) output channels are available for reading the signal patterns. The arithmetically significant logic gates mentioned above are all included. PET and EET are involved in the fluorescence quenching by the open-shell Fe^{3+}. EET is also present to take away pyrene's blue fluorescence and producing green fluorescence instead. Such EET collapses in media acidic enough so as to convert fluorescein into a less-delocalised π-system. Another critical point is that Fe^{3+} loses its usual quenching ability towards fluorescein in basic solution. Ligation of the base to Fe^{3+} will reduce its ability to engage in PET from the fluorophore. It is notable that the XOR logic function is constructed by summing two complementary INH functions arising in the blue and green output channels. This algebraic option has been used by two other groups.[304,305] We are also delighted to note that arithmetic operations with numbers larger than 2 have also recently been accomplished by using ideas from small-molecule logic[221] and implemented in DNA oligomers, but using strand scission, instead of fluorescence, as the output channel.[305]

An excellent example of the potential of fluorescent molecular logic is found in the work of Stojanovic and Stefanovic.[306] The analysis of the board game of tic-tac-toe in terms of all possible moves to produce a tree is available.[307] In the spirit of the famed chess contests between Kasparov and the Deep Blue computer, Stojanovic and Stefanovic implement ideas of small-molecule logic[221] in DNA oligonucleotides to play tic-tac-toe against a human opponent on a 3×3 array of wells. Twenty three logic gates are involved, the most complex being a 3-input INH, like our old case.[232] However, as done by children everywhere, the network of molecular logic systems makes the first move and thus avoids defeat. The moves available to the human opponent are thus restricted to sides or corners of the 3×3 array. As in any productive communication between individuals, the language must be agreed beforehand. Each human move is coded in terms of a DNA oligonucleotide, which is applied to all wells. The molecular logic network responds with its countermove after 15 min by switching 'on' fluorescence in a particular well. What is the molecular basis here? The substrate is designed as a DNA oligonucleotide with one key ribonucleotide as the weakest link, as well as a fluorescein and a tetramethylrhodamine at its termini. The latter pair serves as fluorophore and quencher in an EET process. A stem-loop deoxyribozyme (a DNA catalyst called E6) cleaves this substrate following hybridisation to separate the fluorophore–quencher pair. The fluorescence is recovered by stopping EET in the sense of a molecular beacon.[300] We note that an element of irreversibility is unavoidable in this approach, though its elegance is not in doubt. The logic gates are constructed by adding to this basic structure, e.g. a YES gate has part of E6 protected with a new stem-loop. Addition of the correct input oligonucleotide opens this stem-loop to expose the E6 structure to do its hydrolytic work. A carefully chosen combination of logic gates is

placed in a given well. The network arises, since the combination differs from well to well.

To summarise, EET systems have plenty to offer chemically-oriented designers, whether they are focussed on small molecules or large. As alluded to at the start of this section, EET has largely been the preserve of biochemists, so far. The bunch of examples above are a clear signal to chemists that they, too, are welcome in this domain.

5. Excimer and exciplex systems

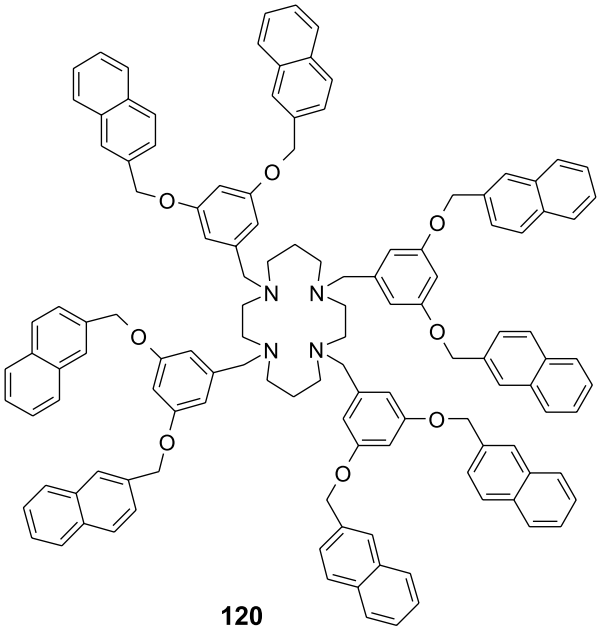
5.1. Brief theory[293]

To put it bluntly, excited states are sticky. In more scientific language, excited states possess half-filled orbitals that can interact attractively with other ground-state orbitals. This leads to π–π overlap and these delocalised states show up in red-shifted and broad emission signatures. The latter are due to the instability of the corresponding ground states, i.e. extremely short lifetimes. Heisenberg's uncertainty principle does the rest. The energy of the state becomes ill-defined. The cliché that excimers are excited dimers and that exciplexes are excited complexes is true. Naturally, the latter have dipoles owing to their hetero nature. We can then appreciate exciplexes as frustrated PET systems.

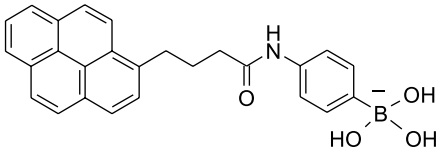
5.2. Various targets

Dendrimeric sensor **120**[308] has a cyclam core built up with naphthalene fluorophores via 1,3-dimethoxybenzene branching units. Emissions corresponding to excimer, exciplex and locally excited fluorophore are seen in acetonitrile:dichloromethane (1:1) solution. The medium is not polar enough to support PET processes in this multi-'fluorophore-spacer-receptor' system. Naturally, fast EET from the dimethoxybenzene to naphthalene units precludes any emission from the former. Protonation of **120** with trifluoroacetic acid eliminates exciplex formation with a concomitant increase in the naphthyl LE emission at 340 nm. Similar H^+-induced switching between the exciplex and LE states of naphthalene in a non-dendrimeric case is available from 1984.[174] Only two of the basic sites in the cyclam core are protonated because of the low polarity there.

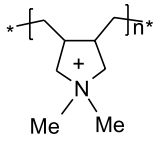
The excimer-based sensor **121**[309] for ATP effectively targets the high charge on ATP, which, with its charge of −4 is electrostatically bound to a polycation **122**. This mediates the association of the fluorophore-derivatised boronic acid **121** with ATP, which contains the monosaccharide with the crucial diol, via a boronic ester bond. The aggregation of monomer subunits leads to an excimer. Selectivity is only slightly greater for ATP over trianionic ADP, but much greater over dianionic AMP. The high pH needed for the phenomenon is another limitation. The concentration of **122** also needs careful optimisation, since an excess would dilute the **121** along the **122** chains, as seen before in DNA sensing.[310] It is important to note a pyrene–guanidinium system with related design ideas targetting pyrophosphate reported by Nishizawa et al.[311]



120

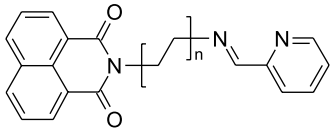


121



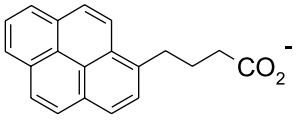
122

Instead of wrapping one long ligand around a metal ion,[312] two shorter ligands can be assembled around the metal to bring the π-systems within reach of each other. Licchelli et al. use the 'fluorophore-spacer-receptor' system **123**.[313] The naphthalimide fluorophore has seen successful use as excimer systems.[314,315] Compounds **123** ($n=3$–5) exhibit 1:3 metal/ligand ratios at low metal concentration. Fluorescence quenching is observed with d^6 to d^9 transition metal ions, due to the usual PET/EET effects. On addition of Zn^{2+} and Cd^{2+}, an additional long-wavelength emission is seen, due to excimer formation. However, as the concentration of Zn^{2+} and Cd^{2+} increases, the excimer band decreases to almost nothing. The excess metal ions result in the consecutive formation of complexes with 1:2 and then 1:1 metal/ligand stoichiometries. Compound **123** ($n=2$) does not show excimers, due to limitations of the short connecting chains but it also exhibits fluorescence quenching with d^6 to d^9 transition metal ions in acetonitrile. However, at higher concentrations of Zn^{2+} and Cd^{2+} the equilibrium shifts to form a 1:1 complex and, a FE of 6 is then observed. This can arise from the interaction of the metal with the carbonyl groups, which increases the energy of the $n\pi^*$ excited state, and thus prevents intersystem crossing.[14,18,293]

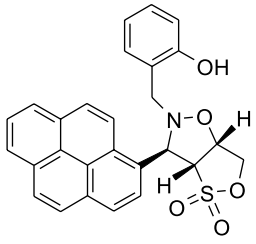


123

Functionalised pyrenes can produce 2:1 complexes with divalent analytes. This gives enhanced excimer emission which permits ratiometric detection, e.g. Prodi's compound **124**[316] targets Zn^{2+}. Compound **125**[317] in 50% aqueous ethanol does the same for Ag^{+} and, to a lesser extent, for Hg^{2+}. The necessity of the rather elaborate functionality in the present case is not proven, though the phenol moiety is shown to be essential. Transition metal ions quench the monomer fluorescence, but with no enhancement in the excimer emission. Interestingly, the lifetimes of the pyrene monomer and excimer are similar, with values of 11.6 and 14.4 ns, respectively. No excimer emission is observed with Ag^{+} in aqueous solutions in excess of 70% ethanol suggesting that hydrophobic stacking[318] of the pyrene units is important.

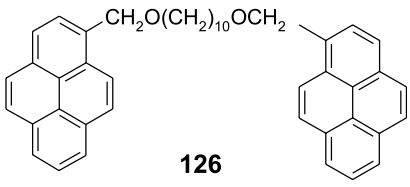


124



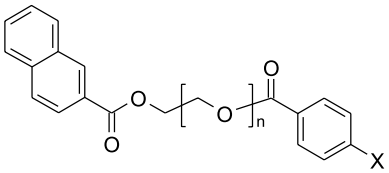
125

Guo et al.[319] continue their combination of ion-sensitive photochromic switches[282] with other photochemical phenomena by addressing excimer formation. The output is the intensity of the intramolecular excimer band of **126**. The classical spiropyran photochrome[228] ring opens to the merocyanine on irradiation with UV light, and can then complex with Zn^{2+}. Previous work on metal-complexing photochromics is available.[229,286,287,320] EET occurs from the excimer to the metal complex, due to good spectral matching of the excimer emission to acceptor absorption. A significant decrease in the excimer emission is seen. Other logical combinations of the two inputs (UV light and Zn^{2+}) do not show this effect. This corresponds to molecular NAND logic.[321] The third input is visible light, which converts the complex back into the initial spiropyran, but the output excimer state is not affected by visible irradiation. We also note that a simpler fluorophore emitting similar wavelengths (around 478 nm) should have achieved the same end result,[238] even though a broader band and a longer excited-state lifetime are available from **126**.

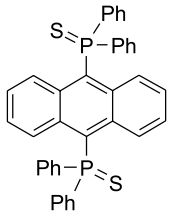


126

In the presence of Ca^{2+} and Ba^{2+}, **127–130**[322] exhibit intramolecular exciplexes in acetonitrile, but not when metal free. Compound **130** displays the greatest FE and selectivity for Ca^{2+}. The length of the polyether spacer is chosen to form a suitable podand receptor for these alkali earth cations, with the carbonyl oxygens also playing a ligating role. Wrapping the podand around the cation will bring the terminal π-systems within reach of each other. The main fluorophore is the naphthoate, with the benzoate serving an auxiliary capacity. In the absence of cations, the fluorescence quantum yield of **130** is the lowest in the set by far, due to the considerable electron deficiency of the cyanobenzoate moiety leading to PET. This work is the most recent in a long line of papers from this laboratory, beginning in 1996.[323]



127; X = H
128; X = Cl
129; X = CF_3
130; X = CN



131

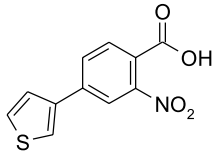
Compound **131**[324] and its O (but not Se) derivative exhibit fluorescence in the dry solid state, and **131** exhibits a significant FE when exposed with toluene, but not other organic solvents including benzene. Its X-ray crystal structure has a groove suitable for binding toluene by a C–H···π interaction, which allows for exciplex formation. The corresponding O and Se derivatives are devoid of this feature and hence do not display the fluorescence effect. Fluorescence of **131** in solution is negligible. Rationalisation of solid-state luminescence phenomena is still not easy, however. Nevertheless, selective and reversible detection of an organic vapour by fluorescence of single crystals remains appealing.

6. Systems based on metal-containing excited states

6.1. Brief theory

We cannot hope to do justice where entire books on inorganic photochemistry[325–327] struggle to cope with the diversity of phenomena thrown up by the various metal complexes. All we can do is to offer a few pointers. Polypyridyl-Ru(II)/Re(I) continues to exercise many researchers, but there are not many sensors and switches that have not been reviewed before.[1–24] The metal-to-ligand charge transfer (MLCT) excited states in these metals with low oxidation states is an axiom in many studies.[327] As has been noted often,[328–331] the luminescence of lanthanide complexes has the appeal of rather long lifetimes and line-like emissions which allows easy extraction from contaminant fluorescence signals and so it is perhaps not surprising that a large fraction of the following discussion concerns these.

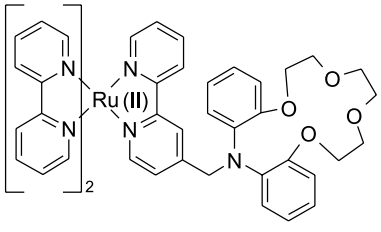
New antennae are always welcome in lanthanide luminescence research. Compound **132**[332] is interesting, since it is a nitro derivative, which is unusual in emissive work. Unfortunately for the sensor community, Eu^{3+} and Tb^{3+} complexes of **132** are not emissive in water. However, switches that can tolerate ethanol could benefit. The spectra show poor EET from the antenna to Eu^{3+}, since some ligand emission survives. Metal-centred luminescence from more unusual lanthanide emitters such as Nd^{3+}, Yb^{3+} and Er^{3+} has recently been reported when complexed with a calix[4]arenetriamide receptor carrying a quinoline-based fluorophore in acetonitrile following fast EET.[333]



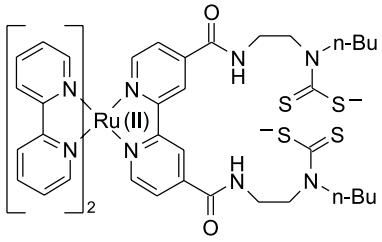
132

6.2. Various targets

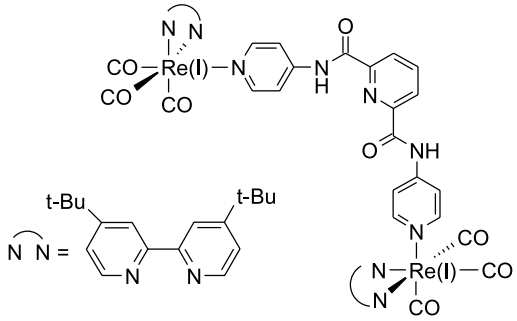
To begin with polypyridyl-Ru(II)/Re(I) systems, Charbonniere et al.[334] find Li^+-induced luminescence enhancement of ca. 2 for **133** with a small binding constant of 23, even in acetonitrile. As noted above,[61] Li^+ sensing is still a very hard target. This is a clear 'lumophore-spacer-receptor' system with PET known in a closely related pH sensor,[335] and so this example could have equally well belonged in Section 2.3. Pratt and Beer[336] find a significant (88%) reduction in luminescence of **134** when confronted with $H_2PO_4^{2-}$ in acetonitrile. DMSO is too competitive and destroys the luminescence effect. As the authors note, small luminescence effects of metal complexes are still difficult to rationalise, but the MLCT nature of the excited state must play some part. The bipyridyl-Re(I) tricarbonyl lumophore is coupled to an amide hydrogen bonding site in Sun et al.'s compound **135**.[337] The triplet MLCT luminescence is quenched when presented with CN^- or halides in dichloromethane. This is probably due to the coupling of the halide···H–N vibrator to the excited state, as seen in organic systems.[271]



133

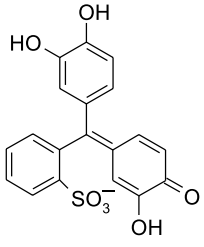


134



135

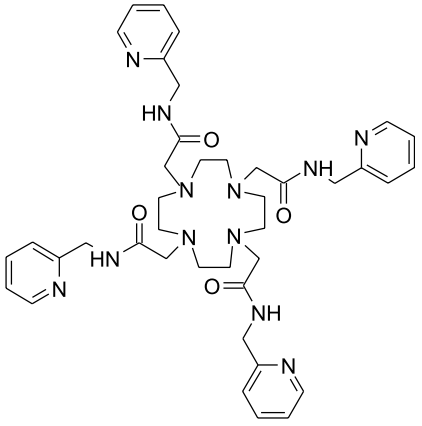
Pyrocatechol violet **136** complexes with Yb^{3+} in neutral aqueous solution, provoking a colour change from yellow to blue.[338] Upon addition of ATP (or HPO_4^{2-}), the colour change reverses as it competes for Yb^{3+} and **136** is liberated. The ensemble demonstrates good selectivity towards phosphate anions and ATP over many common anions, as well as AMP and to some extent, ADP. Though this competitive displacement assay[101] does not use luminescence, **136** can probably be replaced with fluorescent versions. Also, a venerable detector for oligonucleotides and DNA is based on luminescence switching 'on' of Tb^{3+},[339] where the use of Yb^{3+} may extend the method to give emission in a different wavelength region.



136

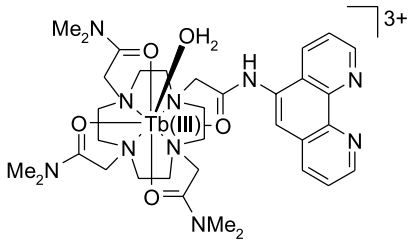
The macrocycle with the trivial name $12aneN_4$ has a good history of supplying ligands for lanthanide ions. Luminescent sensors and switches built from such complexes have arisen particularly from the laboratories of Parker[329] and Gunnlaugsson.[340–342] Tetra-amide ligands **137** in the form of their Eu^{3+} complexes show the typical luminescence spectrum. The intensity ratio of the peaks at 613 and 594 nm can be used for pH determination, since the coordination sphere for Eu^{3+} changes as the bound water molecule is deprotonated under alkaline conditions. The 613 nm band is known to be sensitive to the nature of the

coordination sphere,[328] whereas the 594 nm band is not. Modifying the pyridyl pendants to hydroxypyridyl produces clear PET effects, due to the electron richness of the phenolate derivative, i.e. luminescence switches 'off' at alkaline pH. Related lanthanide-based PET sensors for pH are known.[330]



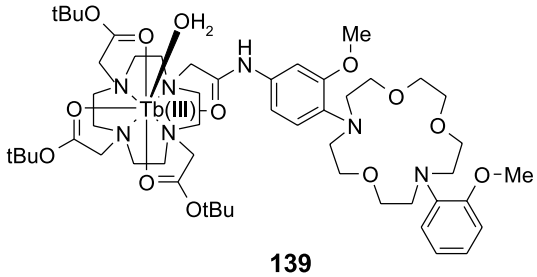
137

Gunnlaugsson et al.'s compound **138**[340] nicely extends the known fluorescent pH-controlled 'off–on–off' switches[84,96] to lanthanide luminescence to result in added advantages. Though still few in number, 'off–on–off' systems[84,96] continue to illustrate how relatively complex switching behaviour can be built up rationally and logically. At pH 11, the sensor is in an 'off' mode, since the metal-bound amide N–H is deprotonated and the amide anion launches PET to Eu^{3+}. Upon acidification, the Eu^{3+} emission becomes switched 'on' with a pK_a of 8.1, increasing to a maximum at pH 6, since the amide anion regains a proton. On further acidification, the emission switches 'off' with a pK_a of 3.8 and levels off at pH 2, since the aminophenanthroline monoprotonates, lowering its triplet energy and losing EET efficiency towards Eu^{3+}. Thus, a well-defined bell-shaped curve results. We note that H^+-driven fluorescent 'off–on–off' systems have been enabled with Na^+[343] or, later, with Ca^{2+}.[154] Gunnlaugsson et al.[341] use **138** again to demonstrate a Cu^{2+} 'on–off' sensor, since the phenanthroline unit is a venerable receptor for the transition metals. The Irving–Williams series ensures a good binding for Cu^{2+}. The Cu^{2+}-induced quenching can be attributed to PET/EET, though detailed experiments would be worthwhile to test this sensory mixed-metal system.[331] Compound **138** is thought to form a 3:1 complex, due to Cu^{2+}'s demand for an octahedral coordination sphere.



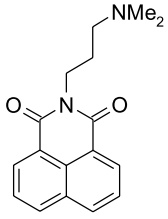
138

Gunnlaugsson and Leonard[342] use their experience of lanthanide-based sensors to build efficient 'off–on' sensors for alkali cations in water in simulated physiological conditions, e.g. **139** produces an FE of 40 for Na^+. The corresponding diaza-18-crown-6 derivative targets K^+ nicely. These are PET systems,[330,331] even though the spacer is hard to pinpoint.



139

The growing collection of INH logic gates[230,232–236,278,304,306] is expanded with **140** in an interesting way.[242] It receives Eu^{3+} as an input above a certain threshold concentration if we observe the emission from the bound lanthanide itself as the output. Compound **140** has had a previous life as a fluorescent PET sensor.[344] Eu^{3+} complexes with it in acetonitrile to give an FE of 3.5 in the fluorophore's normal fluorescence, as well switching 'on' its triplet–triplet absorption. The latter observation in particular, suggests complexation, given that FE can arise from protonation due to the acidic hydration sphere of trivalent cations.[129] The complexes **140**·Eu^{3+}, but not **140**$_2$. Eu^{3+}, luminesces following EET from the **140** triplet to the lanthanide, provided that O_2 is excluded. Such quenching by O_2 is known to arise in lanthanide complexes because of the energetic proximity of the emitting state of the metal to the triplet state of the antenna ligand and so O_2 becomes the disabling input of the INH gate.



140

We close this general section concerning metal-based systems by pointing out a consequence of the long-lived emissive excited states of polypyridyl-Ru(II) and porphyrin-Pd(II) complexes: the major success in sensing O_2 in real-life situations, i.e. critical care diagnostics in hospitals[345] as well as wide-area barometry in wind tunnels.[346,347] Another observation concerning metal ions is that they can serve as the lumophore[348] or the receptor site[164,185,268,348] or the stimulus,[348] a versatility which is hard to match.

7. Perspectives

We hope the reader will be left with the same feeling that we were left with: this field has a wealth of examples to admire and learn from, even though only five years of the new

century have gone by. We can all build on that feeling by constructing new luminescent sensors and switches equally or more deserving of similar feelings of admiration and education. After all, many targets yet lie unfulfilled without a suitable sensor or switch system. Even the targets which have begun to be addressed still exist in other situations for which the corresponding systems have not been constructed. Also, some of these constructions will need new design principles, like those developed recently for thermometry.[349–351] Additionally, logic and gaming, as well as other computational systems, have only begun to explore the possibilities with only a few target species. Surely, these issues will be interesting challenges for us all as the 21st century rolls on.

Acknowledgements

We thank DEL, InvestNI (RTD COE 40), ESF, JSPS, EC (HPRN-CT-2000-00029), J. Stevenson and P. Marr for support and help.

References and notes

1. Bryan, A. J.; de Silva, A. P.; de Silva, S. A.; Rupasinghe, R. A. D. D.; Sandanayake, K. R. A. S. *Biosensors* **1989**, *4*, 169.
2. Krasovitski, B. M.; Bolotin, B. M. *Org. Lumin. Mater.*; VCH: Weinheim, 1989.
3. Bissell, R. A.; de Silva, A. P.; Gunaratne, H. Q. N.; Lynch, P. L. M.; Maguire, G. E. M.; Sandanayake, K. R. A. S. *Chem. Soc. Rev.* **1992**, *21*, 187.
4. Tsien, R. Y. *Am. J. Physiol.* **1992**, *263*, C723.
5. Bissell, R. A.; de Silva, A. P.; Gunaratne, H. Q. N.; Lynch, P. L. M.; Maguire, G. E. M.; McCoy, C. P.; Sandanayake, K. R. A. S. *Top. Curr. Chem.* **1993**, *168*, 223.
6. Czarnik, A. W. *Adv. Supramol. Chem.* **1993**, *3*, 131.
7. *Fluorescent Chemosensors of Ion and Molecule Recognition*; Czarnik, A. W., Ed.; ACS Symp. Ser. 538; American Chemical Society: Washington, DC, 1993.
8. Czarnik, A. W. *Acc. Chem. Res.* **1994**, *27*, 302.
9. Fabbrizzi, L.; Poggi, A. *Chem. Soc. Rev.* **1995**, *24*, 197.
10. de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515.
11. de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Adv. Supramol. Chem.* **1997**, *4*, 1.
12. *Chemosensors of Ion and Molecule Recognition*; Czarnik, A. W., Desvergne, J.-P., Eds.; Kluwer: Dordrecht, 1997.
13. Fabbrizzi, L.; Licchelli, M.; Pallavicini, P. *Acc. Chem. Res.* **1999**, *32*, 846.
14. Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 2nd ed.; Plenum: New York, 1999.
15. Special issue (Ed. Fabbrizzi, L.) *Coord. Chem. Rev.* **2000**, *205*, 1.
16. Kojima, H.; Nagano, T. *Adv. Mater.* **2000**, *12*, 763.
17. Rurack, K. *Spectrochim. Acta A* **2001**, *57*, 2161.
18. Valeur, B. *Molecular fluorescence*; Wiley-VCH: Weinheim, 2001.
19. *Optical Sensors and Switches*; Ramamurthy, V., Schanze, K. S., Eds.; Dekker: New York, 2001.
20. Rurack, K.; Resch-Genger, U. *Chem. Soc. Rev.* **2002**, *31*, 116.
21. de Silva, A. P.; McClean, G. D.; Moody, T. S.; Weir, S. M. In *Handbook of Photochemistry and Photobiology*; Nalwa, H. S., Ed.; American Scientific: Stevenson Ranch, CA, 2003; p 217.
22. Martínez-Máñez, R.; Sancenon, F. *Chem. Rev.* **2003**, *103*, 4419.
23. Balzani, V.; Venturi, M.; Credi, A. *Molecular Devices and Machines*; Wiley-VCH: Weinheim, 2003.
24. Special issue (Ed. Anslyn, E. V.) *Tetrahedron* **2004**, *60*, 11055.
25. He, H.; Mortellaro, M.; Leiner, M. J. P.; Young, S. T.; Fraatz, R. J.; Tusa, J. *Anal. Chem.* **2003**, *75*, 549.
26. He, H.; Mortellaro, M.; Leiner, M. J. P.; Fraatz, R. J.; Tusa, J. *J. Am. Chem. Soc.* **2003**, *125*, 1468.
27. Klahr, S. In *Textbook of Primary Care Medicine*; Noble, J., Ed.; Mosby: St. Louis, MO, 1996.
28. Gokel, G. W. *Crown Ethers and Cryptands*; Royal Society of Chemistry: Cambridge, 1991.
29. Pedersen, C. J. *Science* **1988**, *241*, 536.
30. Siegerman, H. In *Techniques of Electroorganic Synthesis. Part 2*; Weinberg, N. L., Ed.; Wiley: New York, 1975.
31. *Photoinduced Electron Transfer*; Fox, M. A., Chanon, M., Eds.; Elsevier: Amsterdam, 1988.
32. *Photoinduced Electron Transfer* (Ed. Mattay, J.) *Top. Curr. Chem.* **1990**, *156*, *158*; **1991**, *159*; **1992**, *163*; **1993**, *168*.
33. Wasielewski, M. R. *Chem. Rev.* **1992**, *92*, 435.
34. Kavarnos, G. J. *Fundamentals of Photoinduced Electron Transfer*; VCH: Weinheim, New York, 1993.
35. *Electron Transfer. Part 1* (Ed. Mattay, J.) *Top. Curr. Chem.* **1994**, *169*.
36. *Electron Transfer*; Balzani, V., Ed.; Wiley-VCH: Weinheim, 2003.
37. Beer, P. D.; Gale, P. A. *Angew. Chem., Int. Ed.* **2001**, *40*, 487.
38. Kenmoku, S.; Urano, Y.; Kanda, K.; Kojima, H.; Kikuchi, K.; Nagano, T. *Tetrahedron* **2004**, *60*, 11067.
39. Zang, L.; Liu, R.; Holman, M. W.; Nguyen, K. T.; Adams, D. M. *J. Am. Chem. Soc.* **2002**, *124*, 10640.
40. Wang, Y. C.; Morawetz, H. *J. Am. Chem. Soc.* **1976**, *98*, 3611.
41. Brasselet, S.; Moerner, W. E. *Single Mol.* **2000**, *1*, 17.
42. Fahrni, C. J.; Yang, L.; VanDerveer, D. G. *J. Am. Chem. Soc.* **2003**, *125*, 3799.
43. de Silva, A. P.; Gunaratne, H. Q. N. *J. Chem. Soc., Chem. Commun.* **1990**, 186.
44. de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T. *Chem. Commun.* **1996**, 1967.
45. Pragst, F.; Weber, E. *J. Prakt. Chem.* **1976**, *318*, 51.
46. Pina, F.; Lima, J. C.; Lodeiro, C.; de Melo, J. S.; Diaz, P.; Albelda, M. T.; García-España, E. *J. Phys. Chem. A* **2002**, *106*, 8207.
47. Closs, G. L.; Miller, J. R. *Science* **1988**, *240*, 440.
48. Turro, C.; Chang, C. K.; LeRoi, G. E.; Cukier, R. I.; Nocera, D. G. *J. Am. Chem. Soc.* **1992**, *114*, 4013.
49. Tian, H.; Gan, J.; Chen, K.; Song, Q. L.; Hou, X. Y. *J. Mater. Chem.* **2002**, *12*, 1262.
50. de Silva, A. P.; Gunaratne, H. Q. N.; Habib-Jiwan, J.-L.; McCoy, C. P.; Rice, T. E.; Soumillion, J.-P. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1728.
51. de Silva, A. P.; Rice, T. E. *Chem. Commun.* **1999**, 163.

52. Gan, J.; Chen, K.; Chang, C.-P.; Tian, H. *Dyes Pigment* **2003**, *57*, 21.
53. Gunnlaugsson, T.; Nieuwenhuyzen, M.; Richard, L.; Thoss, V. *J. Chem. Soc., Perkin Trans. 2* **2002**, 141.
54. Geue, J. P.; Head, N. J.; Ward, D. L.; Lincoln, S. F. *Aust. J. Chem.* **2003**, *56*, 301.
55. Geue, J. P.; Head, N. J.; Ward, D. L.; Lincoln, S. F. *Aust. J. Chem.* **2003**, *56*, 917.
56. de Silva, A. P.; Rupasinghe, R. A. D. D. *J. Chem. Soc., Chem. Commun.* **1985**, 1669.
57. Geue, J. P.; Head, N. J.; Ward, D.; Lincoln, S. F. *Dalton Trans.* **2003**, 521.
58. Bissell, R. A.; Calle, E.; de Silva, A. P.; de Silva, S. A.; Gunaratne, H. Q. N.; Habib-Jiwan, J. L.; Peiris, S. L. A.; Rupasinghe, R. A. D. D.; Samarasinghe, T. K. S. D.; Sandanayake, K. R. A. S.; Soumillion, J.-P. *J. Chem. Soc. Perkin Trans. 2* **1992**, 1559.
59. Kollmannsberger, M.; Rurack, K.; Resch-Genger, U.; Rettig, W.; Daub, J. *J. Chem. Phys. Lett.* **2000**, *329*, 363.
60. de Silva, A. P.; Sandanayake, K. R. A. S. *J. Chem. Soc., Chem. Commun.* **1989**, 1183.
61. Gunnlaugsson, T.; Bichell, B.; Nolan, C. *Tetrahedron* **2004**, *60*, 5799.
62. Cha, N. R.; Moon, S. Y.; Chang, S. K. *Tetrahedron Lett.* **2003**, *44*, 8265.
63. Beer, G.; Rurack, K.; Daub, J. *J. Chem. Commun.* **2001**, 1138.
64. Baki, C. N.; Akkaya, E. U. *J. Org. Chem.* **2001**, *66*, 1512.
65. DiCesare, N.; Lakowicz, J. R. *Tetrahedron Lett.* **2001**, *42*, 9105.
66. Haugland, R. P. *Handbook of Fluorescent Probes and Research Chemicals*, 10th ed.; Molecular Probes: Eugene, OR, 2005.
67. Gareis, T.; Huber, C.; Wolfbeis, O. S.; Daub, J. *Chem. Commun.* **1997**, 1717.
68. McKervey, M. A.; Mulholland, D. L. *J. Chem. Soc., Chem. Commun.* **1977**, 438.
69. Gee, K. R.; Rukavishnikov, A.; Rothe, A. *Comb. Chem. High Throughput Screening* **2003**, *6*, 363.
70. Tsien, R. Y. *Biochemistry* **1980**, *19*, 2396.
71. Pearson, A. J.; Xiao, W. *J. Org. Chem.* **2003**, *68*, 5369.
72. Pearson, A. J.; Hwang, J.-J.; Ignatov, M. E. *Tetrahedron Lett.* **2001**, *42*, 3537.
73. Pearson, A. J.; Hwang, J.-J. *Tetrahedron Lett.* **2001**, *42*, 3541.
74. Martin, M. M.; Plaza, P.; Meyer, Y. H.; Badaoui, F.; Bourson, J.; Lefebvre, J. P.; Valeur, B. *J. Phys. Chem.* **1996**, *100*, 6879.
75. Létard, J.-F.; Lapouyade, R.; Rettig, W. *Pure Appl. Chem.* **1993**, *65*, 1705.
76. Brimage, D. R. G.; Davidson, R. S. *J. Chem. Soc., Chem. Commun.* **1971**, 1385.
77. Nakahara, Y.; Kida, T.; Nakatsuji, Y.; Akashi, M. *Chem. Commun.* **2004**, 224.
78. Fendler, J. H. *Membrane Mimetic Chemistry*; Wiley: New York, 1982.
79. Bissell, R. A.; Bryan, A. J.; de Silva, A. P.; McCoy, C. P. *J. Chem. Soc., Chem. Commun.* **1994**, 405.
80. Bhattacharya, S.; Gulyani, A. *Chem. Commun.* **2003**, 1158.
81. Clapham, B.; Sutherland, A. J. *Chem. Commun.* **2003**, 84.
82. de Silva, A. P.; de Silva, S. A. *J. Chem. Soc., Chem. Commun.* **1986**, 1709.
83. Chang, C. J.; Nolan, E. M.; Jaworski, J.; Burdette, S. C.; Shang, M.; Lippard, S. *J. Chem. Biol.* **2004**, *11*, 203.
84. de Silva, S. A.; Zavaleta, A.; Baron, D. E.; Allam, O.; Isidor, E. V.; Kashimura, N.; Percarpio, J. M. *Tetrahedron Lett.* **1997**, *38*, 2237.
85. Walkup, G. K.; Burdette, S. C.; Lippard, S. J.; Tsien, R. Y. *J. Am. Chem. Soc.* **2000**, *122*, 5644.
86. Hirano, T.; Kikuchi, K.; Urano, Y.; Nagano, T. *J. Am. Chem. Soc.* **2002**, *124*, 6555.
87. Tsien, R. Y. *Chem. Eng. News* **1994**, *72*(29), 34.
88. Lim, N. C.; Brückner, C. *Chem. Commun.* **2004**, 1094.
89. de Silva, A. P.; Goligher, A.; Gunaratne, H. Q. N.; Rice, T. E. *ARKIVOC, Part 7* **2003**, 229.
90. Gao, Y. Q.; Marcus, R. A. *J. Phys. Chem. A* **2002**, *106*, 1956.
91. Woodroofe, C. C.; Lippard, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 11458.
92. Adams, S. R.; Harootunian, A.; Buechler, Y. J.; Taylor, S. S.; Tsien, R. Y. *Nature* **1991**, *349*, 694.
93. Cain, C. C.; Murphy, R. F. *J. Cell Biol.* **1988**, *106*, 269.
94. de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Lynch, P. L. M. *New J. Chem.* **1996**, *20*, 871.
95. Bhattacharya, S.; Mandal, S. S. *Chem. Commun.* **1996**, 1515.
96. de Silva, A. P.; Gunaratne, H. Q. N.; McCoy, C. P. *Chem. Commun.* **1996**, 2399.
97. Gunnlaugsson, T.; Lee, T. C.; Parkesh, R. *Org. Biomol. Chem.* **2003**, *1*, 3265.
98. Gunnlaugsson, T.; Lee, T. C.; Parkesh, R. *Org. Lett.* **2003**, *5*, 4065.
99. Gunnlaugsson, T.; Lee, T. C.; Parkesh, R. *Tetrahedron* **2004**, *60*, 11239.
100. Kumar, S.; Kaur, S.; Singh, G. *Supramol. Chem.* **2003**, *15*, 65.
101. Wiskur, S. L.; Ali-Haddou, H.; Lavigne, J. J.; Anslyn, E. V.; Anslyn, E. V. *Acc. Chem. Res.* **2001**, *34*, 963.
102. Prodi, L.; Montalti, M.; Zaccheroni, N.; Dallavalle, F.; Folesani, G.; Lanfranchi, M.; Corradini, R.; Pagliari, S.; Marchelli, R. *Helv. Chim. Acta* **2001**, *84*, 690.
103. Ahn, K. H.; Ku, H. Y.; Kim, Y.; Kim, S. G.; Kim, Y. K.; Son, H. S.; Ku, J. K. *Org. Lett.* **2003**, *5*, 1419.
104. Sandell, E. B. *Colorimetric Determination of Traces of Metals*, 3rd ed.; Interscience: London, 1959.
105. Moon, S. Y.; Cha, N. R.; Kim, Y. H.; Chang, S. K. *J. Org. Chem.* **2004**, *69*, 181.
106. Zheng, Y.; Orbulescu, J.; Ji, X.; Andreopoulos, F. M.; Pham, S. M.; Leblanc, R. M. *J. Am. Chem. Soc.* **2003**, *125*, 2680.
107. Zheng, Y. J.; Cao, X. H.; Orbulescu, J.; Konka, V.; Andreopoulos, F. M.; Pham, S. M.; Leblanc, R. M. *Anal. Chem.* **2003**, *75*, 1706.
108. Grandini, P.; Mancin, F.; Tecilla, P.; Scrimin, P.; Tonellato, U. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 3061.
109. Díaz-Fernández, Y.; Pérez-Gramatges, A.; Amendola, V.; Foti, F.; Mangano, C.; Pallavacini, P.; Patroni, S. *Chem. Commun.* **2004**, 1650.
110. Díaz-Fernández, Y.; Pérez-Gramatges, A.; Rodríguez-Calvo, S.; Mangano, C.; Pallavacini, P. *Chem. Phys. Lett.* **2004**, *398*, 245.
111. Russell, J. C.; Costa, S. B.; Seiders, R. P.; Whitten, D. G. *J. Am. Chem. Soc.* **1980**, *102*, 5678.
112. Brasola, E.; Mancin, F.; Rampazzo, E.; Tonellato, U. *Chem. Commun.* **2003**, 3026.
113. Prodi, L.; Montalti, M.; Zaccheroni, N.; Falini, G. *J. Am. Chem. Soc.* **2002**, *124*, 13540.
114. Meallet-Renault, R.; Pansu, R.; Amigoni-Gerbier, S.; Larpent, C. *Chem. Commun.* **2004**, 2344.
115. Fabbrizzi, L.; Licchelli, M.; Pallavicini, P.; Perotti, A.; Taglietti, A.; Sacchi, D. *Chem. Eur. J.* **1996**, *2*, 75.

116. Crego-Calama, M.; Reinhoudt, D. N. *Adv. Mater.* **2001**, *13*, 1171.
117. Cao, Y. D.; Zheng, Q. Y.; Chen, C. F.; Huang, Z.-T. *Tetrahedron Lett* **2003**, *44*, 4751.
118. Nolan, E. M.; Lippard, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 14270.
119. de Silva, A. P.; Gunaratne, H. Q. N.; McCoy, C. P. *J. Am. Chem. Soc.* **1997**, *119*, 7891.
120. Rurack, K.; Kollmannsberger, M.; Resch-Genger, U.; Daub, J. *J. Am. Chem. Soc.* **2000**, *122*, 968.
121. Guo, X.; Qian, X.; Jia, L. *J. Am. Chem. Soc.* **2004**, *126*, 2272.
122. Lieu, V. T.; Handy, C. A. *Anal. Lett.* **1974**, *7*, 267.
123. Rettig, W. *Top. Curr. Chem.* **1994**, *169*, 253.
124. Bronson, R. T.; Montalti, M.; Prodi, L.; Zaccheroni, N.; Lamb, R. D.; Dally, N. K.; Izatt, R. M.; Bradshaw, J. S.; Savage, P. B. *Tetrahedron* **2004**, *60*, 11139.
125. Prodi, L.; Bargossi, C.; Montalti, M.; Zaccheroni, N.; Su, N.; Bradshaw, J. S.; Izatt, R. M.; Savage, P. B. *J. Am. Chem. Soc.* **2000**, *122*, 6769.
126. Jimenez, D.; Martínez-Máñez, R.; Sancenon, F.; Soto, J. *Tetrahedron Lett.* **2004**, *45*, 1257.
127. Martin, M. M.; Plaza, P.; Meyer, Y. H.; Badaoui, F.; Bourson, J.; Lefebvre, J. P.; Valeur, B. *J. Phys. Chem.* **1996**, *100*, 6879.
128. Xiao, Y.; Qian, X. *Tetrahedron Lett.* **2003**, *44*, 2087.
129. Callan, J. F.; de Silva, A. P.; Ferguson, J.; Huxley, A. J. M.; O'Brien, A. M. *Tetrahedron* **2004**, *60*, 11125.
130. Grabchev, I.; Chovelon, J.-M.; Qian, X. *New J. Chem.* **2003**, *27*, 337.
131. Grabchev, I.; Chovelon, J.-M.; Qian, X. *J. Photochem. Photobiol. A: Chem.* **2003**, *158*, 37.
132. de Silva, A. P.; Fox, D. B.; Moody. T. S.; Weir, S. M. In Ref. 19, p 93.
133. Bissell, R. A.; de Silva, A. P.; Fernando, W. T. M. L.; Patuwathavithana, S. T.; Samarasinghe, T. K. S. D. *Tetrahedron Lett.* **1991**, *32*, 425.
134. Sankaran, N. B.; Banthia, S.; Samanta, A. *Proc. Ind. Acad. Sci. Chem. Sci.* **2002**, 539.
135. Pardo, A.; Poyato, J. M. L.; Martin, E.; Camacho, J. J.; Reyman, D. *J. Lumin.* **1990**, *46*, 381.
136. Daffy, L. M.; de Silva, A. P.; Gunaratne, H. Q. N.; Huber, C.; Lynch, P. L. M.; Werner, T.; Wolfbeis, O. S. *Chem. Eur. J.* **1998**, *4*, 1810.
137. Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*; Wiley: New York, 1966.
138. Okamoto, K.; Fukuzumi, S. *J. Am. Chem. Soc.* **2004**, *126*, 13922.
139. Marcus, R. A. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1111.
140. Ojida, A.; Mito-oka, M.; Sada, K.; Hamachi, I. *J. Am. Chem. Soc.* **2004**, *126*, 2454.
141. Ojida, A.; Mito-oka, M.; Inouye, M.; Hamachi, I. *J. Am. Chem. Soc.* **2002**, *124*, 6256.
142. de Silva, A. P.; Sandanayake, K. R. A. S. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1173.
143. Czarnik, A. W. *ACS Symp. Ser.* **1994**, *561*, 314.
144. James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2207.
145. Koulov, A. V.; Stucker, K. A.; Lakshmi, C.; Robinson, J. P.; Smith, B. D. *Cell Death Differ.* **2003**, *10*, 1357.
146. Lakshmi, C.; Haneshaw, R. G.; Smith, B. D. *Tetrahedron* **2004**, *60*, 11307.
147. Mukherjee, S.; Chattopadhyay, A.; Samanta, A.; Soujanya, T. *J. Phys. Chem.* **1994**, *98*, 2809.
148. Uchiyama, S.; Takehira, K.; Kohtani, S.; Imai, K.; Nakagaki, R.; Tobita, S.; Santa, T. *Org. Biomol. Chem.* **2003**, *1*, 1067.
149. Gunnlaugsson, T.; Davis, A. P.; Glynn, M. *Chem. Commun.* **2001**, 2556.
150. Gunnlaugsson, T.; Davis, A. P.; Hussey, G. M.; Tierney, J.; Glynn, M. *Org. Biomol. Chem.* **2004**, *2*, 1856.
151. Gunnlaugsson, T.; Davis, A. P.; O'Brien, J. E.; Glynn, M. *Org. Lett.* **2002**, *4*, 2449.
152. Kim, S. K.; Yoon, J. *J. Chem. Commun.* **2002**, 770.
153. Sook, K. K.; Singh, N. J.; Kim, S. J.; Kim, H. G.; Kim, J. K.; Lee, J. W.; Kim, K. S.; Yoon, J. *Org. Lett.* **2003**, *5*, 2083.
154. Callan, J. F.; de Silva, A. P.; McClenaghan, N. D. *Chem. Commun.* **2004**, 2048.
155. Yoon, J.; Kim, S. K.; Singh, N. J.; Lee, J. W.; Yang, Y. J.; Chellappan, K.; Kim, K. S. *J. Org. Chem.* **2004**, *69*, 581.
156. Cho, E. J.; Moon, J. W.; Ko, S. W.; Lee, J. Y.; Kim, S. K.; Yoon, J.; Nam, K. C. *J. Am. Chem. Soc.* **2003**, *125*, 12376.
157. Xu, G.; Tarr, M. A. *Chem. Commun.* **2004**, 1050.
158. Kwon, J. Y.; Singh, N. J.; Kim, H. N.; Kim, K. S.; Yoon, J. *J. Am. Chem. Soc.* **2004**, *126*, 8892.
159. Aslan, K.; Lakowicz, J. R.; Szmacinski, H.; Geddes, C. D. *J. Fluoresc.* **2004**, *14*, 677.
160. Otón, F.; Tàrraga, A.; Velasco, M. D.; Espinosa, A.; Molina, P. *Chem. Commun.* **2004**, 1658.
161. Ikeda, A.; Shinkai, S. *Chem. Rev.* **1997**, *97*, 1713.
162. Shinkai, S.; James, T. D. In Ref. 19, p 429.
163. Arimori, S.; Davidson, M. G.; Fyles, T. M.; Hibbert, T. G.; James, T. D.; Kociok-Köhn, G. I. *Chem. Commun.* **2004**, 1640.
164. Bonizzoni, M.; Fabbrizzi, L.; Piovani, G.; Taglietti, A. *Tetrahedron* **2004**, *60*, 11159.
165. Yalow, R. S. *J. Chem. Educ.* **1999**, *76*, 767.
166. Connors, K. A. *Chem. Rev.* **1997**, *97*, 1325.
167. Nelissen, H. F. M.; Schut, A. F. J.; Venema, F.; Feiters, M. C.; Nolte, R. J. M. *J. Chem. Soc., Chem. Commun.* **2000**, 577.
168. Inouye, M.; Hashimoto, K.; Isagawa, K. *J. Am. Chem. Soc.* **1994**, *116*, 5517.
169. Koh, K. N.; Araki, K.; Ikeda, A.; Otsuka, H.; Shinkai, S. *J. Am. Chem. Soc.* **1996**, *118*, 755.
170. Sousa, L. R.; Son, B.; Trehearne, T. E.; Stevenson, R. W.; Ganion, S. J.; Beeson, B. E.; Barnell, S.; Mabry, T. E.; Yao, M.; Chakarabarty, C.; Bock, P. L.; Yoder, C. C.; Pope, S. In Ref. 7, p 10.
171. Galindo, F.; Becerril, M.; Burgette, I.; Luis, S. V.; Vigara, L. *Tetrahedron Lett.* **2004**, *45*, 1659.
172. Birks, J. B. *Photophysics of Aromatic Molecules*; Wiley: London, 1970.
173. Ide, R.; Sakata, Y.; Misumi, S.; Okada, T.; Mataga, N. *Chem. Commun.* **1972**, 1009.
174. Cox, G. S.; Turro, N. J.; Yang, N.-C.; Chen, M.-J. *J. Am. Chem. Soc.* **1984**, *106*, 422.
175. Lynam, C.; Jennings, K.; Nolan, K.; Kane, P.; McKervey, M. A.; Diamond, D. *Anal. Chem.* **2002**, *74*, 59.
176. Lin, J.; Li, Z. B.; Zhang, H. C.; Pu, L. *Tetrahedron Lett.* **2003**, *45*, 103.
177. Diehl, H. *Calmagite and o,o′-Dihydroxyazobenzene: Titrimetric, Colorimetric and Fluorometric Reagents for Calcium and Magnesium*; The G. Frederick Smith Chemical Company: Colombus, OH, 1964.
178. Dean, K. E. S.; Klein, G.; Renaudet, O.; Reymond, J.-L. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1653.

179. Arimori, G. A.; Sonsiglio, A.; Phillips, D.; James, T. D. *Tetrahedron Lett.* **2003**, *44*, 4789.
180. Kim, D. H.; Han, M. S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2453.
181. de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T. *Tetrahedron Lett.* **1998**, *39*, 5077.
182. Weltman, J. K.; Szaro, R. P.; Frackelston, A. R.; Dowben, R. M.; Bunting, J. R.; Cathou, R. E. *J. Biol. Chem.* **1973**, *218*, 3173.
183. Kimura, E.; Koike, T. *Chem. Soc. Rev.* **1998**, *27*, 179.
184. Kaletas, B. K.; Williams, R. M.; Konig, B.; De Cola, L. *Chem. Commun.* **2002**, 776.
185. De Santis, G.; Fabbrizzi, L.; Licchelli, M.; Poggi, A.; Taglietti, A. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 202.
186. Whiten, D.; Chen, L.; Jones, R.; Bergstedt, T.; Heeger, P.; McBranch, D. In Ref. 19, p 189.
187. Yang, W.; Yan, J.; Fang, H.; Wang, B. *Chem. Commun.* **2003**, 792.
188. De Silva, A. P.; Gunaratne, H. Q. N.; McVeigh, C.; Maguire, G. E. M.; Maxwell, P. R. S.; O'Hanlon, E. *Chem. Commun.* **1996**, 2191.
189. Cooper, C. R.; James, T. D. *Chem. Commun.* **1997**, 1419.
190. Takeuchi, M.; Yamamoto, M.; Shinkai, S. *Chem. Commun.* **1997**, 1731.
191. Santa, T.; Matsumura, D.; Huang, C.; Kitada, C.; Imai, K. *Biomed. Chromatogr.* **2002**, *16*, 523.
192. Sangoe, C.; Zimerson, E. *J. Liq. Chromatogr.* **1980**, *3*, 971.
193. Uzu, S.; Kanda, S.; Nakashima, K.; Imai, K. *Analyst* **1990**, *115*, 1477.
194. Lytton, S. D.; Cabantchik, Z. I.; Libman, J.; Shanzer, A. *Mol. Pharmacol.* **1991**, *40*, 584.
195. Street, K. W.; Krause, S. A. *Anal. Lett.* **1986**, *19*, 735.
196. Forst, H.; Pacey, R. E. *Talanta* **1989**, *36*, 335.
197. Resch, U.; Rurack, K.; Bricks, J. L.; Slominski, J. L. *J. Fluoresc.* **1997**, *7*, 231S.
198. Boiocchi, M.; Fabbrizzi, L.; Licchelli, M.; Sacchi, D.; Vasquez, M.; Zampa, C. *Chem. Commun.* **2003**, 1812.
199. Meijler, M. M.; Arad-Yellin, R.; Cabantchik, Z. I.; Shanzer, A. *J. Am. Chem. Soc.* **2002**, *124*, 12666.
200. Onoda, M.; Uchiyama, S.; Endo, A.; Tokuyama, H.; Santa, T.; Imai, K. *Org. Lett.* **2003**, *5*, 1459.
201. Akasaka, K.; Suzuki, T.; Ohnai, H.; Meguro, H. *Anal. Lett.* **1987**, *20*, 731.
202. Onoda, M.; Uchiyama, S.; Santa, T.; Imai, K. *Anal. Chem.* **2002**, *74*, 4089.
203. Plater, M. J.; Greig, I.; Helfrich, M. H.; Ralston, S. H. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2553.
204. Sasaki, E.; Kojima, H.; Nishimatsu, H.; Urano, Y.; Kikuchi, K.; Hirata, Y.; Nagano, T. *J. Am. Chem. Soc.* **2005**, *127*, 3684.
205. Ozmen, B.; Akkaya, E. U. *Tetrahedron Lett.* **2000**, *41*, 9185.
206. Tanaka, F.; Mase, N.; Barbas, C. F. *J. Am. Chem. Soc.* **2004**, *126*, 3692.
207. Murov, S. L.; Carmichael, I.; Hug, G. L. *Handbook of Photochemistry*, 2nd ed.; Marcel Dekker: New York, 1993.
208. de Silva, A. P.; de Silva, S. A.; Dissanayake, A. S.; Sandanayake, K. R. A. S. *J. Chem. Soc., Chem. Commun.* **1989**, 1054.
209. Zweig, A. *Pure Appl. Chem.* **1973**, *33*, 389.
210. Reddy, D. R.; Maiya, B. G. *J. Phys. Chem. A* **2003**, *107*, 6326.
211. Perez, E. M.; Dryden, D. T. F.; Leigh, D. A.; Teobaldi, G.; Zerbetto, F. *J. Am. Chem. Soc.* **2004**, *126*, 12210.
212. Qu, D.-H.; Wang, Q.-C.; Ren, J.; Tian, H. *Org. Lett.* **2004**, *6*, 2085.
213. Chen, B.; Wang, M.; Wu, Y.; Tian, H. *Chem. Commun.* **2002**, 1060.
214. Thomas, K. G.; Kamat, P. V. *Acc. Chem. Res.* **2003**, *36*, 888.
215. de Silva, A. P.; McClenaghan, N. D.; McCoy, C. P. In Ref. 36, Vol. 5, p 156.
216. de Silva, A. P.; McClenaghan, N. D.; McCoy, C. P. In *Molecular switches*; Feringa, B. L., Ed.; Wiley-VCH: Weinheim, 2001; p 339.
217. Raymo, F. M. *Adv. Mater.* **2002**, *14*, 401.
218. de Silva, A. P.; McClenaghan, N. D. *Chem. Eur. J.* **2004**, *10*, 574.
219. Balzani, V.; Venturi, M.; Credi, A. *Chem. Phys. Chem.* **2003**, *3*, 49.
220. Malvino, A. P.; Brown, J. A. *Digital Computer Electronics*, 3rd ed.; Glencoe: Lake Forest, 1993.
221. de Silva, A. P.; Gunaratne, H. Q. N.; McCoy, C. P. *Nature* **1993**, *364*, 42.
222. de Silva, A. P.; McClean, G. D.; Pagliari, S. *Chem. Commun.* **2003**, 2010.
223. Koskela, S. J. M.; Fyles, T.; James, T. D. *Chem. Commun.* **2004**, 945.
224. Bag, B.; Bharadwaj, P. K. *Chem. Commun.* **2005**, 5133.
225. Schneider, H.-J.; Liu, T. J.; Lomadze, N.; Palm, B. *Adv. Mater.* **2004**, *16*, 613.
226. Hunter, C. A.; Sarson, L. D. *Tetrahedron Lett.* **1996**, *37*, 699.
227. Guo, X.; Zhang, D.; Zhu, D. *Adv. Mater.* **2004**, *16*, 125.
228. Hirshberg, Y. *J. Am. Chem. Soc.* **1956**, *78*, 2304.
229. Wojtyk, J. T. C.; Kazmaier, P. M.; Buncel, E. *Chem. Commun.* **1998**, 1703.
230. Bu, J.-H.; Zheng, Q.-Y.; Chen, C.-F.; Huang, Z.-T. *Org. Lett.* **2004**, *6*, 3301.
231. Casnati, A.; Pochini, A.; Ungaro, R.; Bocchi, C.; Ugozzoli, F.; Egberink, R. J. M.; Struijk, H.; Lugtenberg, R.; de Jong, F.; Reinhoudt, D. N. *Chem. Eur. J.* **1996**, *2*, 436.
232. de Silva, A. P.; Dixon, I. M.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Maxwell, P. R. S.; Rice, T. E. *J. Am. Chem. Soc.* **1999**, *121*, 1393.
233. Gunnlaugsson, T.; MacDonaill, D. A.; Parker, D. *Chem. Commun.* **2000**, 93.
234. Gunnlaugsson, T.; MacDonaill, D. A.; Parker, D. *J. Am. Chem. Soc.* **2001**, *123*, 12866.
235. Montenegro, J.-M.; Perez-Inestrosa, E.; Collado, D.; Vida, Y.; Suau, R. *Org. Lett* **2004**, *6*, 2353.
236. de Silva, A. P.; McClenaghan, N. D. *Chem. Eur. J.* **2002**, *8*, 4935.
237. Margulies, D.; Melman, G.; Felder, C. E.; Arad-Yellin, R.; Shanzer, A. *J. Am. Chem. Soc.* **2004**, *126*, 15400.
238. Raymo, F. M.; Giordani, S. *J. Am. Chem. Soc.* **2002**, *124*, 2004.
239. Alves, S.; Pina, F.; Albelda, M. T.; Garcia-España, E.; Soriano, C.; Luis, S. V. *Eur. J. Inorg. Chem.* **2001**, 405.
240. Lee, S. H.; Kim, S. K.; Lee, J. H.; Kim, J. H. *Tetrahedron* **2004**, *60*, 5171.
241. Credi, A.; Balzani, V.; Langford, S. J.; Stoddart, J. F. *J. Am. Chem. Soc.* **1997**, *119*, 2679.
242. Turfan, B.; Akkaya, E. U. *Org. Lett.* **2002**, *4*, 2857.
243. Asakawa, M.; Ashton, P. R.; Balzani, V.; Credi, A.; Mattersteig, G.; Matthews, O. A.; Montalti, M.; Spencer, N.; Stoddart, J. F.; Venturi, M. *Chem. Eur. J.* **1997**, *3*, 1992.
244. De Sousa, M.; Kluciar, M.; Abad, S.; Miranda, M. A.; de

Castro, B.; Pischel, U. *Photochem. Photobiol. Sci.* **2004**, *3*, 639.
245. Mohr, G. J. *Chem. Eur. J.* **2004**, *10*, 1082.
246. Yang, R.; Li, K.; Liu, F.; Li, N.; Zhao, F.; Chan, W. *Anal. Chem.* **2003**, *75*, 3908.
247. Raymo, F. M.; Giordani, S. *Org. Lett.* **2001**, *3*, 3475.
248. Guo, X.; Zhang, D.; Zhou, Y.; Zhu, D. *Chem. Phys. Lett.* **2003**, *375*, 484.
249. Hughes, E. *Electrical Technology*, 6th ed.; Longman: Burnt Mill, 1990.
250. Matsui, J.; Mitsuishi, M.; Aoki, A.; Miyashita, T. *Angew. Chem., Int. Ed.* **2003**, *42*, 2272.
251. Birge, R. R. In *Nanotechnology; Research and Perspectives*; Crandall, B. C., Lewis, B. C., Eds.; MIT: Cambridge, MA, 1992; p 156.
252. Matsui, J.; Mitsuishi, M.; Aoki, A.; Miyashita, T. *J. Am. Chem. Soc.* **2004**, *126*, 3708.
253. Kirkbright, G. F. In *Indicators*; Bishop, E., Ed.; Pergamon: Oxford, 1972; p 685.
254. Benniston, A. C.; Harriman, A.; Lawrie, D. J.; Mayeux, A.; Rafferty, K.; Russell, O. D. *Dalton Trans.* **2003**, 4762.
255. Barigelletti, F.; Flamigni, L.; Calogero, G.; Hammarstrom, L.; Sauvage, J.-P.; Collin, J.-P. *Chem. Commun.* **1998**, 2333.
256. Descalzo, A. B.; Martínez-Máñez, R.; Radeglia, R.; Rurack, K.; Soto, J. *J. Am. Chem. Soc.* **2003**, *125*, 3418.
257. Métivier, R.; Leray, I.; Valeur, B. *Chem. Commun.* **2003**, 996.
258. Dutta, S. K.; Perkovic, M. W. *Inorg. Chem.* **2002**, *41*, 6938.
259. Ford, P. C.; Vogler, A. *Acc. Chem. Res.* **1993**, *26*, 220.
260. Belyi, M. U.; Kushnirenko, I. *Zh. Prikl. Spectrosk.* **1968**, *9*, 272.
261. Fletcher, M. H.; White, C. E.; Sheftel, M. S. *Ind. Eng. Chem. Anal. Ed.* **1946**, *18*, 179.
262. Capitan, F.; Roman, M.; Guiraum, A. *An. Quim.* **1974**, *70*, 508.
263. Quinti, L.; Allen, N. S.; Edge, M.; Murphy, B. P.; Perotti, A. *J. Photochem. Photobiol. A: Chem.* **2003**, *155*, 93.
264. Malval, J. P.; Lapouyade, R.; Léger, J.-M.; Jarry, C. *Photochem. Photobiol. Sci.* **2003**, *2*, 259.
265. Morozumi, T.; Hiraga, H.; Nakamura, H. *Chem. Lett.* **2003**, *32*, 146.
266. de Silva, A. P.; Gunaratne, H. Q. N.; Kane, A. T. M.; Maguire, G. E. M. *Chem. Lett.* **1995**, 125.
267. Vázquez, M.; Fabbrizzi, L.; Taglietti, A.; Pedrido, R. M.; González-Noya, A. M.; Bermejo, M. R. *Angew. Chem., Int. Ed.* **2004**, *43*, 1962.
268. Zhang, T.; Anslyn, E. V. *Tetrahedron* **2004**, *60*, 11117.
269. Wisner, J. A.; Beer, P. D.; Berry, N. G.; Tomapatanaget, B. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4983.
270. *Supramolecular Chemistry of Anions*; Bianchi, A., Bowman-James, K., García-España, E., Eds.; Wiley-VCH: New York, 1997.
271. de Costa, M. D. P.; de Silva, A. P.; Pathirana, S. T. *Can. J. Chem.* **1987**, *69*, 1416.
272. DiCesare, N.; Lakowicz, J. R. *J. Phys. Chem.* **2001**, *105*, 6834.
273. Yoon, J.; Czarnik, A. W. *J. Am. Chem. Soc.* **1992**, *114*, 5874.
274. LePecq, J. B.; Paoletti, C. A. *J. Mol. Biol.* **1967**, *27*, 87.
275. Tse, W. C.; Boger, D. L. *Acc. Chem. Res.* **2004**, *37*, 61.
276. Boger, D. L.; Fink, B. E.; Hedrick, M. P. *J. Am. Chem. Soc.* **2000**, *122*, 6382.
277. Komura, T.; Niu, G. Y.; Yamaguchi, T.; Asano, M. *Electrochim. Acta* **2003**, *48*, 631.
278. Langford, S.; Yann, T. J. *J. Am. Chem. Soc.* **2003**, *125*, 11198.
279. de Silva, A. P.; McClenaghan, N. D. *J. Am. Chem. Soc.* **2000**, *122*, 3965.
280. Remacle, F.; Speiser, S.; Levine, R. D. *J. Phys. Chem. B* **2001**, *105*, 5589.
281. Guo, X.; Zhang, D.; Zhang, G.; Zhu, D. *J. Phys. Chem. B* **2004**, *108*, 11942.
282. Raymo, F. M.; Giordani, S. *J. Am. Chem. Soc.* **2001**, *123*, 4651.
283. *Indicators*; Bishop, E., Ed.; Pergamon: Oxford, 1972.
284. Kalyanasundaram, K. *Photochemistry of Polypyridine and Porphyrin Complexes*; Academic: London, 1992.
285. Sivan, S.; Tuchman, S.; Lotan, N. *Biosystems* **2003**, *70*, 21.
286. Tamaki, T.; Ichimura, K. *J. Chem. Soc., Chem. Commun.* **1989**, 1477.
287. Inouye, M.; Akamatsu, K.; Nakazumi, H. *J. Am. Chem. Soc.* **1997**, *119*, 9160.
288. Gobbi, L.; Seiler, P.; Diederich, F. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 674.
289. Inouye, M.; Noguchi, Y.; Isagawa, K. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1163.
290. Tian, H.; Qin, B.; Yao, R.-X.; Zhao, X.-L.; Yang, S.-J. *Adv. Mater.* **2003**, *15*, 2104.
291. Special Issue (Ed. Irie, M.) *Chem. Rev.* **2000**, *100*, 1683.
292. Valeur, B. In *Fluorescent Biomolecules: Methodologies and Applications*; Jameson, D. M., Reinhart, G. D., Eds.; Plenum: New York, 1989; p 269.
293. Turro, N. J. *Modern Molecular Photochemistry*; University Science Books: Mill Valley, CA, 1991.
294. Fernandez-Gutierrez, A.; Munoz de la Pena, A. In *Molecular Luminescence Spectroscopy*; Schulman, S. G., Ed.; Methods and Applications Part 1; Wiley: New York, 1985; p 371.
295. Arduini, M.; Felluga, F.; Mancio, F.; Rossi, P.; Tecilla, P.; Tonellato, U.; Valentinuzzi, N. *Chem. Commun.* **2003**, 1606.
296. De Costa, M. D. P.; Jayasinghe, W. A. P. A. *J. Photochem. Photobiol. A: Chem.* **2004**, *162*, 591.
297. Wagner, R. W.; Johnson, T. E.; Lindsey, J. S. *J. Am. Chem. Soc.* **1996**, *118*, 11166.
298. Otsuki, J.; Yasuda, A.; Takido, T. *Chem. Commun.* **2003**, 608.
299. Drake, T. J.; Tan, W. *Appl. Spectrosc.* **2004**, *58*, 269A.
300. Tyagi, S.; Kramer, F. R. *Nature Biotechnol.* **1996**, *14*, 303.
301. Guo, X.; Zhang, D.; Zhou, Y.; Zhu, D. *J. Org. Chem.* **2003**, *68*, 5681.
302. Krafft, G. A.; Sutton, W. R.; Cummings, R. T. *J. Am. Chem. Soc.* **1988**, *110*, 301.
303. de Silva, A. P. *Nat. Mater.* **2005**, *4*, 15.
304. Stojanovic, M. N.; Stefanovic, D. *J. Am. Chem. Soc.* **2003**, *125*, 6673.
305. Okamoto, A.; Tanaka, K.; Saito, I. *J. Am. Chem. Soc.* **2004**, *126*, 9458.
306. Stojanovic, M. N.; Stefanovic, D. *Nat. Biotechnol.* **2003**, *21*, 1069.
307. Mealy, G. H. *Bell Systems Tech. J.* **1955**, *34*, 1045.
308. Saudan, C.; Balzani, V.; Ceroni, P.; Gorka, M.; Maestri, M.; Vicinelli, V.; Vögtle, F. *Tetrahedron* **2003**, *59*, 3845.
309. Kanekiyo, Y.; Naganawa, R.; Tao, H. *Chem. Commun.* **2004**, 1006.
310. Lee, H. C.; Forte, J. G.; Epel, D. In *Intracellular pH: its Measurement, Regulation and Utilization in Cellular Functions*; Nuccitelli, R., Deamer, D. W., Eds.; Liss: New York, 1982; p 136.

311. Nishizawa, S.; Kato, Y.; Teramae, N. *J. Am. Chem. Soc.* **1999**, *121*, 9463.
312. Kakizawa, Y.; Akita, T.; Nakamura, H. *Chem. Lett.* **1993**, 1671.
313. Licchelli, M.; Biroli, A. O.; Poggi, A.; Sacchi, D.; Sangermani, C.; Zema, M. *Dalton Trans.* **2003**, 4537.
314. Ashikaga, K.; Ito, S.; Yamamoto, M.; Nishijima, Y. *J. Am. Chem. Soc.* **1988**, *110*, 198.
315. Barros, T. C.; Filho, P. B.; Toscano, V. G.; Politi, M. J. *J. Photochem. Photobiol. A: Chem.* **1995**, *89*, 141.
316. Prodi, L.; Ballardini, R.; Gandolfi, M. T.; Roverai, R. *J. Photochem. Photobiol. A: Chem.* **2000**, *136*, 49.
317. Yang, R.-H.; Chan, W.-H.; Lee, A. W. M.; Xia, P.-F.; Zhang, H.-K.; Li, K. *J. Am. Chem. Soc.* **2003**, *125*, 2884.
318. Tanford, C. *The Hydrophobic Effect*, 2nd ed.; Wiley: New York, 1980.
319. Guo, X.; Zhang, D.; Wang, T.; Zhu, D. *Chem. Commun.* **2003**, 914.
320. Winkler, J. D.; Bowen, C. M.; Kim, H. S. *Abstr. Am. Chem. Soc.* **1996**, *212*, 136. -ORGN Part 2.
321. Parker, D.; Williams, J. A. G. *Chem. Commun.* **1998**, 245.
322. Kawakami, J.; Kimura, H.; Nagaki, M.; Kitahara, H.; Ito, S. *J. Photochem. Photobiol. A: Chem.* **2004**, *161*, 141.
323. Kawakami, J.; Komai, Y.; Ito, S. *Chem. Lett.* **1996**, 617.
324. Fei, Z.; Kocher, N.; Mohrschladt, C. J.; Ihmels, H.; Stalke, D. *Angew. Chem., Int. Ed.* **2003**, *42*, 783.
325. Roundhill, D. M. *Photochemistry and Photophysics of Metal Complexes*; Plenum: New York, 1994.
326. Horvath, O.; Stevenson, K. L. *Charge Transfer Photochemistry of Coordination Compounds*; VCH: New York, 1993.
327. Balzani, V.; Scandola, F. *Supramolecular Photochemistry*; Ellis-Horwood: Chichester, 1991.
328. Richardson, F. H. *Chem. Rev.* **1982**, *82*, 541.
329. Parker, D.; Williams, J. A. G. *J. Chem. Soc., Dalton Trans.* **1996**, 3613.
330. de Silva, A. P.; Gunaratne, H. Q. N.; Rice, T. E. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2116.
331. de Silva, A. P.; Gunaratne, H. Q. N.; Rice, T. E.; Stewart, S. *Chem. Commun.* **1997**, 1891.
332. De Bettencourt Dias, A.; Viswanathan, S. *Chem. Commun.* **2004**, 1024.
333. Casnati, A.; Sansone, F.; Sartori, A.; Prodi, L.; Montalti, M.; Zaccheroni, N.; Ugozzoli, F.; Ungaro, R. *Eur. J. Org. Chem.* **2003**, 1475.
334. Charbonniere, L. J.; Ziessel, R. F.; Sams, C. A.; Harriman, A. *Inorg. Chem.* **2003**, *42*, 3466.
335. Grigg, R.; Norbert, W. D. J. A. *J. Chem. Soc., Chem. Commun.* **1992**, 1298.
336. Pratt, M. D.; Beer, P. D. *Tetrahedron* **2004**, *60*, 11227.
337. Sun, S.-S.; Lees, A. J.; Zavalij, P. Y. *Inorg. Chem.* **2003**, *42*, 3445.
338. Yin, C.; Gao, F.; Huo, F.; Yang, P. *Chem. Commun.* **2004**, 934.
339. Stern, K. G.; Steinberg, M. A. *Biochem. Biophys. Acta* **1953**, *2*, 553.
340. Gunnlaugsson, T.; Leonard, J. P.; Sénéchal, K.; Harte *J. Am. Chem. Soc.* **2003**, *125*, 12062.
341. Gunnlaugsson, T.; Leonard, J. P.; Sénéchal, K.; Harte, A. J. *Chem. Commun.* **2004**, 782.
342. Gunnlaugsson, T.; Leonard, J. P. *Chem. Commun.* **2004**, 782.
343. de Silva, S. A.; Amorelli, B.; Isidor, D. C.; Loo, K. C.; Crooker, K. E.; Pena, Y. E. *Chem. Commun.* **2002**, 1360.
344. Ramachandram, B.; Saroja, G.; Sankaran, N. B.; Samanta, A. *J. Phys. Chem. B* **2000**, *104*, 11824.
345. Amao, Y. *Microchim. Acta* **2003**, *143*, 1.
346. Puklin, E.; Carlson, B.; Gouin, S.; Costin, C.; Green, E.; Ponomarev, S.; Tanji, H.; Gouterman, M. *J. Appl. Polym. Sci.* **2000**, *77*, 2795.
347. Lu, K.; Manners, I.; Winnik, M. A. In *New trends in fluorescence spectroscopy*; Valeur, B., Brochon, J.-C., Eds.; Springer: Berlin, 2001; p 229.
348. de Silva, A. P.; McCaughan, B.; McKinney, B. O. F.; Querol, M. *Dalton Trans.* **2003**, 1902.
349. Uchiyama, S.; Matsumura, Y.; de Silva, A. P.; Iwai, K. *Anal. Chem.* **2003**, *75*, 5926.
350. Uchiyama, S.; Matsumura, Y.; de Silva, A. P.; Iwai, K. *Anal. Chem.* **2004**, *76*, 1793.
351. Uchiyama, S.; Kawai, N.; de Silva, A. P.; Iwai, K. *J. Am. Chem. Soc.* **2004**, *126*, 3032.

Biographical sketch

John F. Callan was born in Strabane, Northern Ireland and received his B.Sc. (Hons) degree from Queen's University of Belfast in 1995. He remained at Queen's and completed his PhD in the area of polymeric liquid crystals in 1999. Following four years working in the pharmaceutical industry, he returned to Queen's to work as a Research Fellow under the direction of A. P. de Silva. After a very enjoyable year working in the area of molecular logic, he moved to the Robert Gordon University in Scotland, where he currently works as a lecturer.

A. Prasanna de Silva received his early education in chemistry at the University of Colombo in Sri Lanka. This was followed by PhD and postdoctoral research in organic photochemistry at the Queen's University of Belfast in Northern Ireland. After spending six years lecturing in chemistry at Colombo, he returned to Belfast where he has been lecturer, reader and professor. In both these cities and countries, he has learned to live with diversity and the conflicts it sometimes causes. He has also enjoyed visiting professorships in other parts of the world—Louvain-la-Neuve, Cachan, Bordeaux, Kandy, Nara, Strasbourg, Shanghai and Peradeniya. On the scientific front, he and his co-workers have had the chance to publish the first experimental molecular logic gates in the primary literature and to establish the generality of one of the main principles underlying luminescent sensors, that of photoinduced electron transfer (PET). The development of molecular computation and medical diagnostics are two roads he and his co-workers are travelling.

David C. Magri was born and raised in the small manufacturing city of St. Thomas located in the heart of southwestern Ontario, Canada. He attended the University of Western Ontario, London, Canada as an undergraduate, and continued his education at the same institution as a graduate student under the supervision of Dr. Mark S. Workentin. In 2004, he received his PhD for work on the electrochemical and photochemical dissociative electron transfer reduction of peroxides and endoperoxides. Currently, he is a research fellow at Queen's University of Belfast working with A. P. de Silva on luminescent sensors and molecular logic gates.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
ELSEVIER
Tetrahedron 61 (2005) 8589–8597

A stereoselective route to multi-substituted tetrahydropyrans by vinyl radical cyclization

Naoki Hiramatsu,[a] Natsuko Takahashi,[a] Ryoji Noyori[b,†] and Yuji Mori[a,*]

[a]*Faculty of Pharmacy, Meijo University, 150 Yagotoyama, Tempaku, Nagoya 468-8503, Japan*
[b]*Department of Chemistry and Research Center for Materials Science, Nagoya University, Chikusa, Nagoya 464-8606, Japan*

Received 13 June 2005; accepted 6 July 2005

Available online 21 July 2005

Abstract—The tin-mediated 6-*exo-trig* radical cyclization of the acetylenic β-alkoxy acrylates proceeded smoothly to give fully substituted tetrahydropyrans in good yields with high equatorial selectivity irrespective of the stereochemistry of the propargylic position.

1. Introduction

The tetrahydropyran nucleus is an integral structural component of an increasing number of biologically significant marine polycyclic ether toxins.[1] The synthesis of such a fused ring system is receiving a great deal of attention, and a variety of approaches have been developed.[2] Among them, radical cyclization methods developed for the stereoselective synthesis of trans-fused tetrahydropyrans include the intramolecular addition of acyl,[3] ketyl,[4] and vinyl radicals[5] to β-alkoxy acrylates. However, these methods were limited to the construction of the general structure **I**, and the formation of fully substituted tetrahydropyrans such as structure **II** has not been studied (Fig. 1). The ring system **II** would serve as a potent precursor of 4-hydroxy-3-methyl-tetrahydropyrans **III** and **IV**,[6] which correspond to the I ring of yessotoxin[7] and adriatoxin[8] and the H ring of gambieric acids,[9] respectively. An efficient and straightforward construction of **II** is envisioned by the 6-*exo-trig* cyclization of vinyl radical **V** generated from a propargyl alcohol derivative.

Of particular concern is the influence of the alkoxy group adjacent to the vinyl radical on the cyclization. The stereochemistry and the protecting group of the hydroxy group might influence the reaction course of the radical reaction, such as cyclization, premature reduction (hydrostannation), and elimination.[10] We report here a tin-mediated radical reaction of propargyl alcohol derivatives to construct multi-substituted tetrahydropyrans.

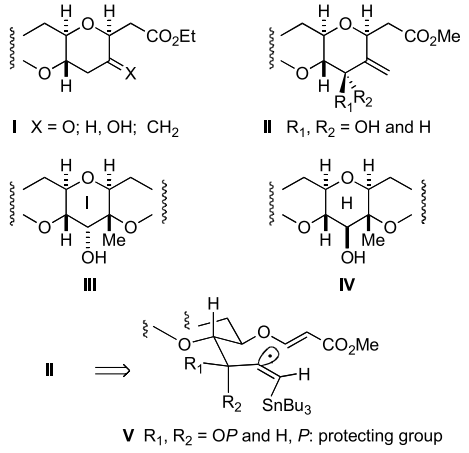


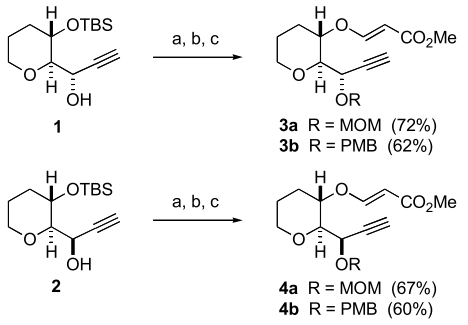
Figure 1.

2. Results and discussion

The synthesis of radical cyclization precursors is summarized in Scheme 1. Treatment of (2*S*,3*S*)-3-(*tert*-butyldimethylsilyloxy)tetrahydropyran-2-carboxaldehyde[11] with ethynylmagnesium bromide gave a separable mixture of propargyl alcohols **1** and **2** in 63 and 33% yield, respectively. The protection of each alcohol with methoxymethyl or *p*-methoxybenzyl groups, desilylation, and the hetero-Michael reaction with methyl propiolate in the presence of *N*-methylmorpholine afforded (*E*)-alkoxy acrylates **3** and **4**.

Keywords: Radical cyclization; Tetrahydropyrans; Polycyclic ethers; Alkoxy acrylates.
* Corresponding author. Tel.: +81 52 832 1781; fax: +81 52 834 8090; e-mail: mori@ccmfs.meijo-u.ac.jp
† Nagoya University. Visiting Professor at Meijo University.

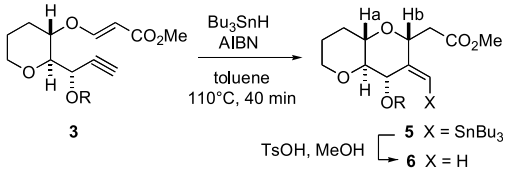
0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.07.007



Scheme 1. Reagents and conditions: (a) MOM–Cl, NaH, DMF or PMB–Cl, NaH, DMF; (b) Bu_4NF, THF, rt; (c) methyl propiolate, NMM, CH_2Cl_2, rt.

Reaction of the acetylenic β-alkoxy acrylates **3** with Bu_3SnH and AIBN in toluene at a concentration of 0.05 M and at 110 °C for 40 min and the subsequent acidic destannylation of the products gave the 6,6-bicyclic ethers **6** having equatorial CH_2CO_2Me and OR groups in good yields (Table 1). The radical cyclization of the other isomers **4** also proceeded under the same reaction conditions to provide the bicyclic products **8**, which have equatorial CH_2CO_2Me and axial OR substituents (Table 2). The stereochemistry of the cyclization products was determined by an NOE interaction between the axial Ha and Hb protons of the newly formed tetrahydropyran ring (9% NOE for **6a**, 10% NOE for **8a**). Interestingly, the 6-*exo-trig* radical cyclization reaction of both isomers **3** and **4** proceeded with high diastereoselectivity and in good yields in such a way as to give products with an equatorial CH_2CO_2Me, irrespective of the stereochemistry of the alkoxy group at the propargylic position. It is worthwhile noting that the present reaction conditions (a 0.05 M solution in toluene at 110 °C) led the cyclization to be completed within 60 min. The reported radical cyclizations of β-alkoxy acrylates were carried out by the slow addition of a benzene solution of Bu_3SnH and AIBN over a period of hours by a syringe pump (80 °C, ~12 h).[3,5]

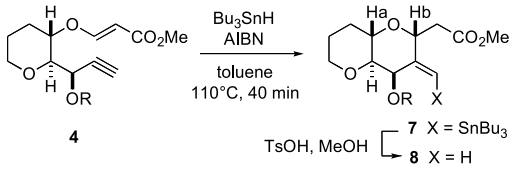
Table 1. Radical cyclization of **3**



Substrate	**5**/Yield (%)[a]	**6**/Yield (%)[a]	ds[b]
3a R=MOM	**5a**/90	**6a**/91	95:5
3b R=PMB	**5b**/75	**6b**/72	94:6

[a] Isolated yield after flash chromatography.
[b] Determined by ^{1}H NMR analysis.

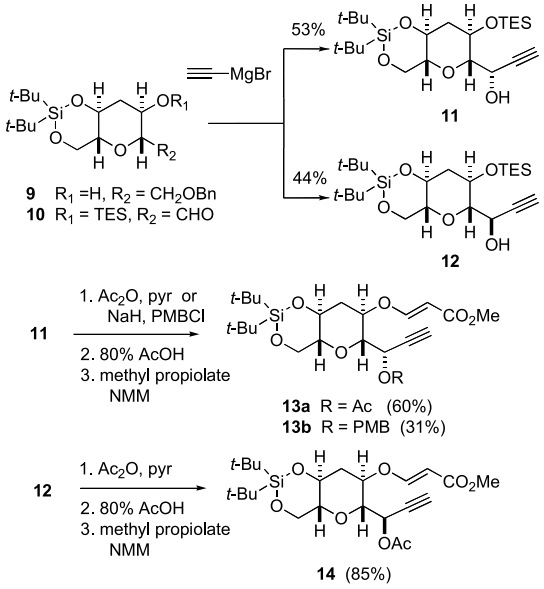
Table 2. Radical cyclization of **4**



Substrate	**7**/Yield (%)[a]	**8**/Yield (%)[a]	ds[b]
4a R=MOM	**7a**/87	**8a**/92[c]	95:5
4b R=PMB	**7b**/90	**8b**/69	94:6

[a] Isolated yield after flash chromatography.
[b] Determined by ^{1}H NMR analysis.
[c] Isolated as R=H.

The high stereoselectivity and good yields of the radical cyclization prompted us to extend this study to more functionalized precursors. The synthesis started with the known bicyclic alcohol **9**,[12] which was prepared in five steps according to our oxiranyl anion strategy.[13] Protection of the alcohol with the triethylsilyl group, hydrogenolysis of the benzyl ether, and oxidation with Dess–Martin periodinane afforded aldehyde **10** (Scheme 2).

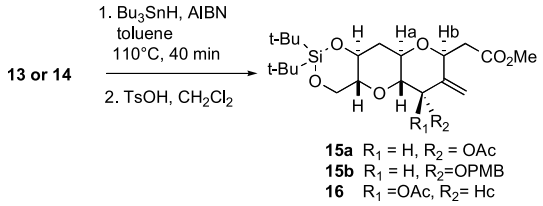


Scheme 2.

The reaction with ethynylmagnesium bromide gave a mixture of diastereoisomers **11** and **12**, which were easily separated by flash chromatography. The stereochemistry of both isomers was determined by the ^{1}H NMR analysis of the corresponding acetonide derivatives. After protection of the propargyl alcohol with an acetyl group, the triethylsilyl group was removed and then an acrylate moiety was introduced with methyl propiolate to give acetylenic β-alkoxy acrylates **13a** and **14** in 60 and 85% yield, respectively. A partial 1,3-migration of the acetyl group was observed during the hetero-Michael reaction with methyl propiolate: the extent of the migration was 1:3.5 for **13a** and 1:9 for **14**. The p-methoxybenzylation of **11** resulted in a low yield of the product due to the 1,3-migration of the triethylsilyl group and the partial decomposition of the silylene group under the reaction conditions.

Treatment of **13** and **14** with Bu_3SnH and AIBN in refluxing toluene furnished the tricyclic ethers **15** and **16** in good yields with high stereoselectivity (Table 3). The stereochemistry of **15a** and **16** was determined by the NOE experiments. An 11.6% NOE was observed between the Ha and Hb of **15a**, and in the case of **16**, 10.2 and 5.1% NOEs were detected between Ha and Hb and between Hb and Hc, respectively. The results showed again that the

Table 3. Radical cyclization of **13** and **14**

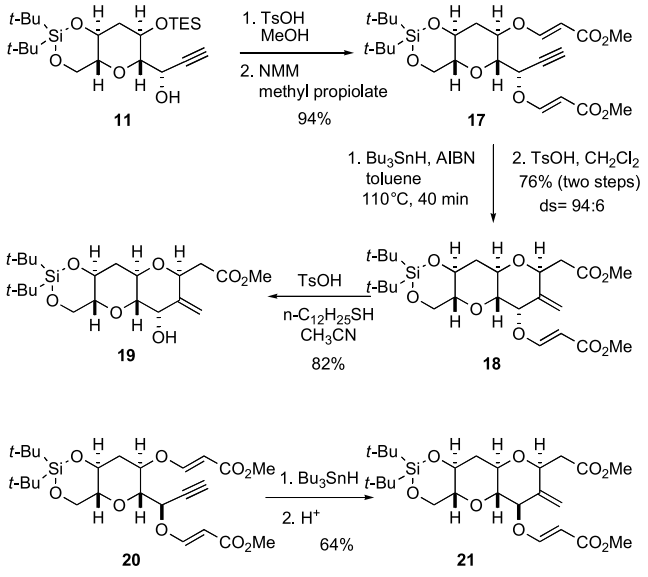


Substrate	Product	Yield (%)[a]	ds[b]
13a	**15a**	78[c]	92:8
13b	**15b**	82	96:4
14	**16**	81	93:7

[a] Isolated yield after flash chromatography.
[b] Determined by ^{1}H NMR analysis.
[c] Corrected yield based on pure **13a**.

stereochemistry of the substituent at the propargylic position did not affect the radical cyclization.

We next examined the protecting group of a propargyl alcohol to prevent the 1,3-migration of the acetyl and triethylsilyl groups in the synthesis of **13** and **14**. As the hetero-Michael reaction of an alcohol with methyl propiolate proceeded in an excellent yield, we decided to introduce two acrylate groups, one for a protecting group of a propargyl alcohol and the other for a radical acceptor. Removal of the triethylsilyl group of **11** followed by the reaction of the resulting diol with methyl propiolate in the presence of *N*-methylmorpholine gave bis(β-alkoxy acrylate) **17** in 94% yield (Scheme 3).



Scheme 3.

Although **17** could suffer radical cyclization in 4-*exo* and 6-*exo* fashions, leading to an oxetane and a tetrahydropyran, respectively, we anticipated that a group-selective cyclization via a 6-*exo* pathway would occur for an obvious steric reason. Indeed, the treatment of **17** with Bu_3SnH and AIBN in refluxing toluene followed by acidic destannylation furnished the desired tetrahydropyran **18** in good yields with high diastereoselectivity.

The following hydrolysis of the β-alkoxy acrylate group of **18** in the presence of the silylene group was carried out effectively with *p*-TsOH in the presence of *n*-dodecanthiol[14] in acetonitrile at 80 °C, giving the desired tetrahydropyran **19** in 82% yield. The principle of the group-selective radical cyclization also applied to the other isomer **20** and the functionalized tetrahyropyran **21** was constructed in good yield.

3. Conclusion

In summary, we developed a straightforward approach towards multi-substituted tetrahydropyrans via a vinyl radical generated from an acetylenic β-alkoxy acrylate moiety. We also demonstrated interesting group-selective radical cyclizations and the efficient thiol-mediated deprotection of β-alkoxy acrylate group. The tetrahydropyran ring constructed contains methoxycarbonylmethyl, hydroxy, and exocyclic methylene groups, which are useful for the partial and total synthesis of the related structurally and biologically interesting class of polycyclic ethers.

4. Experimental

4.1. General

IR spectra were recorded in $CHCl_3$ solution on a JASCO FTIR-420 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL A-400 or A-600 spectrometer in $CDCl_3$ solution using TMS and $CDCl_3$ (77.00 ppm) as internal standards, respectively. EI and FAB mass spectra were obtained on JEOL JMS-700 and HX-110 mass spectrometers, respectively. Optical rotations were determined on a JASCO DIP-370 digital polarimeter. All air- and moisture-sensitive reactions were carried out under an argon atmosphere in dry, freshly distilled solvents under anhydrous conditions. Flash chromatography was carried out with E. Merck silica gel 60 (230–400 mesh). The term 'dried' refers to the drying of an organic solution over $MgSO_4$ followed by filtration.

4.1.1. (*S*)-1-[(2*R*,3*S*)-3-(*tert*-Butyldimethylsilyloxy)tetrahydropyran-2-yl]prop-2-yn-1-ol (1) and (*R*)-1-[(2*R*,3*S*)-3-(*tert*-butyldimethylsilyloxy)tetrahydropyran-2-yl]-prop-2-yn-1-ol (2). To a stirred solution of 0.5 M ethynylmagnesium bromide in Et_2O (14.5 mL, 10.5 mmol) at 0 °C was added a solution of (2*S*,3*S*)-3-(*tert*-butyldimethylsilyloxy)tetrahydropyran-2-carboxaldehyde[11] (857 mg, 3.51 mmol) in THF (18 mL), and the reaction mixture was stirred for 1 h. The reaction was quenched with saturated aqueous NH_4Cl and the mixture was extracted with EtOAc. The extract was washed with water and brine, dried, and concentrated. Purification by flash chromatography (20% EtOAc in hexane) gave alcohols **1** (599 mg, 63%) and **2** (313 mg, 33%). Compound **1**: colorless oil; $[\alpha]_D^{25}$ +25.4 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3564, 3306, 1472, 1234, 1162, 837 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.10 (3H, s), 0.12 (3H, s), 0.89 (9H, s), 1.49 (1H, m), 1.62–1.71 (2H, m), 2.07 (1H, m), 2.45 (1H, d, J=2.0 Hz), 2.76 (1H, d, J=10.2 Hz, OH), 3.19 (1H, dd, J=8.8, 1.9 Hz), 3.45 (1H, m), 3.77 (1H, ddd, J=10.7, 8.8, 4.4 Hz), 4.00 (1H, m), 4.58

(1H, ddd, J=10.7, 1.9, 1.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.8, −4.0, 17.8, 25.2, 25.6, 25.7 (2×C), 33.0, 61.5, 67.3, 68.2, 72.6, 83.2, 83.6; HREIMS *m/z* calcd for $C_{14}H_{26}O_3Si$ 270.1650, found 270.1692. Compound **2**: colorless oil; $[\alpha]_D^{25}$ +65.2 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3563, 3306, 1472, 1254, 1104, 861 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.08 (3H, s), 0.10 (3H, s), 0.87 (9H, s), 1.47 (1H, dddd, J=13.2, 13.2, 10.7, 4.4 Hz), 1.62–1.79 (2H, m), 2.05 (1H, m), 2.49 (1H, d, J=2.4 Hz), 2.87 (1H, d, J=10.2 Hz, OH), 3.26 (1H, dd, J=9.3, 3.4 Hz), 3.37 (1H, ddd, J=11.7, 11.7, 2.9 Hz), 3.64 (1H, ddd, J=10.7, 9.3, 4.9 Hz), 4.02 (1H, m), 4.65 (1H, ddd, J=10.2, 3.4, 2.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.9, −4.0, 17.8, 25.0, 25.7 (3×C), 33.1, 62.8, 68.0, 68.4, 74.1, 81.7, 83.7; HREIMS *m/z* calcd for $C_{14}H_{26}O_3Si$ 270.1650, found 270.1269.

4.1.2. (*E*)-3-{(2*S*,3*S*)-2-[(*S*)-1-(Methoxymethoxy)prop-2-ynyl]tetrahydropyran-3-yloxy}acrylic acid methyl ester (3a). (*i*) *O-Methoxymethylation*. To a stirred solution of **1** (76 mg, 0.281 mmol) in THF were added NaH (60% in mineral oil, 79 mg, 1.976 mmol) and MOM–Cl (0.28 mL, 3.670 mmol), and the reaction mixture was refluxed for 1 h. After cooling to rt, the mixture was extracted with EtOAc. The extract was washed with water and brine, dried, and concentrated. Purification by flash chromatography (20% EtOAc in hexane) gave methoxymethyl ether (72 mg, 81%) as a colorless oil; $[\alpha]_D^{25}$ +101.6 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3306, 1464, 1253, 1104, 1029, 838 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.087 (3H, s), 0.094 (3H, s), 0.89 (9H, s), 1.45 (1H, dddd, J=13.2, 13.2, 10.7, 4.4 Hz), 1.62–1.79 (2H, m), 2.12 (1H, m), 2.45 (1H, d, J=2.0 Hz), 3.24 (1H, dd, J=8.8, 1.5 Hz), 3.42 (1H, ddd, J=11.7, 11.7, 2.4 Hz), 3.43 (3H, s), 3.80 (1H, ddd, J=10.7, 8.8, 4.4 Hz), 4.06 (1H, m), 4.71 and 4.97 (each 1H, d, J=6.8 Hz), 4.75 (1H, t, J=2.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.7, −3.3, 17.9, 25.0, 25.8 (3×C), 33.5, 56.4, 64.5, 66.4, 68.5, 73.6, 81.3, 84.3, 95.3; EIMS *m/z* 314 (M^+).

(*ii*) *Desilylation*. The product obtained above (72 mg, 0.228 mmol) was dissolved in THF (2.3 mL), and 1.0 M TBAF in THF (0.3 mL, 0.300 mmol) was added at 0 °C. The reaction mixture was stirred at rt for 1.5 h and then concentrated. Purification by flash chromatography (50–60% EtOAc in hexane) gave alcohol (42.3 mg, 93%) as a colorless oil; $[\alpha]_D^{25}$ +157.1 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3536, 3306, 1465, 1348, 1153, 1099, 1026 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (1H, dddd, J=12.7, 12.7, 10.7, 4.4 Hz), 1.63–1.81 (2H, m), 2.17 (1H, m), 2.52 (1H, d, J=2.0 Hz), 2.63 (1H, br, OH), 3.27 (1H, dd, J=9.3, 3.4 Hz), 3.40 (1H, ddd, J=11.7, 11.7, 3.0 Hz), 3.42 (3H, s), 3.89 (1H, ddd, J=10.7, 9.3, 4.9 Hz), 4.02 (1H, dddd, J=11.2, 4.4, 1.5, 1.5 Hz), 4.67 and 4.98 (each 1H, d, J=6.8 Hz,), 4.75 (1H, dd, J=3.4, 2.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.9, 31.8, 56.0, 66.5, 66.7, 68.2, 75.3, 79.6, 81.7, 94.4; EIMS *m/z* 201 (MH^+).

(*iii*) *Reaction with methyl propiolate*. To a stirred solution of the alcohol obtained above (42.3 mg, 0.212 mmol) in CH_2Cl_2 (2.1 mL) were added methyl propiolate (76 μL, 0.846 mmol) and *N*-methylmorpholine (NMM) (47 μL, 0.423 mmol). After stirring at rt for 4 h, the reaction mixture was extracted with EtOAc and the extract was washed with water and brine, dried, and concentrated. Purification by flash chromatography (40% EtOAc in hexane) gave **3** (58 mg, 96%) as a colorless oil; $[\alpha]_D^{25}$ +114.5 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3306, 1705, 1645, 1623, 1438, 1335, 1147, 1070, 1022 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.56 (1H, dddd, J=17.6, 12.7, 11.2, 5.4 Hz), 1.73–1.85 (2H, m), 2.32 (1H, m), 2.49 (1H, d, J=2.0 Hz,), 3.34 (3H, s,), 3.47 (1H, ddd, J=11.2, 11.2, 3.4 Hz), 3.48 (1H, dd, J=5.4, 2.0 Hz), 3.69 (3H, s), 4.10 (1H, m), 4.16 (1H, ddd, J=10.7, 9.3, 4.9 Hz), 4.61 and 5.01 (each 1H, d, J=6.8 Hz), 4.67 (1H, t, J=2.2 Hz), 5.32 and 7.51 (each 1H, d, J=12.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.6, 29.1, 51.1, 56.2, 63.4, 68.4, 74.9, 75.6, 79.3, 80.8, 94.3, 98.1, 160.8, 167.9; HREIMS *m/z* calcd for $C_{14}H_{20}O_6$ 284.1259, found 284.1265.

4.1.3. (*E*)-3-{(2*S*,3*S*)-2-[(*S*)-1-(*p*-Methoxybenzyloxy)prop-2-ynyl]tetrahydropyran-3-yloxy}acrylic acid methyl ester (3b). To a solution of alcohol **1** (100 mg, 0.37 mmol) in DMF (1.8 mL) were added NaH (60% in mineral oil, 45 mg, 1.85 mmol) and PMB–Cl (0.15 mL, 1.11 mmol), and the reaction mixture was stirred at 0 °C for 17 h. The reaction was quenched with saturated aqueous NH_4Cl and the mixture was extracted with Et_2O. The extract was washed with water and brine, dried, and concentrated. Purification by flash chromatography (30% EtOAc in hexane) gave PMB ether (95 mg, 65%). The desilylation of the PMB ether and the reaction with methyl propiolate were carried out in the same manner as described for **3a** to give **3b** (82 mg, 95% for two steps) as a colorless oil; $[\alpha]_D^{25}$ +124.2 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3306, 1705, 1643, 1622, 1514, 1157, 1076 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49 (1H, dddd, J=12.7, 12.7, 12.7, 5.4 Hz), 1.62–1.81 (2H, m), 2.02 (1H, m), 2.53 (1H, d, J=2.4 Hz), 3.37 (1H, dd, J=8.8, 2.4 Hz), 3.41 (1H, ddd, J=11.2, 11.2, 3.4 Hz), 3.72 (3H, s), 3.78 (3H, s), 4.05–4.18 (2H, m), 4.26 (1H, t, J=2.4 Hz), 4.44 and 5.77 (each 1H, d, J=11.7 Hz), 5.16 and 7.33 (each 1H, d, J=12.2 Hz), 6.83 and 7.24 (each 2H, d, J=8.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.7, 29.2, 51.1, 55.1, 65.0, 68.4, 70.4, 74.9, 75.8, 79.8, 80.9, 97.7, 113.7 (2×C), 128.6, 130.5 (2×C), 159.5, 161.2, 168.0; HREIMS *m/z* calcd for $C_{20}H_{24}O_6$ 360.1571, found 360.1554.

4.1.4. (*E*)-3-{(2*S*,3*S*)-2-[(*R*)-1-(Methoxymethoxy)prop-2-ynyl]tetrahydropyran-3-yloxy}acrylic acid methyl ester (4a). The procedure used for the preparation of **3a** was employed. An experiment starting with alcohol **2** (102 mg, 0.378 mmol) provided **4a** (72 mg, 67%) as a colorless oil; $[\alpha]_D^{25}$ −56.0 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3305, 1701, 1645, 1623, 1438, 1150, 1028 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.59 (1H, dddd, J=17.5, 12.2, 10.7, 5.4 Hz), 1.73–1.85 (2H, m), 2.30 (1H, m), 2.53 (1H, d, J=2.4 Hz), 3.40 (3H, s), 3.45 (1H, m), 3.57 (1H, dd, J=9.3, 2.4 Hz), 3.70 (3H, s), 4.04 (1H, ddd, J=10.6, 9.3, 4.9 Hz), 4.08 (1H, m), 4.64 (1H, t, J=2.4 Hz), 4.68 and 5.98 (each 1H, d, J=6.8 Hz), 5.33 and 7.50 (each 1H, d, J=12.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.5, 29.2, 51.1, 55.8, 65.1, 67.8, 76.0, 77.1, 78.0, 80.4, 94.4, 98.3, 160.9, 168.0; HREIMS *m/z* calcd for $C_{14}H_{20}O_6$ 284.1259, found 284.1231.

4.1.5. (*E*)-3-{(2*S*,3*S*)-2-[(*R*)-1-(*p*-Methoxybenzyloxy)prop-2-ynyl]tetrahydropyran-3-yloxy}acrylic acid methyl ester (4b). The procedure used for the preparation

of **3b** was employed. An experiment starting with alcohol **2** (100 mg, 0.37 mmol) provided **4b** (80 mg, 60%) as a colorless oil; $[\alpha]_D^{25}$ −79.8 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3305, 1705, 1644, 1623, 1438, 1148, 1073 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.55 (1H, dddd, J=18.0, 12.3, 10.7, 5.8 Hz), 1.70–1.81 (2H, m), 2.23 (1H, m), 2.57 (1H, d, J=2.0 Hz), 3.41 (1H, m), 3.54 (1H, dd, J=9.3, 2.4 Hz), 3.70 (3H, s), 3.81 (3H, s), 3.98–4.07 (2H, m), 4.33 (1H, t, J=2.4 Hz), 4.51 and 4.81 (each 1H, d, J=11.2 Hz, OCH_2Ph), 5.25 and 7.45 (each 1H, d, J=12.2 Hz), 6.87 and 7.28 (each 2H, d, J=8.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.7, 29.4, 51.4, 55.5, 67.9, 68.0, 71.0, 77.5, 77.6, 78.8, 80.9, 98.4, 114.1, 128.5, 130.3 (2×C), 159.7, 161.4 (2×C), 168.4; HREIMS *m*/*z* calcd for $C_{20}H_{24}O_6$ 360.1571, found 360.1538.

4.2. General procedure for radical cyclization and destannylation

(*i*) *Radical cyclization*. A solution of an alkynyl β-alkoxy acrylate (0.30 mmol, 1.0 equiv), Bu_3SnH (0.9 mmol, 1.3 equiv), and AIBN (5 mg, catalytic amount) in toluene (6 mL, 0.05 M solution) was heated at 110 °C for 30–60 min. After cooling to rt, the reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (EtOAc/hexane) to give cyclization product.

(*ii*) *Destannylation*. The cyclization product (0.2 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (2 mL) or MeOH (2 mL) and *p*-TsOH·H_2O (0.3 mmol, 1.5 equiv) was added. The reaction mixture was stirred at rt for 1 h and the reaction was quenched with Et_3N (0.2 mL). The mixture was concentrated and purified by flash chromatography (EtOAc/hexane) to give a product.

4.2.1. (2*R*,4*S*,4a*S*,8a*S*)-(4-Methoxymethoxy-3-methyleneoctahydropyrano[3,2-*b*]pyran-2-yl)acetic acid methyl ester (6a). A colorless oil; $[\alpha]_D^{25}$ +83.2 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 1737, 1439, 1103, 1039 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.45 (1H, dddd, J=12.2, 12.2, 12.2, 5.4 Hz), 1.65–1.80 (2H, m), 2.06 (1H, m), 2.71 (1H, dd, J=15.1, 8.3 Hz), 2.78 (1H, dd, J=15.1, 5.4 Hz), 2.95 (1H, t, J=9.3 Hz), 3.30 (1H, ddd, J=11.2, 9.3, 4.4 Hz), 3.36 (1H, ddd, J=11.2, 11.2, 3.4 Hz), 3.43 (3H, s), 3.71 (3H, s), 3.95 (1H, m), 4.16 (1H, br d, J=9.3 Hz), 4.29 (1H, dd, J=8.3, 5.4 Hz), 4.74 and 4.88 (each 1H, d, J=6.8 Hz), 4.96 (1H, s,), 5.32 (1H, d, J=2.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.2, 29.3, 36.9, 51.9, 55.8, 67.7, 74.2, 76.0, 77.3, 84.1, 96.4, 107.4, 144.4, 171.4; HREIMS *m*/*z* calcd for $C_{14}H_{22}O_6$ 286.1415, found 286.1453.

4.2.2. (2*R*,4*S*,4a*S*,8a*S*)-[4-(*p*-Methoxybenzyloxy)-3-methyleneoctahydropyrano[3,2-*b*]pyran-2-yl]acetic acid methyl ester (6b). A colorless oil; $[\alpha]_D^{25}$ +34.6 (*c* 0.82, CH_3Cl); IR ($CHCl_3$) 1735, 1613, 1514, 1439, 1248, 1102 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.45 (1H, dddd, J=12.2, 12.2, 12.2, 5.4 Hz), 1.66–1.79 (2H, m), 2.05 (1H, m), 2.71 (1H, dd, J=15.6, 8.3 Hz), 2.77 (1H, dd, J=15.6, 5.4 Hz), 3.03 (1H, t, J=9.3 Hz), 3.28 (1H, ddd, J=11.2, 9.3, 4.4 Hz), 3.38 (1H, ddd, J=11.7, 11.7, 3.4 Hz), 3.71 (3H, s), 3.80 (3H, s), 3.93–4.01 (2H, m), 4.22 (1H, dd, J=8.3, 5.4 Hz), 4.65 and 4.71 (each 1H, d, J=11.7 Hz), 4.95 (1H, s), 5.38 (1H, d, J=2.0 Hz), 6.87 and 7.32 (each 2H, d, J=8.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.3, 29.3, 36.9, 51.8, 55.2, 67.7, 73.2, 74.2, 76.0, 80.3, 84.6, 107.7, 113.7 (2×C), 129.2 (2×C), 130.6, 144.3, 159.1, 171.5; HREIMS *m*/*z* calcd for $C_{20}H_{26}O_6$ 362.1727, found 362.1768.

4.2.3. (2*R*,4*R*,4a*R*,8a*S*)-(4-Hydroxy-3-methyleneoctahydropyrano[3,2-*b*]pyran-2-yl)acetic acid methyl ester (8a). A colorless oil; $[\alpha]_D^{25}$ +75.1 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3567, 1738, 1438, 1099, 960 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.43 (1H, dddd, J=12.2, 12.2, 12.2, 5.4 Hz), 1.65–1.78 (2H, m), 2.09 (1H, m), 2.42 (1H, s, OH), 2.63 (1H, dd, J=15.1, 8.8 Hz), 2.73 (1H, dd, J=15.1, 4.9 Hz), 3.06 (1H, dd, J=9.3, 2.9 Hz), 3.46 (1H, m), 3.71 (3H, s), 3.74 (1H, ddd, J=11.2, 9.3, 4.4 Hz), 3.92 (1H, m), 4.41 (1H, d, J=2.9 Hz), 4.71 (1H, dd, J=8.8, 4.9 Hz), 4.91 (1H, d, J=1.5 Hz), 5.16 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.3, 29.2, 36.8, 51.8, 68.1, 70.8, 72.5, 81.0, 113.1, 145.3, 171.4, 187.0; HREIMS *m*/*z* calcd for $C_{12}H_{18}O_5$ 242.1153, found 242.1178.

4.2.4. (2*R*,4*R*,4a*S*,8a*S*)-{4-(*p*-Methoxybenzyloxy)-3-methyleneoctahydropyrano[3,2-*b*]pyran-2-yl}acetic acid methyl ester (8b). A colorless oil; $[\alpha]_D^{25}$ +40.8 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 1736, 1612, 1514, 1439, 1247, 1099 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.37 (1H, dddd, J=12.7, 12.2, 12.2, 4.4 Hz), 1.64 (1H, m), 1.79 (1H, m), 2.08 (1H, m), 2.67 (1H, dd, J=15.6, 8.8 Hz), 2.72 (1H, dd, J=15.6, 3.4 Hz), 3.06 (1H, dd, J=9.3, 3.4 Hz), 3.37 (1H, ddd, J=11.7, 11.7, 2.4 Hz), 3.72 (3H, s), 3.80 (3H, s), 3.87 (1H, ddd, J=11.2, 9.3, 4.4 Hz), 4.01 (1H, m), 4.14 (1H, d, J=2.9), 4.37 and 4.59 (each 1H, d, J=12.2 Hz), 4.62 (1H, t, J=6.8 Hz), 5.03 (1H, d, J=2.0 Hz), 5.21 (1H, s), 6.86 and 7.27 (each 2H, d, J=8.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.0, 29.7, 36.6, 51.8, 55.2, 68.4, 69.0, 70.5, 71.2, 78.2, 81.5, 113.1, 113.7 (2×C), 129.3 (2×C), 130.1, 143.8, 159.0, 171.4; HREIMS *m*/*z* calcd for $C_{20}H_{26}O_6$ 362.1727, found 362.1751.

4.2.5. (4a*R*,6*R*,7*R*,8a*S*)-2,2-Di(*tert*-butyl)-7-(triethylsilyloxy)hexahydro-1,3,5-trioxa-2-silanaphthalene-6-carbaldehyde (10). (*i*) *Triethylsilylation*. To a stirred solution of alcohol **9** (1.95 g, 4.79 mmol) and 2,6-lutidine (1.4 mL, 12.0 mmol) in CH_2Cl_2 (32 mL) was added TESOTf (1.30 mL, 5.75 mmol) at 0 °C, and the reaction mixture was stirred for 1 h. The reaction was quenched with saturated aqueous $NaHCO_3$ and the mixture was extracted with EtOAc. The extract was washed with water and brine, dried, and concentrated. Purification by flash chromatography (5% EtOAc in hexane) gave triethylsilyl ether (2.35 g, 94%) as a colorless oil; $[\alpha]_D^{25}$ −31.1 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 1473, 1365, 1091, 826 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.56 (6H, m), 0.91 (9H, t, J=6.7 Hz,), 0.98 (9H, s), 1.04 (9H, s), 1.53 (1H, q, J=11.2 Hz,), 2.38 (1H, ddd, J=11.2, 4.4, 4.4 Hz), 3.31 (1H, ddd, J=10.2, 10.2, 4.9 Hz), 3.35 (1H, ddd, J=9.3, 5.9, 2.0 Hz), 3.51 (1H, dd, J=10.3, 5.9 Hz), 3.61 (1H, ddd, J=11.2, 9.3, 4.9 Hz), 3.70 (1H, dd, J=10.3, 2.0 Hz), 3.75 (1H, ddd, J=11.2, 8.8, 4.4 Hz), 3.83 (1H, t, J=10.2 Hz), 4.19 (1H, dd, J=10.2, 4.9 Hz), 4.51 and 4.61 (each 1H, d, J=12.2 Hz), 7.23–7.34 (5H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.9 (3×C), 6.8 (3×C), 19.9, 22.6, 27.0 (3×C), 27.4

(3×C), 42.5, 66.3, 66.9, 69.5, 72.0, 73.4, 77.1, 82.0, 127.5, 127.8 (2×C), 128.3 (2×C), 138.1; HREIMS *m/z* calcd for $C_{28}H_{50}O_5Si_2$ 522.3197, found 522.3210.

(*ii*) *Hydrogenolysis*. A mixture of the product (2.35 g, 4.51 mmol) and $Pd(OH)_2$/C (259 mg) in EtOAc (30 mL) was stirred under a hydrogen atmosphere for 1 h. The reaction mixture was filtered through a short column of Celite and the filtrate was concentrated. Purification by flash chromatography (10–30% EtOAc in hexane) gave the alcohol (1.77 g, 91%) as a colorless oil; $[\alpha]_D^{25}$ −45.9 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3596, 1473, 1098 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.63 (6H, q, J=7.8 Hz), 0.97 (9H, t, J=7.8 Hz), 0.99 (9H, s), 1.04 (9H, s), 1.56 (1H, q, J=11.7 Hz), 1.91 (1H, t, J=6.3 Hz, OH), 2.39 (1H, ddd, J=12.2, 4.4, 4.4 Hz), 3.25 (1H, ddd, J=8.8, 5.4, 2.9 Hz), 3.33 (1H, ddd, J=10.3, 9.3, 4.9 Hz,), 3.58–3.67 (2H, m), 3.73 (1H, ddd, J=11.2, 9.3, 4.4 Hz), 3.78 (1H, t, J=10.3 Hz), 3.83 (1H, m), 4.14 (1H, dd, J=10.2, 4.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.9 (3×C), 6.8 (3×C), 19.9, 22.6, 27.0 (3×C), 27.4 (3×C), 42.3, 62.4, 66.4, 66.8, 72.2, 76.9, 82.1; HREIMS *m/z* calcd for $C_{21}H_{44}O_5Si_2$ 432.2727, found 432.2656.

(*iii*) *Oxidation*. To a solution of the alcohol (1.94 g, 4.49 mmol) in CH_2Cl_2 (45 mL) was added Dess–Martin periodinane (2.10 g, 4.94 mmol) at 0 °C, and the reaction mixture was stirred at rt for 1.5 h. The reaction was quenched with saturated aqueous $Na_2S_2O_3$ and the mixture was extracted with CH_2Cl_2. The extract was washed with saturated aqueous $NaHCO_3$, water, and brine, dried, and concentrated. Purification by flash chromatography (30% EtOAc in hexane) gave aldehyde **10** (1.76 g, 91%) as a colorless oil; $[\alpha]_D^{25}$ −42.5° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 1737, 1473, 1100 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.62 (6H, q, J=7.8 Hz), 0.96 (9H, t, J=7.8 Hz), 0.99 (9H, s), 1.04 (9H, s), 1.65 (1H, q, J=11.7 Hz), 2.47 (1H, ddd, J=12.2, 4.4, 4.4 Hz), 3.33 (1H, ddd, J=10.3, 10.3, 4.4 Hz), 3.77 (1H, dd, J=9.3, 1.0 Hz,), 3.78–3.87 (2H, m), 3.88 (1H, t, J=10.3 Hz), 4.19 (1H, dd, J=10.3, 4.4 Hz), 9.71 (1H, d, J=1.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.9 (3×C), 6.7 (3×C), 19.9, 22.6, 27.0 (3×C), 27.4 (3×C), 42.9, 66.5, 66.9, 71.4, 76.6, 84.8, 198.1; HREIMS *m/z* calcd for $C_{21}H_{42}O_5Si_2$ 430.2568, found 362.2561.

4.2.6. (1*S*)-1-[(4a*R*,6*S*,7*R*,8a*S*)-2,2-Di(*tert*-butyl)-7-(triethylsilyloxy)hexahydro-1,3,5-trioxa-2-silanaphthalen-6-yl]prop-2-yn-1-ol (11) and (1*R*)-1-[(4a*R*,6*S*,7*R*,8a*S*)-2,2-Di(*tert*-butyl)-7-(triethylsilyloxy)hexahydro-1,3,5-trioxa-2-silanaphthalen-6-yl]prop-2-yn-1-ol (12). The procedure used for the preparation of **1** was employed. An experiment starting with aldehyde **10** (988 mg, 2.29 mmol) provided the crude products, which were purified by flash chromatography (15–30% EtOAc in hexane). The first eluate gave alcohol **12** (454 mg, 44%) and the second eluate gave alcohol **11** (552 mg, 53%). Compound **11**: colorless oil; $[\alpha]_D^{25}$ −53.1 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3567, 3307, 1473, 1098, 1067, 1006 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.64 (6H, q, J=8.3 Hz), 0.97 (9H, t, J=8.3 Hz), 0.99 (9H, s), 1.05 (9H, s), 1.57 (1H, q, J=11.7 Hz,), 2.41 (1H, ddd, J=11.7, 4.4, 4.4 Hz), 2.45 (1H, d, J=2.4 Hz), 2.65 (1H, d, J=10.2 Hz, OH), 3.33 (1H, ddd, J=10.2, 10.2, 4.9 Hz), 3.36 (1H, dd, J=9.3, 3.4 Hz), 3.73–3.81 (2H, m), 3.85 (1H, t, J=10.2 Hz), 4.17 (1H, dd, J=10.2, 4.9 Hz), 4.63 (1H, ddd, J=10.2, 2.4, 2.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 5.0 (3×C), 6.8 (3×C), 20.0, 22.6, 27.0 (3×C), 27.4 (3×C), 42.2, 42.9, 62.6, 66.6, 67.3, 71.9, 74.5, 77.1, 83.3; HREIMS *m/z* calcd for $C_{23}H_{44}O_5Si_2$ 456.2727, found 456.2749. Compound **12**: colorless oil; $[\alpha]_D^{25}$ −42.3 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3570, 3307, 1473, 1095, 1063 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.66 (6H, q, J=8.3 Hz), 0.98 (9H, t, J=8.3 Hz), 0.99 (9H, s), 1.05 (9H, s), 1.57 (1H, q, J=11.2 Hz), 2.43 (1H, d, J=2.0 Hz), 2.44 (1H, m), 2.58 (1H, d, J=10.7 Hz, OH), 3.28 (1H, dd, J=9.3, 2.0 Hz), 3.39 (1H, ddd, J=9.3, 9.3, 4.9 Hz), 3.74 (1H, ddd, J=11.2, 9.3, 4.4 Hz), 3.81 (1H, t, J=10.2 Hz), 3.89 (1H, ddd, J=11.2, 6.3, 4.9 Hz), 4.18 (1H, dd, J=10.2, 4.9 Hz), 4.56 (1H, ddd, J=10.7, 2.0, 2.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 5.0 (3×C), 6.7 (3×C), 19.9, 22.6, 27.0 (3×C), 27.4 (3×C), 42.0, 61.1, 66.0, 66.7, 71.9, 72.9, 77.3, 82.8, 83.1; HREIMS *m/z* calcd for $C_{23}H_{44}O_5Si_2$ 456.2727, found 456.2758.

4.2.7. (*E*)-(4a*R*,6*R*,7*R*,8a*S*)-3-{6-[(1*S*)-1-(Acetoxy)propynyl]-2,2-di(*tert*-butyl)hexahydro-1,3,5-trioxa-2-silanaphthalen-7-yloxy}acrylic acid methyl ester (13a). (*i*) *Acetylation*. To a stirred solution of alcohol **11** (294 mg, 0.645 mmol) in CH_2Cl_2 (3.0 mL) were added pyridine (0.2 mL) and acetic anhydride (0.2 mL), and the reaction mixture was stirred at rt for 3 h. The solvent was evaporated azeotropically with heptane three times. The residue was purified by flash chromatography (7% EtOAc in hexane) to give acetate (274 mg, 85%) as a colorless oil; $[\alpha]_D^{25}$ −21.4 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3307, 1743, 1473, 1366, 1237, 1099, 1070 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.66 (6H, q, J=7.8 Hz), 0.99 (9H, t, J=7.8 Hz), 0.99 (9H, s), 1.05 (9H, s), 1.57 (1H, q, J=11.7 Hz), 2.13 (3H, s), 2.43 (1H, ddd, J=11.7, 4.4, 4.4 Hz), 2.47 (1H, d, J=2.4 Hz), 3.33 (1H, ddd, J=10.2, 10.2, 4.9 Hz), 3.39 (1H, dd, J=9.3, 2.4 Hz), 3.73–3.81 (2H, m), 3.86 (1H, t, J=10.2 Hz), 4.22 (1H, dd, J=10.2, 4.9 Hz), 5.77 (1H, t, J=2.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 5.0 (3×C), 6.8 (3×C), 19.9, 21.0, 22.6, 27.1 (3×C), 27.4 (3×C), 42.2, 60.1, 63.6, 66.6, 66.7, 71.8, 75.4, 77.1, 82.2, 169.6; EIMS *m/z* 498 (M^+).

(*ii*) *Detriethylsilylation*. A solution of the acetate (281 mg, 0.565 mmol) in THF (3.0 mL) and 80% acetic acid (7.5 mL) was stirred for 5.5 h. The solution was neutralized with 15% NH_4OH to pH 8.0 and extracted with EtOAc. The extract was washed with water and brine, dried, and concentrated. Purification by flash chromatography (30% EtOAc in hexane) gave the alcohol (216 mg, 100%) as a colorless oil; $[\alpha]_D^{25}$ +2.4 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3601, 3306, 1741, 1473, 1366, 1235, 1104 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.99 (9H, s), 1.05 (9H, s), 1.56 (1H, q, J=11.4 Hz), 1.87 (1H, d, J=5.5 Hz, OH), 2.14 (3H, s), 2.51 (1H, d, J=2.2 Hz), 2.53 (1H, ddd, J=11.4, 4.8, 4.8 Hz), 3.34 (1H, ddd, J=10.3, 9.2, 4.8 Hz), 3.44 (1H, dd, J=9.5, 2.6 Hz), 3.80 (1H, ddd, J=11.4, 9.2, 4.4 Hz), 3.83 (1H, m), 3.85 (1H, t, J=10.3 Hz), 4.20 (1H, dd, J=10.3, 4.8 Hz), 5.79 (1H, t, J=2.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.9, 20.9, 22.6, 27.0 (3×C), 27.4 (3×C), 41.6, 63.7, 66.1, 66.6, 71.7, 75.8, 77.1, 77.2, 81.6, 169.8; EIMS *m/z* 384 (M^+).

(*iii*) *Reaction with methyl propiolate*. The procedure used for the preparation of **3a** was employed. An experiment

starting with the above alcohol (216 mg, 0.563 mmol) provided acetate (237 mg, 90%), which was assigned to be a 78:22 mixture of **13a** and its regioisomer by ^{1}H NMR analysis; IR ($CHCl_3$) 3306, 1741, 1706, 1645, 1235, 1104 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) (signals of the major isomer **13a**) δ 0.98 (9H, s), 1.05 (9H, s), 1.63 (1H, q, J=11.4 Hz), 2.14 (3H, s), 2.50 (1H, d, J=1.8 Hz), 2.68 (1H, ddd, J=12.1, 4.8, 4.8 Hz), 3.38 (1H, ddd, J=9.9, 9.9, 4.1 Hz), 3.65 (1H, dd, J=9.5, 2.2 Hz), 3.71 (3H, s), 3.83 (1H, m), 3.86 (1H, t, J=10.2 Hz), 4.10 (1H, ddd, J=11.4, 9.5, 4.8 Hz), 4.23 (1H, dd, J=10.2, 5.1 Hz), 5.37 and 7.48 (each 1H, d, J=12.5 Hz, CH=CH), 5.63 (1H, t, J=2.2 Hz); HREIMS m/z calcd for $C_{23}H_{36}O_8Si$ 468.2179, found 456.2184.

4.2.8. (*E*)-(4a*R*,6*R*,7*R*,8a*S*)-3-{2,2-Di(*tert*-butyl)-6-[(1*S*)-1-(*p*-methoxybenzyloxy)-2-propynyl]hexahydro-1,3,5-trioxa-2-silanaphthalen-7-yloxy}acrylic acid methyl ester (13b). The procedures of **3b** (i) and **13a** (ii and iii) were employed. An experiment starting with alcohol **11** (18 mg, 0.039 mmol) provided **13b** (6.7 mg, 31%) as a colorless oil; $[\alpha]_D^{25}$ −60.6 (c 0.55, $CHCl_3$); IR ($CHCl_3$) 3307, 1705, 1644, 1622, 1516, 1235, 1100 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.97 (9H, s), 1.04 (9H, s), 1.61 (1H, q, J=11.7 Hz), 2.53 (1H, d, J=2.0 Hz), 2.62 (1H, ddd, J=11.7, 4.4, 4.4 Hz), 3.36 (1H, ddd, J=10.3, 10.3, 4.9 Hz), 3.62 (1H, dd, J=9.8, 2.0 Hz), 3.70 and 3.81 (each 3H, s), 3.80 (1H, m), 3.86 (1H, t, J=10.3 Hz), 4.09 (1H, ddd, J=11.2, 9.8, 4.9 Hz), 4.23 (1H, dd, J=10.3, 4.9 Hz), 4.30 (1H, t, J=2.0 Hz), 4.47 and 4.80 (each 1H, d, J=11.7 Hz), 5.27 and 7.42 (each 1H, d, J=12.2 Hz), 6.87 and 7.27 (each 2H, d, J=9.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.9, 22.6, 27.0 (3×C), 27.4 (3×C), 38.2, 51.2, 55.2, 66.6, 67.3, 70.8, 71.4, 75.7, 76.6, 77.2, 78.1, 80.5, 98.5, 113.8 (2×C), 129.0, 129.9 (2×C), 159.6, 160.7, 178.1; HREIMS m/z calcd for $C_{29}H_{42}O_8Si$ 546.2646, found 546.2684.

4.2.9. (*E*)-(4a*R*,6*R*,7*R*,8a*S*)-3-{6-[(1*R*)-1-(Acetoxy)prop-2-ynyl]-2,2-di(*tert*-butyl)hexahydro-1,3,5-trioxa-2-silanaphthalen-7-yloxy}acrylic acid methyl ester (14). The procedure used for the preparation of **13a** was employed. An experiment starting with alcohol **12** (106 mg, 0.232 mmol) provided acetate **14** (92 mg, 85% as a 9:1 mixture of regioisomers) as a colorless oil; $[\alpha]_D^{25}$ −49.8 (c 1.0, $CHCl_3$); IR ($CHCl_3$) 3307, 1745, 1707, 1645, 1625, 1220, 1137, 1105 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (9H, s), 1.05 (9H, s), 1.63 (1H, q, J=11.7 Hz), 2.10 (3H, s), 2.50 (1H, d, J=2.4 Hz), 2.66 (1H, ddd, J=11.7, 4.9, 4.9 Hz), 3.39 (1H, ddd, J=10.2, 9.8, 4.9 Hz), 3.61 (1H, dd, J=9.3, 2.4 Hz), 3.70 (3H, s), 3.84 (1H, ddd, J=11.7, 9.3, 4.4 Hz), 3.90 (1H, t, J=10.2 Hz), 4.05 (1H, ddd, J=11.7, 9.8, 4.9 Hz), 4.21 (1H, dd, J=10.2, 4.9 Hz), 5.31 and 7.43 (each 1H, d, J=12.2 Hz), 5.58 (1H, t, J=2.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.9, 20.7, 22.6, 27.0 (3×C), 27.4 (3×C), 38.0, 51.2, 62.0, 66.4, 71.4, 74.5, 75.2, 77.7, 77.8, 79.0, 98.5, 160.3, 167.7, 169.3; HREIMS m/z calcd for $C_{23}H_{36}O_8Si$ 468.2179, found 468.2202.

4.2.10. (4a*R*,5*S*,7*S*,8a*R*,9a*S*,10a*R*)-[5-Acetoxy-2,2-di(*tert*-butyl)-6-methylenetetradecahydro-1,3,8,10-tetraoxa-2-silaanthracen-7-yl]acetic acid methyl ester (15a). According to the general procedure, a 78:22 mixture of **13a** and its regioisomer (257 mg, 0.550 mmol) was subjected to the radical cyclization and destannylation to give **15a** (155 mg, 61% as a 92:8 mixture of diastereoisomers, 78% corrected yield based on pure **13a**) as a colorless oil; $[\alpha]_D^{25}$ −27.5 (c 0.34, $CHCl_3$); IR ($CHCl_3$) 1741, 1473, 1439, 1371, 1236, 1107, 1047 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96 (9H, s), 1.04 (9H, s), 1.49 (1H, q, J=11.7 Hz), 2.08 (3H, s), 2.47 (1H, ddd, J=11.7, 4.4, 4.4 Hz), 2.61 (1H, dd, J=15.1, 9.3 Hz), 2.70 (1H, dd, J=15.1, 4.4 Hz), 3.22 (1H, dd, J=9.8, 3.4 Hz), 3.33 (1H, ddd, J=10.3, 10.3, 4.9 Hz), 3.72 (3H, s), 3.79 (1H, t, J=10.3 Hz), 3.80 (2H, m), 4.11 (1H, dd, J=10.3, 4.9 Hz), 4.54 (1H, dd, J=8.8, 4.9 Hz), 5.03 (1H, d, J=1.5 Hz), 5.29 (1H, s), 5.63 (1H, d, J=3.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.9, 21.3, 22.6, 27.0 (3×C), 27.4 (3×C), 36.7, 38.5, 51.9, 66.7, 67.0, 71.3, 72.2, 72.6, 77.8, 79.1, 115.3, 141.7, 169.9, 171.4; HREIMS m/z calcd for $C_{23}H_{38}O_8Si$ 470.2335, found 470.2312.

4.2.11. (4a*R*,5*S*,7*S*,8a*R*,9a*S*,10a*R*)-[2,2-Di(*tert*-butyl)-5-(*p*-methoxybenzyloxy)-6-methylenetetradecahydro-1,3,8,10-tetraoxa-2-silaanthracen-7-yl]acetic acid methyl ester (15b). According to the general procedure, **13b** (6.7 mg, 0.012 mmol) was subjected to the radical cyclization and destannylation to give **15b** (5.6 mg, 82% as a 96:4 mixture of diastereoisomers) as a colorless oil; $[\alpha]_D^{25}$ −30.6 (c 1.0, $CHCl_3$); IR ($CHCl_3$) 1738, 1513, 1102, 1054 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96 (9H, s), 1.03 (9H, s), 1.46 (1H, q, J=11.7 Hz), 2.45 (1H, ddd, J=11.7, 4.4, 4.4 Hz), 2.66 (1H, dd, J=15.1, 5.8 Hz), 2.72 (1H, dd, J=15.1, 5.8 Hz), 3.17 (1H, dd, J=9.8, 2.9 Hz), 3.31 (1H, ddd, J=9.8, 9.8, 4.4 Hz), 3.71 (3H, s), 3.84 (3H, s), 3.72–3.95 (2H, m), 3.92 (1H, t, J=10.3 Hz), 4.14 (1H, dd, J=10.3, 4.9 Hz), 4.15 (1H, d, J=2.9 Hz), 4.34 and 4.56 (each 1H, d, J=12.2 Hz), 4.60 (1H, br t, J=7.3 Hz), 5.00 (1H, s), 5.02 (1H, d, J=1.5 Hz), 6.86 and 7.24 (each 2H, d, J=8.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.9, 22.5, 27.0 (3×C), 27.4 (3×C), 36.6, 38.7, 51.8, 55.2, 66.7, 69.2, 69.6, 70.6, 72.1, 77.9, 78.0, 81.2, 113.3, 113.7 (2×C), 129.2 (2×C), 130.1, 143.5, 159.1, 171.4; HREIMS m/z calcd for $C_{29}H_{44}O_8Si$ 548.2802, found 548.2857.

4.2.12. (4a*R*,5*R*,7*S*,8a*R*,9a*S*,10a*R*)-[5-Acetoxy-2,2-di(*tert*-butyl)-6-methylenetetradecahydro-1,3,8,10-tetraoxa-2-silaanthracen-7-yl]acetic acid methyl ester (16). According to the general procedure, **14** (90 mg, 0.192 mmol, a 9:1 mixture of regioisomers) was subjected to the radical cyclization and destannylation to give **16** (73 mg, 81% as a 93:7 mixture of diastereoisomers) as a colorless oil; $[\alpha]_D^{25}$ −27.5 (c 0.25, CH_3Cl); IR ($CHCl_3$) 1741, 1238, 1104, 1054 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.97 (9H, s), 1.02 (9H, s), 1.55 (1H, q, J=11.7 Hz), 2.17 (3H, s), 2.47 (1H, ddd, J=11.7, 4.4, 4.4 Hz), 2.70 (1H, dd, J=15.1, 9.3 Hz), 2.77 (1H, dd, J=15.1, 4.9 Hz), 3.13 (1H, t, J=9.8 Hz), 3.29 (1H, ddd, J=10.2, 10.2, 4.9 Hz), 3.41 (1H, ddd, J=11.7, 9.3, 4.4 Hz), 3.71 (3H, s), 3.81 (1H, t, J=10.2 Hz), 3.84 (1H, ddd, J=11.7, 10.2, 4.4 Hz), 4.11 (1H, dd, J=10.2, 4.9 Hz), 4.35 (1H, dd, J=9.3, 4.9 Hz), 4.94 (1H, d, J=2.0 Hz), 5.00 (1H, d, J=2.0 Hz), 5.40 (1H, d, J=9.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.9, 20.8, 22.6, 27.0 (3×C), 27.4 (3×C), 36.9, 38.3, 51.9, 66.6, 72.2, 73.3, 74.30, 74.33, 77.4, 81.2, 107.7, 141.9, 169.9, 170.9; HREIMS m/z calcd for $C_{23}H_{38}O_8Si$ 470.2336, found 470.2345.

4.2.13. (*E*,*E*)-(4a*R*,6*R*,7*R*,8a*S*)-3-{2,2-Di(*tert*-butyl)-6-[(1*S*)-1-(2-methoxycarbonylvinyloxy)-2-propynyl]-hexahydro-1,3,5-trioxa-2-silanaphthalen-7-yloxy}acrylic acid methyl ester (17). (*i*) *Detriethylsilylation*. A solution of **11** (240 mg, 0.527 mmol) and *p*-TsOH·H_2O (5.0 mg, 0.026 mmol) in MeOH (5.3 mL) was stirred at rt for 1 h. The reaction was quenched with Et_3N (0.2 mL) and the reaction mixture was concentrated. Purification by flash chromatography (40% EtOAc in hexane) gave diol (169 mg, 94%) as a solid. Mp 190–191 °C; $[\alpha]_D^{25}$ −31.4 (*c* 1.0, CH_3Cl); ^1H NMR (400 MHz, acetone-d_6) δ 1.00 (9H, s), 1.42 (9H, s), 1.56 (1H, q, J=11.7 Hz), 2.44 (1H, ddd, J=11.7, 4.9, 4.9 Hz), 2.80 (1H, d, J=2.4 Hz), 3.33 (1H, dd, J=9.3, 3.4 Hz), 3.35 (1H, ddd, J=10.3, 10.3, 4.9 Hz), 3.70 (1H, m), 3.78 (1H, t, J=10.3 Hz), 3.80 (1H, ddd, J=11.2, 9.3, 4.9 Hz), 4.11 (1H, dd, J=10.3, 4.9 Hz), 4.25 (1H, d, J=5.4 Hz, OH), 4.54 (1H, d, J=8.3 Hz, OH), 4.62 (1H, ddd, J=8.3, 3.4, 2.4 Hz); ^{13}C NMR (100 MHz, acetone-d_6) δ 20.4, 23.1, 27.4 (3×C), 27.7 (3×C), 42.5, 63.3, 67.5, 73.2, 74.7, 77.7, 79.1, 83.1, 84.8; HREIMS *m*/*z* calcd for $C_{17}H_{30}O_5Si$ 342.1863, found 342.1852.

(*ii*) *Reaction with methyl propiolate*. The diol (507 mg, 1.48 mmol) dissolved in THF (10 mL), and treated with methyl propiolate (0.53 mL, 5.94 mmol) and NMM (0.65 mL, 5.94 mmol). The reaction mixture was stirred at rt for 17 h and extracted with EtOAc. The extract was washed with water and brine, dried, and concentrated. Purification by flash chromatography (20% EtOAc in hexane) gave **17** (756 mg, 100%) as a colorless oil; $[\alpha]_D^{25}$ −3.3 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3305, 1710, 1645, 1625, 1438, 1137 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.98 (9H, s), 1.05 (9H, s), 1.65 (1H, q, J=11.7 Hz), 2.66 (1H, d, J=2.4 Hz), 2.69 (1H, ddd, J=11.7, 4.4, 4.4 Hz), 3.39 (1H, ddd, J=10.2, 9.8, 4.9 Hz), 3.71 and 3.72 (each 3H, s), 3.74 (1H, dd, J=9.8, 2.0 Hz), 3.83 (1H, ddd, J=11.2, 9.8, 4.4 Hz), 3.86 (1H, t, J=10.2 Hz), 4.10 (1H, ddd, J=11.2, 9.8, 4.9 Hz), 4.20 (1H, dd, J=10.2, 4.9 Hz), 4.81 (1H, t, J=2.4 Hz), 5.37 and 7.46 (each 1H, d, J=12.7 Hz), 5.41 and 7.61 (each 1H, d, J=12.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.9, 22.6, 26.9 (3×C), 27.4 (3×C), 38.0, 51.2, 51.3, 66.3, 70.1, 71.2, 75.1, 75.3, 77.2, 78.9, 79.5, 99.1, 99.3, 159.8, 159.9, 167.5, 167.6; HREIMS *m*/*z* calcd for $C_{25}H_{38}O_9Si$ 510.2285, found 510.2297.

4.2.14. (*E*)-(4a*R*,5*S*,7*S*,8a*R*,9a*S*,10a*R*)-[2,2-Di(*tert*-butyl)-5-(2-methoxycarbonylvinyloxy)-6-methylenetetradecahydro-1,3,8,10-tetraoxa-2-silaanthracen-7-yl]acetic acid methyl ester (18). According to the general procedure, **17** (756 mg, 1.48 mmol) was subjected to the radical cyclization and destannylation to give **18** (577 mg, 76% as a 94:6 mixture of diastereoisomers) as a colorless oil; $[\alpha]_D^{25}$ −35.7 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 1739, 1707, 1473, 1438, 1139, 1107, 1047 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.97 (9H, s), 1.03 (9H, s), 1.48 (1H, q, J=11.7 Hz), 2.48 (1H, ddd, J=11.7, 4.4, 4.4 Hz), 2.63 (1H, dd, J=15.1, 8.8 Hz), 2.71 (1H, dd, J=15.1, 4.9 Hz), 3.26 (1H, dd, J=9.8, 2.9 Hz), 3.34 (1H, ddd, J=10.2, 10.2, 4.9 Hz), 3.68 and 3.71 (each 3H, s), 3.79–3.86 (2H, m), 3.85 (1H, t, J=10.2 Hz), 4.12 (1H, dd, J=10.2, 4.9 Hz), 4.50 (1H, dd, J=8.8, 4.9 Hz), 4.63 (1H, d, J=2.9 Hz), 5.11 (1H, d, J=1.5 Hz), 5.24 (1H, s), 5.35 and 7.45 (each 1H, d, J=12.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.9, 22.5, 27.0 (3×C), 27.4 (3×C), 36.6, 38.4, 51.1, 51.9, 66.5, 69.4, 70.8, 71.9, 72.9, 77.9, 79.9, 82.3, 98.9, 115.2, 141.9, 160.0, 170.1; HREIMS *m*/*z* calcd for $C_{25}H_{40}O_9Si$ 512.2442, found 512.2425.

4.2.15. (4a*R*,5*S*,7*S*,8a*R*,9a*S*,10a*S*)-[2,2-Di(*tert*-butyl)-5-hydroxy-6-methylenetetradecahydro-1,3,8,10-tetraoxa-2-silaanthracen-7-yl]acetic acid methyl ester (19). A solution of **18** (479 mg, 0.936 mmol, a 94:6 mixture of diastereoisomers), *n*-dodecanethiol (0.90 mL, 3.743 mmol), and TsOH·H_2O (53 mg, 0.281 mmol) in CH_3CN (9.4 mL) was heated at 80 °C for 1.5 h. After cooling to rt, Et_3N (0.2 mL) was added to the solution and the reaction mixture was concentrated. Purification by flash chromatography (30% EtOAc in hexane) gave pure **19** (322 mg, 82%) as a solid. Mp 155–156 °C; $[\alpha]_D^{25}$ −47.5 (*c* 0.28, $CHCl_3$); IR ($CHCl_3$) 3574, 1738, 1473, 1438, 1100, 1048 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.97 (9H, s), 1.04 (9H, s), 1.52 (1H, q, J=11.7 Hz), 2.16 (1H, s, OH), 2.47 (1H, ddd, J=11.7, 4.4, 4.4 Hz), 2.63 (1H, dd, J=15.1, 8.8 Hz), 2.73 (1H, dd, J=15.1, 4.9 Hz), 3.18 (1H, dd, J=9.3, 2.9 Hz), 3.41 (1H, ddd, J=10.3, 10.3, 4.9 Hz), 3.71 (3H, s), 3.82 (2H, ddd, J=9.3, 9.3, 4.4 Hz), 3.85 (1H, t, J=10.3 Hz), 4.14 (1H, dd, J=10.3, 4.9 Hz), 4.46 (1H, d, J=2.9 Hz), 4.68 (1H, dd, J=8.8, 4.9 Hz), 4.98 (1H, d, J=1.5 Hz), 5.17 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.9, 22.6, 27.0 (3×C), 27.4 (3×C), 36.8, 38.3, 51.9, 66.7, 69.0, 70.9, 72.2, 72.4, 77.8, 80.7, 113.7, 144.8, 171.4; HREIMS *m*/*z* calcd for $C_{21}H_{36}O_7$ 428.2230, found 428.2238.

4.2.16. (*E*,*E*)-(4a*R*,6*R*,7*R*,8a*S*)-3-{2,2-Di(*tert*-butyl)-6-[(1*R*)-1-(2-methoxycarbonylvinyloxy)-2-propynyl]-hexahydro-1,3,5-trioxa-2-silanaphthalen-7-yloxy}acrylic acid methyl ester (20). The procedure used for the preparation of **17** was employed. An experiment starting with alcohol **12** (111 mg, 0.243 mmol) provided acetate **20** (123 mg, 97%) as a colorless oil; $[\alpha]_D^{25}$ −97.4 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3305, 1711, 1646, 1626, 1483, 1137 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.98 (9H, s), 1.04 (9H, s), 1.63 (1H, q, J=11.7 Hz, H4ax), 2.65 (1H, d, J=2.4 Hz), 2.68 (1H, ddd, J=11.7, 4.4, 4.4 Hz), 3.39 (1H, ddd, J=10.3, 9.3, 4.9 Hz), 3.60 (1H, dd, J=9.3, 2.0 Hz), 3.69 and 3.70 (each 3H, s), 3.84 (1H, ddd, J=11.2, 9.3, 4.4 Hz), 3.89 (1H, t, J=10.3 Hz), 4.11 (1H, ddd, J=11.2, 9.3, 4.9 Hz), 4.21 (1H, dd, J=10.3, 4.9 Hz), 4.79 (1H, t, J=2.2 Hz), 5.33 and 7.41 (each 1H, d, J=12.2 Hz), 5.41 and 7.57 (each 1H, d, J=12.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.9, 22.6, 27.0 (3×C), 27.4 (3×C), 38.0, 51.2 (2×C), 66.3, 68.4, 71.3, 73.9, 76.5, 77.2, 77.9, 79.9, 99.1, 99.5, 159.5, 160.0, 167.4, 167.5; HREIMS *m*/*z* calcd for $C_{25}H_{38}O_9Si$ 510.2285, found 510.2296.

4.2.17. (*E*)-(4a*R*,5*R*,7*S*,8a*R*,9a*S*,10a*R*)-[2,2-Di(*tert*-butyl)-5-(2-methoxycarbonylvinyloxy)-6-methylenetetradecahydro-1,3,8,10-tetraoxa-2-silaanthracen-7-yl]acetic acid methyl ester (21). According to the general procedure, **20** (121 mg, 0.238 mmol) was subjected to the radical cyclization and destannylation to give **21** (90 mg, 74% as a 94:6 mixture of diastereoisomers) as a colorless oil; $[\alpha]_D^{25}$ −18.1 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 1734, 1706, 1473, 1438, 1139, 1104, 1054 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.96 (9H, s), 1.03 (9H, s), 1.55 (1H, q, J=11.7 Hz), 2.48 (1H, ddd, J=11.7, 4.4, 4.4 Hz), 2.72 (1H, dd, J=15.1, 4.4 Hz), 2.78 (1H, dd, J=15.1, 5.4 Hz), 3.18 (1H,

t, $J=9.3$ Hz), 3.29–3.38 (2H, m), 3.69 and 3.72 (each 3H, s), 3.80 (1H, t, $J=10.2$ Hz), 3.83 (1H, m), 4.15 (1H, dd, $J=10.2$, 5.4 Hz), 4.29–4.35 (2H, m), 5.03 (1H, t, $J=2.0$ Hz), 5.20 (1H, d, $J=2.0$ Hz), 5.37 and 7.53 (each 1H, d, $J=12.2$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.9, 22.6, 27.0 (3×C), 27.4 (3×C), 36.7, 38.1, 51.1, 52.0, 66.5, 72.2, 74.2, 74.3, 77.2, 81.7, 83.3, 98.2, 109.5, 141.3, 162.6, 168.0, 170.1; HREIMS *m/z* calcd for $C_{25}H_{40}O_9Si$ 512.2442, found 512.2473.

Acknowledgements

This work was supported by the Uehara Memorial Foundation and by a Grant-in Aid for Creative Scientific Research (No. 14GS0214) from the Japan Society for the Promotion of Science.

References and notes

1. For reviews, see: (a) Yasumoto, T.; Murata, M. *Chem. Rev.* **1993**, *93*, 1897–1909. (b) Shimizu, Y. *Chem. Rev.* **1993**, *93*, 1685–1698. (c) Scheuer, P. J. *Tetrahedron* **1994**, *50*, 3–18.
2. For reviews on cyclic ether synthesis, see: (a) Mori, Y. *Chem. Eur. J.* **1997**, *3*, 849–852. (b) Marmsater, F. P.; West, F. G. *Chem. Eur. J.* **2002**, *8*, 4347–4353. (c) Fujiwara, K.; Murai, A. *Bull. Chem. Soc. Jpn.* **2004**, *77*, 2129–2146.
3. (a) Evans, P. A.; Roseman, J. D. *Tetrahedron Lett.* **1995**, *36*, 31–34. (b) Evans, P. A.; Roseman, J. D. *J. Org. Chem.* **1996**, *61*, 4880–4881. (c) Evans, P. A.; Raina, S.; Ahsan, K. *Chem. Commun.* **2001**, 2504–2505.
4. (a) Lee, E.; Tae, J. S.; Chong, T. H.; Park, Y. C. *Tetrahedron Lett.* **1994**, *35*, 129–132. (b) Sakamoto, Y.; Matsuo, G.; Matsukura, H.; Nakata, T. *Org. Lett.* **2001**, *3*, 2749–2752. (c) Hori, N.; Matsukura, H.; Nakata, T. *Org. Lett.* **1999**, *1*, 1099–1101. (d) Matsuo, G.; Hori, N.; Nakata, T. *Tetrahedron Lett.* **1999**, *40*, 8859–8862.
5. Leeuwenburgh, M. A.; Litjens, R. E. J. N.; Codeé, J. D. C.; Overkleeft, H. S.; van der Marel, G. A.; van Boom, J. H. *Org. Lett.* **2000**, *2*, 1275–1277.
6. Suzuki, K.; Nakata, T. *Org. Lett.* **2002**, *4*, 2739–2741.
7. (a) Murata, M.; Kumagai, M.; Lee, J. S.; Yasumoto, T. *Tetrahedron Lett.* **1987**, *28*, 5869–5872. (b) Satake, M.; Terasawa, K.; Kadowaki, Y.; Yasumoto, T. *Tetrahedron Lett.* **1996**, *37*, 5955–5958. (c) Takahashi, H.; Kusumi, T.; Kan, Y.; Satake, M.; Yasumoto, T. *Tetrahedron Lett.* **1996**, *37*, 7087–7090.
8. Ciminiello, P.; Fattorusso, E.; Forino, M.; Magno, S.; Poletti, R.; Viviani, R. *Tetrahedron Lett.* **1998**, *39*, 8897–8900.
9. Nagai, H.; Murata, M.; Torigoe, K.; Satake, M.; Yasumoto, T. *J. Org. Chem.* **1992**, *57*, 5448–5453.
10. Curran, D.; Porter, N. A.; Giese, B. *Stereochemistry of Radical Reactions*; VCH: Weinheim, 1996.
11. Nicolaou, K. C.; Hwang, C.-K.; Maron, B. E.; deFrees, S. A.; Couladouros, E. A.; Abe, Y.; Carroll, P. J.; Snyder, J. P. *J. Am. Chem. Soc.* **1990**, *112*, 3040–3054.
12. Mori, Y.; Hayashi, H. *J. Org. Chem.* **2001**, *66*, 8666–8668.
13. (a) Mori, Y.; Yaegashi, K.; Furukawa, H. *Tetrahedron Lett.* **1999**, *40*, 7239–7242. (b) Mori, Y.; Yaegashi, K.; Furukawa, H. *J. Am. Chem. Soc.* **1996**, *118*, 8158–8159.
14. Node, M.; Kumar, K.; Nishide, K.; Ohsugi, S.; Miyamoto, T. *Tetrahedron Lett.* **2001**, *42*, 9207–9210.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron 61 (2005) 8598–8605
Tetrahedron

Michael additions catalyzed by phosphines. An overlooked synthetic method

Carolina Gimbert, Maria Lumbierres, Caroline Marchi,† Marcial Moreno-Mañas,* Rosa M. Sebastián and Adelina Vallribera*

Department of Chemistry, Universitat Autònoma de Barcelona, 08193 Cerdanyola, Barcelona, Spain

Received 25 May 2005; revised 30 June 2005; accepted 5 July 2005

Available online 20 July 2005

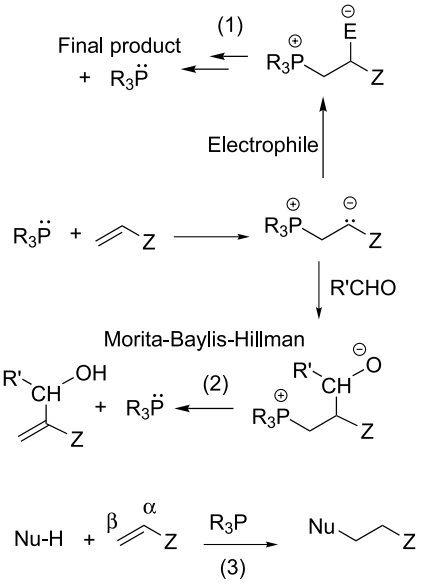
Dedicated to Professor Joaquín Plumet (Univ. Complutense de Madrid) on the occasion of his 60th birthday

Abstract—Triphenylphosphine and tributylphosphine are excellent catalysts for Michael additions. Many β-dicarbonyl compounds and electron-poor olefins, including sterically demanding partners, react successfully. The Michael addition catalyzed by phosphines deserves attention in its own right as a useful synthetic method.

1. Introduction

Organocatalysis induced by phosphines is a topic of increasing interest.[1] The most generally applied phosphine-based catalysis is also known as nucleophilic phosphine catalysis (NPC) and is initiated by the nucleophilic attack of the phosphine to the β-position of an electronically poor alkene or alkyne. The generated α-carbanion reacts as a nucleophile or as a base in many different ways. A common feature is a final step, in which phosphine is recovered thanks to its excellent leaving group properties, thus, permitting a catalytic cycle (Scheme 1, Eq. 1). The Morita–Baylis–Hillman α-hydroxyalkylation of activated olefins is perhaps the best known reaction catalyzed by nucleophilic phosphines (Eq. 2).[1e] Moreover, many different electrophiles have been used in the place of aldehydes. Thus, α,β-unsaturated ketones in intermolecular[2a] and intramolecular[2b,c] manners; π-allylpalladium complexes in intramolecular reaction;[2d] aldehydes[2e] and ketones[2f] in the intramolecular version of the Morita–Baylis–Hillman reaction; and intramolecular attack on vinylsulfones.[2g] A related reaction is the phosphine-catalyzed nucleophilic substitution of acetoxy in allylic systems.[2h] If Z=CO-R in Scheme 1 still another possibility arises: oxygen acting as nucleophile instead of carbon. At least one reaction has been described fulfilling this condition, although, in this case the phosphine was used stoichiometrically.[2i]

The stabilized α-carbanion can also react as a base. This basicity is the origin of the isomerization of acetylenic ketones into dienyl ketones under triphenylphosphine



Scheme 1. Nucleophilic phosphine catalysis (NPC).

Keywords: Organocatalysis; Phosphines; Conjugate addition; β-Dicarbonyl compounds.
* Corresponding authors. Tel./fax: +34 935811254 (M.M.-M.); tel.: +34 935813045; fax: +34 935811265 (A.V.);
e-mail addresses: marcial.moreno@uab.es; adelina.vallribera@uab.es
† Present address: Université Paul Cézanne, 13397 Marseille Cedex 20, France.

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.07.005

catalysis, pioneered by Trost[3a] and reinvestigated by Lu.[3b] Moreover, basicity at C-2 or C-α has been fundamental for the, in principle, unexpected introduction of nucleophiles at C-α,[4] at C-γ,[5] or at both[6] in activated acetylenes and allenes.

This wealth of data is in sharp contrast with the attention paid to a conceptually more simple reaction, the conjugate addition to the β-position of activated alkenes or alkynes (Eq. 3). Thus, activated monosubstituted acetylenes (Z= COOR, COMe) react with thiols,[7] with alcohols,[8] and with oximes[9] to afford the products on conjugate addition at C-β. Heavily fluorinated phosphines were tested for addition of alcohols to acetylenes with the aim of recovering the catalyst in fluorous solvents.[8b,c]

Some other reactions have been recently described: the conjugate addition of water and alcohols to activated olefins under phosphine catalysis,[10] the additions of oximes to simple activated monosubstituted olefins catalyzed by triphenylphosphine,[11] and the aza-Michael addition of carbamates to unsaturated ketones, albeit in this case the presence of trimethylsilyl chloride, together with the phosphine, was essential for the outcome of the reaction.[12]

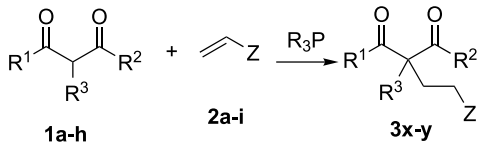
The use of phosphines as catalysts for the conventional Michael addition of compounds with active methylene groups has not been universally recognized as an interesting synthetic method. However, White and Baizer described, in a 1973 note, the reactions of sterically non-demanding nucleophiles (nitromethane, methyl malonate, acetylacetone) to simple activated olefins catalyzed by tertiary phosphines.[13] In 1982, Yoshida and Saito described the examination of the effect of several catalysts in the Michael addition of methyl (phenylsulfinyl)acetate to 1-octen-3-one.[14] Among the tested catalysts, tributylphosphine as well as triphenylphosphine gave good results.

Since then, it seems that phosphines have been forgotten in as far as Michael additions are concerned. This situation is in sharp contrast with the attention paid to transition metals and lanthanides species as catalysts for the same type of reaction[15] in spite of the inherent advantages of phosphines over metals (vide infra).

A few years ago, when examining ruthenium(II) species as possible catalysts for the Michael reaction, we rediscovered that triphenylphosphine, used as a metal stabilizing agent, was active enough to deserve attention in its own right.[16] We now want to describe a full study on the use of tertiary phosphines as excellent catalysts in the Michael addition.

2. Results and discussion

We have tested two phosphines, triphenylphosphine and tributylphosphine, in the Michael additions of Scheme 2. Both phosphines have nucleophilicity parameters *N* differing by slightly more than one unit: Ph_3P (14.33) and Bu_3P (15.49).[17] However, this apparently minimal difference is not trivial since the scale is logarithmic. Nucleophiles **1** were chosen as to embrace a broad diversity of structural types: diesters, ketoesters, diketones, ketoamides, substituted and not substituted at the intercarbonylic position, as



Scheme 2. Michael addition catalyzed by phosphines.

well as cyclic and open-chain compounds. Similar ideas decided the selection of electrophiles **2**: olefins activated by ketone, ester, nitrile, pyridine, phosphonate, as well as disubstituted olefins, and two azodicarboxylates. We considered a priori that if phosphines were successful in reactions combining both selections, they should be considered as general catalysts in their own right.

Indeed, this was the result (Table 1). Some general trends emerge from a perusal of the Table.

First, tributylphosphine is more active than triphenylphosphine as evidenced in entries 2 and 3 as well as in entries 8 and 9. The reactions of entries 2 and 3 failed in the presence of triphenylphosphine, whereas reaction 8 catalyzed with triphenylphosphine and gave total polymerization of the acrylate **2c**. On the contrary, tributylphosphine gave a practically quantitative yield of **3bc** (entry 9).

Second, in some cases the product was unstable, and reverted back to the starting materials on purification (entries 1, 6, and 11). Obviously, this does not imply a violation of the principle of microscopic reversibility, since some compounds **3** decompose in the absence of catalysts by a non-catalyzed process probably involving a six-membered cyclic transition state, whereas Michael addition is initiated by nucleophilic addition of the phosphine to the olefin (Scheme 3). However, the formation of unstable products **3** proves that the method works well even for intrinsically unstable Michael adducts.

Third, the reaction is quite general as is evidenced by the more than 20 different combinations of Table 1. This is in sharp contrast with the limited performances of transition metals and lanthanides in the same type of reactions.[15] In our opinion, phosphines are a better choice than metals.

Whereas some results with metals are remarkable,[15] our attempts to induce enantioselectivity failed. Thus, reaction of 1-adamantyl ester of 2-oxocyclopentanecarboxylic acid with 2-butenone catalyzed by (*R*)-Tol-BINAP gave the Michael adduct in 82% yield and 0% ee. Although negative, this result reinforces our mechanistic hypothesis since according to it (vide infra), the phosphine does not act in the key step when formation of the new chiral center occurs. Catalysis by metals can be a better alternative for induction of enantioselectivity.

Better results were produced when inducing diastereoselectivity. Thus, diastereomeric excesses (de) were sometimes moderate (entries 18, 19, and 21–23), but for product **3gb**, for which an interesting de of 86% was determined (entry 20).

Mechanistically, the phosphine-catalyzed Michael addition probably requires initiation and propagation steps

Table 1. Preparation of products **3** (Scheme 2)

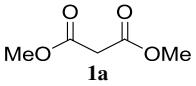
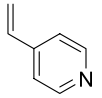
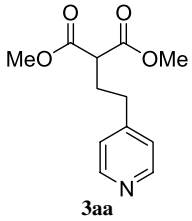
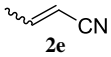
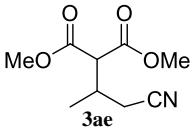
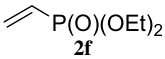
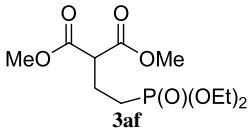
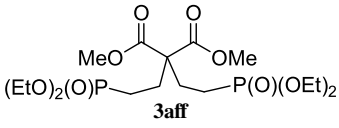
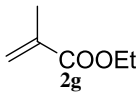
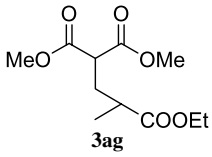
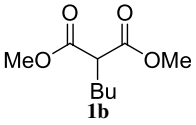
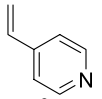
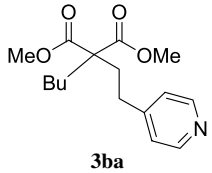
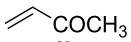
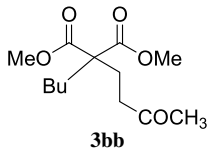
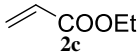
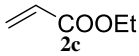
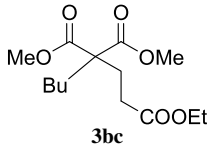
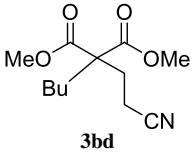
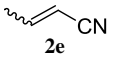
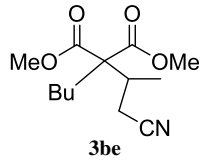
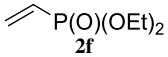
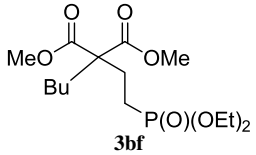
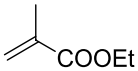
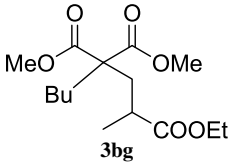
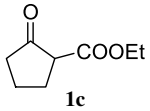
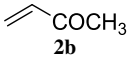
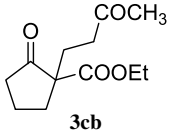
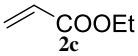
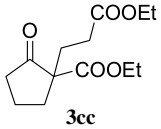
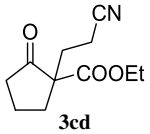
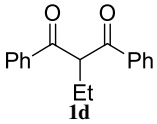
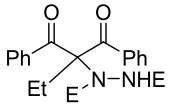
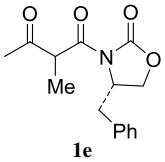
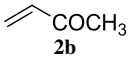
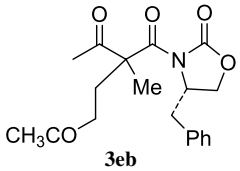
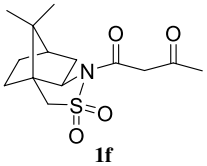
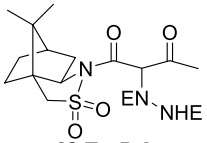
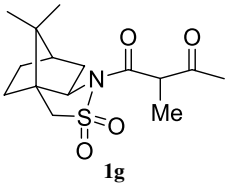
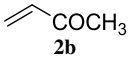
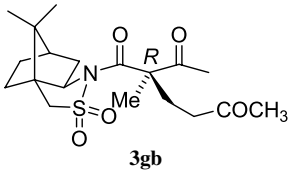
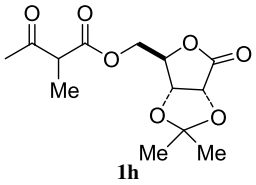
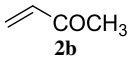
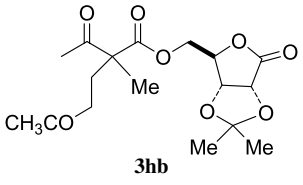
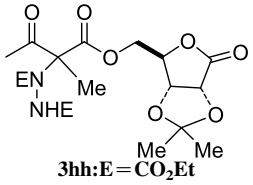
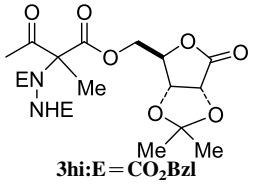
Entry	Nucleophile	Electrophile	R_3P	Product
1	 **1a**	 **2a**	Ph_3P	 **3aa**
2	**1a**	 **2e**	Bu_3P	 **3ae**
3	**1a**	 **2f**	Bu_3P	 **3af**
4	**1a**	**2f**	Bu_3P	 **3aff**
5	**1a**	 **2g**	Bu_3P	 **3ag**
6	 **1b**	 **2a**	Ph_3P	 **3ba**
7	**1b**	 **2b**	Ph_3P	 **3bb**
8	**1b**	 **2c**	Ph_3P	Polyacrylate
9	**1b**	 **2c**	Bu_3P	 **3bc**
10	**1b**	 **2d**	Ph_3P	 **3bd**

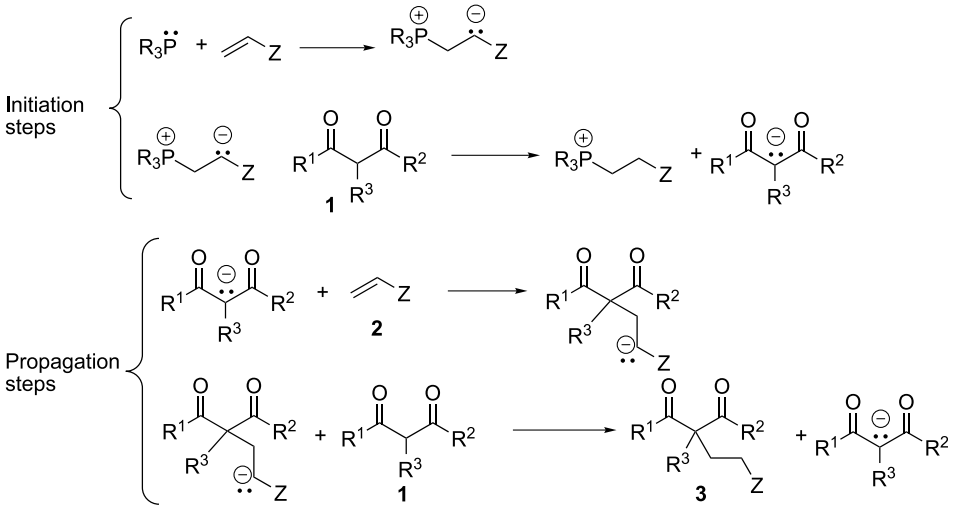
Table 1 (*continued*)

Entry	Nucleophile	Electrophile	R_3P	Product
11	**1b**	 **2e**	Ph_3P	 **3be**
12	**1b**	 **2f**	Bu_3P	 **3bf**
13	**1b**	 **2g**	Bu_3P	 **3bg**
14	 **1c**	 **2b**	Ph_3P	 **3cb**
15	**1c**	 **2c**	Ph_3P	 **3cc**
16	**1c**	 **2d**	Ph_3P	 **3cd**
17	 **1d**	E−N=N−E **2h:E=CO_2Et**	Ph_3P	 **3dh:E=CO_2Et**
18	 **1e**	 **2b**	Ph_3P	 **3eb**
19	 **1f**	E−N=N−E **2i:E=Bzl**	Ph_3P	 **3fi:E=Bzl**
20	 **1g**	 **2b**	Ph_3P	 **3gb**

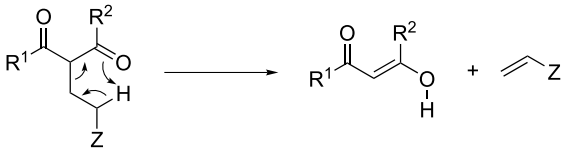
Entry	Nucleophile	Electrophile	R_3P	Product
21	 **1h**	 **2b**	Ph_3P	 **3hb**
22	**1h**	E−N=N−E **2h:E = CO_2Et**	Ph_3P	 **3hh:E = CO_2Et**
23	**1h**	E−N=N−E **2i:E = Bzl**	Ph_3P	 **3hi:E = CO_2Bzl**

(Scheme 3). Initial attack of the phosphine on the olefin generates a phosphonium β-ylid that deprotonates a molecule of the active dicarbonyl compound. The conjugate base of the dicarbonyl triggers the propagation steps as indicated. Another possibility can be envisaged; thus, the β-ylid can be converted into the α-ylid (Wittig reagent, not represented), that can give rise to side reactions. No attempts have been made to obtain more mechanistic information.

Retro-Michael reaction is probably a non-catalyzed process, particularly favorable in some cases as with



Probable mechanism for the catalyzed Michael Addition



Probable mechanism for the non-catalyzed retro-Michael Addition

Scheme 3. Possible mechanisms for the Michael addition catalyzed by phosphines and for the non-catalyzed retro-Michael reaction.

Table 2. Experimental conditions for the preparation of compounds **3** in CH_3CN

Entry	3	Molar ratio 2:1	[1]	R_3P (Molar%)	Temperature °C	Time (h)[a]	Yield%
1	**3aa**	6.0	3.3	Ph_3P (10)	120[b]	44	[c]
2	**3ae**	5.0	2.9	Bu_3P (10)	Room temperature	38	90
3	**3af**	1.1	2.9	Bu_3P (10)	Room temperature	4	60
4	**3aff**	2.0	2.9	Bu_3P (10)	Room temperature	46	78
5	**3ag**	1.1	2.9	Bu_3P (10)	Room temperature	21	73
6	**3ba**	2.2	2.7	Ph_3P (20)	100[b]	300	42[c]
7	**3bb**	4.0	2.5	Ph_3P (18)	Room temperature	24	62
8	**3bc**	1.4	2.7	Ph_3P (10)	100[b]	96	0
9	**3bc**	1.5	2.2	Bu_3P (10)	Room temperature	15.5	99
10	**3bd**	6.3	2.5	Ph_3P (18)	Room temperature	72	78
11	**3be**	5.0	2.2	Ph_3P (10)	Room temperature	165	[c]
12	**3bf**	1.1	2.2	Bu_3P (10)	Room temperature	6.5	82
13	**3bg**	1.3	2.2	Bu_3P (10)	Room temperature	12.5	60
14	**3cb**	3.0	3.8	Ph_3P (10)	Room temperature	5.5	89
15	**3cc**	3.0	4.0	Ph_3P (10)	Reflux	21	89
16	**3cd**	3.0	3.8	Ph_3P (10)	Reflux	5.5	71
17	**3dh**	1.5	2.5	Ph_3P (10)	Reflux	24	100
18	**3eb**	1.5	2.5	Ph_3P (10)	Room temperature	24	57 de 36%
19	**3fi**	1.2	0.6	Ph_3P (18)	Reflux	24	86 de 22%
20	**3gb**	2.4	2.0	Ph_3P (18)	Room temperature	24	63 de 86%[d]
21	**3hb**	2.4	2.0	Ph_3P (9–18)	Room temperature	24	90–100 de 30–42%
22	**3hh**	1.2	2.0	Ph_3P (9)	Room temperature	24	83 de 48%
23	**3hi**	1.2	2.0	Ph_3P (9)	Room temperature	24	65 de 0%

[a] Not optimized.
[b] Closed reactor.
[c] Product **3** reverted to starting materials upon purification.
[d] Diastereoisomer *R* in the new stereocenter was predominant.[16]

4-vinylpyridine, **2a**, and sterically encumbered Michael adducts.

3. Conclusion

Phosphine-catalyzed Michael additions are a useful and general alternative to reactions catalyzed by bases and by metals. The scope is broader than for the metal-catalyzed variant, the yields are generally good, and the process occurs in neutral media.

4. Experimental

4.1. General remarks

Melting points were determined with a Kofler apparatus and are uncorrected. IR Spectra were recorded either by transmission or by attenuated total reflectance mode (ATR). NMR Spectra were recorded with a Brucker AC250 or a Brucker AM400; ^{1}H NMR chemical shifts are reported relative to tetramethylsilane at $\delta=0.00$; coupling constants are reported in Hz. ^{13}C NMR chemical shifts are expressed relative to tetramethylsilane at $\delta=0.0$. Mass spectra (EIMS) were obtained with a Hewlett-Packard 5989A spectrometer and determined at an ionizing voltage of 70 eV; relevant data are listed as *m/z* (%). Elemental analyses were performed at 'Servei d'Anàlisi Química de la Universitat Autònoma de Barcelona'. The given data are the average of two satisfactory determinations.

Compounds **3cb**, **3cc**, and **3cd** were prepared by ourselves under copper catalysis and fully described.[18] Compounds **3bb**, **3bd**, **3dh**, **3eb**, **3fi**, **3gb**, **3hb**, **3hh**, and **3hi** have been previously reported.[16]

4.1.1. Dimethyl butyl-(2-diethoxyphosphoryl)ethylmalonate (3bf, entry 12); general method. A mixture of dimethyl 2-butylmalonate (**1b**) (1.02 g, 5.4 mmol), diethyl vinylphosphonate (**2f**) (0.9 mL, 5.5 mmol), tributylphosphine (140 μL, 0.6 mmol), and anhydrous acetonitrile (2.5 mL) was magnetically stirred in a Schlenk tube under nitrogen atmosphere at room temperature for 6.5 h. The solvent was evaporated and the residue was chromatographed through silica gel with diethyl ether as eluent. Pure **3bf** (187 mg) and **3bf** contaminated with **2f** were isolated. The last fraction was distilled at 80–90 °C/1.1–1.5 mmHg (oven temperature) to afford more pure **3bf** as an oil, 1.5 g, 82%; IR (ATR): 1729, 1260, 1205, 1161, 1058, 1018, 964 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$): $\delta=0.85$ (t, $J=7.2$ Hz, 3H), 1.03–1.31 (m, 4H), 1.28 (t, $J=7.0$ Hz, 6H), 1.53–1.68 (m, 2H), 1.78–1.85 (m, 2H), 2.07–2.15 (m, 2H), 3.68 (s, 6H), 4.00–4.12 (m, 4H); ^{13}C NMR (62.5 MHz, $CDCl_3$): $\delta=14.1$, 16.7 (d, $J=5.7$ Hz), 21.3 (d, $J=142.1$ Hz), 23.1, 26.1 (d, $J=3.8$ Hz), 26.4, 32.8, 52.7, 57.8 (d, $J=18.1$ Hz), 62.0 (d, $J=6.7$ Hz), 171.8; HRMS: *m/z* calcd for [M]: 352.1651 Da; found: 352.1655 Da.

All other compounds were prepared by the same general method under the specific experimental conditions of Table 2.

4.1.2. Dimethyl(4-pyridyl)ethylmalonate (3aa, entry 1). ^{1}H NMR (250 MHz, $CDCl_3$): $\delta=2.24$ (m, 2H), 2.66 (m, 2H), 3.37 (t, $J=7$ Hz, 1H), 3.75 (s, 6H), 7.12 (dd, $J=4.5$, 1.6 Hz, 2H), 8.51 (dd, $J=4.5$, 1.6 Hz, 2H). NMR data were taken from the crude and practically pure product that reverted to starting materials upon attempted purification.

4.1.3. Dimethyl(2-cyano-1-methyl)ethylmalonate (3ae, entry 2).[19] Bp 60–75 °C/1.5–1.7 mmHg (oven temperature); IR (ATR): 2247, 1729 cm^{-1}; ^{1}H NMR (250 MHz,

$CDCl_3$): δ=1.15 (d, J=6.9 Hz, 3H), 2.44–2.65 (m, 1H), 2.56 (d, J=7.0 Hz, 2H), 3.40 (d, J=7.2 Hz, 1H), 3.73 (s, 6H); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ=16.6, 21.4, 29.6, 51.9, 54.5, 117.1, 167.3. Anal. calcd for $C_9H_{13}O_4N$: C 54.26, H 6.58, N 7.03; found: C 54.21, H 6.72, N 7.12.

4.1.4. Dimethyl(diethoxyphosphoryl)ethylmalonate (3af, entry 3). Bp 150 °C (1.7 mmHg, oven temperature); IR (ATR): 2987, 2953, 1730, 1241, 1145, 1018, 959 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$): δ=1.32 (t, J=7.2 Hz, 6H), 1.70–1.87 (m, 2H), 2.12–2.26 (m, 2H), 3.50 (t, J=7.4 Hz, 1H), 3.74 (s, 6H), 4.10 (broad dq, J=9.3, 7.2 Hz, 4H); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ=16.5 (d, J=5.7 Hz), 22.4, 23.5 (d, J=147.3 Hz), 51.7 (d, J=16.2 Hz), 52.9, 62.0 (d, J=6.7 Hz), 169.4. Anal. calcd for $C_{11}H_{21}O_7P$: C 44.60, H 7.14; found: C 44.63, H 7.43.

4.1.5. Dimethyl bis-[(diethoxyphosphoryl)ethyl]malonate (3aff, entry 4). Oil; IR (ATR): 2984, 1730, 1243, 1164, 1017, 940 cm^{-1}; ^{1}H NMR (250 MHz, CD_3OD): δ=1.37 (t, J=7.0 Hz, 12H), 1.67–1.82 (m, 4H), 2.10–2.20 (m, 4H), 3.79 (s, 6H), 4.15 (broad dq, J=8.9, 7.0 Hz, 8H); ^{13}C NMR (62.5 MHz, CD_3OD): δ=17.6 (d, J=6.7 Hz), 22.0 (d, J=142.1 Hz), 27.6 (d, J=2.9 Hz), 54.2, 59.2 (apparent t, J=17.6 Hz), 172.8; HRMS: m/z calcd for [M]: 460.1627 Da; found: 460.1639 Da.

4.1.6. Dimethyl 2-(ethoxycarbonyl)propylmalonate (3ag, entry 5). Bp 75–90 °C/0.9–1.2 mmHg (oven temperature); IR (ATR): 1728 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$): δ=1.18 (d, J=7.0 Hz, 3H), 1.24 (t, J=7.1 Hz, 3H), 2.01 (ddd, J=14.4, 8.8, 6.0 Hz, 1H), 2.23 (ddd, J=14.4, 8.8, 6.3 Hz, 1H), 2.46 (apparent sextet, J=7.1 Hz, 1H), 3.47 (dd, J=8.8, 6.3 Hz, 1H), 3.72 (s, 3H), 4.12 (q, J=7.1 Hz, 2H); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ=14.4, 17.7, 32.5, 37.6, 49.8, 52.8, 52.9, 60.8, 169.7, 169.8, 175.7. Anal. calcd for $C_{11}H_{18}O_6$: C 53.65, H 7.37; found: C 53.47, H 7.50.

4.1.7. Dimethyl butyl-[2-(4-pyridyl)ethyl]malonate (3ba, entry 6). Oil; IR (ATR): 1729, 1205, 1125, 807 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$): δ=0.90 (t, J=7.2 Hz, 3H), 1.12–1.38 (m, 4H), 1.91–1.98 (m, 2H), 2.14–2.21 (m, 2H), 2.47–2.54 (m, 2H), 3.74 (s, 6H), 7.10 (d, J=5.2 Hz, 2H), 8.49 (d, J=5.2 Hz, 2H); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ=13.9, 23.0, 26.4, 30.2, 32.8, 33.5, 57.6, 123.9, 149.8, 150.4, 171.9; MS (70 eV): m/z (%)=294 (M+1, 6), 262 (11), 234 (22), 202 (23), 188 (69), 146 (22), 145 (100), 128 (31); HRMS: m/z calcd for [M+H]: 294.1705 Da; found: 294.1721 Da.

4.1.8. Dimethyl butyl-(2-ethoxycarbonyl)ethylmalonate (3bc, entry 9). Bp 120–150 °C (0.4–0.5 mmHg, oven temperature); IR (ATR): 1730 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$): δ=0.87 (t, J=7.2 Hz, 3H), 1.06–1.34 (m, 4H), 1.24 (t, J=7.1 Hz, 3H), 1.82–1.88 (m, 2H), 2.15–2.30 (m, 4H), 3.70 (s, 6H), 4.11 (q, J=7.1 Hz, 2H); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ=14.1, 14.5, 23.1, 26.5, 28.1, 29.9, 33.2, 52.6, 57.2, 60.8, 172.1, 173.0; MS (70 eV): m/z (%)=288 (M, 8), 257 (51), 243 (81), 232 (94), 188 (43), 183 (66), 172 (61), 158 (34), 145 (100), 141 (39); HRMS: m/z calcd for [M]: 288.1537 Da; found: 288.1614 Da.

4.1.9. Dimethyl butyl-(2-cyano-1-methyl)ethylmalonate (3be, entry 11). Oil; IR (ATR): 2246, 1727, 1209 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$): δ=0.85 (t, J=7.2 Hz, 3H), 1.07 (d, J=6.9 Hz, 3H), 1.00–131 (m, 4H), 1.86 (t, J=8.3 Hz, 2H), 2.30 (dd, J=16.5, 9.3 Hz, 1H), 2.44–2.58 (m, 1H), 2.70 (dd, J=16.5, 3.7 Hz, 1H), 3.69 (s, 3H), 3.70 (s, 3H); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ=13.9, 15.0, 21.9, 23.0, 26.5, 33.4, 33.6, 52.5, 52.6, 60.8, 119.1, 170.4, 171.2; MS (70 eV): m/z (%)=256 (M+1, 22), 224 (61), 196 (34), 188 (40), 171 (26), 159 (100), 145 (51), 127 (53); HRMS: m/z calcd for [M+H]: 256.1549 Da; found: 256.1517 Da.

4.1.10. Dimethyl butyl-(2-ethoxycarbonyl)propylmalonate (3bg, entry 13). Oil; IR (ATR): 1729 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$): δ=0.85 (t, J=7.2 Hz, 3H), 0.93–1.35 (m, 4H), 1.13 (d, J=6.9 Hz, 3H), 1.22 (t, J=7.1 Hz, 3H), 1.71–1.98 (m, 3H), 2.34–2.48 (m, 2H), 3.65 (s, 6H), 4.05 (q, J=7.1 Hz, 2H); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ=13.9, 14.2, 19.6, 23.0, 26.4, 33.1, 35.9, 36.2, 52.3, 52.4, 57.1, 60.5, 171.9, 172.0, 176.3.

Acknowledgements

Financial support from 'Ministerio de Ciencia y Tecnología' of Spain (Project BQU 2002-04002) and 'DURSI—Generalitat de Catalunya' (Project SGR 2001-00181) is gratefully acknowledged.

References and notes

1. For recent reviews see: (a) Buono, G.; Chiodi, O.; Wills, M. *Synlett* **1999**, 377–388. (b) Vedejs, E.; Daugulis, O.; MacKay, J. A.; Rozners, E. *Synlett* **2001**, 1499–1505. (c) Lu, X.; Zhang, C.; Xu, Z. *Acc. Chem. Res.* **2001**, *34*, 535–544. (d) Valentine, D. H., Jr.; Hillhouse, J. H. *Synthesis* **2003**, 317–334. (e) Basavaiah, D.; Rao, A. J.; Satyanarayana, T. *Chem. Rev.* **2003**, *103*, 811–891. (f) Methot, J. L.; Roush, W. R. *Adv. Synth. Catal.* **2004**, *346*, 1035–1050.
2. (a) Du, Y.; Lu, X.; Yu, Y. *J. Org. Chem.* **2002**, *67*, 8901–8905. (b) Wang, L.-C.; Luis, A. L.; Agapiou, K.; Jang, H.-Y.; Krische, M. J. *J. Am. Chem. Soc.* **2002**, *124*, 2402–2403. (c) Frank, S. A.; Mergott, D. J.; Roush, W. R. *J. Am. Chem. Soc.* **2002**, *124*, 2404–2405. (d) Jellerichs, B. G.; Kong, J.-R.; Krische, M. J. *J. Am. Chem. Soc.* **2003**, *125*, 7758–7759. (e) Dinon, F.; Richards, E.; Murphy, P. J.; Hibbs, D. E.; Hursthouse, M. B.; Malik, K. M. A. *Tetrahedron Lett.* **1999**, *40*, 3279–3282. (f) Agapiou, K.; Cauble, D. F.; Krische, M. J. *J. Am. Chem. Soc.* **2004**, *126*, 4528–4529. (g) Luis, A. L.; Krische, M. J. *Synthesis* **2004**, 2579–2585. (h) Cho, C.-W.; Kong, J.-R.; Krische, M. J. *Org. Lett.* **2004**, *6*, 1337–1339. (i) Kuroda, H.; Hanaki, E.; Izawa, H.; Kano, M.; Itahashi, H. *Tetrahedron* **2004**, *60*, 1913–1920.
3. (a) Trost, B. M.; Kazmaier, U. *J. Am. Chem. Soc.* **1992**, *114*, 7933–7935. (b) Guo, C.; Lu, X. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1921–1923.
4. (a) Trost, B. M.; Dake, G. R. *J. Am. Chem. Soc.* **1997**, *119*, 7595–7596. (b) Yavari, I.; Adib, M.; Hojabri, L. *Tetrahedron* **2002**, *58*, 6895–6899. (c) Hanédanian, M.; Loreau, O.; Taran, F.; Mioskowski, C. *Tetrahedron Lett.* **2004**, *45*, 7035–7038.
5. (a) Trost, B. M.; Li, C.-J. *J. Am. Chem. Soc.* **1994**, *116*, 3167–3168. (b) Trost, B. M.; Li, C.-J. *J. Am. Chem. Soc.* **1994**,

116, 10819–10820. (c) Zhang, C.; Lu, X. *Synlett* **1995**, 645–646. (d) Trost, B. M.; Dake, G. R. *J. Org. Chem.* **1997**, *62*, 5670–5671. (e) Álvarez Ibarra, C.; Csákÿ, A. G.; Gómez de la Oliva, C. *Tetrahedron Lett.* **1999**, *40*, 8465–8467. (f) Lu, C.; Lu, X. *Tetrahedron* **2004**, *60*, 6575–6579.

6. (a) Zhang, C.; Lu, X. *J. Org. Chem.* **1995**, *60*, 2906–2908. (b) Xu, Z.; Lu, X. *Tetrahedron Lett.* **1997**, *38*, 3461–3466. (c) Xu, Z.; Lu, X. *J. Org. Chem.* **1998**, *63*, 5031–5041. (d) Liu, B.; Davis, R.; Joshi, B.; Reynolds, D. W. *J. Org. Chem.* **2002**, *67*, 4595–4598. (e) Lu, C.; Lu, X. *Org. Lett.* **2002**, *4*, 4677–4679.
7. Kuroka, H.; Tomita, I.; Endo, T. *Polymer* **1997**, *38*, 6049–6054.
8. (a) Inanaga, J.; Baba, Y.; Hanamoto, T. *Chem. Lett.* **1993**, 241–244. (b) Wende, M.; Meier, R.; Gladysz, J. A. *J. Am. Chem. Soc.* **2001**, *123*, 11490–11491. (c) Wende, M.; Gladysz, J. A. *J. Am. Chem. Soc.* **2003**, *125*, 5861–5872.
9. Yavari, I.; Ramazani, A. *Synth. Commun.* **1997**, *27*, 1449–1454.
10. (a) Jenner, G. *Tetrahedron* **2002**, *58*, 4311–4317. (b) Stewart, I. C.; Bergman, R. G.; Toste, F. D. *J. Am. Chem. Soc.* **2003**, *125*, 8696–8697.
11. Bhuniya, D.; Mohan, S.; Narayanan, S. *Synthesis* **2003**, 1018–1024.
12. Xu, L.-W.; Xia, C.-G. *Tetrahedron Lett.* **2004**, *45*, 4507–4510.
13. White, D. A.; Baizer, M. M. *Tetrahedron Lett.* **1973**, 3597–3600.
14. Yoshida, T.; Saito, S. *Chem. Lett.* **1982**, 1587–1590.
15. For reviews see: (a) Christoffers, J. *Eur. J. Org. Chem.* **1998**, 1259–1266. (b) Comelles, J.; Moreno-Mañas, M.; Vallribera, A. *Arkivoc* **2005**, (part ix), 207–238. Available from the web page: http://www.arkat-usa.org/ark/journal/2005/I09_molina-elguero/1302/me-1302r.pdf.
16. Lumbierres, M.; Marchi, C.; Moreno-Mañas, M.; Sebastián, R. M.; Vallribera, A.; Lago, E.; Molins, E. *Eur. J. Org. Chem.* **2001**, 2321–2328.
17. Kempf, B.; Mayr, H. *Chem. Eur. J.* **2005**, *11*, 917–927.
18. Comelles, J.; Moreno-Mañas, M.; Pérez, E.; Roglans, A.; Sebastián, R. M.; Vallribera, A. *J. Org. Chem.* **2004**, *69*, 6834–6842.
19. (a) Elinson, M. N.; Feducovich, S. K.; Zakharenkov, A. A.; Ugrak, B. I.; Nikishin, G. I.; Lindeman, S. V.; Struchkov, J. T. *Tetrahedron* **1995**, *51*, 5035–5046. (b) Palombi, L.; Feroci, M.; Orsini, M.; Inesi, A. *Chem. Commun.* **2004**, 1846–1847.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron 61 (2005) 8606–8610
Tetrahedron

Isolation and structure elucidation of a new amphidinol with a truncated polyhydroxyl chain from *Amphidinium klebsii*

Nagy Morsy,[a] Shigeru Matsuoka,[a] Toshihiro Houdai,[a] Nobuaki Matsumori,[a] Seiji Adachi,[a] Michio Murata,[a,*] Takashi Iwashita[b] and Tsuyoshi Fujita[b]

[a]*Department of Chemistry, Graduate School of Science, Osaka University, Machikaneyama, Toyonaka, Osaka 560-0043, Japan*
[b]*Suntory Institute for Bioorganic Research, 1-1-1 Wakayamadai, Shimamotocho, Osaka 618-8503, Japan*

Received 25 May 2005; revised 26 June 2005; accepted 5 July 2005

Available online 26 July 2005

Abstract—A new member of amphidinols (AM7) possessing a polyene-polyhydroxyl structure with the shortest carbon backbone and sulfate ester was isolated from the cultured dinoflagellate, *Amphidinium klebsii*. AM7 showed hemolytic and antifungal activities. The structure was elucidated on the basis of 2D NMR data in combination with CID MS/MS experiments.

1. Introduction

Dinoflagellates are an important source of structurally and biologically intriguing natural products,[1,2] for example, amphidinolides,[3] amphidinols,[4–8] brevetoxins, ciguatoxins, maitotoxin, okadaic acid, saxitoxins, and symbioimine. Amphidinols isolated from *Amphidinium klebsii* (NIES 613) show potent membrane-disrupting effects such as antifungal and hemolytic activities.[4–6] The first member of the amphidinol family, amphidinol 1 (AM1), was isolated and characterized in 1991[4] and the absolute configuration of AM3 was determined in 1999 by our group.[7] The structures of amphidinols are characterized by a common part, which comprises a linear polyhydroxyl moiety, two tetrahydropyran rings, and a polyolefinic chain of C_{14} or C_{16}. The closely related analogues bearing different names, luteophanols and lingshuiols, were reported from the same genus of dinoflagellate.[9–11] We have disclosed that the potent biological action by AMs can be accounted for by their membrane permeabilizing actions. Moreover, ion channels formed by AMs appeared to possess unique features;[12,13] the central part involving two tetrahydropyran rings comprises a large hydrophilic part with a hairpin conformation, the polyolefin moiety penetrates deep into membrane, and the polyhydroxyl part is thought to form channel lining. For further investigating the mode of action of these unique membrane-active agents, we continued the screening of the same strain of *A. klebsii* (NIES 613), and isolated a new homologue named amphidinol 7 (AM7, **1**, Fig. 1) possessing the smallest molecular weight among the known AM homologues and a truncated polyhydroxyl chain, which is expected to provide new information on structure–activity relationship.

2. Results and discussion

2.1. Structure elucidation based on NMR

AM7 was isolated as a pale yellow amorphous solid (11 mg), $[\alpha]_D^{20}$ −14.1° (*c* 0.15, MeOH). Electrospray ionization (ESI) MS *m/z* 1253 $[M+Na]^+$ and 1207 $[M-Na]^-$; UV λ_{max} (MeOH), 279 (ε 19,900), 269 (ε 25,200) and 258 (ε 19,900). HRFABMS showed the molecular formula of $C_{59}H_{99}O_{23}SNa$ [*m/z* 1253.6089, $(M+Na)^+$, Δ −0.4 mmu], which corresponded to the molecular weight of 1230 and revealed that AM7 is the smallest AM ever reported. The UV spectrum was indicative of the presence of a conjugated triene chromophore, which is characteristic of AM homologues (Fig. 1).

After mild hydrolysis, AM7 was converted to a less polar compound with *m/z* 1151. A loss of 102 mass unit for the hydrolysate suggested the presence of a sulfate ester in AM7. The unsaturation number of 10 was accounted for by eight carbon=carbon double bonds and two rings. Detailed analysis of DQF-COSY and TOCSY spectra of **1** disclosed the proton-connectivities of the following three structural units: (**A**) from C-1 to C-19 (Fig. 2a), (**B**) from C-21 to C-31 (Fig. 2b), and (**C**) from C-33 to C-55 (Fig. 2c). For partial

Keywords: Amphidinol; Amphidinium; AM7; AM3; CID MS/MS.
* Corresponding author. Tel./fax: +81 668505774;
e-mail: murata@ch.wani.osaka-u.ac.jp

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.07.004

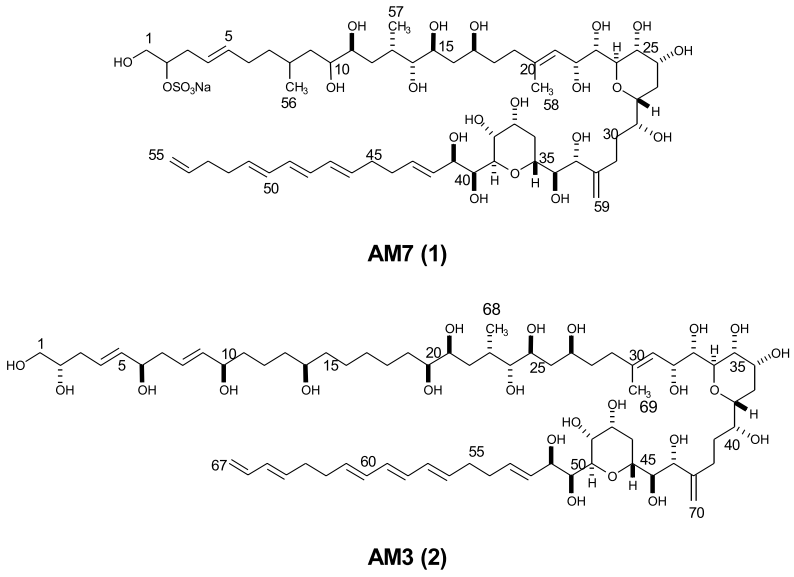


Figure 1. Structures of amphidinol 7 (**1**, AM7) and amphidinol 3 (**2**, AM3).

structure **A**, the proton-connectivity was evident from the DQF-COSY spectrum. The geometry of Δ^4 was assigned as **E** from the ^{13}C NMR chemical shift of the allylic carbons C-3 (δ 35.73) and C-6 (δ 30.79). For partial structure **B**, the DQF-COSY showed the correlation of H-21 to H-31. The presence of two tetrahydropyran rings was indicated by the HMBC correlations for H-24/C-28 and H-39/C-35. For partial structure **C**, the ^{1}H and ^{13}C NMR data from C-44 to C-52 agreed quite well with those for C-54 to C-62 of AM3 (Fig. 1), which indicated that they share the same structure for the polyolefinic part except for a vinyl terminus in lieu of a butadiene of AM3. The connection between **A** and **B** was indicated to be through the sp^2 quaternary carbon (C-20, δ 138.72) bearing the methyl group. This was revealed by the HMBC correlations for H-19/C-58 and H-21/C-58. The connection between **B** and **C** through sp^2 quaternary carbon (C-32, δ 151.95) bearing *exo*-methylene was evidenced by HMBC correlations for H-31/C-32, H-33/C-32 and H-59/C-32. The assignments of the proton and carbon resonances for compound **1** are shown in Table 1. The position of the sulfate ester was deduced to be C2 since ^{1}H NMR chemical shifts for C1–C3 were virtually identical with those of AM1, which possessed the same partial structure for the C1–C5 part.[4]

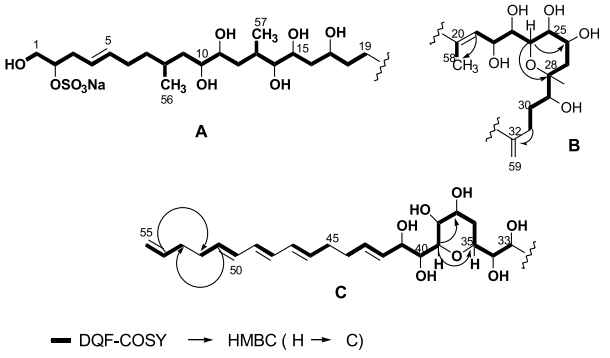


Figure 2. Partial structures of AM7.

^{13}C NMR data of AM7 were essentially identical with those of AM3 for the common structural part (C11–C52 of AM7), which should have the same stereochemistry as that of AM3 (Fig. 1); Kishi's group has reported that alteration in configuration of methyl or hydroxyl substituted alkyl chains causes more than 1 ppm difference in ^{13}C NMR.[14] Three asymmetric centers in the terminal part (C2, C8 and C10) remain unassigned; the *J*-based configuration analysis[15] could not be applied for AM7 since the chemical shifts of C10/C11 and H10/H11 were very close, which prevented the accurate measurements of $^{2,3}J_{C,H}$ or $^3J_{H,H}$ values.

2.2. Structure confirmation using CID MS/MS

Further, verification of the NMR-derived structure was carried out by tandem mass spectrometry.[16] Collision-induced dissociation (CID) is know to produce fragment ions due to cleavages at α and β positions to hydroxyl groups.[4] Negative ion FAB CID MS/MS spectra of **1** (Fig. 3) for the precursor ion at *m*/*z* 1207 $[M-Na]^-$ showed the characteristic patterns of charge-remote fragmentation due to the presence of a sulfate group.[17] Product ion peaks generated by fissions at α positions to a hydroxyl group or at allylic or homoallylic sites were prominently observed (Fig. 3).

The presence of the sulfate ester in **1** was supported by the intense negative ion peaks at *m*/*z* 80 (SO_3^-) and 97 (HSO_4^-).[17a] The position of the sulfate ester was confirmed by the product ion peaks *m*/*z* 123 and 140. The tetrahydropyran rings gave some characteristic fragmentation product ion peaks at *m*/*z* 613, 627, 699, 871 and 943. The series of substantial product ion peaks at *m*/*z* 193, 207, 235, 249, 279, 293, 309, 323, 351, 365, 381, 395 and 469 correlates the aliphatic structure C7–C19. The presence of the terminal polyene structure was supported by the product ion peaks at *m*/*z* 1059, 1073, 1125, 1151, 1166 and 1179. Unaccountable ion peaks at *m*/*z* 453 and 1043 may be the result of oxidation (losing of two protons) before

Table 1. ^{1}H and ^{13}C chemical shift data of AM7 and AM3[12] in CD_3OD–C_5D_5N (2:1)

AM7			AM3		
Pstn	^{1}H	^{13}C	Pstn	^{1}H	^{13}C
			1	3.53, 3.58	66.9
			2	3.74	73.0
			3	2.22, 2.31	37.7
			4	5.76	128.5
			5	5.61	136.5
			6	4.11	73.1
			7	2.25, 2.29	41.6
			8	5.69	128.1
			9	5.56	137.2
			10	4.06	73.4
1	3.78, 3.80	64.48	11	1.52, 1.57	38.5
2	4.54	80.57	12	1.43, 1.60	22.8
3	2.47	35.73	13	1.43	38.5
4	5.49	134.72	14	3.54	72.1
5	5.47	126.33	15	1.43	38.5
6	1.92, 1.95	30.79	16	1.34, 1.45	26.8
7	1.16, 1.28	38.91	17	1.25, 1.33	31.0
8	1.76	29.73	18	1.38, 1.56	27.1
9	1.20, 1.56	41.46	19	1.48, 1.56	34.1
10	3.56	73.63	20	3.46	76.0
11	3.60	73.74	21	3.63	73.1
12	1.58, 1.63	38.91	22	1.58, 1.63	38.9
13	2.34	31.68	23	2.32	31.6
14	3.44	79.79	24	3.45	79.7
15	3.86	72.88	25	3.86	72.8
16	1.63, 2.08	41.46	26	1.63, 2.08	41.4
17	3.95	71.84	27	3.95	71.8
18	1.62	36.91	28	1.65	36.9
19	2.08, 2.22	36.75	29	2.10, 2.21	36.7
20	—	138.72	30	—	138.6
21	5.63	126.81	31	5.63	126.8
22	4.72	67.85	32	4.72	67.8
23	3.84	72.59	33	3.83	72.6
24	4.22	79.26	34	4.22	79.2
25	4.30	68.97	35	4.29	68.9
26	4.12	67.45	36	4.12	67.4
27	1.92, 2.00	31.18	37	1.92, 2.01	30.6
28	3.60	75.84	38	3.60	75.8
29	3.70	74.54	39	3.71	74.5
30	1.67, 2.07	32.63	40	1.68, 2.05	32.6
31	2.22, 2.60	28.04	41	2.23, 2.59	28.0
32	—	151.95	42	—	151.9
33	4.38	76.75	43	4.38	76.7
34	3.50	75.25	44	3.50	75.3
35	4.19	70.62	45	4.21	70.6
36	1.64, 2.32	31.90	46	1.64, 2.30	31.8
37	4.17	67.34	47	4.17	67.3
38	4.30	68.77	48	4.30	68.7
39	3.98	80.68	49	3.98	80.6
40	4.19	72.19	50	4.18	72.1
41	4.60	74.17	51	4.59	74.1
42	5.76	129.76	52	5.75	129.7
43	5.82	134.28	53	5.80	134.2
44	2.04	33.58	54	2.05	33.5
45	2.04	33.58	55	2.09	33.5
46	5.62	134.51	56	5.59	134.1
47	6.00	132.11	57	6.05	132.4
48	6.04	132.26	58	5.97	132.3
49	5.99	132.36	59	5.98	132.2
50	6.00	132.26	60	5.99	132.0
51	5.60	134.30	61	5.62	134.5
52	2.07	33.23	62	2.06	33.4
53	2.04	34.61	63	2.08	33.3
54	5.72	139.25	64	5.62	135.3
55	4.87, 4.95	115.42			
			65	6.01	132.6
			66	6.25	138.4
			67	4.89, 5.05	115.5
56	0.83	19.54			
57	1.02	13.24	68	1.01	13.2
58	1.70	17.44	69	1.71	17.4
59	5.00, 5.12	112.86	70	5.00, 5.13	112.8

detection, which is often observed for polyoxygenated compounds.

2.3. Biological activities of amphidinol 7

AM7 showed antifungal activity against *Aspergillus nigar* with MEC of 10 μg/disk and it showed hemolytic activity with EC_{50} of 3 μM. In comparison with AM3, the most potent homologue in hemolytic and antifungal activities,[8] AM7 showed less potent antifungal and hemolytic activities (Table 2). These differences in bioactivities may provide new insights into the structure–activity relationship for the unique membrane-permeabilizing action of AMs since the polyhydroxyl chain is thought to be important for channel size and conductance.[13] AM7 may, therefore, serve as a useful tool for investigating a role of the polyhydroxyl chain in membrane permeabilization action of AMs.

AM7, the third member of sulfate-bearing AMs after AM1 and lingshuiol B, possesses the shortest carbon chain of all the AMs. The structure variations among the known AMs are concentrated in a C1–C22 part of AM7. As AMs is known to be biosynthesized from the polyhydroxyl end to the polyolefin terminus,[18] the great variation in the structures of the polyhydroxyl part may be attributable to the truncation of a polyketide chain during biosynthesis as suggested for okadaic acid analogues.[19]

3. Conclusion

A new member of amphidinols, amphidinol 7, which possesses a sulfate ester on the shortest carbon backbone and moderate biological activities, was isolated from the cultured cells of the dinoflagellate, *A. klebsii*. The structure was elucidated on the basis of 2D NMR data in combination with CID MS/MS. The stereochemistry of the common part (C11–C52) was deduced to be identical with that of AM3. This analogue may provide new insights into the mode of action of amphidinol homologues.

Table 2. Biological activities of AM3 and AM7

Activity	AM3	AM7
Antifungal (MEC[a] in μg/disk; *A. nigar*)	6	10
Hemolysis (EC_{50} in μM; human erythrocytes)	0.4	3

[a] Minimum effective concentration.

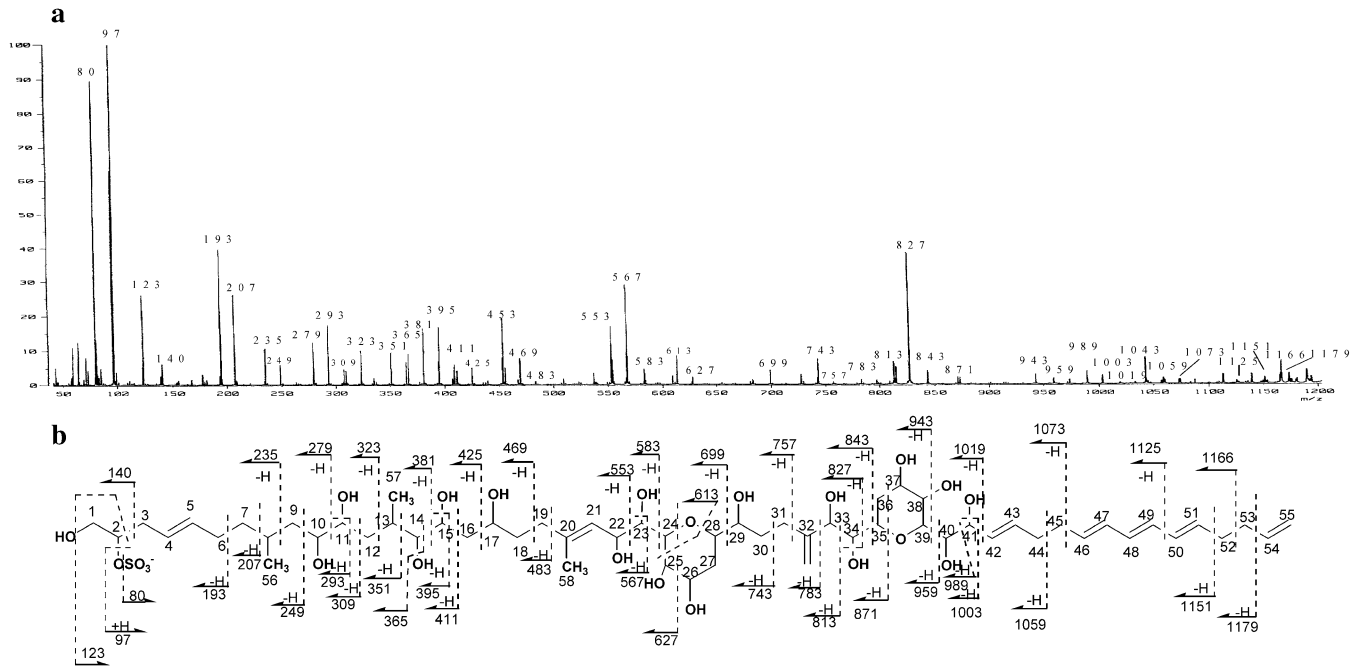


Figure 3. CID MS/MS spectrum (a) and fragmentation patterns (b) observed in negative ion FAB MS/MS spectra of AM7 (**1**) for precursor ion at *m/z* 1207.

4. Experimental

4.1. Chemical and instruments

^{1}H, ^{13}C and 2D NMR spectra were recorded on an INOVA-600 spectrometer with the sample dissolved in 0.65 ml of CD_3OD/pyridine-d_5 (2:1). MS/MS spectra were measured with a JMS HX-110/HX-110A four sector tandem mass spectrometer equipped with 6 kV Xe beam FAB gun and the MS-ADS11 variable mass dispersion array detector. The CID spectra were recorded with 10 kV acceleration voltage (MS1), electrically 8 kV floated collision cell and 10% dispersion array detector conditions. 2,2′-Dithiodiethanol was used as a matrix. Ar used as collision gas was introduced at the rate that precursor ion intensity was attenuated to ca. 30%. UV spectra were measured on a Shimadzu UV-2500 spectrometer.

4.2. Culture of *A. klebsii* and isolation of amphidinol 7

The dinoflagellate *A. klebsii* was isolated from the surface wash from several species of seaweed that were abundant at the collection site near the shore of Aburatsubo Bay, Japan and deposited in National Institute for Environmental Studies as NIES 613. The unialgal culture was grown in a 3-liter Fernbach flask containing artificial seawater (Marin Art Hi, Tomita Pharmaceutical, 3% w/v) enriched with 2% ES-1 supplement (Provasoli 1968) at 25 °C for 3 to 4 weeks under illumination with a 16-8 light–dark photocycle. The cultured cells (60 L) were harvested by filtration with glass filters under reduced pressure. The algal biomass was extracted with methanol (2 L×3) and then with 2 L of 50% aqueous MeOH. Purification of amphidinols was carried out using lead acetate.[20–22] Briefly, the combined extracts, after the solvents were removed, was subjected to ethyl acetate/H_2O partition, the resultant aqueous layer was subjected to lead acetate purification, and the excess of lead acetate was eliminated using Na_2HPO_4 The extract was dried and dissolved in distilled water and applied to an ODS open column and then to HPLC (YMC-Pack, ODS-AM, 250× 10 mm, MeCN/H_2O gradient elution, UV: 270 nm) to furnish 11 mg of AM7 (**1**).

4.3. Desulfation of amphidinol 7

The hydrolysis of sulfate esters was elaborated using a mild hydrolysis method;[23] 100 μg of the sample in 100 μL dioxane was added with 3 mg of PTS·H_2O (*p*-toluene sulphonic acid) in 50 μL dioxane. The solution was stirred for 30 min then Na_2CO_3 as a 10% aqueous solution was added to neutralize the solution. The product was extracted with ethyl acetate. The extract was evaporated and dried with N_2 gas. The reactant was dissolved in MeOH and checked by HPLC (Cosmosil, 5C18-AR-II, Waters, 4.6× 150 mm, 270 nm, 28.57% MeCN in H_2O).

4.4. Biological assays

The fungus *A. nigar* was cultured in a glucose peptone liquid medium (2% glucose, 0.2% yeast extract, 0.5% polypeptone, 0.05% $MgSO_4$, and 0.1% KH_2PO_4) at 25 °C for 2 days. An aliquot of the broth was then spread onto an agar plate (1.5% agar). Each sample dissolved in MeOH was spotted on a paper disk (8 mm in diameter), which were then placed on an agar plate spread with *A. nigar* mycelia. After incubation at 25 °C for 2 days, the diameter of an inhibitory zone on each paper disk was measured.

For hemolytic assays, human blood cells in 3.13% sodium citrate were immediately separated from the plasma by centrifugation at 1000×g for 5 min. Sedimented cells were washed three times with PBS buffer, containing 137 mM NaCl, 2.68 mM KCl, 8.10 mM Na_2HPO_4, and 1.47 mM KH_2PO_4 at pH 7.4. A sample dissolved in methanol (20 μL)

was added to 190 μL of the blood cell suspension in 1% hematocrit PBS buffer and incubated for 6 h at 37 °C. After incubation, the resultant supernatant was subjected to colorimetric measurements at 450 nm on microplate reader (Molecular devices).The percentage amount of hemoglobin released from erythrocytes was calculated. From dose-response curves, the concentration that caused 50% hemolysis (EC_{50}) was determined.

Acknowledgements

We are grateful to Prof. Shoichi Kusumoto, Suntory Institute for Bioorganic Research for encouragement and discussion; and Prof. Tohru Oishi and Ms. Megumi Mori, Osaka University, for their help and support. This study was supported by Yamada Science Foundation. S. M. and T. H among the authors have been supported by 21 century COE program 'Creation of Integrated Ecochemistry'.

References and notes

1. Shimizu, Y. *Chem. Rev.* **1993**, *5*, 1685–1698.
2. Yasumoto, T.; Murata, M. *Chem. Rev.* **1993**, *5*, 1897–1909.
3. Kobayashi, J.; Tsuda, M. *Nat. Prod. Rep.* **2004**, *21*, 77–93.
4. Satake, M.; Murata, M.; Yasumoto, T.; Fujita, T.; Naoki, H. *J. Am. Chem. Soc.* **1991**, *113*, 9859–9861.
5. (a) Paul, G. K.; Matsumori, N.; Murata, M.; Tachibana, K. *Tetrahedron Lett.* **1995**, *36*, 6279–6282. (b) Echigoya, R.; Rhodes, L.; Oshima, Y.; Satake, M. *Harmful Algae* **2005**, *4*, 383–389.
6. Paul, G. K.; Matsumori, N.; Konoki, K.; Sasaki, M.; Murata, M.; Tachibana, K. *Harmful and Toxic Algal Blooms* **1996**, 503–506.
7. Murata, M.; Matsuoka, S.; Matsumori, N.; Paul, G. K.; Tachibana, K. *J. Am. Chem. Soc.* **1999**, *121*, 870–871.
8. Paul, G. K.; Matsumori, N.; Konoki, K.; Murata, M.; Tachibana, K. *J. Mar. Biotechnol.* **1997**, *5*, 124–128.
9. Huang, X.; Zhao, D.; Guo, Y.; Wu, H.; Trivellone, E.; Cimino, G. *Tetrahedron Lett.* **2004**, *45*, 5501–5504.
10. Doi, Y.; Ishibashi, M.; Nakamichi, H.; Kosaka, T.; Ishikawa, T.; Kobayashi, J. *J. Org. Chem.* **1997**, *62*, 3820–3823.
11. Kubota, T.; Tsuda, M.; Doi, Y.; Takahashi, A.; Nakamichi, H.; Ishibashi, M.; Fukushi, E.; Kawabata, J.; Kobayashi, J. *Tetrahedron* **1998**, *54*, 14455–14464.
12. Houdai, T.; Matsuoka, S.; Matsumori, N.; Murata, M. *Biochim. Biophys. Acta* **2004**, *1667*, 91–100.
13. Houdai, T.; Matsuoka, S.; Morsy, N.; Matsumori, N.; Satake, M.; Murata, M. *Tetrahedron* **2005**, *61*, 2795–2802.
14. (a) Lee, J.; Kobayashi, Y.; Tezuka, K.; Kishi, Y. *Org. Lett.* **1999**, *1*, 2177–2180. (b) Kobayashi, Y.; Tan, C.-H.; Kishi, Y. *Angew. Chem., Int. Ed.* **2000**, *39*, 4279–4281. (c) Kobayashi, Y.; Tan, C.-H.; Kishi, Y. *J. Am. Chem. Soc.* **2001**, *123*, 2076–2078. (d) Higashibayashi, S.; Czechtizky, W.; Kobayashi, Y.; Kishi, Y. *J. Am. Chem. Soc.* **2003**, *125*, 14379–14393.
15. Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866–876.
16. Biemann, K.; Hubert, H. A. *Science* **1987**, *237*, 992–998.
17. (a) Kobayashi, J.; Kubota, T.; Takahashi, M.; Ishibashi, M.; Tsuda, M.; Naoki, H. *J. Org. Chem.* **1999**, *64*, 1478–1482. (b) Doi, Y.; Ishibashi, M.; Nakamichi, H.; Kosaka, T.; Ishikawa, T.; Kobayashi, J. *J. Org. Chem.* **1997**, *62*, 3820–3823.
18. Houdai, H.; Matsuoka, S.; Murata, M.; Satake, M.; Ota, S.; Oshima, Y.; Rhodes, L. *Tetrahedron* **2001**, *57*, 5551–5555.
19. (a) Needham, J.; Hu, T.; McLachlan, J. L.; Walter, J. A.; Wright, J. L. C. *J. Chem. Soc., Chem. Commun.* **1995**, 1623–1624. (b) Wright, J. L. C.; Hu, T.; McLachlan, J. L.; Needham, J.; Walter, J. A. *J. Am. Chem. Soc.* **1996**, *118*, 8757–8758.
20. Nelson, C. J.; Seiber, J. N.; Brower, L. P. *J. Chem. Ecol.* **1981**, *7*, 981–1010.
21. Karawya, M. S.; Abdel-Wahab, S. M.; Niazi, H. M. *Planta Med.* **1974**, *25*, 17–21.
22. Wall, M. E.; Taylor, H.; Ambrosio, L.; Davis, K. *J. Pharm. Sci.* **1969**, *58*, 839–841.
23. Singh, S. B. *Tetrahedron Lett.* **2000**, *41*, 6973–6976.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
ELSEVIER
Tetrahedron 61 (2005) 8611–8615

Lissoclibadins 1–3, three new polysulfur alkaloids, from the ascidian *Lissoclinum* cf. *badium*

Hongwei Liu,[a,†] Takeshi Fujiwara,[a] Teruaki Nishikawa,[b] Yuri Mishima,[a] Hiroshi Nagai,[a] Takeshi Shida,[c] Kazuo Tachibana,[c] Hisayoshi Kobayashi,[d] Remy E. P. Mangindaan[e] and Michio Namikoshi[a,*]

[a]*Department of Ocean Sciences, Tokyo University of Marine Science and Technology, Minato-ku, Tokyo 108-8477, Japan*
[b]*The Nagoya University Museum, Chikusa-ku, Nagoya 464-8601, Japan*
[c]*School of Science, The University of Tokyo, Bunkyo-ku, Tokyo 113-0033, Japan*
[d]*Institute of Molecular and Cellular Biosciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-0032, Japan*
[e]*Faculty of Fisheries and Marine Science, Sam Ratulangi University, Kampus Bahu, Manado 95115, North Sulawesi, Indonesia*

Received 24 May 2005; revised 28 June 2005; accepted 5 July 2005

Available online 21 July 2005

Dedicated to the memory of Dr. Kenneth L. Rinehart and Dr. Katsumi Kakinuma

Abstract—Three new polysulfur alkaloids, lissoclibadins 1 (**1**)–3 (**3**), were isolated from the ascidian *Lissoclinum* sp. (cf. *L. badium* Monniot, F. and Monniot, C., 1996). The structures of **1**–**3** were assigned on the basis of their spectral data, and the computational modeling study was utilized for **1**. Compound **1** had a trimeric structure of three identical aromatic anime moieties connected through two sulfide and a disulfide bonds. Compounds **2** and **3** were dimeric structures of the same unit as that of **1** connected through a sulfide and disulfide bonds (**2**) and two sulfide bonds (**3**). Compounds **1** and **2** inhibited the growth of the marine bacterium *Ruegeria atlantica* (15.2 mm at 20 μg/disk and 12.2 mm at 5 μg/disk, respectively) and **2** showed antifungal activity to *Mucor hiemalis* (13.8 mm at 50 μg/disk). Compounds **1**–**3** were cytotoxic against HL-60 (IC_{50}=0.37, 0.21, and 5.5 μM, respectively).

1. Introduction

Many interesting bioactive compounds have been obtained from ascidians (tunicates). Aromatic alkaloids possessing polysulfide structures have been isolated from ascidians of the genera *Lissoclinum*,[1–6] *Eudistoma*,[4] and *Polycitor*.[7] More than 10 monomeric cyclic polysulfides[1–4,6,7] and four dimeric polysulfides[2,5,8] have been reported. These compounds presented various biological activities, for example, antifungal activity,[1–3,7] antibacterial activity,[3,7] cytotoxicity,[1,3,8] antimalarial (*Plasmodium falciparum*) activity,[3] inhibition of protein kinase C,[4,5] and inhibition of IL-8 Rα and Rβ receptors.[5]

In the course of our study on the bioactive metabolites from marine organisms, we found that the ethanol extract of the ascidian *Lissoclinum* sp. (cf. *L. badium* Monniot, F. and Monniot, C., 1996)[9] collected at Manado, Indonesia, showed strong antimicrobial activity against the fungus *Mucor hiemalis* and the marine bacterium *Ruegeria atlantica*. Bioassay-guided separation gave three new polysulfide aromatic alkaloids, named lissoclibadins 1 (**1**),[10] 2 (**2**), and 3 (**3**), together with two known dimeric alkaloids, lissoclinotoxins E (**4**) and F (**5**), and two known monomeric compounds, 3,4-dimethoxy-6-(2′-*N*,*N*-dimethylaminoethyl)-5-(methylthio)benzotrithiane (**6**) and *N*,*N*-dimethyl-5-(methylthio)varacin (**7**). We have reported the structure of **1** in the previous communication[10] and describe here the isolation, structure elucidation, and biological activity of three new lissoclibadins 1–3 (**1**–**3**) and compounds **4**–**7**.

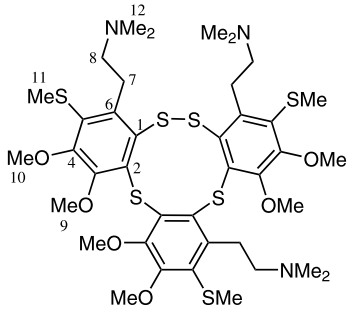
2. Results and discussion

2.1. Ascidian and isolation of alkaloids

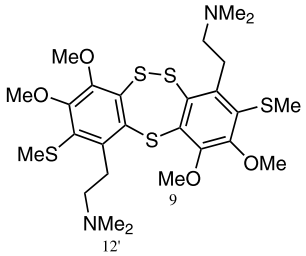
Lissoclinum cf. *badium* was collected at Manado, Indonesia

Keywords: Tunicate; *Lissoclinum* sp.; Lissoclinotoxin; Polysulfur compound; Structure assignment.
* Corresponding author. Tel.: +81 3 5463 0451; fax: +81 3 5463 0398; e-mail: namikosh@s.kaiyodai.ac.jp
† Present address: Tsinghua University, Tsinghua Campus, The University Town, Shenzhen, People's Republic of China.

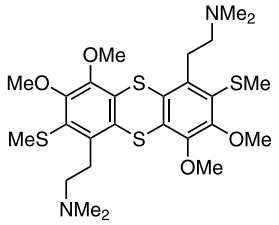
0040–4020/$ - see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.tet.2005.07.002



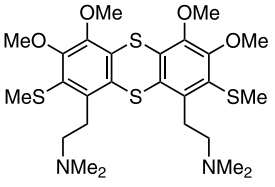
Lissoclibadin 1 (**1**)



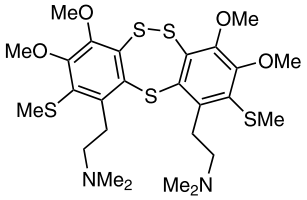
Lissoclibadin 2 (**2**)



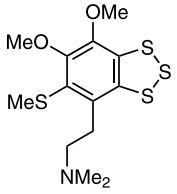
Lissoclibadin 3 (**3**)



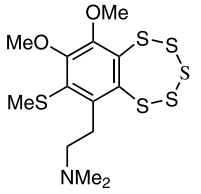
Lissoclinotoxin E (**4**)



Lissoclinotoxin F (**5**)



6



7

in 2003 and 2004 and extracted with ethanol. The ethanol extract was redissolved in MeOH–H_2O (9/1) and extracted with *n*-hexane. The aqueous MeOH layer was diluted with water and extracted with *n*-BuOH. The BuOH extract showed moderate antimicrobial activity and gave **1**, **2**, **3**, and **5** by ODS and SiO_2 column chromatographies followed by HPLC. The hexane extract revealed strong antifungal and antibacterial activities and was separated by SiO_2 column chromatography followed by HPLC to yield **6** and **7**. Compound **4** was isolated from the ascidian collected in 2004 together with **1**–**3** and **5**–**7**.

Structures of three known compounds **5**–**7** were assigned on the basis of their spectral data and comparison with those for the reported values.[4,8]

2.2. Structure of lissoclibadin 1

Lissoclibadin 1 (**1**) was isolated as a Tris–TFA salt. The molecular weight (887) and formula ($C_{39}H_{57}N_3O_6S_7$) were deduced from HRFABMS and NMR data (Table 1). Three sets of ^1H and ^{13}C NMR signals were observed in the NMR spectra of **1** and assigned to three identical aromatic amine moieties by the analysis of ^1H–^1H COSY, HMQC, HMBC, ROESY, and NOESY spectra.

The geminal couplings and connectivity of two methylene groups at the 7 and 8 positions were revealed by ^1H–^1H COSY spectrum. HMBC correlations were detected from H_2-7 to three aromatic carbon signals (C-1, 5, and 6) and C-8. The ^{13}C signal assigned as C-5 showed an HMBC correlation from a methyl singlet of SMe (H_3-11). This SMe revealed an NOE with one of two methoxy methyl singlets due to H_3-10 in the NOESY spectrum. The ^1H signal of H_3-10 showed an HMBC correlation to an aromatic carbon signal (C-4) and an NOE with the H_3-9 (OMe) singlet, which had an HMBC correlation to an aromatic carbon signal (C-3). HMBC correlations were observed from H_2-8 to NMe_2 (C-12) and vice versa. Therefore, three sets of ^1H and ^{13}C NMR signals were assigned except each one aromatic carbon signal, which had no cross peak in any 2D NMR spectra and was deduced as C-2.

^1H and ^{13}C NMR data for three identical aromatic units

Table 1. ^{13}C (150 MHz) and ^1H NMR (600 MHz) data for the three units (TFA salts) in **1** (CD_3OD)

C#	Unit 1		Unit 2		Unit 3		HMBC
	^{13}C (δ)	^1H (δ, m)	^{13}C (δ)	^1H (δ, m)	^{13}C (δ)	^1H (δ, m)	
1	142.2[a]		140.3[b]		140.3[c]		
2	142.3[d]		141.2[d]		137.9[d]		
3	156.9		158.8		159.0		
4	153.6		153.5		158.2		
5	135.1		134.4		133.6		
6	136.9[a]		134.9[b]		135.0[c]		
7	31.3	3.80, m	30.7	3.71, m	30.6	3.68, m	1, 5, 6, 8
		4.01, m		3.92, m		3.85, m	1, 5, 6, 8
8	58.3	3.00, m	58.3	3.25, m	58.2	3.23, m	7, 12
		3.27, m		3.36, m		3.32, m	7, 12
9	61.1	3.99, s	60.5	3.70, s	60.8	3.67, s	3
10	60.8	3.34, s	60.4	3.15, s	62.3	3.88, s	4
11	19.3	2.53, s	19.1	2.46, s	19.1	2.44, s	5
12	43.4	3.06, br s	43.4	3.06, br s	43.4	2.96, br s	8, 12

[a,b,c,d] Signals are interchangeable within the same letters.

(Table 1) assigned as above were similar to those for **6** and **7**. Subtraction of the sum of three aromatic units from the molecular formula of **1** remained four sulfur atoms. Therefore, each aromatic unit was connected through one disulfide and two sulfide bonds. Thus, the gross structure of **1** was assigned to have a tetracyclic ring composed of trimeric aromatic amines and a 10-membered polysulfur ring.

Four geometric isomers were possible for lissoclibadin 1 with the orientation of three aromatic units, cyclos(-1-S-S-1-2-S-1-2-S-2-) (clockwise, structure **1** shown in scheme), cyclo(-1-S-S-2-1-S-1-2-S-2-) (**1a**), cyclo(-2-S-S-1-2-S-1-2-S-1-) (**1b**), and cyclo(-2-S-S-2-1-S-1-2-S-1-2-) (**1c**). An NOE was detected between two OMe signals at δ_H 3.99 and 3.67 due to two 9 positions in the different aromatic units. Therefore, **1b** and **1c** were eliminated from the isomer of lissoclibadin 1.

Although the geometry of two dimeric compounds, lissoclinotoxin D[2] and lissoclin disulfoxide,[5] was not discussed in the original papers, these compounds were subjected to the computational energy minima calculations together with two new dimeric compounds, lissoclinotoxins E and F, by Ireland and co-workers.[8] We have, therefore, employed the same calculation method to two possible isomers (**1** and **1a**) of lissoclibadin 1.

Montecarlo conformational analysis in vacuo was performed on noncharged isomers (**1**, **1a**, **1b**, and **1c**) with MM2 force field utilizing MacroModel® software.[11] The global energy minima calculations revealed that the isomer shown as **1** had the lowest global energy minimum value, and the isomer **1a** showed 0.8 kcal/mol higher value than **1** (isomers **1b** and **1c** had, respectively, 3.7 and 4.6 kcal/mol higher values than **1**).[10] We also tried to assign the connectivity by a linear dithioether compound produced from **1** by the reduction of disulfide bond followed by methylation. The linear product, however, did not show good ^{13}C and 2D NMR spectra probably because of the nature of linear structure and the small sample size. Since we used the sample for bioassays, a further reaction could not be done. Therefore, we have further performed the energy minima calculations in water on noncharged and charged (tri-cation) isomers. The isomer **1** had the lowest energy minimum values in both cases as shown in Table 2.

Table 2. Relative energy value of energy minima of four possible isomers of lissoclibadin 1

MM2	**1**	**1a**	**1b**	**1c**
In vacuo	0.0[a]	+3.2	+15.3	+19.4
Noncharged	(0.0)[b]	(+0.8)	(+3.7)	(+4.6)
In H_2O	0.0	+2.2	+3.6	+0.2
Noncharged	(0.0)	(+0.5)	(+0.9)	(+0.0)
In H_2O	0.0	+25.5	+26.3	+26.6
Tri-cation	(0.0)	(+6.1)	(+6.3)	(+6.4)

[a] kJ/mol.
[b] kcal/mol.

From these results, we have selected the isomer **1** as the most probable structure of lissoclibadin 1. However, the other isomer (**1a**) cannot be excluded since biosynthetic enzymes may construct a thermodynamically more unfavorable structure.[8,10]

2.3. Structure of lissoclibadin 2

Lissoclibadin 2 (**2**) was isolated as a bis-TFA salt. The molecular weight (602) and formula ($C_{26}H_{38}N_2O_4S_5$) were the same as those of lissoclinotoxin F (**5**). ^1H and ^{13}C NMR data for **2** was assigned by ^1H–^1H COSY, HMQC, HMBC, and NOEY spectra and showed two sets of signals ascribable to two identical aromatic amine units (Table 3), which had the same structure as those in **1** and **5**. Compound **5** has symmetric structure of the *cis*-orientation, and, accordingly, one set of signals due to the aromatic amine units was detected in the ^1H and ^{13}C NMR spectra of **5**. The other *cis*-isomer, which has not been detected so far, also has a symmetric structure. Therefore, **2** should have asymmetric structure of the *trans*-orientation.

Moreover, **2** showed an NOE correlation between an OMe at δ 3.93 (H_3-9 of unit 1) and NMe_2 at δ 3.08 (H_6-12′ of unit 2) in the NOESY spectrum. Consequently, the structure of lissoclibadin 2 was definitely assigned as **2**.

2.4. Structures of lissoclibadin 3 and lissoclinotoxin E

Lissoclibadin 3 (**3**) was isolated as a bis-TFA salt. ^1H and ^{13}C NMR data for **3** revealed one set of signals ascribed to

Table 3. ^{13}C (150 MHz) and ^1H NMR (600 MHz) data for the two units (TFA salts) in **2** (CD_3OD)

C#	Unit 1		Unit 2		HMBC	NOESY
	^{13}C (δ)	^1H (δ, m)	^{13}C (δ)	^1H (δ, m)		
1	140.9[a]		127.3[b]			
2	134.1[c]		133.5[c]			
3	155.7		151.2			
4	157.8		156.1			
5	135.8		133.5			
6	136.9[a]		139.0[b]			
7	29.7	3.66, m	29.3	3.87, m	1, 5, 6, 8	8, 12
8	57.7	3.24, m	57.9	3.34, m	7, 12	7, 12
9	62.4	3.93, s	61.2	3.82, s	3	10
10	61.2	3.94, s	61.1	3.88, s	4	9, 11
11	19.1	2.49, s	19.3	2.45, s	5	10
12	43.6	3.03, s	43.6	3.08, s	8, 12	7, 8

[a,b,c] Signals are interchangeable within the same letters.

the aromatic amine units (Table 4), which had the same structure as those in **1**, **2**, and **5**. MS and NMR data for **3** suggested that **3** has a symmetric structure. The molecular weight (570) and formula ($C_{26}H_{38}N_2O_4S_4$) of **3** were identical to those of lissoclinotoxin E.[8] Lissoclinotoxin E gave one set of ^1H and ^{13}C NMR signals and, therefore, was assigned to have the symmetric structure. Since ^1H and ^{13}C NMR data for **3** were not identical with those for lissoclinotoxin E,[8] these two compounds have different structures. Compound **3** showed an NOE correlation between an OMe (H_3-9) and NMe_2 (H_6-12) in the NOESY spectrum. The orientation of two aromatic amine units in **3** was, therefore, deduced as trans. This was first assigned as a structure of lissoclinotoxin E.[8] Accordingly, the structure of lissoclinotoxin E should be revised to **4**. Lissoclinotoxin E (**4**) was also isolated from *L.* cf. *badium* used in the present study and showed the identical spectral data to those for the reported values.[8]

Table 4. ^{13}C (150 MHz) and ^1H NMR (600 MHz) data for **3** (CD_3OD)

C#	^{13}C (δ)	^1H (δ, m)	HMBC	NOESY
1	132.6			
2	133.1			
3	152.1			
4	155.9			
5	134.0			
6	134.9			
7	29.3	3.71, m	1, 5, 6, 8	8, 12
8	57.7	3.25, m	6, 7, 12	7, 12
9	61.9	3.97, s	3	10, 12
10	61.3	3.92, s	4	9, 11
11	19.3	2.44, s	5	10
12	43.6	3.08, s	8, 12	7, 8, 9

2.5. Biological activity

Compounds **1–7** showed different magnitudes of antimicrobial activity (Table 5). Two monomeric compounds (**6** and **7**) had stronger and wider antimicrobial spectra among them. Interestingly, lissoclibadin 3 (**3**) and lissoclinotoxin E (**4**) did not inhibit the growth of five test microorganisms. These compounds have two sulfide bonds that the two aromatic moieties are connected.

Three new compounds **1–3** showed cytotoxicity against the human leukemia cell line HL-60 at IC_{50} values of 0.37 (0.33), 0.21 (0.13), and 5.5 (3.16) μM (μg/mL), respectively. Compounds **5–7** revealed the IC_{50} values of 0.23 (0.14), 6.2 (2.17), and 2.9 (1.20) μM (μg/mL), respectively, in the same experiment.

3. Experimental

NMR spectra were measured on either a JEOL JNM A-500 or ECP-600 NMR spectrometer. Mass spectra were obtained by a JEOL HX-110 mass spectrometer (FAB mode, *m*-nitrobenzylalcohol as matrix). UV and IR spectra were recorded on a Shimadzu UV-300 and on a JASCO A-102, respectively.

3.1. Ascidian

Lissoclinum sp. (most probably *L. badium* Monniot, F. and Monniot, C., 1996)[9] was collected by SCUBA diving at −15 to −20 m of the coral reef in Manado, Indonesia in November, 2003. The voucher specimen is deposited at the Nagoya University Museum as NUM-Az0391. The ascidian was collected again at the same site in September, 2004.

3.2. Isolation of alkaloids

The ascidian was immediately cut into small pieces and soaked in ethanol on a boat. The ethanol extract (14.0 g) was redissolved in MeOH–H_2O (9/1, 100 mL) and extracted with *n*-hexane. The aqueous MeOH layer was diluted with water (60% MeOH/H_2O) and extracted with *n*-BuOH. The BuOH extract (2.3 g) showed moderate antimicrobial activity and was separated into 13 fractions (B-1–B-13) by an ODS column (MeOH–H_2O gradient). The fraction B-4 (0.05 g) was chromatographed on a SiO_2 column ($CHCl_3$/MeOH/NH_4OH, 20:1:0.05, 10:1:0.1, 80:20:1, 70:30:5) to yield fractions B-4-1–B-4-9. The HPLC separation (ODS, 70% MeOH/H_2O containing 0.1% TFA, 254 nm) of the fraction B-4-9 afforded compound **1** (5.4 mg). Compounds **2** (9.3 mg) and **3** (4.5 mg) were isolated from the fraction B-4-3 by HPLC (ODS, 65% MeOH/H_2O containing 0.1% TFA, 254 nm). The fraction B-8 gave compound **5** (5.0 mg) and a mixture of **6** and **7** by SiO_2 column chromatography. The hexane extract (0.15 g) showed a strong antifungal and antibacterial activities and was subjected to SiO_2 column followed by HPLC (ODS, 70% MeOH/H_2O containing 0.1% TFA, 254 nm) to give compounds **6** (10 mg) and **7** (10 mg).

The ethanol extract (12.0 g), obtained from the ascidian

Table 5. Antimicrobial activity of compounds **1–7**

Compound	*M. hiemalis*[a]		*R. atlantica*			*S. cerevisiae*		*S. aureus*	*E. coli*	
	50[b]	20	50	20	5	50	20	50	50	20
1	—[c]	—	23.4[d]	15.2	—	—	—	—	—	—
2	13.8	—	28.2	21.2	12.2	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	—
5	18.0	10.5	20.0	12.1	—	—	—	—	—	—
6	23.0	17.4	32.4	23.3	14.2	11.8	—	10.3	17.8	14.4
7	26.2	19.6	30.0	24.5	15.8	15.2	10.5	14.2	17.1	13.1

[a] Test microorganisms: see Section 3.
[b] Amount (μg/disk).
[c] Not active.
[d] Inhibition zone (mm).

collected in 2004, was subjected to similar separation procedures to afford **1** (11.2 mg), **2** (9.0 mg), **3** (4.6 mg), **4** (12.7 mg), **5** (16.5 mg), **6** (6.8 mg), and **7** (5.0 mg).

3.2.1. Lissoclibadin 1 (1). Compound **1** was isolated as a Tris–TFA salt: UV λ_{max} nm (ε): 279 (134,300), 318 (sh, 26,500); IR ν_{max} (KBr) cm^{-1}: 3440, 2928, 2851, 2816, 2775, 1635, 1446, 1381, 1268, 1059, 1024, 962; HRFABMS [$(M+H)^+$, *m/z* 888.2347, Calcd for $C_{39}H_{58}N_3O_6S_7$, 888.2371]; ^1H and ^{13}C NMR data are listed in Table 1.

3.2.2. Lissoclibadin 2 (2). Compound **2** was isolated as a bis-TFA salt: UV λ_{max} nm (ε): 270 (14,300), 316 (6500); IR ν_{max} (KBr) cm^{-1}: 2941, 1681, 1453, 1387, 1202, 1132, 1061, 1015, 955; HRFABMS [$(M+H)^+$, *m/z* 603.1531, Calcd for $C_{26}H_{39}N_2O_4S_5$, 603.1513]; ^1H and ^{13}C NMR data are listed in Table 2.

3.2.3. Lissoclibadin 3 (3). Compound **3** was isolated as a bis-TFA salt: UV λ_{max} nm (ε): 274 (13,300), 318 (4500); IR ν_{max} (KBr) cm^{-1}: 2934, 1681, 1451, 1397, 1205, 1132, 1023, 958; HRFABMS [$(M+H)^+$, *m/z* 571.1768, Calcd for $C_{26}H_{39}N_2O_4S_4$, 571.1793]; ^1H and ^{13}C NMR data are listed in Table 3.

3.3. Reduction and methylation of 1

Lithium tri-*tert*-butoxyaluminohydride (20 mg) in dry THF (0.2 mL) was added to a solution of **1** (4.6 mg) in dry THF (2 mL), and the mixture was stirred under N_2 at room temperature for 6 h. Methyl iodide (0.1 mL) was added to the mixture and further stirred for 2 h, and 10% NH_4Cl (2 mL) was added to the reaction mixture and extracted with CH_2Cl_2 (5 mL×3). The organic extract was dried over Na_2SO_4 and evaporated to give the reaction product (3.2 mg): ^1H NMR (800 MHz, CD_3OD): δ 2.46, 2.49, 2.51, 2.52, and 2.53 (SMe×5), 3.3–3.5 (NMe_2×3 and OMe×1), 3.40–3.45 (H_2-8×2), 3.63 (H_2-8), 3.83, 3.86, and 4.03 (H_2-7×3).

3.4. Antimicrobial activity

The growth inhibitory activity of compounds **1–7** was examined by the paper disk method against *M. hiemalis* IAM 6088 (fungus), *R. atlantica* TUF-D (marine bacterium),[12] *Saccharomyces cerevisiae* IAM 1438T (yeast), *Staphylococcus aureus* IAM 12544T (Gram+ bacterium), *Escherichia coli* IAM 12119T (Gram− bacterium) as test microorganisms. The results are listed in Table 4.

3.5. Cytotoxicity

The human leukemia cell line HL-60 was incubated in RPMI 1640 using 24-well assay plates. Samples were dissolved in MeOH, and 10 μL of each sample was poured in a well and the solvent evaporated in a clean bench. The suspension (1 mL, 4×10^4 cells/mL) of HL-60 was added to each well and incubated at 37 °C for 72 h in a CO_2 incubator. The shape of the cells was observed after 72 h under an inverted microscope. The number of vital cells in the sample wells after 72 h was compared with those in the control wells using XTT [2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide] (cell proliferation kit II®).

Acknowledgements

We thank Dr. T. Oda and Ms. J. Yamada of Kyoritsu College of Pharmacy for measuring some NMR spectra and Dr. H. Hirota for 800 MHz NMR. We also thank Mr. S. B. Pratasik for his help in sample collection. This work was supported in part by a grant-in-aid for scientific research ('Creation of Biologically Functional Molecules', Group 4, Subject no. 17035029) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan to M. N.

References and notes

1. Davidson, B. S.; Molinski, T. F.; Barrows, L. R.; Ireland, C. M. *J. Am. Chem. Soc.* **1991**, *113*, 4709–4710.
2. Searle, P. A.; Molinski, T. F. *J. Org. Chem.* **1994**, *59*, 6600–6605.
3. Litaudon, M.; Trigalo, F.; Martin, M.-T.; Frappier, F.; Guyot, M. *Tetrahedron* **1994**, *50*, 5323–5334.
4. Compagnone, R. S.; Faulkner, D. J.; Carte, B. K.; Chan, G.; Hemling, M. A.; Hofmann, G. A.; Mattern, M. R. *Tetrahedron* **1994**, *50*, 12785–12792.
5. Patil, A. D.; Freyer, A. J.; Killmer, L.; Zuber, G.; Carte, B.; Jurewicz, A. J.; Johnson, R. K. *Nat. Prod. Lett.* **1997**, *10*, 225–229.
6. Appleton, D. R.; Pearce, A. N.; Lambert, G.; Babcock, R. C.; Copp, B. R. *Tetrahedron* **2002**, *58*, 9779–9783.
7. Makarieva, T. N.; Stonik, V. A.; Dmitrenok, A. S.; Grebnev, B. B.; Iskov, V. V.; Rebachyk, N. M. *J. Nat. Prod.* **1995**, *58*, 254–258.
8. Davis, R. A.; Sandoval, I. T.; Concepcion, G. P.; da Rocha, R. M.; Ireland, C. M. *Tetrahedron* **2003**, *59*, 2855–2859.
9. Monniot, F.; Monniot, C. *Micronesica* **1996**, *29*, 133–279.
10. Liu, H.; Pratasik, S. B.; Nishikawa, T.; Shida, T.; Tachibana, K.; Fujiwara, T.; Nagai, H.; Kobayashi, H.; Namikoshi, M. *Tetrahedron Lett.* **2004**, *45*, 7015–7017.
11. *MacroModel® software, version 6.0*; Department of Chemistry, Columbia University: New York, 1995.
12. Namikoshi, M.; Negishi, R.; Nagai, H.; Dmitrenok, A.; Kobayashi, H. *J. Antibiot.* **2003**, *56*, 755–761.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron 61 (2005) 8616–8624
Tetrahedron

Synthesis and properties of 5-[bis(1-heteroazulen-3-yl)-methylidene]pyrimidine-2,4,6(1,3,5*H*)-triones

Shin-ichi Naya, Kyosuke Yoda and Makoto Nitta*

Department of Chemistry, School of Science and Engineering, Waseda University, Shinjuku-ku, Tokyo 169-8555, Japan

Received 20 May 2005; revised 28 June 2005; accepted 5 July 2005

Available online 26 July 2005

Abstract—The synthesis and properties of a novel type of 5-[bis(1-heteroazulen-3-yl)methylidene]pyrimidine-2,4,6(1,3,5*H*)-triones (**13a–c**) and (**14a–c**) are studied. The synthetic procedure is based on addition of bis(1-heteroazulen-3-yl)methyl cations with barbituric acid and subsequent oxidation by *o*-chloranil. Structural characteristics of **13a–c** and **14a–c** were clarified on inspection of the ^{13}C NMR spectral data and X-ray crystal analysis. Based on the investigation of the UV–vis spectra of **13a–c** and **14a–c** and their protonated cations, conformational change of the heteroazulene-moiety and the barbituric acid-moiety is suggested. In the CV measurements of **13a–c** and **14a–c**, two reversible reduction waves are observed, indicating the stabilizing ability of heteroazulenes toward the corresponding radical and anion species. Furthermore, **13a–c** and **14a–c** exhibit one irreversible oxidation wave and the corresponding reduction wave appearing in a far negative region, which suggested a conformational change in the radical cation during the redox process. The conformational change is rationalized on the basis of the MO calculations.

1. Introduction

Conjugated π-electron chromophores containing a donor and an acceptor group have attracted current interest in terms of optoelectronic materials[1] such as nonlinear optics[2] and near-infrared dyes.[3] Among numerous classes of these chromophores, compounds containing a quinonoid unit as a spacer are especially promising,[4] because such conjugated systems have generally been recognized to possess marked push–pull electronic effects, which induce large dipole moments leading to high nonlinear response[5] and ready intramolecular charge-transfer transitions resulting in deep coloration.[6] Gompper and co-workers have reported the synthesis of a number of dicyanoquinodimethanes including 7,7-dicyano-8,8-diphenyl-1,4-quinodimethane (**1**) (Fig. 1).[7] Furthermore, Oda and co-workers have reported recently the synthesis and properties of **1** and its derivatives.[8] Thus, benzoquinonoid compounds have hitherto played a most important role in the development of organic redox chemistry due to their multistage redox properties.[9]

On the other hand, we have studied the synthesis and properties of heteroazulene analogues of the triphenylmethyl cation, that is, tris(2-oxo-2*H*-cyclohepta[*b*]furan-3-yl)methyl cation and pyrrole analogues,[10] as well as bis(2-oxo-2*H*-cyclohepta[*b*]furan-3-yl)phenylmethyl cations (**2a–e**) and their pyrrole analogues (**3a–e**).[11] Through these

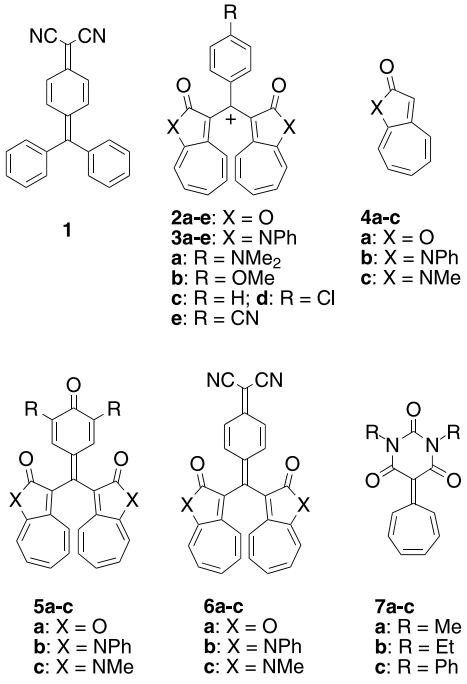
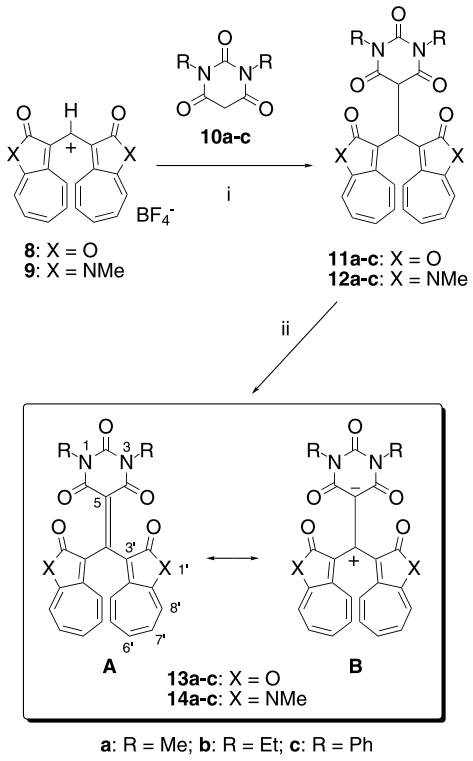


Figure 1.

Keywords: 7,7-Bis(heteroazulen-3-yl)-8,8-dicyano-1,4-quinodimethanes; Heteroazulene; Redox potential; Conformational change.
* Corresponding author. Tel.: +81 3 5286 3236; fax: +81 3 3208 2735; e-mail: nitta@waseda.jp

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.07.001



Scheme 1. Reagents and conditions: (i) Et_3N, CH_3CN, rt 5 min; (ii) *o*-chloranil, $CHCl_3$, rt 48 h.

studies, we clarified the stabilizing ability of heteroazulenes such as **4a–c** toward the methyl cations and the anomalous substituent effect of the substituted phenyl groups of **2a–e** and **3a–e** arising from their conformational change.[11] In this context, we also reported the synthesis and properties of heteroazulene-substituted benzene-1,3-bismethylium derivatives[12] and benzene-1,3,5-trismethylium derivatives.[13] In these cations, two or three methylium units are twisted against the central phenyl group, respectively, and conjugation among the methylium units is not observed. Furthermore, heteroazulenes such as **4a–c** are demonstrated to stabilize not only cations but also radical and anion species based on the studies of the pK_{R+} values and reduction potentials.[11,14] From this viewpoint, we have recently reported the synthesis and properties of α,α-bis(heteroazulen-3-yl)-1,4-benzoquinonemethides (**5a–c**)[15] as well as 7,7-bis(heteroazulen-3-yl)-8,8-dicyano-1,4-quinodimethanes (**6a–c**),[16] which are expected to have multistage redox properties. The polarized structural nature of **5a–c** and **6a–c** was suggested on inspection of their ^{13}C NMR and IR spectra. In addition, their remarkable redox properties were also clarified by the CV measurement. Furthermore, we have recently reported the synthesis of the novel heptafulvene **7a–c**, and their structural detail and redox properties were clarified.[17] Since the nonlinear optical properties of the compound having π-conjugation between the azulene- and the thiobarbituric acid-moiety have been reported,[18] compounds having a π-conjugation between the heteroazulene- and the barbituric acid-moiety are very interesting for the exploration of novel functions. Based on this concept, we have now investigated the synthesis and properties of 5-[bis(1-heteroazulen-3-yl)methylidene]pyrimidine-2,4,6(1,3,5*H*)-triones (**13a–c**) and (**14a–c**) to involve the barbituric acid-moiety instead of the 4-dicyanomethylidene-2,5-cyclohexadienylidene-moiety. To gain insight into the polarized structural nature of **13a–c** and **14a–c**, their ^{13}C NMR spectra and X-ray crystal analysis are studied. Based on a measurement of the CV, the remarkable redox property of **13a–c** and **14a–c** is also demonstrated. We report, herein the results in detail.

2. Results and discussion

2.1. Synthesis

The preparation of 5-[bis(1-heteroazulen-3-yl)methylidene] pyrimidine-2,4,6(1,3,5*H*)-triones was accomplished by the addition of bis(1-heteroazulen-3-yl)methyl cations with barbituric acid and subsequent oxidation. Reactions of *N*,*N*-disubstituted barbituric acids (**10a–c**)[19] with each of the bis(2-oxo-2*H*-cyclohepta[*b*]furan-3-yl)methyl cation (**8**)[20] and the bis(1,2-dihydro-*N*-methyl-2-oxocyclohepta[*b*]-pyrrol-3-yl)methyl cation (**9**)[20] in CH_3CN at rt for 5 min afforded 5-[bis(1-heteroazulen-3-yl)methyl]pyrimidine-2,4,6(1,3,5*H*)-triones (**11a–c**) and (**12a–c**) in good yields, respectively (Scheme 1, Table 1). Compounds **11a–c** and **12a–c** are yellow and orange crystals, the structures of which were assigned on the basis of their IR, ^1H and ^{13}C NMR spectral data as well as the mass spectral data and elemental analyses. Cations **8** and **9** were synthesized easily by the hydride abstraction of the corresponding methane derivatives by using DDQ;[20] however, the reaction of **11a** with DDQ afforded a complex mixture containing cation **8** derived from the C–C bond cleavage. Thus, the dehydrogenation reaction of **11a–c** and **12a–c** was carried out by using 2.2 mol equiv amounts of *o*-chloranil in $CHCl_3$ at rt for 48 h to give **13a–c** and **14a–c** in modest yields (Scheme 1, Table 1).

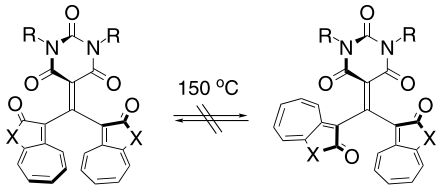
2.2. Properties

The structures of **13a–c** and **14a–c** were assigned based on their spectral data and elemental analyses. In the ^1H NMR spectra at room temperature, two sets of proton signals were

Table 1. Results for the preparation of methanes **11a–c** and **12a–c** and dehydrogenated compounds **13a–c** and **14a–c**

			Addition		Dehydrogenation	
Run	Cation	Barbituric acid	Product	Yield/%	Product	Yield/%
1	**8**	**10a**	**11a**	80	**13a**	48
2	**8**	**10b**	**11b**	95	**13b**	31
3	**8**	**10c**	**11c**	79	**13c**	35
4	**9**	**10a**	**12a**	93	**14a**	31
5	**9**	**10b**	**12b**	76	**14b**	49
6	**9**	**10c**	**12c**	83	**14c**	50

observed for **13a–c** and **14a–c** in the ratio of 5:1. In each set of signals, two heteroazulene-moieties appeared equivalent. Thus, these signals suggest that **13a–c** and **14a–c** exist as mixtures of *syn*- and *anti*-conformers (Fig. 2). Although the ^{1}H NMR spectra of **13a–c** and **14a–c** at higher temperature exhibited a broadening of signals, coalescence of signals was not observed even at 150 °C. Thus, an exchange of *syn*- and *anti*-conformers does not occur at that temperature on the NMR time scale. The calculated heats of formation of *anti*-**13a** and *syn*-**13a** by the AM1 method (MOPAC)[22] are listed in Figure 2, suggesting that *anti*-**13a** is more stable than *syn*-**13a**. Thus, the major and minor conformers seem to be *anti*-**13a** and *syn*-**13a**, respectively. The ^{13}C NMR spectra of **13a–c** and **14a–c** were recorded and the chemical shifts of the C5 are summarized in Table 2. The chemical shifts of the C5 of **13a–c** are lower than those of **14a–c**. Thus, the contribution of the charge-separated ionic structures **13a–c-B** (Scheme 1) is slightly smaller as compared with the contribution of **14a–c-B**, depending on the electron-donating ability of the X:O < NMe. However, the chemical shifts of the C5 of **13a–c** and **14a–c** are lower than those of **7a–c** (δ_C 101.9–103.1), and thus, the contribution of the polarized structures **13a–c-B** and **14a–c-B** seems to be smaller than those of **7a–c**.



	Anti-13a	Syn-13a
Heat of formation kcalmol^{-1} (AM1)	-54.13	-50.53

Figure 2.

Table 2. ^{13}C NMR data and the longest wavelength absorption maxima (λ_{max}) of **13a–c** and **14a–c**

Compd	^{13}C NMR[a]/δ	λ_{max}/nm	
		CH_3CN	$CH_3CN+H_2SO_4$
13a	117.4 (118.2)	380, 509	—[b]
13b	117.2 (117.2)	380, 510	—[b]
13c	119.2 (119.3)	379, 517	—[b]
14a	115.7 (116.0)	412, 537	643[c]
14b	115.7 (116.4)	412, 539	643[c]
14c	115.5 (115.4)	410, 546	642[c]

[a] Chemical shift of C5-position; chemical shifts for minor conformer are shown in parentheses.
[b] No change was observed.
[c] Generation of cations **16a–c**.

A single crystal of a major conformer, *anti*-**13a**, was obtained by recrystallization of a mixture of *anti*-**13a** and *syn*-**13a** from $CHCl_3$. Thus, X-ray crystal analysis of **13a** was accomplished for the first time as the series of the heteroazulene-substituted methanes and methyl cations. The ORTEP drawing is shown in Figure 3 (contained solvent, $CHCl_3$, is omitted for clarity). The barbituric acid-moiety shows a small deformation from planarity to a boat-shape. The dihedral angles, θ_1, θ_2, and θ_3, express deviation of the plane of the barbituric acid-moiety and heteroazulenes from the cationic plane (the plane is defined by the C5 of the barbituric acid-moiety and two arylic ipso carbons as shown in Fig. 4). While the twist angle of barbituric acid-moiety is small ($\theta_1 = 18.5°$), the heteroazulene-moieties are twisted against the cationic plane (θ_2, $\theta_3 = 47.5$, 39.4°) due to steric hindrance. The bond length of C1–C2 is 1.371 Å, which is slightly longer than the simple C=C bond length (1.337 Å),[21] suggesting the small contribution of the polarized structure **13a-B**.

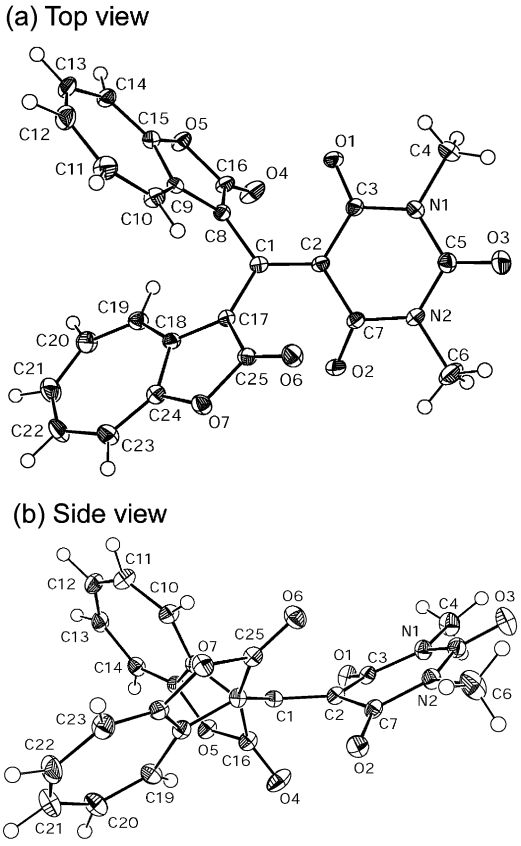
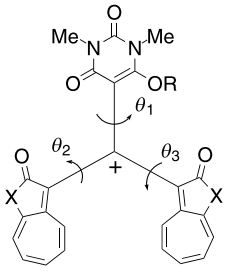


Figure 3. ORTEP drawing of **13a** with thermal ellipsoid plot (50% probability).



(R = -) (R = H) (R = •)
13a: X = O **15a**: X = O **23a** (**23'a**): X = O
14a: X = NMe **16a**: X = NMe **24a** (**24'a**): X = NMe

Figure 4.

The UV–vis spectra of **13a–c** and **14a–c** in CH_3CN are shown in Figures 5 and 6. The spectra of **13a–c** are similar, and two absorption maxima were observed in the visible region as summarized in Table 2. A similar feature is observed in the spectra of **14a–c**. By adding a drop of H_2SO_4, compounds **14a–c** seemed to be completely protonated to give cations **16a–c** (Scheme 2). The longest wavelength absorption maxima of **16a–c** are also summarized in Table 2. In contrast, addition of H_2SO_4 to the solution of **13a–c** did not cause change of the visible region, and thus, cations **15a–c** would not be generated.

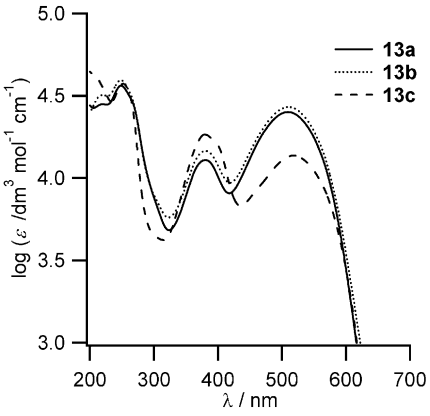


Figure 5. UV–vis spectra of **13a–c** in CH_3CN.

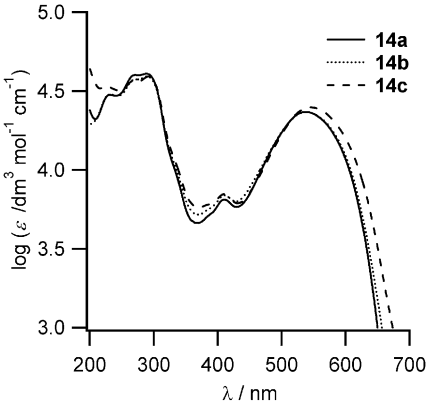
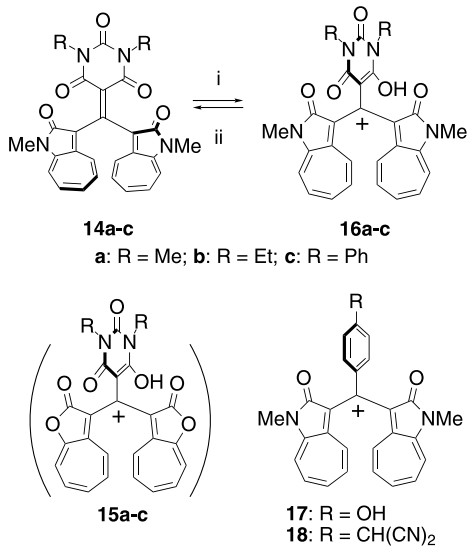


Figure 6. UV–vis spectra of **14a–c** in CH_3CN.



Scheme 2. Reagents and conditions: (i) H_2SO_4, CH_3CN, rt; (ii) Et_3N, CH_3CN, rt.

The feature is rationalized from the larger stability of **9** ($pK_{R+}=10.3$) as compared with that of **8** ($pK_{R+}=2.6$). Upon addition of a drop of Et_3N to the cations **16a–c**, **14a–c** were regenerated quantitatively (confirmed by the UV–vis measurement). Thus, the protonation–deprotonation cycle is completely reversible. The absorption maxima of **16a–c** are similar to those of **17** (646 nm)[15] and **18** (647 nm),[16] suggesting that the electronic effect of the barbituric acid-moiety is less important as in the case of the phenyl group in **17** and **18**. This feature seems to be reasonable based on consideration of the stable conformation of **17**[15] and **18**,[16] in which the phenyl group is twisted and the heteroazulene moieties exist in a cationic plane (the plane is defined by three arylic ipso carbons, cf. Fig. 4). Regarding the charge-separated ionic structures **14a–c-B**, the 2,4(1,3*H*)-dioxopyrimizine-5-oxylide-moiety has a higher electron-donating ability than the heteroazulene-moiety. Thus, the 2,4(1,3*H*)-dioxopyrimizine-5-oxylide-moiety exists in a more planar conformation and the heteroazulene-moiety is twisted against the cationic plane due to steric hindrance. On the contrary, the heteroazulene-moiety has a higher electron-donating ability than the barbituric acid-moiety in cations **16a–c**. Since the heteroazulene-moiety has a more planar conformation and the barbituric acid-moiety is twisted against the cationic plane, conjugation between the barbituric acid-moiety and the methylium carbon becomes

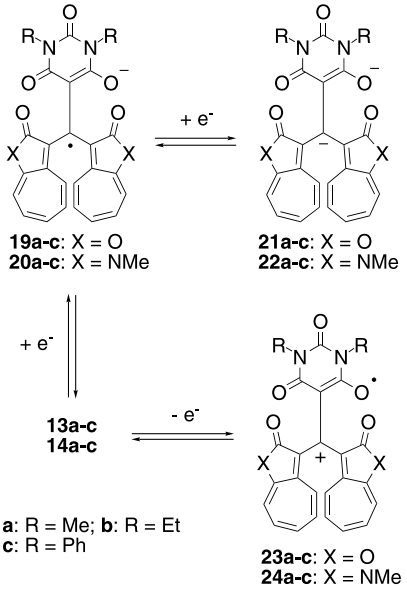
Table 3. Reduction and oxidation potentials of compounds **13a–c** and **14a–c** and reference compounds **6a**,**c**

	Reduction potential[a]/V		Oxidation potential[a]/V		
Compd	$E1_{red}$	$E2_{red}$	$E1_{ox}$	$[E_{red}]^b$	$E2_{ox}$
13a	−0.88	−1.44	(+1.10)	[(+0.33)]	—
13b	−0.89	−1.46	(+1.09)	[(+0.32)]	—
13c	−0.85	−1.44	(+1.09)	[(+0.30)]	—
14a	−1.13	−1.68	(+0.78)	[(+0.08)]	—
14b	−1.10	−1.70	(+0.77)	[(+0.10)]	—
14c	−1.07	−1.68	(+0.81)	[(+0.09)]	—
6a[c]	−0.70	−1.28	(+0.51)	[(−0.15)]	(+1.55)
6c[c]	−0.98	−1.54	(+0.23)	[(−0.58)]	(+1.21)

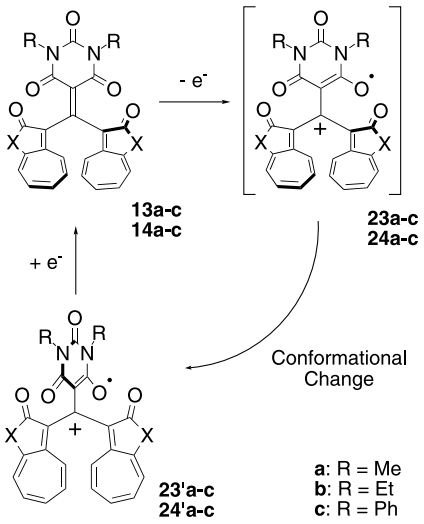
[a] V versus $Ag/AgNO_3$; mean value of the cathodic and anodic peaks. Irreversible processes are shown in parentheses.
[b] Corresponding reduction wave.
[c] See Ref. 16.

smaller. Thus, the effect of the barbituric acid-moiety becomes less important. The electron-donating ability of these moieties was confirmed by MO calculations and CV measurement (vide infra).

The reduction and oxidation potentials of **13a–c** and **14a–c** determined by cyclic voltammentry (CV) in CH_3CN are summarized in Table 3, along with those of reference compounds **6a**,**c**.[16] The two reduction waves of **13a–c** and **14a–c** are reversible under the conditions of the CV measurements. The two waves ($E1_{red}$ and $E2_{red}$) are explained by the formation of stable radical anions **19a–c** and **20a–c** and dianions **21a–c** and **22a–c**, respectively (Scheme 3). These characteristics are due to the hetero-azulenes, which stabilize the radical species and the anion species.[11,14] The values ($E1_{red}$ and $E2_{red}$) of **13a–c** and **14a–c** are more negative than those of **6a** and **6c**, respectively. The feature suggests the smaller electron affinity of **13a–c** and **14a–c** as compared with **6a** and **6c**, respectively. On the other hand, one oxidation wave was recorded also in the measurements of **13a–c** and **14a–c** as summarized in Table 3. The oxidation waves ($E1_{ox}$) of **13a–c** and **14a–c** are irreversible, and can be explained by the formation of radical cations **23a–c** and **24a–c**, respectively (Scheme 3). The values ($E1_{ox}$) of **13a–c** and **14a–c** are more positive than those of **6a** and **6c**, respectively. The more negative reduction potentials and the more positive oxidation potentials of **13a–c** and **14a–c** would be ascribed to the smaller contribution of the polarized structure **13a–c-B** and **14a–c-B** as compared with those of **6a**,**c**. A remarkable observation is the reduction waves corresponding to the first oxidation waves of **13a–c** and **14a–c** appearing in a far negative region (**13a–c**: +0.30–+0.33 V, **14a–c**: +0.08–+0.10 V). These reduction waves are suggested to correspond to the reduction waves of **23′a–c** and **24′a–c**, which are generated by the conformational change of **23a–c** and **24a–c**, respectively, under CV measurement (Scheme 4). In the radical cations **23a–c** and **24a–c** generated by the one-electron oxidation of **13a–c** and **14a–c**, the heteroazulene-



Scheme 3.



Scheme 4.

moiety has a larger electron-donating ability than the 2,4(1,3*H*)-dioxopyrimizine-5-oxyl radical-moiety. Thus, the two heteroazulene units come to have a more planar conformation and the 2,4(1,3*H*)-dioxopyrimizine-5-oxyl radical-moiety would be twisted against the cationic plane as depicted by **23′a–c** and **24′a–c**. By this conformational change giving **23′a–c** and **24′a–c**, the heteroazulene-moiety comes to stabilize **23a–c** and **24a–c** more effectively, and thus, the energy level of the SOMO of **23′a–c** and **24′a–c** (HOMO of **13a–c** and **14a–c**) is raised and the corresponding reduction waves would be shifted to the far negative region. In order to confirm this speculation, MO calculations of heteroazulenes (**4a–c**), **10a′**, **25**, and **26** (Fig. 7) were carried out by using the AM1 method (MOPAC),[22] and their energy levels of HOMOs and LUMOs are summarized in Table 4, together with the oxidation potentials ($E1_{red}$) obtained by CV. The value ($E1_{red}$) of **25** was determined by the CV measurement of the *tert*-butylammonium salt of **25** obtained by the reaction of **10a** with *tert*-butylamine. The calculated energy levels of HOMO of **4a–c** are much lower

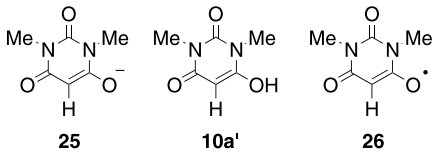


Figure 7.

Table 4. Energy levels of HOMO and LUMO and oxidation potentials of **4a–c**, **25**, **10a′** and **26**

Compd.	Energy level		Oxidation potential[a]
	HOMO/eV	LUMO/eV	$E1_{ox}$/V
4a	−7.891	1.361	+1.13
4b	−7.461	1.641	+0.91
4c	−7.410	1.741	+0.83
25[b]	−3.808	8.895	+0.40
10a′	−9.422	3.312	>+2.00
26	−11.283	−10.765	—

[a] V versus $Ag/AgNO_3$; irreversible process.
[b] Salt **25** $Bu^tNH_3^+$ was used for the CV measurement.

than that of **25**, and they correlate well with the oxidation potentials obtained by CV, respectively. Furthermore, the energy levels of HOMO of **4a–c** are much higher than those of **10a′** and **26**. Thus, the feature suggests that the electron-donating ability is in the order **25** > **4a–c** > **10a′**, **26**. Consequently, the MO calculations and CV measurement of **4a–c**, **10a′**, **25**, and **26** would support the conformational change during the protonation–deprotonation cycle of **14a–c** as well as the redox process of **13a–c** and **14a–c**.

3. Conclusion

A convenient preparation of novel 5-[bis(1-heteroazulen-3-yl)methylidene]pyrimidine-2,4,6(1,3,5*H*)-triones (**13a–c**) and (**14a–c**) was accomplished. The properties of **13a–c** and **14a–c** were clarified by inspection of the ^{13}C NMR and X-ray analysis as well as UV–vis spectra. The contribution of the polarized structures **13a–c-B** and **14a–c-B** was suggested to be smaller than those of **6a**,**c** and **7a–c**. Owing to the stabilizing ability of heteroazulenes toward the radical and anion species, **13a–c** and **14a–c** exhibited two reversible reduction waves in the CV measurements. Furthermore, **13a–c** and **14a–c** exhibited one irreversible oxidation wave, which suggested a conformational change during the redox process. The conformational change is rationalized on the basis of MO calculations. Further studies concerning the synthesis and properties of heteroazulene-substituted methane analogues are under way.

4. Experimental

4.1. General

IR spectra were recorded on a HORIBA FT-710 spectrometer. UV–vis spectra were recorded on a Shimadzu UV-3101PC spectrometer. Mass spectra and high-resolution mass spectra were run on JMS-AUTOMASS 150 and JMS-SX102A spectrometers. Unless otherwise specified, ^{1}H and ^{13}C NMR spectra were recorded on JNM-AL400 and AVANCE600 spectrometers, and the chemical shifts are given relative to internal $SiMe_4$ standard; *J*-values are given in Hz. Mps were recorded on a Yamato MP-21 apparatus and were uncorrected. Bis(2-oxo-2*H*-cyclohepta[*b*]furan-3-yl)methyl tetrafluoroborate (**8**) and bis(1,2-dihydro-*N*-methyl-2-oxocyclohepta[*b*]pyrrol-3-yl)methyl tetrafluoroborate (**9**) were prepared as described previously.[20] Compound **10a** is commercially available, and desired compounds **10b**,**c** were prepared as described in the literature.[19]

4.2. General synthetic procedure for 11a–c and 12a–c

A solution of each barbituric acid **10a–c** (0.1 mmol) and Et_3N (0.11 mg, 0.11 mmol) in CH_3CN (20 mL) was stirred at rt for 5 min. To the solution was added **8** or **9** (0.1 mmol) and the mixture was stirred at rt for 2 h. After the reaction was completed, the mixture was concentrated in vacuo. The resulting residue was poured into H_2O and the mixture was extracted with CH_2Cl_2. The extract was dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by column chromatography on SiO_2 (AcOEt) to give the products **11a–c** and **12a–c** (Table 1).

4.2.1. [5-[Bis(2-oxo-2*H*-cyclohepta[*b*]furan-3-yl)]methyl]-1,3-dimethylpyrimidine-2,4,6(1,3,5*H*)-trione (11a). Orange needles; mp 182–184 °C dec (from CH_2Cl_2/Et_2O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.29 (6H, s, Me), 4.21 (1H, d, J=4.3 Hz, H-5), 5.14 (1H, d, J=4.3 Hz, CH), 6.83 (2H, dd, J=10.7, 9.5 Hz, H-6′), 6.94 (2H, dd, J=11.4, 10.7 Hz, H-5′), 7.02 (2H, dd, J=10.4, 9.5 Hz, H-7′), 7.05 (2H, d, J=10.4 Hz, H-8′), 7.10 (2H, d, J=11.4 Hz, H-4′); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ 28.8, 30.4, 49.0, 105.0, 114.6, 127.4, 131.3, 132.8, 135.5, 148.9, 151.0, 157.5, 166.9, 169.1; IR (KBr) ν 1732, 1683, 1266 cm^{-1}; MS (FAB) *m*/*z* 459 (M^++H). HRMS calcd for $C_{25}H_{18}N_2O_7$: 459.1192 (M+H). Found: 459.1210 (M^++H). Anal. Calcd for $C_{25}H_{18}N_2O_7\cdot CH_2Cl_2$: C, 57.47; H, 3.71; N, 5.15. Found: C, 57.23; H, 3.70; N, 4.91.

4.2.2. [5-[Bis(2-oxo-2*H*-cyclohepta[*b*]furan-3-yl)]methyl]-1,3-diethylpyrimidine-2,4,6(1,3,5*H*)-trione (11b). Orange prisms; mp 190–192 °C dec (from CH_2Cl_2/Et_2O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.19 (6H, t, J=6.9 Hz, CH_3), 3.83–3.96 (4H, m, CH_2), 4.23 (1H, d, J=4.8 Hz, H-5), 5.10 (1H, d, J=4.8 Hz, CH), 6.82 (2H, dd, J=11.2, 10.2 Hz, H-6′), 6.94 (2H, dd, J=11.5, 11.2 Hz, H-5′), 7.01 (2H, dd, J=10.2, 9.2 Hz, H-7′), 7.04 (2H, d, J=9.2 Hz, H-8′), 7.14 (2H, d, J=11.5 Hz, H-4′); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ 13.0, 30.7, 37.4, 49.1, 105.2, 114.6, 127.3, 131.1, 132.7, 135.3, 148.8, 151.0, 157.4, 166.5, 169.0; IR (KBr) ν 1734, 1706, 1685, 1672, 1269 cm^{-1}; MS (FAB) *m*/*z* 487 (M^++H). HRMS calcd for $C_{27}H_{22}N_2O_7$: 487.1505 (M+H). Found: 487.1476 (M^++H). Anal. Calcd for $C_{27}H_{22}N_2O_7\cdot H_2O$: C, 64.28; H, 4.80; N, 5.55. Found: C, 64.52; H, 4.61; N, 5.40.

4.2.3. [5-[Bis(2-oxo-2*H*-cyclohepta[*b*]furan-3-yl)]methyl]-1,3-diphenylpyrimidine-2,4,6(1,3,5*H*)-trione (11c). Yellow needles; mp 190–192 °C dec (from $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.64 (1H, d, J=3.9 Hz, H-5), 5.26 (1H, d, J=3.9 Hz, CH), 6.79 (2H, dd, J=11.0, 9.5 Hz, H-6′), 6.89 (2H, dd, J=11.7, 11.0 Hz, H-5′), 6.99 (2H, d, J=9.2 Hz, H-8′), 7.04 (2H, dd, J=9.5, 9.2 Hz, H-7′), 7.06 (2H, d, J=11.7 Hz, H-4′), 7.28–7.48 (10H, m, Ph); ^{13}C NMR (150.9 MHz, DMSO-d_6) δ 29.9, 49.4, 79.1, 104.3, 114.7, 126.8, 128.4, 128.6, 128.9, 131.4, 133.2, 135.3, 135.8, 148.0, 151.0, 156.3, 166.8, 168.1; IR (KBr) ν 1734, 1717, 1700, 1269 cm^{-1}; MS (FAB) *m*/*z* 583 (M^++H). HRMS calcd for $C_{35}H_{22}N_2O_7$: 583.1505 (M+H). Found: 583.1520 (M^++H). Anal. Calcd for $C_{35}H_{22}N_2O_7\cdot 1/2CHCl_3$: C, 66.39; H, 3.53; N, 4.36. Found: C, 66.29; H, 3.58; N, 4.19.

4.2.4. [5-[Bis(1,2-dihydro-*N*-methyl-2-oxocyclohepta[*b*]pyrrol-3-yl)]methyl]-1,3-dimethylpyrimidine-2,4,6(1,3,5*H*)-trione (12a). Orange needles; mp 148–150 °C dec (from CH_2Cl_2/Et_2O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.29 (6H, s, Me), 3.57 (6H, s, N1′Me), 4.39 (1H, d, J=4.2 Hz, H-5), 5.50 (1H,d, J=4.2 Hz, CH), 6.74–6.84 (4H, m, H-5′, H-6′), 6.91 (2H, d, J=9.2 Hz, H-8′), 7.04 (2H, dd, J=9.8, 9.2 Hz, H-7′), 7.22 (2H, d, J=11.7 Hz, H-4′); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ 26.7, 28.6, 31.3, 49.8, 110.0, 111.7, 127.6, 128.9, 130.4, 130.8, 141.2, 144.6, 152.4, 167.6,

168.2; IR ($CDCl_3$) ν 1696, 1675, 1654 cm^{-1}; MS (FAB) *m/z* 485 ($M^+ + H$). HRMS calcd for $C_{27}H_{24}N_4O_5$: 485.1825 (M+H). Found: 485.1829 ($M^+ + H$). Anal. Calcd for $C_{27}H_{24}N_4O_5 \cdot CH_2Cl_2$: C, 59.06; H, 4.60; N, 9.84. Found: C, 59.30; H, 4.81; N, 9.81.

4.2.5. [5-[Bis(1,2-dihydro-*N*-methyl-2-oxocyclohepta[*b*]pyrrol-3-yl)]methyl]-1,3-diethylpyrimidine-2,4,6(1,3,5*H*)-trione (12b). Orange powder; mp 166–168 °C dec (from CH_2Cl_2/Et_2O); ^1H NMR (600 MHz, $CDCl_3$) δ 1.14 (6H, t, J=7.2 Hz, CH_3), 3.56 (6H, s, N1′Me), 3.83–3.96 (4H, m, CH_2), 4.59 (1H, d, J=4.6 Hz, H-5), 5.43 (1H,d, J=4.6 Hz, CH), 6.79 (2H, dd, J=10.8, 10.0 Hz, H-6′), 6.84 (2H, dd, J=11.0, 10.8 Hz, H-5′), 6.89 (2H, d, J=9.2 Hz, H-8′), 7.03 (2H, dd, J=10.0, 9.2 Hz, H-7′), 7.40 (2H, d, J=11.0 Hz, H-4′); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ 13.2, 26.6, 32.3, 37.0, 49.7, 110.5, 111.6, 127.7, 128.9, 130.4, 130.9, 141.3, 144.6, 151.5, 167.2, 168.2; IR (KBr) ν 1696, 1669, 1653 cm^{-1}; MS (FAB) *m/z* 513 ($M^+ + H$). HRMS calcd for $C_{29}H_{28}N_4O_5$: 513.2138 (M+H). Found: 513.2165 ($M^+ + H$). Anal. Calcd for $C_{29}H_{28}N_4O_5 \cdot 1/2H_2O$: C, 66.78; H, 5.60; N, 10.74. Found: C, 66.70; H, 5.22; N, 10.74.

4.2.6. [5-[Bis(1,2-dihydro-*N*-methyl-2-oxocyclohepta[*b*]pyrrol-3-yl)]methyl]-1,3-diphenyl pyrimidine-2,4,6(1,3,5*H*)-trione (12c). Orange powder; mp 203–205 °C dec (from CH_2Cl_2/Et_2O); ^1H NMR (600 MHz, $CDCl_3$) δ 4.89 (1H, d, J=4.2 Hz, H-5), 5.61 (1H,d, J=4.2 Hz, CH), 6.70–6.78 (4H, m, H-5′, H-6′), 6.90 (2H, d, J=9.2 Hz, H-8′), 7.01 (2H, t, J=9.2 Hz, H-7′), 7.23 (2H, d, J=10.8 Hz, H-4′), 7.33 (6H, t, J=7.2 Hz, *o*-Ph, *p*-Ph), 7.40 (4H, t, J=7.6 Hz, *m*-Ph); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ 26.7, 32.0, 50.2, 109.0, 111.7, 127.6, 128.5, 128.8, 128.9, 129.0, 130.4, 130.9, 135.3, 141.5, 144.6, 167.2, 168.5 (one carbon overlapping); IR (KBr) ν 1700, 1685, 1652 cm^{-1}; MS (FAB) *m/z* 609 ($M^+ + H$). HRMS calcd for $C_{37}H_{28}N_4O_5$: 609.2138 (M+H). Found: 609.2175 ($M^+ + H$). Anal. Calcd for $C_{37}H_{28}N_4O_5 \cdot 1/2H_2O$: C, 71.95; H, 4.73; N, 9.07. Found: C, 72.02; H, 4.43; N, 9.14.

4.3. Preparation of 13a–c and 14a–c

To a stirred solution of each compound **11a–c** and **12a–c** (0.1 mmol) in $CHCl_3$ (20 mL) was added *o*-chloranil (54 mg, 0.22 mmol), and the mixture was stirred at rt for 48 h. After the reaction was completed, the mixture was concentrated in vacuo. The resulting residue was purified by column chromatography on SiO_2 (acetone/AcOEt=1:1) to give the products **13a–c** and **14a–c** (Table 1).

4.3.1. [5-[Bis(2-oxo-2*H*-cyclohepta[*b*]furan-3-yl)]methylidene]-1,3-dimethyl-pyrimidine-2,4,6(1,3,5*H*)-trione (13a). Dark red plates; mp 196–199 °C dec (from CH_2Cl_2/Et_2O); ^1H NMR (600 MHz, $CDCl_3$) (*anti*-**13a**) δ 3.33 (6H, s, Me), 6.98 (2H, dd, J=11.2, 11.0 Hz, H-6′), 7.08 (2H, dd, J=11.5, 11.2 Hz, H-5′), 7.19 (2H, d, J=11.5 Hz, H-4′), 7.20 (2H, dd, J=11.0, 9.2 Hz, H-7′), 7.25 (2H, d, J=9.2 Hz, H-8′); (*syn*-**13a**) δ 3.34 (6H, s, Me), 7.13–7.17 (2H, m, H-6′), 7.25–7.29 (2H, m, H-5′), 7.33–7.39 (6H, m, H-4′, H-7′, H-8′); ^{13}C NMR (150.9 MHz, $CDCl_3$) (*anti*-**13a**) δ 28.7, 109.5, 117.4, 127.9, 129.3, 132.8, 134.9, 137.5, 145.9, 148.8, 149.2, 150.8, 160.9, 165.6; (*syn*-**13a**) δ 28.7, 109.5, 118.2, 127.9, 129.3, 133.3, 135.7, 137.8, 145.9, 148.8, 150.8, 159.1, 160.9, 165.6; IR ($CDCl_3$) ν 1751, 1665, 1268 cm^{-1}; MS (FAB) *m/z* 457 ($M^+ + H$). HRMS calcd for $C_{25}H_{16}N_2O_7$: 457.1036 (M+H). Found: 457.1043 ($M^+ + H$). Anal. Calcd for $C_{25}H_{18}N_2O_7 \cdot H_2O$: C, 63.29; H, 3.82; N, 5.90. Found: C, 62.96; H, 4.00; N, 5.59.

4.3.2. [5-[Bis(2-oxo-2*H*-cyclohepta[*b*]furan-3-yl)methylidene]-1,3-diethyl-pyrimidine-2,4,6 (1,3,5*H*)-trione (13b). Dark red needles; mp 247–250 °C dec (from CH_2Cl_2/Et_2O); ^1H NMR (400 MHz, $CDCl_3$) (*anti*-**13b**) δ 1.22 (6H, t, J=6.8 Hz, CH_3), 3.87–4.05 (4H, m, CH_2), 6.96 (2H, t, J=9.5 Hz, H-6′), 7.07 (2H, dd, J=11.2, 9.5 Hz, H-5′), 7.15–7.25 (4H, m, H-4′, H-7′), 7.34 (2H, d, J=9.5 Hz, H-8′); (*syn*-**13b**) δ 1.24 (6H, t, J=6.8 Hz, CH_3), 3.87–4.05 (4H, m, CH_2), 7.11–7.39 (10H, m, H-4′, H-5′, H-6′, H-7′, H-8′); ^{13}C NMR (150.9 MHz, $CDCl_3$) (*anti*-**13b**) δ 13.4, 37.4, 109.7, 117.2, 127.9, 129.3, 132.7, 134.8, 137.4, 145.6, 148.7, 150.0, 158.6, 160.5, 165.6; (*syn*-**13b**) δ 13.6, 37.4, 109.7, 118.0, 127.9, 129.3, 133.2, 135.7, 137.6, 146.2, 148.7, 150.1, 159.2, 160.5, 165.6; IR (KBr) ν 1751, 1720, 1649, 1263 cm^{-1}; MS (FAB) *m/z* 485 ($M^+ + H$). HRMS calcd for $C_{27}H_{20}N_2O_7$: 485.1349 (M+H). Found: 485.1336 ($M^+ + H$). Anal. Calcd for $C_{27}H_{20}N_2O_7 \cdot 1/2H_2O$: C, 65.72; H, 4.29; N, 5.68. Found: C, 65.67; H, 4.15; N, 5.51.

4.3.3. [5-[Bis(2-oxo-2*H*-cyclohepta[*b*]furan-3-yl)methylidene]-1,3-diphenyl-pyrimidine-2,4,6(1,3,5*H*)-trione (13c). Dark brown prisms; mp 198–201 °C dec (from CH_2Cl_2/Et_2O); ^1H NMR (400 MHz, $CDCl_3$) (*anti*-**13c**) δ 6.98 (2H, t, J=9.3 Hz, H-7′), 7.10 (2H, dd, J=11.7, 11.0 Hz, H-5′), 7.15–7.25 (6H, m, H-4′, H-6′, H-8′), 7.30–7.50 (10H, m, Ph); (*syn*-**13c**) δ 7.14–7.50 (20H, m, H-4′, H-5′, H-6′, H-7′, H-8′, Ph); ^{13}C NMR (150.9 MHz, DMSO-d_6) (*anti*-**13c**) δ 106.3, 119.2, 128.0, 128.5, 129.0, 129.7, 130.3, 134.6, 135.8, 136.6, 139.2, 146.6, 149.8, 151.3, 158.2, 160.8, 165.0; (*syn*-**13c**) δ 106.8, 119.3, 128.0, 128.6, 129.0, 129.7, 130.3, 134.8, 135.9, 136.7, 139.3, 147.1, 150.0, 151.3, 158.2, 161.0, 165.6; IR (KBr) ν 1739, 1671, 1267 cm^{-1}; MS (FAB) *m/z* 581 ($M^+ + H$). HRMS calcd for $C_{35}H_{20}N_2O_7$: 581.1349 (M+H). Found: 581.1371 ($M^+ + H$). Anal. Calcd for $C_{35}H_{20}N_2O_7 \cdot CH_2Cl_2$: C, 64.97; H, 3.33; N, 4.21. Found: C, 64.91; H, 3.48; N, 4.08.

4.3.4. [5-[Bis(1,2-dihydro-*N*-methyl-2-oxocyclohepta[*b*]pyrrol-3-yl)methylidene]-1,3-dimethylpyrimidine-2,4,6(1,3,5*H*)-trione (14a). Dark brown powder; mp >300 °C dec (from CH_2Cl_2/Et_2O); ^1H NMR (600 MHz, $CDCl_3$) (*anti*-**14a**) δ 3.31 (6H, s, Me), 3.66 (6H, s, N1′Me), 6.85–6.97 (4H, m, H-5′, H-6′), 7.06 (2H, d, J=9.4 Hz, H-8′), 7.20 (2H, dd, J=9.8, 9.4 Hz, H-7′), 7.28 (2H, d, J=11.8 Hz, H-4′); (*syn*-**14a**) δ 3.31 (6H, s, Me), 3.52 (6H, s, N1′Me), 7.11–7.16 (4H, m, H-5′, H-6′), 7.28 (2H, d, J=9.8 Hz, H-8′), 7.35 (2H, dd, J=10.0, 9.8 Hz, H-7′), 7.58 (2H, d, J=11.0 Hz, H-4′); ^{13}C NMR (150.9 MHz, $CDCl_3$) (*anti*-**14a**) δ 27.2, 28.5, 114.1, 115.7, 128.2, 130.3, 132.9, 133.7, 142.9, 146.4, 149.6, 151.5, 161.5, 165.8 (one carbon overlapping); (*syn*-**14a**) δ 26.9, 28.5, 114.6, 116.0, 128.8, 130.8, 133.7, 134.4, 142.9, 147.0, 149.6, 151.5, 161.5, 165.8 (one carbon overlapping); IR (KBr) ν 1716, 1678, 1658 cm^{-1}; MS (FAB) *m/z* 483 ($M^+ + H$). HRMS calcd for $C_{27}H_{22}N_4O_5$: 483.1668 (M+H). Found: 483.1661 ($M^+ + H$). Anal. Calcd

for $C_{27}H_{22}N_4O_5 \cdot H_2O$: C, 64.79; H, 4.83; N, 11.19. Found: C, 64.39; H, 4.58; N, 10.92.

4.3.5. [5-[Bis(1,2-dihydro-*N*-methyl-2-oxocyclohepta[*b*]pyrrol-3-yl)methylidene]-1,3-diethylpyrimidine-2,4,6(1,3,5*H*)-trione (14b). Dark brown powder; mp 255–258 °C dec (from CH_2Cl_2/Et_2O); ^{1}H NMR (400 MHz, $CDCl_3$) (*anti*-**14b**) δ 1.20 (6H, t, J=7.1 Hz, CH_3), 3.65 (6H, s, N1′Me), 3.87–4.03 (4H, m, CH_2), 6.86–6.97 (4H, m, H-5′, H-6′), 7.05 (2H, d, J=9.5 Hz, H-8′), 7.12–7.22 (2H, m, H-7′), 7.29 (2H, d, J=11.4 Hz, H-4′); (*syn*-**14b**) δ 1.20 (6H, t, J=7.1 Hz, CH_3), 3.52 (6H, s, N1′Me), 3.87–4.03 (4H, m, CH_2), 7.07–7.17 (4H, m, H-5′, H-6′), 7.29 (2H, d, J=10.0 Hz, H-8′), 7.35 (2H, dd, J=10.4, 10.0 Hz, H-7′), 7.56 (2H, d, J=11.0 Hz, H-4′); ^{13}C NMR (150.9 MHz, $CDCl_3$) (*anti*-**14b**) δ 13.5, 27.1, 37.1, 114.0, 115.7, 128.3, 130.2, 132.7, 133.5, 142.8, 146.4, 149.3, 150.6, 161.2, 165.8, (one carbon overlapping); (*syn*-**14b**) δ 13.6, 26.9, 37.1, 114.6, 116.4, 128.8, 130.7, 133.7, 134.2, 142.8, 147.0, 149.3, 150.6, 161.2, 165.8 (one carbon overlapping); IR (KBr) ν 1701, 1678, 1652 cm^{-1}; MS (FAB) *m*/*z* 511 (M^++H). HRMS calcd for $C_{29}H_{26}N_4O_5$: 511.2012 (M+H). Found: 511.1996 (M^++H). Anal. Calcd for $C_{29}H_{26}N_4O_5 \cdot 1/4CH_2Cl_2$: C, 66.06; H, 5.02; N, 10.54. Found: C, 66.25; H, 4.86; N, 10.54.

4.3.6. [5-[Bis(1,2-dihydro-*N*-methyl-2-oxocyclohepta[*b*]pyrrol-3-yl)methylidene]-1,3-dipheylpyrimidine-2,4,6(1,3,5*H*)-trione (14c). Dark brown needles; mp >300 °C dec (from CH_2Cl_2/Et_2O); ^{1}H NMR (400 MHz, $CDCl_3$) (*anti*-**14c**) δ 3.60 (6H, s, N1′Me), 6.84–6.96 (4H, m, H-5′, H-6′), 7.01 (2H, d, J=9.5 Hz, H-8′), 7.10 (2H, dd, J=9.5, 9.2 Hz, H-7′), 7.16 (2H, d, J=11.2 Hz, H-4′), 7.28–7.48 (10H, m, Ph); (*syn*-**14c**) δ 3.49 (6H, s, N1′Me), 7.03–7.15 (4H, m, H-5′, H-6′), 7.28–7.48 (14H, m, H-7′, H-8′, Ph), 7.70 (2H, d, J=11.0 Hz, H-4′); ^{13}C NMR (150.9 MHz, $CDCl_3$) (*anti*-**14c**) δ 27.2, 114.1, 115.5, 128.2, 128.3, 128.9, 130.3, 133.1, 133.8, 135.5, 143.2, 146.4, 146.6, 150.7, 150.9, 162.6, 165.2 (one carbon overlapping); (*syn*-**14c**) δ 26.9, 114.9, 115.4, 128.2, 128.3, 129.0, 130.9, 134.0, 134.6, 135.7, 143.2, 146.4, 147.3, 150.9, 151.0, 162.6, 165.2 (one carbon overlapping); IR (KBr) ν 1728, 1670, 1648 cm^{-1}; MS (FAB) *m*/*z* 607 (M^++H). HRMS calcd for $C_{37}H_{26}N_4O_5$: 607.1981 (M+H). Found: 607.2019 (M^++H). Anal. Calcd for $C_{37}H_{26}N_4O_5 \cdot 1/3CH_2Cl_2$: C, 70.62; H, 4.23; N, 8.82. Found: C, 70.54; H, 4.25; N, 8.74.

4.4. Cyclic voltammetry of 13a–c and 14a–c

The reduction potential of **13a–c** and **14a–c** was determined by means of CV-27 voltammetry controller (BAS Co). A three-electrode cell was used, consisting of Pt working and counter electrodes and a reference Ag/$AgNO_3$ electrode. Nitrogen was bubbled through an acetonitrile solution (4 mL) of **13a–c** and **14a–c** (0.5 mmol dm^{-3}) and Bu_4NClO_4 (0.1 mol dm^{-3}) to deaerate it. The measurements were made at a scan rate of 0.1 V s^{-1} and the voltammograms were recorded on a WX-1000-UM-019 (Graphtec Co) X-Y recorder. Immediately after the measurements, ferrocene (0.1 mmol) ($E_{1/2}$=+0.083) was added as the internal standard, and the observed peak potential was corrected with reference to this standard. Compounds **13a–c** and **14a–c** exhibited two reversible reduction waves and one irreversible oxidation wave, and they are summarized in Table 3.

4.5. X-ray structure determination of 13a[†]

Dark reddish prisms, $C_{25}H_{16}N_2O_7+CHCl_3$, M=575.79, monoclinic, space group $P2_1/c$, a=9.177(2) Å, b=12.835(4) Å, c=21.019(6) Å, β=99.80(1)°, V=2439(1) Å^3, Z=4, D_c=1.567 g mL^{-1}, crystal dimensions 0.60×0.60×0.40 mm^3. Data were measured on a Rigaku RAXIS-RAPID radiation diffractomater with graphite monochromated Mo Kα radiation. Total 24009 reflections were collected, using the $\omega-2\theta$ scan technique to a maximum 2θ value of 55.0°. The structure was solved by direct methods and refined by a full-matrix least-squares method using SIR92 structure analysis software,[23] with 360 variables and 4303 observed reflections [$I>3.00\sigma(I)$]. The non-hydrogen atoms were refined anisotropically. The weighting scheme $w=[0.5000\times\sigma_c(F_0^2)+0.0030\times F_0^2+0.5000]^{-1}$ gave satisfactory agreement analysis. The final R and R_w values were 0.0360 and 0.0600. The maximum peak and minimum peak in the final difference map were 0.50 and −0.42 e$^-$/Å^3.

Acknowledgements

Financial support from a Waseda University Grant for Special Research Project and 21COE 'Practical Nanochemistry' from MEXT, Japan, is gratefully acknowledged. We thank the Materials Characterization Central Laboratory, Waseda University, for technical assistance with the spectral data, elemental analyses and X-ray analysis.

References and notes

1. Gompper, R.; Wagner, H.-U. *Angew. Chem., Int. Ed. Engl.* **1988**, *100*, 1492–1511.
2. (a) Kaino, T.; Tomaru, S. *Adv. Mater.* **1993**, *5*, 172–178. (b) Nie, W. *Adv. Mater.* **1993**, *5*, 520–545. (c) Marder, S. R.; Perry, J. W. *Adv. Mater.* **1993**, *5*, 804–815.
3. Fabian, J.; Nakazumi, H.; Matsuoka, M. *Chem. Rev.* **1992**, *92*, 1197–1226.
4. Takahashi, K. *J. Synth. Org. Chem. Jpn.* **1986**, *44*, 806–818. *Chem. Abstr.* **1987**, *107*, 115439v and references cited therein.
5. (a) Lalama, S. J.; Singer, K. D.; Garito, A. F.; Desai, K. N. *Appl. Phys. Lett.* **1981**, *39*, 940–942. (b) Marder, S. R.; Beratam, D. N.; Cheng, L.-T. *Science* **1991**, *252*, 103–106. (c) Higuchi, H.; Nakayama, T.; Shimizu, K.; Koyama, H.; Ojima, J.; Wada, T.; Sasabe, H. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 2363–2377.
6. Kubo, Y.; Kuwana, M.; Okamoto, K.; Yoshida, K. *J. Chem. Soc., Chem. Commun.* **1989**, 855.
7. Gompper, R.; Wagner, H.-U.; Kutter, K. *Chem. Ber.* **1968**, *101*, 4123–4143 and 4144–4151.
8. Kawase, T.; Wakabayashi, M.; Takahashi, C.; Oda, M. *Chem. Lett.* **1997**, 1055–1056.
9. Chambers, J. Q. In *The Chemistry of the Quinonoid Compounds*; Pata, S., Ed.; Wiley, 1974; Chapter 14, p 737.

[†] CCDC reference number 271949.

10. Naya, S.; Nitta, M. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2777–2781.
11. Naya, S.; Nitta, M. *J. Chem. Soc., Perkin Trans. 2* **2000**, 2427–2435.
12. Naya, S.; Nitta, M. *J. Chem. Soc., Perkin Trans. 2* **2001**, 275–281.
13. Naya, S.; Isobe, M.; Hano, Y.; Nitta, M. *J. Chem. Soc., Perkin Trans. 2* **2001**, 2253–2262.
14. Naya, S.; Sakakibara, T.; Nitta, M. *J. Chem. Soc., Perkin Trans. 2* **2001**, 1032–1037.
15. Naya, S.; Watano, T.; Nitta, M. *Bull. Chem. Soc. Jpn.* **2003**, *76*, 2035–2043.
16. Naya, S.; Yoda, K.; Nitta, M. *Tetrahedron* **2004**, *60*, 4953–4958.
17. Naya, S.; Nitta, M. *Tetrahedron* **2003**, *59*, 3709–3718.
18. Asato, A. E.; Liu, R. S. H.; Rao, V. P.; Cai, Y. M. *Tetrahedron Lett.* **1996**, *37*, 419–422.
19. Ridi, M.; Aldo, G. *Gazz. Chim. Ital.* **1952**, *82*, 13–22.
20. Naya, S.; Nitta, M. *Tetrahedron* **2003**, *59*, 4157–4165.
21. Dean, J. A. *Lange's Handbook of Chemistry*, 11th ed.; McGraw-Hill: New York, 1973; pp 3-119–3-123.
22. Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902. Dewar, M. J. S.; Zoebisch, E. G. *THEOCHEM* **1988**, *180*, 1.
23. Altomare, A.; Burla, M. C.; Camalli, M.; Cascarano, M.; Giacovazzo, C.; Guagliardi, A.; Polidori, G. *J. Appl. Crystallogr.* **1994**, *27*, 435.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 8625–8632

Synthesis and characterization of novel fluorescent *N*-glycoconjugates

Ana P. Esteves,[a,*] Lígia M. Rodrigues,[a] Marília E. Silva,[a] Sanjay Gupta,[a] Ana M. F. Oliveira-Campos,[a] Oldrich Machalicky[b] and António J. Mendonça[c]

[a]*Department of Chemistry, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal*
[b]*Department of Organic Technology, Faculty of Chemical Technology, University of Pardubice, Czech Republic*
[c]*Department of Chemistry, University of Beira Interior, Covilhã, Portugal*

Received 29 March 2005; revised 30 June 2005; accepted 5 July 2005

Available online 26 July 2005

Abstract—Novel fluorescent *N*-glycoconjugates containing D-glucose, glycine and coumarin or naphthalenetriazole derivatives were prepared by peptide synthesis type methods. The fluorescence properties (spectra, quantum yields) of the compounds were evaluated.

1. Introduction

The glycoconjugates have an enormous potential in drug design.[1] Glycoproteins are widely distributed in nature and the sugar fragment influences their conformation and folding,[2] their properties such as solubility, bioavailability, thermal and proteolytical stability,[3] and enhances the transport through cell membranes.[2,4–6]

Glycopeptides, (which retain the carbohydrate–peptide linkage of a glycoprotein but lack its size and complexity) can mimic natural fragments of glycoproteins and have been largely used as targets for therapeutic agents and as models for biologically relevant systems.[7–11]

Another important field that has registered development is the application of fluorescent labels for several compounds with potential biological activity.[12,13] Among them, the fluorescent peptides[7,14–17] have a large number of applications in biochemistry and biology, namely in studies of protein interactions and conformational analysis.

Fluorescent markers are also being investigated for in vivo imaging studies, for example, in Alzheimer disease.[18] The most used fluorescent markers for peptides are rhodamine, fluorescein, coumarin and their derivatives.[16,19]

Sugars have also been used in the development of fluorescent reagents because they confer water solubility to organic fluorophores with no significant change in absorption and fluorescence properties.[20]

The need for homogeneous samples of the desired glycopeptides leads to significant development of several synthetic strategies.[7,8] The glycoamino acids are the building blocks for glycopeptide synthesis and several routes for their preparation have been reported.[21–25] The most commonly employed methods for the preparation of *N*-glycopeptides proceed through reduction of glycosyl azide to a labile intermediate glycosylamine that is subsequently condensed with the appropriately protected amino acid derivative.[15,21,26]

The work presented in this paper, is part of an ongoing project towards of the synthesis of fluorescent *N*-glycopeptides. Model compounds with a fluorescent aminoacid (the fluorescent labels were introduced at the C-terminus of glycine through an amide bond) were prepared to test the methodologies of synthesis and evaluate the influence either of the amino acid or/and the sugar in the fluorescent properties of the fluorophore (coumarin-3-carboxilic acid and 4-(naphtho[1,2-*d*][1,2,3]triazol-2-yl)benzoic acid). The labeled amino acid will be used in further work as building block for sequence analogues of RGD (arginine-glycine-aspartic acid) motif for binding essays. The absorption and fluorescence properties of these compounds were determined, in acetonitrile, and compared.

Keywords: *N*-Glycoconjugates; Fluorescent; Coumarin; Naphthalenetriazole.
* Corresponding author. Tel.: +351253604373; fax: +351253678983; e-mail: aesteves@quimica.uminho.pt

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.07.006

2. Results and discussion

In this work, the preparation of new fluorescent *N*-glycoconjugates based on acetylated D-glucose, glycine and coumarin or naphthalenetriazole derivatives was studied (Scheme 1). Evaluation of the fluorescence properties shown by different substrates was also carried out.

Treatment of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with sodium azide[27] gave the corresponding β-D-glucopyranosyl azide in 86% yield after recrystallisation. Catalytic hydrogenation of the azide[28] with Pd/C afforded the glucosylamine as the β anomer (based on ^{1}H NMR; $J_{H1H2}=8.7$ Hz) in almost quantitative yield (94%) and it was used with no further purification. Compounds **4** and **5** were obtained by the mixed anhydride method in 13 and 42% yield, respectively, but the same method was unsuccessful for the synthesis of compounds **6** and **7**. Therefore, glycine was coupled to the fluorescent dye by the HBTU method (*O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′,-tetramethyl-uroniumhexa-fluorophosphate) for compound **6** and by the DCC/HOBt (*N*,*N*′-dicyclohexylcarbodiimide/1-hydroxy-benzotriazole) method for **7** with yields of 45%. Hydrolysis of compounds **6** and **7** afforded the corresponding acids **8** and **9** in yields over 80%. Glycoconjugates **10** and **11** were synthesised by DCC/HOBt method in moderate yields, 45 and 46%, respectively. The synthesis of **10** was attempted by the Staudinger reaction between glycosyl azide and amino acid-dye substrate **8** in the presence of tributyl phosphine.[29] This method afforded the α-anomer, as deduced by ^{1}H NMR spectrum ($J=3.6$ Hz for the anomeric proton), instead of the β-anomer obtained by the DCC/HOBt method.

The compounds were isolated and characterized by NMR spectroscopy (^{1}H, ^{13}C, HMQC, HMBC) and elemental analysis or HRMS.

As the compounds prepared differ markedly in water solubility, acetonitrile, a common polar aprotic solvent, was chosen to measure their spectral properties. Besides, acetonitrile avoids changes in spectral curves resulting from dissociation of carboxylic hydrogen in non conjugated probes. Then it was possible to compare a substitution effect on fluorescence properties of starting dyes and their conjugates.

The spectra for compounds **2** and **10** and **3** and **11** are shown on Figures 1 and 2, respectively.

UV–vis absorption spectrum of **2** shows vibrational structure at 340 ($\varepsilon_{max}\approx 24{,}500$ dm^3 mol^{-1} cm^{-1}) and 360 nm, while for compound **3**, only a broad band at 300 nm ($\varepsilon_{max}\approx 11{,}400$ dm^3 mol^{-1} cm^{-1}) and a shoulder around 330 nm are observed. Replacement of the carboxyl group by amido groups (such as in **4**, **6** and **10**) has no significant effect on the position or molar absorptivity of the long-wavelength absorption bands (Fig. 1). A similar observation may be made for the position of the long-wavelength absorption bands of **3**, **5**, **7** and **11** (Fig. 2). However, the carboxylic acid **3** has a lower molar absorptivity ($\varepsilon_{max}\approx 11{,}400$ dm^3 mol^{-1} cm^{-1}) than the corresponding amides ($\varepsilon_{max}\approx 13{,}200$ dm^3 mol^{-1} cm^{-1}).

The steady-state fluorescence spectrum of **2** shows an emission band also with vibrational structure (maxima at 370 and 380 nm). The transformation of the carboxyl group of **2** to amido groups (**4**, **6** and **10**) does not affect either the shape or position of the fluorescence band but it has considerable effect on fluorescence quantum yield (53% for **2** and close to 100% for the other compounds, standard deviation is $\leq$2%). The relatively high absorption coefficient of the first absorption band of **2** and high fluorescence quantum yield lead to the conclusion that the first absorption band of the studied triazole derivatives has the character of S_0–$S_1(\pi\pi^*)$ transition. Moreover, the high quantum yield shows that none of possible triplet $n\pi^*$ states (nonbonding electrons on two heterocyclic nitrogens and nonbonding electrons on carbonyl group) is situated below $S_1(\pi\pi^*)$ state.

The steady-state fluorescence spectrum of **3** displays a broad emission band with maximum at 400 nm (shoulder at 370 nm) similar to the emission spectra of derivatives **5**, **7** and **11**. Fluorescence quantum yields were 1.8% (standard deviation is 0.1%) for **3** and 2.1–2.4% for compounds **5**, **7** and **11**.

As the spectra of compounds **2**, **4**, **6** and **10** and **3**, **5**,**7** and **11** show the same patterns, it is possible to conclude that the substitution on carboxylic groups of both tested fluorescence probes does not affect the π-electronic structure of fluorophores. Therefore, character, energy and sequence of excited states do not change.

3. Conclusions

The fluorescent probes studied show that the transformation of the carboxylic group into amido group in the different *N*-conjugates does not affect either the shape or the position of their fluorescence bands. On the other hand, in case of naphthalenetriazole derivatives, it has considerable effect on fluorescence quantum yield ~50% and almost 100% for the original carboxylic acid and amides, respectively. No appreciable changes in low quantum fluorescence yields (2%) were detected among original coumarin-3-carboxylic acid and its amido *N*-conjugates.

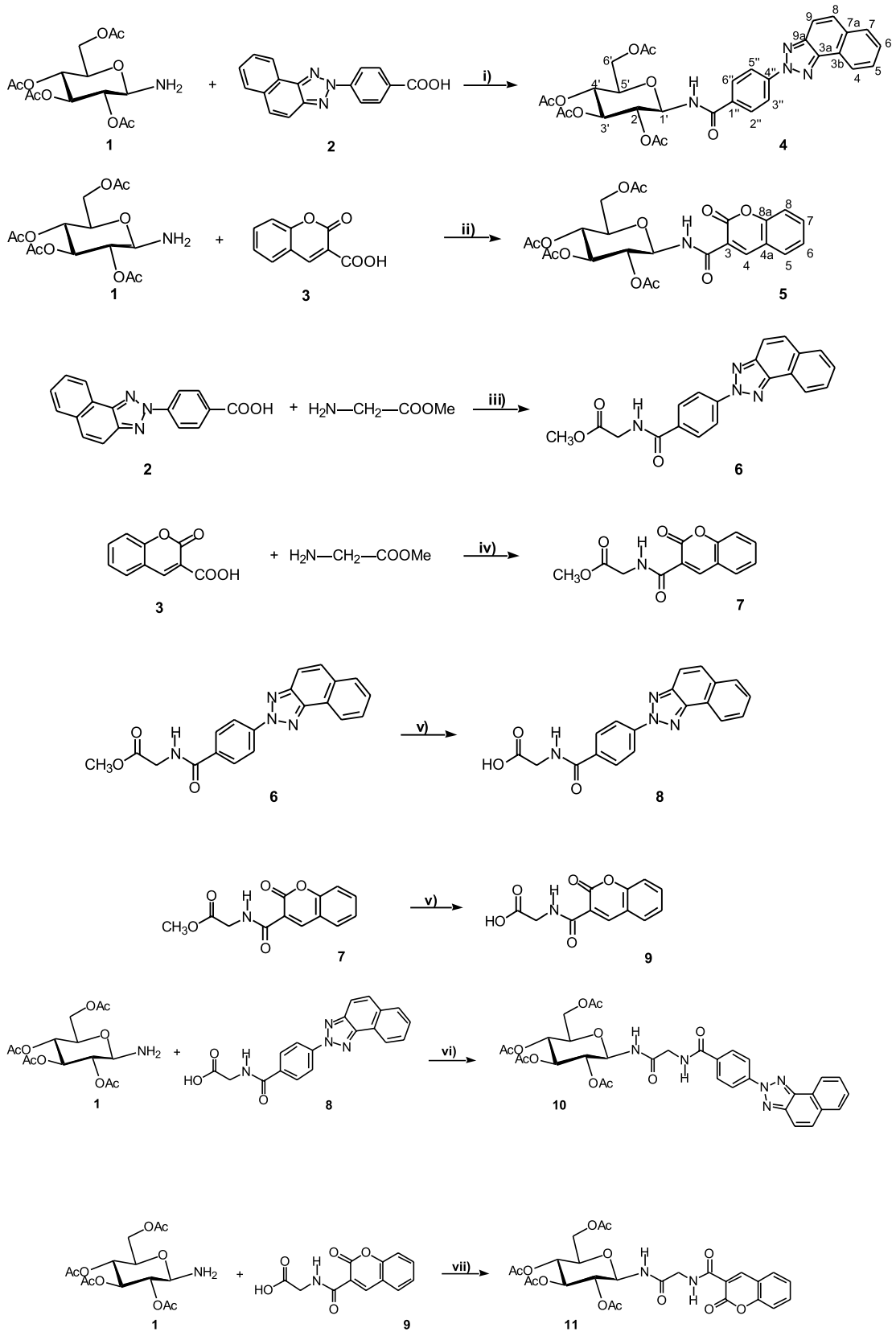
Our findings showed that the usual methods used in peptide synthesis gave acceptable results for the coupling of the glycosylamine to a fluorescent carboxylic dye or to fluorescent amino acid.

This methodology may provide a useful contribution for the design of new fluorescent glycopeptides.

4. Experimental

4.1. General

Dry solvents, referred to in the ensuing experiments, were prepared as follows: Acetone was refluxed over magnesium sulphate, decanted, stirred overnight with calcium chloride, decanted, refluxed over fresh magnesium sulphate, distilled from it and stored over 4 Å molecular sieves;



Scheme 1. Reagents and conditions: (i) $ClCO_2Et$, Et_3N, DMF, rt, 48 h; (ii) $ClCO_2Et$, Et_3N, acetone, reflux, 3 h; (iii) HBTU, DIPEA, DMF, rt, 12 h; (iv) DCC/HOBt, Et_3N, EtOAc, rt, 12 h; (v) (1) NaOH, MeOH, rt, 4 h; (2) HCl; (vi) DCC/HOBt, DMF, rt, 12 h; (vii) DCC/HOBt, DMF, rt, 48 h.

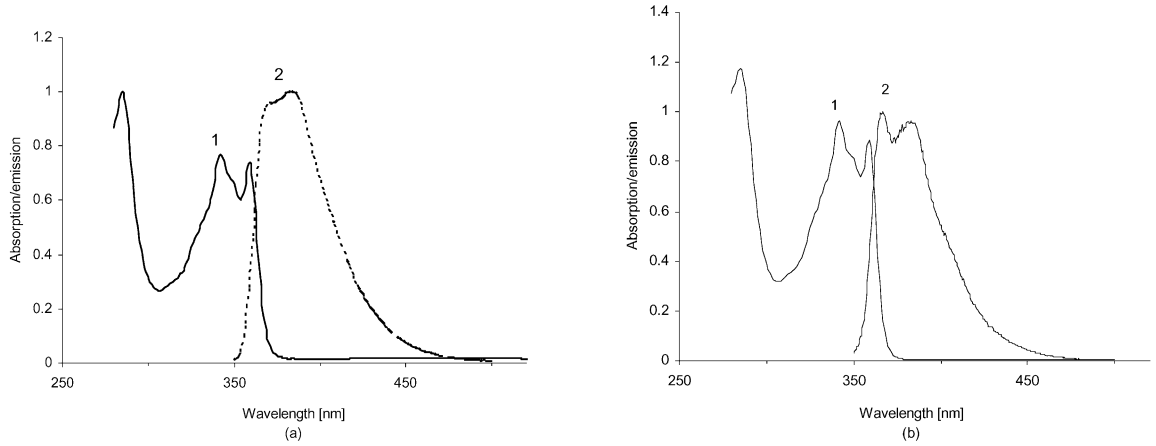


Figure 1. Normalised absorption (curve 1) and fluorescence emission (curve 2) spectra of naphthalenetriazole derivative **2** (a) and **10** (b).

dichloromethane was refluxed over phosphorus pentoxide, distilled from it and stored over 4 Å molecular sieves. Light petroleum refers to the fraction boiling in the range 40–60 °C. Sodium azide was dried in vacuo overnight.

The reactions were followed by thin layer chromatography, which was carried out on pre-coated plates (Merck Kieselgel 60F_{254}) and compounds were visualized by UV-light and exposure to vaporized iodine. Merck Kieselgel 60 (230–400 mesh) was used in column chromatography. Melting points were determined on a Stuart SMP3 melting point apparatus and are uncorrected. Specific optical rotations were calculated from measurements carried out with an Optical Activity-AA-1000 Polarimeter at 27 °C. IR spectra were recorded on a FTIR/Diffus Bomem MB spectrometer. ^{1}H and ^{13}C NMR data were recorded on a Varian Unity Plus 300 Spectrometer in $CDCl_3$ and DMSO-d_6; δ ppm were measured versus residual peak of the solvent and J values are given in Hz. HMQC and HMBC experiments were carried out for complete assignment of proton and carbon signals in the NMR spectra (in descripton of ^{1}H signals app means apparent). Elemental analyses were carried out on a Leco CHNS 932 instrument. MS spectra were obtained in a VG Autospec mass spectrometer. Absorption spectra were recorded using a Perkin-Elmer Lambda 35 spectrophotometer. Steady-state fluorescence spectra were measured on a Perkin-Elmer LS-5 spectrofluorometer. Quantum yields were determined in acetonitrile using quinine sulphate as the fluorescence standard. The path length was 1 cm in quartz cells.

4.1.1. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosylazide.[26] Dry sodium azide (0.163 g, 2.5 mmol) was added to a solution of α-acetobromoglucose (0.411 g, 1.0 mmol) in dry acetone (40 mL) and the mixture refluxed for 7 h. Then it was portioned between dichloromethane (40 mL) and cold water (30 mL). The aqueous phase was extracted with dichloromethane, dried ($MgSO_4$) and evaporated affording the impure azide as a light yellow solid in quantitative yield. Recrystallisation from dichloromethane/diethyl ether/light petroleum afforded the azide as a crystalline white solid in 86% yield, mp 125.6–126.5 °C (lit.[30] 126–127 °C). $[\alpha]_D$ −29 (c 2.0, $CHCl_3$); ν_{max} (Nujol) 2115; 1746; 1213; 1105–1042 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.02, 2.04, 2.09 and 2.11 (4s, 12H, 4×OCH_3), 3.80 (ddd, J=10.0, 4.8, 2.4 Hz, 1H, H-5), 4.18 (dd, J=12.6, 2.4 Hz, 1H, H_a-6), 4.29 (dd, J=12.6, 4.8 Hz, 1H, H_b-6), 4.66 (d, J=9.0 Hz, 1H, H-1), 4.98 (app t, J=9.3, 9.6 Hz, 1H, H-2), 5.11 (app t, J=9.6 Hz, 1H, H-4), 5.23 (app t, J=9.3, 9.6 Hz, 1H, H-3).

4.1.2. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosylamine[28] **(1).** To a solution of the 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide (0.336 g, 0.9 mmol) in ethyl acetate/methanol 1:1 (30 mL), Pd/C 5% (0.119 g) was added. The

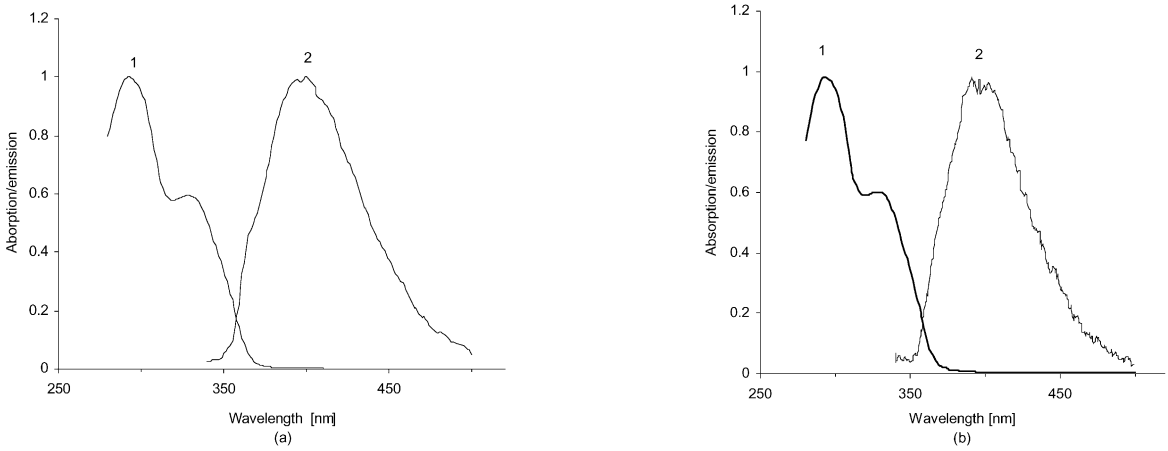


Figure 2. Normalised absorption (curve 1) and fluorescence emission (curve 2) spectra of coumarin derivatives **3** (a) and **11** (b).

resulting mixture was deaerated for 30 min under a nitrogen stream. Then it was submitted to a hydrogen atmosphere, at normal pressure, for 3 h. The final mixture was filtered through a pad of Celite and concentrated affording the product as a yellowish solid in 94% that was used with no further purification, mp 105–106 °C, mp 115–116 °C after crystallization from methanol/light petroleum (lit.,[31] mp not reported, unstable, mp[32] 138–140 °C). $[\alpha]_D+16$ (*c* 2.0, $CHCl_3$); ν_{max} (Nujol) 3410; 3337; 2725; 2668; 2257; 1754; 1639; 1377; 1224; 1095–1062 cm^{-1}. ^1H NMR ($CDCl_3$) δ (ppm): 2.02, 2.04, 2.08 and 2.10 (4s, 12H, $4\times OCH_3$), 2.02–2.10 (br s, 2H, NH_2), 3.70 (ddd, $J=10.0$, 4.8, 2.4 Hz, 1H, H-5), 4.12 (dd, $J=12.3$, 2.4 Hz, 1H, H_a-6), 4.20 (d, $J=8.7$ Hz, 1H, H-1), 4.24 (dd, $J=12.3$, 4.8 Hz, 1H, H_b-6), 4.84 (app t, $J=9.3$, 9.6 Hz, 1H, H-2), 5.05 (app t, $J=9.6$ Hz, 1H, H-4), 5.25 (app t, $J=9.3$, 9.6 Hz, 1H, H-3). Anal. Calcd for $C_{14}H_{21}NO_9$: C, 48.41; H, 6.10; N, 4.03. Found: C, 48.44; H, 6.09; N, 4.07.

4.1.3. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl) naphtho[1,2-*d*][1,2,3]triazol-2-yl-benzamide (4). To a solution of compound **2** (0.280 g, 0.969 mmol) in DMF (200 mL) at −5 °C, triethylamine (0.098 g, 0.134 mL, 0.969 mmol) and ethyl chloroformate (0.108 g, 0.096 mL, 0.969 mmol) were added. The solid precipitated was filtered off and the filtrate was maintained at −5 °C. Then, it was added to the amine (**1**) (0.337 g, 0.969 mmol). The resulting mixture was stirred at rt for 48 h. After evaporation, the crude residue (0.404 g) was purified by flash column chromatography using diethyl ether/light petroleum 2:1–2.5:1 as eluent. The isolated fraction (0.078 g, 13% yield) was recrystallised from ethyl acetate/light petroleum affording the amide **4** as a light pink solid, mp 230–233 °C (dec). $[\alpha]_D-53$ (*c* 1.0, $CHCl_3$); ν_{max} (Nujol) 3430; 1748; 1680; 1607; 1370; 1291; 1228; 1217 cm^{-1}. ^1H NMR ($CDCl_3$) δ (ppm): 2.07, 2.08, 2.09 and 2.11 (4s, 12H, $4\times OCH_3$), 3.95 (ddd, $J=10.0$, 4.0, 1.8 Hz, 1H, H-5′), 4.15 (dd, $J=12.6$, 1.8 Hz, 1H, H_a-6′), 4.40 (dd, $J=12.6$, 4.0 Hz, 1H, H_b-6′), 5.11 (app t, $J=9.6$, 9.9 Hz, 1H, H-2′ or H-4′), 5.15 (app t, $J=9.3$, 10.2 Hz, 1H, H-2′ or H-4′), 5.45 (app t, $J=9.9$, 9.6 Hz, 1H, H-3′), 5.48 (t, $J=9.0$ Hz, 1H, H-1′), 7.16 (d, $J=9.0$ Hz, 1H, NH), 7.62–7.82 (m, 4H, H-5, H-6, H-9, H-8), 7.92 (dd, $J=8.1$, 1.8 Hz, 1H, H-4), 7.98 (d, $J=9.0$ Hz, 2H, H-3″ and H-5″), 8.50 (d, $J=9.0$ Hz, 2H, H-2″ and H-6″), 8.65 (dd, $J=7.5$, 1.8 Hz, 1H, H-7). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.77, 20.74, 20.60 ($4\times CH_3$), 61.58 (C-6′), 68.16 (C-2′ or C-4′), 70.87 (C-2′ or C-4′), 72.51 (C-3′), 73.65 (C-5′), 79.03 (C-1′), 116.31 (C-8 or C-9), 119.96 (C-2″ and C-6″), 123.40 (C-7), 124.77 (C-3b), 127.73 (C-5), 127.92 (C-6), 128.70 (C-3″and C-5″), 129.00 (C-4), 130.42 (C-8 or C-9), 131.95 (C-4″), 132.51 (C-7a), 143.03 (C-1″), 143.42 (C-3a), 144.03 (C-9a), 166.10 (C=O from dye), 169.60, 169.88, 170.64 and 171.74 ($4\times$C=O, acetyl). HRMS (FAB) calcd for $C_{31}H_{30}N_4O_{10}$ ($M^+ +1$) 619.2040, found 619.2051.

4.1.4. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(2-oxo-2*H*-chromene)-3-carboxamide (5). To a solution of compound **3** (0.138 g, 0.723 mmol) in dry acetone (20 mL) at −5 °C, triethylamine (0.146 g, 0.200 mL, 1.45 mmol) and ethyl chloroformate (0.157 g, 0.143 mL, 1.45 mmol) were added. The solid precipitated was filtered off and the filtrate was maintained at −5 °C. Then, it was added to the amine (**1**) (0.251 g, 0.723 mmol) and the resulting mixture was refluxed for 3 h and evaporated. The crude residue (0.357 g) was purified by flash column chromatography using ethyl acetate/diethyl ether 1:5 as eluent. The isolated fraction (0.158 g, 42% yield) was recrystallised from dichloromethane/diethyl ether/light petroleum affording the amide **5** as a white solid, mp 160.0–162.3 °C. $[\alpha]_D-26$ (*c* 2.0, $CHCl_3$); ν_{max} (Nujol) 3306; 2725; 2670; 1748; 1722; 1672; 1610; 1376; 1214; 1159–1034 cm^{-1}. ^1H NMR ($CDCl_3$) δ (ppm): 2.01, 2.04, 2.05 and 2.10 (4s, 12H, $4\times OCH_3$), 3.90 (ddd, $J=10.0$, 4.0, 2.1 Hz, 1H, H-5′), 4.14 (dd, $J=12.6$, 2.1 Hz, 1H, H_a-6′), 4.30 (dd, $J=12.6$, 4.0 Hz, 1H, H_b-6′), 5.17 (app q, $J=9.6$, 9.9, 9.6 Hz, 2H, H-2′ and H-4′), 5.36 (app t, $J=9.3$, 9.6 Hz, 1H, H-3′), 5.50 (app t, $J=9.0$, 9.3 Hz, 1H, H-1′), 7.38–7.44 (m, 2H, H-8 and H-7), 7.68–7.73 (m, 2H, H-6 and H-5), 8.90 (s, 1H, 4-H), 9.31 (d, $J=9.6$ Hz, 1H, NH). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.47, 20.54, 20.56 and 20.69 ($4\times CH_3$), 61.27 (C-6′), 68.04 (C-2′ or C-4′), 70.30 (C-2′ or C-4′), 73.01 (C-3′), 73.63 (C-5′), 78.16 (C-1′), 116.75 (C-7), 117.43 (C-3), 118.28 (C-8a), 125.36 (C-8), 129.95 (C-5), 134.65 (C-6), 149.48 (C-4), 154.62 (C-4a), 160.76 (C=O, coumarin), 162.25 (C=O, amide), 169.39, 169.73, 170.03 and 170.65 ($4\times$C=O, acetyl). Anal. Calcd for $C_{24}H_{25}NO_{12}$: C, 55.48; H, 4.86; N, 2.70. Found: C, 55.47; H, 5.08; N, 2.56. FAB ($M^+ +1$) 520.22.

4.1.5. 4-Naphtho[1,2-*d*][1,2,3]triazol-2-yl-benzoylamino acetic acid methyl ester (6). To a solution of **2** (0.145 g, 0.5 mmol), HGlyOMe, HCl (0.063 g, 0.5 mmol) and DIPEA (0.170 mL, 1 mmol) in DMF (10 mL), cooled to +5 °C, HBTU (0.190 g, 0.5 mmol) was added. The reaction mixture was stirred at rt overnight and diluted with water (50 mL). After cooling for 3 h the solid precipitated was filtered and washed with water. The crude solid was purified by column chromatography using ethyl acetate/dichloromethane 5:1 as eluent. Recrystallisation from methanol/dichloromethane/light petroleum afforded the pure compound as an orange solid in 45% yield, mp 229–231 °C. $[\alpha]_D-2$ (*c* 1.0, DMF); ν_{max} (KBr) 3362; 1754; 1643; 1606; 1367; 1295; 1206; 1179 cm^{-1}. ^1H NMR (DMSO-d_6) δ (ppm): 3.67 (s, 3H, OCH_3), 4.06 (d, $J=5.7$ Hz, 2H, CH_2), 7.69–7.79 (m, 2H, H-5 and H-6), 7.91 (s, 2H, H-8 and H-9), 8.07 (dd, $J=6.9$, 2.0 Hz, 1H, H-4), 8.15 (d, $J=8.7$ Hz, 2H, H-2″ and H-6″), 8.42 (d, $J=8.7$ Hz, 2H, H-3″and H-5″), 8.55 (dd, $J=7.2$, 2.0 Hz, 1H, H-7), 9.19 (t, $J=5.7$ Hz, 1H, NH). ^{13}C NMR (DMSO-d_6) δ (ppm): 41.35 (CH_2), 51.87 (OCH_3), 116.27 (C-8 or C-9), 119.46 (C-3″ and C-5″), 122.88 (C-7), 123.87 (C-3b), 128.18 (C-5), 128.30 (C-6), 129.18 (C-2″ and C-6″), 129.39 (C-4), 130.55 (C-8 or C-9), 132.17 (C-7a), 133.38 (C-4″), 141.49 (C-1″), 142.53 (C-3a), 143.40 (C-9a), 165.66 (C=O, amide), 170.38 (C=O, ester). Anal. Calcd for $C_{20}H_{16}N_5O_3$: C, 66.66; H, 4.48; N, 15.55. Found: C, 66.25; H, 4.57; N, 15.03. FAB ($M^+ +1$) 360.15.

4.1.6. [(2-Oxo-2*H*-chromene-3-carbonyl)-amino]-acetic acid methyl ester (7). To a solution of **3** (1.90 g, 10 mmol) in ethyl acetate (100 mL) HOBt (1.70 g, 10 mmol) was added. After cooling to 0 °C, DCC (2.16 g, 10.5 mmol) was added followed by addition of the HGlyOMe, HCl (1.26 g, 10 mmol) and triethylamine (1.4 mL, 10 mmol). The reaction mixture was stirred at rt overnight and the insoluble materials were filtered off. The

organic layer was successively washed with 5% $NaHCO_3$, water, 5% citric acid, water and dried. The solvent was removed under reduced pressure and the resulting product (1.17 g, 45%) crystallized from ethyl acetate/light petroleum, mp 184.8–187.4 °C. $[\alpha]_D$ −2 (*c* 1.0, DMF); ν_{max} (KBr) 3332; 1748; 1713; 1607; 1372; 1294; 1218 cm^{-1}. ^1H NMR ($CDCl_3$) δ (ppm): 3.81 (s, 3H, OCH_3), 4.27 (d, J= 6.0 Hz, 2H, CH_2), 7.37–7.44 (m, 2H, H-8 and H-6), 7.67–7.72 (m, 2H, H-7and H-5), 8.92 (s, 1H, 4-H), 9.26 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ (ppm): 41.62 (CH_2), 52.35 (OCH_3), 116.64 (C-8), 117.90 (C-3), 118.44 (C-8a), 125.28 (C-6), 129.84 (C-5), 134.23 (C-7), 148.69 (C-4), 154.48 (C-4a), 161.16 (C=O, coumarin), 161.83 (C=O, amide), 169.67 (C=O, ester). Anal. calcd for $C_{13}H_{11}NO_5$: C, 59.76; H, 4.25; N, 5.36. Found: C, 59.93; H, 4.46; N, 5.18. FAB (M^+ + 1) 262.05.

4.1.7. 4-Naphtho[1,2-*d*][1,2,3]triazol-2-yl-benzoylamino acetic acid (8). The ester **6** (0.268 g, 0.744 mmol) was dissolved in methanol (1 mL) and 1 M NaOH (1.9 mL, 1.861 mmol) added. The mixture was stirred at rt for 4 h and 1 M HCl (0.744 mL, 0.744 mmol) was added. The methanol was removed under reduced pressure and the residue thus, obtained cooled in an ice bath and acidified to pH ~ 2–3 with 1 M HCl with vigorous stirring for 1 h. The solid precipitated was filtered, washed with water and dried. Compound **8** was isolated in 87% yield and it was used without further purification, mp 209–213 °C. $[\alpha]_D$ −4 (*c* 1.0, DMF); ν_{max} (KBr) 3403; 2554; 1941; 1681; 1633; 1605; 1366; 1311; 1289; 1228; 1167 cm^{-1}. ^1H NMR (DMSO-d_6) δ (ppm): 3.97 (d, J=5.7 Hz, 2H, CH_2), 7.68–7.80 (m, 2H, H-5 and H-6), 7.92 (s, 2H, H-8 and H-9), 8.08 (br d, J= 7.5 Hz, 1H, H-4), 8.15 (d, J=8.4 Hz, 1H, H-2″ or H-6″), 8.20 (d, J=8.7 Hz, 1H, H-2″ or H-6″), 8.42 (d, J=8.7 Hz, 2H, H-3″ and H-5″), 8.55 (br d, J=7.2 Hz, 1H, H-7), 9.05 (t, J=5.7 Hz, 1H, NH), 12.80 (br s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ (ppm): 41.34 (CH_2), 116.35 (C-8 or C-9), 119.59 (C-3″ or C-5″), 119.70 (C-3″ or C-5″), 123.02 (C-7), 123.98 (C-3b), 128.34 (C-5), 128.44 (C-6), 129.25 (C-2″ or C-6″), 129.50 (C-4), 130.68 (C-9 or C-8), 131.34 (C-2″ or C-6″), 132.29 (C-7a), 133.70 (C-4″), 141.57 (C-1″), 142.66 (C-3a), 143.52 (C-9a), 165.77 (C=O, amide), 171.32 (C=O, acid). HRMS (EI) calcd for $C_{19}H_{14}N_4O_3$ 346.1066, found 346.1062.

4.1.8. [(2-Oxo-2*H*-chromene-3-carbonyl)-amino]-acetic acid (9). The ester **7** (0.769 g, 3 mmol) was dissolved in methanol (5 mL) and 1 M NaOH (7.5 mL, 7.5 mmol) added. The mixture was stirred at rt for 4 h and 1 M HCl (3.0 mL, 3 mmol) was added. The methanol was removed under reduced pressure and the residue thus, obtained cooled in an ice bath and acidified to pH ~ 2–3 with 1 M HCl with vigorous stirring for 1 h. The solid precipitated was filtered, washed with water and dried. Compound **9** was isolated in 86% yield and it was used without further purification, mp 238–239 °C (dec). $[\alpha]_D$ −4 (*c* 1.0, DMF); ν_{max} (KBr) 3315; 2683; 1760; 1712; 1634; 1608; 1372; 1294; 1228; 1162 cm^{-1}. ^1H NMR (DMSO-d_6) δ (ppm): 4.05 (d, J= 5.7 Hz, 2H, CH_2), 7.44 (t, J=7.4 Hz, 1H, H-6), 7.51 (d, J= 8.4 Hz, 1H, H-8), 7.75 (dt, J=1.5, 8.0 Hz, 1H, H-7), 7.99 (dd, J=1.5, 7.8 Hz, 1H, H-5), 8.90 (s, 1H, H-4), 9.03 (t, J= 5.7 Hz, 1H, NH), 12.8 (br s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ (ppm): 41.52 (CH_2), 116.18 (C-8), 118.19 (C-3), 118.43 (C-8a), 125.19 (C-6), 130.43 (C-5), 134.32 (C-7), 148.12 (C-4), 154.00 (C-4a), 160.39 (C=O, coumarin), 161.18 (C=O, amide), 170.81 (C=O, acid). Anal. Calcd for $C_{12}H_9NO_5$: C, 58.18; H, 3.66; N, 5.65. Found: C, 58.10; H, 3.93; N, 5.60.

4.1.9. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-naphtho[1,2-*d*][1,2,3]triazol-2-yl-benzoylamino acetamide (10). To a solution of **8** (0.258 g, 0.744 mmol) in DMF (10 mL), glycosylamine **1** (0.258 g, 0.744 mmol) and HOBt (0.111 g, 0.818 mmol) were added. After cooling to +5 °C, DCC (0.161 g, 0.781 mmol) was added and the resulting mixture stirred at rt overnight. Then, water (50 mL) was added and the mixture cooled for 3 h. The solid precipitated was filtered, dried (0.414 g, 82%) and purified by flash column chromatography using ethyl acetate/dichloromethane 5:1 as eluent. The isolated fraction (0.226 g, 45%) was recrystallised from dichloromethane/light petroleum, affording pure **10** as an orange solid, mp 232.2–233.0 °C. $[\alpha]_D$ +5 (*c* 2.0, $CHCl_3$); ν_{max} (neat) 3413; 1745; 1607; 1370; 1227; 1160 cm^{-1}. ^1H NMR ($CDCl_3$) δ (ppm): 2.01, 2.03, 2.07 and 2.09 (4s, 12H, 4×OCH_3), 3.90 (ddd, J=12.6, 4.0, 2.1 Hz, 1H, H-5′), 4.12–4.19 (m, 3H, H_a-6′ and CH_2Gly), 4.31 (dd, J=12.6, 4.0 Hz, H_b-6′), 5.02 (t, J=9.6 Hz, 1H, H-4′), 5.10 (app t, J=9.6, 10.0 Hz, 1H, H-2′), 5.34 (app t, J=9.3, 9.6 Hz, 2H, H-1′ and H-3′), 7.30 (t, J=5.1 Hz, 1H, NHGly), 7.51 (d, J=9.0 Hz, 1H, NH), 7.82 (app d, 1H, J=7.4 Hz, H-4), 8.00 (d, J=8.7 Hz, 2H, H-2″ and H-6″), 8.38 (d, J=8.7 Hz, 2H, H-3″ and H-5″), 8.53 (app d, 1H, J=8.0 Hz, H-7), 7.60–7.70 (m, 4H, H-5, H-6, H-8 and H-9). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.72, 20.60, 20.53 (4×CH_3), 43.74 (CH_2Gly), 61.58 (C-6′), 68.05 (C-2′ or C-4′), 70.34 (C-2′ or C-4′), 72.73 (C-3′), 73.63 (C-5′), 78.21 (C-1′), 116.17 (C-8 or C-9), 119.67 (C-3″and C-5″), 123.28 (C-7), 124.65 (C-3b), 127.58 (C-5), 127.74 (C-6), 128.57 (C-2″ and C-6″), 128.87 (C-4), 130.20 (C-8 or C-9), 132.36 (C-7a), 132.44 (C-4″), 142.55 (C-1″), 143.18 (C-3a), 143.80 (C-9a), 166.85 (C=O from dye), 169.52 (C=O, acetyl), 169.91 (C=O, acetyl), 169.97 (C=O, aminoacid), 170.72 (C=O, acetyl), 170.94 (C=O, acetyl). Anal. Calcd for $C_{33}H_{33}N_5O_{11}$: C, 58.66; H, 4.92; N, 10.37. Found: C, 58.84; H, 5.06; N, 10.24.

4.1.10. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-[(2-oxo-2*H*-chromene-3-carbonyl)-amino]-acetamide (11). To a solution of **9** (0.1236 g, 0.5 mmol) in DMF (10 mL), glycosylamine **1** (0.1737 g, 0.5 mmol) and HOBt (0.086 g, 0.55 mmol) were added. After cooling to +5 °C, DCC (0.108 g, 0.525 mmol) was added and the resulting mixture stirred at rt for 48 h. Then, water (50 mL) was added and the mixture cooled for 3 h. The solid precipitated was filtered, dried (0.131 g, 46%) and purified by flash column chromatography using ethyl acetate as eluent. The isolated fraction (0.067 g, 23%) was recrystallised from dichloromethane/light petroleum, affording pure **11** as a white solid, mp 245–246 °C. $[\alpha]_D$ −11 (*c* 2.0, $CHCl_3$); ν_{max} (neat) 3413; 1746; 1610; 1370; 1229 cm^{-1}. ^1H NMR ($CDCl_3$) δ (ppm): 1.99, 2.03 and 2.09 (3s, 12H, 4×OCH_3), 3.84 (ddd, J=12.6, 4.5, 2.4 Hz, 1H, H-5′), 4.08–4.15 (m, 1H, Ha-6′), 4.14 (d, J=5.7 Hz, 1H, CH_2Gly), 4.30 (dd, J= 12.6, 4.5 Hz, 1H, Hb-6′), 4.90 (t, J=9.6 Hz, 1H, H-4′), 5.05 (app t, J=9.6, 10.0 Hz, 1H, H-2′), 5.24 (app t, J=9.3, 9.6 Hz, 1H, H-1′), 5.30 (t, J=9.6 Hz, 1H, H-3′), 7.01 (d,

J=9.3 Hz, 1H, NH), 7.40 (d, J=7.8 Hz, 1H, H-8), 7.44 (d, J=7.8 Hz, 1H, H-5), 7.71 (t, J=7.8 Hz, 2H, H-6 and H-7), 8.91 (s, 1H, H-4), 9.26 (t, J=4.8 Hz, 1H, NHGly). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.50, 20.55 and 20.70 (4×CH_3), 43.89 (CH_2Gly), 61.58 (C-6′), 68.09 (C-2′ or C-4′), 70.26 (C-2′ or C-4′), 72.55 (C-3′), 73.56 (C-5′), 78.22 (C-1′), 116.76 (C-7), 117.79 (C-3), 118.44 (C-8a), 125.41 (C-8), 129.92 (C-5), 134.44 (C-6), 148.85 (C-4), 154.54 (C-4a), 161.30 (C=O, coumarin), 162.62 (C=O, coumarin amide), 169.28 (C=O, aminoacid), 169.50, 169.80, 170.63 and 171.10 (4×C=O, acetyl). Anal. Calcd for $C_{26}H_{28}N_2O_{13}$: C, 54.16; H, 4.90; N, 4.86. Found: C, 53.85; H, 5.19; N, 4.72. HRMS (FAB) calcd for $C_{26}H_{29}N_2O_{13}$ (M^++1) 577.1670, found 577.1671.

4.1.11. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-naphtho[1,2-*d*][1,2,3]triazol-2-yl-benzoylamino-acetamide (10) (α-anomer). To a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide (0.195 g, 0.52 mmol), in anhydrous CH_2Cl_2 (20 mL), under a stream of argon, compound **8** (0.20 g, 0.58 mmol) was added. The mixture was cooled to −78 °C, stirred for 10 min followed by the addition of tributylphosphine (0.129 mL, 0.522 mmol). After 10 min, the stream of argon was cut off and the reaction mixture was stirred at −78 °C for 8 h and at rt overnight. Then, it was kept in the refrigerator for 3 h. The precipitate (tributylphosphine oxide) was filtered off and the solution concentrated affording an oily residue, which was purified by column chromatography (eluent: methanol/dichloromethane 3:97). The isolated fraction (0.307 g) was further purified by preparative layer chromatography, using as eluents, successively, diethyl ether/dichloromethane 1:1 (two runs) and dichloromethane (three runs). The required band was removed and extracted with dichloromethane. The solvent was evaporated yielding a white solid (0.024 g, 7%, contaminated with traces of the β-anomer), mp 228.7–230.4 °C; ν_{max} (neat) 3413; 1745; 1607; 1370; 1227; 1160 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.07, 2.10, 2.11 and 2.12 (4s, 12H, 4×OCH_3), 4.21–4.77 (m, 7H, CH_2 Gly, 2×H-6′, H-5′, H-4′and H-3′), 5.24 (app d, J=4.2 Hz, 1H, H-2′), 5.57 (d, J=3.6 Hz, 1H, H-1′), 6.78 (t, J=4.8 Hz, 1H, NHGly), 7.63–7.81 (m, 5H, H-9, H-8, H-6, H-5, NH), 7.91 (dd, J=7.5, 1.5 Hz, 1H, H-4), 8.03 (d, J=9.0 Hz, 2H, H-2″ and H-6″), 8.49 (d, J=8.7 Hz, 2H, H-3″ and H-5″), 8.65 (dd, J=7.8, 1.8 Hz, 1H, H-7). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.48, 20.71, 20.75 (4×CH_3), 41.99 (CH_2Gly), 60.94 (C-3′ and C-4′), 61.80 (C-6′), 66.32 (C-1′), 67.46 (C-2′), 74.12 (C-5′), 116.32 (C-5 or C-6 or C-8 or C-9), 119.88 (C-3″ and C-5″), 123.40 (C-7), 124.82 (C-3b), 127.70 (C-5 or C-6 or C-8 or C-9), 127.86 (C-5 or C-6 or C-8 or C-9), 128.51 (C-2″ and C-6″), 129.00 (C-4), 130.33 (C-5 or C-6 or C-8 or C-9), 132.50 (C-7a or C-4″), 133.06 (C-7a or C-4″), 142.64 (C-1″), 143.35 (C-3a), 143.96 (C-9a), 169.48 (C=O from dye), 169.56, 170.05 and 170.12 (C=O, acetyl), 170.49 (C=O, aminoacid).

Acknowledgements

The authors thank the University of Minho and the Foundation of Science and Technology (FCT) for financial support; Lumir Lichy for absorption and fluorescence measurements, and Dr. Radim Hrdina for a gift of dye **2**.

References and notes

1. Böttcher, C.; Spengler, J.; Essawy, S. A.; Burger, K. *Monatshefte für Chemie* **2004**, *135*, 853–863.
2. Tarasenko, O.; Islam, S.; Paquiot, D.; Levon, K. *Carbohydr. Res.* **2004**, *339*, 2859–2870 and references cited therein.
3. Kihlberg, J.; Åhman, J.; Walse, B.; Drakenberg, T.; Nilsson, A.; Söderberg-Ahlm, C.; Bengtsson, B.; Olsson, H. *J. Med. Chem.* **1995**, *38*, 161–169.
4. Arsequell, G.; Valencia, G. *Tetrahedron: Asymmetry* **1999**, *10*, 3045–3094.
5. Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683–720.
6. Reuter, G.; Gabius, H. *J. Cell Mol. Life Sci.* **1999**, *55*, 368–422.
7. Danishefsky, S. J.; Allen, J. R. *Angew. Chem., Int. Ed.* **2000**, *39*, 836–863.
8. Lohof, E.; Planker, E.; Mang, C.; Burkhart, F.; Dechantsreiter, M. A.; Haubner, R.; Wester, H.-J.; Schwaiger, M.; Hölzemann, G.; Goodman, S. L.; Kessler, H. *Angew. Chem., Int. Ed.* **2000**, *39*, 2761–2764.
9. Coutrot, F.; Grison, C.; Coutrot, P. *C.R. Chimie* **2004**, *7*, 3–13.
10. Davis, B. G. *Chem. Rev.* **2002**, *102*, 579–602.
11. Meinjohanns, E.; Meldal, M.; Paulsen, H.; Dwek, R. A.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1998**, 549–560.
12. Cuppoletti, A.; Younjin, C.; Jin-Seong, P.; Strässler, C.; Kool, E. T. *Bioconjugate Chem.* **2005**, *16*, 528–534.
13. Wetlz, B.; Gruber, M.; Oswald, B.; Dürkop, A.; Weidgans, B.; Probst, M.; Wolfbeis, O. S. *J. Chromatogr. B* **2003**, *793*, 83–92.
14. Fernandez-Carteado, J.; Kogan, M. J.; Castel, S.; Giralt, E. *Angew. Chem., Int. Ed.* **2004**, *43*, 1811–1814.
15. David, R.; Machova, Z.; Beck-Sickinger, A. G. *Biol. Chem.* **2003**, *384*, 1619–1639.
16. Faure, M.-P.; Gaudreau, P.; Shaw, I.; Cashman, N. R.; Beaudet, A. *J. Histochem. Cytochem.* **1994**, *42*, 755–763.
17. Fuchs, S.; Otto, H.; Jehle, S.; Henklein, P.; Schüter, A. D. *Chem. Commun.* **2005**, 1830–1832.
18. Mayo, V. J.; Prabhakaran, J.; Mann, J. J.; Kumar, J. S. D. *Tetrahedron Lett.* **2003**, *44*, 8535–8537.
19. Bennett, F. A.; Barlow, D. J.; Dodoo, A. N. O.; Hider, R. C.; Lansley, A. B.; Lawrence, M. J.; Marriott, C.; Bansal, S. S. *Anal. Biochem.* **1999**, *270*, 15–23.
20. Reddington, M. V. *J. Chem. Soc., Perkin Trans. 1* **1998**, 143–147.
21. He, Y.; Hinklin, R. J.; Chang, J.; Kiessling, L. L. *Org. Lett.* **2004**, *6*, 4479–4482 and references cited therein.
22. Bejugam, M.; Flitsch, S. L. *Org. Lett.* **2004**, *6*, 4001–4004.
23. Huo, C.; Wang, C.; Zhao, M.; Peng, S. *Chem. Res. Toxicol.* **2004**, *17*, 1112–1120.
24. Simo, O.; Lee, V. P.; Davies, A. S.; Kreutz, C.; Gross, P. H.; Jones, P. R.; Michael, K. *Carbohydr. Res.* **2005**, *340*, 557–566.
25. Monsigny, M.; Quétard, C.; Bourgerie, S.; Delay, D.; Pichon, C.; Midoux, P.; Mayer, R.; Roche, A. C. *Biochemie* **1998**, *80*, 99–108.
26. Boons, G.-J.; Polt, R. L. In *Carbohydrate Chemistry*; Boons, G.-J., Ed.; Blackie Academic: London, 1998; pp 223–242.
27. Michael, F.; Klemer, A. *Adv. Carbohydr. Chem.* **1961**, *16*, 95.
28. Paulsen, H.; Pflughaupt, K.-W., 2nd ed. In *The Carbohydrates, Chemistry and Biochemistry*, *Vol. IB*; Academic: New York, 1980; pp 881–927.
29. (a) Inaku, T.; Kobayashi, K. *Synlett* **1993**, 869–870. (b) Mizuno, M.; Muramoto, I.; Kobayashi, K.; Yaginuma, H.; Inazu, T. *Synthesis* **1999**, 162–165.

30. Soli, D. E.; Manoso, A. S.; Patterson, M. C.; DeSong, P. *J. Org. Chem.* **1999**, *64*, 3171–3177.
31. von Roedern, E. G.; Lohof, E.; Hessler, G.; Hoffmann, M.; Kessler, H. *J. Am.Chem. Soc.* **1996**, *118*, 10156–10167.
32. Kondratenko, R. M.; Baltina, L. A.; Mustafina, S. R.; Vasiléva, E. V.; Pompei, R.; Deidda, D.; Plyasunova, O. A.; Pokrovskii, A. G.; Tolstikov, A. *Russ. J. Bioorg. Chem.* **2004**, *30*, 308–315.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 8633–8640

Application of recoverable nanosized palladium(0) catalyst in Sonogashira reaction

Pinhua Li,[a] Lei Wang[a,b,*] and Hongji Li[a]

[a]*Department of Chemistry, Huaibei Coal Teachers College, Huaibei, Anhui 235000, People's Republic of China*
[b]*State Key Laboratory of Organometallic Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, People's Republic of China*

Received 18 March 2005; revised 1 July 2005; accepted 5 July 2005

Available online 26 July 2005

Abstract—In the absence of ligand, copper and amine, a recoverable nanoparticle palladium(0) catalyzed Sonogashira reaction of aryl iodides and bromides with terminal alkynes was developed. The protocol involved the use of an environmental-friendly reaction system with ethanol as the solvent, potassium carbonate as a base, and poly(vinylpyrrolidone) (PVP) supported nanosized palladium metal colloids as the catalyst. The palladium metal was recovered and recycled by a simple decantation of the reaction solution and used for eight consecutive trials without significant loss of its reactivity.

1. Introduction

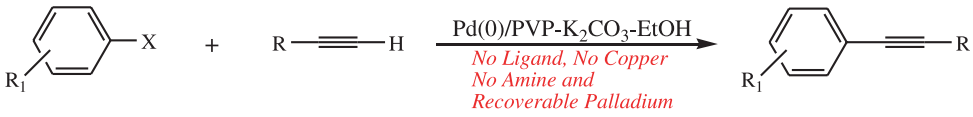
The palladium complexes in the presence of a catalytic amount of CuI and an amine (as a solvent or in large excess) catalyzed coupling of terminal alkynes with aryl and alkenyl halides (the Sonogashira reaction) is one of the most powerful and straightforward methods for the formation of sp^2–sp carbon–carbon bonds in organic synthesis.[1] This method has been widely used for the synthesis of natural products,[2] biologically active molecules,[3] nonlinear optical materials and molecular electronics,[4] dendrimeric and polymeric materials,[5] macrocycles with acetylene links,[6] and polyalkynylated molecules.[7] In general, the traditional palladium-catalyzed reaction conditions are mild, and many reactions can be accomplished at ambient temperature. However, the Sonogashira reaction often generates homo-coupling products of terminal alkynes (Glaser coupling or Hay coupling[8]) along with the main reaction in considerable yields owing to the addition of CuI and these undesirable by-products are generally not easy to be separated from the desired products due to very similar chromatographic mobility.[9] Furthermore, the reaction generally proceeds in the presence of a homogeneous more expensive palladium complex catalyst, which makes the recovery of the metal tedious if not impossible, and might result in high palladium contamination of the products. In addition, amines, such as triethylamine and piperidine, required in most Sonogashira reactions, have a bad smell and add to the environmental burden.

A number of modifications, such as copper-free catalyst systems,[10] amine- and copper-free systems,[11] ligand-free systems,[12] palladium powder systems,[13] nickel systems,[14] environmental-friendly reaction media (including aqueous,[10n,15] solventless,[13] and ionic liquid[10m,16] systems), phase-transfer reaction conditions and hydrogen atmosphere conditions,[17] have been used to solve one or two of the above problems, but none of them can solve all of the problems. Last year, a transition-metal free Sonogashira coupling was developed by Leadbeater using PEG–NaOH (aqueous) under microwave irradiation conditions,[18] but this approach was limited by the narrow choice of reactants (only to aryl iodides and aromatic terminal alkynes). Most recently, ligand-, copper- and amine-free Sonogashira reactions in DMF (not an environmental benign solvent) or in *iso*-propanol were demonstrated.[19] Nevertheless, the development of simpler, more practical, economic, and efficient catalyst system is still an important topic in this area.

Herein, we report a Sonogashira protocol only in the presence of PVP-supported nanoparticle palladium metal, potassium carbonate in ethanol (ligand-free, co-catalyst-free, and amine-free in environmental-friendly solvent),

Keywords: Nanoparticle palladium metal; Sonogashira reaction; Ligand free; Copper free; Amine free.
* Corresponding author. Address: Department of Chemistry, Huaibei Coal Teachers College, Huaibei, Anhui 235000, People's Republic of China. Tel.: +86 561 380 2069; fax: +86 561 309 0518; e-mail: leiwang@hbcnc.edu.cn

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.07.013



Scheme 1.

which generates the corresponding coupling products in excellent yields. It can solve some of the above problems in the reaction. Moreover, the palladium metal can be recovered and recycled by a simple decantation of the reaction solution and used for eight consecutive trials without significant loss of its reactivity (Scheme 1).

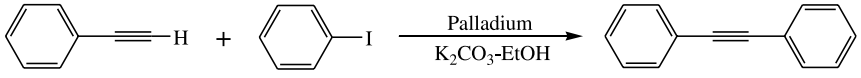
2. Results and discussion

2.1. Optimization of reaction conditions for Sonogashira reaction

Although, P-ligands stabilize palladium and influence its reactivity, the simplest and cheapest Pd-catalysts are of course ligand-free systems, specifically when used in low loading. Such an experimental protocol would constitute a significant advancement, especially if generally applicable. In our initial screening experiments, the Sonogashira reaction catalyzed by palladium in the absence of copper, ligand and amine was our goal. When we searched for a cross-coupling protocol of iodobenzene and phenylacetylene, we observed that iodobenzene could react with phenylacetylene in the presence of 3 mol% of $Pd(OAc)_2$ and 2 equiv of K_2CO_3 in ethanol under ligand-, copper- and amine-free reaction conditions at reflux temperature for 6 h to afford the desired cross-coupling product as the sole product in 52% yield (no homo-coupling product, entry 1, Table 1). Encouraged by this result, we continued to improve the yield by using the most efficient palladium source. In the last decade, Reetz et al. reported that palladium metal colloids could be prepared size-selectively in the range of 1–5 nm in organic solvents or in water by reducing palladium salts chemically or electrochemically in the presence of polymers (such as polyvinylpyrrolidone) or surfactants (e.g., tetraalkyl ammonium salts) and subsequently used for Heck and Suzuki reactions.[20] On the other hand, an atom-economical Pd/Ni bimetallic nanoparticles, a layered double hydroxide supported nanopalladium, has been tested as the catalyst for the Sonogashira reaction.[12,21] As stabilizers and supporting materials, polyvinylpyrrolidone (PVP) and tetraalkylammonium salts can prevent undesired agglomeration by forming a monomolecular layer around the palladium metal core. We used Pd/PVP[22] or $Pd(OAc)_2$/*n*-Bu_4NBr (Jeffery's catalyst),[23] in place of $Pd(OAc)_2$ to perform the model Sonogashira reaction. We were delighted that the desired products were obtained almost quantitatively (96% yield by using Pd/PVP and 90% yield by using $Pd(OAc)_2$/*n*-Bu_4NBr, entries 5 and 6, Table 1). However, only trace of the cross-coupling product was isolated when the reaction was carried out in the presence of submicron palladium or palladium sponge (entries 3 and 4, Table 1). The optimized loading of nano-palladium particles on PVP was found to be in the range of 0.5–5.0 mol% (entries 7–12, Table 1).

During the course of our further optimization of the reaction conditions, we examined several different bases for the Sonogashira coupling reactions. K_2CO_3 was found to be the most effective one (entry 5, Table 2). Other bases such as KOAc, KF, and K_3PO_4, were substantially less effective, KOH failed to promote the reaction, and piperidine, diethylamine, dibenzylamine, and triethylamine were no longer the effective bases in this catalyst system (entries 1–4 and 6–9, Table 2). Because of the polar properties of PVP, the reaction conducted in EtOH and MeOH were most effective (entries 1 and 2, Table 3). The use of DMSO and DMF as solvents led to slower reactions (entries 7 and 8, Table 3), and no desired cross-coupling products were observed while reactions were carried out in toluene, dichloromethane, THF, and acetonitrile (entries 3–6, Table 3). When 1 mol% loading of palladium was used, the reactions were generally completed in a matter of hours

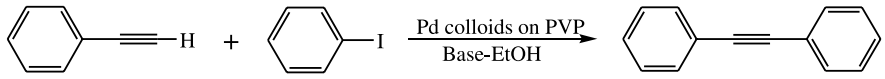
Table 1. Effect of palladium source on the Sonogashira coupling reaction[a]



Entry	Palladium source/amount	Yield[b] (%)
1	$Pd(OAc)_2$ (3mol%)	52
2	$Pd(PPh_3)_4$ (3 mol%)	48
3	Palladium powder (submicron) (5 mol%)	Trace
4	Palladium sponge (5 mol%)	0
5	Palladium colloids (nanosize) on PVP (3 mol%)	96
6	$Pd(OAc)_2$ (3 mol%)/*n*-Bu_4NBr (1.0 mol)	90
7	Palladium colloids (nanosize) on PVP (5 mol%)	93
8	Palladium colloids (nanosize) on PVP (4 mol%)	94
9	Palladium colloids (nanosize) on PVP (2 mol%)	95
10	Palladium colloids (nanosize) on PVP (1 mol%)	95
11	Palladium colloids (nanosize) on PVP (0.75 mol%)	92
12	Palladium colloids (nanosize) on PVP (0.5 mol%)	90[c]

[a] Phenylacetylene (1.00 mmol), iodobenzene (1.00 mmol), Palladium, K_2CO_3 (2.00 mmol) in EtOH (4 mL) at 80 °C stirring for 6 h.
[b] Isolated yields.
[c] The reaction was performed for 10 h.

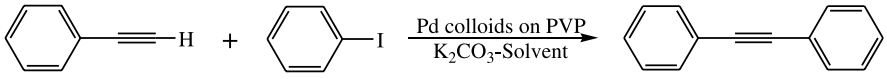
Table 2. Effect of base on the Sonogashira coupling reaction[a]



Entry	Base	Yield[b] (%)
1	Piperidine	40
2	K_3PO_4	42
3	KF	54
4	KOAc	79
5	K_2CO_3	95
6	KOH	Trace
7	Triethylamine	38
8	Diethylamine	27
9	Dibenzylamine	21

[a] Phenylacetylene (1.00 mmol), iodobenzene (1.00 mmol), Pd(0) colloids (7 nm diameter size, 1.06 mg, 0.01 mmol, on PVP), base (2.00 mmol) in EtOH (4 mL) at 80 °C stirring for 6 h.
[b] Isolated yields.

Table 3. Effect of solvent on the Sonogashira coupling reaction[a]



Entry	Solvent	Yield[b] (%)
1	CH_3OH	95
2	CH_3CH_2OH	95
3	$C_6H_5CH_3$	0
4	THF	0
5	CH_2Cl_2	0
6	CH_3CN	Trace
7	DMSO	90
8	DMF	90

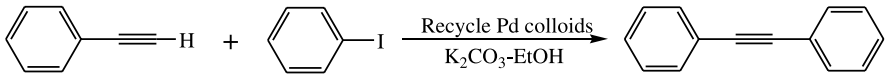
[a] Phenylacetylene (1.00 mmol), iodobenzene (1.00 mmol), Pd(0) colloids (7 nm diameter size, 1.06 mg, 0.01 mmol, on PVP), K_2CO_3 (2.00 mmol) in EtOH (4 mL) at 80 °C stirring for 6 h.
[b] Isolated yields.

Table 4. Nanoparticle palladium metal catalyzed Sonogashira coupling reaction[a]

Entry	Organic halide	Terminal alkyne	Yield[b] (%)
1	C_6H_5I	$C_6H_5C{\equiv}CH$	95
2	*p*-$CH_3OC_6H_4I$	$C_6H_5C{\equiv}CH$	92 90[c]
3	*p*-$CH_3C_6H_4I$	$C_6H_5C{\equiv}CH$	94
4	*p*-$CH_3COC_6H_4I$	$C_6H_5C{\equiv}CH$	98
5	*p*-$O_2NC_6H_4I$	$C_6H_5C{\equiv}CH$	93
6	*m*-$O_2NC_6H_4I$	$C_6H_5C{\equiv}CH$	91
7	*p*-$CF_3C_6H_4I$	$C_6H_5C{\equiv}CH$	96
8	*m*-CNC_6H_4I	$C_6H_5C{\equiv}CH$	90
9	*o*-$CF_3C_6H_4I$	$C_6H_5C{\equiv}CH$	83
10	C_6H_5I	*p*-$BrC_6H_4C{\equiv}CH$	92
11	C_6H_5I	*p*-$FC_6H_4C{\equiv}CH$	94
12	C_6H_5I	*p*-$CH_3C_6H_4C{\equiv}CH$	91
13	C_6H_5I	*p*-$C_6H_5C_6H_4C{\equiv}CH$	93
14	C_6H_5I	*n*-$C_8H_{17}C{\equiv}CH$	92
15	C_6H_5I	*n*-$C_6H_{13}C{\equiv}CH$	90
16	*p*-$CH_3OC_6H_4I$	*n*-$C_8H_{17}C{\equiv}CH$	92
17	*p*-$CH_3C_6H_4I$	*n*-$C_8H_{17}C{\equiv}CH$	94
18	*p*-CNC_6H_4Br	$C_6H_5C{\equiv}CH$	80
19	*m*-$O_2NC_6H_4Br$	$C_6H_5C{\equiv}CH$	86
20	*p*-$CH_3COC_6H_4Br$	$C_6H_5C{\equiv}CH$	78
21	2-Bromopyridine	$C_6H_5C{\equiv}CH$	82
22	*p*-$CH_3C_6H_4Br$	$C_6H_5C{\equiv}CH$	43 94[d]

[a] Terminal alkyne (1.00 mmol), organic halide (1.00 mmol), Pd(0) colloids (7 nm diameter size, 1.06 mg, 0.01 mmol, on PVP), K_2CO_3 (2.00 mmol) in EtOH (4 mL) at 80 °C stirring for 6 h.
[b] Isolated yields.
[c] Up to 10 mmol scales.
[d] In the presence of PPh_3.

Table 5. Successive trials by using recoverable nanosized palladium on PVP[a]



Trial	Yield[b] (%)	Trial	Yield[b] (%)
1	95	5	92
2	94	6	91
3	92	7	90
4	93	8	90

[a] Phenylacetylene (1.00 mmol), iodobenzene (1.00 mmol), Pd(0) colloids (7 nm diameter size, 1.06 mg, 0.01 mmol, on PVP), K_2CO_3 (2.00 mmol) in EtOH (4 mL) at 80 °C stirring for 6 h.
[b] Isolated yields.

but the time, as expected, was inversely proportional to the temperature. A reaction temperature of 80 °C was found to be optimal. Thus, the optimized reaction conditions for this Sonogashira reaction were Pd/PVP (1 mol%), K_2CO_3 (2 equiv) in ethanol under reflux temperature for 6 h.

2.2. The Sonogashira coupling reaction of aryl halides with terminal alkynes

To survey the generality of this Sonogashira-type reaction, we investigated the reaction using a variety of aryl iodides and bromides, with a wide range of terminal alkynes as substrates under the optimized conditions. The results are shown in Table 4. The electron-neutral, electron-rich and electron-poor aryl iodides reacted with phenylacetylene very well to generate the corresponding cross-coupling products in excellent yields under the standard reaction conditions (entries 1–8, Table 4). This cross-coupling was also tolerant of *ortho* substitution in aryl iodides and led to good yields (entry 9, Table 4). Regardless of their electronic characters, both of the aromatic terminal alkynes and aliphatic terminal alkynes component coupled smoothly with iodobenzene to produce the desired products in excellent yields (entries 10–17, Table 4). Activated aryl bromide reacted with phenylacetylene to generate the corresponding products in good yields (entries 18–21, Table 4). For electron-rich aryl bromide, relatively lower yield was obtained under the present reaction conditions (entry 22, Table 4). When the simplest and cheapest P-ligand PPh_3 was added to the reaction mixture, an excellent yield of the product was isolated (entry 22, Table 4). Further investigation of ligands to the coupling reactions is currently underway and will be reported in due course.

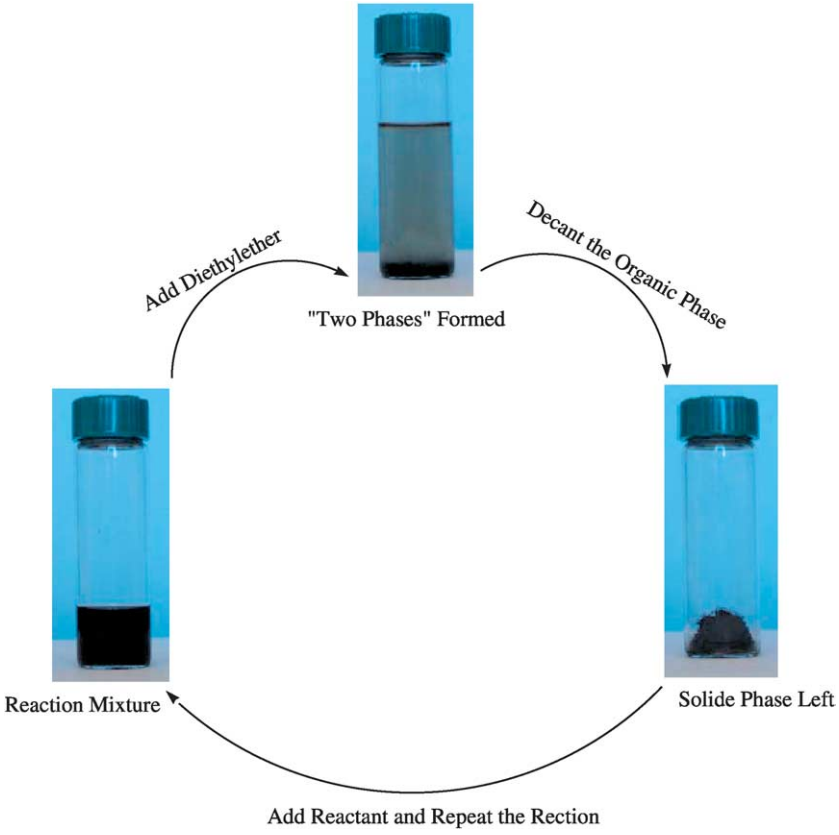


Figure 1. The simple decantation procedure for recycling palladium metal collide on PVP in solution.

2.3. The recyclable of the nanoparticle palladium(0) on PVP

The recyclable of the palladium on PVP was also surveyed. After carrying out the reaction, Et_2O was added to the reaction mixture. The product was extracted into organic layer, Pd–PVP, and other inorganics precipitated and agglomerated to the bottom of the flask. After decantation of the reaction solution, additional K_2CO_3 was added to the palladium on PVP and the reaction was repeated. Only minor decreases in reaction yields were observed after eight repetitive cycles (Table 5). The simple decantation procedure for recycling palladium metal collide on PVP in solution is stated in Figure 1. Alternatively, the product might be distilled directly from the reaction mixture. Palladium, PVP and other inorganics were left at the bottom of flask, and they could be reused directly without purification.

3. Conclusion

In conclusion, we have developed an efficient, simple, practical, and economic catalyst system for the Sonogashira reaction using PVP supported nanosized palladium metal (1 mol% loading) in ethanol under ligand-, copper-, and amine-free reaction conditions. Furthermore, palladium on PVP can be recycled at least eight times without loss of activity via a simple decantation procedure. In addition, the protocol uses environmentally benign reagents and solvents.

4. Experimental

4.1. General

Melting points were recorded on a melting point apparatus and were uncorrected. All ^1H and ^{13}C NMR spectra were recorded at 300 or 250 MHz, and 75 or 62.5 MHz, respectively. Chemical shifts were given as δ value with reference to tetramethylsilane (TMS) as the internal standard. GC/MS data were obtained by using a Hewlett-Packard 6890 series GC equipped with a 5983 mass selective detector. The chemicals were purchased from commercial suppliers and were used without further purification. Products were purified by flash column chromatography.

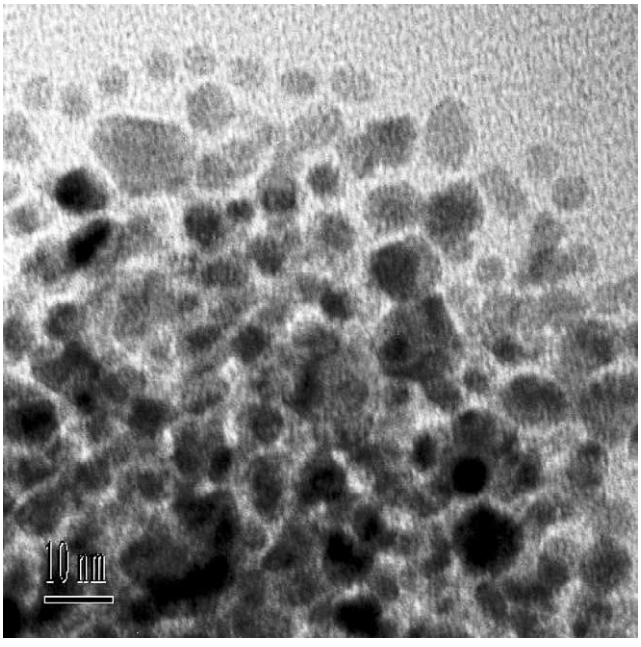


Figure 2. TEM of palladium metal colloids on PVP (Measured by Philips Tecnai 200).

4.2. Preparation of palladium metal colloids on PVP

According to the literature,[22] palladium acetate (50 mg) was added to a solution of PVP (M_W 40,000, 2.5 g) in methanol (150 mL) and the mixture was refluxed under nitrogen for 3 h. The resulting brown liquid was filtered through 0.2 μm Teflon Millipore, and methanol was removed under reduced pressure. This procedure reproducibly yielded colloidal palladium particles in the form of well-formed micro-crystallites with a mean diameter of 7 nm (Fig. 2).

4.3. General procedure for the Sonogashira reaction using $Pd(OAc)_2$/*n*-Bu_4NBr (Jeffery's catalyst)

Under nitrogen atmosphere, an oven-dried round-bottomed flask was charged with $Pd(OAc)_2$ (6.7 mg, 0.03 mmol), *n*-Bu_4NBr (322 mg, 1.0 mmol), K_2CO_3 (272 mg, 2.0 mmol), aryl halide (1.0 mmol), terminal alkyne (1.0 mmol), and ethanol (4 mL). The reaction mixture was refluxed at 80 °C for 6 h. After cooling to the room temperature, Et_2O (25 mL) was added. The organic layer was successively washed with water (2×10 mL) and brine (10 mL) and dried over Na_2SO_4. The solution was filtered, concentrated, and the residue was purified by flash chromatography on silica gel to give the desired cross-coupling product.

4.4. General procedure for recycling of palladium metal on PVP

After carrying out a reaction, Et_2O (10 mL) was added to the reaction mixture. The product was extracted into organic layer, Pd–PVP and other inorganics precipitated and agglomerated to the bottom of the flask. After decantation of the reaction solution, additional K_2CO_3 (136 mg, 1.0 mmol) was added to the palladium on PVP and the reaction was repeated following the general procedure for the Sonogashira reaction using Pd/PVP as described below.

4.5. General procedure for the Sonogashira reaction using Pd/PVP

Under nitrogen atmosphere, an oven-dried round-bottomed flask was charged with Pd/PVP (0.106 g of 1% Pd on PVP, containing 1.06 mg Pd), K_2CO_3 (272 mg, 2.0 mmol), aryl halide (1.0 mmol), terminal alkyne (1.0 mmol), and ethanol (4 mL). The reaction mixture was refluxed at 80 °C for 6 h. After cooling to the room temperature, Et_2O (10 mL) was added and stirred for 10 min to ensure product removal from Pd/PVP. As Pd/PVP and other inorganics precipitated and agglomerated to the bottom of the flask, the organic layer was decanted and the residue was washed using Et_2O (2× 5 mL). The combined organic layers were dried over

Na_2SO_4, filtered, concentrated, and the residue was purified by flash chromatography on silica gel to give the desired cross-coupling product.

4.5.1. Diphenylacetylene. Mp 60–61 °C (lit.[24] 60–62 °C). ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 7.54–7.50 (m, 4H), 7.32–7.28 (m, 6H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ: 131.6, 128.3, 128.2, 123.3, 89.4; MS *m/z* (relative intensity, %): 178 (M^+, 100), 152 (14), 126 (7), 89 (13).

4.5.2. Phenyl-*p*-tolyacetylene. Mp 71–72.5 °C (lit.[25] 72–73 °C). ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 7.52–7.48 (m, 2H), 7.41 (d, J=7.80 Hz, 2H), 7.29–7.26 (m, 3H), 7.10 (d, J=7.81 Hz, 2H), 2.30 (s, 3H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ: 138.3, 131.5, 129.0, 128.2, 128.0, 123.4, 120.2, 89.6, 88.7, 21.4; MS *m/z* (relative intensity, %): 192 (M^+, 100), 165 (15), 139 (4), 115 (6), 94 (10).

4.5.3. (4-Acetylphenyl)phenylacetylene. Mp 95–96 °C (lit.[26] 94–96 °C). ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 7.91 (d, J=8.41 Hz, 2H), 7.58 (d, J=8.41 Hz, 2H), 7.54–7.52 (m, 2H), 7.36–7.33 (m, 3H), 2.57 (s, 3H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ: 197.1, 136.1, 131.7, 131.6, 128.7, 128.4, 128.2, 128.1, 122.6, 92.6, 88.6, 26.5; MS *m/z* (relative intensity, %): 220 (M^+, 60), 205 (100), 176 (48), 151 (18), 102 (10), 88 (19).

4.5.4. (4-Nitrophenyl)phenylacetylene. Mp 120–122 °C (lit.[27] 120–121 °C). ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 8.19 (d, J=8.83 Hz, 2H), 7.64 (d, J=8.73 Hz, 2H), 7.56–7.52 (m, 2H), 7.39–7.37 (m, 3H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ: 146.9, 132.2, 131.8, 130.2, 129.2, 128.5, 123.6, 122.0, 94.7, 87.5; MS *m/z* (relative intensity, %): 223 (M^+, 100), 193 (40), 176 (80), 165 (33), 151 (40), 139 (8), 126 (14).

4.5.5. (3-Nitrophenyl)phenylacetylene. Mp 67–69 °C (lit.[28] 68–70 °C). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.31 (s, 1H), 8.11 (d, J=8.10 Hz, 1H), 7.76 (d, J=7.80 Hz, 1H), 7.51–7.47 (m, 3H), 7.31–7.35 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 148.3, 137.2, 131.8, 129.3, 129.0, 128.5, 126.4, 125.2, 122.8, 122.3, 92.0, 86.9; MS *m/z* (relative intensity, %): 223 (M^+, 100).

4.5.6. (4-Fluorophenyl)phenylacetylene. Mp 108–110 °C (lit.[29] 108–109 °C). ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 7.53–7.47 (m, 4H), 7.35–7.31 (m, 3H), 7.06–7.00 (m, 2H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ: 162.5 (d, J=248.1 Hz), 133.5 (d, J=8.9 Hz), 131.5, 128.3, 123.1, 119.3 (d, J=5.6 Hz), 115.6 (d, J=22.4 Hz), 89.0, 88.3; MS *m/z* (relative intensity, %): 196 (M^+, 100), 175 (7), 144 (5), 98 (15).

4.5.7. (4-Bromophenyl)phenylacetylene. Mp 82–84 °C (lit.[29] 82–84 °C). ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 7.53–7.44 (m, 4H), 7.38–7.31 (m, 5H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ: 133.0, 131.6, 128.5, 128.4, 122.9, 122.5, 122.2, 90.5, 88.3; MS *m/z* (relative intensity, %): 258, 256 (M^+, 97, 100), 176 (88), 151 (37), 128 (17), 88 (61).

4.5.8. 1-Phenyl-1-decyne. Oil.[30] ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 7.40–7.36 (m, 2H), 7.28–7.22 (m, 3H), 2.38 (t, J=6.96 Hz, 2H), 1.64–1.53 (m, 2H), 1.45–1.28 (m, 10H), 0.88 (t, J=6.54 Hz, 3H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ: 131.5, 128.1, 127.3, 124.2, 90.3, 80.6, 31.9, 29.2, 29.1, 28.9, 28.8, 22.7, 19.4, 14.1; MS *m/z* (relative intensity, %): 214 (M^+, 13), 171 (3), 157 (17), 143 (60), 129 (66), 115 (100), 91 (41).

4.5.9. 1-Phenyl-1-octyne. Oil.[31] ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 7.40–7.37 (m, 2H), 7.30–7.23 (m, 3H), 2.39 (t, J=6.97 Hz, 2H), 1.65–1.54 (m, 2H), 1.50–1.26 (m, 6H), 0.90 (t, J=6.68 Hz, 3H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ: 131.5, 128.1, 127.4, 124.2, 90.4, 80.6, 31.4, 28.7, 22.6, 19.4, 14.0; MS *m/z* (relative intensity, %): 186 (M^+, 26), 157 (15), 143 (55), 129 (54), 115 (100), 91 (31).

4.5.10. 1-(4-Methoxylphenyl)-1-decyne. Oil.[32] ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 7.32 (d, J=8.72 Hz, 2H), 6.80 (d, J=8.73 Hz, 2H), 3.78 (s, 3H), 2.37 (t, J=7.00 Hz, 2H), 1.64–1.54 (m, 2H), 1.46–1.28 (m, 10H), 0.88 (t, J=6.00 Hz, 3H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ: 159.0, 132.8, 116.3, 113.8, 88.7, 80.2, 55.2, 31.8, 29.2, 29.1, 28.9, 22.2, 19.4, 14.1; MS *m/z* (relative intensity, %): 244 (M^+, 19), 201 (4), 188 (23), 173 (30), 159 (25), 147 (100), 121 (33), 115 (21), 91 (15).

4.5.11. 1-(4-Methylphenyl)-1-decyne. Oil.[32] ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 7.27 (d, J=8.00 Hz, 2H), 7.04 (d, J=7.93 Hz, 2H), 2.38 (t, J=7.56 Hz, 2H), 2.29 (s, 3H), 1.63–1.52 (m, 2H), 1.45–1.28 (m, 10H), 0.88 (t, J=6.46 Hz, 3H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ: 137.2, 131.4, 128.8, 121.1, 89.5, 80.6, 31.9, 29.2, 29.1, 28.9, 28.8, 22.7, 21.3, 19.4, 14.0; MS *m/z* (relative intensity, %): 228 (M^+, 16), 185 (4), 171 (13), 157 (36), 143 (32), 131 (100), 115 (24), 91 (11).

4.5.12. (4-Methoxyphenyl)phenylacetylene. Mp 58–60 °C (lit.[33] 57–61 °C). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.53–7.46 (m, 4H), 7.37–7.30 (m, 3H), 6.87 (dd, J=8.70, 2.10 Hz, 2H), 3.82 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 159.5, 133.0, 131.4, 128.3, 127.9, 123.5, 115.3, 113.9, 89.3, 88.0, 55.3; MS *m/z* (relative intensity, %): 208 (M^+, 100), 193 (48), 165 (39), 139 (8).

4.5.13. (4-Trifluoromethylphenyl)phenylacetylene. Mp 103–105 °C (lit.[34] 104–106 °C). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.63–7.60 (m, 4H), 7.57–7.54 (m, 2H), 7.39–7.35 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 131.8, 131.7, 129.9 (J=32.8 Hz), 128.8, 128.4, 127.1 (J=1.2 Hz), 125.3 (J=3.7 Hz), 123.9 (J=270.7 Hz), 122.5, 91.7, 87.9; MS *m/z* (relative intensity, %): 246 (M^+, 100), 227 (9), 196 (8), 176 (10), 98 (8).

4.5.14. 4-(Phenylethynyl)-1,1′-biphenyl. Mp 163–164 °C (lit.[28] 162–163 °C). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.63–7.54 (m, 7H), 7.48–7.43 (m, 2H), 7.38–7.34 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 140.9, 140.3, 132.0, 131.6, 128.8, 128.34, 128.25, 127.6, 127.0, 123.2, 122.1, 90.0, 89.3; MS *m/z* (relative intensity, %): 254 (M^+, 100), 255 (23), 252 (26), 127 (9).

4.5.15. (4-Cyanophenyl)phenylacetylene. Mp 109–110 °C (lit.[35] 108.5–109.5 °C). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.65–7.59 (m, 4H), 7.56–7.53 (m, 2H), 7.40–7.37 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 132.0 (2C), 131.8, 129.1, 128.5, 128.2, 122.2, 118.5, 111.4, 93.7, 87.7; MS *m/z* (relative intensity, %): 203 (M^+, 100), 176 (8), 151 (5), 75 (5).

4.5.16. (3-Cyanophenyl)phenylacetylene. Mp 69–71 °C (lit.[35] 70–71 °C). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.79 (t, J= 1.50 Hz, 1H), 7.73 (dt, J=7.80, 1.50 Hz, 1H), 7.59 (dt, J= 7.80, 1.50 Hz, 1H), 7.55–7.52 (m, 2H), 7.45 (t, J=7.80 Hz, 1H), 7.39–7.36 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 135.6, 134.8, 131.7, 131.3, 129.2, 128.9, 128.4, 124.8, 122.2, 118.0, 112.8, 91.7, 86.8; MS *m*/*z* (relative intensity, %): 203 (M^+, 100), 176 (7), 151 (5), 75 (5).

4.5.17. (2-Trifluoromethylphenyl)phenylacetylene. Oil.[36] ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.67–7.62 (m, 2H), 7.57–7.53 (m, 2H), 7.49–7.44 (m, 1H), 7.39–7.33 (m, 4H); MS *m*/*z* (relative intensity, %): 246 (M^+, 100), 225 (17), 202 (12), 196 (8), 176 (5), 98 (4). Anal. Calcd for $C_{15}H_9F_3$: C, 73.22; H, 3.68. Found: C, 73.12; H, 3.77.

4.5.18. 2-(2-Phenylethynyl)pyridine. Oil.[37] ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.60 (dd, J=5.10, 0.90 Hz, 1H), 7.68–7.59 (m, 3H), 7.51 (dd, J=8.10, 0.90 Hz, 1H), 7.37–7.33 (m, 3H), 7.23–7.19 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 149.9, 143.2, 136.0, 131.9, 128.8, 128.2, 127.0, 122.6, 122.0, 89.1, 88.4; MS *m*/*z* (relative intensity, %): 179 (M^+, 100), 180 (17), 178 (40), 151 (13), 126 (8), 76 (9).

Acknowledgements

We gratefully acknowledge financial support by the National Natural Science Foundation of China (No. 20372024), the Excellent Scientist Foundation of Anhui Province, China (No. 04046080), the Natural Science Foundation of the Education Department of Anhui Province, China (No. 2002kj254zd), the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry, China (No. 2002247), the Excellent Young Teachers Program of MOE, China (No. 2024), and the Key Project of Science and Technology of State Education Ministry, China (No. 0204069).

References and notes

1. (a) Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, *16*, 4467–4470. (b) Burnagin, N. A.; Sukhomlinova, L. I.; Luzikova, E. V.; Tolstaya, T. P.; Beletskaya, I. P. *Tetrahedron Lett.* **1996**, *37*, 897–900. (c) Hundertmark, T.; Littke, A. F.; Buchwald, S. L.; Fu, G. C. *Org. Lett.* **2000**, *2*, 1729–1731. (d) Erdelyi, M.; Gogoll, A. *J. Org. Chem.* **2001**, *66*, 4165–4169. For selected reviews on Sonogashira reaction, see: (e) Sonogashira, K. In Trost, B. M., Fleming, I., Eds.; Comprehensive Organic Synthesis; Pergamon: Oxford, 1991; Vol. 3, pp 521–549. (f) Sonogashira, K. In *Metal-Catalyzed Cross-Coupling Reactions*; Diederich, F., Stang, P. J., Eds.; Wiley-VCH: Weinheim, 1998; pp 203–229. (g) Sonogashira, K. In *Handbook of Organopalladium Chemistry for Organic Synthesis*; Negishi, E., Meijere, A., Eds.; Wiley-VCH: New York, 2002. (h) Sonogashira, K. *J. Organomet. Chem.* **2002**, *653*, 46–49. (i) Negishi, E.; Anastasia, L. *Chem. Rev.* **2003**, *103*, 1979–2018 and references cited therein.
2. (a) Paterson, I.; Davies, R. D. M.; Marquez, R. *Angew. Chem., Int. Ed.* **2001**, *40*, 603–607. (b) Toyota, M.; Komori, C.; Ihara, M. *J. Org. Chem.* **2000**, *65*, 7110–7113. (c) Ioshimura, F.; Kawata, S.; Hirama, M. *Tetrahedron Lett.* **1999**, *40*, 8281–8285. (d) Nicolaou, K. C.; Dai, W.-M. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1387–1416.
3. (a) Cosford, N. D. P.; Tehrani, L.; Roppe, J.; Schweiger, E.; Smith, N. D.; Anderson, J.; Bristow, L.; Brodkin, J.; Jiang, X.; McDonald, I.; Rao, S.; Washburn, M.; Varney, M. A. *J. Med. Chem.* **2003**, *46*, 204–206. (b) Taylor, E. C.; Dowling, J. E. *J. Org. Chem.* **1997**, *62*, 1599–1603. (c) Nakamura, H.; Aizawa, M.; Takeuchi, D.; Murai, A.; Shimoura, O. *Tetrahedron Lett.* **2000**, *41*, 2185–2188. (d) Amiet, G.; Hügel, H. M.; Nurlawis, F. *Synlett* **2002**, 495–497. (e) Liu, T.-Z.; Isobe, M. *Synlett* **2000**, 266–268. (f) Kort, M.; Correa, V.; Valentijin, A. R. P. M.; Marel, G. A.; Potter, B. V. L.; Taylor, C. W.; Boom, J. H. *J. Med. Chem.* **2000**, *43*, 3295–3303.
4. (a) Nalwa, H. S.; Miyata, S. *Nonlinear Optics of Organic Molecules and Polymers*; CRC: Boca Raton, FL, 1997. (b) Mongin, O.; Porres, L.; Moreaux, L.; Merta, J.; Blanchard-Desce, M. *Org. Lett.* **2002**, *4*, 719–722. (c) Brunsveld, L.; Meijer, E. W.; Prince, R. B.; Moore, J. S. *J. Am. Chem. Soc.* **2001**, *123*, 7978–7984. (d) Pérez-Balderas, F.; Santoyo-González, F. *Synlett* **2001**, 1699–1702. (e) Sonoda, M.; Inaba, A.; Itahashi, K.; Tobe, Y. *Org. Lett.* **2001**, *3*, 2419–2421. (f) Wong, K.-T.; Hsu, C. C. *Org. Lett.* **2001**, *3*, 173–175. (g) Torruellas, W. E.; Neher, D.; Zanoni, R.; Stegeman, G. I.; Kajzar, F.; Leclerc, M. *Chem. Phys. Lett.* **1990**, *175*, 11–16. (h) Lee, C.-H.; Yamamoto, T. *Tetrahedron Lett.* **2001**, *42*, 3993–3996. (i) Matsumi, N.; Naka, K.; Chujo, Y. *J. Am. Chem. Soc.* **1998**, *120*, 5112–5113. (j) Martin, R. E.; Diederich, F. *Angew. Chem., Int. Ed.* **1999**, *38*, 1350–1377. (k) Inouye, M.; Takahashi, K.; Nakazumi, H. *J. Am. Chem. Soc.* **1999**, *121*, 341–345.
5. (a) Wegner, G.; Müllen, K. *Electronic Materials—the Oligomer Approach*; Wiley-VCH: Weinheim, 1998. (b) Li, J.; Ambroise, A.; Yang, S. I.; Diers, J. R.; Seth, J.; Wack, C. R.; Bocian, D. F.; Holten, D.; Lindsey, J. S. *J. Am. Chem. Soc.* **1999**, *121*, 8927–8940. (c) Solomin, V. A.; Heitz, W. *Macromol. Chem. Phys.* **1994**, *195*, 303–314. (d) Strachan, J.-P.; Gentemann, S.; Seth, J.; Kalsbeck, W. A.; Lindsey, J. S.; Holten, D.; Bocian, D. F. *Inorg. Chem.* **1998**, *37*, 1191–1201. (e) Wagner, R. W.; Seth, J.; Yang, S. I.; Kim, D.; Bocian, D. F.; Holten, D.; Lindsey, J. S. *J. Org. Chem.* **1998**, *63*, 5042–5049.
6. (a) Höger, S.; Rosselli, S.; Ramminger, A.-D.; Enkelmann, V. *Org. Lett.* **2002**, *4*, 4269–4272. (b) Li, C.-J.; Slaven, W. T., IV; John, V. T.; Banerjee, S. *Chem. Commun.* **1997**, 1569–1570.
7. (a) Mongin, O.; Papamicael, C.; Hoyler, N.; Gossauer, A. *J. Org. Chem.* **1998**, *63*, 5568–5580. (b) Tobe, Y.; Utsumi, N.; Nagano, A.; Naemura, K. *Angew. Chem., Int. Ed.* **1998**, *37*, 1285–1287. (c) Onitsuka, K.; Fujimoto, M.; Ohshiro, N.; Takahashi, S. *Angew. Chem., Int. Ed.* **1999**, *38*, 689–692.
8. (a) Glaser, C. *Ber. Dtsch. Chem. Ges.* **1869**, *2*, 422–424. (b) Glaser, C. *Ann. Chem. Pharm.* **1870**, *154*, 137–171. (c) Hay, A. S. *J. Org. Chem.* **1962**, *27*, 3320–3321. (d) Rossi, R.; Carpita, A.; Bigelli, C. *Tetrahedron Lett.* **1985**, *26*, 523–526. (e) Liu, Q.; Burton, D. J. *Tetrahedron Lett.* **1997**, *38*, 4371–4374. (f) For a review of acetylenic coupling, see: Siemsen, P.; Livingston, R. C.; Diederich, F. *Angew. Chem., Int. Ed.* **2000**, *39*, 2632–2657.
9. (a) Ziener, U.; Godt, A. *J. Org. Chem.* **1997**, *62*, 6137–6143. (b) Kukula, H.; Veit, S.; Godt, A. *Eur. J. Org. Chem.* **1999**, 277–286.

10. (a) Leadbeater, N. E.; Tominack, B. J. *Tetrahedron Lett.* **2003**, *44*, 8653–8656. (b) Soheili, A.; Albaneze-Walker, J.; Murry, J. A.; Dormer, P. G.; Hughes, D. L. *Org. Lett.* **2003**, *5*, 4191–4194. (c) Heuzé, K.; Méry, D.; Gauss, D.; Astruc, D. *Chem. Commun.* **2003**, 2274–2275. (d) Méry, D.; Heuzé, K.; Astruc, D. *Chem. Commun.* **2003**, 1934–1935. (e) Ma, Y.; Song, C.; Jiang, W.; Wu, Q.; Wang, Y.; Liu, X.; Andrus, M. B. *Org. Lett.* **2003**, *5*, 3317–3319. (f) Djakovitch, L.; Rollet, P. *Tetrahedron Lett.* **2004**, *45*, 1367–1370. (g) Uozumi, Y.; Kobayashi, Y. *Heterocycles* **2003**, *59*, 71–74. (h) Pal, M.; Parasuraman, K.; Gupta, S.; Yeleswarapu, K. R. *Synlett* **2002**, 1976–1982. (i) Fu, X.; Zhang, S.; Yin, J.; Schumacher, D. P. *Tetrahedron Lett.* **2002**, *43*, 6673–6676. (j) Böhm, V. P. W.; Herrmann, W. A. *Eur. J. Org. Chem.* **2000**, 3679–3681. (k) Fu, X.; Zhang, S.; Yin, J.; Schumacher, D. *Tetrahedron Lett.* **2002**, *43*, 6673–6676. (l) Alonso, D. A.; Nájera, C.; Pacheco, M. C. *Tetrahedron Lett.* **2002**, *43*, 9365–9368. (m) Fukuyama, T.; Shinmen, M.; Nishitani, S.; Sato, M.; Ryu, I. *Org. Lett.* **2002**, *4*, 1691–1694. (n) Nájera, C.; Gil-Moltó, J.; Karlström, S.; Falvello, L. R. *Org. Lett.* **2003**, *5*, 1451–1454. (o) Alonso, D. A.; Nájera, C.; Pacheco, M. C. *Adv. Synth. Catal.* **2003**, *345*, 1146–1158. (p) Buchmeiser, M. R.; Schareina, T.; Kempe, R.; Wurst, K. *J. Organomet. Chem.* **2001**, *634*, 39–46. It should be noted that the copper-free Sonogashira coupling reactions are representative and not comprehensive in this paper.
11. (a) Cheng, J.; Sun, Y.; Wang, F.; Guo, M.; Xu, J.-H.; Pan, Y.; Zhang, Z. *J. Org. Chem.* **2004**, *69*, 5428–5432. (b) Arques, A.; Aunon, D.; Molina, P. *Tetrahedron Lett.* **2004**, *45*, 4337–4340. (c) Mori, A.; Kawashima, J.; Shimada, T.; Suguro, M.; Hirabayashi, K.; Nishihara, Y. *Org. Lett.* **2000**, *2*, 2935–2937. (d) Alonso, D. A.; Najera, C.; Pacheco, M. C. *Tetrahedron Lett.* **2002**, *43*, 9365–9368. (e) Gelman, D.; Buchwald, S. L. *Angew. Chem., Int. Ed.* **2003**, *42*, 5993–5996.
12. Choudary, B. M.; Madhi, S.; Chowdari, N. S.; Kantam, M. L.; Sreedhar, B. *J. Am. Chem. Soc.* **2002**, *124*, 14127–14136.
13. Kabalka, G. W.; Wang, L.; Namboodiri, V.; Pagni, R. M. *Tetrahedron Lett.* **2000**, *41*, 5151–5154.
14. (a) Beletskaya, I. P.; Latyshev, G. V.; Tsvetkov, A. V.; Lukashev, N. V. *Tetrahedron Lett.* **2003**, *44*, 5011–5013. (b) Wang, L.; Li, P.; Zhang, Y. *Chem. Commun.* **2004**, 514–515.
15. (a) Bong, D. T.; Ghadiri, M. R. *Org. Lett.* **2001**, *3*, 2509–2511. (b) Casalnuovo, A. L.; Caladrese, J. C. *J. Am. Chem. Soc.* **1990**, *112*, 4324–4330. (c) Dibowski, H.; Schmidtchen, F. P. *Tetrahedron Lett.* **1998**, *39*, 525–528. (d) Genet, J.-P.; Blart, E.; Savignac, M. *Synlett* **1992**, 715–717.
16. Park, S. B.; Alper, H. *Chem. Commun.* **2004**, 1306–1307.
17. (a) Using an atmosphere of hydrogen gas diluted with nitrogen or argon to diminish homo-coupling in Sonogashira reaction, see: Elangovan, A.; Wang, Y.-H.; Ho, T.-I. *Org. Lett.* **2003**, *5*, 1841–1844. (b) Use of phase-transfer conditions to diminish homo-coupling, see: Chow, H.-F.; Wan, C.-W.; Low, K.-H.; Yeung, Y.-Y. *J. Org. Chem.* **2001**, *66*, 1910–1913. (c) With slow addition of the acetylene to diminish homo-coupling, see: Thorand, S.; Krause, N. *J. Org. Chem.* **1998**, *61*, 8551–8553.
18. Leadbeater, N. E.; Marco, M.; Tominack, B. J. *Org. Lett.* **2003**, *5*, 3919–3922.
19. (a) Urgaonkar, S.; Verkade, J. G. *J. Org. Chem.* **2004**, *69*, 5752–5755. (b) Zhang, G. *Synlett* **2005**, 619–622.
20. Son, S. U.; Jang, Y.; Park, J.; Na, H. B.; Park, H. M.; Yun, H. J.; Lee, J.; Hyeon, T. *J. Am. Chem. Soc.* **2004**, *126*, 5026–5027.
21. (a) Reetz, M. T.; Helbig, W.; Quaiser, S. A.; Stimming, U.; Breuer, N.; Vogel, R. *Science* **1995**, *267*, 367–369. (b) Reetz, M. T.; Winter, M.; Breinbauer, R.; Thurn-Albrecht, T.; Vogel, W. *Chem. Eur. J.* **2001**, *7*, 1084–1094. (c) Reetz, M. T.; Maase, M. *Adv. Mater.* **1999**, *11*, 773–777. (d) Reetz, M. T.; Westermann, E. *Angew. Chem., Int. Ed.* **2000**, *39*, 165–168. (e) Reetz, M. T.; Westermann, E.; Lohmer, R.; Lohmer, G. *Tetrahedron Lett.* **1998**, *39*, 8449–8452. (f) Reetz, M. T.; Breinbauer, R.; Wanninger, K. *Tetrahedron Lett.* **1996**, *37*, 4499–4502. (g) Narayanan, R.; EI-Sayed, M. A. *J. Am. Chem. Soc.* **2003**, *125*, 8340–8347. (h) Reetz, M. T.; Vries, J. G. *Chem. Commun.* **2004**, 1559–1563 and references cited therein. (i) Reetz, M. T.; Helbig, W. *J. Am. Chem. Soc.* **1994**, *116*, 7401–7402. (j) Reetz, M. T.; Quaiser, S. A.; Breinbauer, R.; Tesche, B. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2728–2730.
22. The palladium colloid on poly(vinylpyrrolidone) (PVP) was prepared according to a simple and convenient procedure, see: Bradley, J. S.; Millar, J. M.; Hill, E. W. *J. Am. Chem. Soc.* **1991**, *113*, 4016–4017.
23. For Jeffery's catalyst systems (Pd/QX) and their applications, see (a) Jeffery, T. *J. Chem. Soc., Chem. Commun.* **1984**, 1287–1289. (b) Jeffery, T. *Tetrahedron Lett.* **1985**, *26*, 2667–2670. (c) Jeffery, T. *Tetrahedron Lett.* **1994**, *35*, 3051–3054. (d) Jeffery, T.; Galland, J. C. *Tetrahedron Lett.* **1994**, *35*, 4103–4106. (e) Jeffery, T. *Tetrahedron* **1996**, *52*, 10113–10130. (f) Jeffery, T. In *Advances in Metal-Organic Chemistry*; Liebeskind, L. S., Ed.; Jai: London, 1996; pp 153–260.
24. Carpino, L. A.; Chen, H.-W. *J. Am. Chem. Soc.* **1979**, *101*, 390–394.
25. Paquett, L. A.; Wittenbrook, L. S. *J. Am. Chem. Soc.* **1967**, *89*, 4483–4487.
26. Arcadi, A.; Cacchi, S.; Fabrizi, G.; Marinelli, F.; Pace, P. *Eur. J. Org. Chem.* **1999**, *12*, 3305–3313.
27. Hunter, D. H.; Cram, D. J. *J. Am. Chem. Soc.* **1966**, *88*, 5765–5776.
28. Chow, H.-F.; Wan, C.-W.; Low, K.-H.; Yeung, Y.-Y. *J. Org. Chem.* **2001**, *66*, 1910–1913.
29. Redenko, A. P.; Vasil'ev, A. V.; Russ *J. Org. Chem.* **1995**, *31*, 1360.
30. Sato, H.; Isono, N.; Miyoshi, I.; Mori, M. *Tetrahedron* **1996**, *52*, 8143–8158.
31. Masuda, Y.; Hoshi, M.; Arase, A. *Chem. Lett.* **1980**, 413.
32. Kabalka, G. W.; Wang, L.; Pagni, R. M. *Tetrahedron* **2001**, *57*, 8017–8028.
33. Huggins, J. M.; Bergman, R. G. *J. Am. Chem. Soc.* **1981**, *103*, 3002–3011.
34. Kiplinger, J. L.; King, M. A.; Fechtenkötter, A.; Arif, A. M.; Richmond, T. G. *Organometallics* **1996**, *15*, 5292–5301.
35. Yoshida, K.; Fueno, T. *J. Org. Chem.* **1973**, *38*, 1045–1046.
36. Feuerstein, M.; Berthiol, F.; Doucet, H.; Santelli, M. *Synthesis* **2004**, 1281–1289.
37. Agawa, T.; Miller, S. I. *J. Am. Chem. Soc.* **1961**, *83*, 449–453.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 8641–8647

The fluoride ion-induced intramolecular conjugate addition of propargylsilanes to dihydropyridones. A novel method for the stereoselective construction of azabicyclic ring systems

Magdalena Dziedzic,[a] Grzegorz Lipner,[a] José M. Illangua[b] and Bartłomiej Furman[a,*]

[a]*Institute of Organic Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland*
[b]*Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, Apartado 553, E-41071 Sevilla, Spain*

Received 29 April 2005; revised 13 June 2005; accepted 30 June 2005

Available online 20 July 2005

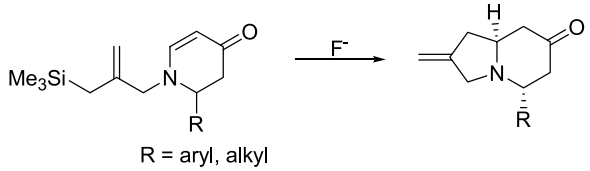
Abstract—The fluoride ion-induced intramolecular conjugate addition of propargylsilanes to dihydropyridones is reported. Our results revealed that tetrabutylammonium triphenyldifluorosilicate (TBAT), an air-stable, non-hygroscopic fluoride ion source, catalyzes cyclocondensation to provide the corresponding 1-vinylidene indolizidines in a high yield as single isomers, while Lewis acid catalysts were ineffective. The scope of this method was further investigated in the reactions leading to compounds with larger ring size. In these cases dihydropyridones with the propargylsilane located in the side-chain underwent cyclization to give 9-vinylidene quinolizidines with significantly lower yields.

1. Introduction

The chemistry of propargylsilanes continues to be widely exploited in organic synthesis. The main interest in propargylsilanes relates to their ability to react with various electrophiles activated by a Lewis acid to form a new C–C bond.[1] With propargylsilanes, the addition of an electrophile provides a quite general route to allenes, which are emerging as versatile building blocks in organic synthesis.[2] The intramolecular cyclizations, involving a terminal propargylsilane function have been successfully employed in the stereospecific synthesis of polycyclic compounds.[3] The proper choice of Lewis acid can, in some cases, enable control of the stereoselectivity and prevent protodesilylation, which is a common drawback of such a reaction.

The fluoride ion is generally known to be a good activating reagent for organosilicon reagents, which readily generates reactive pentacoordinate silicates. In 1978, Hosomi and Sakurai[4] found that a fluoride ion source—tetrabutylammonium fluoride (TBAF) catalyzed the allylation of carbonyl compounds with allylsilanes to give the corresponding homoallyl alcohols. Recently, Wang et al.,[5] have found that TBAF (1 mol%) in the presence of 4 Å MS is an effective catalyst for the allylation of aromatic imines. Majetich et al.,[6] reported that the fluoride ion-catalyst is effective in the conjugate allylation of α,β-unsaturated esters, nitriles, and amides, a reaction, which cannot be achieved by the $TiCl_4$-promoted procedure. Occasionally, intramolecular conjugate allylation of α,β-unsaturated ketones is also catalyzed efficiently by TBAF.[7] In contrast to the reaction of allylsilanes, little is known about the reaction of propargylsilanes with electrophiles under fluoride ion catalysis. To our knowledge, the fluoride ion-promoted 1,4-addition of propargylsilanes to the α,β-unsaturated compounds has never been reported.

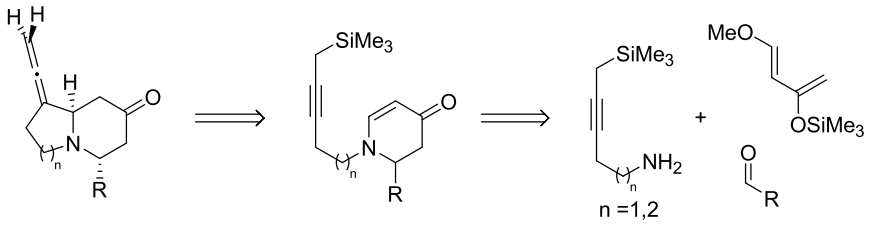
Recently,[8] we reported that the intramolecular fluoride-promoted allylation of the appropriate dihydropyridones proceeds in a conjugate fashion in good yield (Scheme 1). The Lewis acid catalyzed allylation fails, in marked contrast to the known efficiency of this method in the intermolecular allylation of dihydropyridones.[9]



Scheme 1.

Keywords: Conjugate addition; Fluoride ions; Propargylsilanes; Dihydropyridones.
* Corresponding author. Tel.: +48 223 432 128; fax: +48 226 326 681; e-mail: furbar@icho.edu.pl

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.06.105



Scheme 2. Synthetic strategy.

We now report that the similar cyclocondensation of dihydropyridones with propargylsilanes located in the side-chain provides a concise protocol for the highly stereoselective construction of the vinylidene azabicyclic skeleton. The proposed reaction sequence consists of the Lewis acid-mediated addition of an imine to the silyloxy-diene followed by the fluoride ion-initiated intramolecular 1,4-addition of propargylsilanes to dihydropyridones (Scheme 2).

2. Results and discussion

The two amines **1**[10] and **2**[11] were employed for the construction of the desired dihydropyridones (Fig. 1).

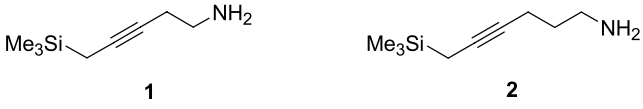


Figure 1.

These substrates were easily prepared in five steps using literature methods from 3-butyn-1-ol and 4-butyn-1-ol in 52 and 35% overall yield, respectively.

The propargylamine **1** was converted into the required Schiff bases **3** by treatment with series of different aldehydes in CH_2Cl_2 in the presence of molecular sieves (4 Å). These intermediates were not isolated. After exchange of the solvent, they were immediately subjected to the Lewis acid-mediated reaction with Danishefsky's diene **4**. We found that for aromatic and aliphatic imines of the type **3** the highest yield of dihydropyridones **5** were attained using $Yb(OTf)_3$ (0.1 equiv) as a Lewis acid with CH_3CN as a solvent (Table 1).

The reactions of the imines **3** with dienes **4** shown in Table 1 take place smoothly to afford the corresponding adducts **5** in good yield. The aliphatic imines worked well, however, the dihydropyridones **5h** and **5i** were obtained in a slightly decreased yield.

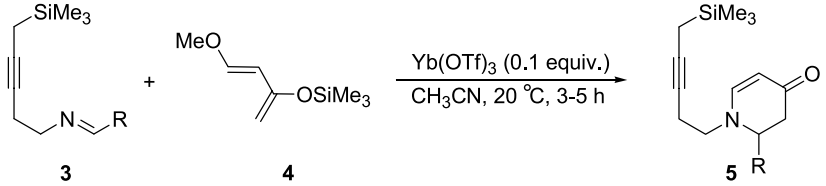
Since imines, particularly those derived from aliphatic aldehydes, are usually not stable, it is synthetically useful when they are generated in situ and allowed to react under one-pot reaction conditions. Recently, Kobayashi et al.[12] reported that $Yb(OTf)_3$ catalyzes the three-component coupling reaction between aldehydes, amines, and Danishefsky's diene to afford dihydropyridone derivatives.

Following this report, we studied the direct synthesis of dihydropyridones as one-pot reaction (Table 2).

As shown in Table 2, the modified procedure afforded the corresponding adducts in yields comparable to those obtained by the reaction of the pre-formed and isolated aldimines. The one-pot reaction can be carried out with various aldehydes, including aliphatic, aromatic, and heteroaromatic compounds.

Very recently we reported[8] that tetrabutylammonium triphenyldifluorosilicate (TBAT)[13] efficiently catalyzes the

Table 1. Lewis acid mediated synthesis of 2-substituted dihydropyridones[a]



Entry	R	Product	Yield (%)[b]
1	Ph	**5a**	82
2	*p*-MePh	**5b**	80
3	*p*-MeOPh	**5c**	78
4	*p*-ClPh	**5d**	86
5	2-Furyl	**5e**	81
6	2-Pyridyl	**5f**	76
7	$PhCH_2OCH_2$	**5g**	89
8	$CH_3(CH_2)_5$	**5h**	77
9	*c*-C_6H_{11}	**5i**	70

[a] All reactions were conducted in CH_3CN at 20 °C in the presence of 0.1 equiv $Yb(OTf)_3$.
[b] Isolated yield after chromatographic purification.

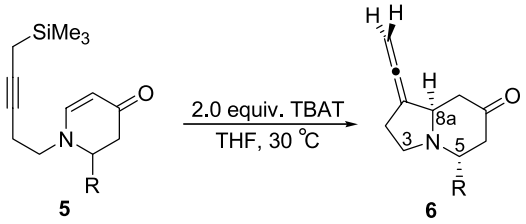
Table 2. Results of direct synthesis of 2-substituted dihydropyridones[a]

Entry	R	Product	Time/h	Yield[b] (%)
1	Ph	**5a**	5	80
2	*p*-MePh	**5b**	6	83
3	*p*-MeOPh	**5c**	4	81
4	*p*-ClPh	**5d**	5	79
5	2-Furyl	**5e**	8	74
6	2-Pyridyl	**5f**	9	72
7	$PhCH_2OCH_2$	**5g**	2	88
8	$CH_3(CH_2)_5$	**5h**	3	80
9	*c*-C_6H_{11}	**5i**	3	77

[a] All reactions were conducted in CH_3CN at 20 °C in the presence of 0.1 equiv $Yb(OTf)_3$.
[b] Isolated yield after chromatographic purification.

intramolecular conjugate allylation of various 2-substituted dihydropyridones with allylsilanes. To our satisfaction we found that the use of 2.0 equiv of TBAT resulted in a nearly quantitative formation of the corresponding vinylidene indolizidines (Table 3). Other fluoride ion sources such as CsF, KF or TBAF failed to promote the cyclization at all. It is important to note that none of the desired products could be obtained by treatment of respective dihydropyridones with Lewis acids such as $EtAlCl_2$, $TiCl_4$, $SnCl_4$, TMSOTf or $BF_3 \cdot Et_2O$.

Table 3. TBAT-initiated intramolecular conjugate addition of propargylsilane to dihydropyridones[a]



5 6

Entry	R	Product	Time/h	Yield[b] (%)
1	Ph	**6a**	1	95
2	*p*-MePh	**6b**	1	93
3	*p*-MeOPh	**6c**	2	97
4	*p*-ClPh	**6d**	2	98
5	2-Furyl	**6e**	5	89
6	2-Pyridyl	**6f**	5	92
7	$PhCH_2OCH_2$	**6g**	2	89
8	$CH_3(CH_2)_5$	**6h**	2	83
9	*c*-C_6H_{11}	**6i**	2	86

[a] All reactions were conducted in THF at 30 °C in the presence of 2.0 equiv of TBAT.
[b] Isolated yield after chromatographic purification.

For all entries in Table 3, treatment of the 2-substituted dihydropyridones with 2.0 equiv of TBAT in THF at 30 °C led to the desired indolizidines (single diastereomer), as estimated from the ^1H and ^{13}C NMR data. It was observed that, in the case of the reaction of dihydropyridones **6e** and **6f** (R = furyl or pyridyl), longer reaction times (4–5 h) were needed to afford products in a high yield. When we used compounds containing aliphatic substituents **6h** and **6i** the yield was slightly decreased, compared to other dihydropyridones.

The relative stereochemistry at C5 and C8a was established with the aid of NOE experiments. Furthermore, the structure and relative stereochemistry of the cyclized product was unambiguously established by the single-crystal X-ray analysis.[14] As revealed in Figure 2, compound **6a** possesses the indolizidine skeleton and two hydrogens at C5 and C8a are trans to each other.

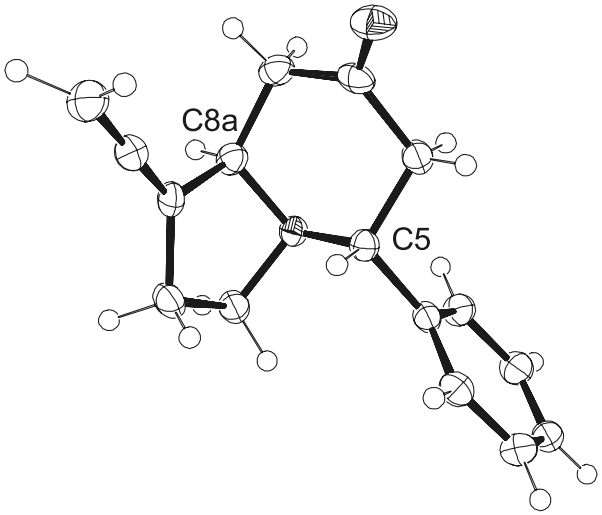


Figure 2. The X-ray structure of **6a**.

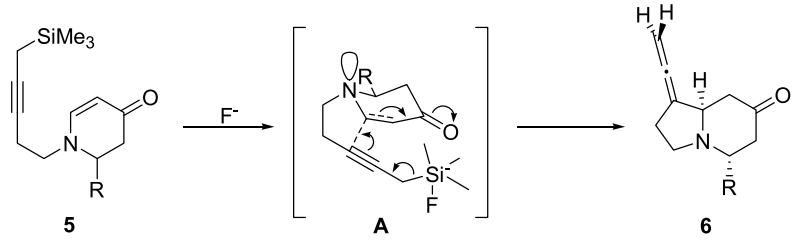
The stereochemical outcome of this cyclocondensation is rationalized in Scheme 3.

We propose that the cyclization proceeds through the transition state **A**, where the substituent R is located in the equatorial position while the nitrogen electron pair is in the axial orientation. The nucleophilic terminal of the double bond approaches exclusively anti to the R substituent leading to the compound **6** with trans-orientation between C5 and C8a protons.

The generality of this transformation was further tested with different ring size. The enaminones of the type **7** were prepared by the similar strategy as described earlier, starting from amine **2**.

For the compounds with a longer side chain the intramolecular 1,4-addition, under standard cyclization conditions (2.0 equiv TBAT in THF at 30 °C), led to the 9-vinylidene quinolizidines **8a** and **8b**, obtained as single diastereomers in 35 and 27% yield, respectively (Table 4). However, significant amounts of protodesilylation products were also observed. No cyclization occurred when compound containing an aliphatic substituent **7c** was used. Numerous attempts to affect the reaction outcome, such as applying alternative fluoride sources or selected Lewis acids, were unsuccessful and only protodesilylation products were observed.

The relative stereochemistry of **8a** was assigned by the NOE experiments. Upon irradiation of the proton at C4 no enhancement was observed for the proton at C9a. This clearly reveals that the two hydrogens at C4 and C9a are trans to each other. The 9-vinylidene quinolizidines have similar relative stereochemistry at bridgehead carbon atoms



Scheme 3. Fluoride ion-initiated intramolecular 1,4-addition of propargylsilane to dihydropyridones.

Table 4. TBAT-initiated synthesis of 9-vinylidene quinolizidines[a]

Entry	R	Product	Time/h	Yield[b] (%)
1	(**7a**) Ph	**8a**	2	35
2	(**7b**) *p*-MeOPh	**8b**	2	27
3	(**7c**) $CH_3(CH_2)_5$	**8c**	2	0[c]

[a] All reactions were conducted in THF at 30 °C in the presence of 2.0 equiv of TBAT.
[b] Isolated yield after chromatographic purification.
[c] Only protodesilylation products were observed.

as indolizidines discussed earlier. Thus, the similar rationalization for the stereochemical outcome observed in these cyclizations should be applicable as well.

3. Conclusions

A novel method for the stereoselective construction of vinylidene azabicyclic compounds has been described. It is based on the fluoride ion-induced intramolecular 1,4-addition of propargylsilanes to dihydropyridones. The structure and relative stereochemistry of the cyclization product, 1-vinylidene indolizidine, was confirmed by the NMR and X-ray analysis. The scope of this method was further investigated in the reactions leading to the compounds with larger ring size. However, the one carbon homologated compounds did not show the same cyclization behavior, and the expected 9-vinylidene quinolizidines were obtained in low yields.

4. Experimental

4.1. General

Column chromatography was performed on silica gel, Merck grade 60 (230–400 mesh). TLC plates were visualized with UV and staining with phosphomolybdic acid. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AM500 (500 MHz) and Varian (400 MHz) spectrometers and the chemical shifts are reported in ppm from the solvent resonans ($CDCl_3$ 7.26 ppm). Infrared (IR) spectra were measured with a Perkin-Elmer FT-IR-1600 infrared spectrophotometer. High-resolution mass spectra were taken on a Mariner PerSeptive Biosystems mass spectrometer with time-of-flight (TOF) detector. Unless stated otherwise, all reagents and solvents were purchased from commercial sources and used without additional purification.

4.2. General procedure for the synthesis of imines

To a solution of amine **1** or **2** (0.5 mmol) in CH_2Cl_2 (5 mL) was added the corresponding aldehyde (0.5 mmol) and activated 4 Å molecular sieves (200 mg). The mixture was stirred for 12 h at ambient temperature, filtered through a Celite pad and the solvent was evaporated in vacuo. The resulting imines were used directly in the subsequent reactions without further purification.

4.3. General procedure for the aza-Diels–Alder reaction of Danishefsky's diene with imines

To a solution of the respective imine (0.5 mmol) in MeCN (5 mL) was added $Yb(OTf)_3$ (0.05 mmol) followed by diene **4** (0.6 mmol, 1.2 equiv). The reaction mixture was stirred for 3–5 h at room temperature. After addition of saturated $NaHCO_3$ (10 mL), the aqueous phase was extracted with CH_2Cl_2 (3×10 mL), the combined organic phases were dried over $MgSO_4$, and the solvent was evaporated in vacuo. The crude material was purified by flash column chromatography on silica gel.

4.3.1. 2-Phenyl-1-(5-trimethylsilanyl-pent-3-ynyl)-2,3-dihydro-1*H*-pyridin-4-one (5a). Chromatography (80:20 Et_2O/hexane) afforded 0.127 g (82%) of a colorless oil: IR (neat) 2219, 1639, 1593, 1579, 849 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.08 (s, 9H), 1.42 (t, J=2.6 Hz, 2H), 2.24–2.42 (m, 2H), 2.69 (dd, J=16.4, 8.6 Hz, 1H), 2.86 (dd, J=16.4, 6.7 Hz, 1H), 3.21 (m, 2H), 4.69 (dd, J=8.6, 6.7 Hz, 1H), 5.04 (d, J=7.7 Hz, 1H), 7.23 (d, J=7.7 Hz, 1H), 7.30–7.39 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ −2.0, 6.9, 19.4, 43.9, 52.8, 60.9, 74.2, 81.3, 98.4, 126.9, 128.3, 129.1, 138.7, 154.5, 190.3; HRMS calcd for $C_{19}H_{25}NOSi$ (M^+) 311.1705, found 311.1716.

4.3.2. 2-*p*-Tolyl-1-(5-trimethylsilanyl-pent-3-ynyl)-2,3-dihydro-1*H*-pyridin-4-one (5b). Chromatography (80:20 Et_2O/hexane) afforded 0.130 g (80%) of a colorless oil: IR (neat) 2220, 1642, 1591, 849 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.08 (s, 9H), 1.42 (t, J=2.6 Hz, 2H), 2.24–2.33 (m, 2H), 2.34 (s, 3H), 2.68 (dd, J=16.4, 9.2 Hz, 1H), 2.81 (dd, J=16.4, 6.6 Hz, 1H), 3.18 (m, 2H), 4.69 (dd, J=9.2, 6.6 Hz, 1H), 5.03 (d, J=7.6 Hz, 1H), 7.15 (d, J=7.6 Hz,

1H), 7.18–7.23 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ −2.0, 6.9, 19.4, 21.1, 44.1, 52.7, 60.8, 74.3, 81.3, 98.3, 126.9, 129.7, 135.7, 138.1, 154.4, 190.5; HRMS calcd for $C_{20}H_{27}NOSi$ 325.1862, found 325.1870.

4.3.3. 2-(4-Methoxyphenyl)-1-(5-trimethylsilanyl-pent-3-ynyl)-2,3-dihydro-1*H*-pyridin-4-one (5c). Chromatography (80:20 Et_2O/hexane) afforded 0.133 g (78%) of a yellow oil: IR (neat) 2220, 1640, 1591, 848 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.08 (s, 9H), 1.42 (t, J=2.6 Hz, 2H), 2.22–2.40 (m, 2H), 2.69 (dd, J=16.4, 9.3 Hz, 1H), 2.80 (dd, J=16.4, 6.6 Hz, 1H), 3.18 (m, 2H), 3.81s, 3H), 4.62 (dd, J=9.3, 6.6 Hz, 1H), 5.02 (d, J=7.6 Hz, 1H), 6.88 (m, 2H), 7.21 (d, J=7.6 Hz, 1H), 7.24 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ −2.0, 6.9, 19.4, 30.3, 44.1, 52.6, 55.3, 81.3, 98.2, 114.4, 120.2, 128.2, 130.6, 154.5, 159.5, 190.7; HRMS calcd for $C_{20}H_{27}NNaO_2Si$ (M+Na) 364.1703, found 364.1698.

4.3.4. 2-(4-Chlorophenyl)-1-(5-trimethylsilanyl-pent-3-ynyl)-2,3-dihydro-1*H*-pyridin-4-one (5d). Chromatography (90:10 Et_2O/hexane) afforded 0.127 g (86%) of a colorless oil: IR (neat) 2220, 1639, 1589, 1574, 849 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.08 (s, 9H), 1.42 (t, J=2.6 Hz, 2H), 2.25–2.41 (m, 2H), 2.62 (dd, J=16.4, 8.0 Hz, 1H), 2.88 (dd, J=16.4, 6.9 Hz, 1H), 3.15–3.25 (m, 2H), 4.67 (dd, J=8.0, 6.9 Hz, 1H), 5.06 (d, J=7.7 Hz, 1H), 7.18 (d, J=7.7 Hz, 1H), 7.25 (m, 2H), 7.32 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ −2.0, 7.0, 19.5, 43.7, 52.9, 60.4, 74.1, 81.5, 98.6, 128.3, 129.3, 134.2, 137.3, 154.1, 189.8; HRMS calcd for $C_{19}H_{24}$-ClNNaOSi (M+Na) 368.1208, found 368.1207.

4.3.5. 2-Furan-2-yl-1-(5-trimethylsilanyl-pent-3-ynyl)-2,3-dihydro-1*H*-pyridin-4-one (5e). Chromatography (70:30 EtOAc/hexane) afforded 0.120 g (81%) of a yellow oil: IR (neat) 2217, 1644, 1589, 848 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.08 (s, 9H), 1.42 (t, J=2.6 Hz, 2H), 2.35–2.51 (m, 2H), 2.78 (dd, J=16.4, 6.5 Hz, 1H), 2.85 (dd, J=16.4, 6.6 Hz, 1H), 3.27 (m, 1H), 3.38 (m, 1H), 4.76 (m, 1H), 4.99 (d, J=7.6 Hz, 1H), 6.30 (d, J=3.3 Hz, 1H), 6.33 (dd, J=3.3, 1.8 Hz, 1H), 7.03 (d, J=7.6 Hz, 1H), 7.39 (d, J=1.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ −2.0, 6.9, 20.0, 39.9, 53.5, 54.1, 74.4, 81.1, 98.0, 108.5, 110.4, 142.6, 151.2, 152.8, 190.3; HRMS calcd for $C_{17}H_{23}NO_2Si$ (M^+) 301.1498, found 301.1494.

4.3.6. 1-(5-Trimethylsilanyl-pent-3-ynyl)-2,3-dihydro-1*H*-[2,2′]bipyridinyl-4-one (5f). Chromatography (30:70 *i*-PrOH/hexane) afforded 0.118 g (76%) of a brown oil: IR (neat) 2219, 1640, 1594, 1584, 850 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.08 (s, 9H), 1.43 (t, J=2.6 Hz, 2H), 2.33–2.50 (m, 2H), 2.85 (dd, J=16.5, 5.6 Hz, 1H), 2.99 (dd, J=16.4, 7.5 Hz, 1H), 3.33 (m, 2H), 4.83 (dd, J=7.5, 5.5 Hz, 1H), 5.01 (d, J=7.6 Hz, 1H), 7.21 (d, J=7.6 Hz, 1H), 7.22 (ddd, J=7.7, 4.8, 1.1 Hz, 1H), 7.33 (d, J=7.7 Hz, 1H), 7.67 (m, 1H), 8.6 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ −2.0, 6.9, 19.8, 41.5, 53.8, 61.8, 74.2, 81.2, 98.3, 121.0, 122.9, 136.9, 150.0, 153.7, 157.9, 189.9; HRMS calcd for $C_{18}H_{24}N_2OSi$ (M^+) 312.1658, found 312.1652.

4.3.7. 2-Benzyloxymethyl-1-(5-trimethylsilanyl-pent-3-ynyl)-2,3-dihydro-1*H*-pyridin-4-one (5g). Chromatography (70:30 Et_2O/hexane) afforded 0.150 g (89%) of a yellow oil: IR (neat) 2220, 1642, 1580, 849 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.07 (s, 9H), 1.42 (t, J=2.6 Hz, 2H), 2.36 (dd, J=16.8, 2.3 Hz, 1H), 2.45 (m, 2H), 2.78 (dd, J=16.4, 6.8 Hz, 1H), 3.29 (m, 1H), 3.46 (m, 1H), 3.55 (dt, J=13.0, 6.0 Hz, 1H), 3.82 (m, 2H), 4.49 (s, 2H), 4.87 (d, J=7.4 Hz, 1H), 6.97 (d, J=7.4 Hz, 1H), 7.27–7.36 (m, 5H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ −2.0, 6.9, 20.6, 37.7, 54.5, 56.2, 68.7, 73.6, 74.4, 80.9, 96.8, 127.6, 127.8, 128.4, 137.6, 152.4, 190.1; HRMS calcd for $C_{21}H_{29}NO_2Si$ (M^+) 355.1968, found 355.1979.

4.3.8. 2-Hexyl-1-(5-trimethylsilanyl-pent-3-ynyl)-2,3-dihydro-1*H*-pyridin-4-one (5h). Chromatography (60:40 Et_2O/hexane) afforded 0.112 g (77%) of a colorless oil: IR (neat) 2220, 1640, 1588, 851 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.08 (s, 9H), 0.87 (t, J=7.1 Hz, 3H), 1.20–1.35 (m, 6H), 1.43 (t, J=2.6 Hz, 2H), 1.57–1.75 (m, 4H), 2.32 (ddd, J=16.3, 3.2, 1.0 Hz, 1H), 2.45 (m, 2H), 2.76 (dd, J=16.3, 6.8 Hz, 1H), 3.30 (m, 2H), 3.51 (m, 1H), 4.89 (dd, J=7.4, 1.1 Hz, 1H), 6.95 (dd, J=7.4, 1.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ −2.0, 6.9, 14.0, 20.7, 22.5, 25.6, 28.9, 29.2, 31.7, 39.5, 53.6, 56.6, 74.3, 81.0, 96.9, 152.2, 190.7; HRMS calcd for $C_{19}H_{34}NOSi$ (M+H) 320.2404, found 320.2400.

4.3.9. 2-Cyclohexyl-1-(5-trimethylsilanyl-pent-3-ynyl)-2,3-dihydro-1*H*-pyridin-4-one (5i). Chromatography (60:40 Et_2O/hexane) afforded 0.100 g (70%) of a colorless oil: IR (neat) 2220, 1638, 1586, 850 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.08 (s, 9H), 0.86–1.31 (m, 6H), 1.42 (t, J=2.7 Hz, 2H), 1.69–1.93 (m, 5H), 2.41–2.49 (m, 3H), 2.76 (dd, J=16.5, 7.4 Hz, 1H), 3.26–3.39 (m, 3H), 4.87 (dd, J=7.3, 1.1 Hz, 1H), 7.02 (dd, J=7.3, 1.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ −2.0, 6.9, 21.0, 26.1, 28.8, 30.1, 37.4, 38.7, 54.8, 61.4, 74.3, 80.7, 97.2, 152.8, 192.2; HRMS calcd for $C_{19}H_{32}NOSi$ (M+H) 318.2248, found 318.2239.

4.3.10. 2-Phenyl-1-(6-trimethylsilanyl-hex-4-ynyl)-2,3-dihydro-1*H*-pyridin-4-one (7a). Chromatography (70:30 Et_2O/hexane) afforded 0.123 g (78%) of a colorless oil: IR (neat) 2219, 1641, 1593, 1579, 850 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.06 (s, 9H), 1.39 (t, J=2.6 Hz, 2H), 1.60–1.72 (m, 2H), 2.12–2.23 (m, 2H), 2.67 (dd, J=16.4, 7.7 Hz, 1H), 2.88 (dd, J=16.4, 7.1 Hz, 1H), 3.18 (m, 1H), 3.27 (m, 1H), 4.61 (t, J=7.4 Hz, 1H), 5.01 (d, J=7.6 Hz, 1H), 7.20 (d, J=7.6 Hz, 1H), 7.28–7.37 (m, 5H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ −2.0, 6.9, 16.0, 28.1, 43.7, 52.3, 61.0, 76.5, 79.3, 98.3, 126.9, 128.2, 129.0, 138.7, 154.1, 190.0; HRMS calcd for $C_{20}H_{28}NOSi$ (M+H) 326.1935, found 326.1940.

4.3.11. 2-(4-Methoxyphenyl)-1-(6-trimethylsilanyl-hex-4-ynyl)-2,3-dihydro-1*H*-pyridin-4-one (7b). Chromatography (70:30 Et_2O/hexane) afforded 0.125 g (71%) of a colorless oil: IR (neat) 2219, 1641, 1593, 1579, 850 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.06 (s, 9H), 1.39 (t, J=2.6 Hz, 2H), 1.58–1.68 (m, 2H), 2.10–2.23 (m, 2H), 2.66 (dd, J=16.4, 8.3 Hz, 1H), 2.82 (dd, J=16.4, 6.8 Hz, 1H), 3.17 (m, 1H), 3.23 (m, 1H), 3.80 (s, 3H), 4.55 (dd, J=8.3, 6.8 Hz, 1H), 5.01 (d, J=7.6 Hz, 1H), 6.88 (m, 2H), 7.17 (d, J=7.6 Hz, 1H), 7.22 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ −2.0, 6.9, 16.0, 28.1, 43.9, 52.0, 55.3, 60.5, 76.5, 79.2,

98.1, 114.4, 128.2, 130.8, 154.1, 159.5, 190.4; HRMS calcd for $C_{21}H_{29}NNaO_2Si$ (M+Na) 378.1860, found 378.1852.

4.3.12. 2-Hexyl-1-(6-trimethylsilanyl-hex-4-ynyl)-2,3-dihydro-1*H*-pyridin-4-one (7c). Chromatography (60:40 Et_2O/hexane) afforded 0.107 g (65%) of a colorless oil: IR (neat) 2220, 1641, 1587, 850 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.1 (s, 9H), 0.88 (t, J=7.0 Hz, 3H) 1.15–1.37 (m, 7H), 1.44 (t, J=2.6 Hz, 2H), 1.53–1.62 (m, 2H), 1.70–1.80 (m, 3H), 2.20–2.28 (m, 2H), 2.33 (dd, J=16.4, 3.0 Hz, 1H), 2.73 (dd, J=16.4, 6.8 Hz, 1H), 3.32 (m, 2H), 3.44 (m, 1H), 4.88 (d, J=7.4 Hz, 1H), 6.94 (d, J=7.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ −2.0, 6.9, 14.0, 16.0, 22.5, 25.6, 28.6, 29.1, 29.2, 31.7, 39.4, 52.6, 56.7, 76.5, 79.3, 96.7, 152.3, 190.4; HRMS calcd for $C_{20}H_{36}NOSi$ (M+H) 334.2561, found 334.2575.

4.4. General procedure for the direct aza-Diels–Alder reaction

The aldehyde (0.1 mmol), amine **1** or **2** (0.1 mmol) and Danishefsky's diene (0.11 mmol) were dissolved in MeCN (5 mL) at room temperature and $Yb(OTf)_3$ (0.01 mmol) was added in one portion. The reaction mixture was stirred for 2–9 h. Then water was added and the mixture was extracted with CH_2Cl_2 (3×5 mL) and the combined organic layers were washed with brine and dried ($MgSO_4$). The residue was purified by flash column chromatography.

4.5. General procedure for the TBAT-mediated cyclocondensation

The dihydropyridones (0.1 mmol) and TBAT (0.2 mmol) were dissolved in THF (5 mL) at 30 °C. After 20 min, the reaction turned orange, and after 1–5 h the reaction was complete (TLC monitoring). The THF was removed under reduced pressure, the residue was redissolved in Et_2O (10 mL), and filtered. The filtrate was concentrated, and the crude material obtained was purified by flash column chromatography.

4.5.1. (5*R,8a*R**)-5-Phenyl-1-vinylidene-hexahydro-indolizin-7-one (6a).** Chromatography (50:50 Et_2O/hexane) afforded 0.023 g (95%) of white needles. Recrystallisation from TBME/pentane afforded **6a** as white needles: mp 118–119 °C; IR (neat) 1966, 1718 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.52 (m, 1H), 2.57–2.75 (m, 6H), 2.88 (m, 1H), 3.85 (m, 1H), 4.12 (dd, J=7.1, 5.5 Hz, 1H), 4.81 (dq, J=9.9, 4.4 Hz, 1H), 4.89 (dq, J=10.0, 4.4 Hz, 1H), 7.23–7.36 (m, 5H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 28.3, 43.1, 47.0, 49.9, 58.3, 60.7, 79.1, 103.1, 127.7, 127.9, 128.4, 139.8, 201.4, 208.6; HRMS calcd for $C_{16}H_{17}NO$ (M^+) 239.1310, found 239.1320.

4.5.2. (5*R,8a*R**)-5-*p*-Tolyl-1-vinylidene-hexahydro-indolizin-7-one (6b).** Chromatography (50:50 Et_2O/hexane) afforded 0.023 g (93%) of a colorless oil: IR (neat) 1966, 1715 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.35 (s, 3H), 2.47–2.77 (m, 7H), 2.89 (m, 1H), 3.85 (m, 1H), 4.06 (t, J=6.1 Hz, 1H), 4.81 (dq, J=9.9, 4.4 Hz, 1H), 4.89 (dq, J=10.0, 4.4 Hz, 1H), 7.10–7.18 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.0, 28.2, 43.2, 46.9, 49.7, 58.1, 60.2, 79.1, 103.1, 127.9, 129.1, 136.4, 137.4, 201.4, 208.9; HRMS calcd for $C_{17}H_{19}NO$ (M^+) 253.1467, found 253.1468.

4.5.3. (5*R,8a*R**)-5-(4-Methoxyphenyl)-1-vinylidene-hexahydro-indolizin-7-one (6c).** Chromatography (40:60 Et_2O/hexane) afforded 0.025 g (97%) of a yellow oil: IR (neat) 1965, 1718, 1512, 1251 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.47–2.76 (m, 7H), 2.88 (ddd, J=10.9, 7.7, 3.3 Hz, 1H), 3.80 (s, 3H), 3.81 (m, 1H), 4.10 (t, J=6.1 Hz, 1H), 4.81 (dq, J=10.0, 4.5 Hz, 1H), 4.89 (dq, J=10.0, 4.4 Hz, 1H), 6.89 (m, 2H), 7.14 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 28.3, 43.3, 47.1, 49.8, 55.3, 58.1, 59.9, 79.1, 103.2, 113.8, 129.1, 131.7, 159.1, 201.4, 208.8; HRMS calcd for $C_{17}H_{20}NO_2$ (M+H) 270.1489, found 270.1501.

4.5.4. (5*R,8a*R**)-5-(4-Chlorophenyl)-1-vinylidene-hexahydro-indolizin-7-one (6d).** Chromatography (60:40 Et_2O/hexane) afforded 0.026 g (98%) of a yellow oil: IR (neat) 1965, 1719 cm^{-1}; ^1H NMR (C_6D_6, 500 MHz) δ 2.20–2.35 (m, 6H), 2.43 (dd, J=15.4, 8.5 Hz, 1H), 2.54 (dd, J=15.4, 4.8 Hz, 1H), 3.53 (t, J=6.5 Hz, 1H), 3.64 (m, 1H), 4.70 (m, 1H), 4.78 (m, 1H), 6.82 (m, 2H), 7.09 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 28.3, 42.8, 46.9, 50.1, 58.4, 60.3, 79.2, 102.9, 128.6, 129.1, 133.4, 138.6, 201.4, 208.2; HRMS calcd for $C_{16}H_{17}ClNO$ (M+H) 274.0993, found 274.1006.

4.5.5. (5*R,8a*R**)-5-Furan-2-yl-1-vinylidene-hexahydro-indolizin-7-one (6e).** Chromatography (40:60 *t*-BuOMe/hexane) afforded 0.020 g (89%) of a yellow oil: IR (neat) 1972, 1722 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.42 (m, 1H), 2.50–2.77 (m, 5H), 2.88 (dd, J=15.3, 6.9 Hz, 1H), 3.10 (m, 1H), 3.34 (m, 1H), 4.47 (dd, J=6.9, 2.3 Hz, 1H), 4.79 (dq, J=9.8, 4.8 Hz, 1H), 4.89 (dq, J=9.9, 4.6 Hz, 1H), 6.13 (d, J=3.2 Hz, 1H), 6.31 (dd, J=3.2, 1.8 Hz, 1H), 7.36 (d, J=1.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 28.4, 44.0, 45.3, 49.1, 53.2, 57.3, 79.2, 103.5, 109.1, 109.9, 142.4, 152.1, 201.1, 207.5; HRMS calcd for $C_{14}H_{15}NO_2$ (M^+) 229.1103, found 229.1106.

4.5.6. (5*R,8a*R**)-5-Pyridin-2-yl-1-vinylidene-hexahydro-indolizin-7-one (6f).** Chromatography (50:50 *t*-BuOMe/hexane) afforded 0.022 g (92%) of a yellow oil: IR (neat) 1967, 1712 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.44–2.54 (m, 3H), 2.65–2.80 (m, 4H), 3.01 (m, 1H), 3.75 (m, 1H), 4.37 (t, J=4.8 Hz, 1H), 4.79 (dq, J=9.9, 4.7 Hz, 1H), 4.87 (dq, J=9.8, 4.6 Hz, 1H), 7.17 (ddd, J=1.1, 4.8, 7.7 Hz, 1H), 7.21 (d, J=7.7 Hz, 1H), 7.66 (m, 1H), 8.57 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 28.3, 44.0, 44.6, 49.3, 57.0, 61.1, 79.0, 103.3, 122.3, 122.8, 136.1, 149.2, 157.9, 201.1, 207.6; HRMS calcd for $C_{15}H_{16}N_2NaO$ (M+Na) 263.1155, found 263.1152.

4.5.7. (5*R,8a*R**)-5-Benzyloxymethyl-1-vinylidene-hexahydro-indolizin-7-one (6g).** Chromatography (40:60 *t*-BuOMe/hexane) afforded 0.025 g (89%) of a colorless oil: IR (neat) 1965, 1713 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.36 (m, 1H), 2.41–2.51 (m, 2H), 2.59–2.71 (m, 3H), 3.03 (m, 2H), 3.44 (m, 1H), 3.51 (dd, J=9.7, 4.6 Hz, 1H), 3.58 (dd, J=9.7, 4.6 Hz, 1H), 3.95 (m, 1H), 4.49 (d, J=2.2 Hz, 2H), 4.80 (m, 1H), 4.84 (m, 1H), 7.27–7.36 (m, 5H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 28.7, 42.2, 44.0, 49.6, 56.2, 58.4, 70.3, 73.4, 78.9, 103.9, 127.5, 127.6, 128.4, 137.9,

201.0, 208.3; HRMS calcd for $C_{18}H_{21}NO_2$ (M^+) 283.1572, found 283.1578.

4.5.8. (5*S,8a*R**)-5-Hexyl-1-vinylidene-hexahydro-indolizin-7-one (6h).** Chromatography (40:60 Et_2O/hexane) afforded 0.020 g (83%) of a colorless oil: IR (neat) 1967, 1715 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.87 (t, J= 7.1 Hz, 3H), 1.20–1.56 (m, 10H), 2.28 (ddd, J=14.2, 4.0, 1.5 Hz, 1H), 2.34–2.44 (m, 2H), 2.62 (dd, J=14.2, 5.6 Hz, 1H), 2.68 (m, 2H), 2.92–3.03 (m, 2H), 3.21 (m, 1H), 3.79 (m, 1H), 4.80 (m, 1H), 4.84 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.0, 22.6, 26.5, 28.8, 29.3, 29.8, 31.7, 43.6, 44.3, 48.9, 57.1, 57.9, 78.8, 103.9, 201.1, 209.3; HRMS calcd for $C_{16}H_{26}NO$ (M+H) 248.2009, found 248.2009.

4.5.9. (5*R,8a*R**)-5-Cyclohexyl-1-vinylidene-hexahydro-indolizin-7-one (6i).** Chromatography (40:60 Et_2O/hexane) afforded 0.021 g (86%) of a colorless oil: IR (neat) 1966, 1714 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.83–0.96 (m, 2H), 1.09–1.25 (m, 4H), 1.37 (m, 1H), 1.63–1.86 (m, 4H), 2.30 (ddd, J=15.0, 4.5, 1.5 Hz, 1H), 2.36–2.42 (m, 2H), 2.49 (dd, J=14.4, 5.1 Hz, 1H), 2.64 (m, 1H), 2.74 (m, 1H), 2.90 (m, 1H), 3.00 (m, 1H), 3.07 (m, 1H), 4.05 (m, 1H), 4.79–4.83 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 26.2, 26.5, 29.2, 30.4, 39.3, 40.2, 43.2, 50.5, 58.8, 63.2, 78.4, 104.3, 201.1, 209.9; HRMS calcd for $C_{16}H_{24}NO$ (M+H) 246.1852, found 246.1853.

4.5.10. (4*R,9a*R**)-4-Phenyl-9-vinylidene-octahydro-quinolizin-2-one (8a).** Chromatography (40:60 Et_2O/hexane) afforded 0.008 g (35%) of a colorless oil: IR (neat) 1968, 1720 cm^{-1}; ^1H NMR (C_6D_6, 500 MHz) δ 0.88–0.95 (m, 1H), 1.58–1.70 (m, 1H), 1.91 (m, 1H), 2.30–2.41 (m, 2H), 2.47 (dd, J=14.6, 9.5 Hz, 1H), 2.53–2.62 (m, 2H), 2.74 (ddd, J=14.8, 3.6, 2.1 Hz, 1H), 2.82 (m, 1H), 3.61 (m, 1H), 4.12 (dd, J=9.5, 4.2 Hz, 1H), 4.76 (dt, J=10.1, 4.1 Hz, 1H), 4.84 (dq, J=13.7, 3.5 Hz, 1H), 7.13–7.25 (m, 5H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 20.4, 29.9, 44.0, 49.0, 49.8, 57.7, 59.4, 77.5, 98.8, 127.8, 127.7, 140.8, 203.7, 207.4; HRMS calcd for $C_{17}H_{19}NO$ (M^+) 253.1467, found 253.1462.

4.5.11. (4*R,9a*R**)-4-(4-Methoxyphenyl)-9-vinylidene-octahydro-quinolizin-2-one (8b).** Chromatography (40:60 Et_2O/hexane) afforded 0.006 g (27%) of a brown oil: IR (neat) 1972, 1718 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.83–0.94 (m, 1H), 1.70–1.80 (m, 1H), 2.10–2.21 (m, 1H), 2.38–2.43 (m, 1H), 2.49–2.54 (m, 2H), 2.57–2.64 (m, 1H), 2.68 (dd, J=15.2, 3.8 Hz, 1H), 2.73 (dd, J=15.2, 6.0 Hz, 1H), 2.86–2.92 (m, 1H), 3.81 (s, 3H), 3.84–3.89 (m, 1H), 4.13–4.17 (m, 1H), 4.74–4.81 (m, 2H), 6.88 (d, J=8.7 Hz, 2H) 7.23 (d, J=8.7 Hz, 2H); HRMS calcd for $C_{18}H_{21}NO_2$ (M^+) 283.1573, found 283.1582.

Acknowledgements

The authors would like to express our sincere gratitude to Prof. Z. Lipkowska (Institute of Organic Chemistry PAS) for X-ray crystallographic analysis. We also thank the State Committee for Scientific Research (Grant 4 T09A 064 25) for financial support.

References and notes

1. (a) Marshal, J. A.; Adams, N. D. *J. Org. Chem.* **1997**, *62*, 8976–8977. (b) Kobayashi, S.; Nishio, K. *J. Am. Chem. Soc.* **1995**, *117*, 6392–6393. (c) Santelli, M.; Abed, D. E.; Jellal, A. *J. Org. Chem.* **1986**, *51*, 1199–1206. (d) Pornet, J.; Miginiac, L. *Tetrahedron* **1986**, *42*, 2017–2024. (e) Pornet, J.; Miginiac, L.; Jaworski, K.; Randrianoelina, B. *Organometallics* **1985**, *4*, 333–338. (f) Pornet, J.; Mesnard, D.; Miginiac, L. *Tetrahedron Lett.* **1982**, *23*, 4083–4086. (g) Pornet, J.; Kolani, N.; Mesnard, D.; Miginiac, L.; Jaworski, K. *J. Organomet. Chem.* **1982**, *236*, 177–183. (h) Pornet, J. *Tetrahedron Lett.* **1981**, *22*, 453–454. (i) Pornet, J.; Randrianoelina, B. *Tetrahedron Lett.* **1981**, *22*, 1327–1328. (j) Pornet, J.; Kolani, N. *Tetrahedron Lett.* **1981**, *22*, 3609–3610. (k) Pornet, J. *Tetrahedron Lett.* **1980**, *21*, 2049–2050.
2. For reviews, see: (a) Shuster, H. F.; Coppola, G. M. *Allenes in Organic Synthesis*; Wiley: New York, 1984. (b) Landor, S. R. *The Chemistry of the Allenes*; Academic: New York, 1982. (c) Patai, S. *The Chemistry of Ketenes, Allenes and Related Compounds*; Wiley: Chichester, 1980. (d) Hashmi, A. S. K. *Angew. Chem., Int. Ed.* **2000**, *39*, 3590–3593.
3. (a) Karstens, W. F. J.; Moolenaar, M. J.; Rutjes, F. P. J. T.; Grabowska, U.; Speckamp, W. N.; Hiemstra, H. *Tetrahedron Lett.* **1999**, *40*, 8629–8632. (b) Luker, T.; Koot, W-J. ; Speckamp, W. N.; Hiemstra, H. *J. Org. Chem.* **1998**, *63*, 220–221. (c) Schinzer, D.; Ruppelt, M. *Chem. Ber.* **1991**, *124*, 247–248. (d) Guay, D.; Johnson, W. S.; Schubert, U. *J. Org. Chem.* **1989**, *54*, 4731–4732. (e) Johnson, W. S.; Elliott, J. D.; Hanson, G. J. *J. Am. Chem. Soc.* **1984**, *106*, 1138–1139. (f) Schinzer, D. *Synthesis* **1988**, 263–273. (g) Schinzer, D.; Dettmer, G.; Ruppelt, M.; Solyom, S.; Steffen, J. *J. Org. Chem.* **1988**, *53*, 3823–3828. (h) Schinzer, D.; Solyom, S.; Steffen, J. *J. Chem. Soc., Chem. Commun.* **1986**, 829–830. (i) Schinzer, D.; Solyom, S.; Becker, M. *Tetrahedron Lett.* **1985**, *26*, 1831–1834.
4. Hosomi, A.; Shirahata, A.; Sakurai, H. *Tetrahedron Lett.* **1978**, *19*, 3043–3046.
5. Wang, D.-K.; Zhou, Y.-G.; Tang, Y.; Hou, X.-L.; Dai, L.-X. *J. Org. Chem.* **1999**, *64*, 4233–4237.
6. Majetich, G.; Casares, A.; Chapman, D.; Behnke, J. *J. Org. Chem.* **1986**, *51*, 1745–1753.
7. (a) Majetich, G.; Desmond, R. W., Jr.; Soria, J. J. *J. Org. Chem.* **1986**, *51*, 1753–1769. (b) Majetich, G.; Hull, K.; Casares, A. M.; Khateni, V. *J. Org. Chem.* **1991**, *56*, 3958–3973.
8. Furman, B.; Dziedzic, M. *Tetrahedron Lett.* **2003**, *44*, 6629–6631.
9. Sato, M.; Aoyagi, S.; Yago, S.; Kibayashi, Ch. *Tetrahedron Lett.* **1996**, *37*, 9063–9066.
10. Hiemstra, H.; Fortgens, H. P.; Stegenga, S.; Speckamp, W. N. *Tetrahedron Lett.* **1985**, *26*, 3151–3154.
11. Wasserman, H. H.; Vu, Ch. B.; Cook, J. D. *Tetrahedron* **1992**, *48*, 2101–2112.
12. Kobayashi, S.; Ishitani, H.; Nagayama, S. *Synthesis* **1995**, 1195–1202.
13. Pilcher, A. S.; Ammon, H.; DeShong, P. *J. Am. Chem. Soc.* **1995**, *117*, 5166–5167.
14. Crystallographic data for the structure **6a** in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 270291. 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].

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron 61 (2005) 8648–8655
Tetrahedron

New domino reaction. One pot synthesis of 4,7-dihydroxythioaurone derivatives from benzaldehydes and 4-acetyl-2-oxo-benz[1,3]oxathiole

Marek T. Konieczny,[a,*] Wojciech Konieczny,[a] Stefan Wolniewicz,[a] Konstanty Wierzba,[b] Yoshimitsu Suda[b] and Paweł Sowiński[c]

[a]*Department of Organic Chemistry, Medical University of Gdańsk, 80-416 Gdańsk, Poland*
[b]*Hanno Research Center, Taiho Pharmaceutical Company, Hanno-city, Saitama 357-8527, Japan*
[c]*Laboratory of Nuclear Magnetic Resonance Spectroscopy, Gdańsk University of Technology, 80-952 Gdańsk, Poland*

Received 27 April 2005; revised 13 June 2005; accepted 30 June 2005

Available online 15 July 2005

Abstract—A convenient synthesis of 4,7-dihydroxythioaurone derivatives by a one pot reaction of benzaldehydes with 4-acetyl-2-oxo-benz[1,3]oxathioles and piperidine acetate in DMSO is described. The structures of the compounds, including double bond geometry were proved unequivocally by NMR methods. The thioaurone ring system seems to be formed by three consecutive reactions: opening of the oxathiolone ring with piperidine, oxidation of the formed mercapto group with DMSO or/and air to disulfide, and condensation with aldehyde.

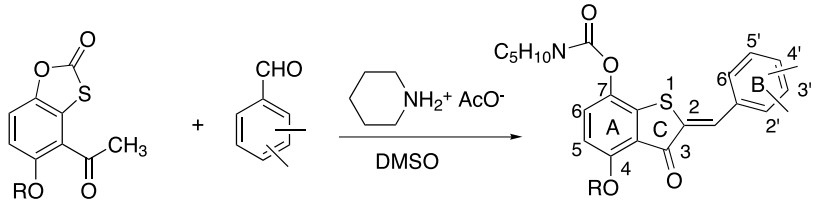
1. Introduction

Thioaurones constitute a relatively unexplored class of compounds. More than 50 years ago they were intensively studied as thioindigo-like dyes, and next as photochromic compounds.[1] Lately, they attracted some attention as photoswitchable molecules[2–4] and their chemistry was recently reviewed.[1] The most important method of preparation of the compounds is the condensation of 1-benzothiophene-3-ones with benzaldehydes,[5] although several other methods are also available.[1]

2. Results and discussion

Continuing our work on thio analogs of flavonoids of biological interest bearing *p*-hydroquinone functionality,[6,7] we now find that 4,7-dihydroxythioaurone derivatives can be prepared in one step from 4-acetyl-5-alkoxy-1,3-benzoxathiol-2-ones (**1**–**3**) and aromatic aldehydes (Scheme 1).

The reaction provided a convenient access to a broad range of 4,7-dihydroxythioaurone derivatives, as listed in Table 1.



1 R = CH_3
2 R = $CH_2CH_2N(C_2H_5)_2$
3 R = $CH_2CH{=}CH_2$

4 - 23

Scheme 1. Formation of thioaurones **4**–**23**.

Keywords: Thioaurones; Synthesis of; Structure of; Domino reaction.
* Corresponding author. Tel.: +48 58 349 3148; fax: +48 58 349 3206; e-mail: markon@amg.gda.pl

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.06.107

Table 1. Thioaurones prepared according to Scheme 1

Compound no.	Yield (%)	Substituents
4	62	3′,4,4′-triOCH$_3$
5	21	4-OCH$_3$; 3′,4′-diOH
6[a]	8	4-OCH$_3$; 3′,4′-diOH; 7-OH
7	29	4-OCH$_3$; 4′-Br
8	51	4-OCH$_3$;
9	19	4-OCH$_3$; pyridinyl-4 ring
10	57	2′,3′,4-triOCH$_3$
11	29	4,4′-diOCH$_3$
12	58	4-OCH$_3$; 4′-OH
13	27	4-OCH$_3$; 3′-OH
14	48	3′,4-diOCH$_3$; 4′-OH
15	36	4-OCH$_3$; 4′-Cl
16	42	4-OCH$_3$; 3′-Cl
17	30	4-OCH$_3$; 2′-Cl
18	52	4,5′-diOCH$_3$; 3′-Br; 4′-OH
19	55	4-OCH$_3$; 4′-N(CH$_3$)$_2$
20	10	4-OCH$_3$; 4′-NO$_2$
21	29	4,4′,5′-triOCH$_3$; 3′-Br
22	35	4-OCH$_2$CH$_2$N(C$_2$H$_5$)$_2$; 3′-Cl
23	42	4-OCH$_2$CH=CH$_2$; 3′-Cl

[a] Formed by hydrolysis of compound **5**. Traces of analogous 7-hydroxy derivatives were present in crude products of all reactions.

The structures of the compounds were proved by elemental analyses, IR and NMR spectra. The NMR gHMBC spectrum of compound **4** revealed long range couplings of the exocyclic hydrogen α with carbons 2′ and 6′, which confirmed the aurone structure. For the alternative flavone structure, such coupling seems to be improbable (Fig. 1).

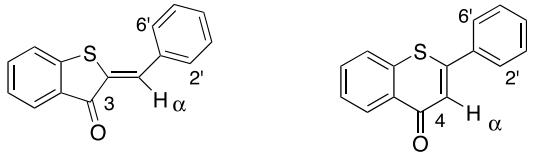


Figure 1. Comparison of thioaurone and thioflavone structures.

Measurement of coupling constants between the carbonyl carbon (carbon 3) and α-proton gave value $J=5$–6 Hz, which proved that the carbonyl group and the α-proton are located cis, as for the trans isomer the value of the coupling constant should be much larger ($J=12$–15 Hz).[8,9] The result is in agreement with previous observations that *Z* isomers of thioaurones are thermodynamically more stable than the *E* isomers, which can be prepared by irradiation of the *Z* form with UV–vis light.[1–4,10] The applied method of determination of double bond geometry seems to be more reliable than the earlier methods, based on chemical shift of the α-proton[2,10] or UV spectra.[2,3,11]

The described reaction seems to be general and takes place with a variety of benzaldehydes (Table 1). The piperidine acetate used as a catalyst and co-reactant could be replaced by the free amine or other amine acetates, including ammonium acetate. However, use of the piperidine acetate resulted in well crystallizing derivatives and for this reason all syntheses were performed with this particular salt, as ultimately we were interested in compounds with free hydroxy groups and the structure of the amine was not of primary importance for us. In most cases, the reactions were performed under argon but the inert atmosphere was not essential. However, the reaction should be run under anhydrous conditions to prevent hydrolysis of the carbamoyl group at the position 7 of the product. If present in larger amount, the product of hydrolysis (e.g., **6**) can only with difficulty be removed by crystallization and had to be separated by low temperature extraction with dilute sodium hydroxide or chromatography.

The mechanism of the reaction is still tentative but a few details are already clarified. Formation of thioaurone from compound **1** has to involve an oxidation step, and air or DMSO appeared as the two possible oxidants. In a related experiment, the reaction flask was flushed with a slow stream of argon and the gases were bubbled through a solution of methyl iodide in acetone, resulting in precipitation of a colorless solid, which was identified by IR as trimethylsulfonium iodide (yield 29 and 48% in relation to the isolated thioaurone; the experiment was not intended to be quantitatively valid, and probably more dimethyl sulfide was formed in the reaction). The result proved that DMSO indeed acted as the oxidant. However, reaction of the compound **1** with 3,4-dimethoxybenzaldehyde and piperidine run in methanol instead of DMSO also resulted in formation of thioaurone **4**, but the reaction time was longer, the yield of the product was lower (10% in comparison to 57% in DMSO), and the thioaurone **4** was accompanied by a complicated mixture of other compounds. One of them was isolated, and identified as 8-hydroxy-3′,4′,5-trimethoxythioflavanone (**24**) (yield 9%) (Fig. 2). The result suggests that oxidation with DMSO was advantageous but not necessary.

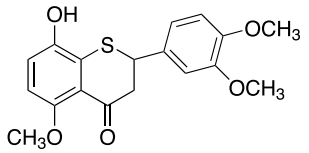
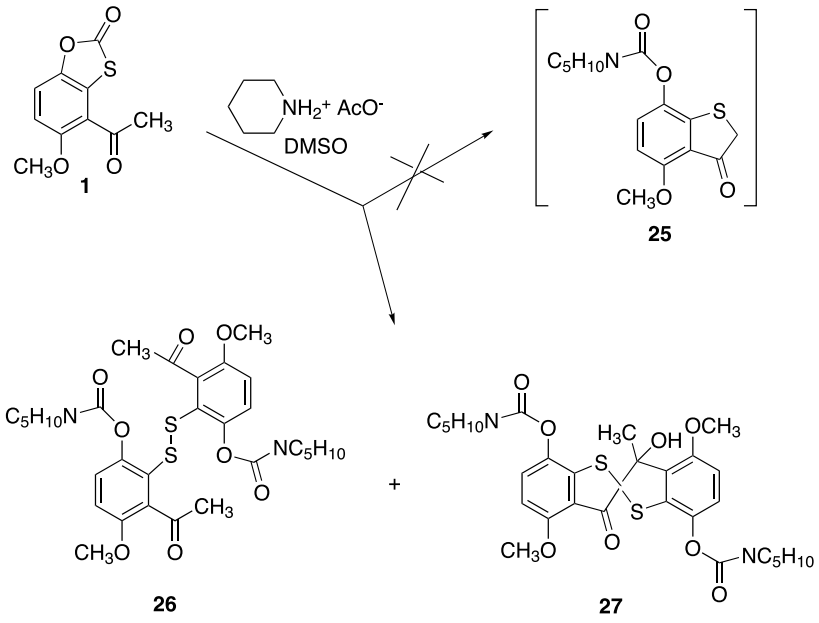


Figure 2. Compound **24**.

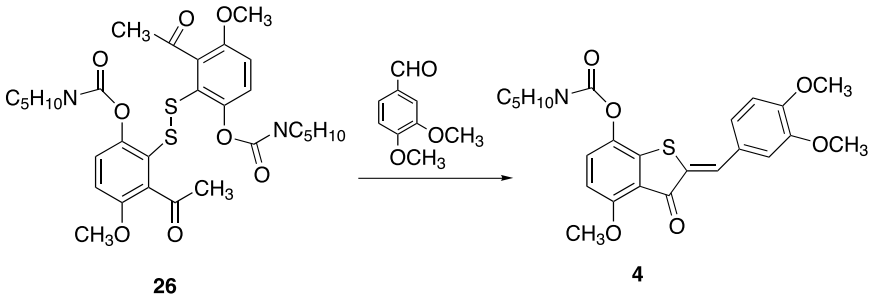
We speculated that formation of thioaurones goes via benzothiophen **25**. To check if compound **25** is indeed formed as an intermediate, reaction of benzoxathiol-2-one (**1**) with piperidine acetate in DMSO was run in the absence of benzaldehyde, but instead of the expected product **25**, disulfide **26** and spiro compound **27** were isolated in the ratio 1:4, respectively, (Scheme 2). Prolongation of the time of the reaction lead exclusively to **27**. The structure of the unusual spiro compound **27** was proved based on MS, IR and NMR ROESY, gHMBC, and gHSQC spectra. The NMR data demonstrated that the compound **27** existed as a mixture of two diastereoisomers.

Formation of the disulfide suggested, that this can be the sought after intermediate, as similar formation of thiaurones from disulfides was already reported by Samogyi.[12] Indeed, under the reaction conditions the disulfide **26** reacts cleanly with 3,4-dimethoxybenzaldehyde to give thioaurone **4** (Scheme 3).

The prepared compounds and their derivatives were tested as potential antitumor agents, the results will be published elsewhere.



Scheme 2. Formation of disulfide **26** and the spiro compound **27**.



Scheme 3. Transformation of disulfide **26** into thioaurone.

3. Conclusion

The described, one pot reaction provides a convenient access to 4,7-dihydroxy derivatives of thioaurones. It can be expected, that the reaction can be extended to other 4-acetyl-1,3-benzoxathiol-2-ones, and that preparatively useful synthesis of thioaurones, starting from 2-acetylthiophenoles or related disulfides and benzaldehydes in DMSO, should be possible. The presented, NMR methods of elucidation of structures of thioaurones seem to be superior to the previous ones, and applicable to aurones.

4. Experimental

4.1. General

Melting points are uncorrected. Infrared spectra were obtained from KBr pellets on Thermo Mattson Satellite instrument. The ^{1}H and ^{13}C NMR spectra were recorded on 200 MHz (Varian Gemini) or 500 MHz (Varian Unity Plus) spectrometers. Elemental analyses were performed on Carlo-Erba 1108 instrument. MALDI TOF mass spectra were obtained with a Bruker Biflex III instrument. Flash column chromatography was performed on silica gel (Merck, less than 230 mesh). TLC was carried out on Merck 0.2 mm silica gel 60 F254 aluminum plates. Commercially available chemicals were reagent grade, and DMSO was dried (Al_2O_3), distilled and stored over molecular sieves.

4.1.1. 4-Acetyl-5-methoxy-1,3-benzoxathiol-2-one (1). 4-Acetyl-5-hydroxy-1,3-benzoxathiol-2-one[13] (11.62 g, 0.055 mol), iodomethane (14 mL, 0.22 mol), dry potassium carbonate (20 g, 0.15 mol) in anhydrous acetone (150 mL) were refluxed, with stirring for 3 h. Water (300 mL) was added to the cooled mixture, and the precipitated solid was filtered off and washed with water. The wet product was dissolved in hot acetone, decolorized with charcoal, and precipitated by addition of water, to give 9.1 g (73%) of 4-acetyl-5-methoxy-1,3-benzoxathiol-2-one (**1**) as brightly yellow crystals, mp 140–142 °C. Anal. Calcd for $C_{10}H_8O_4S_1$: C, 53.57; H, 3.60; S, 14.27. Found: C, 53.40; H, 3.43; S, 14.17. IR (KBr, cm^{-1}): 1745, 1650, 1592, 1467, 1276, 1059. NMR (200 MHz, $CDCl_3$): δ 7.40 (d, 1H, $J=$ 9 Hz, H-7), 6.96 (d, 1H, $J=9$ Hz, H-6), 4.00 (s, 3H, OCH_3), 2.73 (s, 3H, $COCH_3$).

4.1.2. 4-Acetyl-5-[2′-(*N*,*N*-diethylamino)ethoxy]-1,3-benzoxathiol-2-one (2). 4-Acetyl-5-hydroxy-1,3-benzoxathiol-2-one (2.11 g, 0.01 mol), 2-chlorotriethylamine hydrochloride (3.44 g, 0.02 mol), dry potassium carbonate

(8.3 g, 0.06 mol) in anhydrous DMF (20 mL) were stirred at room temperature for 1.5 h. Water and ice were added to the cooled mixture, and the precipitated solid was filtered off and washed with water to give 2.4 g (79%) of crude product. Crystallization from methanol gave 1.93 g (62%) of 4-acetyl-5-[2′-(*N*,*N*-diethylamino)ethoxy]-1,3-benzoxathiol-2-one (**2**) as a gray solid, mp 98–99 °C. Anal. Calcd for $C_{15}H_{19}N_1O_4S_1$: C, 58.23; H, 6.19; N, 4.53; S, 10.34. Found: C, 58.18; H, 6.21; N, 4.52; S, 10.38. IR (KBr, cm^{-1}): 1744, 1662, 1594, 1460, 1274, 1051. NMR (500 MHz, DMSO): δ 7.72 (d, 1H, $J=9$ Hz, H-7), 7.31 (d, 1H, $J=9$ Hz, H-6), 4.23 (t, 2H, $J=6$ Hz, OCH_2), 2.85 (t, 2H, $J=6$ Hz, NCH_2), 2.72 (s, 3H, $COCH_3$), 2.54 (q, 4H, $J=7$ Hz, $2\times CH_2CH_3$), 0.95 (t, 6H, $J=7$ Hz, $2\times CH_2CH_3$).

4.1.3. 4-Acetyl-5-allyloxy-1,3-benzoxathiol-2-one (3). 4-Acetyl-5-hydroxy-1,3-benzoxathiol-2-one (2.11 g, 0.01 mol), allyl bromide (1.73 mL, 0.02 mol), dry potassium carbonate (4.15 g, 0.03 mol) in anhydrous DMF (20 mL) were stirred at room temperature for 1.5 h. Water and ice were added to the cooled mixture, and the precipitated solid was filtered off and washed with water to give 2.3 g (92%) of crude product. Crystallization from toluene–cyclohexane gave 1.8 g (73%) of 4-acetyl-5-allyloxy-1,3-benzoxathiol-2-one (**3**) as a gray solid, mp 120–123 °C. Anal. Calcd for $C_{12}H_{10}O_4S_1$: C, 57.59; H, 4.03; S, 12.79. Found: C, 57.38; H, 3.99; S, 12.85. IR (KBr, cm^{-1}): 1740, 1656, 1593, 1466, 1421, 1270, 1049. NMR (500 MHz, DMSO): δ 7.73 (d, 1H, $J=9$ Hz, H-7), 7.28 (d, 1H, $J=9$ Hz, H-6), 6.15 (m, 1H, =CH–), 5.49 (d, 1H, $J=17$ Hz, H_2C=), 5.34 (d, 1H, $J=11$ Hz, H_2C=), 4.80 (d, 2H, $J=5$ Hz, OCH_2), 2.69 (s, 3H, $COCH_3$).

4.2. General procedure for reaction of 4-acetyl-1,3-benzoxathiol-2-ones (1–3) with benzaldehydes

4-Acetyl-1,3-benzoxathiol-2-ones (**1**–**3**) (4 mmol), a benzaldehyde (6 mmol), piperidine acetate (870 mg, 6 mmol) in anhydrous DMSO (4 mL) were heated at 100–110 °C, with stirring for 1–2 h. The reaction mixture was cooled down and elaborated as described for particular benzaldehydes.

4.2.1. 2-(3′,4′-Dimethoxybenzylidene)-4-methoxy-7-piperidinocarbonyloxy-2,3-dihydro-benzo[*b*]thiophen-3-one (4). Reaction of **1** with 3,4-dimethoxybenzaldehyde. The product precipitated after cooling. The suspension was diluted with methanol and filtered. The yellow solid was washed with methanol, and crystallized from 2-methoxyethanol to give **4**, (62%) as yellow solid, mp 173–175 °C. Anal. Calcd for $C_{24}H_{25}O_6S_1N_1$: C, 63.28; H, 5.53; N, 3.07; S, 7.04. Found: C, 63.39; H, 5.38; N, 3.15; S, 6.87. IR (KBr, cm^{-1}): 1720, 1662, 1584, 1509, 1223, 1040. NMR (200 MHz, $CDCl_3$): δ 7.91 (s, 1H, H-α), 7.35–7.50 (m, 2H, H-6, H-6′), 7.3 (br s, 1H, H-2′), 7.05 (d, 1H, $J=8.4$ Hz, H-5′), 6.82 (d, 1H, $J=8.9$ Hz, H-5), 4.07 (s, 3H, OCH_3), 4.04 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 3.75 (br s, 2H, piperidine), 3.60 (br s, 2H, piperidine), 1.77 (br s, 6H, piperidine).

4.2.2. 2-(3′,4′-Dihydroxybenzylidene)-4-methoxy-7-piperidinocarbonyloxy-2,3-dihydro-benzo[*b*]thiophen-3-one (5) and 2-(3′,4′-dihydroxybenzylidene)-7-hydroxy-4-methoxy-2,3-dihydro-benzo[*b*]thiophen-3-one (6). Reaction of (**1**) with 3,4-dihydroxybenzaldehyde. The products were precipitated by addition of cold water, filtered, dried and separated on silica gel column in chloroform–methanol 20:1 solution to give **5**, (21%) as a yellow solid, mp 255–260 °C dec. Anal. Calcd for $C_{22}H_{21}O_6S_1N_1$: C, 61.81; H, 4.95; N, 3.28; S, 7.50. Found: C, 61.90; H, 5.14; N, 3.03; S, 7.61. IR (KBr, cm^{-1}): 1701, 1664, 1567, 1430, 1229, 1039. NMR (200 MHz, acetone): δ 8.5 (br s, 1H, OH), 8.4 (br s, 1H, OH), 7.70 (s, 1H, H-α), 7.43 (d, 1H, $J=8.9$ Hz, H-6), 7.29 (d, 1H, $J=2.1$ Hz, H-2′), 7.20 (dd, 1H, $J_1=8.7$ Hz, $J_2=2.1$ Hz, H-6′), 6.97 (m, 2H, H-5′, H-5), 3.96 (s, 3H, OCH_3), 3.70 (br s, 2H, piperidine), 3.51 (br s, 2H, piperidine), 1.71 (br s, 6H, piperidine), and **6**, (8%) as a brick-red solid, mp 174–178 °C. Anal. Calcd for $C_{16}H_{12}O_5S_1$: C, 60.75; H, 3.82; S, 10.14. Found: C, 60.57; H, 3.99; S, 10.02. IR (KBr, cm^{-1}): 3394, 1545, 1508, 1233, 1040. NMR (200 MHz, DMSO): δ 10.1 (br s, 1H, OH), 9.8 (br s, 1H, OH), 9.5 (br s, 1H, OH), 7.57 (s, 1H, H-α), 7.26 (d, 1H, $J=2.1$ Hz, H-2′), 7.12 (dd, 1H, $J_1=2.1$ Hz, $J_2=8.4$ Hz, H-6′), 7.05 (d, 1H, $J=8.7$ Hz, H-6), 6.87 (d, 1H, $J=8.1$ Hz, H-5′), 6.78 (d, 1H, $J=8.8$ Hz, H-5), 3.80 (s, 3H, OCH_3).

4.2.3. 2-(4′-Bromobenzylidene)-4-methoxy-7-piperidinocarbonyloxy-2,3-dihydro-benzo[*b*]thiophen-3-one (7). Reaction of (**1**) with 4-bromobenzaldehyde. The product precipitated after cooling. The suspension was diluted with methanol and filtered. The crude product was crystallized from 2-methoxyethanol to give **7**, (29%) as a yellow solid, mp 185–188 °C. Anal. Calcd for $C_{22}H_{20}O_4S_1N_1Br_1$: C, 55.70; H, 4.25; N, 2.95; S, 6.76. Found: C, 55.42; H, 4.20; N, 2.82; S, 6.50. IR (KBr, cm^{-1}): 1710, 1683, 1487, 1417, 1219, 1034. NMR (500 MHz, DMSO): δ 7.77 (s, 1H, H-α), 7.76 (d, 2H, Ar′), 7.69 (d, 2H, $J=8.3$ Hz, Ar′), 7.53 (d, 1H, $J=8.8$ Hz, H-6), 7.01 (d, 1H, $J=9.3$ Hz, H-5), 3.93 (s, 3H, OCH_3), 3.62 (br s, 2H, piperidine), 3.42 (br s, 2H, piperidine), 1.64 (br s, 4H, piperidine), 1.56 (br s, 2H, piperidine).

4.2.4. 2-Benzylidene-4-methoxy-7-piperidinocarbonyloxy-2,3-dihydro-benzo[*b*]thiophen-3-one (8). Reaction of (**1**) with benzaldehyde. The product precipitated after cooling. The suspension was diluted with methanol and filtered. The crude product was purified on silica gel column in chloroform to give **8**, (51%) as a yellow solid, mp 180–181 °C. Anal. Calcd for $C_{22}H_{21}O_4S_1N_1$: C, 66.82; H, 5.35; N, 3.54; S, 8.11. Found: C, 66.99; H, 5.51; N, 3.76; S, 8.34. IR (KBr, cm^{-1}): 1715, 1681, 1585, 1492, 1425, 1220, 1031, NMR (200 MHz, DMSO): δ 7.81 (s, 1H, H-α), 7.42–7.80 (m, 6H, Ar′, H-6), 7.00 (d, 1H, $J=9.0$ Hz, H-5), 3.93 (s, 3H, OCH_3), 3.62 (br s, 2H, piperidine), 3.44 (br s, 2H, piperidine), 1.64 (br s, 6H, piperidine).

4.2.5. 2-[(Pyridin-4yl)-methylene]-4-methoxy-7-piperidinocarbonyloxy-2,3-dihydro-benzo[*b*]thiophen-3-one (9). Reaction of (**1**) with 4-pyridinecarboxaldehyde. Water was added to the cooled reaction mixture, the precipitated oil was dissolved in methylene chloride, the solution was washed with water, dried and evaporated to dryness. The residue was purified on silica gel column in methylene chloride–ethyl acetate 3:1 solution to give 30%, and after crystallization from 2-methoxyethanol 19% of **9** as a yellow solid, mp 203–205 °C. Anal. Calcd for $C_{21}H_{20}N_2O_4S_1$: C, 63.62; H, 5.08; N, 7.07; S, 8.09. Found: C, 63.47; H, 5.20;

N, 7.28; S, 8.37. IR (KBr, cm^{-1}): 1720, 1978, 1583, 1427, 1226, 1034. NMR (200 MHz, $CDCl_3$): δ 8.73 (d, 2H, J= 4 Hz, H-2′,H-6′), 7.73 (s, 1H, H-α), 7.56 (d, 2H, J=6 Hz, H-3′, H-5′), 7.41 (d, 1H, J=8.9 Hz, H-6), 6.77 (d, 1H, J= 8.9 Hz, H-5), 4.01 (s, 3H, OCH_3), 3.69 (br s, 2H, piperidine), 3.55 (br s, 2H, piperidine), 1.71 (br s, 6H, piperidine).

4.2.6. 2-(2′,3′-Dimethoxybenzylidene)-4-methoxy-7-piperidinocarbonyloxy-2,3-dihydro-benzo[*b*]thiophen-3-one (10). Reaction of (**1**) with 2,3-dimethoxybenzaldehyde. The product precipitated after cooling. The suspension was diluted with methanol and filtered. The yellow solid was crystallized from 2-methoxyethanol to give **10** (57%) as a yellow solid, mp 166–167 °C. Anal. Calcd for $C_{24}H_{25}N_1O_6S_1$: C, 63.28; H, 5.53; N, 3.07; S, 7.04. Found: C, 63.10; H, 5.58; N, 3.00; S, 7.17. IR (KBr, cm^{-1}): 1724, 1681, 1582, 1488, 1425, 1224, 1084, 1034. NMR (200 MHz, DMSO): δ 8.00 (s, 1H, H-α), 7.51 (d, 1H, J= 8.9 Hz, H-6), 7.18–7.30 (m, 3H, Ar′), 7.00 (d, 1H, J= 8.9 Hz, H-5), 3.93 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.62 (br s, 2H, piperidine), 3.42 (br s, 2H, piperidine), 1.62 (br s, 6H, piperidine).

4.2.7. 2-(4′-Methoxybenzylidene)-4-methoxy-7-piperidinocarbonyloxy-2,3-dihydro-benzo[*b*]thiophen-3-one (11). Reaction of (**1**) with 4-methoxybenzaldehyde. Water was added to the cooled reaction mixture, the precipitated oil was washed with water, dissolved in chloroform, the solution was washed with water, dried and evaporated to dryness. The residue was purified on silica gel column in chloroform–toluene 3:1 solution and crystallized from 2-methoxyethanol to give 29% of **11** as a yellow solid, mp 156–157 °C. Anal. Calcd for $C_{23}H_{23}N_1O_5S_1$: C, 64.92; H, 5.45, N, 3.29; S, 7.54. Found: C, 64.67; H, 5.33; N, 3.11; S, 7.31. IR (KBr, cm^{-1}): 1727, 1673, 1571, 1509, 1426, 1225, 1176, 1029. NMR (200 MHz, DMSO): δ 7.77 (s, 1H, H-α), 7.72 (d, 2H, J=8.8 Hz, H-2′, H-6′), 7.49 (d, 1H, J= 8.9 Hz, H-6), 7.12 (d, 2H, J=8.7 Hz, H-3′, H-5′), 6.98 (d, 1H, J=9.0 Hz, H-5), 3.91 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.62 (br s, 2H, piperidine), 3.44 (br s, 2H, piperidine), 1.64 (br s, 6H, piperidine).

4.2.8. 2-(4′-Hydroxybenzylidene)-4-methoxy-7-piperidinocarbonyloxy-2,3-dihydro-benzo[*b*]thiophen-3-one (12). Reaction of (**1**) with 4-hydroxybenzaldehyde. The product precipitated after cooling. The suspension was diluted with methanol and filtered. The yellow solid was washed with methanol, crystallized from 2-methoxyethanol and washed with methanol to give **12**, (58%) as a yellow solid, mp 238–240 °C. Anal. Calcd for $C_{22}H_{21}N_1O_5S_1$: C, 64.22; H, 5.14; N, 3.40; S, 7.79. Found: C, 64.11; H, 5.00; N, 3.27; S, 7.53. IR (KBr, cm^{-1}): 3205, 1719, 1659, 1561, 1512, 1426, 1226, 1173, 1040. NMR (200 MHz, DMSO): δ 10.35 (br s, 1H, OH), 7.72 (s, 1H, H-α), 7.62 (d, 2H, J= 8.6 Hz, H-2′, H-6′), 7.47 (d, 1H, J=8.9 Hz, H-6), 6.96 (d, 1H, J=8.9 Hz, H-5), 6.94 (d, 2H, J=8.7 Hz, H-3′, H-5′), 3.91 (s, 3H, OCH_3), 3.62 (br s, 2H, piperidine), 3.54 (br s, 2H, piperidine), 1.63 (br s, 6H, piperidine).

4.2.9. 2-(3′-Hydroxybenzylidene)-4-methoxy-7-piperidinocarbonyloxy-2,3-dihydro-benzo[*b*]thiophen-3-one (13). Reaction of (**1**) with 3-hydroxybenzaldehyde. Water was added to the cooled reaction mixture, the precipitated oil was washed with water, dissolved in chloroform, the solution was washed with water, dried [$(Na)_2SO_4$] and evaporated to dryness. The residue was crystallized from 2-methoxyethanol and washed with methanol to give **13**, (27%) as a yellow solid, mp 255–258 °C. Anal. Calcd for $C_{22}H_{21}N_1O_5S_1$: C, 64.22; H, 5.14; N, 3.40; S, 7.79. Found: C, 64.47; H, 5.11; N, 3.23; S, 7.54. IR (KBr, cm^{-1}): 3322, 1711, 1670, 1578, 1489, 1227, 1042, NMR (200 MHz, DMSO): δ 9.86 (br s, 1H, OH), 7.70 (s, 1H, H-α), 7.50 (d, 1H, J=8.9 Hz, H-6), 7.32 (t, 1H, J=7.7 Hz, H-5′), 7.16 (m, 2H, H-2′, H-6′), 6.99 (d, 1H, J=9. 0 Hz, H-5), 6.88 (dd, 1H, J=7.8 Hz, H-4′), 3.92 (s, 3H, OCH_3), 3.53 (br s, 2H, piperidine), 3.43 (br s, 2H, piperidine), 1.63 (br s, 6H, piperidine).

4.2.10. 2-(4′-Hydroxy-3′-methoxybenzylidene)-4-methoxy-7-piperidinocarbonyloxy-2,3-dihydro-benzo[*b*]thiophen-3-one (14). Reaction of (**1**) with 4-hydroxy-3-methoxybenzaldehyde. The product precipitated after cooling and addition of methanol. The suspension was filtered, the yellow solid was washed with methanol, crystallized twice from 2-methoxyethanol and washed with methanol to give **14**, (48%) as an orange solid, mp 195–196 °C. Anal. Calcd for $C_{23}H_{23}N_1O_6S_1$: C, 62.57; H, 5.25; N, 3.17; S, 7.26. Found: C, 62.78; H, 5.39; N, 3.33; S, 7.02. IR (KBr, cm^{-1}): 3324, 1700, 1670, 1570, 1512, 1428, 1232, 1038. NMR (200 MHz, DMSO): δ 10.00 (br s, 1H, OH), 7.74 (s, 1H, H-α), 7.48 (d, 1H, J=8.8 Hz, H-6), 7.33 (d, 1H, J=2.0 Hz, H-2′), 7.26 (dd, 1H, J_1=8.2 Hz, J_2=2.0 Hz, H-6′), 6.97 (d, 1H, J=8.9 Hz, H-5), 6.94 (d, 1H, J=8.2 Hz, H-5′), 3.91 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.62 (br s, 2H, piperidine), 3.43 (br s, 2H, piperidine), 1.63 (br s, 6H, piperidine).

4.2.11. 2-(4′-Chlorobenzylidene)-4-methoxy-7-piperidinocarbonyloxy-2,3-dihydro-benzo[*b*]thiophen-3-one (15). Reaction of (**1**) with 4-chlorobenzaldehyde. The product precipitated after cooling. The suspension was diluted with methanol and filtered. The yellow solid was washed with methanol and dried to give 44% of crude product. The product was purified on silica gel column in chloroform to give **15**, (36%) as a yellow solid, mp 189–190 °C. Anal. Calcd for $C_{22}H_{20}N_1O_4S_1Cl_1$: C, 61.46; H, 4.69; N, 3.26; S, 7.46. Found: C, 61.27; H, 4.81; N, 3.13; S, 7.21. IR (KBr, cm^{-1}): 1714, 1681, 1582, 1492, 1425, 1221, 1027. NMR (500 MHz, DMSO): δ 7.79 (s, 1H, H-α), 7.76 (d, 2H, J= 8.3 Hz, H-2′, H-6′), 7.63 (d, 2H, J=8.3 Hz, H-3′, H-5′), 7.53 (d, 1H, J=8.9 Hz, H-6), 7.01 (d, 1H, J=8.9 Hz, H-5), 3.93 (s, 3H, OCH_3), 3.62 (br s, 2H, piperidine), 3.43 (br s, 2H, piperidine), 1.64 (br s, 4H, piperidine), 1.56 (br s, 2H, piperidine).

4.2.12. 2-(3′-Chlorobenzylidene)-4-methoxy-7-piperidinocarbonyloxy-2,3-dihydro-benzo[*b*]thiophen-3-one (16). Reaction of (**1**) with 3-chlorobenzaldehyde. The product precipitated after cooling. The suspension was diluted with methanol and filtered. The yellow solid was washed with methanol, crystallized from 2-methoxyethanol and washed with methanol to give **16**, (42%) as a yellow solid, mp 170–172 °C. Anal. Calcd for $C_{22}H_{20}N_1O_4S_1Cl_1$: C, 61.46; H, 4.69; N, 3.26; S, 7.46. Found: C, 61.25; H, 4.45; N, 3.08; S, 7.67. IR (KBr, cm^{-1}): 1714, 1683, 1582, 1490,

1425, 1222, 1026. NMR (500 MHz, DMSO): δ 7.81 (s, 1H, H-2′), 7.79 (s, 1H, H-α), 7.71 (d, 1H, J=7.8 Hz, H-2′), 7.59 (t, 1H, J=7.8 Hz, H-5′), 7. 55–7.57 (m, 2H, H-4′, H-6), 7.02 (d, 1H, J=8.9 Hz, H-5), 3.93 (s, 3H, OCH_3), 3.62 (br s, 2H, piperidine), 3.43 (br s, 2H, piperidine), 1.65 (br s, 4H, piperidine), 1.56 (br s, 2H, piperidine).

4.2.13. 2-(2′-Chlorobenzylidene)-4-methoxy-7-piperidinocarbonyloxy-2,3-dihydro-benzo[*b*]thiophen-3-one (17). Reaction of (**1**) with 2-chlorobenzaldehyde. Water was added to the cooled reaction mixture, the precipitated oil was washed with water, dissolved in chloroform, the solution was washed with water, dried ($MgSO_4$) and evaporated to dryness. The residue was purified on silica gel column in chloroform–toluene 3:1 solution to give 30% of **17** as a yellow solid, mp 170 171 °C. Anal. Calcd for $C_{22}H_{20}N_1O_4S_1Cl_1$: C, 61.46; H, 4.69; N, 3.26; S, 7.46. Found: C, 61.68; H, 4.62; N, 3.39; S, 7.24. IR (KBr, cm^{-1}): 1722, 1681, 1582, 1490, 1424, 1218, 1145, 1030. NMR (200 MHz, DMSO): δ 7.99 (s, 1H, H-α), 7.79 (dd, 1H, J_1=7.2 Hz, J_2= 1.9 Hz, H-6′), 7.44–7.70 (m, 4H, H-6, H-3′, H-4′, H-5′), 7.02 (d, 1H, J=9.1 Hz, H-5), 3.93 (s, 3H, OCH_3), 3.59 (br s, 2H, piperidine), 3.42 (br s, 2H, piperidine), 1.61 (br s, 6H, piperidine).

4.2.14. 2-(3′-Bromo-4′-hydroxy-5′-methoxybenzylidene)-4-methoxy-7-piperidinocarbonyloxy-2,3-dihydro-benzo[*b*]thiophen-3-one (18). Reaction of (**1**) with 3-bromo-4-hydroxy-5-methoxybenzaldehyde. The product precipitated after cooling. The suspension was diluted with methanol and filtered. The yellow solid was washed with methanol, crystallized from 2-methoxyethanol and washed with methanol to give **18**, (52%) as a yellow solid, mp 227–229 °C. Anal. Calcd for $C_{23}H_{22}N_1O_6S_1Br_1$: C, 53.08; H, 4.26; N, 2.69; S, 6.16. Found: C, 52.87; H, 4.45; N, 2.41; S, 6.00. IR (KBr, cm^{-1}): 3388, 1715, 1667, 1579, 1500, 1427, 1226, 1038. NMR (500 MHz, DMSO): δ 10.46 (br s, 1H, OH), 7.73 (s, 1H, H-α), 7.55 (d, 1H, J=1.9 Hz, H-2′), 7.51 (d, 1H, J=8.8 Hz, H-6), 7.39 (d, 1H, J=small, H-6′), 7.00 (d, 1H, J=9.3 Hz, H-5), 3.92 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.62 (br s, 2H, piperidine), 3.44 (br s, 2H, piperidine), 1.66 (br s, 4H, piperidine), 1.56 (br s, 2H, piperidine).

4.2.15. 2-(4′-Dimethylaminobenzylidene)-4-methoxy-7-piperidinocarbonyloxy-2,3-dihydro-benzo[*b*]thiophen-3-one (19). Reaction of (**1**) with 4-dimethylaminobenzaldehyde. Water was added to the cooled reaction mixture, the precipitated oil was washed with water, dissolved in chloroform, the solution was washed with water, dried ($MgSO_4$) and evaporated to dryness. The residue was washed with methanol, crystallized twice from 2-methoxyethanol and washed with methanol to give **19**, (55%) as a red solid, mp 218–222 °C. Anal. Calcd for $C_{24}H_{26}N_2O_4S_1$: C, 65.73; H, 5.98; N, 6.39; S, 7.31. Found: C, 65.82; H, 5.92; N, 6.30; S, 7.18. IR (KBr, cm^{-1}): 1723, 1665, 1561, 1522, 1369, 1228, 1033. NMR (200 MHz, DMSO): δ 7.70 (s, 1H, H-α), 7.60 (d, 2H, J=9.0 Hz, H-2′, H-6′), 7.45 (d, 1H, J=8.9 Hz, H-6), 6.94 (d, 1H, J=8.9 Hz, H-5), 6.85 (d, 2H, J=8.9 Hz, H-3′, H-5′), 3.90 (s, 3H, OCH_3), 3.62 (br s, 2H, piperidine), 3.43 (br s, 2H, piperidine), 3.03 (s, 6H, $N(CH_3)_2$), 1.64 (br s, 6H, piperidine).

4.2.16. 2-(4′-Nitrobenzylidene)-4-methoxy-7-piperidinocarbonyloxy-2,3-dihydro-benzo[*b*]thiophen-3-one (20). Reaction of (**1**) with 4-nitrobenzaldehyde. The product precipitated after cooling. The suspension was diluted with methanol and filtered. The yellow solid was washed with methanol, purified on silica gel column in chloroform, crystallized from 2-methoxyethanol and washed with methanol to give **20**, (10%) as a yellow solid, mp 242–244 °C. Anal. Calcd for $C_{22}H_{20}N_2O_6S_1$: C, 59.99; H, 4.58; N, 6.36; S, 7.28. Found: C, 60.23; H, 4.41; N, 6.53; S, 7.07. IR (KBr, cm^{-1}): 1715, 1688, 1582, 1512, 1490, 1427, 1339, 1226, 1031. NMR (200 MHz, DMSO): δ 8.37 (d, 2H, J= 8.7 Hz, H-2′, H-6′), 7.99 (d, 2H, J=8.7 Hz, H-3′, H-5′), 7.88 (s, 1H, H-α), 7.56 (d, 1H, J=8.9 Hz, H-6), 7.04 (d, 1H, J=8.9 Hz, H-5), 3.94 (s, 3H, OCH_3), 3.62 (br s, 2H, piperidine), 3.46 (br s, 2H, piperidine), 1.64 (br s, 6H, piperidine).

4.2.17. 2-(3′-Bromo-4′,5′-dimethoxybenzylidene)-4-methoxy-7-piperidinocarbonyloxy-2,3-dihydro-benzo[*b*]thiophen-3-one (21). Reaction of (**1**) with 3-bromo-4,5-dimethoxybenzaldehyde. The product precipitated after cooling. The suspension was diluted with methanol and filtered. The crude product was purified on silica gel column in chloroform–ethyl acetate 100:1 solution and crystallized from chloroform–methanol mixture to give **21**, (29%) a yellow solid, mp 194–196 °C. Anal. Calcd for $C_{24}H_{24}N_1O_6S_1Br_1$: C, 53.94; H, 4.53; N, 2.62; S, 6.00. Found: C, 53.90; H, 4.40; N, 2.54; S, 5.85. IR (KBr, cm^{-1}): 1716, 1675, 1582, 1489, 1428, 1229, 1039. NMR (200 MHz, DMSO): δ 7.73 (s, 1H, H-α), 7.55 (d, 1H, J=1.8 Hz, H-2′), 7.51 (d, 1H, J=9.0 Hz, H-6), 7.44 (d, 1H, J=1.9, H-6′), 6.99 (d, 1H, J=9.0 Hz, H-5), 3.92 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.61 (br s, 2H, piperidine), 3.45 (br s, 2H, piperidine), 1.64 (br s, 6H, piperidine).

4.2.18. 2-(3′-Chlorobenzylidene)-4-[2″-(*N*,*N*-diethylamino)ethoxy]-7-piperidinocarbonyloxy-2,3-dihydro-benzo[*b*]thiophen-3-one (22). Reaction of (**2**) with 3-chlorobenzaldehyde. The reaction mixture was poured into solution of potassium carbonate (2 g) in water (80 mL) to give a dark oil. Water was decanted and the oil was washed several time with water to remove DMSO. The residue was dissolved in ethyl acetate, washed with water, dried (sodium sulfate) and evaporated. The residue was purified on silica gel column in chloroform–methanol 10:1 solution and crystallized from methanol to give **22**, (35%) as a yellow solid, mp 122–123 °C. Anal. Calcd for $C_{27}H_{31}N_2O_4S_1Cl_1$: C, 62.96; H, 6.07; N, 5.44; S, 6.23. Found: C, 62.86; H, 5.91; N, 5.45; S, 6.16. IR (KBr, cm^{-1}): 1721, 1682, 1580, 1426, 1223, 1035. NMR (500 MHz, DMSO): δ 7.79 (br s, 2H, H-α, H-2′), 7.70 (d, 1H, J=7.8 Hz, H-6′), 7.59 (t, 1H, J=7.8 Hz, H-5′), 7.54 (d, 1H, J=7.8 Hz, H-4′), 7.50 (d, 1H, J=9.3 Hz, H-6), 7.02 (d, 1H, J=9.3 Hz, H-5), 4.19 (t, 2H, J=4.9 Hz, OCH_2), 3.62 (br s, 2H, piperidine), 3.43 (br s, 2H, piperidine), 2.84 (t, 2H, J=5.4 Hz, NCH_2), 2.60 (q, 4H, J=7.0 Hz, 2×CH_2), 1.65 (br s, 4H, piperidine), 1.56 (br s, 2H, piperidine), 0.99 (t, 6H, J= 7.3 Hz, 2×CH_3).

4.2.19. 2-(3′-Chlorobenzylidene)-4-allyloxy]-7-piperidinocarbonyloxy-2,3-dihydro-benzo[*b*]thiophen-3-one (23). Reaction of (**3**) with 3-chlorobenzaldehyde. The

product precipitated after cooling. The suspension was diluted with methanol and filtered. The crude product was crystallized from 2-methoxyethanol to give **23**, (42%) as a yellow solid, mp 144–146 °C. Anal. Calcd for $C_{24}H_{22}N_1O_4S_1Cl_1$: C, 63.22; H, 4.86; N, 3.07; S, 7.03. Found: C, 62.96; H, 4.73; N, 3.10; S, 7.13. IR (KBr, cm^{-1}): 1721, 1679, 1580, 1424, 1221, 1011. NMR (500 MHz, DMSO): δ 7.79 (s, 2H, H-α, H-2′), 7.70 (d, 1H, $J=7.3$ Hz, H-6′), 7.59 (t, 1H, $J=7.8$ Hz, H-5′), 7.54 (br d, 1H, $J=8.3$ Hz, H-4′), 7.50 (d, 1H, $J=9.3$ Hz, H-6), 7.00 (d, 1H, $J=8.8$ Hz, H-5), 6.07 (m, 1H, =CH–), 5.61 (d, 1H, $J=16.2$ Hz, H_2C=), 5.32 (d, 1H, $J=10.2$ Hz, H_2C=), 4.75 (d, 2H, $J=3.9$ Hz, OCH_2), 3.62 (br s, 2H, piperidine), 3.43 (br s, 2H, piperidine), 1.65 (br s, 4H, piperidine), 1.56 (br s, 2H, piperidine).

4.2.20. Reaction of 4-acetyl-5-methoxy-1,3-benzoxathiol-2-one (1) with 3,4-dimethoxybenzaldehyde in methanol. 4-Acetyl-5-methoxy-1,3-benzoxathiol-2-one (**1**) (224 mg, 1 mmol), 3,4-dimethoxybenzaldehyde (250 mg, 1.5 mmol) and piperidine (0.15 mL, 1.5 mmol) in methanol (2 mL) were stirred at 60 °C for 5 h, and the solution was left for cooling. The precipitated solid was filtered off, and washed with methanol, to give 2-(3′,4′-dimethoxybenzylidene)-4-methoxy-7-piperidinocarbonyloxy-2,3-dihydro-benzo[*b*]thiophen-3-one (**4**) (36 mg, 8%). The combined filtrates were evaporated and the residue was separated on silica gel column in chloroform, to give a second fraction of **4** (10 mg, 2%), and 8-hydroxy-3′,4′,5-trimethoxythioflavanone (**24**) (31 mg, 9%) as a yellow solid, mp 180–182 °C. Anal. Calcd for $C_{18}H_{18}O_5S_1$: C, 62.41; H, 5.24; S, 9.24. Found: C, 62.19; H, 5.30; S, 9.02. IR (KBr, cm^{-1}): 3285, 1646, 1570, 1518, 1466, 1246, 1037. NMR (500 MHz, DMSO): δ 9.76 (s, 1H, OH), 7.07 (d, 1H, $J=2$ Hz, H-2′), 6.99 (dd, 1H, $J_1=8.3$ Hz, $J_2=1.9$ Hz, H-6′), 6.94 (d, 1H, $J=8.8$ Hz, H-7), 6.93 (d, 1H, $J=8.3$ Hz, H-5′), 6.73 (d, $J=9.3$ Hz, H-6), 4.65 (dd, 1H, $J_1=13.1$ Hz, $J_2=3.0$ Hz, H-2), 3.76 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 3.32 (dd, 1H, $J_1=15.6$ Hz, $J_2=13.6$ Hz, H-3), 2.86 (dd, 1H, $J_1=15.2$ Hz, $J_2=3.0$ Hz, H-3).

4.2.21. Reaction of 4-acetyl-5-methoxy-1,3-benzoxathiol-2-one (1) with piperidine acetate in DMSO. Solution of 4-acetyl-5-methoxy-1,3-benzoxathiol-2-one (**1**) (224 mg, 1 mmol) and piperidine acetate (218 mg, 1.5 mmol) in dry DMSO (2 mL) was deoxygenated and stirred under argon at 60 °C for 5 h. The reaction mixture was cooled in ice, and products were precipitated by addition of water. The crude mixture (280 mg) was separated on silica gel column in chloroform–ethyl acetate 3:1 solution to give 2-acetyl-3-methoxy-6-piperidinocarbonyloxyphenyl disulfide (**26**) (20 mg, 6%) as a beige solid, mp 159–161 °C. Anal. Calcd for $C_{30}H_{36}O_8N_2S_2$: $M=616.726$; C-58.42; H-5.89; N-4.55; S-10.38. Found: C-58.75; H, 5.98; N, 4.44; S, 10.55. MS MALDI TOF: 639 (M^+ + Na), 655 (M^+ + K). IR (KBr, cm^{-1}): 1720, 1582, 1420, 1224, 1138, 1044, 1022. NMR (500 MHz, $CDCl_3$): δ 7.21 (d, 1H, $J=9.3$ Hz, H-4), 6.93 (d, 1H, $J=9.3$ Hz, H-5), 3.80 (s, 3H, OCH_3), 3.48 (br s, 4H, H-piperidine), 2.16 (s, 3H, $COCH_3$), 1.61 (br m, 6H, H-piperidine); As the second product was eluted 4,4′-dimethoxy-7,7′-di(piperidinocarbonyloxy)-3′-hydroxy-3′-methyl-3-keto-[3,3′-spirobi(2*H*,2′*H*,3*H*,3′*H*-benzo[*b*]thiophen)] (**27**), (150 mg, 50%) as a cream solid, mp 140–147 °C. Anal. Calcd for $C_{30}H_{34}O_8N_2S_2\times H_2O$: $M=632.75$; C, 56.95; H, 5.73; N, 4.43; S, 10.14. Found: C, 56.98; H, 5.63; N, 4.30; S, 10.13. MS MALDI TOF: 597 (M^+ − OH), 614 (M^+), 637 (M^+ + Na), 653 (M^+ + K). IR (KBr, cm^{-1}): 3442, 1722, 1580, 1488, 1425, 1225, 1141, 1048, 1020. NMR (500 MHz, DMSO) (the compound exists as a mixture of two diastereoisomers. Full NMR data, including ^{13}C, ROESY, gHSQC, and gHMBC spectra, are given in the Supplementary data, the following list presents only ^1H data for the main isomer): δ 7.48 (d, 1H, $J=8.8$ Hz, H-6), 7.09 (d, 1H, $J=9.3$ Hz, H-6′), 6.88 (d, 1H, $J=7.8$ Hz, H-5), 6.82 (d, 1H, $J=9.3$ Hz, H-5′), 6.46 (s, 1H, OH), 3.81 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3′), 3.34–3.60 (br m, 8H, piperidine), 1.42–1.65 (br m, 12H, piperidine), 1.64 (s, 3H, CH_3).

4.2.22. Reaction of 2-acetyl-3-methoxy-6-piperidinocarbonyloxyphenyl disulfide (26) with 3,4-dimethoxybenzaldehyde. 2-Acetyl-3-methoxy-6-piperidinocarbonyl-oxyphenyl disulfide (**26**) (11 mg, 0.028 mmol), 3,4-dimethoxybenzaldehyde (7 mg, 0.042 mmol) and piperidine acetate (6 mg, 0.04 mmol) in anhydrous DMSO (0.5 mL) were stirred at 90 °C for 1 h. The solution was cooled, the product was precipitated with water, filtered, washed with water and methanol, to give thioaurone **4** (4 mg).

Acknowledgements

The authors thank Taiho Pharmaceutical Company, Japan for financial support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2005.06.107

Table 1—NMR data of the spiro compound **27**; Figure 1—low field part of ^1H NMR spectrum of **27**; Figure 2—high field part of ^1H NMR spectrum of **27**; Figures 3 to 7—^{13}C NMR of **27**; Figure 8—ROESY spectrum of **27**; Figure 9—gHMBC spectrum of **27**; Figure 10—gHSQC spectrum of **27**.

References and notes

1. Konieczny, M. T.; Konieczny, W. *Heterocycles* **2005**, *2*, 451–464.
2. Eggers, K.; Fyles, T. M.; Montoya-Pelaez, P. J. *J. Org. Chem.* **2001**, *66*, 2966–2977.
3. Steinle, W.; Rück-Braun, K. *Org. Lett.* **2003**, *5*, 141–144.
4. Lougheed, T.; Borisenko, V.; Hennig, T.; Rück-Braun, K.; Woolley, G. A. *Org. Biomol. Chem.* **2004**, *2*, 2798–2801.
5. Auwers, K.; Arndt, F. *Chem. Ber.* **1909**, *42*, 537.
6. Konieczny, M. T.; Horowska, B.; Kunikowski, A.; Konopa, J.;

Wierzba, K.; Yamada, Y.; Asao, T. *J. Org. Chem.* **1999**, *64*, 359–364.
7. Konieczny, M. T.; Horowska, B.; Kunikowski, A.; Konopa, J.; Wierzba, K.; Yamada, Y.; Asao, T. *Synthesis* **2001**, 1363–1367.
8. Kalinowski, H. O.; Berger, S.; Braun, S. *Carbon 13 NMR Spectroscopy*; Wiley: NY, 1991; p 526.
9. Marshall, J. L. *Carbon–Carbon and Carbon–Hydrogen NMR Couplings*; Verlag Chemie Int.: Deerfield Beach, 1983; p 33.
10. Réamonn, L. S. S.; O'Sullivan, W. I. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1009–1012.
11. Mostoslavskii, M. A.; Izmail'skii, V. A. *J. Gen. Chem. USSR* **1961**, *17*, 21–31.
12. Somogyi, L. *Can. J. Chem.* **2001**, *79*, 1159–1165.
13. Lau, P. T. S.; Kestner, M. *J. Org. Chem.* **1968**, *33*, 4426–4431.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron 61 (2005) 8656–8662
Tetrahedron

New diterpenoids from stems and roots of *Caesalpinia crista*

Sarot Cheenpracha,[a] Rattikan Srisuwan,[a] Chatchanok Karalai,[a,*] Chanita Ponglimanont,[a] Suchada Chantrapromma,[a] Kan Chantrapromma,[b] Hoong-Kun Fun,[c] Shazia Anjum[d] and Atta-ur-Rahman[d]

[a]*Department of Chemistry, Faculty of Science, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand*
[b]*Research Unit of Natural Products Utilization, School of Science, Walailak University, Thasala, Nakhon Si Thammarat 80160, Thailand*
[c]*X-ray Crystallography Unit, School of Physics, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia*
[d]*H.E.J. Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Karachi-75270, Pakistan*

Received 22 April 2005; revised 10 June 2005; accepted 30 June 2005

Available online 21 July 2005

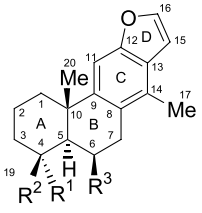
Abstract—Nine new cassane-type diterpenes, named taepeenin A–I, and two new norcassane-type diterpenes, named nortaepeenin A–B, were isolated from the stems and roots of *Caesalpinia crista* along with three known diterpenes: vinhaticoic acid, methyl vinhaticoate and ent-11β-hydroxy-rosa-5,15-diene. Their structures were elucidated on the basis of spectroscopic analysis. In addition, the structure of taepeenin A was confirmed by X-ray diffraction analysis.

1. Introduction

Cassane-type diterpenoids are characteristic components of the genus *Caesalpinia* of the Leguminosae family. One of them, the plant *Caesalpinia crista* L., known locally as 'Taepee' in Thai, is a climber distributed from India and Ceylon through most of Southeast Asia to the Ryu-Kyu Islands, Queensland, and Caledonia.[1] The leaves, roots, and fruits of this plant are used as a tonic and an antiperiodic.[2] The seed kernels of *C. crista* displayed interesting antimalarial activity.[3] Previous chemical investigations of the seed kernels have revealed the presence of several cassane-type diterpenoids.[3–5] We report herein the isolation and structure elucidation of 11 new (**1**–**11**) along with three known diterpenes, vinhaticoic acid (**12**),[6] methyl vinhaticoate (**13**),[7] and ent-11β-hydroxy-rosa-5,15-diene (**14**)[8] from the stems and roots of *C. crista*.

2. Results and discussion

The basic skeleton of **1**–**11** was identified to be a cassane diterpene on the basis of their IR and UV spectroscopic studies. The absorptions of **1**–**5** (λ_{max} 211–293 nm) were characteristic of a benzofuran moiety,[9] whereas the structures of **7** and **8** showed absorption bands of a conjugated carbonyl chromophore in the UV spectrum.



1 : $R^1 = CO_2Me$, $R^2 = Me$, $R^3 = H$
2 : $R^1 = CO_2H$, $R^2 = Me$, $R^3 = H$
3 : $R^1 = CO_2Me$, $R^2 = Me$, $R^3 = OH$
4 : $R^1 = CO_2Me$, $R^2 = Me$, $R^3 = OCOMe$
5 : $R^1 = CO_2Me$, $R^2 = CHO$, $R^3 = H$

Taepeenin A (**1**) was recrystallized from CH_2Cl_2 to yield colorless crystals. The subsequent X-ray structure (Fig. 1, see Section 3) suggested a cassane-type diterpene with a molecular formula $C_{21}H_{26}O_3$ ($[M]^+$ *m*/*z* 326.1887) by HREIMS. Its structure was conclusively established by ^1H and ^{13}C NMR (Tables 1 and 3). The ^1H NMR spectral data (Table 1) displayed two tertiary methyl groups at δ 1.27 (Me-20) and 1.31 (Me-19) and one methyl ester group at δ 3.70 (OMe). In addition, the trisubstituted benzofuran moiety resonated the downfield signals at δ 6.72 (dd, J= 2.1, 0.9 Hz, H-15), 7.53 (d, J=2.1 Hz, H-16), 7.32 (br s, H-11) and one aromatic methyl group at δ 2.35 (Me-17).

Keywords: *Caesalpinia crista*; Taepeenin; Nortaepeenin; Cassane diterpenes.
* Corresponding author. Tel.: +66 7428 8444; fax: +66 7421 2918; e-mail addresses: chatchanok.k@psu.ac.th; chatchanok_k@yahoo.com

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.06.109

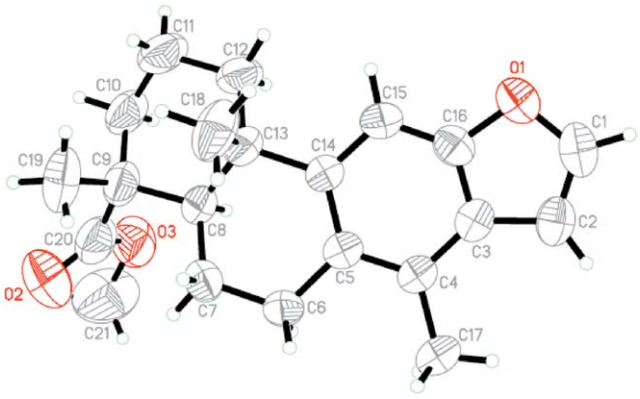


Figure 1. ORTEP drawing of **1**.

The methine proton H-5 showed a doublet of doublet signal at δ 2.27 (J=12.9, 2.4 Hz). On the basis of X-ray analysis and NOESY data, two tertiary methyl groups on C-4 and C-10 were shown to be β-axial orientation, whereas a methine proton H-5 should be α-axial oriented. From these data, taepeenin A was deduced to be **1**.

Taepeenin B (**2**) showed the molecular ion $[M]^+$ at m/z 312.1733 in HRFABMS spectrum in agreement with the formula $C_{20}H_{24}O_3$. The presence of carboxyl (3418 and 1691 cm^{-1}) functionality was evident from IR absorptions. The ^1H and ^{13}C NMR spectral data (Tables 1 and 3) of **2** showed characteristics similar to those of **1** except for the disappearance of the OMe signal at δ 3.70, thus indicating a presence of a free carboxylic acid instead of the methyl ester at C-18. This finding was supported by HMBC spectrum of **2**, in which the methyl protons at δ 1.31 (Me-19) were correlated with the carbons at δ 36.7 (C-3), 44.1 (C-5), 47.4 (C-4) and 184.2 (C-18). Therefore, taepeenin B was determined to be **2**.

Taepeenin C (**3**) exhibited the molecular formula $C_{21}H_{26}O_4$ ($[M]^+$ m/z 342.1825) as determined by HREIMS. The ^1H and ^{13}C NMR spectral data (Tables 1 and 3) revealed the same cassane-type skeleton as **1** and its IR spectrum

Table 1. ^1H NMR (300 MHz) spectral data of **1–6** in $CDCl_3$ (δ in ppm, multiplicities, J in Hz)[a]

Position	1	2	3	4	5	6
1	2.38 m, 1.55 m	2.38 m, 1.60 m	2.26 m, 1.53 m	2.31 m, 1.58 m	2.32 m, 1.55 m	2.27 m, 1.47 m
2	1.81 m, 1.74 m	1.84 m	1.91 m	1.92 m, 1.76 m	1.75 m	1.79 m
3	1.80 m, 1.68 m	1.87 m, 1.77 m	1.85 m, 1.70 m	1.79 m, 1.67 m	2.47 m	1.78 m, 1.65 m
5	2.27 dd (12.9, 2.4)	2.27 dd (12.6, 2.1)	2.33 br s	2.50 br s	2.48 dd (12.9, 1.8)	2.19 dd (12.6, 2.1)
6	1.92 m, 1.55 m	1.95 m, 1.70 m	4.26 br dt (5.4, 1.8)	5.30 dt (5.7, 1.5)	2.01 br dd (12.9, 7.2)	1.54 m
7	2.83 m	2.94 m, 2.84 m	3.10 dd (17.4, 5.4), 2.87 br d (17.4)	3.12 dd (18.3, 5.4), 2.96 br d (18.3)	2.99 br dd (16.5, 5.4), 2.86 m	2.74 m, 2.64 m
11	7.32 br s	7.31 br s	7.35 br s	7.38 s	7.30 br s	6.90 s
15	6.72 dd (2.1, 0.9)	6.71 dd (2.1, 0.9)	6.71 dd (2.4, 0.6)	6.73 dd (2.4, 0.9)	6.72 d (2.1)	3.61 s
16	7.53 d (2.1)	7.52 d (2.1)	7.52 d (2.4)	7.54 d (2.4)	7.53 d (2.1)	
17	2.35 s	2.34 s	2.34 s	2.33 s	2.36 s	2.10 s
19	1.31 s	1.31 s	1.64 s	1.45 s	9.95 s	1.25 s
20	1.27 s	1.27 s	1.64 s	1.64 s	1.11 s	1.28 s
18-O*Me*	3.70 s		3.70 s	3.71 s	3.76 s	3.68 s
6-CO*Me*				2.00 s		

[a] Assignments were made using HMQC and HMBC data.

Table 2. ^1H NMR (300 MHz) spectral data of **7–11** in $CDCl_3$ (δ in ppm, multiplicities, J in Hz)[a]

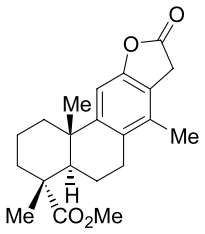
Position	7	8	9	10	11
1	1.74 m, 1.14 m	1.72 m, 1.19 m	1.43 m, 1.18 m	1.79 m, 1.14 m	1.77 m, 1.25 m
2	1.69 m	1.78 m	1.63 m, 1.45 m	1.62 m	1.62 m
3	1.62 m	1.78 m, 1.56 m	1.78 m, 0.94 m	2.38 m, 1.51 m	2.12 m, 1.59 m
5	1.78 dd (12.3, 2.4)	1.80 br s	0.91 m	2.05 dd (12.9, 2.1)	1.79 m
6	1.49 m, 1.29 m	4.03 m	1.29 m, 0.93 m	1.99 m, 1.67 m	1.58 m, 1.28 m
7	2.46 m, 1.31 m	2.48 dt (14.4, 3.6), 1.50 m	2.30 m, 1.08 m	1.80 m, 1.51 m	1.68 m, 1.33 m
8	2.31 td (12.0, 4.2)	2.76 td (12.0, 3.6)	2.26 m	1.85 m	1.73 m
9	1.88 td (12.0, 5.1)	1.91 td (12.0, 5.4)	0.96 m	1.62 m	1.58 m
11	2.90 dd (17.1, 5.1), 2.66 dd (17.1, 12.0)	2.93 dd (17.1, 5.4), 2.76 dd (17.1, 12.0)	1.75 m, 1.39 m	2.60 dd (16.8, 6.6), 2.32 dd (16.8, 10.2)	2.58 dd (16.8, 6.9), 2.36 dd (16.8, 10.2)
12			2.39 m, 2.10 m	2.64 m	2.60 m
14				2.64 m	2.60 m
15	6.63 d (1.8)	6.64 d (1.8)	6.86 dd (17.1, 10.8)	6.18 d (1.8)	6.17 d (1.8)
16	7.30 d (1.8)	7.31 d (1.8)	5.23 d (17.1), 5.07 d (10.8)	7.21 d (1.8)	7.21 d (1.8)
17			4.33 d (11.7), 4.26 d (11.7)	0.99 d (6.9)	0.96 d (6.9)
18			0.83 s		
19	1.21 s	1.59 s	0.85 s	9.90 s	3.96 d (11.7), 3.84 d (11.7)
20	1.01 s	1.35 s	0.84 s	0.76 s	0.89 s
18-O*Me*	3.65 s	3.68 s		3.73 s	3.74 s

[a] Assignments were made using HMQC and HMBC data.

displayed an additional hydroxyl (3419 cm^{-1}) stretching. The ^1H NMR spectral data (Table 1) exhibited a signal due to an oxymethine proton at δ 4.26 (br dt, $J=5.4$, 1.8 Hz) for H-6, which was connected to the oxymethine carbon at δ 68.7 (C-6) in the HMQC spectrum. This proton signal showed HMBC correlations with carbons at δ 37.8 (C-10), 48.5 (C-4) and 124.2 (C-8), confirming the location of the hydroxyl group at C-6. The α-orientation of both protons at C-5 and C-6 was determined from the results of small coupling constants of protons H-5 (δ 2.33, br s) and H-6 (δ 4.26, br dt, $J=5.4$, 1.8 Hz) and the observed cross-peaks between these protons and H-7α (δ 3.10) from NOESY experiments. This result suggested that H-5 and H-6 should be α-axial and α-equatorial oriented, respectively. Therefore, taepeenin C was deduced to be **3**.

Taepeenin D (**4**) had the molecular formula $C_{23}H_{28}O_5$ ($[M]^+$ at *m/z* 384.1936), based on HREIMS. The ^1H and ^{13}C NMR spectral data (Tables 1 and 3) of **4** were closely related to those of **3**, except for the presence of an additional acetyl group (δ_H 2.00 and δ_C 170.7, 21.7). The oxymethine proton H-6 of **4** appeared at δ 5.30 (dt, $J=5.7$, 1.5 Hz), more downfield than that of **3** (δ 4.26, br dt, $J=5.4$, 1.8 Hz) as a result of the deshielding effect of the OAc group and showed HMBC correlations with the carbons at δ 38.0 (C-10), 46.1 (C-5), 48.0 (C-4), 123.8 (C-8) and 170.7 (–O*CO*Me) confirming the location of the OAc group at C-6. Thus, taepeenin D was characterized as **4**.

Taepeenin E (**5**) was deduced as $C_{21}H_{24}O_4$ from an exact mass measurement ($[M]^+$ *m/z* 340.1671 by HREIMS). Its ^1H and ^{13}C NMR spectral data (Tables 1 and 3) of **5** were closely related to those of **1**. The major difference was the replacement of the ^1H NMR signal of Me-19 at δ 1.31 with an aldehydic proton at δ 9.95 (s). The latter was connected to an aldehydic carbon at δ 199.8 from the HMQC experiment and showed HMBC correlations to carbons at δ 29.4 (C-3), 61.1 (C-4) and 173.5 (C-18). Therefore, the structure of taepeenin E was assigned as **5**.



6

Taepeenin F (**6**), $C_{21}H_{26}O_4$ ($[M]^+$ *m/z* 342.1836 by HREIMS), exhibited additional IR absorption band at 1811 (lactone carbonyl) cm^{-1}. The ^1H and ^{13}C NMR spectral data (Tables 1 and 3) of **6** were comparable to those of **1**, except for the presence of a singlet signal of methylene protons (δ 3.61) instead of the two proton signals of a 1,2-disubstituted furan. These methylene protons showed the HMBC correlations with the carbons at δ 119.8 (C-13), 132.9 (C-14), 152.7 (C-12) and lactone carbonyl at δ 174.7 (C-16), indicating them (2H-15) to be connected to the lactone carbonyl and aromatic carbons at C-16 and C-13, respectively. Thus, taepeenin F was assigned to be **6**.

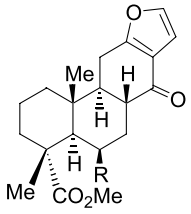
Nortaepeenin A (**7**) had the molecular formula $C_{20}H_{26}O_4$ ($[M]^+$ *m/z* 330.1832) as determined by HREIMS. The ^{13}C NMR (Table 3) and DEPT spectrum exhibited 20 carbons, two of these were a conjugated carbonyl (δ 195.7) and an ester carbonyl (δ 178.9). Excluding the signal due to the methoxy substituent, **7** contained only 19 carbons in the main carbon framework, suggesting it to be a norditerpene. The ^1H and ^{13}C NMR spectral data (Tables 2 and 3) of **7** revealed that nortaepeenin A had the same A and B rings as **1**. The difference was found in ring C, which was aliphatic in **7**. This was supported by the absence of one aromatic proton at δ 7.32 (H-11) in the ^1H NMR spectrum and the presence of methylene protons at δ 2.66 (dd, $J=17.1$, 12.0 Hz, H-11β), 2.90 (dd, $J=17.1$, 5.1 Hz, H-11α) and two

Table 3. ^{13}C NMR (75 MHz) spectral data of **1–11** in $CDCl_3$

Position	1	2	3	4[a]	5	6[a]	7	8[a]	9	10	11
1	38.9	38.8	42.2	42.1	38.6	38.4	37.9	40.6	42.2	38.2	38.6
2	18.8	18.7	19.1	19.0	18.9	18.5	17.9	18.1	19.0	18.1	17.6
3	36.6	36.7	38.1	38.4	29.4	36.5	36.7	38.6	38.7	29.6	29.9
4	47.7	47.4	48.5	48.0	61.1	47.6	47.3	48.2	36.7	60.7	53.5
5	44.4	44.1	47.5	46.1	46.8	44.2	49.0	51.0	55.3	51.1	50.1
6	21.8	21.8	68.7	70.7	21.8	21.4	23.5	69.3	21.6	23.8	23.9
7	27.6	27.5	38.8	34.8	28.4	27.4	26.8	37.1	31.8	31.3	31.3
8	127.5	128.3	124.2	123.8	126.9	129.2	45.0	40.8	38.8	35.8	35.5
9	147.2	147.0	146.1	145.5	144.7	150.7	52.9	53.3	53.8	44.4	46.0
10	37.8	37.8	37.8	38.0	37.9	37.8	36.9	37.5	33.2	37.0	36.9
11	104.3	104.3	105.1	105.0	105.0	104.1	22.8	22.6	21.0	22.4	22.2
12	153.6	153.5	153.8	153.7	153.5	152.7	166.3	166.6	26.9	149.1	149.2
13	125.4	125.4	125.7	125.8	125.8	119.8	119.8	119.9	133.5	122.3	122.4
14	128.3	127.4	128.7	128.7	128.5	132.9	195.7	196.1	139.3	31.4	31.4
15	105.0	105.0	105.0	105.0	104.9	32.5	106.5	106.5	134.4	109.5	109.5
16	144.2	144.2	144.3	144.5	144.5	174.7	142.8	142.8	113.4	140.5	140.4
17	15.9	15.9	16.1	16.1	15.9	16.4			59.1	17.5	17.6
18	179.2	184.2	179.3	178.6	173.5	179.0	178.9	179.0	22.2	173.7	178.6
19	16.6	16.3	18.6	18.1	199.8	16.5	16.8	19.0	33.4	199.9	61.5
20	25.6	25.6	27.5	27.6	24.5	25.1	14.8	17.8	14.2	14.2	15.1
–O*Me*	52.0		52.1	52.3	52.6	52.0	52.0	52.1		52.5	52.1
–OCO*Me*				21.7							
–O*CO*Me				170.7							

[a] The ^{13}C NMR spectra were measured at 125 MHz.

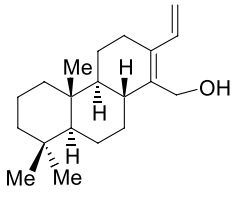
methine protons at δ 1.88 (td, $J=12.0$, 5.1 Hz, H-9) and 2.31 (td, $J=12.0$, 4.2 Hz, H-8). The observed HMBC correlations between a methine proton at δ 1.88 (H-9) with carbons at δ 14.8 (C-20), 36.9 (C-10) 49.0 (C-5), 166.3 (C-12) and 195.7 (C-14), of a methine proton at δ 2.31 (H-8) with carbons at δ 22.8 (C-11), 23.5 (C-6) 52.9 (C-9) and 195.7 (C-14) and of a methine proton at δ 6.63 (H-15) with carbons at δ 166.3 (C-12) and 195.7 (C-14), indicated that the conjugated carbonyl should be C-14. The relative stereochemistry of **7** was determined on the basis of coupling constants and the results of NOESY experiments. The large J values for H-8 and H-9 ($J=12.0$ Hz) indicated that H-8 and H-9 should be axial protons. From the NOESY correlations, the methyl group at δ 1.01 (Me-20) showed a cross-peak with the methyl protons at δ 1.21 (Me-19) and a methine proton at δ 2.31 (H-8), suggesting that Me-19, Me-20, and H-8 should be in β-axial orientation. From these data, the new nortaepeenin A was characterized as **7**.



7 : R = H

8 : R = OH

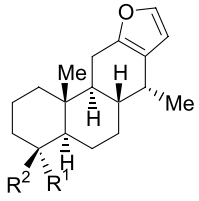
Nortaepeenin B (**8**) showed the $[M]^+$ at m/z 346.1787 ($C_{20}H_{26}O_5$) in HREIMS. Its IR spectrum displayed a hydroxyl stretching at 3415 cm^{-1}. The ^1H and ^{13}C NMR spectral data (Tables 2 and 3) revealed that **8** had the same norcassane-type diterpene as **7**. The minor difference was found on replacement of a multiplet signal of methylene protons at δ 1.49 and 1.29 (2H-6) with an oxymethine proton at δ 4.03, which gave the HMQC cross-peak to the carbon at δ 69.3. This proton showed HMBC correlations with the carbons at δ 37.1 (C-7), 37.5 (C-10), 40.8 (C-8), and 48.2 (C-4). The configuration at C-6 was determined as β-OH by the cross-peak between H-5 (δ 1.80), H-6 (δ 4.03) and H-7α (δ 2.48) in NOESY experiments and the small coupling constant of H-5 (br s) and H-6 (m). Thus, nortaepeenin B was identified as **8**.



9

Taepeenin G (**9**) was deduced as $C_{20}H_{32}O$ ($[M]^+$ m/z 288.2455 by HREIMS). The hydroxyl functionality was shown in IR absorption at 3396 cm^{-1}. The ^1H NMR spectral data (Table 2) showed three singlets of three methyl groups at δ 0.83 (Me-18), 0.84 (Me-20) and 0.85 (Me-19), a typical pattern of vinyl protons at δ 6.86 (dd, $J=17.1$, 10.8 Hz, H-15), 5.23 (d, $J=17.1$ Hz, H-16a) and 5.07 (d, $J=10.8$ Hz, H-16b). The presence of oxymethylene protons were revealed by ^1H NMR signals at δ 4.26 and 4.33 (each d, $J=11.7$ Hz, 2H-17) connecting to carbon at δ 59.1. The ^{13}C NMR spectrum (Table 3) with analysis of DEPT experiments displayed 20 carbons; four of these were sp^2 carbons: attributable to two quaternary carbons (δ 133.5, 139.3), one methine carbon (δ 134.4) and a methylene carbon (δ 113.4). From the HMBC experiments, the oxymethylene protons at δ 4.33 and 4.26 (2H-17) showed long-range correlations to the carbons at δ 38.8 (C-8), 133.5 (C-13) and 139.3 (C-14). The olefinic proton at δ 6.86 (H-15) also showed long-range correlations to the carbons at δ 26.9 (C-12), 133.5 (C-13) and 139.3 (C-14). Methylene olefinic protons at δ 5.23 and 5.07 (2H-16) gave a cross-peak with the carbon at δ 133.5 (C-13). This result suggested that the vinyl substituent was at sp^2 carbon C-13. The relative stereochemistry of **9** was determined from the results of NOESY experiments and compared with cassa-13(14),15-dien-19-oic acid[10] in which the methyl group at δ 0.84 (Me-20) showed cross-peak with a methine proton at δ 2.26 (H-8). Thus, the relative stereostructure of **9** was confirmed and assigned for taepeenin G.

Taepeenin H (**10**) showed the molecular formula $C_{21}H_{28}O_4$ ($[M]^+$ m/z 344.1942) by HREIMS. Its ^1H and ^{13}C NMR spectral data (Tables 2 and 3) revealed that taepeenin H had the same A and B rings as **5**. The ring C was comparable to that of **7** with additional ^1H NMR signals of one methine and one methyl doublet protons at δ 2.64 (m, H-14) and 0.99 (d, $J=6.9$ Hz, Me-17). An analysis of the COSY, HMQC, and HMBC spectra led to structure **10** for this compound. The HMBC correlations, of a furan proton at δ 6.18 (H-15) with the carbon at δ 31.4 (C-14), of methyl protons at δ 0.99 (Me-17) with the carbons at δ 31.4 (C-14), 35.8 (C-8) and 122.3 (C-13) and of methylene protons at δ 2.32 and 2.60 (2H-11) with carbons at δ 35.8 (C-8), 122.3 (C-13) and 149.1 (C-12) suggested that the Me-17 should be attached to C-14. In the NOESY spectrum, correlations between the methine proton at δ 1.62 (H-9) displayed a cross-peak with the methyl protons at δ 0.99 (Me-17), indicating that this methyl group was α-oriented. Thus, the stereostructure of taepeenin H was concluded to be **10**.



10 : R^1 = CO_2Me, R^2 = CHO

11 : R^1 = CO_2Me, R^2 = CH_2OH

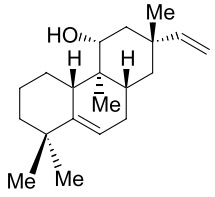
12 : R^1 = CO_2H, R^2 = Me

13 : R^1 = CO_2Me, R^2 = Me

Taepeenin I (**11**), $C_{21}H_{30}O_4$ ($[M]^+$ m/z 346.2111 by HREIMS), was found to be a derivative of **10**. The ^1H NMR spectral data (Table 2) of **11** was comparable to those of **10**, except the aldehydic proton (δ 9.90) in **10** was replaced by oxymethylene protons at δ_H 3.84 and 3.96 (each

d, $J=11.7$ Hz), δ_C 61.5. These proton signals showed correlations with carbons at δ 29.9 (C-3), 50.1 (C-5), 53.5 (C-4) and 178.6 (C-18) in the HMBC spectrum, suggesting of their attachment at C-4. Thus the structure of taepeenin I was concluded to be **11**.

The effective doses (ED_{50}) of cassane and norcassane type diterpenes, taepeenin A–F (**1**–**6**), teapeenin G–I (**9**–**11**), vinhaticoic acid (**12**), methyl vinhaticoate (**13**) and nortaepeenin A, B (**7**, **8**) for antimalaria were more than 50 μg/mL), hence inactive, whereas rosane-type diterpene (**14**) exhibited significant antimalaria with ED_{50} values of 4.1 μg/mL.



14

Cassane-type diterpenoids were found in several plants, which belong to the genus *Caesalpinia*, for example, *C. bonducella*,[9,11] *C. minax*,[12–14] *C. pulcherrima*.[15] However, there have been only two reports of norcassane-types diterpenoids from seed kernels of *C. crista*.[3,4] We now add two new members to the list of compounds of this type and X-ray diffraction analysis of benzofuranoid diterpene structure of **1** is reported here for the first time.

3. Experimental

3.1. General experimental procedures

Melting points were determined on the Fisher-John melting point apparatus. The optical rotation $[\alpha]_D$ values were determined with an AUTOPOL^R II automatic polarimeter. UV spectra were measured with a SPECORD S 100 (Analytikjena). The IR spectra were measured with a Perkin-Elmer FTS FT-IR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded using 500 MHz Varian UNITY INOVA and 300 MHz Bruker FTNMR Ultra Shield™ spectrometers. Chemical shifts are recorded in parts per million (δ) in $CDCl_3$ with tetramethylsilane (TMS) as an internal reference. The EIMS and FABMS were obtained from a MAT 95 XL mass spectrometer. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 F_{254} (Merck) and silica gel 100 (Merck), respectively. Precoated plates of silica gel 60 F_{254} and reversed-phase (RP-18 F_{254S}) were used for analytical purposes. Sephadex LH-20 (Fluka) was used for purification.

3.2. Plant material

Stems and roots of *C. crista* L. were collected from Trang province, Thailand in May 2004. Identification was made by Prof. Puangpen Sirirugsa, Department of Biology, Faculty of Science, Prince of Songkla University and a specimen (No. SC03) deposited at Prince of Songkla University Herbarium.

3.3. Extraction and isolation

Ground-dried stems (7.5 kg) of *C. crista* were extracted with hexane and CH_2Cl_2 (each 2×7.5 L, for 5 days) at room temperature. The crude extracts were evaporated under reduced pressure to afford a brownish crude hexane (29.4 g) and crude CH_2Cl_2 (70.2 g) extracts. The crude hexane extract was further purified by QCC using hexane as eluent and increasing polarity with EtOAc to give six fractions (H1–H6). Fraction H2 (4.2 g) was recrystallized from CH_2Cl_2 to give **1** (692.8 mg) and mother liquor (3.5 g). This mother liquor was further subjected to CC with $CHCl_3$–hexane (1/9, v/v) to afford four subfractions (H2a–H2d). Subfraction H2a (1.1 g) was separated by CC with acetone–hexane (1/1, v/v) and followed by prep TLC with CH_2Cl_2–hexane (9/11, v/v) to give **4** (3.3 mg) and **9** (9.5 mg). Subfraction H2b (100 mg) was purified by prep TLC with $CHCl_3$–hexane (1/9, v/v) to afford **13** (10.1 mg). Subfraction H2c (148.0 mg) was purified by CC with acetone–hexane (1/19, v/v) and followed by prep TLC with EtOAc–hexane (1:19, v/v) to give **14** (31.8 mg). Fraction H4 (432.8 mg) was separated by Sephadex LH-20 with CH_2Cl_2 to afford **3** (72.5 mg). Fraction H6 (248.3 mg) was subjected to CC with acetone–$CHCl_3$ (3/97, v/v) and followed by prep TLC with acetone–hexane (1/5, v/v) to give **2** (5.9 mg) and **8** (4.1 mg). The crude CH_2Cl_2 extract was fractionated by QCC with hexane and increasing polarity with EtOAc to give six fractions (C1–C6). Fraction C2 (1.59 g) was subjected to CC with EtOAc–hexane (1/9, v/v) to afford **6** (3.7 mg). Fraction C3 (1.28 g) was purified by CC with acetone–hexane (1/9, v/v) and followed by recrystallization from CH_2Cl_2 to give **7** (19.5 mg).

Chopped-dried roots of *C. crista* (4.2 kg) were extracted with hexane (2×5 L, for 5 days) at room temperature. The mixture was filtered and the filtrate was evaporated under reduced pressure to give brownish crude hexane extract. This crude extract (47.5 g) was subjected to QCC with hexane and increasing polarity with EtOAc to afford five fractions (R1–R5). Fraction R2 (25.4 mg) was purified by prep TLC with EtOAc–hexane (1/19, v/v) to give **5** (3.4 mg) and **10** (8.2 mg). Fraction R3 (966.1 mg) was separated by flash CC with CH_2Cl_2–hexane (2:3, v/v) and followed by prep TLC with CH_2Cl_2–hexane (9:11, v/v) to afford **12** (16.4 mg). Fraction R5 (778.0 mg) was subjected to CC with EtOAc–hexane (3/17, v/v) and followed by reversed-phase prep TLC with MeOH–H_2O (17/3, v/v) to give **11** (31.2 mg).

3.3.1. Taepeenin A (1). White solid, mp 156–157 °C; $[\alpha]_D^{27}$ +56.6 (*c* 0.005, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 212 (3.56), 260 (3.00), 282 (2.62), 292 (2.61) nm; IR (KBr) ν_{max} 1723, 771 cm^{-1}; HREIMS *m/z* [M]$^+$326.1887 (calcd for $C_{21}H_{26}O_3$, 326.1882); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 3.

3.3.2. Taepeenin B (2). White solid, mp 221–222 °C; $[\alpha]_D^{27}$ +11.8 (*c* 0.002, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 212 (3.34), 263 (2.94), 282 (2.42), 292 (2.41) nm; IR (KBr) ν_{max} 3418, 1691, 761 cm^{-1}; HRFABMS *m/z* [M]$^+$312.1733

(calcd for $C_{20}H_{24}O_3$, 312.1725); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 3.

3.3.3. Taepeenin C (3). White solid, mp 154–155 °C; $[\alpha]_D^{27}$ −2.6 (*c* 0.008, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 212 (3.42), 260 (2.89), 281 (2.29), 291 (2.26) nm; IR (KBr) ν_{max} 3419, 1719, 769 cm^{-1}; HREIMS *m/z* [M]$^+$342.1825 (calcd for $C_{21}H_{26}O_4$, 342.1831); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 3.

3.3.4. Taepeenin D (4). White solid, mp 118.5–119 °C; $[\alpha]_D^{27}$ −29.4 (*c* 0.003, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 212 (3.32), 263 (2.80), 281 (2.43), 291 (2.42) nm; IR (KBr) ν_{max} 1736, 764 cm^{-1}; HREIMS *m/z* [M]$^+$384.1936 (calcd for $C_{23}H_{28}O_5$, 384.1937); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 3.

3.3.5. Taepeenin E (5). White solid, mp 57–58 °C; $[\alpha]_D^{27}$ +22.7 (*c* 0.004, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 211 (3.64), 260 (2.96), 281 (2.52), 293 (2.48) nm; IR (KBr) ν_{max} 2880, 1718, 766 cm^{-1}; HREIMS *m/z* [M]$^+$340.1671 (calcd for $C_{21}H_{24}O_4$, 340.1675); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 3.

3.3.6. Taepeenin F (6). White solid, mp 194–195 °C; $[\alpha]_D^{27}$ +23.3 (*c* 0.003, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 203 (3.13), 235 (2.55), 280 (2.18) nm; IR (KBr) ν_{max} 1811, 1727 cm^{-1}; HREIMS *m/z* [M]$^+$342.1836 (calcd for $C_{21}H_{26}O_4$, 342.1831); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 3.

3.3.7. Nortaepeenin A (7). White solid, mp 157–158 °C; $[\alpha]_D^{27}$ −5.7 (*c* 0.003, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 217 (3.59), 258 (3.67) nm; IR (KBr) ν_{max} 1724, 1668, 762 cm^{-1}; HREIMS *m/z* [M]$^+$330.1832 (calcd for $C_{20}H_{26}O_4$, 330.1831); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 3.

3.3.8. Nortaepeenin B (8). White solid, mp 145–146 °C; $[\alpha]_D^{27}$ −4.2 (*c* 0.002, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 211 (3.69), 257 (3.67) nm; IR (KBr) ν_{max} 3415, 1721, 751 cm^{-1}; HREIMS *m/z* [M]$^+$346.1787 (calcd for $C_{20}H_{26}O_5$, 346.1780); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 3.

3.3.9. Taepeenin G (9). Viscous oil; $[\alpha]_D^{27}$ +4.7 (*c* 0.002, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 205 (3.67), 239 (4.09) nm; IR (neat) ν_{max} 3396, 758 cm^{-1}; HREIMS *m/z* [M]$^+$288.2455 (calcd for $C_{20}H_{32}O$, 288.2453); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 3.

3.3.10. Taepeenin H (10). White solid, mp 164.5–165 °C; $[\alpha]_D^{27}$ +20.8 (*c* 0.005, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 222 (3.40) nm; IR (KBr) ν_{max} 2870, 1748, 1714, 734 cm^{-1}; HREIMS *m/z* [M]$^+$344.1942 (calcd for $C_{21}H_{28}O_4$, 344.1988); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 3.

3.3.11. Taepeenin I (11). White solid, mp 83–84 °C; $[\alpha]_D^{27}$ +90.1 (*c* 0.011, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 225 (2.84) nm; IR (KBr) ν_{max} 3451, 1715, 755 cm^{-1}; HREIMS *m/z* [M]$^+$346.2111 (calcd for $C_{21}H_{30}O_4$, 346.2144); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 3.

3.4. X-ray crystallographic analysis of taepeenin A (1)

The data were collected using a 4 K SMART CCD diffractometer with a graphite monochromated Mo Kα radiation (λ=0.71073 Å). The data were collected at a temperature of 293(2) K to a maximum 2θ value of 56.44°. The collected data were reduced using SAINT program,[16] and the empirical absorption corrections were performed using SADABS program.[17] Crystal data: orthorhombic, $C_{21}H_{26}O_3$ (M_r=326.42), space group $P2_12_12_1$ with a=13.135(5) Å, b=7.293(3) Å, c=18.296(7) Å, $\alpha=\beta=\gamma$=90.0°, V=1752.6(1) Å^3, Z=4, and D_{calcd}=1.237 g/cm^3. The structure was solved by direct method and was refined by least-squares using the SHELXTL software package.[18] All non-hydrogen atoms were located and refined anisotropically with SHELXTL using full-matrix least-squares procedure, whereas the hydrogen atom positions were geometrically idealized and allowed to ride on their parent atoms and refined isotropically with fixed displacement parameters. The final cycle of full matrix least-squares refinement was based on 3088 observed reflections ($I>2\sigma(I)$, $2\theta\leq50.0°$) and 222 variable parameters and converged with unweighted and weighted agreement factors of R=0.0693 and R_w=0.1897.

The crystallographic-information file for **1** has been deposited with the Cambridge Crystallographic Data Centre, CCDC deposition number 262744. The supplementary crystallographic data for **1** can be obtained free of charge from CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1233 336033; e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk/).

Acknowledgements

We are grateful to the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (Grant No. PHD/0157/2547), the Higher Education Development Project: Postgraduate Education and Research Program in Chemistry, the Biodiversity Research and Training Program (BRT) and Prince of Songkla University through Natural Products from Mangrove Plants and Synthetic Materials Research Unit (NSU) and Graduate School for financial support. Bioassays research facility of BIOTEC is also gratefully acknowledged for bioactivity tests.

References and notes

1. Tomlinson, P. B. *The Botany of Mangroves*; Cambridge University Press: UK, 1986; pp 257–258.
2. Uphof, J. C. TH. *Dictionary of Economic Plants*; Verlag von Cramer, Lehre: New York, 1968; p 93.
3. Banskota, A. H.; Attamimi, F.; Usia, T.; Linn, T. Z.; Tezuka, Y.; Kalauni, S. K.; Kadota, S. *Tetrahedron Lett.* **2003**, *44*, 6879–6882.

4. Kalauni, S. K.; Awale, S.; Tezuka, Y.; Banskota, A. H.; Linn, T. Z.; Kadota, S. *J. Nat. Prod.* **2004**, *67*, 1859–1863.
5. Jadhav, A. N.; Kaur, N.; Bhutani, K. K. *Phytochem. Anal.* **2003**, *14*, 315–318.
6. Narayanan, C. R.; Venkatasubramanian, N. K. *Tetrahedron Lett.* **1965**, *41*, 3639–3646.
7. Matos, F. J. A.; Craveiro, A. A.; De Maurera, M. A. M. A. *J. Nat. Prod.* **1984**, *47*, 581–584.
8. Alvarez, M. C. G.; Rodriguez, B.; Valverde, S.; Fraga, B. M.; Gonzalez, A. G. *Phytochemistry* **1981**, *20*, 167–169.
9. Lyder, D. L.; Peter, S. R.; Tinto, W. F.; Bissada, S. M.; McLean, S.; Reynolds, W. F. *J. Nat. Prod.* **1998**, *61*, 1462–1465.
10. Kido, T.; Taniguchi, M.; Baba, K. *Chem. Pharm. Bull.* **2003**, *51*, 207–208.
11. Peter, S.; Tinto, W. F.; McLean, S.; Reynolds, W. F.; Yu, M. *Phytochemistry* **1998**, *47*, 1153–1155.
12. Jiang, R. W.; Ma, S. C.; He, Z. D.; Huang, X. S.; But, P. P. H.; Wang, H.; Chan, S. P.; Ooi, V. E. C.; Xu, H. X.; Mak, T. C. W. *Bioorg. Med. Chem.* **2002**, *10*, 2161–2170.
13. Jiang, R. W.; But, P. P. H.; Ma, S. C.; Ye, W. C.; Chan, S. P.; Mak, T. C. W. *Tetrahedron Lett.* **2002**, *43*, 2415–2418.
14. Jiang, R. W.; But, P. P. H.; Ma, S. C.; Mak, T. C. W. *Phytochemistry* **2001**, *57*, 517–521.
15. Ragasa, C. Y.; Hofilena, J. G.; Rideout, J. A. *J. Nat. Prod.* **2002**, *65*, 1107–1110.
16. Siemens, *SMART and SAINT*; Siemens Analytical X-ray Instruments Inc., Madison: Wisconsin, USA, 1997.
17. Sheldrick, G. M. *SADABS: Program for Empirical Absorption of Area Detector Data*; University of Göttingen: Germany, 1996.
18. Sheldrick, G. M. *SHELXTL* Version 5.1; Bruker AXS Inc., Madison: Wisconsin, USA, 1997.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 8663–8668

Reductive metalation of 1,2-diaryl-substituted ethenes: synthetic applications

Ugo Azzena,* Giovanna Dettori, Caterina Lubinu, Alberto Mannu and Luisa Pisano

Dipartimento di Chimica, Università di Sassari, via Vienna 2, I-07100 Sassari, Italy

Received 22 April 2005; revised 10 June 2005; accepted 30 June 2005

Available online 19 July 2005

Abstract—Reduction of 1,2-diaryl-substituted ethenes with Na metal in dry THF allowed easy access to a variety of 1,2-diaryl-1,2-disodiumethanes. These diorganometallic intermediates were elaborated into the corresponding 1,2-diarylethanes (aqueous work up), or cycloalkylated with 1,3-dichloropropanes. The last reaction led to a highly diastereoselective synthesis of *trans*-1,2-diaryl-substituted cyclopentanes.

1. Introduction

Reduction of stilbene with alkali metals in aprotic solvents is a well known reaction, allowing easy access to the corresponding dianions.[1] Despite the relatively high stabilities of the resulting diorganometals, this reaction was investigated mainly from a mechanistic point of view.[2–5] Indeed, reaction of these intermediates with alkyl halides, phosphonates and sulfates, is of limited synthetic usefulness, due to the formation of diastereoisomeric mixtures of alkylated products,[2–5] whilst reaction with carbonyl compounds, under Barbier-type conditions, afforded 1,2-diphenylethane.[6]

At odds with these results, the reaction of 1,2-disodium-1,2-diphenylethane (1,2-disodiumstilbene) with 1,3-dichloropropane or 1,4-dichlorobutane in 1,2-dimethoxyethane (DME), afforded good yields of the corresponding *trans*-1,2-diphenyl cyclopentane or -cyclohexane, respectively, with very high diastereoselectivity.[3] Surprisingly, the latter reaction was not synthetically exploited, despite the potential usefulness of *trans*-1,2-diaryl-substituted cyclopentanes as C_2-symmetric ligands, for employment in catalysis.[7–11]

Furthermore, it is worth noting that only few reports concern the reductive metalation of functionalized stilbenes.[12,13]

Following on from our interest in the application of reductive metalation reactions to the regioselective preparation of substituted aromatic derivatives, we have previously reported that reduction of several 3,4,5-trimethoxystilbenes, with Na metal in THF, results in regioselective cleavage of the methoxy group at the 4-position and, depending upon reaction conditions, to reduction of the olefinic bond.[14,15]

As a further useful application to this approach, we investigated the reductive metalation of several 1,2-diarylethenes in THF, currently the most commonly used solvent for this kind of reaction,[16,17] with the purpose to apply this procedure to a general synthesis of *trans*-1,2-diarylcyclopentanes.

2. Results and discussion

2.1. Synthesis of starting materials

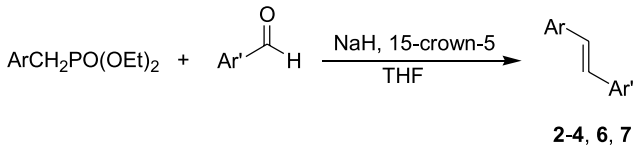
Compounds (*E*)-**1** and (*Z*)-**1**, as well as (*E*)-**5**, are commercially available. Other starting materials were synthesized according to reported procedures,[18,19] by the deprotonation of either diethyl benzylphosphonate, or diethyl 2-methoxybenzylphosphonate, with NaH in THF, in the presence of the appropriate aldehyde and a catalytic amount of 15-crown-5. The corresponding (*E*)-stilbenes **2–4**, **6** and **7** were recovered in good to satisfactory yields (Scheme 1).

2.2. Reductive metalation reactions

Reductive metalations of stilbenes **1–7** were run in the

Keywords: Dicarbanions; Stilbenes; Metalation; Reduction; Cycloalkylation.
* Corresponding author. Tel.: +39 79229549; fax: +39 79229559; e-mail: ugo@uniss.it

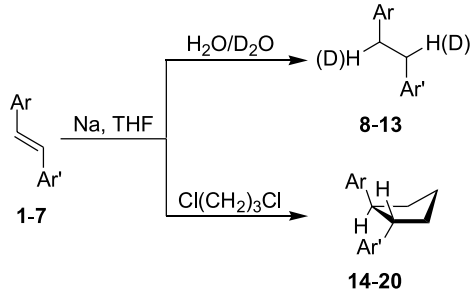
0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.06.108



2-4, **6**, **7**

Scheme 1. Synthesis of 1,2-diarylethenes: **2**: Ar=Ph, Ar′=1-naphtyl, 85%; **3**: Ar=Ph, Ar′=4-pyridyl, 57%; **4**: Ar=Ph, Ar′=2-pyridyl, 61%; **6**: Ar=Ar′=2-$(CH_3O)C_6H_4$, 92%; **7**: Ar=Ph, Ar′=3,4,5-$(CH_3O)_3C_6H_2$, 90%.

presence of an excess of the freshly cut metal, in dry THF, under argon gas. Reaction mixtures were quenched either with H_2O, or with D_2O to provide evidence for the intermediate formation of dicarbanions. $Cl(CH_2)_3Cl$ quenching was performed by adding 2 equiv of the electrophile to the reduction mixtures at −80 °C, and stirring the resulting mixtures for 12 h whilst allowing it to reach 0 °C, before aqueous work up (Scheme 2).



Scheme 2. Reductive metalation of stilbenes; **1**, **8**, **14**: Ar=Ar′=Ph; **2**, **9**, **15**: Ar=Ph, Ar′=1-naphtyl, **3**, **10**, **16**: Ar=Ph, Ar′=4-C_5H_4N; **4**, **11**, **17**: Ar=Ph, Ar′=2-C_5H_4N; **5**, **12**, **18**: Ar=Ar′=2-C_5H_4N; **6**, **13**, **19**: Ar=Ar′=2-$(CH_3O)C_6H_4$; **7**: Ar=C_6H_5, Ar′=3,4,5-$(CH_3O)_3C_6H_2$; **20**: Ar=Ph, Ar′=3,5-$(CH_3O)_2C_6H_3$.

We initially investigated the reduction of (*E*)- and (*Z*)-stilbene **1** under the above reaction conditions.[20] Indeed, literature data do not agree on the relative reactivities of diastereoisomeric stilbenes towards alkali metals.[1,2] It was also necessary to check the stereochemical outcome of the reaction of the resulting diorganometals with $Cl(CH_2)_3Cl$ in THF; several authors reported a solvent effect on the diastereoselectivities of several alkylation reactions of these intermediates.[2,3] Selected results are reported in Table 1.

Reduction of (*E*)-**1** with 3 equiv of Na metal during 5 h, followed by aqueous work up, afforded bibenzyl, **8**, as the only detectable reaction product (Table 1, entry 1). Intermediate, quantitative formation of 1,2-diphenyl-1,2-disodiumethane resulted by quenching the reduction mixture with D_2O (Table 1, entry 2). Under these conditions, quenching the reduction mixture with $Cl(CH_2)_3Cl$, at −80 °C, allowed the recovery of 1,2-diphenylcyclopentane, **14**, as a single diastereoisomer, in 72% yield (Table 1, entry 3). According to the literature, we assigned a relative trans-configuration to cyclopentane **14**.[21]

The same results were obtained using Li in reductive metalations of (*E*)-**1** quenched with H_2O or D_2O (Table 1, entries 4 and 5), whilst quenching the reduction mixture with $Cl(CH_2)_3Cl$ led to recover cyclopentane **14** with a slightly improved yield (Table 1, entry 6).

Reduction of (*Z*)-**1** with Na metal, followed by aqueous work up, led to the recovery of 1,2-diphenylethane, although, a relatively longer reaction time (7 h) was required (Table 1, entry 7). Furthermore, a similar reaction, quenched after 1 h reaction time, led to recover bibenzyl **8** and (*E*)-stilbene **1** in 41 and 59% yield, respectively (Table 1, entry 8), thus, confirming fast isomerization of the starting material, in agreement with original observations of Schlenk and Bergmann.[1,2]

We next, extended our investigation to the reductive metalation of differently substituted 1,2-diarylethenes **2**–**7**, to check the applicability of this reaction to the synthesis of an ecletic array of trans-1,2-diarylcyclopentanes. Attention was focused on the employment of Na metal as a reducing agent as it is, by far, the most economical of the alkali metals. The results are reported in Table 2 (Scheme 2).

Reduction of naphthyl derivative **2** with an excess of Na metal in THF at 25 °C, afforded a complex reaction mixture (not reported in Table 2). A much better result was obtained performing this reduction at −20 °C, in the presence of 6 equiv of the metal: under these conditions, aqueous work up afforded 1-(1-naphthyl)-2-phenylethane, **9**, in almost quantitative yield, via quantitative intermediate formation of the corresponding dianion (Table 2, entries 1 and 2).

Quenching with $Cl(CH_2)_3Cl$ afforded a single diastereoisomer of the corresponding 1-(1-naphthyl)-2-phenylcyclopentane, **15**, in good yield (Table 2, entry 3).

Reductive metalation of pyridyl-substituted derivates **3**–**5** were run at 0 °C, in the presence of variable amounts

Table 1. Reductive metalation of (*E*)-**1** and (*Z*)-**1**[a]

Entry	Substrate	Metal	*t* (h)	Quencher	Product	Yield (%)[b]
1	(*E*)-**1**	Na	5	H_2O	**8** (H)	>95
2	(*E*)-**1**	Na	5	D_2O	**8**-d_2 (D)	>95
3	(*E*)-**1**	Na	5	$Cl(CH_2)_3Cl$	**14**	72[c]
4	(*E*)-**1**	Li	5	H_2O	**8** (H)	>95
5	(*E*)-**1**	Li	5	D_2O	**8**-d_2 (D)	>95
6	(*E*)-**1**	Li	5	$Cl(CH_2)_3Cl$	**14**	76[c]
7	(*Z*)-**1**	Na	7	H_2O	**8** (H)	>95
8	(*Z*)-**1**	Na	1	H_2O	**8** (H)	41[d]

[a] All reductions were run at rt in the presence of 3 equiv of the metal.
[b] Yields calculated by ^1H NMR spectroscopy, unless otherwise indicated.
[c] Yields calculated on isolated products.
[d] (*E*)-**1** (59%) was also recovered.

Table 2. Reductive metalation of 1,2-diarylethenes **2–7**

Entry	Substrate	Na (equiv)	*T* (°C)	*t* (h)	Quencher	Product	Yield (%)[a]
1	**2**	6	−20	7	H_2O	**9** (H)	81
2	**2**	6	−20	7	D_2O	**9**-d_2 (D)	>95[b]
3	**2**	6	−20	7	$Cl(CH_2)_3Cl$	**15**	87
4	**3**	6	0	12	H_2O	**10** (H)	73
5	**3**	6	0	12	D_2O	**10**-d_2 (D)	88[b]
6	**3**	6	0	12	$Cl(CH_2)_3Cl$	**16**	64
7	**4**	8	0	12	H_2O	**11** (H)	78
8	**4**	8	0	12	D_2O	**11**-d_2 (D)	82[b]
9	**4**	8	0	12	$Cl(CH_2)_3Cl$	**17**	81
10	**5**	8	0	12	H_2O	**12** (H)	83
11	**5**	8	0	12	D_2O	**12**-d_2 (D)	90[b]
12	**5**	8	0	12	$Cl(CH_2)_3Cl$	**18**	78
13	**6**	3	0	7	H_2O	**13** (H)	70
14	**6**	3	0	7	D_2O	**13**-d_2 (D)	80[b]
15	**6**	3	0	7	$Cl(CH_2)_3Cl$	**19**	62
16	**7**	6	20	7	$Cl(CH_2)_3Cl$	**20**	59

[a] Yields calculated on isolated products, unless otherwise indicated.
[b] Yields calculated by ^1H NMR spectroscopy.

(6–8 equiv) of Na metal. Under these conditions, the corresponding heteroaryl-substituted ethanes, **10–12**, were recovered, and intermediate formations of 1,2-disodium derivatives was evidenced by quenching the reduction mixtures with D_2O (Table 2, entries 4 and 5, 7 and 8, 10 and 11).

Cycloalkylation reactions, performed as described above, afforded the desired pyridyl-substituted cyclopentanes, **16–18**, as single diastereoisomers, in good to satisfactory yields (Table 1, entries 6, 9 and 12).

Good results were obtained in the reduction of dimethoxy-substituted stilbene **6** with 3 equiv of Na metal at 0 °C during 7 h. Under these conditions, aqueous work up afforded the dihydrostilbene **13** in good yield, with no evidence of cleavage of carbon–oxygen bonds[14] (Table 2, entry 13). Intermediate formation of a diorganometallic derivative was evidenced, as quenching the reduction mixture with D_2O (Table 2, entry 14), whilst cycloalkylation afforded 1,2-di-(2-methoxy-phenyl)cyclopentane, **19**, as a single diastereoisomer, in 62% yield (Table 2, entry 15). The relative trans-configuration of this compound was confirmed by a comparison of its ^1H and ^{13}C NMR spectra with literature data.[8]

Reductive metalation of 3,4,5-trimethoxystilbene, **7**, was performed under previously optimized reaction conditions (6 equiv of Na metal, 20 °C, 7 h);[14] quenching with $Cl(CH_2)_3Cl$ afforded, after aqueous work up and flash chromatography, 1-(3,5-dimethoxyphenyl)-2-phenylcyclopentane, **20**, as a single diastereoisomer, in 59% yield (Table 1, entry 16).

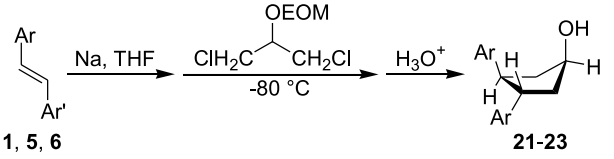
To extend the scope of this reductive cycloalkylation, as well as to confirm the trans stereochemistry of this reaction, we investigated the reactivity of symmetrically-substituted 1,2-disodium intermediates towards a functionalized 1,3-dichloropropane, namely 1,3-dichloro-2-ethoxy-methoxypropane.[22]

Reductive metalations were performed as described above, and reduction mixtures were chilled to −80 °C and quenched with 2 equiv of the electrophile, as described above. Aqueous work up, followed by acidic hydrolyses, afforded the corresponding 3,4-diaryl-1-cyclopentanols (Table 3, Scheme 3).

Table 3. Synthesis of *trans*-3,4-diaryl-1-hydroxycyclopentanes

Entry	Substrate [Ar=]	Product	Yield (%)[a]
1	**1** [Ph]	**21**	60
2	**5** [2-C_5H_4N]	**22**	35
3	**6** [2-$(CH_3O)C_6H_4$]	**23**	32

[a] Yields calculated on isolated products.



Scheme 3. Synthesis of *trans*-3,4-diaryl-1-hydroxycyclopentanes: **1**, **21**: Ar=Ph; **5**, **22**: Ar=2-C_5H_4N; **6**, **23**: Ar=2-$(CH_3O)C_6H_4$; EOM=$CH_2OCH_2CH_3$.

Results obtained with stilbenes **1**, **5** and **6** are reported in Table 3: cyclopentanols **21–23** were recovered in 60, 35 and 32% isolated yield, respectively, as single diastereoisomers (Table 3, entries 1–3). Besides showing the possibility to synthesize *vic*-diarylcyclopentanes functionalized on the alicyclic moiety, these results support the relative trans orientation of aryl substituents at C3 and C4; indeed, a relative cis orientation of these substituents would result in the presence of a stereocenter at C1, with formation of a couple of diastereoisomers.

3. Conclusions

The reported synthetic procedure allows for the generation of several 1,2-disodium-1,2-diarylethanes, bearing an array of different aromatic rings. Interestingly, this procedure was successfully applied to ethenes substituted either with electron-rich or with electron-poor aromatic rings. In both cases, the corresponding diorganometal derivatives were generated under particularly mild reaction conditions.

Reaction of intermediate diorganometals with 1,3-dichloropropanes allowed a simple and highly diastereoselective approach to the synthesis of several trans-1,2-diaryl-substituted cyclopentanes. The employment of some of these derivatives (possessing C_2-symmetry and/or suitable functional groups) as ligands for transition metals, is under investigation in our laboratories.[23]

4. Experimental

4.1. General

Boiling and melting points are uncorrected; the air bath temperature on bulb-to-bulb distillation are given as boiling points. Starting materials were of the highest commercial quality and were purified by distillation immediately prior to use. Na metal (stick) was 99% purity, and Li metal (wire, diameter 3.2 mm) was 99.9% purity. D_2O was 99.8% isotopic purity. THF was distilled from Na/K alloy under N_2 immediately prior to use. ^{1}H NMR spectra were recorded at 300 MHz and ^{13}C NMR spectra were recorded at 75 MHz in $CDCl_3$ with $SiMe_4$ as internal standard. Deuterium incorporation was calculated by monitoring the ^{1}H NMR spectra of crude reaction mixtures, and by comparing the integration of the signal corresponding to protons in the arylmethyl position with that of known signals. Flash chromatography were performed on Merck silica gel 60 (40–63 μm), and TLC analyses on Macherey-Nagel silica gel pre-coated plastic sheets (0.20 mm). Elemental analyses were performed by the microanalytical laboratory of the Dipartimento di Chimica, Università di Sassari.

4.2. Starting materials

Compounds **1** and **5** are commercially available. Compounds **2**,[24] **3**,[25] **4**,[26] and **7**[14] were synthesized according to a general procedure,[16] by the reaction of commercially available diethyl benzylphosphonate with the appropriate arylaldehyde in the presence of 15-crown-5 in dry THF, and characterized according to the literature. Compund **6**[27] was similarly obtained by the reaction of diethyl 2-methoxybenzylphosphonate[28] with 2-methoxybenzaldehyde. Isolated yields are reported in Scheme 1.

4.3. Reductive metalation of 1,2-diarylethenes 1–8, and reaction with electrophiles. General procedure

Two to three pieces of freshly cut metal (3–9 equiv) were placed under Ar in a 50 mL two-necked flask equipped with reflux condenser and magnetic stirrer, and suspended in THF (10 mL) at the reported temperature (Tables). The appropriate 1,2-diarylethene (5 mmol), dissolved in dry THF (2 mL), was added dropwise, and each metal piece was cut into 2–3 smaller pieces with a spatula, and the mixture was vigorously stirred for the reported time at the reported temperature (Tables).

Aqueous work-up was performed by slow dropwise addition of 10 mL of H_2O (caution!) the cold bath removed, and the resulting mixture extracted with Et_2O (3×10 mL). The organic phase was washed with brine (10 mL), dried (K_2CO_3) and the solvent evaporated.

D_2O quenching was performed by slow dropwise addition of 0.75 mL of the electrophile dissolved in dry THF (2 mL), followed by aqueous work-up as described above.

Quenching with $Cl(CH_2)_3Cl$ was performed by adding the appropriate electrophile (2 equiv) to the reduction mixture chilled to −80 °C. The reaction mixture was allowed to warm to 0 °C during 12 h, before aqueous work-up as described above.

Quenching with 1,3-dichloro-2-ethoxymethoxypropane was performed by adding the appropriate electrophile (2 equiv) to the reduction mixture chilled to −80 °C. The reaction mixture was allowed to warm to 0 °C during 12 h, before aqueous work-up as described above. Crude products were added under Ar to a stirred 0.6 M solution of HCl in MeOH [obtained by adding AcCl (0.5 mL) to MeOH (10 mL)] chilled to 0 °C. The mixture was stirred at rt for 3 h, until complete disappearance of starting material, as determined by TLC. The mixture was diluted with H_2O (10 mL), and the MeOH evaporated under reduced pressure. The resulting mixture was basified (1N NaOH), extracted with Et_2O (4× 10 mL), and the organic phase dried (K_2CO_3) and evaporated.

1,2-Diphenylethane, **8**, was purified by flash chromatography (petroleum ether), and characterized by comparison with a commercial sample. Compounds **9**,[29] **10**,[30] **11**,[31] **12**,[32] **13**,[33] **14**,[21] and **19**[8] were purified by flash chromatography (petroleum ether/AcOEt or petroleum ether/AcOEt/Et_3N), and characterized by comparison with literature data. Deuterated compounds were characterised by ^{1}H and ^{13}C NMR spectroscopy: the resonances of arylmethyl CHD protons appear as unresolved broad triplets shifted 0.02–0.05 ppm (δ) upfield relatively to the corresponding CH_2 protons; the resonances of arylmethyl CHD carbions appear as triplets (J=18–21 Hz) shifted 0.3–0.5 ppm (δ) upfield relatively to the corresponding CH_2 carbons.

Other products were purified and characterized as follows.

4.3.1. *trans*-1-(1-Naphthyl)-2-phenylcyclopentane (15). Purified by flash chromatography (petroleum ether/AcOEt=9.5:0.5), white solid; mp 74–75 °C (EtOH); R_f= 0.50 (petroleum ether/AcOEt=9.5:0.5). Anal. Found: C, 92.30; H, 7.56; $C_{21}H_{20}$ requires: C, 92.58; H, 7.42%. ^{1}H NMR: δ 1.71–1.9 (1H, m, CH), 1.91–2.11 (3H, m, CH), 2.30–2.44 (1H, m, CH), 2.44–2.61 (1H, m, CH), 3.46–3.60 (1H, m, CH), 3.98 (1H, q, J=8.4 Hz, CH), 6.42–7.11 (1H, m, ArH), 7.11–7.24 (4H, m, 4×ArH), 7.32–7.50 (4H, m, 4×ArH), 7.64 (1H, d, J=8.1 Hz, ArH), 7.74–7.83 (1H, m, ArH), 7.98–8.06 (1H, m, ArH). ^{13}C NMR: δ 24.3, 35.5, 35.6, 47.8, 51.3, 122.7, 123.7, 125.1, 125.4, 125.6, 125.9, 126.3, 127.4, 128.2, 128.7, 132.4, 133.9, 140.6, 144.3.

4.3.2. *trans*-1-(4-Pyridyl)-2-phenylcyclopentane (16). Purified by flash chromatography (petroleum ether/AcOEt=1:4), yellow oil; R_f=0.53 (petroleum ether/AcOEt=3:7). Anal. Found: C, 85.76; H, 7.94; N, 6.26 $C_{16}H_{17}N$ requires: C, 86.04; H, 7.69; N, 6.27%. ^{1}H NMR: δ

1.81–2.04 (4H, m, 2×CH_2), 2.19–2.34 (2H, m, CH_2), 2.90–3.15 (2H, m, CH_2), 7.00 (2H, dd, J=4.5, 1.8 Hz, 2×ArH), 7.07–7.25 (5H, m, 5×ArH), 8.39 (2H, dd, J=4.5, 1.8 Hz, 2×ArH). ^{13}C NMR: δ 24.5, 34.4, 35.3, 53.2, 53.8, 122.7, 126.2, 127.2, 128.3, 143.3, 149.5, 153.4.

4.3.3. *trans*-1-(2-Pyridyl)-2-phenylcyclopentane (17). Purified by flash chromatography (petroleum ether/AcOEt=3:2), white solid; mp 41–43 °C (Et_2O); R_f=0.67 (petroleum ether/AcOEt=3:2). Anal. Found: C, 85.81; H, 7.96; N, 6.20 $C_{16}H_{17}N$ requires: C, 86.04; H, 7.69; N, 6.27%. ^{1}H NMR: δ 1.81–2.19 (4H, m, 2×CH_2), 2.20–2.36 (2H, m, CH_2), 3.18–3.31 (1H, m, CH), 3.32–3.45 (1H, m, CH), 6.91 (1H, dt, J=7.8, 0.9 Hz, ArH), 7.03 (1H, ddd, J=7.2, 4.8, 0.9 Hz, ArH), 7.07–7.16 (3H, m, 3×ArH), 7.16–7.24 (2H, m, 2×ArH), 7.44 (1H, td, J=7.8, 1.8 Hz, ArH), 8.53–8.56 (1H, m, ArH). ^{13}C NMR: δ 24.7, 34.0, 35.3, 52.8, 55.9, 121.0, 122.8, 125.8, 127.3, 128.2, 135.9, 144.4, 149.4, 163.6.

4.3.4. *trans*-1,2-Di-(2-pyridyl)cyclopentane (18). Purified by flash chromatography (petroleum ether/AcOEt/Et_3N=3:7:1), white solid; mp 76–78 °C; R_f=0.60 (petroleum ether/AcOEt/Et_3N=3:7:1). Anal. Found: C, 80.11; H, 7.41; N, 12.23 $C_{15}H_{16}N_2$ requires: C, 80.31; H, 7.20; N, 12.49%. ^{1}H NMR: δ 1.94–2.19 (4H, m, 2×CH_2), 2.21–2.36 (2H, m, CH_2), 3.52–3.66 (2H, m, CH), 6.97 (2H, dt, J=7.8, 0.9 Hz, ArH), 7.02–7.08 (2H, m, ArH), 7.46 (2H, td, J=7.8, 2.1 Hz, ArH), 8.54 (2H, dq, J=4.8, 0.9 Hz, ArH). ^{13}C NMR: δ 24.9, 34.1, 54.4, 121.0, 122.9, 136.0, 149.2, 163.5.

4.3.5. *trans*-1-(3,5-Dimethoxyphenyl)-2-phenylcyclopentane (20). Purified by flash chromatography (petroleum ether/AcOEt=9:1), colourless oil; R_f=0.4 (petroleum ether/AcOEt=9:1). Anal. Found: C, 80.63; H, 8.03; $C_{19}H_{22}O_2$ requires: C, 80.80; H, 7.87%. ^{1}H NMR: δ 1.77–1.98 (4H, m, 2×CH_2), 2.18–2.31 (2H, m, CH), 2.96–3.14 (2H, m, CH), 3.69 (6H, s, 2×CH_3), 6.24 (1H, t, J=2.1 Hz, ArH), 6.27 (2H, d, J=2.1 Hz, 2×ArH), 7.09–7.17 (3H, m, 3×ArH), 7.18–7.26 (2H, m, 2×ArH). ^{13}C NMR: δ 24.3, 34.9, 35.2, 53.5, 54.0, 54.9, 97.5, 105.4, 125.8, 127.3, 128.1, 144.4, 146.9, 160.4.

4.3.6. *trans*-3,4-Diphenylcyclopentan-1-ol (21). Purified by flash chromatography (gradient elution, from petroleum ether/AcOEt=9:1 to petroleum ether/AcOEt=1:1), white solid; mp 70–72 °C (petroleum ether); R_f=0.63 (petroleum ether/AcOEt=3:7). IR (neat) 3186 cm^{-1}. Anal. Found: C, 85.38; H, 7.83; $C_{17}H_{18}O$ requires: C, 85.66; H, 7.61%. ^{1}H NMR: δ 1.65 (1H, br s, OH), 1.85–1.95 (1H, m, CH), 2.09–2.28 (2H, m, 2×CH), 2.70 (1H, ddd, J=14.1, 9.0, 6.0 Hz, CH), 3.40–3.66 (1H, dd, J=20.1, 9.8 Hz, CH), 3.48 (1H, td, J=10.8, 7.8 Hz, CH), 4.58–4.63 (1H, m, CHO), 7.08–7.25 (10H, m, 10×ArH). ^{13}C NMR: δ 44.9, 44.9, 51.5, 52.6, 72.1, 126.1, 126.1, 127.3, 127.5, 128.2, 128.2, 143.0, 143.7.

4.3.7. *trans*-3,4-Di-(2-pyridyl)cyclopentan-1-ol (22). Purified by flash chromatography (gradient elution, from petroleum ether/AcOEt/Et_3N=5:5:1 to petroleum ether/AcOEt/Et_3N=3:2:1), yellow oil; R_f=0.24 (petroleum ether/AcOEt/Et_3N=3:2:1). IR (neat) 3300 cm^{-1}. Anal. Found: C, 74.69; H, 6.91; N, 11.45; $C_{15}H_{16}N_2O$ requires: C, 74.96; H, 6.72; N, 11.66%. ^{1}H NMR: δ 2.02–2.12 (1H, m, CH), 2.14–2.20 (1H, m, CH), 2.17 (1H, br s, OH), 2.30–2.39 (1H, m, CH), 2.50–2.61 (1H, m, CH), 3.60 (1H, dt, J=11.7, 6.9 Hz, CH), 3.79 (1H, dd, J=9.6, 6.3, 1.8 Hz, CH), 4.51 (1H, t, J=3.6 Hz, CHO), 6.97 (1H, dt, J=7.8, 0.9 Hz, ArH), 7.02 (1H, dt, J=7.8, 0.9 Hz, ArH), 7.10 (1H, ddd, J=5.1, 2.4, 0.9 Hz, ArH), 7.13 (1H, ddd, J=5.1, 2.4, 0.9 Hz, ArH), 7.54 (1H, ddd, J=7.8, 4.2, 1.8 Hz, ArH), 7.52 (1H, ddd, J=7.8, 4.2, 1.8 Hz, ArH), 8.57 (1H, ddd, J=5.1, 1.8, 0.9 Hz, ArH), 8.60 (1H, ddd, J=5.1, 1.8, 0.9 Hz, ArH). ^{13}C NMR: δ 41.7, 46.0, 51.8, 54.7, 74.2, 121.1, 121.3, 122.5, 122.9, 136.2, 136.8, 149.3, 149.6, 163.4, 165.5.

4.3.8. *trans*-3,4-Di-(2-methoxyphenyl)cyclopentan-1-ol (23). Purified by flash chromatography (petroleum ether/AcOEt=2:3), colourless oil; R_f=0.69 (petroleum ether/AcOEt=2:3). IR (neat) 3230 cm^{-1}. Anal. Found: C, 76.21; H, 7.73; $C_{19}H_{22}O_3$ requires: C, 76.47; H, 7.45%. ^{1}H NMR: δ 1.75 (1H, dddd, J=13.5, 9.6, 4.2, 1.5 Hz, CH), 1.99 (1H, ddd, J=13.5, 11.7, 6.6 Hz, CH), 2.05 (1H, br s, OH), 2.24 (1H, ddt, J=13.5, 7.2, 1.5 Hz, CH), 2.73 (1H, ddd, J=13.5, 9.0, 6.6 Hz, CH), 3.55–3.75 (1H, m, CH), 3.68 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 4.00–4.10 (1H, m, CH), 4.50–4.56 (1H, m, CHO), 6.74–6.85 (4H, m, 4×ArH), 7.06–7.13 (2H, m, 2×ArH), 7.16–7.22 (2H, m, 2×ArH). ^{13}C NMR: δ 41.6, 43.5, 43.5, 55.1, 55.3, 72.3, 110.4, 110.5, 120.3, 120.6, 126.7, 126.7, 127.2, 128.0, 131.5, 131.8, 157.2, 157.6.

References and notes

1. Schlenk, W.; Bergmann, E. *Liebigs Ann. Chem.* **1928**, *463*, 1–322. (particularly pages 106–117).
2. Wright, G. F. *J. Am. Chem. Soc.* **1939**, *61*, 2106–2110.
3. Reesor, J. W. B.; Smith, J. G.; Wright, G. F. *J. Org. Chem.* **1954**, *19*, 940–956.
4. Smith, J. G.; Wikman, R. T. *J. Organomet. Chem.* **1973**, *49*, 91–101.
5. Smith, J. G.; Oliver, E.; Boettger, T. J. *Organometallics* **1983**, *2*, 1577–1582 and references therein.
6. Yus, M.; Martínez, P.; Guijarro, D. *Tetrahedron* **2001**, *57*, 10119–10124.
7. Yang, J.-S.; Kondo, K.; Murrakami, Y. *Collect. Czech. Chem. Commun.* **2000**, *65*, 549–554.
8. Whitesell, J. K.; Apodaca, R. *Tetrahedron Lett.* **1997**, *38*, 2589–2592.
9. Kranenburg, M.; Delis, J. G. P.; Kamer, P. C. J.; van Leeuwen, P. W. N. M.; Vrieze, K.; Veldman, N.; Spek, A. L.; Goubitz, K.; Fraanje, J. *J. Chem. Soc., Dalton Trans.* **1997**, 1839–1849.
10. Kanemasa, S.; Yamamoto, H.; Kobayashi, S. *Tetrahedron Lett.* **1996**, *37*, 8505–8506.
11. Fukuda, N.; Mashima, K.; Matsumura, Y.-I.; Takaya, H. *Tetrahedron Lett.* **1990**, *31*, 7185–7188.
12. Platzek, J.; Snatzke, G. *Tetrahedron* **1987**, *43*, 4947–4968.
13. Jeanes, A.; Adams, R. *J. Am. Chem. Soc.* **1937**, *59*, 2608–2622.
14. Azzena, U.; Dettori, G.; Idini, M. V.; Pisano, L.; Sechi, G. *Tetrahedron* **2003**, *59*, 7961–7966.
15. Azzena, U.; Melloni, G.; Piroddi, A. M.; Azara, E.; Contini, S.; Fenude, E. *J. Org. Chem.* **1992**, *57*, 3101–3106.
16. Yus, M. In Rappoport, Z., Marek, I., Eds.; The Chemistry of Organolithium Compounds; Wiley: Chichester, 2004; Vol. 1; Part 2, Chapter 11.

17. For an alternative solvent (dimethyl ether), see: Cohen, T.; Kreethadumrongdat, T.; Liu, X.; Kulkarni, V. *J. Am. Chem. Soc.* **2001**, *123*, 3478.
18. Wadsworth, W. S.; Emmons, W. D. *J. Am. Chem. Soc.* **1961**, *83*, 1733–1738.
19. Baker, R.; Sims, R. J. *Synthesis* **1981**, 117.
20. To the best of our knowledge, there is no report concerning reduction of (*E*)- or (*Z*)-stilbene with alkali metals in THF, followed by H_2O or D_2O quenching, or alkylation with dihaloalkanes. However, in a mechanistic study concerning the reduction of aromatic esters, it was reported that reduction of (*E*)-stilbene, with an excess of Li metal and a catalytic amount (10 mol%) of DBB, under sonication, afforded 1,2-diphenylethane after aqueous work up: Karaman, R.; Fry, J. L. *Tetrahedron Lett.* **1989**, *30*, 4931–4934.
21. Léonel, E.; Paugam, J. P.; Nédélec, J.-Y.; Périchon, J. *J. Chem. Res. (S)* **1995**, 278–279.
22. Gu, X. P.; Ikeda, I.; Okahara, M. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 397.
23. Azzena, U.; Cinellu, M. A.; Dettori, G. in progress.
24. Bergbreiter, D. E.; Osburn, P. L.; Wilson, A.; Sink, E. M. *J. Am. Chem. Soc.* **2000**, *122*, 9058–9064.
25. Lorance, E. D.; Kramer, W. H.; Gould, I. R. *J. Am. Chem. Soc.* **2002**, *124*, 15225–15238.
26. Chen, C.; Wang, B.; Munoz, B. *Synlett* **2003**, 2404–2406.
27. Wallace, T. W.; Wardell, I.; Li, K.-D.; Leeming, P.; Redhouse, A. D.; Challand, S. R. *J. Chem. Soc., Perkin Trans. 1* **1995**, 2293–2308.
28. Vande Velde, C. M. L.; Blockhuys, F.; Van Alsenoy, C.; Lenstra, A. T. H.; Geise, H. J. *J. Chem. Soc., Perkin Trans. 2* **2002**, 1345–1351.
29. Givens, R. S.; Matuszewski, B.; Neywick, C. V. *J. Am. Chem. Soc.* **1974**, *96*, 5547–5552.
30. Katritzky, A. R.; Shipkova, P. A.; Qi, M.; Nichols, D. A.; Burton, R. D.; Watson, C. H.; Eyler, J. R.; Tamm, T.; Karelson, M.; Zerner, M. C. *J. Am. Chem. Soc.* **1996**, *118*, 11905–11911.
31. Tilley, J. W.; Zawoiski, S. *J. Org. Chem.* **1988**, *53*, 386–390.
32. Vedernikov, A. N.; Pink, M.; Caulton, K. G. *J. Org. Chem.* **2003**, *68*, 4806–4814.
33. Tashiro, M.; Yamato, T.; Fukata, G. *J. Org. Chem.* **1978**, *43*, 1413–1420.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
ELSEVIER
Tetrahedron 61 (2005) 8669–8676

4-Substituted prolines as organocatalysts for aldol reactions

Evagelos Bellis and George Kokotos*

Laboratory of Organic Chemistry, Department of Chemistry, University of Athens, Panepistimiopolis, Athens 15771, Greece

Received 11 April 2005; revised 16 June 2005; accepted 30 June 2005

Available online 21 July 2005

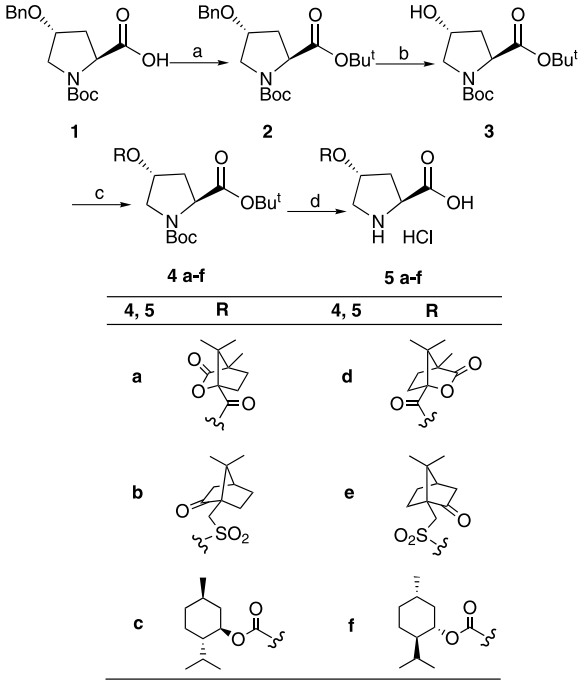
Abstract—A series of 4-substituted prolines were prepared and evaluated as organocatalysts for asymmetric aldol reactions. Using (2*S*,4*R*)-4-camphorsulfonyloxy-proline, the aldol products were obtained in much higher enantiomeric excess in comparison to that observed using proline itself. In addition, the improved solubility of these new catalysts in organic solvents permits their use in lower sub-stoichiometric amounts compared to proline.

1. Introduction

The capability of simple organic molecules from the 'chiral pool' to act like an enzyme represents a remarkable synthetic alternative to many established asymmetric transformations. Among the various enantiomerically pure small organic molecules, amino acids and peptides as well as cinchona alkaloids are extremely interesting asymmetric organocatalysts[1] demonstrating useful levels of enantioselectivity for a wide range of transformations. In particular, the natural amino acid L-proline acts as an enzyme mimic of the type I aldolase, catalysing one of the most important organic asymmetric transformations, the aldol reaction.[2] In addition, proline has shown excellent catalytic activity in catalysing a wide variety of reactions[3] such as Mannich[4] and Michael[5] reactions, Robinson annulation,[6] synthesis of amino acids,[7] α-amination of aldehydes and ketones,[8] α-oxidation[9] and α-alkylation[10] of aldehydes.

2. Results and discussion

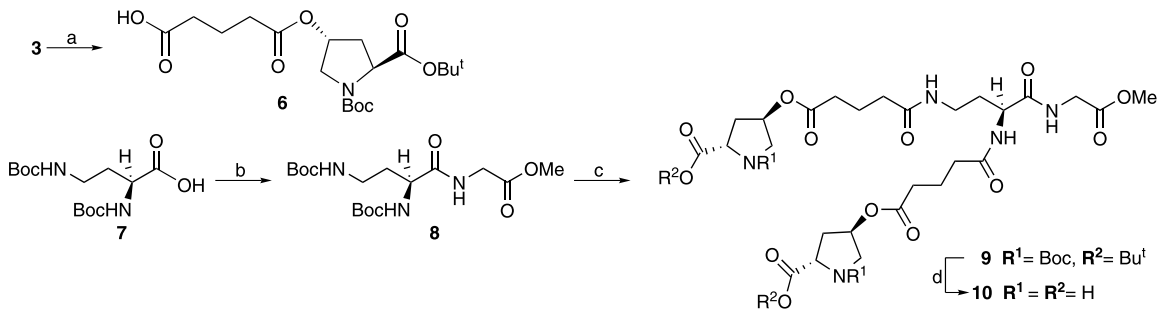
As part of our program aimed at developing methodology for the synthesis of non-natural amino acids and exploring their applications,[11] we synthesized various 4-substituted prolines. In this article we report our study on their catalytic effect on the direct asymmetric aldol reaction.[12] To develop improved proline-based catalysts, we decided to maintain the proline backbone, since both the carboxylic acid group and the pyrrolidine group are essential for effective asymmetric induction, and to introduce a chiral bulky substituent at the 4-position. The rationale behind the design was to induce steric hindrance from one side of the pyrrolidine ring. L-4-Hydroxyproline, which has already



Scheme 1. Reagents and conditions: (a) $CCl_3C(=NH)OBu^t$, $BF_3 \cdot Et_2O$, CH_2Cl_2/C_6H_{12}, 84%; (b) H_2, 10% Pd/C, 1,4-dioxane, rt, 24 h, 84%; (c) (i) (1*S*)-(−)-camphanic acid or (1*R*)-(+)-camphanic acid, DCC, DMAP, CH_2Cl_2, rt, 24 h, 80% for **4a** and 82% for **4d**, or (ii) (1*R*)-(−)-camphor-10-sulfonyl chloride or (1*S*)-(+)-camphor-10-sulfonyl chloride, NMM, THF, 0 °C, 30 min, rt, 24 h, 85% for **4b** and 88% for **4e**, or (iii) NaH, THF, 0 °C, then rt, 30 min, and then (−)-menthyl chloroformate or (+)-menthyl chloroformate, rt, 22 h, 90% for **4c** and 93% for **4f**; (d) 5 N HCl/Et_2O, rt, 4 h, 94–96%.

Keywords: Aldol reactions; Amino acids; Asymmetric catalysis; Organocatalysts; 4-Substituted proline.
* Corresponding author. Tel.: +30 210 7274462; fax: +30 210 7274761; e-mail: gkokotos@cc.uoa.gr

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.06.113

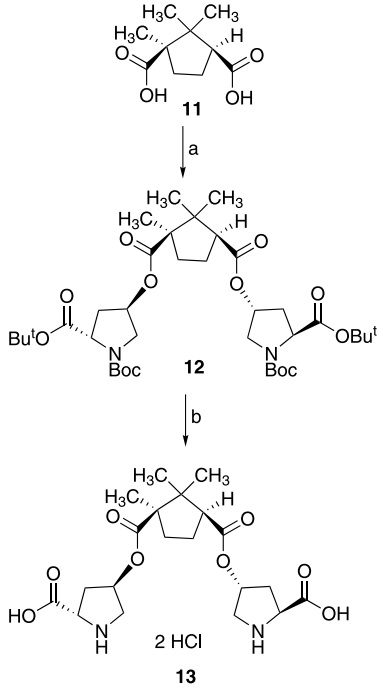


Scheme 2. Reagents and conditions: (a) glutaric anhydride, DMAP, CH_2Cl_2, rt, 36 h, 75%; (b) $HCl \cdot H_2NCH_2COOCH_3$, EDC, HOBt, Et_3N, 0 °C, 1 h, rt, 16 h, 75%; (c) (i) 5 N HCl/Et_2O, rt, 1 h, (ii) **6**, EDC, HOBt, Et_3N, 0 °C, 1 h, rt, 30 h, 70% (overall); (d) 5 N HCl/Et_2O, rt, 4 h, 96%.

been used successfully as a catalyst of the aldol reaction,[2b] seemed an ideal template. Furthermore, it has been recently reported that *trans*-4-*tert*-butyldimethylsiloxy-L-proline efficiently catalyses α-aminoxylation of carbonyl compounds, as well as *O*-nitroso-aldol/Michael, and Mannich reactions.[13]

Thus, commercially available (2*S*,4*R*)-*N*-(*tert*-butoxycarbonyl)-4-benzyloxy-proline (**1**) was converted into compound **3** (Scheme 1). Compounds **4a**,**d** were prepared by coupling **3** with (1*R*)-(+)- and (1*S*)-(−)-camphanic acid using *N*,*N*′-dicyclohexylcarbodiimide (DCC) as coupling agent in the presence of 4-dimethylamino-pyridine (DMAP).[14] Sulfonates **4b**,**e** were prepared by treatment of **3** with the corresponding sulfonyl chlorides in the presence of *N*-methyl-morpholine (NMM) in dry THF and carbonates **4c**,**f** were prepared by treatment of **3** with the corresponding chloroformates, as depicted in Scheme 1. Deprotected derivatives **5a–f** were prepared by treatment of **4a–f** with 5 N HCl/Et_2O.

Derivative **3** was glutarylated and coupled with methyl



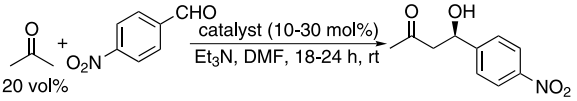
Scheme 3. Reagents and conditions: (a) **3**, DCC, DMAP, CH_2Cl_2, rt, 36 h, 74%; (b) 5 N HCl/Et_2O, rt, 4 h, 98%.

α,γ-diaminobutyryl-glycinate using 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide (EDC) in the presence of 1-hydroxybenzotriazole (HOBt) (Scheme 2). Dendron-like derivative **10** was obtained by deprotection of compound **9**. As depicted in Scheme 3 (1*R*,3*S*)-(+)-camphoric acid (**11**) was coupled with compound **3**, using the DCC/DMAP method, and deprotected to produce compound **13**.

The aldol reaction is one of the most important carbon–carbon bond-forming reactions in organic synthesis. List and Barbas have extensively studied the direct asymmetric aldol reaction of both acyclic and cyclic ketones as aldol donors with aromatic and aliphatic aldehydes and they have demonstrated that the most powerful catalysts are L-proline and 5,5′-dimethyl thiazolidinium-4-carboxylate.[2b] We have chosen in our preliminary investigations the reaction between acetone and 4-nitro-benzaldehyde and we have studied the catalytic effect of derivatives **5a–f**, **10** and **13** on this reaction. The results of our studies are summarized in Table 1.

The substituents at the 4-position have a significant effect on

Table 1. Direct asymmetric aldol reaction of acetone and 4-nitrobenzaldehyde using various proline-based catalysts



Entry[a]	Catalyst	Catalyst loading (%)	Yield (%)[b]	ee (%)[c]
1	**5a**	30	62	80
2	**5a**	20	58	73
3	**5b**	30	62	75
4	**5b**	20	60	81
5	**5c**	30	65	77
6	**5d**	30	66	44
7	**5e**	30	61	82
8	**5e**	20	59	84
9	**5e**	10	71	90
10	**5f**	30	65	71
11	**10**	30	61	68
12	**10**	15	64	70
13	**13**	30	60	69
14	**13**	15	62	71
15	L-Pro·HCl	20	60	63
16	L-Pro[d]	20	63	69
17	L-HyPro[d]	30	71	70

[a] Reactions carried out for 16–18 h at rt.
[b] Isolated yields after column chromatography.
[c] The ee was determined by HPLC on a Daicel Chiralpak AD-RH column.
[d] In the absence of Et_3N.

both the enantioselectivity and yield of the reaction. Under the conditions used (Table 1) the aldol product was obtained in 69 and 70% ee, when L-proline and L-4-hydroxyproline were used as catalysts (entries 16 and 17, respectively). However, when camphanic acid derivative **5a** was used ee values up to 80% were observed (entry 1). A decrease in the catalyst loading of **5a** led to decrease of both chemical yield and ee value (entry 2). It is interesting to note that (1*S*)-(−)-camphanic acid derivative (**5a**) led to high enantioselectivity, while the (1*R*)-(+)-camphanic acid derivative (**5d**) led to much lower enantioselectivity (entries 1 and 6, respectively). Menthyl derivatives **5c** and **5f** (entries 5 and 10, respectively), gave comparable ee values and the result for **5f** was almost similar to those acquired when proline and L-4-hydroxyproline were used as catalysts. The application of the camphorsulfonyl derivatives **5b** and **5e** led to very interesting results. The camphorsulfonyl derivative **5e** produced the aldol product in high ee (90%) when used in 10 mol% catalyst loading (entry 9). It should be noticed that although the loading of the catalyst (30 and 20%) did not considerably influence both the enantioselectivity (82 and 84%, entries 7 and 8, respectively), and the chemical yield (61 and 59%, entries 7 and 8, respectively), better chemical yield (71%) was obtained when derivative **5e** was employed in 10 mol% loading (entry 9). Dendron-like derivatives **10** and **13** containing two pyrrolidine rings per molecule led to chemical yields and ee values almost similar to those obtained by proline itself, probably because proline residues act as non interacting moieties and their catalytic potencies are neither enhanced nor cancelled out by each other.

In an attempt to test the efficacy of catalyst **5e**, we submitted 4-bromo-benzaldehyde and 2-chloro-benzaldehyde as acceptor substrates to the aldol process. The results are presented in Table 2. When proline was used as catalyst in the reaction between acetone and 4-bromo-benzaldehyde in 20 mol% loading, the aldol product was obtained in moderate ee values (62%), whilst in 10 mol% proline loading practically no aldol product was acquired (entry 2). However, when catalyst **5e** was employed in 10 mol% catalyst loading, the aldol product was isolated in much higher enantiomeric excess (80%) (entry 2). In a similar manner, in the reaction between acetone and 2-chloro-benzaldehyde, catalyst **5e** in 10 mol% loading led to a higher ee value (74%) in comparison to that observed by using proline itself (64% ee for 20 mol% proline and almost no aldol product for 10 mol%) and in a comparable chemical yield (entry 3).

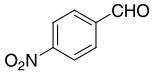
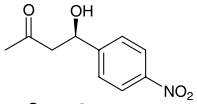
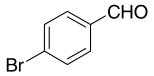
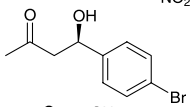
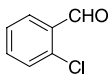
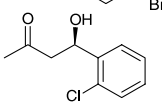
In conclusion, the results of our study indicate that for high enantioselectivity the catalyst should possess a chiral bulky group at the 4-position of the pyrrolidine ring in addition to carboxylic acid and secondary amine groups. In particular, 4-camphorsulfonyloxy-proline derivative **5e**, easily synthesized from proline derivative **1**, compares favourably to proline for the direct asymmetric aldol reaction offering: (a) higher enantioselectivity in comparison to proline and (b) a decrease of the required amount (10%) in comparison to proline (20–30%).

3. Experimental

3.1. General

Melting points were determined on a melting point apparatus and are uncorrected. Specific rotations were measured on a polarimeter using a 10 cm cell. NMR spectra were recorded on 200 and 300 MHz Varian spectrometers. Where applicable, structural assignments were based on COSY experiments. Where rotamers are apparent peaks for major and minor rotamers are reported, when resolved. IR spectra were recorded on a Perkin-Elmer 841 Spectrophotometer. Analytical TLC plates (silica gel 60 F_{254}) and silica gel 60 (70–230 or 230–400 mesh) for column chromatography were purchased from Merck. Visualisation of spots was effected with UV light and/or phosphomolybdic acid and/or ninhydrin both in ethanol stain. THF and 1, 4-dioxane were freshly distilled from sodium-benzophenone ketyl radical under an argon atmosphere immediately prior to use. Et_2O was treated with calcium chloride and stored over Na. DMF was stirred in the presence of P_2O_5 for 15 h and distilled under reduced pressure. Acetone was dried overnight over 3 Å activated molecular sieves (10% w/v) and then distilled. All other solvents and chemicals were of reagent grade and used without further purification. The samples for elemental analyses were dried over P_2O_5 under high vacuum for 48 h.

Table 2. Comparison between proline and catalyst **5e** in the direct asymmetric aldol reaction

Entry[a]	Substrate	Product	Proline (10 mol%)		Proline (20 mol%)		**5e** (10 mol%)	
			Yield (%)[b]	ee (%)	Yield (%)[b]	ee (%)[c]	Yield (%)[b]	Ee (%)[c]
1			<5	n.d.[d]	63	69	71	90
2			<5	n.d.[d]	71	62	74	80
3			<5	n.d.[d]	80	64	78	74

[a] Reactions carried out for 16–18 h at rt.
[b] Isolated yields after column chromatography.
[c] The ee was determined by HPLC on a Daicel Chiralpak AD-RH column.
[d] Not determined.

3.1.1. (2*S*,4*R*)-Di-*tert*-butyl 4-(benzyloxy)pyrrolidine-1,2-dicarboxylate (2). To a stirred solution of Boc-L-Pro(Bn)–OH (322 mg, 1.00 mmol) in CH_2Cl_2 (1 mL), a solution of *tert*-butyl-2,2,2-trichloroacetimidate (440 mg, 2.00 mmol) in C_6H_{12} (2 mL) was added, followed by $BF_3 \cdot Et_2O$ (20 μL). The stirring was continued for 24 h at rt. Work-up involved filtration of the reaction mixture through a pad of Celite® to remove trichloroacetamide and removal of the solvent under reduced pressure. The residue was purified by column chromatography using a mixture of $CHCl_3$/MeOH 95:5 as eluent to afford **2**. Yellowish oil (315 mg, 84%); $[\alpha]_D$ −23.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.45 [br, 18H, 2×C$(CH_3)_3$], 2.05 (m, 1H, C*H*HCHN), 2.35 (m, 1H, CH*H*CHN), 3.45–3.75 (m, 2H, CH_2N), 4.10–4.38 (m, 2H, CHN, OCH), 4.42–4.60 (m, 2H, CH_2Ph), 7.25–7.42 (m, 5H, Ph); ^{13}C NMR (50 MHz, $CDCl_3$) δ 27.9, 28.3, 35.4, 36.7, 51.2, 51.8, 58.6, 71.1, 75.7, 76.7, 79.7, 79.9, 81.0, 127.6, 127.7, 128.4, 137.7, 154.0, 172.1; IR [film, (cm^{-1})]: 1730 and 1698 (C=O); MS (ESI): *m*/*z* (%): 378 (23) [M+H$^+$], 400 (100) [M+Na$^+$]. Anal. Calcd for $C_{21}H_{31}NO_5$: C, 66.82; H, 8.28; N, 3.71. Found: C, 66.59; H, 8.38; N, 3.68.

3.1.2. (2*S*,4*R*)-Di-*tert*-butyl 4-hydroxypyrrolidine-1,2-dicarboxylate (3). To a stirred solution of **2** (380 mg, 1.00 mmol) in anhydrous 1,4-dioxane (10 mL), 10% Pd/C (40 mg) was added. The reaction mixture was stirred under H_2 for 24 h at rt. After filtration through a pad of Celite®, the solvent was removed and the residue was purified by column chromatography using EtOAc as eluent to give **3**. Colourless oil (240 mg, 84%); $[\alpha]_D$ −68.0 (*c* 1.0, MeOH); [Lit.[15] $[\alpha]_D^{21}$ −68.9 (*c* 1.06, MeOH)]; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.45 [br, 18H, 2×C$(CH_3)_3$], 2.01 (m, 1H, C*H*HCHN), 2.25 (m, 1H, CH*H*CHN), 2.92 (br, 1H, OH), 3.33–3.68 (m, 2H, CH_2N), 4.28 (m, 1H, CHN), 4.45 (m, 1H, OCH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 27.9, 28.3, 38.3, 39.1, 54.5, 58.5, 69.1, 70.0, 79.9, 80.2, 81.1, 154.3, 172.2; IR [film, (cm^{-1})]: 1745 and 1690 (C=O); MS (FAB): *m*/*z* (%): 288 (100) [M+H$^+$]. Anal. Calcd for $C_{14}H_{25}NO_5$: C, 58.52; H, 8.77; N, 4.87. Found: C, 58.65; H, 7.69; N, 4.88.

3.2. General procedure for the preparation of the carboxylates 4a,d

To a solution of **3** (290 mg, 1.00 mmol) in dichloromethane (8 mL) was added (1*S*)-(−)-camphanic acid or (1*R*)-(+)-camphanic acid (200 mg, 1.00 mmol) followed by DCC (227 mg, 1.10 mmol) and DMAP (12 mg, 0.10 mmol). The mixture was stirred for 24 h. The dicyclohexylurea was filtered off, the solvent was removed, water (5 mL) was added and the product was then extracted with EtOAc (3× 10 mL). The combined organic layers were washed consecutively with 1 M $KHSO_4$ (1×25 mL), H_2O (1× 30 mL), $NaHCO_3$ 10% (1×25 mL), H_2O (1×30 mL) and dried over Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography using a mixture of EtOAc/petroleum ether 40–60 1:1 as eluent to give **4a**,**d**.

3.2.1. (2*S*,4*R*)-Di-*tert*-butyl 4-[(1*S*,4*R*)-4,7,7-trimethyl-3-oxo-2-oxa-bicyclo[2.2.1]heptane-1-carbonylox]pyrrolidine-1,2-dicarboxylate (4a). Pale yellow solid (374 mg, 80%); mp 173–175 °C; $[\alpha]_D$ −61.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.90 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 1.10 (s, 3H, CH_3), 1.44 [br, 18H, 2×C$(CH_3)_3$], 1.50–2.50 (series of m, 6H, 2×CH_2, CH_2CHN), 3.40–3.80 (m, 2H, CH_2N), 4.23 (m, 1H, CHN), 5.28 (m, 1H, OCH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 9.5, 16.5, 16.6, 27.8, 28.1, 28.2, 28.7, 29.9, 30.2, 31.9, 32.0, 32.1, 32.6, 35.2, 36.2, 52.0, 54.3, 54.7, 58.1, 73.4, 74.1, 80.1, 80.3, 81.4, 90.6, 153.6, 166.9, 171.2, 178.0; IR [KBr, (cm^{-1})]: 1795, 1738 and 1704 (C=O); MS (FAB): *m*/*z* (%): 468 (5) [M+H$^+$]. Anal. Calcd for $C_{24}H_{37}NO_8$: C, 61.65; H, 7.98; N, 3.00. Found: C, 61.80; H, 8.32; N, 3.21.

3.2.2. (2*S*,4*R*)-Di-*tert*-butyl 4-[(1*R*,4*S*)-4,7,7-trimethyl-3-oxo-2-oxa-bicyclo[2.2.1]heptane-1-carbonyloxy]pyrrolidine-1,2-dicarboxylate (4d). White solid (384 mg, 82%); mp 153–155 °C; $[\alpha]_D$ −18.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.91 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 1.08 (s, 3H, CH_3), 1.44 [br, 18H, 2×C$(CH_3)_3$], 1.50–2.48 (series of m, 6H, 2×CH_2, CH_2CHN), 3.49–3.78 (m, 2H, CH_2N), 4.11 (m, 1H, CHN), 5.34 (m, 1H, OCH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 9.5, 16.5, 16.6, 27.8, 28.1, 28.8, 29.9, 30.4, 31.9, 32.0, 32.1, 32.5, 35.2, 36.3, 52.0, 52.1, 54.1, 54.6, 58.2, 73.2, 74.1, 80.3, 81.4, 90.5, 153.5, 166.7, 171.2, 177.8; MS (ESI): *m*/*z* (%): 490 (100) [M+Na$^+$]. Anal. Calcd for $C_{24}H_{37}NO_8$: C, 61.65; H, 7.98; N, 3.00. Found: C, 61.78; H, 8.30; N, 3.26.

3.3. General procedure for the preparation of the sulfonates 4b,e

To a stirred solution of **3** (290 mg, 1.00 mmol) in anhydrous THF (8 mL) were added NMM (0.14 mL, 1.25 mmol) and (1*R*)-(−)-camphor-10-sulfonyl chloride or (1*S*)-(+)-camphor-10-sulfonyl chloride (315 mg, 1.25 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and at rt for 24 h. The solvent was removed, water (15 mL) was added and the product was then extracted with EtOAc (3×10 mL). The combined organic layers were washed consecutively with 1 M $KHSO_4$ (1×25 mL), H_2O (1×30 mL), $NaHCO_3$ 10% (1×25 mL), H_2O (1×30 mL) and dried over Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography using initially a mixture of EtOAc/petroleum ether 40–60 1:1 and finally EtOAc as eluents to give **4b**,**e**.

3.3.1. (2*S*,4*R*)-Di-*tert*-butyl 4-{[(1*R*,4*S*)-7,7-dimethyl-2-oxobicyclo[2.2.1]heptan-1-yl]methylsulfonyloxy}pyrrolidine-1,2-dicarboxylate (4b). Colourless oil (426 mg, 85%); $[\alpha]_D$ −44.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.80–2.56 (series of m, 35H, 2×CH_3, 2×C$(CH_3)_3$, 3×CH_2, CH, CH_2CHN, CH_2SO_2) 3.40–3.80 (m, 2H, CH_2N), 4.30 (m, 1H, CHN), 5.29 (m, 1H, OCH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.6, 19.7, 25.2, 26.8, 27.9, 28.0, 28.3, 33.1, 35.5, 36.6, 42.3, 42.7, 51.9, 52.1, 58.4, 64.2, 71.9, 72.8, 80.1, 80.3, 81.4, 153.8, 171.6, 172.3, 212.8; MS (ESI): *m*/*z* (%): 524 (100) [M+Na$^+$]. Anal. Calcd for $C_{24}H_{39}NO_8S$: C, 57.46; H, 7.84; N, 2.79. Found: C, 57.70; H, 7.55; N, 2.76.

3.3.2. (2*S*,4*R*)-Di-*tert*-butyl 4-{[(1*S*,4*R*)-7,7-dimethyl-2-oxobicyclo[2.2.1]heptan-1-yl]methylsulfonyloxy}pyrrolidine-1,2-dicarboxylate (4e). White solid (442 mg, 88%); mp 93–95 °C; $[\alpha]_D$ −10.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.76 (s, 3H, CH_3), 0.96 (s, 3H, CH_3),

1.37 [br, 18H, 2×C(CH$_3$)$_3$], 1.40–2.60 (series of m, 9H, 3×CH$_2$, CH, C*H*$_2$CHN), 2.91 (d, *J*=14.9 Hz, 1H, C*H*HSO$_2$), 3.40–3.83 (m, 3H, CH$_2$N, CH*H*SO$_2$), 4.16 (m, 1H, CHN), 5.17 (m, 1H, OCH); ^{13}C NMR (50 MHz, CDCl$_3$) δ 19.2, 19.3, 24.5, 24.6, 26.5, 27.6, 27.9, 35.9, 37.2, 42.1, 42.3, 47.7, 52.1, 52.4, 57.6, 57.7, 57.8, 78.2, 78.9, 80.0, 81.2, 153.2, 170.8, 170.9, 213.8; IR [KBr, (cm^{-1})]: 1749, 1728 and 1700 (C=O), 1365 and 1160 (S=O); MS (ESI): *m/z* (%): 524 (100) [M+Na$^+$]. Anal. Calcd for C$_{24}$H$_{39}$NO$_8$S: C, 57.46; H, 7.84; N, 2.79. Found: C, 57.71; H, 7.60; N, 2.74.

3.4. General procedure for the preparation of the carbonates 4c,f

To an ice-cold solution of **3** (290 mg, 1.00 mmol) in dry THF (5 mL), NaH (24 mg, 1.00 mmol) was added. The mixture was warmed up to rt and stirred for 30 min, after which (−)- or (+)-menthyl chloroformate (240 mg, 235 μL, 1.10 mmol) was added. The stirring was continued at rt for 22 h. The solvent was removed under reduced pressure, water (7 mL) was added and the product was then extracted with EtOAc (3×6 mL). The combined organic layers were washed consecutively with 1 M KHSO$_4$ (1×20 mL), H$_2$O (1×25 mL), NaHCO$_3$ 10% (1×20 mL), H$_2$O (1×25 mL) and dried over Na$_2$SO$_4$. The solvent was evaporated and the residue was purified by column chromatography using a mixture of EtOAc/petroleum ether 40–60 1:1 as eluent to give **4c**,**f**.

3.4.1. (2*S*,4*R*)-Di-*tert*-butyl 4-{[(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyloxy]carbonyloxy}pyrrolidine-1,2-dicarboxylate (4c). Pale yellow oil (423 mg, 90%); [α]$_D$ −66.0 (*c* 1.0, CHCl$_3$); ^{1}H NMR (200 MHz, CDCl$_3$) δ 0.70–2.50 (series of m, 38H, 3×CH$_3$, 2×C(CH$_3$)$_3$, 3×CH$_2$, 3×CH, C*H*$_2$CHN), 3.50–3.80 (m, 2H, CH$_2$N), 4.22 (m, 1H, CHN), 4.48 (m, 1H, CHC*H*O), 5.17 (m, 1H, OCH); ^{13}C NMR (50 MHz, CDCl$_3$) δ 16.1, 20.6, 21.8, 23.1, 25.9, 27.8, 27.9, 28.2, 31.3, 33.9, 35.4, 36.5, 40.6, 46.8, 51.9, 58.2, 75.0, 75.8, 78.8, 80.0, 80.2, 81.3, 153.5, 154.0, 171.5; MS (ESI): *m/z* (%): 470 (45) [M+H$^+$], 492 (100) [M+Na$^+$]. Anal. Calcd for C$_{25}$H$_{43}$NO$_7$: C, 63.94; H, 9.23; N, 2.98. Found: C, 64.10; H, 9.45; N, 2.79.

3.4.2. (2*S*,4*R*)-Di-*tert*-butyl 4-{[(1*S*,2*R*,5*S*)-2-isopropyl-5-methylcyclohexyloxy]carbonyloxy}pyrrolidine-1,2-dicarboxylate (4f). Pale yellow oil (437 mg, 95%); [α]$_D$ +25.0 (*c* 1.0, CHCl$_3$); ^{1}H NMR (200 MHz, CDCl$_3$) δ 0.68–2.53 (series of m, 38H, 3×CH$_3$, 2×C(CH$_3$)$_3$, 3×CH$_2$, 3×CH, C*H*$_2$CHN), 3.50–3.77 (m, 2H, CH$_2$N), 4.25 (m, 1H, CHN), 4.50 (m, 1H, CHC*H*O), 5.12 (m, 1H, OCH); ^{13}C NMR (50 MHz, CDCl$_3$) δ 16.1, 20.6, 21.8, 23.1, 25.9, 27.8, 28.2, 31.3, 33.9, 35.4, 36.5, 40.6, 46.9, 51.8, 52.0, 58.1, 74.9, 75.8, 78.7, 79.9, 80.1, 81.2, 153.5, 154.0, 171.4, 171.5; IR [film, (cm^{-1})]: 1742 and 1709 (C=O); MS (ESI): *m/z* (%): 470 (40) [M+H$^+$], 492 (100) [M+Na$^+$]. Anal. Calcd for C$_{25}$H$_{43}$NO$_7$: C, 63.94; H, 9.23; N, 2.98. Found: C, 64.08; H, 9.45; N, 2.76.

3.4.3. 5-[(3*R*,5*S*)-1,5-Bis(*tert*-butoxycarbonyl)pyrrolidin-3-yloxy]-5-oxopentanoic acid (6). To a stirred solution of **3** (287 mg, 1.00 mmol) in CH$_2$Cl$_2$ (7 mL), a solution of glutaric anhydride (224 mg, 2.00 mmol) in CH$_2$Cl$_2$ (3 mL) was added, followed by DMAP (16 mg, 0.13 mmol). The stirring was continued for 36 h at rt. The solvent was then removed under reduced pressure, water (8 mL) was added and the product was extracted with EtOAc (3×8 mL). The combined organic layers were washed consecutively with 1 M KHSO$_4$ (1×20 mL) and H$_2$O (1×25 mL) and dried over Na$_2$SO$_4$. The solvent was evaporated and the residue was purified by column chromatography using EtOAc as eluent to give **6**. Light yellowish oil (300 mg, 75%); [α]$_D$ −37.3 (*c* 1.1, CHCl$_3$); ^{1}H NMR (200 MHz, CDCl$_3$) δ 1.47 [br, 18H, 2×C(CH$_3$)$_3$], 1.92–2.08 (m, 2H, CH$_2$C*H*$_2$CH$_2$), 2.17 (m, 1H, C*H*HCHN), 2.27–2.51 (m, 5H, CH*H*CHN, C*H*$_2$-CH$_2$C*H*$_2$), 3.40–3.78 (m, 2H, CH$_2$N), 4.25 (m, 1H, CHN), 5.27 (m, 1H, OCH); ^{13}C NMR (50 MHz, CDCl$_3$) δ 19.6, 27.8, 27.9, 28.2, 29.6, 32.8, 33.1, 35.5, 36.5, 51.9, 52.1, 58.4, 71.9, 72.8, 80.3, 80.5, 81.5, 153.9, 171.6, 172.3, 177.9; IR [film, (cm^{-1})]: 1738 and 1705 (C=O); MS (FAB): *m/z* (%): 402 (10) [M+H$^+$], 424 (2.5) [M+Na$^+$]. Anal. Calcd for C$_{19}$H$_{31}$NO$_8$: C, 56.84; H, 7.78; N, 3.49. Found: C, 56.65; H, 7.69; N, 3.48.

3.4.4. (*S*)-Methyl 2-[2,4-bis(*tert*-butoxycarbonyl)butanamido]acetate (8). To a stirred solution of (*S*)-2,4-bis(*tert*-butoxycarbonyl)butanoic acid (**7**) (318 mg, 1.00 mmol) in CH$_2$Cl$_2$ (10 mL) were added methyl glycinate hydrochloride (126 mg, 1.00 mmol), 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (211 mg, 1.10 mmol), 1-hydroxybenzotriazole (135 mg, 1.00 mmol) and Et$_3$N (0.16 mL, 1.10 mmol). The reaction mixture was stirred for 1 h at 0 °C and for 16 h at rt. The solvent was removed, water (8 mL) was added and the product was extracted with EtOAc (3×8 mL). The combined organic layers were washed consecutively with 1 M KHSO$_4$ (1×10 mL), H$_2$O (1×10 mL), 5% aqueous NaHCO$_3$ (1×10 mL), H$_2$O (1×10 mL), dried (Na$_2$SO$_4$), and the solvent was evaporated to give **8**. White solid (292 mg, 75%); mp 100–101 °C; [α]$_D$ −29.4 (*c* 1.0, CHCl$_3$); ^{1}H NMR (200 MHz, CDCl$_3$) δ 1.40 [br, 18H, 2×C(CH$_3$)$_3$], 1.70–2.00 (m, 2H, CHC*H*$_2$), 3.05 (m, 1H, C*H*HNHOCO), 3.45 (m, 1H, CH*H*NHOCO), 3.69 (s, 3H, OCH$_3$), 3.85–4.35 (m, 3H, CH, C*H*$_2$COOCH$_3$), 5.22 (m, 1H, CH$_2$N*H*OCO), 5.50 (d, *J*=8 Hz, 1H, CHN*H*OCO), 8.64 (m, 1H, NHCO); ^{13}C NMR (50 MHz, CDCl$_3$) δ 28.2, 28.3, 34.2, 36.7, 41.1, 51.6, 52.2, 79.5, 79.9, 155.6, 156.7, 170.1, 172.1; IR [KBr, (cm^{-1})]: 3331 (N–H), 1754, 1705 and 1689 (C=O), 1519 (N–H); MS (ESI): *m/z* (%): 389 (25) [M$^+$]. Anal. Calcd for C$_{17}$H$_{31}$N$_3$O$_7$: C, 52.43; H, 8.02; N, 10.79. Found: C, 52.56; H, 7.98; N, 10.80.

3.4.5. (2*S*,4*R*)-Di-*tert*-butyl 4-(5-((*S*)-3-(5-((3*R*,5*S*)-1,5-bis(*tert*-butoxycarbonyl)pyrrolidin-3-yloxy)-5-oxopentanamido)-4-(2-methoxy-2-oxoethylamino)-4-oxobutylamino)-5-oxopentanoyloxy)pyrrolidine-1,2-dicarboxylate (9). The *tert*-butoxycarbonyl groups of **8** (390 mg, 1.00 mmol) were removed by treatment with 5 N HCl in Et$_2$O (14 mL) for 1 h at rt. After evaporation, Et$_2$O was added and the product was filtered and recrystallised from MeOH/Et$_2$O. The product was suspended in CH$_2$Cl$_2$ (8 mL) and to the solution were added **6** (802 mg, 2.00 mmol), EDC (422 mg, 2.20 mmol), HOBt (270 mg, 2.00 mmol) and Et$_3$N (0.70 mL, 5.00 mmol). The reaction mixture was stirred for 1 h at 0 °C and for 30 h at rt. The solvent was removed, water (15 mL) was added and the product was extracted with EtOAc (3×10 mL). The combined organic layers were

washed consecutively with 1 M $KHSO_4$ (1×25 mL), H_2O (1×30 mL), 5% aqueous $NaHCO_3$ (1×25 mL), H_2O (1×30 mL), dried (Na_2SO_4), and the solvent was evaporated to give a residue, which was further purified by column chromatography using initially $CHCl_3$ and finally $CHCl_3$/MeOH 95:5 as eluents to give **9**. Colourless oil (670 mg, 70%); $[\alpha]_D$ −48.0 (*c* 1.0, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 1.48 [br, 36H, 4×$C(CH_3)_3$], 1.68–2.75 (m, 18H, 2×$CH_2CH_2CH_2$, $CHCH_2CH_2$, 2×$OCHCH_2CHN$), 2.92 (m, 1H, $CHCH_2CHH$), 3.40–4.08 (m, 9H, $CHCH_2CHH$, 2×CH_2N, OCH_3, $CHHCOOCH_3$), 4.10–4.35 (m, 3H, $CHHCOOCH_3$, 2×$OCHCH_2CHN$), 4.47 (m, 1H, $CHCH_2$-CH_2), 5.17–5.35 (m, 2H, 2×OCH), 6.68–7.00 (m, 2H, 2×NHCO), 8.26 (m, 1H, NHCO); ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.4, 20.8, 28.0, 28.3, 33.3, 33.5, 34.2, 35.2, 35.3, 35.5, 35.7, 36.3, 36.5, 41.1, 49.9, 51.8, 52.0, 52.1, 52.3, 58.4, 71.7, 72.0, 72.7, 80.4, 81.5, 153.9, 170.1, 171.6, 171.7, 171.9, 172.5, 172.9, 173.7; IR [film, (cm^{-1})]: 3315 (N–H), 1735, 1696, 1683 and 1650 (C=O), 1535 (N–H); MS (FAB): *m/z* (%): 956 (47) [M+H$^+$]. Anal. Calcd for $C_{45}H_{73}N_5O_{17}$: C, 56.53; H, 7.70; N, 7.33. Found: C, 56.55; H, 7.71; N, 7.35.

3.4.6. Compound 12. To a solution of **3** (575 mg, 2.00 mmol), in dichloromethane (10 mL), (1*R*,3*S*)-(+)-camphoric acid (200 mg, 1.00 mmol) was added, followed by DCC (454 mg, 2.20 mmol) and DMAP (24 mg, 0.20 mmol). The mixture was stirred for 36 h at rt. The dicyclohexylurea was filtered off, the solvent was removed, water (12 mL) was added and the product was then extracted with EtOAc (3×10 mL). The combined organic layers were washed consecutively with 1 M $KHSO_4$ (1×25 mL), H_2O (1×30 mL), $NaHCO_3$ 10% (1×25 mL), H_2O (1×30 mL) and dried over Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography using a mixture of EtOAc/petroleum ether 40–60 1:1 as eluent to give **12**. Colourless oil (547 mg, 74%); $[\alpha]_D$ −32.8 (*c* 1.0, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 0.92 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 1.38 [br, 36H, 4×$C(CH_3)_3$], 1.75–2.40 (series of m, 8H, 2×CH_2, 2×CH_2CHN), 2.75 (m, 1H, CHCO), 3.30–3.70 (m, 4H, 2×CH_2N), 4.10–4.26 (m, 2H, 2×CHN), 5.10–5.25 (m, 2H, 2×OCH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.9, 19.5, 19.9, 20.5, 24.2, 27.6, 27.7, 28.0, 28.1, 32.8, 33.2, 35.2, 36.3, 43.4, 51.7, 51.9, 54.0, 58.1, 71.7, 72.5, 79.8, 80.0, 81.1, 153.6, 153.8, 171.2, 171.3, 172.0; IR [film, (cm^{-1})]: 1806, 1762, 1741 and 1703 (C=O); MS (ESI): *m/z* (%): 739 (15) [M+H$^+$]. Anal. Calcd for $C_{38}H_{62}N_2O_{12}$: C, 61.77; H, 8.46; N, 3.79. Found: C, 61.90; H, 8.50; N, 3.82.

3.5. General procedure for the removal of Boc and Bu^t protecting groups

Boc and Bu^t groups of **4a–f**, **9**, **12** (1.00 mmol) were removed by treatment with 5 N HCl in Et_2O (14 mL, 70 mmol) for 4 h at rt. After evaporation under reduced pressure to a small volume (1 mL), anhydrous Et_2O was added (5 mL) and the precipitated product was afforded through decantation.

3.5.1. (2*S*,4*R*)-4-[(1*S*,4*R*)-4,7,7-Trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carbonyloxy]pyrrolidine-2-carboxylic acid hydrochloride (5a). White solid (334 mg, 96%); mp 219–221 °C; $[\alpha]_D$ −6.4 (*c* 1.0, DMF); ^1H NMR (200 MHz, CD_3OD) δ 0.97 (s, 3H, CH_3), 1.10 (s, 3H, CH_3), 1.12 (s, 3H, CH_3), 1.57–2.75 (series of m, 6H, 2×CH_2, CH_2CHN), 3.58 (m, 1H, $CHHN$), 3.83 (dd, *J*=5.0, 13.6 Hz, 1H, $CHHN$), 4.63 (m, 1H, CHN), 5.67 (m, 1H, OCH); ^{13}C NMR (50 MHz, CD_3OD) δ 9.9, 17.0, 17.2, 28.1, 29.8, 31.6, 35.9, 52.2, 55.6, 56.1, 59.7, 75.3, 92.4, 167.9, 170.2, 179.9; MS (ESI): *m/z* (%): 312 (100) [M+H$^+$]. Anal. Calcd for $C_{15}H_{21}NO_6 \cdot HCl$: C, 51.80; H, 6.38; N, 4.03. Found: C, 52.01; H, 6.47; N, 4.11.

3.5.2. (2*S*,4*R*)-4-{[(1*R*,4*S*)-7,7-Dimethyl-2-oxobicyclo[2.2.1]heptan-1-yl]methylsulfonyloxy}pyrrolidine-2-carboxylic acid hydrochloride (5b). White solid (363 mg, 95%); mp 164–166 °C; $[\alpha]_D$ −26.2 (*c* 1.0, MeOH); ^1H NMR (200 MHz, CD_3OD) δ 0.91 (s, 3H, CH_3), 1.10 (s, 3H, CH_3), 1.47–2.90 (series of m, 9H, 3×CH_2, CH_2CHN, CH), 3.35 (m, 1H, $CHHSO_2$), 3.62–3.87 (m, 3H, CH_2N, $CHHSO_2$), 4.64 (m, 1H, CHN), 5.59 (m, 1H, OCH); ^{13}C NMR (50 MHz, CD_3OD) δ 19.7, 19.9, 26.3, 27.7, 37.0, 43.4, 44.1, 49.1, 53.0, 59.2, 59.4, 80.8, 170.3, 216.6; MS (ESI): *m/z* (%): 346 (100) [M+H$^+$]. Anal. Calcd for $C_{15}H_{23}NO_6S \cdot HCl$: C, 47.18; H, 6.33; N, 3.67. Found: C, 47.02; H, 6.48; N, 3.85.

3.5.3. (2*S*,4*R*)-4-{[(1*R*,2*S*,5*R*)-2-Isopropyl-5-methylcyclohexyloxy]carbonyloxy}pyrrolidine-2-carboxylic acid hydrochloride (5c). White solid (330 mg, 94%); mp 195–197 °C (dec); $[\alpha]_D$ −22.0 (*c* 1.0, H_2O); ^1H NMR (200 MHz, CD_3OD) δ 0.75–2.15 (series of m, 18H, 3×CH_3, 3×CH_2, 2×CH, $CHHCHN$), 2.35–2.72 (m, 2H, CH, $CHHCHN$), 3.58 (m, 1H, $CHHN$), 3.74 (dd, *J*=4.4, 13.6 Hz, 1H, $CHHN$), 4.45–4.66 (m, 2H, CHN, $CHCHO$), 5.37 (m, 1H, OCH); ^{13}C NMR (50 MHz, CD_3OD) δ 16.7, 21.0, 22.4, 24.4, 27.4, 32.6, 35.2, 35.8, 41.8, 48.4, 52.3, 59.5, 77.1, 80.3, 155.1, 170.4; MS (ESI): *m/z* (%): 314 (100) [M+H$^+$]. Anal. Calcd for $C_{16}H_{27}NO_5 \cdot HCl$: C, 54.93; H, 8.07; N, 4.00. Found: C, 54.80; H, 8.15; N, 4.15.

3.5.4. (2*S*,4*R*)-4-[(1*R*,4*S*)-4,7,7-Trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carbonyloxy]pyrrolidine-2-carboxylic acid hydrochloride (5d). White solid (330 mg, 95%); mp 234–235 °C (dec); $[\alpha]_D$ −15.0 (*c* 1.0, DMF); ^1H NMR (200 MHz, CD_3OD) δ 0.96 (s, 3H, CH_3), 1.09 (s, 3H, CH_3), 1.11 (s, 3H, CH_3), 1.58–2.70 (series of m, 6H, 2×CH_2, CH_2CHN), 3.56 (m, 1H, $CHHN$), 3.80 (dd, *J*=4.8, 13.6 Hz, 1H, $CHHN$), 4.53 (m, 1H, CHN), 5.62 (m, 1H, OCH); ^{13}C NMR (50 MHz, CD_3OD) δ 9.8, 17.0, 17.1, 28.1, 29.8, 31.6, 36.0, 52.2, 55.7, 56.1, 60.2, 75.3, 92.4, 168.0, 168.3, 179.9; MS (FAB): *m/z* (%): 312 (97) [M+H$^+$]. Anal. Calcd for $C_{15}H_{21}NO_6 \cdot HCl$: C, 51.80; H, 6.38; N, 4.03. Found: C, 52.08; H, 6.50; N, 4.09.

3.5.5. (2*S*,4*R*)-4-{[(1*S*,4*R*)-7,7-Dimethyl-2-oxobicyclo[2.2.1]heptan-1-yl]methylsulfonyloxy}pyrrolidine-2-carboxylic acid hydrochloride (5e). White solid (363 mg, 95%); mp 184–186 °C; $[\alpha]_D$ +15.2 (*c* 1.0, DMF); ^1H NMR (200 MHz, CD_3OD) δ 0.91 (s, 3H, CH_3), 1.11 (s, 3H, CH_3), 1.42–2.87 (series of m, 9H, 3×CH_2, CH_2CHN, CH), 3.35 (m, 1H, $CHHSO_2$), 3.64–3.83 (m, 3H, $CHHSO_2$, CH_2N), 4.65 (m, 1H, CHN), 5.59 (m, 1H, OCH); ^{13}C NMR (50 MHz, CD_3OD) δ 19.7, 19.9, 26.4, 27.7, 36.7, 43.4,

44.1, 49.2, 53.1, 59.3, 59.4, 80.7, 170.3, 216.4; MS (FAB): *m/z* (%): 346 (100) [M+H$^+$]. Anal. Calcd for $C_{15}H_{23}NO_6S\cdot HCl$: C, 47.18; H, 6.33; N, 3.67. Found: C, 46.98; H, 6.42; N, 3.81.

3.5.6. (2*S*,4*R*)-4-{[(1*S*,2*R*,5*S*)-2-Isopropyl-5-methylcyclohexyloxy]carbonyloxy}pyrrolidine-2-carboxylic acid hydrochloride (5f). White solid (333 mg, 95%); mp 120–122 °C; $[\alpha]_D$ −22.0 (*c* 1.0, H_2O); ^{1}H NMR (200 MHz, CD_3OD) δ 0.70–2.15 (series of m, 18H, 3×CH_3, 3×CH_2, 2×CH, C*H*HCHN), 2.35–2.74 (m, 2H, CH, CH*H*CHN), 3.53 (m, 1H, C*H*HN), 3.74 (dd, *J*=4.4, 13.6 Hz, 1H, CH*H*N), 4.44–4.65 (m, 2H, CHN, CHC*H*O), 5.38 (m, 1H, OCH); ^{13}C NMR (50 MHz, CD_3OD) δ 16.6, 21.1, 22.4, 24.3, 27.2, 32.6, 35.2, 35.9, 41.7, 48.4, 52.3, 59.6, 77.1, 80.3, 155.1, 170.6; MS (FAB): *m/z* (%): 314 (60) [M+H$^+$]. Anal. Calcd for $C_{16}H_{27}NO_5\cdot HCl$: C, 54.93; H, 8.07; N, 4.00. Found: C, 54.83; H, 8.17; N, 4.19.

3.5.7. (2*S*,4*R*)-4-(5-((*S*)-3-(5-((3*R*,5*S*)-5-Carboxypyrrolidin-3-yloxy)-5-oxopentanamido)-4-(2-methoxy-2-oxoethylamino)-4-oxobutylamino)-5-oxopentanoyloxy)pyrrolidine-2-carboxylic acid dihydrochloride (10). Colourless viscous oil (690 mg, 96%); $[\alpha]_D$ −24.5 (*c* 1.0, H_2O); ^{1}H NMR (200 MHz, D_2O) δ 1.75–2.10 (m, 6H, 2×$CH_2CH_2CH_2$, CH_2CH_2CH), 2.17–2.50 (m, 10H, 2×$CH_2CH_2CH_2$, 2×OCHC*H*HCHN), 2.50–2.74 (m, 2H, 2×OCHCH*H*CHN), 3.20–3.75 (series of m, 9H, CH_2CH_2CH, CH_3O, 2×$OCHCH_2N$), 3.94–4.05 (m, 2H, CH_2COOCH_3), 4.35 (m, 1H, CH_2CH_2CH), 4.49–4.65 (m, 2H, 2×$OCHCH_2CHN$), 5.44–5.50 (m, 2H, 2×OCH); ^{13}C NMR (50 MHz, D_2O) δ 22.9, 23.1, 33.1, 35.5, 37.0, 37.3, 37.5, 38.3, 39.7, 43.8, 53.7, 54.0, 56.0, 61.5, 72.4, 76.1, 174.3, 175.7, 177.0, 177.1, 178.6, 178.8. Anal. Calcd for $C_{27}H_{41}N_5O_{13}\cdot 2HCl$: C, 45.26; H, 6.05; N, 9.77. Found: C, 45.27; H, 6.12; N, 9.80.

3.5.8. Compound 13. White solid (490 mg, 98%); mp 115–117 °C; $[\alpha]_D$ −4.1 (*c* 1.0, MeOH); ^{1}H NMR (200 MHz, CD_3OD) δ 1.03 (s, 3H, CH_3), 1.06 (s, 3H, CH_3), 1.24 (s, 3H, CH_3), 1.70–2.90 (series of m, 9H, 2×CH_2, 2×CH_2CHN, CHCO), 3.50–3.75 (m, 4H, 2×CH_2N), 4.60 (m, 2H, 2×CHN), 5.45 (m, 2H, 2×OCH); ^{13}C NMR (50 MHz, CD_3OD) δ 14.4, 20.3, 20.6, 20.8, 25.3, 33.8, 34.6, 34.7, 35.8, 52.3, 55.6, 59.7, 74.1, 170.7, 173.7; MS (FAB): *m/z* (%): 411 (100) [M$^+$ −CH_3]. Anal. Calcd for $C_{20}H_{30}N_2O_8\cdot 2HCl$: C, 48.10; H, 6.46; N, 5.61. Found: C, 47.99; H, 6.52; N, 5.81.

3.6. General procedure for the preparation of aldol products

To a mixture of anhydrous DMF (1.60 mL) and anhydrous acetone (0.40 mL) was added the corresponding aldehyde (0.20 mmol) followed by the catalysts **5a–f** or **10** or **13** (10–30 mol%) and an equivalent amount of Et_3N. The resulting mixture was stirred at rt for 18–24 h. Following aqueous workup with saturated ammonium chloride solution and extraction several times with EtOAc, the combined organic layers were dried (Na_2SO_4), and the solvent was evaporated. The pure aldol products were obtained by column chromatography using a mixture of EtOAc/petroleum ether 40–60 1:1 as eluent.

3.6.1. (4*R*)-(4-Nitrophenyl)-4-hydroxy-2-butanone[2b] (14) ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.21 (s, 3H, CH_3), 2.83 (m, 2H, CH_2), 3.56 (d, *J*=3.2 Hz, 1H, OH), 5.25 (m, 1H, CH), 7.52 (d, *J*=7.0 Hz, 2H, C_6H_4), 8.20 (d, *J*=7.0 Hz, 2H, C_6H_4); HPLC (Daicel Chiralpak AD-RH, CH_3CN/H_2O 30:70, flow rate 0.5 mL/min, λ=254 nm): t_R (major)= 16.58 min, t_R (minor)=20.26 min.

3.6.2. (4*R*)-(4-Bromophenyl)-4-hydroxy-2-butanone[2b] (15) ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.20 (s, 3H, CH_3), 2.82 (m, 2H, CH_2), 3.40 (d, *J*=3.0 Hz, 1H, OH), 5.12 (m, 1H, CH), 7.24 (d, *J*=8.4 Hz, 2H, C_6H_4), 7.47 (d, *J*=8.4 Hz, 2H, C_6H_4); HPLC (Daicel Chiralpak AD-RH, CH_3CN/H_2O 30:70, flow rate 0.5 mL/min, λ=254 nm): t_R (major)= 27.31 min, t_R (minor)=30.77 min.

3.6.3. (4*R*)-(2-Chlorophenyl)-4-hydroxy-2-butanone[2b] (16) ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.22 (s, 3H, CH_3), 2.64–3.03 (m, 2H, CH_2), 3.61 (br, 1H, OH), 5.56 (m, 1H, CH), 7.19–7.34 (m, 3H, C_6H_4), 7.64 (d, *J*=7.7 Hz, 1H, C_6H_4); HPLC (Daicel Chiralpak AD-RH, CH_3CN/H_2O 30:70, flow rate 0.5 mL/min, λ=254 nm): t_R (major)= 15.63 min, t_R (minor)=18.07 min.

Acknowledgements

This research was supported by Herakleitos EPEAEK programme.

References and notes

1. For reviews see: (a) Gröger, H.; Wilken, J. *Angew. Chem., Int. Ed.* **2001**, *40*, 529–532. (b) Javro, E. R.; Miller, S. J. *Tetrahedron* **2002**, *58*, 2481–2495. (c) Yoon, T. P.; Jacobsen, E. N. *Science* **2003**, *299*, 1691–1693. (d) Dalko, P. I.; Moisan, L. *Angew. Chem., Int. Ed.* **2001**, *40*, 3726–3748. (e) Dalko, P. I.; Moisan, L. *Angew. Chem., Int. Ed.* **2004**, *43*, 5138–5175.
2. (a) List, B.; Lerner, R. A.; Barbas, C. F., III *J. Am. Chem. Soc.* **2000**, *122*, 2395–2396. (b) Sakthivel, K.; Notz, W.; Bui, T.; Barbas, C. F., III *J. Am. Chem. Soc.* **2001**, *123*, 5260–5267. (c) List, B.; Pojarlief, P.; Castello, C. *Org. Lett.* **2001**, *3*, 573–575. (d) Córdova, A.; Notz, W.; Barbas, C. F., III *Chem. Commun.* **2002**, 3024–3025. (e) Northrup, A. B.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2002**, *124*, 6798–6799.
3. For reviews see: (a) List, B. *Synlett* **2001**, 1675–1686. (b) List, B. *Tetrahedron* **2002**, *58*, 5573–5590. (c) Paraskar, A. S. *Synlett* **2003**, 582–583.
4. (a) List, B.; Pojarlief, P.; Biller, W. T.; Martin, H. J. *J. Am. Chem. Soc.* **2002**, *124*, 827–833. (b) Córdova, A.; Barbas, C. F., III *Tetrahedron Lett.* **2003**, *44*, 1923–1926. (c) Hayashi, Y.; Tsuboi, W.; Shoji, M.; Suzuki, N. *J. Am. Chem. Soc.* **2003**, *125*, 11208–11209. (d) Córdova, A. *Chem. Eur. J.* **2004**, *10*, 1987. For a review see: (e) Córdova, A. *Acc. Chem. Res.* **2004**, *37*, 102–112.
5. (a) Hanessian, S.; Pham, V. *Org. Lett.* **2000**, *2*, 2975–2978. (b) List, B.; Pojarliev, P.; Martin, H. J. *Org. Lett.* **2001**, *3*, 2423–2425. (c) Betancort, J. M.; Sakthivel, K.; Thayumanavan, R.; Barbas, C. F., III *Tetrahedron Lett.*

2001, *42*, 4441–4444. (d) Enders, D.; Seki, A. *Synlett* **2002**, 26–28.
6. Bui, T.; Barbas, C. F., III *Tetrahedron Lett.* **2000**, *41*, 6951–6954.
7. (a) Córdova, A.; Notz, W.; Zhong, G.; Betancort, J. M.; Barbas, C. F. *J. Am. Chem. Soc.* **2002**, *124*, 1842–1843. (b) Córdova, A.; Watanabe, S.; Tanaka, F.; Notz, W.; Barbas, C. F. *J. Am. Chem. Soc.* **2002**, *124*, 1866–1867.
8. (a) List, B. *J. Am. Chem. Soc.* **2002**, *124*, 5656–5657. (b) Kumaragurubaran, N.; Juhl, K.; Zhuang, W.; Bøgevig, A.; Jørgensen, K. A. *J. Am. Chem. Soc.* **2002**, *124*, 6254–6255. (c) Bøgevig, A.; Juhl, K.; Kumaragurubaran, N.; Zhuang, W.; Jørgensen, K. A. *Angew. Chem., Int. Ed.* **2002**, *41*, 1790–1793. For a review see: (d) Duthaler, R. O. *Angew. Chem., Int. Ed.* **2003**, *42*, 975–978.
9. (a) Brown, S. P.; Brochu, M. P.; Sinz, C. J.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2003**, *125*, 10808–10809. (b) Zhong, G. *Angew. Chem., Int. Ed.* **2003**, *42*, 4247–4250. (c) Hayashi, Y.; Yamaguchi, J.; Hibino, K.; Shoji, M. *Tetrahedron Lett.* **2003**, *44*, 8293–8296. (d) Hayashi, Y.; Yamaguchi, J.; Sumiya, T.; Shoji, M. *Angew. Chem., Int. Ed.* **2004**, *43*, 1112–1115. (e) Córdova, A.; Sundén, H.; Bøgevig, A.; Johansson, M.; Himo, F. *Chem. Eur. J.* **2004**, *10*, 3673–3684. (f) Bøgevig, A.; Sundén, H.; Córdova, A. *Angew. Chem., Int. Ed.* **2004**, *43*, 1109–1112.
10. Vignola, N.; List, B. *J. Am. Chem. Soc.* **2004**, *126*, 450–451.
11. (a) Kokotos, G.; Padron, J. M.; Noula, C.; Gibbons, W. A.; Martin, V. S. *Tetrahedron: Asymmetry* **1996**, *7*, 857–866. (b) Kokotos, G.; Padron, J. M.; Martin, V. S. *J. Org. Chem.* **1998**, *63*, 3741–3744. (c) Padron, J. M.; Kokotos, G.; Martin, T.; Markidis, T.; Gibbons, W. A.; Martin, V. S. *Tetrahedron: Asymmetry* **1998**, *9*, 3381–3394. (d) Markidis, T.; Kokotos, G. *J. Org. Chem.* **2002**, *67*, 1685–1688. (e) Kokotos, G.; Kotsovolou, S.; Six, D. A.; Constantinou-Kokotou, V.; Beltzner, C. C.; Dennis, E. A. *J. Med. Chem.* **2002**, *45*, 2891–2893. (f) Bellis, E.; Markidis, T.; Kokotos, G. *Synthesis* **2002**, 1359–1364. (g) Kokotos, G.; Six, D. A.; Loukas, V.; Smith, T.; Constantinou-Kokotou, V.; Hadjipavlou-Litina, D.; Kotsovolou, S.; Chiou, A.; Beltzner, C. C.; Dennis, E. A. *J. Med. Chem.* **2004**, *47*, 3615–3628. (h) Müller, E.; Péczely, G.; Skoda-Földes, R.; Takáks, E.; Kokotos, G.; Bellis, E.; Kollár, L. *Tetrahedron* **2005**, *61*, 797–802.
12. Presented in part at the '1st Hellenic Symposium on Organic Synthesis', Athens, 4–6 November; 2004.
13. Hayashi, Y.; Yamaguchi, J.; Hibino, K.; Sumiya, T.; Urushima, T.; Shoji, M.; Hashizume, D.; Koshino, H. *Adv. Synth. Catal.* **2004**, *346*, 1435–1439.
14. Neises, B.; Steglich, W. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 522–524.
15. Tanaka, K.; Sawanishi, H. *Tetrahedron: Asymmetry* **1998**, *9*, 71–77.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 8677–8685

X=Y–ZH systems as potential 1,3-dipoles. Part 61: Metal exchanged zeolites, silver(I) oxide, Ni(II) and Cu(I) complexes as catalysts for 1,3-dipolar cycloaddition reactions of imines generating proline derivatives

Ronald Grigg,[a,*] Daniel M. Cooper,[a] Stephen Holloway,[b] Simon McDonald,[b] Emma Millington[a] and Mohammed A. B. Sarker[a]

[a]*Molecular Innovation, Diversity and Automated Synthesis (MIDAS) Centre, School of Chemistry, Leeds University, Leeds LS2 9JT, UK*
[b]*Discovery Chemistry, GlaxoSmithKline, Gunnels Wood Road, Stevanage, Herts, SG1 2NY, UK*

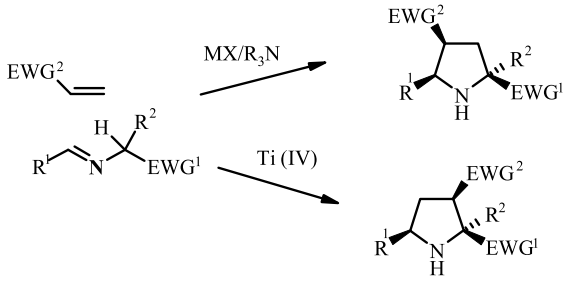
Received 11 April 2005; revised 16 June 2005; accepted 30 June 2005

Abstract—A range of regio- and stereo-selective 1,3-dipolar reactions of imines of α-amino esters, generating polysubstituted prolines, catalysed by silver(I) exchanged zeolites or silver(I) supported on titania, both in combination with DBU, are described. The use of a catalytic amount of silver(I) oxide, Ni(II) complexes and cuprous iodide as catalysts for the cycloaddition reactions are also disclosed.

1. Introduction

Metal exchanged zeolites are widely used for the catalysis of a variety of reactions.[1–9] For example, combination of Co(II), Mn(II) and Ni(II) with certain types of zeolites, for example, ZSM-5 and mordenite are active catalysts for the reduction of NO_X with methane in an oxidising atmosphere[1] and Ag-ZSM-5 catalyst, prepared by an ion exchange method, is an effective catalyst for the catalytic decomposition of NO_X in flue gases.[2] Multifunctional metal-exchanged zeolites are active catalysts for the conversion of methyl halides to ethylene[3] whilst complete catalytic oxidations of low molecular weight chlorinated hydrocarbons such as CH_2Cl_2, trichloroethane and CCl_4 in air over several cation-exchanged Y zeolites (Co–Y, Cr–Y and Mn–Y) have been reported.[4] Catalysts prepared by impregnating samples of neutral chabazite and mordenite zeolites with silver nitrate are active and selective for the epoxidation of ethane[5] whilst Cu(II) or Zn(II) exchanged zeolites catalyse the rearrangement[6] of (+)-pinene oxides. Silver(I) exchanged zeolite Y catalyses the dimerization of alkanes under UV–vis photochemical conditions at room temperature[7] and AgX and AgY are effective catalysts for the formation of glycosyl linkages.[8,9] However, to our knowledge, the use of silver exchanged zeolites as catalysts for 1,3-dipolar cycloaddition reactions has not been reported.

We introduced a facile and wide ranging Bronsted acid[10] or metal salt-tertiary amine catalysed 1,3-dipolar cycloaddition reaction of imines, activated by an appropriately located carbanion stabilising substituent, with electronegative alkenes. The reaction, which proceedes via in situ formation of metalloazomethine ylides, occurs at room temperature or below, is highly regio- and stereo-selective (Scheme 1) and furnishes polysubstituted pyrrolidines in excellent yield.[11]



Scheme 1.

In the case of Bronsted acids the rate in toluene correlates with p*K*a of a range of carboxilic acids.[10] The metal salt

Keywords: Metallo-azomethine ylides; Cycloaddition; Silver-exchanged zeolites; Silver oxide; Ni(II) catalysis; Cu(I) catalysis.
* Corresponding author. Tel.: +44 11334336501; fax: +44 1133436530; e-mail: r.grigg@chem.leeds.ac.uk

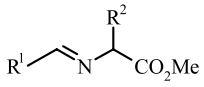
0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.06.110

mediated process is compatible with a range of solvents (toluene, CH_2Cl_2, THF, MeCN, DMF, DMSO), metal salts [Ag, Li, Tl(I), Zn, Mg, Mn(II), Co(II), Ni(II)(vide infra), Cu(I)(vide infra)][11] and bases (tertiary amines, DBU, guanidines) and the regiochemistry is precisely reversed, for room temperature reactions, when Ti(IV) catalysts are employed[12] whilst maintaining the stereoselectivity (Scheme 1). The reaction tolerates a wide variation in EWG[1] [ester, nitrile, COR, $P(O)(OR)_2$, 2-pyridyl, 9-thiazolyl, 9-fluorenyl][11] and EWG[2]. Asymmetric versions of the reaction have been developed, which employ either a chiral catalyst[13] or a chiral auxiliary.[13b,14,15]

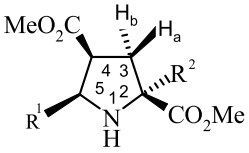
The mild reaction conditions, regio- and stereo-selectivity, simple experimental protocol and high yields ensured it was one of the first ring syntheses to be exploited by solid phase combinatorial chemistry.[16] Since then many further combinatorial chemistry applications have been developed[17] including combinations with other core reactions such as the Pictet–Spengler reaction and palladium catalysis.[18] Very large numbers of pyrrolidines have been made for biological screening by pharmaceutical and agrochemical companies using this chemistry. Recent applications in medicinal chemistry include inhibitions of hepatitis C virus RNA-dependent RNA polymerase[19] and inhibitors of $\alpha_4\beta_1$-integrin-mediated hepatic melanoma metastasis.[20]

An area experiencing rapid recent growth is the use of solid phase reagents,[21] which has many applications in solution phase combinatorial chemistry. This encouraged us to evaluate metal exchanged zeolites as potential heterogeneous catalysts for our pyrrolidine synthesis. This paper describes our studies in this area and also reports the first applications of Ni(II) and Cu(I) complexes/salts as catalysts for Scheme 1.

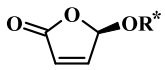
The 1,3-dipolar cycloaddition reaction of methyl (2-naphthylidene)alanate **3** and methyl acrylate, which is known to procede rapidly and in high yield with other Ag(I) salts, was chosen as the standard reaction. The first reaction (MeCN, DBU, 4 h, 25 °C) with a commercial 100-mesh silver exchanged zeolite[22] afforded only 12% of the cycloadduct **4**. When the reaction solvent was changed to dichloromethane the yield of the cycloadduct increased to 56% and when toluene was used as solvent, the product **4** was isolated in 82% yield (Scheme 2). Two further commercial silver exchanged zeolites (20-mesh and 1/16″ pellets) gave yields of 54 and 42%, respectively, under the optimised conditions.



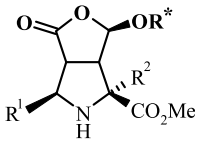
5a-l



6a-l



7



8a-c

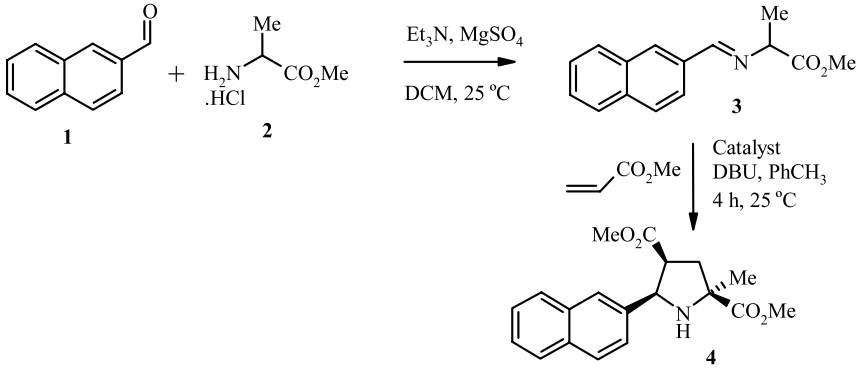
R* = menthyl

The applicability of the 100-mesh silver exchanged zeolite catalyst was demonstrated by using it in a number of other cycloaddition reactions involving imines **3** and **5a–i**. The cycloadditions proceeded regio- and stereo-specifically with methyl acrylate in toluene at room temperature in the presence of commercial silver exchanged zeolite and DBU to give single cycloadducts **6a–i** in 50–96% yield (Table 1). Cycloaddition of imines **3** and **5g** with the chiral dipolarophile (*R*)-5(1*R*)-menthyloxy-2-(5*H*)-furanone **7** resulted in cycloadducts in good yield and diastereoselectivity (Table 1, entries 10 and 11). The lower de in the case of the glycine imine **5g** reflects the well known sensitivity of azomethine ylides derived from glycine imines to stereomutation.[11]

The reaction of imine **3** with methyl acrylate was next investigated with a combination of 20% silver chloride on titania (donated by Johnson Matthey) and DBU in toluene (25 °C, 5 h), which furnished an 85% yield of **4**. Powdered silver chloride alone was then tested, to see whether it was effective in the absence of titania and was found to give a quantitative yield of cycloadduct **4** in a few minutes. Thus, the reaction was retarded by the titania support.

1.1. Silver oxide catalysis

Silver oxide (1 mol equiv) was examined as a potential heterogeneous catalyst in the standard imine cycloaddition reaction (Scheme 2) (toluene, DBU, 2 h, 25 °C). It was found that when 1 mol equiv of silver oxide was used, the



Scheme 2.

Table 1. Cycloaddition of imines **5a–i** using AgA[a] as catalyst[b]

Entry	Imine	Dipolarophile[c]	R^1	R^2	Cycloadduct	Yield (%)
1	**5a**	MA	2-Naphthyl	CH_2Ph	**6a**	96
2	**5b**	MA	2-Naphthyl	CH_2OH	**6b**	50
3	**5c**	MA	2-Naphthyl	CH_2-2-indolyl	**6c**	93
4	**5d**	MA	2-Pyridyl	Me	**6d**	50
5	**5e**	MA	*p*-$MeOC_6H_4$	CH_2Ph	**6e**	53
6	**5f**	MA	Phenyl	Me	**6f**	90
7	**5g**	MA	2-Naphthyl	H	**6g**	50
8	**5h**	MA	Cyclohexyl	Me	**6h**	68
9	**5i**	MA	Cyclohexyl	$CH_2CH_2SCH_3$	**6i**	92
10	**3**	7	2-Naphthyl	Me	**8a**	73 (100% de)[14b d]
11	**5g**	7	2-Naphthyl	H	**8b**	97 (90% de)[14b d]

[a] The amount of commercial zeolite (100 mesh, 20% Ag) used was equivalent to 1 mol equiv of silver.
[b] Reaction conditions: imine (1 equiv), dipolarophile (1 equiv), DBU (1 equiv), toluene, 25 °C, 5 h.
[c] MA = methyl acrylate.
[d] Determined by chiral HPLC—see Section 3.

reaction was over within 5 min and gave a quantitative yield of cycloadduct **4**. Moreover when 10 mol% silver oxide was employed, the reaction was over within 2 h, affording **4** in quantitative yield. Several reactions with different dipolarophiles were conducted to further test the efficacy of silver oxide as a catalyst. Imines **5g**,**h** and **5j–k** reacted with methyl acrylate in the presence of 10 mol% of Ag_2O to afford the corresponding cycloadducts in 92–100% yield. Reactions with chiral dipolarophile (*R*)-5(1*R*)-menthyloxy-2-(5*H*)-furanone **7** afforded the cycloadducts **8a–c** in excellent yield (95–100%) and diastereoselectivity (90–100% de) (Table 2).

1.2. Heterogeneous versus homogeneous nature of the catalysis

It was important to find out whether the silver zeolite and silver oxide were acting in a heterogeneous or a homogeneous manner. Calculations indicated the cycloadduct was larger than the pore size of the zeolite. Hence reactions inside the zeolite cavity would be non-productive. However, heterogeneous catalysis by silver might occur by coordination of the imine to silver ions located on the outer surface of the zeolite. Alternatively, the reactants might leach the silver from the zeolite affording a soluble silver species, which catalyses the cycloaddition in the usual way. It was observed that if the silver exchanged zeolite was filtered from the reaction mixture after the reaction was complete, then washed and reactivated by heating at 40 °C in a high vacuum (1 mmHg) for 24 h and reused, its catalytic activity was reduced. Thus, only 52% of **4** was obtained with recycled catalyst in the same reaction time. Deactivation of the zeolite might be due to reduction of silver (I) to silver metal during the reaction/regeneration cycle or it might arise from soluble silver species leaching from the zeolite. Leaching of silver species may originate in a number of ways, for example, in the preparation of the silver exchanged zeolite, some of the silver nitrate, used in the preparation of the silver exchanged zeolites will be sorbed onto the zeolite and hence more readily transferred to the bulk solution. Another possibility is that silver within the exchanged zeolite is released due to some kind of structural breakdown. For example, Kim and Seff[23] discovered that when Br_2 is sorbed onto zeolite AgA it interacts with the framework oxygen atoms and causes the decomposition of hexasilver clusters with the oxidised silver ion migrating out of the zeolite cavities.

It appeared likely that a leaching mechanism was responsible for the observed catalysis in both the Ag zeolite and Ag_2O cases. Silver oxide is prepared from silver nitrate and it generally contains traces of silver nitrate. We demonstrated that 10 mol% silver nitrate catalysed the 1, 3-dipolar cycloaddition reaction under the same conditions although the yields were lower and the time taken for the completion of the reaction was longer. Fortunately, we were able to obtain some silver oxide from Johnson Matthey, which contained only 5 ppm of silver nitrate. At this level, it was unlikely to be the major catalytic species when the silver oxide was used as a catalyst.

The standard imine cycloaddition reaction (Scheme 2) was carried out in toluene using 10 mol% silver oxide containing 5 ppm silver nitrate. The reaction was followed by thin-layer chromatography and was completed in 2 h. The reaction mixture was filtered through acid washed Celite to

Table 2. Cycloaddition of imines **3** and **5g–k** with dipolarophiles using Ag_2O as catalyst[a]

Entry	Imine	Dipolarophile[b]	R^1	R^2	Cycloadduct	Yield (%)
1	**3**	MA	2-Naphthyl	Me	**4**	100
2	**5g**	MA	2-Naphthyl	H	**6g**	100
3	**5h**	MA	Cyclohexyl	Me	**6h**	95
4	**5j**	MA	Cyclohexyl	H	**6j**	100
5	**5k**	MA	2-Naphthyl	$CH_2CH_2SCH_3$	**6k**	100
6	**3**	7	2-Naphthyl	Me	**8a**	100 (100% de)[14b c]
7	**5g**	7	2-Naphthyl	H	**8b**	97 (90% de)[14b c]
8	**5h**	7	Cyclohexyl	Me	**8c**	95 (99% de)[14b c]

[a] Reaction conditions: DBU/imine/dipolarophile = 1:1:2, Ag_2O (10 mol%), toluene, 25 °C, 2 h.
[b] MA = methyl acrylate.
[c] Determined by chiral HPLC—see Section 3.

Table 3. Filtration experiments for the reaction shown in Scheme 2

Reaction	Dipolarophile	DBU	Imine	Silver oxide	Time (h)	Conversion (%)
1	Yes	Yes	No	Yes	72	50
2	Yes	No	Yes	Yes	72	30
3	No	Yes	Yes	Yes	2	100
4[a]	Yes	Yes	Yes	Yes	2	100

[a] No filtration.

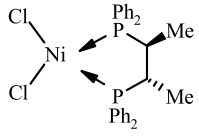
remove all the solids. If any silver was passed through the Celite, it was assumed to be a soluble silver species. The toluene filtrate was then evaporated to dryness under reduced pressure and the residue sent for accurate silver analysis. It was found that soluble silver had passed through the Celite filtration and that the amount (0.44% Ag) exceeded that attributable to silver nitrate alone (11×10^{-5} % Ag).

Using the same filtration technique the mode of solubilisation was examined in more detail. A series of reactions was carried out for 2 h omitting one of the reagents. The reaction mixture was then filtered through Celite and the reagent, which was left out in the first stage was added to the filtrate (Table 3). The reaction was then allowed to proceed for the time shown in Table 3 and progress was followed by thin layer chromatography.

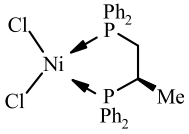
The results in Table 3 demonstrate that both imine and DBU are required for any appreciable solubilisation of silver species from the silver oxide catalyst, and therefore rate enhancement (reactions 3 and 4). When either DBU or imine was omitted (reactions 1 and 2) the reaction was substantially retarded and DBU alone effected a greater solubilisation of Ag(I) than the imine alone. In conclusion, both DBU and imine are required for effective solubilisation of Ag(I) and catalysis.

1.3. Ni(II)–phosphine complexes as catalysts

Nickel(II) catalysed 1,3-dipolar cycloadditions of azomethine ylides were studied. It was found that both $NiCl_2$(dppe), or $NiCl_2(PPh_3)_2$, in combination with Et_3N (1.5 mol equiv) efficiently promoted the cycloaddition reactions of imine **3** with methyl acrylate to give the cycloadduct **4** in 97% yield over 3 h (Scheme 2). The reactions were performed in dichloromethane using equimolar amounts of the Ni(II) complex and imine.



9



10

Previous success with chiral Co(II)[13] and Ag(I)[14] complexes as catalysts for Scheme 2 encouraged us to explore chiral Ni(II)–phosphine complexes. Such complexes have been widely used in a range of catalytic reactions.[24] Nickel(II) halides are known to form a large number of complexes with nitrogen or phosphorus donor ligands[25] and Ni(II) aldimine complexes are known.[26] The square planar chiral nickel(II) complexes **9** and **10** were prepared[27] and evaluated in Scheme 2. Reactions were carried out in dichloromethane using equimolar amounts of the chiral Ni(II) complexes **9** and **10** and imine. When **9** was employed racemic **4** was isolated in 49% yield after 27 h. The dramatic increase in the reaction time, the large reduction in yield of the cycloadduct and lack of enantioselectivity clearly relates to the coordination chemistry of Ni(II).[28] Octahedral and distorted octahedral complexes are the most common but numerous four-coordinate tetrahedral and square planar complexes of Ni(II) are known, together with five-coordinate square pyramidal and trigonal bipyramidal complexes.[29] Equilibria exist between the different structural types in solution and are frequently temperature and concentration dependent. The absence of enantioselectivity and the long reaction time indicates that the cycloaddition reaction is catalysed by 'free Ni(II)' ions resulting from equilibria involving dissociation of the chiral phosphine. Similarly when equimolar amounts of chiral Ni(II) complex **10** and imine were reacted under the same reaction conditions the product, isolated in 92% yield after 24 h, was essentially racemic (4% ee).

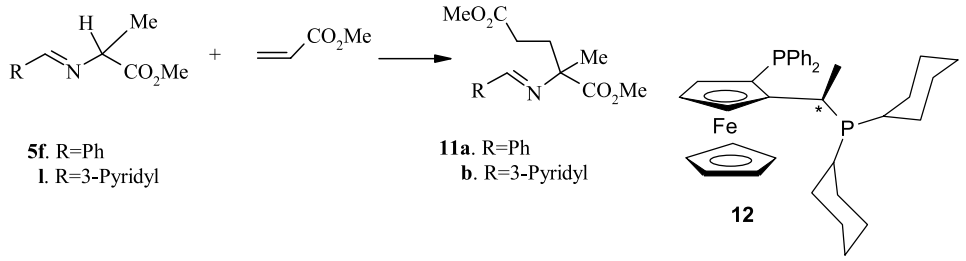
1.4. Cu(I) salts as catalysts

The success of the Ag_2O catalysed processes encouraged us to evaluate Cu_2O and other simple Cu(I) salts as catalysts for Scheme 2. When imines **5f** and **5l** were reacted separately with methyl acrylate in dichloromethane in the presence of Cu_2O (1 mol equiv) and DBU (1.12 mol equiv) the products were the Michael adducts **11a**[30] and **11b** (82–83%) (Scheme 3).

When the reaction was repeated with imine **5l** and a stoichiometric amount of CuCN replacing the Cu_2O the desired cycloadduct **6l**[31] was obtained in 72% yield. However, the same reaction with 10 mol% of CuCN afforded a 1:1 mixture of Michael adduct **11** and cycloadduct **6l**.

We then turned our attention to CuI as a possible catalyst. Cuprous iodide has found numerous applications in organic synthesis including asymmetric Kharasch reactions,[32] asymmetric conjugate addition of Grignard reagents,[33] enantioselective oxidative biaryl coupling,[34] and as an additive in many Pd(0) catalysed cross-coupling processes, for example, the Sonogashira rection.[35]

When Scheme 2 was carried out in dichloromethane with 10 mol% CuI in combination with DBU as base the desired cycloadduct **4** was isolated in 87% yield after 4 h. Analogous cycloadditions of imines **5a**, **5c**, and **5f** afforded the expected cycloadducts **6a**, **6c** and **6f** in 83–92% yield over 4–6 h. Repeating Scheme 2 with 10 mol% CuBr afforded **4** in 64% yield. The coordination chemistry of CuI with N-ligands is often complex[36] and frequently involve



Scheme 3.

aggregates.[37] However, in our studies up to 80% ee was achieved with CuI and ligand **12**.[38] Asymmetric versions of this process employing chiral $Cu(OTf)_2$[13d,e] complexes were reported after our own work was completed. Thus, it would appear that both Cu(I) and Cu(II) salts are effective catalysts with soft anions most effective for Cu(I) and hard anions for Cu(II).

2. Summary

Silver exchanged zeolites have been demonstrated to function as imine cycloaddition catalysts via leaching of silver species and are thus sources of of homogeneous catalytic silver salts. A leaching mechanism also operates in the case of 20% AgCl on titania. In this case, the titania support retards catalysis. The use of catalytic Ag_2O in toluene also involves soluble silver species and the solid→solution phase transfer involves both the imine and the amine base. Ni(II) complexes as shown to promote the imine cycloaddition for the first time. However, the use of chiral Ni(II)–phosphine complexes gives racemic cycloadducts. This together with the rate retarding effects of some chelating phosphines suggest the active Ni(II) catalyst is essentially phosphine free. Cu(I) salts are also shown, for the first time, to catalyse the imine cycloaddition. These salts show interesting selectivity with respect to their counterion. Thus, Cu_2O gives Michael addition products in good yield whilst CuCN gives either cycloadduct, when used in stoichiometric amount, or a 1:1 mixture of cycloadduct and Michael addition product when a substoichiometric amount (10 mol%) is employed. In contrast 10 mol% CuI proved an excellent catalyst whilst CuBr was somewhat less effective. The catalytic efficacy of Cu(I) salts of soft anions contrasts with that of the reported use of Cu(II) salts of hard anions as catalysts for analogous processes. The latter work[13d,e] appeared after completion of our own studies.

3. Experimental

General methods have been described previously.[39] Analytical grade anhydrous silver salts were used as purchased. Silver exchanged zeolites (20-mesh, 1/16″ pellets and 100-mesh) were purchased from Aldrich and were dried at 40 °C and 1 mmHg for 24 h before use. AgCl/TiO_2 (loading, 20% Ag) and Ag_2O containing 5 ppm $AgNO_3$ were supplied by Johnson Matthey. In all reactions involving silver(I) salts the reaction flask was covered with aluminium foil. Chiral HPLC was performed on a Chiralcel AD column (Daicel) eluting with 15% isopropanol in hexane, a flow rate of 1 mL/min and UV detection. All compounds were named using the ACD software version 8.0.

3.1. A. General procedure for preparation of imines

A mixture of amino acid methyl ester hydrochloride (22 mmol), aldehyde (20 mmol), triethylamine (20 mmol) and anhydrous magnesium sulfate (4 g) in dry DCM (50 mL) was stirred at room temperature for 16 h. On completion of the reaction (NMR monitoring), the mixture was diluted with DCM (100 mL), washed with water (2× 100 mL), dried ($MgSO_4$), filtered and the filtrate concentrated in vacuo to give the imine. Solid imines were purified by crystallisation from ether–hexane, and liquid imines were used in the next step without further purification because attempted purification led to decomposition. Imines **3** and **5a**,**d**–**h**,**l** were prepared following the literature methods.[14c,30,31,40]

3.1.1. Methyl (2*E*)-3-hydroxy-2-[(2-naphthylmethyl) imino]propanoate 5b. Prepared by general procedure A from serine methyl ester hydrochloride (3.42 g, 22 mmol), 2-naphthaldehyde (3.10 g, 20 mmol) and triethylamine (2.8 mL, 20 mmol). Crystallisation from dichloromethane afforded **5b** (2.67 g, 52%) as colourless prisms, mp 79–80 °C. (Found: C, 70.05; H, 5.75; N, 5.60. $C_{15}H_{15}NO_3$ requires: C, 70.05; H, 5.85; N, 5.45%); δ ($CDCl_3$, 250 MHz): 8.39 (s, 1H, N=CH), 8.00–7.96 (m, 2H, Ar–H), 7.82–7.67 (m, 3H, Ar–H), 7.53–7.43 (m, 2H, Ar–H), 4.22–4.02 (m, 3H, C*H*CO_2Me and CH_2OH) and 3.73 (s, 3H, OMe); *m/z* (%): 257 (M^+, 30), 198(100), 167(12) and 103(20).

3.1.2. Methyl (2*E*)-3-(2,3-dihydro-1*H*-indol-2-yl)-2-[(2-naphthylmethyl)imino]propanoate 5c. Prepared by general procedure A from tryptophan methyl ester hydrochloride (5.60 g, 22 mmol), 2-naphthaldehyde (3.10 g, 20 mmol) and triethylamine (2.8 mL, 20 mmol). Crystallisation from ether–hexane afforded **5c** (5.41 g, 76%) as colourless plates, mp 155–157 °C. (Found: C, 77.30; H, 5.55; N, 7.65. $C_{23}H_{20}N_2O_2$ requires: C, 77.50; H, 5.60; N, 7.85%); δ ($CDCl_3$, 250 MHz): 7.93 (s, 1H, N=CH), 8.16–7.23 (m, 12H, ArH), 4.36 (dd, 1H, J=7.6, 4.7 Hz, C*H*CO_2Me), 3.81 (s, 3H, OMe), and 3.41 and 3.18 (2× dd, 2×1H, J=13.3, 4.7, 13.3, 7.6 Hz, CH_2); *m/z* (%): 356 (M^+, 48), 297(75), 202(24) and 130(100).

3.1.3. Methyl (2*E*)-*N*-(cyclohexylmethylene)methionate 5i. Prepared by general procedure A from methionine

methyl ester hydrochloride (4.39 g, 22 mmol), cyclohexyl carboxaldehyde (2.26 g, 20 mmol) and triethylamine (2.8 mL, 20 mmol). The product (2.83 g, 55%) was obtained as a colourless oil, which was used without further purification. Found (HRMS, $M^+ + H$): 258.1525. $C_{13}H_{23}O_2SN$ requires: 258.1528. δ ($CDCl_3$, 250 MHz): 7.55 (d, 1H, $J=5.6$ Hz, N=CH), 3.90 (dd, 1H, $J=8.6$, 5.0 Hz, C*H*CO_2Me), 3.74 (s, 3H, OMe), 2.63–2.32 (m, 2H, CH_2), 212–210 (m, 2H, CH_2), 2.08 (s, 3H, SMe) and 1.82–1.21 (m, 11H, cyclohexyl–H); *m/z* (ES, %): 258 ($M^+ + H$).

3.1.4. Methyl (2*E*)-*N*-(cyclohexylmethylene)glycinate 5j. Prepared by general procedure A from glycine methyl ester hydrochloride (3.07 g, 22 mmol), cyclohexyl carboxaldehyde (2.26 g, 20 mmol) and triethylamine (2.8 mL, 20 mmol). The product (2.27 g, 62%) was obtained as a colourless oil, which was used without further purification. Found (HRMS, $M^+ + H$): 184.1336. $C_{10}H_{17}O_2N$ requires: 184.1337. δ ($CDCl_3$, 250 MHz): 7.54 (d, 1H, $J=5.1$ Hz, N=CH), 4.16 (s, 2H, CH_2), 3.75 (s, 3H, OMe) and 2.35–1.21 (m, 11H, cyclohexyl–H); *m/z* (ES, %): 184 ($M^+ + H$).

3.1.5. Methyl (2*E*)-*N*-(2-naphthylmethylene)methionate 5k. Prepared by general procedure A from methionine methyl ester hydrochloride (4.39 g, 22 mmol), 2-naphthaldehyde (3.10 g, 20 mmol) and triethylamine (2.8 mL, 20 mmol). Crystallisation from dichloromethane afforded **5k** (5 g, 83%) as colourless prisms, mp 52–54 °C. (Found: C, 67.90; H, 6.05; N, 4.15; S, 10.90. $C_{17}H_{19}NO_2S$ requires: C, 67.75; H, 6.30; N, 4.65; S, 10.65%); δ ($CDCl_3$, 250 MHz): 8.47 (s, 1H, N=CH), 8.09–7.47 (m, 7H, ArH), 4.28 (dd, 1H, $J=7.9$, 5.4 Hz, C*H*CO_2Me), 3.76 (s, 3H, OMe), 2.39 (m, 2H, CH_2S), 2.31 (m, 2H, CH_2CH_2S) and 2.09 (s, 3H, SMe); *m/z* (%): 301 (M^+, 67), 242(100), 195(37) and 181(14).

3.2. B. General procedure for silver exchanged zeolite catalysed 1,3-dipolar cycloaddition reactions

Dried silver exchanged zeolite (9.72 g or 4.32 g based on 20 or 45% silver in partially and fully exchanged zeolite, respectively) was added to a solution of imine (12 mmol) in toluene (20 mL). The mixture was stirred for 5 min before the dipolarophile (24 mmol) and DBU (12 mmol) were added and stirring was continued until the starting materials had disappeared (TLC). The reaction mixture was quenched with saturated aqueous ammonium chloride, filtered and the filtrate extracted with dichloromethane (3×20 mL). The combined extracts were dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to afford the pyrrolidine.

3.3. C. General procedure for silver(I) oxide catalysed 1, 3-dipolar cycloaddition reactions

A mixture of imine (12 mmol) and silver oxide (0.28 g, 1.2 mmol, 10 mol%) in toluene (20 mL) was stirred for 5 min, dipolarophile (24 mmol) and DBU (12 mmol) added and stirring continued until TLC monitoring showed all the starting materials had disappeared. The reaction mixture was quenched with a saturated solution of ammonium chloride (20 mL) and extracted with dichloromethane (3× 20 mL). The combined extracts were dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to afford the pyrrolidine.

3.4. D. General procedure for cycloaddition reactions catalysed by Ni(II)–phosphine complexes

Ni(II)–phosphine complex (1 equiv) was stirred for 5 min in dichloromethane. Imine (1 equiv) was added and the reaction mixture was stirred for 15 min. Methyl acrylate (3 equiv) and triethylamine (1.5 equiv) were then added and the reaction was stirred at room temperature until the reaction was complete (TLC). The reaction mixture was washed with water, the aqueous layer extracted with dichloromethane and the combined fractions were dried ($MgSO_4$), filtered and evaporated under vacuum. Flash chromatography afforded the cycloadducts.

3.5. E. General procedure for copper(I) iodide catalysed 1,3-dipolar cycloaddition reactions

Cuprous iodide (0.112 mmol, 10 mol%) was added to a stirred solution of imine (1.12 mmol) in dichloromethane (15 mL). Methyl acrylate (1.68 mmol) and DBU (1.12 mmol) were then added dropwise over 5 min. The reaction was followed by TLC until the starting materials had disappeared (4–6 h), then quenched (saturated aqueous NH_4Cl) and extracted with dichloromethane (3×10 mL). The combined extracts were dried ($MgSO_4$), filtered and the solvent removed in vacuo. The crude pyrrolidine was purified by flash column chromatography.

3.5.1. Dimethyl 2-methyl-5-(2-naphthyl)pyrrolidine-2,4-dicarboxylate 4. Prepared by general procedure B from **3** (2.89 g, 12 mmol) and methyl acrylate (2.24 g, 24 mmol). Work up afforded a colourless solid, which was purified by column chromatography eluting with 3:2 v/v ether–hexane to give **4** (3.73 g, 100%), which crystallised from dichloromethane–hexane as colourless needles, mp 83–85 °C. (Found: C, 69.50; H, 6.25; N, 4.55. $C_{19}H_{21}NO_4$ requires: C, 69.70; H, 6.45; N, 4.30%); δ ($CDCl_3$, 250 MHz): 7.84–7.76 (m, 4H, ArH), 7.48–7.37 (m, 3H, ArH), 4.81 (d, 1H, $J=7.3$ Hz, 5-H), 3.85 (s, 3H, OMe), 3.44 (ddd, 1H, $J=7.5$, 7.3, 4.8 Hz, 4-H), 3.12 (s, 3H, OMe), 2.79 (dd, 1H, $J=13.5$, 4.8 Hz, 3-H_a), 2.11 (dd, 1H, $J=13.5$, 7.5 Hz, 3-H_b) and 1.55 (s, 3H, Me); *m/z* (%): 327 (M^+, 61), 269(13), 268(62) and 209(100).

3.5.2. Dimethyl 2-benzyl-5-(2-naphthyl)pyrrolidine-2,4-dicarboxylate 6a. Prepared by general procedure B from **5a** (3.8 g, 12 mmol) and methyl acrylate (2.24 g, 24 mmol). Work up afforded a colourless solid, which was purified by column chromatography eluting with 1:1 v/v ether–hexane to give **6a** (4.64 g, 96%), which crystallised from dichloromethane–hexane as colourless prisms, mp 108–109 °C. (Found: C, 74.50; H, 6.05; N, 3.55. $C_{25}H_{25}NO_4$ requires: C, 74.45; H, 6.20; N, 3.45%); δ ($CDCl_3$, 250 MHz): 7.81–7.22 (m, 13H, ArH), 4.64 (d, 1H, $J=$ 7.5 Hz, 5-H), 3.74 (s, 3H, OMe), 3.28 (m, 1H, 4-H), 3.16 (d, 1H, $J=13.1$ Hz, CH_2Ph), 3.09 (s, 3H, OMe), 2.95 (d, 1H, $J=13.1$ Hz, CH_2Ph), 2.79 (dd, 1H, $J=13.6$, 4.8 Hz, 3-H_a) and 2.25 (dd, 1H, $J=13.6$, 7.5 Hz, 3-H_b); *m/z* (%): 403 (M^+, 67), 344(100), 285(45) and 194(54).

3.5.3. Dimethyl 2-hydroxymethyl-5-(2-naphthyl)pyrrolidine-2,4-dicarboxylate 6b. Prepared by general procedure B from **5b** (3.08 g, 12 mmol) and methyl acrylate (2.24 g, 24 mmol). Work up afforded a colourless solid, which was purified by column chromatography eluting with 4:1 v/v ether–hexane to give **6b** (2.06 g, 50%), which crystallised from ether as colourless needles, mp 117–119 °C. (Found: C, 66.30; H, 6.30; N, 3.90. $C_{19}H_{21}NO_5$ requires: C, 66.45; H, 6.10; N, 4.10%); δ ($CDCl_3$, 250 MHz): 7.82–7.75 (m, 4H, ArH), 7.50–7.33 (m, 3H, ArH), 5.29 (br, 1H, OH), 4.65 (d, 1H, $J=6.8$ Hz, 5-H), 3.87 (s, 3H, OMe), 3.79 and 3.54 (2×d, 2×1H, $J=10.7$ Hz, CH_2OH), 3.35 (m, 1H, 4-H), 3.14 (s, 3H, OMe), 2.62 (dd, 1H, $J=13.9$, 3.3 Hz, 3-H_a) and 2.09 (dd, 1H, $J=13.9$, 7.4 Hz, 3-H_b); *m/z* (%): 343 (M^+, 57), 284(100), 225(58) and 194(39).

3.5.4. Dimethyl 2-(1*H*-indol-2-ylmethyl)-5-(2-naphthyl) pyrrolidine-2,4-dicarboxylate 6c. Prepared by general procedure B from **5c** (3.85 g, 12 mmol) and methyl acrylate (2.24 g, 24 mmol). Work up afforded a colourless solid, which was purified by column chromatography eluting with 3:2 v/v ether–hexane to give **6c** (5.05 g, 93%), which crystallised from dichloromethane–hexane as colourless prisms, mp 116–118 °C. (Found: C, 73.20; H, 5.70; N, 6.50. $C_{27}H_{26}N_2O_4$ requires: C, 73.30; H, 5.90; N, 6.35%); δ ($CDCl_3$, 250 MHz): 8.10 (br, 1H indole NH), 7.79–7.65 (m, 4H, ArH), 7.44–7.06 (m, 7H, ArH), 4.71 (d, 1H, $J=7.2$ Hz, 5-H), 3.70 (s, 3H, OMe), 3.47 (m, 1H, 4-H), 3.22 (s, 2H, CH_2), 3.09 (s, 3H, OMe), 2.78 (dd, 1H, $J=13.4$, 5.5 Hz, 3-H_a) and 2.28 (dd, 1H, $J=13.4$, 7.6 Hz, 3-H_b); *m/z* (%): 443 (M^+, 53), 383(100), 324(19) and 194(33).

3.5.5. Dimethyl 2-methyl-5-pyridin-2-ylpyrrolidine-2,4-dicarboxylate 6d. Prepared by general procedure B from **5d** (2.3 g, 12 mmol) and methyl acrylate (2.24 g, 24 mmol). Work up afforded the crude product, which was purified by column chromatography eluting with ether to 10% methanol in ether to give **6d** (2.77 g, 80%) as a yellow oil. (Found: C, 60.30; H, 6.25; N, 10.30. $C_{14}H_{18}N_2O_4$ requires: C, 60.45; H, 6.45; N, 10.05%); δ ($CDCl_3$, 250 MHz): 8.52–5.16 (m, 4H, ArH), 4.68 (d, 1H, $J=7.6$ Hz, 5-H), 3.81 (s, 3H, OMe), 3.41 (m, 1H, 4-H), 3.27 (s, 3H, OMe), 2.70–2.75 (m, 2H, 3-H_a/H_b) and 1.51 (s, 3H, Me); *m/z* (%): 278 (M^+, 32), 219(100), 160(17) and 145(28).

3.5.6. Dimethyl 2-benzyl-5-(4-methoxyphenyl)pyrrolidine-2,4-dicarboxylate 6e. Prepared by general procedure B from **5e** (3.4 g, 12 mmol) and methyl acrylate (2.24 g, 24 mmol). Work up afforded a colourless solid, which was purified by column chromatography eluting with 1:1 v/v ether–hexane to give **6e** (3.81 g, 86%) as a colourless viscous oil. (Found: C, 68.40; H, 6.35; N, 3.60. $C_{21}H_{23}NO_5$ requires: C, 68.30; H, 6.25; N, 3.80%); δ ($CDCl_3$, 250 MHz): 7.26–7.15 (m, 7H, ArH), 6.81 (m, 2H, ArH), 4.47 (d, 1H, $J=7.5$ Hz, 5-H), 3.77, 3.75 and 3.22 (3×s, 3×3H, 3×OMe), 3.15 (m, 1H, 4-H), 3.10 and 2.92 (2×d, 2×1H, $J=13.1$ Hz, $ArCH_2$), 2.74 (dd, 1H, $J=5.2$, 13.7 Hz, 3-H_a) and 2.18 (dd, 1H, $J=7.5$, 13.7 Hz, 3-H_b); *m/z* (%): 369 (M^+, 53), 310(100), 262(19) and 251(33).

3.5.7. Dimethyl 2-methyl-5-phenylpyrrolidine-2,-4-dicarboxylate 6f. Prepared by general procedure B from **5f** (2.29 g, 12 mmol) and methyl acrylate (2.24 g, 24 mmol). Work up afforded a colourless solid, which was purified by column chromatography eluting with 1:2 v/v ether–hexane to give **6f** (2.99 g, 90%) as a colourless oil. (Found: C, 64.95; H, 6.90; N, 5.15. $C_{15}H_{19}NO_4$ requires: C, 65.00; H, 6.85; N, 5.05%); δ ($CDCl_3$, 250 MHz): 7.31–7.22 (m, 5H, ArH), 4.65 (d, 1H, $J=7.5$ Hz, 5-H), 3.82 (s, 3H, OMe), 3.52 (m, 1H, 4-H), 3.20 (s, 3H, OMe), 2.72 (dd, 1H, $J=13.6$, 5.3 Hz, 3-H_a), 2.05 (dd, 1H, $J=13.6$, 7.5 Hz, 3-H_b) and 1.50 (s, 3H, Me); *m/z* (%): 277 (M^+, 61), 269(13), 268(61) and 209(100).

3.5.8. Dimethyl 5-(2-naphthyl)pyrrolidine-2,4-dicarboxylate 6g. Prepared by general procedure C from **5g** (2.24 g, 12 mmol) and methyl acrylate (2.24 g, 24 mmol). Work up afforded a colourless solid, which was purified by column chromatography eluting with 1:1 v/v ether–hexane to give **6f** (3.57 g, 100%) as colourless plates, mp 161–163 °C. (Found: C, 68.90; H, 6.20; N, 4.40. $C_{18}H_{19}NO_4$ requires: C, 69.00; H, 6.10; N, 4.45%); δ ($CDCl_3$, 250 MHz): 7.85–7.26 (m, 7H, ArH), 4.65 (d, 1H, $J=$ 8.1 Hz, 5-H), 4.04 (dd, 1H, $J=8.8$, 7.3 Hz, 2-H), 3.82 (s, 3H, OMe), 3.38 (m, 1H, 4-H), 3.12 (s, 3H, OMe) and 2.43–2.85 (m, 2H, 3-H_a/H_b); *m/z* (%): 313 (M^+, 60), 254(100), 240(62) and 195(10).

3.5.9. Dimethyl 5-cyclohexyl-2-methylpyrrolidine-2,4-dicarboxylate 6h. Prepared by general procedure C from **5h** (2.36 g, 12 mmol) and methyl acrylate (2.24 g, 24 mmol). Work up afforded a colourless solid, which was purified by column chromatography eluting with 1:1 v/v ether–hexane to give **6h** (3.26 g, 95%) as colourless plates, mp 125–127 °C. (Found: C, 63.35; H, 9.00; N, 4.70. $C_{15}H_{25}NO_4$ requires: C, 63.60; H, 8.85; N, 4.95%); δ ($CDCl_3$, 250 MHz): 3.72 and 3.61 (2×s, 2×3H, 2×OMe), 2.90–2.96 (m, 2H, 5-H and 4-H), 2.68 (m, 1H, 3-H_a), 2.57 (m, 1H, 3-H_b), 1.40 (s, 3H, Me) and 2.05–0.90 (m, 11H, cyclohexyl-H); *m/z* (%): 283 (M^+, 21), 224(100), 165(47) and 150(17).

3.5.10. Dimethyl 5-cyclohexyl-2[2-(methylthioethyl]pyrrolidine-2,4-dicarboxylate 6i. Prepared by general procedure B from **5i** (3.08 g, 12 mmol) and methyl acrylate (2.24 g, 24 mmol). Work up afforded a colourless solid, which was purified by column chromatography eluting with 3:2 v/v ether–hexane to give **6i** (3.79 g, 92%) as a colourless viscous oil. (Found: C, 59.50; H, 8.50; N, 4.15; S, 9.05. $C_{17}H_{29}NO_4S$ requires: C, 59.45; H, 8.45; N, 4.10; S, 9.350%); δ ($CDCl_3$, 250 MHz): 3.77 and 3.63 (2×s, 2×3H, OMe), 2.95–2.83 (m, 2H, 5-H and 4-H), 2.65–2.50 (m, 3H. SCH_2 and 3-H_a), 2.26 (m, 1H, 3-H_b), 2.07 (s, 3H, SMe), 2.04–0.84 (m, 13H, CH_2 and cyclohexyl-H); *m/z* (%): 343 (M^+, 16), 296(80), 284(100) and 225(21).

3.5.11. Dimethyl 5-cyclohexylpyrrolidine-2,4-dicarboxylate 6j. Prepared by general procedure C from **5j** (2.2 g, 12 mmol) and methyl acrylate (2.24 g, 24 mmol). Work up afforded a colourless solid, which was purified by column chromatography eluting with 1:1 v/v ether–hexane to give **6j** (3.07 g, 95%) as colourless plates, mp 35–37 °C. (Found: C, 62.35; H, 8.75; N, 5.05. $C_{14}H_{23}NO_4$ requires: C, 62.45; H, 8.55; N, 5.20%); δ ($CDCl_3$+2 drops C_6D_6, 250 MHz): 3.83 (dd, 1H, $J=5.5$, 10.0 Hz, 2-H), 3.76 and 3.64 (2×s, 2×3H, 2×OMe), 2.94 (ddd, 1H, $J=1.7$, 5.8,

7.3 Hz, 4-H), 2.82 (dd, 1H, $J=5.8$, 9.5 Hz, 5-H), 2.39–2.15 (m, 2H, 3-H_a/H_b), and 2.20–1.15 (m, 11H, cyclohexyl-H); *m/z* (%): 269 (M^+, 21), 210(100), 186(47) and 150(17).

3.5.12. Dimethyl 2-(2-methylthioethyl)-5-(2-naphthyl) pyrrolidine-2,4-dicarboxylate 6k. Prepared by general procedure C from **5k** (3.61 g, 12 mmol) and methyl acrylate (2.24 g, 24 mmol). Work up afforded a colourless solid, which was purified by column chromatography eluting with 1:1 v/v ether–hexane to give **6h** (4.5 g, 100%) as colourless needles, mp 176–178 °C. (Found: C, 65.00; H, 6.50; N, 3.70; S, 8.35. $C_{21}H_{25}NO_4S$ requires: C, 65.10; H, 6.45; N, 3.60; S, 8.250%); δ ($CDCl_3$, 250 MHz): 7.75–7.68 (m, 4H, ArH), 7.39–7.27 (m, 3H, ArH), 4.63 (d, 1H, $J=6.9$ Hz, 5-H), 3.79 (s, 3H, OMe), 3.28 (ddd, 1H, $J=7.4$, 6.9, 3.9 Hz, 4-H), 3.04 (s, 3H, OMe), 2.71–2.60 (m, 2H, CH_2SMe and 3-H_a), 2.32 (m, 1H, CH_2SMe), 2.17–2.01 (m, 2H, CH_2CH_2SMe and 3-H_b), 2.03 (s, 3H, SMe) and 1.88 (m, 1H, CH_2CH_2SMe); *m/z* (%): 387 (M^+, 56), 328(100), 369(31) and 222(46).

3.5.13. Methyl (1*S*,2*R*,4*S*,5*R*,8*R*)-2-methyl-4-(2′-naphthyl)-3-aza-6-oxo-7-oxa-8-(1′*R*,2′*S*,5′*R*-menthyloxy)-bicyclo[3.3.0]octane-2-carboxylate 8a. Prepared by general procedure B and C. The product showed identical spectral data as described in literature.[14b]

3.5.14. Methyl (1*S*,2*R*,4*S*,5*R*,8*R*)-4-(2′-naphthyl)-3-aza-6-oxo-7-oxa-8-(1′*R*,2′*S*,5′*R*-menthyloxy)-bicyclo[3.3.0] octane-2-carboxylate 8b. Prepared by general procedure B and C. The product showed identical spectral data as described in literature.[14b]

3.5.15. Methyl (1*S*,2*R*,4*S*,5*R*,8*R*)-2-methyl-4-(cyclohexyl)-3-aza-6-oxo-7-oxa-8-(1′*R*,2′*S*,5′*R*-menthyloxy)-bicyclo[3.3.0]octane-2-carboxylate 8c. Prepared by general procedure B and C. The product showed identical spectral data as described in literature.[14b]

3.5.16. 2-Methyl-2-{[1-pyridin-3-yl-methylidene]-amino}-pentanedioic acid dimethyl ester 11b. Prepared by general procedure E from imine **5l** (215 mg, 1.12 mmol) and methyl acrylate (0.15 mL, 1.68 mmol). Purification by flash column chromatography (ether) afforded the product (21 mg, 82%) as a colourless gum. HRMS: $[M+H]^+C_{14}H_{19}N_2O_4$ requires 279.1345; found 279.1347. δ (500 MHz): 8.87 (br, m, 1H, PyH), 8.66 (br, s, 1H, PyH), 8.34 (s, 1H, N=CH), 8.15 (dt, 1H, $J=8.2$, 1.8 Hz, PyH), 7.37 (dd, 1H, $J=8.2$, 5.2 Hz, PyH), 3.76 (s, 3H, CO_2CH_3), 3.65 (s, 3H, CO_2CH_3), 2.54–2.34 (m, 3H, C$HCH_2CO_2CH_3$), 2.17 (ddd, 1H, $J=13.6$, 9.3, 6.6 Hz, C$HCH_2CO_2CH_3$) and 1.54 (s, 3H, CH_3). δ ^{13}C: 191.2 (C=N), 2×174.1 (C=O), 157.7, 152.2, 150.9, 135.0 and 124.0 (PyC), 68.1 (4 °C), 52.7 and 52.0 (CO_2CH_3), 35.4 and 29.8 (CH_2) and 23.7 (CH_3). ν (film, cm^{-1}): 3055 (C–$H_{stretch}$), 1732 (C=O_{ester}), 1647 (C=N) and 1591 (C=$C_{aromatic}$); *m/z* (ES^+, %): 279 ($[M+H]^+$, 100).

Acknowledgements

We thank the Mary and Alice Smith Memorial Fund (E.M.) and the Commonwealth Scholarship Commision in the UK (M.A.B.S.) for scholarships and Leeds University, GlaxoSmithKline and the EPSRC for support. We thank the EPSRC, Leeds University and the Royal Society for support.

References and notes

1. Li, Y.; Armor, J. N. *Appl. Catal., B* **1993**, *2*, 239–256. Li, Z.; Flytzani-Stephanopoulos, M. *Appl. Catal., A* **1997**, *165*, 15–34. Matsuoka, M.; Ju, W.-S.; Anpo, M. *Chem. Lett.* **2000**, 626–627.
2. Teng, J. W.; Cai, T. X.; Bao, X. H. *Chin. Chem. Lett.* **1999**, *10*, 83–86. Martens, J. A.; Cauvel, A.; Francis, A.; Hermans, C.; Jayat, F.; Remy, M.; Keung, M.; Lievens, J.; Jacobs, P. A. *Angew. Chem., Int. Ed.* **1998**, *37*, 1901–1903.
3. Murray, D. K.; Howard, T.; Goguen, P. W.; Krawietz, T. R.; Haw, J. F. *J. Am. Chem. Soc.* **1994**, *116*, 6354–6360.
4. Chatterjee, S.; Greene, H. L.; Park, Y. J. *J. Catal.* **1992**, *138*, 179–194.
5. Bagnasco, G.; Ciamgelli, P.; Czaran, E.; Rapp, J.; Russo, G. *Metal Microstrutures in Zeolites*; Elsevier: Amsterdam, 1982.
6. Nomura, M.; Fujihara, Y. *Chem. Express* **1992**, *7*, 121–124.
7. Ozin, G. A.; Hugues, F. *J. Phys. Chem.* **1982**, *86*, 5174–5179. Ozin, G. A.; Hugues, F.; Matta, S. M.; McIntosh, D. F. *J. Phys. Chem.* **1983**, *87*, 3445–3450.
8. Thomas, R. L.; Sarker, A. K.; Kohata, K.; Abbas, S. A.; Matta, K. *Tetrahedron Lett.* **1990**, *31*, 2825–2828.
9. Probert, M. A.; Zhang, J.; Bundle, D. R. *Carbohydrate Res.* **1996**, *296*, 149–170. Zhang, J.; Otter, A.; Bundle, D. R. *Bioorg. Med. Chem.* **1996**, *4*, 1989–2001. Garegg, P. J.; Henrichson, C.; Norberg, T.; Ossowski, P. *Carbohydrate Res.* **1983**, *119*, 95–100.
10. Grigg, R.; Gunaratne, H. Q. N.; Sridharan, V. *Tetrahedron* **1987**, *43*, 5887–5898.
11. Grigg, R.; Sridharan, V. *Adv. Cycloaddition* **1993**, *3*, 161–204.
12. Barr, D. A.; Grigg, R.; Sridharan, V. *Tetrahedron Lett.* **1989**, *30*, 4727–4730.
13. (a) Allway, P.; Grigg, R. *Tetrahedron Lett.* **1991**, *32*, 5817–5820. (b) Grigg, R. *Tetrahedron: Asymmetry* **1995**, *6*, 2475–2486. (c) Longmire, J. M.; Wang, B.; Zhang, X. *J. Am. Chem. Soc.* **2002**, *124*, 13400–13401. (d) Gothelf, K. V.; Hazell, R. G.; Jorgensen, K. A. *Angew. Chem., Int. Ed.* **2002**, *41*, 4236–4238. (e) Oderaotoshi, Y.; Cheng, W.; Fujitomi, S.; Kasano, Y.; Minakata, S.; Komatsu, M. *Org. Lett.* **2003**, *5*, 5043–5046. (f) Chen, C.; Li, X.; Schreiber, S. L. *J. Am. Chem. Soc.* **2003**, *125*, 10174–10175.
14. (a) Barr, D. A.; Dorrity, M. J.; Grigg, R.; Malone, J. F.; Montgomery, J.; Rajviroongit, S.; Stevenson, P. *Tetrahedron Lett.* **1990**, *31*, 6569–6572. (b) Cooper, D. M.; Grigg, R.; Hargreaves, S.; Kennewell, P.; Redpath, J. *Tetrahedron* **1995**, *51*, 7791–7808. (c) Barr, D. A.; Dorrity, M. J.; Grigg, R.; Hargreaves, S.; Malone, J. F.; Montgomery, J.; Redpath, J.; Stevenson, P.; Thornton-Pett, M. *Tetrahedron* **1995**, *51*, 273–294. (d) Coulter, T.; Grigg, R.; Malone, J. F.; Sridharan, V. *Tetrahedron Lett.* **1991**, *32*, 5417–5420. (e) Grigg, R.; Sridharan, V.; Suganthan, S.; Bridge, A. W. *Tetrahedron* **1995**, *51*, 295–306. (f) Grigg, R.; Rankovic, Z.; Thornton-Pett, M.; Somasunderam, A. *Tetrahedron* **1993**, *49*, 8679–8690. (g) Grigg, R.; Thornton-Pett, M.; Yoganathan, G. *Tetrahedron* **1999**, *55*, 8129–8140.
15. Pyne, S. G.; Safaei, J.; Schafer, A. K.; Javidan, A.;

Skelton, B. W.; White, A. H. *Aust. J. Chem.* **1998**, *51*, 137–158. Pyne, S. G.; Safaei-G, J.; Koller, F. *Tetrahedron Lett.* **1995**, *36*, 2511–2514. Pyne, S. G.; Dikic, B.; Gordon, P.; Skelton, B. W.; White, A. H. *Aust. J. Chem.* **1993**, *46*, 73–93. Waldmann, H.; Blaeser, E.; Jansen, M.; Letschert, H. P. *Angew. Chem., Int. Ed. Engl.* **1994**, *106*, 717–719. Waldmann, H.; Blaeser, E.; Jansen, M.; Letschert, H.-P. *Chem. Eur. J.* **1995**, *1*, 150–154. Annunziata, R.; Cinquini, M.; Cozzi, F.; Raimondi, L.; Pilati, T. *Tetrahedron: Asymmetry* **1991**, *2*, 1329–1342. Galley, G.; Liebscher, J.; Paetzel, M. *J. Org. Chem.* **1995**, *60*, 5005–5010. Patzel, M.; Galley, G.; Jones, P. G.; Chrapkowsky, A. *Tetrahedron Lett.* **1993**, *34*, 5707–5710. Garcia Ruano, J. L.; Tito, A.; Peromingo, M. T. *J. Org. Chem.* **2002**, *67*, 981–987. Viso, A.; Fernandez de la Pradilla, R.; Guerrero-Strachan, C.; Alonso, M.; Martinez-Ripoll, M.; Andre, I. *J. Org. Chem.* **1997**, *62*, 2316–2317.

16. Murphy, M. M.; Schullek, J. R.; Gordon, E. M.; Gallop, M. A. *J. Am. Chem. Soc.* **1995**, *117*, 7029–7030.
17. Hollinshead, S. P. *Tetrahedron Lett.* **1996**, *37*, 9157–9160. Marx, M. A.; Grillot, A.-L.; Louer, C. T.; Beaver, K. A.; Bartlett, P. A. *J. Am. Chem. Soc.* **1997**, *119*, 6153–6167. Gong, Y. D.; Najdi, S.; Olmstead, M. M.; Kurth, M. J. *J. Org. Chem.* **1998**, *63*, 3081–3086. Peng, G.; Sohn, A.; Gallop, M. A. *J. Org. Chem.* **1999**, *64*, 8342–8349.
18. Dondas, H. A.; Grigg, R.; MacLachlan, W. S.; MacPherson, D. T.; Markandu, J.; Sridharan, V.; Suganthan, S. *Tetrahedron Lett.* **2000**, *41*, 967–970.
19. Burton, G.; Ku, T. W.; Carr, T. K.; Kiesow, T.; Sarisky, R. T.; Lin-Goerke, J.; Baker, A.; Earnshaw, D. L.; Hofmann, G. A.; Keenan, R. M.; Dhanak, D. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1553–1556.
20. Zubia, A.; Mendoza, L.; Vivanco, S.; Aldaba, E.; Carrascal, T.; Lecea, B.; Arrieta, A.; Zimmerman, T.; Vidal-Vanaclocha, F.; Cossio, F. P. *Angew. Chem., Int. Ed.* **2005**, *44*, 2903–2907.
21. Booth, R. J.; Hodges, J. C. *Acc. Chem. Res.* **1999**, *32*, 18–26. Grabowska, U.; Rizzo, A.; Farnell, K.; Quibell, M. *J. Comb. Chem.* **2000**, *2*, 475–490. Ley, S. V.; Massi, A. *J. Comb. Chem.* **2000**, *2*, 104–107. Golisade, A.; Bressi, J. C.; Van, C. S.; Gelb, M. H.; Link, A. *J. Comb. Chem.* **2000**, *2*, 537–544. Bhattacharyya, S. *Comb. Chem. High Throughout Screen.* **2000**, *3*, 65–92.
22. Commercial 100-mesh silver exchanged zeolite contains 20% Ag (Aldrich) and 1 mol equiv Ag was used in the reaction.
23. Kim, Y.; Seff, K. *J. Phys. Chem.* **1978**, *82*, 925–929.
24. Consiglio, G.; Indolese, A. *J. Organomet. Chem.* **1993**, *463*, 23–27. Consiglio, G.; Indolese, A. *Organometallics* **1991**, *10*, 3425–3427.
25. Griswold, E.; Kleinberg, J.; Lee, R. H. *Inorg. Chem.* **1964**, *3*, 1278–1283. Venanzi, L. M. *J. Chem. Soc.* **1958**, 719–724.
26. Chakravorty, A.; Everett, G. W.; Holm, R. H. *Prog. Inorg. Chem.* **1966**, *7*, 83–214.
27. van Hecke, G. R.; Horrocks, W. D. *Inorg. Chem.* **1966**, *5*, 1968–1974. Consiglio, G.; Morandini, F.; Piccolo, O. *Inorg. Chim. Acta* **1982**, *57*, 15–19.
28. Kubiak, C. P. In Abel, E. W., Stone, F. G. A., Wilkinson, G., Eds.; Comprehensive Organometallic Chemistry II; Pergamon: New York, 1995; Vol. 9, pp 1–14.
29. Nicholls, D. In Trotman-Dickenson, A. F., Ed.; Comprehensive Inorganic Chemistry; Pergamon, 1975; Vol. 3, p 1109.
30. Barr, D. A.; Grigg, R.; Gunaratne, H. Q. N.; Kemp, J.; McMeekin, P.; Sridharan, V. *Tetrahedron* **1988**, *44*, 557–570.
31. Grigg, R.; Kemp, J.; Malone, J. F.; Rajviroongit, S.; Tangthongkum, A. *Tetrahedron* **1988**, *44*, 5361–5374.
32. Sekar, G.; DattaGupta, A.; Singh, V. K. *J. Org. Chem.* **1998**, *63*, 2961–2967.
33. Kanai, M.; Tomioka, K. *Tetrahedron Lett.* **1995**, *36*, 4275–4278.
34. Li, X.; Yang, J.; Kozlowski, M. C. *Org. Lett.* **2001**, *3*, 1137–1140.
35. Sonogashira, K. In Trost, B. M., Fleming, I., Eds.; Comprehensive Organic Synthesis; Pergamon: New York, 1991; Vol. 3, pp 521–549.
36. Janiak, C.; Vehlin, L.; Wu, H.-P.; Klufers, P.; Piotrowski, H.; Sharmann, T. G. *J. Chem. Soc., Dalton Trans.* **1999**, 3121–3131.
37. Victoriano, L. I.; Garkand, M. T.; Vega, A.; Lopez, C. *Inorg. Chem.* **1998**, *37*, 2060–2062. Alemany, P.; Bengtsson-Kloo, L.; Holmberg, B. *Acta Chem. Scand.* **1998**, *52*, 718–727.
38. Millington, E. Ph.D. Thesis, Leeds University, 2003.Togni, A.; Breutel, C.; Schnyder, A.; Spindler, F.; Landert, H.; Tijani, A. *J. Am. Chem. Soc.* **1994**, *116*, 4062–4066.
39. Dondas, H. A.; Fishwick, C. W. G.; Grigg, R.; Kilner, C. *Tetrahedron* **2004**, *60*, 3473–3485.
40. Grigg, R.; Montgomery, J.; Somasunderam, A. *Tetrahedron* **1992**, *48*, 10431–10442.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron 61 (2005) 8686–8693
Tetrahedron

Multiple hydrogen-bond-induced supramolecular nanostructure from a pincer-like molecule and a [60]fullerene derivative

Junpeng Zhuang, Weidong Zhou, Xiaofang Li, Yongjun Li, Ning Wang, Xiaorong He, Huibiao Liu, Yuliang Li,* Li Jiang, Changshui Huang, Shuang Cui, Shu Wang and Daoben Zhu*

CAS Key Laboratory of Organic Solids, Center for Molecular Sciences, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, People's Republic of China

Received 11 April 2005; revised 27 June 2005; accepted 29 June 2005

Abstract—A new supramolecular self-assembled system between a perylene bisimide bearing diaminopyridine-substituted isophthalamide groups (**PP**) and a [60]fullerene containing barbituric acid moiety (**C_{60}bar**) through a complementary six-point hydrogen-bonding interaction was constructed. The formation of hydrogen bonding was confirmed by ^{1}H NMR spectra studies in $CDCl_3$. Fluorescence quenching experiments indicated that the fluorescence of **PP** was greatly quenched by the hydrogen-bonded **C_{60}bar** ($K_{sv}=2.71\times10^4$ M^{-1}). A steady and rapid cathodic 0.15 μA cm^{-2} photocurrent response of the **PP/C_{60}bar** film deposited onto an ITO electrode was produced under the irradiation of 20 mW cm^{-2} white light, indicating the presence of photo-induced electron transfer between **PP** and **C_{60}bar**. TEM images showed that spherical particles were fabricated by the self-assembly of **PP** and **C_{60}bar** through hydrogen-bonding interaction.

1. Introduction

The [60]fullerene molecule has unique and attractive electronic, optical, and mechanical properties owing to its chemical properties and uniform, spherical, and nanoscale physical structure.[1] In order to develop fullerene-containing supramolecular assemblies and advanced materials,[2] [60]fullerene has been incorporated into multi-component molecular systems such as porphyrins,[3] rotaxanes,[4] and catenanes.[5] The encouraging properties of these [60]fullerene hybrids have attracted much more interests in the construction of even more complex two- and three-dimensional systems. And the self-assembly of [60]fullerene-based molecules has been found to be an efficient way to construct nanoscale aggregates with specific properties.[6]

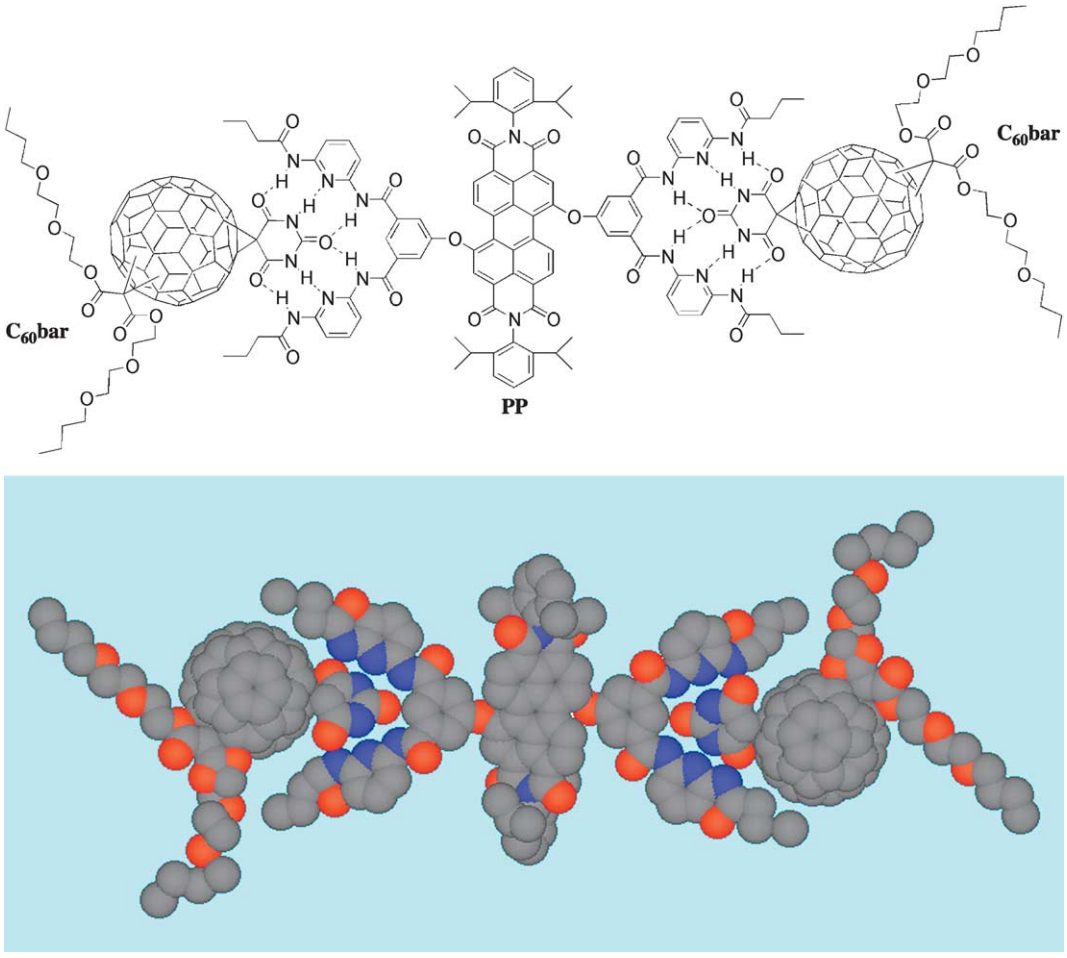
π-Conjugated perylene bis-imides represent one of the most thoroughly studied classes of organic semiconductors with a variety of different structures,[7] with possible applications such as fluorescent solar collectors,[8] photovoltaic devices,[9] dye lasers,[10] and molecular switches.[11] These dyes have outstanding chemical, thermal, and photochemical stability, which offers great opportunities for preparing new materials.

Hydrogen bonds are among the most useful interactions to encourage the self-assembly of molecules into well-defined aggregates and to order an ensemble of molecules spontaneously into larger and more complex structure.[12] The strength and selectivity of hydrogen bonds can be improved by introducing arrays of donor (D) and acceptor (A) sites. Arrays of two and three hydrogen bonds have already been studied, and the self-complementary quadruple hydrogen bonds (DDAA) derived from diaminotriazines or diaminopyrimidines have also been studied for their high dimerization constants.[13] The self-assembly between cyanuric or barbituric acid wedge (ADA–ADA array) and a corresponding diaminopyridine-substituted isophthalamide receptor (DAD–DAD array) provided six-point hydrogen bonds, which made them attractive for molecular recognition studies, supramolecular polymer, and self-assembly of multi-chromophores.[14]

In our previous work based on the hydrogen-bonded supramolecular systems of [60]fullerene, we reported the self-assembly of 2,6-di(acylamino)pyridine-substituted [60]fullerene derivative with 1-dodecyluracil,[15a,b] perylene bisimide derivative[15c] and PPV derivative[15d] by three-point hydrogen-bonding interactions. These molecules tended to form stable nanoscale aggregates. Their energy and electron-transfer processes were investigated. Bassani et al. reported the facile synthesis of a fullerene-barbituric acid from C_{60} and 5-bromobarbituric acid by a modified

Keywords: Hydrogen bond; Fullerene; Supramolecular.
* Corresponding authors. Tel.: +86 10 62588934; fax: +86 10 82616576; e-mail: ylli@iccas.ac.cn

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.06.112



Scheme 1. Supramolecular system of hydrogen-bonded self-assembly of **PP/C_{60}bar** with a 3D model.

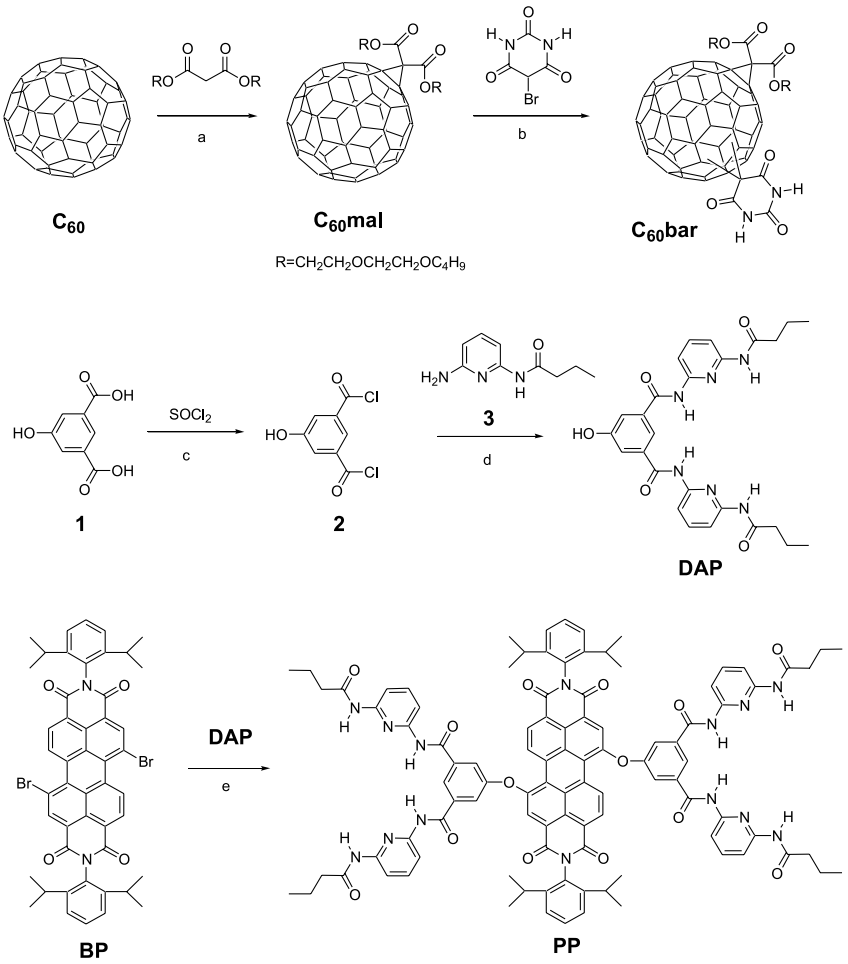
Bingel reaction.[16] This reaction introduced six hydrogen-bonding sites of a barbituric acid into [60]fullerene in one step. These sites were non-self-complementary, and their spatial arrangement made the fullerene-barbituric acid well suitable for the construction of fullerene-containing architectures. Although the structure of this fullerene-barbituric acid was novel, the poor solubility of this compound limited its application in supramolecular chemistry studies. In this paper, we introduced barbituric acid into [60]fullerene-di[2-(2-butoxy-ethoxy)-ethyl] malonate (**C_{60}mal**) to form a novel fullerene derivative (**C_{60}bar**) with excellent solubility in organic solvents. We also synthesized a perylene bisimide (**PP**) with two diaminopyridine-substituted isophthalamide groups for the formation of a pincer-like structure in order to increase hydrogen-bonding interaction. Because the association constant between the barbituric acid ring and the diaminopyridine-substituted isophthalamide receptors was in the range of 10^4–10^5 M^{-1},[14] the supromolecular assembly of **C_{60}bar** and **PP** exhibited strong interaction and formed uniform aggregates. (Scheme 1)

2. Results and discussion

The synthesis of **PP** and **C_{60}bar** was sketched in Scheme 1. **C_{60}mal** was synthesized from C_{60} and di[2-(2-butoxy-ethoxy)-ethyl] malonate by Bingel reaction using CBr_4 and DBU in toluene.[17] **C_{60}bar** was synthesized from **C_{60}mal** and 5-bromobarbituric acid according to the literature method.[16] The crude product was worked up on silica gel using a mixture of toluene/MeOH (v/v, 10:1) as the eluent, and the second band was collected to give **C_{60}bar**. The **C_{60}bar** had excellent solubility in toluene and $CHCl_3$, owing to the introduction of di[2-(2-butoxy-ethoxy)-ethyl] malonate into the fullerene-barbituric acid structure, therefore, greatly facilitated its application Scheme 2.

The cyclopropanation of C_{60} with two addends afforded mixed bisadducts, where *e* and *trans*-3 isomers were the main products.[18] In this work, the barbituric acid ring was introduced into C_{60} as the second addend. The product **C_{60}bar** contained position isomers. However, here we try to use **C_{60}bar** as a building block for the construction of supramolecular assemblies, the *trans*-3 and *e* isomers could form same supramolecular structure with diaminopyridine-substituted isophthalamide group by hydrogen-bonding interaction.

MALDI-TOF MS spectra of **C_{60}bar** showed three peaks, 1237.3 (M^+), 1110.4 (M^+ − barbituric acid ring) and 720.1 (M^+ − barbituric acid ring-bis[2-(2-butoxy-ethoxy)-ethyl] malonate). ^{1}H NMR spectra showed two broad peaks at δ 9.3 and 11.1, which were assigned to the resonance of the



Scheme 2. Synthesis of C_{60}**bar** and **PP**. (a) CBr_4, DBU, toluene, rt, 2 h, 44%; (b) DBU, toluene/DMSO (v/v, 95:5), rt, 7 h, 12%; (c) THF, reflux, 4 h; (d) pyridine, THF, 0–10 °C, 7 h, 56%; (e) K_2CO_3, 18-crown-6, toluene, 80 °C, 4 h, 88%.

imidic protons of barbituric acid ring. Because C_{60}**bar** was an isomeric mixture, the signals of imidic protons appeared at different positions and are rather broad.

N,*N*′-di(6-butyrylamino-pyridin-2-yl)-5-hydroxy-isophthalamide (**DAP**) was synthesized from *N*-butyryl-2,6-diaminopyridine, 5-hydroxy-benzene-1,3-dicarbonyl chloride and pyridine in anhydrous THF. 1,7-dibromo-*N*,*N*′-di(2,6-diisopropyl-phenyl)perylenediimine (**BP**) was synthesized according to a known method.[19] **PP** was obtained by the nucleophilic reaction of **BF**, **DAP** and K_2CO_3 in anhydrous toluene in the presence of 18-crown-6.[20]

The formation of hydrogen-bonded self-assembly of C_{60}**bar** and **PP** was demonstrated by ^{1}H NMR spectroscopic studies. Titration experiment in $CDCl_3$ between the diaminopyridine-substituted isophthalamide group and complementary barbiturate substrate led to the characteristic changes in the ^{1}H NMR spectra of the two components, which confirmed the formation of hydrogen-bonded self-assembly.[14] The ^{1}H NMR spectra changes of C_{60}**bar** with increasing **PP** concentration were recorded. Figure 1A showed the partial ^{1}H NMR spectra of C_{60}**bar** (12 mmol/L) and C_{60}**bar**+**PP** (12 and 6 mmol/L). Two broad peaks at δ 9.3 and 11.1 were observed, which were assigned to the resonance of the imidic protons of the barbituric ring of C_{60}**bar**. When C_{60}**bar** was mixed with 0.5 equiv **PP** (stoichiometric ratio), significant downfield shifts were observed for the resonance of the imidic protons of C_{60}**bar** (from δ 9.3 and 11.1 to 13.2). These shifts showed that strong hydrogen bonding took place between C_{60}**bar** and **PP**.

The ^{1}H NMR spectra changes of **PP** with increasing the concentration of C_{60}**bar** were also recorded in $CDCl_3$. The concentration of **PP** was kept constant (6 mmol/L), and the concentration of C_{60}**bar** was increased gradually (from 0 to 12 mmol/L). As shown in Figure 1B, the signals at δ 8.5 and 7.8, assigned for the two amidic protons of the diaminopyridine-substituted isophthalamide unit, were observed before the addition of C_{60}**bar**. When the concentration of C_{60}**bar** was increased, the signals for the two amidic protons became broad and shifted to lower magnetic fields. When the concentration of C_{60}**bar** was increased to 12 mmol/L, the signals for the two amidic protons shifted downfield to δ 9.8 and 8.8, respectively. And the 2-proton of the isophthalic acid group, which positioned close to the 2-CO of the barbiturate ring, also shifted downfield by 0.25 ppm from δ 8.20 to 8.45. All the spectra changes of the C_{60}**bar** and **PP** in the titration experiment showed the formation of multiple hydrogen-bonding interaction between **PP** and C_{60}**bar**.

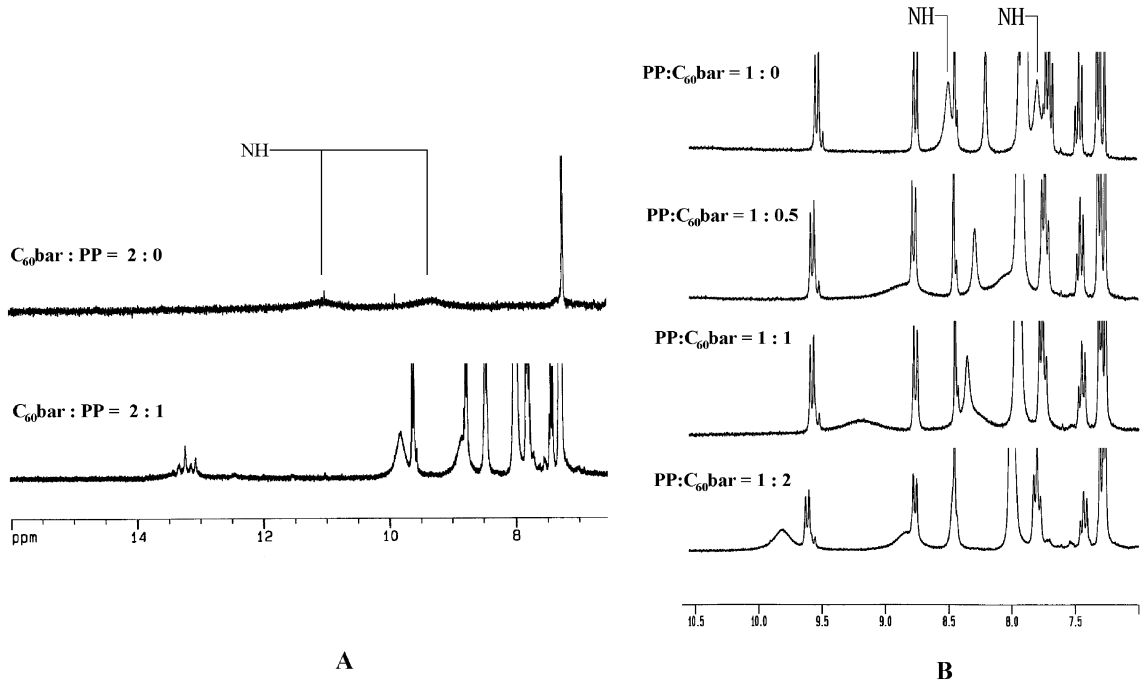


Figure 1. Partial ^{1}H NMR spectra of **PP**/C_{60}**bar** supramolecular system in $CDCl_3$ at rt. (A) the concentration of C_{60}**bar** was kept constant (12 mmol/L), and the concentration of **PP** was 0 and 6 mmol/L, respectively; (B) the concentration of **PP** was kept constant (6 mmol/L), and the concentration of C_{60}**bar** was increased gradually (from 0 to 12 mmol/L).

The fluorescence quenching experiment of **PP** by C_{60}**bar** was carried out in chloroform. The concentration of **PP** was kept constant at 1×10^{-5} mol/L and the concentration of C_{60}**bar** was increased gradually. Because of the competition absorptions of C_{60}**bar** at the excitation and emission wavelength of **PP**, the fluorescence intensities were calibrated according to the literature method.[21] The dependence of the fluorescence intensity of **PP** on the concentration of C_{60}**bar** follows the Stern–Volmer equation. [Eq. 1],[22] in which F_0 is the fluorescence intensity of **PP** without the addition of C_{60}**bar**, F is the calibrated fluorescence intensity of **PP** upon the addition of C_{60}**bar**, K_{sv} is the quenching constant, and $[Q]$ is the concentration of C_{60}**bar**.

$$\frac{F_0}{F} = 1 + K_{sv}[Q] \quad (1)$$

As shown in Figure 2A, **PP** showed a strong emission band at 561 nm and a shoulder band at 600 nm with the excitation wavelength at 490 nm. The fluorescence of **PP** was strongly quenched with the addition of C_{60}**bar**. The Stern–Volmer constant (K_{sv}) is 2.71×10^4 M^{-1}.

For comparison, the fluorescence quenching experiment of **PP** by C_{60}**mal**, which would not form hydrogen bonds with **PP** was also performed. Figure 2B showed the fluorescence spectra of **PP** (1×10^{-5} M, solid line) and **PP**+C_{60}**mal** (1×10^{-5} and 2×10^{-5} M, dash line). We found the

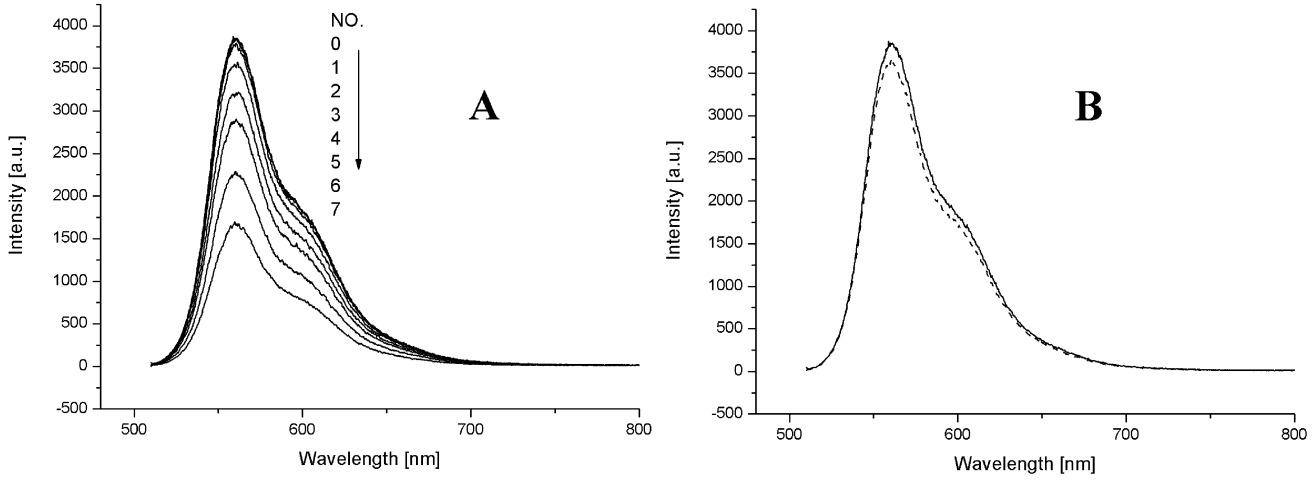


Figure 2. (A) The fluorescence spectra of **PP** ([**PP**]$=1\times10^{-5}$ M) in chloroform with increasing concentration of C_{60}**bar**: 0.0 (0), 1×10^{-6} M (1), 2×10^{-6} M (2), 4×10^{-6} M (3), 8×10^{-6} M (4), 1.2×10^{-5} M (5), 2×10^{-5} M (6), 4×10^{-5} M (7); (B) The fluorescence spectra of **PP** (1×10^{-5} M, solid line) and a mixture of **PP**+C_{60}**mal** (1×10^{-5} M and 2×10^{-5} M, dash line) in chloroform. Excitation wavelength was 490 nm.

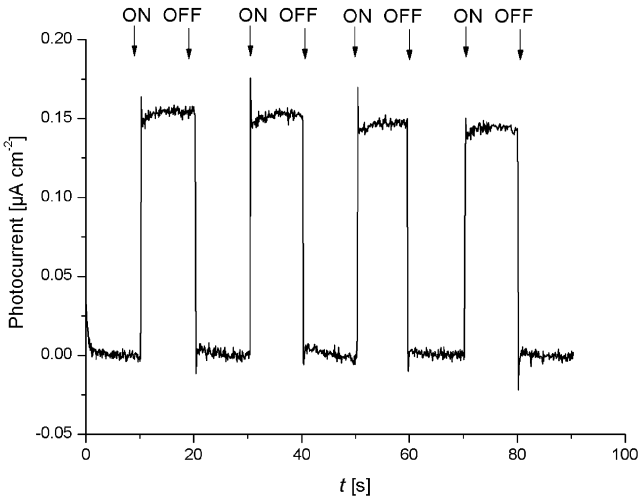


Figure 3. Photocurrent generation of the **PP/C_{60}bar** supramolecular assembly film upon the irradiation of white light (20 mW/cm^2) in 0.5 M KCl solution without bias voltage.

fluorescence of **PP** was almost unchanged upon the addition of 2 equiv **C_{60}mal**. Consider the competition absorptions of **C_{60}mal** at the excitation and emission wavelength of **PP**, we might conclude that the fluorescence of **PP** was not affected with the addition of **C_{60}mal**. This suggested that the hydrogen-bonding interaction between **PP** and **C_{60}bar** played an important role in the fluorescence quenching. These results indicated that there existed intermolecular charge transfer between the supramolecular system of **PP** and **C_{60}bar** by hydrogen-bonding interaction.

A conventional three-electrode cell was used to measure the photoelectrochemical properties of the self-assembled **PP/C_{60}bar** film.[23] The photocurrent generation of **PP/C_{60}bar** film deposited onto an ITO electrode was measured at 20 mW cm^{-2} white light irradiation. When the irradiation was switched on and off, a steady and rapid cathodic 0.15 μA cm^{-2} photocurrent response of the **PP/C_{60}bar** film was produced. Four cycles of the photocurrent generation were shown in Figure 3, which showed that the response to on–off cycling was prompt and reproducible. The photocurrent stability in the system was rather good during the monitored time.

Our group has reported the self-assembly of [60]fullerene containing 2,6-di(acylamino) pyridine unit with 1-dodecyluracil and perylene bis-imides derivative. These hydrogen-bonded supramolecular systems formed spherical nanoaggregates observed by SEM and TEM.[15a,b,c] Some hydrogen-bonded supramolecular system containing perylene derivatives were also reported to form well-defined

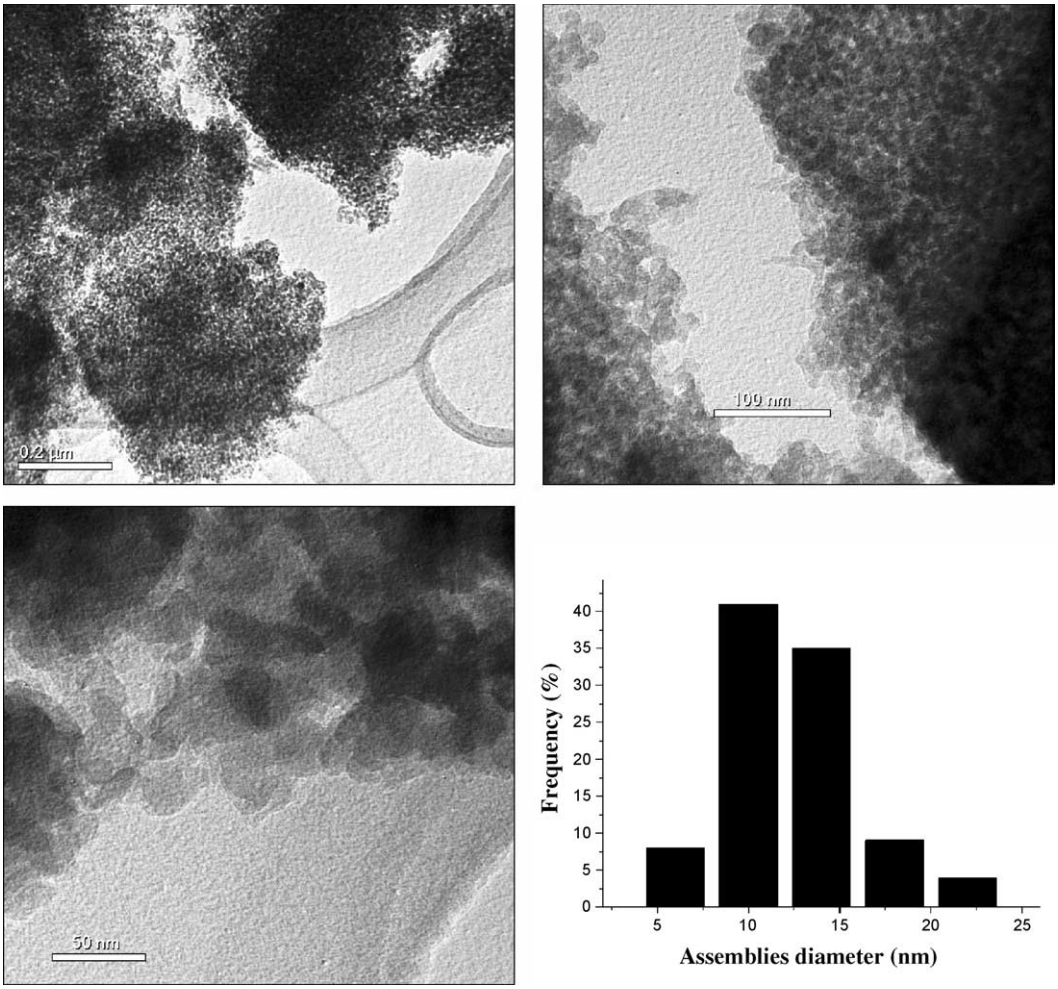


Figure 4. Transmission electron micrograph and size distribution histogram of assemblies resulting from evaporation of 10^{-5} M **PP/C_{60}bar** in $CHCl_3$.

nano-fibrous structures.[24] Consider the self-assembly behavior of **PP** with **C_{60}bar**, we can assume that certain three-dimensional supramolecular structures stabilized by the intermolecular hydrogen bonding through barbituric acid groups and diaminopyridine-substituted isophthalamide moieties was formed. Figure 4 showed the transmission electron micrographs (TEM) of **C_{60}bar/PP** self-assembled on a carbon-coated copper grid after evaporation of $CHCl_3$ solvent and the corresponding size distribution histogram. Spherical particles could be observed and the diameters of these particles were in the range of 5–25 nm with a mean diameter of 12 nm. [60]Fullerene derivatives had high aggregation tendency because of the strong π–π stacking of the carbon cage,[25] and the perylene ring also showed strong intermolecular π–π stacking,[24] which led to the formation of spherical particle aggregates of the hydrogen-bonded **PP/C_{60}bar** supramolecular structure.

3. Conclusions

In conclusion, a novel pincer-like molecule **PP** was synthesized and the supramolecular system between **PP** and **C_{60}bar** through a six-point hydrogen-bonding interaction was fabricated. ^{1}H NMR and fluorescence measurements indicated that strong hydrogen-bonding interaction took place between **PP** and **C_{60}bar**. The photocurrent generated by the self-assembled film was measured, and a cathodic photocurrent response of 0.15 μA cm^{-2} at 20 mW cm^{-2} white light irradiation was obtained. TEM images of **PP/C_{60}bar** aggregates showed spherical particles having a mean diameter of 12 nm. These bichromophoric assemblies could be of importance for potential applications in photovoltaic and nanoscale devices.

4. Experimental

4.1. General

Reagents were purchased from Acros or Aldrich Corporation and were utilized as received unless indicated otherwise. All solvents were purified using standard procedures.

UV–vis and fluorescence spectra were measured on a Hitachi U-3010 and Hitachi F-4500 spectrometer, respectively. IR spectra were recorded as KBr pellets on a Perkin-Elmer System 2000 spectrometer. 300 MHz ^{1}H NMR spectra were recorded on a Bruker dm×300 spectrometers. MALDI-TOF mass spectrometric measurements were performed on Bruker Biflex III MALDI-TOF. Transmission electron micrographs (TEM) were collected on a JEOL JEM 2010 instrument.

The photocurrent generation measurement was carried out using a platinum wire as a counter electrode and the saturated calomel electrode as a reference electrode. A solution of 0.5 M KCl was selected as the supporting electrolyte in the measurements. The ITO plates were cleaned by repeated sonication in isopropyl alcohol and in deionized water. The dry ITO plates were immersed into a chloroform solution of **PP/C_{60}bar** (1×10^{-3} mol/L, molar ratio of **PP** and **C_{60}bar** is 1:2) for 30 min and dried in N_2 stream.

4.1.1. Synthesis of DAP. *N*-butyryl-2,6-diaminopyridine (600 mg, 3.35 mmol) and freshly distilled pyridine (323 μL, 4 mmol) were dissolved in anhydrous THF (30 mL). The reaction mixture was cooled to 0–10 °C with ice water bath. After degassing for 10 min, 5-hydroxy-benzene-1,3-dicarbonyl chloride (360 mg, 1.65 mmol) dissolved in 10 mL anhydrous THF was added dropwise over 1 h. After stirring for 6 h at rt, 20 mL water was added and the reaction mixture was stirred for additional 20 min. THF was then removed under reduced pressure and the resulting precipitate was filtered, washed several times with water, dried at rt, and purified by column chromatography using $CHCl_3$/MeOH (v/v, 100:1) as the eluent to give **DAP** as a white powder. Yield: 463 mg, 56%; IR (KBr): 3370, 3280, 2965, 2875, 1672, 1586, 1516, 1450, 1295, 1242, 1156, 1084, 801 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 10.30 (s, 2H), 10.15 (s, 1H), 10.05 (s, 2H), 7.97 (s, 1H), 7.81–7.72 (m, 6H), 7.49 (s, 2H), 2.36 (t, J=7.2 Hz, 4H), 1.62–1.55 (m, 4H), 0.88 (t, J=7.2 Hz, 6H) ppm MS: *m/z* (EI, 70 eV) 504 [M$^+$]. Anal. Calcd for $C_{26}H_{28}N_6O_5$: C, 61.89; H, 5.59; N, 16.66; Found C, 61.64; H, 5.65; N, 16.59%.

4.1.2. Synthesis of PP. BP (104 mg, 0.12 mmol), **DAP** (140 mg, 0.278 mmol), K_2CO_3 (100 mg, 0.73 mmol) and 18-crown-6 (50 mg, 0.19 mmol) were dissolved in anhydrous toluene (20 mL). The reaction mixture was degassed for 10 min and then heated to 80 °C. After stirring for 4 h, nearly all the **BP** was consumed (monitored by TLC), the solvent was removed under reduced pressure and the residue was purified by column chromatograph on silica gel using CH_2Cl_2/MeOH (v/v, 100:1) as the eluent to give **PP** as a red solid. Yield: 181 mg, 88%. UV–vis ($CHCl_3$): $\lambda_{max}(\varepsilon)$=302 (70220), 389 (8610), 491 (36600), 525 nm (55510 mol^{-1} dm^3 cm^{-1}); fluorescence ($CHCl_3$): λ_{max}=561 nm; IR (KBr): 3305, 3067, 2965, 2931, 1704, 1667, 1588, 1511, 1448, 1404, 1338, 1306, 1247, 1156, 1078, 961, 902, 799, 739 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 9.53 (d, J=8.4 Hz, 2H), 8.75 (d, J=8.4 Hz, 2H), 8.52 (br s, 4H), 8.44 (s, 2H), 8.21 (s, 2H), 7.93–7.87 (m, 12H), 7.81 (br s, 2H), 7.69 (t, J=8.1 Hz, 4H), 7.46 (t, J=7.8 Hz, 2H), 7.30 (d, J=7.5 Hz, 4H), 2.77–2.72 (m, 4H), 2.29 (t, J=7.2 Hz, 8H), 1.75–1.67 (m, 8H), 1.15–1.11 (m, 24H), 0.97 (t, J=7.2 Hz, 12H) ppm; MALDI-TOF MS: Calcd for $C_{100}H_{94}N_{14}O_{14}$ (M$^+$), *m/z*=1714.7; found 1714.8.

4.1.3. Synthesis of C_{60}mal. C_{60} (250 mg, 0.35 mmol) and CBr_4 (166 mg, 0.5) were dissolved in anhydrous toluene (150 mL) with vigorous stirring. After degassing for 15 min, di[2-(2-butoxy-ethoxy)-ethyl] malonate (196 mg, 0.5 mmol) and DBU (150 μL, 1 mmol) were added and the mixture color changed gradually from purple to dark red. After stirring for 2 h, the solvent was removed under reduced pressure. The residue was purified by column chromatography using toluene/ethyl acetate (v/v, 10:1) as the eluent to give **C_{60}mal** as a brown solid. Yield: 170 mg, 43.8%; IR (KBr): 2954, 2927, 2864, 1746, 1429, 1378, 1232, 1113, 1031, 706, 527 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 4.65 (t, J=4.8 Hz, 4H), 3.88 (t, J=4.8 Hz, 4H), 3.68 (t, J=4.8 Hz, 4H), 3.57 (t, J=4.8 Hz, 4H), 3.45 (t, J=6.8 Hz, 4H), 1.58–1.53 (m, 4H), 1.39–1.33 (m, 4H), 0.91 (t, J=7.2 Hz, 6H)

ppm; MALDI-TOF MS: Calcd for $C_{79}H_{34}O_8$ (M^+), m/z= 1110.2; found 1110.1.

4.1.4. Synthesis of C_{60}bar. C_{60}**mal** (111 mg, 0.1 mmol) and 5-bromobarbituric acid (21 mg, 0.1 mmol) were dissolved in toluene/DMSO (v/v, 95:5, 150 mL) with vigorous stirring. After degassing for 15 min, DBU (20 μL, 0.2 mmol) was added. After stirring at rt for 2 h, the reaction mixture was washed with water (60×3 mL). The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was then removed under reduced pressure. The residue was purified by column chromatography on silica gel using $CHCl_3$/MeOH (v/v, 10:1) as the eluent to give C_{60}**bar** as a brown solid. Yield: 17 mg, 12%; IR (KBr): 3173, 2955, 2925, 2857, 1744, 1663, 1541, 1457, 1377, 1245, 1114, 1031, 880, 766 cm^{-1}; ^1H NMR ($CDCl_3$): δ 11.1 (br), 9.3 (br), 4.6–4.5 (br, 4H), 3.7–3.4 (br, 16H), 1.5–0.8 (br, 14H) ppm^{-1}. MALDI-TOF MS: Calcd for $C_{83}H_{36}O_{11}$ (M^+), m/z=1236.23; found 1237.3 (M^++H), 1110.4 (M^+−barbituric acid), 720.1 (M^+−barbituric acid ring-bis[2-(2-butoxy-ethoxy)-ethyl] malonates).

4.2. Supporting information available

^1H NMR and MS spectra of **PP**, C_{60}**mal** and C_{60}**bar**.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (20131040, 50372070, 20418001 and 20473102) and Major State Basic Research Development Program.

References and notes

1. (a) Mirkin, C. A.; Caldwell, W. B. *Tetrahedron* **1996**, *52*, 5113–5130. (b) Prato, M. *Top. Curr. Chem.* **1999**, *199*, 173–187. (c) Yu, G.; Gao, J.; Hummelen, J. C.; Wudl, F.; Heeger, A. J. *Science* **1995**, *270*, 1789–1791.
2. (a) Prato, M. *J. Mater. Chem.* **1997**, *7*, 1097–1109. (b) Imahori, H.; Sakata, Y. *Adv. Mater.* **1997**, *9*, 537–546. (c) Liu, Y.; Wang, H.; Liang, P.; Zhang, H. Y. *Angew. Chem., Int. Ed.* **2004**, *43*, 2690–2694.
3. (a) Shirakawa, M.; Fujita, N.; Shinkai, S. *J. Am. Chem. Soc.* **2003**, *125*, 9902–9903. (b) Yamaguchi, T.; Ishii, N.; Tashiro, K.; Aida, T. *J. Am. Chem. Soc.* **2003**, *125*, 13934–13935.
4. (a) Diederich, F.; Dietrich-Buchecker, C.; Nierengarten, J.-F.; Sauvage, J.-P. *J. Chem. Soc., Chem. Commun.* **1995**, 781–782. (b) Armaroli, N.; Diederich, F.; Dietrich Buchecker, C. O.; Flamigni, L.; Marconi, G.; Nierengarten, J.-F.; Sauvage, J.-P. *Chem. Eur. J.* **1998**, *4*, 406–416. (c) Da Ros, T.; Guldi, D. M.; Morales, A. F.; Leigh, D. A.; Prato, M.; Turco, R. *Org. Lett.* **2003**, *5*, 689–691. (d) Li, K.; Schuster, D. I.; Guldi, D. M.; Herranz, M. A.; Echegoyen, L. *J. Am. Chem. Soc.* **2004**, *126*, 3388–3389. (e) Nakamura, Y.; Minami, S.; Iizuka, K.; Nishimura, J. *Angew. Chem., Int. Ed.* **2003**, *42*, 3158–3162.
5. (a) Ashton, P. R.; Diederich, F.; Gómez-López, M.; Nierengarten, J.-F.; Preece, J. A.; Raymo, F. M.; Stoddart, J. F. *Angew. Chem., Int. Ed.* **1997**, *36*, 1448–1451. (b) Schuster, D. I.; Li, K.; Guldi, D. M.; Ramey, J. *Org. Lett.* **2004**, *6*, 1919–1922.
6. (a) Kimura, M.; Saito, Y.; Ohta, K.; Hanabusa, K.; Shirai, H.; Kobayashi, N. *J. Am. Chem. Soc.* **2002**, *124*, 5274–5275. (b) Carano, M.; Corvaja, C.; Garlaschelli, L.; Maggini, M.; Marcaccio, M.; Paolucci, F.; Pasini, D.; Righetti, P. P.; Sartori, E.; Toffoletti, A. *Eur. J. Org. Chem.* **2003**, 374–384. (c) Nierengarten, J.-F. *Chem. Eur. J.* **2000**, *6*, 3667–3670. (d) Hasobe, T.; Kashiwagi, Y.; Absalom, M. A.; Sly, J.; Hosomizu, K.; Crossley, M. J.; Imahori, H.; Kamat, P. V.; Fukuzumi, S. *Adv. Mater.* **2004**, *16*, 975–979. (e) Sun, D.; Tham, F. S.; Reed, C. A.; Chaker, L.; Boyd, P. D. W. *J. Am. Chem. Soc.* **2002**, *124*, 6604–6612.
7. (a) Müller, G. R. J.; Meiners, C.; Enkelmann, V.; Geerts, Y.; Müllen, K. *J. Mater. Chem.* **1998**, *8*, 61–64. (b) van de Craats, A. M.; Warman, J. M.; Schlichting, P.; Rohr, U.; Geerts, Y.; Müllen, K. *Synth. Met.* **1999**, *102*, 1550–1551. (c) Schneider, M.; Müllen, K. *Chem. Mater.* **2000**, *12*, 352–362. (d) Wehmeier, M.; Wagner, M.; Müllen, K. *Chem. Eur. J.* **2001**, *7*, 2197–2205.
8. Seybold, G.; Wagenblast, G. *Dyes Pigm.* **1989**, *11*, 303–317.
9. Schlettwein, D.; Wöhrle, D.; Karmann, E.; Melville, U. *Chem. Mater.* **1994**, *6*, 3–6.
10. Sadrai, M.; Hadel, L.; Sauers, R. R.; Husain, S.; Krogh-Jespersen, K.; Westbrook, J. D.; Bird, G. R. *J. Phys. Chem.* **1992**, *96*, 7988–7996.
11. O'Neil, M. P.; Niemczyk, M. P.; Svec, W. A.; Gosztola, D.; Gaines, G. L., III; Wasielewski, M. R. *Science* **1992**, *257*, 63–65.
12. (a) Prins, L. J.; Reinhoudt, D. N.; Timmerman, P. *Angew. Chem., Int. Ed.* **2001**, *40*, 2382–2426. (b) Ajayaghosh, A.; George, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 5148–5149.
13. (a) Beijer, F. H.; Kooijman, H.; Spek, A. L.; Sijbesma, R. P.; Meijer, E. W. *Angew. Chem., Int. Ed.* **1998**, *37*, 75–78. (b) Schmuck, C.; Wienand, W. *Angew. Chem., Int. Ed.* **2001**, *40*, 4363–4369. (c) El-ghayoury, A.; Schenning, A. P. H. J.; van Hal, P. A.; van Duren, J. K. J.; Janssen, R. A. J.; Meijer, E. W. *Angew. Chem., Int. Ed.* **2001**, *40*, 3660–3663.
14. (a) Chang, S. K.; Engen, D. V.; Fan, E.; Hamilton, A. D. *J. Am. Chem. Soc.* **1991**, *113*, 7640–7645. (b) Berl, V.; Schmutz, M.; Krische, M. J.; Khoury, R. G.; Lehn, J.-M. *Chem. Eur. J.* **2002**, *8*, 1227–1244. (c) Collinson, S. R.; Gelbrich, T.; Hursthouse, M. B.; Tucker, J. H. R. *Chem. Commun.* **2001**, 555–556. (d) Tecilla, P.; Dixon, R. P.; Slobodkin, G.; Alavi, D. S.; Waldeck, D. H.; Hamilton, A. D. *J. Am. Chem. Soc.* **1990**, *112*, 9408–9410. (e) Salameh, A. S.; Ghaddar, T.; Isied, S. S. *J. Phys. Org. Chem.* **1999**, *12*, 247–254. (f) Binder, W. H.; Kunz, M. J.; Kluger, C.; Hayn, G.; Saf, R. *Macromolecules* **2004**, *37*, 1749–1759.
15. (a) Shi, Z. Q.; Li, Y. L.; Gong, H. F.; Liu, M. H.; Xiao, S. X.; Liu, H. B.; Li, H. M.; Xiao, S. Q.; Zhu, D. B. *Org. Lett.* **2002**, *4*, 1179–1182. (b) Xiao, S. Q.; Li, Y. L.; Fang, H. J.; Li, H. M.; Liu, H. B.; Shi, Z. Q.; Jiang, L.; Zhu, D. B. *Org. Lett.* **2002**, *4*, 3063–3066. (c) Liu, Y.; Xiao, S. Q.; Li, H. M.; Li, Y. L.; Liu, H. B.; Lu, F. S.; Zhuang, J. P.; Zhu, D. B. *J. Phys. Chem. B* **2004**, *108*, 6256–6260. (d) Fang, H. J.; Wang, S.; Xiao, S. Q.; Yang, J. L.; Li, Y. L.; Shi, Z. Q.; Li, H. M.; Liu, H. B.; Xiao, S. X.; Zhu, D. B. *Chem. Mater.* **2003**, *15*, 1593–1597.
16. McClenaghan, N. D.; Absalon, C.; Bassani, D. M. *J. Am. Chem. Soc.* **2003**, *125*, 13004–13005.
17. (a) Camps, X.; Hirsch, A. *J. Chem. Soc., Perkin Trans. 1* **1997**, *11*, 1595–1596. (b) Richardson, C. F.; Schuster, D. I.; Wilson, S. R. *Org. Lett.* **2000**, *2*, 1011–1014.

18. (a) Hirsch, A.; Lamparth, I.; Karfunkel, H. R. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 437–438. (b) Hirsch, A.; Lamparth, I.; Grösser, T.; Karfunkel, H. R. *J. Am. Chem. Soc.* **1994**, *116*, 9385–9386. (c) Lamparth, I.; Maichle-Mössmer, C.; Hirsch, A. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1607–1609. (d) Lamparth, I.; Herzog, A.; Hirsch, A. *Tetrahedron* **1996**, *52*, 5065–5075. (e) Djojo, F.; Herzog, A.; Lamparth, I.; Hampel, F.; Hirsch, A. *Chem. Eur. J.* **1996**, *2*, 1537–1547. (f) Camps, X.; Schönberger, H.; Hirsch, A. *Chem. Eur. J.* **1997**, *3*, 561–567. (g) Djojo, F.; Hirsch, A. *Chem. Eur. J.* **1998**, *4*, 344–356.
19. Rohr, U.; Kohl, C.; Müllen, K.; van de Craats, A.; Warman, J. *J. Mater. Chem.* **2001**, *11*, 1789–1799.
20. Xiao, S. Q.; Li, Y. L.; Li, Y. J.; Zhuang, J. P.; Wang, N.; Liu, H. B.; Ning, B.; Liu, Y.; Lu, F. S.; Fan, L. Z.; Yang, C. H.; Li, Y. F.; Zhu, D. B. *J. Phys. Chem. B* **2004**, *108*, 16677–16685.
21. Zheng, M.; Bai, F. L.; Li, F. Y.; Li, Y. L.; Zhu, D. B. *J. Appl. Polym. Sci.* **1998**, *70*, 599–603.
22. Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum: New York, 1993.
23. Li, H. M.; Li, Y. L.; Zhai, J.; Cui, G. L.; Liu, H. B.; Xiao, S. Q.; Liu, Y.; Lu, F. S.; Jiang, L.; Zhu, D. B. *Chem. Eur. J.* **2003**, *9*, 6031–6038.
24. (a) Liu, Y.; Zhuang, J. P.; Liu, H. B.; Li, Y. L.; Lu, F. S.; Gan, H. Y.; Jiu, T. G.; Wang, N.; He, X. R.; Zhu, D. B. *Chem. Phys. Chem.* **2004**, *5*, 1210–1215. (b) Thalacker, C.; Würthner, F. *Adv. Funct. Mater.* **2002**, *12*, 209. (c) Schenning, A. P. H. J.; Herrikhuyzen, J. V.; Jonkheijm, P.; Chen, Z.; Würthner, F.; Meijer, E. W. *J. Am. Chem. Soc.* **2002**, *124*, 10252.
25. (a) Mirkin, C. A.; Caldwell, W. B. *Tetrahedron* **1996**, *52*, 5113–5130. (b) Nath, S.; Pal, H.; Palit, D. K.; Sapre, A. V.; Mittal, J. P. *J. Phys. Chem. B* **1998**, *102*, 10158–10164.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron 61 (2005) 8694–8698
Tetrahedron

A chitosan-based chemomechanical polymer triggered by stacking effects with aromatic effectors including aminoacid derivatives

Nino Lomadze and Hans-Jörg Schneider*

FR Organische Chemie and Institut für Neue Materialien, Universität des Saarlandes, D 66041 Saarbrücken, Germany

Received 6 May 2005; revised 24 June 2005; accepted 27 June 2005

Available online 26 July 2005

Abstract—Chitosan with a covalently attached anthrylunit is used as chemomechanical polymer, in which stacking and cation–π interactions allow aromatic effector compounds with positively charged nitrogen centers to trigger macroscopic motions in aqueous surrounding. Thus, only protonated heterocycles such as imidazole or histamine lead at pH 5 to expansion, in contrast to toluenesulfonic acid, or pyrazole and pyrimidine. Inorganic salts and pH influence the polymer swelling, and must be taken into account for the calculation of net effects induced by organic effectors. Reversible volume expansions on the top of the swelling effect of water alone are observed as function of different effector structures, showing, for example, 45% net effect with imidazole and 66% with benzimidazole. Aminoacids, for solubility reasons measured in the form of their methylesters, yield smaller expansions, showing, however, a regular and selective increase with the lipophilicity of the residues. The kinetics of effector uptake, which relates to the velocity of expansion, are measured with histamine and follow first order, with $t_{1/2}=2.7$ min for 50% absorption.

1. Introduction

The aminosaccharide polymer chitosan has been used to a great extend for biocompatible films or fibers,[1] in particular for drug delivery systems,[2] also after chemical modification. Specific supramolecular binding units have been rarely implemented until now into chitosan. Such polymers can lead to intelligent materials, which could show selective dimension changes under the influence of external effector molecules in solution. Modified chitosan derivatives were shown early to respond to pH changes;[3] non-covalent 'cross-linking' by salt bridges also lead to swelling by added pyrophosphate anions as function of pH and of ionic strength, if the amount of phosphate anion was not too high.[4] Metal binding units[5] and cyclodextrins[6] were also successfully introduced into chitosan based polymers.

Chemomechanical polymers hold much promise for the development of artificial muscles, new array sensors, and in particular for drug delivery. Recently, we have shown how implementation of supramolecular binding sites into flexible polymers allows selective recognition of external substrates in aqueous solution, leading to large volume changes as function of the effector structure.[7] The hitherto used underlying polyacrylates were equipped with polyamine functions as binding site for anions and/or for transition metal ions, and with alkyl groups for lipophilic interactions. With these polymers, selective macroscopic motions could be triggered by, for example, aromatic anions, nucleotides, copper or zinc ions, aminoacids, peptides, etc. The advantage of chitosan derivatives is, that such materials would be better biocompatible than the polyacrylates which we have used until now.

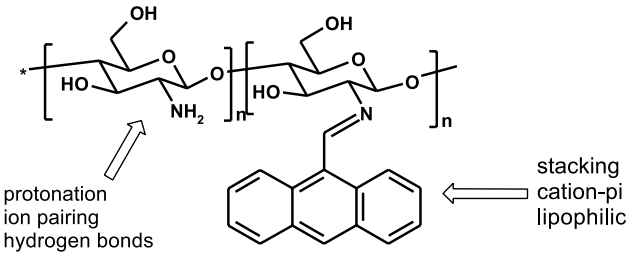
2. Results

2.1. Materials

As a first step to implement supramolecular binding sites into chitosan we have used anthryl units covalently attached to the polysaccharide (Scheme 1). Large aromatic units can be expected to recognize lipophilic substrates, and in particular all kind of aromatic effectors by well-documented stacking effects;[8] in addition one can expect non-covalent binding contributions by cation–π interactions if the effector or the chemomechanical polymer such as chitosan contains positively charged nitrogen atoms. Anthryl units were introduced into chitosan with slight modification of literature protocols;[9] reactions of chitosan (average $M_w \sim 400{,}000$) with 9-anthrylaldehyde furnishes the desired material, which from solutions could be poured into flat gel films. The degree of substitution was described in the

Keywords: Supramolecular complexes; Aminoacids; Chitosan; Chemomechanical polymers; Hydrogels.
* Corresponding author. Tel./fax: +49 681 302 3209;
e-mail: ch12hs@rz.uni-sb.de

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.06.092



Scheme 1. Basic chitosan structure with anthryl units and possible binding mechanisms.

literature,[9] where ^{13}C NMR-spectra are also given, to amount to about 70% on the basis of elemental analysis. After swelling in water the gel took up about 60–70% water, as determined by weight difference. Additional increase of water content after exposure to the effectors was too small for accurate measurements. Reduction of the Schiff bases with sodium cyanoborohydride yielded as described in the literature[9] the corresponding amine. In contrast to the imine derivative, the amine gels were not stable enough for mechanical handling and therefore were not further investigated. The amine gel showed promising swelling triggered, for example, by 0.1 M imidazole at pH 3.0 (up to about 80% elongation in one dimension in comparison to neutral condition), but became too fragile for exact measurements, even more so at lower pH. In double or triple runs with the same material the dimension changes, always measured in two dimensions, were as expected isotropic, and reproducible within $\pm 1\%$; they also were fully reversible.

2.2. Salt and pH effects

Polymeric materials which are sensitive towards pH and unspecific salt concentration changes were until recently the only well-studied chemomechanical polymers,[10] comprising also some chitosan derivatives.[4,11]

The pH-dependent dimension changes are due to repulsion of ionic groups and/or to osmotic pressure changes within the polymer, which occur upon protonation or deprotonation. Corresponding viscosity increases of polyacrylate solutions have been reported already in 1950.[12] It should be noted, that before deacetylation of chitin no swelling by water or by pH changes alone was observed, due to the absence of ionizable groups in the network.

In contrast to our polyacrylate–amine derived material, which is the first chemomechanical polymer with a symmetric pH-profile around pH 7, the anthryl-chitosan derivative shows as most pH-triggered systems a proton-sensitive dimension change only in one direction (Fig. 1, Supplementary data, Tables 1 and 2). At pH 3 there is sharp dimension increase, which at this pH also depends on the salt (NaCl) concentration present in solution. The reason for this becomes clear by a separate investigation of the salt effect at different pH. At pH values below 3 the ionic strength produced by the acid itself becomes so large, that the presence of additional salts has only small effects. Thus, at pH 2 the addition of 0.03 M NaCl has a negligible effect ($<1\%$ volume change) on the dimension, whereas at pH 3 the salt triggers a volume expansion by 66%, always in

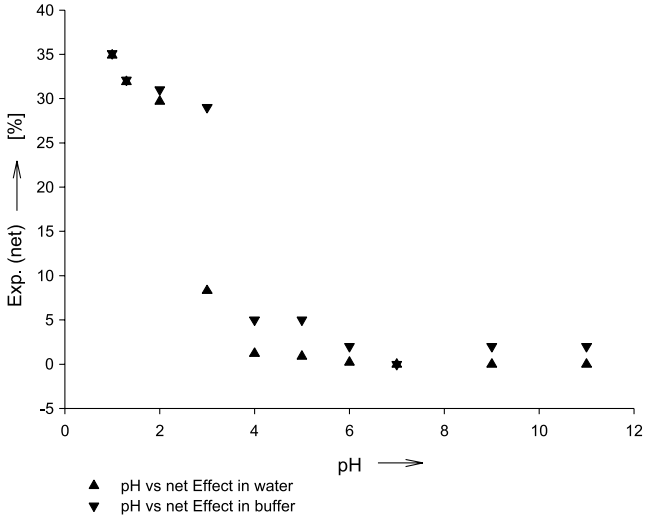


Figure 1. Expansions (% in one dimension) induced by pH changes; details see Supplementary data, Tables 1 and 2).

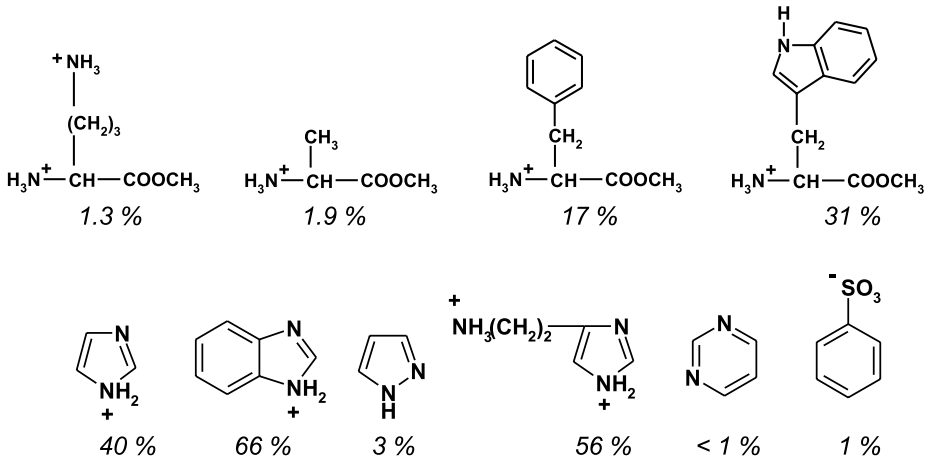
comparison to the effect of the pH alone (Supplementary data, Table 3). Similarly, the presence of 0.1 M phosphate produces at pH 1.3 no significant change, whereas at pH 3 one finds 83% volume increase (Supplementary data, Table 4). That at pH 5 the expansion triggered by the salt is with 32% again smaller than at pH 3 (Supplementary data, Table 4) is in line with the observation, that under neutral conditions the swelling of the gel network becomes generally smaller. For example, 0.1 M imidazole at pH 7 produces no change ($<2\%$), whereas at pH 5 one observes 45% (net volume effect). At pH values above 7 the effect of 0.1 M imidazole and other effectors is marginal ($<2\%$).

Obviously, in order to see the net effect of any organic effector molecules one has to subtract from the observed total dimension change of the gel in water the changes produced by the acidity, and also by the salts present in the solution (Supplementary data, Table 5).

2.3. Organic effectors (Scheme 2)

As expected, only aromatic substrates lead to sizeable volume changes, in particular if they carry positively charged nitrogen atoms which can generate large cation–π interactions. In line with this, anionic effectors such as *p*-toluenesulfonic acid or non-protonated heteroarenes show almost negligible effects (Scheme 2, Supplementary data, Table 9).

With imidazole the concentration dependence of the expansion was determined at pH 5 both in presence and absence of NaCl (Figs. 2 and 3; Supplementary data, Tables 6 and 7). Expansions were also measured at high and constant ionic strength ($I=0.3$ M, attained by addition of various amount of NaCl (Supplementary data, Table 8). The profiles indicate saturation at about 0.1 M effector concentration. In contrast to our amine acrylate-based polymer[13] one cannot decrease the concentration necessary for a given dimension change significantly by using smaller polymer pieces. Thus, a film piece of $2\times2\times0.4$ mm showed with 0.01 M imidazole within the error the same 65% volume expansion as a smaller $1\times1\times0.4$ mm piece. This, as well



Scheme 2. Volume expansions % with different organic effectors (at 0.1 M concentration, pH 5.0); net effects: pH and salt effects deducted, see Table 9, Supplementary data.

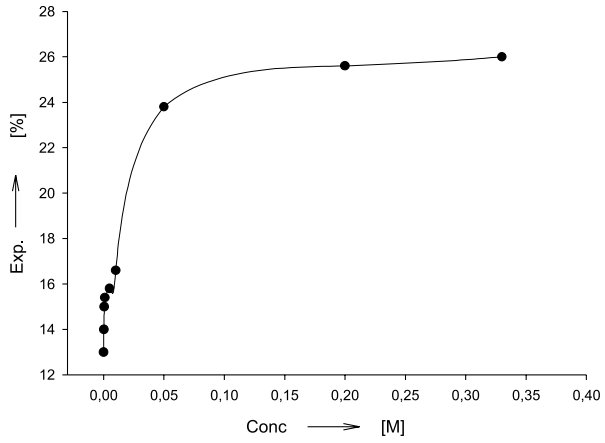


Figure 2. Expansion (% in one dimension) as function of effector imidazole concentration; no further expansion (<3%) at higher concentrations (details see Supplementary data, Tables 6 and 7).

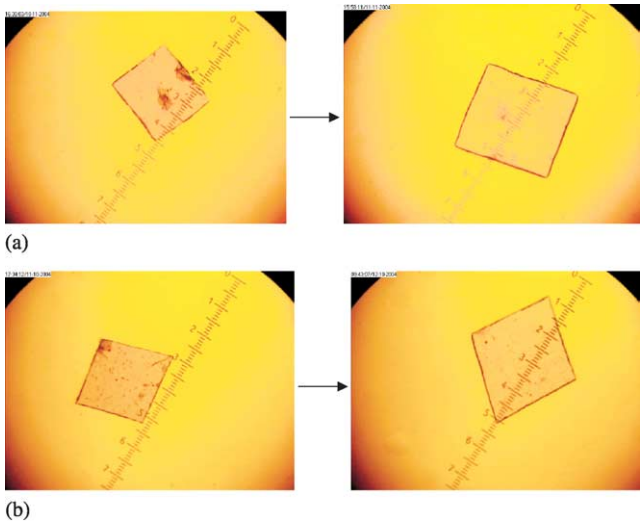


Figure 3. Visualization/measuring of expansions: (a) with benzimidazole; 0.1 M at pH 5.0; expansion in one dimension 29%; (b) with imidazole 0.1 M pH 5.0; expansion in one dimension 26%.

as the smaller expansions with the anthryl chitosan in comparison to our polyamine–polyacrylate system can be ascribed to a smaller binding capacity, but in particular a smaller affinity between the effector and the polymer. That the latter factor is predominant is visible in the above-mentioned independence of expansion on the film size, and in the generally higher effector concentration needed for full expansion. Noticeably, the chitosan-based polymer shows a continuous expansion–concentration profile, in contrast to the polyamine–polyacrylate polymer, where also the dependence on the presence of a second effector leads to a chemomechanical logical gate function.[7b]

In spite of the relatively small expansions selective and characteristic volume changes were triggered by different organic effector compounds, for which we selected some of also biological interest (Scheme 2, Supplementary data, Table 9). Most promising are results obtained with aminoacids as effectors, which for solubility reasons had to be used in form of their methyl esters. Here, the volume change pattern generated by the different side chains indicates the operation of lipophilicity and of stacking effects; this could be the basis of new chemomechanical polymers triggered also by corresponding peptides.

The rate of the volume changes will depend primarily on the rate of effector uptake, and depends also on the surface to volume ratio of the polymer particles. The depletion of effector as a function of time was followed by measuring the UV absorption decrease of histamine (Fig. 4). As observed with the polyamine–polyacrylate polymer[7a] the adsorption followed first order kinetics, allowing to extract a 'half life' time $t_{1/2}$ needed for 50% uptake of the effector. The time constant agrees roughly with the average time needed for expansion, which in view of the relatively small effects was not measured accurately, also since they are also a function of the surface to volume ratio of the used polymer piece. With film piece dimensions of $3\times3\times0.4$ mm full expansions, as illustrated in Figure 2, take about 10–20 min, whereas 50% expansions are already reached after about 2–3 min. As expected and demonstrated with our polyamine–polyacrylate polymer, the response time can be considerably lowered by using thinner films, or generally smaller polymer particles.

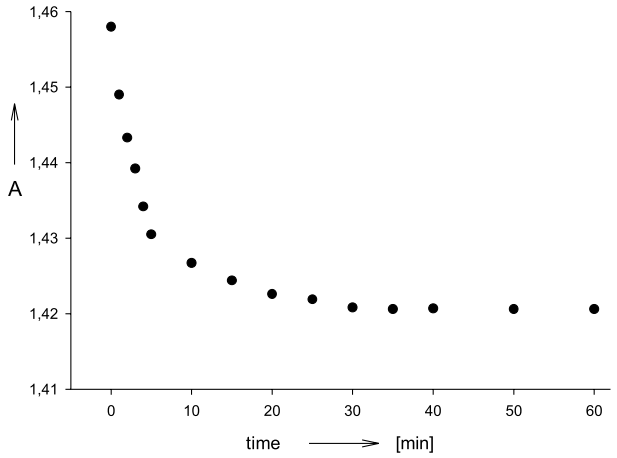


Figure 4. Absorption kinetics with effector histamine ($\varepsilon=5950$) in water at pH 5.0. From first order non-linear least square fit ($r=0.996$; see Figure 1 in Supplementary data): $k=0.256\ \text{min}^{-1}$, $t_{1/2}=2.7$ min. Dry material of the polymer was 1.0 mg; measured after swelling in 3 ml solution; loading of effector from extinction change: 1.89×10^{-8} mol per 1 mg dry mass.

3. Conclusions

The results show that small synthetic variations of natural polymers can already lead to significant volume changes of such chemomechanical polymers, triggered by biologically interesting effector compounds. The observed macroscopic motions of the polymer follow the mechanistic rules for supramolecular chemistry in solution for corresponding complexes between effectors and the polymer binding elements. Selectivity with respect the effector structure can be further increased by implementation of additional binding sites into the polymer. Higher sensitivity, allowing the use of smaller effector concentrations can be achieved with stronger binding elements and larger capacity in terms of the number of freely accessible binding sites. Optimized chemomechanical polymers on the basis of biocompatible materials with implemented supramolecular binding functions hold promise for many applications, in particular for drug release and for actuators as implants.

4. Experimental

4.1. Polymer gel preparation

Chitosan was purchased from Fluka ($M_r\sim400{,}000$) and further deacetylated similar to literature protocols, yielding about 80% free amino groups.[14] The commercial material was dissolved in 2% acetic acid, then precipitated out by adding a concentrated NaOH solution. The precipitate was washed several times with a large amount of deionized water, until the pH had reached 7, and allowed to dry in the air. Similar to the literature[9] 1.0 g (6.0 mM) of the dried chitosan was then dissolved in 60 ml of 1% acetic acid and diluted with 40 ml methanol; 2.0 g (10.0 mmol) of 9-antrylaldehyde in 50 ml methanol were added dropwise and stirred for 24 h to obtain a yellow gel,[9] which was washed repeatedly with methanol and diethylether. Of the gel-like material 1 g was stirred in 20 ml hot DMSO (about 50% of the material remained insoluble); the solution was poured on glass vessel with flat bottom of about 8 cm diameter; the solution was then dried for 24 h in a vacuum oven at 60 °C and 16 mm Hg. After swelling in water the films had an average thickness of 0.4 mm. The water content after complete swelling was 60–70%, as determined by weight in wet stage and after drying in vacuum. The film looses slowly some of the chemomechanical activity; this can be avoided if the film is kept in a dry state, or in methanol.

4.2. Measurements of the dimension changes

Expansions are measured by immersing film pieces in appropriate solutions of the effectors after taking out the pieces from the solution. Film pieces of, for example, 1×1 mm and 0.4 mm thickness are cut from the cast polymer; the exact size (only in the dimensions length, l, and width, w, as the thickness was less accurate to measure) is determined with a measuring microscope equipped with a digital camera system and suitable evaluation software (MOTIC) on a PC (Fig. 3). The average deviation between duplicate measurements with the same film charge is in one dimension (l or w) is usually within $\pm1\%$; the resulting error in volume is about $\pm3\%$.

Kinetics of absorption were followed by measuring the optical density decrease at $\lambda=210$ nm in a 3 ml cuvet equipped with a magnetic stirrer, containing a polymer piece of $3\times3\times0.4$ mm and a solution with 2.45×10^{-4} M, or 7.35×10^{-7} mol in 3 ml solution. The density changed from 1.4580 at the begin to 1.4204 at the end (Fig. 3), corresponding to 2.39×10^{-4} M concentration or 7.16×10^{-7} Mol (extinction coefficient $\varepsilon=5950$ at $\lambda=210$ nm calculated from measurements between 0.1 and 0.4 mM concentration in neat water). The decrease amounts to 1.89×10^{-8} Mol, which is only a small part of the available total effector material. On the basis of, for example, 50% occupation of the chitosan aminogroups the total amount of available anthryl groups would be 2.9×10^{-6} mol per mg dry polymer, which was immersed in 3 ml of a 2.45×10^{-4} M effector solution; the absorbed material would then be only 0.65% of the total effector, but still enough to generate about 11% volume expansion. However, the high amount of substitution (70%) given in the literature for the anthryl polymer preparation[9] is only a rough estimate based on combustion analysis data.

Supplementary data

Supplementary data associated with this article can be found at 10.1016/j.tet.2005.06.092

References and notes

1. Dutta, P. K.; Ravikumar, M. N.; Dutta, J. *J. Macromol. Sci. Polym. Rev.* **2002**, *C42*, 307–354. Kumar, M. *React. Funct. Polym.* **2000**, *46*, 1–27. Knaul, J. Z.; Hudson, S. M.; Creber, K. A. M. *J. Appl. Polym. Sci.* **1999**, *72*, 1721–1732.

Rathke, T. D.; Hudson, S. M. *J. Macromol. Sci.-Rev. Macromol. Chem. Phys.* **1994**, *C34*, 375–437.

2. Park, K.; Shalaby, W. S. W.; Park, H. *Biodegradable Hydrogels for Drug Delivery*; Technomic: Lancaster, 1993. Ganguly, S.; Dash, A. K. *Int. J. Pharm.* **2004**, *276*, 83–92. Dufes, C.; Muller, J. M.; Couet, W.; Olivier, J. C.; Uchegbu, I. F.; Schatzlein, A. G. *Pharm. Res.* **2004**, *21*, 101–107. Kockisch, S.; Rees, G. D.; Young, S. A.; Tsibouklis, J.; Smart, J. D. *J. Pharm. Sci.* **2003**, *92*, 1614–1623. Morimoto, M.; Saimoto, H.; Shigemasa, Y. *Trends Glycosci. Glycotechnol.* **2002**, *14*, 205–222. Singh, D. K.; Ray, A. R. *J. Macromol. Sci.-Rev. Macromol. Chem. Phys.* **2000**, *C40*, 69–83. Paul, W.; Sharma, C. P. *STP Pharma Sci.* **2000**, *10*, 5–22. Gupta, K. C.; Kumar, M. *J. Macromol. Sci.-Rev. Macromol. Chem. Phys.* **2000**, *C40*, 273–308. Bernkop-Schnurch, A. *Int. J. Pharm.* **2000**, *194*, 1–13. Kubota, N.; Tatsumoto, N.; Sano, T.; Toya, K. *Carbohydr. Res.* **2000**, *324*, 268–274. Skaugrud, O.; Hagen, A.; Borgersen, B.; Dornish, M. *Biotechnol. Genet. Eng. Rev.* **1999**, *16*, 23–40. Felt, O.; Buri, P.; Gurny, R. *Drug Dev. Ind. Pharm.* **1998**, *24*, 979–993. Dodane, V.; Vilivalam, V. D. *Pharm. Sci. Technol. Today* **1998**, *1*, 246–253. For combination of chitosan and cyclodextrin see: Jimenez, V.; Belmar, J.; Alderete, J. B. *J. Incl. Phenom. Macrocycl. Chem.* **2003**, *47*, 71–75.
3. Yao, K. D.; Yin, Y. J.; Xu, M. X.; Wang, Y. F. *Polym. Int.* **1995**, *38*, 77. Wang, T.; Turhan, M.; Gunasekaran, S. *Polym. Int.* **2004**, *53*, 911 and references cited therein.
4. Mi, F.-L.; Shyu, S.-S.; Lee, S.-T-.; Wong, T.-B. *J. Polym. Sci.* **1999**, *37*, 1551–1564.
5. Tang, X. H.; Tan, S. Y.; Wang, Y. T. *J. Appl. Polym. Sci.* **2002**, *83*, 1886. Tang, X. H.; Tan, S. Y.; Wang, Y. T. *J. Appl. Polym. Sci.* **2002**, *83*, 1886.
6. Wang, H. F.; Li, W. J.; Lu, Y. H.; Wang, L. *J. Appl. Polym. Sci.* **1997**, *65*, 1445.
7. (a) Schneider, H.-J.; Tianjun, L.; Lomadze, N. *Angew. Chem., Int. Ed. Engl.* **2003**, *42*, 3544–3546. (b) Schneider, H.-J.; Tianjun, L.; Lomadze, N.; Palm, B. *Adv. Mater.* **2004**, *16*, 613–615.(c) Lomadze, N.; Schneider, H.- J. *Tetrahedron Lett.* **2005**, *46*, 751–754. (d) Schneider, H.-J.; Tianjun, L. *Chem. Commun.* **2004**, 100–101.
8. Meyer, E. A.; Castellano, R. K.; Diederich, F. *Angew. Chem., Int. Ed.* **2003**, *42*, 1210–1250 and references cited therein.
9. Yalpani, M.; Hall, D. *Can. J. Chem.* **1981**, *59*, 2934–2939.
10. See: e.g. Roux, E.; Lafleur, M.; Lataste, E.; Moreau, P.; Leroux, J. C. *Biomacromolecules* **2003**, *4*, 240–248. Robinson, D. N.; Peppas, N. A. *Macromolecules* **2002**, *35*, 3668–3674. Suzuki, K.; Yumura, T.; Tanaka, Y.; Akashi, M. *J. Bioact. Compat. Polym.* **2001**, *16*, 409–418. Kuckling, D.; Adler, H. J. P.; Arndt, K. F.; Ling, L.; Habicher, W. D. *Macromol. Symp.* **1999**, *145*, 65–74. Kaneko, M.; Fukui, M.; Takashima, W.; Kaneto, K. *Synth. Met.* **1997**, *84*, 795–796. Firestone, B. A.; Siegel, R. A. *J. Biomater. Sci.-Polym. Ed.* **1994**, *5*, 433–450 and references cited therein. For pH controlled release e.g. from capsules: Kanekiyo, Y.; Naganawa, R.; Tao, H. *Angew. Chem., Int. Ed.* **2003**, *42*, 3014–3016. Zhang, L.; Jin, Y.; Liu, H. Q.; Du, Y. M. *J. Appl. Polym. Sci.* **2001**, *82*, 584–592. Kono, K.; Kawakami, K.; Morimoto, K.; Takagishi, T. *J. Appl. Polym. Sci.* **1999**, *72*, 1763–1773. Kidchob, T.; Kimura, S.; Imanishi, Y. *J. Appl. Polym. Sci.* **1997**, *63*, 453–458.
11. Kockisch, S.; Rees, G. D.; Young, S. A.; Tsibouklis, J.; Smart, J. D. *J. Pharm. Sci.* **2003**, *92*, 1614–1623. Patel, V. R.; Amiji, M. M. In *Hydrogels and Biodegradable Polymers for Bioapplications*, *Vol. 627,* 1996, pp 209–220. Okhamafe, A. O.; Amsden, B.; Chu, W.; Goosen, M. F. A. *J. Microencapsul.* **1996**, *13*, 497–508.
12. Kuhn, W.; Hargitay, B.; Katchalsky, A.; Eisenberg, H. *Nature* **1950**, *165*, 514–516.
13. Schneider, H.-J.; Tianjun, L.; Lomadze, N. *Chem. Commun.* **2004**, 2436–2437.
14. Hsu, S.-C.; Don, T.-M.; Chiu, W.-Y. *J. Appl. Polym. Sci.* **2002**, *86*, 3047–3056.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
ELSEVIER
Tetrahedron 61 (2005) 8699–8704

Stereoselective synthesis of a 12-acetoxyazadiradione analogue

Alfonso Fernández-Mateos,* Gustavo Pascual Coca and Rosa Rubio González

Dpto. Química Orgánica, Facultad de C. Químicas, Universidad de Salamanca, Plaza de los Caídos1-5, Salamanca 37008, Spain

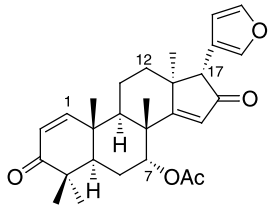
Received 5 May 2005; revised 22 June 2005; accepted 27 June 2005

Abstract—The synthesis of the 12-acetoxy enone **15** related to the limonoid azadiradione has been achieved in 12 steps (16% overall yield) starting from tricyclic diester **1**. The key steps involve intramolecular 1,3-dipolar cycloaddition of a nitrile oxide and a Stille coupling reaction of a vinyl iodide with a stannylfuran.

1. Introduction

Limonoids are degraded triterpenes,[1] that have attracted scientific attention because of their remarkably broad spectrum of biological activity as insect antifeedants,[1,2] their insect growth regulating properties,[3] and their antimalarial,[4] antiarthritic, anti-inflammatory and anticancer activities.[5] Within this family of naturally occurring compounds of special interest are those with C-12 oxygenated function and a 14,15-double bond or 14,15-epoxide in a 4,4,8-trimethyl-17-furyl androstane skeleton, not only because of their bioactivity but also because they are considered to be precursors of the C-seco limonoides, which are the most active group of the limonoid family, (Fig. 1).[1,2]

To our knowledge only the total synthesis of azadiradione, a member of the limonoid group of havanensin, has been reported, based on stereocontrolled polyalkene cyclization and further elaboration of ring D.[6] Other groups have reported approaches to the limonoid model based mainly on AB ring and CDE ring systems.[7] An extensive study has been carried out in our laboratory towards the synthesis of limonoid model compounds based on CDE and BCDE rings system in order to find a simple structural fragment with high bioactivity.[8] We have recently reported a short route to the CDE structural fragment of 12-hydroxy azadiradione,[8c] and here, we prepared a limonoid analogue, that contains some of the key features of 12-acetoxy azadiradione. The approach used for the synthesis is based upon the previously described strategy developed for the synthesis of the 12-hydroxy CDE-model and is a proof of the versatility of the method reported by us.[8c]



Azadiradione

Figure 1.

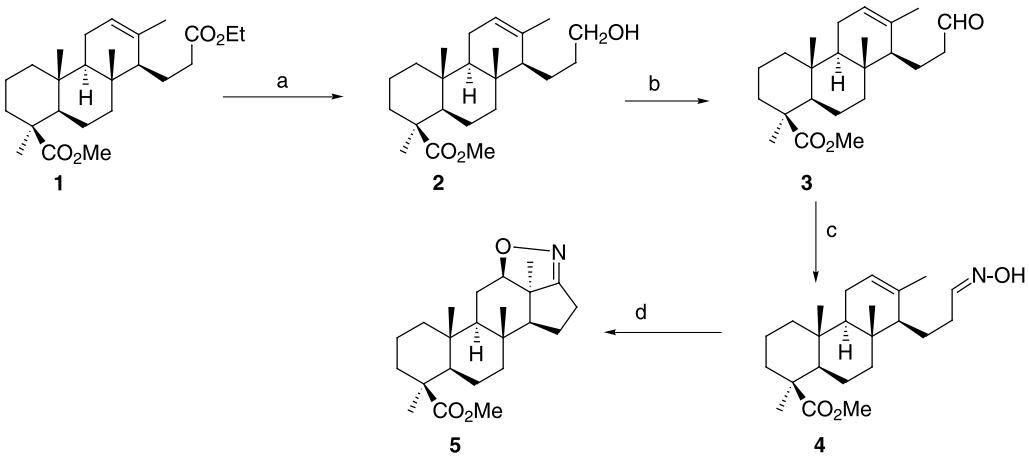
2. Results and discussion

The tricyclic diester **1**, readily available from agathic acid, was selected as the starting material.[9] The first part of the synthesis, which consisted in the transformation of diester **1** into the isoxazoline **5**, was carried out in a four step sequence: selective reduction with LAH to the hydroxyester **2**; Swern oxidation to oxoester **3**; oxime formation with hydroxylamine to **4**, and intramolecular dipolar cyclization, through intermediate nitrile oxide,[8c] with sodium hypochlorite to give **5**. The overall yield of the sequence was the 73%. The β-orientation of the side chain in the oxime **4** guarantees a C-18 methyl angular α-orientation in the pentacyclic isoxazoline **5** after cyclization. The configuration of carbon C-13 in isoxazoline **5** is unequivocally the same as in azadiradione (Scheme 1).

Cleavage of the heterocyclic ring of compound **5** was carried out with hydrogen saturated palladium on carbon

Keywords: Limonoids; Antifeedant; Azadiradione; 1,3-Dipolar cycloaddition; Stille coupling.
* Corresponding author. Tel.: +34 923 294481; fax: +34 923 294574; e-mail: afmateos@usal.es

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.06.088

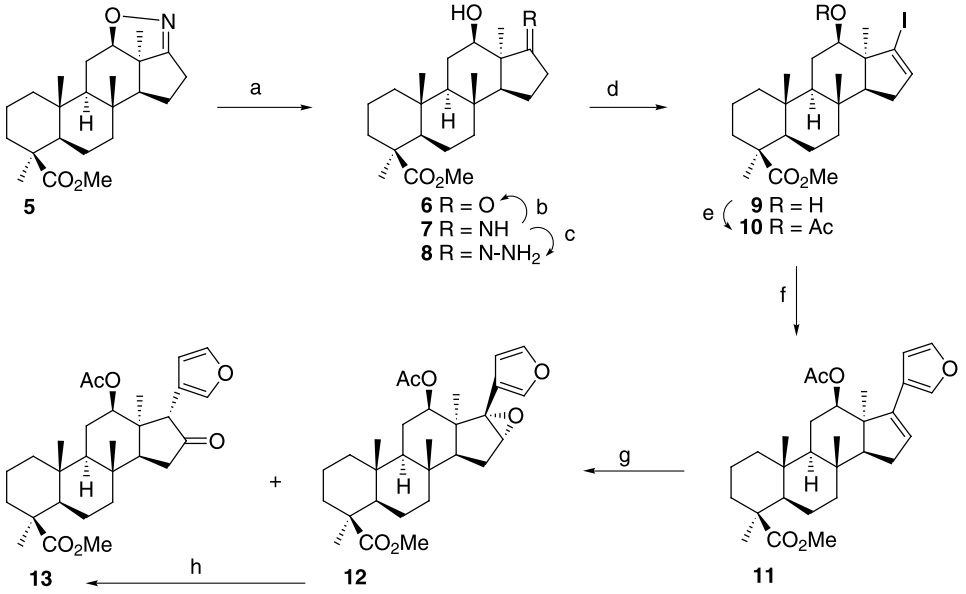


Scheme 1. Reaction conditions: (a) $LiAlH_4$, ether; (b) Swern, CH_2Cl_2; (c) $HCl{\cdot}NH_2OH$, Na_2CO_3, CH_2Cl_2; (d) NaClO, CH_2Cl_2.

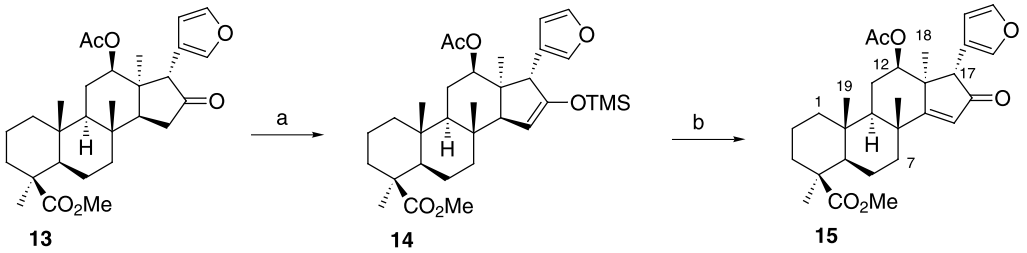
(5%) suspended in a boric acid methanol–water solution.[10] A mixture of the hydroxy ketone **6** and the hydroxyimine **7** was obtained in 75% yield at a ratio 1:3, respectively. The main difference between the two compounds in the ^{13}C NMR spectra was the C-17 carbon signal, which appeared at 225 ppm for the ketone and 196 ppm for the imine. Hydrolysis of the imine function was a very hard reaction. The reaction conditions (2 M HCl/MeOH/reflux) used by Zard[11] were unfruitful; and also the treatment with KOH in MeOH at reflux. Finally, the hydrolysis could be successfully achieved with a solution of *p*TsOH in a water–acetone mixture at reflux after 7 days. The inertia to reaction at carbon C-17 must be due to the severe steric hindrance exerted by the C-13α and the C-8β methyl groups.

The key compound necessary for coupling with the furane ring was the vinyl iodide **9**. This compound was obtained through the hydrazone **8**, which in turn was easily obtained from the ketone **6** by treatment with hydrazine. It is of interest to note the easy and smooth formation of hydrazone from the sterically hindered carbonyl group of **6**. The same must be the case of the imine substitution of **7** by the hydrazone. In this way, it would be possible to avoid the hard hydrolysis of the imine group. Indeed, the hydrazinolysis of the imine compound of **7** occurred readily in quantitative yield.

Transformation of the hydrazone **8** into the vinyl iodide **9** was accomplished by Barton's method,[12] with iodide and triethylamine in THF in 74% yield. The signal at 6.18 ppm in the ^1H NMR assigned to the vinyl proton and the two signals at 102 and 140 ppm assigned to the C=C of the vinyl function confirmed the proposed structure for compound **9**. Coupling of the iodo hydroxy ester **9** with the furane as 3-furyl tributylstannane was attempted with the Stille reaction method,[13] using Pd(0) as catalyst in DMF at 70 °C, but without success. The influence of the near hydroxyl group in the coupling reaction was unknown to us; we believe that some kind deactivation of the catalyst by hydrogen bonding could be involved. To avoid this inhibition, we used an acetoxyl group to replace the hydroxyl group. In the



Scheme 2. Reaction conditions: (a) H_2, Pd/C, MeOH, H_3BO_3; (b) *p*TsOH, acetone, H_2O; (c) $NH_2{-}NH_2.H_2O$, Et_3N, EtOH; (d) I_2, Et_3N, THF; (e) Ac_2O, pyr, DMAP; (f) 3-FurylBu_3Sn, $Pd(Ph_3)_4$, DMF; (g) mCPBA, CH_2Cl_2, −40 °C; (h) $BF_3{\cdot}Et_2O$, CH_2Cl_2, −40 °C.



Scheme 3. Reaction conditions: (a) TfOTMS, Et_3N, CH_2Cl_2; (b) (i) mCPBA, CH_2Cl_2, −40 °C; (ii) *p*TsOH, toluene, reflux.

Stille reaction,[13] the acetoxy derivative **10** was obtained satisfactorily to afford the expected furyl derivate **11** in good yield (80%) after 42 h.

To accomplish the transformation of the D cyclopentene ring of the limonoid **11** into a cyclopentenone, three steps were required: epoxidation, rearrangement and dehydrogenation.

Treatment of the furylandrostane **11** with 3 equiv of mCPBA in CH_2Cl_2 at −40 °C to avoid the oxidation of furane afforded two products in 83% yield: the expected epoxide **12** and the ketone **13** at a ratio 3:1, respectively. A α-configuration was assigned to the oxiranic oxygen of the epoxide **12**, assuming that the β face was more sterically hindered for the peracid approach, as shown in Scheme 2. Formation of ketone **13** was easily explained in terms of relief of the strong interaction between the methyl group in C-8 position and the furane. (Epoxide **12** was difficult to purify due to the easy rearrangement to **13**, even with SiO_2 chromatography). Rearrangement of **12** to **13** (78% yield) with $BF_3{\cdot}Et_2O$ at −40 °C was totally stereoselective after the regioselective heterolytic cleavage of the oxirane, followed by the migration of H-16 to C-17. In this manner the relative orientation of the furan respect to the C-13 methyl group must be *cis*, as is found in the limonoid azadiradione. The cis relation was corroborate by diamagnetic shielding effect caused in the methyl group by the furan ring ($\delta = 1.00$ ppm), as has been observed for related compounds[14] (see Table 1 and Scheme 3).

The final step of the synthesis was dehydrogenation of **13**, which was first attempted by treatment of the sylil enol ether **14** with palladium acetate in acetonitrile.[15] Although the procedure has been applied with success in the preparation of cyclohexenones, it failed in our case. Fortunately, a variation of the above method was satisfactory: epoxidation at low temperature to avoid furan degradation, and subsequent treatment of the resulting mixture with *p*TsOH in toluene at reflux afforded exclusively the 12-acetoxy-azadiradione analog **15** in good yield (62% from **13**).

3. Experimental

3.1. General methods

When required, all solvents and reagents were purified by standard techniques. Reactions were monitored by TLC on silica 60 F245. Organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure with the aid of a rotary evaporator. Column chromatographyc was performed on silica gel 60 (0.040–0.063 mm).

3.1.1. Ent-methyl 15-(2-hydroxyethyl)-isocopal-12-en-19-oate 2. $LiAlH_4$ (282 mg, 7.42 mmol) was added to a solution of diester **1** (1.19 g, 2.97 mmol) in diethyl ether (25 mL) at 0 °C. The reaction mixture was vigorously stirred under argon for 1 h, after, which it was quenched with $Na_2SO_4{\cdot}10H_2O$. The resulting mixture was filtered, and then the filtrate was evaporated under reduced pressure to afford the unsaturated alcohol **2** (1.0 g, 92%) as a white solid, mp 135–137 °C. $[\alpha]_D$ +35.3 (*c* 0.45, $CHCl_3$). IR, ν: 3376, 1726 cm^{-1}. ^1H NMR $CDCl_3$, δ: 0.67 (3H, s), 0.72 (3H, s), 1.15 (3H, s), 1.65 (3H, s), 3.60 (3H, s), 3.61 (2H, m), 5.33 (1H, br s) ppm. HRMS (EI): 362.2845 (M^+, $C_{23}H_{38}O_3$), calcd 362.2821. Anal. Calcd for $C_{23}H_{38}O_3$: C, 76.20; H, 10.56. Found: C, 76.41; H, 10.65.

3.1.2. Ent-methyl 15-(formylmethyl)-isocopal-12-en-19-oate 3. A solution of dimethyl sulfoxide (0.57 mL, 7.22 mmol) in CH_2Cl_2 (2 mL) was added dropwise to a stirred solution of oxalyl chloride (0.35 mL, 3.83 mmol) in CH_2Cl_2 (12.2 mL) under argon at −60 °C. After 5 min, a solution of the alcohol **2** (950 mg, 2.62 mmol) in CH_2Cl_2–DMSO (3:1, 1.8 mL) was added dropwise. The reaction mixture was stirred for a further 20 min, triethylamine (2.36 mL, 17.75 mmol) was added at −60 °C, and stirring was continued for a further 10 min. Then, it was allowed to warm to room temperature, and water was added. The organic layer was separated, and the aqueous phase was extracted with CH_2Cl_2. The combined extracts were washed with water, dried and filtered. The solvent was removed to afford the aldehyde **3** (869 mg, 92%) as a crystalline solid: mp 91–93 °C. IR, ν: 2930, 1720 cm^{-1}. ^1H NMR $CDCl_3$, δ: 0.69 (3H, s), 0.77 (3H, s), 1.15 (3H, s), 1.65 (3H, s), 3.63 (3H, m), 5.45 (1H, br s), 9.79 (1H, br s) ppm. ^{13}C NMR $CDCl_3$, δ: 201.9, 177.6, 133.8, 123.0, 57.0, 54.6, 54.3, 50.9, 45.8, 43.6, 41.1, 40.2, 38.0, 37.7, 37.0, 28.6, 22.8, 21.9, 20.2, 19.1, 18.9, 14.1, 13.5 ppm. HRMS (EI): 360.2691 (M^+, $C_{23}H_{36}O_3$), calcd 360.2664. Anal. Calcd for $C_{23}H_{36}O_3$: C, 76.62; H, 10.06. Found: C, 76.49; H, 10.11.

3.1.3. Oxime of ent-methyl 15-(formylmethyl)-isocopal-12-en-19-oate 4. A solution of hydroxylamine hydrochloride (1.73 g, 25.18 mmol) in water (6.5 mL) was added to a solution of the aldehyde **3** (830 mg, 2.31 mmol) in ether (28 mL). The mixture was vigorously stirred at room temperature for 5 min, and a solution of sodium carbonate (2.67 g, 9.72 mmol) in water (6.5 mL) was added dropwise. After the mixture was stirred for an additional 1 h, the layers were separated. The aqueous phase was extracted with ether. The combined extracts were washed with brine, dried,

filtered and evaporated to give an oily product identified as a mixture of the oximes **4** (795 mg, 92%): IR, ν: 3312, 2947, 1724 cm^{-1}. ^1H NMR $CDCl_3$, δ: 0.70 (6H, s), 0.75 (6H, s), 1.17 (6H, s), 1.67 (3H, s), 1.75 (3H, s), 3.62 (3H, s), 3.63 (3H, s) 5.40 (2H, br s), 6.74 (1H, t, $J=6$ Hz), 7.41 (1H, t, $J=6$ Hz) ppm.

3.1.4. 4β-Methoxycarbonyl-4α,8β-dimethyl-13α-androstane[12,17-*d*,*c*]-1,2-oxazoline 5. To a solution of the oximes **4** (760 mg, 2.03 mmol) in CH_2Cl_2 (80 mL) at 0 °C was added dropwise a solution of aqueous sodium hypochlorite (5%, 5.5 mL). After the mixture was stirred for 30 min, it was added water, and the organic layer was separated. The aqueous phase was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried and filtered. The solvent was removed to afford the isoxazoline **5** (707 mg, 93%): $[\alpha]_D$ +116.1 (*c* 0.20, $CHCl_3$). IR, ν: 2880, 1730 cm^{-1}. ^1H NMR $CDCl_3$, δ: 0.65 (3H, s), 0.89 (3H, s), 1.17 (3H, s), 1.22 (3H, s), 3.62 (3H, s), 4.27 (1H, t, $J=9$ Hz) ppm. ^{13}C NMR $CDCl_3$, δ: 177.5, 175.3, 85.5, 61.0, 57.3, 57.0, 51.0 (2C), 43.9, 43.7, 40.2, 37.8, 37.5, 36.2, 29.0, 28.6, 25.9, 25.0, 19.8, 19.4, 19.0, 15.4, 13.6 ppm. MS *m*/*z* (relative intensity) 373 (56, M^+), 356 (26), 314 (21), 235 (13), 206 (15), 192 (33), 161 (19), 107 (59), 81 (76), 55 (100). HRMS (EI): 373.2651 (M^+, $C_{23}H_{35}NO_3$), calcd 373.2617.

3.1.5. Reduction of isoxazoline 5. A solution of the isoxazoline **5** (675 mg, 1.8 mmol) in methanol–water (5:1, 13 mL) was added boric acid (233 mg, 1.28 mmol) and Pd/C of 10% (60 mg). The resulting mixture was hydrogenated for 54 h at 40 °C and atmospheric pressure. After filtration of the catalyst, the solvent was removed in vacuo and the residue was extracted with ether and washed with saturated aqueous $NaHCO_3$. The dried organic phase was concentrated at reduce pressure to give a crude residue, which was purified by flash chromatography. Eluting with hexane–ether (70:30) furnished 12β-hydroxy-4β-methoxycarbonyl-4α,8β-dimethyl-13α-androstan-17-one **6** (132 mg, 19%), as a white solid, mp 203–206 °C. $[\alpha]_D$ +27.2 (*c* 0.53, $CHCl_3$). IR, ν: 3490, 2940, 1720, 1710 cm^{-1}. ^1H NMR $CDCl_3$, δ: 0.66 (3H, s), 0.74 (3H, s), 1.17 (3H, s), 1.34 (3H, s), 3.53 (1H, m), 3.63 (3H, s) ppm. ^{13}C NMR $CDCl_3$, δ: 225.2, 177.5, 77.9, 59.3, 57.3, 54.9, 51.3, 51.1, 43.9, 42.0, 40.3, 38.0, 37.8, 37.6, 35.6, 29.1, 28.7, 24.8, 19.6, 19.1, 18.3, 16.0, 14.4 ppm. HRMS (EI): 376.2634 (M^+, $C_{23}H_{36}O_4$), calcd 376.2614. Anal. Calcd for $C_{23}H_{36}O_4$: C, 73.37; H, 9.64. Found: C, 73.21; H, 9.55. Eluting with hexane–ether (30:70) furnished 12β-hydroxy-4β-methoxycarbonyl-4α,8β-dimethyl-13α-androstan-17-imine **7** (400 mg, 56%), as a white solid, mp 182–185 °C. ^1H NMR $CDCl_3$, δ: 0.65 (3H, s), 0.76 (3H, s), 1.15 (3H, s), 1.32 (3H, s), 3.50 (1H, m), 3.62 (3H, s) ppm. ^{13}C NMR $CDCl_3$, δ: 196.1, 177.3, 78.4, 60.6, 57.0, 55.0, 50.8, 48.3, 43.6, 42.0, 40.0, 37.8, 37.6, 37.3, 34.3, 28.6, 28.4, 26.7, 19.9, 19.3, 18.9, 15.5, 14.1 ppm. HRMS (EI): 375.2761 (M^+, $C_{23}H_{37}NO_3$), calcd 375.2773. Anal. Calcd for $C_{23}H_{37}NO_3$: C, 73.56; H, 9.93; N, 3.73. Found: C, 73.84; H, 10.14; N, 3.79.

3.1.6. Reaction of imine 7 with *p*-toluenesulphonic acid. A solution of **7** (50 mg, 0.13 mmol) in acetone (3 mL) was added water (0.5 mL) and *p*-$TsOH\cdot H_2O$ (5 mg, 0.02 mmol). The reaction mixture was stirred under argon at reflux for 7 days. Then, the mixture was cooled to room temperature and the acetone was removed in vacuo. Water was added and the residue was extracted with ether, the organic layer was washed with an aqueous solution of 5% $NaHCO_3$ and brine. Removal of the solvent afforded the hydroxy ketone **6** (38 mg, 75%).

3.1.7. Reaction of hydroxy ketone 6 with NH_2–NH_2. A solution of the hydroxy ketone **6** (152 mg, 0.40 mmol) in ethanol (8 mL) was treated with triethylamine (0.5 mL) and hydrazine hydrate (1.0 mL), and the solution was heated under reflux for 3 days. The solvent was evaporated, the residue was dissolved in ether and the solution was washed with water to neutrality. Then the organic phase was dried and evaporated to give the corresponding hydrazone **8** (157 mg, 100%): IR, ν: 3328, 1726, 1469, 1110 cm^{-1}. ^1H NMR $CDCl_3$, δ: 0.59 (3H, s), 0.61 (3H, s), 0.93 (3H, s), 1.14 (3H, s), 3.59 (3H, m) ppm.

3.2. Reaction of imine 7 with NH_2–NH_2

A solution of the imine **7** (230 mg, 0.60 mmol) in ethanol (12 mL) was treated with triethylamine (0.8 mL) and hydrazine hydrate (1.5 mL), and the solution was heated under reflux for 4 days. The solvent was evaporated, the residue was dissolved in ether and the solution was washed with water to neutrality. Then the organic phase was dried and evaporated to give the corresponding hydrazone **8** (235 mg, 100%).

3.2.1. 12β-Hydroxy-17-iodo-4β-methoxycarbonyl-4α,8β-dimethyl-13α-androst-16-ene 9. A solution of the above hydrazone **8** (350 mg, 0.89 mmol) in THF (14 mL) and triethylamine (2.0 mL) was treated with iodine until a slight excess was present (cessation of nitrogen evolution, brown color not discharged) and then was added ether. The ethereal solution was washed successively with aqueous 2 N HCl, water to neutrality, aqueous $NaHSO_3$ (10%), water, saturated aqueous $NaHCO_3$, and brine. Removal of the solvent afforded a crude residue, which was purified by flash chromatography. Eluting with hexane/Et_2O (90:10) furnished vinyl iodide **9** (320 mg, 74%), as a white solid, mp 140–141 °C. $[\alpha]_D$ +47.9 (*c* 1.86, $CHCl_3$). IR, ν: 3354, 2924, 1715 cm^{-1}. ^1H NMR $CDCl_3$, δ: 0.73 (3H, s), 0.92 (3H, s), 1.12 (3H, s), 1.16 (3H, s), 3.63 (3H, s), 3.76 (1H, m), 6.18 (1H, t, $J=3$ Hz) ppm. ^{13}C NMR $CDCl_3$, δ: 177.4, 140.6, 102.6, 73.5, 57.5, 57.4, 54.7, 53.3, 50.9, 44.5, 43.7, 40.1, 37.9, 37.7, 36.3, 34.3, 28.8, 28.6, 19.8, 18.9, 17.1, 16.8, 13.6 ppm. HRMS (EI): 486.1675 (M^+, $C_{23}H_{35}IO_3$), calcd 486.1631.

The second fraction was the hydroxy ketone **6** (74 mg, 22%).

3.2.2. 12β-Acetoxy-17-iodo-4β-methoxycarbonyl-4α,8β-dimethyl-13α-androst-16-ene 10. A solution of vinyl iodide **9** (236 mg, 0.48 mmol) in CH_2Cl_2 (3 mL) at 0 °C was added pyridine (0.5 mL, 5.0 mmol), acetic anhydride (0.25 mL, 2.50 mmol) and DMAP (5 mg). The reaction mixture was stirred at room temperature for 48 h, and then poured in water. The mixture was extracted with ether and the organic phase was washed successively with 2 N HCl, aqueous 5% $NaHCO_3$ (5%), and brine. The organic layer

was dried and concentrate to afford the acetate **10** (246 mg, 96%) as a white solid: mp 153–154 °C. IR, ν: 2962, 1736 cm^{-1}. ^1H NMR $CDCl_3$, δ: 0.67 (3H, s), 0.85 (3H, s), 1.08 (3H, s), 1.16 (3H, s), 2.03 (3H, s), 3.62 (3H, s), 4.83 (1H, dd, $J=6$ Hz, $J'=8$ Hz), 6.08 (1H, t, $J=3$ Hz) ppm. ^{13}C NMR $CDCl_3$, δ: 177.5, 170.1, 140.3, 102.3, 74.4, 57.7, 57.6, 53.5, 53.0, 51.0, 44.3, 43.8, 40.0, 37.9, 37.6, 36.3, 34.2, 29.2, 28.6, 25.3, 21.2, 19.8, 18.9, 16.4, 13.7 ppm. HRMS (EI): 528.1796 (M^+, $C_{25}H_{37}IO_4$), calcd 528.1737.

3.2.3. 12β-Acetoxy-17-(3′-furyl)-4β-methoxycarbonyl-4α,8β-dimethyl-13α-androst-16-ene 11. To a solution of $Pd(PPh_3)_4$ (10 mg, 2% mol) in DMF (0.8 mL) was added a solution of vinyl iodide **10** (212 mg, 0.40 mmol) and 3-(tributylstannyl)furan (157 mg, 0.44 mmol) in DMF (4 mL). This mixture was heated under reflux for 42 h, cooled to room temperature, and diluted with pentane. The resulting solution was washed sequentially with water, 10% ammonium hydroxide, water and brine. This solution was dried and filtered. Removal of the solvent afforded a residue, which was chromatographed using hexane/ether (90:10) to yield the acetate **11** (150 mg, 80%) as a colorless oil: $[\alpha]_D$ +53.0 (*c* 0.26, $CHCl_3$). IR, ν: 2951, 1740, 1717 cm^{-1}. ^1H NMR $CDCl_3$, δ: 0.67 (3H, s), 0.88 (3H, s), 1.17 (3H, s), 1.23 (3H, s), 1.81 (3H, s), 3.63 (3H, s), 5.03 (1H, t, $J=8$ Hz), 5.71 (1H, t, $J=3$ Hz), 6.41 (1H, m), 7.32 (2H, m) ppm. ^{13}C NMR $CDCl_3$, δ: 177.6, 170.2, 141.7, 139.8, 138.2, 128.1, 121.9, 110.7, 76.1, 61.6, 57.5, 53.3, 52.1, 50.9, 44.1, 43.8, 39.9, 37.9, 37.8, 36.6, 31.2, 29.2, 28.6, 25.9, 20.9, 19.6, 18.8, 16.4, 13.7 ppm. HRMS (EI): 486.2842 (M^+, $C_{29}H_{40}O_5$), calcd 468.2876. Anal. Calcd for $C_{29}H_{40}O_5$: C, 74.33; H, 8.60. Found: C, 74.51; H, 8.49.

3.2.4. Reaction of 11 with mCPBA. To a stirred mixture of **11** (130 mg, 0.28 mmol) and Na_2CO_3 (5 mg) in CH_2Cl_2 (7 mL) at −40 °C was added mCPBA (145 mg, 0.84 mmol). The reaction mixture was stirred under argon at this temperature for 3 h. Then, Na_2SO_3 (10%) was added and the resulting heterogeneous mixture was vigorously stirred for 15 min. The organic layer was separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic extracts were washed with $NaHCO_3$ (5%) and brine. Removal of the solvent afforded a crude residue, which was purified by flash chromatography. Eluting with hexane–Et_2O (98:2) furnished 12β-acetoxy-16α,17-epoxy-17β-(3′-furyl)-4β-methoxycarbonyl-4α,8β-dimethyl-13α-androstane **12** (84 mg, 62%), as a colorless oil. IR, ν: 2964, 1751 cm^{-1}. ^1H NMR $CDCl_3$, δ: 0.71 (3H, s), 1.02 (3H, s), 1.17 (3H, s), 1.34 (3H, s), 1.99 (3H, s), 3.63 (3H, s), 3.69 (1H, s), 5.83 (1H, m), 6.37 (1H, m), 7.34 (1H, m), 7.36 (1H, m) ppm. HRMS (EI): 484.2855 (M^+, $C_{29}H_{40}O_6$), calcd 484.2825. Anal. Calcd for $C_{29}H_{40}O_6$: C, 71.87; H, 8.32. Found: C, 71.72; H, 8.26.

Eluting with hexane/Et_2O (90:10) furnished 12β-acetoxy-17α-(3′-furyl)-4β-methoxycarbonyl-4α,8β-dimethyl-13α-androstan-16-one **13** (28 mg, 21%), as a crystalline solid, mp 185–187 °C. $[\alpha]_D$ +26.4 (*c* 1.7, $CHCl_3$). IR, ν: 2980, 1720 cm^{-1}. ^1H NMR $CDCl_3$, δ: 0.69 (3H, s), 0.90 (3H, s), 1.00 (3H, s), 1.18 (3H, s), 1.80 (3H, s), 3.64 (3H, s), 3.78 (1H, s), 4.94 (1H, dd, $J=16$ Hz, $J'=7$ Hz), 6.16 (1H, m), 7.21 (1H, m), 7.33 (1H, m) ppm. ^{13}C NMR $CDCl_3$, δ: 218.1, 177.5, 170.4, 142.3, 141.6, 120.4, 112.6, 80.1, 56.9, 56.0, 55.4, 51.2, 50.6, 47.2, 43.8, 42.0, 40.1, 38.4, 37.9, 37.6, 37.5, 28.5, 25.9, 23.9, 20.6, 19.3, 18.9, 16.2, 14.2 ppm. HRMS (EI): 484.2820 (M^+, $C_{29}H_{40}O_6$), calcd 484.2825. Anal. Calcd for $C_{29}H_{40}O_6$: C, 71.87; H, 8.32. Found: C, 71.82; H, 8.26.

3.3. Reaction of 12 with $BF_3 \cdot Et_2O$

To a solution of epoxide **12** (63 mg, 0.13 mmol) in dry CH_2Cl_2 (5 mL) was added boron trifluoride–diethyl ether (0.05 mL, of the solution of 0.25 mL of BF_3–Et_2O in 10 mL of CH_2Cl_2) at −40 °C, and the mixture was stirred for 5 min. Then, it was diluted with ether and water was added. The layers were separated, and the aqueous phase was extracted with ether. The organic layer was washed with $NaHCO_3$ (5%), and brine. Evaporation of the solvent afforded the ketone **13** (49 mg, 78%).

3.3.1. 12β-Acetoxy-17α-(3′-furyl)-4β-methoxycarbonyl-4α,8β-dimethyl-16-trimethylsililoxy-13α-androst-15-ene 14. To a solution of the ketone **13** (49 mg, 0.10 mmol) in CH_2Cl_2 (0.5 mL) under argon and at 0 °C, was added dropwise triethylamine (11 mL, 0.11 mmol) and then TMSOTf (0.10 mL, 0.10 mmol, 1 M in CH_2Cl_2). The mixture reaction was stirred at room temperature for 3 h and then poured in $NaHCO_3$ (5%). The layers were separated, and the aqueous phase was extracted with ether. The organic layer was washed with $NaHCO_3$ (5%), and brine. Evaporation of the solvent afforded the compound **14** (43 mg, 77%) as a colorless oil. ^1H NMR $CDCl_3$, δ: 0.03 (9H, s), 0.69 (3H, s), 0.94 (3H, s), 0.98 (3H, s), 1.17 (3H, s), 1.43 (3H, s), 3.63 (3H, s), 3.92 (1H, m), 4.72 (2H, m), 6.19 (1H, m), 7.18 (1H, m), 7.26 (1H, m) ppm. ^{13}C NMR $CDCl_3$, δ: 177.8, 172.7, 155.0, 142.1, 140.5, 122.1, 113.7, 103.6, 80.8, 64.4, 57.1, 54.4, 51.1, 47.1, 46.3, 43.8, 43.2, 40.3, 39.7, 38.0, 37.7, 30.3, 28.6, 28.4, 24.8, 19.7, 18.9, 17.6, 14.4–1.40 (3C) ppm. HRMS (EI): 556.3293 (M^+, $C_{32}H_{48}O_6Si$), calcd 556.3220.

3.3.2. Synthesis of 12β-acetoxy-17α-(3′-furyl)-4β-methoxycarbonyl-4α,8β-dimethyl-13α-androst-14-en-16-one 15. (A) Epoxidation of **14**. To a stirred mixture of **14** (40 mg, 0.07 mmol) in CH_2Cl_2 (2 mL) at −40 °C was added mCPBA (36 mg, 0.21 mmol). The reaction mixture was stirred under argon at this temperature for 15 min. Then, $Na_2S_2O_3$ (10%) was added and the resulting heterogeneous mixture was vigorously stirred for 15 min. The organic layer was separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic extracts were washed with $NaHCO_3$ (5%) and brine. The solvent was removed to afford a crude product, which was used in the next reaction. (B) Reaction with *p*-TsOH. To a solution of the above mixture in deoxygenated toluene (2 mL) at reflux and under argon was added a catalytic amount of *p*-TsOH·H_2O The reaction mixture was stirred under argon at reflux for 30 min. Then, the mixture was cooled to room temperature and then poured in $NaHCO_3$ (5%). The layers were separated, and the aqueous phase was extracted with ether. The organic layer was washed with $NaHCO_3$ (5%), and brine. Evaporation of the solvent afforded the compound **15** (27 mg, 80%) as a colorless oil. $[\alpha]_D$ +25.5 (*c* 0.20, $CHCl_3$). IR, ν: 2928, 1723, 1694 cm^{-1}. ^1H NMR $CDCl_3$, δ: 0.76 (3H, s), 1.06 (3H, s), 1.19 (3H, s), 1.33 (3H,

s), 2.10 (3H, s), 3.67 (3H, s), 3.76 (1H, s), 5.17 (1H, d, J= 9 Hz), 6.01 (1H, s), 6.22 (1H, m), 7.40 (1H, m), 7.44 (1H, m) ppm. ^{13}C NMR $CDCl_3$, δ: 205.6, 194.2, 177.5, 169.9, 144.8, 142.0, 124.9, 118.0, 112.6, 70.6, 57.3, 52.9, 51.3, 51.0, 47.5, 43.7, 40.8, 39.7, 39.5, 38.0, 37.8, 28.6, 25.8, 25.4, 25.1, 21.3, 20.2, 18.8, 13.5 ppm. HRMS (EI): 482.2649 (M^+, $C_{29}H_{38}O_6$), calcd 482.2668. Anal. Calcd for $C_{29}H_{38}O_6$: C, 72.17; H, 7.94. Found: C, 72.21; H, 7.87.

Acknowledgements

Financial support for this work from the Ministerio de Ciencia y Tecnología of Spain (PPQ2002-00290) and the Junta de Castilla y León (SA027/03) are gratefully acknowledged.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2005.06.088

References and notes

1. (a) Taylor, D. H. A. *Prog. Chem. Org. Nat. Prod.* **1984**, *45*, 1. (b) Champagne, D. E.; Koul, O.; Isman, M. B.; Scudder, G. G. E.; Towers, G. H. N. *Phytochemistry* **1992**, *31*, 377. (c) Akhila, A.; Rani, K. *Prog. Chem. Org. Nat. Prod.* **1999**, *78*, 48.
2. (a) *Neem: A Tree for Solving Global Problems*; National Research Council, National Academy: Washington DC, 1992; p 28. (b) Mordue, A. J.; Blackwell, A. *J. Insect Physiol.* **1993**, *39*, 903. (c) Rembold, H. In *Focus on Phytochemical Pesticides*; Jacobson, M., Ed.; The Neem Tree; CRC: Boca Raton, 1988; Vol. 1, p 47. (d) Kraus, W. *Chem. Biotech. Biol. Act. Nat. Prod. Prod.* 2nd International Conference Budapest **1983**, 331. (e) Ley, S. V.; Denholm, A. A.; Wood, A. *Nat. Prod. Rep.* **1993**, 109. (f) Nanduri, S.; Thunuguntla, S. S. R.; Nyavanandi, V. K.; Kasu, S.; Kumar, P. M.; Ram, P. S.; Rajagopal, S.; Kumar, R. A.; Deevi, D. S.; Rajagopalan, R.; Venkateswarlu, A. *Biorg. Med. Chem. Lett.* **2003**, *13*, 4111.
3. Jones, I.; Ley, S. V.; Denholm, A. A.; Lovell, H.; Wood, A.; Sieden, R. E. *FEMS Microbiol. Lett.* **1994**, *120*, 267.
4. Nair, M. S.; Gopal, S.; Issac, D. *Phytochemistry* **1997**, *46*, 1177.
5. Brahmachari, G. *ChemBioChem* **2004**, *5*, 408.
6. (a) Corey, E. J.; Hahl, R. W. *Tetrahedron Lett.* **1989**, *30*, 3023. (b) Corey, E. J.; Reid, J. G.; Myers, A. G.; Hahl, R. W. *J. Am. Chem. Soc.* **1987**, *109*, 918.
7. (a) Ley, S. V.; Denholm, A. A.; Wood, A. *Nat. Prod. Rep.* **1993**, 109. (b) Ley, S. V.; Denholm, A. A.; Wood, A.; Jennens, L. *Tetrahedron* **1995**, *51*, 6591. (c) Ley, S. V.; Gutteridge, C. E.; Pape, A. R.; Spilling, C. D.; Zumbrunn, C. *Synlett* **1999**, 1295. (d) Ishihara, J.; Ikuma, Y.; Hatakeyama, S.; Suzuki, T.; Murai, A. *Tetrahedron* **2003**, *59*, 10287. (e) Paquette, L. A.; Hong, F. T. *J. Org. Chem.* **2003**, *68*, 6905.
8. (a) Fernández Mateos, A.; López Barba, A. *J. Org. Chem.* **1995**, *60*, 3580. (b) Fernández Mateos, A.; Pascual Coca, G.; Rubio González, R.; Tapia Hernández, C. *J. Org. Chem.* **1996**, *61*, 9097. (c) Fernández Mateos, A.; Martín de la Nava, E. M.; Pascual Coca, G.; Rubio González, R.; Ramos Silvo, A. I.; Simmonds, M. S. J.; Blaney, W. M. *J. Org. Chem.* **1998**, *63*, 9440. (d) Fernández Mateos, A.; Eva Martín de la Nava, E. M.; Rubio González, R. *Synlett* **2001**, 1399. (e) Fernández Mateos, A.; Eva Martín de la Nava, E. M.; Rubio González, R. *J. Org. Chem.* **2001**, *66*, 7632. (f) Fernández Mateos, A.; Eva Martín de la Nava, E. M.; Rubio González, R. *Synthesis* **2002**, 1728. (g) Fernández Mateos, A.; Eva Martín de la Nava, E. M.; Rubio González, R. *J. Org. Chem.* **2003**, *68*, 7632.
9. Fernández-Mateos, A.; Pascual Teresa, J. de; Rubio González, R. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2429.
10. Curran, D. P. *J. Am. Chem. Soc.* **1998**, *105*, 5826.
11. Boivin, J.; Schiano, A. M.; Zard, S. Z. *Tetrahedron Lett.* **1992**, *33*, 7849.
12. Barton, D. H. R.; Bashiardes, G.; Fourrey, J. L. *Tetrahedron* **1988**, *44*, 147.
13. Stille, J. K. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 508.
14. (a) Kraus, W.; Cramer, R. *Tetrahedron Lett.* **1978**, *19*, 2395. (b) Gaikward, B. R.; Mayelvaganan, T.; Vyas, B. A.; Bhat, S. V. *Phytochemistry* **1990**, *29*, 3963.
15. Ito, Y.; Hirao, T.; Saegusa, T. *J. Org. Chem.* **1978**, *43*, 1011.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron 61 (2005) 8705–8710
Tetrahedron

Facile synthesis of α,β-acetylenic ketones and 2,5-disubstituted furans: consecutive activation of triple and double bond with $ZnBr_2$ toward the synthesis of furan ring

Ka Young Lee, Mi Jung Lee and Jae Nyoung Kim*

Department of Chemistry and Institute of Basic Science, Chonnam National University, Gwangju 500-757, South Korea

Received 1 June 2005; accepted 24 June 2005

Available online 18 July 2005

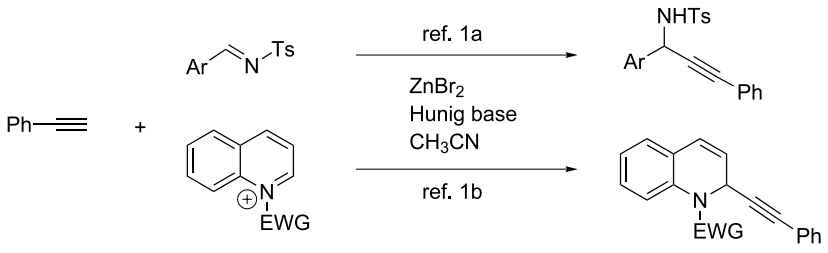
Abstract—α,β-Acetylenic ketones were synthesized from the reaction of acid chlorides and acetylenic compounds in the presence of $ZnBr_2$ and DIEA in acetonitrile. From the acetylenic ketones having nearby methylene unit, 2,5-disubstituted furan derivatives could be synthesized under the same reaction conditions.

1. Introduction

Recently, we reported the alkynylation of *N*-tosylimines[1a] and quinolinium salts[1b] with aryl acetylenes promoted by $ZnBr_2$ and *N*,*N*-diisopropylethylamine (DIEA, Hunig base) in acetonitrile. In the reactions, we obtained *N*-tosyl propargylamines[1a] and 1-acyl-1,2-dihydroquinolines[1b] in moderate to good yields by the addition of in situ generated zinc acetylide (Scheme 1). On the other hand, Carreira and co-workers have reported a mild procedure for the addition of acetylenic compounds to nitrones,[2a] *N*-acyliminium salts,[2b] and aldehydes[2c] involving the in situ generated zinc acetylide in the presence of zinc triflate and tertiary amine. The two procedures were similar conceptually, however, the latter procedure used hygroscopic and expensive zinc triflate instead of $ZnBr_2$.

α,β-Acetylenic ketones are very important synthetic intermediates[3] and numerous synthetic methods have been reported.[3,4] Among them the use of copper(I) salt in combination of a tertiary amine is widely used.[4] However, the method used carcinogenic triethylamine as the solvent and required long time (30 h) for the reaction.[4a,b] Very recently, the combination of palladium catalyst and CuI has been published.[4d,e] However, most of the reported methods have some problems such as low yields, use of toxic or expensive reagents, and long reaction time. Moreover, to the best of our knowledge, the use of zinc triflate and amine system of Carreira[2] has not been reported for the synthesis of α,β-acetylenic ketones. In searching for the usefulness of our reaction conditions,[1] we envisioned that the reaction of aryl acetylene **1** and acid chloride **2** under the conditions could afford valuable α,β-acetylenic ketones **3** (Scheme 2).

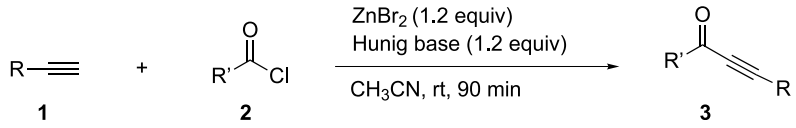
As expected the reaction of phenylacetylene (**1a**) and benzoyl chloride (**2a**) in acetonitrile in the presence of



Scheme 1.

Keywords: Acetylenic ketones; Phenylacetylene; Furans.
* Corresponding author. Tel.: +82 62 530 3381; fax: +82 62 530 3389; e-mail: kimjn@chonnam.ac.kr

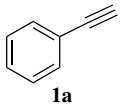
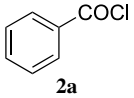
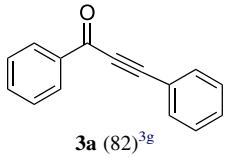
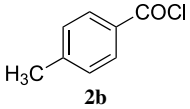
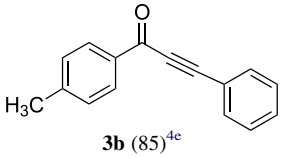
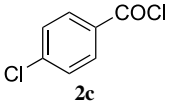
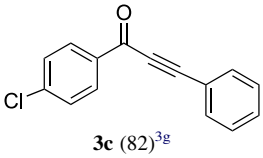
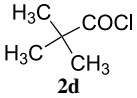
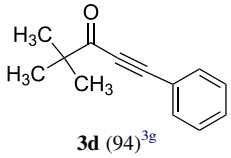
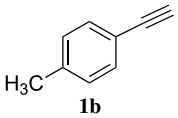
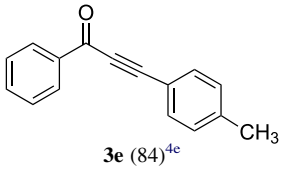
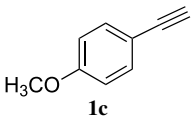
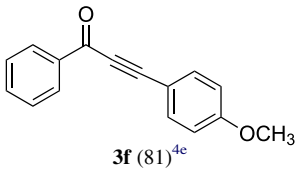
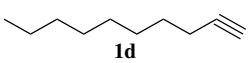
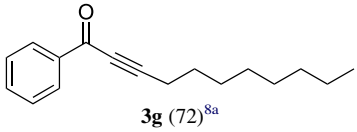
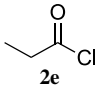
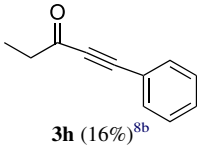
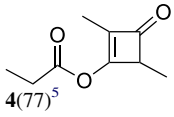
0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.06.104



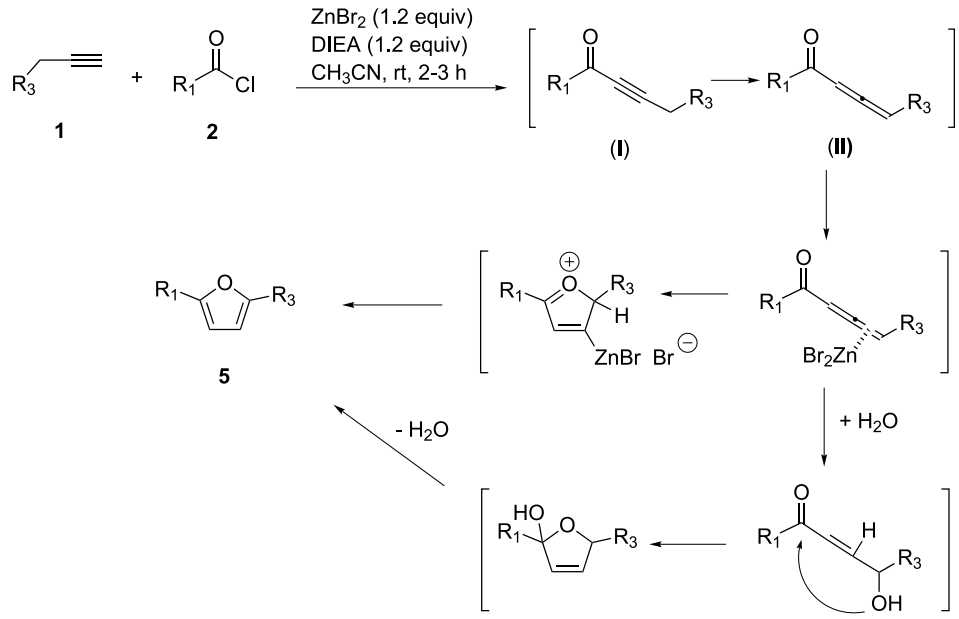
Scheme 2.

$ZnBr_2$ and DIEA gave the desired compound **3a** in high yield (82%). The yield was satisfactory and the reaction conditions were very convenient to carry out in a practical sense. Encouraged by the results we examined the reactions between **1a–d** and **2a–e** and the results are summarized in Table 1. As shown, substituted benzoyl chlorides (entries 2 and 3) and pivaloyl chloride (entry 4) could be used as the electrophilic components. The reaction with benzoic

Table 1. Synthesis of α,β-acetylenic ketones[a,b]

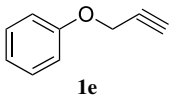
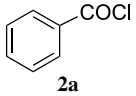
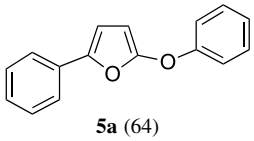
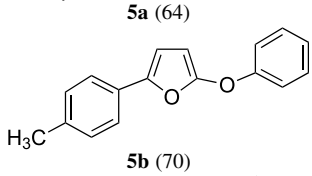
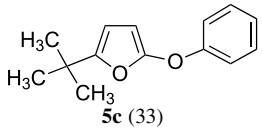
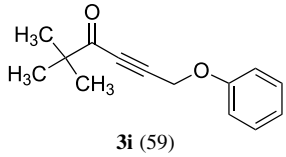
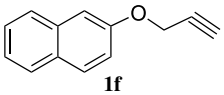
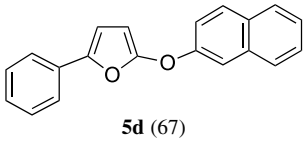
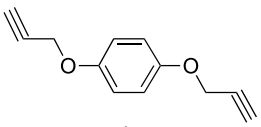
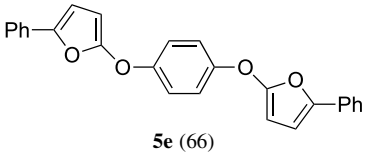
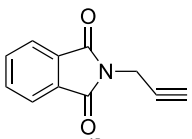
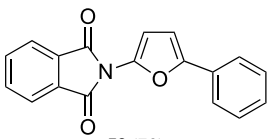
Entry	Acetylenes	Acid chlorides	Products (%)
1	 **1a**	 **2a**	 **3a** (82)[3g]
2	**1a**	 **2b**	 **3b** (85)[4e]
3	**1a**	 **2c**	 **3c** (82)[3g]
4	**1a**	 **2d**	 **3d** (94)[3g]
5	 **1b**	**2a**	 **3e** (84)[4e]
6	 **1c**	**2a**	 **3f** (81)[4e]
7	 **1d**	**2a**	 **3g** (72)[8a]
8	**1a**	 **2e**	 **3h** (16%)[8b]  **4**(77)[5]

[a] Conditions: acetylenic compound **1** (1 equiv), acid chloride **2** (1.2 equiv), $ZnBr_2$ (1.2 equiv), DIEA (1.2 equiv), rt, 90 min, CH_3CN.
[b] The reaction of **1a** and **2a** in the presence of $Zn(OTf)_2$ (1.2 equiv) and DIEA (rt, 90 min) gave **3a** in a similar yield (80%). The use of $ZnCl_2$, ZnI_2, or catalytic amounts of $ZnBr_2$ did not give **3a** in appreciable yield.



Scheme 3.

Table 2. Synthesis of 2,5-disubstituted furans[a]

Entry	Acetylenes	Acid chlorides	Time (h)	Products (%)
1	 **1e**	 **2a**	2	 **5a** (64)
2	**1e**	**2b**	3	 **5b** (70)
3	**1e**	**2d**	3	 **5c** (33)
				 **3i** (59)
4	 **1f**	**2a**	3	 **5d** (67)
5	 **1g**	**2a**	8	 **5e** (66)
6[b]	 **1h**	**2a**	20	 **5f** (70)

[a] Conditions: acetylenic compound **1** (1 equiv), acid chloride **2** (1.2 equiv), $ZnBr_2$ (1.2 equiv), DIEA (1.2 equiv), rt, times given in that table.
[b] Conditions: **2a** (2 equiv), $ZnBr_2$ (2 equiv). DIEA (2 equiv), rt, 20 h.

anhydride instead of benzoyl chloride failed. The acid chloride bearing α-proton showed different reactivity under the reaction conditions. As an example (entry 8), for the reaction of propionyl chloride (**2e**) and phenylacetylene we obtained the desired acetylenic ketone **3h** in low yield (16%) together with ketene dimer derivative **4** as the major product (77%).[5]

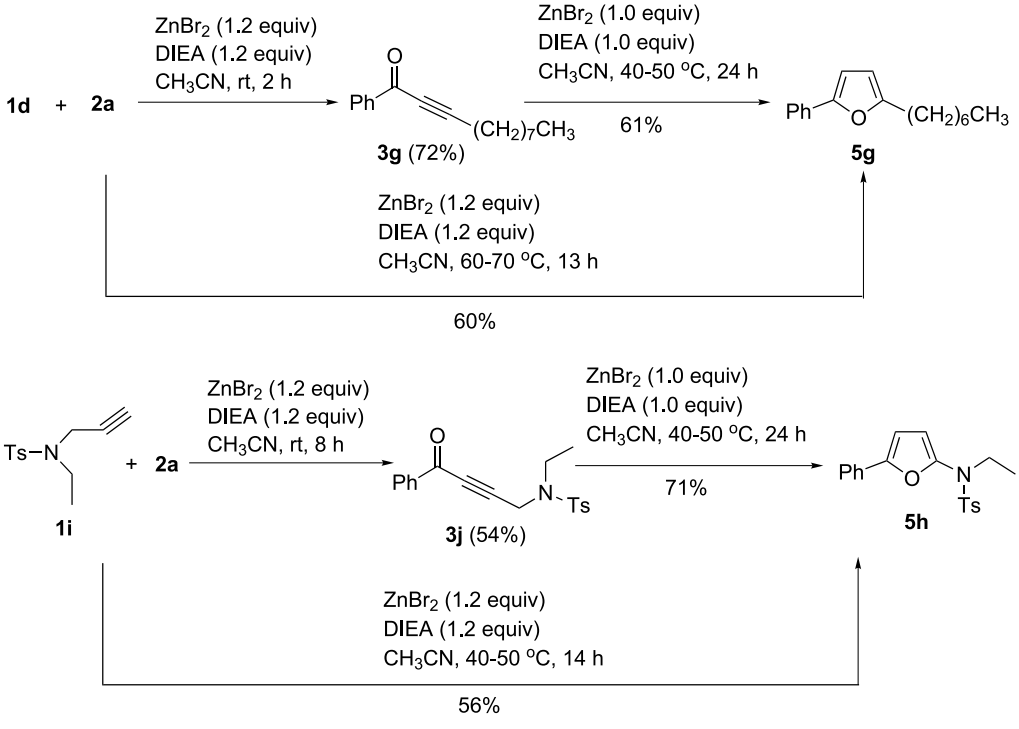
Aryl acetylenes **1a–c** gave the desired α,β-acetylenic ketones in good to excellent yields. However, the situations were different for the alkyl group-attached alkynes (vide infra). In our previous synthesis of *N*-tosylpropargylamines, alkyl group-substituted acetylenic compounds failed completely presumably due to the low acidity of the acetylenic hydrogen and the low electrophilicity of *N*-tosylimine.[1a] However, the reaction of benzoyl chloride and 1-decyne (**1d**) gave the desired α,β-acetylenic ketone **3g** in moderate yield.

Moreover, very interesting results were observed when we used aryl propargyl ethers **1e–g** or *N*-propargylphthalimide (**1h**) in the same reaction. 5-Phenyl-2-phenoxyfuran (**5a**) was obtained in 64% yield in a one-pot reaction from the reaction of **1e** and **2a**.[6,7] This compound was generated definitively from the corresponding α,β-acetylenic ketone intermediate with $ZnBr_2$ assistance. The plausible reaction mechanism is suggested in Scheme 3 (vide infra). The $ZnBr_2$-catalyzed and base-assisted propargyl-allenyl isomerization occurred to allene derivative (**II**), which immediately undergoes sequential transformation into furans as reported in a similar system.[6a,d,7] At this stage, we could not rule out the possibility of involvement of moisture in the reaction. Synthesis of furan derivatives from acetylenic ketones or allenic ketones has been accomplished with the aid of CuI/Et_3N or $AuCl_3$.[7] In our reaction, $ZnBr_2$ played the same role of CuI or $AuCl_3$ without any problems.

Although, the convertibility of acetylenic ketone into furan was known,[7] our findings suggest many important scientific issues: (1) furan could be synthesized in a one-pot from benzoyl chlorides and phenyl propargyl ethers in short time in high yield and (2) $ZnBr_2$ could act well for the acetylene–allene isomerization and concomitant activation of the allene moiety toward cyclization. In other words, triple bond of acetylenic compound was activated by $ZnBr_2$ to generate efficiently the corresponding zinc acetylide species.[1a] After the formation of α,β-acetylenic ketones $ZnBr_2$ played the role of activation of triple bond once more to be isomerized into the corresponding allenic ketone derivatives. Finally, during the formation of furan skeleton $ZnBr_2$ activates the double bond of allene moiety to facilitate the formation of furan ring. The results for the synthesis of furans are illustrated in Table 2. For the reaction of **1e** and **2d** (entry 3) the yield of **5c** was low. Instead acetylenic ketone **3i** was isolated as the major product. During the cyclization stage for furan the bulky *tert*-butyl group might affect. For the reaction of **1h** (entry 6), long reaction time was required in order to obtain moderate yield of **5f**.

The formation of furan derivatives occurs definitively from the corresponding α,β-acetylenic ketones (vide supra). When we carried out the reaction of **1d** and **2a** at rt we obtained only **3g**. The reaction of **3g** under the same conditions at elevated temperature afforded **5g** in 61% yield. The reaction of **1d** and **2a** at elevated temperature gave **5g** directly (Scheme 4) in 60% yield. We obtained same results from the reaction of **1i** and **2a**. From the experiments, we could conclude that the furan compounds **5a–h** were formed via the intermediacy α,β-acetylenic ketone compounds.

In conclusion, we found that zinc acetylide species can react with acyl chlorides to give α,β-acetylenic ketone derivatives in high yields in short time. The zinc acetylides can be



Scheme 4.

generated efficiently from the corresponding acetylenic compounds with the aid of $ZnBr_2$ and DIEA in acetonitrile. For the acetylenic ketones bearing nearby methylene unit, concomitant isomerization to allene and $ZnBr_2$-assisted cyclization occurred to afford 2,5-disubstituted furan derivatives.

2. Experimental

2.1. General procedure

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded in $CDCl_3$. The signal positions are reported in ppm relative to TMS (δ scale) used as an internal standard. The separations were carried out by flash column chromatography over silica gel (230–400 mesh ASTM). Organic extracts were dried over anhydrous $MgSO_4$ and the solvents were evaporated on a rotary evaporator under water aspirator pressure. IR spectra are reported in cm^{-1}. Mass spectra were obtained from the Korea Basic Science Institute (Gwangju branch). Melting points are uncorrected. The combustion analyzes were carried out at Korea Research Institute of Chemical Technology, Taejon, Korea. The starting materials **1a–d** were obtained from commercial sources. Compounds **1e–h** were prepared from phenol, 2-naphthol, 1,4-dihydroxybenzene, and phthalimide with propargyl bromide in the presence of K_2CO_3 in DMF. Identification of starting materials **1e–h** was carried out with their ^{1}H and/or ^{13}C NMR spectra simply.

2.2. Typical procedure for the synthesis of 3a

To a stirred solution of benzoyl chloride (**2a**, 169 mg, 1.2 mmol) in CH_3CN (3 mL) was added phenylacetylene (**1a**, 102 mg, 1.0 mmol), $ZnBr_2$ (270 mg, 1.2 mmol), and *N*,*N*-diisopropylethylamine (DIEA, 155 mg, 1.2 mmol). The reaction mixture was stirred for 90 min at rt. After the usual aqueous workup and column chromatographic purification process (hexanes/ether, 10:1) we obtained **3a**, 169 mg (82%). Identification of prepared compounds **3a–h** and **4** was carried out with their melting points, ^{1}H and/or ^{13}C NMR spectra simply in comparison with the reported data (**3a**,[3g] **3b**,[4e] **3c**,[3g] **3d**,[3g] **3e**,[4e] **3f**,[4e] **3g**,[8a] **3h**,[8b] and **4**[5]). Spectroscopic data of compounds **3i** and **3j** are as follows.

2.2.1. Compound 3i. Oil (59%); IR (KBr) 2970, 2214, 1674, 1493 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.13 (s, 9H), 4.87 (s, 2H), 6.95–7.04 (m, 3H), 7.28–7.34 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.70, 44.69, 55.68, 83.97, 87.77, 115.05, 121.94, 129.53, 157.22, 193.36; ESIMS *m*/*z* 217 ($M^+ + H$). Anal. Calcd for $C_{14}H_{16}O_2$: C, 77.75; H, 7.46. Found: C, 77.92; H, 7.59.

2.2.2. Compound 3j. Oil (54%); IR (KBr) 2229, 1647, 1346, 1265, 1161 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, J=7.2 Hz, 3H), 2.21 (s, 3H), 3.38 (q, J=7.2 Hz, 2H), 4.44 (s, 2H), 7.18–7.22 (m, 2H), 7.37–7.46 (m, 2H), 7.57–7.64 (m, 1H), 7.73–7.84 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.21, 21.29, 36.05, 41.79, 83.06, 86.99, 127.54, 128.49, 129.31, 129.69, 134.25, 135.41, 135.94, 143.90, 176.77; ESIMS *m*/*z* 342 ($M^+ + H$). Anal. Calcd for $C_{19}H_{19}NO_3S$: C, 66.84; H, 5.61; N, 4.10. Found: C, 66.95; H, 5.59, N, 4.00.

2.3. Typical procedure for the synthesis of 5a

To a stirred solution of benzoyl chloride (**2a**, 169 mg, 1.2 mmol) in CH_3CN (3 mL) was added phenyl propargyl ether (**1e**, 132 mg, 1.0 mmol), $ZnBr_2$ (270 mg, 1.2 mmol), and *N*,*N*-diisopropylethylamine (DIEA, 155 mg, 1.2 mmol). The reaction mixture was stirred for 2 h at rt. After the usual aqueous workup and column chromatographic purification process (hexanes/ether, 10:1) we obtained **5a**, 152 mg (64%). The spectroscopic data of the prepared furans **5a–h** are as follows.

2.3.1. Compound 5a. White solid (64%), mp 66–67 °C; IR (KBr) 1547, 1485, 1242 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.63 (d, J=3.3 Hz, 1H), 6.57 (d, J=3.3 Hz, 1H), 7.08–7.22 (m, 4H), 7.29–7.35 (m, 4H), 7.56–7.58 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 91.26, 106.09, 116.93, 122.97, 123.90, 126.89, 128.62, 129.69, 130.49, 146.28, 156.04, 156.85; ESIMS *m*/*z* 237 ($M^+ + H$). Anal. Calcd for $C_{16}H_{12}O_2$: C, 81.34; H, 5.12. Found: C, 81.28; H, 5.17.

2.3.2. Compound 5b. White solid (70%), mp 46–47 °C; IR (KBr) 1554, 1489, 1250 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.19 (s, 3H), 5.50 (d, J=3.3 Hz, 1H), 6.38 (d, J=3.3 Hz, 1H), 6.94–7.02 (m, 5H), 7.14–7.22 (m, 2H), 7.33–7.36 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.14, 91.23, 105.24, 116.82, 122.96, 123.78, 127.82, 129.28, 129.65, 136.66, 146.55, 155.64, 156.94. Anal. Calcd for $C_{17}H_{14}O_2$: C, 81.58; H, 5.64. Found: C, 81.50; H, 5.59.

2.3.3. Compound 5c. Oil (33%); IR (KBr) 2966, 1566, 1489, 1250 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26 (s, 9H), 5.44 (d, J=3.3 Hz, 1H), 5.89 (d, J=3.3 Hz, 1H), 6.98–7.11 (m, 3H), 7.28–7.35 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.83, 32.50, 89.32, 102.58, 116.46, 123.37, 129.57, 154.27, 156.62, 157.38. Anal. Calcd for $C_{14}H_{16}O_2$: C, 77.75; H, 7.46. Found: C, 77.45; H, 7.38.

2.3.4. Compound 5d. White solid (67%), mp 109–110 °C; IR (KBr) 1547, 1250 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.72 (d, J=3.3 Hz, 1H), 6.64 (d, J=3.3 Hz, 1H), 7.19–7.24 (m, 1H), 7.31–7.49 (m, 6H), 7.58–7.62 (m, 2H), 7.72–7.85 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 91.49, 106.16, 112.42, 118.02, 123.02, 125.02, 126.73, 126.95, 127.29, 127.75, 128.66, 129.92, 130.48, 130.50, 134.02, 146.42, 154.60, 156.06. Anal. Calcd for $C_{20}H_{14}O_2$: C, 83.90; H, 4.93. Found: C, 83.95; H, 4.92.

2.3.5. Compound 5e. White solid (66%), mp 129–130 °C; IR (KBr) 1493, 1176 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.63 (d, J=3.3 Hz, 2H), 6.60 (d, J=3.3 Hz, 2H), 7.10 (s, 4H), 7.20–7.25 (m, 2H), 7.33–7.38 (m, 4H), 7.57–7.60 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 89.81, 105.10, 117.37, 121.97, 125.95, 127.65, 129.43, 145.26, 151.87, 155.35; ESIMS *m*/*z* 395 ($M^+ + H$). Anal. Calcd for $C_{26}H_{18}O_4$: C, 79.17; H, 4.60. Found: C, 79.34; H, 4.72.

2.3.6. Compound 5f. White solid (70%), mp 161–162 °C; IR (KBr) 1736, 1369 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.53 (d, J=3.3 Hz, 1H), 6.77 (d, J=3.3 Hz, 1H), 7.24–7.30

(m, 1H), 7.34–7.40 (m, 2H), 7.64–7.68 (m, 2H), 7.80–7.83 (m, 2H), 7.95–7.98 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 106.28, 108.74, 123.89, 124.11, 127.80, 128.62, 130.06, 131.57, 134.76, 137.03, 152.92, 166.09; ESIMS *m/z* 290 ($M^+ + H$). Anal. Calcd for $C_{18}H_{11}NO_3$: C, 74.73; H, 3.83; N, 4.84. Found: C, 74.75; H, 3.95; N, 4.89.

2.3.7. Compound 5g. Oil (60%); IR (KBr) 2927, 1546, 1461 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (t, J= 6.9 Hz, 3H), 1.25–1.40 (m, 8H), 1.64–1.74 (m, 2H), 2.68 (t, J=7.5 Hz, 2H), 6.06 (d, J=3.3 Hz, 1H), 6.55 (d, J= 3.3 Hz, 1H), 7.18–7.26 (m, 1H), 7.33–7.38 (m, 2H), 7.62–7.65 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.15, 22.70, 28.13, 28.22, 29.09, 29.21, 31.82, 105.64, 106.82, 123.31, 126.71, 128.60, 131.27, 152.07, 156.52. Anal. Calcd for $C_{17}H_{22}O$: C, 84.25; H, 9.15. Found: C, 83.99; H, 9.21.

2.3.8. Compound 5h. White solid (56%), mp 140–150 °C (dec); IR (KBr) 1681, 1354, 1169 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.56 (t, J=7.2 Hz, 3H), 2.44 (s, 3H), 3.59 (q, J=7.2 Hz, 2H), 6.30 (d, J=3.3 Hz, 1H), 6.62 (d, J=3.3 Hz, 1H), 7.26–7.38 (m, 5H), 7.46–7.50 (m, 2H), 7.67–7.71 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.94, 20.54, 44.28, 105.02, 108.57, 122.56, 126.60, 126.76, 127.62, 128.50, 129.27, 134.96, 142.77, 143.42, 150.54; ESIMS *m/z* 342 ($M^+ + H$). Anal. Calcd for $C_{19}H_{19}NO_3S$: C, 66.84; H, 5.61; N, 4.10. Found: C, 66.81; H, 5.85; N, 4.33.

Acknowledgements

This work was supported by Korea Research Foundation Grant (KRF-2002-015-CP0215). Spectroscopic data was obtained from the Korea Basic Science Institute, Gwangju branch.

References and notes

1. (a) Lee, K. Y.; Lee, C. G.; Na, J. E.; Kim, J. N. *Tetrahedron Lett.* **2005**, *46*, 69. (b) Lee, K. Y.; Lee, M. J.; Kim, J. N. *Bull. Korean Chem. Soc.* **2005**, *26*, 665.
2. (a) Frantz, D. E.; Fassler, R.; Carreira, E. M. *J. Am. Chem. Soc.* **1999**, *121*, 11245. (b) Fischer, C.; Carreira, E. M. *Org. Lett.* **2004**, *6*, 1497. (c) Anand, N. K.; Carreira, E. M. *J. Am. Chem. Soc.* **2001**, *123*, 9687.
3. (a) For the synthesis of α,β-acetylenic ketones and their usefulness as synthetic intermediates, see: (a) Auge, J.; Lubin-Germain, N.; Seghrouchni, L. *Tetrahedron Lett.* **2003**, *44*, 819. (b) Marko, I. F.; Southern, J. M. *J. Org. Chem.* **1990**, *55*, 3368. (c) Vasilyev, A. V.; Walspurger, S.; Pale, P.; Sommer, J. *Tetrahedron Lett.* **2004**, *45*, 3379. (d) Rossi, E.; Abbiati, G.; Canevari, V.; Nava, D.; Arcadi, A. *Tetrahedron* **2004**, *60*, 11391. (e) Jeevanandam, A.; Narkunan, K.; Ling, Y.-C. *J. Org. Chem.* **2001**, *66*, 6014. (f) Maeda, Y.; Kakiuchi, N.; Matsumura, S.; Nishimura, T.; Kawamura, T.; Uemura, S. *J. Org. Chem.* **2002**, *67*, 6718. (g) Alonso, D. A.; Najera, C.; Pacheco, M. C. *J. Org. Chem.* **2004**, *69*, 1615 and further references cited therein.
4. For the synthesis of α,β-acetylenic ketones involving the use of CuI, see: (a) Chowdhury, C.; Kundu, N. G. *Tetrahedron Lett.* **1996**, *37*, 7323. (b) Chowdhury, C.; Kundu, N. G. *Tetrahedron* **1999**, *55*, 7011. (c) Wang, J.-X.; Wei, B.; Hu, Y.; Liu, Z.; Fu, Y. *Synth. Commun.* **2001**, *31*, 3527. (d) Cox, R. J.; Ritson, D. J.; Dane, T. A.; Berge, J.; Charmant, J. P. H.; Kantacha, A. *Chem. Commun.* **2005**, 1037. (e) Chen, L.; Li, C.-J. *Org. Lett.* **2004**, *6*, 3151. (f) Guo, M.; Li, D.; Zhang, Z. *J. Org. Chem.* **2003**, *68*, 10172. (g) Tohda, Y.; Sonogashira, K.; Hagihara, N. *Synthesis* **1977**, 777.
5. For the generation and dimerization of ketene species from propionyl chloride, please see: Farnum, D. G.; Johnson, J. R.; Hess, R. E.; Marshall, T. B.; Webster, B. *J. Am. Chem. Soc.* **1965**, *87*, 5191.
6. For the synthesis and biological importance of 2,5-disubstituted furan derivatives, see: (a) Marshall, J. A.; Van Devender, E. A. *J. Org. Chem.* **2001**, *66*, 8037. (b) Padwa, A.; Rashatasakhon, P.; Rose, M. *J. Org. Chem.* **2003**, *68*, 5139. (c) Pohmakotr, M.; Takampon, A.; Ratchataphusit, J. *Tetrahedron* **1996**, *52*, 7149. (d) Takai, K.; Morita, R.; Sakamoto, S. *Synlett* **2001**, 1614. (e) Kolasa, T.; Stewart, A. O.; Brooks, C. D. W. *Tetrahedron: Asymmetry* **1996**, *7*, 729. (f) Stoner, E. J.; Roden, B. A.; Chemburkar, S. *Tetrahedron Lett.* **1997**, *38*, 4981. (g) Dickman, D. A.; Ku, Y.-Y.; Morton, H. E.; Chemburkar, S. R.; Patel, H. H.; Thomas, A.; Plata, D. J.; Sawick, D. P. *Tetrahedron: Assymmetry* **1997**, *8*, 1791.
7. For the synthesis of furan derivatives by cycloisomerization of alkyne- and allene-containing compounds, see: (a) Patil, N. T.; Wu, H.; Yamamoto, Y. *J. Org. Chem.* **2005**, *70*, 4531. (b) Sniady, A.; Wheeler, K. A.; Dembinski, R. *Org. Lett.* **2005**, *7*, 1769. (c) Brown, R. C. D. *Angew. Chem., Int. Ed.* **2005**, *44*, 850. (d) Hoffmann-Roder, A.; Krause, N. *Org. Biomol. Chem.* **2005**, *3*, 387. (e) Hashmi, A. S. K.; Schwarz, L.; Choi, J.-H.; Frost, T. M. *Angew. Chem., Int. Ed.* **2000**, *39*, 2285. (f) Marshall, J. A.; Robinson, E. D. *J. Org. Chem.* **1990**, *55*, 3450. (g) Karpov, A. S.; Merkul, E.; Oeser, T.; Muller, T. J. *J. Chem. Commun.* **2005**, 2581.
8. (a) Chen, D.-W.; Ochiai, M. *J. Org. Chem.* **1999**, *64*, 6804. (b) Katritzky, A. R.; Lang, H. *J. Org. Chem.* **1995**, *60*, 7612.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 8711–8717

Synthesis, antitumor and DNA photocleaving activities of novel naphthalene carboxamides: effects of different thio-heterocyclic rings and aminoalkyl side chains

Zhigang Li,[a] Qing Yang[b] and Xuhong Qian[a,c,*]

[a]*State Key Lab. of Fine Chemicals, Dalian University of Technology, Dalian 116024, China*
[b]*Department of Bioscience and Biotechnology, Dalian University of Technology, Dalian 116024, China*
[c]*Shanghai Key Lab. of Chemical Biology, School of Pharmacy, East China University of Science and Technology, Shanghai 200237, China*

Received 28 March 2005; revised 21 June 2005; accepted 24 June 2005

Available online 19 July 2005

Abstract—Two kinds of thio-heterocyclic fused naphthalene carboxamides, **3a–b**, **4a–b**, were designed, synthesized and quantitatively evaluated as efficient antitumor and DNA photocleaving agents. Compound **3a** or **3b**, having the thiophene ring, intercalated into DNA more strongly than compound **4a** or **4b**, having the thioxanthene ring. Compound **4a** or **4b**, photocleaved DNA more efficiently than **3a** or **3b** via superoxide anion. Compound **4a** was the strongest inhibitor for P388 (murine leukemia cell), while **3a** was the most cytotoxic one against A549 (human lung cancer cell). Each new compound showed stronger DNA photocleaving activity than corresponding naphthalimide.

1. Introduction

DNA intercalating agents form an important class of drugs in anticancer therapy.[1] To study the interaction between such agents and DNA, in particular, effects of structural characters of them on the interaction, could offer novel insights into the design of new DNA targeting antitumor drugs.[2] Naphthalimides are significant examples of DNA-intercalating agents,[1,3–5] many of, which have shown efficient antitumor activities upon a variety of murine and human tumor cells,[4] and/or DNA photocleaving activities.[5] They are generally characterized by the presence of a planar tri- or tetracylic aromatic chromophore and one or two flexible basic side chains.

To promote the antitumor and/or DNA photocleaving activities, previous attempts were focused on incorporating substituents or fusing aromatic rings to the naphthalimide skeletons.[4,5] The presence of a larger aromatic chromophore was proved to improve the affinity of the intercalator for the DNA molecule, consequently to a greater cytotoxic and/or DNA photocleaving activity.[4,5] We have ever reported a series of thio-heterocyclic fused naphthalimides, **1a–d**[5f] and **2a–d**,[5e] as efficient DNA photocleavers (Fig. 1). In our continued efforts for simple but efficient antitumor and/or DNA photocleaving agents, we report here, the molecular design and chemical synthesis of a novel heterocyclic family of **3a–b** and **4a–b** (Fig. 1) by modification of the naphthalimide skeletons via mercuric oxide mediated decarbonylation. Compounds **3a–b** or **4a–b**, which could be considered as ring opened models of **1a–b** or **2a–b**, were expected to intercalate into DNA more strongly to shown

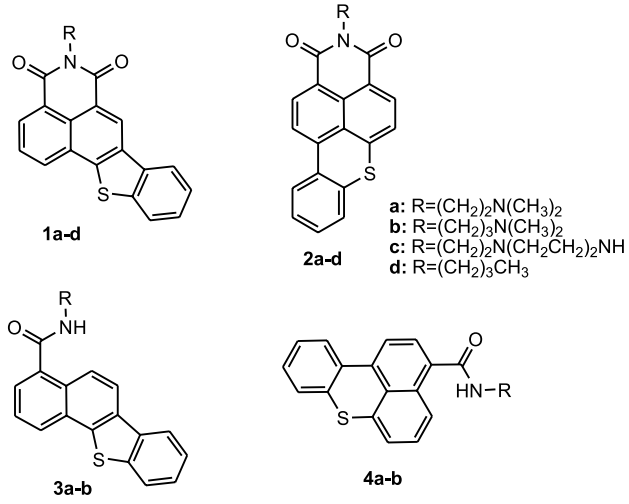
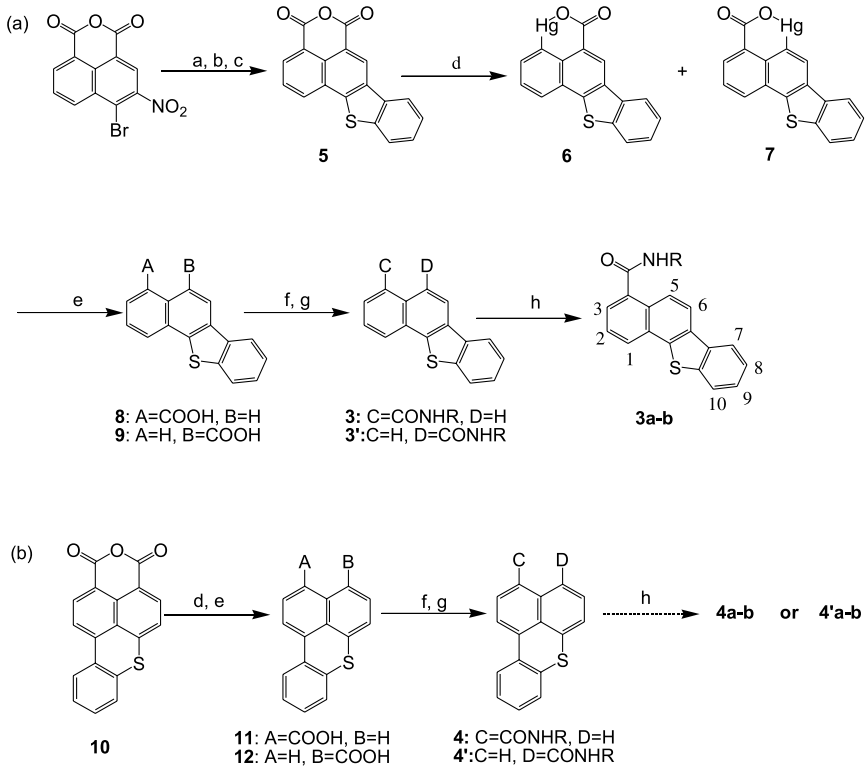


Figure 1. Structures of the reported naphthalimides (**1**, ,**2**) and novel designed naphthalene carboxamides (**3**, **4**).

Keywords: DNA photocleavage; Antitumor; Intercalating.
* Corresponding author. Address: State Key Lab. of Fine Chemicals, Dalian University of Technology, Dalian 116024, China. Tel.: +86 411 83673466; fax: +86 411 83673488; e-mail: xhqian@ecust.edu.cn

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.06.097



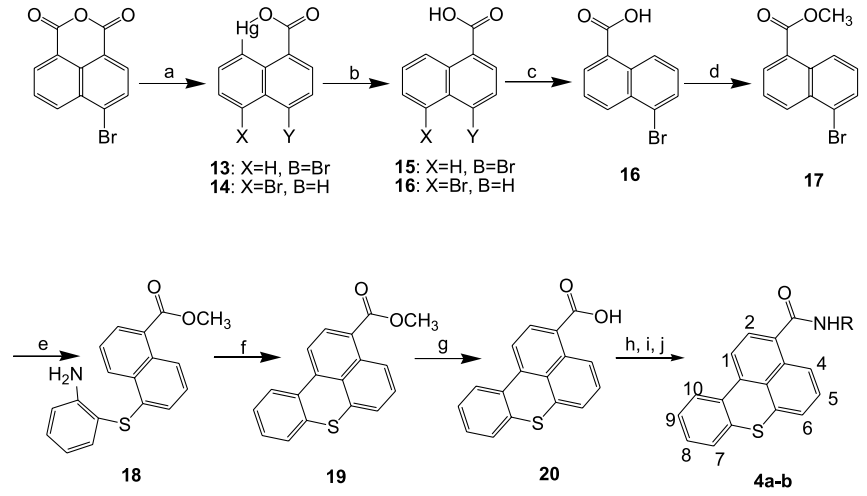
Scheme 1. (a) PhSH, EtOH, reflux, 4 h, 59% yield; (b) $SnCl_2$/concentrated HCl, 65% yirld; (c) Pochorr cyclization: $NaNO_2$, H_2O–HCl–HOAc, 0–5 °C, 2 h; $CuSO_4$, HOAc, reflux, 2 h; (d) HgO (yellow), NaOH, AcOH, H_2O, reflux, 4 days, 95% yield; (e) concentrated HCl, reflux, 2 h, 75% yield; (f) $SOCl_2$, $CHCl_3$, Et_3N, reflux, 20 h; (g) propitiate amine, CH_2Cl_2, room temperature, 24 h; (h) careful column chromatography.

efficient medical and biological use due to the reduction of the steric effect of carbonyl anchor and the flexibility of the aminoalkyl side chains.

2. Results and discussion

2.1. Synthesis and spectra

The mercuric oxide (yellow or red) mediated decarbonylation of (substituted) naphthalic anhydrides has been reported.[6] In our efforts to synthesize target compounds **3a**–**b**, we found that only yellow mercuric oxide successfully mediated the decarbonylation of benzothiophenonaphthalic anhydride **5**, synthesized from 4-bromo-3-nitro anhydride through Pochorr intramolecular cyclization.[5f,7] Two benzothiophenonaphthoic acid isomers, **8** and **9**, were obtained after the reaction, which were then converted to a pair of isomers, **3a**, **3a′** or **3b**, **3b′**. However, only pure **3a** or **3b** was obtained after careful column chromatography due to the slight amount of **3a′** or **3b′** (Scheme 1a).



Scheme 2. (a) HgO (yellow or red), NaOH, AcOH, H_2O, reflux, 4 days, 94% yield; (b) concentrated HCl, reflux, 2 h, 70% yield; (c) recrystalized with AcOH, 40% yield; (d) $SOCl_2$, reflux, 5 h; methanol, reflux, 2 h; (e) 2-aminobenzenethiol, K_2CO_3, DMF, reflux, 80 h; (f) Pochorr cyclization: $NaNO_2$, HOAc H_2SO_4, 0–5 °C, 12 h; $CuSO_4$, HOAc, reflux, 2 h; (g) NaOH, Methanol, reflux, 2 h; HCl; (h) $SOCl_2$, $CHCl_3$, Et_3N, reflux 20 h; (i) propitiate amine, CH_2Cl_2, room temperature, 24 h; (j) careful column chromatography.

The decarbonylation of benzothioxanthenenaphthalic anhydride **10**[5e] was also only mediated by yellow mercuric oxide. The pair of isomer mixtures, **4a**, **4a′** or **4b**, **4b′** was obtained. However, neither pure product could be separated after careful column chromatography due to the similar molecular polarities between **4a**, **4a′** or **4b**, **4b′** (Scheme 1b). Then we tried the other way outlined in Scheme 2. Either yellow or red mercuric oxide was found to mediate the decarbonylation of 4-bromo-1,8-naphthalic anhydride to obtain a pair of isomers of **15** and **16** with the ratio of 2:3 via ^{1}H NMR. Pure 5-bromo-1-naphthaloic acid **16** was obtained after recrystallization from acetic acid. It was transformed to methyl-5-bromo-1-naphthoate **17**, which was then condensed with 2-aminobenzenthiol to obtain **18**. Followed by the Pochorr intramolecular cyclization, methyl benzothioxanthenenaphthoate **19** was obtained, which was converted to corresponding acid **20**, then to target product **4a** or **4b**, which was purified by careful column chromatography.

Structures of all the final products were well confirmed by ^{1}H NMR, HRMS and IR. Furthermore, the above experiments provided two different ways to synthesize side-armed heterocyclic fused naphthalene derivatives, which may have potentials in other fields.

Spectra data of these new compounds were measured and summarized in Table 1. Compared with those of their corresponding naphthalimides,[5e–f] values of both the emission wavelength and the absorption wavelength of **3a–b**, **4a–b**, were blue-shifted due to the reduction of their conjugation areas and electronic pushing–pulling ICT (intramolecular charge transfer) effects caused by the decarbonylation reaction. Slight effect of the side chains was found on spectra data of these compounds.

Table 1. Spectra data,[a,b] and Scatchard binding constants of compounds **1–4**

Compd	UV λ_{max}/nm (log ε)	FL λ_{max}/nm (Φ)	Scatchard binding constants (M^{-1})
3a	350 (3.32)	423 (0.009)	4.46×10^5
3b	350 (3.42)	421 (0.011)	6.86×10^5
4a	389 (3.73)	455 (0.013)	1.95×10^4
4b	388 (3.91)	454 (0.014)	4.02×10^4
1a[4f]	384 (4.23)	437 (0.0215)	2.8×10^5
2a[4e]	462 (4.42)	521 (0.51)	8.27×10^3

[a] In absolute ethanol.
[b] With quinine sulfate in sulfuric acid as quantum yield standard ($\phi=0.55$).

2.2. DNA intercalation

The fluorescences of these compounds were quenched upon addition of Calf thymus DNA. The Scatchard binding constants[8] were calculated and summarized in Table 1 with the orders of **3b**>**3a**, **4b**>**4a**, clearly indicating the importance of the aminoalkyl side chains serving as DNA groove binders and/or external electrostatic binders. The chain length is important in placing the protonated side chain nitrogen in proximity to functional groups suitable for hydrogen bonding on the DNA double helix after the thioheterocyclic fused naphthalene chromophore has intercalated. In our cases, the side chain with three methylene units between two nitrogen atoms can significantly enhance the intercalating abilities of the thiazonaphthalimides. As to the intercalation orders of **3a**>**4a**, **3b**>**4b**, it is obvious that the chromophore of **3a** or **3b**, having the thiophene ring could intercalated more strongly than that of **4a** or **4b**, having the thioxanthene ring, in agreement with the comparison of **1a** ($2.8\times10^5\ M^{-1}$)[5f] and **2a** ($8.27\times10^3\ M^{-1}$).[5e] As expected, **3a** or **4a**, exhibited greater affinity for DNA than **1a** or **2a**, indicating that the reduction of the steric effect of carbonyl anchor and/or the higher flexibility of the side chain facilitated its intercalation with DNA (Fig. 2).

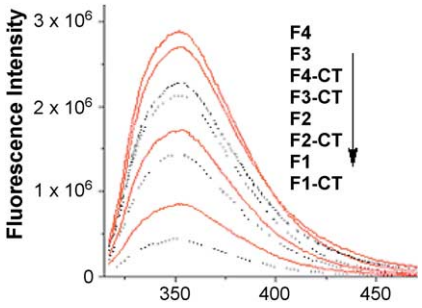


Figure 2. Fluorescence spectra before and after interaction of compound **3b** and CT-DNA. Curves F and F-CT corresponded to compound **3b** before and after mixed with DNA. Numbers 1–4 indicated the concentration of **3b**, 5, 10, 20, 40 μM, respectively. DNA applied was 50 μM (bp).

2.3. Antitumor evaluation

The antitumor activities of these compounds were evaluated in vitro (under scattered light) against A549 (human lung cancer cell) and P388 (murine leukemia cell) cell lines, respectively. As shown in Table 2, all these compounds exhibited efficient antitumor activities with IC_{50} ranging from 0.176 to 29 μM. Also, all of them were more cytotoxic against P388 than against A549, reflecting an excellent selectivity for a special murine or leukemia cell type. Cytotoxic potencies of these compounds against two tumor cells were highly dependent on the length of side chains. The compound with two methylene units in the side chain between two nitrogen atoms was more cytotoxic than corresponding homologue with one more methylene unit, as indicated by the cytotoxicity orders of **3a**>**3b**, **4a**>**4b**. Compound **4a** was found to be the strongest inhibitor for P388 with IC_{50} of 0.176 μM, while **3a** was the most cytotoxic one against A549 with IC_{50} of 1.16 μM.

Table 2. Cytotoxicity of these compounds against A549 [a] and P388 [b] cells

Compounds	Cytotoxicity (IC_{50}, μM)	
	A549[a]	P388[b]
3a	1.16	0.428
3b	19.1	0.89
4a	20.5	0.176
4b	29	0.295

[a] Cytotoxicity (CTX) against human lung cancer cell (A549) was measured by sulforhodamine B dye-staining method.[9]
[b] CTX against murine leukemia cells (P388) was measured by microculture tetrazolium–formazan method.[10]

2.4. DNA photocleavage

The photocleavage of these compounds to supercoiled plasmid pBR322 DNA were evaluated by 1% agarose gel electrophoresis. The reaction mixture containing each

compound and plasmid DNA was put under photo-irradiation (2300 W/cm^2) through a transluminator (360 nm) at a distance of 20 cm at 0 °C for 3 h under aerobic conditions. The photocleavage efficiency was defined by the conversion ratio from supercoiled pBR322 DNA (form I) to relaxed circular DNA (form II) and linear DNA (form III).

As shown in Figure 3a, all these compounds efficiently cleaved DNA from form I to form II and form III at the concentration of 100 μM with an order of **4b** > **4a** > **3b** > **3a**. Compound **4b** was more active than its analogues in that it could produce more percentage of form III (36%), generally, the result from double-strand cuts or proximal single-strand cuts on opposite strands. The concentration-dependent experiment showed that **4b** exhibited detectable cleavage (21% form II) even at 0.5 μM (Fig. 3b). No damage was observed in the absence of either compound (Fig. 3c, lane 2) or light (lane 3), indicating that they were obligate factors for DNA strand scission. In this case, UV light actually functioned as a trigger to initiate the strand scission.

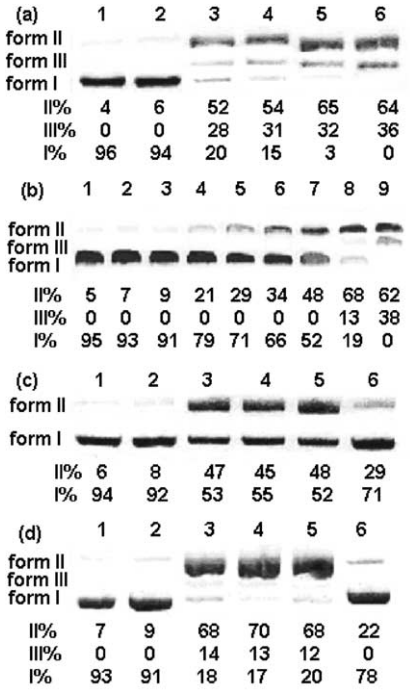


Figure 3. Photocleavage of closed supercoiled pBR322 DNA (200 μM/bp) in the buffer of Tris–HCl (20 mM, pH 7.5). (a) Photocleavage of plasmid DNA by different compounds (100 μM) for 3 h. Lane 1, DNA alone; lane 2, DNA with UV; lane 3–6, each compound of **3a**, **3b**, **4a**, **4b**, and DNA, respectively. (b) Photocleavage of plasmid DNA by **4b** at various concentrations for 3 h. Lane 1, DNA alone; lane 2, DNA with UV; lane 3, DNA with **4b** (100 μM) without UV; Lane 4–9, DNA with **4b** at concentration of 0.5, 5, 10, 25, 50, 100 μM, respectively, following UV irradiation. (c) and (d) Effect of additives on the photocleavage by compound **3b** or **4b** (50 μM) for 3 h. Lane 1, DNA alone; lane 2, DNA with UV; lanes 3 DNA and compound **3b** (c), **4b** (d) with UV; lane 4–6, DNA and compound in the presence of histidine (6 mM), ethanol (1.7 M), dithiothreitol (DTT, 30 mM), respectively, following UV irradiation.

Photocleavage can be via a variety of mechanisms involving free radical, electron transfer and singlet oxygen.[11] In order to establish the reactive species responsible for cleavage of the plasmid, mechanistic experiments were performed by addition of histidine (singlet oxygen quencher), dithiothreitol (DTT, superoxide anion scavenger) and ethanol (hydroxyl radical scavenger), respectively. As shown in Figure 3c or d, with **3b**, **4b** as examples, histidine, and ethanol clearly had no obvious effects on the photocleavage, indicating that singlet oxygen and hydroxyl radical were not likely to be cleavage contributors. However, the DNA cleaving activities both decreased greatly in the presence of DTT (lane 6), indicating superoxide anion was most likely to be the reactive specie responsible for plasmid cleavage. Under identical conditions, DTT inhibited the photocleavage activity of **4b** (form II: 68% to 22%) more efficiently than that of **3b** (form II: 47% to 29%), indicating superoxide anion was more easily produced in the photocleaving reaction of **4b**.

The effect of side chains on the order of photocleavage abilities was parallel to that of their DNA intercalating abilities (**3b** > **3a**, **4b** > **4a**), but anti-parallel to that of their antitumor activities (**3a** > **3b**, **4a** > **4b**). The reason accounting for it probably lies on that: the cytotoxicity of one compound is determined by two conflicting factors including cell membrane crossing ability and DNA binding ability. The side chains could be protonated to different extent under physiological pH due to their different basicity of corresponding nitrogen atoms. The protonation extent significantly affected the ability of the molecule to pass through lipophilic memebranes to bind to DNA. Lower degree of protonation is desirable for cell penetration, but higher degree of protonation favored DNA binding. Once these two factors effectively compromise, the higher antitumor potency is possible, such as **3a**, **4a**. Also, the selective cytotoxicities of these compounds were possibly determined by their different memebrane crossing abilities for different cell lines.

The effect of heterocyclic rings on the order of photocleavage abilities (**4a** > **3a**, **4b** > **3b**) was anti-parallel to that of their DNA intercalating abilities (**3a** > **4a**, **3b** > **4b**). In our case, the five-membered thiophene ring was better conjugated to the naphthalene skeleton than the six-membered thioxanthene ring, resulting in the better planar chromophore of **3a** or **3b**. The better planar chromophore of **3** in turn accounted for the more efficient intercalating activity of **3**. As to **4a** or **4b**, its worse conjugating structure made it less stable under photo-irradiation. Then electrons were more easily transferred from the chromophore of **4** to oxygen to generate superoxide anions responsible for DNA cleavage to lead to its stronger photocleaving activity.

Furthermore, **3a–b** or **4a–b** photocleaved DNA more efficiently than corresponding napthalimides **1a–b** or **2a–b**.[5e–f] Electron densities of the thio-heterocyclic fused naphthalene chromophores were relatively higher than those of their corresponding naphthalimides due to the reduction of one strong electron-withdrawing carbonyl group. The higher chromophores' electron densities were inferred to grant **3a–b** or **4a–b** stronger abilities of transferring electrons, which were from their chromophores to oxygen to form more superoxide anions for photocleavage under photo-activation, consequently to higher photocleavage abilities than **1a–b** or **2a–b**.

3. Conclusion

In summary, the present work demonstrated the design, synthesis and quantitative evaluation of two kinds of thio-heterocyclic fused naphthalene carboxamides as efficient antitumor and DNA photocleaving agents. All these compounds were found to be more cytotoxic to P388 than to A549. Compounds with the five-membered thiophene rings were proved to be more efficient DNA intercalators while those with the six-membered thioxanthene rings were more efficient DNA photocleavers. The side chains played different roles in intercalating/photocleaving and antitumor activities.

4. Experimental

4.1. Materials

All the solvents were of analytic grade. ^{1}H NMR were measured on a Bruker AV-400 spectrometer with chemical shifts reported as ppm (in DMSO/$CDCl_3$-d_6, TMS as internal standard). Mass spectra were measured on a HP 1100 LC–MS spectrometer. Melting points were determined by an X-6 micro-melting point apparatus and uncorrected. Absorption spectra were determined on PGENERAL TU-1901 UV–VIS Spectrophotometer.

4.2. Synthesis

4.2.1. Benzothiophenonaphthalic anhydride 5. 4-Bromo-3-nitro-1,8-naphthalic anhydride (6.44 g) was stirred under reflux in ethanol (60 mL) with thiophenol (2.6 mL) for 5 h. The liquor was reduced in volume to 30 mL and filtered, washed with some ethanol, dried, giving 6.83 g (97.3%) of 3-nitro-4-phenylthio-1,8-naphthalic anhydride. The recrystallization from glycol monomethyl ether gave long golden needles. Mp: 177–178 °C. The above nitro compound (2.7 g) was stirred into a mixture of stannous chloride (8.81 g) and concentrated hydrochloric acid (12 mL). After warming to 40 °C, the temperature rose spontaneously to 85 °C and was maintained at 85 °C for 1 h (color change from orange to olive-green). The suspension was cooled and filtered to give 2.4 g crude product. Recrystallization from pyridine gave greenish-yellow needles of 3-amino-4-phenylthio-1,8-naphthalic anhydride. Mp: 224–225 °C ^{1}H NMR (d_6-$CDCl_3$) δ (ppm): 8.69 (d, J=8.5 Hz, 1H, 7-H), 8.37 (d, J=7.6 Hz, 1H, 5-H), 8.15 (s, 1H, 2-H), 8.15 (t, J_1=7.6 Hz, J_2=8.5 Hz, 1H, 6-H), 7.25–7.20 (m, 2H, 3′-H, 5′-H), 7.17–7.13 (m, 1H, 4′-H), 7.06–7.02 (m, 2H, 2′-H, 6′-H), 5.08 (s, 2H, NH_2). EI-MS: (m/z): 321.3 (M^+). Sodium nitrite (0.7 g) and glacial acid (2 mL) were added dropwise to concentrated sulfuric acid (10 mL). The mixture was cooled to 0–5 °C and 3-amino-4-phenylthio-1,8-naphthalic anhydride (3.2 g) was slowly added over 1 h. After stirring for 1 h, the dark red viscous liquor was added over 90 min to a boiling solution of copper sulfate (70 g) in water (1000 mL) and glacial acetic acid (120 mL). After the addition was complete, the liquor was refluxed for 30 min, cooled, filtered, dried and an orange solid (2.63 g, 85%) was collected. Recrystallization from DMF gave deep red needles. Mp: 284–286 °C. ^{1}H NMR (d_6-DMSO) δ (ppm): 9.4 (s, 1H, 7-H), 8.77–8.73 (m, 2H, 3-H, 1-H), 8.59 (d, J_1=1.0 Hz, J_2=7.4 Hz, 1H, 11-H), 8.30–8.26 (m, 1H, 2-H), 8.04 (d, J_1=8.2 Hz, J_2=7.4 Hz, 1H, 8-H), 7.70–7.66 (m, 2H, 9-H, 10-H). HRMS (ESI): Calcd for $C_{18}H_9O_3S$ $(M+H)^+$: 305.0272. Found: 305.0281.

4.2.2. Anhydro-8-hydroxylmercuri-1-benzothiophenonaphthoic acids 8 and 9. The obtained precipitate **5** (2.63 g) was suspend in 60 mL aqueous sodium hydroxide (1 g) and refluxed until the solid material dissolved, a solution of HgO (yellow) (2.34 g) in a mixture of H_2O (10 mL) and AcOH (7 mL) was added with stirring to result in slow evolution of carbon dioxide. The reaction mixture refluxed for 96 h, then cooled and filtered. The highly insoluble yellow solid was washed with water and dried under vacuum at 50 °C over night to give the mixture of **6** and **7** (4.11 g, 95% yield). Attempts to purify and separate the anhydro compounds were unsuccessful. It is insoluble in organic solvents. The above obtained solid (4.11 g) were suspended in 30 mL concentrated HCl, stirred, heated under reflux for 3 h. Hot filtration gave the mixture thio-heterocyclic fused naphthoic acids **8** and **9** (1.86 g, 76% yield). HRMS (ESI): Calcd for $C_{17}H_{12}O_2S$ $(M+H)^+$: 279.0480. Found: 279.0472.

4.2.3. *N*-(2-(Dimethylamino)ethyl)benzothiophenonaphalene-4-carboxamide 3a. The acids **9** and **10** (0.6 g) were treated with thionyl chloride (10 mL) and DMF (1 drop) in $CHCl_3$ (10 mL) under reflux for 20 h. After removal of the solvent and excess thionyl chloride, the crude solid and *N*,*N*-dimethyl ethyl diamine (0.4 mL) were combined in 25 mL CH_2Cl_2. The mixture cooled in an ice bath while Et_3N (0.45 mL) was added dropwise with stirring. The stirring continued for 20 h at room temperature. Removal of solvent and separated on silica gel chromatography (CH_2Cl_2:MeOH=6:1, v/v) to get pure **3a** (0.32 g, 42% yield). Mp: 194–195 °C. ^{1}H NMR (d_6-DMSO) δ (ppm): 2.42 (s, 6H, NCH_3), 2.75 (t, J_1=J_2=6.4 Hz, 2H, NCH_2), 3.55 (s, 2H, J_1=4 Hz, J_2=6.8 Hz, $CONHCH_2$), 7.58 (t, J_1=6.8 Hz, J_2=6.4 Hz, 2H, 8-H, 9-H), 7.72 (t, J_1=6.0 Hz, J_2=7.2 Hz, 2H, 2-H, 3-H), 8.13 (t, J_1=5.2 Hz, J_2=3.6 Hz, 1H, 10-H), 8.22 (t, J_1=4 Hz, J_2=5.2 Hz, 1H, 1-H), 8.30 (d, J=8 Hz, 1H, 6-H), 8.42 (s, 1H, 7-H), 8.44 (s, 1H, 5-H). HRMS (ESI): Calcd for $C_{21}H_{21}N_2OS$ $(M+H)^+$: 349.1375. Found: 349.1368. IR (KBr): 3268, 2921, 2815, 1634, 1555, 768 cm^{-1}.

4.2.4. *N*-(2-(Dimethylamino)propyl)benzothiophenonaphthalene-4-carboxamide 3b. Prepared and purified in a similar manner as that in **3a**, *N*,*N*-dimethylpropyl diamine was used here, instead of *N*,*N*-dimethylethyl diamine and separated on silica gel chromatography (CH_2Cl_2:MeOH=5:1, v/v) to get pure **3b** (39% yield). Mp: 183–184 °C. ^{1}H NMR (d_6-DMSO) δ (ppm): 1.82 (t, J_1=6.4 Hz, J_2=7.2 Hz, 2H, CH_2), 2.42 (s, 6H, NCH_3), 3.16 (s, 2H, NCH_2), 4.06 (t, J_1=7.2 Hz, J_2=7.2 Hz, 2H, $CONCH_2$), 7.56 (t, J_1=4.4 Hz, J_2=4 Hz, 2H, 8-H, 9-H), 7.69 (d, J=7.8 Hz, 1H, 3-H), 7.74 (t, J_1=J_2=8 Hz, 1H, 2-H), 8.17 (t, J_1=4 Hz, J_2=4.8 Hz, 1H, 1-H), 8.23 (d, J=8.8 Hz, 2H, 7-H, 10-H), 8.47 (t, J_1=8.8 Hz, J_2=7.8 Hz, 2H, 5-H, 6-H). HRMS (ESI): calcd for $C_{22}H_{23}N_2OS$ $(M+H)^+$: 363.1531, Found: 363.1523. IR (KBr): 3257, 2922, 2853, 1632, 1544, 767 cm^{-1}.

4.2.5. 5-Bromo-1-naphthoic acid 16. 4-Bromo-1,8-naphthalic anhydride (2.77 g) was suspend in 50 mL aqueous sodium hydroxide (1.25 g) and refluxed until the solid material dissolved, a solution of HgO (red or yellow) (2.2 g) in a mixture of H_2O (2 mL) and AcOH (2 mL) was added with stirring to result in slow evolution of carbon dioxide. The reaction mixture refluxed for 96 h, then cooled and filtered. The highly insoluble yellow solid was washed with water and dried under vacuum at 100 °C over night to give the mixture of **13** and **14** (4.9 g, 96% yield). The obtained precipitates were suspended in 25 mL concentrated HCl, stirred, heated under reflux for 3 h. Hot filtration gave the mixture of 4-bromo-1-naphthoic acid **15** and 5-bromo-1-naphthoic acid **16** with ratio of 2:3 via ^{1}H NMR. Pure 5-bromo-1-naphthoic acid **16** was obtained after recrystallization from acetic acid (1.1 g, 42% yield), mp: 205–206 °C, ^{1}H NMR (d_6-DMSO) δ (ppm): 7.57 (t, J_1= 8 Hz, J_2=7.6 Hz, 1H, 7-H), 7.77 (t, J_1=8 Hz, J_2=8.4 Hz, 1H, 3-H), 8.00 (d, J=7.6 Hz, 1H, 6-H), 8.23 (d, J=7.2 Hz, 1H, 4-H), 8.43 (d, J=8.4 Hz, 1H, 2-H), 8.88 (d, J=8.8 Hz, 1H, 8-H). HRMS (ESI): Calcd for $C_{11}H_8BrO_2$ $(M+H)^+$: 250.9708. Found: 250.9715.

4.3. Methyl-5-bromo-1-naphthoate 17

The pure 5-bromo-1-naphthoic acid **16** (4.16 g) was combined with thionyl chloride (30 mL) and DMF (1 drop) under reflux for 20 h. After removal of the excess thionyl chloride, 30 mL methanol was added, reacted for 1 h under reflux. The crude methyl-5-bromo-1-naphthoate was obtained after removal of the excess methanol. ESI-MS (positive) *m/z*: 266.5 $(M+H)^+$.

4.3.1. Benzothioxanthenenaphthoic acid 20. Methyl-5-bromo-1-naphthoate **17** (3.75 g) was stirred under reflux in DMF (40 mL) with 2-aminobenzenethiol (2.12 g) and K_2CO_3 (1.035 g) for 80 h. Cooled and poured into 100 mL water, filtered and dried under vacuum to obtain the crude solid of **18** (3.56 g, 74%). ESI-MS (positive) *m/z*: 310.1 $(M+H)^+$. 11 mL aqueous solution of sodium nitrite (8.35 g) was slowly added into mixture of **18** (3.56 g), HCl (3 mL) glacial acetic acid (30 mL) and H_2O (5 mL) within 1 h at 0–5 °C. After stirring for 12 h, the dark red viscous liquor was added over 90 min to a boiling solution of copper sulfate (8.17 g) in water (120 mL) and glacial acetic acid (7 mL). After the addition was complete, the liquor was refluxed for 30 min, cooled, filtered, dried and an dark-orange solid **19** (3.23 g, 95%) was collected. ESI-MS (positive) *m/z*: 293.1 $(M+H)^+$. The above obtained solid **19** was added into the mixture of 20%NaOH (50 mL) and methanol (50 mL), then the mixture was refluxed for 1 h, removal of excess methanol, cooled, filtered, dried under vacuum to obtain **20** (1.95 g, 62%), mp: >300 °C. HRMS (ESI): Calcd for $C_{17}H_{12}O_2S$ $(M+H)^+$: 279.0480. Found: 279.0489.

4.3.2. *N*-(2-(Dimethylamino)ethyl) benzothioxanthenenaphthalene-3-carboxamide 4a. The acid **20** (0.6 g) was treated with thionyl chloride (10 mL) and DMF (1 drop) in $CHCl_3$ (10 mL) under reflux for 20 h. After removal of the solvent and excess thionyl chloride, the crude solid and *N*,*N*-dimethyl ethyl diamine (0.4 mL) were combined in 25 mL CH_2Cl_2. The mixture cooled in an ice bath while Et_3N (0.45 mL) was added dropwise with stirring. The stirring continued for 20 h at room temperature. Removal of solvent and separated on silica gel chromatography to afford the pure products. Separated on silica gel chromatography (CH_2Cl_2:MeOH=6:1, v/v) to get pure **4a** (0.30 g, 42% yield). Mp: 223–224 °C. ^{1}H NMR (d_6-$CDCl_3$) δ (ppm): 2.88 (s, 6H, NCH_3), 3.05 (t, J_1=J_2=3.6 Hz, 2H, NCH_2), 3.67 (d, 2H, J=5.6 Hz, $CONHCH_2$), 7.40 (t, J_1=J_2=3.6 Hz, 2H, 8-H, 9-H), 7.49 (d, J=3.2 Hz, 1H, 1-H), 7.61 (t, J_1=J_2=6.4 Hz, 1H, 5-H), 7.85 (s, 1H, 2-H), 8.17 (d, J=8 Hz, 1H, 10-H), 8.23 (d, J=6.8 Hz, 1H, 6-H). 8.30 (d, J=8 Hz, 1H, 7-H), 8.94 (t, J_1=J_2=3.6 Hz, 1H, 4-H). HRMS (ESI): Calcd for $C_{21}H_{21}SN_2O$ $(M+H)^+$: 349.1375. Found: 349.1378. IR (KBr): 3266, 2918, 2849, 1638, 1548, 762 cm^{-1}.

4.3.3. *N*-(2-(Dimethylamino)propyl) benzothioxanthenenaphthalene-3-carboxamide 4b. Prepared and purified in a similar manner as that in **4a**, *N*,*N*-dimethylpropyl diamine was used here, instead of *N*,*N*-dimethylethyl diamine and separated on silica gel chromatography (CH_2Cl_2: MeOH= 4:1, v/v) to get pure **4b** (36% yield). Mp: 217–218 °C. ^{1}H NMR (d_6-$CDCl_3$) δ (ppm): 1.89 (t, J_1=6.4 Hz, J_2=7.2 Hz, 2H, CH_2), 2.58 (s, 6H, NCH_3), 2.62 (s, 2H, NCH_2), 3.03 (t, J_1=7.2 Hz, J_2=7.2 Hz, 2H, $CONCH_2$), 7.42 (t, J_1=J_2= 6.4 Hz, 2H, 8-H, 9-H), 7.48 (d, J=3.2 Hz, 1H, 1-H), 7.65 (t, J_1=J_2=6.8 Hz, 1H, 5-H), 7.81 (s, 1H, 2-H), 8.16 (d, J= 7.8 Hz, 2H, 7-H, 10-H), 8.247 (d, J=6.4 Hz, 1H, 6-H). 8.29 (d, J=6.4 Hz, 1H, 4-H). HRMS (ESI): Calcd for $C_{22}H_{23}N_2OS$ $(M+H)^+$: 363.1531. Found: 363.1535. IR (KBr): 3274, 2940, 2856, 1634, 1541, 763 cm^{-1}.

4.4. Intercalation studies of compounds to CT-DNA

0.1 mL solution of a compound in DMSO (10^{-3}–10^{-4} M) mixed with 20 mM Tris–HCl (pH=7.5) to 5 mL. Then, two groups of samples were prepared in the concentration of chemical at 5, 10, 20, 40 μM, one contained Calf-thymus DNA 50 μM, the other contained no DNA but had the same concentration of chemical as control. All the above solution was shaken for 3 days at 25 °C in the dark. Fluorescence wavelength and intensity area of samples were measured.

4.5. Cytocoxicity in vitro evaluation

The prepared compounds have been submitted to Shanghai Institute of Materia Medica for testing their cytotoxicities.

4.6. Photocleavage of supercoiled pBR322 DNA

250 mg pBR322DNA (form I), 1 μL solution of chemical and 20 mM Tris–HCl (pH=7.5) were mixed to 10 μL, then irradiated for 3 h with light (360 nm) using lamp placed at 20 cm from sample. Supercoiled DNA runs at position I, nicked DNA at position II, linear DNA at position III. The samples were analyzed by gel electrophoresis in 1% Agarose and gel was stained with ethidium bromide.

Acknowledgements

Financial support by the National Key Project for Basic Research (2003CB114400) and under the auspices of

National Natural Science Foundation of China is greatly appreciated.

References and notes

1. Braña, M. F.; Cacho, M.; Gradillas, A.; Pascual-Teresa, B.; Ramos, A. *Curr. Pharm. Des.* **2001**, *7*, 1745–1780.
2. Demeunynck, M.; Bailly, C.; Wilson, W. D. *DNA and RNA Binders*; Wiley-VCH: Weinheim, 2003.
3. Braña, M. F.; Ramos, A.; et al *Curr. Med. Chem.: Anti-cancer Agents* **2001**, *1*, 237–255.
4. (a) Sami, S. M.; Dorr, R. T.; Alberts, D. S.; Remers, W. A. *J. Med. Chem.* **1993**, *36*, 765–770. (b) Sami, S. M.; Dorr, R. T.; Alberts, D. S. *J. Med. Chem.* **2000**, *43*, 3067–3073. (c) Braña, M. F.; Cacho, M.; Garcia, M. A. *J. Med. Chem.* **2002**, *45*, 5813–5816. (d) Braña, M. F.; Cacho, M.; Garcia, M. A. *J. Med. Chem.* **2004**, *47*, 1391–1399. (e) Antonini, I.; Polucci, P.; Magnano, A.; Sparapani, S.; Martelli, S. *J. Med. Chem.* **2004**, *47*, 5244–5250. (f) Wakelin, L. P. G.; Adams, A.; Denny, W. A. *J. Med. Chem.* **2002**, *45*, 894–901. (g) Li, Z.; Yang, Q.; Qian, X. *Bioorg. Med. Chem.* **2005**, *13*, 3149–3155.
5. (a) Saito, I.; Takayama, M. *J. Am. Chem. Soc.* **1995**, *117*, 5590–5591. (b) Saito, I.; Takayama, M.; Sugiyama, H.; Nakatani, K. *J. Am. Chem. Soc.* **1995**, *117*, 6406–6408. (c) Li, Y.; Xu, Y.; Qian, X. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3513–3515. (d) Li, Y.; Xu, Y.; Qian, X. *Tetrahedron Lett.* **2004**, *45*, 1247–1251. (e) Qian, X.; Li, Y.; Xu, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2665–2668. (f) Xu, Y.; Qu, B.; Qian, X.; Li, Y. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1139–1142. (g) Li, Z.; Yang, Q.; Qian, X. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1769–1772.
6. (a) Leuck, B. G.; Perkins, R. P.; Whitmore, F. C. *J. Am. Chem. Soc.* **1929**, *51*, 1831–1836. (b) Whitmore, F. C.; Fox, A. L. *J. Am. Chem. Soc.* **1929**, *51*, 3363–3367. (c) Bailey, R. J.; Schechter, H. *J. Am. Chem. Soc.* **1983**, *105*, 6096–6103. (d) Rosowsky, A.; Wright, J. E.; Vaidya, C. M.; Forsch, R. A.; Bader, H. *J. Med. Chem.* **2000**, *43*, 1620–1634.
7. Peters, A. T.; Bide, M. J. *Dyes and Pigments* **1985**, *6*, 267.
8. Gupta, M.; Ali, R. *J. Biochem.* **1984**, *95*, 1253–1257.
9. Skehan, P.; Storeny, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesc, H.; Kenney, S.; Boyd, M. R. *J. Nat. Cancer Inst.* **1990**, *82*, 1107–1112.
10. Kuroda, M.; Mimaki, Y.; Sashida, Y.; Hirano, T.; Oka, K.; Dobashi, A.; Li, H.; Harada, N. *Tetrahedron.* **1997**, *53*, 11549–11562.
11. Armitage, B. *Chem. Rev.* **1998**, *98*, 1171–1200.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
ELSEVIER
Tetrahedron 61 (2005) 8718–8721

Janoxepin and brevicompanine B: antiplasmodial metabolites from the fungus *Aspergillus janus*

Kennett Sprogøe,[a,*] Søren Manniche,[a] Thomas Ostenfeld Larsen[b] and Carsten Christophersen[c]

[a]*Department of Medicinal Chemistry, The Danish University of Pharmaceutical Sciences, Universitetsparken 2, DK-2100 Copenhagen, Denmark*
[b]*Mycology Group, Department of Biotechnology and Department of Organic Chemistry, Technical University of Denmark, DK-2800 Lyngby, Denmark*
[c]*Marine Chemistry Section, Department of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark*

Received 21 April 2005; revised 8 June 2005; accepted 23 June 2005

Available online 21 July 2005

Abstract—Two compounds janoxepin (**1**) and brevicompanine B (**2**) were isolated from the fungus *Aspergillus janus* and the structures elucidated by one- and two-dimensional NMR spectroscopic methods and mass spectrometry. Janoxepin is a novel oxepin derivative with a rare D-leucine incorporated. Brevicompanine B has previously only been isolated from *Penicillium brevicompactum*. Both compounds were tested in antimicrobial assays and found to be active against the malaria parasite *Plasmodium falciparum* 3D7 (IC_{50}-values of 28 and 35 mg/ml, respectively). However, no activity was observed in antifungal or antibacterial assays.

1. Introduction

UV-guided screening is a powerful tool for identifying fungal metabolites, either for taxonomic purposes or for the isolation of new compounds. By this method, a fungal extract subjected to analysis with a HPLC system hyphenated with a diode array detector, DAD, give rise to UV-spectra of all metabolites. The spectra, in conjunction with retention times, may be compared with established profiles in a database, and thus serve as a powerful tool for dereplication of the constituents. The metabolite profile may be decisive in the classification of the fungus in question.[1] However, every now and then spectra with unfamiliar patterns arise, which after interpretation can be attributed to a novel compound. Further, investigation into novelty of a compound include, fractionation of the raw extract, followed by analytical scale HPLC to identify fractions containing compounds of interest. Finally, the compounds are isolated by preparative scale RP-HPLC and subjected to structure elucidation by MS and 1D and 2D NMR spectroscopy.

In this paper, we report the isolation and structure elucidation of a novel oxepin-containing natural product and identification of brevicompanine B in extracts of *Aspergillus janus*. Brevicompanine B has previously only been isolated from *Penicillium brevicompactum*. Both metabolites exhibit activity in an antimalarial assay against *Plasmodium falciparum*, and approximate IC_{50} values are reported.

2. Results and discussion

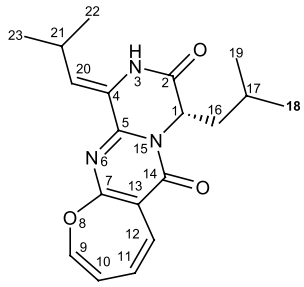
Janoxepin (**1**) and brevicompanine B (**2**) (Fig. 1) were isolated from an organic extract (ethyl acetate) of *A. janus*, cultivated on yeast extract sucrose (YES) agar and separated using a multi-step fractionation scheme, including defatting by liquid–liquid partition and C-18 reverse phase vacuum chromatography. Analytical scale RP-HPLC-DAD was used to identify those fractions suitable for further investigation on the basis of UV spectra. Final purification was performed on reverse phase preparative HPLC.

Compound **1** is an orange crystalline powder with a melting point of 88–89 °C. The structure was inferred from NMR spectroscopic data in conjunction with HRMS. Cinereain, a structure previously reported,[2] shares the ring structure of **1**. The ring system of **1** is numbered in accordance with that of cinereain.[2]

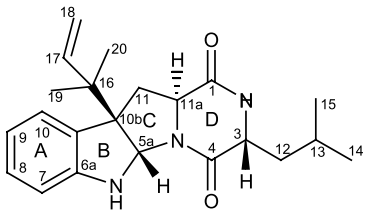
The HRMS data from both positive and negative mode

Keywords: UV-guided screening; NMR; Janoxepin; Brevicompanine B; Antiplasmodial; Fungal metabolites.
* Corresponding author. Tel.: +45 35306254; fax: +45 35306040; e-mail: kes@dfuni.dk

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.06.086



Janoxepin (**1**)



Brevicompanine B (**2**)

Figure 1. The novel compound janoxepin (**1**) and brevicompanine B (**2**).

clearly indicated the molecular formula $C_{19}H_{23}N_3O_3$ due to the presence of base peaks at *m/z* 342.1785 $(M+H)^+$ and *m/z* 340.1696 $(M-H)^-$. This information was supported by the ^1H and ^{13}C NMR data altogether showing a molecule with 10 degrees of saturation.

In the ^1H NMR spectrum signals appear from four methyl, one methylene, and three methine groups, and there are seven signals ranging from δ 5.31–8.68 due to deshielded protons. A broad singlet at 8.68 was assigned to a NH proton. The HMBC data reveals this proton to have $^2J_{CH}$-correlations to C-4 and C-2, $^3J_{CH}$-correlations to C-1 and C-5, thus establishing the position of N-3 in the ring system.

The signal at δ 5.31 originates from H-1 and appears at low field due to deshielding from the C-2 carboxy group and N-15. This assignment is supported by the COSY data, where H-1 couples with H-16. The remaining signals originate from protons attached to unsaturated carbons. COSY data and ^1H NMR coupling pattern suggest, that four of these signals are part of the same spin system, which is part of the oxepin ring in accordance with HMBC data analysis (Fig. 2).

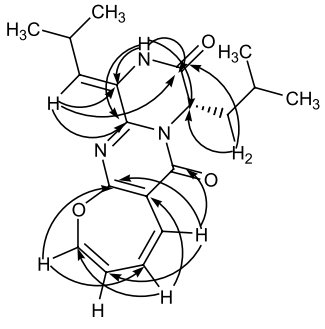


Figure 2. Selected HMBC correlations for **1**.

The last proton attached to an unsaturated carbon, H-20, appear at 6.31 ppm. The signal appear as a douplet due to coupling with H-21, which itself splits into a double heptet, indicating coupling to two methyl groups as well as H-20. This conclusion is fully supported by HMBC experiments.

The structure of the leucine residue was determined through coupling pattern analysis and HMBC data, and is consistent with experimental values previously described.[3] The configuration of the chiral C-1 was determined as R by Marfey's method.[4] The leucine residue thus originates from the rare D-leucine, in contrast to cinereain, which contain an L-amino acid.[2]

The configuration of the double bond was established as *Z* from NOE data, where H-3 and H-21 exhibit NOE (Fig. 3).

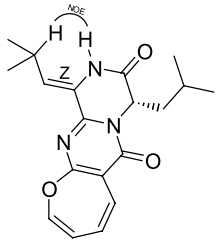


Figure 3. The NOE between H-3 and H-21 determines the double bond configurations as *Z*.

The ^{13}C NMR spectrum has 11 signals in the unsaturated region, six of, which are quaternary.

Three of the quaternary carbons resonate at low field (160–165 ppm) typical of amide carbons, accounting for C-2 and C-14 at 165.9 and 160.4 ppm, respectively. The signal at 162.7 ppm originates from C-7, in accordance with the expected value for a sp^2-hybridized carbon bearing two heteroatoms. All NMR-values are listed in Table 1.

In analogy with albonoursin from *Streptomyces noursei* janoxepin may originate from the diketopiperazine formed between two leucine residues. This condensation reaction is catalyzed by enzymes, which are not related to non-ribosomal peptide syntethases.[5]

The oxidation following the diketopiperazine biosynthesis is catalyzed by a cyclic dipeptide oxidase enzyme. In the case of janoxepin the diketopiperazine might suffer benzoylation and subsequent condensation with anthranilic acid followed by oxidation of the benzoyl derivative to the oxepin derivative.

Diketopiperazines are well known in *Aspergillus* spp. The fumiquinazolines are isolated from *A. fumigatus* and resembles **1** structurally. However, no oxidation of the benzoyl moiety has taken place. The distribution of compounds containing oxepin as well as diketopiperazine

Table 1. NMR data for janoxepin (**1**) (400 MHz (^{1}H NMR) and 100.6 MHz (^{13}C NMR))

#	δ ^{13}C	δ ^{1}H (J in Hz)	HMBC	COSY
1	54.1	5.31 (ddd, 9.1/5.5/0.9)	C-2, C-5, C-14, C-16	H-16
2	165.9			
3	—	8.68 (s (broad)	C-1, C-2, C-4, C-5	
4	123.9			
5	149.0			
7	162.7			
9	142.9	6.01 (d, 5.3)	C-7, C-10, C-11, C-12	H-10
10	117.1	5.55 (t, 5.3)	C-12, C-13	H-9, H-11
11	127.6	6.06 (dd, 10.7/5.3)	C-7, C-9, C-10	H-10, H-12
12	125.5	6.67 (d, 10.7)	C-7, C-9, C-10, C-13, C-14	H-11
13	110.3			
14	160.4			
16	42.6	1.61 (m)	C-1, C-2, C-17, C-18, C-19	H-1, H-17
17	24.8	1.75 (m)	C-1, C-16, C-18, C-19	H-16, H-18, H-19
18[a]	22.1	0.87 (d, 6.5)	C-16, C-17, C-19	H-17
19[a]	231	1.02 (d, 6.4)	C-16, C-17, C-18	H-17
20	129.0	6.31 (d, 10.2)	C-2, C-4, C-5, C-21, C-22, C-23	H-21
21	26.3	2.66 (dh, 10.2/6.5)	C-4, C-20, C-22, C-23	H-20, H-22, H-23
22[a]	21.1	1.08 (d, 6.7)	C-20, C-21, C-23	
23[a]	21.9	1.07 (d, 6.5)	C-20, C-21, C-22	

The HMBC experiment is optimized for couplings of 7 Hz.
[a] Signals may be interchanged.

entities, are very limited. Apart from cinereain, only three other fungal compounds, oxepinamid A–C, are described in the literature, all isolated from a marine fungus of the genus *acremonium*.[6]

The structure of **2** was inferred from the complete agreement of all spectroscopic data with those reported for brevicompanin B.[7]

Both **1** and **2** were found to exhibit antiplasmodial activity against the malaria parasite *Plasmodium falciparum* 3D7 in the radio isotope assay.[8] Compounds **1** and **2** exhibited IC_{50}-values of 28 and 35 mg/ml, respectively, but the actual IC_{50}-values are lower since the compounds precipitated in the test media. In comparison cinchonin, which also precipitated, has an IC_{50}-value of 126 μg/ml. Under the same conditions, chloroquine and quinine have IC_{50}-values of 15 and 129 ng/ml, respectively. Further, antimalarial testing is necessary to establish the exact IC_{50}-values of **1** and **2**.

Neither of the compounds showed any activity in an in-house antifungal disc diffusion assay nor antibacterial agar overlay assay.

3. Conclusion

It is evident that fungal sources continue to provide novel and interesting chemical structures and that UV-guided screening is a powerful tool for identifying interesting compounds for further investigations on the basis of the compound chromophores. The occurrence of janoxepin, an oxepin derivative with a D-leucine incorporated, in *A. janus* is interesting, due to the limited distribution of D-leucine as well as compounds containing a oxepin ring, in natural sources. Furthermore, brevicompanin B was also isolated from *A. janus*. Brevicompanin B has previously only been observed in *P. brevicompactum*.

4. Experimental

4.1. Fungal material and fermentation

The isolate of *Aspergillus janus* (IBT 22274) was obtained from the IBT Culture Collection at BioCentrum-DTU, The Technical University of Denmark. Isolates were cultured as three-point mass inoculations on 200 YES agar plates for 14 days in the dark, as previously described.[9]

4.2. Extraction and separations

Agar plates were extracted twice overnight with EtOAc (2.4 L) to give a crude extract of 5.5 g. This was partitioned between hexane and 90% aqueous MeOH followed by 50% aqueous MeOH and dichloromethane giving three fractions. The dichloromethane phase (2.9 g) was coated onto 3.0 g of Celite, which was placed on top of a Sep-Pak Vac 35 cm^3 (10 g) Florisil Cartridge column and separated into four fractions (H_2O/MeOH (75:25), (50:50), (25:75) and 100% MeOH) by vacuum liquid chromatography. The 75% MeOH fraction was subjected to HPLC on a Waters Prep Nova-Pak C18 cartridge (25 mm×100 mm, 6 μm) using a H_2O:CH_3CN gradient starting at (70:30) running to 100% CH_3CN in 15 min as mobile phase at 20 mL/min flow rate, to give **2** at 6.8 min (16 mg) and **1** at 8.5 min (52 mg).

4.3. Determination of stereochemistry at C-22

Compound **1** (3.1 mg) was hydrolyzed with 1 mL 6 M HCl for 20 h at 120 °C in a sealed glass vial. The content was filtered through a Waters 0.45 μm filter and evaporated to dryness in vacuo. The remanence was dissolved in 0.2 mL milli-Q water. This solution was subjected to the following treatment: 50 μL is mixed with 100 μL 1% solution of Marfey's reagent in acetone and 20 μL 1 M $NaHCO_3$. The mixture is placed in an oil bath thermostated to 40 °C for 1 h with frequent shaking. 10 μL 2 M HCl is added and the mixture is evaporated in a vacuum exicator over NaOH

pellets. The remanence is dissolved in 0.5 mL DMSO and used for HPLC.

50 μL of 50 μM of D- and L-leucin were subjected to the same treatment and injected in the same chromatographic system as references.

4.4. Antimicrobial assays

Compounds **1** and **2** were tested for antifungal activity in a modified version of the assay specified by Hadacek and Greger.[10] The compounds were tested in the following concentrations 100, 50, and 25 μg per filter disc. The assay involved the following fungi; *Alternaria infectoria*, *A. fumigatus*, *Botrytis cinerea*, *Cladosporium* sp., *Fusarium avaenceum*, *F. culmorum*, *F. oxysporum*, *F. solari*, *F. sparatrichoides*, *Penicillium digitatum*, *P. expansum* and *P. italicum*.

The tests for antibacterial activity of **1** and **2** were carried out in an agar overlay method involving gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative (*Escherichia coli* and *Pseudomanas aeruginosa*) bacteria. 100, 50, and 25 μg of **1** and **2** were added to a TLC-plate, covered with inoculated agar and incubated at 37 °C for 24 h. Neither **1** nor **2** showed any antibacterial activity in the tested concentrations.

4.5. Janoxepin (1)

Orange red crystalline solid; mp 88–89 °C; $[\alpha]_D^{25}$ −3.4 (MeOH; *c* 1.46); EIMS 70 eV *m/z* (rel. int.): 341 $[M]^+$(100), 324 $[M-17]^+$(14), 284 $[M-C_4H_9]^+$(52). HRMS obsd $(M+H)^+$ at *m/z* 342.1785, calcd for $C_{19}H_{24}N_3O_3$ 342.1818, and obsd $(M-H)^-$ at *m/z* 340.1696, calcd for $C_{19}H_{22}N_3O_3$ 340.1661. NMR values are given in Table 1.

Acknowledgements

The authors thank Dorte Brix, the Institute of Medicinal Chemistry, The Danish University of Pharmaceutical Sciences, for kindly performing the malaria assay. The support of the Danish Research Councils (grant No. 9901295) and the Center for Advanced Food Studies is acknowledged.

References and notes

1. Nielsen, K. F.; Smedsgaard, J.; Larsen, T. O.; Lund, F.; Thrane, U.; Frisvad, J. C. In *Chemical Identification of Fungi: Metabolite Profiling and Metabolomics*; Arora, D. K., Ed.; Fungal Biotechnology in Agricultural, Food, and Environmental Applications; Marcel Dekker: New York, 2003; pp 19–35.
2. Cutler, H. G.; Springer, J. P.; Arrendale, R. F.; Arison, B. H.; Cole, P. D.; Roberts, R. G. *Agric. Biol. Chem.* **1988**, *52*, 1725–1733.
3. Breitmaier, E.; Wolfgang, V. *Carbon-13 NMR Spectroscopy. High-Resolution Methods and Applications in Organic Chemistry and Biochemistry*, 3rd ed.; VCH: Weinheim, 1987; pp 414–422.
4. Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591–596.
5. Lautru, S.; Gondry, M.; Genet, R.; Pernodet, J.-L. *Chem. Biol.* **2002**, *9*, 1355–1364.
6. Belofsky, G. N.; Anguera, M.; Jensen, P. R.; Fenical, W.; Kock, M. *Chem. Eur. J.* **2000**, *6*, 1355–1360.
7. Kusano, M.; Sotoma, G.; Koshino, H.; Uzawa, J.; Chijimatsu, M.; Fujioka, S.; Kawano, T.; Kimura, Y. *J. Chem. Soc., Perkins Trans. 1* **1998**, *17*, 2823–2826.
8. Zeigler, H. L.; Stærk, D.; Christensen, J.; Hviid, L.; Hägerstrand, H.; Jaroszewski, J. *Antimicrob. Agents Chemother.* **2002**, *46*, 1441–1446.
9. Svendsen, A.; Frisvad, J. C. *Mycol. Res.* **1994**, *98*, 1317–1328.
10. Hadacek, F.; Greger, H. *Phytochem. Anal.* **2000**, *11*, 137–147.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
ELSEVIER
Tetrahedron 61 (2005) 8722–8739

The synthetic versatility of alkoxycarbonyl- and hydroxymethyl-piperazine-2,5-diones

Christina L.L. Chai,* John A. Elix and Paul B. Huleatt

Department of Chemistry, The Faculties, Australian National University, Canberra, ACT 0200, Australia

Received 25 March 2005; revised 8 June 2005; accepted 23 June 2005

Available online 26 July 2005

Abstract—Alkoxycarbonylpiperazine-2,5-diones are versatile precursors for the α-functionalisation of piperazine-2,5-diones. The alkoxycarbonyl group activates the α-carbon position to alkylation reactions and this provides a mild and selective method for the extension of the carbon framework of piperazine-2,5-diones. In addition, the alkoxycarbonyl group can be converted to the carboxy group, which in turn can be 'deleted' or manipulated for the installation of carbon and/or heteroatom substituents where desired, the latter via *N*-acyliminium chemistry. We also demonstrate that hydroxymethylpiperazine-2,5-diones complement carboxypiperazinediones as precursors for the generation of *N*-acyliminium ions.

1. Introduction

The ubiquity of the piperazine-2,5-dione motif in a number of biologically active natural products[1] has encouraged research into the development of methods for the selective functionalisation of piperazine-2,5-diones. Although piperazine-2,5-diones are cyclic dipeptides and can formally be synthesized from the condensation of the requisite α-amino acids,[2] in practice complex piperazine-2,5-diones are accessed by the functionalisation of readily available piperazine-2,5-dione precursors.[3] This approach has the advantage of enabling the assembly of the carbon and heteroatom framework of piperazine-2,5-diones without the need to develop individual strategies for the synthesis of the requisite α-amino acids.

The existing methods for *C*-functionalisation are limited in scope due to the necessity of using strong bases in cases where the generation of the enolate of the piperazinedione is required.[4] Regioselectivity is also a problem with unsymmetrical piperazine-2,5-diones. Other methods for *C*-functionalisation of piperazinediones include using radical addition chemistry[5] and Diels–Alder reactions.[6] The former relies on the ability to synthesise suitable radical precursors and/or substrates and is sensitive to polar and steric effects while the latter is more suitable for the synthesis of bicyclic frameworks. Due to the limitations of the aforementioned methodologies, we sought to develop complementary synthetic routes for the *C*-functionalisation of piperazinediones. In addition we also recognized the need to be able to install heteroatom functionalities on the α-carbon positions of the piperazine-2,5-dione ring as this substitution is present in numerous bioactive piperazine-2,5-diones. These heteroatom functionalised piperazine-2,5-diones can also serve as radical or cationic precursors in subsequent chemical transformations.

Our plan involved the use of an alkoxycarbonyl group as a 'traceless' substituent on the piperazine-2,5-dione ring. The choice of the alkoxycarbonyl group was expected to enhance the reactivity of the α-carbon center of the piperazine-2,5-dione ring towards alkylation reactions. In addition, we envisage that the alkoxycarbonyl group can be converted to the carboxy group, which in turn can be 'deleted' or manipulated for the installation of carbon and/or heteroatom substituents where desired.

Following on from our previous communication,[7] the studies reported herein detail the synthetic utility of alkoxycarbonyl piperazine-2,5-diones in the functionalisation of the piperazine-2,5-dione nucleus. In the course of our studies we also discovered that the readily accessible hydroxymethylpiperazine-2,5-diones complement the chemistry of alkoxycarbonyl derivatives.

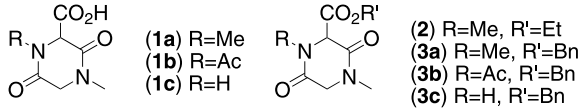
Keywords: *N*-Acyliminium ions; Alkylations; Amino acids and derivatives; Trapping reactions.
* Corresponding author at present address: Institute of Chemical and Engineering Sciences, 1 Pesek Road, Jurong Island, Singapore 627833. Tel.: +65 6796 3902; fax: +65 6316 6184;
e-mail: christina_chai@ices.a-star.edu.sg

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.06.084

2. Results and discussion

2.1. Synthesis of alkoxycarbonyl- and carboxy-piperazine-2,5-diones

In order to examine the effects of various substitution patterns on the chemistry of carboxy piperazine-2,5-diones, the acids **1a**–**c** derived from the esters of types **2** and **3**, were targeted.



Our initial work was directed towards the synthesis of the target, acid **1a**. A three step synthesis of diethyl *N*-methylaminomalonate (**6**) was carried out using modification of literature procedures.[8–10] Diethyl malonate was readily converted to diethyl α-bromomalonate **4** in 70% yield using bromine in carbon tetrachloride.[8] However, when the bromide **4** was treated with *N*-methylbenzylamine using conditions reported in the literature,[9,10] only diethyl malonate was recovered. After much experimentation, the synthesis of the desired diethyl *N*-methylbenzylaminomalonate (**5**) was achieved by treatment of the bromide **4** with *N*-methylbenzylamine in chloroform at reflux. Hydrogenolytic cleavage of the benzyl group from amine **5** then cleanly afforded diethyl *N*-methylaminomalonate (**6**) in 95% yield. With the aminomalonate **6** in hand, DCC mediated coupling of the amine with CBZ-sarcosine afforded the dipeptide **7** in good yield. Hydrogenolytic cleavage of the CBZ protecting group from the dipeptide **7**, followed by thermally promoted cyclisation of the deprotected dipeptide afforded the α-ethoxycarbonyl piperazinedione **2** (see Scheme 1).

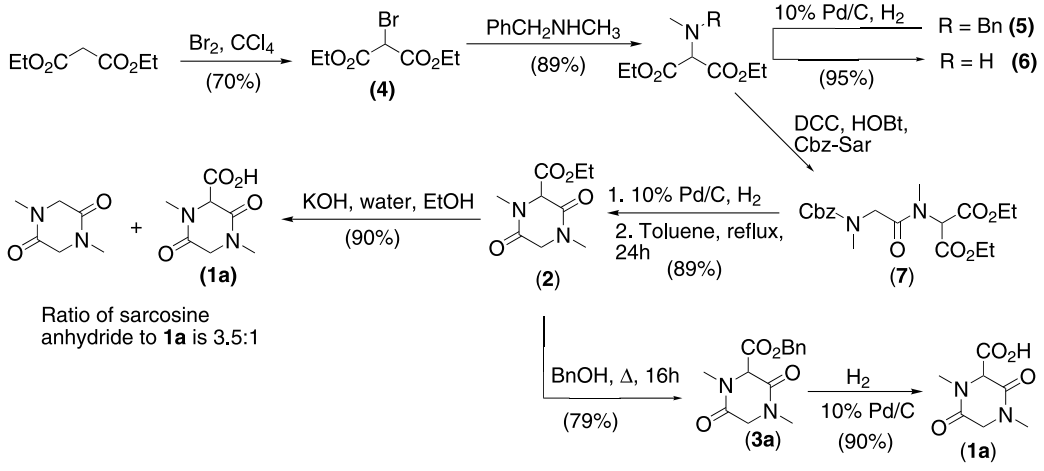
With the α-ethoxycarbonyl piperazinedione **2** in hand, saponification of the ethyl ester moiety using potassium hydroxide in aqueous ethanol was carried out as reported in the literature.[11] Disappointingly, we were unable to achieve the smooth conversion of the ester **2** to the acid **1a**. Approximately 70% of the material recovered from the reaction was identified as sarcosine anhydride, clearly resulting from the premature decarboxylation of the acid **1a**. Our attempts to minimise the premature decarboxylation of acid **1a** by numerous modifications to the work-up procedure were unsuccessful and sufficient quantities of the acid **1a** could not be obtained. Consequently, a milder method of generating the acid **1a** was desired. We envisaged that the acid **1a** may be attainable via hydrogenolysis of the benzyl ester **3a**. Thus, ester **2** was successfully transesterified with benzyl alcohol and the desired benzyl ester **3a** was obtained in good yield.

Subsequent hydrogenolysis of the ester **3a** using 10% palladium on carbon under a hydrogen atmosphere proceeded smoothly and the desired acid **1a** was obtained in excellent yield. Premature decarboxylation of the acid **1a** was suppressed and sarcosine anhydride was only observed as a minor byproduct.

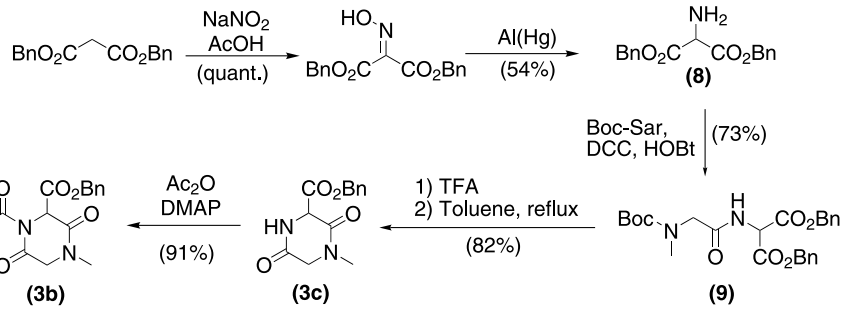
From the studies above it is evident that the benzyloxycarbonyl piperazine-2,5-diones of type **3** are suitable precursors to the corresponding acids. With this in mind, a synthetic route to the esters was undertaken. The synthesis of dibenzyl aminomalonate (**8**) was carried out following literature procedures[12] starting from dibenzyl malonate. Peptide coupling of dibenzyl aminomalonate (**8**) with BOC-sarcosine, deprotection of the resultant dipeptide **9** and subsequent cyclisation gave the piperazinedione **3c** in good yields. *N*-acetylation of piperazinedione **3c** using acetic anhydride and DMAP under standard conditions gave the corresponding ester **3b** (see Scheme 2).

With the two esters **3b** and **3c** in hand, the target acids **1b** and **1c** were accessed via hydrogenolysis. When the *N*-acetyl ester **3b** was treated with hydrogen and 10% palladium on carbon, as described above, a 2:1 mixture of the desired acid **1b** and 1-acetyl-4-methylpiperazine-2,5-dione was formed (Scheme 3). Unfortunately, this mixture could not be separated because of the instability of the acid **1b** and in subsequent experiments this mixture of compounds was utilised without purification.

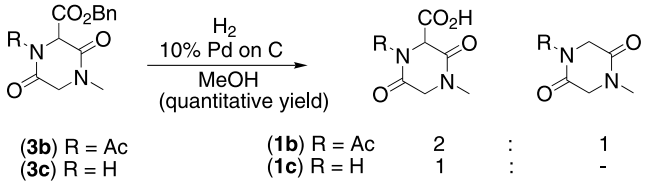
When the free amido ester **3c** was reacted under similar conditions only the desired acid **1c** was obtained. The greater stability of the acid **1c** as compared with the acid **1b** maybe due to the relief of steric strain upon decarboxylation of acid **1b**. Since analogous steric effects are not present in



Scheme 1.



Scheme 2.



Scheme 3.

1c, the latter is relatively stable. Whilst the acid **1c** showed greater stability than **1b**, it was highly insoluble in organic solvents including dichloromethane and chloroform and only showed appreciable solubility in more polar solvents such as methanol and DMSO.

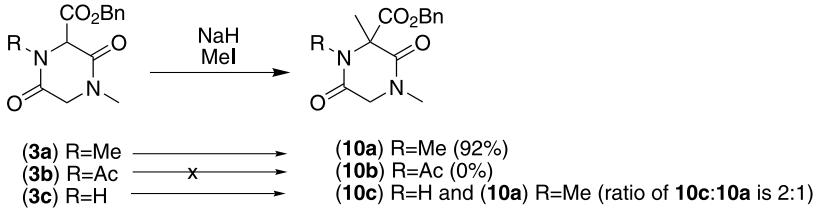
2.2. Extension of the carbon framework of alkoxycarbonyl piperazine-2,5-diones

The α-alkylation reactions of piperazine-2,5-diones are usually accomplished using strong bases such as *n*-butyl lithium or sodamide.[4] In our studies, the α-methylation of piperazinedione **3a** was effected in excellent yields using NaH and MeI. This is due to the presence of the activating alkoxycarbonyl group at the α-carbon center of the piperazine-2,5-dione ring. When the *N*-acetyl ester **3b** was reacted with NaH and MeI under similar conditions, only unreacted starting material was recovered. The failure of this system to undergo α-alkylation is surprising and may be related to subtle conformational effects associated with the substitution pattern of the piperazinedione ring.

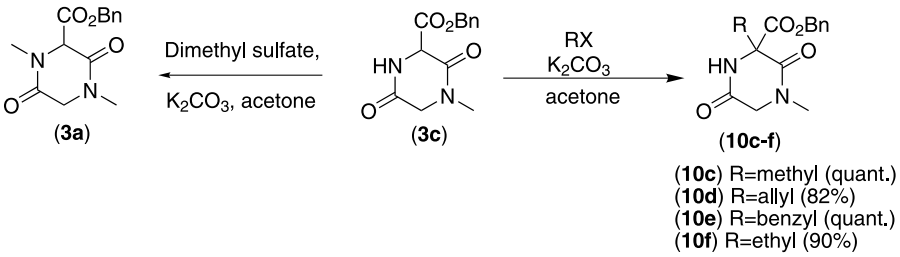
Alkylation of piperazinedione **3c** with one equivalent of NaH yielded a mixture of products comprising the α-methylated ester **10c** as the major component and equal amounts of the trimethyl compound **10a** and the starting material **3c** (Scheme 4). Thus the use of NaH as base does not discriminate between *C*- and *N*-alkylation. With excess NaH and MeI, complete conversion of piperazine-2,5-dione **3c** and **10c** to the trimethyl compound **10a** was observed.

When the ester **3c** was reacted with one equivalent of potassium carbonate and excess methyl iodide, α-methyl piperazinedione **10c** was formed exclusively and in excellent yields. The excellent regioselectivity observed in the alkylation of piperazinedione **3c** is general for a number of alkyl halides studied. Scheme 5 summarises the range of alkyl substituents that can be selectively installed at the α-carbon position of piperazinedione **3c** under these mild alkylation conditions.

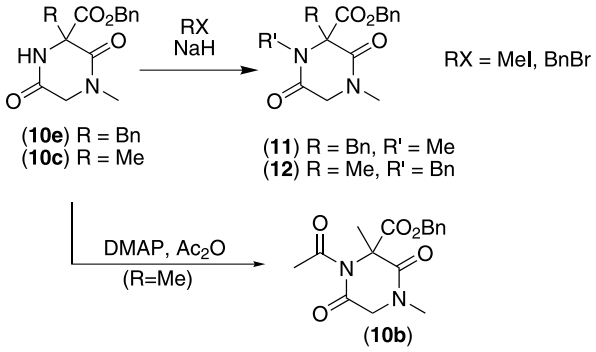
Surprisingly when dimethyl sulfate was used as the alkylating agent instead of MeI, only ester **3a** was obtained (Scheme 5). Thus the choice of alkylating agents provides complementary routes to the α-methylated derivative **10c** as well as to the *N*-methylated piperazine-2,5-dione **3a**.



Scheme 4.



Scheme 5.



Scheme 6.

The potential of this selective alkylation methodology is highlighted when sequential *C*- and *N*-functionalisation steps are carried out. For example, ester **3c** can be selectively *C*-benzylated to give piperazinedione **10e**. This compound is then readily *N*-methylated with methyl iodide and sodium hydride in DMF to yield the *N*-methylated, *C*-benzylated piperazinedione **11** in 85% yield. Alternatively, by simply changing the sequence of addition of the alkylating agents, the ester **3c** can be *C*-methylated and subsequently *N*-benzylated to afford piperazinedione **12** (Scheme 6).

The methodology to selectively install *C*-alkyl functionality onto the piperazinedione ring can also be used for the synthesis of piperazinedione **10b**. As previously outlined in Scheme 4, the synthesis of piperazinedione **10b** could not be achieved via α-methylation of the *N*-acetyl piperazinedione **3b**. However, with the *C*-methylated piperazinedione **10c** in hand, *N*-acetylation was readily achieved using acetic anhydride and DMAP to afford piperazinedione **10b** (Scheme 6).

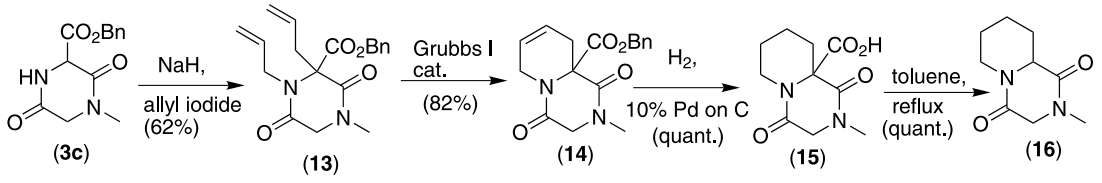
2.3. Applications to the synthesis of ring fused piperazinediones

The methodology described above can be applied to the synthesis of conformationally constrained α-amino acid derivatives such as that derived from pipecolic acid and baikiain.[13] Two approaches are possible for the synthesis of these fused ring systems, both of which involve a sequence of *N*- and *C*-alkylation reactions. In the first approach (Scheme 7), allylation of **3c** afforded the 3,4-diallyl piperazinedione **13** in moderate yield. The fused six-membered ring of the baikiain derivative **14** was then formed efficiently via a ring closing metathesis reaction using either Grubb's type I or type II catalysts. Hydrogenolysis of the benzylic ester **14** is accompanied by the concomitant reduction of the alkene to give the carboxylic acid **15**. Finally, the known pipecolic acid derivative **16**[14] was prepared via the quantitative thermal decarboxylation of the acid **15**.

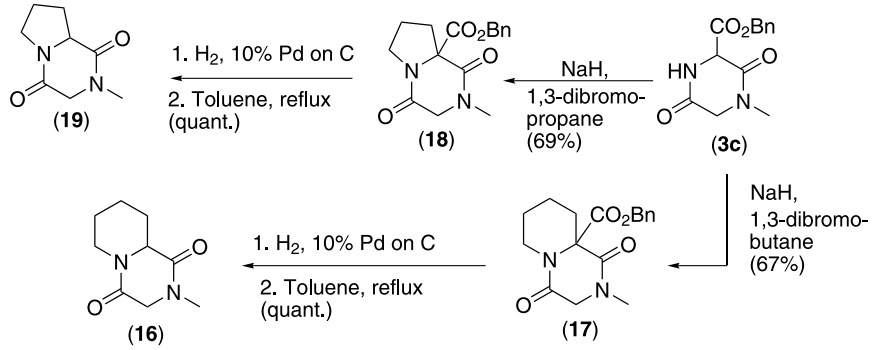
The second approach, outlined in Scheme 8, to the pipecolic acid derivative **16** is more direct. When the key precursor **3c** was treated with 2 equiv of sodium hydride and 1 equiv of 1,4-dibromobutane the desired ester **17** was obtained in moderate yield. The identity of the ester **17** was confirmed by quantitative conversion to piperazinedione **16** using the standard decarboxylation conditions.

Similarly the five membered ring analogue of **16** was also be synthesized in this manner using 1,3-dibromopropane as the alkylating agent (Scheme 8). The piperazine-2,5-dione formed was subsequently converted to the known derivative **19**[14] via ester cleavage and decarboxylation.

This study into the functionalisation of ester **3c** resulted in the development of a versatile synthetic route to piperazinediones with varying *N*- and α-substitution patterns. Our subsequent studies focussed on sequential decarboxylation oxidation nucleophilic addition (DONA) reactions of carboxy piperazine-2,5-diones. We envisaged that in conjunction with the mild, efficient and selective alkylation procedures described above, the DONA protocol could lead to piperazinediones with greater functional diversity.



Scheme 7.



Scheme 8.

2.4. Decarboxylation oxidation nucleophilic addition reactions (DONA) of carboxy piperazine-2,5-diones

In 1999, Suarez et al. reported the synthesis of α-functionalised pyrrolidines from proline derivatives, in the presence of iodine and diacetoxyiodobenzene (DIB).[15] The transformation is believed to occur via the intermediacy of the *N*-acyliminium ion. Although the synthetic potential of acyl iminium ion chemistry has long been recognized,[16] the study by Suarez provides a mild and efficient method for the generation and trapping of *N*-acyl iminium ion species.

This methodology offered an attractive means for preparing α-functionalised piperazinediones from the corresponding acids. Thus treatment of acid **1a** with 1 equiv of diacetoxyiodobenzene (DIB) and 0.5 equiv iodine gave the α-acetoxy derivative **22a**. The mechanism, as outlined in Scheme 9, is likely to proceed through the intermediacy of the carboxy radical which then undergoes rapid decarboxylation to the α-carbon centred radical **20**. DIB mediated one electron oxidation of this radical species would give the *N*-acyliminium ion intermediate **21a**. In the absence of an added nucleophile, the acetate ions (or acetic acid) would add to the *N*-acyliminium ion **21a** to yield the α-acetoxy piperazinedione **22a**. The simplicity and versatility of this sequence is demonstrated when the acid **1a** was treated with DIB and iodine in the manner described above and methanol was added as an external nucleophile. This resulted in the efficient conversion of the acid **1a** into the corresponding α-methoxy product **22b**, a compound which displayed spectroscopic data identical to that reported in the literature.[17]

The successful use of the acid **1a** as a substrate for such DONA reactions prompted us to assess the relative reactivity of the acids **1b** and **1c**. In view of the proposed mechanism of the reaction, it is evident that the ease of generation as well as the stability of the *N*-acyliminium ion may be strongly influenced by electronic and/or steric effects. For example, the generation of the *N*-acyliminium ion **21b** may be more difficult than **21a** and **21c** as the radical intermediate derived from acid **1b** would be expected to be more difficult to oxidise to the highly electron deficient species **21b**. If formed, the *N*-acyliminium ion **21b** will also be destabilised by electronic effects and would be expected to be more reactive with nucleophiles.

In contrast to the *N*-methyl carboxylic acid **1a**, which showed poor solubility in dichloromethane, the *N*-acetyl acid **1b** was very soluble in chlorinated solvents. Thus, when the acid **1b** was treated with DIB and I_2 in the absence and in the presence of methanol, respectively, the α-acetoxy piperazinedione **22c** and the α-methoxy piperazinedione **22d** were obtained in excellent yields.

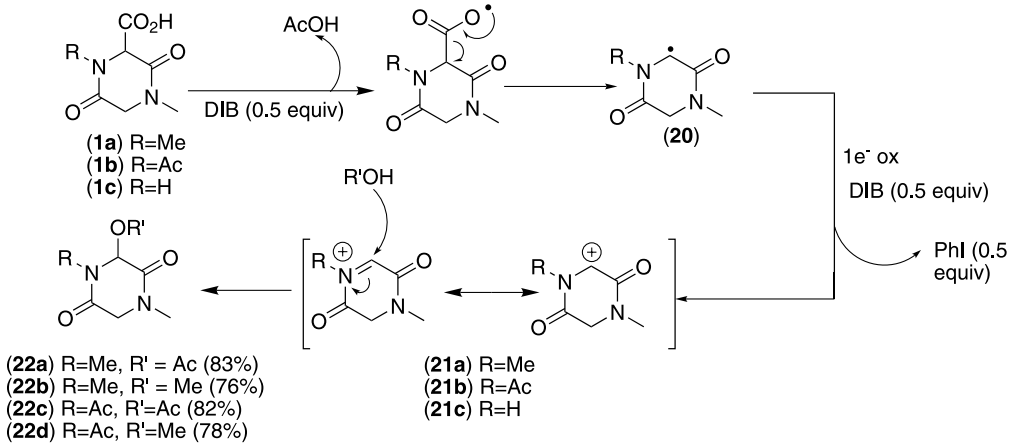
These experiments indicate that under these conditions, the acid **1b** appears to have similar reactivity to the acid **1a** despite the expected disparity in the ease of generation and stability of the reactive *N*-acyliminium ion intermediates, **21a** and **21b**. This result is noteworthy as *N*-acyliminium ions such as **21b** have not been available for synthetic purposes prior to this work.[16]

In contrast to the *N*-acetyl acid **1b**, the free amido acid **1c** was sparingly soluble in solvents suitable for use in the DONA reaction, namely dichloromethane and acetonitrile. As a result, when the acid **1c** was subjected to the DONA protocol outlined previously no reaction was observed and in all cases the starting material was recovered quantitatively.

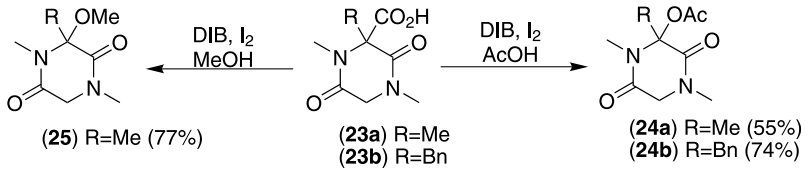
2.5. DONA reactions of α,α-disubstituted piperazine-2,5-diones

The effect of an α-substituent on the formation and reactivity of the *N*-acyliminium ion intermediates generated using the DONA strategy was examined using the α-methyl acid **23a** obtained from the hydrogenolysis of the corresponding ester **10a**. It is interesting to note that in contrast to the acid **1a**, no premature decarboxylation of the acid **23a** was detected. This suggests that the α-substituted acid **23a** is more stable than the unsubstituted acid, **1a**, presumably because the presence of the α-methyl substituent hinders the ability of the acid **23a** to adopt the planar six-membered ring transition state required for the decarboxylation to occur.

Treatment of the α-methyl carboxylic acid **23a** with DIB and iodine in the presence of either acetic acid or methanol



Scheme 9.



Scheme 10.

gave rise to the α-acetoxy (**24a**) and the α-methoxy (**25**) piperazinediones, respectively in yields comparable to those obtained for acids **1a** and **1b**. Similarly, conversion of the acid **23b** (which was obtained from the ester **11**) to the corresponding α-acetoxy compound **24b** was also achieved (Scheme 10).

The synthetic sequence described above led to piperazinedione derivatives which cannot be readily accessed using other synthetic methods. Studies carried out within our group have established that selective heteroatom α-functionalisation of piperazinediones can be difficult in some cases.[18] For example, the α-methoxy compound **25** cannot be prepared by the established protocols for the α-functionalisation of piperazinediones. 3-Bromo-1,3,4-trimethylpiperazine-2,5-dione cannot be accessed through established methods as radical bromination of 1,3,4-trimethylpiperazine-2,5-dione with NBS leads to exclusive formation of the regioisomer, 6-bromo-1,3,4-trimethylpiperazine-2,5-dione.[18b] This in turn means that the formation of the α-methoxy compound **25**, via nucleophilic displacement of the bromide, cannot be obtained by this route.

2.6. Carbon–carbon bond forming reactions via *N*-acyliminium ions derived from piperazine-2,5-diones

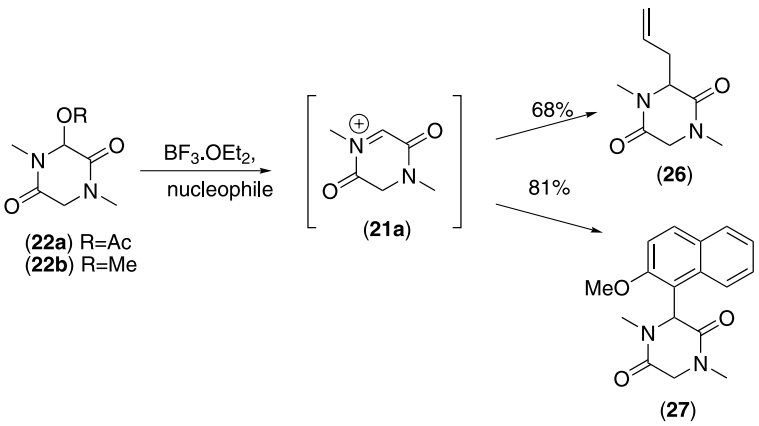
In order to increase the repertoire of synthetic transformations that can be achieved with *N*-acyliminium ions generated from piperazinedione precursors, carbon–carbon bond forming reactions were investigated. The use of such *N*-acyliminium ions as glycine cation equivalents in carbon chain extension reactions has been reported.[19] Our initial studies focussed on the use of 3-methoxy-1,4-dimethylpiperazine-2,5-dione (**22b**) as an *N*-acyliminium ion precursor. Thus, treatment of piperazinedione **22b** with boron trifluoride diethyl etherate in the presence of allyltrimethylsilane afforded the desired 3-allyl-1,4-dimethylpiperazine-2,5-dione (**26**) in 68% yield (Scheme 11).

Similarly when 2-methoxynaphthalene was used as the carbon nucleophile in the presence of boron trifluoride diethyl etherate, Friedel–Crafts alkylation of the intermediate **21a** occurred to yield the α-aryl piperazinedione **27** in 81% yield (Scheme 11).

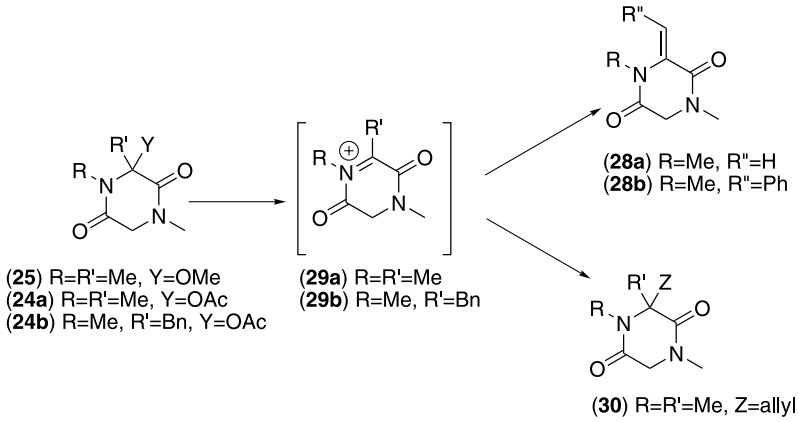
In contrast to the stability of the α-acetoxy and α-methoxy compounds, **22a** and **22b**, respectively, the C-methyl piperazinediones **24a** and **25**, were unstable. Upon standing, these piperazinediones are converted to the methylidene piperazinedione **28a**. Although Schmidt et al. have reported the synthesis of methylidene piperazinediones from α-methyl-α-hydroxypiperazinediones under acidic conditions,[20] there is no literature precedence for a conversion of this type under neutral conditions. This elimination process probably proceeds through the intermediacy of an α-carbocation/*N*-acyliminium ion intermediate **29a** as outlined in Scheme 12.

Due to the competing elimination process, efficient *C*-allylation of the *N*-acyliminium ion **29a** (generated from α-methoxy piperazinedione **25**) was difficult to achieve. Treatment of the piperazinedione **25** with boron trifluoride diethyl etherate and allyltrimethylsilane gave a 1:1 mixture of the desired product **30** and the methylidene piperazinedione **28a**. The formation of the desired product **30** was favoured when 10 equiv of allyltrimethylsilane was used. Using these optimised conditions, the conversion of the α-methoxy piperazinedione **25** to the α-allyl piperazinedione **30** was achieved in 66% yield.

The conversion of the *N*-acyliminium ion **29a** to the methylidene piperazinedione **28a** can be exploited for the synthesis of a variety of alkylidene piperazinediones



Scheme 11.

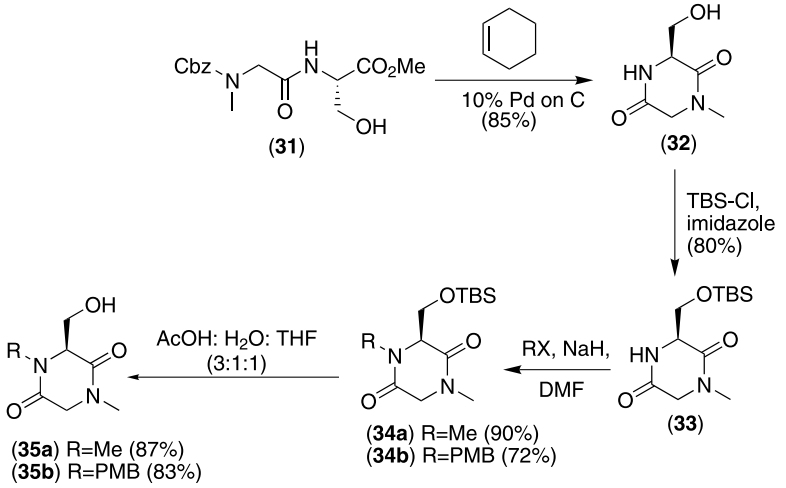


Scheme 12.

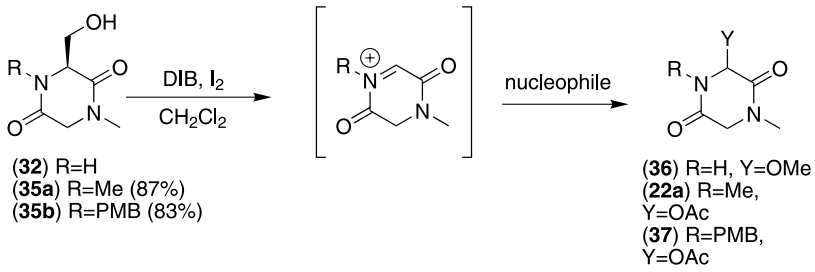
including natural products. For example, dehydrophenylahistin contains a *Z*-benzylidene piperazinedione moiety.[21] Thus, to demonstrate the generality of the elimination process, a conversion of the piperazinedione **24b** to the benzylidene piperazinedione **28b** was attempted. Treatment of the α-acetoxypiperazinedione **24b** with boron trifluoride diethyl etherate afforded the benzylidene piperazinedione **28b** as a single stereoisomer in 76% yield. The stereochemistry of the product was assigned as (*Z*) by comparison of the ^{1}H NMR data observed for piperazinedione **28b** with data reported in the literature.[22] It is likely that formation of the *E*-isomer is disfavoured due to steric interaction between the phenyl ring and the carbonyl group of the piperazinedione ring.

2.7. Alternative piperazine-2,5-dione precursors for modified DONA type reactions: the use of hydroxymethylpiperazinediones

In order to extend the utility of the DONA methodology, we sought to examine alternative substrates for the DONA type reactions. The work of Suarez et al. on the synthesis of α-aryl amino acids from serine derivatives provided the impetus for our studies.[23] In that study Suarez et al. demonstrated that the hydroxylmethyl moiety can act as a latent functionality for the *N*-acyliminium ion via a sequence of radical deformylation and oxidation steps. We envisaged that such a modified DONA protocol could be applied to serine derived piperazinediones in which the α-hydroxymethyl moiety could be employed as a latent functionality for the *N*-acyliminium ion in the same manner as the carboxylic acid group in the previous study. This is an attractive alternative as the serine based piperazinediones are readily accessible using peptide coupling procedures. For example as outlined in Scheme 13, the dipeptide **31** can be readily converted to piperazinedione **32** using the standard two step hydrogenolysis/thermal cyclisation protocol. More efficient conversion was achieved using catalytic transfer hydrogenolysis conditions. Using 10% palladium on carbon and cyclohexene in methanol at reflux, cleavage of the CBZ protecting group was followed by the in situ cyclisation of the free amine to afford the piperazinedione **32** in 85% yield.[24] The piperazinedione **32** was only sparingly soluble in most organic solvents, however, protection of the hydroxy functionality as a silyl ether afforded the readily soluble piperazinedione **33**. Piperazinedione **33** is a key synthetic intermediate that can be selectively *N*-alkylated with a wide variety of alkyl halides in a combinatorial fashion. In this study, the *N*-methyl **34a** and *N*-*p*-methoxybenzyl **34b** derivatives were targeted. Treatment of piperazinedione **33** with sodium hydride and either methyl iodide or *p*-methoxybenzyl chloride afforded piperazinediones **34a** and **34b**, respectively. Acidic cleavage of the *t*-butyldimethylsilyl group



Scheme 13.



Scheme 14.

then yielded the piperazinediones **35a** and **35b** in excellent yield.

Our initial work in the study focussed on the DONA reactions of piperazinedione **32**. A suspension of the hydroxymethyl piperazinedione **32** in dichloromethane was treated with iodine and DIB, followed by addition of excess methanol to give the desired α-methoxy piperazinedione **36** in a moderate yield of 44%.

In contrast to the solubility and stability complications encountered with the carboxylic acid **1a**, the *N*-methylated serine derived piperazinedione **35a** was stable and readily soluble in dichloromethane. Furthermore, using the standard DONA reaction conditions, complete conversion of the alcohol **35a** to the α-acetoxy compound **22a** was achieved (Scheme 14). Similarly, the *N*-*p*-methoxybenzyl compound **35b** underwent the modified DONA reaction to give the α-acetoxy compound **37** in excellent yield.

3. Conclusion

Our studies have demonstrated that carboxy- and hydroxymethyl-piperazinediones are complementary precursors for the generation of *N*-acyliminium ions. The carboxy piperazinediones potentially suffer from problems with premature decarboxylation and lack of solubility in the solvents typically used in these reactions. On the other hand, the carboxy piperazinediones (with the exception of the *N*-acetylated systems) allow for mild *C*-alkylation reactions by activating the α-carbon positions of the piperazine-2,5-dione. By contrast the hydroxymethyl piperazinediones are readily accessible from commercially available serine and derivatives, have a much better solubility profile in organic solvents but cannot be *C*-functionalised under mild conditions.

4. Experimental

4.1. General

^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini II NMR spectrometer at 300 and 75.4 MHz, respectively. $CDCl_3$ was used as the solvent unless otherwise indicated. The chemical shifts (δ) are reported as the shift in ppm from tetramethylsilane (TMS, 0.00 ppm). ^{1}H spectra were appropriately referenced to either the $CHCl_3$ singlet (7.26 ppm), CHD_2OD quintet (3.31 ppm), $CHD_2S(O)CD_3$ quintet (2.50 ppm), $CHD_2(CD_3)NC(O)D$ quintet (2.90 ppm) or to TMS. ^{13}C spectra were appropriately referenced to either $CDCl_3$ (77.0 ppm), CD_3OD (49.0 ppm), $(CD_3)_2SO$ (39.5 ppm). Infrared spectra were recorded on a Perkin Elmer 1800 or a Shimadzu FTIR-8400 Fourier Transform Infrared Spectrometer. Samples were analysed as KBr discs (for solids) or as thin liquid films (for oils and liquids) on NaCl plates. Low and high resolution mass spectra were recorded on a VG Micromas 7070F double focussing mass spectrometer using positive ion electron impact techniques. Melting points are uncorrected and were recorded on a Leica Galen III microscope. Flash chromatography utilised Merck silica gel 60 (200–400 mesh ASTM) and analytical reagent (AR) grade solvents as indicated. Unless stated otherwise, reagents were purchased from Aldrich, Merck, Fluka, AJAX or BDH Chemicals. All solvents were of AR grade, purified by literature procedures[25] and where appropriate, stored over molecular sieves. All reactions were carried out under an atmosphere of dry, oxygen-free nitrogen unless otherwise specified. Reactions which involved moisture sensitive compounds were carried out using oven-dried or flame-dried apparatus with dry solvents.

4.2. Syntheses of alkoxycarbonyl- and carboxy-piperazine-2,5-diones

4.2.1. Ethyl *N*-[*N*-benzyloxycarbonylsarcosyl]-2-ethoxycarbonylsarcosinate (7). To a stirred solution of CBZ-sarcosine (5.58 g, 25.0 mmol) in DMF (60 mL) at 0 °C was added DCC (5.21 g, 25.0 mmol) and HOBt (3.38 g, 25.0 mmol). The resultant reaction mixture was stirred at 0 °C for 2 h and filtered to remove the DCU byproduct. To the filtrate was added a solution of diethyl (*N*-methylamino)malonate (**6**)[8–10] (4.73 g, 25.0 mmol) in DMF (20 mL). The reaction mixture was allowed to warm to room temperature and stirred for a further 16 h. After this time, the reaction mixture was filtered and the solvent was removed in vacuo. The residue was taken up in ethyl acetate (100 mL) and washed successively with saturated sodium carbonate solution (50 mL) and water (50 mL). The organic phase was separated, dried over magnesium sulfate and the solvent was removed in vacuo to give the crude product. Purification via flash column chromatography (7:3, ethyl acetate–petroleum spirit, R_f: 0.58) afforded the title compound **7** as a thick, clear oil (8.06 g, 81%). ν_{max} film/cm^{-1} 2981 m, 2938 m, 1740 vs, 1709 vs, 1675 vs, 1478 m, 1456 m, 1399 m, 1368 m, 1308 s, 1233 s, 1180 s, 1154 s, 1113 m, 1034 m. δ_H (300 MHz, $CDCl_3$) 1.30 (m, 6H, 2× $CO_2CH_2CH_3$), 2.99 (s, 3H, NCH_3), 3.04, 3.09 (rotamers 2× s, 3H, NCH_3), 4.14–4.25 (m, 6H, 2×$CO_2CH_2CH_3$ and $CH_2C(O)NCH_3$), 5.11, 5.14 (rotamers 2×s, 2H, CH_2Ar), 5.92 (s, 1H, $CH(CO_2Et)_2$), 7.30–7.36 (m, 5H, C_6H_5). δ_C

(75 MHz, $CDCl_3$) 13.7 ($2\times CO_2CH_2CH_3$), 32.3 (NCH_3), 35.0, 35.7 (rotamers CBZ-NCH_3), 50.0, 50.2 (rotamers $CH_2C(O)NCH_3$), 59.9, 59.9 (rotamers $CH(CO_2Et)_2$), 61.8 ($2\times CO_2CH_2CH_3$), 66.9, 67.1 (rotamers CH_2Ar), 127.3, 127.4, 127.5, 127.6, 128.1, 128.1 (rotamers aromatic *C*H), 136.3 (2×quaternary aromatic *C*), 155.9, 156.5 (rotamers *C*(O)), 165.9 (*C*(O)), 168.9 (*C*(O)). *m/z* (EI) 394.1741 ($M^{+\cdot}$. $C_{19}H_{26}N_2O_7$ requires 394.1740), 349 ($[M-OEt]^+$, 5%), 259 ($[M-Cbz]^+$, 10%), 188 (15%), 116 (33%), 91 ($[C_7H_7]^+$, 100%).

4.2.2. 3-Ethoxycarbonyl-1,4-dimethylpiperazine-2,5-dione (2). A solution of ethyl *N*-[*N*-benzyloxycarbonylsarcosyl]-2-ethoxycarbonylsarcosinate (**7**) (4.14 g, 10.4 mmol) in degassed methanol (100 mL) was stirred in the presence of 10% palladium on carbon (450 mg) under an atmosphere of hydrogen for 16 h. The reaction mixture was filtered through celite and the solvent evaporated under reduced pressure. The oily residue was taken up in toluene (50 mL) and the resultant solution was heated at reflux for 48 h. After this time the solvent was removed in vacuo and the crude product was recrystallised from ethyl acetate/petroleum spirit affording 3-ethoxycarbonyl-1,4-dimethylpiperazine-2,5-dione (**2**) as colourless crystals (1.99 g, 89%). The product may also be purified by column chromatography (5:1, ethyl acetate–methanol, R_f: 0.48). Mp 66–68 °C. ν_{max} KBr/cm^{-1} 2980 m, 2941 m, 1738 vs, 1678 vs, 1479 s, 1404 s, 1323 m, 1229 s, 1196 s, 1045 s, 746 w, 648 w. δ_H (300 MHz, $CDCl_3$) 1.31 (t, 3H, $J=6$ Hz, $CO_2CH_2CH_3$), 2.94 (s, 3H, NCH_3), 2.98 (s, 3H, NCH_3), 3.84 (d, 1H, $J_{AB}=17$ Hz, ring CH_aH_b), 4.18 (d, 1H, $J_{AB}=17$ Hz, ring CH_aH_b), 4.28 (m, 2H, $CO_2CH_2CH_3$), 4.55 (s, 1H, $CHCO_2Et$). δ_C (75 MHz, $CDCl_3$) 13.9 ($CO_2CH_2CH_3$), 32.7 (NCH_3), 33.7 (NCH_3), 51.5 (ring CH_2), 62.9 ($CO_2CH_2CH_3$), 65.9 ($CHCO_2Et$), 160.0 (*C*(O)), 164.5 (*C*(O)), 166.3 (*C*(O)). *m/z* (EI) 214.0953 ($M^{+\cdot}$. $C_9H_{14}N_2O_4$ requires 214.0954), 171 (12%), 141 ($[M-CO_2Et]^+$, 67%), 113 (100%).

4.2.3. 3-Benzyloxycarbonyl-1,4-dimethylpiperazine-2,5-dione (3a). *Method A*. A stirred solution of 3-ethoxycarbonyl-1,4-dimethylpiperazine-2,5-dione (**2**) (203 mg, 0.95 mmol) in benzyl alcohol (2 mL) was heated at 180 °C for 16 h. The solvent was then removed by distillation and the residue purified by column chromatography (ethyl acetate, R_f: 0.45) to afford 3-benzyloxycarbonyl-1,4-dimethylpiperazine-2,5-dione (**3a**) as a colourless solid (207 mg, 79%).

Method B. To a stirred solution of 3-benzyloxycarbonyl-1-methylpiperazine-2,5-dione (**3c**) (978 mg, 3.7 mmol) in acetone (20 mL) was added anhydrous K_2CO_3 (1.031 g, 7.46 mmol) and dimethyl sulfate (1.4 mL, 14.9 mmol). The reaction mixture was heated at reflux under nitrogen for 48 h. After this time the inorganic salts were filtered off and the filtrate was concentrated under reduced pressure. Trituration with ether (10 mL) followed by filtration afforded 3-benzyloxycarbonyl-1,4-dimethylpiperazine-2,5-dione (**3a**) as a colourless solid (909 mg, 89%). If necessary the product may be purified by column chromatography (ethyl acetate, R_f: −0.45). Mp 110–112 °C. (Found: C 60.6; H, 6.0; N, 10.1. $C_{14}H_{16}N_2O_4$ requires C, 60.9; H, 5.8; N, 10.1%); ν_{max} KBr/cm^{-1} 3449 w, 3039 w, 2969 w, 1740 vs, 1700 vs, 1696 vs, 1405 s, 1278 s, 1189 s, 1035 m, 739 m. δ_H (300 MHz, $CDCl_3$) 2.91 (s, 3H, NCH_3), 2.97 (s, 3H, NCH_3), 3.82 (d, 1H, $J_{AB}=17$ Hz, ring CH_aH_b), 4.13 (d, 1H, $J_{AB}=17$ Hz, ring CH_aH_b), 4.60 (s, 1H, ring C*H*), 5.12 (d, 1H, $J_{AB}=12$ Hz, benzyl CH_aH_b), 5.29 (d, 1H, $J_{AB}=12$ Hz, benzyl CH_aH_b), 7.35 (m, 5H, aromatic C_6H_5). δ_C (75 MHz, $CDCl_3$) 32.7 (NCH_3), 33.6 (NCH_3), 51.5 (ring CH_2), 65.8 (ring *C*H), 68.2 (benzyl CH_2), 128.0 (2×aromatic *C*H), 128.5 (3×aromatic *C*H), 134.4 (quaternary aromatic *C*), 159.8 (*C*(O)), 164.4 (*C*(O)), 166.2 (*C*(O)). *m/z* (EI) 276.1105 ($M^{+\cdot}$. $C_{14}H_{16}N_2O_4$ requires 276.1110), 232 (17%), 185 ($[M-C_7H_7]^+$, 6%), 141 ($[M-CO_2Bn]^+$, 95%), 113 (80%), 91 ($[C_7H_7]^+$, 100%).

4.2.4. 3-Carboxyl-1,4-dimethylpiperazine-2,5-dione (1a). To a solution of 3-benzyloxycarbonyl-1,4-dimethylpiperazine-2,5-dione (**3a**) (920 mg, 3.33 mmol) in degassed methanol (20 mL) was added 10% palladium on carbon (80 mg). The reaction mixture was then stirred under a hydrogen atmosphere for 1 h after which time it was filtered through a bed of celite. The filtrate was reduced in vacuo and following co-evaporation with chloroform (3×5 mL), the acid (**1a**) was obtained as an unstable colourless solid (552 mg, ~90%) which was contaminated with a small amount of sarcosine anhydride. δ_H (300 MHz, CD_3OD) 2.97 (s, 3H, NCH_3), 3.00 (s, 3H, NCH_3), 3.97 (d, 1H, $J=18$ Hz, ring CH_aH_b), 4.20 (d, 1H, $J=18$ Hz, ring CH_aH_b), 4.73 (s, 1H, ring C*H*).

4.2.5. Benzyl *N*-[*N*-*t*-butoxycarbonylsarcosyl]-2-benzyloxycarbonylglycinate (9). To a solution of *N*-*t*-butoxycarbonylsarcosine (227 mg, 1.20 mmol) in DMF (10 mL) at 0 °C was added DCC (248 mg, 1.20 mmol) and HOBt (163 mg, 1.21 mmol). The resultant reaction mixture was stirred at 0 °C for 2 h and filtered to remove the DCU byproduct. To the filtrate was added a solution of dibenzyl aminomalonate (**8**) (360 mg, 1.20 mmol) in DMF (5 mL). The reaction mixture was warmed to room temperature and stirred for a further 16 h. After this time, the reaction mixture was filtered and the solvent was removed in vacuo to give a viscous, pale yellow oil. The residue was taken up in chloroform (20 mL) and washed with saturated sodium carbonate solution (10 mL) and water (10 mL). The organic phase was separated, dried over magnesium sulfate and the solvent was removed in vacuo to give the crude product as a clear oil. Purification via flash column chromatography (4:1, ethyl acetate–petroleum spirit, R_f: 0.55) afforded the title compound **9** as a clear and colourless oil which solidified upon prolonged standing (547 mg, 97%). Mp 82–84 °C. (Found: C, 63.9; H, 6.7; N, 5.7. $C_{25}H_{30}N_2O_7$ requires C, 63.8; H, 6.4; N, 5.95%); ν_{max} KBr/cm^{-1} 3239 s, 3054 w, 2976 m, 1765 vs, 1743 vs, 1708 vs, 1678 vs, 1550 s, 1456 s, 1396 s, 1329 s, 1217 s, 1143, 743 s, 698 s. δ_H (300 MHz, $CDCl_3$) 1.46 (s, 9H, $(CH_3)_3C$), 2.91 (s, 3H, NCH_3), 3.93 (bs, 2H, $NCH_2C(O)$), 5.15 (d, 2H, $J_{AB}=12$ Hz, $2\times CH_aH_bPh$), 5.21 (d, 2H, $J_{AB}=12$ Hz, $2\times CH_aH_bPh$), 5.29 (d, 1H, $J=7$ Hz, NHC*H*C(O)), 7.24–7.33 (m, 11H, N*H* and $2\times C_6H_5$). δ_C (75 MHz, $CDCl_3$) 28.0 ($3\times(CH_3)_3C$), 35.4 (NCH_3), 52–53 (rotamers $CH_2C(O)NH$), 56.0 ($CH(CO_2Bn)_2$), 68.0 ($2\times CH_2Ph$), 80.6 ($C(CH_3)_3$), 128.0 (4×aromatic *C*H), 128.4 (6×aromatic *C*H), 134.4 (2×quaternary aromatic *C*), 165.6 (3×*C*(O)), 169.2 (carbamate *C*(O)). *m/z* (EI) 470.2050 ($M^{+\cdot}$. $C_{25}H_{30}N_2O_7$ requires 470.2053), 414 ($MH-C(CH_3)_3]^+$,

16%), 371 ([M−Boc+2]$^+$, 17%), 279 ([M−Boc–C_7H_7]$^+$, 21%), 91 ([C_7H_7]$^+$, 100%).

4.2.6. 3-Benzyloxycarbonyl-1-methylpiperazine-2,5-dione (3c). To a solution of benzyl *N*-[*N*-*t*-butoxycarbonylsarcosyl]-2-benzyloxycarbonylglycinate (**9**) (8.24 g, 17.5 mmol) in chloroform (125 mL) at 0 °C was added TFA (10 mL). The resultant solution was stirred for 16 h at room temperature. After this time the solvent and excess TFA were removed in vacuo. The resulting residue was taken up in chloroform (100 mL) and washed with saturated sodium bicarbonate solution (50 mL). The separated organic phase was dried ($MgSO_4$) and concentrated in vacuo. The residue was then taken up in toluene (125 mL) and heated at reflux for 24 h. The product crystallised from solution upon cooling and was collected by filtration. The filtrate was evaporated and further product was obtained from the residue via chromatographic purification (5:1, ethyl acetate–methanol, R_f: 0.58). Using a combination of these purification methods, the title compound was obtained as a colourless crystalline solid (4.59 g, 82%). Mp 119–120 °C. (Found C, 59.6; H, 5.4; N, 10.6. $C_{13}H_{14}N_2O_4$ requires C, 59.5; H, 5.4; N, 10.7%). ν_{max} KBr/cm^{-1} 3338 s, 3069 w, 3016 w, 1742 vs, 1702 vs, 1670 vs, 1457 s, 1248 s, 1180 s, 1040 m, 945 m, 718 m, 700 s. δ_H (300 MHz, $CDCl_3$) 2.97 (s, 3H, NCH_3), 3.80 (d, 1H, J_{AB}=18 Hz, ring CH_aH$_b$), 4.12 (d, 1H, J_{AB}=18 Hz, ring CH$_a$$H_b$), 4.70 (d, 1H, J=4 Hz, NHCHC(O)), 5.19 (d, 1H, J_{AB}=12 Hz, benzyl CH_aH$_b$), 5.27 (d, 1H, J_{AB}=12 Hz, benzyl CH$_a$$H_b$), 6.80 (bs, 1H, N$H$), 7.35 (s, 5H, Aromatic C$_6$$H_5$). δ_C (75 MHz, $CDCl_3$) 34.1 (NCH_3), 51.4 (ring CH_2), 59.2 (NHCHC(O)), 68.3 (CH_2Ph), 128.2 (2×aromatic CH), 128.6 (3×aromatic CH), 134.6 (quaternary aromatic CH), 160.2 (C(O)), 166.6 (C(O)), 167.1 (C(O)). *m/z* (EI) 262.0953 (M$^+$. $C_{13}H_{14}N_2O_4$ requires 262.0954), 218 (21%), 171 ([M−C_7H_7]$^+$, 18%), 143 (19%), 127 ([M−CO_2Bn]$^+$, 23%), 99 (26%), 91 ($C_7H_7^+$, 100%).

4.2.7. 4-Acetyl-3-benzyloxycarbonyl-1-methylpiperazine-2,5-dione (3b). To a stirred solution of 3-benzyloxycarbonyl-1-methylpiperazine-2,5-dione (**3c**) (646 mg, 2.46 mmol) in dichloromethane (10 mL) was added DMAP (331 mg, 2.71 mmol) and acetic anhydride (0.26 mL, 2.76 mmol). The resultant solution was stirred under nitrogen until TLC analysis of the reaction mixture showed complete consumption of starting material (typically 2 h). After this time the solvent was removed in vacuo and the residue was purified via column chromatography (4:1, ethyl acetate–petroleum spirit, R_f: 0.49). The title compound was obtained as a colourless oil (682 mg, 91%) which solidified upon standing. Mp 100–101 °C. (Found C, 59.2; H, 5.4; N, 9.1. $C_{15}H_{16}N_2O_5$ requires C, 59.2; H, 5.3; N, 9.2%). ν_{max} KBr/cm^{-1} 3439 w, 2996 w, 1730 vs, 1711 vs, 1676 vs, 1383 s, 1232 s, 1189 s, 998 m, 761 m. δ_H (300 MHz, $CDCl_3$) 2.59 (s, 3H, NC(O)CH_3), 2.98 (s, 3H, NCH_3), 3.86 (d, 1H, J_{AB}=18 Hz, ring CH_aH$_b$), 4.23 (d, 1H, J_{AB}=18 Hz, ring CH$_a$$H_b$), 5.22 (d, 1H, J_{AB}=12 Hz, benzyl CH_aH$_b$), 5.29 (d, 1H, J_{AB}=12 Hz, benzyl CH$_a$$H_b$), 5.68 (s, 1H, C$HCO_2$Bn), 7.34 (m, 5H, aromatic C$_6$$H_5$). δ_C (75 MHz, $CDCl_3$) 26.6 (NC(O)CH_3), 33.8 (NCH_3), 53.2 (ring CH_2), 59.8 (CHCO$_2$Bn), 68.4 (benzyl CH_2), 127.8 (2×aromatic CH), 128.5 (aromatic CH), 128.6 (2×aromatic CH), 134.6 (quaternary aromatic C), 159.8 (C(O)), 165.7 (C(O)), 165.9 (C(O)), 171.3 (ester C(O)). *m/z* (EI) 304.1059 (M$^{+\cdot}$. $C_{15}H_{16}N_2O_5$ requires 304.1059), 262 ([MH−Ac]$^+$, 15%), 213 ([M−C_7H_7]$^+$, 25%), 170 ([MH−CO_2Bn]$^+$, 20%), 127 ([MH−CO_2Bn-Ac]$^+$, 42%), 91 ($C_7H_7^+$, 100%).

4.2.8. 4-Acetyl-3-carboxyl-1-methylpiperazine-2,5-dione (1b). To a solution of 4-acetyl-3-benzyloxycarbonyl-1-methylpiperazine-2,5-dione (**3b**) (241 mg, 0.79 mmol) in degassed methanol (20 mL) was added 10% palladium on carbon (20 mg). The reaction mixture was then stirred under a hydrogen atmosphere for 1 h after which time it was filtered through a bed of celite. The filtrate was reduced in vacuo and following co-evaporation with chloroform (3×5 mL), the acid (**1b**) (~112 mg, 66%) was obtained as an unstable colourless oil which also contained 1-acetyl-4-methylpiperazine-2,5-dione (~45 mg, 33%).

δ_H (300 MHz, $CDCl_3$) 2.54 (s, 3H, NC(O)CH_3), 2.97 (s, 3H, NCH_3), 3.89 (d, 1H, J_{AB}=18 Hz, ring CH_aH$_b$), 4.36 (d, 1H, J_{AB}=18 Hz, ring CH$_a$$H_b$), 5.58 (s, 1H, ring C$H$).

4.3. 3-Carboxyl-1-methylpiperazine-2,5-dione (1c)

To a solution of 3-benzyloxycarbonyl-1-methylpiperazine-2,5-dione (**3c**) (710 mg, 2.71 mmol) in degassed methanol (30 mL) was added 10% palladium on carbon (100 mg). The reaction mixture was then stirred under a hydrogen atmosphere for 1 h after which time it was filtered through a bed of celite. The filtrate was reduced in vacuo and following co-evaporation with chloroform (5×10 mL), the acid (**1c**) was obtained as a colourless gum (468 mg, 100%). δ_H (300 MHz, CD_3OD) 3.01 (s, 3H, NCH_3), 3.94 (d, 1H, J_{AB}=18 Hz, ring CH_aH$_b$), 4.21 (d, 1H, J_{AB}=18 Hz, ring CH$_a$$H_b$), 4.62 (s, 1H, C$HCO_2$H). δ_C (75 MHz, CD_3OD) 34.3 (NCH_3), 52.3 (CH_2), 60.2 (CHCO$_2$H), 163.1 (C(O)), 168.9 (C(O)), 170.0 (C(O)).

4.4. Syntheses of N- and/or C-functionalised piperazine-2,5-diones from alkoxycarbonyl piperazinedione precursors

4.4.1. 3-Benzyloxycarbonyl-1,3,4-trimethylpiperazine-2,5-dione (10a). *Method A*. To a solution of 3-benzyloxycarbonyl-1,4-dimethylpiperazine-2,5-dione (**3a**) (552 mg, 2.00 mmol) in DMF (10 mL) at 0 °C under nitrogen was added sodium hydride (160 mg, 4.00 mmol, 60% in paraffin) and methyl iodide (2 mL, excess). The resultant solution was stirred under a nitrogen atmosphere for 24 h and after this time the solvent was removed under reduced pressure. The residue was taken up in chloroform (20 mL) and washed with water (5 mL), brine (5 mL) and dried ($MgSO_4$). The solvent was removed in vacuo and the crude product was purified by column chromatography (ethyl acetate, R_f: 0.47) giving (**10a**) as a clear and colourless oil which solidified upon prolonged standing (533 mg, 92%).

Method B. The title compound was prepared, as described in *Method A*, from 3-benzyloxycarbonyl-1-methylpiperazine-2,5-dione (**3c**) (820 mg, 3.13 mmol) in DMF (10 mL), with sodium hydride (276 mg, 6.89 mmol, 60% in paraffin) and methyl iodide (2 mL, excess). The title compound was

obtained as a clear and colourless oil which solidified upon prolonged standing (754 mg, 83%).

Mp 86–88 °C. (Found C, 61.7; H, 6.3; N, 9.5. $C_{15}H_{18}N_2O_4$ requires C, 62.1; H, 6.3; N, 9.7%). ν_{max} KBr/cm^{-1} 3451 w, 3000 w, 2943 w, 1746 vs, 1664 vs, 1458 m, 1403 m, 1262 s, 1128 m, 769 m, 710 m. δ_H (300 MHz, $CDCl_3$) 1.79 (s, 3H, C–CH_3), 2.81 (s, 3H, NCH_3), 2.96 (s, 3H, NCH_3), 3.93 (d, 1H, J_{AB}=18 Hz, ring CH_aH$_b$), 4.00 (d, 1H, J_{AB}=18 Hz, ring CH$_a$$H_b$), 5.16 (d, 1H, J_{AB}=12 Hz, benzyl CH_aH$_b$), 5.24 (d, 1H, J_{AB}=12 Hz, benzyl CH$_a$$H_b$), 7.28–7.35 (m, 5H, aromatic C_6H_5). δ_C (75 MHz, $CDCl_3$) 20.1 (α-CH$_3$), 29.1 (NCH$_3$), 33.6 (NCH$_3$), 50.9 (ring CH$_2$), 67.6 (quaternary C–CH$_3$), 67.7 (benzyl CH$_2$), 127.7 (2×aromatic CH), 128.1 (aromatic CH), 128.2 (2×aromatic CH), 134.5 (quaternary aromatic C), 163.0 (C(O)), 163.6 (C(O)), 167.6 (C(O)). *m/z* (EI) 290.1264 ($M^{+\cdot}$. $C_{15}H_{18}N_2O_4$ requires 290.1267), 155 ([M−CO_2Bn]$^+$, 100%), 127 (82%), 91 ([C_7H_7]$^+$, 60%).

4.4.2. 4-Acetyl-3-benzyloxycarbonyl-1,3-dimethylpiperazine-2,5-dione (10b). To a stirred solution of 3-benzyloxycarbonyl-1,3-methylpiperazine-2,5-dione (**10c**) (140 mg, 0.51 mmol) in dichloromethane (10 mL) was added DMAP (63 mg, 0.52 mmol) and acetic anhydride (0.05 mL, 0.530 mmol). The resultant solution was stirred under nitrogen until TLC analysis of the reaction mixture showed complete consumption of starting material (typically 2 h). After this time the solvent was removed in vacuo and the residue was purified via column chromatography (4:1, ethyl acetate–petroleum spirit, R_f: 0.44). The title compound was obtained as a colourless oil (140 mg, 87%). δ_H (300 MHz, $CDCl_3$) 1.82 (s, 3H, C–CH_3), 2.47 (s, 3H, NC(O)CH_3), 3.00 (s, 3H, NCH_3), 4.05 (d, 1H, J_{AB}=18 Hz, ring CH_aH$_b$), 4.18 (d, 1H, J_{AB}=18 Hz, ring CH$_a$$H_b$), 5.12 (d, 1H, J_{AB}=12 Hz, OCH_aH$_b$Ph), 5.16 (d, 1H, J_{AB}=12 Hz, OCH$_a$$H_b$Ph), 7.33 (m, 5H, aromatic H). δ_C (75 MHz, $CDCl_3$) 21.4 (C–CH$_3$), 27.8 (NC(O)CH$_3$), 33.8 (NCH$_3$), 52.1 (ring CH$_2$), 67.1 (quaternary ring C), 67.9 (OCH$_2$Ph), 128.3 (2×aromatic CH), 128.3 (aromatic CH), 128.4 (2×aromatic CH), 135.1 (quaternary aromatic C), 164.3 (C(O)), 165.2 (C(O)), 166.9 (C(O)), 173.4 (C(O)). *m/z* (EI) 318.1207 ($M^{+\cdot}$. $C_{16}H_{18}N_2O_5$ requires 318.1216), 276 ([M−Ac+1]$^+$, 12%), 211 (25%), 141 ([M−Ac–CO_2Bn+1]$^+$, 91%), 91 ([C_7H_7]$^+$, 100%).

4.5. General procedure for the selective α-alkylation of piperazinedione 3c to 10c–10f

To a stirred solution of 3-benzyloxycarbonyl-1-methylpiperazine-2,5-dione (**3c**) (1 mol equiv, typical scales 0.5–2 mmol) in acetone (ca. 10 mL per mmol of **3c**) was added anhydrous K_2CO_3 (1 mol equiv). For the synthesis of **10c**, **10d** and **10f**, this was followed by the addition of the appropriate alkyl halide in excess (5 mol equiv) and the reaction mixture was heated under reflux in a sealed tube. For the synthesis of **10e**, 1 mol equiv of the appropriate alkyl halide was utilized and the reaction mixture was heated at reflux under nitrogen.

After 24 h of reflux, the reaction mixture was cooled and filtered to remove the inorganic salts. The filtrate was then concentrated under reduced pressure.

For **10c** and **10e**, the resulting solid was triturated with ether and the solid was further purified (if necessary) via recrystallisation from the mixed solvent of ethyl acetate/petroleum spirits. For **10d** and **10f**, the residue obtained was purified by column chromatography.

4.5.1. 3-Benzyloxycarbonyl-1,3-dimethylpiperazine-2,5-dione (10c). The title compound **10c** was synthesised from **3c** following the general procedure above. Compound **10c** is a colourless solid which was obtained in quantitative yield. Mp 142–143 °C. (Found C, 60.6; H, 5.9; N, 10.0. $C_{14}H_{16}N_2O_4$ requires C, 60.9; H, 5.8; N, 10.1%). ν_{max} KBr/cm^{-1} 3179 w, 3100 w, 2950 w, 1719 vs, 1675 vs, 1448 m, 1414 m, 1239 vs, 1120 m, 920 w, 761 m, 704 m. δ_H (300 MHz, $CDCl_3$) 1.73 (s, 3H, C–CH_3), 2.95 (s, 3H, NCH_3), 3.78 (d, 1H, J_{AB}=18 Hz, ring CH_aH$_b$), 4.02 (d, 1H, J_{AB}=18 Hz, ring CH$_a$$H_b$), 5.19 (m, 2H, benzyl C$H_2$), 6.97 (bs, 1H, N$H$), 7.32 (m, 5H, aromatic C_6H_5). δ_C (75 MHz, $CDCl_3$) 21.3 (C–CH$_3$), 34.3 (NCH$_3$), 52.1 (ring CH$_2$), 62.6 (quaternary ring C), 68.0 (benzyl CH$_2$), 127.7 (2×aromatic CH), 128.4 (aromatic CH), 128.5 (2×aromatic CH), 134.7 (quaternary aromatic C), 163.7 (C(O)), 167.1 (C(O)), 169.0 (C(O)). *m/z* (EI) 276.1109 ($M^{+\cdot}$. $C_{14}H_{16}N_2O_4$ requires 276.1110), 232 (3%), 185 ([M−C_7H_7]$^+$, 5%), 141 ([M−CO_2Bn]$^+$, 100%), 113 (58%), 91 ([C_7H_7]$^+$, 87%).

4.5.2. 3-Allyl-3-benzyloxycarbonyl-1-methylpiperazine-2,5-dione (10d). The title compound **10d** was synthesized from **3c** following the general procedure outlined. Compound **10d** is a colourless oil which was obtained in 82% yield after column chromatography (ethyl acetate, R_f: −0.55). ν_{max} film/cm^{-1} 3237 m, 3089 w, 2932 w, 1746 s, 1696 vs, 1667 vs, 1499 w, 1455 m, 1435 m, 1406 m, 1219 s, 751 m, 698 m. δ_H (300 MHz, $CDCl_3$) 2.78 (m, 1H, C–CH_aH$_b$CH=CH$_2$), 2.95 (s, 3H, NCH_3), 3.06 (m, 1H, C–CH$_a$$H_b$CH=CH$_2$), 3.79 (d, 1H, J_{AB}=18 Hz, ring CH_aH$_b$), 4.01 (d, 1H, J_{AB}=18 Hz, ring CH$_a$$H_b$), 5.23 (m, 4H, OC$H_2$Ph and C–CH$_2$CH=C$H_2$), 5.60 (m, 1H, C–CH$_2CH$=CH$_2$), 6.32 (bs, 1H, N$H$), 7.34 (m, 5H, aromatic C_6H_5). δ_C (75 MHz, $CDCl_3$) 34.3 (NCH$_3$), 38.8 (C–CH$_2$CH=CH$_2$), 51.9 (ring CH$_2$), 65.2 (quaternary ring C), 68.2 (OCH$_2$Ph), 121.7 (C–CH$_2$CH=CH$_2$), 127.8 (2×aromatic CH), 128.4 (aromatic CH), 128.5 (2×aromatic CH), 130.3 (C–CH$_2$$C$H=CH$_2$), 134.7 (quaternary aromatic C), 162.3 (C(O)), 166.5 (C(O)), 168.1 (C(O)). *m/z* (EI) 302.1266 ($M^{+\cdot}$. $C_{16}H_{18}N_2O_4$ requires 302.1267), 167 ([M−CO_2Bn]$^+$, 100%), 139 (35%), 91 ([C_7H_7]$^+$, 95%).

4.5.3. 3-Benzyl-3-benzyloxycarbonyl-1-methylpiperazine-2,5-dione (10e). The title compound **10e** was synthesized from **3c** following the general procedure outlined. Compound **10e** is a colourless solid which was obtained in quantitative yield. Mp 153–154 °C. (Found C, 68.1; H, 5.9; N, 7.8. $C_{20}H_{20}N_2O_4$ requires C, 68.2; H, 5.7; N, 7.95%). ν_{max} KBr/cm^{-1} 3209 w, 1751 vs, 1696 s, 1661 vs, 1457 m, 1224 s, 735 m, 701 m. δ_H (300 MHz, $CDCl_3$) 2.85 (s, 3H, NCH_3), 3.17 (d, 1H, J_{AB}=18 Hz, ring CH_aH$_b$), 3.44 (d, 1H, J_{AB}=14 Hz, C–CH_aH$_b$Ph), 3.50 (d, 1H, J_{AB}=14 Hz, C–CH$_a$$H_b$Ph), 3.71 (d, 1H, J_{AB}=18 Hz, ring CH$_a$$H_b$), 5.23 (d, 1H, J_{AB}=12 Hz, OCH_aH$_b$Ph), 5.30 (d, 1H, J_{AB}=12 Hz, OCH$_a$$H_b$Ph), 6.32 (bs, 1H, N$H$), 7.10 (m, 2H, aromatic H), 7.25 (m, 3H, aromatic H), 7.36 (s, 5H, aromatic H). δ (75 MHz, $CDCl_3$) 34.1 (NCH$_3$), 41.6

(C–CH_2Ph), 51.3 (ring CH_2), 66.9 (quaternary ring *C*), 68.4 (OCH_2Ph), 127.8 (aromatic *C*H), 128.2 (2×aromatic *C*H), 128.6 (2×aromatic *C*H), 128.7 (3×aromatic *C*H), 130.5 (2×aromatic *C*H), 133.5 (quaternary aromatic *C*), 134.8 (quaternary aromatic *C*), 162.4 (*C*(O)), 165.8 (*C*(O)), 168.0 (*C*(O)). *m/z* (EI) 352.1423 ($M^{+\cdot}$. $C_{20}H_{20}N_2O_4$ requires 352.1423), 217 ($[M-CO_2Bn]^+$, 86%), 189 (27%), 132 (18%), 91 ($[C_7H_7]^+$, 100%).

4.5.4. 3-Benzyloxycarbonyl-3-ethyl-1-methylpiperazine-2,5-dione (10f). The title compound **10f** was synthesized from **3c** following the general procedure outlined. Compound **10f** is a colourless solid which was obtained in 90% yield after purification by flash column chromatography (ethyl acetate, R_f: 0.53). Mp 122–123 °C. ν_{max} KBr/cm^{-1} 3209 m, 3101 m, 2978 w, 1730 vs, 1686 vs, 1670 vs, 1415 s, 1217 s, 1188 s, 1179 s, 917 m, 757 m, 748 m, 699 m. δ_H (300 MHz, $CDCl_3$) 0.91 (t, 3H, $J=7$ Hz, C–CH_2CH_3), 1.96 (m, 1H, C–$CH_aH_bCH_3$), 2.42 (m, 1H, C–$CH_aH_bCH_3$), 2.95 (s, 3H, NCH_3), 3.81 (d, 1H, $J_{AB}=18$ Hz, ring CH_aH_b), 3.99 (d, 1H, $J_{AB}=18$ Hz, ring CH_aH_b), 5.16 (d, 1H, $J_{AB}=12$ Hz, benzyl CH_aH_b), 5.22 (d, 1H, $J_{AB}=12$ Hz, benzyl CH_aH_b), 7.03 (bs, 1H, N*H*), 7.33 (m, 5H, aromatic C_6H_5). δ_C (75 MHz, $CDCl_3$) 7.8 (α-CCH_2CH_3), 27.7 (α-CCH_2CH_3), 34.3 (NCH_3), 52.0 (ring CH_2), 66.8 (quaternary ring *C*), 68.1 (benzyl CH_2), 127.9 (2×aromatic *C*H), 128.5 (aromatic *C*H), 128.6 (2×aromatic *C*H), 134.8 (quaternary aromatic *C*), 162.7 (*C*(O)), 167.1 (*C*(O)), 168.8 (*C*(O)). *m/z* (EI) 290.1270 ($M^{+\cdot}$. $C_{15}H_{18}N_2O_4$ requires 290.1267), 155 ($[M-CO_2Bn]^+$, 100%), 127 ($[MH-Et\text{-}CO_2Bn]^+$, 44%), 91 ($[C_7H_7]^+$, 70%).

4.5.5. 3-Benzyl-3-benzyloxycarbonyl-1,4-dimethylpiperazine-2,5-dione (11). To a stirred solution of 3-benzyl-3-benzyloxycarbonyl-1-methylpiperazine-2,5-dione (**10e**) (449 mg, 1.27 mmol) in DMF (15 mL) at 0 °C under nitrogen was added sodium hydride (51 mg, 1.275 mmol, 60% in paraffin) and methyl iodide (0.8 mL, 12.74 mmol). The reaction mixture was stirred under a nitrogen atmosphere for 24 h and after this time the solvent was removed under reduced pressure. The residue was taken up in chloroform (25 mL) and washed with water (10 mL), brine (10 mL) and dried ($MgSO_4$). The solvent was removed in vacuo and the crude product was purified by column chromatography (3:2, ethyl acetate–petroleum spirit, R_f: 0.49) giving **11** as a clear and colourless oil which solidified upon standing (398 mg, 85%). Mp 96–97 °C. (Found C, 68.5; H, 6.2; N, 7.5. $C_{21}H_{22}N_2O_4$ requires C, 68.8; H, 6.1; N, 7.7%). ν_{max} KBr/cm^{-1} 3037 w, 2945 w, 1755 s, 1672 s, 1399 m, 1224 m, 1043 m, 745 m. δ_H (300 MHz, $CDCl_3$) 2.27 (d, 1H, $J_{AB}=17$ Hz, ring CH_aH_b), 2.70 (s, 3H, NCH_3), 2.82 (s, 3H, NCH_3), 3.24 (d, 1H, $J_{AB}=14$ Hz, C–CH_aH_bPh), 3.40 (d, 1H, $J_{AB}=17$ Hz, ring CH_aH_b), 3.62 (d, 1H, $J_{AB}=14$ Hz, C–CH_aH_bPh), 5.15 (d, 1H, $J_{AB}=12$ Hz, OCH_aH_bPh), 5.39 (d, 1H, $J_{AB}=12$ Hz, OCH_aH_bPh), 7.03 (m, 2H, aromatic *H*), 7.27 (m, 4H, aromatic *H*), 7.36 (m, 4H, aromatic *H*). δ_C (75 MHz, $CDCl_3$) 30.0 (NCH_3), 33.2 (NCH_3), 38.9 (C–CH_2Ph), 50.2 (ring CH_2), 68.3 (OCH_2Ph), 72.9 (quaternary ring *C*), 127.9 (aromatic *C*H), 128.5 (2×aromatic *C*H), 128.6 (2×aromatic *C*H), 128.7 (3×aromatic *C*H), 129.9 (2×aromatic *C*H), 133.7 (quaternary aromatic *C*), 134.7 (quaternary aromatic *C*), 163.0 (*C*(O)), 163.4 (*C*(O)), 167.2 (*C*(O)). *m/z* (EI) 366.1577 ($M^{+\cdot}$. $C_{21}H_{22}N_2O_4$ requires 366.1580), 275 ($[M-C_7H_7]^+$, 33%), 231 ($[M-CO_2Bn]^+$, 93%), 203 (26%), 132 (35%), 91 ($[C_7H_7]^+$, 100%).

4.5.6. 4-Benzyl-3-benzyloxycarbonyl-1,3-dimethylpiperazine-2,5-dione (12). To a stirred solution of 3-benzyloxycarbonyl-1,3-dimethylpiperazine 2,5-dione (**10c**) (174 mg, 0.63 mmol) in DMF (10 mL) at 0 °C under nitrogen was added sodium hydride (27 mg, 0.68 mmol, 60% in paraffin) and benzyl bromide (0.1 mL, 0.84 mmol). The reaction mixture was stirred under a nitrogen atmosphere for 24 h and after this time the solvent was removed under reduced pressure. The residue was taken up in chloroform (15 mL) and washed with water (5 mL), brine (5 mL) and dried ($MgSO_4$). The solvent was removed in vacuo and the crude product was purified by column chromatography (3:2, ethyl acetate–petroleum spirit, R_f: 0.45) giving **12** as a clear and colourless oil (155 mg, 67%). δ_H (300 MHz, $CDCl_3$) 1.76 (s, 3H, CCH_3), 2.98 (s, 3H, NCH_3), 4.01 (d, 1H, $J_{AB}=18$ Hz, ring CH_aH_b), 4.12 (d, 1H, $J_{AB}=18$ Hz, ring CH_aH_b), 4.47 (d, 1H, $J_{AB}=16$ Hz, NCH_aH_bPh), 4.74 (d, 1H, $J_{AB}=16$ Hz, NCH_aH_bPh), 4.82 (d, 1H, $J_{AB}=12$ Hz, OCH_aH_bPh), 5.02 (d, 1H, $J_{AB}=12$ Hz, OCH_aH_bPh), 7.18–7.37 (m, 10H, Aromatic *H*). *m/z* (EI) 366.1581 ($M^{+\cdot}$. $C_{21}H_{22}N_2O_4$ requires 366.1580), 304 (15%), 231 ($[M-CO_2Bn]^+$, 52%), 91 ($[C_7H_7]^+$, 100%).

4.6. General procedure for the α,α-dialkylation of 3-benzyloxycarbonyl-1-methylpiperazine-2,5-dione (3c): conversion of 3c to piperazinediones 13, 17, 18.

To a solution of 3-benzyloxycarbonyl-1-methylpiperazine-2,5-dione (**3c**) (1 mol equiv, typical scale ca. 1 mmol) in DMF (15 mL/mmol) at 0 °C under nitrogen was added sodium hydride (2 mol equiv, 60% in paraffin) and the appropriate alkyl halide. For the synthesis of piperazinedione **13**, excess allyl iodide was added (5 mol equiv) while for the synthesis of piperazinediones **17** and **18**, 1 mol equiv of the appropriate dibromoalkane was utilized. The resultant solution was stirred under a nitrogen atmosphere for 24 h and after this time the solvent was removed under reduced pressure. The residue was taken up in chloroform and washed with water, brine and dried ($MgSO_4$). The solvent was removed in vacuo and the crude product was purified by column chromatography.

4.6.1. 3,4-Diallyl-3-benzyloxycarbonyl-1-methylpiperazine-2,5-dione (13). The title compound **13** was obtained as a clear and colourless oil in 66% yield after purification by column chromatography (4:1, ethyl acetate–petroleum spirit, R_f: 0.51). ν_{max} film/cm^{-1} 3089 w, 2934 w, 1751 vs, 1667 vs, 1455 s, 1407 s, 1340 m, 1275 s, 1224 s, 929 m, 755 m, 699 m. δ_H (300 MHz, $CDCl_3$) 2.88 (dd, 1H, $^2J=15$ Hz, $^3J=7$ Hz, C–$CH_aH_bCH{=}CH_2$), 2.95 (s, 3H, NCH_3), 3.21 (dd, 1H, $^2J=15$ Hz, $^3J=7$ Hz, C–$CH_aH_bCH{=}CH_2$), 3.74 (dd, 1H, $^2J=15$ Hz, $^3J=7$ Hz, $NCH_aH_bCH{=}CH_2$), 3.97–4.05 (m, 3H, ring CH_2 & $NCH_aH_bCH{=}CH_2$), 5.05–5.25 (m, 6H, benzyl CH_2, C–$CH_2CH{=}CH_2$ & $NCH_2CH{=}CH_2$), 5.55 (m, 1H, C–$CH_2CH{=}CH_2$), 5.76 (m, 1H, $NCH_2CH{=}CH_2$), 7.33 (m, 5H, Aromatic C_6H_5). δ_C (75 MHz, $CDCl_3$) 33.4 (NCH_3), 37.0 (C–$CH_2CH{=}CH_2$), 46.5 ($NCH_2CH{=}CH_2$), 50.9 (ring CH_2), 67.7 (quaternary ring *C*), 71.0 (OCH_2Ph), 118.1 (allyl $CH_2CH{=}CH_2$), 121.0 (allyl $CH_2CH{=}CH_2$),

127.9 (2×aromatic *C*H), 128.3 (3×aromatic *C*H), 129.7 (allyl $CH_2CH{=}CH_2$), 131.9 (allyl $CH_2CH{=}CH_2$), 134.4 (quaternary aromatic *C*), 162.5 (*C*(O)), 163.3 (*C*(O)), 167.3 (*C*(O)). *m/z* (EI) 342.1583 ($M^{+\cdot}$. $C_{19}H_{22}N_2O_4$ requires 342.1580), 292 (28%), 251 ($[M-C_7H_7]^+$, 40%), 207 ($[M-CO_2Bn]^+$, 100%), 91 ($[C_7H_7]^+$, 42%).

4.6.2. 9a-Benzyloxycarbonyl-2-methyl-1,4-dioxo-octahydropyrido[1,2-*a*]pyrazine (17). The title compound **17** was obtained as a solid in 67% yield after purification by column chromatography (ethyl acetate, R_f: 0.51), followed by trituration with ether. Mp 107–109 °C. (Found C, 64.5; H, 6.5; N, 8.7. $C_{17}H_{20}N_2O_4$ requires C, 64.5; H, 6.4; N, 8.9%). ν_{max} KBr/cm^{-1} 2949 w, 2856 w, 1751 s, 1665 s, 1427 m, 1209 m, 1122 m, 761 m, 706 w. δ_H (300 MHz, $CDCl_3$) 1.30–1.55 (m, 2H, 7-CH_aH$_b$ and 8-CH_aH$_b$), 1.60–1.70 (m, 2H, 9-CH_aH$_b$ and 7-CH$_a$$H_b$), 1.79–1.83 (m, 1H, 8-CH$_a$$H_b$), 2.35–2.46 (m, 1H, 6-C$H_aH_b$), 2.91–2.97 (m, 1H, 9-CH$_a$$H_b$), 2.92 (s, 3H, NC$H_3$), 3.84 (d, 1H, J_{AB}=17 Hz, 3-CH_aH$_b$), 3.98 (d, 1H, J_{AB}=17 Hz, 3-CH$_a$$H_b$), 4.48 (dm, 1H, ^2J=12 Hz, 6-CH$_a$$H_b$), 5.16 (d, 1H, J_{AB}=12 Hz, OCH_aH$_b$Ph), 5.27 (d, 1H, J_{AB}=12 Hz, OCH$_a$$H_b$Ph), 7.26–7.39 (m, 5H, 5×aromatic C*H*). δ_C (75 MHz, $CDCl_3$) 20.4 (8-*C*H$_2$), 22.8 (7-*C*H$_2$), 31.0 (9-*C*H$_2$), 33.5 (N*C*H$_3$), 40.2 (6-*C*H$_2$), 50.8 (3-*C*H$_2$), 67.1 (9a-*C*), 67.7 (O*C*H$_2$Ph), 127.5 (2×aromatic *C*H), 128.1 (aromatic *C*H), 128.2 (2×aromatic *C*H), 134.5 (quaternary aromatic *C*), 162.1 (*C*(O)), 164.7 (*C*(O)), 167.2 (*C*(O)). *m/z* (EI) 316.1425 ($M^{+\cdot}$. $C_{17}H_{20}N_2O_4$ requires 316.1423), 181 ($[M-CO_2Bn]^+$, 100%), 153 (43%), 91 ($[C_7H_7]^+$, 23%).

4.6.3. 8a-Benzyloxycarbonyl-2-methyl-1,4-dioxo-hexahydropyrrolo[1,2-*a*]pyrazine (18). The title compound **18** was obtained as a colourless oil in 69% yield after purification by column chromatography (ethyl acetate). ν_{max} film/cm^{-1} 2955 w, 1740 s, 1682 vs, 1445 m, 1213 m, 700 w. δ_H (300 MHz, $CDCl_3$) 1.70–2.00 (m, 2H, 7-CH_2), 2.25–2.37 (m, 1H, 8-CH_aH$_b$), 2.67–2.75 (m, 1H, 8-CH$_a$$H_b$), 2.92 (s, 3H, NC$H_3$), 3.50–3.70 (m, 2H, 6-C$H_2$), 3.69 (d, 1H, J_{AB}=17 Hz, 3-CH_aH$_b$), 4.07 (d, 1H, J_{AB}=17 Hz, 3-CH$_a$$H_b$), 5.16 (d, 1H, J_{AB}=12 Hz, OCH_aH$_b$Ph), 5.23 (d, 1H, J_{AB}=12 Hz, OCH$_a$$H_b$Ph), 7.26–7.33 (m, 5H, 5×aromatic C*H*). δ_C (75 MHz, $CDCl_3$) 20.8 (7-*C*H$_2$), 32.7 (8-*C*H$_2$), 33.6 (N*C*H$_3$), 44.9 (6-*C*H$_2$), 53.1 (3-*C*H$_2$), 67.7 (O*C*H$_2$Ph), 70.7 (8a-*C*), 127.4 (2×aromatic *C*H), 128.1 (1×aromatic *C*H), 128.2 (2×aromatic *C*H), 134.4 (quaternary aromatic *C*), 163.1 (*C*(O)), 163.4 (*C*(O)). *m/z* (EI) 302.1270 ($M^{+\cdot}$. $C_{16}H_{18}N_2O_4$ requires 302.1267), 226 (20%), 167 ($[M-CO_2Bn]^+$, 100%), 139 (72%), 108 (65%), 91 ($[C_7H_7]^+$, 43%), 79 (65%).

4.6.4. 9a-Benzyloxycarbonyl-2-methyl-1,4-dioxo-1,2,3,4,6,9-hexahydropyrido[1,2-*a*]pyrazine (14). To a solution of 3,4-diallyl-3-benzyloxycarbonyl-1-methylpiperazine-2,5-dione (**13**) (81 mg, 0.24 mmol) in dichloromethane (3 mL) was added benzylidene-bis(tricyclohexylphosphine)-dichlororuthenium (8 mg, ~10% w/w). The reaction mixture was stirred under nitrogen for 16 h following which the solvent was removed in vacuo. The residue was purified via column chromatography (4:1, ethyl acetate–petroleum spirit) to give the title compound **14** (61 mg, 82%) as a clear and colourless oil. ν_{max} cm^{-1} 3034 w, 2938 w, 1754 vs, 1732 s, 1673 vs, 1499 m, 1428 s, 1330 m, 1247 s, 1195 s, 754 m, 699 m. δ_H (300 MHz, $CDCl_3$) 2.48 (dm, 1H, ^2J=18 Hz, 9-CH_aH$_b$), 2.96 (s, 3H, NCH_3), 3.40 (dd, 1H, ^2J=18 Hz, ^3J=6 Hz, 9-CH$_a$$H_b$), 3.56 (dm, 1H, ^2J=19 Hz, 6-CH_aH$_b$), 3.92 (d, 1H, J_{AB}=18 Hz, 3-CH_aH$_b$), 4.05 (d, 1H, J_{AB}=18 Hz, 3-CH$_a$$H_b$), 4.37 (dm, 1H, ^2J=19 Hz, ^3J=4 Hz, 6-CH$_a$$H_b$), 5.15 (d, 1H, J_{AB}=12 Hz, OCH_aH$_b$Ph), 5.22 (d, 1H, J_{AB}=12 Hz, OCH$_a$$H_b$Ph), 5.62–5.58 (m, 1H, 7 or 8-C*H*), 5.81–5.88 (m, 1H, 7 or 8-C*H*), 7.25–7.35 (m, 5H, C_6H_5). δ_C (75 MHz, $CDCl_3$) 31.4 (9-*C*H$_2$), 33.4 (N*C*H$_3$), 41.9 (6-*C*H$_2$), 51.1 (3-*C*H$_2$), 65.9 (9a-*C*), 68.3 (O*C*H$_2$Ph), 121.9 (7 or 8-*C*H), 122.6 (7 or 8-*C*H), 127.8 (2×aromatic *C*H), 128.4 (aromatic *C*H), 128.5 (2×aromatic *C*H), 134.7 (quaternary aromatic *C*); 162.1 (*C*(O)), 164.5 (*C*(O)), 167.4 (*C*(O)). *m/z* (EI) 315.1351 ($[MH]^+$), $C_{17}H_{19}N_2O_4$ ($[MH]^+$) requires 315.1345), 223 ($[M-Bn]^+$, 19%), 195 (18%), 179 ($[M-CO_2Bn]^+$, 100%), 151 (41%), 91 ($[C_7H_7]^+$, 59%).

4.6.5. 2-Methyl-hexahydropyrido[1,2-*a*]pyrazine-1,4-dione (16). To a solution of 9a-benzyloxycarbonyl-2-methyl-1,4-dioxo-1,2,3,4,6,9-hexahydropyrido[1,2-*a*]pyrazine (**14**) (43 mg, 0.14 mmol) or 9a-benzyloxycarbonyl-2-methyl-1,4-dioxo-octahydropyrido [1,2-a]pyrazine (**17**) in degassed methanol (10 mL) was added 10% palladium on carbon (5 mg). The reaction mixture was then stirred under a hydrogen atmosphere for 1 h after which time it was filtered through a bed of celite. The filtrate was concentrated in vacuo and suspended in toluene (25 mL). The reaction mixture was then heated at reflux for 24 h, following which the solvent was removed in vacuo to give the title compound **16** (25 mg, 100%) as a colourless oil which solidified upon standing. The physical data for **16** is consistent with that previously reported, although the NMR data has not been reported previously.[14] Mp 85–86 (lit.: 84–86 °C).[14] δ_H (300 MHz, $CDCl_3$) 1.35–1.80 (m, 4H, 7-CH_2, 8-CH_2), 1.97 (m, 1H, 9-CH_aH$_b$), 2.34 (m, 1H, 9-CH$_a$$H_b$), 2.49 (dt, 1H, 6-C$H_aH_b$), 2.94 (s, 3H, NC$H_3$), 3.80 (dm, 1H, 9a-C*H*), 3.91 (d, 1H, J_{AB}=18 Hz, 3-CH_aH$_b$), 3.99 (d, 1H, J_{AB}=18 Hz, 3-CH$_a$$H_b$), 4.64 (dm, 1H, J=13 Hz, 6-CH$_a$$H_b$). δ_C (75 MHz, $CDCl_3$) 24.3 (8-*C*H$_2$), 24.5 (7-*C*H$_2$), 31.3 (9-*C*H$_2$), 33.3 (N*C*H$_3$), 42.4 (6-*C*H), 51.2 (3-*C*H$_2$), 59.1 (9a-*C*H), 161.3 (*C*(O), 165.4 (*C*(O). *m/z* (EI) 182.1056 ($M^{+\cdot}$. $C_9H_{14}N_2O_2$ requires 182.1055), 153 (40%), 125 (38%), 97 (70%), 151 (41%), 83 (100%).

4.6.6. 2-Methyl-hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (19). To a stirred solution of 8a-benzyloxycarbonyl-2-methyl-1,4-dioxo-hexahydropyrrolo[1,2-*a*]pyrazine (**18**) (197 mg, 1.17 mmol) in degassed methanol (10 mL) was added 10% palladium on carbon (5 mg). The reaction mixture was then stirred under a hydrogen atmosphere for 1 h after which time it was filtered through a bed of celite. The filtrate was reduced in vacuo and suspended in toluene (25 mL). The reaction mixture was then heated at reflux for 24 h. After this time the solvent was removed in vacuo giving the title compound **19** (197 mg, 100%) as a colourless oil. The NMR data has not been reported previously.[14] δ_H (300 MHz, $CDCl_3$) 1.81–2.05 (m, 3H, 7-CH_2 and 8-CH_aH$_b$), 2.31–2.43 (m, 1H, 8-CH$_a$$H_b$), 2.97(s, 3H, NC$H_3$), 3.51–3.63 (m, 2H, 6-C$H_2$), 3.78 (d, 1H, J_{AB}=17 Hz, 3-CH_aH$_b$), 4.06 (bt, 1H, J=7 Hz, 8a-C*H*), 4.16 (d, 1H, J_{AB}=17 Hz, 3-CH$_a$$H_b$). δ_C (75 MHz, $CDCl_3$) 19.6 (7-*C*H$_2$), 28.4 (8-*C*H$_2$), 33.0 (N*C*H$_3$), 44.8 (6-*C*H$_2$), 53.0

(3-*C*H_2), 58.4 (8a-*C*), 162.2 (*C*(O)), 166.8 (*C*(O)). *m/z* (EI) 168.0897 ($M^{+\cdot}$. $C_8H_{12}N_2O_2$ requires 168.0899), 83 (71%), 42 (100%).

4.7. DONA reactions of carboxypiperazine-2,5-diones

General procedure for the DONA reactions of carboxypiperazine-2,5-diones. To a stirred solution of the appropriate carboxy piperazine-2,5-dione (1 mol equiv) in dichloromethane (ca. 7.5 mL/mmol of carboxypiperazinedione) was added iodine (0.5 mol equiv) and diacetoxyiodobenzene (1 mol equiv). In reactions where external nucleophiles were added, the reaction mixture was stirred for 1 min (for **22a** and **22b**) or 10 min (for **22d**, **24a**, **25**) before the addition of the appropriate additive. For the acetoxy compounds, excess glacial acetic acid (typically 0.4 mL/mmol of carboxypiperazinedione) is added while for methoxy derivatives, excess methanol (typically 2.2 mL/mmol of carboxypiperazinedione) is added.

Stirring under a nitrogen atmosphere was continued for 16 h following which time the reaction mixture was washed with a saturated solution of sodium thiosulfate, dried ($MgSO_4$) and the solvent removed under reduced pressure.

4.7.1. 3-Acetoxy-1,4-dimethylpiperazine-2,5-dione (22a). The title compound **22a** was synthesized from 3-carboxy-1,4-dimethylpiperazine-2,5-dione (**1a**) with acetic acid as additive, using the general procedure outlined above for the DONA reactions. 3-Acetoxy-1,4-dimethylpiperazine-2,5-dione (**22a**) was purified by column chromatography (5:1, ethyl acetate–methanol, R_f: −0.42) and was isolated as a colourless oil which crystallised upon standing (83%). The title compound has also been prepared from (*S*)-hydroxymethyl-1,4-dimethylpiperazine-2,5-dione (**35a**) using the procedure described above (86% yield). Mp 92–94 °C. ν_{max} KBr/cm^{-1} 3429 w, 2981 w, 2935 w, 1757 vs, 1692 vs, 1487 m, 1399 s, 1331 m, 1205 s, 1011 s, 946 s, 750 w. δ_H (300 MHz, $CDCl_3$) 2.11 (s, 3H, OC(O)CH_3), 2.99 (s, 3H, NCH_3), 3.01 (s, 3H, NCH_3), 3.89 (d, 1H, J_{AB}=18 Hz, ring C$H_a$$H_b$), 4.21 (d, 1H, J_{AB}=18 Hz, ring C$H_a$$H_b$), 6.03 (s, 1H, C*H*OAc). δ_C (75 MHz, $CDCl_3$) 20.2 (OC(O)*C*H_3), 31.7 (N*C*H_3), 33.0 (N*C*H_3), 51.0 (ring *C*H_2), 79.4 (*C*HOAc), 160.5 (*C*(O)), 165.1 (*C*(O)), 169.4 (*C*(O)). *m/z* (EI) 201.0874 ($[MH]^+$. $C_8H_{13}N_2O_4$ requires 201.0875), 157 (36%), 142 ($[MH-OAc]^+$, 100%), 113 (65%).

4.7.2. 3-Methoxy-1,4-dimethylpiperazine-2,5-dione (22b). The title compound **22b** was synthesized from 3-carboxy-1,4-dimethylpiperazine-2,5-dione (**1a**) with methanol as additive, using the general procedure outlined above for the DONA reactions. Purification by column chromatography (5:1, ethyl acetate–methanol, R_f: 0.33) gave 3-methoxy-1,4-dimethylpiperazine-2,5-dione (**22b**) as a colourless oil (76%) with physical and spectral properties consistent with data reported previously.[15] δ_H (300 MHz, $CDCl_3$) 2.94 (s, 3H, NCH_3), 3.00 (s, 3H, NCH_3), 3.44 (s, 3H, OCH_3), 3.79 (d, 1H, J_{AB}=18 Hz, ring C$H_a$$H_b$), 4.12 (d, 1H, J_{AB}=18 Hz, ring C$H_a$$H_b$), 4.67 (s, 1H, C*H*OMe).

4.7.3. 3-Acetoxy-4-Acetyl-1-methylpiperazine-2,5-dione (22c). The title compound **22c** was synthesised from 4-acetyl-3-carboxyl-1-methylpiperazine-2,5-dione (**1b**) using the general procedure outlined above for the DONA reactions. No external nucleophile was added. The residue obtained was purified by column chromatography (ethyl acetate, R_f: 0.45) to afford 3-acetoxy-4-acetyl-1-methylpiperazine-2,5-dione (**22c**) as a colourless solid (98 mg, 82%). Mp 156–157 °C. δ_H (300 MHz, $CDCl_3$) 2.08 (s, 3H, OC(O)CH_3), 2.54 (s, 3H, NC(O)CH_3), 2.99 (s, 3H, NCH_3), 4.00 (d, 1H, J_{AB}=18 Hz, ring C$H_a$$H_b$), 4.42 (d, 1H, J_{AB}=18 Hz, ring C$H_a$$H_b$), 6.89 (s, 1H, C*H*OAc). δ_C (75 MHz, $CDCl_3$) 20.6 (OC(O)*C*H_3), 26.7 (NC(O)*C*H_3), 33.3 (N*C*H_3), 53.1 (ring *C*H_2), 73.6 (*C*HOAc), 161.4 (*C*(O)), 166.4 (*C*(O)), 168.6 (*C*(O)), 170.4 (*C*(O)). *m/z* (EI) 229.0825 ($[MH]^+$, $C_9H_{13}N_2O_5$ requires 229.0824), 168 ($[MH-AcOH]^+$, 33%), 126 ($[M-Ac-AcOH]^+$, 100%), 115 (36%), 99 (34%).

4.7.4. 4-Acetyl-1-methyl-3-methoxypiperazine-2,5-dione (22d). The title compound **22d** was synthesized from 4-acetyl-3-carboxyl-1-methylpiperazine-2,5-dione (**1b**) with methanol as additive, using the general procedure outlined above for the DONA reactions. The residue was purified by column chromatography (ethyl acetate, R_f: 0.57) to give 4-acetyl-1-methyl-3-methoxypiperazine-2,5-dione (**22d**) as a colourless solid (74 mg, 78%). Mp 89–91 °C. δ_H (300 MHz, $CDCl_3$) 2.57 (s, 3H, NC(O)CH_3), 3.01 (s, 3H, NCH_3), 3.50 (s, 3H, OCH_3), 3.84 (d, 1H, J_{AB}=18 Hz, ring C$H_a$$H_b$), 4.44 (d, 1H, J_{AB}=18 Hz, ring C$H_a$$H_b$), 5.80 (s, 1H, C*H*OCH$_3$). δ_C (75 MHz, $CDCl_3$) 26.6 (NC(O)*C*H_3), 33.1 (N*C*H_3), 52.9 (*C*H_2), 57.9 (O*C*H_3), 81.5 (*C*HOCH$_3$), 163.0 (*C*(O)), 167.2 (*C*(O)), 171.6 (*C*(O)). *m/z* (EI) 201.0876 ($[MH]^+$. $C_8H_{13}N_2O_4$ requires 201.0875), 145 ($[MH-OMe]^+$, 46%), 128 (31%), 55 (86%).

4.7.5. 3-Carboxyl-1,3,4-trimethylpiperazine-2,5-dione (23a). To a solution of 3-benzyloxycarbonyl-1,3,4-trimethylpiperazine-2,5-dione (**10a**) (503 mg, 1.73 mmol) in degassed methanol (10 mL) was added 10% palladium on carbon (40 mg). The reaction mixture was stirred under a hydrogen atmosphere for 1 h. The reaction mixture was then filtered through a bed of celite. The filtrate was reduced in vacuo and following co-evaporation with chloroform (3× 5 mL), the acid (**23a**) was obtained as a colourless solid (346 mg, 100%). The acid is unstable and was used without purification. δ_H (300 MHz, CD_3OD) 1.74 (s, 3H, α-CH_3), 2.91 (s, 3H, NCH_3), 2.98 (s, 3H, NCH_3), 4.11 (s, 2H, ring CH_2). δ_C (75 MHz, CD_3OD) 20.6 (α-*C*H_3), 30.1 (N*C*H_3), 34.3 (N*C*H_3), 52.0 (ring *C*H_2), 69.3 (quaternary α-*C*CH_3), 165.8 (*C*(O)), 166.5 (*C*(O)), 170.7 (*C*O_2H). *m/z* (EI) 200.0799 ($M^{+\cdot}$. $C_8H_{12}N_2O_4$ requires 200.0797), 156 ($[M-CO_2]^+$, 100%), 141 ($[M-CH_3-CO_2]^+$, 30%), 127 (47%), 113 (85%).

4.7.6. 3-Acetoxy-1,3,4-trimethylpiperazine-2,5-dione (24a). The title compound **24a** was synthesized from 3-carboxyl-1,3,4-trimethylpiperazine-2,5-dione (**23a**) with glacial acetic acid as additive, using the general procedure outlined above for the DONA reactions. However, in this case, stirring after addition of all reaction components was continued for only 6 h before work-up commenced. The residue obtained from the reaction was purified by column chromatography (5:1, ethyl acetate–methanol, R_f: 0.53) to give the title compound **24a** as a thick colourless oil (56 mg, 55%). The compound is unstable and eliminates acetic acid

to give the known 1,4-dimethyl-3-methylidenepiperazine-dione[26] (**28a**) when stored. δ_H (300 MHz, $CDCl_3$) 1.75 (s, 3H, α-CH_3), 2.03 (s, 3H, C(O)CH_3), 2.91 (s, 6H, 2×NCH_3), 3.93 (d, 1H, $J=18$ Hz, ring CH_aH_b), 4.07 (d, 1H, $J=18$ Hz, ring CH_aH_b). δ_C (75 MHz, $CDCl_3$) 20.6 (α-CH$_3$), 24.4 (NCH$_3$), 27.2 (NCH$_3$), 33.7 (C(O)CH$_3$), 51.8 (ring CH$_2$), 86.0 (α-COAc), 163.8 (NC(O)), 164.1 (NC(O)), 169.7 (C(O)CH$_3$).

4.7.7. 3-Acetoxy-3-benzyl-1,4-dimethylpiperazine-2,5-dione (24b). To a solution of 3-benzyl-3-benzyloxycarbonyl-1,4-dimethylpiperazine-2,5-dione (**11**) (265 mg, 0.72 mmol) in degassed methanol (20 mL) was added 10% palladium on carbon (20 mg). The reaction mixture was stirred under a hydrogen atmosphere for 1 h after which time the reaction mixture was filtered through a bed of celite. The filtrate was reduced in vacuo and taken up in dichloromethane (10 mL). DIB (233 mg, 0.72 mmol) and iodine (92 mg, 0.36 mmol) were added and the reaction mixture was stirred under nitrogen for 6 h. After this time, the reaction mixture was washed with saturated sodium thiosulfate solution (5 mL). The organic phase was separated, dried and concentrated in vacuo. The oily residue was purified via column chromatography (ethyl acetate, R_f: 0.61) to give the title compound **24b** as a colourless oil (155 mg, 74%). ν_{max} film/cm^{-1} 3476 w, 3027 w, 2943 w, 1751 s, 1676 vs, 1399 m, 1222 s, 1016 m, 745 m, 703 m. δ_H (300 MHz, $CDCl_3$) 2.15 (s, 3H, OC(O)CH_3), 2.71 (s, 3H, NCH_3), 2.88 (d, 1H, $J_{AB}=18$ Hz, ring CH_aH_b), 3.05 (s, 3H, NCH_3), 3.24 (d, 1H, $J_{AB}=13$ Hz, α-CCH_aH_bPh), 3.45 (d, 1H, $J_{AB}=13$ Hz, α-CCH_aH_bPh), 3.67 (d, 1H, $J_{AB}=18$ Hz, ring CH_aH_b), 7.07 (m, 2H, aromatic H), 7.27 (m, 3H, aromatic H). δ_C (75 MHz, $CDCl_3$) 20.6 (OC(O)CH$_3$), 27.4 (NCH$_3$), 33.0 (NCH$_3$), 41.3 (α-CCH$_2$Ph), 50.7 (ring CH$_2$), 88.5 (quaternary ring C), 127.9 (2×aromatic CH), 128.4 (aromatic CH), 129.9 (2×aromatic CH), 131.8 (quaternary aromatic C), 162.3 (C(O)), 163.3 (C(O)), 168.9 (C(O)). m/z (EI) 290.1273 ($M^{+\cdot}$. $C_{15}H_{18}N_2O_4$ requires 290.1267), 231 ([M−OAc]$^+$, 26%), 199 ([M−C$_7$H$_7$]$^+$, 39%), 157 (100%), 91 ([C$_7$H$_7$]$^+$, 35%).

4.7.8. 3-Methoxy-1,3,4-trimethylpiperazine-2,5-dione (25). The title compound **24a** was synthesized from 3-carboxyl-1,3,4-trimethylpiperazine-2,5-dione (**23a**) with methanol as additive, using the general procedure outlined above for the DONA reactions. However, in this case, stirring after addition of all reaction components was continued for only 6 h before work-up commenced. The residue obtained after work-up was purified by column chromatography (5:1, ethyl acetate–methanol, R_f: −0.35) to give the title compound **25** as a thick colourless oil (79 mg, 77%). The compound is unstable and eliminates acetic acid to give the known 1,4-dimethyl-3-methylidenepiperazine-dione[26] (**28a**) when stored. Data for **25**: δ_H (300 MHz, $CDCl_3$) 1.60 (s, 3H, α-CH_3); 2.93 (s, 3H, NCH_3), 3.00 (s, 3H, NCH_3), 3.09 (s, 3H, OMe), 4.01 (s, 2H, ring CH_2). δ_C (75 MHz, $CDCl_3$) 24.1 (α-CH$_3$), 26.6 (NCH$_3$), 33.6 (NCH$_3$), 51.2 (ring CH$_2$), 51.2 (OCH$_3$), 88.0 (quaternary α-CCH$_3$), 163.5 (C(O)), 164.0 (C(O)). m/z (EI) 187 ([M+1]$^+$, 3%); 186.1001 ($M^{+\cdot}$. $C_8H_{14}N_2O_3$ requires 186.1004), 171 ([M−CH$_3$]$^+$, 20%), 156 ([M−OCH$_3$+1]$^+$, 80%), 127 (100%), 114 (50%), 101 (35%), 87 (30%), 72 (50%).

4.8. Reactions of *N*-acyliminium ions derived from piperazine-2,5-diones

4.8.1. 3-Allyl-1,4-dimethylpiperazine-2,5-dione (26). To a stirred solution of 3-methoxy-1,4-dimethylpiperazine-2,5-dione (**22b**) (587 mg, 3.41 mmol) in dichloromethane (10 mL) at 0 °C was added allyltrimethylsilane (5.5 mL, 35.3 mmol) and boron trifluoride diethyl etherate (0.9 mL, 7.06 mmol). The cooling bath was removed, the reaction mixture was warmed to room temperature and stirring was continued for a further 24 h. After this time the reaction mixture was washed with brine (5 mL), the organic phase was separated, dried and the solvent removed under reduced pressure. The crude residue was purified by column chromatography (5:1, ethyl acetate–methanol, R_f: 0.31) to give the title compound **26** as a colourless oil which crystallized upon prolonged standing (422 mg, 68%). Mp 60–62 °C. ν_{max} KBr/cm^{-1} 3447 m, 2923 m, 1651 vs, 1492 s, 1400 s, 1347 s, 1256 m, 1039 m, 946 m. δ_H (300 MHz, $CDCl_3$) 2.61 (m, 1H, allyl CHCH_aH_bCH=CH$_2$), 2.72 (m, 1H, allyl CHCH_aH_bCH=CH$_2$), 2.96 (s, 3H, NCH_3), 2.98 (s, 3H, NCH_3), 3.81 (d, 1H, $J_{AB}=18$ Hz, ring CH_aH_b), 3.98 (m, 1H, ring CHCH$_2$), 4.04 (d, 1H, $J_{AB}=18$ Hz, ring CH_aH_b), 5.15 (bs, 1H, allyl CHCH$_2$CH=CH_aH_b), 5.20 (d, 1H, $J=9$ Hz, allyl CHCH$_2$CH=CH_aH_b), 5.68 (m, 1H, allyl CHCH$_2$CH=CH$_2$). δ_C (75 MHz, $CDCl_3$) 32.2 (NCH$_3$), 33.4 (NCH$_3$), 36.0 (allyl CHCH$_2$CH=CH$_2$), 51.6 (ring CH$_2$), 62.2 (ring CH), 121.0 (allyl CHCH$_2$CH=CH$_2$), 130.7 (allyl CHCH$_2$$C$H=CH$_2$), 163.8 ($C$(O)), 165.6 ($C$(O)). m/z (EI) 182.1055 ($M^{+\cdot}$. $C_9H_{14}N_2O_2$ requires 182.1055), 141 ([M−allyl]$^+$, 100%), 113 (90%).

4.8.2. 3-(2-Methoxynaphthalen-1-yl)-1,4-dimethylpiperazine-2,5-dione (27). To a stirred solution of 3-acetoxy-1,4-dimethylpiperazine-2,5-dione (**22a**) (181 mg, 0.90 mmol) in dichloromethane (10 mL) was added 2-methoxynaphthalene (286 mg, 1.81 mmol) and boron trifluoride diethyl etherate (0.23 mL, 1.81 mmol). The resultant reaction mixture was stirred under nitrogen for 16 h. After this time, the reaction mixture was washed with brine (5 mL), dried ($MgSO_4$) and the solvent removed in vacuo. The residue was purified via column chromatography (5:1, ethyl acetate–methanol, R_f: 0.42) to afford the title compound **27** (218 mg, 81%). Mp 174–175 °C. ν_{max} KBr/cm^{-1} 2939 w, 1655 vs, 1518 m, 1485 m, 1271 s, 1122 m, 1094 m, 1001 m, 806 m, 750 m, 741 m. δ_H (300 MHz, $CDCl_3$) 2.67 (s, 3H, NCH_3), 3.03 (s, 3H, NCH_3), 3.92 (s, 3H, OCH_3), 4.08 (d, 1H, $J_{AB}=18$ Hz, ring CH_aH_b), 4.19 (d, 1H, $J_{AB}=18$ Hz, ring CH_aH_b), 5.95 (bs, 1H, ring CH), 7.27 (d, 1H, $J=8$ Hz, aromatic CH), 7.38 (t, 1H, $J=7$ Hz, aromatic CH), 7.56 (t, 1H, $J=7$ Hz, aromatic CH), 7.82 (d, 1H, $J=8$ Hz, aromatic CH), 7.88 (d, 1H, $J=9$ Hz, aromatic CH), 8.08 (bd, 1H, $J=8$ Hz, aromatic CH). δ_C (75 MHz, $CDCl_3$) 31.0 (NCH$_3$), 33.2 (NCH$_3$), 51.9 (ring CH$_2$), 56.5 (OCH$_3$), 57.0 (ring CH), 112.7 (aromatic CH), 116.8 (quaternary aromatic C), 121.3 (aromatic CH), 123.5 (aromatic CH), 127.6 (aromatic CH), 128.7 (aromatic CH), 128.9 (quaternary aromatic C), 131.0 (aromatic CH), 133.3 (quaternary aromatic C); 155.0 (quaternary aromatic COCH$_3$), 163.6 (C(O)), 165.0 (C(O)). m/z (EI) 298.1318 ($M^{+\cdot}$. $C_{17}H_{18}N_2O_3$ requires 298.1317); 210 (38%); 198 (63%); 183 (30%); 169 (39%); 113 (48%).

4.8.3. (*Z*)-3-Benzylidene-1,4-dimethylpiperazine-2,5-dione (28b). To a stirred solution of 3-acetoxy-3-benzyl-1,4-dimethylpiperazine-2,5-dione (**24b**) (55 mg, 0.19 mmol) in dichloromethane (5 mL) at 0 °C was added boron trifluoride diethyl etherate (0.03 mL, 0.21 mmol). The reaction mixture was warmed to room temperature and stirring was continued for a further 16 h. After this time the reaction mixture was washed with brine (1 mL), dried and filtered. The solvent was removed in vacuo to give the crude product as a yellow oil. The oil was purified via column chromatography (ethyl acetate, R_f: 0.5) to afford the title compound (33 mg, 76%) as a colourless oil with spectroscopic properties consistent with the *Z*-isomer reported previously.[22]

4.8.4. 3-Allyl-1,3,4-trimethylpiperazine-2,5-dione (30). To a solution of 3-methoxy-1,3,4-trimethylpiperazine-2,5-dione (**25**) (30 mg, 0.16 mmol) in dichloromethane (1 mL) at 0 °C was added allyltrimethylsilane (0.26 mL, 1.61 mmol) and boron trifluoride diethyl etherate (0.04 mL, 0.32 mmol). The cooling bath was removed and the reaction mixture was warmed slowly to room temperature. Stirring was continued for a further 4 h following which time the reaction mixture was washed with brine (1 mL), the organic phase was separated, dried and concentrated in vacuo. The residue was purified by column chromatography (5:1, ethyl acetate–methanol, R_f: 0.46) to give the title compound **30** as a colourless oil (21 mg, 66%). δ_H (300 MHz, $CDCl_3$) 1.56 (s, 3H, α-CH_3), 2.43 (dd, 1H, J=14 Hz, 8 Hz, allyl α-CCH_aH$_b$CH), 2.76 (dd, 1H, J=14 Hz, 7 Hz, allyl α-CCH$_a$$H_b$CH), 2.96 (s, 6H, 2×NC$H_3$), 3.95 (m, 2H, ring C$H_2$), 5.09 (m, 1H, allyl α-CCH$_2$CHCH_aH$_b$), 5.13 (m, 1H, allyl α-CCH$_2$CHCH$_a$$H_b$), 5.55 (m, 1H, allyl α -CCH$_2$CH). δ_C (75 MHz, $CDCl_3$) 24.6 (α-CH$_3$), 27.8 (NCH$_3$), 33.8 (NCH$_3$), 42.0 (allyl CCH$_2$CH), 51.4 (ring CH$_2$), 64.5 (quaternary ring carbon), 120.2 (allyl CCH$_2$CHCH$_2$), 131.4 (allyl CCH$_2$$C$H), 163.5 ($C$(O)), 167.7 ($C$(O)). The by-product 1,4-dimethyl-3-methylidenepiperazine-2,5-dione[26] (**28a**) (~33%) had spectral properties consistent with those reported previously.

4.9. Hydroxymethylpiperazinediones as precursors in modified DONA reactions

4.9.1. (*S*)-3-(*t*-Butyldimethylsilanyloxymethyl)-1-methylpiperazine-2,5-dione (33). To a stirred solution of (*S*)-3-hydroxymethyl-1-methylpiperazine-2,5-dione (**32**)[24] (851 mg, 5.38 mmol) in DMF (15 mL) was added *t*-butyldimethylsilylchloride (983 mg, 6.52 mmol) and imidazole (888 mg, 13.0 mmol). The reaction mixture was stirred for 1 h and the solvent was then removed in vacuo. The residue was taken up in ethyl acetate (20 mL) and washed with water (10 mL). The organic phase was dried ($MgSO_4$) and the solvent removed to afford the title compound **33** as a colourless solid (1.17 g, 80%). Mp 115–117 °C. ν_{max} KBr/cm^{-1} 3213 w, 2932 w, 1670 s, 1443 m, 1335 m, 1250 m, 1096 m, 837 m, 779 m. δ_H (300 MHz, $CDCl_3$) 0.01 (s, 6H, Si(CH_3)$_2$), 0.83 (s, 9H, C(CH_3)$_3$), 2.94 (s, 3H, NCH_3), 3.82 (d, 1H, J=17 Hz, 6-CH_aH$_b$), 3.83–3.87 (m, 1H, CH_aH$_b$OSi), 3.86 (bs, 1H, 3-CH), 3.93–4.00 (m, 1H, CH$_a$$H_b$OSi), 4.02 (d, 1H, J=17 Hz, 6-CH$_a$$H_b$). δ_C (75 MHz, $CDCl_3$) −5.8 (2×Si(CH$_3$)$_2$), 17.9 (C(CH$_3$)$_3$), 25.5 (3×C(CH$_3$)$_3$), 33.4 (NCH$_3$), 51.4 (6-CH$_2$), 57.3 (3-CH), 65.7 (CH$_2$O), 164.9 (C(O)), 166.6 (C(O)). *m/z* (EI) 272.1533 ($M^{+\cdot}$. $C_{12}H_{24}N_2O_3Si$ requires 272.1556), 215 ([M−C(CH$_3$)$_3$]$^+$, 100%), 158 ([M−Si(CH$_3$)$_2$C(CH$_3$)$_3$]$^+$, 30%), 73 (33%).

4.9.2. (*S*)-3-(*t*-Butyldimethylsilanyloxymethyl)-1,4-dimethylpiperazine-2,5-dione (34a). To a stirred solution of (*S*)-3-(*t*-butyldimethylsilanyloxymethyl)-1-methylpiperazine-2,5-dione (**33**) (1.24 g, 4.56 mmol) in DMF (10 mL) at 0 °C under nitrogen was added sodium hydride (182 mg, 4.60 mmol, 60% in paraffin) and methyl iodide (5 mL, excess). The resultant solution was stirred under a nitrogen atmosphere for 24 h and after this time the solvent was removed under reduced pressure. The residue was taken up in chloroform (30 mL) and washed with water (10 mL), brine (10 mL) and dried ($MgSO_4$). The solvent was removed in vacuo and the crude product was purified by column chromatography (ethyl acetate, R_f: 0.49) giving **34a** as a colourless solid (1.17 g, 90%). Mp 147–149 °C. ν_{max} KBr/cm^{-1} 2932 w, 2858 m, 1666 s, 1474 m, 1408 m, 1335 m, 1258 m, 1111 m, 837 m, 783 m. δ_H (300 MHz, $CDCl_3$) 0.01 (s, 3H, SiCH_3), 0.02 (s, 3H, SiCH_3), 0.83 (s, 9H, C(CH_3)$_3$), 2.94 (s, 3H, NCH_3), 2.95 (s, 3H, NCH_3), 3.77 (d, 1H, J=17 Hz, 6-CH_aH$_b$), 3.84 (bs, 1H, 3-CH), 3.90 (dd, 1H, ^2J=10 Hz, ^3J=2 Hz, CH_aH$_b$O), 4.04 (dd, 1H, ^2J=10 Hz, ^3J=2 Hz, CH$_a$$H_b$O), 3.82 (d, 1H, J=17 Hz, 6-CH$_a$$H_b$). δ_C (75 MHz, $CDCl_3$) −5.8 (2×Si(CH$_3$)$_2$), 18.0 (C(CH$_3$)$_3$), 25.5 (3×C(CH$_3$)$_3$), 31.6 (NCH$_3$), 33.2 (NCH$_3$), 51.8 (6-CH$_2$), 62.3 (CH$_2$O), 64.5 (3-CH), 164.7 (C(O)), 165.4 (C(O)). *m/z* (EI) 286.1710 ($M^{+\cdot}$. $C_{13}H_{26}N_2O_3Si$ requires 286.1713); 229 ([M−C(CH$_3$)$_3$]$^+$, 100%); 158 ([M−Si(CH$_3$)$_2$C(CH$_3$)$_3$]$^+$, 30%); 73 (37%).

4.9.3. (*S*)-3-(*t*-Butyldimethylsilanyloxymethyl)-4-(*p*-methoxybenzyl)-1-methylpiperazine-2,5-dione (34b). The title compound was prepared from (*S*)-3-(*t*-butyldimethylsilanyloxymethyl)-1-methylpiperazine-2,5-dione (**33**) (2.18 g, 8.00 mmol) as outlined for **34a**. In this case *p*-methoxybenzyl chloride was used instead of methyl iodide. The compound was purifed via column chromatography (ethyl acetate, R_f: 0.53). Piperazinedione **34b** was obtained as a colourless solid (2.26 g, 72%). Mp 94–97 °C. ν_{max} KBr/cm^{-1} 2932 m, 1666 s, 1647 s, 1512 m, 1466 m, 1331 m, 1246 s, 1115 m. δ_H (300 MHz, $CDCl_3$) 0.00 (s, 3H, SiCH_3), 0.01 (s, 3H, SiCH_3), 0.85 (s, 9H, C(CH_3)$_3$), 2.94 (s, 3H, NCH_3), 3.78 (s, 3H, OCH_3), 3.75–3.85 (m, 3H, CH_aH$_b$O & 6-CH_aH$_b$ & 3-CH), 3.95–3.98 (m, 1H, CH$_a$$H_b$O), 4.00 (d, 1H, J_{AB}=15 Hz, NCH_aH$_b$Ph), 4.21 (d, 1H, J_{AB}=17 Hz, 6-CH$_a$$H_b$), 5.15 (d, 1H, J_{AB}=15 Hz, NCH$_a$$H_b$Ph), 6.83 (d, 2H, J=9 Hz, 2×aromatic CH), 7.17 (d, 2H, J=9 Hz, 2×aromatic CH). δ_C (75 MHz, $CDCl_3$) −5.6 (2×Si(CH$_3$)$_2$), 18.1 (C(CH$_3$)$_3$), 25.7 (3×C(CH$_3$)$_3$), 33.4 (NCH$_3$), 46.4 (NCH$_2$Ph), 52.2 (ring CH$_2$), 55.3 (OCH$_3$), 61.4 (ring CH), 62.8 (CH$_2$OSi), 114.3 (2×aromatic CH), 127.5 (quaternary aromatic), 129.7 (2×aromatic CH), 159.3 (quaternary aromatic), 164.9 (C(O)), 166.0 (C(O)). *m/z* (EI) 393 ([MH]$^+$, 1%), 392.2130 ($M^{+\cdot}$. $C_{20}H_{32}N_2O_4Si$ requires 392.2131), 335 ([M−C(CH$_3$)$_3$]$^+$, 63%), 121 (57%), 61 (100%).

4.9.4. 3-Hydroxymethyl-1,4-dimethylpiperazine-2,5-dione (35a). 3-(*t*-Butyldimethylsilanyloxymethyl)-1,4-dimethylpiperazine-2,5-dione (**34a**) (620 mg, 2.41 mmol) was dissolved in acetic acid (6 mL), THF (2 mL) and water

(2 mL). The resultant solution was heated at reflux for 24 h, following which the solvent was removed in vacuo. The residue was purified via column chromatography (9:1, chloroform–ethyl acetate, R_f: 0.29) to give 3-hydroxymethyl-1,4-dimethylpiperazine-2,5-dione (**35a**) as a colourless solid (361 mg, 87%). Mp 127–129 °C. (Found C, 48.9; H, 7.3; N, 16.5. $C_7H_{12}N_2O_3$ requires C, 48.8; H, 7.0; N, 16.3%). ν_{max} KBr/cm^{-1} 3379 s, 2992 w, 2816 w, 1672 vs, 1647 vs, 1493 s, 1410 s, 1340 s, 1259 s, 1070 m, 1056 m, 1032 m, 833 w. δ_H (300 MHz, $CDCl_3$) 2.97 (s, 3H, NCH_3), 3.00 (s, 3H, NCH_3), 3.17 (bs, 1H, OH), 3.78 (d, 1H, $J_{AB}=17$ Hz, 6-CH_aH$_b$), 3.85 (bs, 1H, 3-CH), 3.96 (dd, 1H, $^2J_{AB}=12$ Hz, $^3J=3$ Hz, CH_aH$_b$OH), 4.05 (dd, 1H, $^2J_{AB}=12$ Hz, $^3J=3$ Hz, CH$_a$$H_b$OH), 4.16 (d, 1H, $J_{AB}=17$ Hz, 6-CH$_a$$H_b$). δ_C (75 MHz, $CDCl_3$) 31.7 (NCH_3), 33.3 (NCH_3), 51.6 (6-CH_2), 61.2 (CH_2OH), 64.5 (3-CH), 165.2 (C(O)), 165.7 (C(O)). *m/z* (EI) 173.0923 ([MH]$^+$. $C_7H_{13}N_2O_3$ requires173.0926), 142 ([MH−CH_2OH]$^+$, 100%), 113 (72%), 71 (32%).

4.9.5. 3-Hydroxymethyl-4-(*p*-methoxybenzyl)-1-methylpiperazine-2,5-dione (35b). The title compound was prepared from (*S*)-3-(*t*-butyldimethylsilanyloxymethyl)-4-(*p*-methoxybenzyl)-1-methylpiperazine-2,5-dione (**34b**) (1.01 g, 2.57 mmol) as outlined for the conversion of **34a** to **35a**. The compound was purifed via column chromatography (5:1, ethyl acetate–methanol, R_f: 0.39). Piperazinedione **35b** was obtained as a colourless solid (594 mg, 83%). Mp 94–97 °C. ν_{max} KBr/cm^{-1} 3368 w, 2936 w, 1666 s, 1647 m, 1512 m, 1246 m. δ_H (300 MHz, $CDCl_3$) 2.97 (s, 3H, NCH_3), 3.79 (s, 3H, OCH_3), 3.82–3.90 (m, 3H, 6-CH_aH$_b$, 3-CH, CH_aH$_b$O), 4.00 (dd, 1H, $^2J=12$ Hz, $^3J=3$ Hz, CH$_a$$H_b$O), 4.07 (d, 1H, $J=15$ Hz, NCH_aH$_b$), 4.27 (d, 1H, $J=17$ Hz, 6-CH$_a$$H_b$), 5.16 (d, 1H, $J=15$ Hz, NCH$_a$$H_b$), 6.85 (d, 2H, $J=9$ Hz, 2×aromatic H), 7.19 (d, 2H, $J=9$ Hz, 2×aromatic H). δ_C (75 MHz, $CDCl_3$) 33.5 (NCH_3), 46.4 (NCH_2), 52.1 (6-CH_2), 55.4 (OCH_3), 61.8 (CH_2OH), 114.4 (2×aromatic CH), 127.2 (quaternary aromatic C), 129.8 (2×aromatic CH), 159.5 (quaternary aromatic C), 165.4 (C(O)), 166.2 (C(O)). *m/z* (EI) 278.1266 (M$^{+\cdot}$. $C_{14}H_{18}N_2O_4$ requires 278.1267), 248 (26%), 121 (100%).

4.9.6. 3-Methoxy-1-methylpiperazine-2,5-dione (36). To a solution of 3-hydroxymethyl-1-methylpiperazine-2,5-dione (**32**) (225 mg, 1.42 mmol) in dichloromethane (10 mL) was added iodine (718 mg, 2.84 mmol) and DIB (462 mg, 1.43 mmol). The reaction mixture was then stirred under a nitrogen atmosphere for 16 h. After this time, methanol (10 mL) was added and the reaction mixture was stirred for a further 24 h. Then reaction mixture was then washed with a saturated solution of sodium thiosulfate (2 mL). The aqueous phase was extracted with dichloromethane (5×5 mL). The organic washings were dried ($MgSO_4$) and the solvent removed under reduced pressure. The residue was purified by column chromatography (5:1, ethyl acetate–methanol, R_f: 0.35) to afford the title compound **36** as a colourless solid (99 mg, 44%). Mp 118–120 °C. ν_{max} KBr/cm^{-1} 3267 w, 1682 s, 1655 s, 1477 m, 1327 m, 1072 s, 1018 m. δ_H (300 MHz, $CDCl_3$) 2.99 (s, 3H, NCH_3), 3.45 (s, 3H, OCH_3), 3.78 (d, 1H, $J_{AB}=18$ Hz, ring CH_aH$_b$), 4.23 (d, 1H, $J_{AB}=18$ Hz, ring CH$_a$$H_b$), 4.74 (d, 1H, $J=4$ Hz, ring CH), 7.84 (bs, 1H, NH). δ_C (75 MHz, $CDCl_3$) 33.7 (NCH_3), 51.3 (ring CH_2), 55.9 (OCH_3), 82.3 (ring CH), 162.4 (C(O)), 167.9 (C(O)). *m/z* (EI) 159.0767 ([MH]$^+$. $C_6H_{11}N_2O_3$ requires 159.0767), 128 ([M−OCH_3+1]$^+$, 71%), 101 (100%), 73 (32%).

4.9.7. 3-Acetoxy-4-(*p*-methoxybenzyl)-1-methylpiperazine-2,5-dione (37). To a solution of **35b** (106 mg, 0.38 mmol) in dichloromethane (10 mL) was added iodine (48 mg, 0.19 mmol) and DIB (125 mg, 0.39 mmol). The reaction mixture was then stirred under a nitrogen atmosphere for 16 h. After this time, the reaction mixture was washed with a saturated solution of sodium thiosulfate (1 mL), dried ($MgSO_4$) and the solvent removed under reduced pressure. The residue was triturated with diethyl ether and collection of the solid afforded the title compound **37** as a colourless solid (110 mg, 94%). Mp 118–120 °C. δ_H (300 MHz, $CDCl_3$) 1.98 (s, 3H, OC(O)CH_3), 2.96 (s, 3H, NCH_3), 3.78 (s, 3H, OCH_3), 3.93 (d, 1H, $J_{AB}=18$ Hz, 6-CH_aH$_b$), 4.18 (d, 1H, $J_{AB}=18$ Hz, NCH_aH$_b$), 4.28 (d, 1H, $J_{AB}=18$ Hz, 6-CH$_a$$H_b$), 4.94 (d, 1H, $J_{AB}=18$ Hz, NCH$_a$$H_b$), 5.98 (s, 1H, 3-C$H$), 6.84 (d, 2H, $J=9$ Hz, 2×aromatic H), 7.23 (d, 2H, $J=9$ Hz, 2×aromatic H). δ_C (75 MHz, $CDCl_3$) 20.5 (OC(O))CH_3), 33.5 (NCH_3), 46.9 (NCH_2), 51.7 (6-CH_2), 55.2 (OCH_3), 77.7 (3-CH), 114.1 (2×aromatic CH), 127.2 (quaternary aromatic C), 130.1 (2×aromatic CH), 159.4 (quaternary aromatic), 161.1 (C(O)), 165.2 (C(O)), 169.7 (C(O)). *m/z* (EI) 306.1219 (M$^{+\cdot}$. $C_{15}H_{18}N_2O_5$ requires 306.1216), 246 ([M−OAc−1]$^+$, 56%), 147 (39%), 136 (29%), 121 (100%).

Acknowledgements

The authors thank the Australian Research Council (ARC) for support for this project.

References and notes

1. (a) Johne, S.; Groeger, D. *Pharmazie* **1977**, *32*, 1–16. (b) Rajappa, S.; Natekar, M. V. *Adv. Heterocycl. Chem.* **1993**, *57*, 187–289. (c) Witiak, D. T.; Wei, Y. *Prog. Drug. Res.* **1990**, *35*, 249–363.
2. For a recent review, see Fischer, P. M. *J. Peptide Sci.* **2003**, *9*, 9–35.
3. For examples, see (a) Hayashi, Y.; Orikasa, S.; Tanaka, K.; Kanoh, K.; Kiso, Y. *J. Org. Chem.* **2000**, *65*, 8402–8405. (b) Williams, R. M.; Glinka, T.; Kwast, E.; Coffman, H.; Stille, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 808–821. (c) Fukuyama, T.; Nakatsuka, S.; Kishi, Y. *Tetrahedron* **1981**, *37*, 2045–2078.
4. For examples, see (a) Bull, S. D.; Davies, S. G.; Epstein, S. W.; Leech, M. A.; Ouzman, J. V. A. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2321–2330. (b) Bull, S. D.; Davies, S. G.; O'Shea, M. D. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3657–3658. (c) Oehler, E.; Poisel, H.; Tataruch, F.; Schmidt, U. *Chem. Ber.* **1972**, *105*, 635–641. (d) Orena, M.; Porzi, G.; Sandri, S. *J. Org. Chem.* **1992**, *57*, 6532–6536.
5. (a) Chai, C. L. L.; Page, D. M. *Tetrahedron Lett.* **1993**, *34*, 4373–4376. (b) Chai, C. L. L.; King, A. R. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1173–1182.
6. (a) Burkett, B. A.; Chai, C. L. L. *Tetrahedron Lett.* **2001**, *42*, 2239–2242. (b) Chai, C. L. L.; Johnson, R. C.; Koh, J. *Tetrahedron* **2002**, *58*, 975–982.

7. Chai, C. L. L.; Elix, J. A.; Huleatt, P. B. *Tetrahedron Lett.* **2003**, *44*, 263–265.
8. Palmer, C. S.; McWherter, P. W.; *Organic Syntheses*; Wiley: New York, 1941; Collect. Vol. I, pp 245–246.
9. Hardegger, E.; Corrodi, H. *Helv. Chim. Acta* **1956**, *39*, 980–983.
10. Hino, T.; Sato, T. *Chem. Pharm. Bull.* **1974**, *22*, 2866–2874.
11. Hino, T.; Sato, T. *Chem. Pharm. Bull.* **1976**, *24*, 285–293.
12. (a) Kissman, H. M.; Witkop, B. *J. Am. Chem. Soc.* **1953**, *75*, 1967–1974. (b) Zaugg, H. E.; Freifelder, M.; Glenn, H. J.; Horrom, B. W.; Stone, G. R.; Vernstein, M. R. *J. Am. Chem. Soc.* **1956**, *78*, 2626–2631.
13. For a recent review on conformationally constrained amino acids, see Park, K.-H.; Kurth, M. J. *Tetrahedron* **2002**, *58*, 8629–8659.
14. Mauger, A. B.; Desai, R. B.; Rittner, I.; Rzeszotarski, W. J. *J. Chem. Soc., Perkin Trans. 1* **1972**, 2146–2148.
15. (a) Boto, A.; Hernandez, R.; Suarez, E. *Tetrahedron Lett.* **1999**, *40*, 5945–5948. (b) Boto, A.; Hernandez, R.; Suarez, E. *J. Org. Chem.* **2000**, *65*, 4930–4937. (c) Boto, A.; Hernandez, R.; Suarez, E. *Tetrahedron Lett.* **2000**, *41*, 2899–2902.
16. Speckamp, W. N.; Moolenar, M. J. *Tetrahedron* **2000**, *56*, 3817–3856.
17. Badran, T. W.; Easton, C. J. *Aust. J. Chem.* **1990**, *43*, 1455–1459.
18. For examples, see (a) Badran, T. W.; Chai, C. L. L.; Easton, C. J.; Harper, J. B.; Page, D. M. *Aust. J. Chem.* **1995**, *48*, 1379–1384. (b) Chai, C. L. L.; Hay, D. B.; King, A. R. *Aust. J. Chem.* **1996**, *49*, 605–610.
19. Bull, S. D.; Davies, S. G.; Garner, A. C.; O'Shea, M. D.; Savory, E. D.; Snow, E. J. *J. Chem. Soc., Perkin Trans. 1* **2002**, 2442–2448.
20. Hausler, J.; Schmidt, U. *Chem. Ber.* **1974**, *107*, 2804–2815.
21. (a) Kanoh, K.; Kohno, S.; Katada, J.; Takahashi, J.; Uno, I.; Hayashi, Y. *Bioorg. Med. Chem. Lett.* **1999**, *7*, 1451–1457. (b) Kanoh, K.; Kohno, S.; Katada, J.; Takahashi, J.; Uno, I. *J. Antibiot.* **2002**, *55*, 1042–1047.
22. (a) Machin, P. J.; Sammes, P. G. *J. Chem. Soc., Perkin Trans. 1* **1976**, 628–634. (b) Shin, C. G.; Sato, Y.; Hayakawa, M.; Kondo, M.; Yoshimura, J. *Heterocycles* **1981**, *16*, 1573–1578.
23. Boto, A.; Hernandez, R.; Suarez, E. *Tetrahedron Lett.* **2002**, *43*, 8269–8272.
24. Falomi, M.; Satta, M.; Conti, S.; Giacomelli, G. *Tetrahedron: Asymmetry* **1993**, *4*, 2389–2398.
25. Armarego, W. L. F.; Chai, C. L. L. *Purification of Laboratory Chemicals*, 5th ed.; Butterworth: Heinemann, 2003.
26. Chai, C. L. L.; King, A. R. *J. Chem. Res. (S)* **1997**, 382.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 8740–8745

Synthesis and interfacial properties of amphiphilic β-cyclodextrins and their substitution at the O-6 position with a mono bio-recognisable galactosyl antenna

Alain Salameh,[a] Adina N. Lazar,[b] Anthony W. Coleman[b] and Hélène Parrot-Lopez[a,*]

[a]*Méthodologie de Synthèse et Molécules Bioactives, UMR-CNRS 5181, Université Claude Bernard-Lyon 1, Domaine Scientifique de la Doua, Bât. J. Raulin, 43 Bd du 11 Novembre 1918, 69622 Villeurbanne cedex, France*
[b]*Institut de Biologie et Chimie des Protéines, CNRS-UMR 5086, 7 passage du Vercors, Lyon cedex 07, 69367, France*

Received 8 April 2005; revised 9 June 2005; accepted 16 June 2005

Abstract—The synthesis of a mono-galactosylated amphiphilic β-cyclodextrin, in five steps from mono-6-azido-6-deoxy-β-cyclodextrin, via coupling to a *N*-β-D-galactopyranosylamino-antenna is described. Both characterization by electrospray mass spectrometry and NMR show the presence of only the mono-substituted product. The Langmuir isotherms of the final product and intermediates are described.

1. Introduction

β-Cyclodextrins (β-CDs) or cyclomaltoheptaoses have long been recognized to have significant potential as drug carriers arising from their ability to form inclusion complexes.[1] Inclusion of the bioactive molecules generates several therapeutic advantages: solubilisation of poorly soluble or insoluble molecules,[2] protection from chemical and enzymatic degradation,[3] transport and time control of release.[4] Currently, in order to reduce the appearance of resistance towards therapeutic agents and to decrease the toxicity of the bioactive molecules, several studies have been undertaken to target such carriers, reducing in this way the quantity of drug used in therapies. Carbohydrates are biocompatible molecules having low immunogenicity and are responsible of recognition between the cells. We have previously demonstrated the capacity of galactosyl-β-cyclodextrin to be recognized by a galactosyl specific cell wall lectin *Kluyveromyces bulgaricus* (KbCWL)[5] and in the literature there exist several reports of the synthesis of cyclodextrin derivatives substituted with mono or poly-saccharides and which have shown to be recognized by lectins.[6] Recently, chemo-enzymatic synthesis of amphiphilic cyclodextrins fully substituted with *N*-acetyl-glucosamine (Glc-*N*Ac) has been described. [7]

Indeed to pass from a system of 1:1 complexation to nanoparticles able to ensure a much higher degree of encapsulation, the use of amphiphilic cyclodextrins has been developed. In the previous studies, amphiphilic cyclodextrins were obtained by the introduction of lipophilic groups at the primary face and/or secondary face.[8] These amphiphilic cyclodextrins are capable of forming liposomes,[9] nanoparticles,[10] vesicles,[11] micellar aggregates [12] and solid lipid nanoparticles.[13]

The objective of the current work resides in the combination of the recognition properties of the mono-galactosyl-β-cyclodextrins and their amphiphilic properties to obtain new carrier molecules containing a galactosyl antenna at the O-6 position and lipophilic ester groups at the O-2 and O-3 positions. The synthesis, characterization and the interfacial properties of these molecules as Langmuir monolayers are described.

2. Results and discussion

2.1. Synthesis and characterization

In previous studies we have demonstrated good recognition capacity (1.75 mmol dm^{-3}) towards a galactose specific yeast lectin *K. bulgaricus* (Kb CWL) of a mono-galactosyl-β-CD derived from mono-6-amino-6-deoxy-β-CD by coupling a galactosyl head group via a spacer chain (9 carbon atoms). In contrast to expectations, a 'clustering

Keywords: Amphiphilic cyclodextrin; Galactosyl antenna; Langmuir isotherms.
* Corresponding author. Tel.: +33 472431532; fax: +33 472448438; e-mail: h.parrot@cdlyon.univ-lyon1.fr

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.06.054

effect' by the heptakis-galactosyl-β-CD was not observed with only a 1.5 fold increase in recognition.[5]

In view of the above, the mono-galactosylated amphiphilic β-cyclodextrin derivative **7** was synthesized from mono-6-azido-6-deoxy-β-cyclodextrin **1**[14] in five steps (Scheme 1). The synthetic procedure for the synthesis of **7** is based on an amide bond between the carbohydrate-antenna and the amphiphilic-β-cyclodextrin moiety.

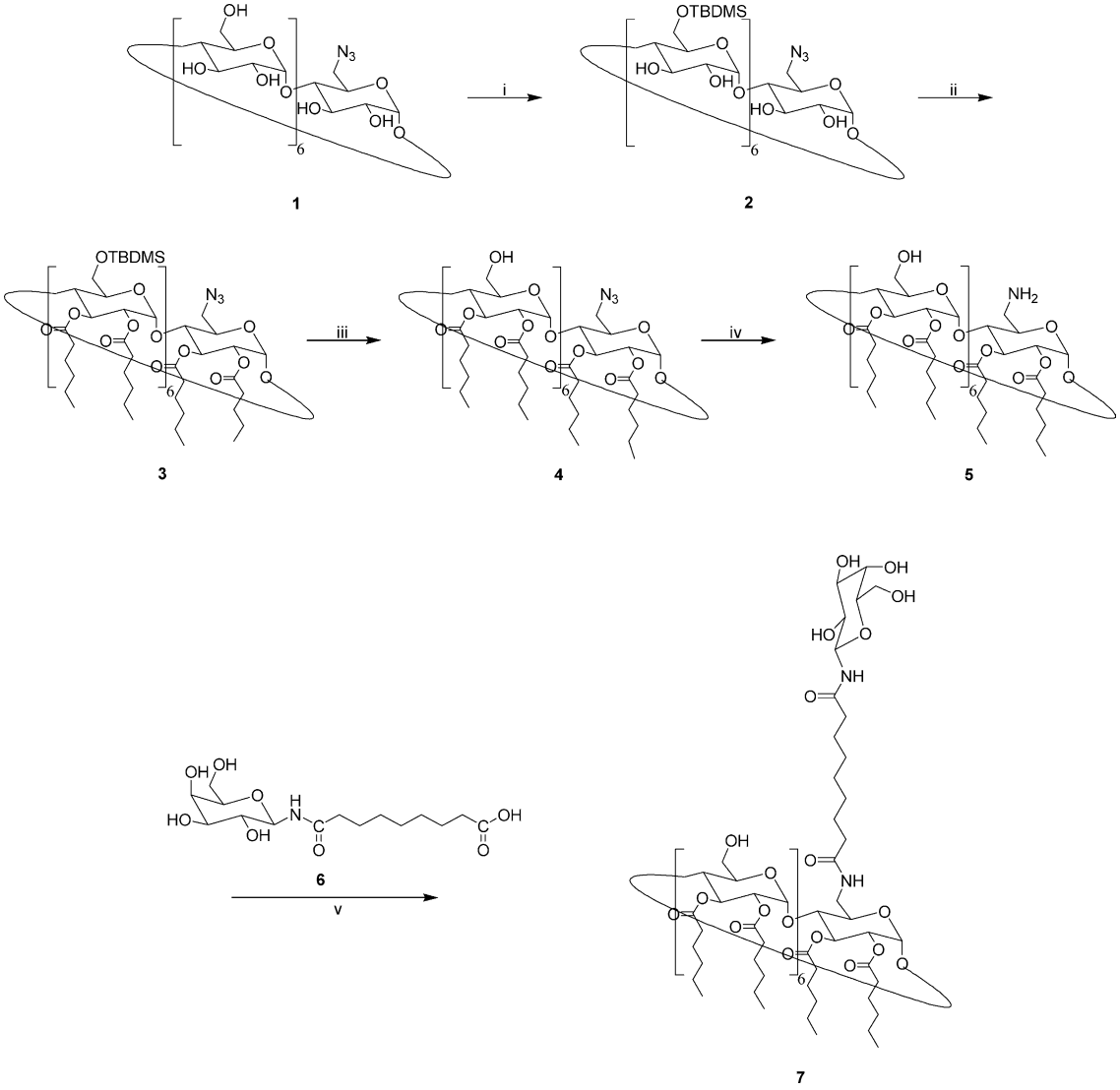
O-acylation at the secondary hydroxyl groups of the β-CD requires protection of the free primary hydroxyl groups at O-6 position of mono-6-azido-6-deoxy-β-cyclodextrin **1** with *tert*-butyldimethylsilyl chloride in dry pyridine. The new intermediate **2** is recrystallized from chloroform (yield 86%). The use of 56 equiv of hexanoic anhydride and 42 equiv of 4-dimethylaminopyridine (DMAP) leads to the pure fully acylated product **3** with 14 hexanoyl chains at secondary face of the β-cyclodextrin, according to the conditions of Lesieur and Dubes.[15] We have applied a new method of purification consisting of simply precipitating **3** from a mixture of methanol/chloroform 95:5 in 66.7% yield.

The degree of substitution is easily verified by electrospray mass-spectrometry (ES-MS, positive mode, *m*/*z*: 1621.4 $[M+H+Na]^{2+}$ and the structure confirmed by ^{1}H and ^{13}C NMR spectroscopy.

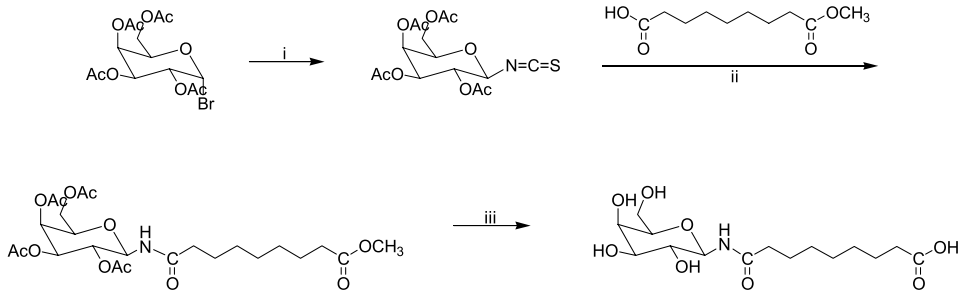
Selective removal of the *tert*-butyldimethylsilyl groups at O-6 position of **3** by boron trifluoride etherate ($BF_3 \cdot Et_2O$) in dry $CHCl_3$ gave the new product **4** with one azido group on the primary face and 14 hexanoyl chains on the secondary hydroxyl groups in 97% yield.

The reduction of the azido group into the corresponding amino group is carried out by catalytic hydrogenation. The presence of the ester groups at the secondary face requires neutral condition; here the use of Staudinger reduction is not successful. The novel mono-6-amino-6-deoxy-amphiphilic cyclodextrin **5** was synthesized from **4** in MeOH in presence of the Pd/C (10%) under hydrogen pressure (2 bar) during 4 h in 95% yield. The absence of the azido band at 2100 cm^{-1} was confirmed by IR spectroscopy.

The covalent amido linkage between the glycoconjugate



Scheme 1. Synthesis of galactosylated amphiphilic cyclodextrin: (i) TBDMSCl, pyridine; (ii) hexanoic anhydride, DMAP, pyridine; (iii) $BF_3 \cdot Et_2O$ $CHCl_3$; (iv) Pd/C, H_2, MeOH; (v) **6**, DCC, HOBT, DMF.



Scheme 2. Synthesis of the glycoconjugate **6**. (i) KSCN, $Bu_4N^+Br^-$, Acetonitrile; (ii) Et_3N, toluene; (iii) NaOH (1 M), MeOH.

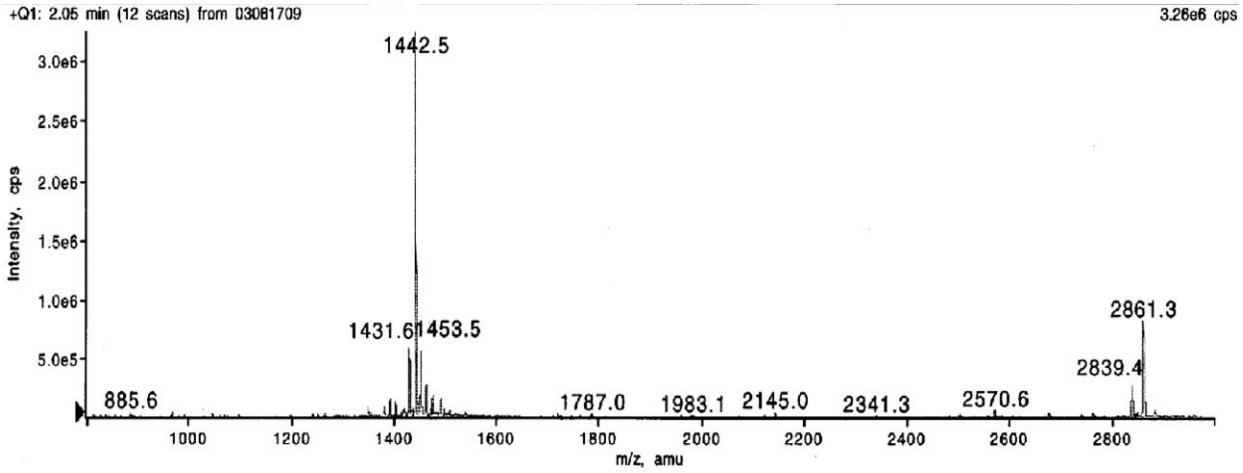


Figure 1. ES-mass spectrometry of the mono-galactosylated amphiphilic β-cyclodextrin **7**.

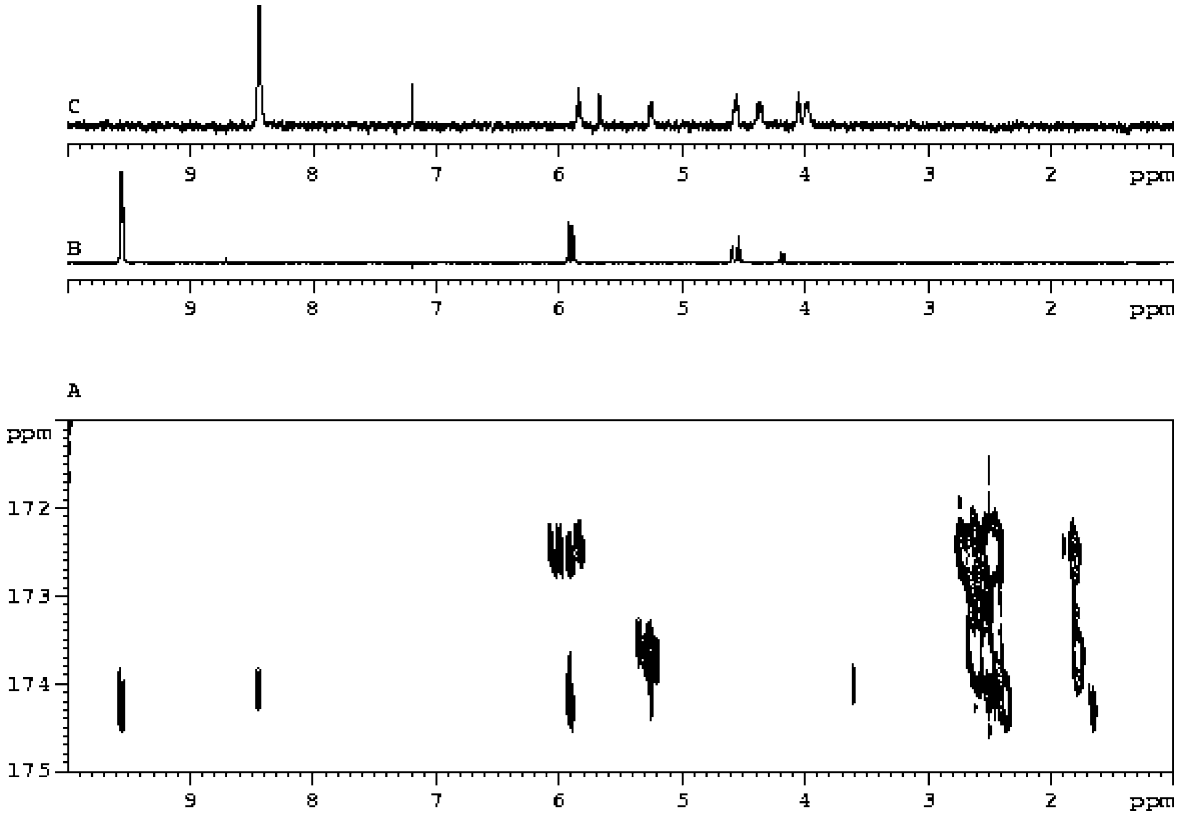


Figure 2. (A) ^1H–^{13}C 2D HMBC NMR spectrum of **7**; (B) ^1H NMR spectrum of galactose from 2D TOCSY; (C) ^1H NMR spectrum of glucopyranose A substituted by galactosyl antenna from 2D TOCSY.

and β-CD requires the synthesis of amphiphilic synthons presenting an amine function at the primary face and terminal carboxylic acid function in the glycoconjugate. We have described the synthesis of 9-(*N*-β-D-galactopyranosyl-amino)azelaïc acid **6**[16] from tetra-*O*-acetyl-α-D-bromo-galactose in three steps via galactosylisothiocyanate (Scheme 2).

The method used to synthesize the final product **7**, is that of a peptide coupling under soft conditions, in the presence of dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBT) in the anhydrous DMF. This method appeared to us most judicious to graft via a covalent bond an unprotected β-D-galactose onto the amphiphilic β-CD derivative. This method avoids working at high temperature in the presence of the galactosylated antenna. Mono-galactosyl amphiphilic-β-cyclodextrin **7** was synthesized by condensation of glycoconjugate **6** with mono-6-amino-6-deoxy-amphiphilic-β-cyclodextrin **5** in dry DMF using DCC/HOBT as the coupling reagents. The product was isolated by chromatography on silica gel with EtOH/toluene 8:2 as eluent in 42% yield. Characterization by mass spectrometry of **7** showed only species corresponding to $[\mathbf{7}+2\mathrm{Na}]^{2+}$: (*m*/*z*: 1442.5) and $[\mathbf{7}+\mathrm{Na}]^{+}$: (*m*/*z*: 2861.3) (Fig. 1). This substitution by one galactosyl antennae at the O-6 position and 14 hexanoyl chains at O-2 and O-3 positions were confirmed by 2D ^{1}H and ^{13}C NMR (COSY, HMBC, TOCSY) at 500 MHz in pyridine-d_5. The ^{13}C–^{1}H HMBC NMR spectrum (Fig. 2A) clearly shows the correlation between the amide proton of the galactosyl residue at 9.55 ppm and the C=O carbon at 174.2 ppm; also a correlation is observed between the amide proton of the substituted glucopyranose A of β-cyclodextrin at 8.44 ppm and the carbonyl carbon at 174.1 ppm. Covalent coupling is confirmed by the presence of a correlation between the amide C=O of the β-cyclodextrin and the CH_2 (α to the C=O) of galactosyl antenna situated at 2.36 ppm. ^{1}H–^{1}H TOCSY has allowed attribution of the protons arising from the galactose (Fig. 2B) and those of glucopyranose A, which are displaced by the substitution (Fig. 2C).The presence of a series of doublets between 5.3 and 5.0 ppm characteristic of the anomeric protons demonstrates the loss of symmetry of the molecule and confirms the monosubstitution at the primary face the β-cyclodextrin molecule.

No interaction is observed between the galactosyl antennae and the H-3 and H-5 protons of β-cyclodextrin. The terminal hydrophilic group of the antennae makes it possible to avoid the well-known phenomenon in the literature of self-inclusion of chains alkyls of the monosubstituted β-CD.[17]

2.2. Interfacial properties

Langmuir compression isotherms were obtained for

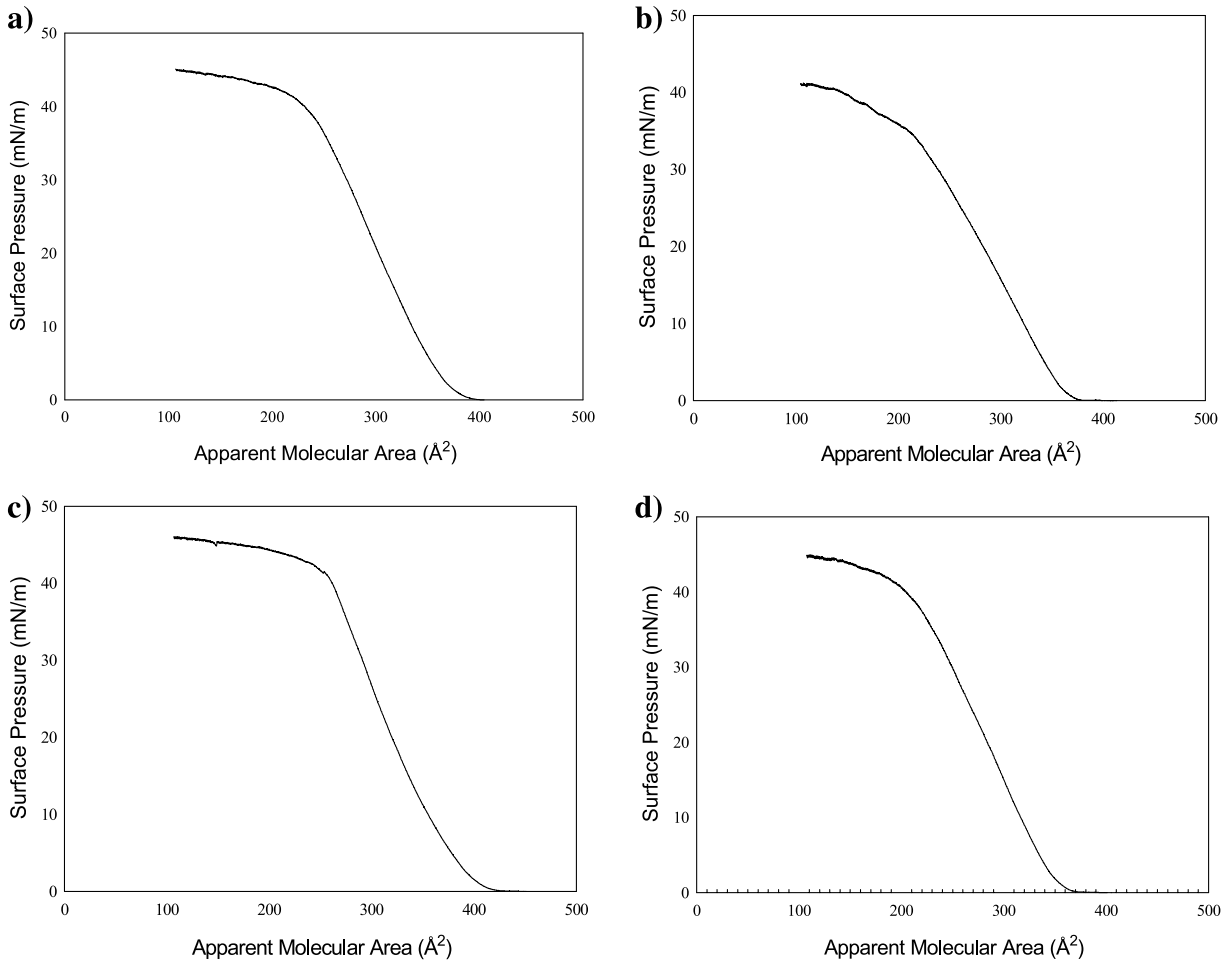


Figure 3. Langmuir isotherms of amphiphilic cyclodextrin derivatives: (a) **4**; (b) **5**; (c) **7** and (d) **8**.

Table 1. Apparent molecular areas, collapse pressures and compressibility of amphiphilic cyclodextrins Langmuir films

Amphiphilic cyclodextrin	A_0 ⟨Å^2⟩	A_1 ⟨Å^2⟩	A_c ⟨Å^2⟩	P_c ⟨mN/m⟩	C_s
Compound **4**	373 (±3)	366 (±3)	214 (±2)	36.6 (±0.2)	55.8 (±1)
Compound **5**	395 (±3)	378 (±3)	220 (±2)	41.3 (±0.2)	59 (±1)
Compound **7**	410 (±3)	390 (±3)	250 (±2)	42 (±0.2)	62 (±1)
Compound **8**	360 (±3)	350 (±3)	209 (±2)	41 (±0.2)	59 (±1)

A_0, apparent molecular area at $p=0.1$ mN/m; A_1, apparent molecular area for pressure = 1 mN/m; A_c, apparent molecular area at collapse; P_c, pressure at collapse; C_s = compressibility.

compounds **4**, **5** and **7** and are given along with that of the parent compound tetradecakis-O2, O3-hexanoyl-β-cyclodextrin **8**, Figure 3a–d, respectively. The relevant isotherm data is summarized in Table 1.

Molecules **4**, **5** and **8** show collapse areas in the range 205–220 Å^2, in close agreement with values previously observed for similar compounds by Lesieur[15a]. This lack of significant variance in the collapse area is in agreement with the fact that the molecular size of cyclodextrins acylated at the secondary face is determined by the area contribution from the 14 acyl chains[18], and not the cyclodextrin core. However, in the case of **7**, the presence of the galactosyl antenna leads to a collapse area of 250 Å^2. The collapse pressures show a variation **7**>**5**≥**8**>**4**, as would be expected from differences in the head group hydrophilicity, where introduction of a charged amino group **5** and especially the carbohydrate antenna **7** increases film stability, while introduction of an apolar, non-hydrogen bonding, azido function in **4** markedly decreases film stability.

3. Conclusion

We have synthesized a new generation of cyclodextrins presenting a galactosylated antenna on the primary face of β-cyclodextrin and *O*-acyls groups on the secondary face. These cyclodextrins are characterised by NMR and mass spectroscopy. The Langmuir isotherms show the amphiphilic character of these new molecules, and work is underway to study the self-assembly and transport properties of these molecules.

4. Experimental

4.1. General

All chemicals were purchased from Aldrich and were used without further purification. β-cyclodextrin was generously provided by Wacker (Lyon, France) and was dried under vacuum (10^{-2} T) at 120 °C for 48 h before use. ^{1}H and ^{13}C NMR experiments were performed at 300 and 75 MHz, respectively, using a Bruker DRX 300 spectrometer. 2D Experiments (COSY, TOCSY, HMBC) were recorded with a Bruker AM 500 spectrometer. Mass spectra were measured using a Perkin-Elmer Sciex spectrometer. IR spectra were recorded on a Perkin-Elmer instrument. Isotherms were carried out on a Langmuir type balance (Nima 610) using MilliQ water (resistivity >18 MΩ) as the subphase. Solutions of the molecules in $CHCl_3$ at suitable concentrations were deposited on the aqueous surface and allowed to equilibrate during 30 min to compression. Compressions were performed continuously at a rate of 20 cm^2 min^{-1} from 510 to 50 cm^2. Each isotherm was run at least three times to ensure areas of reproducibility of results (deviation of area and pressure were less than 3%).

4.1.1. Mono-6-azido-6-deoxy-hexakis(6-*O*-*tert*-butyldimethylsilyl)cyclomaltoheptaose 2. Mono-6-azido-6-deoxy-β-cyclodextrin **1** (5.5 g, 4.74 mmol) in dry pyridine (80 mL) is added a solution of *tert*-butyldimethylsilyl chloride (TBDMSCl) (6.43 g, 42.6 mmol) in 70 mL of dry pyridine at 0 °C. After agitation during 3 h at 0 °C and 20 h at 20 °C, the reaction is stopped by addition of ice (500 g). The crude product precipitated, filtered and recrystallised in water; 86% yield; mp 225 °C; $[\alpha]_D = +13.4$ (*c* 1); ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm): 6.55–6.82 (m, 7H, OH), 5.18–5.3 (m, 7H, OH), 4.87 (d, 1H, *J*=3.8 Hz, H-1), 4.96 (d, 6H, *J*=3.8 Hz, H-1), 4.05–3.6 (m, 42H, H-2, H-3, H-4, H-5, H-6), 0.88 (s, 54H, CH_3–C), 0.04 (s, 36H, CH_3–Si); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) −4.79 (C–Si), 18.68 (*C*–$(CH_3)_3$), 26.28 (C–(*C*$H_3)_3$), 52.51 (C_6–N_3), 62.77 (C_6–Si), 72.63 (C_3), 72.94 (C_2), 73.91 (C_5), 82.16 (C_4), 102.32 (C_1); ES-MS (+) *m/z*: 1867.7 $[M+Na]^+$; $C_{78}H_{153}N_3O_{34}Si_6$.

4.1.2. Mono-6-azido-6-deoxy-hexakis(6-*O*-*tert*-butyldimethylsilyl)-heptakis(2,3-di-*O*-hexanoyl)-cyclomaltoheptaose 3. The use of 56 equiv of hexanoic anhydride (71.7 mmol, 16.6 mL), 42 equiv of 4-dimethylaminopyridine (DMAP) (6.57 g, 53.7 mmol) in dry pyridine (40 mL) and 2.36 g (1.28 mmol) of **2** leads to the fully acylated product **3**. The mixture was stirred under nitrogen pressure at 70 °C for 48 h, then cooled at 25 °C and poured thereafter in 250 mL of distilled water. The organic layer was dried with Na_2SO_4 and concentrated to dryness to yield oil. The MeOH/CH_2Cl_2 95:5 solution is added in oil and the mixture is heated at 70 °C. The product **3** precipitate. 66.7% yield (2.75 g); mp 205 °C; $[\alpha]_D$ 15.3 (*c* 1); ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm): 5.5–5.3 (m, 7H, H-3), 5.2–5.05 (m, 7H, H-1), 4.85–4.59 (m, 7H, H-2), 3.6–4.02 (m, 28H, H-4, H-5, H-6), 2.55–2.1 (m, 28H, CH_2–COO), 1.15 (s, 56H, –C*H*$_2$–C*H*$_2$–CH_2–COO), 1.8 (s, 28H, CH_3–C*H*$_2$–), 1.05–0.75 [m, 96H, CH_3 and C–$(CH_3)_3$], 0.04 (s, 36H, CH_3–Si); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm): −4.6 (C–Si), 14.3 (CH_3), 18.6 (*C*–$(CH_3)_3$, 22.7 (–*C*H_2–CH_3), 24.8 (–CH_2–*C*H_2–CH_3), 26.3 (C–(*C*$H_3)_3$), 31.9 (–*C*H_2–CH_2–COO), 34.5 (–*C*H_2–COO), 52.1 (C_6–N_3), 62.1 (C_6–Si), 70.0–72.2 (C_2 and C_3), 72.4 (C_5), 77.7 (C_4), 96.7 (C_1), 172.1 (–CH2–*C*OO), 174.2 (–CH2–*C*OO); ES-MS (+) *m/z*: 1621.4 [**3**+H+ Na]$^{2+}$, 1629.9 [**3**+H+K]$^{2+}$; $C_{164}H_{293}N_3O_{48}Si_6$.

4.1.3. Mono-6-azido-6-deoxy-heptakis(2,3-di-*O*-hexanoyl)-cyclomaltoheptaose 4. To a stirred solution of **3** (2.75 g, 0.85 mmol) in anhydrous chloroform stabilized on

amylene (63 mL) was added 1.7 mL of $BF_3 \cdot Et_2O$. The mixture was stirred under N_2 at 25 °C for 23 h then poured into cold water (160 mL). The organic layer was removed and washed with water, $NaHCO_3$ and water then dried with Na_2SO_4. The organic layer was then concentrated to dryness and the residue purified by flash chromatography (eluent: cyclohexane/acetone 6:4). 97% Yield; mp 194 °C; $[\alpha]_D$ 12.4 (*c* 1); ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm): 0.9 (s, 42H, CH_3), 1.3 (s, 56H, –C*H*$_2$–C*H*$_2$–CH_2–COO), 1.6 (s, 28H, CH_3–C*H*$_2$–), 2.08–2.42 (m, 28H, CH_2–COO), 3.55–4.15 (m, 28H, H-4, H-5, H-6), 4.25–4.55 (m, 6H, OH), 4.65–4.8 (m, 7H, H-2), 5.01 (d, 1H, J=3.8 Hz, H-1), 5.12 (d, 5H, J=3.8 Hz, H-1), 5.18 (d, 1H, J=3.8 Hz, H-1), 5.29–5.45 (m, 7H, H-3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm): 14.30 (CH_3), 22.79 (–*C*H_2–CH_3), 24.75 (–*C*H_2–CH_2–CH_3), 31.77 (–*C*H_2–CH_2–COO), 34.36 (–*C*H_2–COO), 52.1 (C_6–N_3), 62.10 (C_6), 70.74 (C_2), 72.44 (C_3), 75.20 (C_5), 76.56 (C_4), 96.70 (C_1), 172.16 (–CH2–*C*OO), 173.72 (–CH2–*C*OO); ES-MS (+) *m/z*: 2556.6 [**4**+ Na]$^+$; $C_{128}H_{209}N_3O_{48}$.

4.1.4. Mono-6-amino-6-deoxy-heptakis(2,3-di-*O*-hexanoyl)-cyclomaltoheptaose 5. To a solution of **4** (5 g, 1.97 mmol) in MeOH (200 mL) was added 450 mg of the Pd/C (10%) under hydrogen pressure (2 bar) at 20 °C for 4 h. The mixture was filtered and washed with MeOH. Yield 95%; mp 175 °C; $[\alpha]_D$ 11.6 (*c* 1); IR (KBr): no band azido at ν 2100 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm): 0.9 (s, 42H, CH_3), 1.3 (s, 56H, –C**H$_2$**–C**H$_2$**–CH_2–COO), 1.56 (s, 28H, CH_3–C**H$_2$**–), 2.08–2.37 (m, 28H, CH_2–COO), 3.4–4.2 (m, 34H, H-4, H-5, H-6, OH-6), 4.7–4.79 (m, 7H, H-2), 5.01 (d, 1H, J=3.8 Hz, H-1), 5.12 (d, 5H, J=3.8 Hz, H-1), 5.18 (d, 1H, J=3.8 Hz, H-1), 5.29–5.45 (m, 7H, H-3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm): 14.30 (CH_3), 22.79 (–*C*H_2–CH_3), 24.75 (–*C*H_2–CH_2–CH_3), 31.77 (–*C*H_2–CH_2–COO), 34.36 (–*C*H_2–COO), 62.10 (C_6), 70.69 (C_2), 72.42 (C_3), 75.20 (C_5), 76.56 (C_4), 96.70 (C_1), 172.16 (–CH2–*C*OO), 173.72 (–CH2–*C*OO); ES-MS (+) *m/z*: 2531 [**5**+Na]$^+$; $C_{128}H_{211}NO_{48}$.

4.1.5. Mono-6-deoxy-6[9-(β-D-galactopyranosylamino)-1,9-dioxononanoyl]amino-heptakis(2,3-di-*O*-hexanoyl)-cyclomaltoheptaose 7. To a solution of **5** (0.9 g, 0.36 mmol) in dry DMF (18 mL) were added **6**[16] (1 equiv 0.13 g), hydroxybenzotriazole (HOBT, 1.2 equiv 0.058 g) and dicyclohexylcarbodiimide (DCC, 1.2 equiv 0.088 g). The mixture is then left under agitation at 25 °C, until appearance of a fine precipitate corresponding to the formation of dicyclohexylurea. After filtration, the organic solution was then concentrated to dryness and the residue purified by flash chromatography (eluent: EtOH/toluene 8:2). Yield 42%; mp 192 °C; $[\alpha]_D$ 16.2 (*c* 1); ^{1}H NMR (2D-COSY and TOCSY, pyridine-d_5, 500 MHz): δ (ppm): 0.99 (d, 42H, CH_3), 1.20 (m, 6H, 3 CH_2 antenna), 1.40 (m, 56H, –C**H$_2$**–C**H$_2$**–CH_2–COO), 1.57 (m, 4H, 2 CH_2 antenna), 1.70 (s, 28H, CH_3–C**H$_2$**–), 2.36 (m, 4H, 2 $CH_2\alpha$–C=O antenna), 2.4–2.7 (m, 28H, CH_2–COO), 4.0 (m, 1H, H-5^A), 4.15 (t, 1H, H-6^A), 4.2 (m, 1H, H-5 and H-6-gal), 4.35 (m, 1H, H-4^A), 4.55 (dd, 1H, H-6$'^A$), 4.3–4.5 (m, H-2, H-4, H-6, H-6′, H-5), 4.6–4.72 (m, H-gal), 5.22 (d, 1H, J=3.72 Hz, H-1^A), 5.0–5.3 (an series d, 6H, J=3.72 Hz, H-1), 5.82 (t, 1H, H-3^A), 5.90 (t, 1H, J=7.83 Hz, H-1-gal), 5.96–6.3 (m, 6H, H-3), 8.44 (t, 1H, J=5.8 Hz, NHβ-CD), 9.55 (d, 1H, J=9 Hz, NH-gal); ES-MS (+) *m/z*: 1442.5 [**7**+2Na]$^{2+}$, 2861.3 [**7**+Na]$^+$; $C_{1143}H_{236}N_2O_{55}$.

References and notes

1. Uekama, K.; Hirayama, F.; Irie, T. *Chem. Rev.* **1998**, *98*, 2045–2076.
2. Duchêne, D. *News Trends in Cyclodextrin and Derivatives*; Edition de Santé: Paris, 1991.
3. Szejtli, J. *Med. Res. Rev.* **1994**, *14*, 353–386.
4. Szejtli, J. *Cyclodextrin Technology*; Kluwer Academic: Dordrecht, 1998.
5. Attioui, F.; Al-Omar, A.; Leray, E.; Parrot-Lopez, H.; Finance, C.; Bonaly, R. *Biol. Cell.* **1994**, *82*, 161–167.
6. Ortiz-Mellet, C.; Defaye, J.; Garcia-Fernandez, J. M. *Chem. Eur. J.* **2002**, *8*, 1982–1990. Furuike, T.; Aiba, S.; Nishimura, S. I. *Tetrahedron* **2000**, *56*, 9909–9915.
7. Sallas, F.; Niikura, K.; Nishimura, S. I. *Chem. Commun.* **2004**, 596–597.
8. (a) Tanaka, M.; Azumi, R.; Tachibana, H.; Nakamura, T.; Kawabata, Y.; Matsumoto, M.; Miyasaka, T.; Tagaki, W.; Nakahara, H.; Fukuda, K. *Thin Solid Films* **1994**, *244*, 832–835. (b) Greenhall, M.; Lukes, P.; Kataky, R.; Agbor, N. E.; Badyal, J. P.; Yarwood, J.; Parker, D.; Petty, M. C. *Langmuir* **1995**, *11*, 3997–4000. (c) Zhang, P.; Ling, C. C.; Coleman, A. W.; Parrot-Lopez, H.; Galons, H. *Tetrahedron Lett.* **1991**, *32*, 2769–2770. (d) Mazzaglia, A.; Donohue, R.; Ravoo, B. J.; Darcy, R. *Eur. J. Org. Chem.* **2001**, 1715–1721. (e) Granger, C.; Félix, C.; Parrot-Lopez, H.; Langlois, B. *Tetrahedron Lett.* **2000**, *41*, 9257–9260. (f) Sukegawa, T.; Furuike, T.; Niikura, K.; Yamagishi, A.; Monde, K.; Nishimura, S. I. *Chem. Commun.* **2002**, 430–431. (g) Peroche, S.; Parrot-Lopez, H. *Tetrahedron Lett.* **2003**, *44*, 241–245.
9. Auzély-Velty, R.; Perly, B.; Taché, O.; Zemb, T.; Jéhan, P.; Guenot, P.; Dalbiez, J. P.; Djedaïni-Pilard, F. *Carbohydrate Res.* **1999**, *318*, 82–90.
10. Perrier, E.; Terry, N.; Rival, D.; Coleman, A. W. Jpn. Kokai Tokkyo Koho 2001,323,002, 2001.
11. Ravoo, B. J.; Darcy, R. *Angew. Chem., Int. Ed.* **2000**, *39*, 4324–4326.
12. Mazzaglia, A.; Ravoo, B. J.; Darcy, R.; Gambadauro, P.; Mallamace, F. *Langmuir* **2002**, *18*, 1945–1948.
13. Dubes, A.; Parrot-Lopez, H.; Abdelwahed, W.; Degobert, G.; Fessi, H.; Shahgaldian, P.; Coleman, A. W. *Eur. J. Pharm. Biopharm.* **2003**, *55*, 279–282.
14. Tsujihara, K.; Kurita, H.; Kawazu, M. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 1567.
15. (a) Lesieur, S.; Charon, D.; Lesieur, P.; Ringard-Lefebvre, C.; Muguet, V.; Duchêne, D.; Wouessidjewe, D. *Chem. Phys. Lipids* **2000**, *106*, 127–144. (b) Dubes, A.; Bouchu, D.; Lamartine, R.; Parrot-Lopez, H. *Tetrahedron Lett.* **2001**, *42*, 9147–9151.
16. Kassab, R.; Félix, C.; Parrot-Lopez, H.; Bonaly, R. *Tetrahedron Lett.* **1997**, *38*, 7555–7558.
17. Petter, R. C.; Salek, J. C.; Sikorski, C. T.; Kumaravel, G.; Lin, F. T. *J. Am. Chem. Soc.* **1990**, *112*, 3860–3868.
18. Dubes, A.; Parrot-Lopez, H.; Shahgaldian, P.; Coleman, A. W. *J. Colloid Interface Sci.* **2003**, *259*, 103–111.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron 61 (2005) 8746–8751
Tetrahedron

Ring opening of 2-(bromomethyl)-1-sulfonylaziridines towards 1,3-heteroatom substituted 2-aminopropane derivatives

Matthias D'hooghe, Mario Rottiers, Inge Kerkaert and Norbert De Kimpe*

Department of Organic Chemistry, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium

Received 12 April 2005; revised 10 June 2005; accepted 13 June 2005

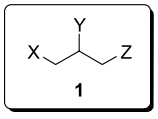
Available online 25 July 2005

Abstract—1,3-Heteroatom substituted 2-aminopropane derivatives have been prepared from 2-(bromomethyl)-1-sulfonylaziridines for the first time using sodium azide or different potassium phenoxides in water in the presence of silica gel. The applicability of 1-arenesulfonyl-2-(bromomethyl)aziridines for the synthesis of functionalized sulfonamides has also been demonstrated towards different 1,3-dialkoxy-2-(tosylamino)propanes and 1,3-dialkylthio-2-(tosylamino)propanes upon treatment with the appropriate sodium alkoxide or sodium alkylthiolate in the corresponding alcohol or in methanol, respectively.

1. Introduction

A key element present in a whole range of physiologically active natural products and their synthetic analogues comprises a 1,2,3-heteroatom substituted three-carbon unit **1** (Fig. 1; X, Y, Z=O, N, S). Many drugs accommodate such a moiety in their structure, hence the interest in the development of new entries towards compounds bearing a 1,2,3-trisubstituted propane skeleton. Aryloxypropanolamines (X=OAr, Y=OH, Z=NHR, Fig. 1) are used as β-blockers for the treatment of hypertension, angina pectoris, glaucoma, obesity, and arrhythmia,[1] but also as antidiabetic,[2] antihypertensive and vasorelaxing agents.[3] Sphingolipids (X=Z=OH, Y=NH_2, Fig. 1), membrane compounds of essentially all eukaryotic cells, comprise a 2-amino-1,3-dihydroxypropane subunit as part of a longer (unsaturated) carbon chain.[4] Also 2-[(arylmethyl)amino]-propanediols (X=Z=OH, Y=NHR, Fig. 1) or shortly AMAP's have been reported as antitumor DNA intercalators with promising prospects in medicine.[5] Moreover, compounds containing a 2-amino-1,3-propanediol subunit are important constituents of broad-spectrum antibiotics such as thiamphenicol and florfenicol.[6] Finally, also some sulfur containing analogues are known for their biological activity, especially in agriculture as pest control agents (acaricides and insecticides).[7–9]

In recent years, the demand for environmentally benign processes has become an important element in the design of new synthetic methodologies. From an ecological point of view, organic reactions in aqueous media constitute an attractive alternative for the use of classical solvents. The present report describes an efficient approach towards 1,3-difunctionalized 2-aminopropane derivatives in water as a solvent in the presence of silica gel, starting from 2-(bromomethyl)-1-sulfonylaziridines, a versatile but fairly unknown class of substrates in organic synthesis. The molecular diversity of these aziridines originates from the presence of two electrophilic moieties, namely two carbon atoms of the aziridine ring on the one hand and the halogenated carbon atom on the other hand, which enables a variety of different synthetic transformations towards cyclic and acyclic target compounds. It has already been demonstrated in the literature that the closely related 1-tosyl-2-(tosyloxymethyl)aziridines suffer from ring opening upon treatment with organocuprate reagents by attack at the least hindered carbon atom of the aziridine moiety, immediately followed by ring closure by displacement of the tosylate in a straightforward manner.[10] Also for *N*,*O*-bis(diphenylphosphinyl)-2-(hydroxymethyl)aziridine, an aziridine with a different electron-withdrawing substituent at nitrogen, comparable results were published.[11]



X, Y, Z = heteroatoms
e.g. O, N, S

Figure 1.

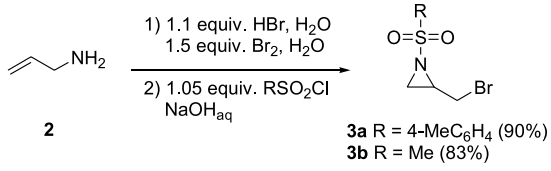
Keywords: 2-(Bromomethyl)aziridines; 2-Aminopropanes; Water; Ring opening; Nucleophiles.
* Corresponding author. Tel.: +32 92645951; fax: +32 92646243; e-mail: norbert.dekimpe@ugent.be

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.06.036

1-Arenesulfonyl-2-(bromomethyl)aziridines can be applied successfully as synthetic equivalents for the 2-aminopropane dication synthon upon treatment with lithium cuprate reagents towards α-branched *N*-tosylamides.[12] In the present report, the 2-aminopropane dication synthon equivalency of 2-(bromomethyl)-1-sulfonylaziridines will be further evaluated upon treatment with heteroatom centered nucleophiles. In the literature, a limited number of reports dealing with ring opening reactions of 2-(alkoxymethyl)aziridines and 2-(hydroxymethyl)aziridines towards 2-amino-1,3-dioxypropanes have been reported up to now.[13] Only one example of the conversion of an aziridine into a 1,3-dialkylthio-2-aminopropane derivative has been published, in which *N*,*O*-bis(diphenylphosphinyl)-2-(hydroxymethyl)aziridine was treated with 3 equiv of lithium thiophenoxide in THF at −42 °C, giving rise to 2-(diphenylphosphinamido)-1,3-bis(thiophenyl)propane in 70% yield.[11]

2. Results and discussion

2-(Bromomethyl)-1-sulfonylaziridines **3** are very easily accessible, and thus very attractive starting materials in organic synthesis.[14] 2-(Bromomethyl)-1-(4-methylbenzenesulfonyl)aziridine **3a** and 2-(bromomethyl)-1-(methanesulfonyl)aziridine **3b** were prepared from allylamine **2** in a very efficient two-step procedure adapted from the literature (Scheme 1).[15]



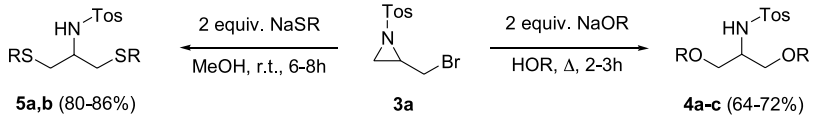
Scheme 1.

When 2-(bromomethyl)-1-tosylaziridine **3a** was treated with 2 equiv of an alkoxide or an alkylthiolate, the corresponding ring opened 1,3-dialkoxy- or 1,3-dialkylthiopropanamines **4** and **5** were isolated in high yield (Scheme 2). *N*-(2-Methoxy-1-(methoxymethyl)ethyl)-4-methylbenzenesulfonamide **4a** and *N*-(2-ethoxy-1-(ethoxymethyl)ethyl)-4-methylbenzenesulfonamide **4b** were obtained upon treatment of aziridine **3a** with 2 equiv of sodium methoxide, respectively, sodium ethoxide, in the corresponding alcohol (2 N) after reflux for 3 h (Table 1). Similarly, *N*-(2-(2-propenyloxy)-1-((2-propenyloxy)methyl)ethyl)-4-methylbenzenesulfonamide **4c** was prepared in 64% yield utilizing 2 equiv of sodium allyloxide in refluxing allyl alcohol (1 N). The sulfur analogues **5a**,**b** of the former dialkoxypropane derivatives were synthesized by treatment of 2-(bromomethyl)-1-tosylaziridine **3a** with 2 equiv of sodium isopropylthiolate or sodium allylthiolate in methanol (0.3 N) at room temperature for 6–8 h, affording *N*-(2-((1-methylethyl)thio)-1-(((1-methylethyl)thio)methyl)ethyl)-4-methylbenzenesulfonamide **5a** and *N*-(2-(2-propenylthio)-1-((2-propenylthio)methyl)ethyl)-4-methylbenzenesulfonamide **5b** in high yields (Table 1).

Very recently, a new protocol for the synthesis of 1-tosylaziridines in water as a solvent in the presence of silica has been reported, exploiting the adsorptive nature of silica gel.[16] Also the ring opening of 1-tosyl-2-hexylaziridine with sodium azide in water in the presence of silica was mentioned in the same reference. This reaction did not proceed in the absence of silica. The applicability of this approach using 2-(bromomethyl)-1-tosylaziridines **3**, however, has not been investigated up to now.

An attempt to prepare 2-(azidomethyl)-1-tosylaziridine **6** selectively by reaction of 1-tosyl-2-bromomethyl)aziridine **3a** with 1 equiv of sodium azide in water failed, since this aziridine **6** was further consumed as a substrate in a ring opening reaction with azide, probably due to activation of the aziridine ring by silica. In this way, a mixture of unreacted 2-(bromomethyl)-1-tosylaziridine **3a** (44%), 2-(azidomethyl)-1-tosylaziridine **6** (25%) and tosylamide **7a** (31%) was obtained (Scheme 3).

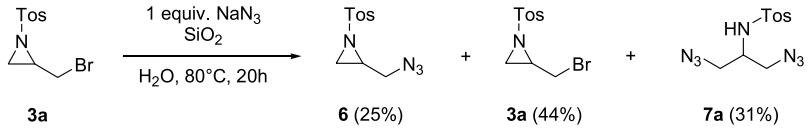
Consequently, the diazido compounds **7a**–**b** were obtained very easily and in high yields after treatment of 2-(bromomethyl)aziridines **3** with 2 equiv of sodium azide in water in the presence of silica (0.5 g for 1 mmol of substrate) (Caution: the azido compounds should be handled with care, utilizing a safety shield). After heating for 16–20 h at 80 °C, *N*-(2-azido-1-(azidomethyl)ethyl)-4-methylbenzenesulfonamide **7a** and *N*-(2-azido-1-(azidomethyl)ethyl)-4-methanesulfonamide **7b** were isolated as the sole reaction products (Scheme 4).



Scheme 2.

Table 1. Ring opening reactions of 2-(bromomethyl)-1-tosylaziridine **3a** with alkoxides and alkylthiolates

Entry	Conditions	Product	R	Yield (%)
1	2 equiv NaOMe/MeOH (2 N), △, 3 h	**4a**	Me	72
2	2 equiv NaOEt/EtOH (2 N), △, 3 h	**4b**	Et	77
3	2 equiv $NaOCH_2CH{=}CH_2$/allyl alcohol (1 N), △, 2 h	**4c**	$CH_2CH{=}CH_2$	64
4	2 equiv NaSiPr/MeOH (0.3 N), rt, 6 h	**5a**	*i*Pr	80
5	2 equiv $NaSCH_2CH{=}CH_2$/MeOH (0.3 N), rt, 8 h	**5b**	$CH_2CH{=}CH_2$	86

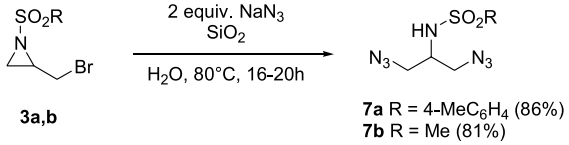


3a — 6 (25%) — 3a (44%) — 7a (31%)

Scheme 3.

A stepwise approach based on this methodology enables the synthesis of 2-aminopropane derivatives with a different substitution pattern at C1 and C3. 2-(Bromomethyl)-1-tosylaziridine **3a** was converted into the corresponding 2-(methoxymethyl)aziridine **8** upon reaction with 1.05 equiv of NaOMe in MeOH (2 N) after stirring at room temperature for 5 h (Scheme 5). This reaction proceeds through ring opening and subsequent ring closure of the resulting β-bromosulfonamide. Treatment of the latter aziridine **8** with 2 equiv of sodium azide in water in the presence of silica gel afforded *N*-(2-azido-1-(methoxymethyl)ethyl)-4-methylbenzenesulfonamide **9** after heating for 20 h at 80 °C (Scheme 5).

Furthermore, also other water-compatible nucleophiles besides sodium azide have been evaluated using this water–silica based methodology. Oxygen-centered nucleophiles such as potassium phenoxides were applied successfully towards 1,3-diaryloxy-2-aminopropane derivatives **10** in good yields upon treatment of 2-(bromomethyl)aziridines **3** with 2.2 equiv of a substituted phenol in water in the presence of silica (0.5 g for 1 mmol of substrate) and 5 equiv of potassium carbonate after heating for 16–20 h at 80 °C (Scheme 6). When the same reactions were performed with less then 5 equiv of K_2CO_3 (e.g. 2 equiv), the intermediate 2-(aryloxymethyl)aziridines were also isolated (25%), besides the desired 1,3-diaryloxy-2-aminopropane derivatives **10** (75%). The use of phenoxides clearly extends the scope of the presented water–silica based methodology towards a large variety of possible target compounds, and water can be used as a suitable green alternative for other solvents in ring opening reactions of 2-(bromomethyl)-1-sulfonylaziridines with heteroatom centered nucleophiles.



3a,b

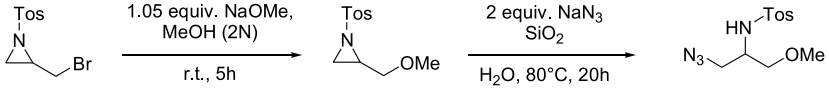
7a R = 4-MeC$_6$H$_4$ (86%)
7b R = Me (81%)

Scheme 4.

In summary, the versatility of 2-(bromomethyl)-1-sulfonylaziridines **3** as substrates in organic synthesis has been demonstrated by the development of a straightforward approach towards different 1,3-heteroatom substituted 2-aminopropane derivatives using water as a solvent in the presence of silica gel. In this way, monoazido- and diazidopropane derivatives have been synthesized using sodium azide in water, as well as 1,3-diaryloxypropane sulfonamides by means of different potassium phenoxides as reagents. The potential of 2-(bromomethyl)-1-sulfonylaziridines has been further demonstrated by the synthesis of 1,3-dialkoxy-2-(tosylamino)propanes and 1,3-dialkylthio-2-(tosylamino)propanes upon treatment with the appropriate sodium alkoxide or sodium alkylthiolate. As plentiful methods are available for *N*-detosylation of sulfonamides, the presented methodology offers a suitable access to the synthesis of the corresponding amines.

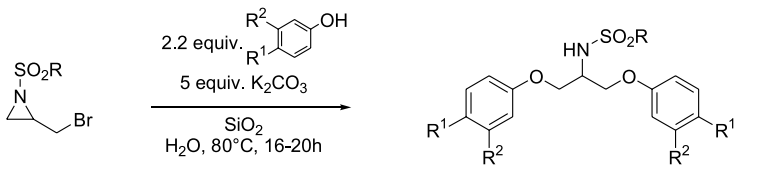
3. Experimental

^{1}H NMR spectra were recorded at 270 MHz (JEOL JNM-EX 270) or at 300 MHz (JEOL ECLIPSE+) with $CDCl_3$ as solvent and tetramethylsilane as internal standard. ^{13}C NMR spectra were recorded at 68 MHz (JEOL JNM-EX 270) or at 75 MHz (JEOL ECLIPSE+) with $CDCl_3$ as solvent. Mass spectra were obtained with a mass spectrometer (VARIAN MAT 112, 70 eV using a GC–MS coupling (RSL 200, 20 m glass capillary column, i.d. 0.53 mm, He carrier gas) or AGILENT 1100, 70 eV. IR spectra were measured with a



3a — 8 (52%) — 9 (74%)

Scheme 5.



3a,b

10a R = Me, R^1 = H, R^2 = Cl (81%)
10b R = 4-MeC$_6$H$_4$, R^1 = F, R^2 = H (84%)
10c R = 4-MeC$_6$H$_4$, R^1 = OMe, R^2 = H (72%)

Scheme 6.

Spectrum One FT-IR spectrophotometer. Dichloromethane was distilled over calcium hydride, other solvents were used as received from the supplier.

3.1. Synthesis of 1,3-dialkoxy-2-(tosylamino)propanes 4a–c

As a representative example, the synthesis of *N*-(2-ethoxy-1-(ethoxymethyl)ethyl)-4-methylbenzenesulfonamide **4b** is described. To 2-(bromomethyl)-1-(4-methylbenzenesulfonyl)aziridine **3a** (0.29 g, 1 mmol) was added sodium methoxide in methanol (1.0 mL, 2 equiv, 2 N in MeOH), and the resulting mixture was heated under reflux for 4 h. The reaction mixture was poured into water (20 mL), extracted with CH_2Cl_2 (3×10 mL) and dried (K_2CO_3). Filtration of the drying agent and evaporation of the solvent afforded *N*-(2-ethoxy-1-(ethoxymethyl)ethyl)-4-methylbenzenesulfonamide **4b** (0.23 g, 77%). These compounds were purified by means of column chromatography: (EtOAc/Hexane 1:4). Purity (NMR) >95%.

3.1.1. *N*-(2-Methoxy-1-(methoxymethyl)ethyl)-4-methylbenzenesulfonamide 4a. Yie1d 72%, colorless liquid. ^{1}H NMR (270 MHz, $CDCl_3$): δ 2.41 (3H, s, CH_3Ar); 3.21 (6H, s, 2×CH_3O); 3.23–3.38 (4H, m, 2×CH_2O); 3.40–3.47 (1H, m, CHN); 5.35 (1H, s(broad), NH); 7.30 and 7.78 (2×2H, 2×d, J=8.3 Hz, C_6H_4). ^{13}C NMR (68 MHz, $CDCl_3$): δ 21.49 (CH_3Ar); 52.36 (CHN); 58.87 (2×CH_3O); 71.18 (2×CH_2O); 127.08 and 129.58 (2×HC_{ortho} and 2×HC_{meta}); 137.93 (CH_3C); 143.30 ($C_{arom,quat}$). IR (NaCl, cm^{-1}): ν=3270 (NH), 1609, 1452, 1330, 1165. MS (70 eV) *m/z* (%): 273 (M^+, 1); 229 (81); 171 (31); 155 (44); 139 (81); 91 (100); 73 (67); 65 (27); 45 (60). Anal. Calcd for $C_{12}H_{19}NO_4S$ (%): C 52.73; H 7.01; N 5.12. Found (%): C 52.90; H 7.19; N 4.90.

3.1.2. *N*-(2-Ethoxy-1-(ethoxymethyl)ethyl)-4-methylbenzenesulfonamide 4b. Yie1d 77%, colorless liquid. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.11 (6H, t, J=7.3 Hz, 2×CH_3CH_2); 2.43 (3H, s, CH_3Ar); 3.29–3.48 (9H, m, 4×CH_2O and CHN); 5.05 (1H, s(broad), NH); 7.30 and 7.79 (2×2H, 2×d, J=8.0 Hz, C_6H_4). ^{13}C NMR (68 MHz, $CDCl_3$): δ 14.99 (2×CH_3CH_2); 21.49 (CH_3Ar); 52.61 (CHN); 66.58 (2×CH_3CH_2O); 69.00 (2×$CH_3CH_2OCH_2$); 127.13 and 129.56 (2×HC_{ortho} and 2×HC_{meta}); 137.84 (CH_3C); 143.29 ($C_{arom,quat}$). IR (NaCl, cm^{-1}): ν=3277 (NH), 2978, 2878, 1598, 1457, 1423, 1332. MS (70 eV) *m/z* (%): 302 (M^+, 7); 245 (26); 244 (50); 243 (94); 215 (21); 210 (12); 186 (17); 185 (80); 157 (38); 156 (23); 155 (90); 146 (11); 141 (16); 140 (32); 139 (94); 133 (12); 117 (16); 107 (11); 106 (13); 105 (10); 102 (52); 100 (13); 92 (57); 91 (100); 87 (92); 86 (87); 77 (21); 65 (83). Anal. Calcd for $C_{14}H_{23}NO_4S$ (%): C 55.79; H 7.69; N 4.65. Found (%): C 55.96; H 7.88; N 4.48.

3.1.3. *N*-(2-(2-Propenyloxy)-1-((2-propenyloxy)methyl)ethyl)-4-methylbenzenesulfonamide 4c. Yie1d 64%, colorless crystals. Mp 42 °C. Flash chromatography on silica gel: EtOAc/Hexane 1:4, R_f=0.25. ^{1}H NMR (270 MHz, $CDCl_3$): δ 2.42 (3H, s, CH_3Ar); 3.30–3.36 (2H, m, 2×NCH(*H*CH)); 3.46–3.49 (3H, m, 2×NCH(HC*H*) and CHN); 3.86 (4H, d, J=5.3 Hz, 2×CH_2CH=CH_2); 5.07 (1H, s(broad), NH); 5.12–5.21 (4H, m, 2×CH=CH_2); 5.71–5.86 (2H, m, 2×C*H*=CH_2); 7.29 and 7.77 (2×2H, 2×d, J=8.1 Hz, C_6H_4). ^{13}C NMR (68 MHz, $CDCl_3$): δ 21.49 (CH_3Ar); 52.60 (CHN); 68.62 (2×NCH*C*H_2); 71.98 (2×*C*H_2CH=CH_2); 117.05 (2×CH=*C*H_2); 127.08 and 129.58 (2×HC_{ortho} and 2×HC_{meta}); 134.23 (2×*C*H=CH_2); 137.73 (CH_3C); 143.30 ($C_{arom,quat}$). IR (NaCl, cm^{-1}): ν=3296 (NH), 1647, 1598, 1485, 1456, 1329, 1161, 1092. MS (70 eV) *m/z* (%): 325 (M^+, 0.3); 254 (100); 195 (16); 170 (6); 155 (60); 91 (96); 82 (19); 71 (4); 65 (13). Anal. Calcd for $C_{16}H_{23}NO_4S$ (%): C, 9.05; H, 7.12; N, 4.30. Found (%): C, 58.83; H, 7.28; N, 4.21.

3.2. Synthesis of 1,3-dialkylthio-2-(tosylamino)propanes 5a,b

As a representative example, the synthesis of *N*-(2-((1-methylethyl)thio)-1-(((1-methylethyl)thio)methyl)ethyl)-4-methylbenzenesulfonamide **5a** is described. To sodium methoxide (12 mL, 4 mmol, 2 equiv, 0.33 N in MeOH) was added propane-2-thiol (0.46 g, 6 mmol, 3 equiv), and the mixture was stirred for 30 min at room temperature. Subsequently, a solution of 2-(bromomethyl)-1-(4-methylbenzenesulfonyl)aziridine **3a** (0.58 g, 2 mmol) in methanol (2 mL) was added to the mixture. After stirring for 6 h at room temperature, the reaction mixture was poured into water (50 mL), extracted with CH_2Cl_2 (3×25 mL) and dried ($MgSO_4$). Filtration of the drying agent and evaporation of the solvent afforded the crude *N*-(2-((1-methylethyl)thio)-1-(((1-methylethyl)thio)methyl)ethyl)-4-methylbenzenesulfonamide **5a**, which was purified by means of column chromatography (EtOAc/Hexane 1:4, R_f=0.43).

3.2.1. *N*-(2-((1-Methylethyl)thio)-1-(((1-methylethyl)thio)methyl)ethyl)-4-methylbenzenesulfonamide 5a. Yie1d 80%, colorless liquid. Flash chromatography on silica gel: EtOAc/Hexane 1:4, R_f=0.43. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.13 and 1.17 (12H, 2×d, J=6.6 Hz, 2×CH(CH_3)$_2$); 2.41 (3H, s, CH_3Ar); 2.62 (2H, d×d, J=13.7, 6.8 Hz, 2×(*H*CH)S); 2.69 (2H, sept, J=6.6 Hz, 2×C*H*(CH_3)$_2$); 2.82 (2H, d×d, J=13.7, 5.4 Hz, 2×(HC*H*)S); 3.35 (1H, m, CHN); 5.42 (1H, d, J=6.6 Hz, NH); 7.31 and 7.80 (2×2H, 2×d, J=8.3 Hz, C_6H_4). ^{13}C NMR (68 MHz, $CDCl_3$): δ 21.47 (CH_3Ar); 23.18 and 23.23 (2×CH(*C*H_3)$_2$); 34.21 (2×CH_2S); 35.74 (CHS); 52.88 (CHN); 127.24 and 129.61 (2×HC_{ortho} and 2×HC_{meta}); 137.10 (CH_3C); 143.47 ($C_{arom,quat}$). IR (NaCl, cm^{-1}): ν=3276 (NH), 1598, 1495, 1452, 1337, 1160. MS (70 eV) *m/z* (%): 361 (M^+, 4); 360 (18); 319 (8); 272 (100); 230 (25); 190 (27); 157 (32); 155 (49); 139 (31); 117 (47); 105 (27); 91 (52); 89 (24); 74 (35); 65 (15); 43 (24). Anal. Calcd for $C_{16}H_{27}NO_2S_3$(%): C, 53.15; H, 7.53; N, 3.87. Found (%): C, 53.31; H, 7.70; N, 3.70.

3.2.2. *N*-(2-(2-Propenylthio)-1-((2-propenylthio)methyl)ethyl)-4-methylbenzenesulfonamide 5b. Yie1d 86%, colorless liquid. Flash chromatography on silica gel: EtOAc/Hexane 1:4, R_f=0.25. ^{1}H NMR (270 MHz, $CDCl_3$): δ 2.44 (3H, s, CH_3Ar); 2.62 and 2.71 (4H, 2×d×d, J=13.8, 6.6, 5.2 Hz, 2×NCH(*HC*H)); 2.89–3.00 (4H, m, 2×SCH_2CH=CH_2); 3.36–3.45 (1H, m, NCH); 5.02–5.12 (5H, m, 2×CH=CH_2 and NH); 5.60–5.75 (2H, m, 2×C*H*=CH_2); 7.33 and 7.78 (2×2H, 2×d, J=8.4 Hz, C_6H_4). ^{13}C NMR (68 MHz, ref=$CDCl_3$): δ 21.44 (CH_3Ar);

34.67 and 35.11 (4×CH_2S); 52.06 (CHN); 117.68 (2×CH=CH_2); 127.21 and 129.60 (2×HC_{ortho} and 2×HC_{meta}); 133.64 (2×CH=CH_2); 137.22 (CH_3C); 143.52 ($C_{arom,quat}$). IR (NaCl, cm^{-1}): ν=3279 (NH), 1635, 1598, 1495, 1407, 1331, 1160. MS (70 eV) *m/z* (%): 357 (M^+, 1); 202 (4); 201 (54); 155 (74); 91 (100); 65 (21). Anal. Calcd for $C_{16}H_{23}NO_2S_3$ (%): C, 53.75; H, 6.48; N, 3.92. Found (%): C, 53.96; H, 6.63; N, 3.81.

3.3. Ring opening of 1-tosylaziridines with sodium azide in water

As a representative example, the synthesis of *N*-(2-azido-1-(azidomethyl)ethyl)-4-methylbenzenesulfonamide **7a** is described. 2-(Bromomethyl)-1-(4-methylbenzenesulfonyl) aziridine **3a** (1.45 g, 5 mmol), sodium azide (0.66 g, 2 equiv) and silica gel (2.5 g) were suspended in water (7.5 mL) and stirred at 80 °C for 16 h. The reaction mixture was filtered over Celite and the filter cake was washed with CH_2Cl_2 (2×20 mL). Isolation of the organic phase, extraction of the water phase with CH_2Cl_2 (20 mL), drying of the combined organic extracts ($MgSO_4$), filtration and evaporation of the solvent afforded the crude *N*-(2-azido-1-(azidomethyl)ethyl)-4-methylbenzenesulfonamide **7a**, which was recrystallized from methanol, yielding pure **7a** (1.27 g, 86%).

3.3.1. *N*-(2-Azido-1-(azidomethyl)ethyl)-4-methylbenzenesulfonamide 7a. Yie1d 86%, colorless crystals. Mp 77.8 °C. Recrystallized from methanol. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.45 (3H, s, CH_3Ar); 3.27–3.50 (5H, m, 2×CH_2N_3 and CHN); 5.06 (1H, s(broad), NH); 7.33–7.36 and 7.76–7.80 (2×2H, 2×m, C_6H_4). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.57 (CH_3Ar); 51.91 (2×CH_2N_3); 52.23 (CHN); 127.11 and 129.98 (2×HC_{ortho} and 2×HC_{meta}); 137.24 (CH_3C); 144.09 ($C_{arom,quat}$). IR (NaCl, cm^{-1}): ν= 3262 (NH), 2106 (N_3), 2936, 2863, 1597, 1449, 1328, 1162, 1093, 733. MS (70 eV) *m/z* (%): 296 (M^++1, 54); 239 (56); 225 (39); 155 (100). Anal. Calcd for $C_{10}H_{13}N_7O_2S$ (%): C, 40.67; H, 4.44; N, 33.20. Found (%): C, 40.85; H, 4.67; N, 33.47.

3.3.2. *N*-(2-Azido-1-(azidomethyl)ethyl)methanesulfonamide 7b. Yie1d 81%, colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.07 (3H, s, CH_3); 3.53–3.55 (4H, m, 2×CH_2N_3); 3.64–3.68 (1H, m, CHN); 5.04 (1H, s(broad), NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 41.73 (CH_3); 52.78 (2×CH_2N_3); 52.97 (CHN). IR (NaCl, cm^{-1}): ν=3277 (NH), 2106 (N_3), 2936, 2874, 1443, 1319, 1154, 993. MS (70 eV) *m/z* (%): no M^+, 218 (7), 149 (30); 87 (63). Anal. Calcd for $C_4H_9N_7O_2S$ (%): C, 21.91; H, 4.14; N, 44.72. Found (%): C, 22.07; H, 4.01; N, 44.93.

3.3.3. *N*-(2-Azido-1-(methoxymethyl)ethyl)-4-methylbenzenesulfonamide 9. Yie1d 74%, colorless crystals. Mp 67–68 °C. Flash chromatography on silica gel: EtOAc/Hexane 1:4, R_f=0.14. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.43 (3H, s, CH_3Ar); 3.18–3.51 (5H, m, CH_2N_3, CH_2O and CHN); 3.24 (3H, s, CH_3O); 5.15–5.27 (1H, m, NH); 7.29–7.33 and 7.74–7.84 (2×2H, 2×m, C_6H_4). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.55 (CH_3Ar); 51.74 (CH_2N_3); 52.51 (CHN); 58.97 (CH_3O); 71.05 (CH_2O); 127.09 and 129.80 (2×HC_{ortho} and 2×HC_{meta}); 137.54 (CH_3C); 143.74 ($C_{arom,quat}$). IR (NaCl, cm^{-1}): ν=3257 (NH), 2102 (N_3), 2935, 2898, 2832, 1596, 1416, 1326, 1162, 1086, 983, 820, 682. MS (70 eV) *m/z* (%): no M^+; 239 (M^+-CH_2OMe, 9); 228 (28); 155 (49); 139 (30); 91 (100); 65 (20); 45 (19). Anal. Calcd for $C_{11}H_{16}N_4O_3S$ (%): C 46.47; H 5.67; N 19.70. Found (%): C 46.61; H 5.83; N 19.59.

3.3.4. 2-(Methoxymethyl)-1-(4-methylbenzenesulfonyl) aziridine 8. To 2-(bromomethyl)-1-(4-methylbenzenesulfonyl)aziridine **3a** (0.29 g, 1 mmol) was added sodium methoxide in methanol (0.53 mL, 1.05 equiv, 2 N in MeOH), and the resulting mixture was stirred for 5 h at room temperature. The reaction mixture was poured into water (20 mL), extracted with CH_2Cl_2 (3×10 mL) and dried ($MgSO_4$). Filtration of the drying agent and evaporation of the solvent afforded the crude 2-(methoxymethyl)-1-(4-methylbenzenesulfonyl)aziridine **8**, which was purified by means of column chromatography (EtOAc/Hexane 1:4, R_f=0.08).

Yie1d 52%, colorless liquid. Flash chromatography on silica gel: EtOAc/Hexane 1:4, R_f=0.08. ^{1}H NMR (270 MHz, $CDCl_3$): δ 2.20 (1H, d, J=4.3 Hz, (H_{trans}CH)N); 2.43 (3H, s, CH_3Ar); 2.61 (1H, d, J=7.2 Hz, (HCH_{cis})N); 2.92–3.01 (1H, m, CHN); 3.29 (3H, s, CH_3O); 3.38 and 3.53 (2H, 2×d×d, J=6.9, 4.3, 1.4 Hz, (HCH)O); 7.32 and 7.81 (2×2H, 2×d, J=8.1 Hz, C_6H_4). ^{13}C NMR (68 MHz, $CDCl_3$): δ 21. 62 (CH_3Ar); 30.85 (CH_2N); 38.40 (CHN); 58.92 (CH_3O); 71.21 (CH_2O); 128.01 and 129.68 (2×HC_{ortho} and 2×HC_{meta}); 134.79 (CH_3C); 144.65 ($C_{arom,quat}$). IR (NaCl, cm^{-1}): ν=2253, 1916, 1596, 1490, 1448, 1322, 1290, 1226. MS (70 eV) *m/z* (%): 241 (M^+, 4); 211 (3); 155 (25); 91 (79); 65 (33); 56 (97); 45 (100). Anal. Calcd for $C_{11}H_{15}NO_3S$ (%): C 54.75; H 6.27; N 5.80. Found (%): C 54.93; H 6.44; N 5.64.

3.4. Synthesis of diaryloxysulfonamides 10

As a representative example, the synthesis of *N*-(2-(3-chlorophenoxy)-1-((3-chlorophenoxy)methyl)ethyl)methanesulfonamide **10a** is described. 2-(Bromomethyl)-1-(4-methanesulfonyl)aziridine **3b** (0.54 g, 2.5 mmol) was added to a mixture of 3-chlorophenol (0.70 g, 2.2 equiv), K_2CO_3 (1.73 g, 5 equiv) and silica gel (1.25 g) and stirred at 80 °C for 16 h. The reaction mixture was filtered over Celite and the filter cake was washed with CH_2Cl_2 (2×20 mL). Isolation of the organic phase, extraction of the water phase with CH_2Cl_2 (20 mL), drying of the combined organic extracts ($MgSO_4$), filtration and evaporation of the solvent afforded the crude *N*-(2-(3-chlorophenoxy)-1-((3-chlorophenoxy)methyl)ethyl)methanesulfonamide **10a**, which was recrystallized from methanol/CH_2Cl_2 (1:1), yielding pure **10a** (0.79 g, 81%).

3.4.1. *N*-(2-(3-Chlorophenoxy)-1-((3-chlorophenoxy) methyl)ethyl)methanesulfonamide 10a. Yie1d 81%, colorless crystals. Mp 99.7–100.7 °C. Recrystallized from methanol/CH_2Cl_2 (1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.09 (3H, s, CH_3); 4.13–4.21 (5H, m, 2×CH_2O and CHN); 5.12–5.14 (1H, m, NH); 6.77–6.81, 6.90–6.91, 6.96–6.99 and 7.19–7.26 (8H, 4×m, HC_{arom}). ^{13}C NMR (75 MHz, $CDCl_3$): δ 41.93 (CH_3); 52.49 (CHN); 67.71 (2×CH_2O); 112.85, 115.06, 121.90 and 130.50 (HC_{arom}); 135.15 (2×

CCl); 158.65 (2×OC_{quat}). IR (NaCl, cm^{-1}): ν=3351 (NH), 3071, 3011, 2962, 2931, 1597, 1486, 1311, 1251, 1151, 770. MS (70 eV) *m/z* (%): 389/91/93 (M^+, 16); 261/3 (10); 167 (22); 153/5 (100); 134 (15); 111 (23); 96 (24). Anal. Calcd for $C_{16}H_{17}Cl_2NO_4S$ (%): C 49.24; H 4.39; N 3.59. Found (%): C 49.41; H 4.52; N 3.44.

3.4.2. *N*-(2-(4-Fluorophenoxy)-1-((4-fluorophenoxy) methyl)ethyl)-4-methylbenzenesulfonamide 10b. Yie1d 84%, colorless crystals. Mp 154.4–155.5 °C. Recrystallized from Hexane/CH_2Cl_2 (1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.40 (3H, s, CH_3Ar); 3.84–3.96 (3H, m, 2×(*H*CH)O and CHN); 4.05–4.09 (2H, m, 2×(HC*H*)O); 5.41 (1H, d, *J*= 7.4 Hz, NH); 6.66–6.73 and 6.87–6.96 (8H, 2×m, 4×$OHC_{ortho}HC_{meta}$); 7.25 and 7.78 (4H, 2×d, *J*=8.3 Hz, 2×$SHC_{ortho}HC_{meta}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.51 (CH_3Ar); 51.91 (CHN); 66.92 (2×CH_2O); 115.54 (d, *J*= 8.1 Hz, 4×OHC_{ortho}); 115.93 (d, *J*=23.1 Hz, 4×OHC_{meta}); 127.20 and 129.78 (2×$SHC_{ortho}HC_{meta}$); 137.55 (CH_3C); 143.75 (SC); 154.10 (2×OC_{quat}); 157.64 (d, *J*=238.8 Hz, 2×CF). IR (NaCl, cm^{-1}): ν=3360 (NH), 3057, 2930, 8875, 1506, 1220, 831, 810. MS (70 eV) *m/z* (%): 434 (M^++1, 100). Anal. Calcd for $C_{22}H_{21}F_2NO_4S$ (%): C, 60.96; H, 4.88; N, 3.23. Found (%): C, 61.13; H, 5.01; N, 3.09.

3.4.3. *N*-(2-(4-Methoxyphenoxy)-1-((4-methoxyphenoxy) methyl)ethyl)-4-methylbenzenesulfonamide 10c. Yie1d 72%, colorless crystals. Mp 91.1–92.3 °C. Recrystallized from methanol. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.41 (3H, s, CH_3Ar); 3.75 (6H, s, 2×CH_3O); 3.85–3.94 (3H, m, 2×(*H*CH)O and CHN); 4.05–4.09 (2H, m, 2×(HC*H*)O); 5.25–5.28 (1H, m, NH); 6.69–6.80 (8H, m, 4×$OHC_{ortho}HC_{meta}$); 7.26 and 7.78 (4H, 2×d, *J*=8.4 Hz, 2×$SHC_{ortho}HC_{meta}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.53 (CH_3Ar); 52.02 (CHN); 55.70 (2×CH_3O); 66.97 (2×CH_2O); 114.68 and 115.47 (4×MeO$HC_{ortho}HC_{meta}$); 127.18 and 129.75 (2×$SHC_{ortho}HC_{meta}$); 137.57 (CH_3C); 143.61 (SC); 152.15 and 154.28 (2×CH_2OC_{quat} and 2×$MeOC_{quat}$). IR (NaCl, cm^{-1}): ν=3307 (NH), 2940, 2834, 1508, 1228, 1162, 1039, 818. MS (70 eV) *m/z* (%): no M^+, 333 ($M^+-MeOC_6H_4OH$, 42); 210 (45); 155 (100); 123 (32); 91 (99). Anal. Calcd for $C_{24}H_{27}NO_6S$ (%): C 63.00; H 5.95; N 3.06. Found (%): C 63.19; H 6.11; N 2.90.

Acknowledgements

The authors are indebted to the 'Fund for Scientific Research—Flanders (F.W.O.-Vlaanderen)' (Belgium) and Ghent University (GOA) for financial support.

References and notes

1. (a) Barrett, C. *Br. J. Pharmacol.* **1968**, *34*, 43. (b) Hansteen, V. *Br. Med. J.* **1982**, *284*, 155. (c) Fitzgerald, J. D. In *Pharmacology of Antihypertensive Drugs*; Scriabine, A., Ed.; Raven: New York, 1980; p 195. (d) Terao, Y.; Murata, M.; Achiwa, K. *Tetrahedron Lett.* **1988**, *29*, 5173. (e) Fuganti, C.; Grasselli, P.; Seneci, P. F.; Servi, S. *Tetrahedron Lett.* **1986**, *27*, 2061. (f) Bevinakatti, H. S.; Banerji, A. A. *J. Org. Chem.* **1992**, *57*, 6003. (g) Aubriot, S.; Nicolle, E.; Lattier, M.; Morel, C.; Cao, W.; Daniel, K. W.; Collins, S.; Leclerc, G.; Faure, P. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 209. (h) Narimatsu, S.; Watanabe, T.; Masubuchi, Y.; Horie, T.; Kumagai, Y.; Cho, A. K.; Imaoka, S.; Funae, Y.; Ishikawa, T.; Suzuki, T. *Chem. Res. Toxicol.* **1995**, *8*, 721. (i) Narimatsu, S.; Arai, T.; Watanabe, T.; Masubuchi, Y.; Horie, T.; Suzuki, T.; Ishikawa, T.; Tsutsui, M.; Kumagai, Y.; Cho, A. K. *Chem. Res. Toxicol.* **1997**, *10*, 289. (j) Teerlink, J.; Massie, B. *Am. J. Cardiol.* **1999**, *84*, 94.
2. Ashwell, M. A.; Solvibile, W. R., Jr.; Han, S.; Largis, E.; Mulvey, R.; Tillet;, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 3123.
3. Wang, L.-W.; Kang, J.-J.; Chen, I.-J.; Teng, C.-M.; Lin, C.-N. *Bioorg. Med. Chem.* **2002**, *10*, 567.
4. (a) Hakomori, S. *J. Biol. Chem.* **1990**, *5*, 878. (b) Van Meer, G.; Burger, K. N. J. *Trends Cell Biol.* **1992**, *2*, 332. (c) Howell, A. R.; So, R.C.; Richardson, S.K. *Tetrahedron* **2004**, *60*, 11327.
5. (a) Bair, K. W.; Tuttle, R. L.; Knick, V. C.; Cory, M.; McKee, D. D. *J. Med. Chem.* **1990**, *33*, 2385. (b) Bair, K. W.; Andrews, C. W.; Tuttle, R. L.; Knick, V. C.; Cory, M.; McKee, D. D. *J. Med. Chem.* **1991**, *34*, 1983.
6. (a) Davis, F. A.; Zhou, P. *Tetrahedron Lett.* **1994**, *35*, 7525. (b) Giordano, C.; Cavicchioli, S.; Levi, S.; Villa, M. *J. Org. Chem.* **1991**, *56*, 6114. (c) Clark, J. E.; Fischer, P. A.; Schumacher, D. P. *Synthesis* **1991**, 891. (d) Schumacher, D. P.; Clark, J. E.; Murphy, B. L.; Fischer, P. A. *J. Org. Chem.* **1990**, *55*, 5291.
7. (a) Sakai, M.; Kato, M.; Hagiwara, H.; Konishi, K. Jpn. Tokkyo Koho JP 51,042,177, Nov 13, 1976; *Chem. Abstr.* **1977**, *86*, 166404.(b) Tohnishi, M.; Nakao, H.; Kohno, E.; Nishida, T.; Furuya, T.; Shimizu, T.; Seo, A.; Sakata, K.; Fujioka, S.; Kanno, H. Eur. Pat. Appl. EP 1,006,107 A2, June 7, 2000; *Chem. Abstr.* **2000**, *133*, 17278.
8. Lee, S. J.; Caboni, P.; Tomizawa, M.; Casida, J. E. *J. Agric. Food Chem.* **2004**, *52*, 95.
9. Wang, Z.; Chen, J.; Zhao, Y. *Huanjing Huaxue* 1997, *16*, 159; *Chem. Abstr.* **1997**, *127*, 61838.
10. Bergmeier, S. C.; Seth, P. P. *J. Org. Chem.* **1997**, *62*, 2671.
11. Sweeney, J. B.; Cantrill, A. A. *Tetrahedron* **2003**, *59*, 3677.
12. D'hooghe, M.; Kerkaert, I.; Rottiers, M.; De Kimpe, N. *Tetrahedron* **2004**, *60*, 3637.
13. (a) Sugiyama, S.; Watanabe, S.; Inoue, T.; Kurihara, R.; Itou, T.; Ishii, K. *Tetrahedron* **2003**, *59*, 3417. (b) Buijnsters, P. J. J. A.; Feiters, M. C.; Nolte, R. J. M.; Sommerdijk, N. A. J. M.; Zwanenburg, B. *Chem. Commun.* **2001**, 269. (c) Choi, S.-K.; Lee, W.-K. *Heterocycles* **1998**, *48*, 1917. (d) Ho, M.; Chung, J. K. K.; Tang, N. *Tetrahedron Lett.* **1993**, *34*, 6513.
14. (a) Thakur, V. V.; Sudalai, A. *Tetrahedron Lett.* **2003**, *44*, 989. (b) Abbaspour Tehrani, K.; Nguyen Van, T.; Karikomi, M.; Rottiers, M.; De Kimpe, N. *Tetrahedron* **2002**, *58*, 7145. (c) Karikomi, M.; De Kimpe, N. *Tetrahedron Lett.* **2000**, *41*, 10295. (d) Ali, S. I.; Nikalje, M. D.; Sudalai, A. *Org. Lett.* **1999**, *1*, 705. (e) Kato, S.; Harada, H.; Morie, T. *J. Heterocycl. Chem.* **1995**, *32*, 637. (f) Gensler, W. J.; Dheer, S. K. *J. Org. Chem.* **1981**, *46*, 4051. (g) Leonard, N. J.; Ning, R. Y.; Booth, R. L. *J. Org. Chem.* **1965**, *30*, 4357. (h) Gensler, W. J.; Koehler, W. R. *J. Org. Chem.* **1962**, *27*, 2754.
15. Gensler, W. J. *J. Am. Chem. Soc.* **1948**, *70*, 1843.
16. Minakata, S.; Kano, D.; Oderaotoshi, Y.; Komatsu, M. *Angew. Chem., Int. Ed.* **2004**, *43*, 79.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron 61 (2005) 8752–8761
Tetrahedron

SG1 based alkoxyamines as radical initiators for the synthesis of lactones and lactames

Denis Bertin, Didier Gigmes,* Sylvain R.A. Marque and Paul Tordo

UMR 6517 case 542, Universités d'Aix-Marseille I, II et III-CNRS, Av. Escadrille Normandie-Niemen, 13397 Marseille Cedex 20, France

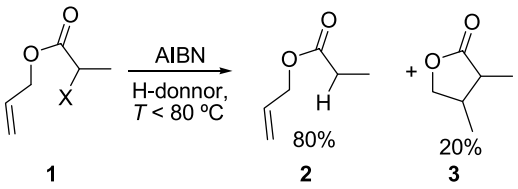
Received 11 February 2005; revised 18 April 2005; accepted 8 June 2005

Available online 20 July 2005

Abstract—Recently, it has been shown that alkoxyamines can be used as radical initiators in tin-free radical chemistry. Thus, it prompted us to develop the preparation of highly valuable alkoxyamines via ionic chemistry and the radical cyclization was triggered by thermal initiation. Following that procedure, bicyclic, spiro and eight-membered lactones were easily prepared in good yields with very high stereoselectivity.

1. Introduction

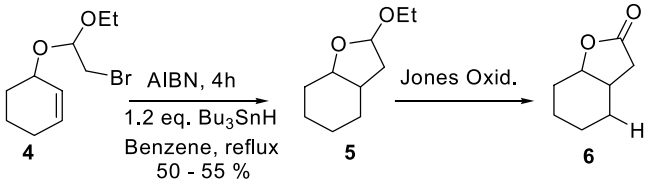
For decades, research has been actively carried out to find new monocomponent initiators for free radical reactions.[1] Such initiators have to fulfill several requirements: they have to be easy to handle and to store, highly selective and not hazardous. At the same time, a number of research teams has developed new hydrogen donors[2,3] to replace the tin derivatives, as they have several drawbacks: they are toxic, environmental harmful, not easily removable and they generate waste.[4] Meanwhile, they are always based on bicomponent systems. In addition, over the past decade, a major challenge has been the preparation of lactone by free radical cyclization from alkoxycarbonylethylene **1** precursors.[5–7] In fact, when **1** was heated below 80 °C in the presence of a H-donor and radical initator (AIBN), only a small amount of the targeted lactone **3** could be obtained while the main product was the reduced radical **2** (Scheme 1).



Scheme 1.

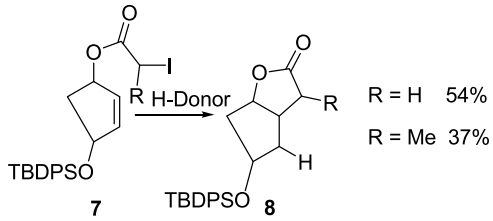
Keywords: Ueno–Stork reaction; Alkoxyamines; Radical cyclization.
* Corresponding author. Fax: +33 4 91 28 87 58;
e-mail: didier.gigmes@up.univ-mrs.fr

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.06.100

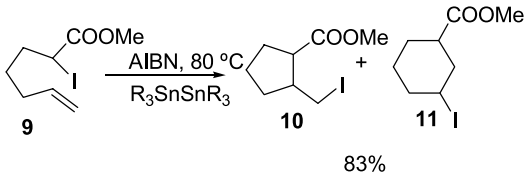


Scheme 2.

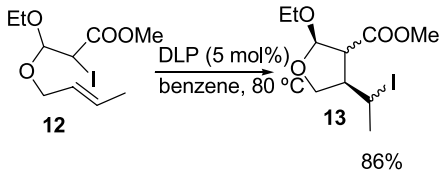
Three solutions have been found to this problem: (i) protection of the carbonyl function (Scheme 2),[8,9] (ii) work above 80 °C and addition of H-donor by small fractions to keep its concentration low (Scheme 3),[10] (iii) use of a degenerative process based on iodine transfer (Scheme 4).[11,12]



Scheme 3.

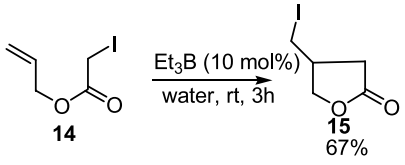


Scheme 4.

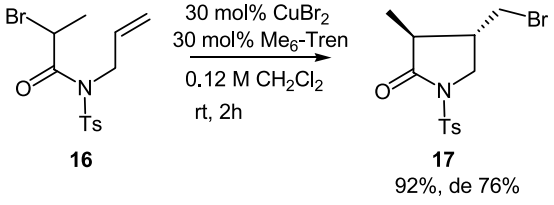


Scheme 5.

Most of the solutions (bicomponent system) involve the presence of tin compounds either as H-donors or as transferring agents. Then, many works have been devoted to develop tin free Ueno–Stork reaction[13] as, for example, the works of the Renaud[14] (Scheme 5) and Oshima[15] (Scheme 6) groups on the iodine atom transfer reaction, and as, for example, the search for less toxical hydrogen donor agents such as Ph_2SiH_2.[16] Beside the development of the Ueno–Stork reaction, the preparation of γ-lactames by radical cyclization interests also many groups.[17] The halogen atom transfer in the presence of metal salts is probably the most suitable approach as exemplified by the work of Clark et al. (Scheme 7).[18]

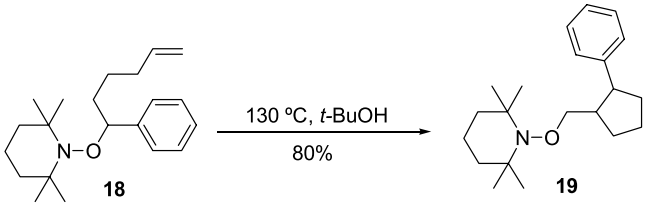


Scheme 6.



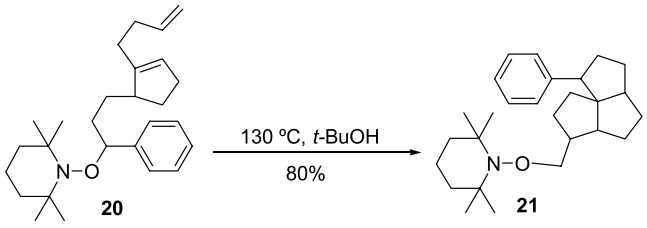
Scheme 7.

However, all these synthetic ways rest on the preparation of halogenated homologues, and often require the presence of catalytic amount of metal salt. Thus, developing new radical cyclization processes free of the drawbacks mentioned above is of high interest. With the introduction, by Rizzardo,[19] of nitroxide in radical chemistry to prepare alkoxyamines by radical addition onto olefins, a new concept was developed. Main of efforts[20] were done on radical polymerization to achieve a controlled process with a living character up to Studer,[21] who showed, five years ago, that alkoxyamines could fulfil almost all the requirements for their applications in free radical organic chemistry. Using TEMPO alkoxyamines **18** (Scheme 8), the corresponding cyclic compounds **19** were obtained in good yields (>80%).[21]



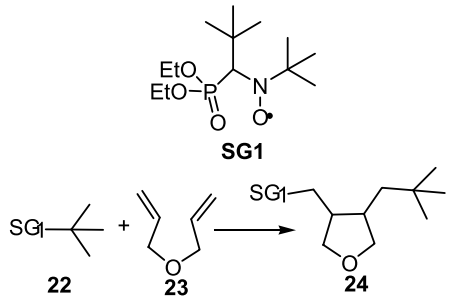
Scheme 8.

The same methodology was applied to the synthesis of triquinane **21** (Scheme 9).[21] However, such TEMPO alkoxyamines are not suitable to prepare lactones or lactams.[21] Even with new technology based on microwave process, the preparation of lactones and lactames from TEMPO alkoxyamines remains still difficult, leading to low yield.[22] Improvements could be achieved in presence of acid additives.

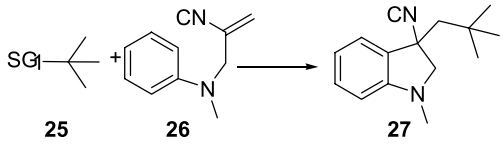


Scheme 9.

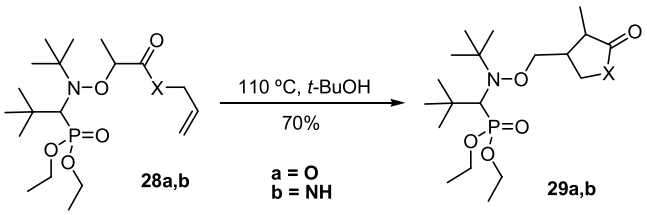
For several years, our research team has been developing with success new nitroxide (SG1 leader) and derivative alkoxyamines as initiators/controllers for nitroxide mediated polymerization (NMP).[23,24] Many improvements were obtained compared to TEMPO such as lower temperature polymerization, application to various monomers, heterogenous systems.[25,26] And recently, Ciufolini et al.[27] have shown that SG1 alkoxyamines are good radical initiators for conjunctive reactions (Scheme 10) and have applied that reaction model to the preparation of isoindoline (Scheme 11).[28] We present hereafter a versatile approach using SG1-alkoxyamines which allows us to overcome the drawbacks mentioned above. The valuable alkoxyamines were prepared by condensation reaction between the easily accessible[29] alkoxyamine **37** and valuable alcohols or amine. It was first applied to the



Scheme 10.

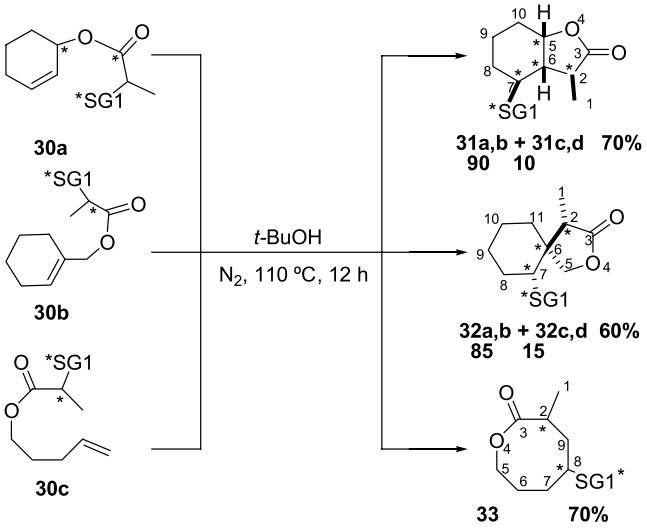


Scheme 11.



Scheme 12.

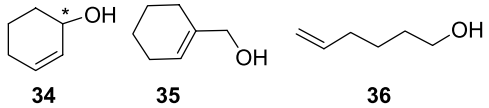
preparation of the simple lactone **29a** and lactame **29b** (Scheme 12). It was then used to prepare the bicyclic-, spiro- and eight-membered ring lactones **31**, **32**, and **33** with good yields and moderate to good stereoselectivity, starting from SG1 alkoxyamines **30a–c**, respectively, without the presence of any tin compounds or other reactants. (Scheme 13).



Scheme 13.

2. Experimental

Solvents, triethylamine, dimethylamino 4-pyridine (DMAP), thionyl chloride ($SOCl_2$), homoallylic alcohol and amine, and alcohols **34**, **35** and **36** (Scheme 14) were purchased from Aldrich and used as received. Alkoxyamine **37** (MONAMS) was provided by ARKEMA. Alkoxyamine **38** was prepared as previously described.[30] NMR experiments were performed on 300 Avance Bruker spectrometer (^1H 300 MHz, ^{13}C 75.48 MHz and ^{31}P 121.59 MHz). Chemical shifts were relative to TMS (internal reference) for ^1H NMR, to $CDCl_3$ (internal reference) for ^{13}C NMR and to H_3PO_4 85% (external reference) for ^{31}P NMR. Elemental analyses were performed in the 'Service Commun de Micro Analyse Université d'Aix-Marseille 3'. Reactions were monitored by TLC (60 F 240 Silicagel plates, eluent: ethyl acetate/pentane 1:1), using UV and phosphomolybdic acid as indicators. Alkoxyamines were purified by chromatography (60 Silicagel, 70–230 mesh, Merck), eluent: ethyl acetate/pentane 3:1.



Scheme 14.

2.1. General procedure 1 (GP1)

In a typical experiment, a solution of **38** (4.0 g, 11.0 mmol) in CH_2Cl_2 was degassed by nitrogen bubbling for 10 min. Then, 3 equiv of $SOCl_2$ (2.4 mL, 33.0 mmol) were added under nitrogen atmosphere. The mixture was stirred for 45 min at rt and the excess of $SOCl_2$ was removed under vacuum (0.1 mbar) to yield alkoxyamine **39**. Under nitrogen atmosphere, crude **39** was diluted in ether, and then a 20 mL ether solution of alcohol (2 equiv), Et_3N (1 equiv, 1.5 mL, 11 mmol) and DMAP (0.4 equiv, 0.3 g, 2.4 mmol) was added. A white solid precipitated and the mixture was stirred for 16 h at rt. The white solid was filtered off and the solvent removed to yield oil. The oil was dissolved in 30 mL of ether, washed three times with 15 mL of an aqueous solution of NH_4Cl 5%, three times with 15 mL of a saturated sodium carbonate aqueous solution, and then with water to reach a neutral pH. The organic layer was dried over $MgSO_4$ and the solvent removed to yield oil, which was purified by chromatography to afford alkoxyamines **28a**,**b** and **30a–c**.

2.2. General procedure 2 (GP2)

A 0.025 M solution of alkoxyamine **28** and **30** in degassed *tert*-BuOH was heated in a Schlenk flask at 110 °C for 12 h. The solvent was removed and the residue was purified by column chromatography to afford the cyclic lactone or lactame aimed at.

2.2.1. Alkoxyamine 28a. Alkoxyamine **28a** was prepared according to GP1 (40% yields). ^{31}P NMR (121.59 MHz, $CDCl_3$, δ, ppm): 23.23 (s, Dia I, 80%). 22.61 (s, Dia II, 20%). ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 5.96–5.87 (m, 2H, dia I+II), 5.37–5.23 (m, 4H, dia I+II), 4.64–4.58 (m, 6H, dia I+II), 4.25–3.93 (m, 8H, dia I+II), 3.37 (d, J= 27 Hz, 1H, dia II), 3.27 (d, 1H, J=24 Hz, dia I), 1.53 (d, J= 9 Hz, 3H, dia I), 1.50 (d, J=6 Hz, 3H, dia II), 1.36–1.27 (m, 12H, dia I+II), 1.17 (s, 9H, dia II), 1.16 (s, 9H, dia I), 1.14 (s, 9H, dia II), 1.11 (s, 9H, dia I). ^{13}C NMR (75.54 MHz, $CDCl_3$, δ, ppm): Dia I. 173.4 (s, *C*O), 131.7 (s, *C*H=CH_2), 118.5 (s, CH=*C*H_2), 82.5 (s, *C*H–ON), 69.5 (d, J= 139.75 Hz, *C*H–P), 64.9 (s, O–*C*H_2–CH), 61.7 (d, J= 6.04 Hz, CH_2), 61.5 (s, N–*C*$(CH_3)_3$), 58.7 (d, J=7.55 Hz, CH_2), 35.5 (d, J=5.28 Hz, CH–*C*$(CH_3)_3$), 29.5 (d, J= 5.28 Hz, CH–C(*C*$H_3)_3$), 27.8 (s, N–C(*C*$H_3)_3$), 19.2 (s, CH–*C*H_3), 16.4 (d, J=5.29 Hz, CH_2*C*H_3), 16.1 (d, J=6.8 Hz, CH_2*C*H_3). Dia II. 172.0 (s, *C*O), 132.1 (s, *C*H=CH_2), 118.0 (s, CH=*C*H_2), 82.5 (s, *C*H–ON),69.2 (d, J=139.75 Hz, *C*H–P), 64.8 (s, O–*C*H_2–CH), 61.8 (d, J=8.3 Hz, CH_2), 61.3 (s, N–*C*$(CH_3)_3$), 58.8 (d, J=6.8 Hz, CH_2), 35.1 (d, J=5.28 Hz, CH–*C*$(CH_3)_3$), 30.2 (d, J=6.04 Hz, CH–C(*C*$H_3)_3$), 27.9 (s, N–C(*C*$H_3)_3$), 17.7 (s, CH–*C*H_3), 15.8 (d, J=6.8 Hz, CH_2*C*H_3), 15.8 (d, J=6.8 Hz, CH_2*C*H_3).

2.2.2. Alkoxyamine 28b. Alkoxyamine **28b** was prepared according to GP1 (50% yields). ^{31}P NMR (121.59 MHz, $CDCl_3$, δ, ppm): 27.42 (s, Dia I, 35%). 27.05 (s, Dia II, 65%). ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): Dia I. 8.61 (b, NH, 1H), 5.96–5.83 (m, 1H), 5.19 (dq, J_{HH}=1.5, 18 Hz, 1H), 5.08 (dq, J_{HH}=1.5, 9 Hz, 1H), 4.48 (q, J=6 Hz, 1H),

4.29–3.97 (m, 5H), 3.67 (m, 1H), 3.35 (d, J=27 Hz, 1H), 1.51 (d, J=6 Hz, 3H), 1.35–1.28 (m, 6H), 1.21 (s, 9H), 1.08 (s, 9H). Dia II. 7.74 (b, NH, 1H), 5.96–5.83 (m, 1H), 5.21 (d, J=18 Hz, 1H), 205.11 (d, J=9 Hz, 1H), 4.51 (q, J=9 Hz, 1H), 4.20–3.95 (m, 5H), 3.88 (t, J=7.5 Hz, 1H), 3.28 (d, J=24 Hz, 1H), 1.63 (d, J=6 Hz, 3H), 1.36–1.28 (m, 6H), 1.25 (s, 9H), 1.24 (s, 9H). ^{13}C NMR (75.54 MHz, $CDCl_3$, δ, ppm): Dia I. 173.6 (s, *C*O), 134.4 (s, *C*H=CH_2), 115.2 (s, CH=*C*H_2), 81.7 (s, *C*H–ON), 68.6 (d, J=137.48 Hz, *C*H–P), 62.2 (s, N–*C*$(CH_3)_3$), 61.6 (d, J=6.04 Hz, CH_2), 59.6 (d, J=7.55 Hz, CH_2), 41.1 (s, N–CH_2), 35.4 (d, J=5.28 Hz, CH–*C*$(CH_3)_3$), 29.7 (d, J=6.04 Hz, CH–C(*C*$H_3)_3$), 28.2 (s, N–C(*C*$H_3)_3$), 19.2 (s, CH–*C*H_3), 16.3 (d, J=6.04 Hz, CH_2*C*H_3), 15.9 (d, J=6.8 Hz, CH_2*C*H_3). Dia II. 173.4 (s, *C*O), 134.3 (s, *C*H=CH_2), 116.3 (s, CH=*C*H_2), 83.1 (s, *C*H–ON), 69.3 (d, J=137.48 Hz, *C*H–P), 62.9 (s, N–*C*$(CH_3)_3$), 61.6 (d, J=6.04 Hz, CH_2), 60.0 (d, J=7.55 Hz, CH_2), 41.5 (s, N–CH_2), 35.3 (d, J=5.28 Hz, CH–*C*$(CH_3)_3$), 30.1 (d, J=5.28 Hz, CH–C(*C*$H_3)_3$), 28.4 (s, N–C(*C*$H_3)_3$), 19.6 (s, CH–*C*H_3), 16.6 (d, J=6.80 Hz, CH_2*C*H_3), 16.3 (d, J=6.8 Hz, CH_2*C*H_3).

2.2.3. Alkoxyamine 29a. Alkoxyamine **29a** was isolated (70% yield) according to GP2. ^{31}P NMR (121.59 MHz, $CDCl_3$, δ, ppm): 4 diastereoisomers 23.11 (s, Dia I, 12%). 22.68 (s, Dia II, 38%), 22.61 (s, Dia III, 12%), 22.49 (s, Dia IV, 38). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): Dia I.4.46–3.95 (m, 7H), 3.55 (m, 1H), 3.18 (d, J=24.0 Hz, 1H), 2.88–2.77 (m, 1H), 2.45–2.31 (m, 1H), 1.31 (t, J=6.0 Hz, 6H), 1.18 (d, J=6 Hz, 3H), 1.15–1.13 (m, 18H). Dia II. 4.33–3.86 (m, 7H), 3.58 (t, J=12.0 Hz, 1H), 3.26 (d, J=27.0 Hz, 1H), 3.00–2.94 (m, 1H), 2.72 (quint, J=9.0 Hz, 1H), 1.31 (td, J_{HH}=6.0 Hz, J_{HP}=3.0 Hz, 6H), 1.18 (d, J=6.0 Hz, 3H), 1.15 (s, 9H), 1.14 (s, 9H). Mixture of Dia III+IV (1/1) δ 4.41–3.7 (m, 16H, III+IV), 3.37 (d, J=27.0 Hz, 1H, III/IV), 3.27 (d, 1H, J=27.0 Hz, III/IV), 2.63–2.30 (m, 4H, III+IV), 1.37–1.26 (m, 18H, III+IV), 1.15–1.14 (m, 36 H, dia III+IV). ^{13}C NMR (75.54 MHz, $CDCl_3$, δ, ppm): Dia I. 179.7 (s, *C*O), 73.0 (s, *C*H–ON), 69.5 (s, *C*H_2O), 69.1 (d, J=137.48 Hz, *C*H–P), 62.1 (s, N–*C*$(CH_3)_3$), 61.0 (d, J=5.3 Hz, O–*C*H_2CH_3), 59.6 (d, J=7.6 Hz, O–*C*H_2CH_3), 38.2 (s, *C*H–CH_3), 36.5 (s, NO–CH_2–*C*H), 35.4 (d, J=5.3 Hz, CH–*C*$(CH_3)_3$), 30.0 (d, J=6.0 Hz, CH–*C*$(CH_3)_3$), 28.0 (s, N–C(*C*$H_3)_3$), 16.6 (d, J=6.0 Hz, CH_2*C*H_3), 16.3 (d, J=6.8 Hz, CH_2*C*H_3), 10.3 (s, *C*H_3CH). Dia II. 179.5 (s, *C*O), 72.7 (s, *C*H–ON), 69.0 (s, *C*H_2O), 69.0 (d, J=139.74 Hz, *C*H–P), 62.0 (s, N–*C*$(CH_3)_3$), 61.0 (d, J=7.6 Hz, O–*C*H_2CH_3), 59.3 (d, J=8.3 Hz, O–*C*H_2CH_3), 38.3 (s, *C*H–CH_3), 36.4 (s, NO–CH_2–*C*H), 35.3 (d, J=5.3 Hz, CH–*C*$(CH_3)_3$), 30.1 (d, J=6.0 Hz, CH–*C*$(CH_3)_3$), 27.8 (s, N–C(*C*$H_3)_3$), 16.6 (d, J=5.3 Hz, CH_2*C*H_3), 16.3 (d, J=6.8 Hz, CH_2*C*H_3), 9.9 (s, *C*H_3CH). Dia III+IV. 179.1 (s, *C*O), 179.1 (s, *C*O), 75.7 (s, *C*H–ON), 75.5 (s, *C*H–ON), 69.9 (s, *C*H_2O), 69.8 (s, *C*H_2O), 68.7 (d, J=139.0 Hz, *C*H–P), 68.6 (d, J=139.8 Hz, *C*H–P), 61.9 (s, N–*C*$(CH_3)_3$), 61.9 (s, N–*C*$(CH_3)_3$), 60.9–60.8 (m, O–*C*H_2CH_3, dia III+IV), 59.5 (d, J=6.8 Hz, O–*C*H_2CH_3, dia III+IV), 42.9 (s, *C*H–CH_3), 42.9 (s, *C*H–CH_3), 37.1 (s, NO–CH_2–*C*H), 36.6 (s, NO–CH_2–*C*H), 35.1 (d, J=5.3 Hz, CH–*C*$(CH_3)_3$), 35.1 (d, J=5.3 Hz, CH–*C*$(CH_3)_3$), 30.1 (d, J=6.0 Hz, CH–C(*C*$H_3)_3$, dia III+IV), 27.7 (s, N–C(*C*$H_3)_3$), 27.6 (s, N–C(*C*$H_3)_3$), 16.4 (d, J=5.3 Hz, CH_2*C*H_3), 16.4 (d, J=6.0 Hz, CH_2*C*H_3), 16.1 (d, J=6.8 Hz, CH_2*C*H_3, dia III+IV), 14.3 (s, *C*H_3CH), 14.1 (s, *C*H_3CH).

2.2.4. Alkoxyamine 29b. Alkoxyamine **29b** was isolated (70% yield) according to GP2. ^{31}P NMR (121.59 MHz, $CDCl_3$, δ, ppm): mixture of 4 diastereoisomers 23.24 (16%), 23.00 (16%), 22.93 (34%), 22.79 (34%). ^{13}C NMR (75.54 MHz, $CDCl_3$, δ, ppm): mixture of 4 diastereoisomers 180.9, 180.4, 180.2, 180.0 (4 s, 4 *C*O), 77.4, 77.1, 74.3, 73.9 (4 s, 4 *C*H–ON), 68.7 (d, J=142.0 Hz, 2 *C*H–P), 68.5 (d, J=139.0 Hz, 2 *C*H–P); 61.6 (s, 2 N–*C*$(CH_3)_3$), 61.6 (s, 2 N–*C*$(CH_3)_3$), 60.8–60.7 (m, 4 O–*C*H_2CH_3), 59.1–58.7 (m, 4 O–*C*H_2CH_3), 44.6, 44.4, 43.8, 43.7 (4s, 4 *C*H_2NH), 42.3, 42.1 (2 s, 2 *C*H–CH_3), 39.0 (s, 2 *C*H–CH_3), 38.4, 37.9, 37.8 (4 s, 4 NO–CH_2–*C*H), 34.9–35.1 (m, CH–*C*$(CH_3)_3$), 29.9–29.6 (m, CH–*C*$(CH_3)_3$), 27.7, 27.6 (2 s, 2 N–C(*C*$H_3)_3$), 27.5 (s, 2 N–C(*C*$H_3)_3$), 16.3–15.9 (m, CH_2*C*H_3), 14.9, 14.7, 10.4, 9.9 (4 s, 4 *C*H_3CH).

2.2.5. Alkoxyamines 30a. Alkoxyamines **30a** were prepared according to GP1 (50% yields). Calcd for $C_{22}H_{42}NO_6P$ (447.55 g mol^{-1}): C, 59.04; H, 9.46; N, 3.13. Found: C, 58.70; H, 9.49; N, 3.11. ^{31}P NMR (121.59 MHz, $CDCl_3$, δ, ppm): 24.77 (s, Dia 1+2, 60%), 24.23 (s, Dia 3, 20%), 24.12 (s, dia 4, 20%). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 6.00–5.66 (m, 8H), 5.27 (m, 4H), 4.71–4.52 (m, 2H), 4.29–3.89 (m, 2H), 3.35 (d, dia 1+dia 2, J=24.0 Hz, 2H), 3.26 (d, dia 3+dia 4, J=27.0 Hz, 2H), 2.13–1.12 (m, 148H). ^{13}C NMR (75.54 MHz, $CDCl_3$, δ, ppm), for diastereoisomers 1 and 2: 173.7 (C), 173.7 (C), 133.0 (CH), 132.8 (CH), 125.3 (CH), 125.1 (CH), 83.2 (CH), 83.1 (CH), 69.7 (d, J=140.4 Hz, 2 CH), 68.1 (2 CH), 61.9 (d, J=6.0 Hz, CH_2), 61.6 (2 C), 58.7 (d, J=8.3 Hz, CH_2), 35.6 (d, J=6.0 Hz, 2 C), 29.5 (d, J=6.0 Hz, 2 CH_3), 28.0 (2 CH_3), 24.8 (CH_2), 24.8 (CH_2), 19.5 (CH_3), 19.5 (CH_3), 18.7 (CH_2), 18.6 (CH_2), 16.5 (d, J=6.0 Hz, CH_3), 16.2 (d, J=7.5 Hz, CH_3); for diastereoisomers 3 and 4: 172.2 (C), 172.2 (C), 133.0 (CH), 132.8 (CH), 125.7 (CH), 125.6 (CH), 76.9 (CH), 76.8 (CH), 69.4 (d, J=138.9 Hz, 2CH), 68.3 (CH), 68.1 (CH), 62.0 (d, J=7.54 Hz, CH_2), 61.5 (C), 61.4 (C), 59.5 (d, J=6.8 Hz, CH_2), 35.3 (d, J=4.5 Hz, C), 35.3 (d, J=4.5 Hz, C), 30.3 (d, J=4.5 Hz, CH_3), 30.2 (d, J=5.3 Hz, CH_3), 28.2 (CH_2), 28.1 (CH_2), 28.0 (2 CH_3), 18.9 (CH_2), 18.8 (CH_2), 17.9 (CH_3), 16.5 (d, J=6.0 Hz, CH_3), 16.2 (d, J=7.5 Hz, CH_3).

2.2.6. Alkoxyamines 30b. Alkoxyamines **30b** were prepared according to GP1 (48% yields). Calcd for $C_{23}H_{44}NO_6P$ (461.57 g mol^{-1}): C, 59.85; H, 9.61; N, 3.03. Found: C, 59.93; H, 9.57; N, 3.07. ^{31}P NMR (121.59 MHz, $CDCl_3$, δ, ppm): 24.69 (s, Dia 1, 50%). 24.03 (s, Dia 2, 50%). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 5.77–5.72 (m, dia 1+dia 2, 2H), 4.69 (q, J=6.0 Hz, dia 1, 1H), 4.58 (q, J=6.0 Hz, dia 2, 1 H), 4.53–4.44 (m, dia 1+dia 2, 2 H), 4.25–3.91 (m, dia 1+dia 2, 8 H), 3.36 (d, J=24.0 Hz, dia1, 1 H), 3.26 (d, J=24.0 Hz, dia 2, 1 H), 2.07–1.92 (m, dia 1+dia 2, 8 H), 1.70–1.65 (m, dia+dia 2, 8 H), 1.53 (t, J=6.0 Hz, dia 1+dia 2, 6 H), 1.27–1.36 (m, dia 1+dia 2, 12 H), 1.17 (s, 9 H), 1.15 (s, 9 H), 1.14 (s, 9 H), 1.10 (s, 9 H). For diastereoisomers 1 and 2, ^{13}C NMR (75.54 MHz, $CDCl_3$, δ, ppm): 173.7 (C), 172.2 (C), 132.6 (C), 132.3 (C), 127.0 (CH), 125.9 (CH), 82.8 (CH), 76.5 (CH), 69.5 (d, J=129.7 Hz, CH), 69.2 (d, J=129.7 Hz,

CH), 68.8 (CH_2), 68.4 (CH_2), 61.7 (d, $J=7.6$ Hz, CH_2), 61.7 (d, $J=6.8$ Hz, CH_2), 61.5 (C), 61.2 (C), 58.8 (d, $J=8.3$ Hz, CH_2), 58.5 (d, $J=8.3$ Hz, CH_2), 35.4 (d, $J=5.3$ Hz, C), 35.0 (d, $J=4.5$ Hz, C), 30.1 (d, $J=6.0$ Hz, CH_3), 29.3 (d, $J=5.3$ Hz, CH_3), 27.8 (CH_3), 27.7 (CH_3), 25.8 (CH_2), 25.6 (CH_2), 22.2 (2 CH_2), 21.9 (CH_2), 21.9 (CH_2), 19.3 (CH_3), 17.6 (CH_3), 16.3 (d, $J=6.0$ Hz, CH_3), 16.0 (d, $J=6.0$ Hz, CH_3).

2.2.7. Alkoxyamines 30c. Alkoxyamines **30c** were prepared according to GP1 (45% yields). Calcd for $C_{21}H_{42}NO_6P$ (435.27 g mol^{-1}): C, 57.61; H, 9.72; N, 3.22. Found: C, 58.26; H, 9.83; N, 3.30. ^{31}P NMR (121.59 MHz, $CDCl_3$, δ, ppm): 25.72 (s, Dia 1, 60%), 25.16 (s, Dia 2, 40%). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 5.90–5.70 (m, 1H), 5.08–4.98 (m, 1H), 4.66 (q, dia 2, $J=6.0$ Hz, 1H), 4.58 (q, dia 1, $J=6$ Hz, 1H), 4.27–3.93 (m, dia 1 and 2, 12H), 3.36 (d, $J=26.0$ Hz, dia 2, 1H), 3.27 (d, $J=24.0$ Hz, dia 2, 1H), 2.19–2.10 (m, dia 1+2, 4H), 1.85–1.69 (m, dia 1+2, 4H), 1.52 (d, $J=26.0$ Hz, dia 1, 3H), 1.49 (d, $J=26.0$ Hz, dia 2, 3H), 1.33–1.26 (m, dia 1+2, 14H), 1.17 (s, dia 1, 9H), 1.16 (s, dia 1, 9H), 1.14 (s, dia 2, 9H), 1.11 (s, dia 2, 9H). ^{13}C NMR (75.54 MHz, $CDCl_3$, δ, ppm), for diasteroisomer 1: 173.9 (C), 137.1 (CH), 115.4 (CH_2), 82.7 (CH), 69.6 (d, $J=139.4$ Hz, CH), 63.7 (CH_2), 61.8 (d, $J=6.0$ Hz, CH_2), 61.6 (C), 58.7 (d, $J=7.5$ Hz, CH_2), 35.5 (d, $J=5.5$ Hz, C), 29.5 (d, $J=6.0$ Hz, CH_3), 27.9 (CH_3), 27.7 (CH_2), 19.3 (CH_3), 16.5 (d, $J=5.5$ Hz, CH_3), 16.1 (d, $J=7.0$ Hz, CH_3); for diasteroisomer 2: 172.5 (C), 137.4 (CH), 115.1 (CH_2), 77.0 (CH), 69.3 (d, $J=139.4$ Hz, CH), 63.7 (CH_2), 61.8 (d, $J=6.54$, CH_2), 61.4 (C), 58.9 (d, $J=7.5$ Hz, CH_2), 35.2 (d, $J=5.0$ Hz, C), 30.2 (d, $J=6.5$ Hz, CH_3), 29.9 (CH_2), 28.0 (CH_3), 19.3 (CH_3), 17.8 (d, $J=5.5$ Hz, CH_3), 16.1 (d, $J=7.0$ Hz, CH_3).

2.2.8. Alkoxyamine 31a. Alkoxyamine **31a** was isolated (31% yield) according to GP2. ^{31}P NMR (121.59 MHz, $CDCl_3$, δ, ppm): 24.70. ^{1}H (300 MHz, $CDCl_3$, δ, ppm): 4.76 (q, $J=6.0$ Hz, 1H), 4.27–3.98 (m, 5H), 3.38 (d, $J=27.0$ Hz, 1 H), 2.92 (m, 1H), 2.01–1.40 (m, 7H), 1.35–1.28 (m, 9H), 1.15 (s, 9H), 1.13 (s, 9H). ^{13}C NMR (75.54 MHz, $CDCl_3$, δ, ppm): 179.9 (C), 78.2 (CH), 76.9 (CH), 69.0 (d, $J=137.4$ Hz, CH), 61.4 (C), 61.2 (d, $J=6.0$ Hz, CH_2), 60.0 (d, $J=7.6$ Hz, CH_2), 46.7 (CH), 38.4 (CH), 35.4 (d, $J=4.5$ Hz, C), 30.6 (d, $J=6.0$ Hz, $3CH_3$), 28.5 ($3CH_3$), 28.3 (CH_2), 26.5 (CH_2), 17.9 (CH_2), 16.5 (d, $J=6.0$ Hz, CH_3), 16.3 (d, $J=3.4$ Hz, CH_3), 14.1 (CH_3). Calcd for $C_{22}H_{42}NO_6P$ (447.55 g mol^{-1}): C, 59.04; H, 9.46; N, 3.13. Found: C, 59.23; H, 9.49; N, 3.17.

2.2.9. Alkoxyamine 31b. Alkoxyamine **31b** was isolated (31% yield) according to GP2. ^{31}P NMR (121.59 MHz, $CDCl_3$, δ, ppm): 22.96. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 4.76–4.65 (m, 1H), 4.20–3.96 (m, 5H), 3.44 (d, $J=27.0$ Hz, 1H), 2.87–2.80 (m, 1H), 2.58–2.48 (m, 1H), 2.23–2.17 (m, 1H), 2.10–2.03 (m, 1H), 1.73–1.39 (m, 4H), 1.36–1.28 (m, 9H), 1.18 (s, 9H), 1.15 (s, 9H). ^{13}C NMR (75.54 MHz, $CDCl_3$, δ, ppm): 179.0 (C), 77.4 (CH), 76.5 (CH), 68.8 (d, $J=139.6$ Hz, CH), 61.4 (C), 61.2 (d, $J=6.0$ Hz, CH_2), 59.4 (d, $J=6.8$ Hz, CH_2), 43.9 (CH), 37.1 (CH), 35.1 (d, $J=3.8$ Hz, C), 31.1 (d, $J=6.0$ Hz, 3 CH_3), 29.0 (CH_2), 27.9 (3 CH_3), 26.1 (CH_2), 17.3 (CH_2), 16.4 (d, $J=6.8$ Hz, CH_3), 16.3 (d, $J=6.0$ Hz, CH_3), 14.5 (CH_3). Calcd for $C_{22}H_{42}NO_6P$ (447.55 g mol^{-1}): C, 59.04; H, 9.46; N, 3.13. Found: C, 59.15; H, 9.48; N, 3.23.

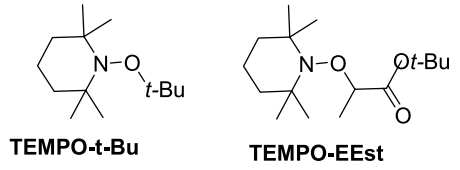
2.2.10. Alkoxyamine 31c,d. Alkoxyamines **31c**,**d** were isolated (10% yield) according to GP2 as a mixture 1:1. Calcd for $C_{23}H_{44}NO_6P$ (461.57 g mol^{-1}): C, 59.85; H, 9.61; N, 3.03. Found: C, 59.93; H, 9.56; N, 3.07. ^{31}P NMR (121.59 MHz, $CDCl_3$, δ, ppm): 24.69 (54%), 24.03 (46%). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 5.77–5.72 (m, **c** or **d**, 2H), 4.69 (q, **c** or **d**, $J=6.0$ Hz, 1H), 4.59 (q, **c** or **d**, $J=6.0$ Hz, 1H), 4.54–4.40 (m, **c**+**d**, 4H), 4.29–3.91 (m, **c**+**d**, 8H), 3.36 (d, **c** or **d**, $J=24.0$ Hz, 1H), 3.26 (d, **c** or **d**, $J=24.0$ Hz, 1H), 2.05–1.95 (m, **c**+**d**, 8H), 1.65–1.49 (m, **c**+**d**, 14H) 1.36–1.27 (m, **c**+**d**, 12H) 1.19 (s, **c** or **d**, 9H), 1.17 (s, **c** or **d**, 9H), 1.15 (s, **c** or **d**, 9H), 1.10 (s, **c** or **d**, 9H). ^{13}C NMR (75.54 MHz, $CDCl_3$, δ, ppm): **31c** or **31d**: 173.7 (C), 132.6 (C), 127.0 (CH), 82.8 (CH), 69.5 (d, $J=138.9$ Hz, CH), 68.8 (CH_2), 61.7 (d, $J=6.0$ Hz, CH_2), 61.5 (C), 58.5 (d, $J=6.0$ Hz, CH_2), 35.4 (d, $J=5.3$ Hz, C), 29.3 (d, $J=5.3$ Hz, 3 CH_3), 27.7 (3 CH_3), 25.8 (CH_2), 24.8 (CH_2), 22.2 (CH_2), 22.0 (CH_2), 19.3 (CH_3), 16.3 (d, $J=6.0$ Hz, CH_3), 16.0 (d, $J=6.0$ Hz, CH_3). **31c** or **31d**: 172.2 (C), 132.3 (C), 125.9 (CH), 76.5 (CH), 69.2 (d, $J=139.6$ Hz, CH), 68.4 (CH_2), 61.8 (d, $J=7.5$ Hz, CH_2), 61.2 (C), 58.8 (d, $J=7.5.0$ Hz, CH_2), 35.0 (d, $J=4.5$ Hz, C), 30.1 (d, $J=6.0$ Hz, $3CH_3$), 27.8 (3 CH_3), 25.6 (CH_2), 24.8 (CH_2), 22.1 (CH_2), 21.9 (CH_2), 17.6 (CH_3), 16.3 (d, $J=6.0$ Hz, CH_3), 16.0 (d, $J=6.0$ Hz, CH_3).

2.2.11. Alkoxyamine 32a. Alkoxyamines **32a**, were isolated according to GP2. Calcd for $C_{23}H_{44}NO_6P$ (461.57 g mol^{-1}): C, 59.85; H, 9.61; N, 3.03. Found: C, 59.94; H, 9.75; N, 3.05. ^{31}P NMR (121.59 MHz, $CDCl_3$, δ, ppm): 24.39 (78%). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 4.89 (bd, $J=9.0$ Hz, 1H), 4.16–3.94 (m, 5H), 3.71 (dd, $J=12.0$, 3.0 Hz, 1H), 3.30 (d, $J=27.0$ Hz, 1H), 2.44 (b, 1H), 1.82–1.10 (m, 35H). ^{13}C NMR (75.54 MHz, $CDCl_3$, δ, ppm): 180.2 (C), 81.7 (CH), 68.2 (d, $J=139.6$ Hz, CH), 69.0 (CH_2), 62.8 (C), 60.9 (d, $J=7.5$ Hz, CH_2), 60.5 (d, $J=6.8$ Hz, CH_2), 47.3 (C), 37.8 (CH), 35.4 (d, J=4.5 Hz, C), 30.6 (d, $J=5.3$ Hz, $3CH_3$), 29.0 (CH_2), 28.4 ($3CH_3$), 28.0 (CH_2), 24.3 (CH_2), 21.3 (CH_2), 16.5–16.1 (m, CH_3), 9.1 (CH_3).

2.2.12. Alkoxyamine 32b,c,d. Alkoxyamines **32b**,**c**,**d** were isolated according to GP2 as a mixture. Calcd for $C_{21}H_{42}NO_6P$ (435.27 g mol^{-1}): C, 57.91; H, 9.72; N, 3.22. Found: C, 58.25; H, 9.82; N, 3.30. ^{31}P NMR (121.59 MHz, $CDCl_3$, δ, ppm): 25.72, 25.16, 23.99. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 5.90–5.70 (m, dia **c**+**d**, 2H), 5.12–4.94 (m, dia **c**+**d**, 4H), 4.71–4.43 (m, dia **b**+**c**+**d**, 3H), 4.32–3.88 (m, dia **b**+**c**+**d**, 12H), 3.36 (d, dia **b**+**d**, $J=26.0$ Hz, 2H), 3.27 (d, dia **c**, $J=24.0$ Hz, 1H), 2.19–2.07 (m, dia **c**+**d**, 2H), 1.84–1.11 (m, 105H). ^{13}C NMR (75.54 MHz, $CDCl_3$, δ, ppm): **32b**: 181.0 (C), 77.5 (CH), 68.8 (d, $J=135.9$ Hz, CH), 68.8 (CH_2), 61.7 (C), 61.3 (d, $J=7.5$ Hz, CH_2), 60.6 (d, $J=6.8$ Hz, CH_2), 47.8 (C), 37.9 (CH), 35.6 (d, $J=6.8$ Hz, C), 30.1 (d, $J=6.0$ Hz, $3CH_3$), 29.1 (CH_2), 28.8 ($3CH_3$), 27.5 (CH_2), 24.0 (CH_2), 21.8 (CH_2), 16.5–16.1 (CH_3), 9.3 (CH_3); **32c**: 173.9 (C), 137.1 (CH), 115.4 (CH_2), 82.7 (CH), 69.6 (d, $J=139.4$ Hz, CH), 63.7 (CH_2), 61.8 (d, $J=6.0$ Hz, CH_2), 61.7 (C), 58.7 (d, $J=7.5$ Hz, CH_2), 35.4 (d, $J=5.5$ Hz, C),

29.9 (CH_2), 29.5 (d, J=5.3 Hz, 3 CH_3), 27.9 (3 CH_3), 27.6 (CH_2), 19.3 (CH_3), 16.5 (d, J=5.5 Hz, CH_3), 16.1 (d, J=7.0 Hz, CH_3); **32d**: 172.5 (C), 137.4 (CH), 115.1 (CH_2), 77.0 (CH), 69.3 (d, J=139.4 Hz, CH), 63.7 (CH_2), 61.8 (d, J=6.5 Hz, CH_2), 61.4 (C), 58.7 (d, J=7.5 Hz, CH_2), 35.2 (d, J=5.0 Hz, C), 30.2 (d, J=6.5 Hz, 3CH_3), 29.9 (CH_2), 28.0 (3 CH_3), 27.6 (CH_2), 17.8 (CH_3), 16.5 (d, J=5.5 Hz, CH_3), 16.1 (d, J=7.0 Hz, CH_3).

2.2.13. Alkoxyamine 33. Alkoxyamines **33** were isolated (70% yield) according to GP2 as a mixture of 2 unseparable diastereoisomers and 2 pure diastereoisomers in the ratio 1:1:1:1. Calcd for $C_{21}H_{42}NO_6P$ (435.27 g mol^{-1}): C, 57.91; H, 9.72; N, 3.22. Found: C, 57.56; H, 9.85; N, 3.13. ^{31}P NMR (121.59 MHz, $CDCl_3$, δ, ppm): 24.28 (dia 1), 24.15 (dia 2), 22.83 (dia 3), 24.88 (dia 4). ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): dia 3: 4.33–3.93 (m, 7H), 3.35 (d, J=27.0 Hz, 1H), 3.05–2.93 (m, 1H), 2.66–2.59 (b, 1H), 2.19–2.10 (m, 1H), 1.95–1.78 (m, 4H), 1.30 (t, J=9.0 Hz, 3H), 1.29 (t, J=9.0 Hz, 3H), 1.24 (d, J=6.0 Hz, 3H), 1.13 (s, 9H), 1.11 (s, 9H). Dia 4: 4.42–3.89 (m, 7H), 3.37 (d, J=30.0 Hz, 1H), 3.07–2.95 (m, 1H), 2.39–2.31 (m, 1H), 2.27–2.17 (m, 1H), 1.84–1.49 (m, 4H), 1.28 (t, J=6.0 Hz, 6H), 1.26 (d, J=6.0 Hz, 3H), 1.14 (s, 9H), 1.11 (s, 9H). ^{13}C NMR (75.54 MHz, $CDCl_3$, δ, ppm): Dia 1. 179.6 (C), 80.1 (CH), 68.7 (d, J=139.6 Hz, CH), 65.9 (CH_2), 61.0 (d, dia 1+dia 2, J=6.8 Hz, CH_2), 60.8 (C), 58.6 (d, J=6.8 Hz, CH_2), 42.9 (CH_2), 36.5 (CH), 34.8 (d, dia 1+dia 2, J=4.5 Hz, C), 32.0 (CH_2), 30.5 (d, J=5.3 Hz, 3 CH_3), 28.0 (CH_2), 27.5 (dia 1, 3 CH_3), 16.8 (CH_3), 16.2 (d, J=6.0 Hz, CH_3), 15.9 (d, J=6.8 Hz, CH_3). Dia 2: 179.2 (C), 81.2 (CH), 68.8 (d, J=140.4 Hz, CH), 65.4 (CH_2), 61.0 (d, dia 1+dia 2, J=6.8 Hz, CH_2), 60.9 (C), 58.8 (d, J=7.5 Hz, CH_2), 43.6 (CH_2), 34.9 (CH), 34.8 (d, dia 1+dia 2, J=4.5 Hz, C), 30.3 (d, J=6.0 Hz, CH_3), 28.5 (CH_2), 28.0 (CH_2), 27.5 (dia 2, 3 CH_3), 17.5 (CH_3), 16.1 (d, J=6.0 Hz, CH_3), 15.9 (d, J=6.0 Hz, CH_3). Dia 3: ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 4.33–3.93 (m, 7H), 3.35 (d, J=27.0 Hz, 1H), 3.05–2.93 (m, 1H), 2.66–2.59 (b, 1H), 2.19–2.10 (m, 1H), 1.95–1.78 (m, 4H), 1.30 (t, J=9.0 Hz, 3H), 1.29 (t, J=9.0 Hz, 3H), 1.24 (d, J=6.0 Hz, 3H), 1.13 (s, 9H), 1.11 (s, 9H). Dia 3: 178.4 (C), 79.4 (CH), 69.3 (d, J=137.4 Hz, CH), 69.0 (CH_2), 61.5 (d, J=6.0 Hz, CH_2), 61.4 (C), 59.7 (d, J=7.5 Hz, CH_2), 41.2 (CH_2), 35.5 (b, CH_2), 35.2 (C), 31.3 (CH), 30.8 (d, J=5.3 Hz, 3 CH_3), 28.9 (CH_2), 28.1 (3CH_3), 17.5 (CH_3), 16.6 (d, J=6.0 Hz, CH_3), 16.3 (d, J=6.8 Hz, CH_3). Dia 4: 178.0 (C), 80.0 (CH), 69.3 (d, J=138.9 Hz, CH), 69.4 (CH_2), 61.4 (d, J=6.8 Hz, CH_2), 61.1 (C), 59.2 (d, J=8.3 Hz, CH_2), 41.6 (CH_2), 35.2 (d, J=5.3 Hz, C), 33.3 (CH_2), 30.9 (d, J=6.0 Hz, 3 CH_3), 30.9 (CH), 29.1 (CH_2), 28.1 (3 CH_3), 17.8 (CH_3), 16.5 (d, J=6.0 Hz, CH_3), 16.3 (d, J=6.8 Hz, CH_3).



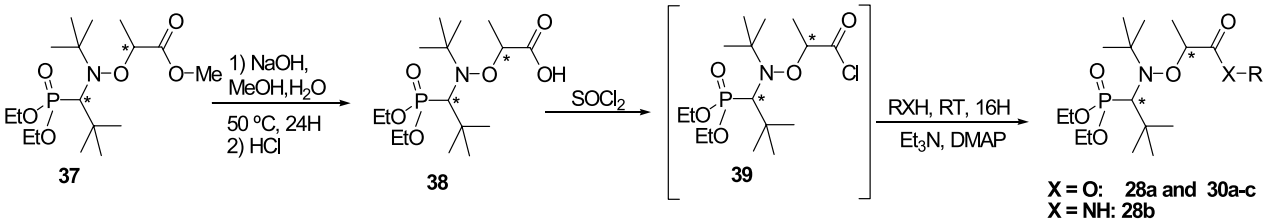
Scheme 15.

3. Results and discussion

As mentioned above, TEMPO-alkoxyamines are not suitable for the preparation of lactones and lactames because the TEMPO nitroxyl radical abstracts readily the H-atom from the alkoxycarbonylethyl-type radical (Scheme 1) and because the intramolecular H-tranfer is facilitated in stable alkoxyamines such as TEMPO-*t*Bu and TEMPO-EEst (Scheme 15).[31]

On the other hand, it has been shown in the case of SG1-alkoxyamines that neither the H-abstraction by the SG1 nitroxyl radical from the alkyl radical nor the intramolecular H-transfer occurred.[32] Consequently, SG1-alkoxyamines look as if they are the most suitable alkoxyamines to carry out radical cyclization with high yields. In general, alkoxyamines are prepared with the Atom Transfer Radical Addition (ATRA) method, which is today the easiest method. However, this method requires the presence of a copper catalyst for the alkyl radical to be generated and a big amount of metal wastes is produced. Furthermore, the metal residues have to be removed since the synthesis of drugs requires a high level of purity.They are difficult to get rid of, except when highly valuable alkoxyamines are prepared as displayed in Scheme 16. To validate our approach lactone **29a** and lactame **29b** were prepared starting from alkoxyamine MONAMS (**37**). It was firstly hydrolyzed into **38**, then the acid function was transformed into acyl chloride (**39** was not isolated) and homoallylic alcohol or amine was added to yield (≈50%) alkoxyamines **28a** or **28b**, respectively (Scheme 16).

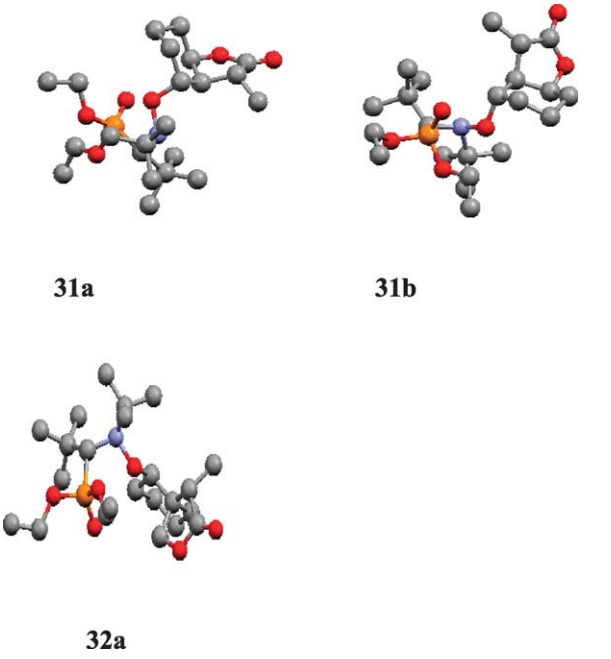
Heating a solution of 0.025 M of **29a** or **29b**, at 120 °C under nitrogen atmosphere for 12 h in a Schlenk flask, afforded lactone **28a** or lactame **28b** in 70% isolated yields (100% conversion, more than 95% of the targeted alkoxyamines by ^{31}P NMR). Alkoxyamines **28a** and **28b** were identified by ^1H, ^{13}C and ^{31}P NMR and Elemental analysis. Alkoxyamine **29a** was obtained as a mixture of four diastereoisomers in the ratio 12, 12, 38 and 38%. Such ratio is expected from the two possible transition states[13,33] and the unselective scavenging[34] of the primary alkyl radical by the nitroxyl radical SG1. Similarly, alkoxyamine



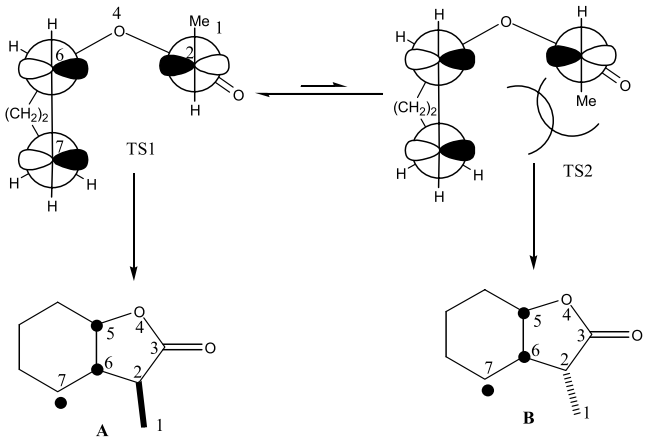
Scheme 16.

29b was obtained as a mixture of four diastereoisomers in the ratio 16, 16, 34 and 34%. To highlight the potentiality of that approach alkoxyamines **31**–**33** were prepared (Scheme 13). Alkoxyamines **30a**–**c** were prepared beforehand (50% yields) as depicted in Scheme 16. Structures were determined by ^{1}H, ^{13}C and ^{31}P NMR. All the cyclizations were carried out to total conversion, and alkoxyamines **31**–**33** were isolated with moderate to good yields (70, 60 and 70%, respectively) as racemics. Good to moderate stereoselectivity is observed (see Scheme 13) since three stereocenters in **30a** were replaced by five stereocenters in **31** but only four diastereoisomers were observed, and two stereocenters in **30b** were replaced by four stereocenters in **31** but only four diastereoisomers were again observed. The relative configurations of diastereoisomers **31a** and **31b**, and **32a** were determined by X-ray crystallography (Scheme 17).[35]

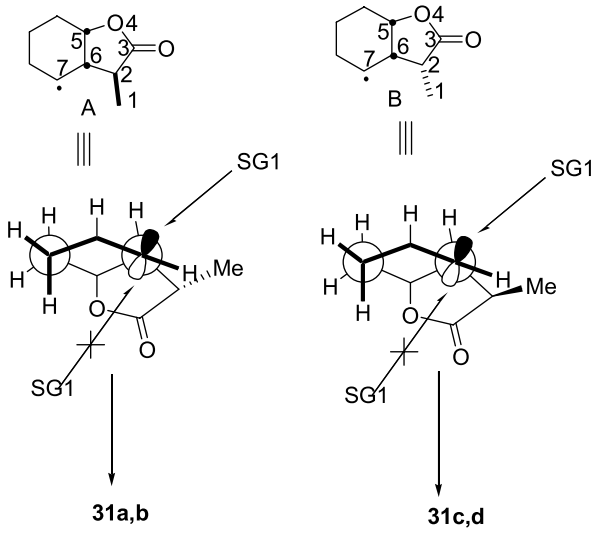
For lactone **31**, in agreement with the Beckwith–Baldwin rules,[36] the 5-*exo-trig* cyclization is favored over the 6-*endo-trig*.[37,38] Due to exclusive cis ring junction the stereochemistry of C_5 controls the stereochemistry of C_6 (Scheme 13).[33] The C_2 stereochemistry is controlled by a late transition state (TS) where the TS1 is favored over TS2, which is more sterically hindered (Scheme 18).[12] As expected from the work of Hannessian et al.[10] a high stereoselectivity (90%, see Scheme 13) in favor of the trans methyl group is observed (intermediate **A**, Scheme 18) and one can assume that the 10% left correspond to the diastereoisomer with the cis methyl group (TS2 leads to intermediate **B**, see Scheme 18).



Scheme 17. X-ray structure for alkoxyamines **31a**,**b**, and **32a**.

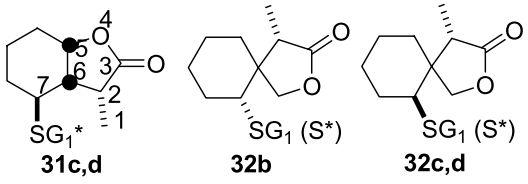


Scheme 18.



Scheme 19.

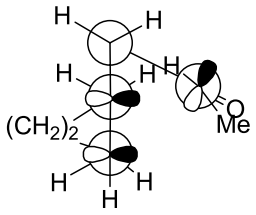
Then, both radicals **A** and **B** are stereoselectively scavenged by the SG1 via the less sterically hindered face to provide **32a**,**b** and **32c**,**d**, respectively (Scheme 19). Diastereoisomers **31a** and **31b** differ only by the chiral carbon on the SG1 moiety. Unfortunately, despite several attempts, it was not possible to isolate **31c** or **31d** sufficiently pure to confirm the stereochemistry given in Scheme 20.



Scheme 20.

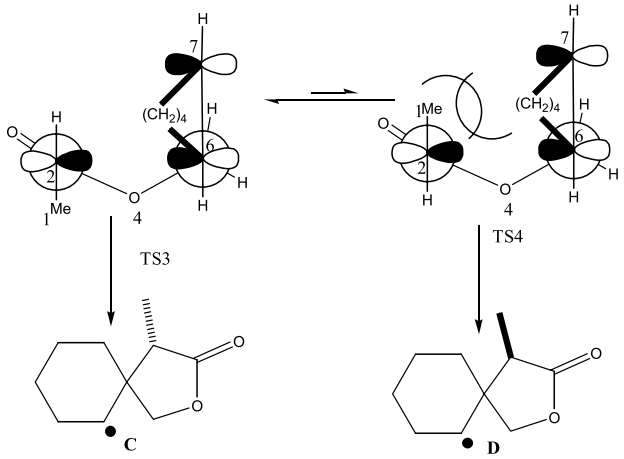
The synthesis of **32** is also highly selective because two new chiral centers are generated in the course of the reaction and the final molecule exhibits four assymetric carbons but only four diastereoisomers are observed (Scheme 13). And by analogy to **31a**,**b**, and because **32b** was obtained in the same amount as **32a** (Scheme 13), one can honestly assume that **32b** differs from **32a** only by the chiral carbon of the nitroxyl moiety.

For lactone **32**, in agreement with the Beckwith–Baldwin rules,[36] the *5-exo-trig* cyclization was favored over the



Scheme 21.

6-endo-trig (no overlap between the odd electron and the double bond, Scheme 21).[12,37,38] Like for lactone **31**, the α-ester alkyl radical was made up of two conformers *Z* and *E* in equilibrium and in roughly comparable amount.[39,40] However, TS3 was strongly preferred over TS4 because of strong steric strain between the methyl group and the six membered ring in TS4 (Scheme 22). The scavenging of the alkyl radical **C** by SG1 to afford **32** occurred through the sterically less hindered face.

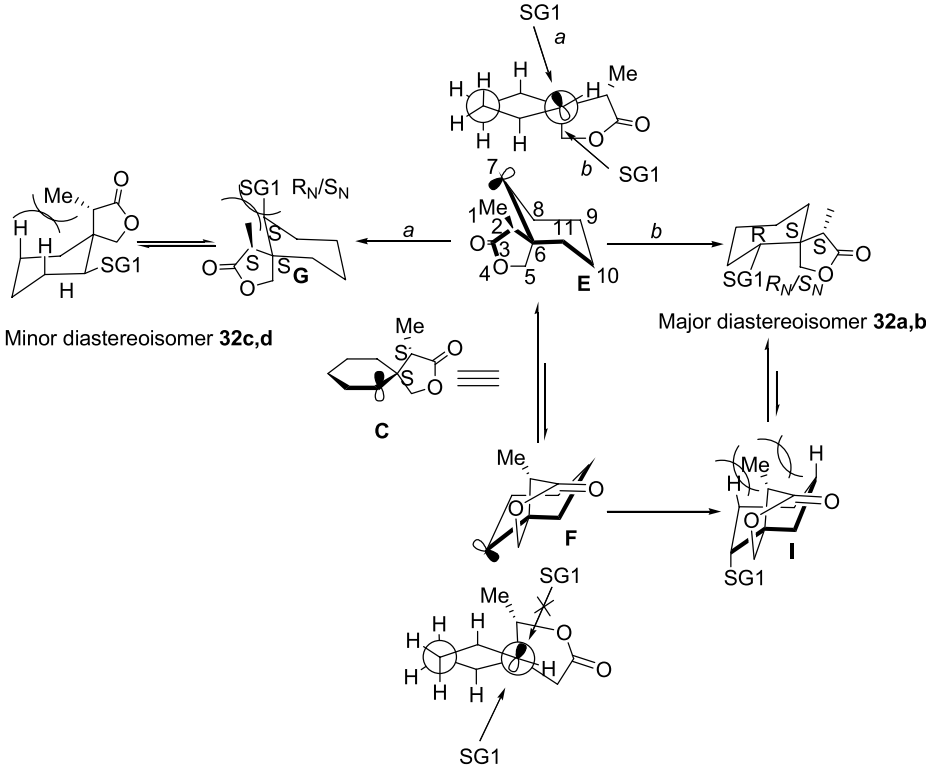


Scheme 22.

However, the conformer **E** should be more stable than the conformer **F**, which exhibit more *syn*-1,3 interactions (Scheme 23). Then, the formation of **32a**,**b** can occur either by the attack of the SG1 onto the more hindered face of the conformer **E** (route b) or by the attack of the SG1 on the more accessible face of the less stable conformer **F** to yield the strongly hindered conformer **I**, which exchange fastly into the most stable conformers **32a**,**b**.

The upper face of the conformer **F** is too sterically hindered to allow the approach of the SG1. Whereas the attack of the SG1 on the less sterically hindered upper face of the conformer **E** (route a, Scheme 23) should afford the probable minor compounds **32c**,**d** because of strong destabilizing *syn*-1,3 interactions. Unfortunately, it was not possible to confirm the structure of **32c**,**d** because all the attempts to separate **32c** or **32d** of **32a**,**b** were unsuccessful.

Eight-membered ring lactones are often difficult to prepare.[41] But Lee et al.[42] have shown that such lactones can be easily prepared via selective 8-*endo* radical cyclization. However, with unsubstituted precursors yields are generally low (<60%), for example, preparation of hydrogenated C_8 of **33** has been done with 13% yields.[42] That challenge has been readily overcome thanks to the Persistent Radical Effect,[43–45] which governs the reactivity of alkoxyamines.[46] Therefore, the synthesis of lactone **33** afforded quantitatively four diastereoisomers (three assymetric carbons in the final alkoxyamine) via 8-*endo* ring closure as expected from the work of Beckwith et al.[38]



Scheme 23.

and Lee et al.[42] The diastereoisomers were not separated but the ^{31}P NMR shifts lay in the alkoxyamine zone and were different from those of the starting materials (see Section 2). The 1:1:1:1 diaseteroisomer ratio delineates unhindered both cyclization and scavenging TSs.[33,34] The ^{13}C NMR analyses showed that the four C_8 carbons are bound to the SG1 fragment, which rules out any 7-*exo* cyclization that would have involved a methylene (C_9) bound to the SG1 fragment (Scheme 13). Although 100% conversion was observed by ^{31}P NMR with at least 95% of the expected compounds **33**, only 70% was recovered after chromatography. The success of the total conversion of **30c** in **33** leads us to expect that this radical generation approach via alkoxyamines would be very efficient to prepare large ring lactones with high yields.

4. Conclusion

In conclusion, this monocomponent system using SG1-based alkoxyamines as radical reaction initiators is very efficient for preparing heterocyclic compounds with good yields (>60%) and high stereoselectivity, that is, four diasteroisomers out of the 16 or 8 expected for lactones **31** and **32**, respectively. Furthermore, the experiments did not take too much time (less than 12 h) at not too high temperatures (110 °C). These syntheses highlight the powerfulness of the Persistent Radical Effect and of the SG1-based alkoxyamines applied to radical synthetic chemistry.

Acknowledgements

The authors thank M. Giorgi for performing X-ray experiments. S. M. is grateful to Pr M. Bertrand (Université d'Aix-Marseille 3) for helpful discussions and advices. The authors thank Université de Provence, CNRS and Arkema for their financial support.

References and notes

1. Renaud, P., Sibi, M., Eds.; Free Radicals in Organic Synthesis; Wiley-VCH: Weinheim, 2003; Vols. 1 and 2.
2. (a) Chatgilialoglu, C.; Griller, D.; Lesage, M. *J. Org. Chem.* **1988**, *53*, 3641–3642. (b) Ballestri, M.; Chatgilialoglu, C.; Seconi, G. *J. Organomet. chem.* **1991**, *408*, C1–C4. (c) Chatgilialoglu, C. *Acc. Chem. Res.* **1992**, *25*, 188–194.
3. Barton, D. H. R.; Jacob, M. *Tetrahedron Lett.* **1998**, *39*, 1331–1334.
4. Baguley, P. A.; Walton, J. C. *Angew. Chem., Int. Ed.* **1998**, *37*, 3072–3082.
5. Curran, D. P.; Kuo, S.-C. *Tetrahedron* **1987**, *43*, 5653–5661.
6. Surzur, J. M.; Bertrand, M.-P. *Pure Appl. Chem.* **1988**, *60*, 1659–1668.
7. Stork, G.; Mah, R. *Heterocycles* **1989**, *28*, 723–727.
8. Stork, G.; Mook, R., Jr.; Biller, S. A. *J. Am. Chem. Soc.* **1983**, *105*, 3741–3742.
9. Ueno, Y.; Chino, K.; Watanabe, M.; Motiya, O.; Okawara, M. *J. Am. Chem. Soc.* **1982**, *104*, 5564–5566.
10. Hanessian, S.; Di Fabio, R.; Marcoux, J.-F.; Prud'homme, M. *J. Org. Chem.* **1990**, *55*, 3436–3438.
11. Curran, D. P.; Chang, C.-T. *Tetrahedron Lett.* **1987**, *28*, 2477–2480.
12. Curran, D. P.; Chang, C.-T. *J. Org. Chem.* **1989**, *54*, 3140–3157.
13. Salom-Roig, X. J.; Dénès, F.; Renaud, P. *Synthesis* **2004**, 1903–1928 and references cited therein.
14. Ollivier, C.; Bark, T.; Renaud, P. *Synthesis* **2000**, 1598–1602.
15. Yorimitsu, H.; Nakamura, T.; Shinokubo, H.; Oshima, K. *J. Org. Chem.* **1998**, *63*, 8604–8605.
16. Chuang, C.-P.; Jiang, V. J. *J. Chin. Chem. Soc.* **1989**, *36*, 177.
17. Clark, A. J. *Chem. Soc. Rev.* **2002**, *31*, 1–11 and references cited therein.
18. Clark, A. J.; De Campo, F.; Deeth, R. J.; Filik, R. P.; Gatard, S.; Hunt, N. A.; Lastécouères, D.; Thomas, G. H.; Verlhac, J.-B.; Wongtap, H. *J. Chem. Soc., Perkin Trans 1* **2000**, 671.
19. Rizzardo, E.; Solomon, D.H.; Cacioli, P. U.S. Patent 4,581, 429, 1986.
20. Georges, M. K.; Veregin, R. P. N.; Kazmaier, P. M.; Hamer, G. K. *Macromolecules* **1993**, *26*, 2987–2988.
21. Studer, A. *Angew. Chem., Int. Ed.* **2000**, *39*, 1108–1111.
22. Wetter, C.; Studer, A. *Chem. Commun.* **2004**, 174–175.
23. Grimaldi, S.; Le Moigne, F.; Finet, J.-P.; Tordo, P.; Nicol, P.; Plechot, M. International Patent WO 96,24620. 15 August 1996.
24. Le Mercier, C.; Acerbis, S.; Bertin, D.; Chauvin, F.; Gigmes, D.; Guerret, O.; Lansalot, M.; Marque, S.; Le Moigne, F.; Fischer, H.; Tordo, P. *Macromol. Symp.* **2002**, *182*, 225–247.
25. Gigmes, D.; Marque, S.; Guerret, O.; Couturier, J.-L.; Chauvin, F.; Dufils, P.-E.; Bertin, D.; Tordo, P. WO. 2004014926 2004.
26. Nicolas, J.; Charleux, B.; Guerret, O.; Magnet, S. *Angew. Chem., Int. Ed.* **2004**, *43*, 6186–6189.
27. Leroi, C.; Fenet, B.; Couturier, J.-L.; Guerret, O.; Ciufolini, M. A. *Org. Lett.* **2003**, *5*, 1079–1081.
28. Leroi, C.; Fenet, B.; Couturier, J.-L.; Guerret, O.; Ciufolini, M.; Bertin, D.; Gigmes, D.; Marque, S.; Tordo, P. *Org. Lett.* **2003**, *5*, 4943–4945.
29. The nitroxyl radical **SG1** or the alkoxyamine **37** can be purchased to Arkema group.
30. Bertin, D.; Gigmes, D.; Marque, S.; Maurin, R.; Tordo, P. *J. Polym. Sci. Part A: Polym. Chem.* **2004**, *42*, 3504–3515.
31. (a) Ananchenko, G. S.; Fischer, H. *J. Polym. Sci: Part A: Polym. Chem.* **2001**, *39*, 3604–3621. (b) Henry-Ryad, H.; Tidwell, T. T. *J. Phys. Org. Chem.* **2003**, *16*, 559–563.
32. Ananchenko, G. S.; Souaille, M.; Fischer, H.; Le Mercier, C.; Tordo, P. *J. Polym. Sci: Part A: Polym. Chem.* **2002**, *4*, 3264–3283.
33. Curran, D. P.; Porter, N. A.; Giese, B. Stereochemistry of Radical Reactions.; VCH: Weinheim, 1996; Chapter 2, pp 23–115.
34. Sobek, J.; Martschke, R.; Fischer, H. *J. Am. Chem. Soc.* **2001**, *123*, 2849–2857.
35. CCDC-245737 for **31a**, CCDC-245738 for **31b**, CCDC-245739 for **32a** contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).
36. Baldwin, J. E. *J. Chem. Soc., Chem. Commun.* **1976**, 734–736.
37. Spellmeyer, D. C.; Houk, K. N. *J. Org. Chem.* **1987**, *52*, 959–974.

38. Beckwith, A. L. J.; Schiesser, C. H. *Tetrahedron* **1985**, *41*, 3925–3941.
39. Beckwith, A. L. J.; Glover, S. A. *Aust. J. Chem.* **1987**, *40*, 157–173.
40. Wu, L.; Fischer, H. *Helv. Chim. Acta* **1983**, *66*, 138–147.
41. Rousseau, G. *Tetrahedron* **1995**, *51*, 2777–2849.
42. Lee, E.; Yoon, C. H.; Lee, T. H.; Kim, S. Y.; Ha, T. J.; Sung, Y.; Park, S. H.; Lee, S. *J. Am. Chem. Soc.* **1998**, *120*, 7469–7478.
43. Fischer, H. *J. Am. Chem. Soc.* **1986**, *108*, 3925–3927.
44. Daikh, B. E.; Finke, R. G. *J. Am. Chem. Soc.* **1992**, *114*, 2938–2943.
45. Fischer, H. *Chem. Rev.* **2001**, *101*, 3581–3610 and references cited therein.
46. Kothe, T.; Marque, S.; Martschke, R.; Popov, M.; Fischer, H. *J. Chem. Soc., Perkin Trans. 2* **1998**, 1553–1559.