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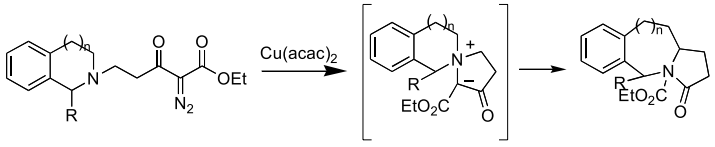
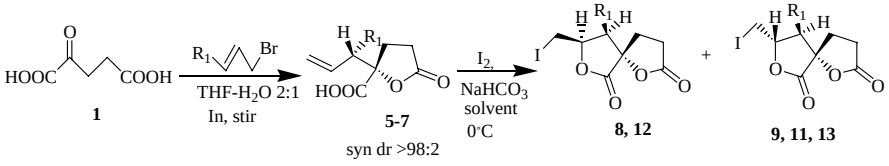
Contents

REPORT

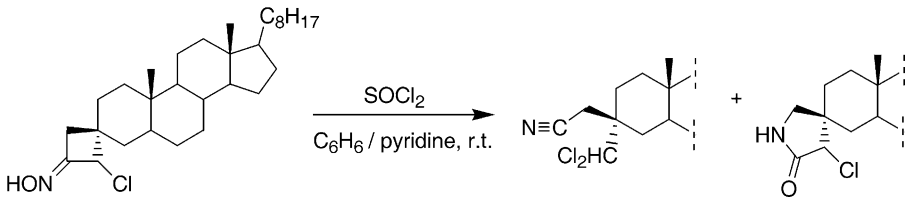


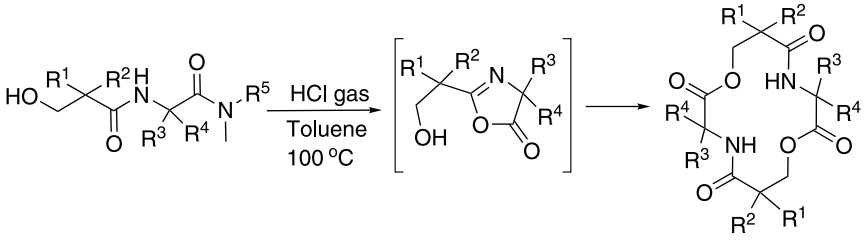
Recent advances in the synthetic applications of the Stevens [1,2]-shift of ammonium ylides are reviewed. Methods for ylide generation, ring-expansion processes, and strategies for stereocontrol are emphasized along with application to alkaloid natural products.

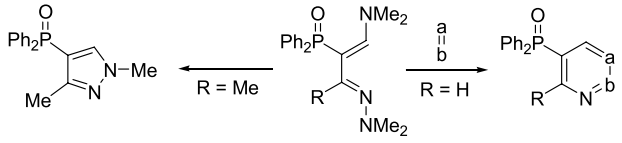
ARTICLES

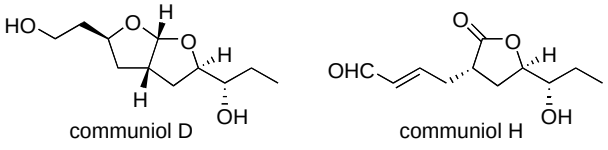


The title spirononanes have been obtained by two step approach involving indium mediated allylation of 2-oxoglutaric acid and subsequent iodocyclization.









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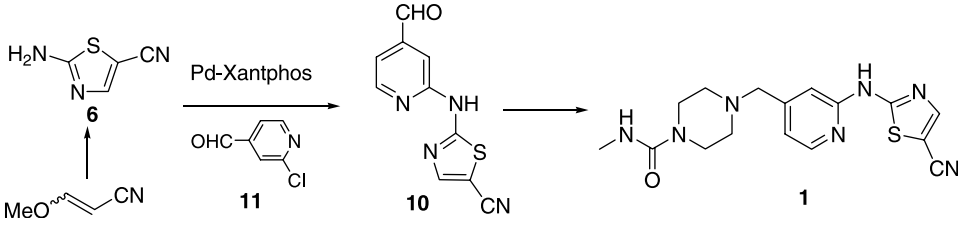
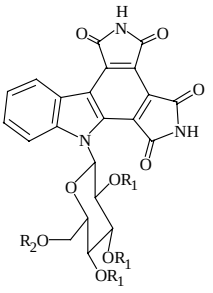
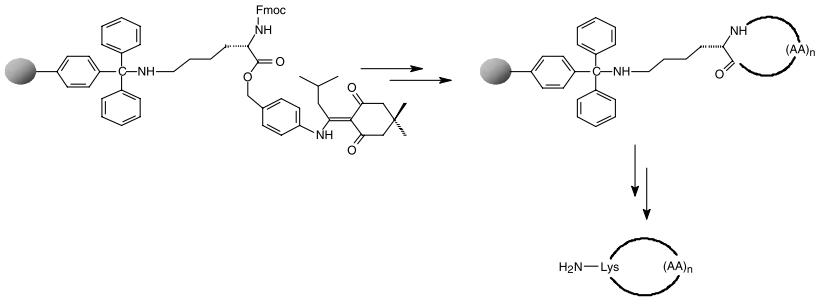
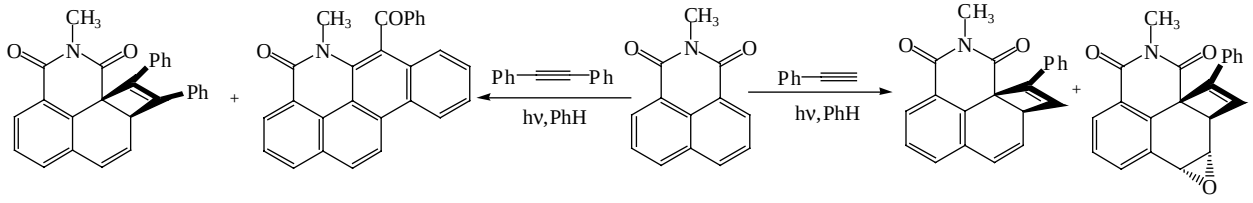
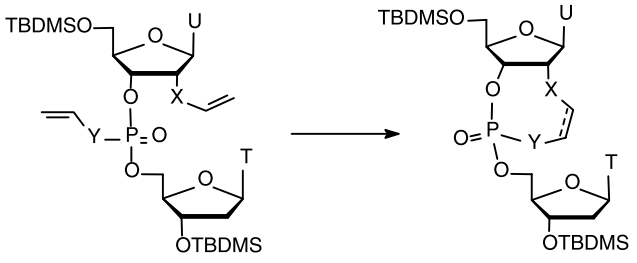
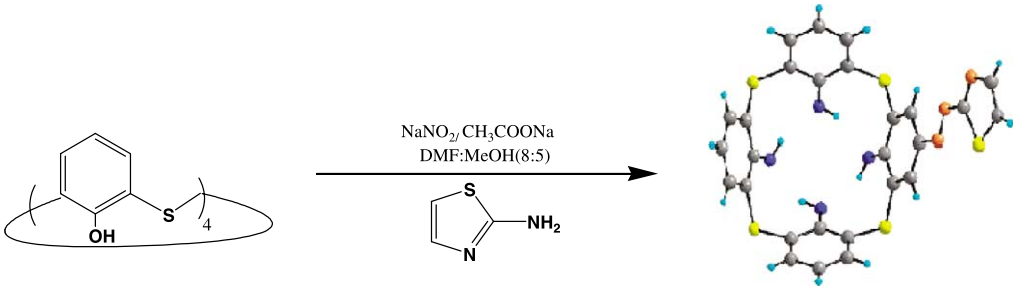


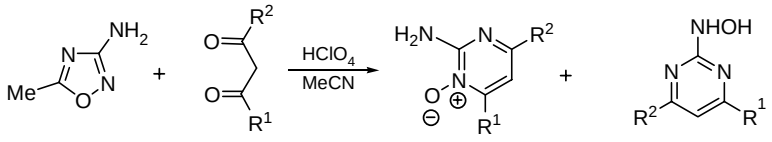


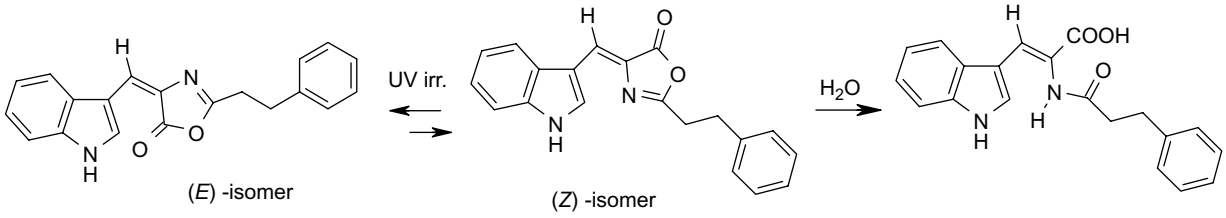


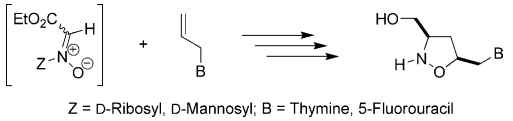


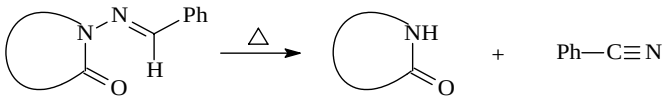


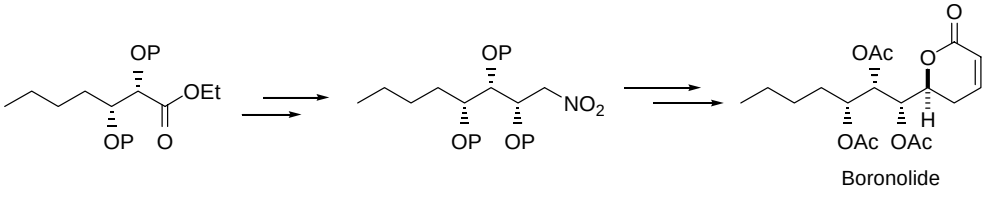


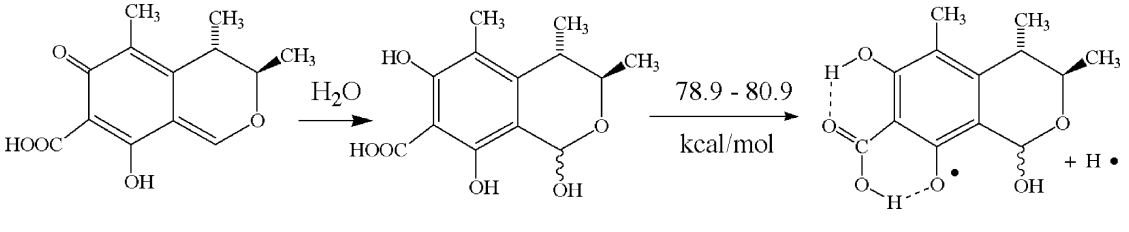


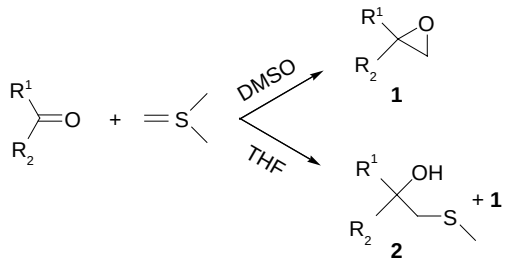


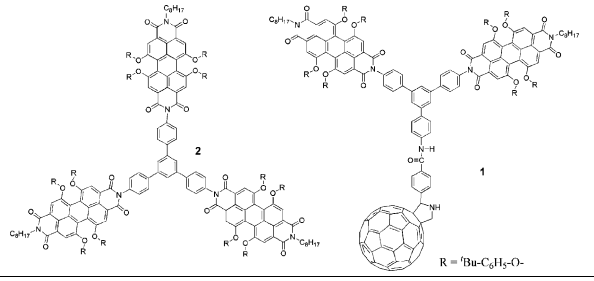


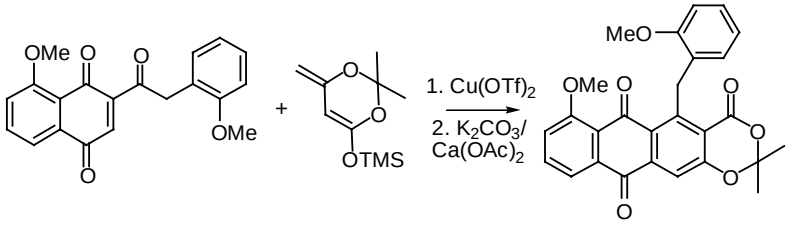


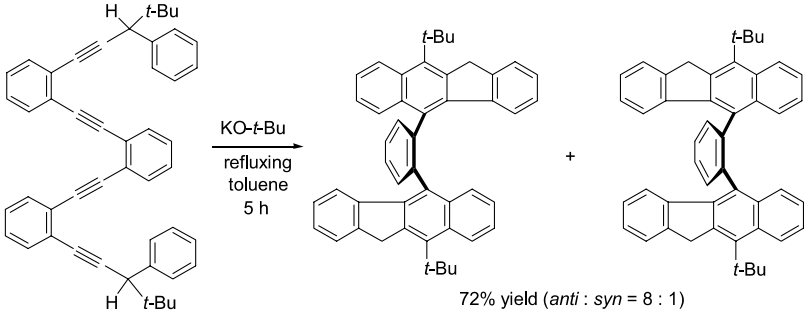


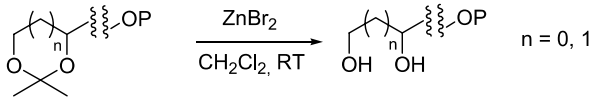


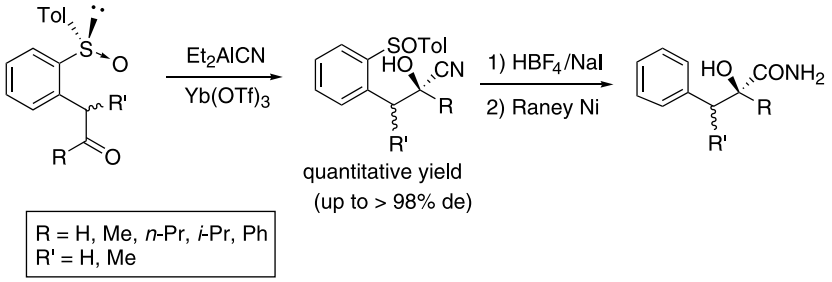




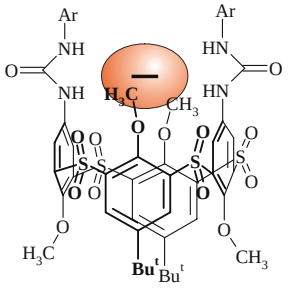


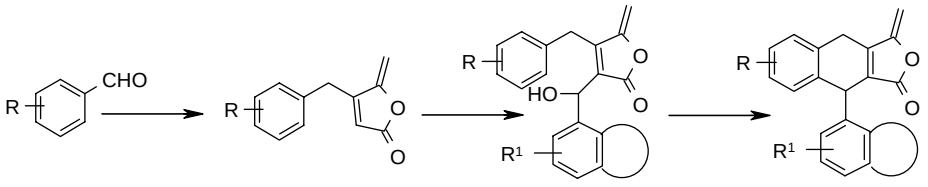


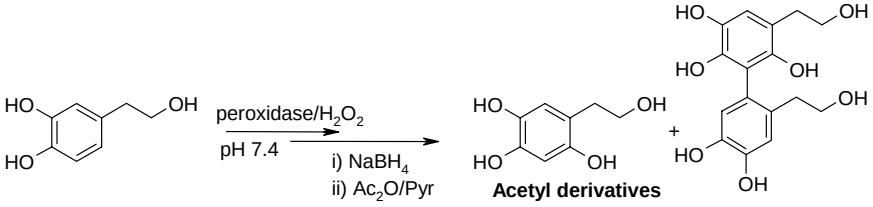


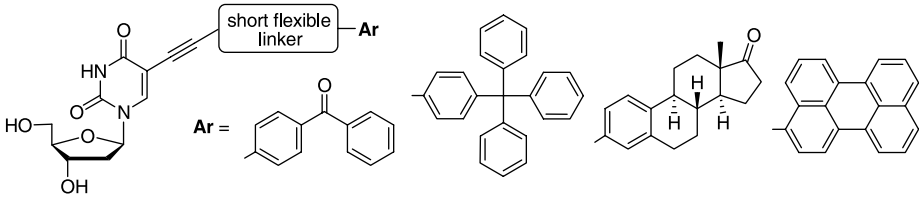


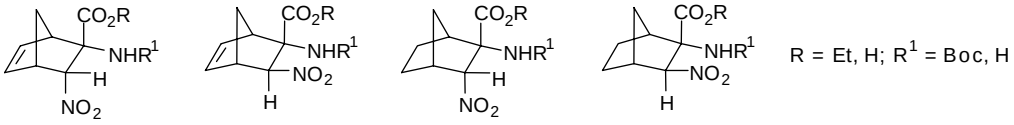
The regioselective *ipso*-nitration of *tert*-butylthiacalix[4]arene-tetrasulfone was used for the construction of thiacalixarene derivatives bearing one or two arylureido functions on the upper rim. The preorganization of ureido units using the thiacalix[4]arene moiety as a molecular scaffold leads to novel anion receptors with good complexation ability in organic solvents.

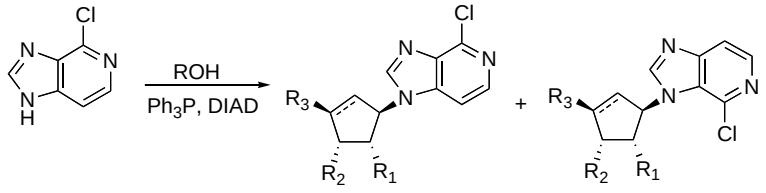


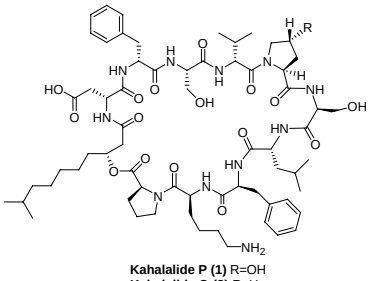


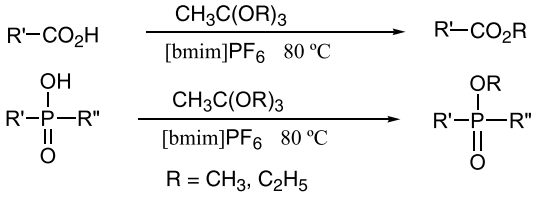












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Recent advances in the Stevens rearrangement of ammonium ylides. Application to the synthesis of alkaloid natural products

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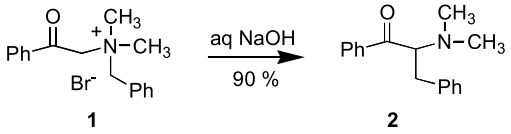
1. Introduction

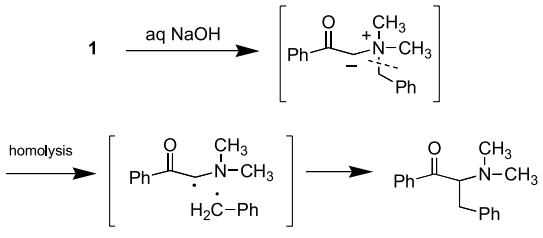
In 1928, Stevens and co-workers[1] discovered a novel [1,2]-shift involving ammonium ylides while investigating amine-protecting groups (Scheme 1). Stevens noticed that under basic conditions, ammonium salt **1** provided the rearranged tertiary amine product **2** in good yield. This type of rearrangement, now commonly referred to as the Stevens rearrangement, or Stevens [1,2]-shift, has the unique ability to generate many different types of nitrogen containing compounds. The utility of such a transformation has encouraged increased study since its discovery, and the reaction has been reviewed several times.[2] This article will provide a summary of the recent advances in the Stevens rearrangement of ammonium ylides since the last review.[2c] Emphasis will be placed on the [1,2]-shift; the [2,3]-shift will only be discussed in cases where both processes are observed. In addition, the synthetic applications of the Stevens rearrangement of ammonium ylides will also be discussed.



Scheme 1. First example of the Stevens rearrangement.

2. Mechanism of the Stevens rearrangement

Three distinct mechanisms have been proposed for the Stevens rearrangement: ion-pair,[3] concerted [1,2]-shift,[4–9] and radical-pair.[2,7,8,10,11] Most evidence suggests that the shift occurs through a radical pair mechanism. This mechanism involves homolytic cleavage of the carbon–nitrogen bond to the most stable potential carbon-centered radical, generating a radical pair that is held tightly together by a solvent cage. This is followed by rapid recombination to provide the Stevens [1,2]-shift product (Scheme 2). This solvent cage/rapid recombination pathway would also account for the high degree of intramolecularity and stereoselectivity observed in these rearrangements.[7a,10e] However, the transition metal involvement in the [1,2]-shift cannot be ruled out in those cases in which the ylide is generated via a metallocarbene (vide infra).

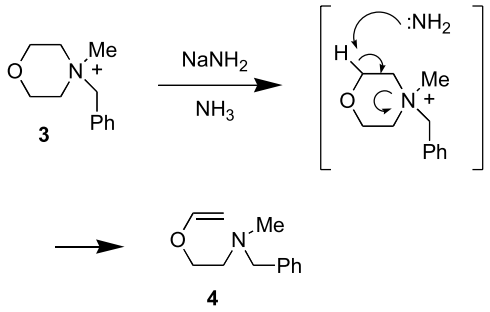


Scheme 2. Radical pair mechanism.

3. Ylide formation

3.1. Base-induced ylide generation

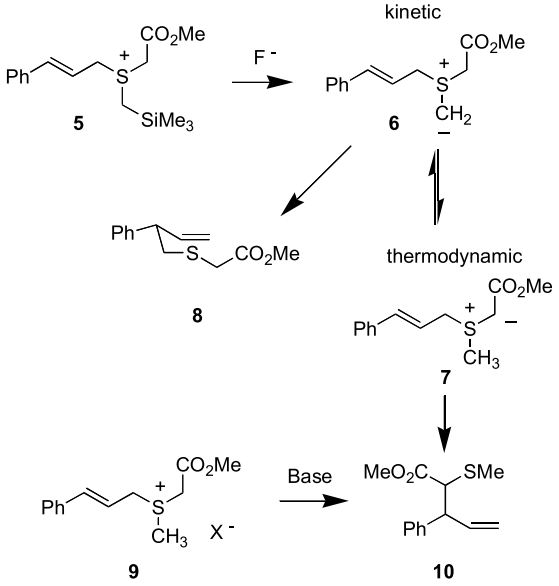
The earliest methods of ylide generation were conducted by treating quaternary ammonium salts with a strong base (see Scheme 1). Inherent to this method is the potential for side reactions in the salt forming step if a nucleophilic counterion is used, as well as Hofmann elimination of the amine (Scheme 3).[12] This elimination process can dominate and greatly decrease the synthetic utility of the Stevens rearrangement. Regioselective ylide formation may also be problematic if there is more than one acidic site in the precursor salt.



Scheme 3. Hofmann elimination to give side product.

3.2. Fluoride-mediated desilylation

Years later, Vedejs[13] and Sato[14] found a solution to this problem by regioselectively generating ylides under nonbasic conditions using fluoride-mediated desilylation of trimethylsilyl-substituted onium salts. This method was especially well suited to ammonium and sulfonium salts, although in some cases both kinetic (**8**) and thermodynamic (**10**) rearranged products were seen (Scheme 4). In contrast, base induced ylide formation led to only the thermodynamic product (**9**→**10**). This process does provide two advantages over the basic method. First, it minimizes the elimination products and second, it usually leads to regioselective ylide formation.

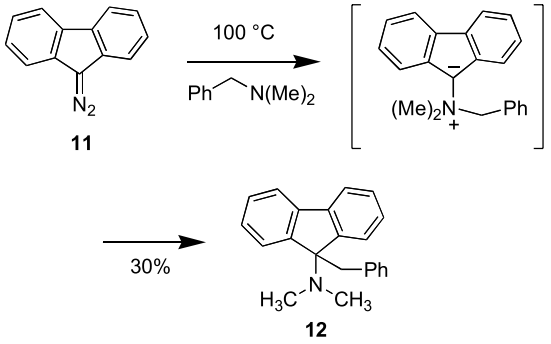


Scheme 4. Fluoride-mediated desilylation ylide formation.

3.3. Ylide generation via carbenes

The use of carbenes as a way to access ammonium ylides was first presented by Stevens in 1952 (Scheme 5).[15] By

heating diazofluorene **11** in the presence of *N*,*N*-dimethylbenzylamine, the tertiary amine product **12** was formed in 30% yield. The use of carbenes quickly became a viable alternative to base-promoted ylide formation.[2]



Scheme 5. Use of carbenes to access ammonium ylides.

Early on, carbenes were generated through photochemical or thermal decomposition of diazo precursors, but the reactivity of these types of carbenes was rather random.[13,16] It was not until the advent of copper catalysts[17–21] and rhodium carboxylates[22,23] in generating metal stabilized carbenes that the Stevens rearrangement began to emerge as a powerful synthetic tool.[24a]

Figure 1 shows how diazo compounds are thought to combine with metal catalysts to form reactive electrophilic carbenoids **15**. Coordinative unsaturation at the metal center allows for addition to the diazo species **13**, to give intermediate **14**. Loss of nitrogen gas then provides metal-stabilized carbene **15**. Attack of the electrophilic carbene by a nucleophilic amine **16** provides the desired metal-associated ylide **17**,[24b] which may undergo rearrangement or first suffer dissociation of the metal to generate the free ylide **18**.

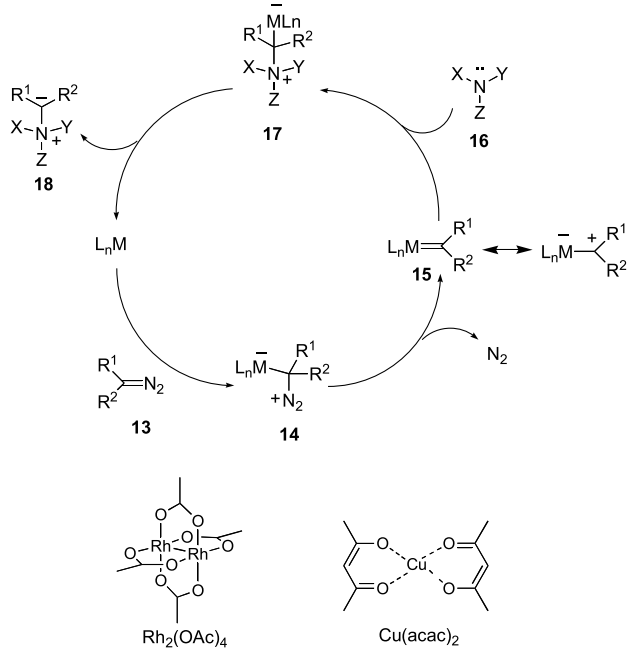


Figure 1. Catalytic cycle for generation of ammonium ylides via metallocarbenes.

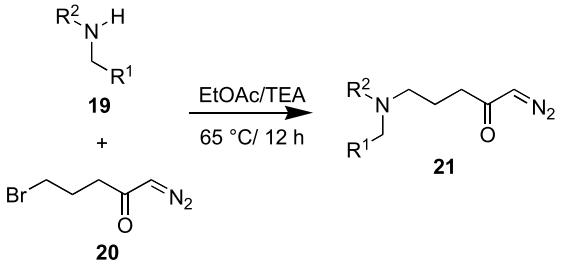
4. New methodology

Recent growth in the application of the Stevens rearrangement in synthesis has been driven by the development of new methods. Section 4.1 will review the advances in methodology.[24c–f]

4.1. Recent advances in ylide generation methodology

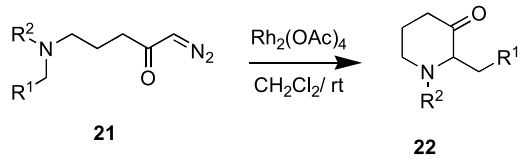
4.1.1. Intramolecular carbenoid-amine reactions. Substituted piperidine-3-ones are useful building blocks in the synthesis of alkaloid natural products. West and Naidu developed a route to these compounds utilizing a domino sequence.[10a] In their approach, diazoketones with pendant tertiary amines are first converted to metallocarbenes, which then undergo intramolecular attack by the amine to form cyclic ammonium ylides. The ylides, in turn, undergo a [1,2]-shift of one of the nitrogen substituents.[25]

Diazoketones containing pendant tertiary amine groups can be obtained simply in a one-step process. Reaction of a variety of secondary amines **19** with bromide **20** provided tertiary amines **21** in good to excellent yields (Scheme 6).



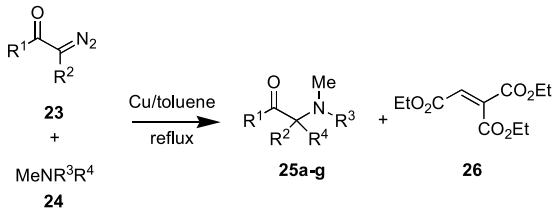
Scheme 6. Synthesis of diazoketones containing pendant tertiary amines.

Subsequent treatment of the tertiary amines (**21**) with $Rh_2(OAc)_4$ then provided the 3-piperidone [1,2]-shift products (Scheme 7). A major concern with the use of $Rh_2(OAc)_4$ was the high affinity of amines for the empty coordination sites on the dimeric catalyst.[26] Prior to the work by West and co-workers, the only other successful example of ammonium ylide generation from Rh-carbenoids to overcome this problem involved use of high dilution and long addition times.[22] This chemistry allows for efficient entry into substituted piperidinones since the starting acyclic 5-amino-1-diazopentan-2-ones are easily prepared. The key transformation is simple and does not require high-dilution or slow addition times. The absence of substrate deactivation of the catalyst in these intramolecular cases may result from internal delivery of the diazo moiety following initial complexation of the tertiary amine to the empty rhodium coordination site. Not long after this approach, Clark and co-workers released results on the use of ammonium ylide [2,3]-shifts from intramolecular



Scheme 7. Formation of 2-substituted piperidin-3-ones.

Table 1. Intermolecular carbenoid–amine reactions



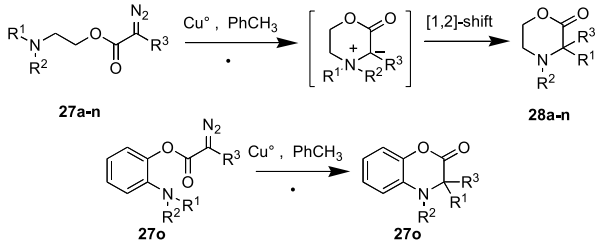
Entry	R^1	R^2	R^3	R^4	Product (% yield)
1	OEt	H	Me	CH_2Ph	**25a** (63)
2	OEt	H	Me	CH_2CO_2Et	**25b** (35)
3	OEt	H	CH_2CO_2Et	CH_2Ph	—
4	Ph	H	Me	CH_2Ph	**25c** (70)
5	Ph	H	Me	CH_2CO_2Et	**25d** (26)
6	Ph	H	CH_2CO_2Et	CH_2Ph	**25e** (70)
7	OEt	CO_2Et	Me	CH_2Ph	**25f** (97)
8	OEt	CO_2Et	Me	CH_2CO_2Et	**26** (90)
9	OEt	CO_2Et	CH_2CO_2Et	CH_2Ph	**25g** (92)
10	OEt	H	Me	$CH(CO_2Et)_2$	**26** (100)

trapping of a metallocarbenoid by an amine.[27] This related study provided similar cyclic amines in good yields employing both rhodium and copper catalysts. Both of these approaches to cyclic amines can be envisioned as rapid entries into alkaloid natural product systems.

4.1.2. Intermolecular carbenoid–amine reactions. Around the same time, West and co-workers also published an efficient one-step synthesis of substituted α-aminocarbonyl compounds from amines and diazocarbonyl compounds.[28] The intermolecular reaction between ethyl diazoacetate (EDA) and tertiary amines was examined. Initial attempts using rhodium(II) acetate dimer were unsuccessful. The need for excess amine and the known affinity of amines for the two empty axial sites on the rhodium dimer was believed to be the problem and even with slow addition times, could not be overcome. Switching to copper catalysts, it was found that [1,2]-shift products could be isolated in good to excellent yields. This prompted the authors to examine a variety of amines and diazo compounds (Table 1). The convergence of this method and the simplicity of the two reactants makes this methodology very convenient as an entry into the synthesis of α-amino ester and α-aminoketone compounds. However, there were a few potential drawbacks to this process. The necessity of more than 1 equiv of amine as well as the unsuitability of the resulting tertiary amines for subsequent transformations prompted further studies in this area.

In 1994, West and co-workers introduced an alternative to their approach above by linking the carbenoid precursor and the nucleophilic amine via an ester, allowing for the

Table 2. Intramolecular carbenoid–amine additions

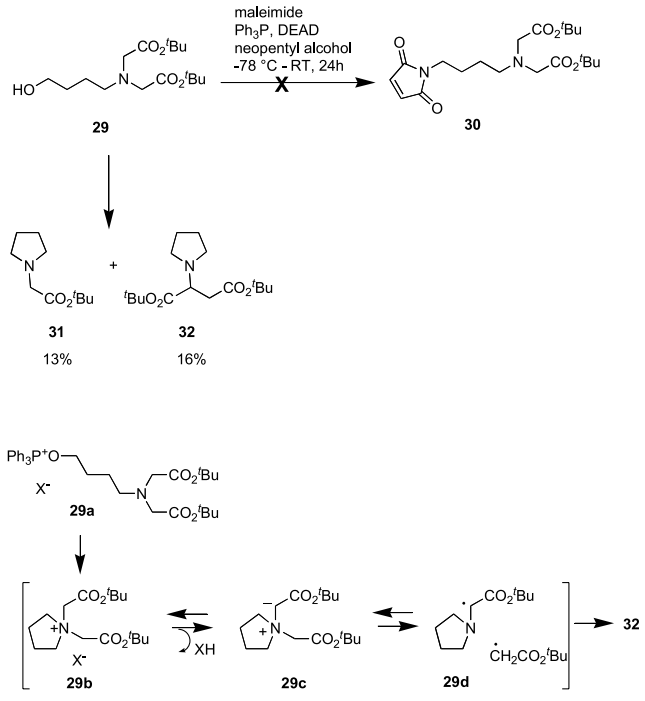


Entry	Reactant	R^1	R^2	R^3	Product (% yield)
1	**27a**	$C_6H_5CH_2$	CH_3	Ac	**28a** (79)
2	**27b**	$C_6H_5CH_2$	$C_6H_5CH_2$	Ac	**28b** (0)
3	**27c**	*p*-NO_2–$C_6H_4CH_2$	CH_3	Ac	**28c** (68)
4	**27d**	*p*-Ac–$C_6H_4CH_2$	CH_3	Ac	**28d** (64)
5	**27e**	*p*-MeO–$_6H_4CH_2$	CH_3	Ac	**28e** (76)
6	**27f**	*p*-Me_2N–$_6H_4CH_2$	CH_3	Ac	**28f** (55)
7	**27g**	$(C_6H_5)_2CH$	CH_3	Ac	**28g** (0)
8	**27h**	$(CH_3)_2CH$	CH_3	Ac	**28h** (0)
9	**27i**	$(CH_3)_3C$	CH_3	Ac	**28i** (10)
10	**27j**	CH_2=$CHCH_2$	CH_2=$CHCH_2$	Ac	**28j** (70)
11	**27k**	CMe_2=$CHCH_2$	CMe_2=$CHCH_2$	Ac	**28k** (72)
12	**27l**	$NCCH_2$	CH_3	H	**28l** (19)[a]
13	**27m**	$C_6H_5CH_2$	CH_3	H	**28m** (64)[a]
14	**27n**	$C_6H_5CH_2$	$C_6H_5CH_2$	H	**28n** (64)[a]
15	**27o**	$C_6H_5CH_2$	CH_3	Ac	**28o** (80)

[a] $Cu(acac)_2$ was used in place of Cu^0.

intervention of cyclic ylides.[29] This would eliminate the need for excess amine and benefit from the high efficiency of [1,2]-shifts of cyclic ammonium ylides derived from intramolecular carbenoid-amine additions.[10a,27] They also envisioned that oxidative cleavage of the amine linker would permit access to versatile secondary amines. To this end, the reaction of **27a** with rhodium and copper catalysts was examined in order to help determine optimal conditions for the subsequent screening of a variety of diazoketones. Though success had been obtained with $Rh_2(OAc)_4$ in similar transformations, treatment of **27a** with $Rh_2(OAc)_4$ in CH_2Cl_2 failed to provide **28a**. The same catalyst did provide small amounts of **28a** when used in toluene at reflux. On the other hand, copper powder and soluble copper catalysts did provide good to excellent yields of **28a**, which may be a consequence of the greater electrophilicity of the derived copper carbenes.[22] For cost efficiency, copper powder was used to examine this transformation on a wide variety of diazoketones since it worked as well as other soluble copper catalysts and is rather inexpensive by comparison (Table 2). For the most part, racemic morpholines **28a–o** could be generated via Stevens [1,2]-shift in good yields. In the case of monostabilized carbenoids (entries 13–18), however, yields were typically lower. Use of readily available starting materials and the absence of high-dilution or slow addition conditions makes this an efficient synthesis of possible amino acid precursors.[30] Similar studies involving [2,3]-shifts were also reported by Clark and co-workers.[31]

4.1.3. Ylide formation under Mitsunobu conditions. In 1996, Walker[10b] discovered a novel method for ylide formation via intramolecular amine quaternization while treating **29** under standard Mitsunobu conditions in an attempt to generate **30** (Scheme 8). Instead compounds **31** and **32** were isolated, albeit in low yields. Upon formation of the phosphonium salt **29a** (where X is $(EtO_2CNHNCO_2Et)^-$), intramolecular attack by nitrogen leads to **29b**. Deprotonation by the conjugate base X^-, leads to ylide **29c**, which upon homolysis, provides biradical **29d**. Rapid recombination would then furnish product **32**.



Scheme 8. Walker's novel ylide generation and Stevens rearrangement under Mitsunobu conditions.

The yield of **32** could be improved upon by running the reaction in the absence of maleimide or neopentyl alcohol. These conditions also did not produce any of compound **31**. An optimized yield of 57% was obtained by increasing the amount of DEAD/Ph_3P to 1.5 equiv and was further improved to 77% when DEAD was replaced with 2.2 equiv of ADDP (1,1′-(azodicarbonyl)dipiperidine). Investigations were then conducted on the length of the alcohol tether and the authors found this methodology to be applicable to five- or six-membered ring synthesis. All other attempts at larger ring systems failed to provide any desired product. Most noteworthy in this sequence is that the reaction is carried out in essentially neutral conditions, whereas basic conditions are typically needed for the initiation of Stevens rearrangement from ammonium salts. Also worth mentioning is that the requisite ammonium salt formation and subsequent ylide generation/Stevens rearrangement occur in one-pot.

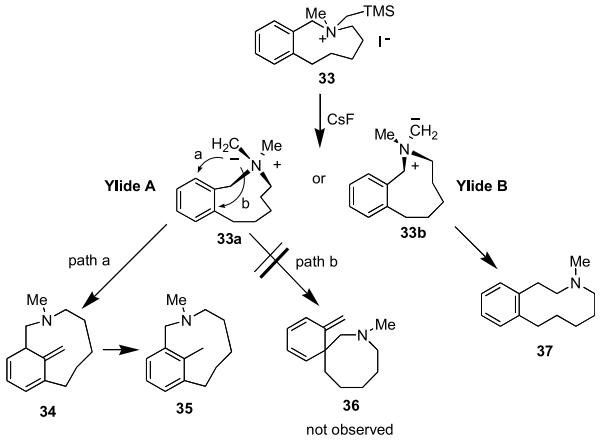


Figure 2. Competing Sommelet–Hauser and Stevens rearrangement of compound **33**.

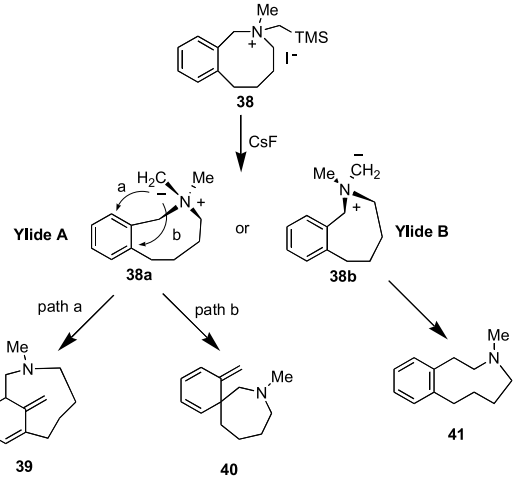


Figure 3. Competing Sommelet–Hauser and Stevens rearrangement of compound **38**.

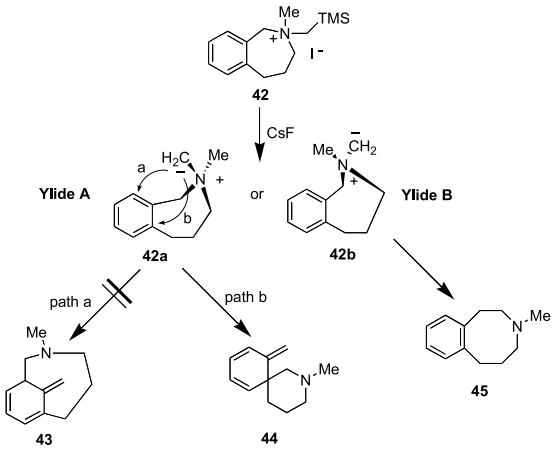


Figure 4. Competing Sommelet–Hauser and Stevens rearrangement of compound **42**.

4.1.4. Selectivity in ylide rearrangements under disilylative conditions. While investigating the synthetic utility of the Sommelet–Hauser rearrangement of cyclic benzylic ammonium salts **33**, **38**, and **42**, Sato observed very different product distributions depending on the ring size of the starting salt (Figs. 2–4; Table 3)[32a] In the case of the larger ring size, **33**, the [1,2]-shift product **37** was isolated as the major product while the isotoluene product, **35**, was obtained in minor amounts (Fig. 2).

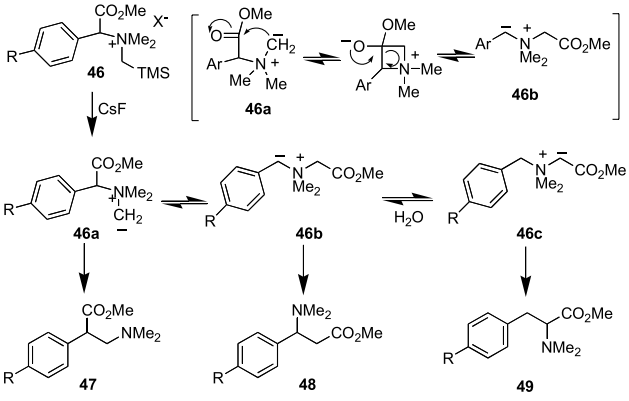
However, as the ring size was decreased, the amount of Sommelet–Hauser (S–H) products were seen in increasing amounts, especially the spirocyclic type as exemplified by **40** (Fig. 3).

The smallest ring size **42** gave almost exclusive formation of the Sommelet–Hauser spiro compound **44** with minor amounts of the Stevens rearrangement product (Fig. 4).

Table 3. Product distribution for reactions of compounds **33**, **38**, and **42**

Entry	Salt	Additive	Total yield of products (%)	S–H	Amines (ratio)[a]		
					Stevens	Isotoluene	Spiro compound
1	**33**		86		**37** (71)	**34** (29)	**36** (0)
2	**33**		82		**37** (73)	**34** (27)	**36** (0)
3	**33**	DBU	79	**35** (14)	**37** (70)	**34** (16)	**36** (0)
4	**38**		46		**41** (48)	**39** (38)	**40** (14)
5	**38**	DBU	40		**41** (47)	**39** (40)	**40** (13)
6	**42**		23		**45** (9)	**43** (0)	**44** (84)

[a] Determined via ^{1}H NMR integration. Compounds **34** and **39** were identified by ^{1}H and ^{13}C NMR and UV of the crude reaction mixture and not isolated. These products tautomerized to the aromatic products on silica gel.



Scheme 9. Treatment of ammonium salts **46** with CsF.

The authors later re-examined these types of reactions in effort to better understand the reaction pathway.[32b] In this study, acyclic ammonium salts of the type **46** were synthesized and treated with CsF in DMF (Scheme 9). A mixture of products **47**, **48**, and **49** was isolated depending on the starting aromatic compound as well as the salt's counterion (Table 4). Isomeric Stevens [1,2]-shift products **47** and **48** were isolated in all cases but one. When the aryl substituent R is methoxy (entry 4), only product **47** was isolated, presumably due to minimal equilibration between ylides **46a** and **46b** in the case of the more electron rich aryl group. The two results (entries 2 and 6) in which a third Stevens rearrangement product **49** was isolated are also of interest. Here, the CsF was not dried prior to the reaction and it is believed a small amount of water may facilitate equilibrium between ylides **46b** and **46c** to furnish

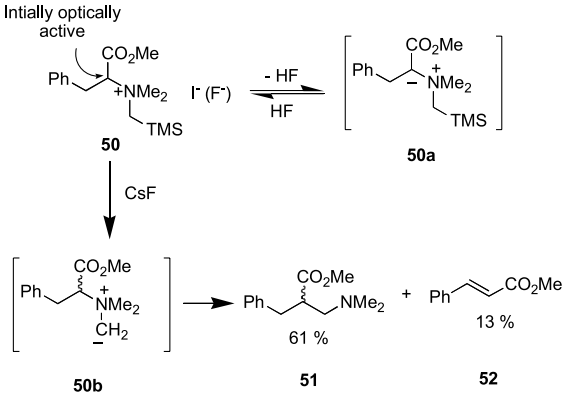
Table 4. Product distribution with compound **46**

Entry	Salt	R	X	Total yield (%)	Product proportions[a]		
					47	**48**	**49**
1	**a**	H	I	81	70	30	0
2	**a**	H	I	27[b]	65	16	19
3	**b**	Me	ClO_4	89	80	20	0
4	**c**	OMe	I	50	100	0	0
5	**d**	F	ClO_4	86	80	20	0
6	**d**	F	ClO_4	33[b]	69	19	12
7	**e**	Cl	ClO_4	95[c]	77	23	0

[a] Proportions of the product were determined by integration of signals in 400 or 500 MHz ^{1}H NMR.
[b] CsF was not predried.
[c] Includes methyl 2-(4-chlorophenyl)propenoate (9%).

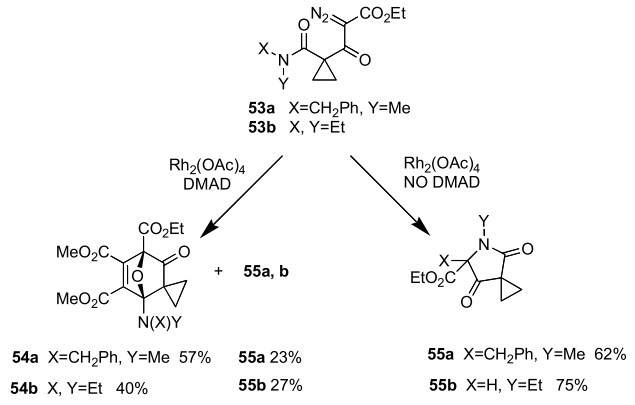
[1,2]-shift product **49**. Support of this is seen in the recent work of Jon'czyk and Zdrojewski.[33]

Sato and co-workers also looked at optically active ammonium salts **50** (Scheme 10). Treatment of **50** with CsF provided the expected tertiary amine product **51** (61%) as well as Hofmann elimination product **52** (13%). While there are many examples of excellent stereochemical retention in Stevens rearrangements of ylides with chiral migrating centers,[2c] product **51** was isolated as a racemic mixture. The authors attributed this to fluoride ion-induced α-deprotonation of **50**, which would give an equilibrium mixture of ylide **50a** and **50** with eventual formation of the desired ylide **50b** as a racemate.



Scheme 10. Treatment of ammonium salt **50** with CsF.

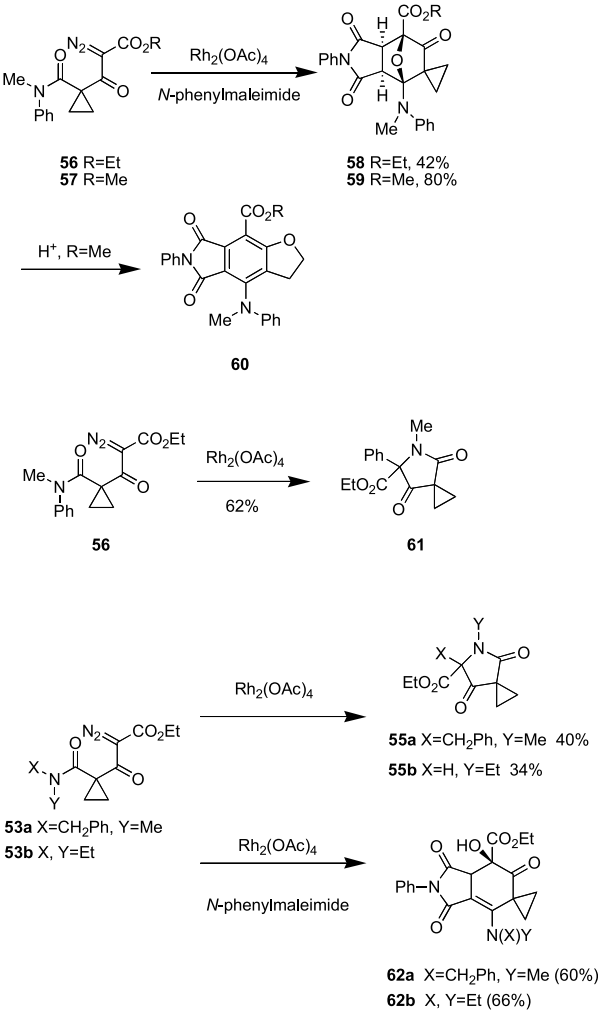
4.1.5. Competing formation of ammonium and carbonyl ylides. Padwa and co-workers recently investigated the α-diazo ketoamide **53a** in an effort to compare product distributions arising from ammonium versus carbonyl ylide formation when both pathways are possible (Scheme 11).[34] They expected to see preferential carbonyl ylide formation followed by reaction with DMAD (dimethyl acetylenedicarboxylate) to provide product **54a**. Instead, a mixture was isolated, consisting of **54a** (57%) and the lactam **55a** (23%). Exclusion of DMAD furnished solely product **55a** in 62% yield. This product was attributed to the formation of an *N*-acylammonium ylide followed by [1,2]-shift of the benzyl group. Switching to diazo compound **53b**, products **54b** (40%) and **55b** (27%) were isolated when DMAD was present. When DMAD was removed from the reaction, only product **55b** was isolated in 75% yield. It is presumed that



Scheme 11. Competing ammonium and carbonyl ylide formation.

55b arises from α′,β-fragmentation of the intermediate ammonium ylide, with consequent generation of ethylene.

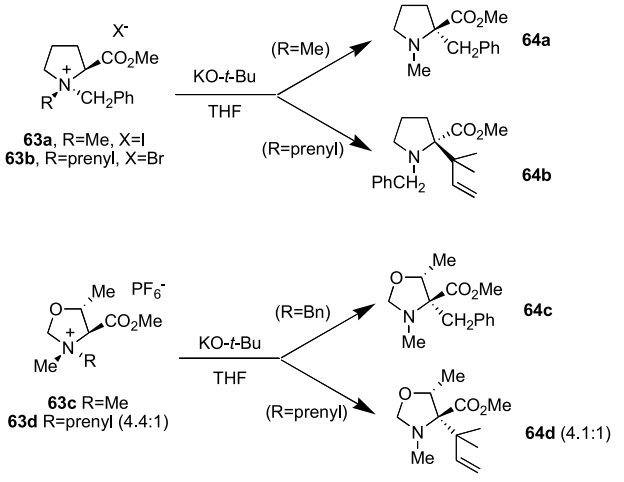
Further investigations into the nature of these reactions provided similar results.[35] Using the trapping agent *N*-phenylmaleimide, compound **56** provided the cycloadduct **58** in 42% yield (Scheme 12). Upon switching to the methyl ester **57**, cycloadduct **59** was isolated in excellent yield (80%). Treatment of **56** under the same reaction conditions in the absence of an external dipolarphile efficiently provided the Stevens [1,2]-shift product **61** in 62% yield.



Scheme 12. Further investigations by Padwa on competing ylide formation.

In a further extension, Padwa and co-workers treated diazo compounds **53a** and **53b** with *N*-phenylmaleimide to give a mixture of the cycloadducts **62a** (60%) and **62b** (66%) as well as [1,2]-shift product **55a** (40%) and fragmentation product **55b** (34%) as shown in Scheme 12. Exclusion of *N*-phenylmaleimide from the reaction conditions furnished **55a** and **55b** in 70 and 93%. The formation of both carbonyl and ammonium ylides is a common feature with compounds **53a**,**b**, **56**, and **57**. Though these ylides are not isolable, the product distributions under various reaction conditions provide strong evidence for their interconversion.

4.1.6. Diastereoselective nitrogen quaternization. West and Glaeske[36] described a stereoselective route to α-quaternary amino acid derivatives through a novel chirality transfer approach. Based on the work of Clark[37] and McMills[38] as well as their own previously published results,[39] the possibility of forming optically active quaternary ammonium salts of a known configuration prior to generating the short-lived ylide was investigated. Proline derived salts **63a**,**b** and threonine derived salts **63c**,**d** were examined and subjected to basic ylide formation conditions (Scheme 13). When salts **63a** and **63b** were treated with *t*-BuOK in THF, products **64a** and **64b** were formed in good to excellent yields. Moderate to good yields of **64c** and **64d** were obtained upon treatment of **63c** and **63d** with *t*-BuOK. Compound **64c** was formed as a 2.8:1 mixture of diastereomers (^{1}H NMR) while **64d** was formed as a 4.1:1 mixture. Important to note is that **63d** was used initially as a 4.4:1 mixture of diastereomers, indicating a very high retention in formation of product **64d** (93%). Mosher ester analysis of products **64a** and **64b** showed a 3.3:1 diastereomeric mixture for **64a** and a single diastereomer for **64b**.



Scheme 13. Examination of optically active ammonium salts.

In all cases, the configuration at the quaternary nitrogen resulted from a cis-selective alkylation controlled by the adjacent center at C-2. This was believed to arise from a preferential N pyramidal form, in which the substituent on nitrogen and the carbomethoxy groups are trans disposed.[36b] With the benzyl shift examples **64a** and **64c**, an average ratio of ca. 3.1:1 was obtained. This suggests that radical pair recombination from the same face is competitive with that of diffusion to the opposite face or out of the solvent cage entirely (Fig. 5). For substrate **63b**, exclusive migration of the prenyl group via the [2,3]-shift was observed,[40] and the product was formed as a single enantiomer. Similar results were observed with substrate **64d**. This process involves the sequential transfer of chirality from C-2 to nitrogen, and back to C-2 again, and is an example the 'self-regeneration of stereocenters' concept first described by Seebach.[41]

Based on previous work, West and co-workers were also

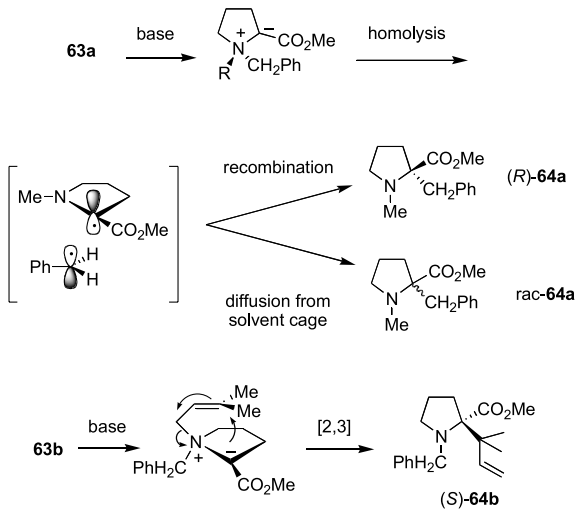
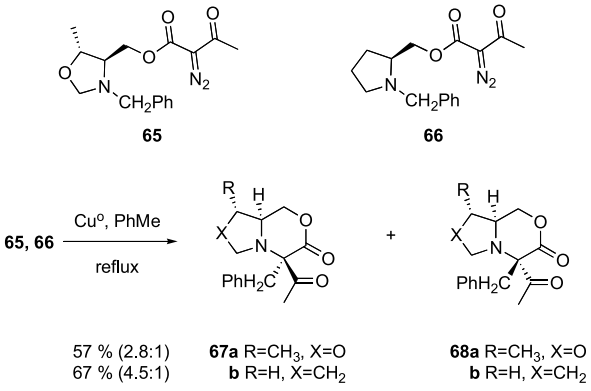


Figure 5. Explanation of preferential product formation.

interested in exploring approaches to morpholinones involving amino alcohols that possessed a chiral backbone. In 2003, they showed that morpholin-2-ones with a quaternary center at C-3 could be obtained from amino alcohol derived diazoacetoacetates in good yields and with moderate to good diastereoselectivity (Scheme 14).[42]



Scheme 14. Stereoselective formation of morpholinones.

The observed stereoselectivity is rationalized by preferential attack of the metallocarbene intermediate from the β-face of the five-membered ring heterocycle producing ylides **69a**,**b**. Benzyl homolysis then occurs, followed by recombination at the adjacent carbon to give **67** and **68** (Fig. 6).

4.1.7. Stevens rearrangement of cyclic hemiacetals. In 2004, Tomooka[43] and co-workers reported a novel Stevens rearrangement of a cyclic hemiacetal system in their diastereoselective approach to chiral α-amino ketones. Utilizing the optically active hemiacetal **71**, and subjecting it to *t*-BuOK in ethanol at room temperature provided the expected hydroxy ketone **72** in 35% yield as a single diastereomer, as well as hemiacetal **73** in 40% yield also as a single diastereomer (Scheme 15). It was also observed that

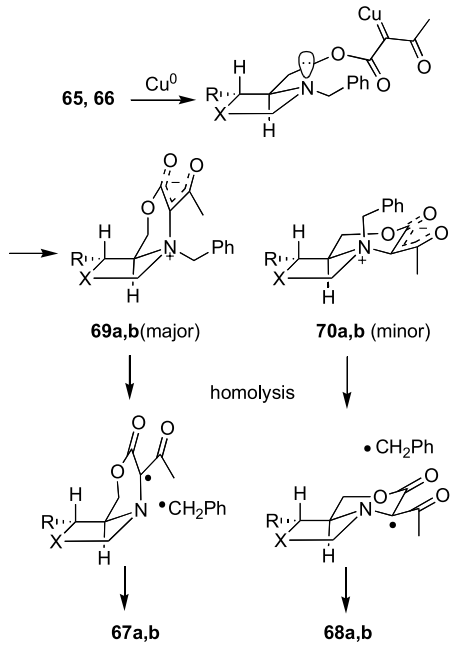
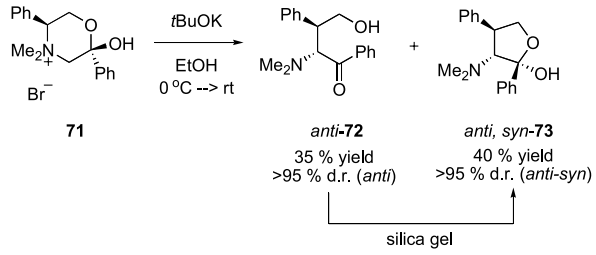


Figure 6. Rationalization for observed stereoselectivity.



Scheme 15. Stevens rearrangement of cyclic hemiacetals.

hydroxy ketone **72** could easily be tautomerized to hemiacetal **73** on silica gel.

4.2. Ring expansion methodology

4.2.1. Synthesis of 5,7-bicyclic systems. The potential to access many different alkaloid natural product ring systems is one of the most appealing aspects of the Stevens rearrangement of ammonium ylides. An efficient way to access a variety of amine natural product ring systems is through the formation of a spirocyclic ylide and subsequent ring-expansion/Stevens rearrangement. A recent application of this type of chemistry is seen in the work of Saba and co-workers during their synthesis of 5,7-fused bicyclic amine ring systems, which are potential precursors to more elaborate polycyclic alkaloid systems (Fig. 7).[44]

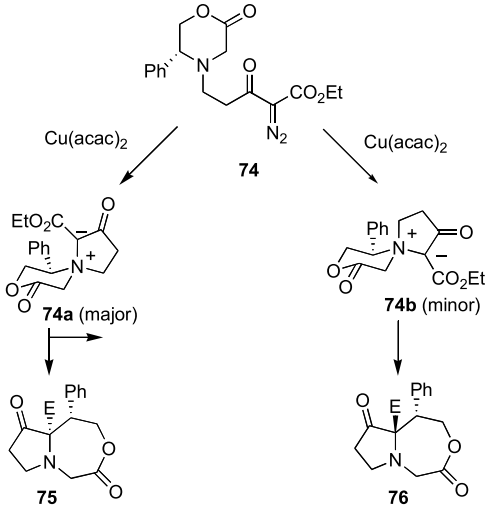
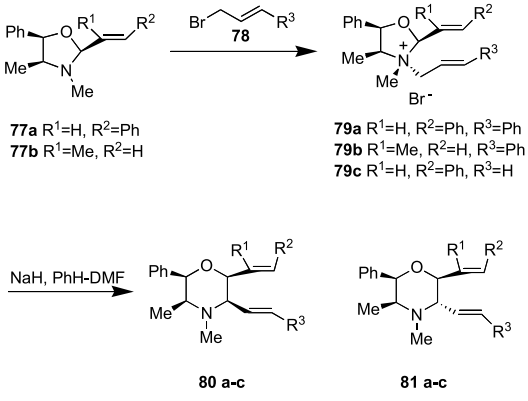


Figure 7. Ring expansion/Stevens rearrangement of diazo compound **74**.

Treatment of **74** with $Cu(acac)_2$ in toluene at reflux provided a 2:1 mixture of desired 5,7-bicycles (**75** and **76**) in quantitative yield, in which the starting morpholinone has undergone a 1-carbon ring expansion. Utilizing $Rh_2(OAc)_4$, however, provided a complex reaction mixture containing the desired ring-expanded products in minor amounts. It is interesting to note that the authors obtained compounds **75** and **76** in enantiomerically pure form, suggesting complete retention of configuration during the ring-expansion. The diastereoselectivity arises from selective formation of ylide **74a**. This is consistent with approach of the carbenoid from the same face of the morpholinone ring as the phenyl substituent, a result that was also seen by West and Naidu in their synthesis of epilupinine (vida infra).[39] This methodology provides a backbone for many entries into alkaloid natural products, some of which are discussed in the next section.

4.2.2. Enantiopure morpholines from oxazolidines. A recent publication by Pedrosa and co-workers[45] provides an excellent example of the Stevens ring-expansion process. High levels of retention of configuration were seen in this study, which provides a novel entry into enantiopure morpholines (Scheme 16, Table 5). Oxazolidines **77a** and **77b** were first converted into their corresponding bromide salts (**79a–c**), and then treated under basic conditions (NaH,



Scheme 16. Stevens rearrangement/ring expansion to access enantiopure morpholines.

Table 5. Ring expansion of salts **79a–c**

Entry	Salt	Solvent	Temperature (°C)	Yield [%] (dr, **80**:**81**)
1	**79a**	THF	−20	[15] (61:39)
2	**79a**	DMF	−55	[18] (63:37)
3	**79a**	DMF	−20	[20] (68:32)
4	**79a**	PhH–MF	20	[60] (68:32)
5	**79a**	PhH–MF	70	[65] (67:33)
6	**79b**	PhH–MF	20	[50] (65:35)
7	**79c**	PhH–MF	20	[45] (67:33)

PhH/DMF) to provide ring-expanded products **80** and **81**. The authors characterized the salts **79a–c** by examination of the *N*-methyl ^{1}H NMR chemical shifts. The major product was believed to arise from an approach of the allyl group trans to the existing groups in the ring. This is in contrast to the results seen by West,[36a] Vedejs,[36b] and Sato[36c] where the preferential N pyramidal form would position the *N*-methyl and C-2 substituent in a trans configuration, favoring approach of the electrophile cis to the neighboring vinyl. However, the more distant stereocenters in these more elaborate cases may alter the usual conformational preferences or invertomer populations, or may slow the rate of cis alkylation.

Treatment in THF or DMF at lower temperatures resulted in low yields of the desired products (Table 5, entries 1–3). When the solvent was switched to a PhH/DMF mixture and the temperature was raised, the yields of the desired products were significantly increased (entries 4–7). It is very interesting to note that none of the [2,3]-shift products were seen in this series. This is rationalized by the formation of a single ylide, arising from deprotonation of the allylic methylene. The trans relationship proposed for the two vinyl moieties in the precursor salts **79a–c** would be expected to disfavor rearrangement by [2,3]-shift due to poor orbital overlap. Also, competing deprotonation at C-2 to form an endocyclic ylide must be unfavorable, due to steric demands.

Though epimeric at C-3, the configuration at C-2 for both isomers is the same and coincident with that of the salts, indicating that the [1,2]-shift occurred with complete retention of configuration (Fig. 8). Initially, homolytic cleavage occurs upon ylide formation to provide diradical **83**. At this point, the authors reasoned that rapid recombination would provide **84**, while bond rotation and subsequent recombination would provide **85**. Though their effect is not discussed, it is reasonable to consider that the substituents at C-5 and C-6 may very well play an important role in the product distribution as well.

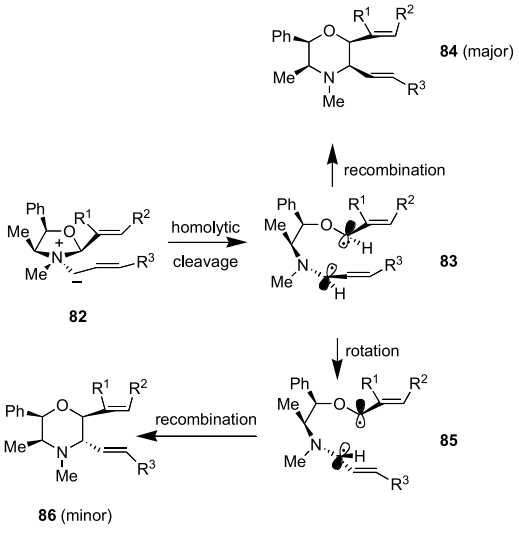
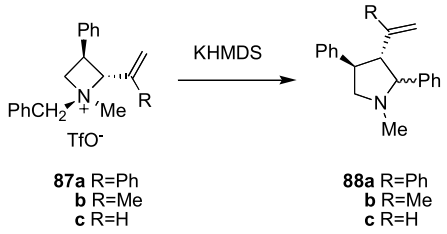


Figure 8. Pedrosa's rationale for formation of major product **84**.

4.2.3. Synthesis of pyrrolidines from enantiomerically pure azetidines. In 2004, Couty[46] and co-workers published results on the synthesis of enantiomerically pure 2-alkenyl azetidines and the regioselective Stevens rearrangement of their ammonium derivatives (Scheme 17, Table 6). Ammonium triflate salts of type **87a–c** were prepared and investigated. When these *N*-benzyl triflates were subjected to KHMDS in THF at −78 °C, a regioselective rearrangement occurred and the corresponding pyrrolidines were obtained in fair to good yields as a mixture of isomers, apparently epimeric at C-2.



Scheme 17. Couty's synthesis of pyrrolidines from 2-alkenyl azetidines.

Table 6. Regioselective rearrangement of ammonium triflate salts, **87a–c**

Substrate	Product	Yield (%)	de (%)
87a	**88a**	39	8
87b	**88b**	94	72
87c	**88c**	83	6

The low diastereoselectivity at C-2 is presumed to result from fast bond rotation by the benzylic radical center relative to radical recombination (Fig. 9). Complete retention of the migrating center (C-3 of the pyrrolidine products) was assumed. It is also notable that none of the alternative tetrahydroazepine product resulting from [2,3]-shift of the azetidinium ylide was isolated. The authors suggest that the trans relationship of the benzylidine ylide carbon and the neighboring alkenyl group prohibits this process.

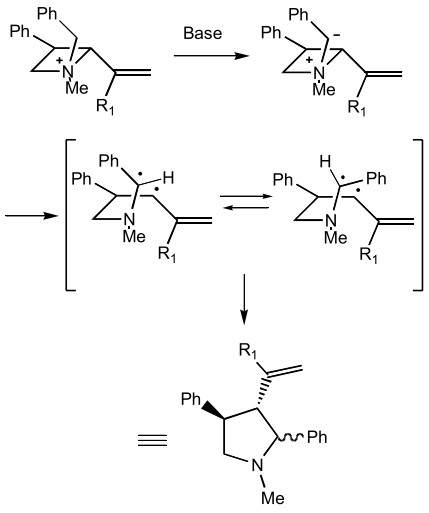
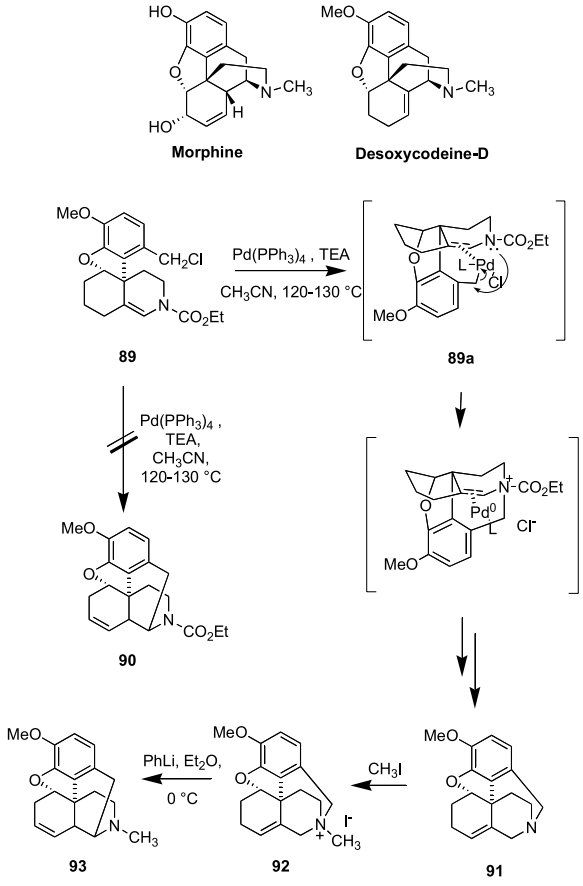


Figure 9. Mechanism of the Stevens rearrangement of azetidines **87a–c**.

5. Synthetic applications of the Stevens rearrangement of ammonium ylides

5.1. Approaches to isopavines and the morphine core

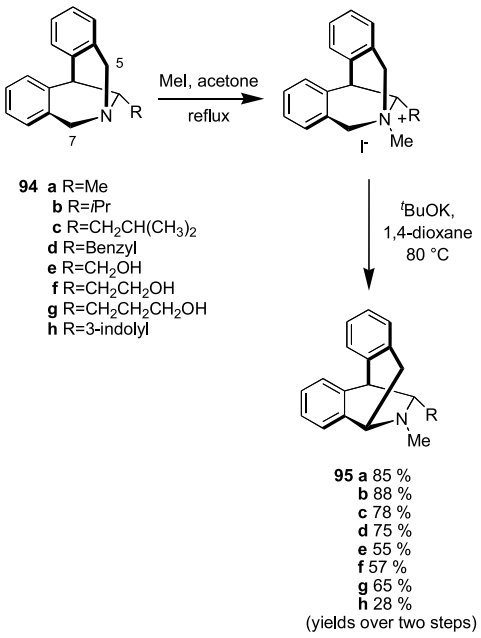
Cheng[47] and Hanessian[48] have employed the Stevens rearrangement in their approaches to morphine and isopavines, respectively. In the recent synthesis of the complete pentacyclic skeleton of morphine, Liao and Cheng subjected compound **89** to Heck reaction conditions in hopes of generating **90**. Much to the authors' surprise, tertiary amine **91** was obtained in high yield (Scheme 18).



Scheme 18. Cheng's approach to the morphine core.

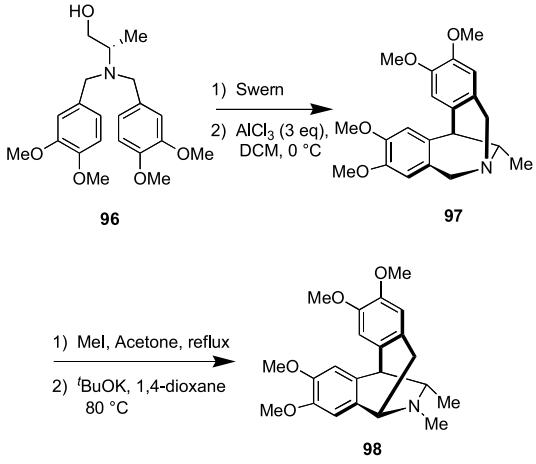
Their mechanistic rationale for the generation of this product involves the attack of nitrogen on the pendent Pd–π species (**89a**). Though this product does not contain the desired connectivity, Kametani's important prior work concerning the Stevens rearrangement of quaternary tetrahydroisoquinoline alkaloids[49] prompted the authors to pursue this methodology in obtaining the correct connectivity seen in morphine. Accordingly, formation of quaternary salt **92** was followed by subjection to phenyl lithium. Much to their delight, the Stevens rearrangement took place in excellent yield (83%, two-steps) to form desoxycodeine-D **93** possessing the morphine core.

Hanessian and Mauduit applied the Stevens rearrangement in a similar manner in their approach to the asymmetric synthesis of isopavines. The authors first chose to examine



Scheme 19. Formation of [1,2]-shift products **95a–h**.

the substrate scope of the desired [1,2]-shift (Scheme 19). Several amine salts were treated with ^{t}BuOK in 1,4-dioxane to provide the desired [1,2]-shift products **95a–h**. In general, moderate to good yields were obtained for the two-step process. Alcohol **96** was then employed to generate a more highly functionalized. substrate **97** in an effort to determine the effect that aromatic substitution would have on the desired transformation (Scheme 20).



Scheme 20. Generation of a more highly functionalized isopavine.

Ammonium salt formation with methyl iodide and subsequent treatment under basic conditions led to the formation of the desired Stevens derived product **98** in 77% overall yield. Notably, though these reactions can proceed through two distinct pathways, giving rise to different rearrangement products, only one product was observed in each case. To explain the product distributions, the authors reasoned an ionic mechanism for the Stevens rearrangement (Fig. 10), despite the strong support for a radical mechanism.[7d,10a,11b] Deprotonation at C-12 leads to formation of ylide **100a** (path a), followed by fragmentation

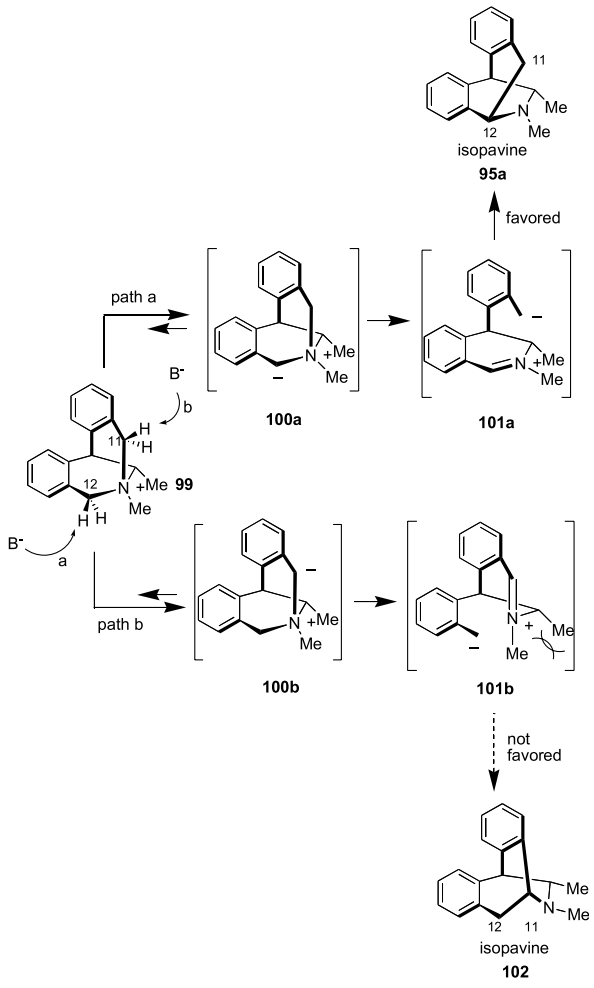
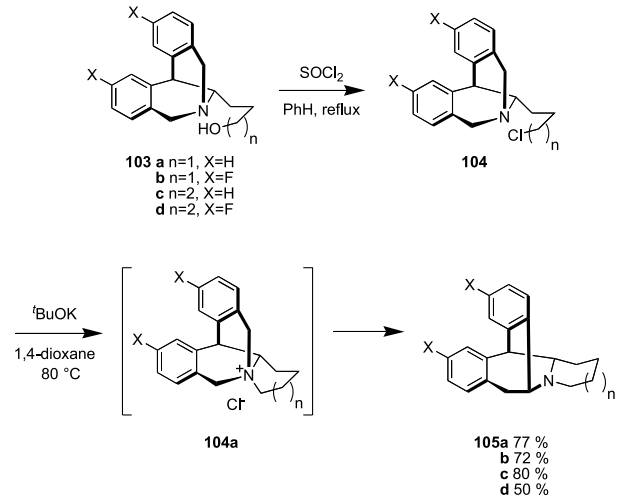


Figure 10. Proposed ion pair Stevens rearrangement.

to **101a** and intramolecular attack of the iminium ion by the benzylic anion to provide the observed product **95a**. Proton abstraction at the other benzylic position (C-12) would lead to ylide **100b** (path b) followed by heterolysis to intermediate **101b**, which is believed to be unfavorable due to 1,2-allylic strain.

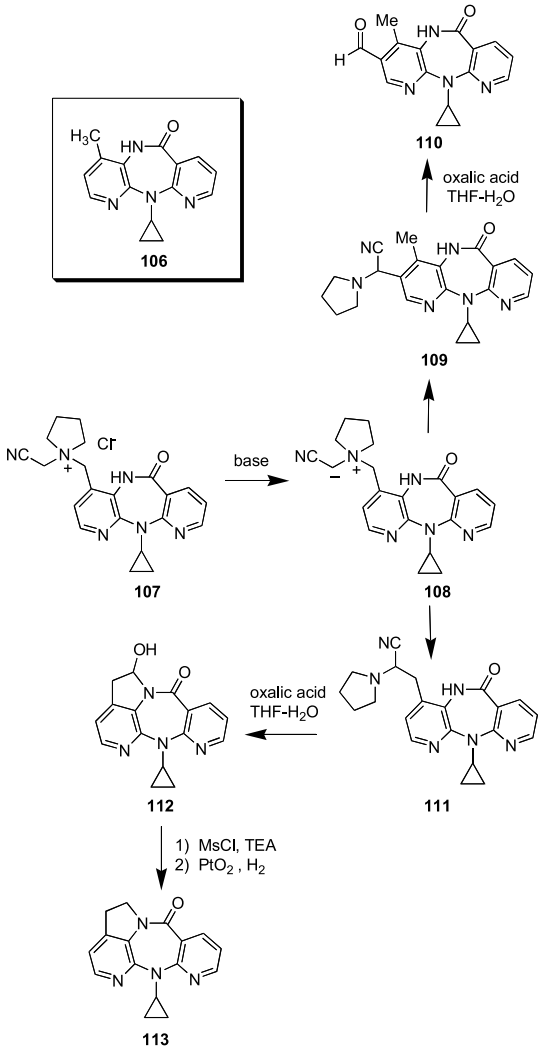


Scheme 21. Synthesis of morphinomimetics.

Due to the structural similarity of the isolated isopavines to the morphine backbone, the authors chose to further extend this chemistry towards the synthesis of morphinomimetics (Scheme 21).[50] Chlorination of alcohol **103a** provided intermediate **104a**, which upon treatment with base then participated in a Stevens rearrangement to provide compounds **105a–d**. The highly diastereoselective nature of the [1,2]-shift seen by Hanessian exemplifies the powerful nature of this rearrangement providing access to pharmacologically interesting alkaloid natural products.[51]

5.2. Synthesis of HIV-1 RT inhibitors

During efforts to synthesize analogs of **106** (Scheme 22), a potent and specific inhibitor of HIV type 1 reverse transcriptase, Klunder isolated an unexpected side product of the intended Sommelet–Hauser (S–H) rearrangement resulting from a competing [1,2]-shift pathway. Manipulation of this product gave rise to a more potent inhibitor.[52] Ylide formation from **107** and subsequent rearrangement provided **110** in 32% yield, after acid hydrolysis of **109**. The major product in this reaction, however, was the Stevens rearrangement product **111**, which upon acid hydrolysis, furnished compound **112** in 58% yield. Compounds derived



Scheme 22. Synthesis of potent HIV-1 RT inhibitors via Stevens [1,2]-shift.

from both the S–H and Stevens rearrangement proved to be poor inhibitors of HIV-1 RT. However, when **112** was treated with mesyl chloride and TEA, followed by hydrogenation, compound **113** was isolated in 18% yield. Both its precursor (elimination product) as well as **113** proved to be potent inhibitors of HIV-1 RT.

5.3. Synthesis of azacene ring systems

McMills and co-workers considered the use of the [2,3]-shift/ring-expansion methodology as an entry into medium sized azacane ring systems and observed some interesting results (Fig. 11).[38] Diazo decomposition of racemic **114** was studied under a variety of conditions (Table 7). Both [2,3]-shift and Stevens [1,2]-shift products were observed in this study. Clark and co-workers had undertaken a similar study in which they observed exclusive formation of the [2,3]-shift products.[37] The high levels of [1,2]-shift products seen are believed to be a consequence of the rapid interconversion of the nitrogen center. This inversion allows for approach of the pendant metal carbenoid from either side of the vinyl group.[53] McMills and co-workers have proposed that approach of the metallocarbene from the bottom provides an ylide (**115a**) that is not close enough to the vinyl group to allow for efficient overlap for [2,3]-shift, so the [1,2]-shift predominates (**116**). Conversely approach from the top face generates an ylide (**115b**) that is close enough for interaction with the alkene and, therefore, proceeds through the intended [2,3]-shift pathway (**117**). Larger nitrogen ring systems (6 vs 5) gave only products from the desired [2,3]-shift. The authors intended to use this methodology towards the synthesis of expanded heterocyclic ring systems.

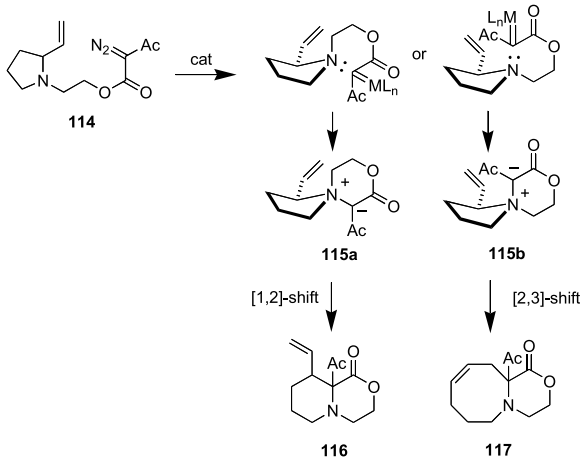


Figure 11. Synthesis of azacene ring systems.

5.4. Synthesis of epilupinine

West and Naidu used the Stevens ring-expansion methodology in an efficient total synthesis of epilupinine.[39] Diazoketone **118**, available in two-steps from proline, was treated with copper or rhodium catalysts (Fig. 12, Table 8). The corresponding methyl ester was also examined, furnishing the desired quinolizidine product in comparable yields but with lower diastereoselectivity.

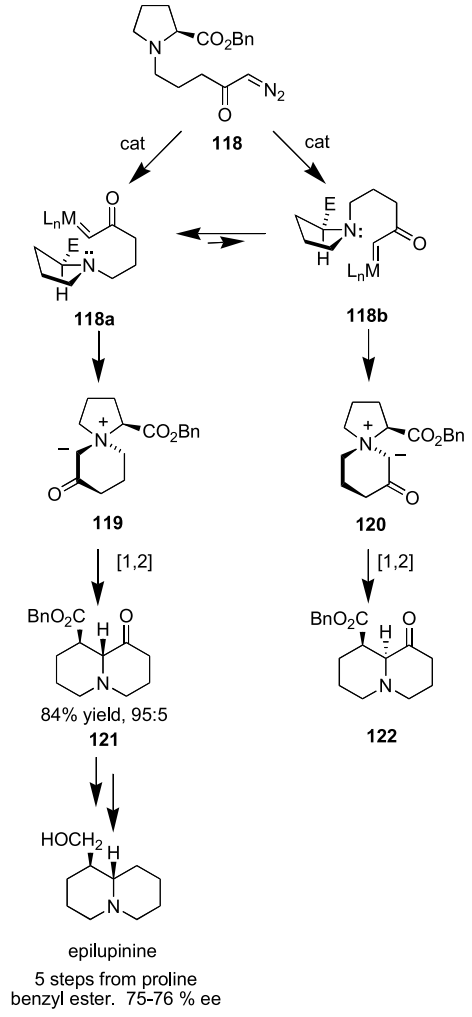


Figure 12. West's synthesis of epilupinine.

Formation of a spiro-cyclic ylide was followed by a [1,2]-shift to provide the desired quinolizidine ring system in high yields and with good diastereoselectivity. Having achieved high levels of diastereoselectivity, the authors examined the

Table 7. Diazo decomposition of **114** to generate **116** and **117**

Entry	Catalyst[a]	Solvent	Temperature	Yield (%)	**116**:**117**[b]
1	$Cu(acac)_2$	$ClCH_2CH_2Cl$	Reflux	53	2.5:1
2	$Cu(acac)_2$	PhH	Reflux	33	4:1
3	$Cu(acac)_2$	$PhCH_3$	Reflux	62	3:1
4	$Cu(hfacac)_2$	$ClCH_2CH_2Cl$	Reflux	48	2.5:1
5	$Cu(hfacac)_2$	PhH	Reflux	62	2.5:1
6	$Cu(hfacac)_2$	$PhCH_3$	Reflux	70	2.5:1

[a] All reactions used 0.15 eq of catalyst.
[b] Some ratios are based on GC-MS analysis of the crude reaction mixture.

Table 8. Catalytic decomposition of **118**

Entry	Catalyst	Solvent	Temperature	Total yield (%)	**122**:**121**
1	$Rh_2(OAc)_4$	CH_2Cl_2	rt	74	25:75
2	$Cu(acac)_2$	$PhCH_3$	Reflux	84	5:95
3	Cu powder[a]	$PhCH_3$	Reflux	87	6:94
4	Cu powder[b]	$PhCH_3$	Reflux	87	7:93

[a] Cu powder (50 mol%) used.
[b] Cu powder (15 mol%) used.

enantiomeric excess of the major ring expansion diastereomer. Using a chiral shift reagent, the ee of the resulting quinolizidine under optimal conditions was determined to be 65–75%. The resulting quinolizidine was carried in three-steps on to give epilupinine with an ee of 75%.

This synthesis raised several key points concerning the ring-expansion methodology. A large change in the de between using a methyl ester and a benzyl ester suggests a steric argument during ylide formation. This was an interesting result considering previously described acyclic ammonium ylides containing optically active starting amines provided only 1:1 ratios of diastereomers.[7d] A closer look at the formation of the ylide shows two possible approaches of the carbenoid (Fig. 12). It is reasonable to consider that the diazoketone exists as two rapidly interconverting invertomers differing at the nitrogen center. This would provide two possible diastereomeric ylides **119** or **120**. The results of this study suggest a preference for the side chain of the diazoketone to be in a trans relationship to the ester group to minimize unfavorable steric interactions.

Ylide formation is then followed by homolysis to give biradical **123** (Fig. 13). The biradical can recombine via two paths: path (a) provides the major product **121** in high ee, or bond rotation can occur leading to achiral biradical **124** (path b) followed by recombination to give racemic products. The high levels of ee observed in the reaction suggest a strong preference for path a. The high overall diastereoselectivity obtained indicates that the racemic pathway must also be selective for **121** over **122**. Stereoselective recombination of **124** is plausible.[54] Biradicals **124a** and **124b** show the two possible approaches of **124** prior to bond formation. Intermediate **124a** requires unfavorable steric interactions between the ester and the pseudoaxial proton of the methylene β to the carbonyl, but this unfavorable interaction is avoided in **124b**.

5.5. Synthesis of benzazepine alkaloids

Padwa and co-workers utilized the ring-expansion methodology in their work towards the synthesis of various benzazepine alkaloids (Scheme 23).[40,55] With (−)-cephalotaxine **125** as their target, the ring-expansion of several model substrates was first examined. Diazoacetoacetates (**127a–d**) were first subjected to $Rh_2(OAc)_4$, but the reactions were observed to be slow and provided a complex mixture of products. Treatment of these diazo compounds with $Cu(acac)_2$ gave the desired ring-expansion products **128a–d** in good yields. The better results seen with

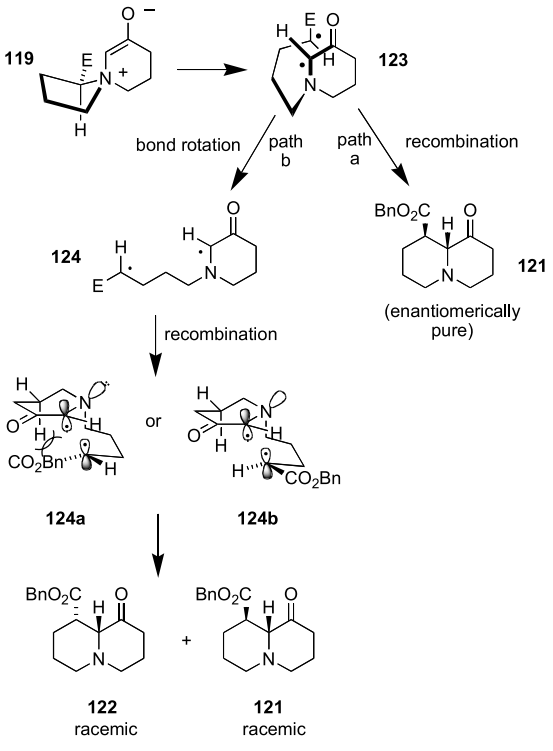
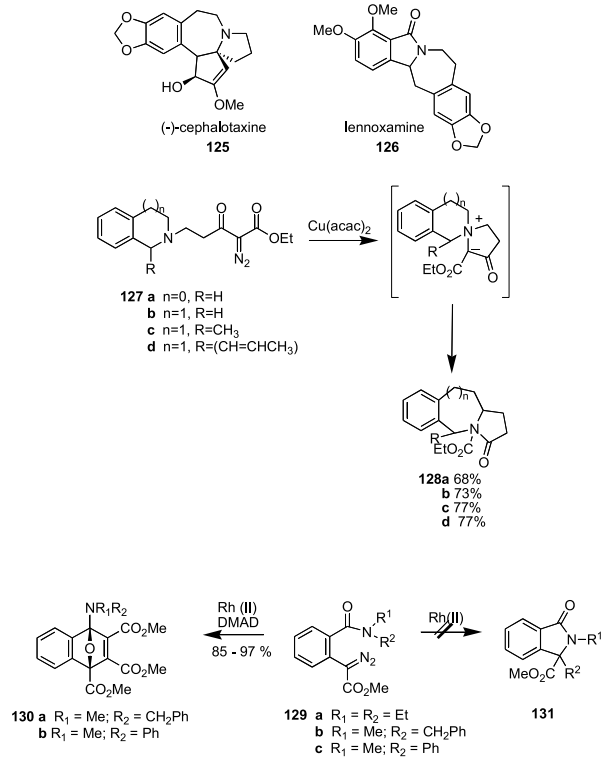


Figure 13. Rationale for diastereoselectivity seen in [1,2]-shift.

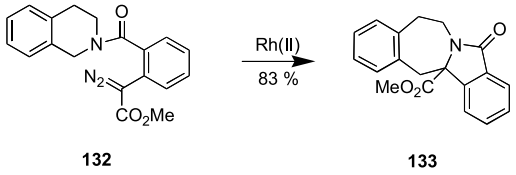


Scheme 23. Padwa's approach to benzazepine alkaloids.

$Cu(acac)_2$ are not surprising since it is known that copper catalysts work far better than rhodium in these types of transformations.[56]

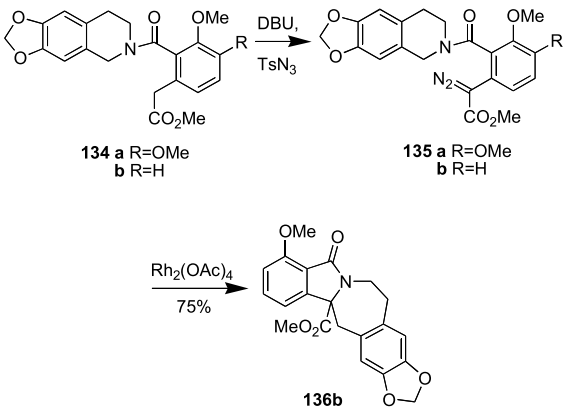
Given the success encountered with the cephalotaxine model study, attention was turned to the possibility of accessing the core of natural product lennoxamine **126**. Unlike the cephalotaxine approach, the lennoxamine ring system would require the rearrangement of substituted amido diazoesters. A potential problem with the rearrangement is the formation of the undesired carbonyl ylide intermediate that could provide isobenzofuran type products in a competing reaction.[57] In order to test the efficiency of the desired ammonium ylide forming step, Padwa and co-workers synthesized diazo compounds **129a–c** (Scheme 23). Treatment of **129a–c** with $Rh_2(OAc)_4$ and DMAD in benzene at reflux gave cycloadducts **130a–c**. Unfortunately, removal of the trapping agent from the reaction provided no rearranged products of the type **131**.

Despite their initial failure with these simple substrates, the authors chose to test a more accurate model system where the required tetrahydroisoquinoline moiety is incorporated into the amide. Therefore, diazo compound **132** was prepared and subsequently subjected to rhodium catalyzed diazo decomposition conditions (Scheme 24). The desired isoindolobenzazepine ring system **133** was isolated in 83% yield. The cause for complete selectivity reversal in ylide formation remains unclear.



Scheme 24. Synthesis of the isoindolobenzazepine ring system.

Padwa and co-workers then directed their attention to the synthesis of the lennoxamine ring system. Initial efforts to convert **134a** to the diazo compound **135a** were unsuccessful (Scheme 25). The authors attribute this to the diminished acidity of the benzylic protons due to the OMe group in the five-position of the aromatic ring. Further evidence of this was shown when the related amine **134b** provided the desired diazo compound **135b** in 88% without any of the difficulties associated with the previous series. Treatment of **135b** with $Rh_2(OAc)_4$ furnished compound **136b** in 75% yield. Although **136b** lacks the additional methoxy group necessary for the synthesis of lennoxamine, the aforementioned examples substantiate the utility of this transformation in the formation of 5,7-fused nitrogen heterocyclic natural products.



Scheme 25. Unsuccessful attempt to convert **134** to diazo compound **135**.

5.6. Hydroxylated quinolizidines via silyl-directed [1,2]-shift

Recently, West and Vanecko[58] developed new methodology derived from previous work in their group concerning the synthesis of epilupinine (Fig. 14).[39] They were interested in finding a directing group for the [1,2]-shift that could subsequently function as a hydroxyl surrogate.

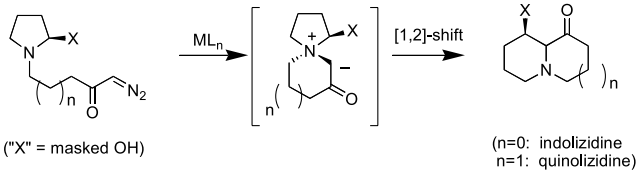
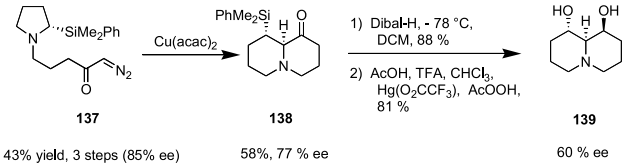


Figure 14. Introduction of a masked hydroxyl group, X.

West and Vanecko chose to examine silyl groups as potential [1,2]-shift directing moieties based on previous suggestions that silyl groups could stabilize adjacent radical centers,[59] along with the efficiency of the Fleming–Tamao[60] oxidation to convert silanes to hydroxyl groups (Scheme 26). Moreover, synthesis of the necessary silyl substituted pyrrolidines could rely on the asymmetric lithiation chemistry of Beak and co-workers.[61]

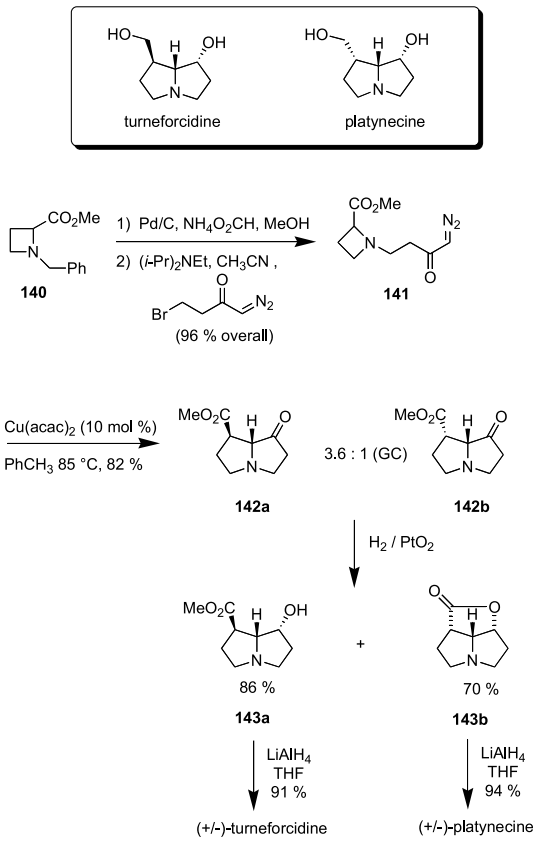


Scheme 26. Silyl groups as potential [1,2]-shift directing groups.

Reaction of **137** with $Cu(acac)_2$ in toluene at reflux provided the desired quinolizidine **138** in 58% yield and as a single diastereomer and with 77% ee. The quinolizidine was formed as the cis compound, a result that is consistent with their previous work.[39] An ee of 77% signifies 91% retention at the migrating center based on the initial 85% ee for **137**. The ketone was then diastereoselectively reduced to the alcohol (Dibal-H, 88%) and subjected to modified Fleming–Tamao conditions[62] to provide the diol **139** in 81% yield. This represents the first application of a silyl directed Stevens rearrangement.

5.7. Synthesis of pyrrolizidine alkaloids turniforcidine and platynecine

West and Vanecko also employed the ring-expansion chemistry in a rapid entry to the pyrrolizidine ring system.[63] The authors envisioned that conversion of known azetidine



Scheme 27. West's synthesis of turneforcidine and platynecine.

140[64] to diazoketone **141** and treatment with rhodium or copper catalysts could provide the pyrrolizidine ring system via [1,2]-shift of the intermediate ylide **142** (Scheme 27). Subsequent elaboration would provide natural products like platynecine or turneforcidine.

Beginning with azetidine **140**, debenzylation under transfer hydrogenation conditions (Pd/C, ammonium formate)[65] followed by immediate coupling with 4-bromo-1-diazo-butan-2-one[66] furnished substrate **141** in excellent yield. Treatment of **141** with $Cu(acac)_2$ furnished pyrrolizidines **142a** and **142b** in excellent yield as a 3.6:1 mixture of diastereomers as determined by GC analysis. This mixture was chemoselectively reduced to provide a separable mixture of alcohol **143a** and lactone **143b**. Further reduction using $LiAlH_4$ provided natural products turneforcidine and platynecine in good overall yields. This represents an extremely efficient (five-steps from readily available **140**) and high yielding approach towards pyrrolizidine natural products.

5.8. Approach to indolizidinone alkaloids

Saba[67] and co-workers recently utilized a tandem carbenoid/ylide/[1,2]-Stevens rearrangement and ring expansion process in their approach to swainsonine analogues (Fig. 15). L-Proline derived substrates (**144a**,**b**) containing a nitrogen tethered to an α-diazoketoester chain were successfully converted into chiral indolizidinones **147a**,**b**.

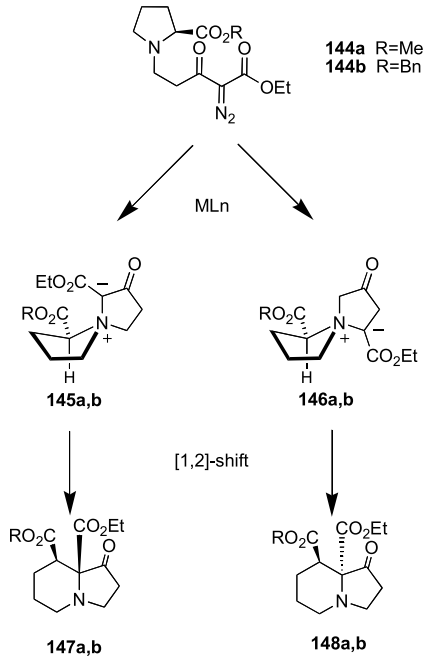


Figure 15. Saba's approach to indolizidinones.

Formation of the target indolizidinones was found to go through the [5,5]-spirocyclic ylides **145a** and **145b**. $Rh_2(OAc)_4$ and $Cu(acac)_2$ were selected as potential catalysts and the reactions were carried out in refluxing toluene (Table 9).

Table 9. Effects of using $Rh_2(OAc)_4$ versus $Cu(acac)_2$

Substrate	Catalyst	Yield (%)	**147a**,**b**:**148a**,**b**	ee of **147a**,**b** (%)
144a	$Rh_2(OAc)_4$	84	60:40	80
144a	$Cu(acac)_2$	90	53:47	68
144b	$Rh_2(OAc)_4$	85	72:28	84
144b	$Cu(acac)_2$	90	65:35	90

It was also discovered that changing the reaction conditions from refluxing toluene to dichloromethane, in the presence of $Rh_2(OAc)_4$, allowed for the isolation of ylides **145a** and **145b** in 91 and 90% yields, respectively, with moderate diastereoselectivity. The authors then reported that heating ylide **145a** in refluxing toluene without a catalyst afforded alkaloid **147a** as a single diastereomer with 95% ee and 83% yield. Subjecting **145b** to the same reaction conditions afforded **147b** as a single diastereomer in 95% ee and 85% yield.

5.9. Approaches toward pyrrolo[1,2-*a*]azepine frameworks

Encouraged by the work reported by West[39,58] and Clark[27,37] on the rearrangement of spiro-ylides, Sweeney

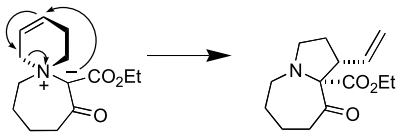
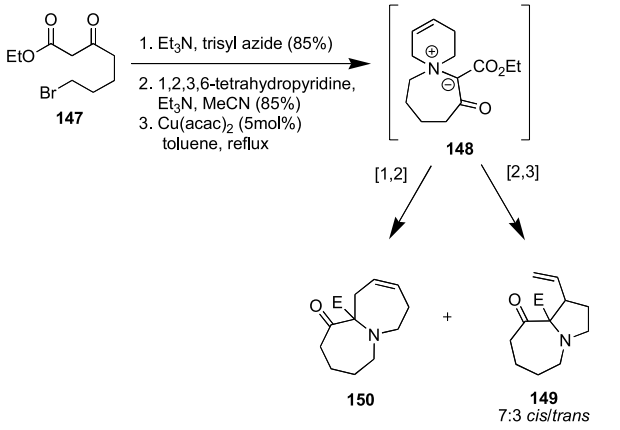


Figure 16. The 'ene-*endo*-spiro ylide' approach.

and co-workers looked at [2,3]-rearrangements of spiro-ammonium ylides. The goal was to involve an endocyclic alkene unit in the [2,3]-shift (Fig. 16); such methodology could be utilized as a route to the pyrroloazepine core of the Stemona alkaloids.

In 2005, Sweeney[68] and co-workers described the first example of [2,3]-endocyclic rearrangements of spirocyclic ammonium ylides. This novel discovery also allowed for the facile introduction of a quaternary center to the ring junction of pyrrolazepine structures (Scheme 28).



Scheme 28. Sweeney's [2,3]-rearrangement of *endo*-spiro-ammonium ylides.

Rearrangement of *endo*-spiro-ammonium ylide **148** proceeded to give azepinones **149** and **150** (the 1,2-shift product) with yields of 60 and 8%, respectively. This work allows for highly efficient and facile entry into the pyrrolazepine frameworks found in some bicyclic alkaloids such as the Stemona alkaloids.

6. Conclusions

The past decade has seen an increased appreciation for the synthetic utility of the Stevens rearrangement. An important component of this growth has been the development of alternative methods for ylide generation. Investigations on the use of fluoride-induced desilylation to form ammonium ylides by Sato provided interesting results with regards to product distributions. Though this method still suffers from many of the inherent problems encountered years before by both Sato and Vedejs, it does provide an alternative route to ylide formation when other methods fail.

The use of metal stablilized carbenes derived from diazo compounds has become the most commonly employed means of ylide formation. Padwa has done extensive studies concerning the formation of ammonium versus carbonyl ylides when both pathways are present in the system. Padwa demonstrated that indeed both ylides are formed in the reaction and depending on the reaction conditions, the outcome could be controlled to provide products from either pathway in excellent yields. This was exemplified in his recent work towards the synthesis of cephalotaxine and lennoxamine.

West and co-workers have used the spirocyclic ylide/Stevens ring-expansion methodology to access quinolizidine and pyrrolizidine natural products in a very efficient manner. Based on their early work in the total synthesis of epilupinine, a novel silyl mediated Stevens rearrangement was reported as rapid entry into the quinolizidine alkaloids. The use of the silyl group is indeed intriguing because it not only directs the Stevens reaction in a highly diastereoselective manner, but also allows access to polyhydroxylated quinolizidines via the Fleming–Tamao oxidation. The same authors used the ring-expansion methodology to synthesize pyrrolizidine natural products platynecine and turneforcidine through the novel use of azetidines. West and co-workers have also reported on their approaches to morpholinones involving amino alcohols that possessed a chiral backbone. Recently, Tomooka and co-workers reported on a novel Stevens rearrangement of cyclic hemiacetal systems during their diastereoselective approach to the synthesis of chiral α-amino ketones. Saba has shown an efficient method to access amine natural product ring systems via the ring expansion/Stevens rearrangement of spirocyclic ylides in the synthesis of 5,7 bicyclic compounds. Pedrosa and Couty have also reported on the use of the Stevens rearrangement in the synthesis of enantiopure morpholines and pyrrolidines, respectively.

A variety of important alkaloid skeletons can be accessed via concise and stereoselective routes using [1,2]-shifts of ammonium ylides. As new methods for ylide generation and novel classes of substrates continue to be disclosed, it seems likely that ever more ambitious applications of this chemistry will be described in the literature.

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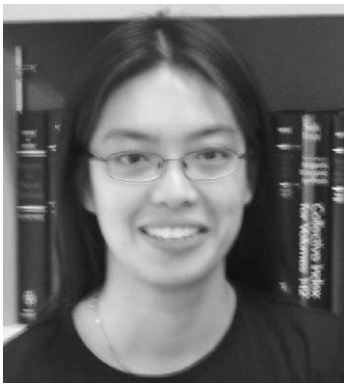
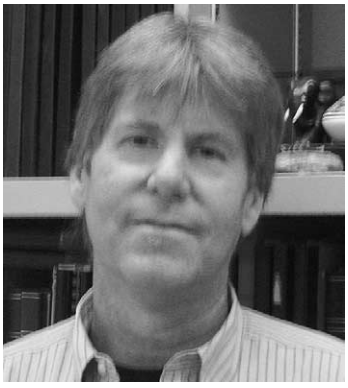
Biographical sketch

John A. Vanecko was born outside of Cleveland, Ohio and raised outside of Chicago, Illinois. He attended Indiana University as an undergraduate where, upon graduation, he entered graduate school at the University of Utah under the direction of F. G. West. In 2003, he received his PhD for work on ammonium and oxonium ylide chemistry, which culminated in research concerning iterative approaches towards polycyclic ethers and the synthesis of pyrrolizidine natural products platynecine and turneforcidine. He then followed his graduate work by completing a postdoctoral fellowship under the direction of Amos B. Smith III where he was involved in efforts towards the total synthesis of sorangicin A. Currently, he is employed at Myriad Genetics of Salt Lake City, Utah as a research scientist 1.



Hayley Wan, born in Salford, UK (1977) received her BSc in 1998 from Manchester Metropolitan University (UK), her MPhil in 1999 from UMIST (UK), and her PhD in 2003 from the University of Durham (UK) where she worked under the supervision of Professor Andrew Whiting on the asymmetric catalysis of nitroso Diels–Alder reactions. After a postdoctoral fellowship at the University of Alberta, Canada (2003–2005) with Professor F. G. West working on iterative and cascade methodology to synthesize polycyclic ether arrays, she moved to the National Research Council of Canada where she currently holds the position of Technical Officer at the National Institute for Nanotechnology. Her interests include organic synthesis, homogeneous catalysis and combinatorial chemistry.



Frederick G. West was born and raised in Safford, Arizona. He received an Honors BSc at the University of Arizona (summa cum laude), and pursued his graduate training at the University of Wisconsin at Madison under the direction of Edwin Vedejs. His research focused on synthetic applications of azomethine ylides. After receiving his PhD in 1986, he spent 2 years at Columbia University as an NIH Postdoctoral Fellow with Gilbert Stork, working on radical cyclization methodology directed towards cardiatonic steroids. He then accepted an appointment as Assistant Professor at the University of Utah, where he spent 14 years. In 2002, he moved to the University of Alberta. Current research interests include metallocarbene chemistry, new domino processes based upon the Nazarov reaction, chemical synthesis of polycyclic ethers, and organic photochemistry.

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De-novo approach for a unique spiro skeleton-1,7-dioxa-2,6-dioxospiro[4.4]nonanes

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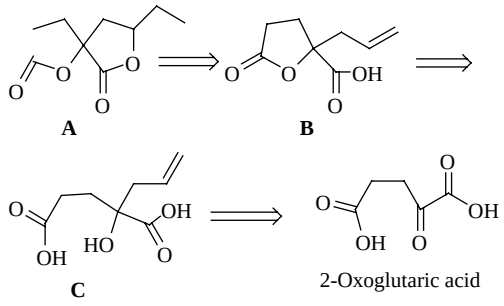
Abstract—2-Oxoglutaric acid (**1**) underwent facile indium mediated allylation with allyl bromide (**2**), and ethyl 4-bromocrotonate (**3**), cinnamyl bromide (**4**) and subsequent in situ dehydration to provide respective 5-oxotetrahydrofuran-2-carboxylic acids **5**–**7** (90–95%). The reaction of **1** with **3** and **4** proceeded with high regio and stereo selectivity to provide only γ-addition products with *syn* stereochemistry as ascertained from their cyclic products. Compounds **5**–**7** underwent diastereoselective iodocyclization to provide respective 1,7-dioxa-2,6-dioxospiro[4.4]nonanes **8**–**13**. The relative stereochemistries have been ascertained by single crystal X-ray structures, NOE experiments and coupling constants in ^{1}H NMR spectra.

1. Introduction

Spiro compounds exhibiting structural rigidity due to conformational locking, play a significant role in the biological systems. 1,7-dioxa-2,6-dioxospiro[4.4]nonane skeleton, present in natural molecules such as leucodrin,[1] conocarpin,[2] piptoside,[3] dihydropiptosidin,[3] leudrin[4] and cinatrin[5] A and B imparts them various biological properties. The cinatrins are used as inhibitors of phosphorylase A_2 (PLA_2).[6] The spiro skeleton acts as synthon[7] for building up other natural systems such as zaragozic acids.[8] In spite of 1,7-dioxa-2,6-dioxospiro[4.4]nonane being present in number of natural products, the literature search on CAS shows it to be one of the least studied spiro derivative.

Of the chiron and de-novo approaches, the chiron approach for the synthesis of 1,7-dioxa-2,6-dioxospiro[4.4]nonanes from 1,2-cyclopentandione,[7,9] D-arabinose[5a] and vitamin C[10] often involves multi-step procedures and is limited to only hydroxy substituted spirononanes. The only reported de-novo approach involves the BF_3 catalysed cyclization of 1,1-diphenylethene with 2-oxoglutaric acid[11] to provide 8,8-diphenyl-1,7-dioxa-2,6-dioxospiro[4.4]nonane in 17% yield.

The retrosynthesis of spiro skeleton shows that spirononane **A** can be obtained by iodocyclization of furan-2-ones **B**, which in turn can be obtained by allylation and subsequent intramolecular dehydration of hydroxy carboxylic acid (Scheme 1).



Scheme 1.

Recently, we have reported[12] that 2-oxocarboxylic acids undergo highly regio and stereo-selective allylation to provide respective 2-allyl-2-hydroxy carboxylic acids, which subsequently undergo iodocyclization to provide respective furan-2-one derivatives.

In the present work, we have performed the indium mediated allylation of 2-oxoglutaric acid to get 2-allyl-, 2-(1-phenylallyl)- and 2-[(1-ethoxycarbonyl)allyl]- derivatives of furanone **5**–**7** (90–95%), which underwent iodocyclization to provide the target spirononanes in 72–83% yields. In comparison to available multi-step procedures, this provides a simple two step approach for the synthesis of 1,7-dioxa-2,6-dioxospiro[4.4]nonanes.

Keywords: 2-Oxoglutaric acid; Indium; Allylation; Iodocyclisation; Diastereoselective.
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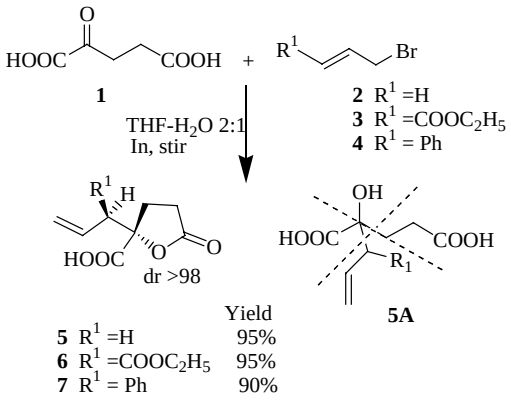
2. Results and discussion

2.1. Allylation of 2-oxoglutaric acids

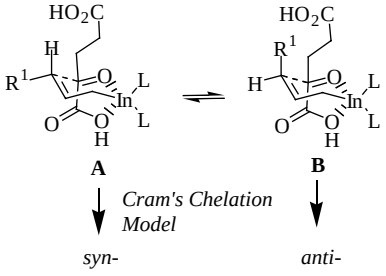
A solution of 2-oxoglutaric acid (**1**), allyl bromide (**2**) and indium metal (suspension) (1:1.5:1) in THF–H_2O (2/1) on stirring at 0 °C, followed by usual work-up and chromatography gave a clear transparent liquid **5** (98%), M^+ *m/z* 170 (Scheme 2). The ^1H and ^{13}C NMR spectra could assign either of the structures **5** and **5A** to this compound. The presence of C=O str at 1775 cm^{-1} due to butyrolactone unit conspicuously assigns the cyclic structure 2-allyl-5-oxo-tetrahydrofuran-2-carboxylic acid (**5**).



Scheme 2.

2-Oxoglutaric acid (**1**) on indium mediated allylation with ethyl 4-bromocrotonate (**3**) gave 2-(1-ethoxycarbonyl-allyl)-5-oxo-tetrahydrofuran-2-carboxylic acid (**6**). Compound **1** on allylation with cinnamyl bromide (**4**) gave 5-oxo-2-(1-phenyl-allyl)-tetrahydrofuran-2-carboxylic acid (**7**), a white crystalline solid (96%), *m/z* 247 ($M^+ + 1$), (Scheme 2). In the ^1H NMR spectra of **6** and **7**, presence of C*H*$COOC_2H_5$ proton as doublet at δ 3.74 in **6** and the presence of 1H doublet at δ 3.82 due to C*H*Ph in **7** and lack of CH_2 signals in the region δ 2.5–3.00 confirmed the formation of only γ-addition products. The presence of only one set of signals in both ^1H and ^{13}C NMR spectra points to a single diastereomer being formed. *syn* Stereochemistries of **6** and **7** have been assigned on the basis of stereochemistries of the cyclic products **8** and **13**.

The formation of only *syn* addition products in the allylation of **1** with **3** and **4** could be explained by the participation of Cram's chelation model **A** (Scheme 3) where the conformation of 2-oxoglutaric acid is locked by

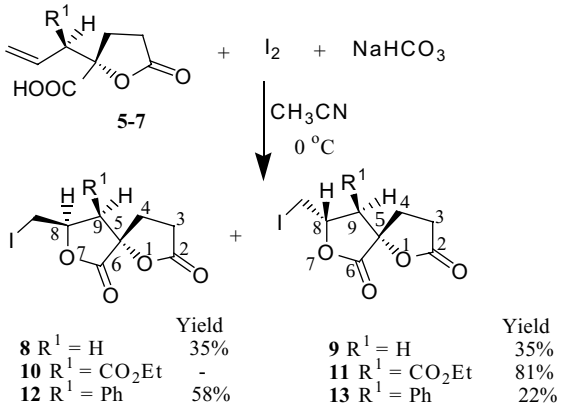


Scheme 3.

complexation with allyl–indium reagent and the allylic anion adds from the sterically less hindered face. The second carboxylic acid group, which is separated by two carbon spacers from carbonyl group, does not seem to participate in allylation process.

2.2. Synthesis of 1,7-dioxa-2,6-dioxospiro[4.4]nonanes

A solution of **5** in dry CH_3CN containing I_2 (3 equiv) and suspended $NaHCO_3$ (3 equiv) on stirring at 0 °C after work-up gave a 1:1 mixture of two diastereomeric products **8** and **9**, M^+ *m/z* 297 (Scheme 4). ^1H NMR spectrum of **9** shows signals at δ 2.31 (dt, 1H of CH_2-4), 2.58–2.67 (m, 4H, 1H of H-4 and 2H of H-9 and 1H of H-3), 2.84 (dt, 1H of CH_2-3), 3.34 (dd, 1H of CH_2–I), 3.49 (dd, 1H of CH_2–I), 4.62–4.69 (m, 1H, CH-8) decoupling of CH-8 multiplet at δ 4.62–4.69 converts two dd's at δ 3.34 and 3.49 into doublets, and also a multiplet at δ 2.58–2.67 shows one singlet at δ 2.62 embedded into symmetrical multiplet. In ^1H–^{13}C HETCOR spectrum the dd's at δ 3.34 and 3.49 show correlations with CH_2 carbon at δ 5.29 and could be assigned as CH_2I. The CH_2 carbon at δ 40.04 shows correlation with doublet at δ 2.62 embedded in multiplet corresponds to CH_2-9. The CH_2 carbon at δ 28.12 corresponds to dt at δ 2.84 and left part of multiplet at δ 2.58–2.67. The CH_2 carbon at δ 30.92 shows correlation with dt at δ 2.31 and right part of multiplet at δ 2.58–2.67. These spectral data assigns the signals to each proton in **9** but the relative stereochemistries at C-5 and C-8 could not be assigned.



Scheme 4.

X-ray crystal structure of **9** (Fig. 1) shows that this molecule has a normal structure. The dihedral angles C(2)–C(3)–C(4)–C(6) and C(8)–O(3)–C(4)–C(5) being 88.6 and 96.4°, show that the two five membered rings are almost perpendicular to each other. The four atoms C(4), C(5), O(1) and C(2) of ring (I) are in plane as shown by the dihedral angle C(2)–O(1)–C(5)–C(4) being 0.7°. C(3) carbon moves out of the plane to make it an envelope type structure with O(1)–C(2)–C(3)–C(4) and C(2)–C(3)–C(4)–C(5) dihedral angles being $31.5 \pm 5°$. Similarly, ring (II) also attains envelope structure with C(4), O(3), C(8) and C(7) atoms in one plane with dihedral angle C(4)–O(3)–C(8)–C(7) being 4.4°. C(6) carbon is placed out of plane with dihedral angle C(4)–C(6)–C(7)–C(8) and O(3)–C(4)–C(6)–C(7) being -25.5 and 28.5°. X-ray structure assigns

configuration (5*R**, 8*R**) to this compound and O(3) and CH_2I are placed *syn* to each other.

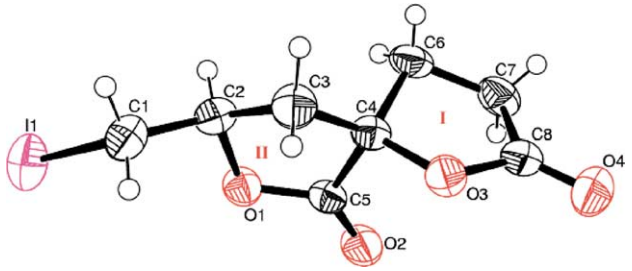


Figure 1. The ORTEP view (50% ellipsoid) of **9**.

Crystal packing of **9** (Fig. 2) shows that the compound **9** is a 1:1 mixture of (5*S**,8*S**)-**9** and (5*R**,8*R**)-**9** enantiomers. Four molecules on an average are packed in one unit cell with a pair of enantiomers placed diagonally to each other and result in formation of center of symmetry. The packing of molecules along side A shows that the rings (II) of molecules in different unit cells are placed parallel to each other and along side B, the rings (I) of molecules are placed parallel to each other.

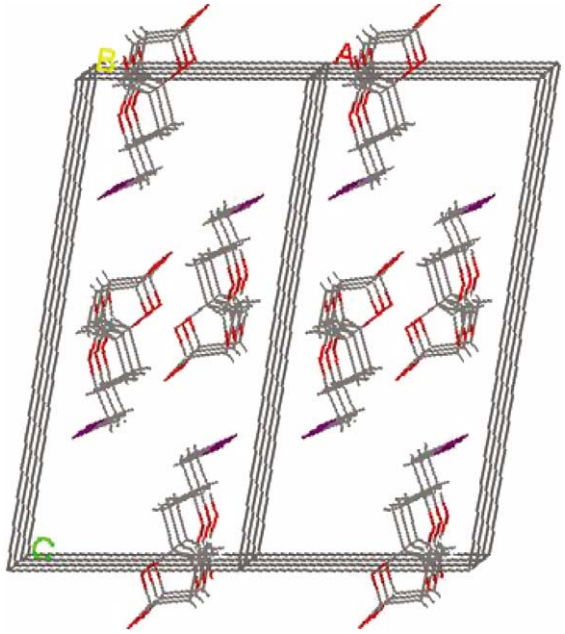


Figure 2. The crystal packing of **9** along side A.

The ^1H and ^{13}C NMR spectral data, the decoupling of various protons in ^1H NMR spectrum and ^1H–^{13}C HETCOR spectrum have been used to assign the chemical shifts of protons and carbons in compound **8**. The relative stereochemistries could not be ascertained at C-8 and C-5 carbons on the basis of these spectral data. However, on the basis of stereochemistries in **9**, the relative stereochemistries (5*S**,8*R**)- have been assigned in compound **8**.

Therefore, **5** undergoes iodocyclization in CH_3CN to provide 1:1 mixture of two diastereomers **8** and **9**. On performing the reaction in DMF, the diastereoselectivity could be increased to 1:2 (S.no. 2, Table 1), which could not be further enhanced by using other solvents and bases (Table 1).

Table 1. Iodocyclisations of **5–7** to spirononanes **8–13**

S.no.	**5–7**	Reaction conditions	Time (h)	dr	%
1	**5**	$NaHCO_3$, CH_3CN	72	8:9 (50:50)	72
2	**5**	$NaHCO_3$, DMF	1–2	8:9 (34:64)	90
3	**5**	DMF[a]	6	8:9 (50:50)	91
4	**5**	THF[a]	6	8:9 (50:50)	68
5	**6**	$NaHCO_3$, CH_3CN	72	10:11 (40:6)	78
6	**6**	$NaHCO_3$, DMF	24	10:11 (2:98)	83
7	**7**	$NaHCO_3$, CH_3CN	72	12:13 (70:30)	75
8	**7**	THF[b]	6	12:13 (84:16)	73
9	**7**	THF[a]	8	12:13 (84:16)	84
10	**7**	DMF[a]	6	12:13 (98:2)	80

[a] Phenylethylamine.
[b] Diisopropylamine.

The iodocyclization of **6** with iodine by using DMF as solvent gave mainly one compound **11**, FAB mass *m*/*z* 369 ($M^+ + 1$); mp 120 °C. ^1H NMR spectrum of **11** shows all the protons to be present in well defined splitting patterns. The decoupling of 1H ddd at δ 4.60 converts doublet at δ 3.61 into singlet and two dd's at δ 3.47 and 3.59 into two doublets and could be assigned as H-8. In ^1H–^{13}C HETCOR spectrum, the correlation of two 1H dd's at δ 3.47 and 3.59 with most upfield carbon at δ 4.97 (due to iodine effect) assigns the CH_2I protons. The coupling constant between H-9 and H-8 ($J=7.5$ Hz) cannot unambiguously assign the relative stereochemistries on these two carbons. The relative stereochemistries at C-5, C-8 and C-9 carbons have been assigned on the basis of X-ray crystal structure.

The dihedral angles O(5)–C(8)–C(11)–C(10) and O(5)–C(9)–C(10)–C(11) 2.0°, C(9)–O(5)–C(8)–C(11) −0.8°, C(8)–O(5)–C(9)–C(10) −0.7°, C(9)–C(10)–C(11)–C(8) −2.3°, show that all the five atoms of ring (I) are in one plane. In ring (II) four atoms C(5), O(3), C(7) and C(8) are in one plane as shown from dihedral angle C(5)–O(3)–C(7)–C(8) 1.4°. C(4) carbon moves out of plane by an angle of 21° (dihedral angles O(3)–C(7)–C(8)–C(4) −21.5° and C(7)–O(3)–C(5)–C(4) −19.3°) and ring (II) attains an envelope type structure. The two rings in this molecule are not exactly perpendicular to each other perhaps due to the strain of the substituents. Ring (II) makes an angle of almost 120° with ring (I) as is clear from dihedral angles C(7)–C(8)–C(11)–C(10) being 121° and C(7)–C(8)–O(5)–C(9) being −120°. X-ray structure shows that O(5) and CH_2I are placed on the same face of the ring (II) and $COOC_2H_5$ is placed on the opposite face. It concludes that O(5) and CH_2I are *syn* to each other and both are *anti* to $COOC_2H_5$. X-ray crystal structure of **11** confirms *syn* addition of allyl group to 2-oxoglutaric acid. On the basis of X-ray structure the configuration (5*R**,8*R**,9*S**)-**11** has been assigned (Fig. 3).

Crystal packing diagram of compound **11** (Fig. 4) shows it to be 1:1 mixture of two enantiomers. Four molecules on the average are packed in one unit cell with a pair of enantiomers placed diagonally to each other and result in formation of center of symmetry. Along side C, rings (II) are packed parallel to each other (Fig. 4) and along side B, rings (I) are packed parallel to each other.

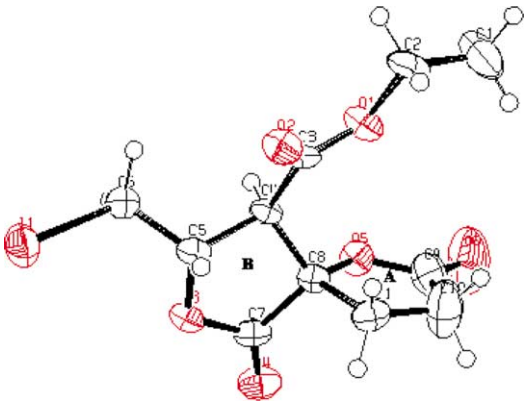


Figure 3. The ORTEP view (50% ellipsoid) of **11**.

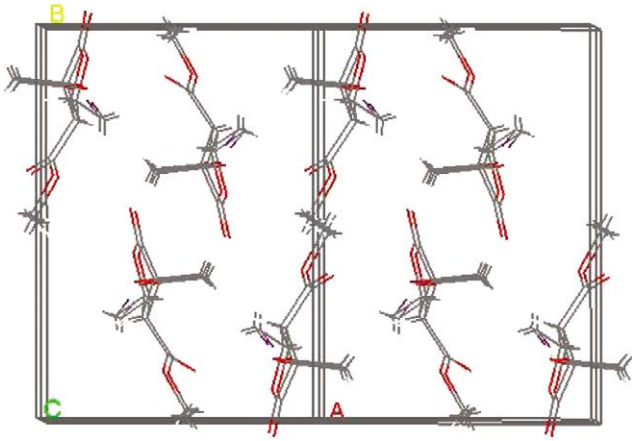


Figure 4. The crystal packing of **11** along side C.

Iodocyclization of **6** in CH_3CN and $NaHCO_3$ gives two diastereomeric products **10** and **11** in 3:4 ratio. However, even on repeated chromatography and crystallization, the minor component could not be isolated (S.no. 5, Table 1).

Furanone **7** on iodocyclisation in CH_3CN–$NaHCO_3$ gave mixture of **12** and **13** in dr 70:30. On using phenyethylamine/diisipropylamine as base in THF (S.no. 8 and 9, Table 1), the diastereoselectivity could be increased to 84:16. On performing iodocyclization of **7** in DMF–$NaHCO_3$–I_2 conditions, diastereoselectivity was increased to 98:2 (S.no. 10, Table 1). The major (fast moving) diastereomer **12**, mp 130 °C; FAB mass 373 ($M^+ + 1$), in its ^1H NMR spectrum shows each proton with well defined splitting pattern. In its ^1H NMR, the decoupling of 1H ddd at δ 5.26 converts the triplet at δ 2.77 into doublet, and two 1H dd's at δ 3.35 and 3.83 to singlets. In ^1H–^{13}C HETCOR spectrum the most upfield CH_2 carbon at δ −0.43 (due to iodine effect) shows correlation with 1H triplet at δ 2.77 and 1H dd at δ 3.35. These data assign 1H signals at δ 2.77, 3.35 as CH_2I, 1H dd at δ 3.83 as C9-H and 1H ddd at δ 5.26 as C8-H. The correlation of CH_2 carbon signal at δ 26.18 with 1H dt at δ 1.83 and 1H ddd at δ 2.29 and correlation of CH_2 carbon at δ 28.17 with 1H ddd at δ 2.52 and 1H dt at δ 2.92 assigns two CH_2 groups in ring (II) of **12**. The irradiation of C9-H shows NOE with C8-H (11%) and does not show NOE with CH_2I and CH_2 groups of ring (I). These data assign the relative stereochemistries as (5*R**,8*S**,9*S**) in **12**. The coupling constant $J_{H\text{-}8,H\text{-}9}$ = 4.8 Hz further support that C8-H and C9-H are on the same face of the furanone ring.

The minor diastereomer (slow moving) **13**, liquid, FAB mass 373 (M + 1), in its ^1H NMR spectrum shows all the protons with well defined stereochemistries except 1H of CH_2-4 and 1H of CH_2-3, which overlap with each other at δ 2.13–2.26. Decoupling of 1H dt at δ 4.72 converts dd's at δ 3.42 and 3.59 into doublets and doublet at δ 3.86 into singlet. ^1H–^{13}C HETCOR spectrum shows the correlation of CH_2 carbon at δ 4.20 with the two dd's at δ 3.42 and 3.59. These data assign C8-H at δ 4.72 and protons at δ 3.42, 3.59 as CH_2I and doublet at δ 3.86 as C9-H. The correlation of CH_2 carbon at δ 26.53 with 1H dt at δ 1.96 and multiplet at δ 2.13–2.26 and CH_2 carbon at δ 28.27 with multiplet at δ 2.13–2.26 and dt at δ 2.77 assign them as CH_2 protons of ring (II). The irradiation of C9-H doublet at δ 3.86 shows NOE with CH_2I protons (1.6%) only. The higher coupling constant $J_{H\text{-}8,H\text{-}9}$ = 7.5 Hz in this diastereomer in comparison with that in **12** (4.8 Hz) points to the presence of C8-H and C9-H protons on the opposite faces of furanone ring (II) of **13**. This coupling constant is parallal with that in case of **11** where the relative stereochemistries have been ascertained by X-ray crystal structure. The up-field shift of C8–H to δ 4.72 in comparison with 5.26 in **12** shows that C8-H proton faces the π-cloud of phenyl ring present on C-9. These data assign the stereochemistries (5*R**,8*R**,9*S**) to **13**.

3. Conclusions

Thus, indium mediated allylation of 2-oxoglutaric acid with allyl bromide, cinnamyl bromide and ethyl 4-bromocrotanate followed by iodocyclization provides a simple two step approach for the synthesis of 1,7-dioxa-2,6-dioxospiro[4.4]nonanes.

4. Experimental

4.1. General details

Melting points were determined in capillaries and are uncorrected. ^1H NMR spectra were recorded on JEOL AL 300 MHz instrument using $CDCl_3$ solution containing tetramethylsilane as an internal standard. The chemical shifts are reported in δ values relative to TMS and coupling constants (J) are expressed in Hz. ^{13}C NMR spectra were recorded at 75 MHz and values are reported relative to $CDCl_3$ signal at δ 77.0. Chromatography was performed with silica gel 100–200 mesh and the reactions were monitored by thin-layer chromatography (TLC) with glass plates coated with silca gel HF-254.

4.2. General procedure

Procedure A. The 2-oxoglutaric acid **1** (5 mmol), allyl bromide (7.5 mmol) and indium metal (fine flakes) (5 mmol) were taken in THF–H_2O (2/1) mixture and the reaction mixture was stirred in an ice bath until the indium metal dissolved (18–24 h). The turbid reaction mixture was

treated with 4 N HCl and extracted with $CHCl_3$. The organic solvent was distilled off and the residue was column chromatographed (silica gel, 100–200 mesh) to isolate the allyl addition product.

4.2.1. 2-Allyl-5-oxo-tetrahydrofuran-2-carboxylic acid (5). Procedure A. 95%; Colourless liquid; FAB mass *m/z* 171 ($M^+ + 1$); ^{1}H NMR ($CDCl_3$): δ 2.28 (dt, $J_1 = 13.5$ Hz, $J_2 = 9.6$ Hz, 1H, CH_2 of ring), 2.46–2.78 (m, 4H, 1H of allylic CH_2, 3H of ring CH_2), 2.82 (dd, $J_1 = 13.5$ Hz, $J_2 = 6.0$ Hz, 1H, allylic CH_2), 4.06 (s, 1H, OH, exchanges with D_2O), 5.18–5.27 (m, 2H, $=CH_2$), 5.72–5.86 (m, 1H, =CH). ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 28.06 (−ve, CH_2), 30.14 (−ve, CH_2), 40.74 (−ve, CH_2), 86.01 (absent, C-5), 120.88 (−ve, CH_2), 130.14 (+ve, CH), 174.40 (absent, C=O), 176.89 (absent, C=O); IR ν_{max} (neat): 1775 (C=O), 3448 (OH) cm^{-1}. (Found C 56.6, H 5.9%. $C_8H_{10}O_4$ requires C 56.47, H 5.92).

4.2.2. (2*S,1′*S**)-2-(1-Ethoxycarbonyl-allyl)-5-oxo-tetrahydrofuran-2-carboxylic acid (6).** Procedure A. 95%; Colourless liquid; FAB mass *m/z* 242 ($M^+ + 1$); ^{1}H NMR ($CDCl_3$): δ 1.25 (t, $J = 7.2$ Hz, 3H, CH_3), 2.36–2.72 (m, 4H, 2×CH_2), 3.74 (d, $J = 9.9$ Hz, 1H, CH), 4.18 (quartet, $J_1 = 7.2$ Hz, 2H, CH_2), 5.45 (dd, $J_1 = 16.5$ Hz, $J_2 = 10.5$ Hz, 2H, $=CH_2$), 5.77 (dt, $J_1 = 16.5$ Hz, $J_2 = 9.9$ Hz, 1H, =CH), 9.07 (b, 1H, OH, exchanges with D_2O); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 13.82 (+ve, CH_3), 27.37 (−ve, CH_2), 28.96 (−ve, CH_2), 55.09 (+ve, CH), 61.85 (−ve, CH_2), 84.81 (absent, C), 123.79 (−ve, CH_2), 128.77 (+ve, CH), 169.81 (absent, C=O), 174.44 (absent, C=O), 176.21 (absent, C=O); IR ν_{max} (neat): 1785 (C=O), 1735 (C=O), 3480 (OH) cm^{-1}. (Found C 54.7, H 5.9%. $C_{11}H_{14}O_6$ requires C 54.54, H 5.83%).

4.2.3. (2*S,1′*R**)-2-(1-Phenyl-allyl)-5-oxo-tetrahydrofuran-2-carboxylic acids (7).** Procedure A. 90%; White solid, mp 60 °C (CH_2Cl_2); FAB mass *m/z* 247 ($M^+ + 1$); ^{1}H NMR ($CDCl_3$): δ 2.41–2.56 (m, 4H, 2×CH_2 of ring), 3.82 (d, 1H, $J = 9.6$ Hz, CH), 5.27–5.32 (m, 2H, $=CH_2$), 6.00 (dt, $J_1 = 16.5$ Hz, $J_2 = 9.9$ Hz, 1H, =CH), 6.1–6.5 (1H, OH, exchanges with D_2O), 7.19–7.36 (m, 5H, ArH); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 27.50 (−ve, CH_2), 29.67 (−ve, CH_2), 55.78 (+ve, CH), 87.92 (absent, C-5), 120.36 (−ve, $=CH_2$), 127.48 (+ve, CH), 128.61 (+ve, CH), 128.73 (+ve, CH), 134.19 (+ve, CH), 138.12 (absent, C), 174.54 (absent, C=O), 175.77 (absent, C=O); IR ν_{max} (neat): 1775 (C=O), 3448 (OH) cm^{-1}. (Found C 67.9, H 6.1%. $C_{14}H_{14}O_4$ requires C 68.28, H 5.7%).

4.3. Iodine mediated cyclization of 2-allyl-5-oxo-tetrahydrofuran-2-carboxylic acids 5–7

Procedure B. Sodium hydrogen carbonate (9 mmol) was added to an ice cold solution of 2-allyl-5-oxo-tetrahydrofuran-2-carboxylic acids **5–7** (3 mmol) in dry acetonitrile and resulting suspension was stirred for 15 min at 0 °C. Iodine (9 mmol) was added and stirring was continued for 7–8 h at 0 °C (TLC monitoring). The reaction mixture was diluted with water and extracted with $CHCl_3$. The organic layer was washed with saturated aqueous sodium thiosulphate to remove excess of iodine. The organic layer was dried over anhydrous sodium sulphate and was distilled off. The residue was column chromatographed (silica gel 100–200) to isolate 1,7-dioxa-2,6-dioxospiro[4,4]nonanes **8–13**.

4.3.1. (5*R,8*S**)-8-Iodomethyl-1,7-dioxa-2,6-dioxospiro-[4.4]nonane (8).** Procedure B. 35%; White solid, mp 58 °C (CH_2Cl_2); FAB mass *m/z* 297 ($M^+ + 1$); ^{1}H NMR ($CDCl_3$): δ 2.12 (dd, $J_1 = 11.4$ Hz, $J_2 = 8.7$ Hz, 1H, 1H of CH_2-9), 2.29 (dt, $J_1 = 16.2$ Hz, $J_2 = 10.0$ Hz, 1H, 1H of CH_2-4), 2.62–2.74 (m, 2H, 1H of CH_2-3 and 1H of CH_2-4), 2.84 (dd, $J_1 = 14.4$ Hz, $J_2 = 6.0$ Hz, 1H, CH_2-9), 3.02 (dt, $J_1 = 18.0$ Hz, $J_2 = 10.0$ Hz, 1H, CH_2-3), 3.48 (dd, $J_1 = 10.8$ Hz, $J_2 = 6.9$ Hz, 1H, CH_2–I), 3.65 (dd, $J_1 = 10.8$ Hz, $J_2 = 3.9$ Hz, 1H, CH_2–I), 4.62–4.68 (m, 1H, CH-8); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 6.20 (−ve, CH_2I), 28.02 (−ve, CH_2-3), 28.83 (−ve, CH_2-4), 41.03 (−ve, CH_2-9), 76.24 (+ve, CH-8), 83.95 (absent, C-5), 172.88 (absent, C=O), 174.52 (absent, C=O); IR ν_{max} (KBr): 1790, 1785 (C=O) cm^{-1}. (Found C 32.4, H 3.3%. $C_8H_9IO_4$ requires C 32.45, H 3.06%).

4.3.2. (5*R,8*R**)-8-Iodomethyl-1,7-dioxa-2,6-dioxospiro-[4.4]nonane (9).** Procedure B. 35%; White solid, mp 118 °C (CH_2Cl_2); FAB mass *m/z* 297 ($M^+ + 1$); ^{1}H NMR ($CDCl_3$): δ 2.31 (dt, $J_1 = 13.5$ Hz, $J_2 = 9.3$ Hz, 1H, 1H of CH_2-4), 2.58–2.67 (m, 4H, 1H of CH_2-4 and 2H of H-9 and 1H of CH_2-3), 2.84 (dt, $J_1 = 18.0$ Hz, $J_2 = 10.2$ Hz, 1H, 1H of CH_2-3), 3.34 (dd, $J_1 = 10.2$ Hz, $J_2 = 8.7$ Hz, 1H, 1H of CH_2–I), 3.49 (dd, $J_1 = 10.2$ Hz, $J_2 = 5.1$ Hz, 1H, 1H of CH_2–I), 4.62–4.69 (m, 1H, CH-8); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 5.29 (−ve, CH_2I), 28.12 (−ve, CH_2-3), 30.92 (−ve, CH_2-4), 40.04 (−ve, CH_2-9), 76.59 (+ve, CH-8), 83.12 (absent, C-5), 173.69 (absent, C=O), 174.90 (absent, C=O); IR ν_{max} (KBr): 1785 (C=O) cm^{-1}. (Found C 32.3, H 3.3%. $C_8H_9IO_4$ requires C 32.45, H 3.06%).

4.3.3. (5*R,8*R**,9*S**)-8-Iodomethyl-1,7-dioxa-2,6-dioxospiro[4.4]nonane-9-carboxylic acid ethyl ester (11).** Procedure B. 81%; White crystalline solid, mp 120 °C (CH_2Cl_2); FAB mass *m/z* 369 ($M^+ + 1$); ^{1}H NMR ($CDCl_3$): δ 1.33 (t, $J = 7.2$ Hz, 3H, CH_3), 2.33 (ddd, 1H, $J_1 = 13.5$ Hz, $J_2 = 9.9$ Hz, $J_3 = 8.7$ Hz, 1H of CH_2-4), 2.46 (ddd, 1H, $J_1 = 13.5$ Hz, $J_2 = 10.2$ Hz, $J_3 = 4.5$ Hz, 1H of CH_2-4), 2.62 (ddd, 1H, $J_1 = 18$ Hz, $J_2 = 9.9$ Hz, $J_3 = 4.5$ Hz, 1H of CH_2-3), 2.89 (ddd, 1H, $J_1 = 18$ Hz, $J_2 = 10.2$ Hz, $J_3 = 9$ Hz, 1H of CH_2-3), 3.47 (dd, $J_1 = 11.1$ Hz, $J_2 = 4.5$ Hz, 1H, 1H of CH_2I), 3.59 (dd, $J_1 = 11.1$ Hz, $J_2 = 5.7$ Hz, 1H, 1H of CH_2I), 3.61 (d, $J = 7.5$ Hz, 1H, CH-9), 4.29 (q, $J_1 = 6.9$ Hz, 2H, OCH_2), 4.60 (ddd, 1H, $J_1 = 7.5$ Hz, $J_2 = 5.7$ Hz, $J_3 = 4.5$ Hz, CH-8); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 4.96 (−ve, CH_2I), 14.00 (+ve, CH_3), 27.18 (−ve, CH_2), 27.24 (−ve, CH_2), 54.30 (+ve, CH-9), 62.57 (−ve, CH_2), 75.85 (+ve, CH), 83.33 (absent, C-5), 167.18 (absent, C=O), 171.27 (absent, C=O), 173.84 (absent, C=O); IR ν_{max} (KBr): 1800, 1795, 1731 (C=O) cm^{-1}. (Found C 35.8, H 3.8%. $C_{11}H_{13}IO_6$ requires C 35.89, H 3.56%).

4.3.4. (5*R,8*S**,9*S**)-8-Iodomethyl-9-phenyl-1,7-dioxa-2,6-dioxospiro[4.4]nonane (12).** Procedure B. 58%, White solid, mp 130 °C (CH_2Cl_2); FAB mass *m/z* 373 ($M^+ + 1$); ^{1}H NMR ($CDCl_3$): δ 1.83 (dt, $J_1 = 9.9$ Hz, $J_2 = 13.5$ Hz, 1H, 1H of CH_2-4), 2.27, 2.32 (ddd's, $J_1 = 13.8$ Hz, $J_2 = 9.9$ Hz, $J_3 = 3.6$ Hz, 1H, 1H of CH_2-4), 2.52 (ddd's,

$J_1=18$ Hz, $J_2=9.9$ Hz, $J_3=3.6$ Hz, 1H, 1H of CH_2-3), 2.77 (t, $J=9.9$ Hz, 1H, 1H of CH_2-I), 2.92 (dt, $J_1=18.0$ Hz, $J_2=$ 9.9 Hz, 1H, 1H of CH_2-3), 3.35 (dd, $J_1=9.9$ Hz, $J_2=$ 5.7 Hz, 1H, 1H of CH_2–I), 3.83 (d, $J=4.8$ Hz, 1H, CH-9), 5.26 (ddd, $J_1=9.9$ Hz, $J_2=5.7$ Hz, $J_3=4.8$ Hz, 1H, CH-8) 7.10–7.13 (m, 2H, ArH), 7.36–7.41 (m, 3H, ArH); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ −0.43 (−ve, CH_2I), 26.18 (−ve, CH_2-4), 28.17 (−ve, CH_2-3), 55.10 (+ve, CH-9), 82.21 (+ve, CH-8), 88.18 (absent, C-5), 129.35 (+ve, CH), 129.64 (+ve, CH), 129.79 (+ve, CH), 134.15 (absent, ArC), 173.63 (absent, C═O), 174.62 (absent, C═O); IR ν_{max} (KBr): 1785 (C═O) cm^{-1}. (Found C 45.2, H 3.4%. $C_8H_9IO_4$ requires C 45.18, H 3.52%).

4.3.5. (5*R,8*R**,9*S**)-8-Iodomethyl-9-phenyl-1,7-dioxa-2,6-dioxospiro[4.4]nonane (13).** Procedure B. 22%, transparent liquid; FAB mass 373 ($M^+ +1$); ^{1}H NMR ($CDCl_3$): δ 1.96 (dt, $J_1=13.5$ Hz, $J_2=9.6$ Hz, 1H, 1H of CH_2-4), 2.13–2.26 (m, 2H, 1H of CH_2-4 and 1H of CH_2-3), 2.77 (dt, $J_1=17.7$ Hz, $J_2=9.6$ Hz, 1H, 1H of CH_2-3), 3.42 (dd, $J_1=$ 11.0 Hz, $J_2=5.4$ Hz, 1H, 1H of CH_2–I), 3.59 (dd, $J_1=$ 11.0 Hz, $J_2=5.4$ Hz, 1H, 1H of CH_2I), 3.86 (d, $J=7.5$ Hz, 1H, CH-9), 4.73 (dt, $J_1=7.5$ Hz, $J_2=5.4$ Hz, 1H, CH-8), 7.18–7.26 (m, 2H, ArH), 7.39–7.47 (m, 3H, ArH); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 4.20 (−ve, CH_2I), 26.53 (−ve, CH_2-4), 28.27 (−ve, CH_2-3), 54.62 (+ve, CH-9), 79.39 (+ve, CH-8), 85.70 (absent, C-5), 128.13 (+ve, CH), 129.05 (+ve, CH), 129.67 (+ve, CH), 132.40 (absent, ArC), 172.54 (absent, C═O), 174.31 (absent, C═O); IR ν_{max} (KBr): 1786 (C═O) cm^{-1}. (Found C 45.4, H 3.4%. $C_8.H_9IO_4$ requires C 45.18, H 3.52%).

4.4. X-ray crystal data collection for 9 and 11

X-ray crystal data was measured by using θ–2θ scan mode. The structures were solved by using direct method SHELX-97.

4.4.1. Compound 9. CCDC no. 279159, Molecular formulae $C_8H_9IO_4$; monoclinic space group $P21/c$, $a=$ 8.3250 Å, $b=6.5600$ Å, $c=17.9240$ Å, $\alpha=90°$, $\beta=$ 98.097(7)°, $\gamma=90°$, $v=969.11(14)$ Å^3, $z=4$, $d_c=$ 2.029 mg/m^3, θ range for data collection 2.29–24.91°. The structure solution is based on 1824 reflections, which converged to $R=0.037$. Refinement method: full-matrix least squares on F^2, goodness of fit = 1.087.

4.4.2. Compound 11. CCDC no. 279160, Molecular formulae $C_{11}H_{13}IO_6$; monoclinic space group P-21/a, $a=$ 9.494(4) Å, $b=12.622(5)$ Å, $c=11.688(9)$ Å, $\alpha=90°$, $\beta=109.88(5)°$, $\gamma=90°$, $v=1317.2(12)$ Å^3 , $z=4$, $d_c=$ 1.856 mg/m^3, θ range for data collection 1.85–24.97°. The structure solution is based on 2141 reflections, which converged to $R=0.066$. Refinement method: full-matrix least squares on F^2, goodness of fit = 1.090.

Acknowledgements

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Abnormal Beckmann rearrangement of steroidal α-chlorocyclobutanone oximes: the fragmentation–substitution reaction☆

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Abstract—α,α-Dichlorocyclobutanones of various substitution patterns, readily available by the thermal cycloaddition of dichloroketene to steroid olefins, were selectively dehalogenated and further transformed to α-chlorocyclobutanone oximes. In contrast to the Beckmann rearrangement of the α-unsubstituted oximes, α-chlorocyclobutanone oximes, on treatment with thionyl chloride in benzene, gave normal and abnormal reaction products. In all reactions studied, the Beckmann fragmentation–substitution was the major process. The rearrangement of α-chlorocyclobutanone oximes is the key step of the novel method for geminal functionalization of carbonyl carbon atom in ketones and regioselective vicinal functionalization of unsymmetrical olefins. The regiochemistry observed in the rearrangements is supported by semiempirical calculations (AM1).

1. Introduction

Substituted cyclobutanones are readily available by synthesis.[1] One of the most frequently used and efficient method for their preparation is the [2+2] thermal cycloaddition of ketenes to alkenes.[2,3] These sterically constrained compounds are versatile intermediates in organic synthesis.[2] In steroids, a series of cyclobutanone derivatives have been synthesized from olefins in the reaction with dichloroketene.[4–8] The transformation of steroidal fused and spiro cyclobutanones has been applied to the synthesis of cyclopropane and cyclopentane derivatives[9] as well as oxa- and aza-heterocyclic derivatives of steroids[10] in high yielding reactions. The fused[11] and spiro[9] dichlorocyclobutanones have been shown to undergo ring opening and tele substitution reactions as well.

The Beckmann rearrangement of cyclobutanone oximes provides an effective method for synthesizing of γ-lactams.[12,13] In steroids, stereoisomeric 5α-spiro-[cholestane-3,1′-cyclobutane]-3′-one oximes have been transformed to spiro-pyrrolidinones and stereospecificity of the process has been clearly established.[10] In the Beckmann rearrangements of oximes bearing electronegative substituents like OH, –OR, $–NR_2$ in position α, stabilized transient cations are formed upon the C(α)–C(sp^2) bond cleavage and abnormal rearrangement has been observed.[14] α-Chloroximes have been synthesized by several approaches[15] and their transformation has been described.[16] In this context, it is interesting to note that in the chemical literature one can find only one paper describing the reaction of an α-chloroxime, the *syn*-2-chloro-1-hydroxyiminocyclododecane, which underwent the Beckmann rearrangement under very drastic conditions (concd H_2SO_4, 110–112 °C) to give the respective α-chlorolactam.[17]

α,α-Dichlorocyclobutanones have been considered unreactive under standard oximation conditions and our attempts to prepare oximes of steroidal dichlorocyclobutanones have also failed. However, an example of Beckmann rearrangement, in which α,α-dichlorocyclobutanone upon reaction with Tamura's reagent[18] is transformed to the respective pyrrolidinone, has been described.[19] Partial dehalogenation of α,α-dichlorocyclobutanones has been previously reported.[20,21] Accordingly, we have found that dehalogenation of steroidal dichlorocyclobutanones results in partial or complete removal of chlorine atoms, depending on the reaction conditions.[8,22,23] The possible synthetic availability of the oximes, derivatives of the strained α-chlorocyclobutanones, raised the question of their reactivity and synthetic utility. While the chemistry of cyclobutanones has been

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Keywords: Cyclobutanones; Oximes; Beckmann rearrangement; Steroids.
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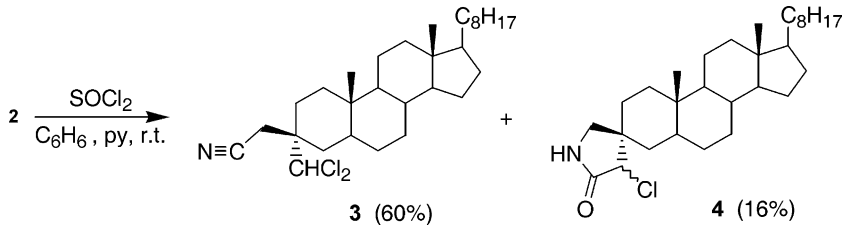
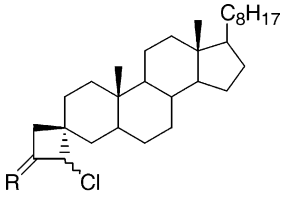


Scheme 1.

thoroughly investigated,[1,2,24] the Beckmann rearrangement of α-chlorocyclobutanone oximes, to our knowledge, has not been previously reported. This reaction was anticipated to give access to steroids with spiro or fused chloropyrrolidinone fragment as well as to azacardenolides, which are also compounds of biological importance.[25–27] The possible formation of abnormal Beckmann products was also considered. As a continuation of our interest in new transformations and synthetic utility of steroidal cyclobutanone derivatives, we have investigated the rearrangements of a series of α-chlorocyclobutanone oximes having different steroid residues attached to the four-membered ring and the following results are presented.

2. Results and discussion

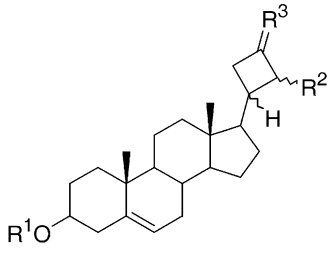
The α-chlorocyclobutanone **1**[22] gave under standard conditions ($NH_2OH\cdot HCl$, pyridine, room temperature) the respective, α-chloroxime **2** that was shown by ^1H NMR spectrum to be a mixture of stereoisomers. It gave one spot on TLC plates developed with several solvent systems. Thus, attempts to separate[10] *syn* and *anti* isomers of **2** by chromatography failed.



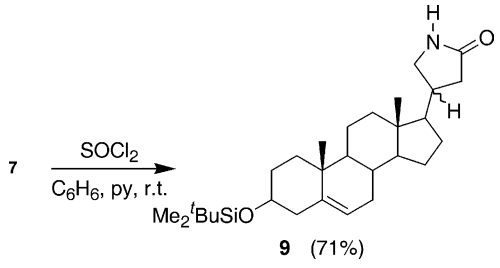
1 R = O
2 R = NOH

The reaction of monochloroxime **2** with thionyl chloride in benzene containing traces of pyridine gave two compounds in the approximately 2:1 ratio (by ^1H NMR spectrum), separated by column chromatography (Scheme 1). The major product of low polarity (60% isolated yield), was assigned structure **3**. It had absorption at $\nu_{max}=2248\ cm^{-1}$ (cyano group) in the IR spectrum and showed characteristic signals in the ^1H NMR spectrum at δ 6.25 and 2.70 assigned to the protons of $CHCl_2$ and CH_2CN groups, respectively. In the ^{13}C NMR spectrum, the corresponding signals were found at δ 77.9 ($CHCl_2$) and 117.7 (CN). The highly polar product, chloropyrrolidinone **4** isolated pure in 16% yield, was characterized by IR, ^1H NMR and mass spectra (see Section 4).

The possible synthesis of aza analogs of cardenolides[25–27] prompted us to investigate the reaction of oximes, derivatives of cyclobutanones **5** and **6**. These ketones were synthesized in few steps from 3β-acetoxypregna-5,20-diene by application of the dichloroketene cycloaddition methodology,[23,28] and gave oximes **7** and **8**, respectively, in excellent yield. The oxime **7**, treated with thionyl chloride in benzene solution at room temperature, gave the expected pyrrolidinone **9** in 71% isolated yield (Scheme 2). It was an approximately 1:1 mixture of C(20)-epimers as indicated by the ^1H NMR spectrum. The attempted dehydrogenation of the saturated lactam **9** to obtain aza-cardenolide analogue **12** with benzeneseleninic anhydride failed, however.



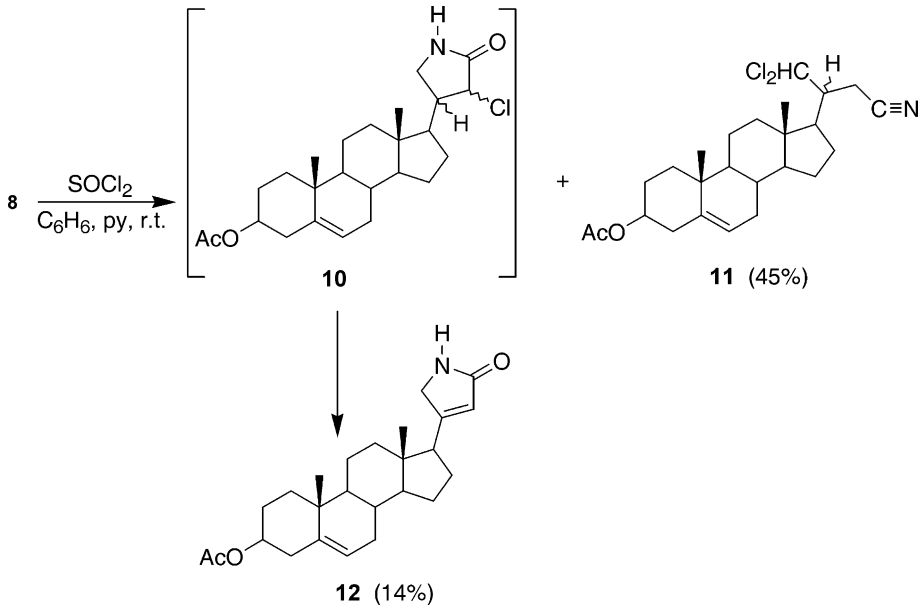
5 $R^1 = Si^tBuMe_2$, R^2 = H, R^3 = O
6 R^1 = Ac, R^2 = Cl, R^3 = O
7 $R^1 = Si^tBuMe_2$, R^2 = H, R^3 = NOH
8 R^1 = Ac, R^2 = Cl, R^3 = NOH



Scheme 2.

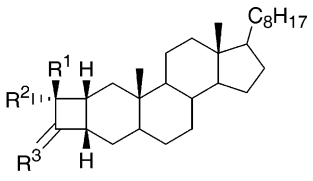
The α-chloroxime **8**, obtained from α-chlorocyclobutanone **6**,[28] was a mixture of stereoisomers. The formation of oxime *anti*-**8** as the major isomer was assumed on the basis of literature data.[16,17] Denmark and co-workers[16a] proposed that the net dipole moment is the major factor responsible for predominant formation of *E* isomers of α-chloroximes, derivatives of 3- and 4-alkyl-2-chlorocyclohexanone. Moreover, our further experiments firmly established the preferred formation of *anti* α-chloroximes from rigid fused cyclobutanones (vide infra).

Under standard rearrangement conditions ($SOCl_2$, benzene, pyridine), α-chloroxime **8** gave two compounds, **10** and **11** in approx. 1:3 ratio estimated from the ^1H NMR spectrum of the crude reaction mixture (Scheme 3). The compounds



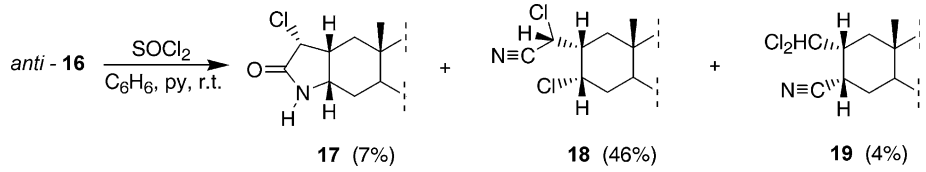
Scheme 3.

formed in the rearrangement slowly decomposed during isolation and chromatography. Thus, the polar product, azacardenolide **12**[29] and unpolar compound **11** were obtained pure in 14 and 45% yield, respectively. The IR, ^{1}H and ^{13}C NMR spectra of **12**[29] confirmed the presence of the pyrrolinone fragment attached to the steroid skeleton[30] (see Section 4). Formation of dichloro-cyano derivative **11** was indicated by the presence of absorption at $\nu_{max}=2243\ cm^{-1}$ in the IR spectrum. Additionally, ^{13}C NMR spectrum revealed signals at δ 118.7 and 76.1 corresponding to carbon atoms in CN and $CHCl_2$ groups, respectively. Compound **11** was a mixture of 20*R*- and 20*S*-diastereoisomers, as evidenced by ^{1}H NMR signals of protons in the $CHCl_2$ group at δ 5.96 and 6.14 and of protons in the 22-CH_2 group at δ 2.81 and 2.72. Tentative assignment of the 20*R* configuration of the major nitrile **11** was based on the assumption that in the course of cycloaddition the approach of dichloroketene to the Δ^{20} double bond in 3β-acetoxypregna-5,20-diene, preferentially existing as 17(20)-*s*-trans rotamer, should occur from the side opposite to the 18-CH_3 group of the steroid.[8,28] The intermediate α-chloropyrrolidinone **10** could not be isolated due to elimination of hydrogen chloride from **10** that occurred during chromatography on silica gel. However, in the ^{1}H NMR spectrum of the crude reaction product, the formation of **10** was clearly evidenced by the signal of proton H-22 at δ 4.11 (d, $J=7.9$ Hz).



13 $R^1=R^2=Cl$, $R^3=O$
14 $R^1=H$, $R^2=Cl$, $R^3=O$
15 $R^1=R^2=H$, $R^3=O$
16 $R^1=H$, $R^2=Cl$, $R^3=NOH$

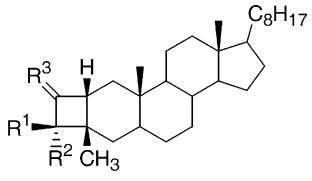
The formation of nitriles **3** and **11** as the major products from oximes **2** and **8**, showed that α-chloroximes, in contrast to the α-unsubstituted cyclobutanone oximes, undergo mainly the Beckmann fragmentation resulting in the cleavage of the C(sp^2)–C(α) bond. In the light of these results it seemed of interest to study the reaction of α-chlorocyclobutanone oximes substituted in α′ position, for example derivatives of cyclobutanones fused to the steroidal ring A. Mild reduction of α,α-dichlorocyclobutanone **13**[5,6] with zinc in acetic acid at room temperature gave **14** in 78% yield. The configuration of the chlorine-bearing carbon atom in α-chlorocyclobutanone **14** was assigned on the basis of the following CD data. The comparison of the $\Delta\varepsilon$ values determined for compounds **13** ($\Delta\varepsilon=+4.1$), **14** ($\Delta\varepsilon=\sim 0.0$) and **15** ($\Delta\varepsilon=+0.7$)[5b] clearly showed that the quasi-axial Cl atom was responsible for the strong positive Cotton effect recorded for **13**. The assignment of configuration of carbon atom C(4′) in **14** was confirmed by the ^{1}H NMR spectrum exhibiting a signal (dd) for proton H-4′ at δ 5.00 with $J=8.7$, 2.2 Hz, the value similar to that (9 Hz) reported for 4β-acetoxy derivative of compound **14**.[11c] It follows then that reduction of compound **13** gave monochloroketone **14** that retained the chlorine atom in pseudoequatorial position. This result was in agreement with our previous finding concerning the selective removal of the quasi axial chlorine atom in the course of mild reduction of α,α-dichlorocyclobutanone.[8] The α-chloroxime **16** was prepared from the ketone **14** by the standard method. The ^{1}H NMR spectrum of the crude oxime showed that it was a mixture of *anti* and *syn* isomers. It has been established that in the pairs of oxime geometric isomers, deshielding of the proton in the α position to the hydroxyimino group and cis to the oxime hydroxyl is observed.[16,17,31] Thus, the 4.5:1 ratio was estimated from the integration of signals at δ 4.84 and 3.16 ascribed to *anti*-**16** and at δ 5.03 and 2.98 ascribed to *syn*-**16**, respectively. Preparative TLC separation of this mixture afforded pure (by ^{1}H NMR) major oxime *anti*-**16** that was slightly more polar than isomer *syn*-**16**.



Scheme 4.

The rearrangement of the oxime *anti*-**16** under the standard conditions afforded a mixture of products (Scheme 4). The three components **X**, **Y**, and **Z** were traced by TLC and the approx. 1:1:8 ratio was evaluated from the ^{1}H NMR spectrum of the crude reaction mixture. The highly polar **X**, insoluble in hexane, was separated by filtration (7% yield). As expected, this was α-chlorolactam **17** whose structure was confirmed by the ^{1}H NMR spectrum (see Section 4). The two other non-polar products **Y** and **Z** were isolated upon chromatography in 4 and 46% yield, respectively. The major product **Z** was assigned structure **18**. The presence of the cyano group was evidenced by the IR absorption at $\nu_{max}=2237\ cm^{-1}$. The three low-field signals of the protons in the C*H*ClCN group (δ 5.61), 3β-H (δ 3.45, $J=2.2$ Hz) and 2β-H (δ 2.21) were in full agreement with the proposed structure **18** and the small coupling constant assigned for the signal of the proton 3β-H confirmed its equatorial position. The configuration of the 2α-chlorocyanomethyl group in compound **18** is the same as that of carbon C-4′ in the oxime **16**. The ^{1}H NMR spectrum of the minor product **Y** showed a characteristic low field signal of the proton in the $CHCl_2$ group at δ 6.13, the value similar to those found for **3** and **11**. In the IR spectrum, absorption at $\nu_{max}=2236\ cm^{-1}$ suggested the presence of the cyano group. Thus, the product **Y** was assigned structure **19**, which was additionally supported by two low field signals at δ 3.46 (3β-H) and 2.96 (2β-H) observed in the ^{1}H NMR spectrum.

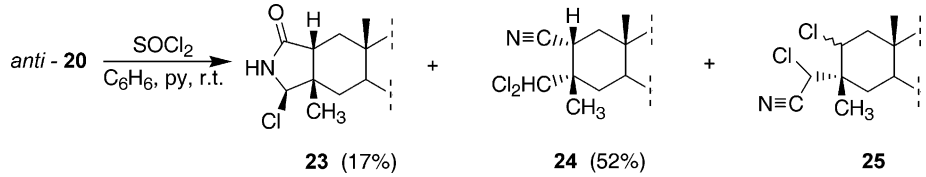
In the rearrangement of oxime **16**, the formation of compound **18**, a new type of the abnormal product resulting from the cleavage of the C(sp^2)–C(α′) bond, was rather a surprise. Therefore, the synthesis of another steroidal fused α-chlorocyclobutanone oxime **20** was of considerable interest.



20 R^1 = Cl, R^2 = H, R^3 = NOH
21 R^1 = R^2 = Cl, R^3 = O
22 R^1 = Cl, R^2 = H, R^3 = O

Thus, dichlorocyclobutanone **21**,[7] prepared according to a slightly modified literature procedure[7] from 3-methyl-5α-cholest-2-ene,[32] was dehalogenated with zinc in acetic acid at room temperature to give α-monochloroketone **22** in 70% isolated yield. The comparison of CD data of the α,α-dichlorocyclobutanone **21** ($\Delta\varepsilon=-1.3$) and of the monochloro derivative **22** ($\Delta\varepsilon=-5.65$) confirmed removal of the α-axial chlorine atom from **21** in the course of reduction. However, some admixture (less than 5%) of 3′-epimeric **22** could be detected in the mono-dehalogenation product obtained from **21**, as evidenced by the ^{1}H NMR spectrum of **22** (see Section 4). In contrast to **13**, dehalogenation of **21** was not entirely stereoselective. The energy-minimized structure **21** shows that the quasi-axial chlorine atom, being in close proximity to the 5α-hydrogen, is strongly sterically hindered and less accessible.

The reaction of the α-chloroketone **22** with hydroxylamine furnished oxime **20** (87% yield), as a mixture of stereoisomers. The 5:1 ratio was determined by the integration of proton signals at δ 4.99 and 4.95 (CHCl) and δ 2.94 and 2.61 (2β-H), characteristic of isomer *anti*-**20** and *syn*-**20**, respectively. The isomers were separated by preparative TLC and the pure (by ^{1}H NMR) major isomer *anti*-**20** thus obtained was subjected to the reaction with thionyl chloride in benzene (Scheme 5). The crude reaction product showed three components on TLC plate and their approx. 3:1:1 ratio was assigned from the ^{1}H NMR spectrum. The most polar compound was insoluble in hexane and was separated from the mixture by filtration (17% yield). It was assigned formula **23** on the basis of the ^{1}H NMR, IR, and MS spectra (see Section 4). The fraction of unpolar products (74% yield) consisted of two components. These were separated by column and preparative TLC to give two compounds. The major product isolated in 52% yield was the cyano derivative **24**, showing absorption at $\nu_{max}=2234\ cm^{-1}$ in the IR spectrum and characteristic signals in the ^{1}H NMR spectrum at δ 6.22 ($CHCl_2$), 2.86 (2β-H) and 1.37 (3β-CH_3). The third compound could not be obtained pure. It probably partly decomposed during chromatography. However, its IR absorption at $\nu_{max}=2230$ and $2245\ cm^{-1}$ and mass spectrum suggested structure **25**. This was also supported by the ^{1}H NMR spectrum, which showed two singlets integrated to one proton at δ 5.60 and 5.41 ascribed to the proton in the CHClCN group, two signals at δ 4.69



Scheme 5.

and 3.45 (2β- and 2α-H) and a signal of the 3β-methyl group at δ 1.43.

The reactions of oximes **2**, **8**, **16** and **20** appear to be the first example of the Beckmann rearrangement of α-chlorocyclobutanone oximes. In all cases investigated, the formation of normal and abnormal reaction product was detected, however, the fragmentation–displacement was found to be the major process. Previously, abnormal Beckmann rearrangements of cyclobutanone oximes have only been observed for substrates that upon cleavage of the C(sp^2)–C(α) bond gave stable tertiary carbocations.[14c,33] In some cases, this stabilization seems to outweigh the stereoelectronic effect as evidenced by the rearrangement of steroidal cyclobutanone oximes. In this reaction the major product was the unsaturated nitrile, which resulted from the α-fission of the bond cis to the oxime leaving group (*N*-nucleofuge).[33] A delicate balance between structural and stereoelectronic effects is often responsible for the formation of normal and abnormal products in the course of the rearrangement. This has also been shown in the recent studies concerning reactions of oximes derived from steroid 17-ketone, which, depending on the reaction conditions, also gave normal[34] or abnormal[35] Beckmann rearrangement products.

The α-chlorocyclobutanones investigated in this work gave two stereoisomeric oximes, while the *anti* isomer always prevailed,[16,17] presumably as a result of a dipole–dipole interaction. Semiempirical calculations (AM1, CAChe, Fujitsu) show, that *anti* isomers of **16** and **20** are more stable than *syn* isomers by 0.28 and 0.16 kcal/mol, respectively. Similarly, the $\Delta\Delta H_f = 0.16$ kcal/mol was calculated for *anti* and *syn* isomers of spirane oxime **2**. We observed that *syn* and *anti* α-chlorooximes were stable in the solid state and in benzene solution. Moreover, the addition of pyridine prevented isomerization under the rearrangement conditions.[10]

The steroidal stereoisomeric spiro-cyclobutanone oximes, unsubstitituted in position α and α′, rearranged stereospecifically with trans bond migration and gave normal products, spiro-pyrrolidinones.[10] Similarly, the oxime **7**, upon rearrangement, gave exclusively the normal product **9**, as expected. The presented results clearly show that the novel rearrangement of α-chlorocyclobutanone oximes strongly depends on the substitution pattern and stereochemistry of the substrate. In the reaction of α-chloroximes **2** and **8**, lactams **4** and **12**, respectively, likely derive from the normal Beckmann rearrangement, while nitriles **3** and **11** are α-scission-substitution reaction (Beckmann fragmentation)[14b,36] products. It appears, therefore, that in reactions of **2** and **8** stereoelectronic effect seems to play the major role. Thus, both reactions of the α′-unsubstituted α-chlorocyclobutanone oximes *syn*-**2** or *syn*-**8** afford normal Beckmann products **4** or **12**, respectively, resulting from the migration of the bond trans to the leaving group. At the same time, the reaction of the more abundant *anti* chloroximes *anti*-**2** or *anti*-**8** results in formation of **3** or **11**, respectively, according to the α-fission-displacement mechanism. The formation of vicinal dichloromethyl-cyano derivatives may well be explained by the stabilization of a transient positive charge resulting from the participation of the α-chlorine atom lone pair, which facilitates the rupture of the C(sp^2)–CCl bond. This is in agreement with the known effect that adjacent atoms with one or more lone electron pairs strongly stabilize carbocations.[37] When the energies of transient carbocations **A** and **B** (Fig. 1) formed upon cleavage of α- or α′-bond in spiro-chloroxime **2** are compared, it is evident that the stabilization of the former by about 18 kcal/mol should favour the α-bond scission leading to dichloromethyl-cyano derivative **3**.

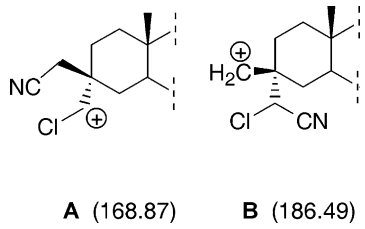


Figure 1. The partial structure (ring A) of the energy minimized transient carbocations resulting from the α- (A) and α′-bond (B) cleavage of the spiro-α-chlorocyclobutanone oxime **2**. AM1 enthalpies are in kcal/mol.

Reactions of α′-substituted-α-chlorocyclobutanone oximes **16** and **20** pointed once again to the importance of the energy of the intermediate carbocations. While an abnormal reaction was again the major process in the reaction of oxime **16**, the rather unexpected formation of **18** occurred, as a consequence of the α′ bond cleavage leading to the intermediate 2° carbocation. The compound **19**, resulting from the α-fission of the C(sp^2)–CCl bond, was only the minor product. Indeed, this experimental result was supported by theoretical considerations. The geometry optimization showed that the most stable rotamer of the intermediate 2° cation **C** was 5.6 kcal/mol lower in energy than the most stable rotamer of the alternative intermediate **D** (Fig. 2). Previously, α-cleavage of the bond cis to the oxime OH group has been observed[33] in steroids and fragmentation of 3-nortricyclanone oxime resulting in the formation of intermediate 2° carbocation has recently been reported.[38] The analysis of the reaction products formed from the oxime **20** leads to the conclusion that the major pathway involves the formation of the intermediate carbocation **E** (Fig. 2) that might be attacked by a nucleophile to form the dichloromethyl-cyano derivative **24**.

The intermediacy of carbocation **F** (Fig. 2) can currently only be inferred from the formation of compound **25**, which

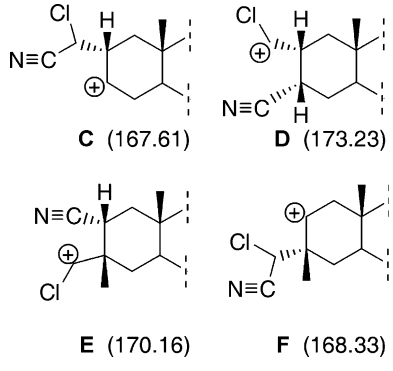


Figure 2. The intermediate carbocationic species **C**, **D** and **E**, **F** postulated in the Beckmann rearrangement of α-chloroximes **16** and **20**, respectively. AM1 enthalpies are in kcal/mol.

is a mixture of 2α- and 2β-chloro isomers (^{1}H NMR evidence). Both cations **E** and **F** are of similar energy.

3. Conclusion

In summary, an unprecedented rearrangement of α-chlorocyclobutanone oximes, derivatives of steroids, is described. All the rearrangements of α-chlorocyclobutanone oximes are characterized by the prevailing abnormal pathway (Beckmann fragmentation–substitution). The results are interpreted in terms of structural and stereoelectronic effects and are supported by AM1 calculations. The stability of the transient carbocations appears to play the major role in reactions leading to the abnormal Beckmann rearrangement products. The reported rearrangement is also of synthetic value. A novel geminal alkylation of alicyclic ketones based upon spiro-annelation procedure is presented. The sequence: ketone → methylene compound → α-chlorocyclobutanone oxime → geminal dichloromethyl–cyanomethyl derivative is a variant of ketones geminal alkylation protocol.[39] Moreover, for non-symmetrical alkenes, regio- and stereoselective cycloaddition of dichloroketen leading to the α,α-dichlorocyclobutanone followed by Beckmann rearrangement of the respective α-chlorocyclobutanone oxime presents a new method for vicinal functionalization[40,41] enabling attachment of the CN group to the less substituted carbon atom of the olefin and of the $CHCl_2$ group to the more substituted one.

The two further examples of mono-dehalogenation of the rigid α,α-dichlorocyclobutanones **13** and **21** confirm, what appears now to be a general rule, that in reduction of α,α-dichlorocyclobutanones carried out under mild conditions, the pseudo-axial α-chlorine atom is selectively removed.

4. Experimental

Mp values were determined on a Kofler hot-stage apparatus and are uncorrected. IR Spectra were determinated with a FT-IR Bruker FS 113V spectrophotometer for solutions in chloroform or as KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded with a Varian Gemini 300 VT spectrometer (300 and 75.5 MHz, respectively) operating in the Fourier transform mode using solutions in deuteriochloroform. Chemical shifts (δ) are expressed in ppm relative to tetramethylsilane as the internal standard. The DEPT technique was used for the assignment of multiplicity of carbon signals in ^{13}C NMR spectra. The additivity rules and comparison with data reported for compounds of similar structure were helpful for signal assignment. Electron impact (ionization energy of 70 eV) and FAB mass spectra were recorded with an AMD 402 or AMD 604 spectrometer. CD spectra were recorded with a JASCO J-810 spectropolarymeter for solutions in acetonitrile. Solvents were dried and distilled according to the standard procedures. Reactions progress and purity of compounds was monitored by TLC using precoated aluminum-backed silica plates (Merck, no. 5554). Silica gel 60 (Merck 70–230 mesh, no. 7734) was used for flash chromatography and silica gel (Merck, no. 13895) for preparative TLC separation.

4.1. Synthesis of oximes 2, 7 and 8. General procedure

To a solution of cyclobutanone **1**,[22] **5**[23,28] or **6**[23,28] (0.2 mmol), dissolved in anhydrous pyridine (2 mL), hydroxylamine hydrochloride (100 mg, 1.44 mmol) was added. After stirring at room temperature for 1 h, the reaction mixture was poured into water and extracted with ether. The organic layer was washed with water, dried with magnesium sulfate, and the solvent was removed in vacuo. The crude product was dried at reduced pressure to give chromatographically pure oxime, which was crystallized.

4.1.1. (3*R*)-2′ξ-Chloro-3′-(hydroxyimino)spiro[5α-cholestane-3,1′-cyclobutane] (2). (95% Yield) oil. R_f=0.42 (PhH/AcOEt, 10:1). IR ($CHCl_3$): ν_{max} 3576, 3302, 2931, 2868, 2853, 1467, 1445, 1383, 1365, 1221, 1216, 914, 763, 715 cm^{-1}. δ_H 0.64 (s, 3H, 18-C*H*$_3$), 0.806 and 0.801 (s, 3H, 19-C*H*$_3$), 0.85 (d, J=1.2 Hz, 3H, 26-C*H*$_3$), 0.87 (d, J=1.5 Hz, 3H, 27-C*H*$_3$), 0.90 (d, J=6.6 Hz, 3H, 21-C*H*$_3$), 2.35–2.53 (m, 1H, C*H*$_2$CN), 2.66–2.76 (m, 1H, C*H*$_2$CN), 4.56–4.62 (m, 1H, C*H*Cl), 7.47 (br s, 1H, O*H*). The 2:1 ratio of isomers was established from the integration of two pairs of signals at δ 4.60 and 4.56 and at δ 0.806 and 0.801. δ_C 11.7, 12.1, 18.7, 22.6, 22.9, 23.9, 24.2, 28.0, 28.3, 28.7, 28.9, 31.9, 35.1, 35.5, 35.8, 36.2, 39.5, 39.9, 40.0, 40.6, 40.8, 42.6, 42.8, 54.2, 54.3, 56.2, 56.4, 62.8, 63.0 (*C*–Cl), 155.6(*C*=NOH). MS (EI): *m*/*z* (%) 475 (88) [M$^+$], 440 (98), 368 (40), 320 (47), 304 (37), 284 (31), 43 (100). HRMS: calcd for $C_{30}H_{50}Cl(35)NO$: 475.3580; found 475.3531, calcd for $C_{30}H_{50}Cl(37)NO$: 477.3551; found 477.3502.

4.1.2. 3ξ-[3′β-*tert*-Butyldimethylsilyloxy-androst-5′-en-17′β-yl]-1-(hydroxyimino)-cyclobutane (7). (96% Yield), mp 192–196 °C (Et_2O). R_f=0.60 (PhH/AcOEt, 2:1). IR ($CHCl_3$): ν_{max} 3586, 3295, 3019, 2936, 2904, 2856, 1471, 1379, 1255, 1215, 1209, 1092, 887, 870, 837 cm^{-1}. δ_H 0.06 [s, 6H, Si(C*H*$_3$)$_2$], 0.64 (s, 3H, 18-C*H*$_3$), 0.89 [s, 9H, (C*H*$_3$)$_3$], 1.00 (s, 3H, 19-C*H*$_3$), 2.46–2.66 (m, 2H, C*H*$_2$CN), 2.89–3.10 (m, 2H, C*H*$_2$CN), 3.48 (m, 1H, 3α-H), 5.32 (d, J=4.9 Hz, 1H, 6-H). δ_C 18.3, 19.5, 20.8, 24.4, 26.0, 26.2, 29.9, 31.8, 32.0, 35.6, 35.8, 36.6, 37.00, 37.4, 38.9, 42.5 (*C*H$_2$CN), 42.8 (*C*H$_2$CN), 50.2, 56.1, 56.6, 72.5, 123.7 (C-6), 141.4 (C-5), 157.8 (*C*=NOH). MS (EI): *m*/*z* (%) 471 (2) [M$^+$], 414 (100), 398 (19), 357 (4), 75 (21). $C_{29}H_{49}NO_2Si$ (471.80): calcd for C 73.83, H 10.47, N 2.97; found C 73.52, H 10.65, N 2.83.

4.1.3. 3ξ-[3′β-Acetoxyandrost-5′-en-17′β-yl]-2ξ-chloro-1-(hydroxyimino)-cyclobutane (8). (91% Yield), mp 195–205 °C (benzene). R_f=0.52 (PhH/AcOEt, 10:1). IR ($CHCl_3$): ν_{max} 3577, 3307, 2947, 2870, 1725, 1467, 1376, 1365, 1254, 1029, 777 cm^{-1}. δ_H 0.67 and 0.75 (two s, integrating to 3H, 18-C*H*$_3$), 1.020 and 1.028 (s, 3H, 19-C*H*$_3$), 2.03 (s, 3H, C*H*$_3$CO), 2.40–2.58 (m, 1H, C*H*$_2$CN), 3.13–3.25 (m, 1H, C*H*$_2$CN), 4.51–4.55 (m, 1H, C*H*Cl), 4.58 (m, 1H, 3α-H), 5.38 (d, J=4.6 Hz, 1H, 6-H), 7.90 (br s, 1H, O*H*). δ_C 13.0, 19.4, 20.8, 21.5, 24.7, 25.7, 27.8, 31.8, 31.9, 32.6, 36.7, 37.1, 38.1, 38.6, 42.8, 44.0, 50.1, 55.6, 55.9, 58.0, 73.9 (*C*–Cl), 122.3 (C-6), 139.5 (C-5), 156.2 (*C*=NOH), 170.4 (CH$_3$*C*OO). MS (FAB): *m*/*z* (%) 434 (62) [M$^+$+H], 391 (41), 374 (55), 149 (100). HRMS: calcd for $C_{25}H_{37}NO_3Cl(35)$ [M$^+$+H]: 434.2461; found

434.2451, calcd for $C_{25}H_{37}NO_3Cl$(37) [$M^+ + H$]: 436.2432; found 436.2423.

4.2. Rearrangemat of oxime 2

To a solution of oxime **2** (140 mg, 0.28 mmol) dissolved in anhydrous benzene (4 mL) and pyridine (132 μL, 1.64 mmol) thionyl chloride (60 μL, 0.82 mmol) was added. The mixture was stirred at room temperature for 15 min, poured into ice-water, and extracted with ether. The organic layer was washed with aqueous sodium carbonate solution, water, dried with magnesium sulfate, and the solvent was removed in vacuo. The crude product consisting mainly of **3** and **4** in approx. 2:1 ratio (estimated from ^{1}H NMR spectrum) was chromatographed on silica gel with benzene–ethyl acetate as eluent to give compounds **3** (88.5 mg, 60% yield) and **4** (22.5 mg, 16% yield).

4.2.1. 3β-Cyanomethyl-3α-dichloromethyl-5α-cholestane (3). Mp 173–175 °C (methanol). R_f=0.52 (PhH/hexane, 2:1). IR ($CHCl_3$): ν_{max} 2936, 2867, 2248 (CN), 1467, 1383, 1207, 908, 777, 680 cm^{-1}. δ_H 0.65 (s, 3H, 18-CH_3), 0.85 (s, 3H, 19-CH_3), 0.87 (d), 0.89 (d) and 0.91 (d) (methyl groups), 2.70 (s, 2H, CH_2CN), 6.25 (s, 1H, CHCl$_2$). δ_C 11.9, 12.0, 18.6, 20.9, 22.5, 22.8, 23.8, 24.1, 24.6, 27.7, 27.9, 28.2, 31.7, 34.3, 35.3, 35.7, 36.1, 39.4, 39.8, 40.9, 42.5, 44.4, 54.3, 56.2, 56.4, 77.9 (*C*Hal$_2$), 117.7 (*C*N). MS (EI): *m*/*z* (%) 493 (47) [M^+], 478 (31), 422 (10), 340 (83), 338 (100), 270 (22). HRMS: calcd for $C_{30}H_{49}Cl_2N$: 493.3242; found 493.3273.

4.2.2. 4′ξ-Chloro-(3*R*)-spiro[5α-cholestane-3,3′-pyrrolidin]-5′-one (4). Oil. R_f=0.27 (AcOEt). IR ($CHCl_3$): ν_{max} 3264, 2931, 2866, 1708, 1467, 1444, 1383, 1096, 723 cm^{-1}. δ_H 0.65 (s, 3H, 18-CH_3), 0.85 (s, 3H, 19-CH_3), 0.87 (d), 0.89 (d) and 0.91 (d) (methyl groups), 3.31–3.41 (m, 1H, CH_2N), 3.58–3.65 (m, 1H, CH_2N), 4.59 and 4.63 (two s, 1H, CHCl), 6.59 (br s, 1H, NH). δ_C 51.5 (*C*H–Cl), 170.1 (*C*=O). MS (EI): *m*/*z* (%) 475 (5) [M^+], 439 (32), 301 (43), 284 (63), 149 (42), 95 (100). HRMS: calcd for $C_{30}H_{49}NO$ [M^+ −HCl]: 439.3814; found 439.3863.

4.3. 4ξ [(3′β-*tert*-Butyldimethylsilyloxy)-androst-5′-en-17′β-yl]-3-pyrrolidin-2-one (9)

To a solution of oxime **7** (80 mg, 0.17 mmol) dissolved in anhydrous benzene (4 mL) thionyl chloride (30 μL, 0.41 mmol) was added and the reaction was carried out in a manner similar to that described above. The usual workup gave the crude product, which was chromatographed on silica gel (benzene/AcOEt, 5:1) to give lactam **9** (58.7 mg, 71% yield), two diastereoisomers in the approx. 1:1 ratio estimated from the integration of the signals at δ 0.69 and 0.68 in the ^{1}H NMR spectrum, mp 260–265 °C (AcOEt). R_f=0.25 (AcOEt). IR ($CHCl_3$): ν_{max} 3441, 3230, 2948, 2856, 1690, 1255, 1092, 887, 869, 836 cm^{-1}. δ_H 0.06 [s, 6H, Si(CH_3)$_2$], 0.68 and 0.69 (two s, 3H, 18-CH_3), 0.89 [s, 9H, C(CH_3)$_3$], 1.00 (s, 3H, 19-CH_3), 2.97–3.14 (m, 1H, CH_2N), 3.11 and 3.00 (two t, 1H, J=9.0 Hz, CH$_A$H$_B$N), 3.36–3.53 (m, 2H, CH$_A$H$_B$N and 3α-H), 5.32 (br d, J=5.0 Hz, 1H, 6-H), 6.02 (br s, 1H, NH). δ_C −4.42, −4.48, 12.6, 19.5, 20.9, 24.5, 26.0, 26.6, 27.5, 31.8, 32.1, 36.6, 37.4, 37.9 and 38.0, 38.8, 42.4 and 42.5, 42.8, 47.6, 47.9, 50.2, 55.3, 56.0 and 56.1, 72.5 (C-3), 120.7 and 120.8 (C-6), 141.3 and 141.4 (C-5), 178.0 and 178.6 (C-23). MS (EI): *m*/*z* (%) 471 (1) [M^+], 414 (100), 338 (3), 75 (14). $C_{29}H_{49}NO_2Si$ (471.80): calcd for C 73.83, H 10.47, N 2.97; found C 73.65, H 10.38, N 2.82.

4.4. Rearrangement of oxime 8

To a solution of oxime **8** (100 mg, 0.23 mmol) in anhydrous benzene (3 mL) and pyridine (66.3 μL, 0.82 mmol), thionyl chloride (30 μL, 0.41 mmol) was added. The reaction mixture was stirred at room temperature for 30 min under argon atmosphere, then poured into ice-water. The usual workup gave a mixture, which was separated by column chromatography (benzene/hexane, benzene, benzene/AcOEt) and preparative TLC on silica gel (benzene) to give compound **12**[29] (14.2 mg, 14% yield) and nitrile **11** (a mixture of two isomers, 47.3 mg, 45% yield). Preparative TLC separation on silica gel (benzene) of the mixture of nitriles gave the pure major nitrile **11A**.

4.4.1. 3β-Acetoxy-21,21-dichloro-20ξ-24-nor-chol-5-en-23-oic acid nitrile (11). (Major isomer A): mp 184–186 °C (methanol). R_f=0.57 (PhH/AcOEt, 5:1). δ_H 0.78 (s, 3H, 18-CH_3), 1.02 (s, 3H, 19-CH_3), 2.03 (s, 3H, CH_3CO$_2$), 2.81 (d, J=4.3 Hz, 2H, 22-H), 4.60 (m, 1H, 3α-H), 5.38 (br d, J=5.2 Hz, 1H, 6-H), 5.96 (d, J=2.1 Hz, 1H, 21-H). δ_C 12.7, 16.6, 19.3, 21.0, 21.5, 23.9, 26.4, 27.7, 31.6, 32.0, 36.5, 36.9, 38.1, 38.8, 47.8, 49.7, 51.8, 55.7, 73.8 (C-3), 76.1 (C-21), 118.7 (C-23), 122.0 (C-6), 139.6 (C-5), 170.3 (CH$_3$*C*OO); (minor isomer B): δ_H 0.75 (s, 3H, 18-CH_3), 1.02 (s, 3H, 19-CH_3), 2.03 (s, 3H, CH_3CO$_2$), 2.72 (m, 2H, 22-H), 4.60 (m, 1H, 3α-H), 5.38 (br d, J=5.2 Hz, 1H, 6-H), 6.14 (d, J=2.1 Hz, 1H, 21-H); (mixture of isomer A and B). IR ($CHCl_3$): ν_{max} 2942, 2850, 2243, 1729, 1436, 1374, 1248, 1034 cm^{-1}. MS (EI): *m*/*z* (%) 391 (100) [M^+ −AcOH], 376 (22), 355 (40), 340 (17), 320 (13), 283 (10), 213 (20), 145 (27), 121 (35). HRMS: calcd for $C_{23}H_{31}Cl_2N$ [M^+ −AcOH]: 391.1833; found 391.1837.

4.4.2. 3β-Acetoxy-17β-[3′-pyrrolin-2′-one-4′-yl]-androst-5-ene (12). Mp 218–220 °C (methanol); lit.[29] mp 255–260 °C (sublim. 220 °C). R_f=0.37 (AcOEt). IR ($CHCl_3$): ν_{max}=3632, 3460, 1725, 1685, 1255, 1017, 788 cm^{-1} (in accordance with data in lit.[29]). δ_H 0.61 (s, 3H, 18-CH_3), 1.02 (s, 3H, 19-CH_3), 3.88 (d, J=19.2 Hz, 1H, 21-H), 4.04 (d, J=19.2 Hz, 1H, 21-H), 4.60 (m, 1H, 3α-H), 5.38 (br d, J=5.2 Hz, 6-H), 5.87 (s, 1H, 22-H), 6.70 (br s, 1H, NH), (signals of protons 21-H and 22-H in accordance with data in lit.[30] for 3β-methoxy-derivative of compound **12**). δ_C 12.9 (C-18), 19.2 (C-19), 20.7 (C-11), 21.3 (*C*H$_3$CO$_2$), 24.2 (C-15), 27.6 (C-2), 25.9 (C-16), 31.6 (C-7), 32.0 (C-8), 36.5 (C-10), 36.9 (C-1), 37.9 (C-4), 38.0 (C-12), 44.0 (C-13), 49.9 (C-9), 51.1 (C-21), 51.5 (C-17), 56.4 (C-14), 73.8 (C-3), 122.1 (C-22), 122.3 (C-6), 139.8 (C-5), 164.1 (C-20), 170.7 (CH$_3$*C*O$_2$), 175.9 (C-23), (signals of C-21, C-22 and C-23 in accordance with data in lit.[30] for 3β-methoxy-derivative of compound **12**). MS (EI): *m*/*z* (%) 397 (1) [M^+], 337 (100), 322 (55), 213 (11), 110 (31). HRMS: calcd for $C_{25}H_{36}NO_3$ [$M^+ + H$]: 398.2695; found 398.2676.

4.5. 4′α-Chloro-2β,3β-dihydrocyclobuta[2,3]-5α-cholestan-3′(4′H)-one (14)

Dichlorocyclobutanone **13** was prepared in the reaction of cholest-2-ene[32,42] (754 mg), CCl_3COCl (1 mL) and activated Zn (1.227 g) in Et_2O (33 mL) solution under sonification conditions. To the crude reaction product (612 mg, 1.27 mmol) dissolved in AcOH (29 mL) activated Zn (238 mg, 3.30 mmol) was added and the mixture was stirred at room temperature. After 0.5 h, an additional portion of zinc (50 mg) was added and the reaction was finished after 75 min (disappearance of the substrate on TLC). The usual workup [C_6H_6/NaCl/NaOH(0.1N)/NaCl(-dil)/$MgSO_4$] afforded the crude cyclobutanone **14** (0.445 g, 78% yield), which had mp 162–164 °C (Me_2CO). R_f=0.3 (hexane/AcOEt, 10:1). CD (CH_3CN): $\Delta\varepsilon$=0.0. IR ($CHCl_3$): ν_{max} 2948, 2933, 2868, 1787, 1467, 1446, 1368, 666 cm^{-1}. δ_H 0.65 (s, 3H, 18-CH_3), 0.75 (s, 3H, 19-CH_3), 0.85 (d), 0.87 (d) and 0.90 (d) (methyl groups), 2.79 (m, 1H, 2-H), 3.27 (t, J=8.8 Hz, 1H, 3-H), 5.00, (dd, J=8.7, 2.2 Hz, 1H, C*H*Cl). δ_C 11.0, 12.1, 18.7, 20.9, 22.6, 22.9, 23.8, 24.2, 24.8, 27.4, 28.0, 28.2, 28.8, 31.4, 34.6, 35.5, 35.7, 35.8, 36.1, 39.3, 39.5, 41.2, 42.3, 51.1, 52.9, 56.1, 56.3, 64.0, 77.4 (*C*Hal), 200.7 (*C*O). MS (EI): *m/z* (%) 446 (14) [M^+], 418 (18), 383 (10) 32.9 (20), 305 (10), 291 (17), 263 (21), 215 (11), 189 (12), 119 (26), 107 (41), 95 (62), 81 (60), 55 (100). HRMS: calcd for $C_{28}H_{47}ClO$: 446.3315; found 446.3271.

4.6. 4′α-Chloro-3′-hydroxyimino(4′H)-2β,3β-dihydrocyclobuta[2,3]-5α-cholestane (16)

To a solution of ketone **14** (138 mg, 0.31 mmol) in pyridine (2.5 mL) hydroxylamine hydrochloride (180 mg, 2.59 mmol) was added and the mixture was stirred at room temperature for 5.5 h. The workup [C_6H_6/H_2O/Hal(5%)/Na_2CO_3(5%)NaCl(dil)/$MgSO_4$] afforded a crude product (120 mg, two isomers in the ratio of 4.5:1). The mixture was separated on a preparative TLC plate (C_6H_6/AcOEt, 15:1) to afford pure oxime *anti*-**16**.

Oxime 16. Isomer *anti*-**16**: mp 189–195 °C ($CHCl_3$/MeOH, 2:1). R_f=0.3 (PhH/AcOEt, 15:1). IR (KBr): ν_{max} 3308, 2942, 2867, 2850, 1467, 1443, 1364, 1367, 958, 935, 765 cm^{-1}. δ_H 0.65 (s, 3H, 18-CH_3), 0.71 (s, 3H, 19-CH_3), 0.85 (d), 0.87 (d) and 0.90 (d) (methyl groups), 2.62 (m, 1H, 2β-H), 3.16 (t, J=7.9 Hz, 1H, 3β-H), 4.84 (d, J=7.7 Hz, 1H, C*H*Cl), 6.97 (s, 1H, O*H*). δ_C 10.9, 12.0, 18.6, 20.8, 22.5, 22.7, 23.8, 24.2, 26.7, 27.9, 28.1, 28.7, 31.41, 31.6, 34.4, 35.5, 35.7, 36.1, 39.4, 39.9, 40.7, 41.9, 42.3, 53.0 (*C*–Cl), 55.3, 56.1, 56.4, 158.3 (*C*=N). MS (EI): *m/z* (%) 461 (27) [M^+], 446 (28), 306 (66). HRMS: calcd for $C_{29}H_{48}ClNO$: 461.3424; found 461.3407, calcd for $C_{29}H_{48}NOCl$(37): 463.3395; found 463.3367. Isomer *syn*-**16**. δ_H 2.98 (t, J=7.9 Hz, 1H, 3β-H), 5.03 (d, J=7.7 Hz, 1H, C*H*Cl), 7.12 (s, 1H, O*H*).

4.7. Rearrangement of oxime *anti*-16

To the solution of oxime *anti*-**16** (96.5 mg, 0.20 mmol) in anhydrous benzene (8 mL) and pyridine (84.1 μL, 1.04 mmol) stirred at room temperature under argon, $SOCl_2$ (38.2 μL, 0.52 mmol) was added and the reaction mixture was stirred for 1.5 h. Then ice-water (4 mL) was added and the mixture stirred for further 15 min. The usual workup [C_6H_6 and Et_2O (1:1)/$NaHCO_3$ (0.1 N)/NaCl (5%)/$MgSO_4$] gave a crude product (110 mg). This was treated with hexane (4 mL). The solid was filtered off to give lactam **17** (7 mg, 7% yield). The fraction soluble in hexane was separated on an SiO_2 column (hexane–benzene) and preparative TLC (hexane/benzene, 2:1, three times) to give nitrile **18** (44.2 mg, 46% yield) and nitrile **19** (3.6 mg, 4% yield).

4.7.1. 3′-Chloro-2′-oxopyrrolidino[4′,5′:2,3](2*R*,3*S*)-5α-cholestane (17). Mp 260–265 °C (hexane). R_f=0.18 (AcOEt). IR (KBr): ν_{max} 3267 (N–H), 2946, 2866, 1712 (C=O), 1672, 1445, 1382, 1245, 755 cm^{-1}. δ_H 0.65 (s, 3H, 18-CH_3), 0.78 (s, 3H, 19-CH_3), 0.85 (d), 0.87 (d) and 0.90 (d) (methyl groups), 2.69 (m, 1H, 2β-H), 3.77 (t, 1H, 3β-H), 4.63 (d, J=6.6 Hz, 1H, C*H*Cl), 5.46 (s, 1H, N*H*). δ_C 11.5, 12.1, 14.2, 18.7, 20.8, 20.9, 22.6, 22.9, 23.9, 24.2, 28.0, 28.2, 29.7, 34.7, 35.3, 35.8, 36.2, 38.0, 38.5, 39.5, 39.9, 49.6, 50.4, 53.7, 56.1, 56.4, 61.9, 178.6. MS (EI): *m/z* (%) 461 (34) [M^+], 308 (53), 306 (100) [M^+-155], 270 (12), 254 (17), 199 (12), 164 (12), 120 (15), 106 (20), 94 (34), 80 (27), 69 (20), 56 (32), 55 (38). HRMS: calcd for $C_{29}H_{48}ClNO$: 461.3424; found 461.3420, calcd for $C_{29}H_{48}NOCl$(37): 463.3395; found 463.3376.

4.7.2. 2α-(*R*)-Chlorocyanomethyl-3α-chloro-5α-cholestane (18). Mp 133–136 °C (hexane). R_f=0.35 (hexane/PhH, 2:1, developed twice). IR (KBr): ν_{max} 2963, 2931, 2868, 2237 (C≡N), 1467, 1447, 1388, 1254, 766 cm^{-1}. δ_H 0.65 (s, 3H, 18-CH_3), 0.82 (s, 3H, 19-CH_3), 0.85 (d), 0.87 (d) and 0.90 (d) (methyl groups), 2.21 (d, J=2.8 Hz, 1H, 2β-H), 3.40 (t, J=2.2 Hz, 1H, 3β-H), 5.61 (d, J=9.6 Hz, 1H, C*H*ClCN). δ_C 12.0, 12.7, 18.6, 20.8, 22.5, 22.8, 23.8, 24.13, 27.4, 27.9, 28.1, 30.7, 31.2, 31.4, 35.7, 35.2, 36.1, 36.3, 38.5, 39.4, 39.7, 42.0, 42.5, 45.8, 53.7, 56.1, 56.2, 75.8 (*C*Cl–CN), 119.1 (*C*≡N). MS (EI): *m/z* (%) 479 (36) [M^+], 443 (2) [M^+-HCl], 325 (80), 323 (100), 255 (7), 123 (13), 95 (23), 55 (40). HRMS: calcd for $C_{29}H_{47}Cl_2N$: 479.3085; found 479.3101.

4.7.3. 2α-Dichloromethyl-3α-cyano-5α-cholestane (19). Oil. R_f=0.25 (hexane/PhH, 2:1, developed twice). IR (KBr): ν_{max} 2928, 2866, 2853, 2236, 1787, 1736, 1731, 1467, 1383, 787 cm^{-1}. δ_H 0.65 (s, 3H, 18-CH_3), 0.69 (s, 3H, 19-CH_3), 0.86 (d), 0.88 (d) and 0.90 (d) (methyl groups), 2.96 (d, J=12.9 Hz, 1H, 3β-H), 3.46 (br s, 1H, 2β-H), 6.13 (d, J=1.7 Hz, 1H, C*H*Cl_2). MS (EI): *m/z* (%) 479 (13) [M^+], 443 (76) [M^+-HCl], 330 (35), 326 (23), 324 (30), 290 (64), 289 (80), 288 (100), 175 (23), 121 (21), 107 (27), 95 (48), 71 (27), 57 (61), 55 (68). HRMS: calcd for $C_{29}H_{47}Cl_2N$: 479.3085; found 479.3061.

4.8. 3′,3′-Dichloro-3β-methyl-2β,3β-dihydrocyclobuta-[2,3]-5α-cholestan-4′ (3′H)-one (21)

To the solution of 3-methyl-5α-cholest-2-ene[32] (1.883 g, 4.9 mmol) in Et_2O (58 mL) activated Zn (1.081 g) was added. The reaction mixture was sonificated and a solution of trichloroacetyl chloride (1.9 mL) in Et_2O (30 mL) was slowly added dropwise. After 5 h the usual workup [C_6H_6/NaOH (5%)/NaCl(5%)/$MgSO_4$] and evaporation of the solvent gave a crude product, which was chromatographed

on an SiO_2 column (hexane) to give pure dichlorocyclobutanone **21** (1.379 g, 57% yield), mp 119–122 °C (ethyl formate/methanol, 3:1). R_f=0.47 (hexane/CH_2Cl_2, 2:1). CD (CH_3CN): $\Delta\varepsilon$=−1.3 (326 nm). δ_H 0.64 (s, 3H, 18-CH_3), 0.70 (s, 3H, 19-CH_3), 0.85 (d), 0.87 (d) and 0.89 (d) (methyl groups), 1.40 (s, 3H, 3β-CH_3), 3.05 (t, J=10.1 Hz, 1H, 2β-H) (in agreement with lit.[7]). δ_C 12.0, 12.1, 18.7, 21.1, 22.6, 22.9, 23.9, 24.2, 28.0, 28.2, 29.3, 29.9, 31.5, 34.6, 34.7, 35.8, 35.9, 36.2, 36.7, 39.5, 39.8, 41.5, 42.2, 42.4, 53.2, 56.2, 56.3, 59.1, 92.9 (*C*Cl_2), 199.4 (*C*=O).

4.9. 3′β-Chloro-3β-methyl-2β,3β-dihydrocyclobuta-[2,3]-5α-cholestan-4′(3′H)-one (22)

To the solution of dichlorocyclobutanone **21** (519 mg, 1.034 mmol) in AcOH (50 mL) activated Zn (307 mg) was added portionwise and the reaction mixture was stirred at room temperature for 2 h. The workup [C_6H_6/$NaHCO_3$(5%)/NaCl(5%)/$MgSO_4$] gave a crude product (428 mg), which was chromatographed on an SiO_2 column (hexane, hexane–benzene mixture) to give compound **22** (335 mg, 70% yield): mp 189–191 °C (HCO_2Et/CH_3OH, 3:1). R_f=0.30 (hexane/CH_2Cl_2, 2:1). CD (CH_3CN): $\Delta\varepsilon$=−5.65 (301 nm). IR ($CDCl_3$): ν_{max} 2933, 1787 (C=O), 1457, 1385, 863 cm^{-1}. δ_H 0.64 (s, 3H, 18-CH_3), 0.70 (s, 3H, 19-CH_3), 0.85 (d), 0.87 (d) and 0.89 (d) (methyl groups), 1.15 (s, 3H, 3-CH_3), 2.72 (dt, J=9.7, 2.0 Hz, 1H, 2β-H), 5.11 (d, J=2.2 Hz, 1H, C*H*Cl, minor isomer, less than 5%), 5.13 (d, J=2.4 Hz, 1H, C*H*Cl). δ_C 10.5, 11.9, 18.6, 21.0, 22.5, 22.7, 23.7, 24.1, 24.9, 27.9, 28.1, 28.8, 31.6, 33.0, 34.6, 35.2, 35.3, 35.7, 36.1, 39.4, 39.8, 40.8, 42.3, 56.2, 56.4, 56.5, 66.0 (*C*–Cl), 202.1 (*C*=O). MS (EI): *m*/*z* (%) 460 (29) [M^+], 425 (100), 384 (74), 316 (53), 161 (42), 121 (37), 109 (57), 107 (54), 95 (75), 91 (35), 79 (43), 69 (46), 55 (94). $C_{30}H_{49}ClO$ (460.34) calcd for C 78.13, H 10.71; found C 78.17, H 10.99.

4.10. 3′β-Chloro-3β-methyl-4′-hydroxyimino(3′H)-2β,3β-dihydrocyclobuta[2,3]-5α-cholestane (20)

To a solution of the ketone **22** (117 mg, 0.25 mmol) in pyridine (5 mL), hydroxylamine hydrochloride (106 mg, 6 equiv) was added and the mixture was stirred at room temperature for 1 h. Additional portion of the amine (100 mg) was added after 2.5 h and the mixture was stirred for an additional 1 h. The workup [C_6H_6/H_2O/Hal(5%)/NaHCO(5%)/NaCl (5%)/$MgSO_4$] gave a crude product (105 mg, 87% yield). This was a 5:1 mixture of isomer *anti*-**20** and *syn*-**20**. The mixture was separated on preparative TLC plates (C_6H_6-AcOEt, 20:1) to give the pure isomer *anti*-**20**.

Oxime 20. Mp 232–234 °C (*n*-heptane). R_f=0.47 (PhH/AcOEt, 20:1). IR ($CHCl_3$): ν_{max} 3578, 3306, 2953, 2868, 1467, 1382, 1366, 788 cm^{-1}. Isomer *anti*-**20**: δ_H 0.65 (s, 3H, 18-CH_3), 0.69 (s, 3H, 19-CH_3), 0.85 (d), 0.87 (d) and 0.90 (d) (methyl groups), 1.17 (s, 3H, 3-CH_3), 2.28 (q, J=4.8, 9.3 Hz, 1H), 2.94 (dt, J=2.4, 9.9 Hz, 1H, 2β-H), 4.99 (d, J=2.5 Hz, 1H, C*H*Cl), 7.08 (br s, 1H, O*H*). δ_C 10.7, 12.1, 18.7, 21.1, 22.6, 22.9, 23.9, 24.3, 25.2, 28.0, 28.2, 29.2, 31.7, 35.2, 35.6, 35.7, 35.8, 36.2, 37.5, 37.8, 39.5, 39.9, 41.1, 42.4, 43.4, 53.2, 56.2, 56.5, 58.7 (*C*Hal), 160.6 (*C*=N). MS (EI): *m*/*z* (%) 475 (10), 459 (40), 424 (100), 384 (32), 304 (56), 284 (46), 268 (58), 109 (47), 95 (79), 55 (79). HRMS: calcd for $C_{30}H_{50}ClNO$: 475.3580; found 475.3546. Isomer *syn*-**20**. δ_H 0.65 (s, 3H, 18-CH_3), 0.69 (s, 3H, 19-CH_3), 1.17 (s, 3H, 3-CH_3), 2.61 (dd, J=8.2, 2.4 Hz, 1H, 2β-H), 4.95 (d, J=2.5 Hz, 1H, C*H*Cl), 7.05 (br s, 1H, O*H*).

4.11. Rearrangement of oxime *anti*-20

To the solution of oxime *anti*-**20** (57 mg, 0.12 mmol) in benzene (8 mL) and pyridine (48.5 μL, 0.60 mmol) thionyl chloride (22 μL, 0.30 mmol) was added dropwise and the mixture was stirred at room temperature for 30 min. An additional portion of $SOCl_2$ (20 μL) was added and the reaction continued for 15 min. Ice-water (4 mL) was added and the product was extracted with a C_6H_6–Et_2O (1:1) mixture. The workup [$NaHCO_3$(5%)/NaCl(5%), $MgSO_4$] gave a crude product. The approx. 1:3:1 ratio of compounds **23**, **24** and **25** was estimated from the ^1H NMR spectrum of the mixture. The fraction insoluble in hexane was filtered off to give pure lactam **23** (10 mg, 17% yield). The fraction soluble in hexane (42.6 mg, 74% yield) was separated on a SiO_2 TLC plate (hexane/benzene, 2:1) to give nitriles **24** (30 mg, 52% yield) and **25** (12 mg).

4.11.1. 5′β-Chloro-2′-oxo-pyrrolidino[3′,4′:2,3](2*R*)-3β-methyl-5α-cholestane (23). Oil. R_f=0.5 (AcOEt). IR (KBr): ν_{max} 3233 (NH), 2932, 2868, 2849, 1704, 1457, 1094 cm^{-1}. δ_H 0.64 (s, 3H, 18-CH_3), 0.72 (s, 3H, 19-CH_3), 1.07 (s, 3H, 3β-CH_3), 0.85 (d), 0.87 (d) and 0.90 (d) (methyl groups), 2.04 (br s, 1H, 2β-H), 4.21 (d, J=1.7 Hz, C*H*Cl), 6.37 (br s, 1H, N*H*). MS (EI): *m*/*z* (%) 439 (78) [M^+−HCl], 424 (45), 284 (100), 270 (27), 119 (21), 95 (19), 60 (66). HRMS: calcd for $C_{30}H_{49}NO$ [M^+−HCl]: 439.3814; found 493.3827.

4.11.2. 2α-Cyano-3α-dichloromethyl-3β-methyl-5α-cholestane (24). Mp 148–155 °C (PhH/hexane). R_f=0.32 (hexane/PhH, 2:1, developed twice). IR (KBr): ν_{max} 2929, 2866, 2234 (C≡N), 1466, 1384, 1366, 726, 592 cm^{-1}. δ_H 0.65 (s, 3H, 18-CH_3), 0.85 (s, 3H, 19-CH_3), 0.88 and 0.90 (methyl groups), 1.37 (s, 3H, 3β-CH_3), 2.86 (dd, J=3.3, 13.5 Hz, 1H, 2β-H), 6.22 (s, 1H, C*H*Cl_2). δ_C 12.1, 12.6, 18.7, 21.1, 22.6, 22.9, 23.9, 24.1, 27.9, 28.0, 28.2, 31.6, 34.9, 35.4, 35.8, 36.1, 36.4, 38.6, 38.7, 39.5, 39.7, 40.7, 42.6, 43.6, 54.0, 56.2, 56.3, 76.8 (*C*Hal_2), 127.4 (*C*≡N). MS (EI): *m*/*z* (%) 493 (63) [M^+], 338 (100), 263 (12), 250 (13), 123 (23), 95 (30), 55 (38). HRMS: calcd for $C_{30}H_{49}Cl_2N$: 493.3242; found 493.3208.

4.11.3. 2ξ-Chloro-3α-chlorocyanomethyl-3β-methyl-5α-cholestane (25). Oil. R_f=0.20 (hexane/PhH, 2:1, developed twice). IR (KBr): ν_{max} 2961, 2926, 2851, 2245 and 2230 (C≡N, two isomers), 1467, 1261, 801 cm^{-1}. δ_H 0.63 (s, 3H, 18-CH_3), 0.78 (s, 3H, 19-CH_3), 0.85 (d), 0.87 (d) and 0.89 (d) (methyl groups), 1.43 (s, 3H, 3β-CH_3), 3.45 (d, J=11.8 Hz, 1H, 2α-H_{eq}), 4.69 (q, J=7.1, 9.1 Hz, 1H, 2β-H), 5.41 and 5.60 (two s, 1H, C*H*ClCN). MS (EI): *m*/*z* (%) 457 (80) [M^+−HCl], 422 (32), 316 (19), 302 (100), 268 (18), 95 (30), 57 (44). HRMS: calcd for $C_{30}H_{48}ClN$ [M^+−HCl]: 457.34753; found 457.34956.

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14-Membered cyclodepsipeptides with alternating β-hydroxy and α-amino acids by cyclodimerization

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Abstract—The cyclodimerization (twinning) of β-hydroxy acid amides of type **1** under 'direct amide cyclization' (DAC) conditions is described. Although other coupling methods also gave moderate results, best yields were obtained via DAC, reaching 88% for the cyclodimer **10**. In all cases, when starting with racemic material, only the trans-substituted cyclodepsipeptides were isolated. Simple molecular modeling revealed that the formation of the cyclodimer is thermodynamically slightly more favorable than that of the cyclomonomer. The proposal that cyclodimer formation is preferred because of the presence of intramolecular H-bonds could not be confirmed by X-ray crystallography. The influence of substituents, both in the amino acid and in the hydroxy acid moieties, was also studied. It is shown, that cyclodimerization was successful only when the hydroxy acid moiety is α,α-disubstituted.

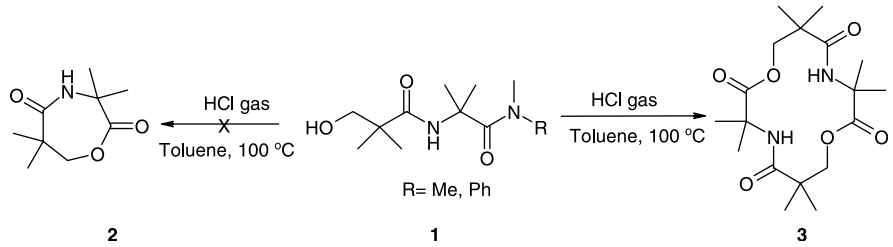
1. Introduction

In a recent paper,[1] we reported that amides **1**, when subjected to the conditions of the 'direct amide cyclization' reaction,[2–9] yielded only the dimerized product **3**. The 14-membered cyclodepsipeptide **3** was isolated as the sole product and none of the expected seven-membered monomer **2** could be detected (Scheme 1).

In order to explain this unexpected result, we investigated some other lactonization methods with derivatives of **1**, including that described by Richard et al.,[10] which, in the case of ethyl 6-hydroxyhexanoates, had resulted in the formation of seven-membered lactones. Again, in all experiments with derivatives of **1**, where a defined product could be isolated, we obtained only the dimeric product **3**.

Cyclic 14-membered depsipeptides with the same ring skeleton as **3** have been known since the 1960s, and the dimerization process is not as surprising as it seems at first. Since the discovery, isolation, and identification of serratamolide (**4**: R=H, R′=$(CH_2)_6CH_3$, Scheme 2) from *Serratia marcescens* in 1961,[11] the proof of its antibiotic activity,[12] and its first total synthesis by Shemyakin et al.,[13] the interest in depsipeptides containing β-hydroxy acids has increased significantly, mainly because of their antibiotic properties. The other important biological role of the same compound is its use as a surface-active agent under the name of serrawettin W1,[14] and it has been patented for use as a pesticide as well.[15]

The synthetic pathways to serratamolide (**4**) and its derivatives are numerous, but the approaches can be divided



Scheme 1.

Keywords: Cyclodepsipeptides; Cyclodimerization; Twinning; 2*H*-Azirin-3-amines; 1,3-Oxazole-5(4*H*)-ones.
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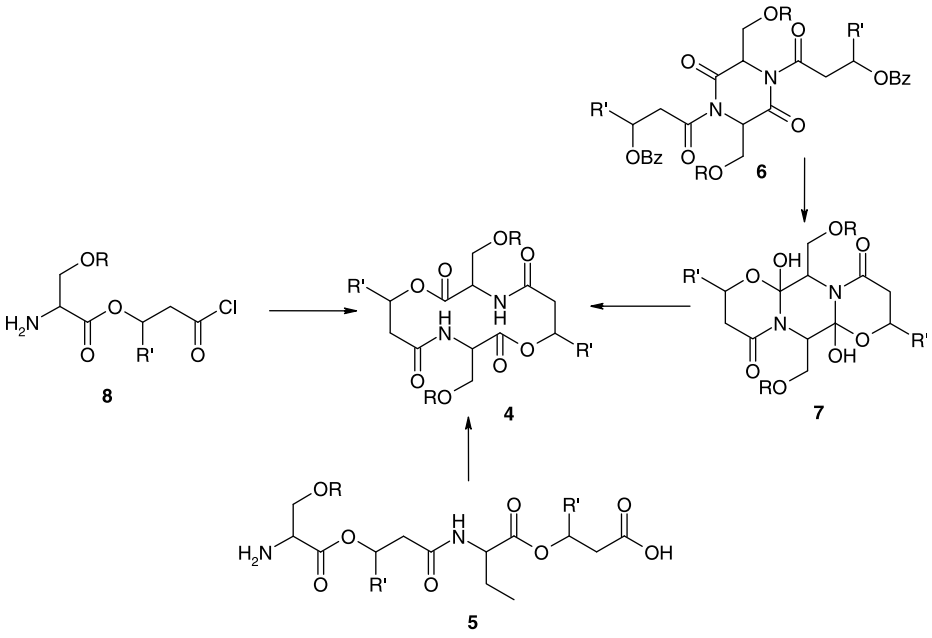


Scheme 2.

into three main groups: classical ring closure of linear precursors, usually by lactam bond formation (**5**→**4**), ring enlargement (**6**→**7**→**4**), and cyclization by twinning (**8**→**4**) (Scheme 2).

The main feature of the last-mentioned method is that the cyclization is performed with the monomeric linear precursor, and the twinning, or cyclodimerization, takes place during the course of the reaction. Some basic theories have been offered as an explanation of this dimerization. One of the most popular proposals states that it is mainly due to intermolecular hydrogen bonding between carbonyl and amine groups that are formed during the reaction.[16,17] In the case of **4**, the two planar *trans*-amide groups form a trans-annular hydrogen bond (intramolecular) and thus favor the formation of a 14-membered ring. That no monomeric product was formed in the reaction can probably be explained by the fact that such an intramolecular interaction would not be possible within a seven-membered ring. However, the contribution of hydrogen bonding tends to be overestimated as some dimerizations also take place in polar solvents, which could interfere with such an intramolecular interaction.[17] Another theory proposed by Ovchinnikov et al.[18] is that the cyclization is preceded by a linear polycondensation, which results in linear dimers and oligomers, which, under the conditions of high dilution, may undergo cyclization. The extent of the ring closure is determined principally by the most stable conformation of a given linear peptide, being close to that of the cyclic product. On the other hand, the formation of trimers seems to be statistically unfavorable.

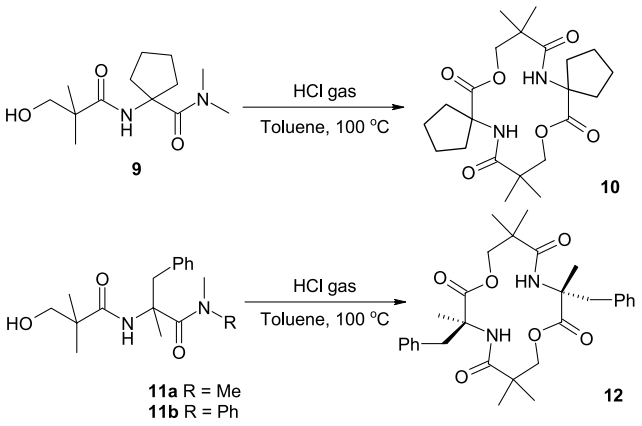
The reason for the preferred dimerization in the case of **1** is most probably a defined conformation of the linear precursor and the stability of the transition state. Another reason could be the rigidity of the amide bond in **1**, although the reactions with an ester analogue of compound **1** showed that again oligomerization occurred to give tri- and tetrameric structures of the β-hydroxy acid.[1] Therefore, most probably the dimerization is not a result of the amide bond rigidity.

In order to further investigate the cyclodimerization of dipeptide analogues of type **1** under the conditions of the 'direct amide cyclization' we synthesized some other amide precursors, which differ in the substitution in the α-amino acid as well as in the β-hydroxy acid moiety, including chiral compounds. Furthermore, we tried to get some information from computer modeling of key compounds.

2. Results and discussion

It is known from previous experiments[5–7,19,20] that dipeptides of type **1**, in which the two methyl groups in the amino acid residue are replaced by a cyclopentane ring (i.e., the compounds contain 1-aminocyclopentane carboxylic acid instead of 1-aminoisobutyric acid (Aib)), react in a very similar way to **1**. Therefore, our first target was amide **9**, which was conveniently prepared by the coupling of 3-hydroxy-2,2-dimethylpropanoic acid with *N*,*N*-dimethyl-1-azaspiro[2.4]hept-1-en-2-amine in analogy to Ref. 1 ('azirine/oxazolone method'; see also Ref. 21). After bubbling HCl gas through a solution of **9** in toluene (20 mM) at 100 °C for 4 min, 88% of the corresponding 14-membered cyclodepsipeptide **10** was obtained (Scheme 3). Similarly, the linear racemic dipeptides **11a**,**b**[22] carrying two different substituents on the C(α) atom, were also synthesized by the 'azirine/oxazolone method'. 'Direct amide cyclization' of **11b** yielded, upon cyclization, 68% of the dimeric *meso*-compound **12** as the sole product.

This was clearly indicated by the ^{1}H and ^{13}C NM spectra, which show only one set of signals. The structures of **10** and **12** were established by X-ray crystallography (Fig. 1).



Scheme 3.

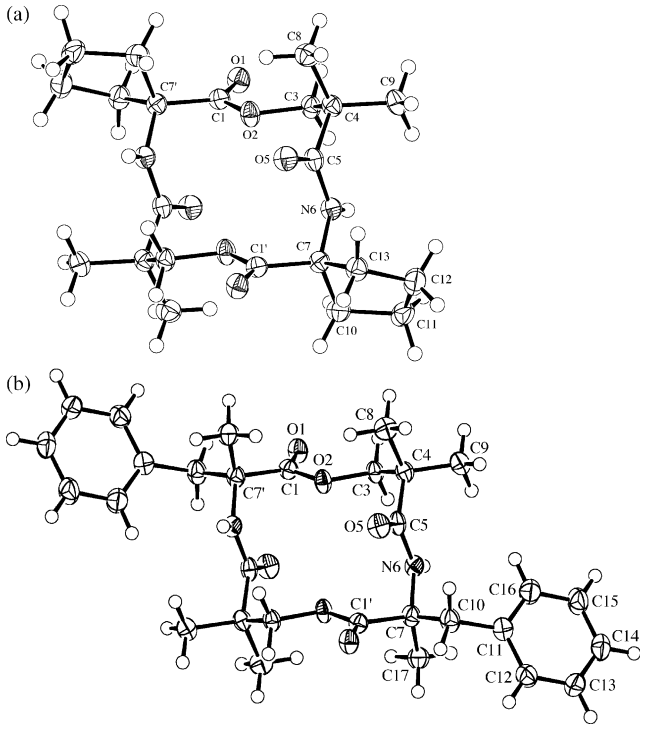
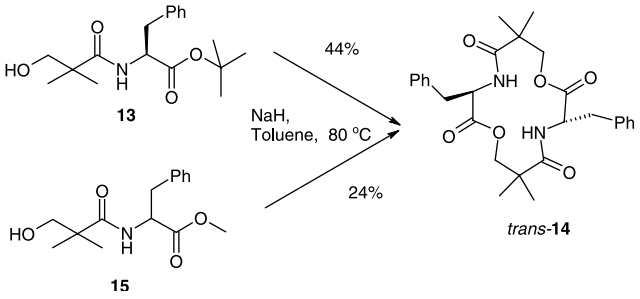


Figure 1. ORTEP plots[23] of the molecular structures of (a) **10** and (b) **12** (50% probability ellipsoids, arbitrary numbering of atoms).

The backbones of the two structures are very similar and also resemble the structures of the previously reported tetramethyl analogue **3**.[1] In the case of **10**, the molecule sits about a crystallographic centre of inversion. The cyclopentane ring has an envelope conformation with the spiro C-atom as the envelope flap. Each amide NH group forms an intermolecular hydrogen bond with a lactone carbonyl O-atom of an adjacent molecule. The molecular symmetry results in there being two parallel hydrogen bonds running in opposite directions between each molecule. These interactions link the molecules into extended double-bridged chains, which run parallel to the [1 0 0] direction. Taken individually, the repeat unit in the chain generated by one of the interactions has a graph set motif[24] of C(5). The pair of hydrogen bonds linking two adjacent molecules forms a loop with a graph set motif of $R_2^2(16)$. In the case of **12**, the space group is centrosymmetric and the molecule again sits about a crystallographic centre of inversion. Therefore, **12** has the (7*RS*,7′*SR*)-configuration, that is, **12** is the trans-isomer. The hydrogen bonding pattern in the crystals of **12** is the same as for **10**.

The nature of the cyclodimerization in the case of **11** is remarkable. Starting with a racemic precursor **11**, one would expect that a mixture of cis- and trans-substituted cyclodimers would be obtained, but only the trans-isomer **12** has been formed in 68% yield.

As mentioned before, factors that might influence the cyclization are not only the type, but also the number of substituents in the amino acid moiety. Therefore, a cyclization experiment was carried out with an analogue of **11**, which contains a monosubstituted amino acid. Direct amide cyclization was not an option in this case, since the intermediate oxazolone does not form smoothly in the case of monosubstituted substrates.[25] Therefore, we used the base catalyzed lactonization described in Ref. 10. When a solution of the enantiomerically pure **13** was heated in toluene (200 mM) in the presence of 1 equiv of NaH, the 14-membered cyclic depsipeptide *trans*-**14** was obtained as the only isolable product in 44% yield. In order to compare the configurations of the two stereogenic centers, the analogous cyclization was performed with a racemic starting material, that is, methyl ester **15** (Scheme 4). Surprisingly, a single cyclodepsipeptide was again obtained in 24% yield and was identical with *trans*-**14** in all respects.



Scheme 4.

The ^{1}H NMR spectrum of the cyclized product obtained from **13** using a shift reagent (Pirkle reagent) and HPLC on a chiral adsorbent (Chiracel OD-H, Merck Whelk-O 1) showed the presence of only one compound, which was identical with the product obtained from the cyclization of **15**. X-ray crystallography of both products confirmed their identical structure and proved that the benzyl groups are trans-oriented (Fig. 2). The space group of *trans*-**14** is centrosymmetric and the molecule sits about a crystallographic centre of inversion, so the two stereogenic centres have inverted configurations, that is, (7*RS*,7′*SR*). The hydrogen bonding pattern is again analogous to that of **12** and **10**.

As the precursor **13** was optically active ($[\alpha]_D^{25}$ +44.6 (*c* 1, $CHCl_3$)), an inversion of the configuration at one of the stereogenic centers has taken place during the cyclization step. With the aim of avoiding this inversion, another cyclization was attempted with the corresponding free hydroxy acid **16**, which was obtained from the basic

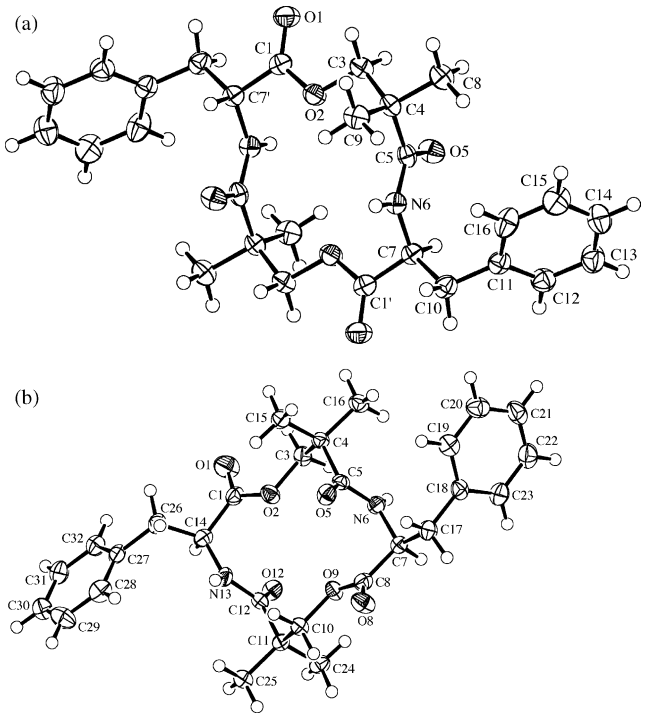


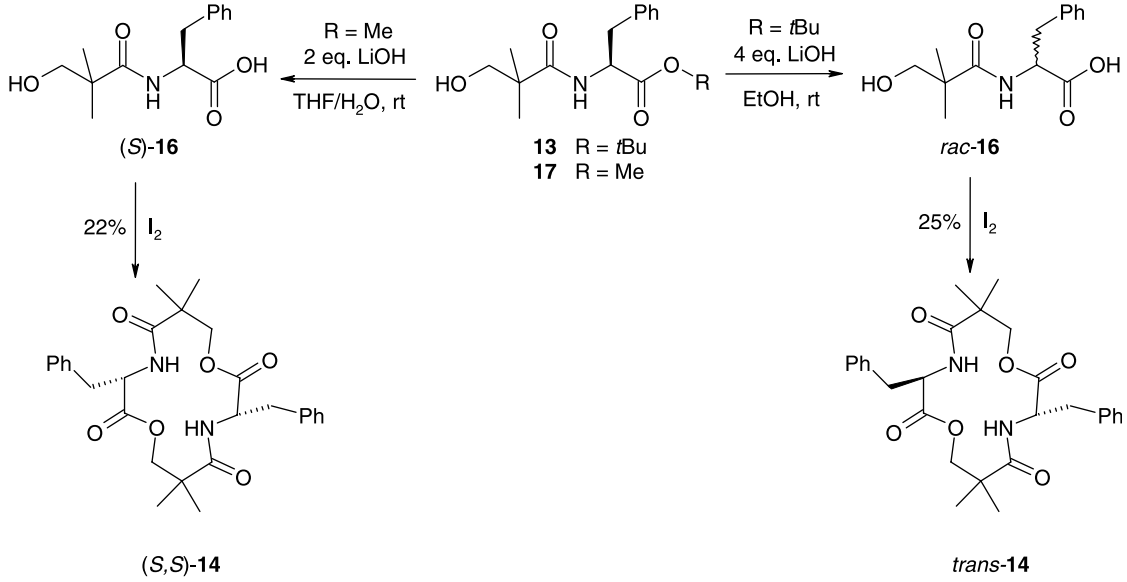
Figure 2. ORTEP plots[23] of the molecular structures of (a) *trans*-**14** and (b) molecule A of (*S*,*S*)-**14** (50% probability ellipsoids, arbitrary numbering of atoms).

hydrolysis of **13**. Cyclization of both *rac*-**16** and (*S*)-**16** was achieved under neutral conditions using I_2 as a catalyst,[26] which has previously proven to be efficient for the synthesis of depsipeptide **3**.[1] The product obtained from *rac*-**16** was again the *meso*-compound *trans*-**14a**, while the enantiomerically pure acid (*S*)-**16**, obtained by a milder hydrolysis of its methyl ester **17**, yielded also only one cyclodepsipeptide, namely (*S*,*S*)-**14** (Scheme 5).

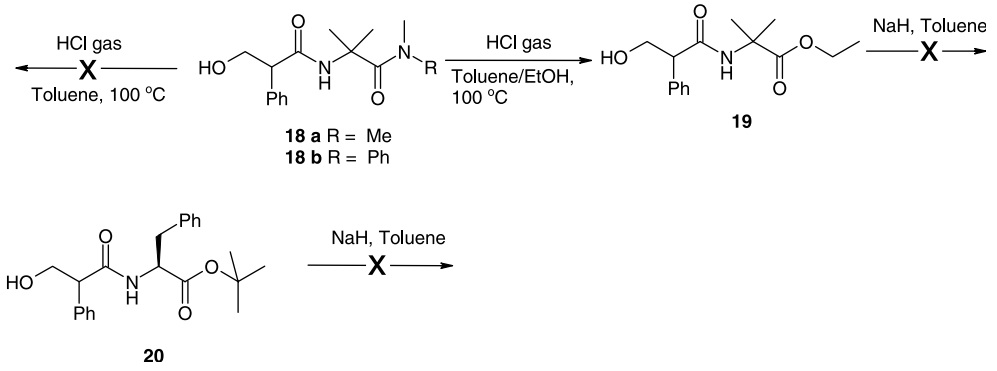
Analytical HPLC of both of these compounds on a chiral column (Whelk-O 1 column, hexane/EtOH 10:1) clearly shows two different peaks with retention times of 10.8 and 10.9 min, respectively. The HPLC diagram of a mixture of the two compounds under the same conditions also showed two peaks, proving once more their different structure. Furthermore, it was shown, that (*S*,*S*)-**14** is optically active. For this reason, we expected that it is the cis-isomer with the (*S*,*S*)-configuration, which was subsequently proven by X-ray crystallography (Fig. 2).

In the crystal structure of (*S*,*S*)-**14**, there are two symmetry-independent molecules A and B in the asymmetric unit, but the conformations of the two molecules are almost identical. The space group permits the compound in the crystal to be enantiomerically pure, but the absolute configuration of the molecule has not been determined. The enantiomer used in the refinement was based on the expected (*S*)-configuration of each chiral centre in the molecule. The crystal is merohedrally twinned, with twin operator [1 0 0/0 −1 0/0 0 −1] and the major twin domain has a volume fraction of 0.640(1). Each amide group in molecule A forms an intermolecular hydrogen bond with an amide O-atom of an adjacent molecule A. This results in there being two parallel hydrogen bonds running in opposite directions between each molecule. The interactions link the molecules into extended double-bridged ···A···A···A··· chains, which run parallel to the [1 0 0] direction. Taken individually, the repeat unit in the chain generated by one of the interactions has a graph set motif[24] of C(4). The pair of hydrogen bonds linking two adjacent molecules forms a loop with a graph set motif of $R_2^2(18)$. The molecules of type B are similarly linked into extended double-bridged ···B···B···B··· chains, which also run parallel to the [1 0 0] direction.

From these results it could be concluded that monosubstituted amino acids in amides of type **1** do not prevent the cyclodimerization. Next, the influence of the disubstitution of C(α) of the β-hydroxy acid should be investigated and, therefore, analogous dipeptides containing a α-monosubstituted β-hydroxy acid were synthesized and subjected to cyclization procedures. Tropic acid (3-hydroxy-2-phenylpropanoic acid) turned out to be an interesting starting material



Scheme 5.



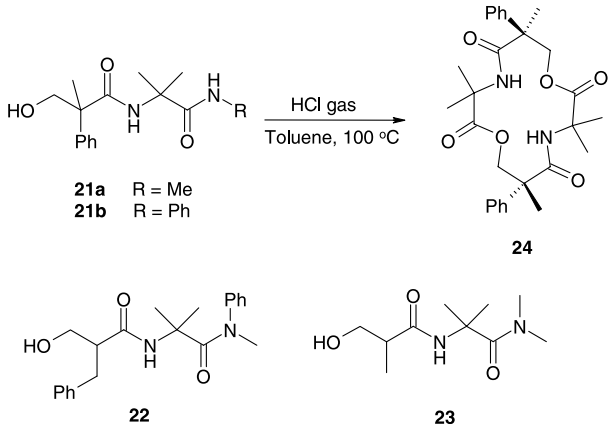
Scheme 6.

for this study. Thus, amides **18a** and **18b** were prepared by coupling tropic acid with 2,2,*N*,*N*-tetramethyl-2*H*-azirin-3-amine and 2,2, *N*-trimethyl-*N*-phenyl-2*H*-azirin-3-amine, respectively, and subjected to the conditions of the 'direct amide cyclization'. Surprisingly, the cyclization failed and only traces of the corresponding 1,3-oxazol-5(4*H*)-one were identified in the crude reaction mixture by IR and NMR spectroscopy. Column chromatography led to no identifiable products. Furthermore, the corresponding ethyl ester **19**, derived from either of the amides **18** by treatment with HCl gas in the presence of EtOH, did not yield cyclic products upon treatment with NaH. Other lactonization procedures, proven to be successful in the case of amide derivatives,[1] also failed to give the desired cyclic depsipeptide in this case (Scheme 6).

When tropic acid was coupled with L-phenylalanine *t*-butylester.HCl, amide **20** was obtained as a mixture of two diastereoisomers. They were separated on a Whelk-O 1 preparative HPLC column, and we attempted to cyclize each of them, in order to determine the stereospecificity of the NaH cyclization. Unfortunately both the racemic and the enantiomerically pure substrates failed to give cyclic depsipeptides (Scheme 6).

The reason for the failure of cyclization of **18**–**20** could be a steric hindrance of the phenyl group in the α-position or its electronic effect. To examine this possibility, 3-hydroxy-2-methyl-2-phenylpropanoic acid was prepared[27] and coupled with the corresponding 2*H*-azirin-3-amines to give **21a**,**b**. The third explanation could be that the cyclization is thermodynamically unfavorable when the substrate is monosubstituted in the α-position of the β-hydroxy acid moiety. Therefore, α-benzyl-β-hydroxypropanoic acid and β-hydroxyisobutyric acid were synthesized according to known procedures[28] and coupled with 2,2,*N*,*N*-tetramethyl-2*H*-azirin-3-amine to give amides **22** and **23**, respectively, as substrates for the cyclization (Scheme 7).

As expected, amide **21b** cyclized under DAC conditions to yield the 14-membered cyclodepsipeptide **24** in moderate yield. This result suggests that amides of type **18/21** bearing a phenyl group at C(α) of the hydroxy acids do cyclize when α,α-disubstituted, that is, the phenyl group in **18** was not the reason for its failure to give cyclic products. Compound **24** was isolated as a colorless solid, which showed only one set of signals in the NMR spectra. Therefore it could be suggested that only one stereoisomer, as a *meso*-compound, has been obtained. Careful crystallization from a mixture of toluene/acetonitrile/acetone gave crystals suitable for X-ray crystallography. The molecular structure of **24** is depicted in Figure 3, showing that again the trans-isomer has been obtained.



Scheme 7.

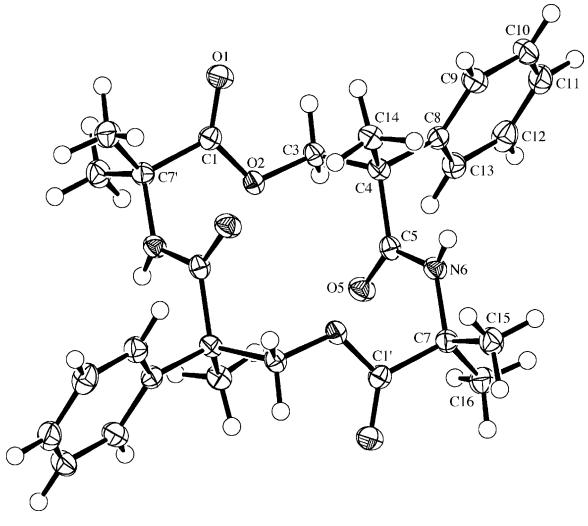


Figure 3. ORTEP plot[23] of the molecular structure of **24** (50% probability ellipsoids, arbitrary numbering of atoms).

Since the space group is centrosymmetric, **24** is a *meso*-compound. The molecule sits about a crystallographic centre of inversion, so therefore has the (4*RS*,4′*SR*)-configuration. Remarkably, the amide groups are not involved in any

hydrogen bonds. Although amide O-atoms in adjacent molecules appear to be positioned correctly to accept a hydrogen bond from the amide H-atom, the H···O distance of 2.98 Å is much too long for it to be considered a hydrogen bond. This is probably the result of molecular bulk preventing the molecules packing close enough together for intermolecular hydrogen bonds to form.

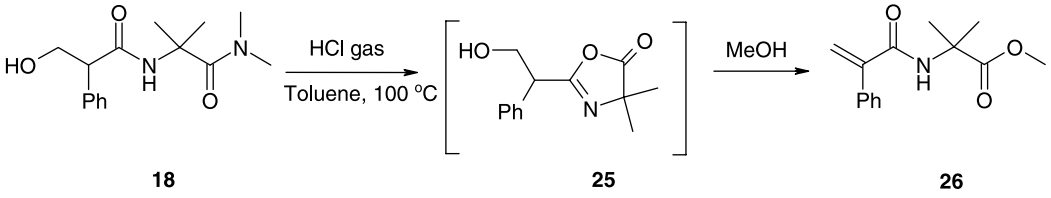
Amides **22** and **23** failed to cyclize under the DAC reaction conditions, as was the case with **18**. Upon monitoring the reaction of **18** by IR spectroscopy, the formation of the corresponding 1,3-oxazol-5(4*H*)-one **25**, which is the expected intermediate in the cyclization reaction, was observed (strong absorption at 1820–1830 cm^{-1}),[29] and after addition of methanol to the reaction mixture, methyl ester **26** was isolated in 58% yield (Scheme 8).

The formation of a methyl ester and the IR spectra strongly suggest the presence of a 1,3-oxazol-5(4*H*)-one as an intermediate. It seems that the oxazolone formation and dehydration in the case of **18** are competitive reactions. In order to get an indication of which reaction takes place first, a similar set of experiments was carried out with benzyl derivative **22**. Upon bubbling HCl gas through a solution of **22** in toluene/methanol (20%), the only product obtained was the corresponding hydroxy ester **27** (Scheme 9). When the reaction was carried out under the conditions described for **18**, that is, oxazolone formation with HCl gas in toluene (monitoring by IR, increasing absorption at 1826 cm^{-1}) and addition of methanol after saturation, the dehydrated ester **28** and the dehydrated amide **29** were obtained in addition to **27** (Scheme 9).

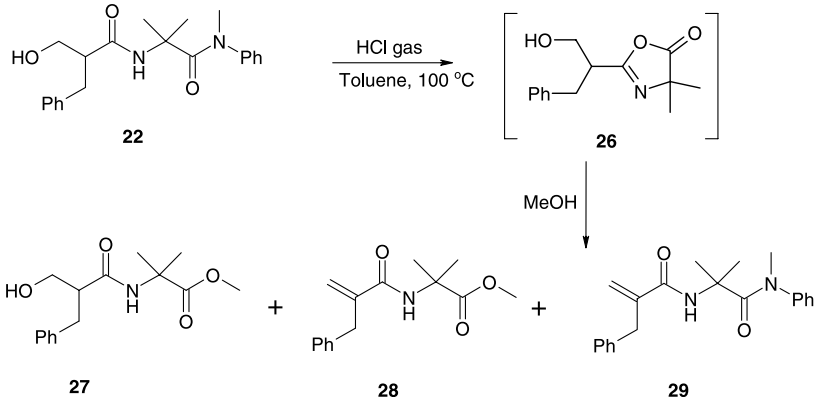
This result suggests that when the reaction is carried out in the presence of a nucleophile, such as methanol, oxazolone **26** is formed first and is immediately transformed into the corresponding hydroxy ester **27**. On the other hand, in the absence of a nucleophile, the intermediate oxazolone **26** eliminates water, leading to 2-vinyl oxazolones and thus preventing further cyclization. The formation of **28** and **29** can be explained by the competitive ring opening of **26** by the nucleophiles methanol and *N*-methylaniline, respectively. This result is an additional indication that the cyclodimerization process occurs via an oxazolone intermediate.

Another variation of the starting materials for the direct amide cyclization (DAC) is the insertion of aromatic β-hydroxy acids or β-hydroxycycloalkane carboxylic acids. The first of these derivatives, salicylamide **30**, was obtained from salicylic acid by coupling with the corresponding 2*H*-azirin-3-amine (Scheme 10).[5,19]

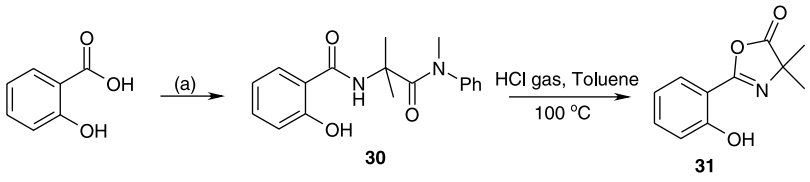
Compound **30** was subjected to the DAC conditions. The starting material disappeared quickly (TLC), but even after



Scheme 8.

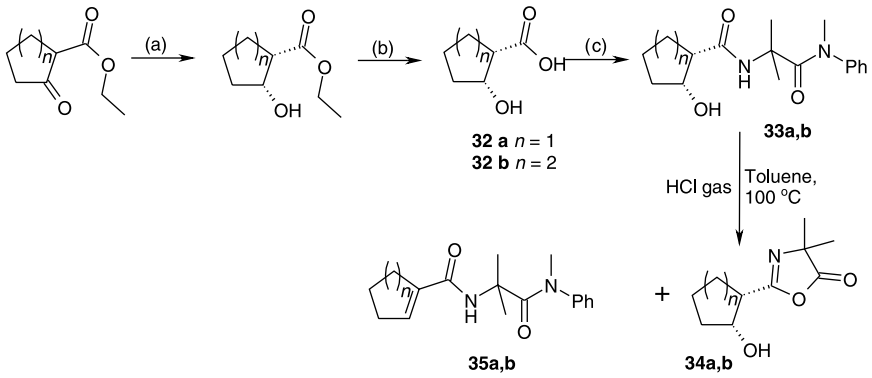


Scheme 9.



(a) 2,2,*N*-Trimethyl-*N*-phenyl-2*H*-azirin-3-amine,THF

Scheme 10.



(a) Yeast, H_2O; Ref. 32; (b) LiOH, THF/H_2O; (c) 2,2,*N*-Trimethyl-*N*-phenyl-2*H*-azirin-3-amine,THF.

Scheme 11.

35 min the only product formed was the corresponding oxazolone **31**. After its isolation, further exposure to the same reaction conditions did not propagate the reaction further, and the oxazolone **31** was recovered. The stability of the oxazolone is in this case extremely high, and apparently a ring enlargement reaction is sterically disfavored.

Crystals of **31** suitable for X-ray crystal structure determination were grown from a mixture of deuterochloroform and dichloromethane by slow evaporation of the solvent. The five-membered heterocycle is planar and the phenyl residue is almost coplanar with the ring. The hydroxy group forms an intramolecular hydrogen bond with the imine N-atom. The interaction can be described by the graph set motif[24] of S(6) (for crystallographic details see Section 4).

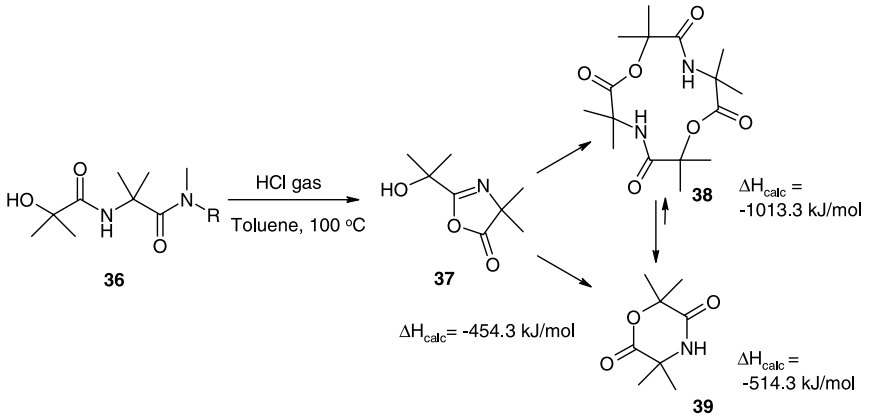
Next, the aromatic ring in **30** was replaced by a cycloaliphatic one. The preparation of the precursors **33a**,**b** was achieved in three steps according to Scheme 11. The cyclization under the standard conditions led to a mixture of two products, the oxazolone **34** and the dehydrated amide **35**, but no cyclodepsipeptide could be detected.

2.1. Computer modeling

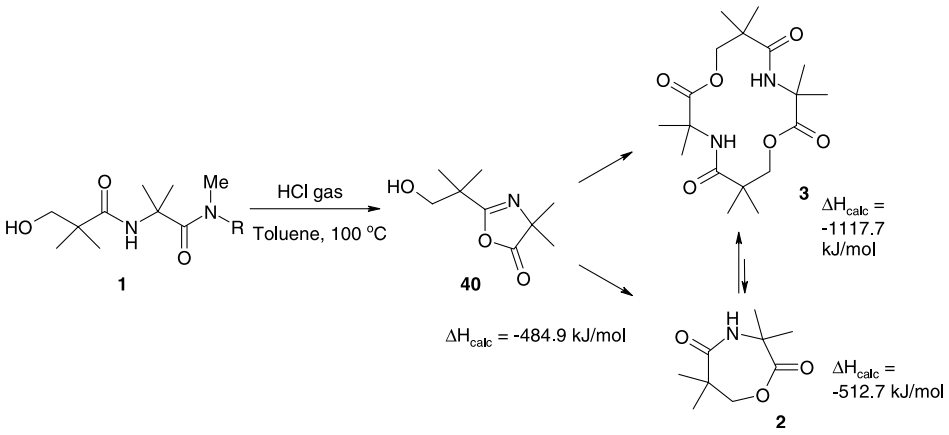
As we were searching for an explanation for the preferred cyclodimerization process of diamides of type **1**, we carried out some simple quantum mechanical calculations. Especially surprising was the discrepancy with the lower homologue **36**, which under the DAC conditions gave the cyclic monomer **39** exclusively. Assuming that the reaction proceeds indeed via the oxazolone intermediates (**37** and **40**, respectively), we compared the energies of the corresponding monomeric and dimeric ring structures, both in the cases of α-hydroxy acids and β-hydroxy acids (Schemes 12 and 13).

AM1 calculations[30] with Ampac v.6.5.5[31] revealed that the transition state required to achieve a nucleophilic attack of the oxazolone hydroxy group on the carbonyl C-atom is more favorable in the case of **40** than in **37**, which is to be expected, having in mind the length of the alkyl chain to which the OH group is bound. Nevertheless, under the DAC conditions, **36** gives **39**, whereas **1** undergoes the twinning process.

Direct comparison of the heats of formation (ΔH) of **38** and **39**, as well as those of **3** and **2**, reveals that in the first case (Scheme 12) the formation of the dimer **38** is energetically more unfavorable than the formation of the monomer **39** ($2E_{39} < E_{38}$). In the second case (Scheme 12), formation of the dimer **3** is energetically more favorable than the formation of the monomer ($2E_2 > E_3$), which suggests that the cause for the different products formed is thermodynamic. This crude modeling helps to understand why the dimers of type **3** could be the main products of the direct



Scheme 12.



Scheme 13.

amide cyclization of **1**, but it does not explain why they are the only products formed. An equilibrium between the monomeric and dimeric forms under the strongly acidic conditions of the DAC is to be expected in both cases (between **2** and **3** on the one hand and between **39** and **38** on the other). This equilibrium might be shifted almost completely in one direction in the case of **3** and in the other in the case of **39**.

3. Conclusions

Upon investigating the reasons for the cyclodimerization of β-hydroxy acid amides of type **1** under various conditions, we were able to isolate five different 14-membered cyclodimers of type **3**. Although other coupling methods also gave moderate results, the best yields were obtained via the 'direct amide cyclization' (DAC), reaching 88% for the cyclodimer **10**. It is worth mentioning that in all cases, when starting with racemic material, only the trans-substituted cyclodepsipeptides were isolated.

The cyclodimerization is most probably a result of the greater thermodynamic stability of the 14-membered ring compared with the seven-membered one. Another factor, which might contribute to the cyclodimerization, as suggested in the literature, is H-bond formation, which would be more pronounced in the 14-membered ring, although the structures of all cyclodepsipeptides, which were characterized by X-ray crystallography, showed no evidence of intramolecular H-bonding.

Molecular modeling using simple AM1 calculations shows in the case of **1** that the formation of the dimeric depsipeptide is indeed thermodynamically favored over the formation of the monomer, but it does not explain why the 14-membered ring is the only product formed. A mixture of the monomeric and dimeric forms is to be expected. Thus, the reason for the exclusive cyclodimerization of compounds of type **1** remains unclear and further investigation in this area is needed.

Variation in the substitution pattern of the starting compounds showed that mono-substitution in the amino acid moiety does not prevent twinning (**13**, **15**, and **16** yielded *trans*-**14**, although not via DAC). Using I_2 mediated lactonization allowed for the selective synthesis of both (*S*,*S*)-**14** (starting with (*S*)-**16**) and *trans*-**14** (starting with *rac*-**16**). If, on the other hand, the hydroxy acid moiety is monosubstituted (as in **18**, **19**, **27**), although the formation of the intermediate 1,3-oxazol-5(*4H*)-one has been monitored by IR, dehydration occurs and no cyclic products are formed. Therefore, the synthesis of β-hydroxy acid containing cyclic depsipeptides via DAC is useful only if the starting amides contain α,α-disubstituted acids.

4. Experimental

4.1. General

Thin-layer chromatography (TLC): Merck TLC aluminium sheets, silica gel 60 F_{254}. Prep. TLC: Merck PLC plates (glass), silica gel 60 F_{254}, 2 mm and 40–63 μm. Flash chromatography (CC): Uetikon-Chemie 'Chromatographiegel' C-560. Mp: Büchi 540 apparatus, uncorrected. IR Spectra: Perkin-Elmer Spectrum one spectrometer; in KBr, unless otherwise stated, absorption bands in cm^{-1}. ^1H NMR (300 MHz) and ^{13}C NMR (75.5 MHz) spectra: Bruker ARX-300 or Bruker DRX-600 instrument; ^1H NMR (600 MHz) and ^{13}C NMR (150 MHz); in $CDCl_3$ at 300 K; TMS as internal standard, unless otherwise stated; δ in ppm, coupling constants J in Hz. Mass spectrometry (MS): Finnigan MAT-90 for electron impact ionization (EI), Finnigan SSQ-700 for chemical ionization (CI, with NH_3) and electrospray ionization (ESI, in MeOH + NaI), unless otherwise stated.

2,2,*N*,*N*-Tetramethyl-2*H*-azirin-3-amine, 2,2,*N*-trimethyl-*N*-phenyl-2*H*-azirin-3-amine, 2-benzyl-2,*N*-dimethyl-*N*-phenyl-2*H*-azirin-3-amine and *N*,*N*-dimethyl-1-azaspiro[2.4]hept-1-en-2-amine were prepared according to standard procedures (Refs. 5 and 7 and references cited therein). 3-Hydroxy-2-benzylpropanoic acid was prepared by the method of Monteil et al.[28] and methyltropic acid from hydroptropic aldehyde by hydroxymethylation, followed by oxidation, according to Geffken.[27] Hydroxy acids **32** were synthesized by the method of Seebach et al.[32] All other products used were commercially available.

General procedure 1 (GP1). To a solution of a hydroxy acid (2–6 mmol) in dry THF (5–20 mL), 1.05 equiv of the corresponding 2*H*-azirin-3-amine were added dropwise. The mixture was stirred at rt for 12–36 h, the solvent evaporated and the remaining solid purified by column chromatography (CC) over silica gel and dried in h.v.

General procedure 2 (GP2). According to GP1, the reaction was stirred overnight, the solvent evaporated, the solid residue washed with Et_2O and recrystallized from AcOEt.

General procedure 3 (GP3). To a solution of a hydroxy acid (3 mmol) in dry THF (10 mL) was added the corresponding phenylalanine ester hydrochloride (3.0 mmol) and 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEPBT, 916 mg, 98%, 3.0 mmol). To the cooled mixture were added dropwise 12 mmol (1.21 g) of Et_3N. The mixture was stirred at rt overnight, the solvent was partially evaporated, AcOEt was added and the solution was washed with 5% aq $KHSO_4$ and with saturated aq $NaHCO_3$ solutions. The combined organic fractions were dried over $MgSO_4$, evaporated, purified by CC, and dried in h.v.

General procedure 4 (GP4). To a solution of an ester (2.0 mmol) in 10 mL EtOH was added $LiOH \cdot H_2O$ (336 mg, 8 mmol). The reaction was stirred overnight at rt, acidified with 6 N HCl, the organic solvent was evaporated in vacuo and the residue extracted with AcOEt. The crude acids were used in the next reaction step without further purification.

General procedure 5 (GP5). A suspension of an amide (1 mmol) in dry toluene (50 mL) was heated to 100 °C, and dry HCl gas was bubbled through the suspension for 5–15 min. Then, the mixture was allowed to cool to rt while bubbling N_2 through it (ca. 20 min). The solvent was evaporated, the white residue was washed with 3×15 mL of CH_2Cl_2 and dried in h.v.

General procedure 6 (GP6). According to GP5, after bubbling N_2 through the reaction mixture (ca. 20 min) the solvent was evaporated, and the residue purified by CC.

General procedure 7 (GP7). To a solution of **13** or **15** (1 mmol) in dry toluene (5 mL), NaH (40 mg of a 60% suspension in mineral oil, 1 mmol) was added slowly at 0 °C and under constant stirring (N_2-atmosphere). After 6 h at 80 °C, the mixture was acidified with 0.1 N HCl ($\sim$5 mL) to pH 5 and extracted with CH_2Cl_2. The combined organic fractions were dried ($MgSO_4$) and evaporated i.v. The crystalline residue was purified by CC (CH_2Cl_2/acetone 200:1) and dried in h.v.

General procedure 8 (GP8). To a solution of a hydroxy acid (1 mmol) in acetonitrile (5 mL), I_2 (25 mg, 0.1 mmol) was added. After 2 days under reflux, the mixture was cooled and the solvent evaporated. After addition of 20 mL of AcOEt and washing with aq $Na_2S_2O_3$, the combined organic fractions were dried ($MgSO_4$) and evaporated i.v. The crystalline residue was purified by CC.

General procedure 9 (GP9). A suspension of an amide (1 mmol) in a toluene/20% ethanol solution (60 mL) was heated to 100 °C, and dry HCl gas was bubbled through the suspension for 10 min. Then, the mixture was allowed to cool to rt while bubbling N_2 through it (ca. 20 min). The solvent was evaporated and the oily residue was purified by CC.

General procedure 10 (GP10). A suspension of an amide (0.5 mmol) in dry toluene (25 mL) was heated to 100 °C, and dry HCl gas was bubbled through the suspension for 10 min. Then, the mixture was allowed to cool to rt while bubbling N_2 through it (ca. 20 min). The solvent was removed i.v. and MeOH (15 mL) was added to the residue and stirred at rt for 1 h in the presence of 500 mg SiO_2. The silica gel was filtered, the solvent was evaporated and the oily residue was purified by CC.

4.2. Preparation of 3-hydroxy-2-methylpropanoic acid

To a solution of methyl (*R*)-3-hydroxy-2-methylpropanoate (1.0 g, 8.38 mmol) in THF/water 75:10 (10 mL) LiOH (1.55 g, 33.5 mmol) was added at 0 °C. The mixture was stirred overnight, the organic solvent evaporated, the residue acidified with 6 N HCl to pH 1 and extracted with AcOEt. The colorless oil was used in the next reaction without further purification. Yield: 768 mg (88%). Spectroscopic data in accordance with previously published data.[33] The optical purity has not been determined. ^{1}H NMR((d_6)-DMSO): 1.04 (d, $J=5.9$ Hz, CH_3); 2.31–2.38 (m, CH); 3.38–3.44, 3.52–3.61 (2m, CH_2); 4.62 (br s, OH); 11.95 (br s, COOH).

4.3. Coupling of β-hydroxy acids with 2*H*-azirin-3-amines

4.3.1. 3-Hydroxy-2,2-dimethyl-*N*-[1-(*N*,*N*-dimethylcarbamoyl)cyclopentyl]propanamide (9). According to GP1, 3-hydroxy-2,2-dimethylpropanoic acid (806 mg, 6.84 mmol) in dry THF (10 mL), *N*,*N*-dimethyl-1-azaspiro[2.4]hept-1-en-2-amine (1.037 g, 7.52 mmol), 18 h, CC (SiO_2, acetone/CH_2Cl_2 1:20). Yield: 1.330 g (76%) of **9**. White solid. Mp 180.6–181.9 °C (AcOEt). IR: 3397vs, 3279s, 2960s, 2873m, 1644vs, 1542vs, 1469m, 1393s, 1309m, 1257m, 1212w, 1164m, 1119w, 1061s, 1011w, 983w, 911w, 817w, 669m. ^{1}H NMR ((d_6)-DMSO): 1.14 (s, Me_2C); 1.71 (m, $2CH_2$); 1.81–2.00, 2.28–2.48 (2m, $2CH_2$); 2.99 (s, Me_2N); 3.56 (br s, OH); 4.46 (s, CH_2O); 7.42 (br s, NH). ^{13}C NMR ((d_6)-DMSO): 22.8 (q, Me_2C); 24.2 (t, CH_2); 37.2 (t, CH_2); 37.7 (q, Me_2N); 42.8, 66.2 (2s, 2C); 69.1 (t, CH_2O); 172.7, 176.9 (2s, 2CO). CI-MS: 257 (21, $[M+H]^+$), 212.3 (100, $[M-NMe_2]^+$). Anal. Calcd for $C_{13}H_{24}N_2O_3$ (256.35): C 60.91, H 9.44, N 10.93; found: C 60.24, H 9.50, N 10.76.

4.3.2. 3-Hydroxy-2,2-dimethyl-*N*-[1-methyl-1-(*N*,*N*-dimethylcarbamoyl)-2-phenylethyl]propanamide (11a). According to GP1, 3-hydroxy-2,2-dimethylpropanoic acid (436 mg, 2.00 mmol) in dry THF (5 mL), 2-benzyl-2,*N*,*N*-trimethyl-2*H*-azirin-3-amine (395 mg, 2.1 mmol), 24 h, CC (SiO_2, acetone/CH_2Cl_2 1:20). Yield: 538 mg (88%) of **11a**. White powder. Mp 92.6–94.9 °C. ^{1}H NMR ((d_6)DMSO): 1.05 (s, Me_2C); 1.29 (s, Me); 3.09 (s, Me_2N); 3.13–3.38 (m, PhCH_2); 3.54 (s, CH_2O); 4.77 (br s, OH); 6.92 (br s, NH); 7.06–7.36 (m, Ph). ^{13}C NMR ((d_6)DMSO): 22.4 (q, Me_2C); 24.8 (q, Me); 37.3 (q, MeN); 41.0 (s, Me_2C); 43.8 (t, PhCH_2);

70.1 (t, CH_2O); 128.3, 128.8, 129.4 (3d, 5 arom. CH); 136.1 (s, arom. C); 173.1, 175.2 (2s, 2CO). CI-MS: 307 (100, $[M+H]^+$), 262 (25, $[M-NMe_2]^+$).

Recrystallization from DMSO/diethyl ether yielded crystals of **11a**, suitable for an X-ray crystal structure determination.

4.3.3. 3-Hydroxy-2,2-dimethyl-*N*-[1-methyl-1-(*N*-methyl-*N*-phenylcarbamoyl)-2-phenylethyl]propanamide (11b). According to GP1, 3-hydroxy-2,2-dimethylpropanoic acid (436 mg, 2.00 mmol) in dry THF (5 mL), 2-benzyl-2,*N*-dimethyl-*N*-phenyl-2*H*-azirin-3-amine (525 mg, 2.1 mmol), 28 h, CC (SiO_2, acetone/CH_2Cl_2 1:30). Yield: 618 mg (84%) of **11b**. White powder. Mp 62.0–64.2 °C. IR: 3385vs, 3291vs, 3060m, 2941vs, 1701vs, 1634vs, 1591s, 1454s, 1386s, 1312s, 1238s, 1193m, 1165m, 1111m, 1053s, 886m, 908w, 834w, 772s, 705s. ^{1}H NMR: 0.98, 1.08 (2s, Me_2C); 1.33 (s, Me); 3.29 (s, MeN); 3.41 (m, PhCH_2); 3.54 (s, CH_2O); 3.77 (br s, OH); 6.89 (br s, NH); 7.12–4.10 (m, 2Ph). ^{13}C NMR: 22.1 (q, *Me*$_2$C); 24.1 (q, Me); 41.8 (q, MeN); 43.4 (s, Me$_2$*C*); 43.7 (t, Ph*C*H$_2$); 70.4 (t, CH_2O); 127.1, 128.3, 128.8, 129.5, 130.1 (5d, 10 arom. CH); 136.1, 144.2 (2s, 2 arom. C); 173.0, 177.1 (2s, 2CO). CI-MS: 369 (30, $[M+H]^+$), 262 (100, $[M-N(Me)Ph]^+$), 234.2 (20), 160.1 (18), 134.1 (36), 107.1 (26). Anal. Calcd for $C_{22}H_{28}N_2O_3$ (368.48): C 71.71, H 7.66, N 7.60; found: C 71.79, H 7.82, N 7.07.

4.3.4. 3-Hydroxy-2-phenyl-*N*-[1-methyl-1-(*N*,*N*-dimethylcarbamoyl)ethyl]propanamide (18a). According to GP2, 3-hydroxy-2-phenylpropanoic acid (tropic acid, 332 mg, 2.00 mmol) in dry THF (5 mL), 2,2,*N*,*N*-tetramethyl-2*H*-azirin-3-amine (249 mg, 2.1 mmol), 8 h. Yield: 440 mg (79%) of **18a**. White powder. Mp 160.8–161.3 °C (AcOEt). IR: 3421m, 3296s, 3060m, 2936m, 1651vs, 1619vs, 1540s, 1491m, 1454w, 1396s, 1271m, 1219m, 1123s, 1068m, 1051m, 913w, 750m, 702m. ^{1}H NMR: 1.88, 1.94 (2s, Me_2C); 3.28 (s, Me_2N); 3.87–4.12 (m, CH_2); 4.42 (br t, J= 6.8 Hz, CH); 6.98 (s, NH); 7.50–7.62 (m, Ph). ^{13}C NMR: 24.3, 24.5 (2q, *Me*$_2$C); 38.0 (q, Me$_2$N); 56.9 (s, Me$_2$*C*); 54.7 (d, CH); 64.9 (t, CH_2O); 127.7, 128.2, 129.0 (3d, 5 arom. CH); 136.7 (s, arom. C); 171.9, 172.6 (2s, 2CO). ^{1}H NMR ((*d*$_6$)DMSO): 1.38, 1.32 (2s, Me_2C); 2.71 (s, Me_2N); 3.42–3.58, 3.60–3.69, 3.87–3.94 (3m, CH, CH_2); 4.78 (t, OH); 7.22–7.36 (m, Ph); 8.31 (s, NH). ^{13}C NMR ((*d*$_6$)DMSO): 25.5, 25.8 (2q, *Me*$_2$C); 37.0 (q, Me$_2$N); 53.9 (s, Me$_2$C); 55.4 (d, CH); 63.2 (t, CH_2O); 126.6, 127.7, 128.0 (3d, 5 arom. CH); 138.1 (s, arom. C); 170.2, 171.7 (2s, 2CO). CI-MS: 279 (80, $[M+H]^+$), 234 (100, $[M-NMe_2]^+$), 206 (22), 157 (18), 104 (8).

4.3.5. 3-Hydroxy-2-phenyl-*N*-[1-methyl-1-(*N*-methyl-*N*-phenylcarbamoyl)ethyl]propanamide (18b). According to GP2, 3-hydroxy-2-phenylpropanoic acid (tropic acid, 332 mg, 2.00 mmol) in dry THF (5 mL), 2,2,*N*-trimethyl-*N*-phenyl-2*H*-azirin-3-amine (365 mg, 2.1 mmol), 9 h. Yield: 578 mg (85%) of **18b**. White powder. Mp 128.6–129.9 °C (AcOEt). IR: 3298s, 3274s, 3055m, 2940m, 1673vs, 16,124vs, 1545s, 1488m, 1462w, 1394s, 1270m, 1124s, 1099s, 1060m, 913m, 745m. ^{1}H NMR: 1.76, 1.89 (2s, Me_2C); 3.31 (s, MeN); 3.91–4.19 (m, CH_2); 4.36–4.42 (m, CH); 7.05 (s, NH); 7.41–7.55, 7.60–7.87 (2m, 2Ph). ^{13}C NMR: 24.6, 24.8 (2q, *Me*$_2$C); 41.1 (*q*, MeN); 57.4 (s, Me$_2$*C*); 55.0 (d, CH); 67.3 (t, CH_2O); 127.6, 127.7, 128.2, 128.5, 128.8, 129.1 (6d, 10 arom. CH); 136.7, 143.8 (2s, 2 arom. C); 173.1, 173.9 (2s, 2CO). ESI-MS: 363 (100, $[M+Na]^+$).

4.3.6. 3-Hydroxy-2-methyl-2-phenyl-*N*-[1-methyl-(*N*,*N*-dimethylcarbamoyl)ethyl]propanamide (21a). According to GP1, 3-hydroxy-2-methyl-2-phenylpropanoic acid (360 mg, 2.00 mmol) in dry THF (5 mL), 2,2,*N*,*N*-tetramethyl-2*H*-azirin-3-amine (249 mg, 2.1 mmol), 36 h, CC (SiO_2, acetone/CH_2Cl_2 1:10). Yield: 455 mg (78%) of **21a**. Colorless crystals. Mp 147.6–148.4 °C (toluene). IR: 3533s, 3280vs, 3054s, 2928vs, 1708vs, 1622vs, 1526vs, 1394s, 1264s, 1208s, 1118s, 1050m, 976m, 900w, 770m, 751m, 802m. ^{1}H NMR: 1.50 (s, Me_2C); 1.61 (s, Me), 3.00 (*s*, Me_2N); 3.55–3.70, 4.05–4.19 (2m, CH_2O); 4.45 (br s, OH); 7.18–7.41 (m, Ph, NH). ^{13}C NMR: 21.4 (q, *Me*$_2$C); 25.0 (q, Me); 37.7 (q, Me$_2$N); 51.5, 56.4 (2s, 2Me$_2$*C*); 68.7 (t, CH_2O); 126.5, 126.9, 128.3 (3d, 5 arom. CH); 141.5 (s, arom. C); 172.6, 175.3 (2s, 2CO). CI-MS: 293 (88, $[M+H]^+$), 248 (100, $[M-NMe_2]^+$), 113 (28). Anal. Calcd for $C_{16}H_{24}N_2O_3$ (292.38): C 65.73, H 8.27, N 9.58; found: C 65.69, H 8.40, N 9.59.

4.3.7. 3-Hydroxy-2-methyl-2-phenyl-*N*-[1-methyl-1-(*N*-methyl-*N*-phenylcarbamoyl)ethyl]propanamide (21b). According to GP1, 3-hydroxy-2-methyl-2-phenylpropanoic acid (360 mg, 2.00 mmol) in dry THF (5 mL) 2-benzyl-2,*N*-dimethyl-*N*-phenyl-2*H*-azirin-3-amine (365 mg, 2.1 mmol), 28 h, CC (SiO_2, acetone/CH_2Cl_2 1:20). Yield: 608 mg (86%) of **21b**. White solid. Mp 132.1–133.0 °C. IR: 3451s, 3282vs, 3057m, 2992s, 1709s, 1635vs, 1592s, 1445s, 1395s, 1262m, 1157m, 1123m, 1092s, 1026s, 921m. ^{1}H NMR: 1.30, 1.38 (2s, Me_2C); 1.48 (s, Me), 3.22 (s, MeN); 3.55–3.61, 4.00–4.10 (2m, CH_2O); 4.63 (br s, OH); 6.61 (br s, NH); 7.18–7.41 (m, 2Ph). ^{13}C NMR: 21.8 (q, *Me*$_2$C); 25.7 (q, Me); 41.5 (q, MeN); 52.3, 58.8 (2s, 2Me$_2$*C*); 69.2 (t, CH_2O); 126.3, 126.6, 126.9, 127.1, 127.2, 128.1, 128.3, 128.4, 128.6, 129.4 (10d, 10 arom. CH); 141.6, 144.2 (2s, 2 arom. C); 173.7, 176.0 (2s, 2CO). ESI-MS: 731 (48, $[2M+Na]^+$), 377 (100, $[M+Na]^+$), 248 (16).

4.3.8. 3-Hydroxy-2-benzyl-*N*-[1-methyl-1-(*N*-methyl-*N*-phenylcarbamoyl)ethyl]propanamide (22). According to GP2, 3-hydroxy-2-benzylpropanoic acid (360 mg, 2.00 mmol) in dry THF (5 mL), 2,2,*N*-trimethyl-*N*-phenyl-2*H*-azirin-3-amine (365 mg, 2.1 mmol), 8 h. Yield: 560 mg (79%) of **22**. White powder. Mp 162.4–163.9 °C. IR: 3409m, 3277s, 3060w, 2932m, 1630vs, 1592s, 1544s, 1493s, 1395s, 1254m, 1188w, 1092s, 1070m, 769m, 743m, 703s, 617m. ^{1}H NMR: 1.27, 1.33 (2s, Me_2C); 2.38–2.42, 2.61–2.73, 2.88–3.01 (3m, CH_2, CH); 3.27 (s, MeN); 3.66–3.73 (m, OH); 6.18 (br s, NH); 7.11–7.41 (m, 2Ph). ^{13}C NMR: 26.2, 27.0 (2q, *Me*$_2$C); 34.3 (t, CH_2); 41.5 (q, MeN); 50.8 (d, CH); 59.0 (*s*, Me$_2$*C*); 63.6 (t, CH_2O); 126.3, 128.2, 128.3, 128.5, 129.0, 129.4 (6d, 10 arom. CH); 139.3, 144.4 (2s, 2 arom. C); 173.8, 174.0 (2s, 2CO). ESI-MS: 377 (100, $[M+Na]^+$). Anal. Calcd for $C_{16}H_{24}N_2O_3$ (354.45): C 65.73, H 8.27, N 9.58; found: C 60.24, H 9.50, N 10.76.

4.3.9. 3-Hydroxy-2-methyl-*N*-[1-methyl-1-(*N*,*N*-dimethylcarbamoyl)ethyl]propanamide (23). According to GP1, 3-hydroxy-2-methylpropanoic acid (416 mg, 4.00 mmol)

in dry THF (5 mL), 2,2,*N*,*N*-tetramethyl-2*H*-azirin-3-amine (498 mg, 4.2 mmol), 38 h, CC (SiO_2, acetone/CH_2Cl_2 1:20). Yield: 790 mg (83%) of **23**. Colorless crystals. Mp 118.7–120.0 °C. IR: 3418s, 1619vs, 1540s, 1397s, 1279m, 1226s, 1124s, 1077m, 1036m. ^{1}H NMR: 1.09 (d, $J=6.1$ Hz, Me); 1.58 (s, Me_2C); 2.59 (m, CH); 3.06 (s, Me_2N); 3.63 (d, CH_2); 4.21 (br s, OH); 7.70 (s, NH). ^{13}C NMR: 13.7 (q, Me); 25.5, 25.6 (2q, Me_2C); 37.9 (q, MeN); 56.3 (*s*, Me_2C); 42.3 (d, CH); 64.8 (t, CH_2O); 173.0, 174.9 (2s, 2CO). ESI-MS: 455 (20, $[2M+Na]^+$), 239 (100, $[M+Na]^+$). Anal. Calcd for $C_{10}H_{20}N_2O_3$ (216.28): C 55.53, H 9.32, N 12.95; found: C 55.74, H 9.71, N 13.12.

4.3.10. 2-Hydroxy-*N*-[1-methyl-1-(*N*-methyl-*N*-phenylcarbamoyl)ethyl]benzamide (30). According to GP1, salicylic acid (276 mg, 2.00 mmol) in dry THF (5 mL), 2,2,*N*-trimethyl-*N*-phenyl-2*H*-azirin-3-amine (365 mg, 2.1 mmol), CC (SiO_2, acetone/CH_2Cl_2 1:20). Yield: 565 mg (92%) of **30**. Colorless crystals. Mp 146.1–147.8 °C. IR: 3533s, 3280vs, 3054s, 2928vs, 1708vs, 1622vs, 1526vs, 1394s, 1264s, 1208s, 1118s, 1050m, 976m, 900w, 770m, 751m, 802m. ^{1}H NMR: 1.43 (s, Me_2C); 3.22 (s, MeN); 6.68–6.74 (m, 1 arom. H); 6.76 (br s, NH); 6.92–7.0 (m, 2H arom);7.11–7.28 (m, 4 arom. H); 7.31–7.42 (m, 2 arom. H); 12.1 (s, OH). ^{13}C NMR: 26.4 (q, Me_2C); 41.4 (q, MeN); 58.4 (s, Me_2C); 119.9 (s, arom. C); 126.6, 126.8, 127.3, 127.9, 128.2, 128.9, 129.3 (7d, 9 arom. CH); 134.1, 142.2 (2s, 2 arom. C); 172.9, 177.5 (2s, 2CO). ESI-MS: 335 (100, $[M+Na]^+$).

4.3.11. (1*R*,2*S*)-2-Hydroxy-*N*-[1-methyl-1-(*N*-methyl-*N*-phenylcarbamoyl)ethyl]cyclopentanecarboxamide (33a). According to GP1, (1*R*,2*S*)-2-hydroxycyclopentanoic acid (**32a**, 260 mg, 2.00 mmol) in dry THF (5 mL), 2,2,*N*-trimethyl-*N*-phenyl-2*H*-azirin-3-amine (365 mg, 2.1 mmol), CC (SiO_2, acetone/CH_2Cl_2 1:10). Yield: 530 mg (86%) of **33a**. White powder. Mp 153.4–155.0 °C. IR: 3289s, 2943s, 1708vs, 1636vs, 1593s, 1494s, 1390s, 1240s, 1092s, 1028m, 919m, 732s. ^{1}H NMR: 1.42 (s, Me_2C); 1.57–1.68, 1.70–1.77, 1.79–1.96 (3m, $3CH_2$, CH); 3.27 (s, MeN); 4.35 (m, CHO); 6.38 (br s, NH); 7.24–7.41 (m, Ph, NH). ^{13}C NMR: 21.9 (t, CH_2); 26.1, 26.4 (2q, Me_2C); 26.8, 33.9 (2t, $2CH_2$); 41.4 (q, MeN); 50.2 (d, CH); 58.6 (s, Me_2C); 74.2 (d, CHO); 128.1, 128.3, 129.4 (3d, 5 arom. CH); 144.2 (s, arom. C); 173.4, 176.1 (2s, 2CO). ESI-MS: 327 (100, $[M+Na]^+$).

4.3.12. (1*R*,2*S*)-2-Hydroxy-*N*-[1-methyl-1-(*N*-methyl-*N*-phenylcarbamoyl)ethyl]cyclohexanecarboxamide (33b). According to GP1, (1*R*,2*S*)-2-hydroxycyclohexanoic acid (**32b**, 288 mg, 2.00 mmol) in dry THF (5 mL), 2,2,*N*-trimethyl-*N*-phenyl-2*H*-azirin-3-amine (365 mg, 2.1 mmol), 12 h, CC (SiO_2, acetone/CH_2Cl_2 1:10). Yield: 566 mg (89%) of **33b**.White powder. Mp 171.2–172.9 °C. IR: 3290s, 3020m, 2948s, 1711vs, 1635vs, 1599s, 1491s, 1421m, 1391s, 1239s, 1091s, 1022m, 919m, 731s. ^{1}H NMR: 1.37, 1.42 (2s, Me_2C); 1.43–1.52, 1.62–1.74, 1.77–1.83, 1.86–1.95 (4m, $4CH_2$, CH); 3.21 (s, MeN); 3.97 (m, CHO); 6.41 (br s, NH); 7.22–7.41 (m, Ph, NH). ^{13}C NMR: 19.2, 24.4, 24.9 (3t, $3CH_2$); 25.9, 26.1 (2q, Me_2C); 31.7 (t, CH_2); 41.4 (q, MeN); 47.9 (d, CH); 59.4 (s, Me_2C); 66.7 (d, CHO); 127.9, 128.2, 129.3 (3d, 5 arom. CH); 142.2 (s, arom. C); 172.1, 177.4 (2s, 2CO). ESI-MS: 341 (100, $[M+Na]^+$).

4.4. Preparation of dipeptide esters

4.4.1. *tert*-Butyl (*S*)-2-(3-hydroxy-2,2-dimethylpropanoylamino)-3-phenylpropanoate (13). According to GP3, 3-hydroxy-2,2-dimethylpropanoic acid (654 mg, 3 mmol), L-phenylalanine *tert*-butyl ester hydrochloride (774 mg, 3.0 mmol), CC (SiO_2, acetone/CH_2Cl_2 1:30). Yield: 857 g (89%) of **13**. Colorless solid. Mp 82.7–84.1 °C. $[\alpha]_D^{25}$ +44.6 (*c* 1, $CHCl_3$). IR: 3265vs, 3006s, 2982vs, 2867s, 1721vs, 1635vs, 1543vs, 1455s, 1392s, 1315s, 1165vs, 1102m, 963s, 850m. ^{1}H NMR: 1.09 (s, Me_2C); 1.45 (s, Me_3C), 3.00–3.17 (m, $PhCH_2$); 3.42–3.51 (m, CH_2O); 3.72 (br s, OH); 4.63–4.78 (m, CH); 6.94 (br s, NH); 7.13–7.36 (m, Ph). ^{13}C NMR: 22.3 (q, Me_2C); 27.8 (q, Me_3C); 37.6 (t, CH_2); 43.1 (s, Me_2C); 53. (d, CH); 69.9 (t, CH_2); 82.3 (s, Me_3C); 126.9, 128.3, 129.3 (3d, 5 arom. CH); 136.1 (s, arom. C); 170.8, 177.1 (2s, 2CO). CI-MS: 322 (88, $[M+H]^+$), 266 (100, $[M-{}^t\mathrm{Bu}]^+$). Anal. calcd for $C_{18}H_{27}NO_4$ (321.42): C 67.26, H 8.47, N 4.36; found: C 67.04, H 8.66, N 4.20.

4.4.2. Methyl (*RS*)-2-(3-hydroxy-2,2-dimethylpropanoylamino)-3-phenylpropanoate (15). According to GP3, 3-hydroxy-2,2-dimethylpropanoic acid (654 mg, 3 mmol), DL-phenylalanine methyl ester hydrochloride (639 mg, 3.0 mmol), CC (SiO_2, acetone/CH_2Cl_2 1:30). Yield: 673 g (80%) of **15**. Pale yellow oil. ^{1}H NMR: 1.03 (s, Me_2C); 2.98–3.12 (m, $PhCH_2$); 3.36–3.49 (m, CH_2O); 3.67 (s, MeO); 4.68–74 (m, CH); 6.50 (br s, NH); 7.13–7.38 (m, Ph). ^{13}C NMR: 22.2 (q, Me_2C); 37.6 (t, CH_2); 43.0 (s, Me_2C); 52.2 (d, CH); 52.9 (q, MeO); 69.5 (t, CH_2); 127.0, 128.4, 129.1 (3d, 5 arom. CH); 135.8 (s, arom. C); 172.2, 177.3 (2s, 2CO). CI-MS: 280 (100, $[M+H]^+$), 162 (18). Anal. Calcd for $C_{15}H_{13}NO_4$ (279.34): C 64.50, H 7.58, N 5.01; found: C 64.15, H 7.73, N 4.89.

4.4.3. Methyl (*S*)-2-(3-hydroxy-2,2-dimethylpropanoylamino)-3-phenylpropanoate (17). According to GP3, 3-hydroxy-2,2-dimethylpropanoic acid (654 mg, 3 mmol), L-phenylalanine methyl ester hydrochloride (639 mg, 3.0 mmol), CC (SiO_2, acetone/CH_2Cl_2 1:30). Yield: 720 g (86%) of **17**. Pale yellow oil. ^{1}H NMR: 1.05 (s, Me_2C); 3.00–3.09 (m, $PhCH_2$); 3.38–3.48 (m, CH_2O); 3.69 (s, MeO); 4.71 (t, $J=6.0$ Hz, CH); 6.52 (s, NH); 7.14–7.36 (m, Ph). ^{13}C NMR: 22.3 (q, Me_2C); 37.7 (t, CH_2); 43.0 (s, Me_2C); 52.1 (d, CH); 53.0 (q, MeO); 69.6 (t, CH_2); 127.0, 128.4, 129.1 (3d, 5 arom. CH); 135.9 (s, arom. C); 172.3, 177.5 (2s, 2CO). CI-MS: 280 (100, $[M+H]^+$), 162 (18).

4.4.4. *tert*-Butyl (*R*,*S*)-2-(3-hydroxy-2-phenylpropanoylamino)-3-phenylpropanoate (20). According to GP3, tropic acid (498 mg, 3 mmol), L-phenylalanine *tert*-butyl ester hydrochloride (774 mg, 3.0 mmol), CC (SiO_2, acetone/CH_2Cl_2 1:30). Yield: 974 mg (88%) of **20**. Pale yellow crystals. Mp 117.4–119.1 °C. IR: 3426s, 3290s, 3059m, 1738vs, 1658vs, 1635s, 1551m, 1454m, 1368s, 1223s, 1154vs, 1059m, 1023m, 845m, 740m, 701s. ^{1}H NMR ((*d*$_6$)DMSO): 1.28, 1.31 (2s, Me_3C); 2.91–3.10 (m, $PhCH_2$); 3.50–3.62, 3.69–3.80 (2m, CH_2O); 4.00–4.16, 4.60–4.78 (2m, 2CH); 5.81–6.00 (m, OH); 6.73 (d, $J=$ 3.7 Hz, NH); 6.93–7.27 (m, 2Ph). ^{13}C NMR: 27.8, 27.9 (2q, Me_3C); 37.7 (t, CH_2); 53.1, 53.5, 54.3, 54.4 (4d, 2CH); 64.7, 64.8 (t, CH_2O); 81.8 (s, Me_3C); 127.7, 128.2, 128.3, 128.4, 129.0, 129.3 (6d, 10 arom. CH); 136.1, 136.8 (2*s*, 2

arom. C); 171.0, 173.2 (2s, 2CO). CI-MS: 370 (85, $[M+H]^+$), 313 (100, $[M-{}^t\mathrm{Bu}]^+$).

4.5. Saponification of dipeptide amides

4.5.1. (*RS*)-2-(3-Hydroxy-2,2-dimethylpropanoylamino)-3-phenylpropanoic acid (*rac*-16). According to GP4, from **13** (642 mg, 2.0 mmol). Yield 454 mg (79%) of **13**. White solid. Mp 91.3–94.9 °C. ^{1}H NMR: 0.99, 1.02 (2s, Me_2C); 2.96–3.02, 3.08–3.14 (2m, PhCH_2); 3.21–3.41 (m, CH_2O); 4.66–4.78 (m, CH); 6.95 (br s, NH); 6.98–7.18 (m, Ph). ^{13}C NMR: 22.0 (q, Me_2C); 37.0 (t, CH_2); 43.3 (s, Me_2C); 53.1 (d, CH); 69.3 (t, CH_2); 127.0, 128.4, 129.3 (3d, 5 arom. CH); 135.8 (s, arom. C); 174.0, 178.1 (2s, 2CO). ESI-MS: 288 (100, $[M+H]^+$).

4.5.2. (*S*)-2-(3-Hydroxy-2,2-dimethylpropanoylamino)-3-phenylpropanoic acid ((*S*)-16). According to GP4, from **17** (558 mg, 2.0 mmol). Yield: 442 mg (79%) of (*S*)-**16**. Colorless oil, $[\alpha]_D^{25}$ +44.1 (*c* 1, $CHCl_3$). IR: 3369vs, 3192s, 2963s, 1723vs, 1643vs, 1529vs, 1455s, 1394m, 1287m, 1254s, 1179m, 1111w, 1049s, 913w, 699s. ^{1}H NMR: 0.99, 1.01 (2s, Me_2C); 2.88–2.94, 3.02–3.14 (2m, PhCH_2); 3.38 (m, CH_2O); 4.71–4.83 (m, CH); 6.71 (br s, NH); 6.98–7.18 (m, Ph). ^{13}C NMR: 21.1 (q, Me_2C); 36.1 (t, CH_2); 42.4 (s, Me_2C); 52.2 (d, CH); 68.5 (t, CH_2); 126.1, 127.6, 128.4 (3d, 5 arom. CH); 134.9 (s, arom. C); 173.2, 177.2 (2s, 2CO). ESI-MS: 288 (100, $[M+Na]^+$).

4.6. Solvolysis of dipeptide amides under DAC conditions

4.6.1. Ethyl 2-(3-hydroxy-2-phenylpropanoylamino)-2-methylpropanoate (19). According to GP9, **18** (278 mg, 1 mmol) in toluene/EtOH 80:20 (60 mL), CC (AcOEt/hexane 1:10). Yield: 209 mg (76%) of **19**. Colorless oil. IR: 3237vs, 3068s, 2980s, 1734vs, 1642vs, 1557s, 1475s, 1383m, 1288s, 1164s, 1070m, 1037s, 872w, 751m, 699s. ^{1}H NMR: 1.10 (t, J=6.1 Hz, Me); 1.42 (s, Me_2C); 3.50–3.60, 3.63–3.72 (2m, CH_2); 3.91–4.13 (m, CH, CH_2); 6.02 (s, OH); 7.11–7.29 (m, Ph, NH). ^{13}C NMR: 13.9 (q, Me); 24.5 (q, Me_2C); 54.4 (d, CH); 56.6 (s, Me_2C); 61.5 (t, CH_2); 65.1 (t, CH_2O); 127.7, 128.3, 129.0 (3d, 5 arom. CH); 136.5 (s, arom. C); 172.8, 174.2 (2s, 2CO). ESI-MS: 280 (100, $[M+H]^+$).

4.6.2. Methyl 2-methyl-2-(2-phenylacryloylamino)-propanoate (26). According to GP10, **18** (139 mg, 0.5 mmol) in toluene (30 mL), CC (AcOEt/hexane 1:10). Yield: 72 mg (58%) of **26**. White crystals. Mp 116.8–117.4 °C. IR: 3328m, 3068m, 2960vs, 2861s, 1727vs, 1657s, 1533s, 1460s, 1382m, 1276vs, 1139s, 1073s, 944w, 731m. ^{1}H NMR: 1.56 (s, Me_2C); 3.77 (s, MeO); 5.60, 6.10 (2*s*, H_2C=); 6.28 (br s, NH); 7.30–7.42 (m, Ph). ^{13}C NMR: 24.6 (q, Me_2C); 52.6 (s, MeO); 56.7 (s, Me_2C); 121.7 (t, H_2C=); 127.9, 128.6, 128.9 (3d, 5 arom. CH); 136.8 (s, C=CH_2); 144.7 (s, arom. C); 166.6, 174.7 (2s, 2CO). CI-MS: 265 (6, $[M+NH_4]^+$), 249 (14), 248 (100, $[M+H]^+$).

4.6.3. Methyl 2-(2-benzyl-3-hydroxypropanoylamino)-2-methylpropanoate (27). According to GP9, **22** (188 mg, 0.5 mmol) in toluene/MeOH 80:20 (30 mL), CC (AcOEt/hexane 1:10). Yield: 81 mg (58%) of **22**. Colorless oil. ^{1}H NMR: 1.41, 1.44 (2s, Me_2C); 2.59 (m, CH); 2.82 (m, CH_2); 3.51, (br s, OH); 3.71 (m, MeO, CH_2); 6.50 (s, NH); 7.11–7.31 (m, Ph). ^{13}C NMR: 26.3 (q, Me_2C); 35.2 (t, CH_2); 48.4 (s, MeO); 51.1 (d, CH); 55.1 (s, Me_2C); 66.0 (t, CH_2); 126.6, 128.4, 128.9 (3d, 5 arom. CH); 138.0 (s, arom. C); 170.9, 172.9 (2s, 2CO). CI-MS: 281 (16), 280 (100, $[M+H]^+$).

4.7. Attempted cyclization reactions

4.7.1. Direct amide cyclization.

4.7.1.1. 8,8,19,19-Tetramethyl-10,21-dioxa-6,17-diazadispiro[4.6.4.6]docosane-7,11,18,22-tetraone (10). According to GP5, **9** (256 mg, 1 mmol) in dry toluene (50 mL) for 15 min. Yield: 186 mg (88%) of **10**. White powder. Mp 344.8–346.4 °C (decomp.). IR: 3386vs, 3029w, 2989s, 2936s, 1715vs, 1668vs, 1519vs, 1470m, 1268vs, 1190m, 1126vs, 1012m, 804w, 699s. ^{1}H NMR ((*d*$_7$)DMF): 1.00 (s, 2Me_2C); 1.46–1.54 (m, 4CH_2); 1.75–1.82, 1.88–1.94 (2m, 4CH_2); 3.94 (s, 2CH_2O); 7.69 (s, 2NH). ^{13}C NMR ((*d*$_7$)DMF): 22.8 (q, 2Me_2C); 24.7, 36.1 (2t, 8CH_2); 41.3, 65.2 (2s, Me_2C, 2C); 70.8 (t, 2CH_2O); 173.9, 174.5 (2s, 4CO). CI-MS: 441 (21), 440 (90, $[M+Na]^+$), 423 (100, $[M+H]^+$). Anal. Calcd for $C_{22}H_{34}N_2O_6$ (422.53): C 62.54, H 8.11, N 6.63; found: C 61.89, H 8.07, N 6.56.

Recrystallization from DMF/toluene/ethyl acetate yielded crystals of **10**, suitable for an X-ray crystal structure determination.

4.7.1.2. 3,10-Dibenzyl-3,6,6,10,13,13-hexamethyl-1,8-dioxa-4,11-diazacyclotetradecane-2,5,9,12-tetraone (12). According to GP 5, **11** (368 mg, 1 mmol) in dry toluene (50 mL), for 15 min. Yield: 177 mg (34%) of **12**. White solid. Mp 288.1–290.5 °C (decomp.). IR: 3520m, 3460vs, 3057w, 2970s, 1744vs, 1660vs, 1527vs, 1483s, 1364s, 1260s, 1236m, 1121vs, 1020w, 719m, 701s. ^{1}H NMR: 1.11, 1.25, 1.46 (3s, 6Me); 3.28–3.38 (m, 2PhCH_2); 4.09 (s, 2CH_2O); 7.07 (s, 2NH); 7.21–7.31 (m, 10 arom. H). ^{13}C NMR: 21.3, 21.8 (2q, 2Me_2C); 28.7 (q, 2Me); 39.7 (s, 2Me_2C); 41.3 (t, 2CH_2); 58.4 (s, 2C); 70.2 (t, 2CH_2O); 126.1, 127.3, 129.6 (3d, 10 arom. CH); 135.0 (s, 2 arom. C); 171.7, 173.2 (2s, 4CO). ESI-MS: 545 (100, $[M+Na]^+$).

Recrystallization from DMF/benzene/CH_2Cl_2/hexane/*i*-PrOH yielded crystals of **12**, suitable for an X-ray crystal structure determination.

4.7.1.3. 3,6,6,10,13,13-Hexamethyl-3,10-diphenyl-1,8-dioxa-4,11-diazacyclotetradecane-2,5,9,12-tetraone (24). According to GP6, **21a** (292 mg, 1 mmol) in dry toluene (50 mL), 15 min, (SiO_2, acetone/CH_2Cl_2 1:60). Yield: 75 mg (30%) of **24**. White solid. Mp 251.6–253.1 °C (decomp.). IR: 3526m, 3435vs, 3023w, 2987s, 1736vs, 1665vs, 1519vs, 1384s, 1276s, 1142vs, 1081w, 998s, 770m, 699s. ^{1}H NMR: 1.30, 1.51, 1.60 (3s, 6Me); 4.41–4.61 (m, 2CH_2O); 7.07 (s, 2NH); 7.28–7.42 (m, 10 arom. H). ^{13}C NMR: 22.6, 24.5 (2q, 2Me_2C); 25.7 (q, 2Me); 50.6 (s, 2Me_2C); 56.1 (s, 2C); 70.0 (t, 2CH_2O); 126.4, 127.7, 128.9 (3d, 10 arom. CH); 141.0 (s, 2 arom. C); 172.9,173.5 (2s, 4CO). CI-MS: 513 (32), 512 (100, $[M+NH_4]^+$), 495 (28, $[M+H]^+$).

Recrystallization from toluene/MeCN/acetone yielded crystals of **24**, suitable for an X-ray crystal structure determination.

4.7.1.4. Reaction of 22 under DAC conditions. A suspension of **22** (188 mg, 0.5 mmol) in toluene (30 mL) was heated to 100 °C, and dry HCl gas was bubbled through the suspension for 20 min (IR monitoring). Then, the mixture was allowed to cool to rt while bubbling N_2 through it (ca. 20 min), MeOH was added to the solution and stirred at rt for 1 h. The solvent was evaporated, the oily residue was purified by CC (AcOEt/hexane 1:10) yielding **27** (13 mg, 9%), **28** (28 mg, 21%) and **29** (36 mg, 30%).

4.7.1.4.1. Methyl 2-(2-benzylacryloylamino)-2-methylpropanoate (28). Colorless oil. IR: 3332m, 3069m, 2954vs, 2861s, 1727vs, 1661s, 1537s, 1454s, 1364m, 1282vs, 1241s, 1144s, 1073s, 1026m, 931w, 731m, 701m. ^{1}H NMR: 1.46, 1.49 (2s, Me_2C); 3.61 (s, CH_2); 3.71 (s, MeO); 5.22, 5.79 (2s, H_2C=); 6.32 (br s, NH); 7.14–7.32 (m, Ph). ^{13}C NMR: 24.5 (q, Me_2C); 38.5 (t, CH_2); 52.4 (s, MeO); 56.4 (s, Me_2C); 119.6 (t, H_2C=); 126.4, 128.4, 128.8 (3d, 5 arom. CH); 138.2 (s, arom. C); 144.4 (s, C=CH_2); 167.3, 174.8 (2s, 2CO). CI-MS: 263 (18), 262 (100, $[M+H]^+$).

4.7.1.4.2. 2-Benzyl-*N*-[1-methyl-1-(*N*-methyl-*N*-phenylcarbamoyl)ethyl]acrylamide (29). White solid. Mp 119.1–121.6 °C. IR: 3282s, 3060w, 2932m, 1634vs, 1598s, 1541s, 1481s, 1421m, 1390s, 1254m, 1172w, 1090s, 1079m, 769m, 703s. ^{1}H NMR: 1.41 (s, Me_2C); 3.20 (s, MeN); 3.46 (s, CH_2); 5.10, 5.53 (2s, H_2C=); 6.18 (br s, NH); 7.09–7.39 (m, 2Ph). ^{13}C NMR: 26.1 (q, Me_2C); 38.4 (t, CH_2); 41.3 (q, MeN); 58.1 (s, Me_2C); 120.0 (t, H_2C=); 126.5, 127.8, 128.1, 128.6, 129.0, 129.4 (6d, 10 arom. CH); 138.4, 143.4 (2s, 2 arom. C); 144.4 (s, C=CH_2); 166.5, 173.1 (2s, 2CO). CI-MS: 237 (52, $[M+H]^+$), 230 (100, $[M-N(Me)Ph]^+$), 108 (18).

4.7.1.5. 2-(2-Hydroxyphenyl)-4,4-dimethyl-1,3-oxazol-5(4*H*)-one (31). According to GP6, **30** (156 mg, 0.5 mmol) in dry toluene (50 mL), 5 min (SiO_2, acetone/CH_2Cl_2 1:40). Yield: 73 mg (74%) of **31**. Colorless crystals. Mp 68.2–69.0 °C. IR: 3079m, 2977s, 1823vs, 1643vs, 1615vs, 1579s, 1478s, 1320vs, 1251s, 1206s, 1090s, 1016s, 915s, 735s. ^{1}H NMR: 1.55 (s, Me_2C); 6.84–7.08, 7.41–7.49, 7.68–7.73 (3m, 4 arom. H). ^{13}C NMR: 24.8 (q, Me_2C); 64.5 (s, Me_2C); 108.8 (s, arom. C); 117.2, 119.3, 128.2, 134.5 (d, 4 arom. CH); 160.0 (s, C=N); 161.8 (s, arom. C); 178.6 (s, CO). CI-MS: 206 (100, $[M+NH_4]^+$).

Recrystallization from $CDCl_3/CH_2Cl_2$ yielded crystals of **31**, suitable for an X-ray crystal structure determination.

4.7.1.6. Reaction of 33a under DAC conditions. According to GP6, **33a** (152 mg, 0.5 mmol) in dry toluene (50 mL), 6 min, CC (SiO_2, acetone/CH_2Cl_2 1:10). Yield: 51 mg (34%) of **35a** and 30 mg (28%) of **34a**.

4.7.1.6.1. *N*-[1-Methyl-1-(*N*-methyl-*N*-phenylcarbamoyl)ethyl]cyclopent-1-enecarboxamide (35a). White solid. Mp 109.6–111.1 °C. ^{1}H NMR: 1.53 (s, Me_2C); 1.84–1.95, 2.22–2.26, 2.37–2.44 (3m, $3CH_2$); 3.26 (s, Me_2N); 5.81 (m, CH=); 6.43 (br s, NH); 7.20–7.23, 7.29–7.34 (m, Ph). ^{13}C NMR: 23.4 (t, CH_2); 26.6 (q, Me_2C); 31.0, 31.1, 32.8 (3t, $3CH_2$); 41.3 (q, MeN); 57.7 (s, Me_2C); 117.9 (s, C=); 127.6, 127.8, 129.1 (d, 5 arom. CH); 139.4 (d, CH=); 144.5 (s, arom. C); 169.0, 173.1 (2s, 2CO). ESI-MS: 202 (100, $[M+Na]^+$).

4.7.1.6.2. 2-(2-Hydroxycyclopentyl)-4,4-dimethyl-1,3-oxazol-5(4*H*)-one (34a). White solid. Mp 98.0–102.1 °C (decomp.). IR: 3080w, 2980m, 1822vs, 1642s, 1597s, 1472m, 1382w, 1216m, 1095s, 1018m, 916s. ^{1}H NMR: 1.56 (s, Me_2C); 1.54–1.75, 1.79–1.98 (2m, $3CH_2$, CH); 4.41 (m, CHO). ^{13}C NMR: 22.4 (t, CH_2); 25.4, 25.8 (2q, Me_2C); 27.8, 31.1 (3t, $3CH_2$); 54.4 (d, CH); 62.3 (s, Me_2C); 76.1 (d, CHO); 162.5 (s, C=N); 173.1 (s, CO). CI-MS: 229 (100, $[M+NH_4]^+$).

4.7.1.7. Reaction of 33b under DAC conditions. According to GP6, **33b** (158 mg, 0.5 mmol) in dry toluene (50 mL), 6 min, CC (SiO_2, acetone/CH_2Cl_2 1:10). Yield: 51 mg (34%) of **35b** and 31 mg (31%) of **34b**.

4.7.1.7.1. *N*-[1-Methyl-1-(*N*-methyl-*N*-phenylcarbamoyl)ethyl]cyclohex-1-enecarboxamide (35b). White crystals. Mp 112.1–113.8 °C. ^{1}H NMR: 1.46 (s, Me_2C); 1.52–1.62, 1.84–1.96, 2.01–2.14 (3m, $4CH_2$); 3.26 (s, MeN); 5.85 (m, CH=); 6.53 (br s, NH); 7.18–7.20, 7.30–7.36 (m, Ph). ^{13}C NMR: 21.4, 21.9, 23.8, 25.2 (4t, $4CH_2$); 26.6 (q, Me_2C); 41.3 (q, MeN); 57.6 (s, Me_2C); 117.1 (s, CH=); 127.7, 129.1, 129.3 (3d, 5 arom. CH); 133.9 (d, CH=); 144.5 (s, arom. C); 169.6, 173.2 (2s, 2CO). ESI-MS: 323 (100, $[M+Na]^+$).

4.7.1.7.2. 2-(2-Hydroxycyclohexyl)-4,4-dimethyl-1,3-oxazol-5(4*H*)-one (34b). White solid. Mp 101.1–105.2 °C (decomp.). IR: 3288w, 3017m, 29,619m, 1824vs, 1638s, 1595s, 1490m, 1421m, 1380m, 1092s, 917s. ^{1}H NMR: 1.39, 1.43 (2s, Me_2C); 1.44–1.56, 1.64–1.89, 1.90–1.98 (3m, $4CH_2$); 4.01 (m, CHO). ^{13}C NMR: 19.8, 24.9, 26.6 (3t, $3CH_2$); 26.0, 26.1 (q, Me_2C); 31.9 (t, CH_2); 49.0 (d, CH); 64.0 (s, Me_2C); 66.9 (d, CHO); 163.1 (s, C=N); 176.8 (s, CO). CI-MS: 215 (100, $[M+NH_4]^+$).

4.7.2. Other cyclizations.

4.7.2.1. *trans*-3,10-Dibenzyl-6,6,13,13-tetramethyl-1,8-dioxa-4,11-diazacyclotetradecane-2,5,9,12-tetraone (*trans*-14). According to GP7, **15** (321 mg, 1 mmol) or **13** (279 mg, 1 mmol). Yield of *trans*-**14**: 108 mg (44%) (from **13**) and 60 mg (24%) (from **15**), respectively. Colorless crystals. Mp 232.9–234.6 °C (decomp.). $[\alpha]_D^{25}$ 0 (*c* 1, $CHCl_3$). IR: 3374vs, 3024w, 2966m, 1732vs, 1645vs, 1531s, 1366m, 1282s, 1179s, 1108w, 1001m, 751m, 700m. ^{1}H NMR: 0.90, 0.98 (2s, Me_2C); 3.06 (d, J= 5.8 Hz, CH_2); 3.91–3.96 (m, CH_2); 4.80–4.84 (m, CH); 5.89 (d, J=3.9 Hz, NH); 6.97–6.99 (m, 6 arom. H); 7.10–7.25 (m, 4 arom. H). ^{13}C NMR: 21.6, 22.7 (2q, Me_2C); 37.6 (t, $2CH_2$); 42.2 (s, 2C); 52.5 (d, 2CH); 70.9 (t, $2CH_2O$); 127.2, 128.6, 129.3 (3d, 10 arom. CH); 135.7 (s, 2 arom. C); 170.0, 174.0 (2s, 4CO). ESI-MS: 517 (100, $[M+Na]^+$), 444 (11). CI-MS: 513 (30), 512 (100, $[M+NH_4]^+$), 496 (12), 495 (30, $[M+H]^+$). Anal. Calcd for $C_{28}H_{34}N_2O_6$ (494.59): C 68.00, H 6.93, N 5.66; found: C 67.69, H 7.08, N 5.48.

Recrystallization from *i*-PrOH/CH_2Cl_2/hexane yielded crystals of *trans*-**14**, suitable for an X-ray crystal structure determination.

According to GP8, *rac*-**16** (287 mg, 1 mmol), CC (CH_2Cl_2/acetone 200:1) yielded *trans*-**14** as white crystals in 25% yield (62 mg). $[\alpha]_D^{25}$ 0 (*c* 1, $CHCl_3$).

4.7.2.2. *cis*-(*S*,*S*)-3,10-Dibenzyl-6,6,13,13-tetramethyl-1,8-dioxa-4,11-diazacyclotetradecane-2,5,9,12-tetraone ((*S*,*S*)-14). According to GP8, (*S*)-**16** (1 mmol, 287 mg), CC (CH_2Cl_2/acetone 200:1). Yield: 54 mg (22%) of (*S*,*S*)-**14b**. Mp 212.1–214.4 °C (decomp.). $[\alpha]_D^{25}$ −26.3 (*c* 1, $CHCl_3$). ^{1}H NMR: 0.88, 1.08 (2s, $2Me_2C$); 3.13 (s, $2CH_2O$); 3.91–4.04, 4.11–4.22 (2m, $2CH_2$); 4.83–5.00 (m, 2CH); 5.83 (br s, 2NH); 7.03–7.14, 7.28–7.42 (2m, 2Ph). ^{13}C NMR: 21.5, 22.7 (2q, $2Me_2C$); 37.6 (t, $2CH_2$); 42.1 (s, 2C); 53.3 (d, 2CH); 71.0 (t, $2CH_2$); 127.3, 128.7, 129.4 (3d, 10 arom. CH); 136.1 (s, 2 arom. C); 169.7, 174.3 (2s, 4CO). ESI-MS: 517 (100, $[M+Na]^+$), 444 (11).

Recrystallization from CH_2Cl_2/*sec*-BuOH yielded crystals of (*S*,*S*)-**14**, suitable for an X-ray crystal structure determination.

4.8. X-ray crystal structure determination of 10, 11a, 12, *trans*-14, (*S*,*S*)-14, 24, and 31

All measurements were made on a Nonius KappaCCD area-detector diffractometer[34] using graphite-monochromated Mo K_α radiation (λ 0.71073 Å) and an Oxford Cryosystems Cryostream 700 cooler. The data collection and refinement parameters are given below[35] and views of the molecules are shown in Figures 1–3. Data reduction was performed with HKL Denzo and Scalepack.[36] The intensities were corrected for Lorentz and polarization effects, but not for absorption. Equivalent reflections were merged. Each structure was solved by direct methods using SIR92,[37] which revealed the positions of all non-hydrogen atoms.

In the case of **10**, **12**, *trans*-**14** and **24**, the molecule sits about a crystallographic centre of inversion.

In the case of (*S*,*S*)-**14**, there are two symmetry-independent molecules in the asymmetric unit. The atomic coordinates of the two molecules were tested carefully for a relationship from a higher symmetry space group using the program PLATON,[38] but none could be found. The crystal is merohedrally twinned. Successful refinement of the structure was achieved using the twin operator [1 0 0/ 0 −1 0/0 0 −1] and the major twin domain has a volume fraction of 0.640(1).

The non-hydrogen atoms were refined anisotropically. Any amide or hydroxy H-atoms in the structures were placed in the positions indicated by a difference electron density map and their positions were allowed to refine together with individual isotropic displacement parameters. All remaining H-atoms were placed in geometrically calculated positions and refined using a riding model where each H-atom was assigned a fixed isotropic displacement parameter with a value equal to $1.2U_{eq}$ of its parent C-atom ($1.5U_{eq}$ for the methyl groups). Except for **11a**, the refinement of each structure was carried out on F^2 by using full-matrix least-squares procedures, which minimized the function $\Sigma w(F_o^2-F_c^2)^2$. The refinement of the structure of **11a** was carried out on *F* by minimizing the corresponding function based of *F*. Corrections for secondary extinction were applied.

Neutral atom scattering factors for non-hydrogen atoms were taken from Ref. 39, and the scattering factors for H-atoms were taken from Ref. 40. Anomalous dispersion effects were included in F_c;[41] the values for f' and f'' were those of Ref. 42. The values of the mass attenuation coefficients are those of Ref. 43. All calculations were performed using the SHELXL97 program[44] with the exception of **11a**, where the teXsan crystallographic software package[45] was used.

Crystal data for **10**. $C_{22}H_{34}N_2O_6$, M=422.52, colorless, plate, crystal dimensions 0.05×0.12×0.30 mm, triclinic, space group *P*bar, Z=1, reflections for cell determination 2354, 2θ range for cell determination 4–55°, a=6.0630(5) Å, b=9.5495(6) Å, c=10.7064(9) Å, α=66.451(3)°, β=83.228(4)°, γ=71.733(5)°, V=539.61(7) Å^3, T=160 K, D_x=1.300 g cm^{-3}, μ(Mo K_α)=0.0941 mm^{-1}, $2\theta_{(max)}$=55°, total reflections measured =11,110, symmetry independent reflections =2457, reflections with $I>2\sigma(I)$=1919, reflections used in refinement =2455, parameters refined =143, $R(F)$ [$I>2\sigma(I)$ reflections] =0.0473, $wR(F^2)$ [all data] =0.1277 ($w=[\sigma^2(F_o^2)+(0.0624P)^2+0.093P]^{-1}$ where $P=(F_o^2+2F_c^2)/3$), goodness of fit =1.059, secondary extinction coefficient 0.09(2), final Δ_{max}/σ=0.001, $\Delta\rho$ (max; min) =0.30; −0.28 *e* Å^{-3}.

Crystal data for **11a**. $C_{17}H_{26}N_2O_3$, M=306.40, colorless, prism, crystal dimensions 0.15×0.15×0.25 mm, monoclinic, space group $P2_1/n$, Z=4, reflections for cell determination 4110, 2θ range for cell determination 4–55°, a=8.1009(1) Å, b=17.1770(3) Å, c=12.6459(2) Å, β=101.112(1)°, V=1726.67(5) Å^3, T=160 K, D_x=1.179 g cm^{-3}, μ(Mo K_α)=0.0806 mm^{-1}, $2\theta_{(max)}$=55°, total reflections measured =37,684, symmetry independent reflections =3973, reflections used in refinement [$I>2\sigma(I)$]=2947, parameters refined =208, $R(F)$ =0.0469, $wR(F)$=0.0479 $w=[\sigma^2(F_o)+(0.005F_o)^2]^{-1}$, goodness of fit =2.962, secondary extinction coefficient =3.3(5)×10^{-6}, final Δ_{max}/σ=0.0005, $\Delta\rho$ (max; min)=0.39; −0.27 *e* Å^{-3}.

Crystal data for **12**. $C_{30}H_{38}N_2O_6$, M=522.64, colorless, tablet, crystal dimensions 0.05×0.10×0.20 mm, monoclinic, space group $P2_1/n$, Z=2, reflections for cell determination 2439, 2θ range for cell determination 4–50°, a=6.0109(2) Å, b=17.3238(5) Å, c=12.9060(5) Å, β=91.855(2)°, V=1343.22(8) Å^3, T=160 K, D_x=1.292 g cm^{-3}, μ(Mo K_α)=0.0896 mm^{-1}, $2\theta_{(max)}$=50°, total reflections measured =17,323, symmetry independent reflections =2359, reflections with $I>2\sigma(I)$ =1731, reflections used in refinement =2358, parameters refined =180, $R(F)$ [$I>2\sigma(I)$ reflections]=0.0540, $wR(F^2)$ [all data] =0.1283 ($w=[\sigma^2(F_o^2)+(0.0395P)^2+0.6836P]^{-1}$ where $P=(F_o^2+2F_c^2)/3$), goodness of fit=1.129, secondary extinction coefficient=0.013(2), final Δ_{max}/σ=0.001, $\Delta\rho$ (max; min)=0.35; −0.18 *e* Å^{-3}.

Crystal data for trans-**14**. $C_{28}H_{34}N_2O_6$, M=494.58, colorless, needle, crystal dimensions 0.05×0.05×0.25 mm, monoclinic, space group $C2/c$, Z=4, reflections for cell determination 2421, 2θ range for cell determination 4–50°,

$a=18.6829(6)$ Å, $b=14.5471(5)$ Å, $c=10.0381(3)$ Å, $\beta=104.943(2)°$, $V=2635.9(2)$ Å^3, $T=160$ K, $D_x=1.246$ g cm^{-3}, μ(Mo K$_\alpha$)$=0.0875$ mm^{-1}, $2\theta_{(max)}=50°$, total reflections measured$=18{,}709$, symmetry independent reflections$=2333$, reflections with $I>2\sigma(I)=1579$, reflections used in refinement$=2333$, parameters refined$=170$, $R(F)$ [$I>2\sigma(I)$ reflections]$=0.0456$, $wR(F^2)$ [all data]$=0.1156$ ($w=[\sigma^2(F_o^2)+(0.041P)^2+1.0127P]^{-1}$ where $P=(F_o^2+2F_c^2)/3$), goodness of fit$=1.040$, secondary extinction coefficient$=0.0040(7)$, final $\Delta_{max}/\sigma=0.001$, $\Delta\rho$ (max; min)$=0.18$; -0.17 e Å^{-3}.

Crystal data for (S,S)-**14**. $C_{28}H_{34}N_2O_6$, $M=494.58$, colorless, prism, crystal dimensions $0.13\times0.13\times0.30$ mm, monoclinic, space group $P2_1$, $Z=4$, reflections for cell determination 7591, 2θ range for cell determination 4–60°, $a=5.4181(2)$ Å, $b=35.569(1)$ Å, $c=13.3108(4)$ Å, $\beta=90.090(1)°$, $V=2565.2(2)$ Å^3, $T=160$ K, $D_x=1.281$ g cm^{-3}, μ(Mo K$_\alpha$)$=0.0899$ mm^{-1}, $2\theta_{(max)}=60°$, total reflections measured$=52{,}489$, symmetry independent reflections$=7611$, reflections with $I>2\sigma(I)=5073$, reflections used in refinement$=7606$, parameters refined$=675$, $R(F)$ [$I>2\sigma(I)$ reflections]$=0.0446$, $wR(F^2)$ [all data]$=0.0899$ ($w=[\sigma^2(F_o^2)+(0.036P)^2]^{-1}$ where $P=(F_o^2+2F_c^2)/3$), goodness of fit$=1.025$, secondary extinction coefficient$=0.012(1)$, final $\Delta_{max}/\sigma=0.001$, $\Delta\rho$ (max; min)$=0.23$; -0.21 e Å^{-3}.

Crystal data for **24**. $C_{28}H_{34}N_2O_6$, $M=494.58$, colorless, prism, crystal dimensions $0.22\times0.25\times0.32$ mm, triclinic, space group $P\bar{1}$, $Z=1$, reflections for cell determination 2078, 2θ range for cell determination 4–50°, $a=5.9467(3)$ Å, $b=10.1233(5)$ Å, $c=10.9664(5)$ Å, $\alpha=73.363(3)°$, $\beta=83.818(3)°$, $\gamma=76.704(3)°$, $V=614.97(5)$ Å^3, $T=160$ K, $D_x=1.335$ g cm^{-3}, μ(Mo K$_\alpha$)$=0.0938$ mm^{-1}, $2\theta_{(max)}=50°$, total reflections measured$=8138$, symmetry independent reflections$=2137$, reflections with $I>2\sigma(I)=1796$, reflections used in refinement$=2136$, parameters refined$=171$, $R(F)$ [$I>2\sigma(I)$ reflections]$=0.0390$, $wR(F^2)$ [all data]$=0.1010$ ($w=[\sigma^2(F_o^2)+(0.043P)^2+0.2587P]^1$ where $P=(F_o^2+2F_c^2)/3$), goodness of fit$=1.067$, secondary extinction coefficient$=0.052(9)$, final $\Delta_{max}/\sigma=0.001$, $\Delta\rho$ (max; min)$=0.18$; -0.25 e Å^{-3}.

Crystal data for **31**. $C_{11}H_{11}NO_3$, $M=205.21$, colorless, prism, crystal dimensions $0.10\times0.17\times0.30$ mm, monoclinic, space group $P2_1/n$, $Z=4$, reflections for cell determination 2470, 2θ range for cell determination 4–55°, $a=5.6230(2)$ Å, $b=8.9884(4)$ Å, $c=20.2034(8)$ Å, $\beta=93.985(3)°$, $V=1018.65(7)$ Å^3, $T=160$ K, $D_x=1.338$ g cm^{-3}, μ(Mo K$_\alpha$)$=0.0982$ mm^{-1}, $2\theta_{(max)}=55°$, total reflections measured$=21{,}205$, symmetry independent reflections$=2333$, reflections with $I>2\sigma(I)=1834$, reflections used in refinement$=2331$, parameters refined$=143$, $R(F)$ [$I>2\sigma(I)$ reflections]$=0.0517$, $wR(F^2)$ [all data]$=0.1359$ ($w=[\sigma^2(F_o^2)+(0.0581P)^2+0.2986P]^{-1}$ where $P=(F_o^2+2F_c^2)/3$), goodness of fit$=1.107$, secondary extinction coefficient$=0.032(6)$, final $\Delta_{max}/\sigma=0.001$, $\Delta\rho$ (max; min)$=0.21$; -0.22 e Å^{-3}.

Acknowledgements

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Preparation and reactions of 3-phosphinyl-1-aza-1,3-butadienes. Synthesis of phosphorylated pyridine and pyrazole derivatives

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Abstract—3-Phosphinyl 1-aza-1,3-butadienes **2** are obtained by aldol condensation between hydrazonoalkyl phosphine oxides and *N*,*N*-dimethylformamide dimethyl acetal. Transamination reaction of these azadienes with amines yields functionalized 1-aza-1,3-butadienes **3**. Cycloaddition processes of these azadienes **2a** with electron-poor dienophiles to give phosphorylated pyridine derivatives **9** and **15** are also reported, while intramolecular cyclization reaction of heterodiene **2b** affords phosphorylated pyrazole **17**.

1. Introduction

Hydrazones constitute an important class of compounds due to the rich chemistry of the hydrazono group and have attracted a great deal of attention in recent years because of their range of applications.[1] They have been extensively used as versatile precursors in acyclic[2] and heterocyclic synthesis,[3] and also form part of the structure of new azapeptides,[4] as well as biologically active compounds.[5]

Aza-Diels–Alder (ADA) reactions[6,7] of 1-azabutadienes are gaining widespread acceptance as tools in heterocyclic synthesis and have found use in the preparation of compounds containing pyridine, quinoline, mono- and diazaanthracene and other nitrogen rings. In particular, α,β-unsaturated dimethylhydrazones have been widely used in hetero Diels–Alder reactions, as 1-azadienes[8] (**I**, $R^1=NMe_2$) (Fig. 1) with electron-deficient partners, as key steps in a variety of syntheses of natural products and other biologically relevant heterocycles.[9]

In this context, we have been involved in the synthesis of 1-aza (**I**),[10] 2-aza-(**II**),[11] and 1,2-diaza-1,3-butadienes (**III**)[12] (Fig. 1) as well as new strategies for the preparation of nitrogen heterocyclic compounds.[13]

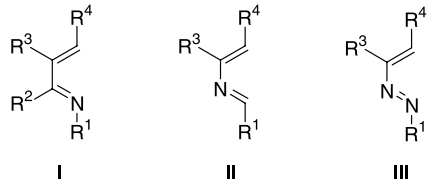

Figure 1.

However, as far as we know no examples of aza-Diels–Alder (ADA) reaction of 1-azadienes containing a phosphorus substituent at *C*-3 position (**I**, $R^1=NMe_2$, $R^3=P(O)Ph_2$, Fig. 1), have been reported. Furthermore, it is known that phosphorus substituents regulate important biological functions,[14] and that molecular modifications involving the introduction of organophosphorus functionalities in simple synthons could be very interesting for the preparation of biologically active compounds.

As a continuation of our work on the cycloaddition reaction of 1-azadienes and on the chemistry of new phosphorus- and nitrogen-substituted heterocycles, here we aim to explore the behaviour of 1-azadienes derived from dimethylhydrazones, such as 3-phosphinyl-1-aza-1,3-butadiene **Ia** (**I**, $R^1=R^4=NMe_2$, $R^3=P(O)Ph_2$) towards dienophiles (a=b), for the preparation of phosphorus-substituted heterocycles **IV** (Scheme 1), as well as the effect of subtituents at *C*-2 position of the azadiene. This strategy could open new entries for the preparation of substituted six-membered heterocycles.

Keywords: Hydrazones; 1-Aza-1, 3-buladienes; Phosphorylated heterocycles; Aza-Diels–Alder.
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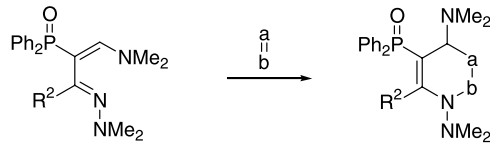


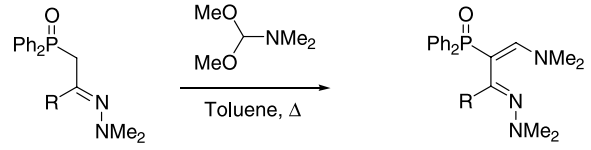
Ia **IV**

Scheme 1.

2. Results and discussion

2.1. Preparation of 3-phosphinyl 1-aza-1,3-butadienes 2

1-Aza-1,3-butadienes **2** (R=H, Me), containing electron-donating groups at *N*-1 and *C*-4 as well as an electron-withdrawing group at *C*-3 position, were prepared by aldol condensation between hydrazonoalkyl phosphine oxides **1** (R=H, Me) and *N*,*N*-dimethylformamide dimethyl acetal (Scheme 2). Thus, reaction of β-hydrazono phosphine oxide **1a** (R=H), prepared from methyl diphenylphosphine oxide, DMF and *N*,*N*-dimethylhydrazine (see Section 3), with *N*,*N*-dimethylformamide dimethyl acetal in refluxing toluene (TLC control) led to the formation of 1-azadiene **2a** (R=H) in good yield (Scheme 2). In the same way, 2-methyl-substituted 1-azadiene **2b** (R=Me) can be obtained by reaction of hydrazonoalkyl phosphine oxide **1b** (R=Me)[10b] with *N*,*N*-dimethylformamide dimethyl acetal. These compounds **2** were characterized by their spectroscopic data, and the vicinal coupling constant ($^3J_{PH}$) in the range of 15.0 Hz indicate a cis-relationship between the phosphorus atom and the vinylic proton, being consistent with an *E*-configuration for the carbon–carbon double bond.[15] ^{31}P NMR spectrum of **2a** showed one absorption at δ_P 33.1 ppm. Likewise, the ^{1}H NMR spectra of **2a** gave a well resolved doublet for the vinylic proton at δ_H 7.12 ppm ($^3J_{PH}$=15.0 Hz), while in ^{13}C NMR a doublet appeared at δ_C 146.0 ppm ($^2J_{PC}$=16.6 Hz) for the methine carbon.



1a R = H
1b R = Me

2a R = H
2b R = Me

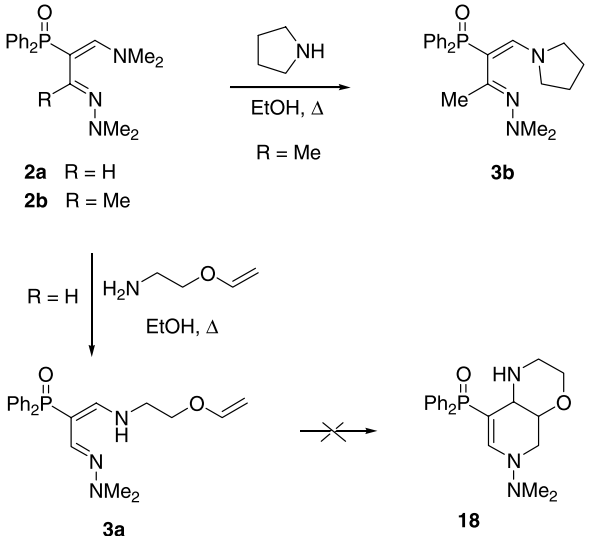
Scheme 2.

As far as we know, this process represents the first example for the preparation of 1-aza-1,3-butadienes containing a phosphorus electron-withdrawing group (Scheme 2).

These results prompted us to extend this reaction and to explore whether other phosphorylated 1-aza-1,3-butadienes can be obtained by transamination reaction of these 1-azadienes **2** with amine derivatives.

2.2. Transamination reaction of 4-dimethylamino 3-phosphinyl 1-aza-1,3-butadienes 2

We studied the transamination reaction between 1-azadienes **2** and simple and functionalized amines. Treatment of 1-azadiene **2b** (R=Me) with pyrrolidine in refluxing EtOH gave the transamination product **3b** in almost quantitative yield (Scheme 3). The spectroscopic data are in agreement with the assigned structure for compound **3b**. This process was extended to other functionalized amine derivatives. Thus, 2-vinyloxy ethylamine reacted with 1-azadiene **2a** (R=H) and gave, after purification, *N*-functionalized 1-aza-1,3-butadiene **3a** in 76% yield (Scheme 3).



2a R = H
2b R = Me

3b **3a** **18**

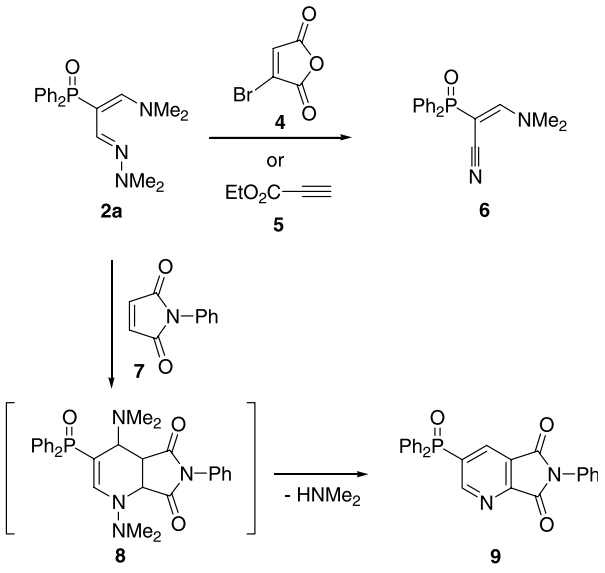
Scheme 3.

Next, we explore whether new phosphorylated 1-aza-1, 3-butadienes could be used as versatile tools for the construction of nitrogen-containing heterocycles through the cycloaddition reaction of these azadienes.

2.3. Cycloaddition reaction of 3-phosphinyl 1-aza-1,3-butadienes 2

The presence of electron-rich groups such as dimethylamino substituents on the terminal nitrogen atom (*N*-1) and on the terminal carbon atom (*C*-4) of the heterodiene system, may favour the Aza-Diels–Alder (ADA) cycloaddition of these substrates. In this way, 1-azadiene systems have been used as building blocks for the preparation of a wide range of heterocycles.[16] However, aza-Diels–Alder (ADA) reaction of 1-aza-1,3-butadienes **2** containing phosphorus substituents has not been reported, although, this strategy could be very useful for the preparation of phosphorylated azaheterocycles.[17]

Initially, we studied the cycloaddition reaction of electron-poor dienophiles such as tetracyanoethylene, naphthoquinone, diethyl azodicarboxylate, tosylisocyanate, diethyl fumarate, diethyl maleate, or maleic anhydride to azadiene **2a**. However, the formation of cyloadducts was not observed and decomposition products were obtained. In the same way, the addition of bromomaleic anhydride **4** or ethyl propiolate **5** to 1-azadiene **2a** gave the phosphorylated α,β-unsaturated nitrile **6** in moderate to good yield (Scheme 4). The formation of this nitrile **6** could be explained by transfer of the dimethylamino group of the azadienic system to the dienophile followed by oxidation to nitrile **6** as reported before for other authors.[18]



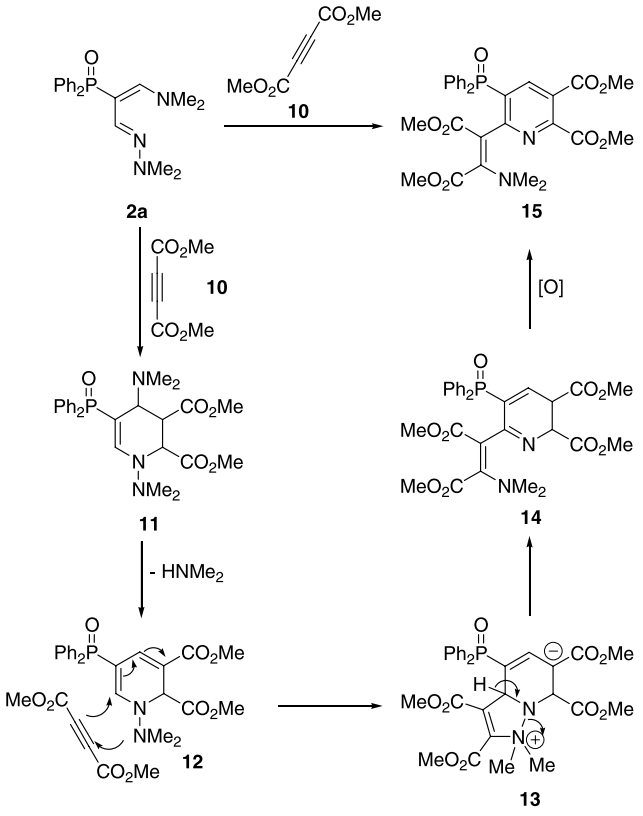
Scheme 4.

The addition of *N*-phenylmaleimide **7** to 1-aza-1,3-butadiene **2a** in the absence of solvent and at 100 °C led to the formation of the functionalized bicyclic cycloadduct **9** (yield of isolated compound 37%, Scheme 4). All attempts to increase the yield of this cycloaddition by addition of Lewis acids such as BF_3, $AlCl_3$, $Cu(OTf)_2$, $InCl_3$ or $LiClO_4$ were unsuccessful giving to the decomposition of the starting 1-azadiene **2a**. As before, the structure of compound **9** was assigned on the basis of NMR spectroscopic data, including MS data and its formation could be rationalized through a initial [4+2] cycloaddition reaction of 1-azadiene **2a** with *N*-phenylmaleimide **7** as dienophile to give tetrahydropyridine **8**, which aromatization with the loss of dimethylamine afforded substituted fused-pyridine **9** (Scheme 4).

A different behaviour was observed when 1-azadiene **2a** (R=H) was treated with an excess of diethyl acetylenedicarboxylate (DEAD) **10** in the absence of solvent and at room temperature to give, in moderate yield the substituted vinyl pyridine **15**, instead of the expected phosphorylated pyridine derivative **11** (Scheme 5).

This substituted vinyl pyridine **15** were characterized by its NMR spectral data, where ^{1}H NMR spectrum of **15** showed a well resolved doublet at δ_H 7.89 ppm with coupling constant $^3J_{PH}=12.8$ Hz corresponding to *H*-4. The structure was finally determined by X-ray study of **15**[19] (Fig. 2). The process could be explained through an initial [4+2] cycloaddition reaction of 1-azadiene **2a** with DEAD **10** to give tetrahydropyridine **11**, followed by elimination of diethylamine and formation of dihydropyridine **12**. Subsequent addition of a second molecule of DEAD to this dihydropyridine **12** may give a bicyclic heterocycle **13**, which five-membered ring opening and aromatization led to the formation of pyridine **15**.

Next, the reaction of 1-azabutadienes **2b** substituted with a methyl group at *C*-2 position as heterodienes with electron-poor dienophiles such as diethyl acetylenedicarboxylate, diethyl azodicarboxylate, or benzoquinone was explored.



Scheme 5.

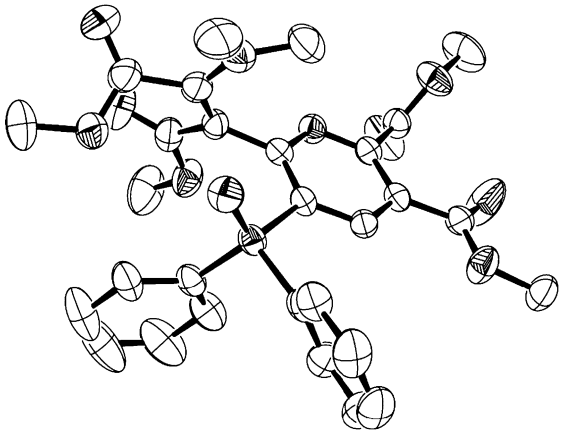
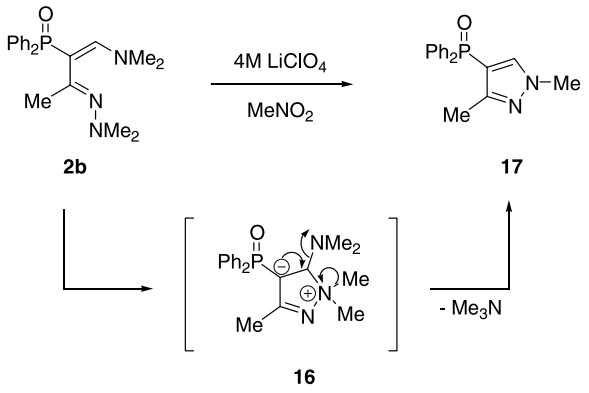


Figure 2. ORTEP view of compound **15**.

However, the formation of cyloadducts was not observed and decomposition products were obtained. As reported by Ghosez et al.,[20] the presence of a methyl group at *C*-2 of the 1-azadiene system **2b** shifts the dimethylamino group at *N*-1 out of the plane (steric inhibition of conjugation), deactivating the diene system and hindering the cycloaddition reaction with dienophiles.

For this reason, we explored the behaviour of these substrates **2b** in the presence of $LiClO_4$ as catalyst. Addition of $LiClO_4$ to methyl-substituted 1-azadiene **2b** in the presence or even in the absence of dienophile and using nitromethane as solvent led to the formation of 4-phosphorylated pyrazole **17** (Scheme 6). Mass spectrometry supported the molecular ion peak, while in the ^{31}P NMR



Scheme 6.

spectrum the phosphinyl group resonated at δ_P 19.2 ppm. The ^1H NMR spectrum showed an absorption at δ_H 7.07 ppm as a singlet for *H*-5 and ^{13}C NMR spectrum showed an absorption at δ_C 137.1 ppm as a doublet with coupling constant $^2J_{PC}=20.6$ Hz for *C*-5 and at δ_C 110.4 ppm as a doublet with coupling constant $^1J_{PC}=125.9$ Hz for the carbon atom directly bonded to the phosphorus atom (*C*-4). The formation of this pyrazole **17** could be explained by intramolecular cyclization involving nucleophilic atack of the dimethylamino group at *N*-1 to the carbon–carbon double bond to give pyrazolidine **16**. Subsequent loss of trimethylamine afforded 4-phosphorylated pyrazole **17** (Scheme 6).

Finally, the behaviour of functionalized 1-aza-1,3-butadiene **3a** (Scheme 3) as heterodiene in intramolecular aza-Diels–Alder (IADA) reaction was explored. However, the formation of cycloadducts **18** was not observed in this reaction and the starting compound **3a** was recovered unchanged. Neither standing the 1-azadiene in refluxing xylene nor the use of Lewis acids as catalyst led to the formation of cycloadducts, probably due to the unfavourable configuration of the amino group in position 4.

In conclusion, the synthesis of 1-dimethylamino-1-aza-1,3-butadienes containing a phosphine oxide group at *C*-3 **2** is described. The process implies aldol condensation between hydrazonoalkyl phosphine oxides and *N*,*N*-dimethylformamide dimethyl acetal. Electron-deficient dienophiles such as *N*-phenylmaleimide or diethyl acetylenedicarboxylate react with *C*-2 unsubstituted 1-azadienes **2a** to afford phosphorylated pyridine derivatives **9** and **15**. However, 1-azadienes **2b** substituted with a methyl group at *C*-2 of the azadiene system do not react with electron-poor dienophiles, and its cyclization in the presence of $LiClO_4$ to give phosphorylated pyrazole **17** has been described. Through these strategies reported in this paper, new access to polysubstituted nitrogen- and phosphorus-containing heterocycles can be designed.

3. Experimental

3.1. General

Solvents for extraction and chromatography were of technical grade. All solvents used in reactions were freshly distilled. All other reagents were recrystallized or distilled as necessary. All reactions were performed under an atmosphere of dry nitrogen. Analytical TLC's were performed with silica gel 60 F_{254} plates. Spot visualization was accomplished by UV light or $KMnO_4$ solution. Flash chromatography was carried out using silica gel 60 (230–400 mesh). Melting points were determined with a Electrothermal IA9100 digital apparatus and are uncorrected. ^1H (300 MHz), ^{13}C (75 MHz) and ^{31}P NMR (120 MHz) spectra were recorded on a Varian Unity Plus 300 MHz spectrometer, using tetramethylsilane (TMS) (0.00 ppm) or chloroform (7.24 ppm) for ^1H NMR spectra, chloroform (77.0 ppm) for ^{13}C NMR spectra, and phosphoric acid (85%) (0.00 ppm) for ^{31}P NMR spectra. Chemical shifts (δ) are given in ppm; multiplicities are indicated by s (singlet), br s (broad singlet), d (doublet), dd (double-doublet), t (triplet), q (quadruplet) or m (multiplet). Coupling constants (*J*) are reported in Hertz. Low-resolution mass spectra (MS) were obtained on a Hewlett Packard 5971 MSD Series spectrometer at 50–70 eV by electron impact (EI) or on a Hewlett Packard 1100 MSD Series spectrometer by chemical ionization (CI). Data are reported in the form *m*/*z* (intensity relative to base peak = 100). Infrared spectra (IR) were taken on a Nicolet FTIR Magna 550 spectrometer, and were obtained as solids in KBr or as neat oils in NaCl. Peaks are reported in cm^{-1}. Elemental analyses were performed in a Leco CHNS-932 instrument. Hydrazonoalkyl phosphine oxide **1b** was synthesized according to literature procedures.[10b]

3.1.1. Synthesis of 1-(dimethylhydrazono)ethenyl diphenylphosphine oxide (1a). To a −60 °C stirred solution of methyl diphenylphosphine oxide (10 mmol, 2.16 g) in THF (40 mL), a solution of butyllithium (1.6 M in hexanes, 11 mmol, 6.9 mL) in THF (2 mL) was added under a nitrogen atmosphere. After 30 min at the same temperature a solution of DMF (15 mmol, 1.16 mL) in THF (5 mL) was added dropwise and the reaction was kept to reach room temperature for 16 h. After this time, H_2O (10 mL) was added to the reaction mixture and stirred for 45 min. HCl (20%) was then added to the reaction mixture until pH = 1 and the mixture was stirred for 30 additional min. Then, the aqueous phase was extracted with CH_2Cl_2 (3×15 mL) and the organic phases were dried over anhydrous $MgSO_4$ and evaporated under vacuum. The obtained aldehyde, without further purifications, was dissolved in dry chloroform (40 mL) and *N*,*N*-dimethylhydrazine (11 mmol, 0.85 mL) was then added at room temperature and stirred for 16 h. The reaction mixture was washed with water (2×10 mL) and the organic phase was dried over anhydrous $MgSO_4$ and evaporated under vacuum. Precipitation of the crude product from ethyl ether and recrystallization from a mixture of hexanes–CH_2Cl_2 (3/1) gave **1a** (2.40 g, 84% two steps) as a white solid: 90–92 °C; ^1H NMR ($CDCl_3$) δ 7.79–7.42 (m, 10H), 6.58–6.53 (m, 1H), 3.37 (dd, $^2J_{PH}=14.2$ Hz, $^3J_{HH}=5.9$ Hz, 2H), 2.66 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 133.1, 131.8, 131.7, 131.6, 131.5, 131.1, 131.0, 130.9, 128.6, 128.5, 128.3, 125.9 (d, $^2J_{PC}=7.5$ Hz), 42.8, 35.7 (d, $^1J_{PC}=68.5$ Hz); ^{31}P NMR ($CDCl_3$) δ 29.9; IR (KBr) 3065, 2959, 2853, 1434, 1182; MS (CI) *m*/*z* 287 (M^+ 1, 100). Anal. Calcd for $C_{16}H_{19}N_2OP$: C, 67.12; H, 6.69; N, 9.78. Found C, 67.10; H, 6.72; N, 9.77.

3.2. General procedure for the synthesis of phosphorylated 1-azadienes (2)

To a stirred solution of β-hydrazono phosphine oxide **1a** or **1b** (1 mmol) in toluene (1 mL), was added *N*,*N*-dimethylformamide dimethyl acetal (1.2 mmol, 0.16 mL) under a nitrogen atmosphere, and the mixture was refluxed for 36 h. Then, the solvent was evaporated under vacuum and the crude product was purified by flash-chromatography (silica gel, AcOEt/MeOH 85:15).

3.2.1. [3-(Dimethylhydrazono)-2-(diphenylphosphinoyl)-propenyl]dimethylamine (2a). The title compound (0.24 g, 69%) obtained as a white solid from 2-(*N*,*N*-dimethylhydrazono)ethyldiphenylphosphine oxide (1 mmol, 0.29 g) as described in the general procedure: mp 92–93 °C; ^{1}H NMR ($CDCl_3$) δ 7.83–7.27 (m, 11H), 7.12 (d, $^{3}J_{PH}$=15.0 Hz, 1H), 3.07 (s, 6H), 2.47 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 146.0 (d, $^{2}J_{PC}$=16.6 Hz), 131.1, 129.7, 129.2 (d, $^{2}J_{PC}$=8.1 Hz), 127.8, 127.7, 127.3, 127.2, 126.1, 126.0, 124.0, 123.9, 123.8, 123.2, 123.0, 87.4 (d, $^{1}J_{PC}$=115.3 Hz), 39.5, 38.7; ^{31}P NMR ($CDCl_3$) δ 33.1; IR (KBr) 3436, 2906, 2793, 1600, 1527, 1122; MS (CI) *m*/*z* 342 (M^++1, 100). Anal. Calcd for $C_{19}H_{24}N_3OP$: C, 66.85; H, 7.09; N, 12.31. Found C, 66.63; H, 7.08; N, 12.34.

3.2.2. [3-(Dimethylhydrazono)-2-(diphenylphosphinoyl) but-1-enyl]dimethylamine (2b). The title compound (0.25 g, 70%) obtained as a white solid from 2-(*N*,*N*-dimethylhydrazono)propyldiphenylphosphine oxide (1 mmol, 0.30 g) as described in the general procedure: mp 78–80 °C; ^{1}H NMR ($CDCl_3$) δ 7.85–7.29 (m, 10H), 6.55 (d, $^{3}J_{PH}$=15.4 Hz, 1H), 2.80 (s, 6H), 2.21 (s, 6H), 1.83 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 163.0, 150.3 (d, $^{2}J_{PC}$=18.6 Hz), 133.8, 132.4, 131.6, 131.4, 130.8, 130.6, 130.4, 130.3, 130.2, 130.1, 127.0, 126.9, 95.6 (d, $^{1}J_{PC}$=115.3 Hz), 45.7, 42.1, 20.8; ^{31}P NMR ($CDCl_3$) δ 30.7; IR (KBr) 3409, 2952, 2653, 1613, 1440, 1241; MS (CI) *m*/*z* 356 (M^++1, 100). Anal. Calcd for $C_{20}H_{26}N_3OP$: C, 67.59; H, 7.37; N, 11.82. Found C, 67.75; H, 7.39; N, 11.83.

3.3. General procedure for the transamination reaction. Synthesis of phosphorylated 1-azadienes (3)

To a stirred solution of 1-azadiene **2a** or **2b** (1 mmol) in dry ethanol (4 mL), the corresponding amine (1.5–2 mmol) was added under a nitrogen atmosphere. The reaction mixture was stirred and refluxing for 8–24 h. The solvent was evaporated under vacuum, and the crude product was purified by flash-chromatography (silica gel, AcOEt/MeOH 95:5).

3.3.1. [3-(Dimethylhydrazono)-2-(diphenylphosphinoyl)-propenyl](2-vinyloxyethyl)amine (3a). The title compound (0.29 g, 76%) obtained as a yellow oil from 1-azadiene **2a** (1 mmol, 0.34 g) and 2-vinyloxy ethylamine (1.5 mmol, 0.17 g) after refluxing for 8 h as described in the general procedure: R_f=0.82, AcOEt/MeOH 3:1; ^{1}H NMR ($CDCl_3$) δ 9.39 (br s, 1H), 7.67–6.78 (m, 12H), 6.37 (dd, $^{3}J_{HH}$=6.1 and 14.0 Hz, 1H), 4.10 (d, $^{3}J_{HH}$=14.0 Hz, 1H), 3.96 (d, $^{3}J_{HH}$=6.1 Hz, 1H), 3.70–3.67 (m, 2H), 3.38–3.36 (m, 2H), 2.58 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 151.0, 149.5 (d, $^{2}J_{PC}$=17.6 Hz), 137.8 (d, $^{2}J_{PC}$=19.5 Hz), 134.5, 133.1, 131.8, 131.7, 131.1, 131.0, 128.2, 128.1, 127.9, 89.6 (d, $^{1}J_{PC}$=126.9 Hz), 87.0, 67.3, 47.4, 43.6; ^{31}P NMR ($CDCl_3$) δ 31.8; IR (NaCl) 3376, 2965, 1619, 1440, 1188; MS (EI) *m*/*z* 383 (M^+, 35). Anal. Calcd for $C_{21}H_{26}N_3O_2P$: C, 65.78; H, 6.83; N, 10.96. Found C, 65.96; H, 6.85; N, 10.93.

3.3.2. [3-(Dimethylhydrazono)-2-(diphenylphosphinoyl)-but-1-enyl]pyrrolidine (3b). The title compound (0.36 g, 94%) obtained as a yellow oil from 1-azadiene **2b** (1 mmol, 0.36 g) and pyrrolidine (2 mmol, 0.17 mL) after refluxing for 24 h as described in the general procedure: R_f=0.84, AcOEt/MeOH 3:1; ^{1}H NMR ($CDCl_3$) δ 7.86–7.21 (m, 10H), 6.57 (d, $^{3}J_{PH}$=15.0 Hz, 1H), 3.16–3.14 (m, 4H), 2.27 (s, 6H), 1.89 (s, 3H), 1.76–1.71 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 164.2 (d, $^{2}J_{PC}$=9.6 Hz), 146.9 (d, $^{2}J_{PC}$=19.6 Hz), 134.0, 132.6, 132.1, 132.0, 131.9, 131.8, 131.0, 130.9, 130.6, 130.5, 130.1, 130.0, 127.5, 127.4, 127.3, 127.2, 97.6 (d, $^{1}J_{PC}$=115.3 Hz), 51.4, 46.4, 24.8, 21.1; ^{31}P NMR ($CDCl_3$) δ 29.7; IR (NaCl) 2945, 2859, 1606, 1175; MS (CI) *m*/*z* 382 (M^++1, 100). Anal. Calcd for $C_{22}H_{28}N_3OP$: C, 69.27; H, 7.40; N, 11.02. Found C, 69.03; H, 7.41; N, 10.98.

3.3.3. Synthesis of 3-dimethylamino-2-(diphenylphosphinoyl)acrylonitrile (6). A mixture of 1-azadiene **2a** (1 mmol, 0.34 g) in xylene (3 mL) and the corresponding dienophile (2 mmol) was stirred at room temperature under a nitrogen atmosphere for 36 h. The solvent was evaporated under vacuum and the crude product was purified by flash-chromatography (silica gel, AcOEt/MeOH 95:5) to afford compound **6** (76–83%) as a yellow oil: R_f=0.65, AcOEt/MeOH 3:1; ^{1}H NMR ($CDCl_3$) δ 7.76–7.33 (m, 11H), 3.31 (s, 3H), 3.11 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 157.0 (d, $^{2}J_{PC}$=12.6 Hz), 133.0, 131.8, 131.7, 131.6, 131.4, 131.2, 128.3, 128.1, 119.0 (d, $^{2}J_{PC}$=12.6 Hz), 62.0 (d, $^{1}J_{PC}$=122.5 Hz), 46.9, 38.0; ^{31}P NMR ($CDCl_3$) δ 28.1; IR (NaCl) 3423, 2925, 2182, 1613, 1434, 1367; MS (EI) *m*/*z* 295 (M^+−1, 100). Anal. Calcd for $C_{17}H_{17}N_2OP$: C, 68.91; H, 5.78; N, 9.45. Found C, 68.78; H, 5.77; N, 9.47.

3.4. General procedure for the cycloaddition reaction. Synthesis of phosphorylated pyridine derivatives (9) and (15)

A mixture of 1-azadiene **2a** (1 mmol, 0.34 g) and the corresponding dienophile (5–6 mmol) was stirred, without solvent, at room temperature or 100 °C under a nitrogen atmosphere. The reaction mixture was stirred for 3–5 h and the crude product was purified by flash-chromatography (silica gel).

3.4.1. 3-(Diphenylphosphinoyl)-6-phenyl pyrrolo[3,4,-*b*]-pyridine-5,7-dione (9). The title compound (0.16 g, 37%) obtained as a white solid from 1-azadiene **2a** and *N*-phenylmaleimide (6 mmol, 1.1 g) after 5 h at 100 °C as described in the general procedure. The crude product was purified by flash-chromatography (silica gel, AcOEt/hexanes 1:1): mp 193–195 °C; ^{1}H NMR ($CDCl_3$) δ 9.18 (d, $^{3}J_{PH}$=6.1 Hz, 1H), 8.34 (d, $^{3}J_{PH}$=10.5 Hz, 1H), 7.59–7.27 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 176.3, 158.7 (d, $^{2}J_{PC}$=12.1 Hz), 135.9, 135.3, 135.2, 134.7, 133.1, 132.0, 131.9, 131.0, 130.8, 129.6, 129.3, 129.2, 129.1, 128.8, 126.4; ^{31}P NMR ($CDCl_3$) δ 25.7; IR (KBr) 3320, 2975, 1689, 1420, 1267; MS (CI) *m*/*z* 425 (M^++1, 100). Anal.

Calcd for $C_{25}H_{17}N_2O_3P$: C, 70.75; H, 4.04; N, 6.60. Found C, 70.66; H, 4.08; N, 6.64.

3.4.2. 6-(2-Dimethylamino-1,2-bis-methoxycarbonyl-vinyl)-5-(diphenylphosphinoyl)pyridine-2,3-dicarboxylic acid dimethyl ester (15). The title compound (0.37 g, 63%) obtained as a white solid from 1-azadiene **2a** and dimethyl acetylenedicarboxylate (5 mmol, 0.62 mL) after 3 h at room temperature as described in the general procedure. The crude product was purified by flash-chromatography (silica gel, AcOEt) and recrystallized from a mixture of CH_2Cl_2–hexanes (1/5): mp 184–186 °C; ^{1}H NMR ($CDCl_3$) δ 7.89 (d, $^3J_{PH}$=12.8 Hz, 1H), 7.61–7.33 (m, 10H), 3.85 (s, 3H), 3.71 (s, 3H), 3.66 (s, 3H), 2.97 (s, 3H), 2.50 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 166.3, 165.7, 164.6, 163.3, 163.2, 155.0, 152.4, 142.5 (d, $^2J_{PC}$=12.6 Hz), 133.2, 132.1, 132.0, 131.9, 131.8, 130.8, 130.6, 129.2, 128.8, 128.6, 127.9, 127.8, 121.6, 121.5, 53.1, 52.9, 52.3, 50.6, 42.6; ^{31}P NMR ($CDCl_3$) δ 25.0; IR (KBr) 3356, 2925, 1739, 1440, 1195; MS (EI) *m/z* 580 (M^+, 3). Anal. Calcd for $C_{29}H_{29}N_2O_9P$: C, 60.00; H, 5.04; N, 4.83. Found C, 59.77; H, 5.02; N, 4.82.

X-ray analysis of compound **15**. A yellow prismatic crystal of $C_{29}H_{29}N_2O_9P$ having approximate dimensions of 0.31×0.22×0.16 mm^3 was mounted on a glass fiber. All measurements were carried out by means of a Nonius KappaCCD diffractometer with graphite monochromated Mo Kα radiation. Crystal data: $C_{29}H_{29}N_2O_9P$, T=293 K, monoclinic, space group $P2_1/n$, with a=8.9660(10) Å, b=28.402(3) Å, c=12.031(9) Å, β=110.111(12)°, V=2877(2) Å^3 and Z=4 (d_{calcd}=1.340 g cm^{-3}), μ(Mo Kα)=0.152 mm^{-1}, no absorption correction; 5072 unique reflections, 3828 with $I>2\sigma(I)$; R=5.7%, R_w=15.4% for reflections with $I>2\sigma(I)$. Crystal data for the structure of this paper have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 283335).

3.4.3. Synthesis of 4-(diphenylphosphinoyl)-1,3-dimethyl-1*H*-pyrazole (17). To a stirred solution of 1-azadiene **2b** (1 mmol, 0.36 g) in nitromethane (3 mL), lithium perchlorate was added until a 4 M solution of the salt was formed under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 16 h, washed with H_2O (2×5 mL) and the aqueous phase was extracted twice with CH_2Cl_2 (5 mL). The organic layer was dried over $MgSO_4$ and evaporated under vacuum, and the crude product was purified by flash-chromatography (silica gel, AcOEt) affording compound **17** (0.22 g, 74%) as a white solid: 176–178 °C; ^{1}H NMR ($CDCl_3$) δ 7.68–7.40 (m, 10H), 7.07 (s, 1H), 3.74 (s, 3H), 2.07 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 152.2 (d, $^2J_{PC}$=9.5 Hz), 137.1 (d, $^2J_{PC}$=20.6 Hz), 134.1, 132.6, 131.8, 131.7, 131.6, 131.4, 128.6, 128.4, 110.4 (d, $^1J_{PC}$=125.9 Hz), 38.7, 13.5; ^{31}P NMR ($CDCl_3$) δ 19.2; IR (KBr) 3104, 3051, 1527, 1440, 1195; MS (EI) *m/z* 295 (M^+-1, 100). Anal. Calcd for $C_{17}H_{17}N_2OP$: C, 68.91; H, 5.78; N, 9.45. Found C, 69.10; H, 5.76; N, 9.43.

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Stereochemical revision of communiols D and H through synthesis

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Abstract—Based on the previously revised stereochemistries for communiols A–C, the *ent*-8-*epi*- and *ent*-6-*epi*-stereoisomers of the original structures proposed for communiols D and H, respectively, were synthesized as highly probable candidates for their genuine structures by using the Sharpless asymmetric dihydroxylation as the source of chirality. Complete accord in spectral properties between each synthetic candidate and the corresponding natural material as well as the fact that communiols A–D and H were all isolated from the same fungal source, led us to the conclusion that the stereochemistries of communiols D and H should also be revised to their (3*S*,5*S*,7*R*,8*S*,11*R*)- and (5*S*,7*R*,8*S*)-forms, respectively.

1. Introduction

In the course of their search for antimicrobial substances produced by coprophilous (dung-colonizing) fungi, Gloer and co-workers isolated four novel polyketide motabolites (communiols A–D, Fig. 1) from horse dung-colonizing *Podospora communis*, and determined their structures by spectroscopic methods including extensive 2D NMR experiments.[1] Quite recently, they also reported the isolation and structural determination of communiol H together with three other structurally-related cyclopentanoids (communiols E–G) from cultures of the same fungus.[2] Communiols A–C (**3**–**5**) showed significant antimicrobial activity against *Bacillus subtilis* and *Staphylococcus aureus*, while communiols D (**1**) and H (**2**) exhibited no such activity. The 2,4-disubstituted tetrahydrofuran substructure incorporated in **3**–**5** is relatively rare as a structural unit of natural products and displays a characteristic difference in substitution pattern from 2,5-disubstituted tetrahydrofurans frequently found in annonaceous acetogenins[3] or ionophores.[4] The 3,7-disubstituted 2,8-dioxabicyclo[3.3.0]-octane structure contained in communiol D (**1**) is also rare, although some related structural units analogous to but different in substitution and/or oxidation patterns from the bicyclic portion of **1** have been found in many natural products such as clerodane diterpenoids and fungal metabolites.[5] The structural uniqueness of communiols A–C (**3**–**5**), coupled with their interesting biological activity, prompted us to undertake the synthesis of **3**–**5**, which recently culminated in their stereochemical revision as described in our previous paper,[6] wherein the genuine stereochemistries of communiols A, B and C were concluded to be represented by structures *ent*-8-*epi*-**3**, *ent*-8-*epi*-**4** and *ent*-6-*epi*-**5**, respectively (Fig. 2). The corrected stereochemistries of **3**–**5**, as well as our presumption that the structurally-related metabolites (communiols D and H) of the same microbial origin should have the same stereochemical arrangement as communiols A–C, led us to

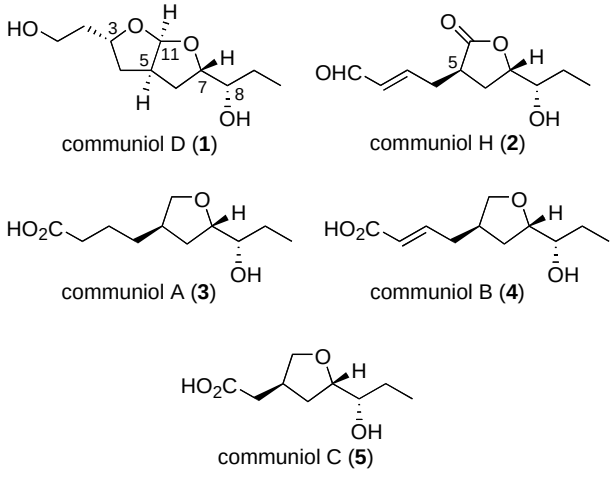

Figure 1. Originally proposed stereochemistries for communiols A–D and H.

Keywords: Communiol; Enantioselective synthesis; Asymmetric dihydroxylation; *Podospora communis*; Dioxabicyclo[3.3.0]octane.
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suppose that the genuine stereochemistries of communiols D and H might be exhibited by structures *ent*-8-*epi*-**1** and *ent*-8-*epi*-**2**, respectively (see Scheme 1). Herein, we describe the enantioselective synthesis of the newly proposed stereoisomers for communiols D and H, which eventually enabled us to revise their stereochemistries as shown later in this paper.

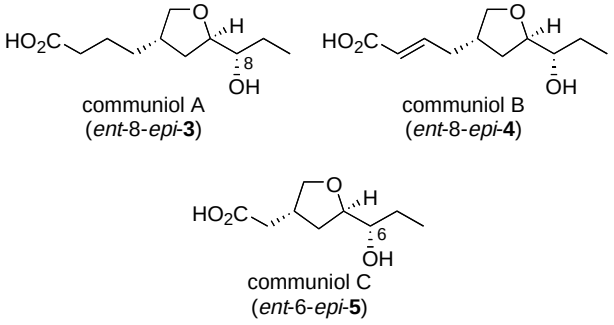
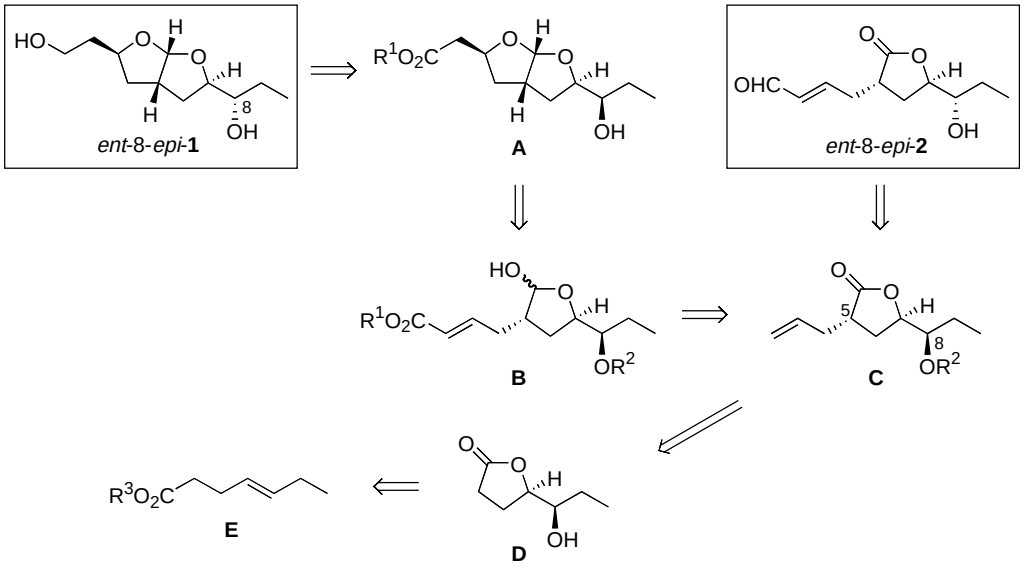


Figure 2. Revised stereochemistries for communiols A–C.

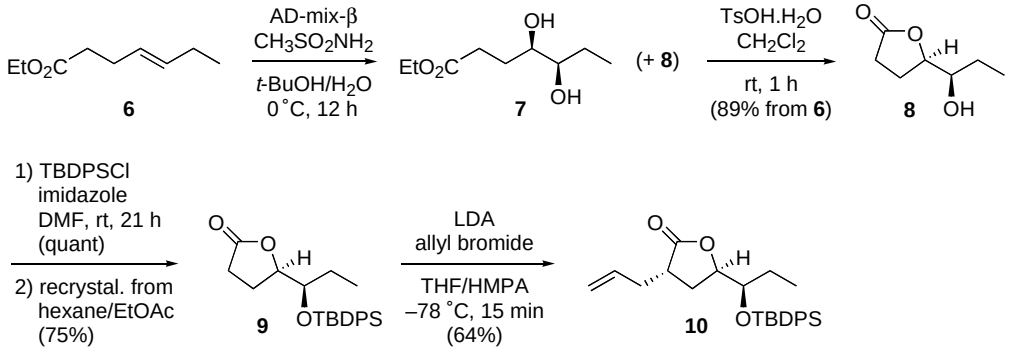
Our retrosynthetic analysis of *ent*-8-*epi*-**1** and *ent*-8-*epi*-**2** is depicted in Scheme 1. The 2,8-dioxabicyclo[3.3.0]octane structural unit in *ent*-8-*epi*-**1** was considered to be installable by the intramolecular Michael addition of lactol **B** into **A**, which, in turn, would readily be converted into the target molecule through inversion of the stereochemistry of the C8–OH and subsequent reduction of the ester group. The intermediate **B** would be obtainable from lactone **C** via elongation of the C5-side chain and reduction of the lactone moiety. Retrosynthetic dissection of the allyl substituent of **C** would lead to known hydroxy lactone **D**, which had previously been prepared from D-glutamic acid in modest yields through four steps.[7] In order to prepare **D**, we adopted the Sharpless asymmetric dihydroxylation of olefinic ester **E** followed by lactonization of the resulting diol, due to its simple experimental operation as compared to the previous procedure. In the synthetic plan presented in Scheme 1, we need to invert the original (*R*)-stereochemistry of the hydroxyl group in **D** (*threo* isomer) at the final stage of the synthesis, which might be avoidable by using *erythro*-**D** possessing the opposite absolute configuration at the OH-bearing stereogenic center. The preparation of the epimeric lactone (*erythro*-**D**) would demand the asymmetric dihydroxylation of the (*Z*)-isomer of olefin **E**. However, utilization of the (*Z*)-isomer as the substrate for the Sharpless asymmetric dihydroxylation was considered to be inappropriate based on a general rule that (*Z*)-olefinic substrates give only modest enantioselectivities on exposure to the asymmetric dihydroxylation conditions, while the (*E*)-olefins can be converted into the corresponding diols with constantly high enantioselectivities.[8] This consideration made us choose **D** as our synthetic intermediate, despite the additional stereoinversion process required at a later stage of the synthesis. As for *ent*-8-*epi*-**2**, transformation of the common intermediate **C** into the target aldehyde seemed to be effected straightforwardly through the inversion of the C8-stereochemistry of **C** and chain-elongation at its C5-side chain.

2. Results and discussion

Our synthesis of the common synthetic intermediate **C** (**10**, in Scheme 2) began with the Sharpless asymmetric dihydroxylation[8,9] of known olefinic ester **6**[10,11] using AD-mix-β as the chiral catalyst. Exposure of the resulting crude product consisting of diol **7** and its lactonization product **8** to acidic conditions brought about complete conversion of **7** into **8**, whose ^{1}H NMR spectrum was in good agreement with that reported for an authentic sample of **8** previously prepared from D-glutamic acid.[7] The absolute stereochemistry of **8** was confirmed by comparison of its specific rotation value ($[\alpha]_D^{22}$ −41.3 (*c* 2.25, CH_2Cl_2)) with the literature value ($[\alpha]_D^{22}$ −46.2 (*c* 2.25, CH_2Cl_2)),[7] and the enantiomeric excess of **8** was estimated to be 96% by analyzing the ^{1}H NMR spectra of the corresponding (*R*)- and (*S*)-MTPA esters.[12] The protection of the hydroxyl group of **8** as its TBDPS ether proceeded uneventfully to



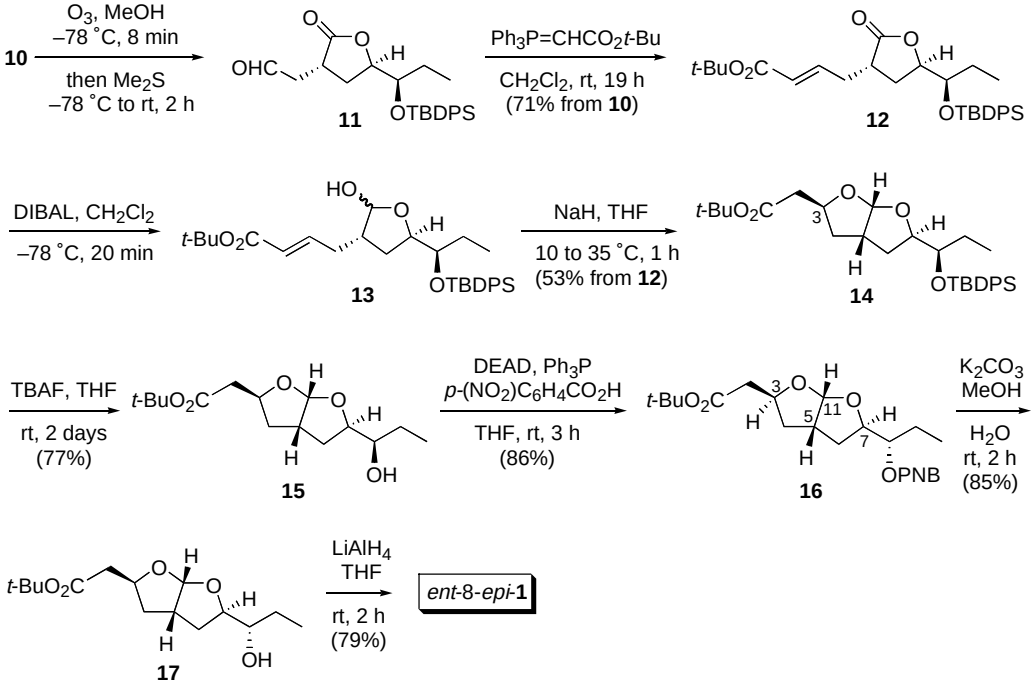
Scheme 1. Retrosynthetic analysis of *ent*-8-*epi*-**1** and *ent*-8-*epi*-**2**.



Scheme 2. Synthesis of common synthetic intermediate **10**.

afford **9** quantitatively. Quite fortunately, and expectedly to some extent, the TBDPS ether **9** was obtained as crystals, which enabled us to enrich the enantiomeric excess of **9** to 100% by a single recrystallization of the crystalline product from hexane/ethyl acetate. The optical integrity of **9** was checked by inspection of the ^{1}H NMR spectra of the corresponding (*R*)- and (*S*)-MTPA esters, which, in turn, were prepared by treatment of the optically enriched silyl ether **9** with TBAF and subsequent (*R*)- and (*S*)-MTPA esterifications of the resulting alcohol **8** ($[\alpha]_D^{22}$ −46.8 (*c* 0.24, CH_2Cl_2)). The protected lactone **9** was then subjected to well-documented trans-selective alkylation with allyl bromide,[13,14] which afforded an 8.3:1 mixture of desired product **10** and the corresponding cis-allylation product in 90% combined yield.[15] Careful chromatographic purification of the mixture, then, furnished diastereomerically pure trans-lactone **10** in 64% isolated yield.

Having secured a sufficient amount of the common synthetic intermediate **10**, we next turned our attention to its elaboration into *ent*-8-*epi*-**1** (Scheme 3), which we considered as the genuine structure for communiol D. Ozonolysis of **10** gave aldehyde **11**, which without purification was subjected to Wittig olefination conditions to afford *t*-butyl ester **12**. Chemoselective reduction of the lactone carbonyl of **12** was achieved by using DIBAL in CH_2Cl_2 to give lactol **13** as an inseparable 2.8:1 mixture of epimers.[15] Upon exposure to NaH in THF, the lactol **13** cyclized intramolecularly into a 6.3:1 mixture of desired bis-THF derivative **14** and its C3-epimer in 53% combined yield from **12**.[15] The preferential formation of the *exo*-stereoisomer **14** could readily be rationalized by considering thermodynamic stability among possible cyclization products (Fig. 3). Of the two possible alkoxide intermediates, **13**α and **13**β, the β-alkoxide intermediate (**13**β) could not cyclize into **14**β due to its extremely strained trans-fused bicyclo[3.3.0]octane system. The intramolecular Michael cyclization of the other alkoxide **13**α, which should also arise from **13**β through an open-chain intermediate, would produce two bis-THF intermediates, *exo*-**14**α and *endo*-**14**α, and higher thermodynamic stability of *exo*-**14**α possessing the C3-substituent on the convex face of the bicyclic system should have driven the equilibrium via retro-Michael reaction from less stable *endo*-**14**α with



Scheme 3. Synthesis of *ent*-8-*epi*-**1**, the newly proposed structure for communiol D.

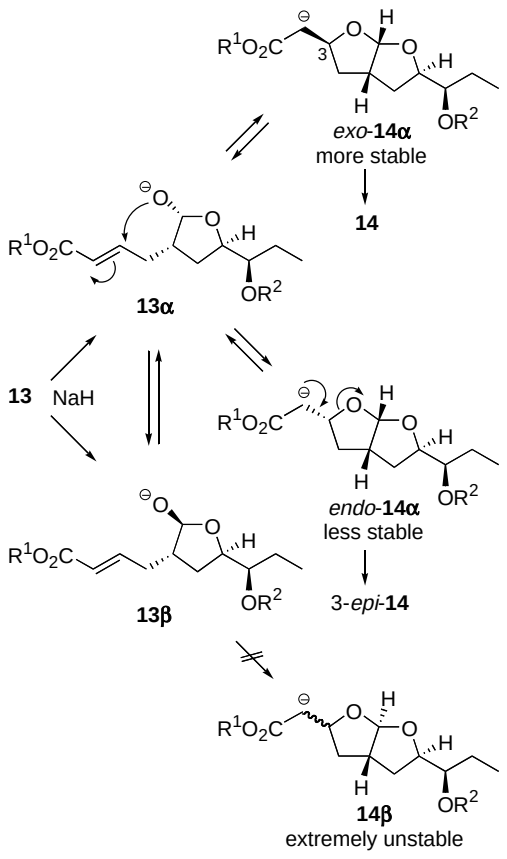


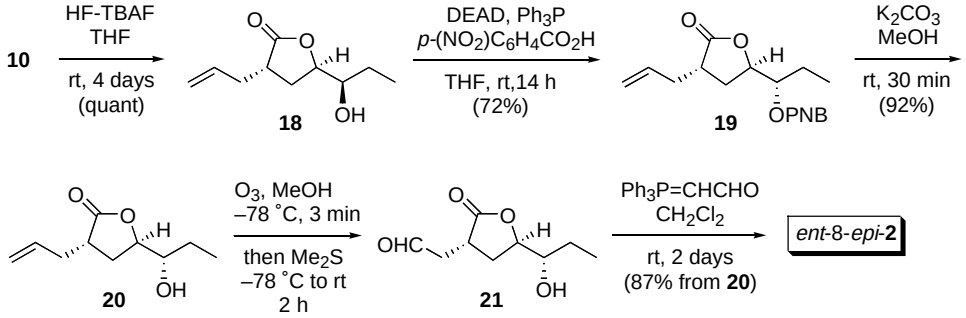
Figure 3. Preferential formation of **14**.

the C3-side chain on the concave face to more stable *exo*-**14α** leading eventually to **14** after aqueous workup. Since the separation of the epimers was difficult at this stage, **14** was subjected, as the mixture, to deprotection of the silyl group followed by the Mitsunobu inversion[16] of the C8-stereochemistry of the resulting alcohol **15** to give diastereomerically pure **16** after simple chromatographic purification. The stereochemistry of **16** was confirmed by observing diagnostic NOE correlations between C3-H and C7-H, and C5-H and C11-H. Finally, methanolysis of the PNB-ester to **17** and subsequent reduction of the *t*-butyl ester group afforded *ent*-8-*epi*-**1**. Direct comparison of the ^{1}H and ^{13}C NMR spectra of *ent*-8-*epi*-**1** with those of natural communiol D assured that these two samples were, as expected, identical, and the optical rotation value of *ent*-8-*epi*-**1** ($[\alpha]_D^{22}$ +3.0 (*c* 0.22, CH_2Cl_2)) also exhibited good agreement with that of natural communiol D ($[\alpha]_D$ +2.7 (*c* 0.3, CH_2Cl_2)).[1] On the basis of these facts, we concluded that the stereochemistry of communiol D, including its absolute configuration, should be represented by *ent*-8-*epi*-**1**.

We next set about the synthesis of *ent*-8-*epi*-**2**, which we presumed to be the real structure of communiol H (Scheme 4). Deprotection of **10** and subsequent inversion of the stereochemistry of the resulting alcohol **18** yielded **19**. Methanolytic removal of its PNB group afforded **20**, the double bond of which was then cleaved by ozonolysis to give aldehyde **21**. Two-carbon elongation of **21** by the Wittig reaction furnished the target molecule, *ent*-8-*epi*-**2**. The ^{1}H and ^{13}C NMR data of *ent*-8-*epi*-**2** were completely identical with those of natural communiol H,[17] which enabled us to revise the relative stereochemistry of communiol C to the 7,8-*erythro*-form of its original structure proposed by Gloer et al. As in the cases of communiols A and B, however, the specific rotation value of synthetic *ent*-8-*epi*-**2** ($[\alpha]_D^{22}$ −12.9 (*c* 0.135, CH_2Cl_2)) was inconsistent with the reported value for natural communiol H ($[\alpha]_D$ −70 (*c* 0.1, CH_2Cl_2)), although they shared the same minus sign. Actually, our synthetic *ent*-8-*epi*-**2** seems to contain a minute amount of dienal (less than 5%, as judged by ^{1}H NMR) generated by an additional two-carbon elongation toward the initially formed enal *ent*-8-*epi*-**2**, but this large difference in specific rotation value seems to be inexplicable by the contamination of such a slight amount of analogous compound. Despite this ambiguity, the complete agreement in ^{1}H and ^{13}C NMR data between synthetic *ent*-8-*epi*-**2** and natural communiol H as well as the consideration of biogenetic similarity among the metabolites isolated from the same fungal source led us to the conclusion that the genuine stereochemistry of communiol H should be depicted as *ent*-8-*epi*-**2**.

3. Conclusion

In summary, the enantioselective total synthesis of *ent*-8-*epi*-**1** and *ent*-8-*epi*-**2**, which we presumed as the genuine structures of communiols D and H, respectively, was accomplished starting from known olefinic ester **6** in 12 and 9 steps, respectively, by using the Sharpless asymmetric dihydroxylation as the source of chirality. The ^{1}H and ^{13}C NMR data of *ent*-8-*epi*-**1** and *ent*-8-*epi*-**2** were identical with those of natural communiols D and H, respectively, and



Scheme 4. Synthesis of *ent*-8-*epi*-**2**, the newly proposed structure for communiol H.

furthermore, the specific rotation value of *ent*-8-*epi*-**1** exhibited good agreement with that of natural communiol D, which allowed us to revise the stereochemistry of communiol D to 3*S*, 5*S*, 7*R*, 8*S* and 11*R*. With regard to communiol H, despite considerable discrepancy in specific rotation value, the complete accord in spectral properties between *ent*-8-*epi*-**2** and natural communiol H as well as the fact that communiol H was isolated together with communils A–D from the same fungal source, strongly supported that genuine structure of commuiols H should be *ent*-8-*epi*-**2**.

4. Experimental

4.1. General

IR spectra were measured as films by a Jasco IR Report-100 spectrometer. ^{1}H NMR spectra (300 or 500 MHz) and ^{13}C NMR spectra (75 or 125 MHz) were recorded with TMS as an internal standard in $CDCl_3$ by a Varian Gemini 2000 spectrometer or a Varian UNITYplus-500 spectrometer. Optical rotation values were measured with a Horiba Septa-300 polarimeter, and mass spectra were obtained with a Jeol JMS-700 spectrometer. Merck silica gel 60 (70–230 mesh) was used for silica gel column chromatography.

4.1.1. (4*R*,5*R*)-5-Hydroxy-4-heptanolide (8). To a stirred mixture of AD-mix-β (53.3 g) and $MeSO_2NH_2$ (3.62 g, 38.0 mmol) in water–*t*-BuOH (1/1, 430 ml) was added a solution **6** (5.94 g, 38.0 mmol) in water–*t*-BuOH (1/1, 30 ml) at 0 °C. After 12 h, $Na_2S_2O_3$ was added, and the resulting mixture was stirred for 3 h at rt. The mixture was extracted with ethyl acetate, and the extract was successively washed with water and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue containing **7** and **8** (1:1, as judged by ^{1}H NMR) was dissolved in CH_2Cl_2 (130 ml), and $TsOH\cdot H_2O$ (1.60 g) was added to the solution. The mixture was stirred for 1.5 h at rt, and then successively washed with satd $NaHCO_3$ aq and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_2 (hexane/ethyl acetate, 3:1) to give 4.88 g (89% from **6**) of **8** as a colorless oil: $[\alpha]_D^{22}$ −41.3 (*c* 2.25, CH_2Cl_2); IR *ν* 3430 (m), 1765 (s), 1190 (s), 750 (s); ^{1}H NMR *δ* 1.03 (3H, t, *J*=7.5 Hz), 1.50–1.70 (2H, m), 1.96 (1H, d, *J*=4.5 Hz, OH), 2.06–2.20 (1H, m), 2.20–2.33 (1H, m), 2.48–2.70 (2H, m), 3.45–3.56 (1H, m), 4.45 (1H, dt, *J*=4.5, 7.5 Hz); ^{13}C NMR *δ* 9.9, 24.1, 26.0, 28.7, 75.0, 82.6, 177.2; HRMS (FAB) *m*/*z* calcd for $C_7H_{13}O_3$ ($[M+H]^+$) 145.0865, found 145.0869.

4.1.2. Determination of the enantiomeric excess of 8. According to the literature,[12] **8** (2.0 mg) was treated with 2 equiv of (*R*)- and (*S*)-MTPA chloride in pyridine to give (*S*)- and (*R*)-MTPA esters, respectively, which were analyzed, without purification, by ^{1}H NMR (500 MHz). The methoxy signal of the (*R*)-MTPA ester derived from **8** was observed at *δ* 3.51 as a singlet, while that from *ent*-**8** contained as an impurity appeared at *δ* 3.58 as a singlet, and each chemical shift was confirmed by the ^{1}H NMR spectrum of the (*S*)-MTPA ester. Calculation of the ratio of the two peak areas revealed the enantiomeric excess of **8** to be 96%.

4.1.3. (4*R*,5*R*)-5-(*t*-Butyldiphenylsilyloxy)-4-heptanolide (9). To a stirred solution of **8** (4.67 g, 32.4 mmol) in DMF (23 ml) was added imidazole (1.22 g, 18.0 mmol) and TBDPSCl (10.95 ml, 42.1 mmol) at rt. After 21 h, satd NH_4Cl aq was added, and the resulting mixture was extracted with ether. The extract was successively washed with water and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_2 (hexane/ethyl acetate, 4:1) to give 12.4 g (quant) of **9** as a white crystalline solid, recystallization of which from hexane/ethyl acetate afforded 9.29 g (75%) of enantiomerically pure **9** as colorless prisms: mp 62.5–63 °C; $[\alpha]_D^{22}$ −23.2 (*c* 1.00, $CHCl_3$); IR (KBr) *ν* 3070 (w), 3050 (w), 1780 (s), 1585 (w); ^{1}H NMR *δ* 0.73 (3H, t, *J*=7.5 Hz), 1.05 (9H, s), 1.35–1.50 (1H, m), 1.58–1.74 (1H, m), 2.07–2.22 (2H, m), 2.40–2.65 (2H, m), 3.65 (1H, ddd, *J*=7.2, 4.8, 3.3 Hz), 4.54 (1H, dt, *J*=3.3, 6.9 Hz), 7.35–7.48 (6H, m), 7.67–7.74 (4H, m); ^{13}C NMR *δ* 9.5, 19.5, 23.2, 25.6, 27.0, 28.5, 76.1, 80.5, 127.5 (2C), 127.7 (2C), 129.7 (1C), 129.8 (1C), 133.2 (1C), 133.9 (1C), 135.8 (4C), 177.4; HRMS (FAB) *m*/*z* calcd for $C_{23}H_{31}O_3Si$ ($[M+H]^+$) 383.2042, found 383.2046. Anal. Calcd for $C_{23}H_{30}O_3Si$: C, 72.21; H, 7.90. Found: C, 72.47; H, 7.63.

4.1.4. Determination of the enentiomeric excess of 9. A solution of the optically enriched silyl ether **9** (1.00 g, 2.61 mmol) in THF (5 ml) was added to a stirred solution of TBAF–HF in THF (prepared by adding several drops of 40% aq HF to 4 ml of 1 M TBAF in THF, pH ca. 7, checked by a pH-test paper). The mixture was stirred for 2 days at rt, diluted with satd $NaHCO_3$ aq and then extracted with ethyl acetate. The extract was successively washed with water and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_2 (hexane/ethyl acetate, 5:1) to give 269 mg (71%) of **8** as a colorless oil ($[\alpha]_D^{22}$ −46.8 (*c* 0.24, CH_2Cl_2)). The enantiomeric excess of **8** just obtained from enriched **9** was determined to be 100% by the same protocol as described above.

4.1.5. (2*S*,4*R*,5*R*)-2-Allyl-5-(*t*-butyldiphenylsilyloxy)-4-heptanolide (10). To a stirred solution of LDA, prepared by treating a solution of diisopropylamine (0.263 ml, 1.88 mmol) and HMPA (0.311 ml, 1.79 mmol) in THF (7 ml) with butyllithium (1.6 M in hexane, 1.12 ml, 1.79 mmol) at −10 °C, was added dropwise a solution of **9** (684 mg, 1.79 mmol) in THF (7 ml) at −78 °C. After 20 min, a solution of allyl bromide (0.155 ml, 1.79 mmol) in THF (3 ml) was added, and the resulting mixture was stirred for 20 min at −78 °C. After the addition of satd NH_4Cl aq, the mixture was extracted with ether. The extract was successively washed with water and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_2 (hexane/ethyl acetate, 20:1) to give 680 mg (90%) of a mixture of **10** and its cis-allylation epimer in a ratio of 8.3:1.[15] Repeated SiO_2-column chromatography of the mixture yielded 481 mg (64%) of pure **10** as a colorless oil: $[\alpha]_D^{22}$ −10.9 (*c* 1.00, $CHCl_3$); IR *ν* 3080 (w), 3050 (w), 1770 (s), 1110 (s), 705 (s); ^{1}H NMR *δ* 0.70 (3H, t, *J*=7.5 Hz), 1.04 (9H, s), 1.39 (1H, ddq, *J*=13.8, 5.4, 7.5 Hz), 1.59–1.74 (1H, m), 1.94 (1H, dt, *J*=13.2, 8.1 Hz), 2.16–2.33 (2H, m), 2.51–2.61 (1H, m), 2.78–2.89 (1H, m), 3.63 (1H, ddd, *J*=7.8, 5.1, 3.3 Hz), 4.48 (1H, ddd, *J*=8.1, 3.9, 3.3 Hz), 5.10 (1H, br d, *J*=11.1 Hz), 5.11 (1H,

dm, J=15.9 Hz), 5.67–5.81 (1H, m), 7.35–7.48 (6H, m), 7.65–7.72 (4H, m); ^{13}C NMR δ 9.6, 19.5, 25.7, 27.0, 28.9, 35.5, 39.1, 76.6, 78.4, 117.7, 127.5 (2C), 127.7 (2C), 129.7 (1C), 129.9 (1C), 133.1 (1C), 133.9 (1C), 134.5, 135.81 (2C), 135.83 (2C), 179.1; HRMS (FAB) *m/z* calcd for $C_{26}H_{35}O_3Si$ ($[M+H]^+$) 423.2355, found 423.2358.

4.1.6. (2*S*,4*R*,5*R*)-2-[3-(*t*-Butoxycarbonyl)-2-propenyl]-5-(*t*-butyldiphenylsilyloxy)4-heptanolide (12). Ozone was bubbled into a stirred solution of **10** (1.00 g, 2.34 mmol) in methanol (10 ml) for 8 min at −78 °C. After the addition of Me_2S (1.7 ml), the mixture was allowed to gradually warm to rt and concentrated in vacuo to give 1.22 g of crude aldehyde **11** as a pale yellow oil, which was then dissolved in CH_2Cl_2 (2.5 ml). To this solution was added $Ph_3P{=}CHCO_2t$-Bu (980 mg, 2.82 mmol), and the mixture was stirred for 19 h at rt, diluted with water and extracted with ethyl acetate. The extract was successively washed with water and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_2 (hexane/ethyl acetate, 15:1) to give 877 mg (71% from **10**) of **12** as a colorless oil: $[\alpha]_D^{22}$ −3.54 (*c* 1.00, $CHCl_3$); IR ν 3060 (w), 3045 (w), 1770 (s), 1710 (s), 1655 (m); ^{1}H NMR δ 0.71 (3H, t, J=7.4 Hz), 1.04 (9H, s), 1.35–1.46 (1H, m), 1.50 (9H, s), 1.62–1.74 (1H, m), 1.90 (1H, dt, J=12.9, 9.0 Hz), 2.21–2.34 (2H, m), 2.67–2.77 (1H, m), 2.83–2.95 (1H, m), 3.62 (1H, ddd, J=8.4, 4.8, 2.7 Hz), 4.50 (1H, ddd, J=9.0, 3.6, 2.7 Hz), 5.80 (1H, d, J=15.6 Hz), 6.74 (1H, dt, J=15.6, 7.5 Hz), 7.36–7.46 (6H, m), 7.64–7.71 (4H, m); ^{13}C NMR δ 9.6, 19.5, 25.8, 27.0, 28.1, 29.3, 33.7, 38.6, 76.6, 78.3, 80.5, 125.7, 127.6 (2C), 127.8 (2C), 129.8 (1C), 130.0 (1C), 133.0 (1C), 133.8 (1C), 135.78 (2C), 135.82 (2C), 143.1, 165.4, 178.4; HRMS (FAB) *m/z* calcd for $C_{31}H_{42}O_5SiNa$ ($[M+Na]^+$) 545.2699, found 545.2703.

4.1.7. *t*-Butyl [(2*S*,3a*S*,5*R*,6a*R*)5-((*R*)-1-(*t*-butyldiphenylsilyloxy)propyl)hexahydrofuro[2,3-*b*]furan-2-yl]acetate (14). To a stirred solution of **12** (169 mg, 0.322 mmol) in CH_2Cl_2 (3 ml) was added dropwise a solution of DIBAL (0.94 M in hexane, 0.377 ml, 0.355 mmol) at −78 °C. After 20 min, the reaction mixture was quenched with a saturated potassium sodium tartrate aqueous solution, stirred for an additional 1.5 h at ambient temperature, and extracted with ethyl acetate. The extract was washed with brine, dried (Na_2SO_4) and concentrated in vacuo to give 185 mg of a 2.8:1 epimeric mixture of lactols **13** as a pale yellow oil,[15] which was then dissolved in THF (3 ml). To the solution was added NaH (0.355 mmol) at −10 °C, and the mixture was stirred for 1 h at 35 °C. The reaction mixture was poured into satd NH_4Cl aq at 0 °C and extracted with ethyl acetate. The extract was washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_2 (hexane/ethyl acetate, 7:1) to give 89.0 mg (53% from **12**) of a 6.3:1 mixture[15] of **14** and its C3-epimer as a colorless oil: $[\alpha]_D^{22}$ +15.8 (*c* 1.00, $CHCl_3$); IR ν 3060 (w), 3045 (w), 1730 (s), 1150 (m), 1110 (s), 1015 (m); ^{1}H NMR δ 0.74 (3H, t, J=7.4 Hz), 1.05 (9H, s), 1.28–1.40 (1H, m), 1.51–1.75 (3H, m), 1.82–1.90 (1H, m), 1.96–2.08 (1H, m), 2.29 (1H, dd, J=15.3, 7.8 Hz), 2.62 (1H, dd, J=15.3, 6.0 Hz), 2.84–2.94 (1H, m), 3.58 (1H, dt, J=3.9, 6.0 Hz), 4.20 (1H, ddd, J=8.1, 6.6, 4.2 Hz), 4.27–4.37 (1H, m), 5.70 (1H, d, J=4.9 Hz), 7.32–7.45 (6H, m), 7.66–7.74 (4H, m); ^{13}C NMR δ 10.0, 19.5, 25.7, 27.1, 28.1, 33.5, 38.9, 41.5, 42.8, 75.3, 76.4, 80.7, 81.1, 109.0, 127.38 (2C), 127.44 (2C), 129.4 (1C), 129.5 (1C), 134.0 (1C), 134.6 (1C), 135.9 (2C), 136.0 (2C), 170.2; HRMS (FAB) *m/z* calcd for $C_{31}H_{44}O_5SiNa$ ($[M+Na]^+$) 547.2856, found 547.2857.

4.1.8. *t*-Butyl [(2*S*,3a*S*,5*R*,6a*R*)-5-((*R*)-1-hydroxypropyl)-hexahydrofuro[2,3-*b*]furan-2yl]acetate (15). To a stirred solution of **14** (112 mg, 0.214 mmol) in THF (0.5 ml) was added a solution of TBAF (1 M in THF, 0.641 ml, 0.641 mmol) at rt. After 2 days, the reaction mixture was diluted with water and extracted with ethyl acetate. The extract was washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_4 (hexane/ethyl acetate, 4:1) to give 47.0 mg (77%) of a 6.3:1 mixture of **15** and its C3-epimer as a colorless oil: $[\alpha]_D^{22}$ −0.92 (*c* 0.50, $CHCl_3$); IR ν 3050 (br w), 1730 (s), 1150 (s), 1020 (s); ^{1}H NMR δ 1.00 (3H, t, J=7.4 Hz), 1.40–1.55 (2H, m), 1.45 (9H, s), 1.70–1.85 (2H, m), 1.89–2.02 (2H, m), 2.14 (1H, d, J=4.0 Hz, OH), 2.32 (1H, dd, J=15.6, 7.5 Hz), 2.64 (1H, dd, J=15.6, 6.0 Hz), 2.95–3.04 (1H, m), 3.24–3.33 (1H, m), 4.06 (1H, dt, J=9.6, 5.7 Hz), 4.39–4.49 (1H, m), 5.75 (1H, d, J=4.8 Hz); ^{13}C NMR δ 10.1, 26.7, 28.1, 34.8, 38.7, 41.5, 43.2, 75.2, 75.9, 80.8, 82.8, 108.8, 170.1; HRMS (FAB) *m/z* calcd for $C_{15}H_{27}O_5$ ($[M+H]^+$) 287.1859, found 287.1861.

4.1.9. *t*-Butyl [(2*S*,3a*S*,5*R*,6a*R*)-5-((*S*)1-(*p*-nitrobenzoyloxy)propyl)hexahydrofuro[2,3-*b*]furan-2-yl]acetate (16). To a stirred solution of **15** (47.2 mg, 0.165 mmol), Ph_3P (130 mg, 0.494 mmol) and *p*-nitrobenzoic acid (83 mg, 0.494 mmol) in THF (2 ml) was added diethylazodicarboxylate (90 μl, 0.494 mmol) at 0 °C, and the mixture was stirred for 3 h at rt. The reaction mixture was quenched with satd $NaHCO_3$ aq and extracted with ethyl acetate. The extract was washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_2 (hexane/ethyl acetate, 5:1) to give 62.0 mg (86%) of **16** as a pale yellow oil: $[\alpha]_D^{22}$ −16.8 (*c* 1.02, $CHCl_3$); IR ν 3110 (w), 1720 (s), 1525 (m), 1270 (s); ^{1}H NMR δ 0.98 (3H, t, J=7.5 Hz), 1.45 (9H, s), 1.72–1.91 (4H, m), 1.98 (1H, ddd, J=12.0, 5.4, 1.8 Hz), 2.08 (1H, dt, J=12.9, 9.2 Hz), 2.32 (1H, dd, J=15.6, 7.5 Hz), 2.65 (1H, dd, J=15.6, 6.0 Hz), 2.93–3.04 (1H, m), 4.34 (1H, dt, J=9.3, 5.7 Hz), 4.39–4.49 (1H, m), 5.16 (1H, dt, J=8.4, 4.8 Hz), 5.72 (1H, d, J=4.9 Hz), 8.21 (2H, d, J=9.0 Hz), 8.30 (2H, d, J=8.7 Hz); ^{13}C NMR δ 9.6, 23.9, 28.0, 34.2, 38.6, 41.4, 42.5, 75.8, 77.7, 80.1, 80.9, 109.0, 123.6, 130.8, 135.8, 150.7, 164.3, 170.3; HRMS (FAB) *m/z* calcd for $C_{22}H_{29}O_8NNa$ ($[M+Na]^+$) 458.1791, found 458.1794.

4.1.10. *t*-Butyl [(2*S*,3a*S*,5*R*,6a*R*)-5-((*S*)-1-hydroxypropyl)hexahydrofuro[2,3-*b*]furan-2-yl]acetate (17). To a stirred solution of **16** (20 mg, 0.047 mmol) in methanol (1 ml) was added 1 M K_2CO_3 aq (1 ml, 1 mmol) at 0 °C, and the mixture was stirred at rt for 2 h. After the addition of satd NH_4Cl aq, the reaction mixture was extracted with ethyl acetate. The extract was washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_2 (hexane/ethyl acetate, 1:1) to give 11 mg (85%) of **17** as a colorless oil: $[\alpha]_D^{22}$ −5.73 (*c* 0.565, $CHCl_3$); IR ν 3470 (br m), 1725 (s), 1150 (s), 1010 (s), 755 (s); ^{1}H NMR δ 0.99 (3H, t, J=7.4 Hz), 1.34–1.46

(2H, m), 1.45 (9H, s), 1.62 (1H, ddd, J=12.9, 6.3, 2.1 Hz), 1.80 (1H, dt, J=12.6, 9.3 Hz), 1.98 (1H, ddd, J=12.9, 5.4, 2.1 Hz), 2.01 (1H, br s, OH), 2.11 (1H, dt, J=12.6, 9.9 Hz), 2.32 (1H, dd, J=15.3, 6.9 Hz), 2.63 (1H, dd, J=15.3, 6.0 Hz), 2.92–3.02 (1H, m), 3.73–3.80 (1H, m), 4.13 (1H, ddd, J=9.9, 5.7, 3.3 Hz), 4.39–4.49 (1H, m), 5.75 (1H, d, J=4.9 Hz); ^{13}C NMR δ 10.2, 25.4, 28.0, 30.9, 38.8, 41.6, 42.8, 72.3, 76.1, 80.8, 82.4, 108.7, 170.3; HRMS (FAB) m/z calcd for $C_{15}H_{27}O_5$ ($[M+H]^+$) 287.1858, found 287.1860.

4.1.11. (*S*)-1-[(2*R*,3a*S*,5*S*,6a*R*)-5-(2-Hydroxyethyl)hexahydrofuro[2,3-*b*]furan2-yl]-1-propanol (*ent*-8-*epi*-1). To a stirred suspension of $LiAlH_4$ (3.5 mg, 0.092 mmol) in THF (0.5 ml) was added dropwise a solution of **17** (11 mg, 0.039 mmol) in THF (0.5 ml) at 0 °C, and the mixture was stirred for 1 h at rt. To the mixture was successively added ethyl acetate and a large amount of SiO_2. The resulting slurry was stirred for 30 min and filtered through a pad of Celite. The filter cake was washed with 2-propanol, and the combined filtrate and washings were concentrated in vacuo. The residue was chromatographed over SiO_2 (hexane/ethyl acetate, 1:5) to give 6.7 mg (79%) of *ent*-8-*epi*-**1** as a colorless oil: $[\alpha]_D^{22}$ +3.0 (c 0.22, CH_2Cl_2); IR ν 3395 (br s), 1090 (m), 1005 (s); ^{1}H NMR δ 0.99 (3H, t, J=7.5 Hz), 1.36–1.46 (2H, m), 1.62 (1H, ddd, J=12.9, 6.5, 2.1 Hz), 1.72 (1H, ddt, J=14.3, 8.4, 6.0 Hz), 1.76–1.88 (2H, m), 1.89 (1H, ddd, J=13.0, 5.9, 2.5 Hz), 1.99 (1H, br s, OH), 2.13 (1H, dt, J=12.6, 9.9 Hz), 2.29 (1H, br s, OH), 2.91–3.02 (1H, m), 3.72–3.83 (3H, m), 4.13 (1H, ddd, J=9.9, 6.0, 3.3 Hz), 4.24–4.34 (1H, m), 5.78 (1H, d, J=4.9 Hz); ^{13}C NMR δ 10.4, 25.4, 31.1, 37.7, 39.4, 42.6, 60.9, 72.4, 79.1, 82.6, 108.9; HRMS (FAB) m/z calcd for $C_{11}H_{21}O_4$ ($[M+H]^+$) 217.1440, found 217.1444.

4.1.12. (2*S*,4*R*,5*R*)-2-Allyl-5-hydroxy-4-heptanolide (18). To a stirred solution of TBAF–HF in THF (prepared by adding a few drops of 40% aq HF to 0.71 ml of 1 M TBAF in THF, pH ca. 7, checked by a pH-test paper) was added a solution of **10** (200 mg, 0.473 mmol) in THF (1.0 ml) at rt. After 4 days, the reaction mixture was quenched with satd $NaHCO_3$ aq and extracted with ethyl acetate. The extract was washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_2 (hexane/ethyl acetate, 1:1) to give 88 mg (quant) of **18** as a colorless oil: $[\alpha]_D^{22}$ −15.4 (c 1.00, $CHCl_3$); IR ν 3440 (br m), 3075 (w), 1755 (s), 1180 (m); ^{1}H NMR δ 1.02 (3H, t, J=7.4 Hz), 1.51–1.69 (2H, m), 1.84 (1H, d, J=6.0 Hz, OH), 2.06 (1H, ddd, J=13.2, 8.4, 7.2 Hz), 2.22–2.35 (2H, m), 2.52–2.62 (1H, m), 2.80–2.91 (1H, m), 3.45–3.54 (1H, m), 4.41 (1H, dt, J=8.4, 4.4 Hz), 5.14 (1H, dt, J=17.1, 2.1 Hz), 5.16 (1H, d, J=10.2 Hz), 5.78 (1H, ddt, J=17.1, 10.2, 6.9 Hz); ^{13}C NMR δ 10.0, 26.2, 29.5, 35.3, 39.1, 75.3, 80.5, 118.0, 134.3, 179.1; HRMS (FAB) m/z calcd for $C_{10}H_{17}O_3$ ($[M+H]^+$) 185.1178, found 185.1178.

4.1.13. (2*S*,4*R*,5*S*)-2-Allyl-5-(*p*-nitrobenzoyloxy)-4-heptanolide (19). To a stirred solution of **18** (90.0 mg, 0.473 mmol), Ph_3P (186 mg, 0.710 mmol) and *p*-nitrobenzoic acid (119 mg, 0.710 mmol) in THF (4 ml) was added diethylazodicarboxylate (0.129 ml, 0.710 mmol) at 0 °C, and the mixture was stirred for 14 h at rt. The reaction mixture was quenched with satd $NaHCO_3$ aq and extracted with ethyl acetate. The extract was washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_2 (hexane/ethyl acetate, 7:1) to give 114 mg (72%) of **19** as a pale yellow oil: $[\alpha]_D^{22}$ +4.54 (c 1.15, $CHCl_3$); IR ν 3080 (w), 1770 (s), 1725 (s), 1525 (s), 1265 (s); ^{1}H NMR δ 1.02 (3H, t, J=7.4 Hz), 1.83 (2H, qui, J=7.5 Hz), 2.13 (1H, ddd, J=13.5, 8.4, 7.8 Hz), 2.26–2.40 (2H, m), 2.52–2.62 (1H, m), 2.74–2.86 (1H, m), 4.66 (1H, dt, J=8.4, 5.1 Hz), 5.15 (1H, dm, J=9.6 Hz), 5.16 (1H, dm, J=17.1 Hz), 5.29 (1H, dt, J=5.1, 6.5 Hz), 5.77 (1H, ddt, J=17.1, 9.6, 6.9 Hz), 8.17–8.22 (2H, m), 8.29–8.34 (2H, m); ^{13}C NMR δ 9.6, 23.3, 28.1, 35.1, 38.5, 76.6, 77.7, 118.4, 123.7, 130.8, 133.9, 135.0, 150.8. 164.1, 177.9; HRMS (FAB) m/z calcd for $C_{17}H_{20}O_6N$ ($[M+H]^+$) 334.1290, found 334.1291.

4.1.14. (2*S*,4*R*,5*S*)-2-Allyl-5-hydroxy-4-heptanolide (20). To a stirred solution of **19** (54.0 mg, 0.163 mmol) in methanol (0.5 ml) was added a catalytic amount of K_2CO_3 at 0 °C, and the mixture was stirred at rt for 30 min. The reaction mixture was filtered through a pad of Celite, and the filter cake was washed with ethyl acetate. The combined filtrate and washings were concentrated in vacuo, and the residue was chromatographed over SiO_2 (hexane/ethyl acetate, 4:1) to give 27.5 mg (92%) of **20** as a colorless oil: $[\alpha]_D^{22}$ −0.84 (c 0.56, $CHCl_3$); IR ν 3445 (br m), 3080 (w), 1750 (s), 1180 (m); ^{1}H NMR δ 1.02 (3H, t, J=7.5 Hz), 1.42–1.54 (2H, m), 1.93 (1H, ddd, J=13.2, 8.1, 6.9 Hz), 2.03 (1H, d, J=4.5 Hz, OH), 2.22–2.34 (1H, m), 2.40 (1H, ddd, J=13.2, 9.9, 5.1 Hz), 2.51–2.61 (1H, m), 2.78–2.89 (1H, m), 3.78–3.86 (1H, m), 4.41 (1H, ddd, J=8.1, 5.1, 3.3 Hz), 5.12 (1H, dm, J=10.2 Hz), 5.14 (1H, dm, J=16.8 Hz), 5.78 (1H, ddt, J=16.8, 10.2, 6.9 Hz); ^{13}C NMR δ 9.9, 25.1, 26.5, 35.3, 39.2, 73.4, 80.6, 118.0, 134.5, 179.4; HRMS (FAB) m/z calcd for $C_{10}H_{17}O_3$ ($[M+H]^+$) 185.1178, found 185.1178.

4.1.15. (2*S*,4*R*,5*S*)-2-(3-Formyl-2-propenyl)-5-hydroxy-4-heptanolide (*ent*-8-*epi*-2). Ozone was bubbled into a stirred solution of **20** (27.5 mg, 0.149 mmol) in methanol (1 ml) for 3 min at −78 °C. After the addition of Me_2S (0.3 ml), the mixture was allowed to gradually warm to rt and concentrated in vacuo. The residue was roughly chromatographed over SiO_2 (hexane/ethyl acetate, 3:1) to give 26.9 mg **21** as a colorless oil, which was immediately dissolved in CH_2Cl_2 (0.5 ml). To this solution was added Ph_3P=CHCHO (87.9 mg, 0.289 mmol), and the mixture was stirred for 2 days at rt. After concentration of the reaction mixture, the residue was chromatographed over SiO_2 (hexane/ethyl acetate, 1:5) to give 27.5 mg (87% from **20**) of *ent*-8-*epi*-**2** as a colorless oil (*ent*-8-*epi*-**2**/two carbon-homologated dienal=95:5): $[\alpha]_D^{22}$ −12.9 (c 0.135, CH_2Cl_2); IR ν 3445 (m), 2750 (w), 1760 (s), 1685 (s), 1170 (m), 975 (m), 805 (m), 745 (m); ^{1}H NMR δ 1.02 (3H, t, J=7.4 Hz), 1.43–1.55 (2H, m), 1.90 (1H, dt, J=13.2, 8.4 Hz), 2.01 (1H, d, J=3.9 Hz, OH), 2.50 (1H, ddd, J=13.2, 9.6, 3.6 Hz), 2.48–2.59 (1H, m), 2.85 (1H, dddd, J=15.0, 6.5, 5.5, 1.5 Hz), 2.95–3.06 (1H, m), 3.80–3.89 (1H, m), 4.45 (1H, dt, J=8.4, 3.5 Hz), 6.20 (1H, ddt, J=15.6, 8.0, 1.5 Hz), 6.84 (1H, dt, J=15.6, 7.1 Hz), 9.55 (1H, d, J=8.0 Hz); ^{13}C NMR δ 10.3, 25.7, 27.4, 34.4, 38.7, 73.8, 80.7, 135.1, 153.5, 178.4, 193.7; HRMS (EI) m/z calcd for $C_{11}H_{16}O_4$ (M^+) 212.1048, found 212.1041.

Acknowledgements

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15. The ratio of **10** and its cis-isomer (8.3:1) was calculated from the peak areas of the proton signals (500 MHz ^{1}H NMR) assignable to 7-H (communiol numbering) of each epimer (δ 4.48 for **10**, and δ 4.37 for the cis-isomer). Comparison of the peak areas of the hemiacetalic proton signals of the epimeric mixture **13** (δ 4.95 and 5.25) indicated their ratio to be 2.8:1. The acetalic protons of **14** and its C3-epimer were observed at δ 5.70 and δ 5.58, respectively, which showed the ratio of them to be 6.3:1.
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17. The ^{1}H and ^{13}C NMR data for *ent*-8-*epi*-**2** shown in the Section 4 were recorded with TMS as an internal standard in $CDCl_3$, while the published data for natural communiol H are reported to have been obtained by using the solvent signals (δ_H 7.24, δ_C 77.0) as internal standards.[2] This difference brought about subtle disagreement in the NMR chemical shifts between synthetic *ent*-8-*epi*-**2** and natural communiol H (see Section 4 and Ref. 2). However, we were able to make sure that the two samples were identical by regarding the chemical shifts due to C*H*Cl_3 and *C*DCl_3 in our measurements as 7.24 and 77.0 ppm, respectively.

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A very concise synthesis of a potent *N*-(1,3-thiazol-2-yl)pyridin-2-amine KDR kinase inhibitor

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Abstract—A very concise synthesis of a potent KDR kinase inhibitor **1** is described. The synthesis features an exceedingly efficient one-pot preparation of the aminothiazole **6** followed by Pd–Xantphos catalyzed cross-coupling with chloropyridine aldehyde **11**. Reductive amination of the resulting aldehyde **10** with the piperazine fragment **9** afforded the final product.

1. Introduction

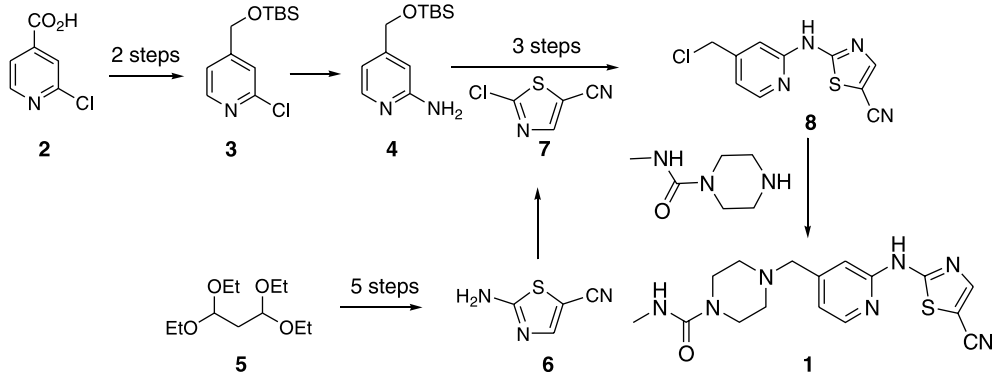
Angiogenesis, the growth of new blood vessels, is promoted by vascular endothelial growth factor (VEGF). The kinase insert domain-containing receptor (KDR) is a tyrosine kinase-linked receptor for VEGF. Inhibition of KDR has been shown to inhibit tumor angiogenesis and the growth of tumors in animal models.[1]

Recent efforts at Merck led to the discovery of a potent and orally active KDR kinase inhibitor **1**.[2] The reported synthesis of the target molecule required a total of 14 steps from commercially available starting materials (Scheme 1).

One feature of the synthesis we wished to avoid was the functional group and protecting group manipulation at the 4-position of the pyridine. Additionally, a reverse polarity coupling of a chloropyridine **3** with aminothiazole **6** would remove an additional two steps. We also sought a shorter synthesis of the thiazole fragment **6**.[3] We envisioned that the most efficient synthetic scheme would involve a direct coupling of the aminothiazole **6** with 2-chloropyridine aldehyde **11** (Scheme 2). The resulting aldehyde **10** could then be directly connected to the piperazine fragment **9** by reductive amination.

2. Results and discussion

Although Pd-catalyzed arylation of amines has attracted a large amount of attention in the past few years,[4] there are few examples of Pd-catalyzed N-arylation of heteroarylamines. Among these examples, no functional groups on the aryl halides or the arylamines were present.[5,6] Additionally,



Scheme 1. Medicinal chemistry synthesis.

Keywords: KDR; Pd-catalysis; Xantphos; Aminothiazole; Chloropyridine; Piperazine.
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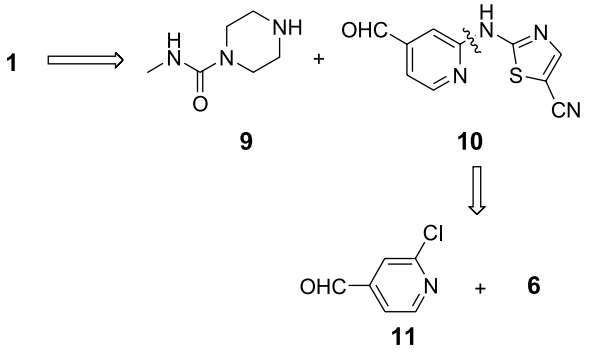


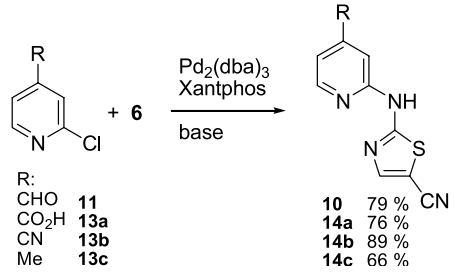
Scheme 2. Retrosynthetic analysis.

N-arylation of an aminothiazole had not been reported at all in the literature despite the important biological activities of 2-arylaminothiazoles.[7] Attempted coupling of aminothiazole **6** with chloropyridine aldehyde **11** under palladium catalysis failed to give any desired coupling product **10** using literature procedures. The reaction conditions were indeed too harsh to tolerate the aldehyde functional group. We were also unsuccessful in attempts using analogous copper catalyzed coupling. Thus, development of a new Pd-catalyzed N-arylation that can tolerate the sensitive aldehyde functional group was required to implement our synthetic strategy.

The main cause for the failure was likely due to decomposition of the aldehyde in the presence of a strong base such as KO^{t}Bu. Thus, screening experiments were carried out with milder bases such as K_3PO_4, Na_2CO_3, K_2CO_3, Cs_2CO_3 etc. We found that many ligands that are generally successful for Pd-catalyzed carbon–nitrogen bond forming reactions with some functional group compatability[4] such as BINAP, DPEphos, DPPF, P(*o*-Tol)$_3$, DPPB, and phosphinobiphenyls[8] gave either no reaction or decomposition due to the presence of the aldehyde group. Only Xantphos[9] gave significant amounts of the desired product **10**. Solvent screening indicated that toluene was the solvent of choice. More polar solvents led to rapid decomposition of **11**. Commercial powdered K_3PO_4 was found to give better results than granular material. Under optimized reaction conditions, the coupling product was obtained in as high as 83% assay yield.

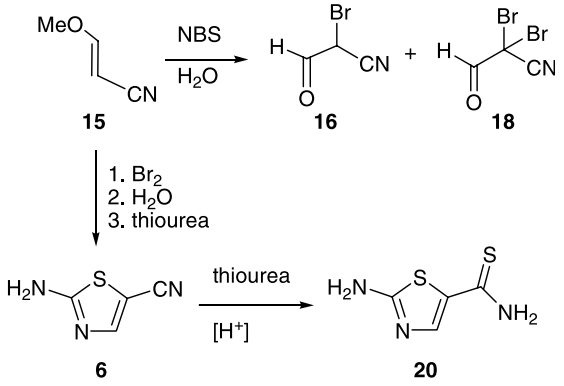
Subsequently, we found that scale up from a sealed Schlenk tube to a septum-capped flask connected to nitrogen line led to incomplete conversion. Rigorous purification of the starting materials and more careful exclusion of air/moisture did not improve the outcome. The only rationale for the low conversion appeared to be the loss of water in an unsealed system. Indeed, addition of 0.5–1.0 equiv of water restored the reaction profile. By maintaining the water content in the reaction vessel, the reaction can be scaled up reproducibly. The role of water in the reaction is unclear. It might retard the formation of imine or make K_3PO_4 a more soluble and more effective base.

2-Chloropyridines with different substituents could also be efficiently coupled with aminothiazole **6** (Scheme 3). Note the carboxylic acid group was also tolerated. The less activated substrate **13c** required a higher catalyst loading. The Pd–Xantphos catalyst system proved to be general for N-arylation of heteroaryl amines, and the results from our laboratories were published recently.[10]



Scheme 3. Pd-catalyzed coupling of 2-chloropyridines.

With the key coupling step established, the search for a more efficient synthesis of the aminothiazole was initiated. We envisioned that bromination of inexpensive 3-methoxyacrylonitrile (**15**) followed by reaction with thiourea and subsequent cyclization should deliver the desired aminothiazole **6** quickly (Scheme 4). The starting material **15** was available as a ~2:1 *E*/*Z* mixture. The presence of *E*/*Z* isomers should be inconsequential, however.



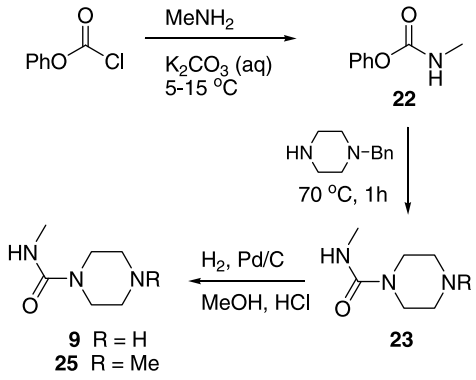
Scheme 4. Preparation of aminothiazole.

Treatment of **15** with NBS in MeOH followed by thiourea and aqueous HCl failed to give any aminothiazole **6**. The surprisingly sluggish hydrolysis of the dimethylacetal intermediate was likely the problem. Thus, the reaction was carried out in aqueous acetonitrile to directly give aldehyde **16**. This approach was, however, complicated by the formation of the dibromo aldehydre **18**. On the other hand, in dry acetonitrile, bromine reacted with **15** almost instantaneously at 0 °C affording the dibromo adduct, which was easily hydrolyzed to **16** in situ by simply adding water. Subsequent reaction with thiourea followed by cyclization at 60 °C afforded aminothiazole **6**. However, the yield was only 30%. The main product was identified to be the thioamide **20**,[11] formed by reaction of the aminothioazole **6** and excess thiourea[12] under strongly acidic conditions. By neutralizing the generated HBr with NaOAc, we were able to improve the yield of **6** to 78% after carbon treatment and recrystallization. Thus, we have developed an efficient, economical and high yielding one-pot synthesis of the aminothiazole **6**.

Although the piperazine fragment **9** can be made from methyl isocyanate[13] and mono-protected piperazine, the toxicity of methyl isocyanate makes it undesirable for large scale use. The most common methods for the

preparation of unsymmetrical ureas use alkyl or aryl chloroformates to take advantage of the substantial reactivity differences of the two reactive sites.[14] Phenyl chloroformate is ideal due to its relatively low cost and enhanced reactivity of the carbamate for the urea formation step.

Methylamine was reacted first to obviate issues with its low boiling point as the urea formation step required somewhat elevated temperature. Thus phenyl chloroformate was added to a biphasic mixture of methylamine, K_2CO_3 in acetonitrile and water to give the carbamate intermediate **22** (Scheme 5). Rapid addition of phenyl chloroformate and efficient cooling were required to avoid significant hydrolysis of **22**.

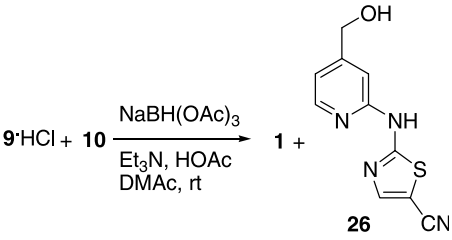


Scheme 5. Piperazine fragment.

The carbamate was not isolated but directly reacted with *N*-benzyl-piperazine. Using unprotected piperazine was not viable due to severe bis-acylation problem. The reaction was carried out at 70 °C furnishing **23** as a crystalline dihydrate in 87% yield. The benzyl protecting group was then removed by a simple hydrogenolysis in MeOH with 5% Pd/C to give the desired product **9**. Interestingly, a small amount of methylation product **25** was observed with higher catalyst loading (20 wt% of 10% Pd/C). Since compound **9** is a viscous oil, it was isolated as its crystalline HCl salt in 83% overall yield based on *N*-benzyl-piperazine.

With both partners **9** and **10** in hand, the final coupling step via a reductive amination was investigated (Eq. 1).[15]

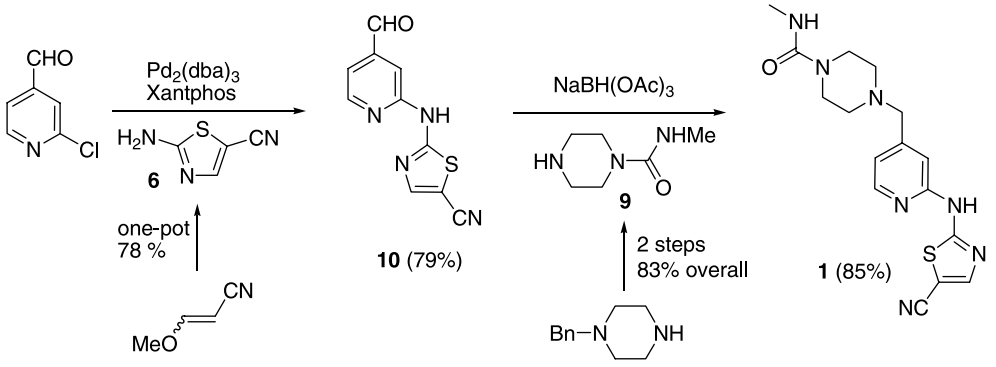
We found that the reaction was very sluggish in THF with $NaBH(OAc)_3$ and 5 equiv of HOAc, giving only 25% conversion after stirring at rt overnight. Furthermore, the undesired alcohol **26** was the main product. It was found that polar solvents such as DMF, DMAc, NMP or DMSO gave much better results. DMAc was selected for better impurity rejection and more controlled crystallization.



(1)

Approximately 4–5 equiv of acetic acid seemed optimal for the reaction. The selectivity for reductive amination versus aldehyde reduction increased from 60:40 to 93:7 as HOAc was increased from 0.5 to 5 equiv. Further increase afforded negligible improvement. Strong acids such as MsOH gave mixed results. With 0.5 equiv of MsOH, the selectivity was 84:15, but with 1.0 equiv of MsOH, the selectivity decreased to 73:27 and the reaction was also slower. Using the HCl salt of piperizine **9** directly instead of the free base for the reductive amination resulted in significantly lower selectivity (**1**:**26**=83:17). Fortunately, this problem was easily remedied by adding 1.1 equiv of Et_3N. The optimal temperature for the reaction was 15–25 °C. Lowering the temperature to 0–5 °C decreased the reaction rate without noticeable selectivity enhancement. Addition of $NaBH(OAc)_3$ in portions provided better results than addition in one portion.[16] With the optimized reaction conditions, the final product **1** was isolated in 85% yield.

Thus, we have developed a very concise and convergent synthesis of a potent thiazolyl pyridine KDR inhibitor **1** (Scheme 6). The longest sequence is only three steps and the total number of steps is only five. The overall yield was 52% from 3-methoxyacrylonitrile or 71% from *N*-benzylpiperazine. Key discoveries include a new Pd catalyzed N-arylation of aminothioazole that can tolerate an aldehyde functional group and an exceedingly efficient one-pot synthesis of the aminothiazole **6**.



Scheme 6. Overall synthesis of **1**.

3. Experimental

3.1. General

All commercial chemicals were used as is unless otherwise noted. ^{1}H and ^{13}C NMR spectra were measured at 400 and 100 MHz, respectively.

3.1.1. 2-Amino-thiazole-5-carbonitrile (6). Bromine (2.88 kg, 18 mol) was added to a solution of 3-methoxy-acrylonitrile (1.50 kg, 18.0 mol, ~2:1 *E*/*Z* mixture) in acetonitrile (3.0 L) at 0–5 °C. The mixture was stirred for 20 min then cold water (~5 , 12 L) was added. After vigorous stirring for 1 h, $NaOAc \cdot 3H_2O$, (2.21 kg, 16.2 mol) was added and the stirring continued for 15 min. Thiourea (1.51 kg, 19.8 mol) was added and the mixture was stirred at 5–10 °C for 2 h. More $NaOAc \cdot 3H_2O$ (1.47 kg, 10.8 mol) was added and the reaction mixture was heated to 60 °C over 1 h and stirred for 3 h. The reaction mixture was cooled to 10 °C and the pH adjusted to 3.8–4.0 with NaOH (10 N, 1.1 L) to crystallize the product. The product was filtered, washed with water and then dried to afford 1.93 kg of the crude aminothiazole as a brown solid in 96.6% purity. It was purified by treatment with Darco KB-B (384 g) in acetone (36 L) at 50 °C. The mixture was filtered at 50 °C and the filtrate was concentrated in vacuo to 6.5 kg. Heptane (9.6 L) was added slowly. The product was filtered, washed with 2:1 heptane/acetone and dried to furnish 1.79 kg of **6** as a pinkish solid, 78% yield corrected for 98.2% purity. Mp 210–230 °C (dec); ^{1}H NMR (DMSO-d_6) δ 8.10 (s, 2H), 7.81 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 173.1, 152.5, 114.2, 89.4; IR (KBr, cm^{-1}) 3346, 3277, 2206, 1636, 1491, 1231, 1060. Anal. Calcd for $C_4H_3N_3S$: C, 38.39; H, 2.42; N, 33.58; S, 25.62. Found: C, 38.28; H, 2.36; N, 33.34; S, 25.27.

3.1.2. 2-Amino-thiazole-5-carbothioic acid amide (20). A mixture of aminothiazole **6** (657 mg, 95%, 5.0 mmol) and thiourea (456 mg, 6.0 mmol) in MeCN (2 mL) and H_2SO_4 (10 N, 2 mL) was stirred at 60 °C for 1.5 h then cooled to rt. It was neutralized with NaOH (10 N) until pH=8–9. The acetonitrile was distilled off. Water (5 mL) was added and the product was filtred and air dried affording 557 mg of **20** dihydrate as a yellow solid (17.5% water). ^{1}H NMR (DMSO-d_6) δ 9.00 (s, 1H), 8.94 (s, 1H), 7.64 (s, overlapping NH_2 and C_4–H, 3H); ^{13}C NMR (DMSO-d_6) δ 187.7, 174.4, 139.8, 129.4.

3.1.3. 5-(4-Formyl-pyridin-2-ylamino)-thiazole-2-carbonitrile (10). A mixture of chloropyridine **11** (1.49 kg, 10.5 mol), 2-aminothiazole **6** (1.27 kg, 10.0 mol), powdered K_3PO_4 (2.34 kg, 11.0 mol), $Pd_2(dba)_3$ (114.5 g, 0.125 mol), Xantphos (159 g, 0.275 mol) and toluene (20 L) was degassed and then heated to 60 °C. After slowly adding degassed water (90 mL, 5.0 mol), the mixture was heated to 90 °C and stirred for 8 h. The reaction mixture was then cooled to rt and the crude product was collected by filtering and washing with toluene. DMAc (24 L) was added to the crude product solid and the insoluble material was filtered off. The filtrate was acidified with concentrated HCl (110 mL) to pH 2.7 and then water (3 L) was added. The mixture was concentrated under vacuum with slow addition of water (3 L) to remove most of the toluene. More water (14 L) was very slowly added to crystallize the product, which was filtered and washed sequentially with 5:4 DMAc/water, water and acetone, and then dried at 40 °C under vacuum to give 1.92 kg of **10** in 79% yield corrected for 94.5% purity. Mp 291–292 °C; ^{1}H NMR (DMSO-d_6) δ 12.51 (br s, 1H), 10.01 (s, 1H), 8.58 (d, J=5.2 Hz, 1H), 8.25 (s, 1H), 7.48 (s, 1H), 7.41 (d, J=5.2 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 193.2, 163.4, 151.8, 150.8, 148.3, 144.2, 115.7, 114.5, 111.5, 95.8; IR (polyethylene, cm^{-1}) 2216, 1708, 1618, 1553, 1438, 1408, 1243, 825. HRMS calcd for $(M+H)^+$ $C_{10}H_7N_4OS$ 231.03351, found 231.03447.

3.1.4. 2-(4′-Carboxy-2′-pyridylamino)-5-cyanothiazole (14a). Under nitrogen, a re-sealable Schlenk tube was charged with $Pd_2(dba)_3$ (13.8 mg, 3% Pd), Xantphos (26.0 mg, 4.5%), aminothiazole **6** (132 mg, 1.05 mmol, 1.05 equiv), 2-chloro-4-pyridine carboxylic acid (159 mg, 1.0 mmol, 1.0 equiv), Na_2CO_3 (256 mg, 2.4 mmol, 2.4 equiv), and dioxane (4 mL). Water (18 mg, 1.0 mmol, 1.0 equiv) was added dropwise while stirring the mixture. The Schlenk tube was quickly sealed and immersed into a 100 °C oil bath. After 16 h, the mixture was cooled, filtered, and washed with toluene and water and 1 N HCl. After drying the crude solid, it was recrystallized from DMAc (acidified with aqueous HCl) and water to give the product as a yellow solid (187 mg, 76%). Mp>300 °C; ^{1}H NMR (DMSO-d_6) δ 13.65 (br s, 1H), 12.39 (br s, 1H), 8.48 (d, J=5.1 Hz, 1H), 7.23 (s, 1H), 7.57 (s, 1H), 7.40 (d, J=5.1 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 166.1, 163.4, 151.5, 150.7, 147.7, 141.0, 116.6, 114.5, 111.7, 95.6; IR (polyethylene, cm^{-1}) 3261, 2223, 1699, 1619, 1551, 1382, 763. Anal. Calcd for $C_{10}H_6N_4O_2S$: C, 48.78; H, 2.46; N, 22.75. Found: C, 48.57; H, 2.22; N, 22.38.

3.1.5. 2-(4′-Cyano-2′-pyridylamino)-5-cyanothiazole (14b). Following similar procedure as **14a** but using K_3PO_4 (1.2 equiv) as base, toluene (4 mL) as solvent, and 0.5 equiv of water (without the HCl wash in the workup) gave compound **14b** as a yellow solid (201 mg, 89%). Mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.56 (s, 1H), 8.59 (dd, J=5.2, 0.6 Hz, 1H), 8.29 (s, 1H), 7.44 (dd, J=5.2, 0.6 Hz, 1H), 7.41 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 163.0, 151.2, 150.8, 148.4, 121.6, 118.6, 117.1, 114.6, 114.3, 96.2; IR (polyethylene, cm^{-1}) 2246, 2220, 1621, 1544, 1435, 1395. HRMS calcd for $(M+H)^+$ $C_{10}H_6N_5S$ 228.0338, found 228.0349.

3.1.6. 2-(4′-Methyl-2′-pyridylamino)-5-cyanothiazole (14c). The reaction was carried out following similar procedure as **14a** but using 8 mol% of Pd, 1.4 equiv of **6**, Na_2CO_3 (2.0 equiv) as base, and toluene (8 mL) as solvent. The reaction mixture was cooled, diluted with THF and then filtered. The filtrate was concentrated and the residue was purified by silica gel column chromatography followed by recrystallization in toluene to give compound **14c** as a brown solid (142 mg, 66%). Mp 292–293 °C; ^{1}H NMR (DMSO-d_6) δ 12.09 (br s, 1H), 8.19 (br s, 2H), 6.89 (s, 1H), 6.87 (d, J=4.9 Hz, 1H), 2.28 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 163.7, 150.8, 150.7, 150.0, 146.2, 119.4, 114.8, 111.8, 95.1, 21.2; IR (polyethylene, cm^{-1}) 2215, 1622, 1440, 1147. HRMS calcd for $(M+H)^+$ $C_{10}H_9N_4S$ 217.0542, found 217.0553.

3.1.7. 4-Benzyl-piperazine-1-carboxylic acid methylamide (23). Phenyl chloroformate (2.59 kg) was added to a mixture of aqueous K_2CO_3 (4.56 kg in 6.0 L water), acetonitrile (12 L) and methylamine (40 wt% in water, 1.40 kg) as rapidly as possible while maintaining the exothermic reaction at 0–15 °C. After stirring for 15 min, 1-benzyl-piperazine (2.65 kg) was added and the mixture was heated to 70 °C and stirred for 1 h. The reaction mixture was concentrated under vacuum to remove the MeCN. NaOH (5 N, 7.5 L) was added and the mixture was seeded and then cooled to rt to crystallize the product. It was filtered and washed with cold 0.5 N aqueous NaOH, ice-cold water and dried to give 3.59 kg of the dihydrate of **23**, 87% yield. Mp 99–100 °C; ^{1}H NMR (DMSO-d_6) δ 7.32–7.24 (m, 5H), 6.37 (q, J=4.4 Hz, 1H), 3.45 (s, 2H), 3.25 (t, J=4.9 Hz, 4H), 2.55 (d, J=4.4 Hz, 3H), 2.82 (t, J=4.9 Hz, 4H); ^{13}C NMR (DMSO-d_6) δ 158.4, 138.4, 129.3, 128.6, 127.4, 62.5, 52.9, 43.8, 27.5. Anal. Calcd for $C_{13}H_{19}N_3O$: C, 66.92; H, 8.21; N, 18.01. Found: C, 66.93; H, 8.14; N, 18.17.

3.1.8. Piperazine-1-carboxylic acid methylamide hydrochloride (9·HCl). HCl (12 N, 74 mL) was added to MeOH (7 L) and then *N*-benzylpiperizine **23** dihydrate (2.69 kg, 10.0 mol) was added. The mixture was hydrogenated using 5% Pd/C (180 g) under 40 psi of hydrogen pressure at 40 °C for 18 h. The mixture was filtered and concentrated. *i*-PrOH (5 L) was added followed by HCl (12 N aqueous, 0.77 L) until the pH of the solution reached ~3. The mixture was then concentrated under vacuum and flushed with more *i*-PrOH until the water content was <1%. After stirring at 15 °C for 5 h, the crystallized product was filtered, washed with *i*-PrOH and dried to give 1.53 kg of **9**·HCl in 95% yield. Mp 185.5–187.0 °C; ^{1}H NMR (DMSO-d_6) δ 9.51 (br s, 2H), 6.79 (q, J=4.3 Hz, 1H), 3.53 (t, J=5.2 Hz, 4H), 2.98 (t, J=5.2 Hz, 4H), 2.55 (d, J=4.3 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 157.6, 42.4, 40.4, 27.1. Anal. Calcd for $C_6H_{14}ClN_3O$: C, 40.11; H, 7.85; N, 23.39; Cl, 19.73. Found: C, 39.99; H, 7.73; N, 23.22; Cl, 19.91.

3.1.9. 4-[2-(2-Cyano-thiazol-5-ylamino)-pyridin-4-ylmethyl]-piperazine-1-carboxylic acid methylamide (1). $NaBH(OAc)_3$ (2.54 kg, 12.0 mol) was added in six portions (0.5 h/portion) to a mixture of pyridine aldehyde **10** (2.44 kg, 94.5 wt%, 10.0 mol), piperazine urea **9**·HCl salt (1.99 kg, 11.0 mol), DMAc (15 L), Et_3N (1.53 L, 11.0 mol) and acetic acid (2.29 L, 40.0 mol) with slight cooling (15 °C). After stirring for 1 h, water (7.5 L) was slowly added to complete the crystallization. The product was filtered, washed sequentially with 2:1 DMAc/water, 1:1 acetone/water, and acetone, dried at 100 °C to give 3.07 kg of **1** in 85% yield. Mp 248–249.5 °C; ^{1}H NMR (DMSO-d_6) δ 12.16 (s, 1H), 8.30 (d, J=5.3 Hz, 1H), 8.24 (s, 1H), 7.12 (s, 1H), 7.01 (dd, J=5.3, 0.9 Hz, 1H), 6.40 (q, J=4.4 Hz, 1H), 3.50 (s, 2H), 3.28 (t, J=4.7 Hz, 4H), 2.55 (d, J=4.3 Hz, 3H), 2.33 (t, J=4.7 Hz, 4H); ^{13}C NMR (DMSO-d_6) δ 163.1, 157.9, 150.4, 150.2, 145.9, 117.7, 114.2, 110.6, 94.6, 60.6, 52.5, 43.3, 27.1; IR (KBr, cm^{-1}): 2209, 1622, 1552, 1446, 1265, 1147, 1007, 882, 786. Anal. Calcd for $C_{16}H_{19}N_7OS$: C, 53.76; H, 5.36; N, 27.43; S, 8.97. Found: C, 53.63; H, 5.27; N, 27.41; S, 8.79.

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Synthesis of dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,3,4,6-tetraones bearing a sugar moiety

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Abstract—The synthesis of a series of dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,3,4,6-tetraones, structurally related to the G2 checkpoint inhibitor granulatimide and bearing a sugar moiety, is described. Substitutions were carried out at the 6-position of the glycosyl unit to lead to an amino substituent as observed in many biologically active compounds such as anthracyclins or staurosporines.

1. Introduction

Many antitumor products from natural sources such as anthracyclins, staurosporine and related compounds, contain a heteroaromatic framework to which a sugar moiety is attached (Fig. 1).[1–6] In daunomycin, the prototype anthracyclin antibiotic, the planar chromophore intercalates into the DNA, whereas the amino-sugar lies in the minor groove.[7] In rebeccamycin, the sugar unit is required for a tight interaction with DNA and for topoisomerase I inhibition.[8] In the case of staurosporine, UCN-01 and K-252a, which are kinase inhibitors interacting with the ATP binding site, the sugar unit mimics the ATP ribose.[9,10] An amino function on the carbohydrate moiety is often present. Its amphiphilic properties may facilitate the access to the different cell compartments.

Granulatimide and isogranulatimide (Fig. 1) are natural products, which inhibit the G2 checkpoint of the cell cycle.[11,12] In response to DNA damage, the cell cycle checkpoints are activated. Their role consists in blocking the cell cycle to allow time for DNA repair. G2 checkpoint inhibition has attracted a widespread interest because, in more than 50% of tumors, the G1 checkpoint is lacking. Therefore, the combination of a G2 checkpoint inhibitor with a DNA damaging agent should force selectively cancer cells into a premature and lethal mitosis, due to an accumulation of DNA lesions. The G2 checkpoint is regulated by various kinases and mainly by the Chk1 kinase. Granulatimide and isogranulatimide, as well as staurosporine and UCN-01, are Chk1 inhibitors. Compounds structurally related to granulatimide have been recently synthesized.[13–21]

In this paper, we report the synthesis of bis-imide granulatimide analogue **9** bearing a maleimide moiety instead of the imidazole heterocycle, and a glucosyl unit attached to the indole nitrogen. The sugar moiety was introduced with the aim of improving the biological activity but also to increase the solubility. Indeed, the bis-imide granulatimide analogues previously synthesized in our laboratory proved to be very insoluble that could reduce cell penetration. Moreover, 6′-chloro then 6′-azido substituents were introduced to finally obtain the 6′-amino derivative **13**.

2. Results and discussion

The first steps of the synthesis were perfected with a methyl protective group on the imide nitrogen, which could be removed via an anhydride according to a method described by Brenner et al.,[22] and, in parallel, with a benzyloxymethyl protective group removable at the end of the synthesis[23,24] to give the free imide nitrogen, necessary for kinase inhibition.

Several methods are described in the literature for the glycosylation of indole derivatives. The Koenigs–Knorr

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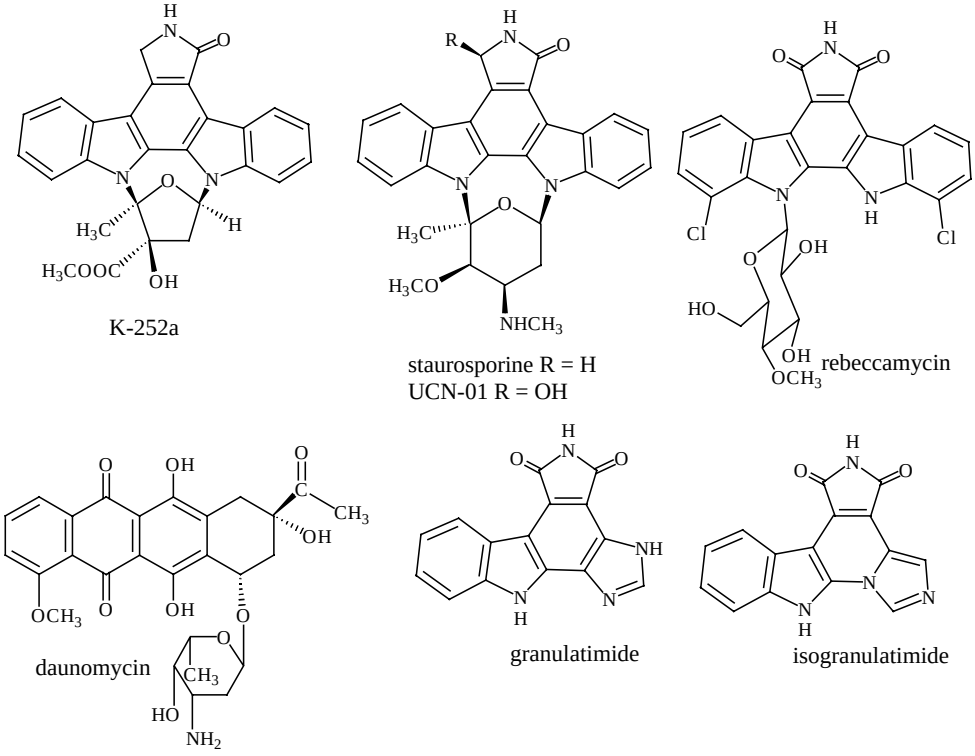


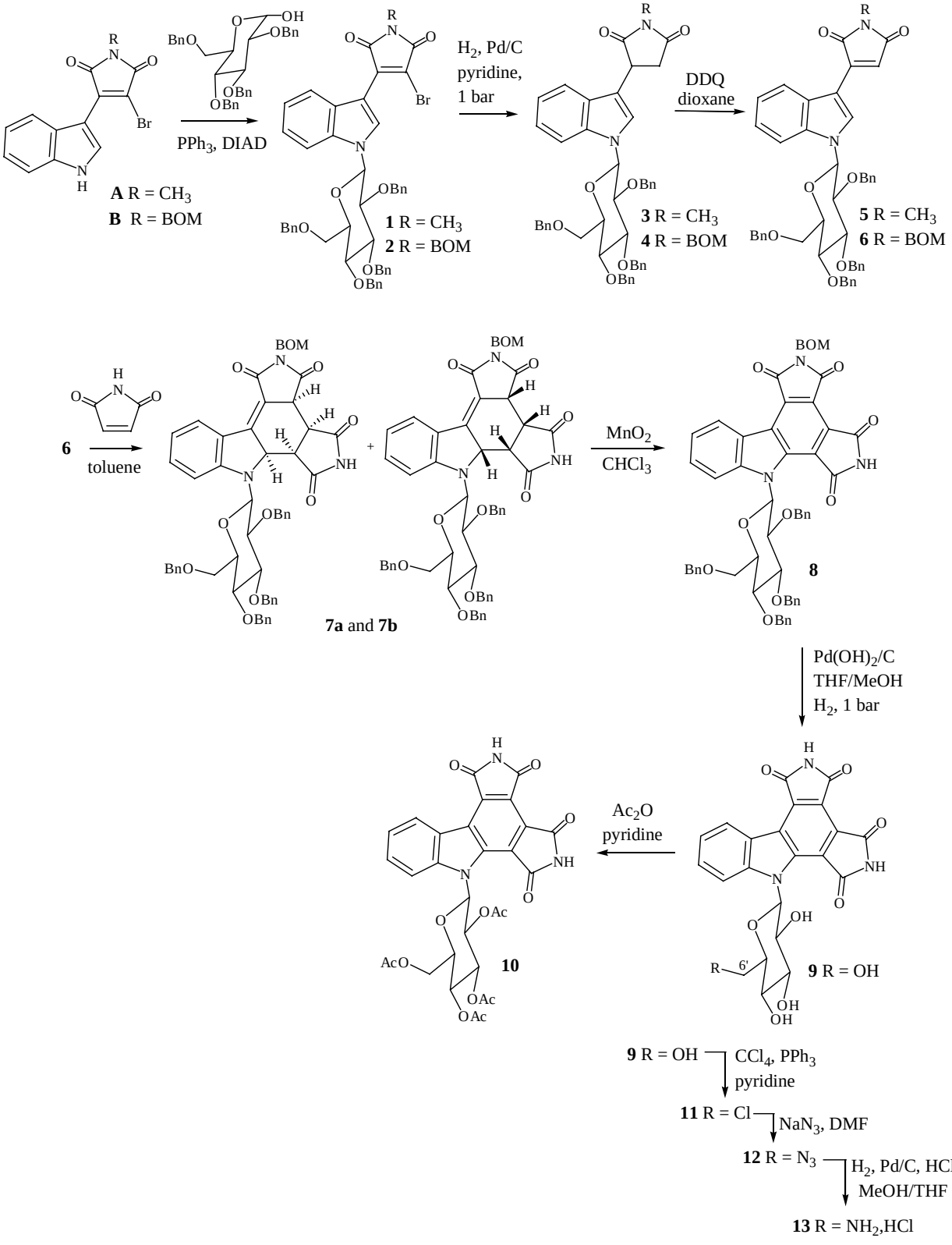
Figure 1. Chemical structures of some natural antitumor compounds.

method was described by Kaneko et al.[25] for the synthesis of rebeccamycin. An anhydrosugar was coupled to a bis-indolylmaleimide or to an indole-3-acetamide in the syntheses of rebeccamycin described by Danishefsky[23] and Faul,[24] respectively. Okhubo et al.[26,27] reported the glycosylation of indolocarbazoles using an α-chlorosugar in a basic heterogeneous medium. A Mitsunobu reaction was performed by Okhubo et al.[28] and Zembower et al.[29] for the glycosylation of bis-indolylmaleimides and indolylbromomaleimides, respectively. For the synthesis of compound **9** (Scheme 1), we chose the Mitsunobu reaction, which was performed on indolylbromomaleimides **A** and **B**. The indolylbromomaleimides **A** and **B**, protected on the imide nitrogen with either a methyl group or benzyloxymethyl substituent, were prepared from the corresponding *N*-protected-dibromomaleimides and indolylmagnesium bromide according to a known procedure.[30]

The glycosylation was carried out using a commercial anomeric mixture of 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose, leading to bromo-indolylmaleimides **1** and **2** in 94 and 92% yields, respectively. Removal of the bromine atom was performed by hydrogenolysis in the presence of pyridine to avoid the hydrogenolysis of the benzyl groups.[31,32] Succinimides **3** and **4** were obtained in 61 and 58% yields, respectively, as mixtures of diastereoisomers in 0.69:1 and 0.82:1 ratios. The diastereoisomeric ratios were determined from ^{1}H NMR spectra on the signals at 3.47 and 3.50 ppm for compound **3** and at 2.85 and 2.92 ppm for compound **4**. The oxydation step to the corresponding maleimides was carried out using DDQ to give compounds **5** and **6** in 62 and 91% yields, respectively. At this stage, the conversion of *N*-methylmaleimide[22] of compound **5** to an anhydride failed. Therefore, the synthesis was continued only from compound **6**. The Diels–Alder reaction with maleimide gave the cycloadduct **7** from compound **6**, as a mixture of diastereoisomers **7a** and **7b**, which could be separated by chromatography. In a previous work, we observed the isomerization of the Diels–Alder cyclo-adduct from indoline to indole.[19] To get an insight into the structures of compounds **7a** and **7b**, complementary NMR studies were carried out. The indoline structure was proved from ^{1}H–^{13}C long range coupling (^3J) between $H_{1'}$ and two carbons of the indoline, one of them (C_{6b}) bearing a hydrogen (Fig. 2). The Diels–Alder reaction has been performed using various amounts of maleimide and various solvents. The best yield (94%) was obtained in toluene with maleimide (5 equiv). The diastereoisomers **7a** and **7b** were further oxidized using either DDQ or manganese dioxide in various solvents to give compound **8** (Table 1). The best yield (75%) was obtained from the isomer **7b** with MnO_2 in chloroform.

Finally, the BOM and benzyl protective groups were removed by hydrogenolysis using 20% palladium hydroxide on carbon affording compound **9** in 71% yield. Compound **10**, bearing four acetyl groups on the sugar moiety, was also prepared to serve as a possible prodrug, which could be hydrolyzed inside the cells to release compound **9**. This kind of prodrug has been previously successfully used for glycosyl-indigo derivatives.[33,34]

Several substituents were introduced in 6′-position on the sugar moiety. In rebeccamycin series, we had observed that



Scheme 1.

such substituents could modify the biological targets, changing topoisomerase I inhibitors to kinases inhibitors.[35] The chloro-derivative **11** was obtained in 76% yield by treatment of compound **9** by CCl_4 in the presence of triphenylphosphine and pyridine. Nucleophilic substitution using sodium azide led to the azido derivative **12** in 60% yield. Compound **13** was obtained in a quantitative yield by catalytic hydrogenation using 10% Pd/C in methanol/THF/HCl. The hydrochloride was directly formed in the reaction mixture.[36]

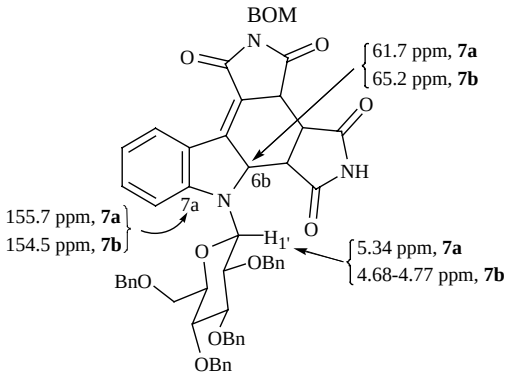


Figure 2.

Table 1. Experimental conditions and yields for the oxidation of **7a** and **7b** to compound **8**

Oxidizing agent	Solvent	Temperature (time of reaction)	Yields
DDQ (2.05 equiv)	Dioxane	Reflux (20 h)	35% from **7a**, 0% from **7b**
DDQ (2.05 equiv)	Toluene	Room temperature (30 h)	47% from **7a**
DDQ (2.5 equiv)	Toluene	Room temperature (24 h) then reflux (1 h)	0% from **7b**
MnO_2 (20 equiv)	CH_2Cl_2	Room temperature (6 h) then 4 days (40 °C)	53% from **7a**
MnO_2 (20 equiv)	$CHCl_3$	Reflux (65 h)	73% from **7a**, 75% from **7b**

3. Conclusion

In conclusion, we have synthesized new bis-imide granulatimide analogues bearing a sugar moiety on the indole nitrogen with the aim of increasing the solubility and the interaction with the target enzyme(s). Moreover, various substituents have been introduced on the sugar unit. The biological activities of these novel granulatimide analogues are under investigation.

4. Experimental

4.1. General

IR spectra were recorded on Perkin-Elmer 881 or Perkin-Elmer Paragon 500 spectrometers (ν in cm^{-1}). NMR spectra were performed on a Bruker AVANCE 400 and AVANCE 500 (chemical shifts δ in ppm, the following abbreviations are used: singlet (s), broad signal (br s), doublet (d), doubled doublet (dd), doubled doubled doublet (ddd), doubled triplet (dt), triplet (t), multiplet (m), tertiary carbons (C *tert*), quaternary carbons (C quat). The signals were assigned from ^1H–^1H COSY and ^{13}C–^1H correlations. Low-resolution mass spectra (ESI+, CI) and HRMS were determined on a Hewlett Packard MS engine. Chromatographic purifications were performed by flash silicagel Geduran SI 60 (Merck) 0.040–0.063 mm column chromatography.

4.1.1. 3-(3-Bromo-2,5-dihydro-1-methyl-2,5-dioxo-pyrrol-4-yl)-1-(1-deoxy-2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranos-1-y1)-indole (1).

To a mixture of **A** (50 mg, 0.164 mmol), PPh_3 (129 mg, 0.49 mmol) and an anomeric mixture of 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (266 mg, 0.49 mmol) in THF (3.2 mL) at −78 °C, DIAD (95 μL) was added dropwise. The mixture was allowed to reach room temperature and stirred for 5 h. 0.2 M HCl (40 mL) was poured into the mixture. After extraction with EtOAc, the organic phase was washed successively with a saturated aqueous $NaHCO_3$ solution and water. The organic phase was dried over $MgSO_4$. The solvent was removed and the residue was purified by flash chromatography (eluent: toluene/EtOAc 50:1) to give **1** as a red solid (128 mg, 0.155 mmol, 94% yield). Mp 55 °C. IR (KBr) $\nu_{C=C}$ 1615 cm^{-1}, $\nu_{C=O}$ 1710, 1770 cm^{-1}. HRMS (FAB+) calcd for $C_{47}H_{43}N_2O_7Br$ $[M]^+$ 826.2254, found 826.2265.

^1H NMR (400 MHz, $CDCl_3$): 3.20 (3H, s), 3.67 (1H, d, J=10.5 Hz), 3.74–3.82 (2H, m), 3.85–3.92 (2H, m), 3.97 (1H, t, J=9.5 Hz), 4.06 (1H, t, J=9.0 Hz), 4.28 (1H, d, J=10.5 Hz), 4.55 (1H, d, J=12.0 Hz), 4.63 (1H, d, J=12.0 Hz), 4.71 (1H, d, J=10.5 Hz), 4.93 (1H, d, J=10.5 Hz), 4.94 (1H, d, J=11.0 Hz), 4.98 (1H, d, J=11.0 Hz), 5.45 (1H, d, J=9.0 Hz), 6.71 (2H, d, J=7.5 Hz), 7.07 (2H, t, J=7.5 Hz), 7.13 (1H, m), 7.23–7.40 (17H, m), 7.67 (1H, m), 8.05–8.09 (2H, m).

^{13}C NMR (100 MHz, $CDCl_3$): 24.9 (CH_3), 68.5, 73.6, 75.1, 75.4, 75.9 (CH_2), 77.5, 78.1, 80.8, 85.5, 86.9 (CH), 112.3, 121.9, 123.4, 123.6, 127.5–128.6, 131.4 (C *tert* arom), 105.6, 115.8, 126.1, 136.1, 136.7, 137.3, 137.9, 138.1, 138.3 (C quat), 166.8, 169.3 (C=O).

4.1.2. 3-(1-Benzyloxymethyl-3-bromo-2,5-dihydro-2,5-dioxo-pyrrol-4-yl)-1-(1-deoxy-2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranos-1-y1)-indole (2).

Identical procedure as described for **1** was used from **B** (302 mg, 0.73 mmol), PPh_3 (578 mg, 2.20 mmol), 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (1.19 g, 2.20 mmol) and DIAD (427 μL) in THF (13.5 mL) to give after purification by flash chromatography (eluent: toluene/EtOAc 50:1 then 50:2) compound **2** as an orange solid (632 mg, 0.68 mmol, 92% yield). Mp 40 °C. IR (film, NaCl) $\nu_{C=O}$ 1725, 1780 cm^{-1}. Mass (ESI+) 955, 957 $[M+Na]^+$.

^1H NMR (400 MHz, $CDCl_3$): 3.64 (1H, d, J=10.5 Hz), 3.72–3.80 (2H, m), 3.83–3.90 (2H, m), 3.96 (1H, t, J=9.5 Hz), 4.03 (1H, t, J=9.0 Hz), 4.26 (1H, d, J=10.5 Hz), 4.54 (1H, d, J=12.0 Hz), 4.61 (1H, d, J=12.0 Hz), 4.69 (2H, s), 4.69 (1H, d, J=10.5 Hz), 4.89–4.97 (3H, m), 5.19 (2H, s), 5.43 (1H, d, J=9.0 Hz), 6.67–6.71 (2H, m), 7.02–7.13 (3H, m), 7.22–7.40 (22H, m), 7.65 (1H, m), 8.03 (1H, m), 8.04 (1H, s).

^{13}C NMR (100 MHz, $CDCl_3$): 67.7, 68.5, 71.9, 73.6, 75.1, 75.4, 75.9 (CH_2), 77.5, 78.1, 80.8, 85.4, 86.8 (CH), 112.4, 122.0, 123.5, 123.7, 127.6–128.7, 131.7 (C *tert* arom), 105.3, 116.1, 126.0, 136.1, 136.6, 137.4, 137.5, 137.9, 138.1, 138.3 (C quat), 166.3, 168.8 (C=O).

4.1.3. 1-(1-Deoxy-2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranos-1-y1)-3-(1-methyl-2,5-dioxo-pyrrolidin-3-yl)-indole (3).

A solution of compound **1** (344 mg, 0.416 mmol)

in methanol (11.5 mL) and THF (4 mL) was hydrogenated (1 atm) for 3 h in the presence of 10% Pd/C (34 mg) and pyridine (39 μL, 0.48 mmol). After filtration over Celite, the filtrate was evaporated, and the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc 8:2) to give **3** as a pale orange solid (191 mg, 0.254 mmol, 61% yield). Compound **3** was isolated as a mixture of diastereoisomers. IR (KBr) $\nu_{C=O}$ 1705, 1775 cm^{-1}. Mass (ESI+) 751 $[M+H]^+$, 773 $[M+Na]^+$.

^1H NMR (400 MHz, $CDCl_3$): (a: major diastereoisomer; b: minor diastereoisomer) 2.86 ($1H^a$, dd, $J_1=18.5$ Hz, $J_2=5.0$ Hz), 2.92 ($1H^b$, dd, $J_1=18.5$ Hz, $J_2=5.0$ Hz), 3.10 ($3H^b$, s), 3.11 ($3H^a$, s), 3.27 ($1H^b$, dd, $J_1=18.5$ Hz, $J_2=9.5$ Hz), 3.29 ($1H^a$, dd, $J_1=18.5$ Hz, $J_2=9.5$ Hz), 3.47 ($1H^a$, d, $J=10.0$ Hz), 3.50 ($1H^b$, d, $J=10.0$ Hz), 3.69–3.74 ($1H^{a+b}$, m), 3.75–3.87 ($3H^{a+b}$, m), 3.92 ($1H^{a+b}$, t, $J=9.5$ Hz), 3.97 ($1H^b$, t, $J=9.0$ Hz), 3.98 ($1H^a$, t, $J=9.0$ Hz), 4.16 ($1H^b$, d, $J=10.5$ Hz), 4.19 ($1H^a$, d, $J=10.0$ Hz), 4.29–4.35 ($1H^{a+b}$, m), 4.54 ($1H^{a+b}$, d, $J=12.0$ Hz), 4.62 ($1H^a$, d, $J=12.0$ Hz), 4.62 ($1H^b$, d, $J=12.0$ Hz), 4.68 ($1H^{a+b}$, d, $J=10.5$ Hz), 4.88–4.98 ($3H^{a+b}$, m), 5.35 ($1H^{a+b}$, d, $J=9.0$ Hz), 6.69–6.75 ($2H^{a+b}$, m), 7.07–7.38 ($21H^{a+b}$, m), 7.45–7.50 ($1H^{a+b}$, m), 7.59–7.63 ($1H^{a+b}$, m).

^{13}C NMR (100 MHz, $CDCl_3$): 25.2 (CH_3), 36.3, 36.6, 68.6, 73.5, 74.6, 74.7, 75.3, 75.8 (CH_2), 38.0, 38.1, 77.5, 77.9, 81.0, 81.1, 85.4, 86.5 (CH), 112.1, 119.0, 119.1, 120.8, 123.0, 123.8, 123.9, 127.7–128.5 (CH arom), 112.0, 112.1, 127.2, 127.3, 136.3, 136.4, 137.0, 138.0, 138.1, 138.4 (C quat arom), 176.3, 176.4, 177.7, 177.8 (C=O).

4.1.4. 3-(1-Benzyloxymethyl-2,5-dioxo-pyrrolidin-3-yl)-1-(1-deoxy-2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranos-1-y1)-indole (4).

Identical procedure as for the preparation of **3** was carried out from a mixture of **2** (559 mg, 0.60 mmol) in methanol (16.6 mL) and THF (6 mL) in the presence of Pd/C (56 mg) and pyridine (56 μL, 0.69 mmol), which was hydrogenated for 8 h. Compound **4** was isolated after purification by flash chromatography (eluent: cyclohexane/EtOAc 8:2) as an orange solid (296 mg, 0.345 mmol, 58% yield). Compound **6** was also obtained in mixture with the starting product **2** (117 mg containing 15% of **2** as determined on ^1H NMR spectrum). Compound **4** was isolated as a mixture of diastereoisomers. IR (KBr) $\nu_{C=O}$ 1715, 1780 cm^{-1}. Mass (ESI+) 879 $[M+Na]^+$, 895 $[M+K]^+$.

^1H NMR (400 MHz, $CDCl_3$): (a: major diastereoisomer; b: minor diastereoisomer) 2.85 ($1H^a$, dd, $J_1=18.5$ Hz, $J_2=5.5$ Hz), 2.92 ($1H^b$, dd, $J_1=18.5$ Hz, $J_2=5.5$ Hz), 3.19 ($1H^b$, dd, $J_1=18.5$ Hz, $J_2=9.5$ Hz), 3.21 ($1H^a$, dd, $J_1=18.5$ Hz, $J_2=9.5$ Hz), 3.51 ($1H^a$, d, $J=10.0$ Hz), 3.52 ($1H^b$, d, $J=10.5$ Hz), 3.72–3.77 ($1H^{a+b}$, m), 3.78–3.83 ($1H^{a+b}$, m), 3.84–3.91 ($2H^{a+b}$, m), 3.95 ($1H^{a+b}$, t, $J=9.0$ Hz), 4.00 ($1H^b$, t, $J=9.0$ Hz), 4.01 ($1H^a$, t, $J=9.0$ Hz), 4.16–4.25 ($2H^{a+b}$, m), 4.56 ($1H^a$, d, $J=12.0$ Hz), 4.57 ($1H^b$, d, $J=12.0$ Hz), 4.64 ($1H^a$, d, $J=12.0$ Hz), 4.65 ($1H^b$, d, $J=12.0$ Hz), 4.68–4.75 ($3H^{a+b}$, m), 4.92–5.02 ($3H^{a+b}$, m), 5.15 ($2H^b$, s), 5.16 ($2H^a$, s), 5.37 ($1H^{a+b}$, d, $J=9.0$ Hz), 6.72–6.76 ($2H^{a+b}$, m), 7.10–7.43 ($26H^{a+b}$, m), 7.48–7.52 ($1H^{a+b}$, m), 7.62–7.66 ($1H^{a+b}$, m).

^{13}C NMR (100 MHz, $CDCl_3$): 36.2, 36.4, 68.0, 68.6, 72.3, 73.5, 74.6, 74.7, 75.3, 75.8 (CH_2), 38.0, 38.1, 77.5, 77.9, 81.0, 81.1, 85.4, 86.4 (CH), 112.1, 118.9, 119.0, 120.8, 123.0, 123.9, 124.0, 127.6–128.7 (C *tert* arom), 111.6, 111.7, 127.1, 127.2, 136.3, 136.4, 136.9, 137.6, 138.0, 138.1, 138.4 (C quat arom), 175.9, 176.0, 177.2, 177.3 (C=O).

4.1.5. 1-(1-Deoxy-2,3,4,6-tetra-*O*-benzyl-β-D-gluco-pyranos-1-y1)-3-(2,5-dihydro-1-methyl-2,5-dioxo–pyrrol-3-yl)-indole (5).

A solution of DDQ (21.7 mg, 0.096 mmol) in dioxane (1 mL) was slowly added to a solution of **3** (65.2 mg, 0.087 mmol) in dioxane (1 mL). The mixture was stirred at room temperature overnight, then it was filtered off. After evaporation of the filtrate, the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc 9:1) to give **5** as a yellow solid (40.4 mg, 0.054 mmol, 62% yield). Mp 97–103 °C. IR (KBr) $\nu_{C=C}$ 1617 cm^{-1}, $\nu_{C=O}$ 1703, 1765 cm^{-1}. Mass (ESI+) 749 $[M+H]^+$.

^1H NMR (400 MHz, $CDCl_3$): 3.11 (3H, s), 3.65 (1H, d, $J=10.5$ Hz), 3.72–3.80 (2H, m), 3.83 (1H, dd, $J_1=11.0$ Hz, $J_2=4.0$ Hz), 3.86 (1H, t, $J=9.0$ Hz), 3.93 (1H, t, $J=9.0$ Hz), 4.00 (1H, t, $J=9.0$ Hz), 4.25 (1H, d, $J=10.5$ Hz), 4.54 (1H, d, $J=12.0$ Hz), 4.60 (1H, d, $J=12.0$ Hz), 4.68 (1H, d, $J=10.5$ Hz), 4.89–4.96 (3H, m), 5.42 (1H, d, $J=9.0$ Hz), 6.66–6.70 (3H, m), 7.03 (2H, t, $J=7.5$ Hz), 7.08–7.13 (1H, m), 7.21–7.36 (17H, m), 7.66 (1H, d, $J=8.0$ Hz), 7.80 (1H, d, $J=8.0$ Hz), 8.51 (1H, s).

^{13}C NMR (100 MHz, $CDCl_3$): 23.8 (CH_3), 68.6, 73.6, 75.0, 75.4, 75.8, (CH_2), 77.5, 78.1, 80.8, 85.5, 86.8 (CH sugar), 112.8, 116.0, 120.6, 122.7, 123.8, 127.7–128.6, 131.8 (CH), 106.8, 127.2, 136.3, 136.7, 137.9, 138.1, 138.3, 139.0 (C quat), 171.8, 172.3 (C=O).

4.1.6. 3-(1-Benzyloxymethyl-2,5-dihydro-2,5-dioxo-pyrrol-3-yl)-1-(1-deoxy-2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranos-1-y1)-1*H*-indole (6).

A solution of DDQ (78.2 mg, 0.344 mmol) in dioxane (3.4 mL) was slowly added to a solution of **4** (281 mg, 0.328 mmol) in dioxane (3.4 mL). The mixture was stirred at room temperature for 3 h then it was filtered off. The filtrate was evaporated and the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc 8:2) to give **6** as a yellow solid (254 mg, 0.297 mmol, 91% yield). Mp 47 °C. IR (KBr) $\nu_{C=C}$ 1615 cm^{-1}, $\nu_{C=O}$ 1710, 1770 cm^{-1}. Mass (ESI+) 877 $[M+Na]^+$, 893 $[M+K]^+$.

^1H NMR (400 MHz, $CDCl_3$): 3.65 (1H, d, $J=10.5$ Hz), 3.72–3.80 (2H, m), 3.83 (1H, dd, $J_1=11.0$ Hz, $J_2=4.0$ Hz), 3.86 (1H, t, $J=9.0$ Hz), 3.93 (1H, t, $J=9.0$ Hz), 3.99 (1H, t, $J=9.0$ Hz), 4.26 (1H, d, $J=10.5$ Hz), 4.53 (1H, d, $J=12.0$ Hz), 4.60 (1H, d, $J=12.0$ Hz), 4.66–4.70 (3H, m), 4.89–4.96 (3H, m), 5.13 (2H, s), 5.43 (1H, d, $J=9.0$ Hz), 6.65–6.69 (2H, m), 6.71 (1H, s), 6.98–7.04 (2H, m), 7.07 (1H, m), 7.21–7.41 (22H, m), 7.66 (1H, d, $J=8.0$ Hz), 7.79 (1H, d, $J=7.5$ Hz), 8.52 (1H, s).

^{13}C NMR (100 MHz, $CDCl_3$): 66.6, 68.5, 71.5, 73.5, 75.0, 75.4, 75.8 (CH_2), 77.5, 78.1, 80.8, 85.4, 86.7 (CH), 106.7, 127.1, 136.3, 136.6, 137.6, 137.9, 138.0, 138.3, 139.2

(C quat), 112.8, 116.0, 120.5, 122.8, 124.0, 127.7–128.6, 132.1 (CH), 171.3, 171.6 (C=O).

4.1.7. 2-Benzyloxymethyl-7-(1-deoxy-2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranos-1-y1)-1,3,3a,3b,4,6,6a,6b-octahydro-dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,3,4,6-tetraone (7a) and (7b). A mixture of **6** (171 mg, 0.20 mmol) and maleimide (97 mg, 1.0 mmol) in toluene (5 mL) was refluxed for 19 h. The solvent was removed and the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc from 7:3 to 4:6) to give **7** (94% yield) as two diasteroisomers **7a** (117 mg, 0.123 mmol) and **7b** (61.4 mg, 0.064 mmol).

Compound **7a**. Mp 95 °C. IR (KBr) $\nu_{C=C}$ 1605 cm^{-1}, $\nu_{C=O}$ 1650, 1710, 1725, 1765 cm^{-1}, ν_{NH} 3150–3550 cm^{-1}. Mass (ESI+) 974 $[M+Na]^+$.

^1H NMR (400 MHz, $CDCl_3$): 2.81 (1H, d, $J=6.5$ Hz), 3.31 (1H, dd, $J_1=8.5$ Hz, $J_2=6.5$ Hz), 3.59–3.66 (2H, m), 3.72–3.89 (6H, m), 4.17 (1H, d, $J=10.5$ Hz), 4.38 (1H, d, $J=12.0$ Hz), 4.45 (1H, d, $J=10.5$ Hz), 4.46 (1H, d, $J=12.0$ Hz), 4.65 (1H, d, $J=12.0$ Hz), 4.66 (1H, d, $J=10.5$ Hz), 4.70 (1H, d, $J=12.0$ Hz), 4.86 (1H, d, $J=11.0$ Hz), 4.87 (1H, d, $J=11.0$ Hz), 4.94 (1H, d, $J=11.0$ Hz), 5.09 (1H, d, $J=10.5$ Hz), 5;15 (1H, d, $J=10.5$ Hz), 5.33 (1H, d, $J=8.5$ Hz), 6.55–6.60 (2H, m), 6.82 (1H, t, $J=7.5$ Hz), 6.93 (1H, d, $J=8.5$ Hz), 7.01–7.09 (3H, m), 7.18–7.35 (19H, m), 7.39 (2H, d, $J=7.5$ Hz), 7.90 (1H, br s, NH), 8.60 (1H, d, $J=7.5$ Hz).

^{13}C NMR (100 MHz, $CDCl_3$): 38.9, 42.1, 43.7, 61.7, 76.7, 77.4, 77.8, 85.7, 86.2 (CH), 67.5, 68.3, 71.8, 73.4, 74.5, 75.2, 75.7 (CH_2), 110.6, 119.3, 127.6–128.8, 134.9 (CH), 110.3, 120.9, 136.7, 137.7, 137.9 (2C), 138.3, 147.7, 155.7 (C quat), 165.9, 172.1, 173.0, 174.4 (C=O).

Compound **7b**. Mp 94 °C. IR (KBr) $\nu_{C=O}$ 1650, 1705, 1720, 1760 cm^{-1}, ν_{NH} 3200–3550 cm^{-1}. Mass (ESI+) 974 $[M+Na]^+$.

^1H NMR (400 MHz, $CDCl_3$): 2.62 (1H, m), 3.58–3.70 (3H, m), 3.80 (1H, t, $J=9.0$ Hz), 3.86–3.89 (2H, m), 3.91 (1H, t, $J=9.5$ Hz), 4.03 (1H, d, $J=11.0$ Hz), 4.15 (1H, t, $J=9.0$ Hz), 4.52–4.57 (2H, m), 4.65 (1H, d, $J=10.5$ Hz), 4.68–4.77 (5H, m), 4.88 (1H, d, $J=10.5$ Hz), 4.90 (1H, d, $J=11.0$ Hz), 4.98 (1H, d, $J=11.0$ Hz), 5.15 (1H, d, $J=10.5$ Hz), 5.20 (1H, d, $J=10.5$ Hz), 6.87–6.96 (3H, m), 7.08–7.14 (3H, m), 7.22–7.39 (20H, m), 7.44 (2H, d, $J=7.5$ Hz), 7.98 (1H, br s, NH), 8.72 (1H, d, $J=8.0$ Hz).

^{13}C NMR (100 MHz, $CDCl_3$): 67.5, 68.5, 71.8, 73.3, 75.2, 75.6, 75.7 (CH_2), 39.9, 41.3, 42.1, 65.2, 77.8, 77.9, 79.2, 86.2, 86.8 (CH), 113.3, 120.4, 127.5–128.7; 129.0, 134.7 (CH), 110.8, 122.6, 137.3, 137.7, 138.0, 138.2, 138.4, 147.5, 154.5 (C quat), 165.9, 172.0, 173.4, 174.5 (C=O).

4.1.8. 2-Benzyloxymethyl-7-(1-deoxy-2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranos-1-y1)-1,3,4,6-tetrahydro-dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,3,4,6-tetraone (8). This procedure is also applicable to stereoisomer **7a**.

A mixture of **7b** (84 mg, 0.088 mmol) and MnO_2 (153 mg, 1.76 mmol) in chloroform (4 mL) was refluxed for 60 h. The solvent was removed and the residue was purified by flash chromatography (eluent: cyclohexane/EtOAc 7:3) to give **8** as an orange solid (62.7 mg, 0.066 mmol, 75% yield). Mp 78–80 °C. IR (KBr) $\nu_{C=O}$ 1722, 1781 cm^{-1}, ν_{NH} 3130–3530 cm^{-1}. Mass (ESI+) 970 $[M+Na]^+$.

^1H NMR (400 MHz, $CDCl_3$): 3.20 (1H, d, $J=11.5$ Hz), 3.90–4.03 (5H, m), 4.09–4.14 (1H, m), 4.21 (1H, d, $J=11.5$ Hz), 4.68 (1H, d, $J=12.0$ Hz), 4.71–4.78 (2H, m), 4.79 (2H, s), 4.85 (1H, d, $J=11.0$ Hz), 4.92 (1H, d, $J=11.0$ Hz), 4.98 (1H, d, $J=11.0$ Hz), 5.26 (1H, d, $J=11.0$ Hz), 5.31 (1H, d, $J=11.0$ Hz), 6.15 (2H, d, $J=7.5$ Hz), 6.68 (2H, t, $J=7.5$ Hz), 6.85 (1H, t, $J=7.5$ Hz), 7.23–7.48 (21H, m), 7.53 (1H, t, $J=7.5$ Hz), 7.61 (1H, d, $J=8.5$ Hz), 8,02 (1H, d, $J=8.5$ Hz), 8.67 (1H, br s, NH), 9.13 (1H, d, $J=8.0$ Hz).

^{13}C NMR (100 MHz, $CDCl_3$): 67.3, 69.0, 72.0, 73.3, 74.5, 75.3, 75.9 (CH_2), 77.5, 77.9, 79.8, 85.7, 87.0 (CH), 115.2, 123.5, 126.7–128.6, 130.7 (C *tert* arom), 118.3, 119.3, 121.7, 126.4, 127.2, 130.0, 136.7, 137.5, 137.9 (2C), 138.1, 140.1, 142.1 (C quat arom), 163.9 (2C), 166.6, 166.9 (C=O).

4.1.9. 7-(1-Deoxy-β-D-glucopyranos-1-y1)-1,3,4,6-tetra-hydro-dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,3,4,6-tetraone (9). A solution of **8** (567 mg, 0.60 mmol) in a mixture THF/MeOH 4:1 (44 mL) was hydrogenated (1 atm) for 24 h in the presence of Pearlman catalyst (20% $Pd(OH)_2$/C, 283 mg). THF and MeOH were added, the reaction mixture was stirred at room temperature for 2 h before filtration over Celite. The filtrate was evaporated and the residue was purified by flash chromatography (eluent: THF/cyclohexane 8:2) leading to **9** as an orange solid, which was further washed with CH_2Cl_2 (199 mg, 0.43 mmol, 71% yield). Mp >300 °C. IR (KBr) $\nu_{C=O}$ 1720, 1755, 1770 cm^{-1}, $\nu_{NH,OH}$ 3100–3600 cm^{-1}. HRMS (ESI+) calcd for $C_{22}H_{17}N_3O_9Na$ $[M+Na]^+$490.0862, found 490.0877.

^1H NMR (400 MHz, DMSO-d_6): 3.40–3.52 (2H, m), 3.59–3.73 (2H, m), 3.84–3.94 (2H, m), 4.74 (1H, t, $J=5.5$ Hz, OH), 5.02 (1H, d, $J=5.5$ Hz), 5.19 (1H, d, $J=5.0$ Hz, OH), 5.23 (1H, d, $J=5.0$ Hz, OH), 7.53 (1H, d, $J=9.0$ Hz), 7.54 (1H, t, $J=7.5$ Hz), 7.74 (1H, t, $J=7.5$ Hz), 8.09 (1H, d, $J=7.5$ Hz), 9.28 (1H, d, $J=8.0$ Hz), 11.62–11.91 (2H, br s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 61.0 (CH_2), 69.9, 70.4, 77.4, 80.3, 87.7 (CH), 115.4, 122.5, 126.0, 129.8 (C *tert* arom), 118.5, 120.5, 121.5, 125.2, 127.8, 131.8, 140.2, 141.8 (C quat arom), 165.6, 165.8, 168.3, 169.0 (C=O).

4.1.10. 7-(1-Deoxy-2,3,4,6-tetra-*O*-acetyl-β-D-gluco-pyranos-1-y1)-1,3,4,6-tetrahydro-dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,3,4,6-tetraone (10). Acetic anhydride (101 μL, 1.07 mmol) was added to a mixture of **9** (50 mg, 0.107 mmol) in pyridine (221 μL, 2.73 mmol) at 0 °C. The mixture was stirred at room temperature for 20 h. Water was added. After extraction with EtOAc, the organic phase was dried over $MgSO_4$, and the solvent was removed. The residue was purified by flash chromatography (eluent: cyclohexane/EtOAc from 5:5 to 4:6) to give **10** as a yellow solid (32.6 mg, 0.051 mmol, 48% yield). Mp 176 °C. IR (KBr) $\nu_{C=O}$ 1685–1800 cm^{-1}, ν_{NH} 3100–3550 cm^{-1}.

HRMS (ESI+) calcd for $C_{30}H_{26}N_3O_{13}$ $[M+H]^+$636.1466, found 636.1495.

^{1}H NMR (400 MHz, $CDCl_3$): 1.53 (3H, s), 2.03 (3H, s), 2.17 (3H, s), 2.20 (3H, s), 4.32–4.44 (2H, m), 4.54 (1H, d, J= 11.5 Hz), 5.50 (1H, t, J=9.5 Hz), 5.67 (1H, t, J=9.5 Hz), 5.89 (1H, t, J=9.5 Hz), 7.38 (1H, t, J=7.5 Hz), 7.71 (1H, t, J=8.0 Hz), 7.99 (1H, d, J=8.5 Hz), 8.07 (1H, d, J= 9.0 Hz), 8.82 (1H, br s, NH), 8.96 (1H, d, J=8.0 Hz), 9.06 (1H, br s, NH).

^{13}C NMR (100 MHz, $CDCl_3$): 20.1, 20.6, 20.7, 20.9 (CH_3), 61.8 (CH_2), 68.2, 69.2, 73.5, 74.8, 85.5 (CH), 114.3, 123.7, 127.0, 131.0 (C *tert* arom), 117.7, 121.2, 121.6, 126.9, 127.4, 131.5, 139.8, 141.5 (C quat arom), 164.5, 165.1, 166.9, 167.4, 169.5, 169.8, 170.3, 171.0 (C=O).

4.1.11. 7-(6-Chloro-1,6-dideoxy-β-D-glucopyranos-1-y1)-1,3,4,6-tetrahydro-dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,3,4,6-tetraone (11). PPh_3 (337 mg, 1.28 mmol) then CCl_4 (62 μL, 0.64 mmol) were successively added to a solution of **9** (150 mg; 0.321 mmol) in pyridine (1.6 mL). The mixture was stirred at room temperature for 8 h. The solvent was removed and the residue was dried under vaccum. CH_2Cl_2 was added to the residue and the mixture was filtered off. The solid was purified by flash chromatography (eluent: cyclohexane/THF from 8:2 to 100% THF then THF/methanol 95:5) to give **11** (118 mg, 0.243 mmol, 76% yield) as an orange solid. Mp >300 °C. IR (KBr) $\nu_{C=O}$ 1725, 1780 cm^{-1}, ν_{NH} 3150–3650 cm^{-1}. HRMS (ESI+) calcd for $C_{22}H_{16}N_3O_8NaCl$ $[M+Na]^+$508.0524, found 508.0544.

^{1}H NMR (400 MHz, DMSO-d_6): 3.46 (1H, dt, J_1=9.0 Hz, J_2=5.5 Hz), 3.59 (1H, dt, J_1=9.0 Hz, J_2=6.0 Hz), 3.84–3.90 (1H, m), 3.92 (1H, dt, J_1=9.0 Hz, J_2=6.0 Hz), 4.02 (1H, dd, J_1=12.0 Hz, J_2=5.0 Hz), 4.08 (1H, dd, J_1= 12.0 Hz, J_2=2.5 Hz), 5.13 (1H, d, J=6.0 Hz), 5.33 (1H, d, J=5.5 Hz), 5.58 (1H, d, J=6.0 Hz), 7.55 (1H, ddd, J_1= 8.0 Hz, J_2=7.0 Hz, J_3=1.0 Hz), 7.58 (1H, d, J=9.0 Hz), 7.75 (1H, ddd, J_1=8.5 Hz, J_2=7.0 Hz, J_3=1.5 Hz), 8.08 (1H, d, J=8.5 Hz), 9.28 (1H, ddd, J_1=8.0 Hz, J_2=1.0 Hz, J_3=0.5 Hz), 11.62–11.97 (2H, br s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 45.1 (CH_2), 70.2 (2C), 76.8, 77.8, 87.6 (CH), 115.0, 122.6, 126.1, 129.8 (C *tert* arom), 118.5, 120.7, 121.6, 125.2, 127.7, 131.8, 140.2, 141.6 (C quat arom), 165.5, 165.7, 168.3, 168.8 (C=O).

4.1.12. 7-(6-Azido-1,6-dideoxy-β-D-glucopyranos-1-y1)-1,3,4,6-tetrahydro-dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,3,4,6-tetraone (12). NaN_3 (95 mg, 1.46 mmol) was added to a solution of compound **11** (71 mg, 0.146 mmol) in anhydrous DMF (3.8 mL). The mixture was heated at 90 °C for 3 days. After evaporation, water was added to the residue. The mixture was filtered off and the solid residue was washed with water. The residue was purified by flash chromatography (eluent: cyclohexane/THF from 8:2). The solid obtained after chromatography was washed with CH_2Cl_2 to give **12** (43.0 mg, 0.087 mmol, 60% yield) as an orange solid. Mp >300 °C. IR (KBr) $\nu_{C=O}$ 1722, 1775 cm^{-1}, ν_{N3} 2104 cm^{-1}, ν_{NH} 3110–3620 cm^{-1}. HRMS (ESI+) calcd for$C_{22}H_{16}N_6O_8Na$ $[M+Na]^+$515.0927, found 515.0934.

^{1}H NMR (400 MHz, DMSO-d_6): 3.40–3.48 (1H, m), 3.50–3.58 (1H, m), 3.65 (1H, dd, J_1=13.0 Hz, J_2=6.0 Hz), 3.76–3.84 (2H, m), 3.93–4.01 (1H, m), 5.13 (1H, d, J=6.0 Hz), 5.33 (1H, d, J=5.5 Hz), 5.52 (1H, d, J=5.5 Hz), 7.52–7.58 (2H, m, $H_{1'}+H_{arom}$), 7.76 (1H, t, J=8.0 Hz), 8.06 (1H, d, J=8.5 Hz), 9.27 (1H, d, J=8.0 Hz), 11.73 (1H, br s, NH), 11.83 (1H, br s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 51.2 (CH_2), 70.1, 70.3, 77.1, 77.9, 87.7 (CH sucre), 114.9, 122.5, 126.0, 128.9 (CH arom), 118.5, 120.7, 121.5, 125.2, 127.7, 131.8, 140.2, 141.5 (C quat arom), 165.4, 165.7, 168.2, 168.8 (C=O).

4.1.13. 7-(6-Amino-1,6-dideoxy-β-D-glucopyranos-1-y1)-1,3,4,6-tetrahydro-dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,3,4,6-tetraone hydrochloride (13). A solution of **12** (20 mg, 0.041 mmol) in MeOH/THF 2:1 (1.5 mL) was hydrogenated for 5 h in the presence of 10% Pd/C (2 mg) concentrated HCl (4 μL). 10% Pd/C (3 mg) was added and the reaction mixture was hydrogenated for 18 h before filtration over Celite. After evaporation on reduced pressure, compound **13** (20 mg, 0.040 mmol, 98% yield) was obtained as a yellow solid. Mp >235 °C (decomposition). IR (KBr) $\nu_{C=O}$ 1725, 1770 cm^{-1}, ν_{NH} 3100–3650 cm^{-1}. HRMS (ESI+) calcd for $C_{22}H_{19}N_4O_8$ $[M+H]^+$467.1203, found 467.1216.

^{1}H NMR (400 MHz, DMSO-d_6): 3.12–3.21 (1H, m), 3.34–3.41 (1H, m), 3.44–3.58 (2H, m), 3.82–3.89 (1H, m), 3.98–4.05 (1H, m), 5.21 (1H, d, J=5.5 Hz, OH), 5.48 (1H, br s, OH), 5.75 (1H, d, J=4.5 Hz, OH), 7.56 (1H, t, J=7.5 Hz), 7.61 (1H, d, J=9.0 Hz, $H_{1'}$), 7.75 (1H, t, J=7.5 Hz), 8.07 (3H, br s, NH_2, HCl), 8.15 (1H, d, J=8.5 Hz), 9.27 (1H, d, J=8.0 Hz), 11.74 (1H, s, NH), 11.88 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 40.5 (CH_2), 70.0, 71.0, 75.6, 76.8, 87.5 (CH), 115.4, 122.6, 126.0, 129.9 (CH arom), 118.3, 120.8, 121.5, 125.1, 127.7, 131.9, 140.2, 141.5 (C quat arom); 165.5, 165.7, 168.2, 168.8 (C=O).

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New strategy towards the efficient solid phase synthesis of cyclopeptides

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Abstract—Synthetic cyclopeptides, and particularly those designed from VEGF structure, present considerable interest for the development of nanodevices devoted to tumor imaging or drug delivery. In order to obtain functionalizable cyclopeptides, we herein present a novel efficient way based on Fmoc-Lys-ODmab use, which was applied to the synthesis of c(PHGRIK) cyclopeptide.

1. Introduction

Angiogenesis is a key process of cancer development and metastasis. Targeting and/or imaging of this phenomenom are of great interest in order to develop or follow-up new therapeutic approaches.

In this way, vascular endothelial growth factor (VEGF), which is the major tumoral angiogenic factor, is a promising target. Its biological properties are mediated through dimerizing interactions with several receptors among which Type 2 VEGF receptor is the most efficient towards tumoral neoangiogenesis. These interactions are mainly achieved through three basic (R,K,H) residues. We have previously demonstrated that synthetic cyclopeptides, designed from the structural requirements for the activity of VEGF, present a very high affinity for VEGFR2 and subsequently a high level of antitumoral in vivo activity.[1–3] One of them, cycloVEGI is presently under preclinical development as anticancer agent and studies to use it as nanodevice for tumor delivery of anticancer drug, gene delivery system or imaging agent are in progress. Within this series, we synthesized, by solution 'head-to-tail' cyclic peptide synthesis, the smaller deprotected 6-mer cyclopeptide c(PHGRIK) **4** (Fig. 1) designed from VEGF.

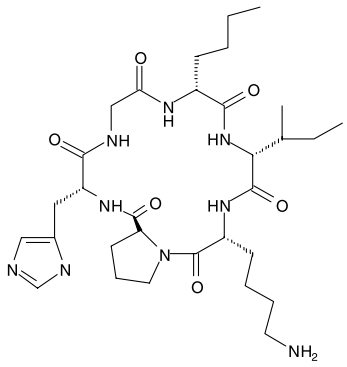

Figure 1. Structure of c(PHGRIK) **4**.

As often cyclic structure by far increase metabolic stability[4–9] but within these series they require synthetic improvements as they embody only a few, but biologically essential basic residus as functional amino acids. Thus, we focused our approach on the synthesis of cyclopeptide **4** which only contents alkyl and basic amino acids. One of them appeared suitable for functionalization, namely lysine.

2. Results and discussion

Actually, on resin preparation of head-to-tail-cyclic peptides requires temporary protection of the α-carboxyl group of resin bound amino acid.[10–14] To date, general synthesis of these peptides has mainly used allyl group as

Keywords: Nanodevice; Cyclopeptides; On-resin cyclization; Dmab ester; Lysine.
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orthogonal α-carboxyl protection[15–21] as Fmoc-Lys-OAll for side-chain anchoring for instance. This strategy presents some disadvantages for peptide synthesis: (i) the selective removal of allyl esters requires palladium chemistry, which results on partial deprotection of α-carboxyl group[22] (ii) acetylation of peptide N-terminal group by acetic acid used for allyl deprotection has been observed and prevents cyclization step.[20] To overcome these drawbacks, we focused our approach on the use of a recently described carboxyl protective group, Dmab (4{*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]-amino} benzyl alcohol) (Fig. 2), based on aminobenzylester moiety. To date, the use of this group has been reported as a temporary α-carboxyl protecting group of acidic (Asp, Glu) and hydroxyl (Tyr) amino acids[23–26] but never for basic amino acid such as lysine.

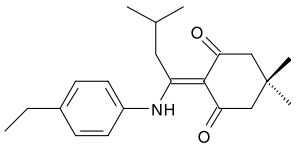


Figure 2. Dmab structure.

Removal of this protecting group does not require any metal catalyst, which avoids side reactions or further biological peptide poisoning. Dmab group is removed using brief treatment with 2% hydrazine in dimethylformamide (DMF), giving the free acid and by-products that are easily washed away from the solid phase (Scheme 1).

Moreover, Dmab protection of the carboxyl groups is orthogonal to the Fmoc/Boc strategy as it is stable to piperidine and TFA used for peptide synthesis.

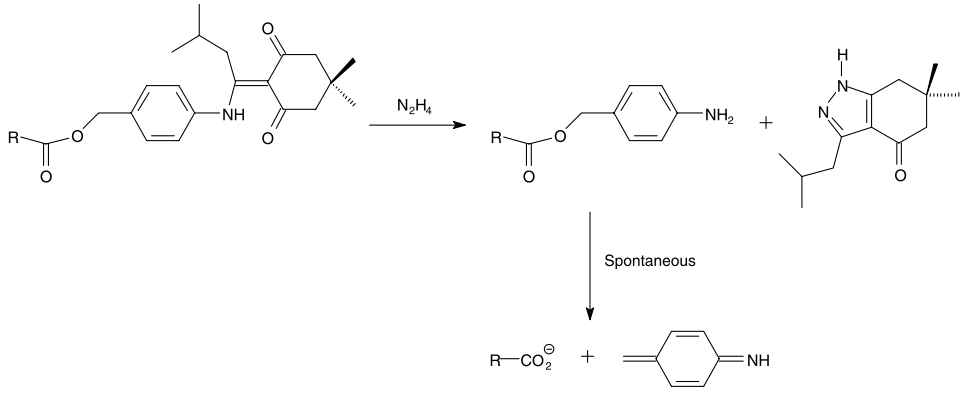
Thus, the use of such orthogonal protecting group for the synthesis of Dmab ester of a Fmoc-lysine yields a novel building block for 'on-resin' head-to-tail peptide cyclization.

For this purpose, esterification of the Fmoc-Lys(Boc)-OH with Dmab-OH was carried out by activation with diisopropylcarbodiimide (DCI) and dimethylaminopyridine (DMAP) in a mixture of dichloromethane (DCM)/acetonitrinile (ACN): 1:1 to yield Fmoc-Lys(Boc)-ODmab **1** (75%). After purification, treatment of Fmoc-Lys(Boc)-ODmab **1** with trifluoroacetic acid (TFA) in DCM gave the required Fmoc-Lys-ODmab **2** with 92% yield (Scheme 2). The structure and purity of these original compounds were confirmed by NMR and mass spectrometry for **1** and by NMR, IR, mass and analytical reversed-phase HLPC (RP-HPLC) for **2**.

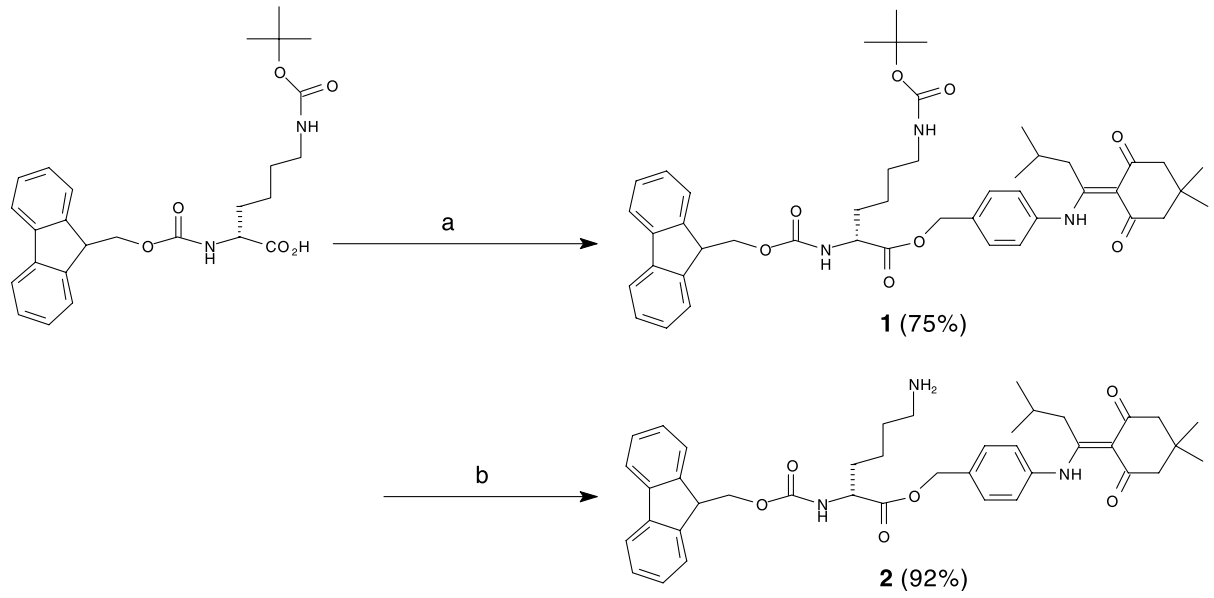
Synthetic usefulness of our approach was highlighted by (on resin) synthesis of **4**. Solid-phase synthesis of the 6-mer cyclic peptide was performed on a 2-chloro chlorotrityl resin (1.5 mmol/g). Loading of the resin was achieved with **2** and an excess of diisopropyldiethylamine (DIEA) in tetrahydrofurane (THF) (Scheme 3). Reaction was stopped after 5 h, resin was filtered off and washed successively with DMF, DCM and methanol. After solvents removal under vacuum, a substitution level of 0.1 mmol/g was estimated for this novel preloaded resin **3** by UV determination of the concentration of liberated dibenzofulvene, resulting from the cleavage of the Fmoc group with piperidine.[27] Low substitution level favors cyclization by pseudodilution, and therefore favors intramolecular reactions over intermolecular side reactions.[28] This resin (0.1 mmol) was then used for linear peptide synthesis on a ABI-431A continuous-flow automated peptide synthesizer using dicyclohexylcarbodiimide (DCC)/*N*-hydroxy-benzotriazole (HOBt) activation. After completion of peptide assembly, resin was treated three times with 2% hydrazine/DMF at room temperature for 3 min. The complete removal of the Dmab group was monitored by UV at 300 nm for each treatment. After the third, no absorbance was detected, which demonstrated that α-COOH group was totally deprotected. Resin was then washed with DMF.

For the intramolecular cyclization, PyBOP and HOBt (5 mol equiv for both) were used along with 10 mol equiv of DIEA for 48 h. Then, the peptide was cleaved from resin using a TFA–triisopropylsilane (TIS)–H2O (95/2.5/2.5) solution affording totally unprotected cyclic peptide **4** (Scheme 4).

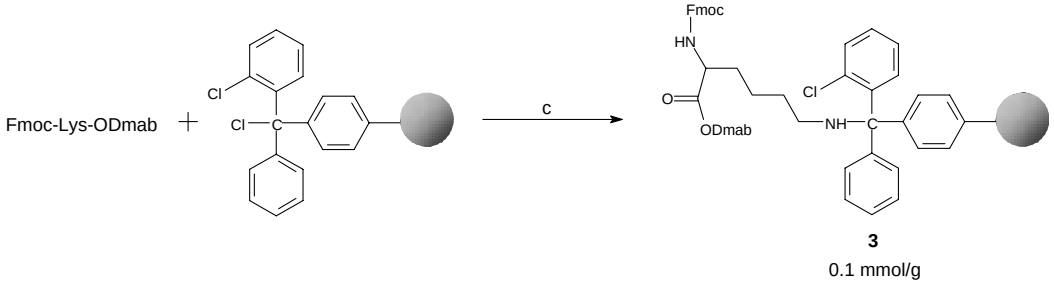
Peptide cyclic structure was confirmed on crude product with MALDI TOF mass spectrometry analysis. Crude product was purified by a semi-preparative RP-HPLC on a C18 column coupled with UV–vis detection (λ amide bond =214 nm) for separation. Purification gave 17 mg of **4** (25%). The structure of cyclic peptide 4 was confirmed by



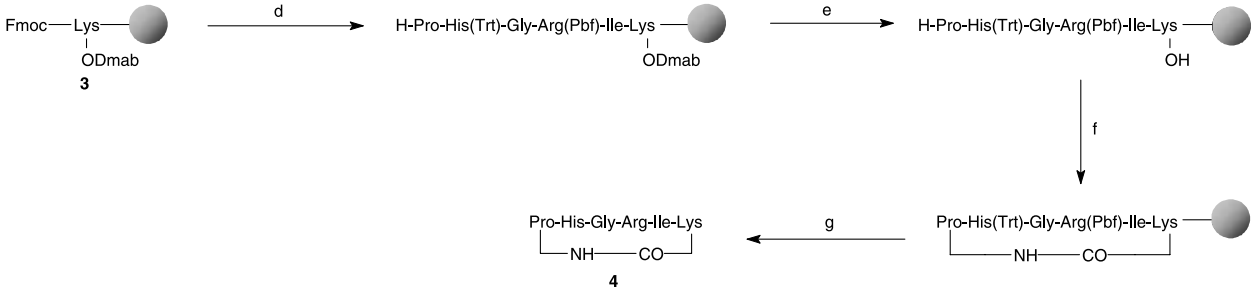
Scheme 1. Hydrazine-mediated cleavage of Dmab group.



Scheme 2. Synthesis of Fmoc-Lys-ODmab. (a) DmabOH, DMAP, DCI, DCM–ACN (1/1), rt, 24 h; (b) TFA–DCM (1/1), rt, 3 h.



Scheme 3. Anchoring of Fmoc-Lys-ODmab to the 2-chloro chlorotrityl resin. (c) DIEA, THF, rt, 5 h.



Scheme 4. Solid phase peptide synthesis of c(PHGRIK) **4**. (d) See Section 3: general procedure; (e) 2% H_4N_2/DMF; (f) PyBOP, HOBt, DIEA, NMP, rt, 48 h; (f) TFA–TIS–H_20 (9.5/0.25/0.25), rt, 3 h.

MALDI mass spectrometry analysis and its purity by analytical RP-HPLC.

Synthesized peptide **4** was then studied for its properties as in vitro inhibitor of the binding of $VEGF_{165}$ to its receptor. To this purpose, Chinese hamster ovary (CHO) cells expressing VEGFR2 were used. Competition between ^{125}I-$VEGF_{165}$ and **4** revealed a fairly high level of inhibition, that is, $IC_{50}=1.9$ μM (Fig. 3). Therefore, when compound with other peptidic inhibitory derivatives[29] our family behaves as one of them, low molecular weight most active derivatives.

Thus, our results highlight that Dmab α carboxyl protection of lysine and further coupling on trityl resin offers an efficient ways to synthesize poorly functionalized basic cyclic peptides, via Fmoc chemistry. As we had previously demonstrated that such derivatives exhibited high affinity for VEGFR2 and were of a great interest towards the generation of VEGF targeting nanodevices, the synthesis of their conjugates is of a great importance. With facile access to further functionalization via the liberated amine function, lysine appeared to be a good candidate. Subsequent functionalization on the side chain amine function resulting in a neutral amide bond, introduction of a supplementary lysine within an active cyclopeptide has to be considered as a key-step to apply this strategy.

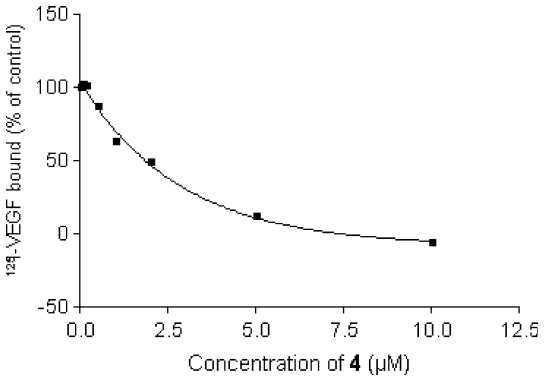


Figure 3. Inhibition of ^{125}I-VEGF$_{165}$ binding to CHO-VEGFR2 cells by cyclo(PHGRIK) **4**.

3. Experimental

3.1. General methods

All standard chemicals and solvents were of analytical grade and purchased from Sigma Aldrich. 2-Chloro chlorotrityl resin and all the Fmoc-amino acids were purchased from Advanced Chemtech. Dmab-OH was purchased from Novabiochem. All chemical reactions were carried out under nitrogen with dry solvents. *N*,*N*-dimethyl formamide (DMF) was refluxed over CaH_2 overnight and distilled, and methylene chloride (DCM) was distilled before use. Thin-layer chromatography (TLC) was performed on aluminium plates precoated with silica gel $60F_{254}$ (Merck) with detection by UV light (365 nm), and column chromatography was performed on silica gel 60, 70–230 mesh (SDS). ^{1}H and ^{13}C NMR spectra were recorded at 298 K on a Brucker Avance 300 MHz spectrometer at 300.13 and 75.46 MHz, respectively. Chemical shifts (δ) are given in parts per million (ppm). IR spectra were recorded on a Perkin-Elmer Spotlight 300 and Spectrum one (point mode 100 μM, 4 scans, 2 cm^{-1}, transmission mode). Solid phase peptide synthesis was carried out on an Applied Biosystems 431A automated peptide synthesizer using dicyclohexylcarbodiimide (DCC)/*N*-hydroxybenzotriazole (HOBt) activation. Reversed phase high pressure liquid chromatography (RP-HPLC) was performed on a Shimadzu instrument equipped with a SCL-10 AVP system controller, LC8A HPLC pumps and SPD-10 AVP UV–vis detector probing at 214, 267 and 254 nm on a SATISFACTION RP18AB 5 μM 250×4.6 mm C18 column (C.L.I Cluzeau). The following solvent systems were used for the elution in a linear gradient mode at a flow rate of 1 or 4 ml/min (for analytical and semi preparative HPLC, respectively): (A) 0.1% aqueous trifluoroacetic acid (TFA) and (B) 0.1% TFA in 70% aqueous acetonitrile (ACN). MALDI-TOF mass spectrometry was performed on a Reflex III Brucker apparatus.

3.1.1. Synthesis of Fmoc-Lys(Boc)ODmab (1). To a solution of Fmoc-lys(Boc)OH (1.3 g, 2.77 mmol) in a mixture of ACN–DCM (1/1.16 ml) was added successively Dmab-OH (0.61 g, 1.85 mmol) and dimethylamino pyridine (DMAP) (52 mg, 0.42 mmol). After complete dissolution, diisopropylcarbodiimide (DIC) (485 μl, 3.1 mmol) was added. Thereafter the solution was stirred at room temperature for 24 h. Then the reaction mixture was filtered, and the solvent was removed under vacuum. The crude product was dissolved in DCM (50 ml) and washed with water (3×50 ml). The organic layer was dried over $MgSO_4$ and filtered. The solvent is evaporated under vacuum to give greenish oil, which was purified by silica gel chromatography (DCM/ethyl acetate, 90:10). Then 1.1 g (1.4 mmol, R_F 0.53 (DCM–ethyl acetate, (80/20)) (75%) of compound **1** were obtained as a yellowish oil. MALDI-TOF mass spectrometry analysis gave the expected result (*m/z* calculated: 779.97 found $(M+Na)^+$: 802.46, $(M+K)^+$: 818.42). ^{1}H NMR ($CDCl_3$) δ ppm: 15.18 (s, 1H, NH Dmab), 7.72 (d, 2H,

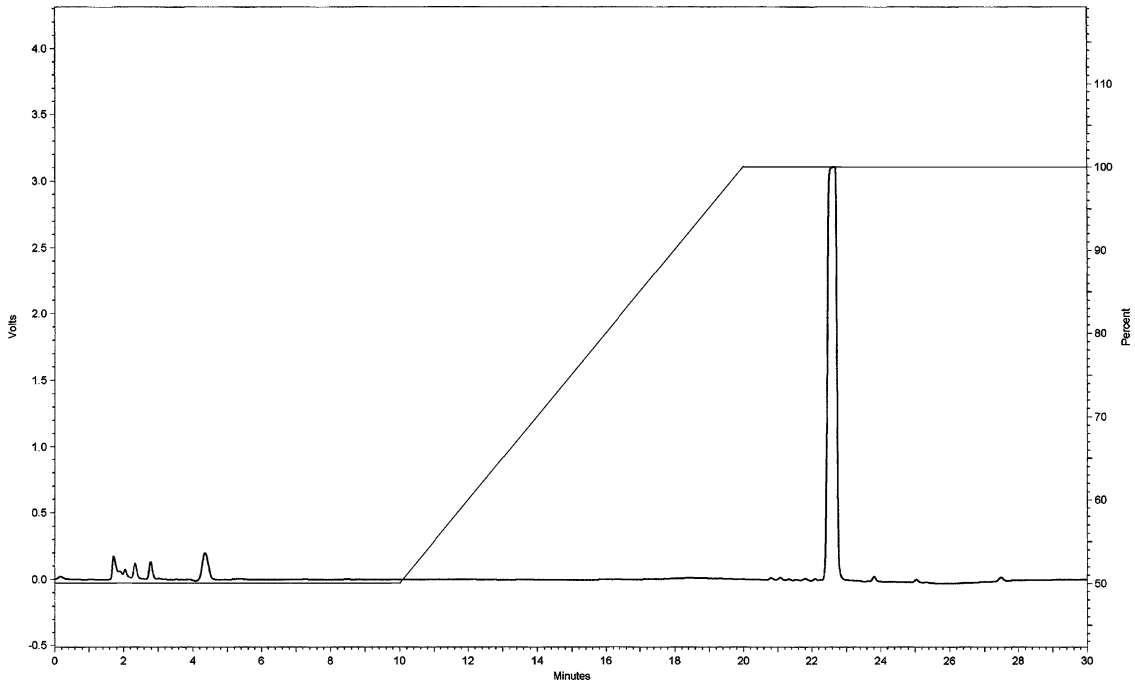


Figure 4. Analytical RP-HPLC of compound **2**. t_R=22.45 min.

CH_{Ar}Fmoc, $^3J_{H-H}$=7.5 Hz), 7.56 (d, 2H, CH_{Ar}Fmoc, $^3J_{H-H}$=7.32 Hz), 7.28–7.36 (m, 4H, CH_{Ar}Fmoc), 7.25 (d, 2H, CH_{Ar}Bzl, $^3J_{H-H}$=7.20 Hz), 7.06 (d, 2H, CH_{Ar}Bzl, $^3J_{H-H}$=8.25), 5.68 (d, 1H, $N_\alpha H$, $^3J_{H-H}$=7.8 Hz), 5.14 (s, 2H, CH_2Bzl), 4.6 (br s, 1H, NH-Boc), 4.34–4.37 (m, 1H, $C_\alpha H$), 4.19 (t, 1H, CH-Fmoc, $^3J_{H-H}$=14.3), 4.04–4.13 (m, 2H, CH_2-Fmoc), 3.15–3.02 (m, 2H, CH_2–NH), 2.94 (d, 2H, CH_2 Dmab, $^3J_{H-H}$=5.73 Hz), 2.49 (s, 4H, 2 CH_2 Dmab), 1.39–1.99 (m, 7H, 3 CH_2, CH *i*propyl), 1.38 (s, 9H, 3 CH_3 (Boc)), 1.03 (s, 6H, 2 CH_3), 0.75 (d, 6H, $(CH_3)_2$CH, $^3J_{H-H}$=6.66 Hz); ^{13}C NMR ($CDCl_3$) δ ppm: 198.82, 176.99, 172.27, 176.10, 156.52, 143.85, 141.50, 137.10, 135.13, 129.32, 128.00, 127.29, 126.88, 125.22, 120.25, 107.95, 78.87, 67.47, 66.55, 53.84, 53.00, 47.27, 39.71, 38.66, 31.96, 30.24, 29.76, 28.42, 27.00, 22.74, 22.27, 13.98.

3.1.2. Synthesis of Fmoc-Lys-ODmab (2). To a solution of compound **1** (1.1 g, 1.4 mmol) in DCM (30 ml) was added trifluoroacetic acid (TFA) (30 ml). The reaction mixture was stirred at room temperature for 3 h. The solution was concentrated to dryness in vacuo, followed by repeated washings and evaporations with diethylether. Then it was dried in dessicator under high vacuum over KOH to give 0.9 g (1.3 mmol, R_F 0.43 (DCM–methanol (90/10)) (92%) of the compound **2** as a pale yellow solid. Purity of compound **2** was determinated by RP-HPLC (Fig. 4) mp 92–94 °C; $[\alpha]_D^{20}$ +5.95 (*c* 0.84 in DCM); HRMS gave the expected result (*m*/*z* (m+1) calcd for $C_{41}H_{50}N_3O_6$ 680.3699 found 680.3673); IR ($cm^{-}1$): 1045, 1550, 1590, 1638, 1687, 2920, 2959; ^{1}H NMR ($CDCl_3$) δ ppm: 15.19 (s, 1H, NH Dmab), 7.97 (br s, 2H, NH_2), 7.71 (d, 2H, CH_{Ar}Fmoc, $^3J_{H-H}$=7.3 Hz), 7.54 (d, 2H, CH_{Ar}Fmoc, $^3J_{H-H}$=6.87 Hz), 7.28–7.34 (m, 4H, CH_{Ar}Fmoc), 7.25 (d, 2H, CH_{Ar}Bzl, $^3J_{H-H}$=7.47 Hz), 7.05 (d, 2H, CH_{Ar}Bzl, $^3J_{H-H}$=8.88 Hz), 5.68 (d, 1H, $N_\alpha H$, $^3J_{H-H}$=7.8 Hz), 5.14 (s, 2H, CH_2Bzl), 4.30–4.35 (m, 3H, CH_2-Fmoc, CH-Fmoc), 4.14–4.17 (m, 1H, $C_\alpha H$), 2.88–2.96 (m, 4H, CH_2–N, CH_2 Dmab), 2.42 (s, 4H, CH_2 Dmab), 1.94–1.37 (m, 7H, 3 CH_2, CH *i*propyl), 1.04 (s, 6H, 2 CH_3), 0.71 (d, 6H, $(CH_3)_2$CH, $^3J_{H-H}$=6.51 Hz); ^{13}C NMR ($CDCl_3$) δ ppm: 198.80, 176.98, 172.21, 176.04, 143.79, 141.36, 137.05, 135.18, 129.28, 128.10, 127.23, 126.88, 125.29, 120.20, 107.98, 67.50, 66.49, 53.91, 53.08, 47.31, 39.76, 38.61, 31.99, 30.27, 29.76, 27.05, 22.79, 22.25, 13.91.

3.1.3. Synthesis of Fmoc-Lys-(Trityl resin)-ODmab (3). Fmoc-Lys-ODmab **2** (1 g, 1.5 mmol) was dissolved in dry tetrahydrofuran (THF) (5 ml). After complete dissolution, diisopropylethylamine (DIEA) (600 µl, 3.46 mmol) was added. After 5 min, 2-chloro chorotrityl resin (500 mg, 1.5 mmol/g) was added. The resulting

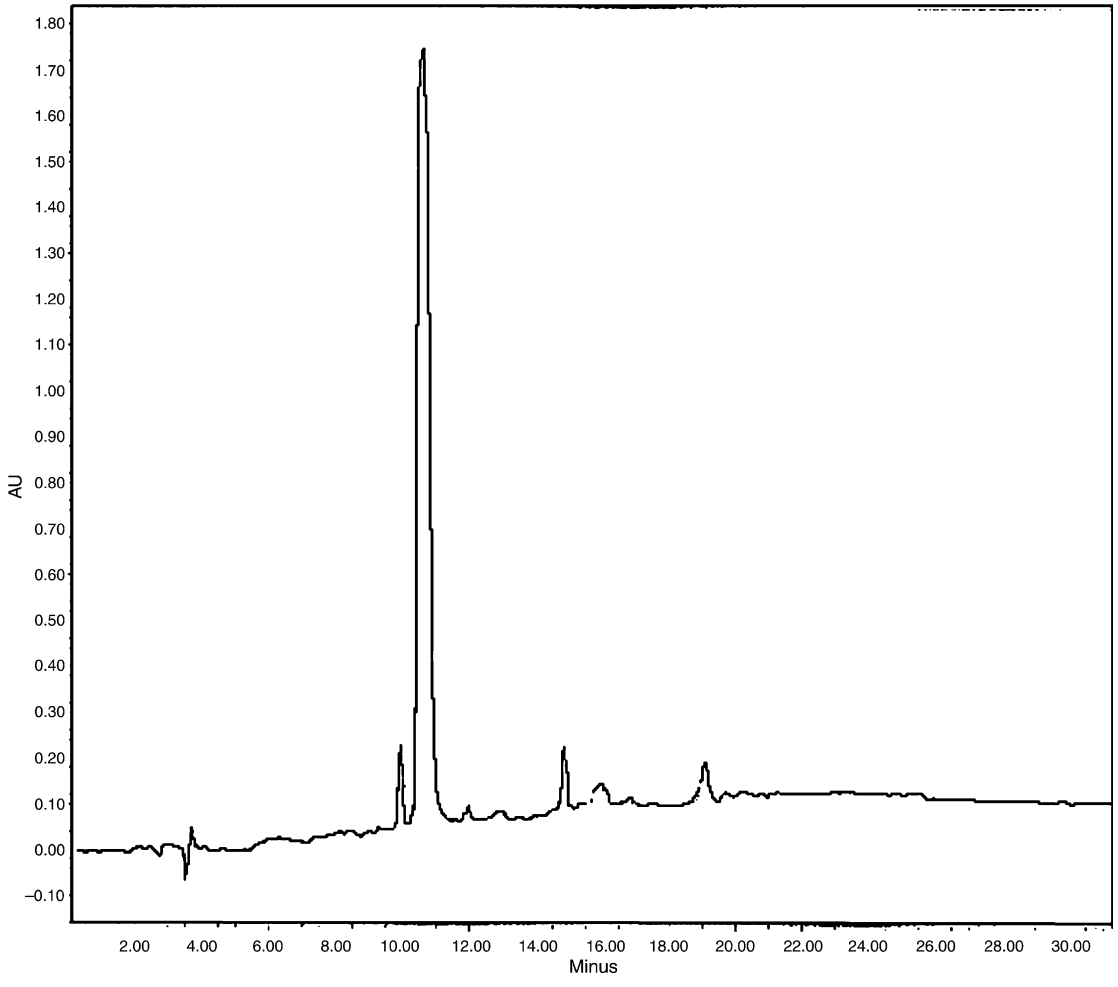


Figure 5. Analytical RP-HPLC of crude compound **4**.

suspension was stirred at room temperature for 5 h. The resin was filtered off and successively washed with DMF (1×20 ml), DCM–methanol–DIEA (17:2:1) (2×20 ml), DMF (1×10 ml) and DCM (2×20 ml). The new preloaded resin was dried in dessicator under high vacuum over KOH. The substitution level was determinated spectrophotometrically by Fmoc cleavage: Fmoc-Lys-(Trityl resin)-ODmab (4.3 mg) was introduced into a test tube and a solution of 20% piperidine in DMF was added (0.5 ml). 20% piperidine in DMF (0.5 ml) was also added to an empty test tube to serve as a blank. Over the next 15 min, the test tube with the resin was swirled to make sure all the resin has come in contact with the cleavage solution. DMF was added to both tubes to bring a volume of 50 ml. The blank was used to zero the UV spectrophotometer at 301 nm. The absorbance of the solution is 0.07. The substitution level was calculated from the formule: $(Abs_{301\ nm}\times vol(ml))/(7800\times m(g))$ and was determinated to be 0.1 mmol/g.

3.1.4. Synthesis of cyclo(PHGRIK) (4). Cyclo(PHGRIK) was synthesized by Fmoc/*t*Bu batch solid phase synthesis on an Applied Biosystems 431A automated peptide synthesizer. Preloaded Fmoc-Lys-(Trityl resin)-ODmab (1 g, 0.1 mmol) was used for the linear chain assembly. Subsequent Fmoc amino acids were coupled using a 10-fold excess of amino acids activated as HOBt ester. Removal of the Dmab group was performed after the N-terminal automated Fmoc deprotection. The peptidyl resin (1.28 g) was placed on a frit syringe barrel and allowed to equilibrate for 5 min in DMF (20 ml/g of resin). The solvent was removed from the resin by applying a nitrogen pressure and the residue was resuspended in a solution of 2% hydrazine monohydrate in DMF (20 ml/g of resin). Reaction was allowed to proceed for 3 min with gentle manual agitation and the hydrazine treatement was repeated a further two times to ensure complete reaction. The resin was washed successively with DMF (2×20 ml), DCM (2×20 ml), methanol (1×20 ml) and DCM (1×20 ml) and dried. Peptidyl resin (1.21 mg) was mixed with a solution of PyBOP (260 mg, 0,5 mmol), HOBt (69 mg, 0.51 mmol) and DIEA (180 µl, 1 mmol) in *N*-methyl-pyrrolidone (NMP) (10 ml). The mixture was swelled at room temperature for 48 h. The peptidyl resin was washed with 30 ml of each DMF, DCM and methanol and was dried over vacuum. Final cleavage from the resin and total deprotection of cyclo(PHGRIK) was performed with TFA–H_2O–Triisopropylsilane (TIS) (95/2.5/2.5) mixture for 2.5 h at room temperature. Then the filtrate was evaporated under reduced pressure to 5% of the volume. The product was precipitated from cold diethyl ether, filtered to give 30 mg of crude product (Fig. 5). MALDI-TOF mass spectrometry analysis of a sample of crude product gave the expected result (*m*/*z* calculated: 687.83 found $(M+H)^+$: 689.34) (Fig. 6). Then it was purified by semi-preparative HPLC to yield 17 mg of pure compound **4** (Fig. 6). MALDI-TOF mass spectrometry analysis of the pure product gave the expected result (*m*/*z* calculated: 687.83 found $(M+H)^+$: 689.34).

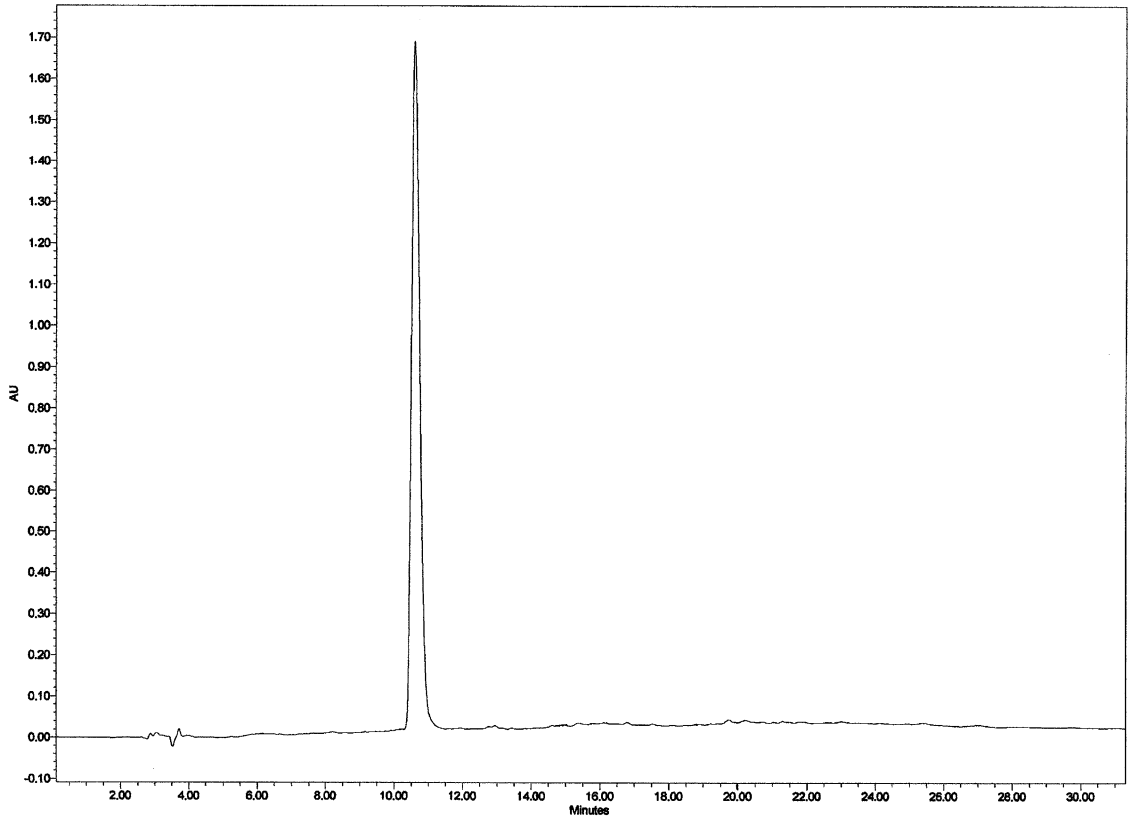


Figure 6. Analytical RP-HPLC of compound **4**. $t_R=10.5$ min.

3.2. Cell culture

CHO-VEGFR2 cells were grown in DMEM supplemented with 10% fetal calf serum, 2 mM L-glutamine, 1% non-essential amino acids and antibiotics (Invitrogen).

3.3. Binding assays

$VEGF_{165}$ was labeled with $Na^{125}I$ using IODO-GEN (Pierce) as coupling agent according to the manufacturer's instructions, CHO-VEGFR2 cells were seeded at 2.5×10^5 density in gelatin-coated 6-well plates and cultured in complete medium for 2 days. Cells were washed twice with ice-cold PBS and incubated with 10 ng/ml ^{125}I-VEGF and cyclo(PHGRIK) **4** at indicated concentrations in binding medium (DMEM; 20 mM Hepes, pH 7.4; 0.15% gelatin) on a shaker at 4 °C. After 2 h, cells were washed three times with PBS and solubilized by the addition of 2% Triton, 10% glycerol, and 1 mg/ml bovine serum albumin prior to γ-counting. Each condition was tested in duplicate and repeated at least two times. Data are expressed as percentage of total radioactivity.

Acknowledgements

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Photoinduced cycloadditions of *N*-methyl-1,8-naphthalenedicarboximides with alkynes

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Abstract—Photoinduced cycloadditions of *N*-methyl-1,8-naphthalenedicarboximide **1** with phenylacetylenes **2a**–**2c**, cyclopropylacetylene **2d**, diphenylacetylenes **2e**–**2f** and 1-phenylpropyne **2g** were investigated. In the case of phenylacetylenes **2a**, **2b** and cyclopropylacetylene **2c**, photoreaction with **1** takes place at the naphthalene C(1)=C(2) bond to give the cyclobutene products. For 4-methoxyphenylacetylene **1c**, the cyclobutene **3c** is obtained together with the 4-benzo[*a*]thebenidinone **4c** derived from a primary oxetene product formed by [2+2] addition of the imide carbonyl with the alkyne. Similar to **2c**, photocycloaddition of **1** with **2e** and **2f** gave the cyclobutenes **7e**, **7f**, **8f** and the 4-benzo[*a*]thebenidinone products **9e**, **9f** and **10f**, respectively, derived from the corresponding oxetenes. Photoreaction of **1** with **2g** gave cyclobutene **7g** and benzo[*a*]thebenidinone **9g**. Sensitization experiment and internal heavy atom effect study showed that these reactions proceed from the $\pi\pi^*$ singlet excited state of **1**. Estimation of the free energy change for electron transfer between ^{1}**1*** and the alkynes and the calculation of charge and spin density distribution in the anion radical of **1** and the cation radical of the alkynes suggested that the cyclobutene products are formed by direct [2+2] cycloaddition of ^{1}**1*** with the alkyne, while the formation of the oxetene products is the result of electron transfer interaction between ^{1}**1*** and the alkyne. The regioselectivity in the oxetene formation is accounted for by charge and spin density distribution in the anion radical of **1** and the cation radical of the alkyne.

1. Introduction

Photoinduced cycloaddition of aromatic imides with alkenes has long been an active research area in organic photochemistry. These reactions are characterized by the diversity in reaction patterns and mechanisms, depending on the structures of the imides and the alkenes. In photoinduced reactions of phthalimide with alkenes,[1] the most common reaction is the insertion of alkene into C(O)–N bond of the imide to give dihydrobenzazepinedione product.[2,3] For alkenes with allylic or benzylic hydrogens, hydrogen abstraction by the excited phthalimide followed by imide ketyl—alkene allylic (benzylic) radical pair recombination takes place, affording the reductive coupling product.[4] For electron rich alkenes of low oxidation potential with a negative free energy change for electron transfer (ΔG_{ET}) with the excited phthalimide, photoreaction in the presence of nucleophilic solvent (such as alcohol) often resulted in the formation of solvent incorporated coupling product.[4b–d,5] The Paterno-Buchi reaction leading to an oxetane product, typical for carbonyl addends with $n\pi^*$ excited state, is seldom observed in photoreactions of phthalimide with alkenes.[6] A current trend in the area of aromatic imide photochemistry is to change the aromatic moiety of the imide in order to tune the properties of the reactive excited state and the reduction potential with the purpose of exploring new reaction modes and searching for the mechanistic origin for the observed reaction pathways. Therefore, photoinduced reactions of 1,2-,[7] 2,3-,[8] 1,8-naphthalimides,[9] 9,10-phenanthrenedicarboximide[10] and 1*H*-dibenz[*c*.*e*]azepine-5,7-dione[11] with alkenes have been studied. In contrast to this situation, photoinduced cycloaddition reactions of aromatic imides with alkynes have not been reported. As these photoreactions between aromatic imides and alkynes may yield cyclobutene[12] and oxetene[13] products, both of which are important and versatile synthetic precursors that undergo diversified transformations to elaborate structures, we investigated the photocycloaddition reactions of *N*-methyl-1,8-naphthalimide **1** with alkynes **2a**–**2g** (Fig. 1).

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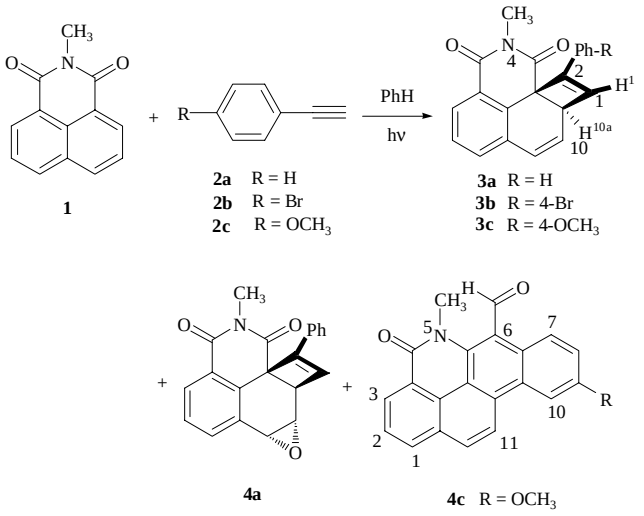

Figure 1.

2. Results and discussion

Photoinduced reaction of **1** (0.03 M) with an excess amount of **2a** in benzene with the irradiation of $\lambda > 330$ nm led to a regioselective [2+2] cycloaddition of **2a** to **1**, giving the cyclobutene product **3a** (85%) and a small amount of the oxirane product **4a** (2%) (Fig. 1). The structures of **3a** and **4a** are determined based on spectral (^{1}H NMR, IR, MS) and elemental analysis data and are further confirmed by X-ray crystallographic analyses.[14,15] In **3a**, the cyclobutene ring is almost perpendicular to the six-member ring. ^{1}H NMR experiment shows the proton at the C=C bond in the cyclobutene (H^1 in formula **3a**) has a small coupling constant (0.9 Hz) with the neighboring proton at the sp^3 carbon atom (C(10a)) in formula **3a** due to an unfavorable dihedral angle close to 90°. The coupling constant between H(10a) and H(10) is 4.2 Hz, typical for vicinal protons at adjacent sp^2 and sp^3 carbon atoms (=CH–CH<). A control experiment showed that the oxirane **4a** is derived from photooxidation of **3a** by a trace amount of oxygen within the solution. Therefore, irradiation of **3a** in an oxygen saturated benzene solution resulted in photooxygenation and partial cycloreversion to give **4a** (35%) and **1** (41%). Since **1** is known an effective singlet oxygen sensitizer,[16] the conversion of **3a** to **4a** in the photoreactions of **1** with **2a** may also be effected by naphthalimide sensitized photooxygenation of **3a**. In a separate experiment, photolysis of a benzene solution of **3a** in the presence of **1** under oxygen atmosphere also resulted in the formation of **4a**. Irradiation of **1** with **2b** in benzene solution similarly gave a cyclobutene product **3b** (82%). The molecular structure of **3b** is shown in Figure 2. On the other hand, photoreaction of **1** with 4-methoxyphenylacetylene **2c** gave, a 6-formyl-4-benzo[*a*]thebenidinone product **4c** (25%) in addition to cyclobutene product **3c**[17] (68%) (Table 1). In the ^{1}H NMR spectrum of **4c**, the aldehydic proton absorbs at δ 10.91, while H(7) (Formula **4c**) resonates at an unusually low field (δ 9.20) by the anisotropic deshielding effect of the nearby carbonyl group. Also, the protons in the 'bay area' (H(10) and H(11)) absorb at rather low field (δ 8.13 and 8.74, respectively).

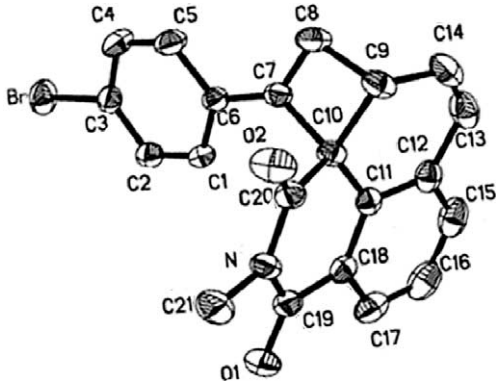

Figure 2. Molecular structure of **3b**.

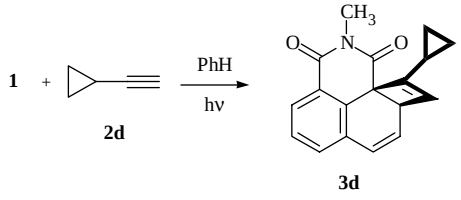

Figure 3.

Photoinduced reaction of **1a** with **2d** in benzene under similar conditions gave the cyclobutene product **3d** in 84% yield (Fig. 3).

The first excited singlet and triplet states (S_1 and T_1) of *N*-methyl-1,8-naphthalimide **1** are both of $\pi\pi^*$ character.[16,18] The photoreactions of **1** are believed to take place via the singlet excited state (S_1).[9c] Fluorescence quenching experiment showed that phenylacetylene quenches the

Table 1. Photoreactions of **1** with alkynes **2a–2g**

Alkyne	$E_{1/2}$ (V, SCE)	G_{ET} (kcal mol^{-1})[a]	Irradiation time (h)	Conversion (%)	Products and yield (%)[b]
2a	2.04	9.0	12	88	**3a** (85) **4a** (2)
2b			20	75	**3b** (82)
2c			10	85	**3c** (68) **4c** (25)
2d			48	48	**3d** (84)
2e	1.81	3.7	15	80	**7e** (40) **9e** (53)
2f	1.97	7.4	15	74	**7f**, **8f** (40, 59:41) **9f**, **10f** (53, 46:54)
2g			36	44	**7g** (52) **9g** (9)

[a] Calculated by the Weller equation.[26]
[b] Yield of isolated pure product based on consumed **1**.

fluorescence of **1** inefficiently. The fluorescence of **1** (2×10^{-5} mol/L, excitation wavelength 334 nm λ^{f}_{max} 380 nm) has no significant change with the addition of phenylacetylene in the concentration range 0.1–0.7 M. Taking into account that **1** has a singlet lifetime of 0.115 ns in benzene,[18c] the quenching rate constant must therefore be $\leq 10^{8}\ M^{-1}\ S^{-1}$. Sensitization experiments with xanthone (E_T 74 kcal/mol) and thioxanthone (E_T 66 kcal/mol)[19] as triplet sensitizer (E_T of **1** is 57 kcal/mol)[9c] did not sensitize the photocycloaddition of **1** with **2a**. Therefore, these photoreactions between **1** and **2** are from S_1 state of **1**.

We have also examined the internal heavy atom effect on the reaction by irradiating a benzene solution of 2-methyl-6-bromo-1,8-naphthalimide **5** with **2a** under the same conditions as for the photolysis of **1** with **2a**. This resulted in a sluggish reaction and only a 20% conversion of **5** was reached after photolysis for 20 h, giving the corresponding cyclobutene product **6a**[20] in 70% yield. This fact further supports the participation of the S_1 state in the photoreactions of **1** with **2** (Fig. 4).

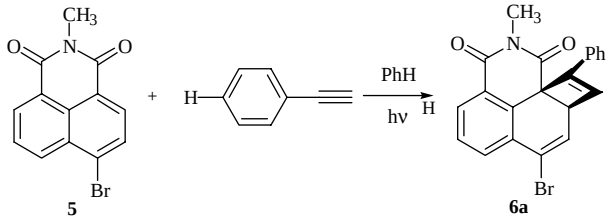

Figure 4.

The [2+2] cycloaddition of **1** with **2a–2c** is highly regioselective, and the corresponding **3a–3c** is the only cyclobutene product detected without their regioisomers. This regioselectivity is similar to that found in the photoreactions of **1** with alkenes, where cyclobutanes was formed regioselectively.[9c] In these photocycloadditions with alkenes, rationalization of the observed regioselectivity was not given. In rationalizing the regioselectivity of photocycloadditions of singlet excited addend with alkenes, consideration based on frontier molecular orbital (FMO) interactions of the two reactants has met some success.[21] However, the FMO interaction consideration is not rewarding here in rationalizing the observed regioselectivity of **3**. For photoreaction of **1** with **2a**, in either of the two FMO interactions ($LSOMO_{(imide)}$–$HOMO_{(2a)}$ and $HSOMO_{(imide)}$–$LUMO_{(2a)}$), requirement of maximum positive orbital overlap at the bonding sites leads to a regioselectivity in contrast to that seen in **3a**.[22] Same situation is also found in the case of **3c**, **3d** and **3g** (vide infra).

While the cyclobutene products **3a–3d** are formed by [2+2] cycloaddition of singlet exicited **1** with **2a–2d**, product **4c**, on the other hand, is derived from an oxetene primary product (**Ia**) formed in a 'Paterno-Buchi type' [2+2] photocycloaddition of **1** with **2c** (Fig. 6). For alkynes, such [2+2] photocycloaddition is typically found in photoinduced reactions with ($n\pi^*$) triplet carbonyl compounds.[23,24] However, oxetene formation between a ($\pi\pi^*$) excited carbonyl addend with alkyne has not been reported before. We believe that electron transfer interaction between ^{1}**1*** and **2c** is the reason for the reaction site switching from the naphthalene

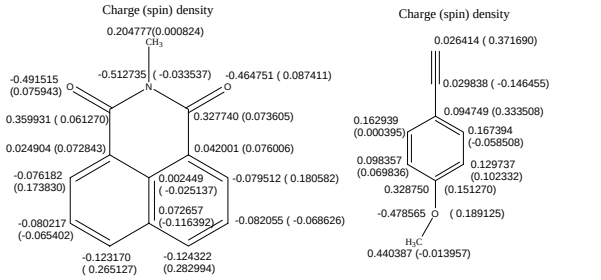

Figure 5. Charge and spin density distribution in the anion radical of **1** (left) and cation radical of **2c** (right) with the charge density on hydrogen atom summed up to their linked carbon atom.

ring to the imide carbonyl group. With the 4-methoxy substituent at the benzene ring, **2c** would have a much lower oxidation potential than **2a** ($E^{ox}_{1/2}=2.04$ V, SCE).[25] Compound **1** has a reduction potential of -1.44 V (SCE).[16] The free energy change for electron transfer between ^{1}**1*** and **2a** in benzene is estimated by the Weller equation[26] to have a positive value of 9 kcal/mol, while ΔG_{ET} between ^{3}**1*** and **2c** is expected to be much smaller. This nearly isothermal nature of the single electron transfer (SET) between ^{1}**1*** and **2c** is in agreement with the observed competition of the non-SET pathway leading to cyclobutene **3c** with the SET pathway leading to **4c** formation. The structure of product **4c** indicated that the Paterno-Buchi reaction of **1** with **2c** is regiospecific, giving the spirooxetene **Ia** as the only primary cycloadduct. SET interaction between ^{1}**1*** and **2c** in the nonpolar solvent benzene results in the formation of highly polar exciplex or contact ion radical pair. We have calculated the charge and spin density distribution of the anion radical of **1** and the cation radical of **2c** by DFT method at the UB3LYP 6-31G level with Gaussian 98 package.[27] The results given in Figure 5 show that, in $\mathbf{1}^{-\cdot}$, the carbonyl oxygen atom is the most heavily negatively charged atom, and also has a significant spin density, while in $\mathbf{2}^{+\cdot}$, C_α and C_β are positively charged with C_β also having a high spin density. These charge and spin density distributions explain the move of the reaction site to carbonyl in **1**. Positive and negative charge combination of $\mathbf{1}^{-\cdot}$ and $\mathbf{2c}^{+\cdot}$ at the carbonyl oxygen atom in **1** and C_β in **2c**, and bond formation between carbonyl carbon atom in **1** and C_α in **2c** leads to the product **4c**. If a singlet 1,4-diradical intermediate **IIa** is supposed as an intervening intermediate to **4c**,[29] the regioselectivity is also in agreement with the formation of a more stable diradical intermediate considering that in **IIa**, the α-phenyl vinyl radical has a linear structure, where the radical center carbon atom is sp hybridized with the unpaired electron in a p orbital.[24c,28] In this π radical, the phenyl π system is parallel with the spin bearing p orbital to provide efficient spin delocalization. The 1,4-diradical **IIa** is therefore more stable than the regioisomeric **IIIa**, in which the β-phenyl vinyl radical is a bent σ radical without spin delocalization (Fig. 6).

We have further investigated the photoreactions of **1** with diphenylacetylenes **2e** and **2f**. In the case of **2e**, the cyclobutene product **7e** (42%) and the 4,5-dihydro-4-benzo[*a*]thebenidinone product **9e** (53%) are formed. The X-ray crystallographic study of **7e** (Fig. 7) showed that, the two phenyls on the cyclobutene C=C bond deviate severely from coplanarity with each other. As a result, no significant deshielding effect to the protons at the two phenyls

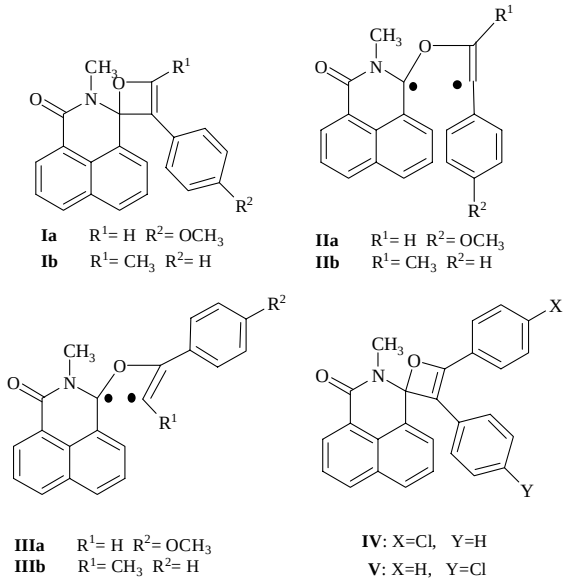

Figure 6.

are observed in the ^{1}H NMR spectrum, and all the phenyl protons resonate at normal field strength at δ 6.8–7.4. Photoreactions of **1** with **2f** gave four products **7f**–**10f** (Fig. 8). The cyclobutene regioisomers **7f** and **8f** are formed in a total yield of 40% with a ratio of 59:41. They cannot be fully separated by column chromatography on silica gel and the product ratio is determined by integration of the corresponding proton signals in the ^{1}H NMR spectrum of the mixed products. The benzothebenidinone regioisomers **9f** and **10f** are formed in 53% total yield in a ratio of 46:54. These two products are derived from the two corresponding regioisomeric oxetene primary products **IV** and **V** (Fig. 6). The concomitant formation of the cyclobutene and oxetene products in the reactions of **1** with **2e** and **2f** further indicates that, the switching of the reaction site from the naphthalene ring to the imide carbonyl is not caused by steric hindrance to the cyclobutene formation but is a manifestation of the occurrence of the parallel and competitive non-SET pathway via the S_1 ($\pi\pi^*$) state of **1** and the SET pathway via the polar exciplex or contact ion radical pair. This situation is in accord with the calculated ΔG_{ET} values for **1** with **2e** and **2f**, which

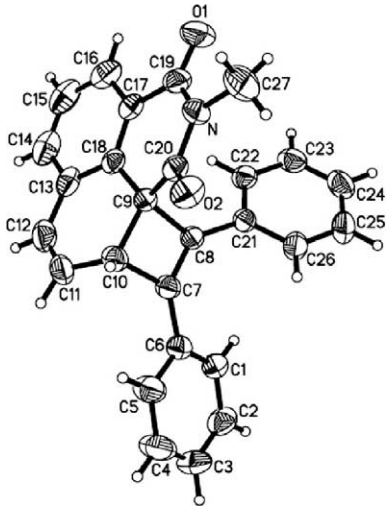

Figure 7. Molecular structure of **7e**.

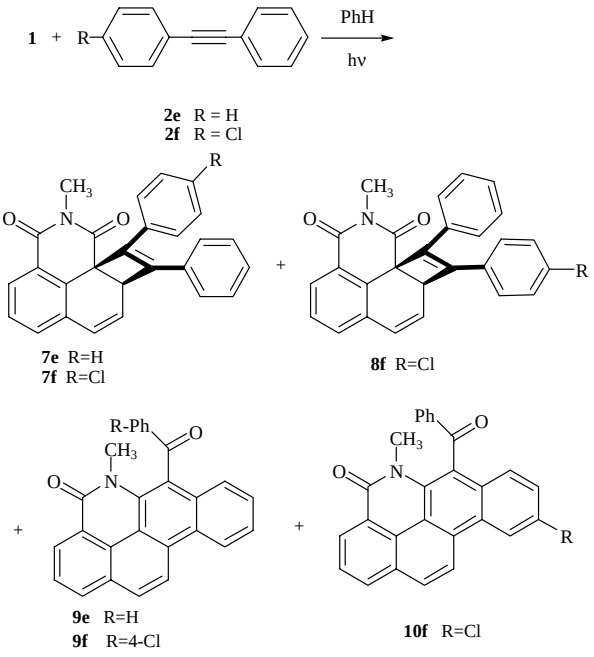

Figure 8.

have rather small positive values of 3.7 and 7.4 kcal/mol, respectively (Table 1).

Photoreactions of **1** with another central alkyne **2g** in benzene similarly afford the cyclobutene product **7g** (52%) and the 4-benzo[*a*]thebenidinone product **9g** (9%) (Fig. 9). Compound **7g** has the same regiochemistry as in **3a**–**3d**, with the phenyl linking to the C(2) atom. Product **9g** is derived from a primary oxetene **Ib**, which in turn is formed by coupling of **1** and **2g** in polar exciplex or contact ion radical pair. Again, the reaction site and regioselectivity in the oxetene formation could be rationalized by the charge and spin density distribution in $\mathbf{1}^{-\cdot}$ and $\mathbf{2g}^{+\cdot}$,[22] and is also in accord with the intervening of the 1,4-diradical intermediate **IIb** (which is more stable than the regioisomeric **IIIb**).

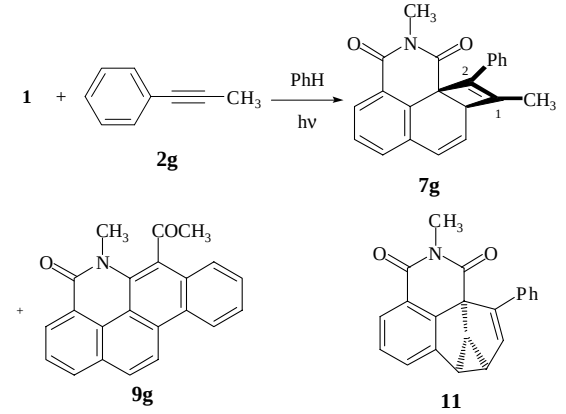

Figure 9.

In the cyclobutene products **3a**–**3d**, the C(1)═C(2) and C(9)═C(10) bonds, together with the C(2a) atom constitute a di-π methane structural unit. We have found that, upon irradiation of **3a** with light of $\lambda > 254$ nm, a di-π methane rearrangement[30] took place to give the semibullvalene product

11 (43%) together with a significant amount of **1** by cycloreversion of **3a**.

3. Conclusion

In summary, photoinduced [2+2] cycloadditions of *N*-methyl-1,8-naphthalenedicarboximide (**1**) with the alkynes **2a**, **2b**, and **2d** take place selectively at the naphthalene C(1)=C(2) bond in **1** to give cyclobutene products **3a–3d**. These reactions have the same regioselectivity, with the phenyl group attached to the C(2) atom in **3**. In photoreactions of **1** with the more electron rich alkynes **2c** and **2e–2g**, where the SET processes with ^{1}**1*** are nearly isothermal, SET and non-SET pathways run competitively to give the cyclobutene and oxetene products simultaneously. The oxetenes undergo spontaneous tandem 4-π electrocyclic ring opening and 6-π electrocyclization to give the 4-benzo[*a*]thebenidinone products. The oxetene formation reactions are also highly regioselective, dictated by the charge and spin density distribution in $\mathbf{1}^{-\cdot}$ and the alkyne cation radical.

4. Experimental

4.1. General

Melting points are uncorrected. ^{1}H NMR spectra were measured on a Bruker DPX 300 spectrometer at 300 MHz with $CDCl_3$ as solvent unless otherwise stated. The chemical shifts (δ) are reported in ppm relative to the residual deuterated solvent signal, and coupling constants (J) are given in Hz. ^{13}C NMR spectra were measured on a Bruker Avance 400 spectrometer at 100 MHz with $CDCl_3$ as solvent. IR spectra were recorded with a Shimadzu IR 440 spectrometer in KBr pallet. Mass spectra were taken on a VG ZAB-HS spectrometer in the electron impact ionization mode. UV–visible spectra were run on a UV-2401 PC spectrophotometer. Elemental analyses were performed with a Perkin-Elmer 240C analyzer. Fluorescence quenching experiments were performed with a Varian Cary Elipse fluorospectrometer. For X-ray crystallographic analysis, the X-ray diffraction intensities and the unit cell parameters were determined on a Siemens P4 diffractometer employing graphite-monochromated (Mo Kα) radiation (λ=0.71073 Å) and operating in the $\omega-2\theta$ scan mode. Data collection and cell refinement were performed with XSCANS. Structures were solved by direct methods and refined by full-matrix least-squares on F^2 with SHELXTL. Non-hydrogen atoms were refined by anisotropic displacement parameters, and the positions of all H-atoms were fixed geometrically and included in estimated positions using a riding model.

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 279548 and CCDC 279549. 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].

4.2. Materials

N-Methyl-1,8-naphthalenedicarboximide **1** was synthesized from acenaphthene by the literature method.[8a] 4-Bromophenylacetylene **2b**,[31] 4-methoxyphenylacetylene **2c**,[31] 4-chlorodiphenylacetylene **2e**,[32] 1-phenylpropyne **2g**[33] and 2-methyl-6-bromo-1,8-naphthelimide **5**[34] were prepared according to literature procedures, respectively. Benzene (A.R.) was refluxed with sodium/benzophenone and distilled before use. Petroleum ether refers to that with boiling points between 60–90 °C.

4.3. Procedures for the photoinduced reactions of 1 with alkynes 2a–2g

Irradiations were carried out with UV light of $\lambda>330$ nm using a medium-pressure mercury lamp (500 W) in a cooling water jacket, which was farther surrounded by a layer of filter solution (10% aq sodium nitrate, 1 cm thickness). The nitrogen-purged benzene solution of **1** (0.03 M) and an excess amount of alkyne was irradiated at ambient temperature, and the reaction course was monitored by TLC. After the photolysis, solvent was removed in vacuo, and the residue was subjected to flash column chromatography on silica gel (300–400 mesh) to afford the products.

4.3.1. Irradiation of 1 with 2a. A solution of **1** (633 mg, 3.0 mmol) and **2a** (6.120 g, 60 mmol) in benzene (100 mL) was irradiated for 12 h to give **3a** (702 mg, 85%), **4a** (17 mg, 2%) and unreacted **1** (76 mg, 88% conversion).

4.3.1.1. 4-Methyl-2-phenyl-3*H*,10a*H*-benzo[*de*]cyclobut[*i*]isoquinoline-3,5-dione (3a). Colorless crystals from petroleum ether–ethyl acetate, mp 154–155.5 °C. δ_H: 8.10 (1H, t, J=7.3 Hz), 7.43 (1H, t, J=7.4 Hz), 7.39 (1H, dd, J=7.6, 1.9 Hz), 7.18–7.21 (3H, m), 6.96–6.99 (2H, m), 6.76 (1H, d, J=0.9 Hz), 6.47 (1H, d, J=9.9 Hz), 6.22 (1H, dd, J=9.9, 4.3 Hz), 4.13 (1H, d, J=4.2 Hz), 3.32 (3H, s). ν_{max}: 1713, 1668, 1357, 1288, 765 cm^{-1}. *m/z*: 313 (M$^+$, 15), 211 (100), 183 (13), 102 (25). Found: C 80.06, H 5.36, N 4.75. $C_{21}H_{15}NO_2$ requires C 80.49, H 4.82, N 4.47.

4.3.1.2. 9,10-Epoxy-4-methyl-2-phenyl-3a*H*,10a*H*-benzo[*de*]cyclobut[*i*]isoquinoline-3,5-dione (4a). Colorless crystals from petroleum ether–ethyl acetate, mp 174–175.5 °C. δ_H: 8.19 (1H, dd, J=7.9, 1.3 Hz), 7.77 (1H, dd, J=7.5, 1.3 Hz), 7.49 (1H, t, J=7.7 Hz), 7.17–7.25 (3H, m), 6.85–6.89 (2H, m), 6.73 (1H, d, J=1.0 Hz), 4.43 (1H, d, J=0.9 Hz), 3.93 (2H, d, J=0.9 Hz), 3.24 (3H, s). ν_{max}: 1716, 1671, 1357, 1293, 768 cm^{-1}. *m/z*: 329 (M$^+$, 100), 313 (22), 312 (87), 301 (35), 300 (23), 227 (21), 215 (23), 211 (7), 199 (33), 142 (66), 102 (62). Found: C 76.52, H 5.25, N 4.62. $C_{21}H_{15}NO_3$ requires C 76.58, H 4.59, N 4.25.

4.3.2. Irradiation of 1 with 2b. A solution of **1** (633 mg, 3.0 mmol) and **2b** (1.086 g, 6.0 mmol) in benzene (100 mL) was irradiated for 20 h to give **3b** (723 mg, 82%) and unreacted **1** (158 mg, 75% conversion).

4.3.2.1. 4-Methyl-2-(4-bromophenyl)-3*H*,10a*H*-benzo[*de*]cyclobut[*i*]isoquinoline-3,5-dione (3b). Colorless crystals from petroleum ether–ethyl acetate, mp 212–213.5 °C.

δ_H: 8.09 (1H, dd, J=7.5, 1.4 Hz), 7.31–7.47 (2H, m), 7.32 (2H, d, J=8.5 Hz), 6.77–6.87 (3H, m), 6.47 (1H, d, J=9.9 Hz), 6.21 (1H, dd, J=9.9, 4.2 Hz), 4.12 (1H, d, J=4.1 Hz), 3.32 (3H, s). ν_{max}: 1719, 1665, 1422, 1361, 1291, 830, 764 cm^{-1}. *m/z* 393 (M^++2, 10.5), 392 (M, 11.4), 391 (M^+, 10.8), 211 (100), 182 (22). Found: C 64.52, H 4.11, N 3.68. $C_{21}H_{14}BrNO_2$ requires C 64.30, H 3.60, N 3.57. X-ray structure analysis: $C_{21}H_{14}BrNO_2$, M=327.37. Triclinic, space group P-1, a=11.327(2), b=9.2270(18), c=16.397(3) Å, α=90.00, β=97.95(3), γ=90.00°, V=1697.2(6) $Å^3$, Z=4, D_c=1.281 g cm^{-3}, $F(000)$=688, absorption coefficient 2.461 mm^{-1}, scan range for data collection $1.82 \le \theta \le 25.00°$, 3150 measured reflections, 2991 independent reflections, 1734 reflections with $I > 2\sigma(I)$, R_{int}=0.0473, 227 refinable parameters, $R[F^2 > 2\sigma(F^2)]$=0.0562, wR_2 (F^2)=0.1579.

4.3.3. Irradiation of 1 with 2c. A solution of **1** (633 mg, 3.0 mmol) and **2c** (792 mg, 6.0 mmol) in benzene (100 mL) was irradiated for 10 h to furnish **3c** (595 mg, 68%), **4c** (217 mg, 25%) and unreacted **1** (95 mg, 85% conversion).

4.3.3.1. 4-Methyl-2-(4-methoxyphenyl)-3*H*,10a*H*-benzo[*de*]cyclobut[*i*]isoquinoline-3,5-dione (3c). Colorless crystals from petroleum ether–ethyl acetate, mp 168–169.5 °C. δ_H: 8.09 (1H, dd, J=7.2, 1.9 Hz), 7.41 (1H, t, J=7.4 Hz), 7.38 (1H, dd, J=7.5, 1.9 Hz), 6.88–6.92 (2H, m), 6.70–6.74 (2H, m), 6.65 (1H, d, J=1.0 Hz), 6.45 (1H, d, J=9.9 Hz), 6.22 (1H, dd, J=9.9, 4.2 Hz), 4.10 (1H, d, J=4.2 Hz), 3.74 (3H, s), 3.32 (3H, s). ν_{max}: 1713, 1666, 1421, 1359, 1290, 833, 764 cm^{-1}. *m/z*: 343 (M^+, 14), 211 (4), 132 (100). Found: C 76.74, H 5.43, N 4.35. $C_{22}H_{17}NO_3$ requires C 76.95, H 4.99, N 4.08.

4.3.3.2. 4,5-Dihydro-5-methyl-6-formyl-9-methoxy-4-benzo[*a*]thebenidinone (4c). Orange powder from chloroform–petroleum ether, mp 227–229 °C. δ_H: 10.9 (1H, s), 9.20 (1H, d, J=9.4 Hz), 8.84 (1H, dd, J=7.5, 1.1 Hz), 8.74 (1H, d, J=9.2 Hz), 8.38 (1H, dd, J=7.5, 1.1 Hz), 8.29 (1H, d, J=9.2 Hz), 8.13 (1H, d, J=2.6 Hz), 8.00 (1H, t, J=7.7 Hz), 7.51 (1H, dd, J=9.4, 2.6 Hz), 4.10 (3H, s), 4.02 (3H, s). ν_{max}: 1657 (C=O), 1620, 1246, 823, 753 cm^{-1}. *m/z*: 341 (M^+, 100), 324 (73), 298 (23), 270 (20), 240 (21), 149 (19). Found: C 81.42, H 4.41, N 4.48. $C_{22}H_{15}NO_3$ requires C 81.21, H 4.65, N 4.30.

4.3.4. Irradiation of 1 with 2d. A solution of **1** (633 mg, 3.0 mmol) and **2d** (3.963 g, 60 mmol) in benzene (100 mL) was irradiated for 48 h to give **3d** (335 mg, 84%), and unreacted **1** (329 mg, 48% conversion).

4.3.4.1. 2-Cyclopropyl-4-methyl-3*H*,10a*H*-benzo[*de*]-cyclobut[*i*]isoquinoline-3,5-dione (3d). White solid from petroleum ether–ethyl acetate, mp 103–105 °C. δ_H: 8.03 (1H, d, J=6.0 Hz), 7.37–7.42 (2H, m), 6.41 (1H, d, J=12.0 Hz), 6.14 (1H, dd, J=9.1, 0.8 Hz), 5.99 (1H, s), 3.99 (1H, d, J=4.0 Hz), 3.41 (3H, s), 0.88 (1H, s), 0.53–0.59 (2H, m), 0.26–0.33 (2H, m). ν_{max}: 3035, 2925, 1713, 1669, 1476, 1286 cm^{-1}. *m/z*: 277 (M^+, 8.4), 211 (100), 183 (19), 167 (16), 127 (14). Found: C, 77.76, H 5.58, N 5.10. $C_{18}H_{15}NO_2$ requires C 77.96, H 5.45, N 5.05.

4.3.5. Irradiation of 1 with 2e. A solution of **1** (633 mg, 3.0 mmol) and **2e** (1.068 g, 6.0 mmol) in benzene (100 mL) was irradiated for 15 h to afford **7e** (373 mg, 40%), **9e** (492 mg, 53%) and unreacted **1** (127 mg, 80% conversion).

4.3.5.1. 4-Methyl-1,2-diphenyl-3*H*,10a*H*-benzo[*de*]-cyclobut[*i*]isoquinoline-3,5-dione (7e). Colorless crystals from petroleum ether–ethyl acetate, mp 170–171 °C. δ_H: 7.97 (1H, dd, J=7.2, 1.9 Hz), 7.37–7.46 (4H, m), 7.19–7.32 (6H, m), 6.81–6.85 (2H, m), 6.49 (1H, d, J=10.1 Hz), 6.40 (1H, dd, J=10.0, 4.3 Hz), 4.77 (1H, d, J=4.3 Hz), 3.16 (3H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ_c: 27.1, 41.4, 55.0, 125.4, 126.4, 126.8, 126.9, 127.4, 127.5, 128.2, 128.5, 128.8, 128.9, 130.4, 131.2, 131.5, 131.8, 132.3, 132.6, 134.0, 142.6, 149.1, 164.6, 173.0. ν_{max}: 1715, 1670, 1418, 1360, 1295, 827, 757 cm^{-1}. *m/z*: 388 (M^+−1, 3), 211 (1), 178 (100). Found: C 83.10, H 5.18, N 4.03. $C_{27}H_{19}NO_2$ requires C 83.27, H 4.92, N 3.60. X-ray structural analysis: $C_{27}H_{19}NO_2$, M=389.43. Monoclinic, space group $P2(1)/n$, a=8.258(1), b=0.465(2), c=23.942(6) Å, α=90, β=93.32(1), γ=90°, V=2065.6(7) $Å^3$, Z=4, D_c=1.252 g cm^{-3}, $F(000)$=816, absorption coefficient 0.079 mm^{-1}, scan range for data collection $1.70 \le \theta \le 25.20°$, 3987 measured reflections, 3710 independent reflections, 1649 reflections with $I > 2\sigma(I)$, R_{int}=0.0221, 273 refinable parameters, $R[F^2 > 2\sigma(F^2)]$=0.0434, wR_2 (F^2)=0.1002.

4.3.5.2. 4,5-Dihydro-5-methyl-6-benzoyl-4-benzo[*a*]thebenidinone (9e). Pale yellow powder from chloroform–petroleum ether, mp 206–208 °C. δ_H: 8.95 (1H, d, J=9.2 Hz), 8.91 (1H, d, J=8.4 Hz), 8.81 (1H, dd, J=7.5, 1.1 Hz), 8.40 (1H, dd, J=7.9, 0.8 Hz), 8.31 (1H, d, J=9.1 Hz), 7.99 (1H, t, J=7.7 Hz), 7.86–7.90 (3H, m), 7.72 (1H, td, J=7.6, 1.2 Hz), 7.57–7.64 (2H, m), 7.44 (2H, t, J=7.9 Hz), 3.82 (3H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ_c: 36.3, 116.0, 120.2, 122.0, 123.1, 124.0, 125.7, 125.8, 126.0, 126.8, 126.9, 127.1, 127.7, 127.8, 128.2, 128.9, 129.2, 129.7, 130.5, 131.7, 133.9, 134.1, 134.5, 138.8, 163.1, 199.4. ν_{max}: 1660 (C=O), 1229, 757 cm^{-1}. *m/z*: 387 (M^+, 100), 370 (73), 310 (51), 282 (22), 252 (34), 226 (11), 193 (8), 105 (23). Found: C 84.06, H 5.03, N 4.17. $C_{27}H_{17}NO_2$ requires C 83.70, H 4.42, N 3.61.

4.3.6. Irradiation of 1 with 2f. A solution of **1** (633 mg, 3.0 mmol) and **2f** (1.272 g, 6.0 mmol) in benzene (100 mL) was irradiated for 15 h to give an isomeric mixture of **7f** and **8f** (376 mg, 40%, 59:41) and an isomeric mixture of **9f** and **10f** (496 mg, 53%, 46:54), and unreacted **1** (165 mg, 74% conversion).

4.3.6.1. 4-Methyl-1-(4-chlorophenyl)-3*H*,10a*H*-2-phenylbenzo[*de*]cyclobut[*i*]isoquinoline-3,5-dione (7f). Colorless crystals from petroleum ether–ethyl acetate, mp 186–188 °C. δ_H: 7.93 (1H, dd, J=7.4, 1.7 Hz), 7.22–7.39 (7H, m), 7.14–7.18 (2H, m), 6.71–6.74 (2H, m), 6.44 (1H, d, J=10.1 Hz), 6.32 (1H, dd, J=10.1, 4.3 Hz), 4.71 (1H, d, J=4.2 Hz), 3.13 (3H, s). ν_{max}: 1717, 1671, 1418, 1357, 1287, 829, 761 cm^{-1}. *m/z*: 425 (M^++2, 1.5), 423 (M^+, 4.8), 214 (56), 213 (32), 212 (100), 176 (17), 151 (11). Found: C 76.82, H 4.61, N 3.10. $C_{27}H_{18}ClNO_2$ requires C 76.50, H 4.28, N 3.34.

4.3.6.2. 4-Methyl-1-phenyl-3*H*,10a*H*-2-(4-chlorophenyl)benzo[*de*]cyclobut[*i*]isoquinoline-3,5-dione (8f). Not fully separated by column chromatography on silica gel from isomer **7f**.

4.3.6.3. 4,5-Dihydro-5-methyl-6-(4-chlorobenzoyl)-4-benzo[*a*]thebenidinone (9f). Pale yellow blocks from chloroform–petroleum ether, mp 281–282 °C. δ_H: 8.91 (1H, d, J=7.5 Hz), 8.87 (1H, d, J=7.5 Hz), 8.78 (1H, d, J=7.5 Hz), 8.37 (1H, d, J=7.8 Hz), 8.27 (1H, d, J=9.1 Hz), 7.97 (1H, t, J=7.9 Hz), 7.80–7.83 (3H, m), 7.71 (1H, t, J=7.4 Hz), 7.60 (1H, t, J=7.6 Hz), 7.41 (2H, d, J=8.7 Hz), 3.78 (3H, s). ν_{max}: 1660, 1220, 755 cm^{-1}. *m/z* 421 (M^+, 100), 404 (91), 369 (15), 310 (64), 282 (26), 252 (46), 226 (11), 139 (26). Found: C 76.31, H 4.28, N 3.04. $C_{27}H_{16}ClNO_2$ requires C 76.87, H 3.82, N 3.32.

4.3.6.4. 4,5-Dihydro-5-methyl-6-benzoyl-9-chloro-4-benzo[*a*]thebenidinone (10f). Pale yellow blocks from chloroform–petroleum ether, mp 243–245 °C. δ_H: 8.86–8.81 (3H, m), 8.41 (1H, d, J=1.0 Hz), 8.32 (1H, d, J=9.2 Hz), 8.02 (1H, d, J=7.7 Hz), 7.85 (2H, d, J=7.2 Hz), 7.81 (1H, d, J=9.1 Hz), 7.61 (1H, t, J=7.4 Hz), 7.53 (1H, dd, J=9.0, 2.1 Hz), 7.44 (2H, t, J=7.5 Hz), 3.80 (3H, s). ν_{max}: 1665, 1233, 756 cm^{-1}. *m/z*: 423 (M^++2, 38), 421 (M^+, 100), 404 (58), 369 (13), 344 (52), 286 (16), 105 (51). Found: C 76.68, H 4.55, N 2.98. $C_{27}H_{16}ClNO_2$ requires C 76.87, H 3.82, N 3.32.

4.3.7. Irradiation of 1 with 2g. A solution of **1** (633 mg, 3.0 mmol) and **2g** (6.96 g, 60 mmol) in benzene (100 mL) was irradiated for 36 h to give **7g** (224 mg, 52%) and **9g** (38 mg, 9%) and unreacted **1** (354 mg, 44% conversion).

4.3.7.1. 1,4-Dimethyl-2-phenyl-3*H*,10a*H*-benzo[*de*]-cyclobut[*i*]isoquinoline-3,5-dione (7g). Colorless crystals from dichloromethane–petroleum ether, mp 164–165 °C. δ_H: 8.02 (1H, dd, J=7.2, 2.0 Hz), 7.36–7.43 (2H, m), 7.16–7.28 (3H, m), 6.80–6.84 (2H, m), 6.48 (1H, d, J=10.0 Hz), 6.26 (1H, dd, J=9.9, 4.8 Hz), 4.15 (1H, d, J=4.1 Hz), 3.24 (3H, s), 2.63 (3H, s). ν_{max}: 3069, 2927, 1717, 1671, 1593, 1474, 1290, 1132 cm^{-1}. *m/z*: 327 (M^+, 6.3), 211 (25), 167 (18), 116 (100), 77 (5.6). Found: C 80.52, H 5.45, N, 4.34, $C_{22}H_{17}NO_2$ requires C 80.71, H 5.23, N 4.28.

4.3.7.2. 4,5-Dihydro-5-methyl-6-acetyl-4-benzo[*a*]-thebenidinone (9g). Yellow solid from petroleum ether–ethyl acetate, mp 146–148 °C. δ_H: 8.92 (2H, dd, J=9.5, 2.9 Hz), 8.85 (1H, dd, J=7.5, 1.2 Hz), 8.40 (1H, d, J=8.0 Hz), 8.30 (1H, d, J=9.2 Hz), 8.10–8.13 (1H, m), 8.02 (1H, t, J=7.7 Hz), 7.76–7.81 (2H, m), 3.99 (3H, s), 2.72 (3H, s). ν_{max}: 3048, 2973, 2920, 1691, 1654, 1599, 1365, 1240 cm^{-1}. *m/z*: 325 (M^+, 64), 310 (100), 252 (45), 163 (7.7), 105 (13), 77 (7.4), 40 (15.8). Found: C 81.52, H 4.69, N 4.32. $C_{22}H_{15}NO_2$ requires C 81.21, H 4.65, N 4.30.

4.3.8. Irradiation of 5 with 2a. A solution of **5** (870 mg, 3.0 mmol) and **2a** (6.120 g, 60 mmol) in benzene (100 mL) was irradiated for 20 h to provide **6a** (183 mg, 78%) and unreacted **5** (696 mg, 20% conversion).

4.3.8.1. 9-Bromo-4-methyl-2-phenyl-3*H*,10a*H*-benzo [*de*]cyclobut[*i*]isoquinoline-3,5-dione (6a). Colorless crystals from petroleum ether–ethyl acetate, mp 200–201 °C. δ_H: 8.19 (1H, d, J=7.8 Hz), 7.98 (1H, d, J=7.8 Hz), 7.53 (1H, t, J=7.8 Hz), 7.24–7.16 (3H, m), 6.97–6.90 (2H, m), 6.76 (1H, s), 6.69 (1H, d, J=4.8 Hz), 4.20 (1H, d, J=4.8 Hz), 3.32 (3H, s). ν_{max}: 1713, 1667, 1360, 1294, 762 cm^{-1}. *m/z*: 392 (M^+, 8.6), 312 (17), 291 (100), 289 (94), 261 (19), 247 (13), 245 (17), 226 (17), 166 (14), 102 (78). Found: C 64.58, H 3.26, N 3.91. $C_{21}H_{14}BrNO_2$ requires C 64.30, H 3.60, N 3.57.

4.3.9. Photolysis of 3a. A solution of **3a** (156 mg, 0.5 mmol) in benzene (25 mL) purged with nitrogen was irradiated with light of λ>254 nm for 15 h to produce **11** (57 mg, 43%) and **1** (41 mg, 46%) and unreacted **3a** (23 mg, 85% conversion).

Compound (**11**). Colorless crystals from acetone, mp 234–236 °C. δ_H: 7.59 (2H, t, J=6.6 Hz), 7.35 (1H, t, J=7.6 Hz), 7.27 (1H, m), 7.18 (2H, t, J=7.4 Hz), 6.66 (2H, d, J=6.9 Hz), 5.46 (1H, d, J=2.7 Hz), 4.10 (1H, d, J=6.3 Hz), 3.27 (1H, t, J=6.6 Hz), 3.14 (1H, dt, J=6.9, 2.7 Hz), 3.06 (3H, s). ν_{max}: 1716, 1672, 1349, 1274, 764 cm^{-1}; *m/z*: 313 (M^+, 63), 256 (49), 228 (41), 226 (51), 211 (100), 102 (9). Found: C 80.62, H 4.61, N 4.75. $C_{21}H_{15}NO_2$ requires C 80.49, H 4.82, N 4.47.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2005.10.076.

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Synthesis of dinucleotides with 2′-C to phosphate connections by ring-closing metathesis

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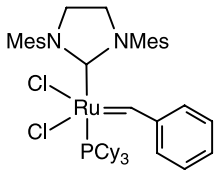
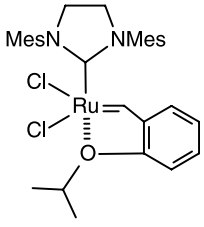
Abstract—Four different nucleosides with olefinic 2′-modifications were prepared; 2′-*C*-methylene, 2′-*C*-(propen-1-yl), 2′-*C*-allyl and 2′-*O*-allyl uridines, respectively. These were incorporated into dinucleotides with allyl phosphate or vinyl phosphonate linkages. Hence, six different dinucleotides were studied as substrates for RCM reactions, and from four of these, cyclic dinucleotides with connections between 2′-C and phosphorus of 3–6 atoms were obtained.

1. Introduction

The ring-closing metathesis (RCM) reaction is generally acknowledged as an extremely powerfull synthetic method for introducing medium to large rings tolerating most functional groups.[1] The method relies on efficient catalysts being able to induce a series of [2+2] cycloadditions combining two olefinic moieties into a new cyclic olefin.[1,2] Two of the most useful catalysts for the purpose are the ruthenium-based **A**[2,3] and **B**[4] (Mes=2,4,6-trimethylphenyl, Cy=cyclohexyl).



A



B

Among many successful applications, the RCM methodology has been used in peptide chemistry for the preparation of cyclic peptide analogues, for example, conformationally restricted β-turn mimics.[5] Also nucleosides and nucleotides have been prepared by the use of RCM or related cross-metathesis methods.[6,7]

We have introduced RCM in nucleic acid chemistry as a general synthetic strategy for the introduction of conformationally restricting internucleotide connections in appropriate dinucleotide substrates.[8,9] Hereby, dinucleotides and, subsequently, oligonucleotides with artificial bends can be obtained as potential tools in targeting and modeling secondary nucleic acid structures like bulges or three-way-junctions.[10] The dinucleotide substrates studied so far have involved nucleoside building blocks with terminal alkenes like 5′-*C*-vinylthymidine,[11] 5′-*C*-allylthymidine,[12] 4′-*C*-vinylthymidine,[12] 2′-*O*-allyluridine,[9] 2′-*O*-allylarabinouridine,[9] 5-allyluridine[8,13] and 5-allyl-2′-deoxyuridine[9,13] in combination with allyl phosphortriester linkages. In several cases, cyclic dinucleotides have been obtained in high yields by RCM on these substrates. Also a tandem RCM-hydrogenation protocol has been applied for the direct introduction of saturated linkages avoiding the *E*/*Z*-isomeri often involved in the production of large cycloalkene structures.[9,13]

In the present study, we focus on the scope for preparing connections between the 2′-C position and the adjacent phosphorus in dinucleotides—in other words the preparation of 2′-3′-fused bicyclic nucleotides with varying ring sizes. Hence, a series of different nucleosides with olefinic 2′-modifications are prepared and incorporated into dinucleotides with allyl phosphate or vinyl phosphonate linkages. Subsequently, 2′-C to P connections of 1–6 atoms can be formed by RCM reactions. The most successfully prepared of these cyclic dinucleotides (containing a 5 atom

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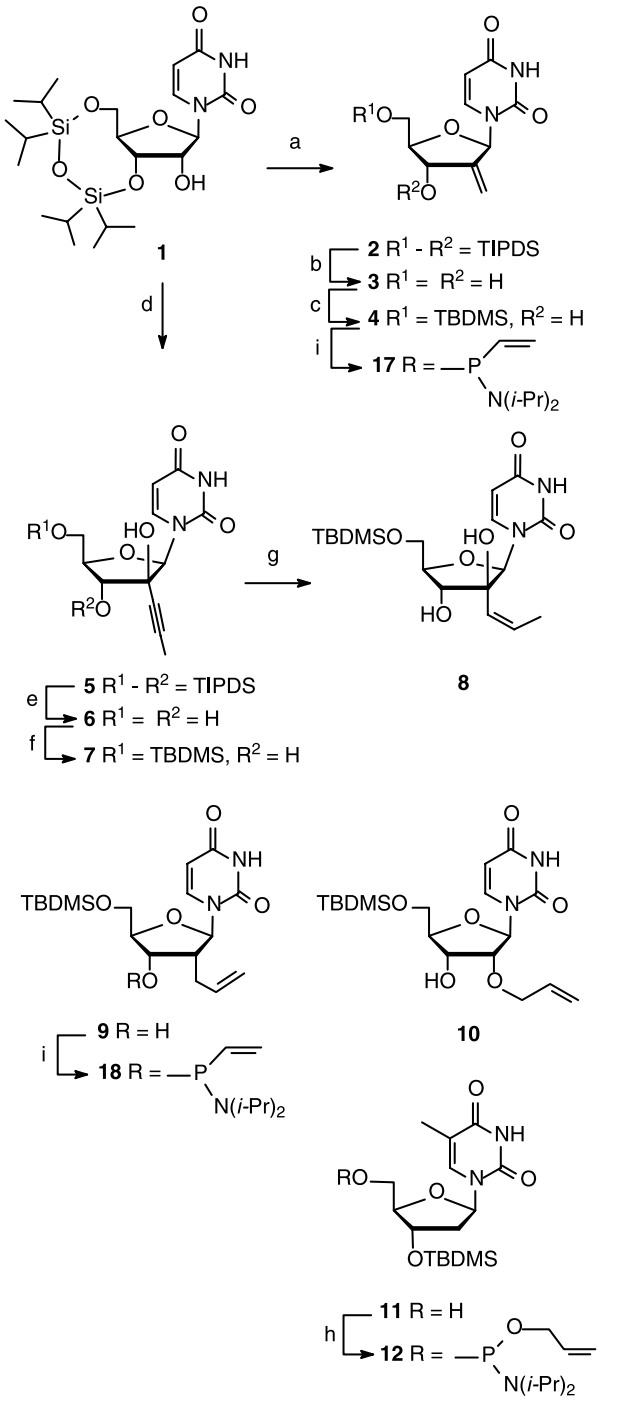
2′-*C*-$(CH_2)_4$O–P connection) has been presented in a separate publication including the incorporation of the dinucleotide into oligonucleotides and the subsequent impact on nucleic acid duplexes, bulged duplexes and three-way junctions.[14] In the present paper, we cover the complete synthetic effort investigating the scope of RCM to produce 2′-C to phosphorus connections in dinucleotides.

2. Results and discussion

Four different 5′-*O*-protected nucleosides with olefin-containing 2′-modifications were prepared. These represent four distances, from 0 to 3 bonds, between the sugar and the olefin moiety; a 2′-*C*-methylene group, a 2′-*C*-(propen-1-yl) group, a 2′-*C*-allyl group and a 2′-*O*-allyl group. All building blocks were made from the widely used TIPDS-protected uridine **1** as the starting material (Scheme 1). The known 2′-methylene derivative **2**[15] was made by an oxidation/Wittig procedure according to recent literature methods[16] and deprotected to give the likewise well-known 2′-deoxy-2′-*C*-methyleneuridine **3**.[16,17] Selective silylation gave the 5′-protected compound **4** in a good yield.

Another oxidation of **1** followed by a stereoselective Grignard reaction afforded the 2′-(propyn-1-yl) derivative **5**. We preferred to use a propyn-1-yl group over an ethynyl group since a controlled reduction to give a double bond could be expected.[18] Moreover, several RCM-reactions with methyl substituated double bonds leading to the release of propylene have been reported.[1] Direct introduction of a vinyl-group was avoided. All though the reaction of VinylMgBr with this substrate has been shown before,[19] low yields with this reagent on related substrates have been observed.[11] A lot of Grignard, organolithium or organo-aluminium reactions with the present substrate have been described in the literature[19–23] including the introduction of other alkynyl groups.[19,22,23] However, the introduction of the propyn-1-yl group has not been shown before. Nevertheless, our first yield of **5** (52% from the ketone) was hampered by the formation of partly desilylated side products as demonstrated similarly with the use of MeMgBr.[20] This problem was solved, however, by the use of a $CeCl_3$ assisted Grignard reaction affording **5** as the only product in 69% yield. Removal of the protecting group afforded **6** and the selective silylation gave the 5′-TBDMS protected derivative **7** in a high yield. As expected, the controlled hydrogenation using the Lindlar catalyst[18] gave in a very high yield the *Z*-configured alkene **8** as the only product. Exact control of the reaction time was found to be crucial, as isomerisation to give the *E*-configured isomer or over reduction to give the propyl group was observed in the reaction mixture after more than 30 min reaction time. No effort towards the deoxygenation of the 2′-tertiary alcohol was undertaken, as similar compounds are well-known to afford the arabino-configured 2′-deoxy-products after radical-type deoxygenations.[19,23,24]

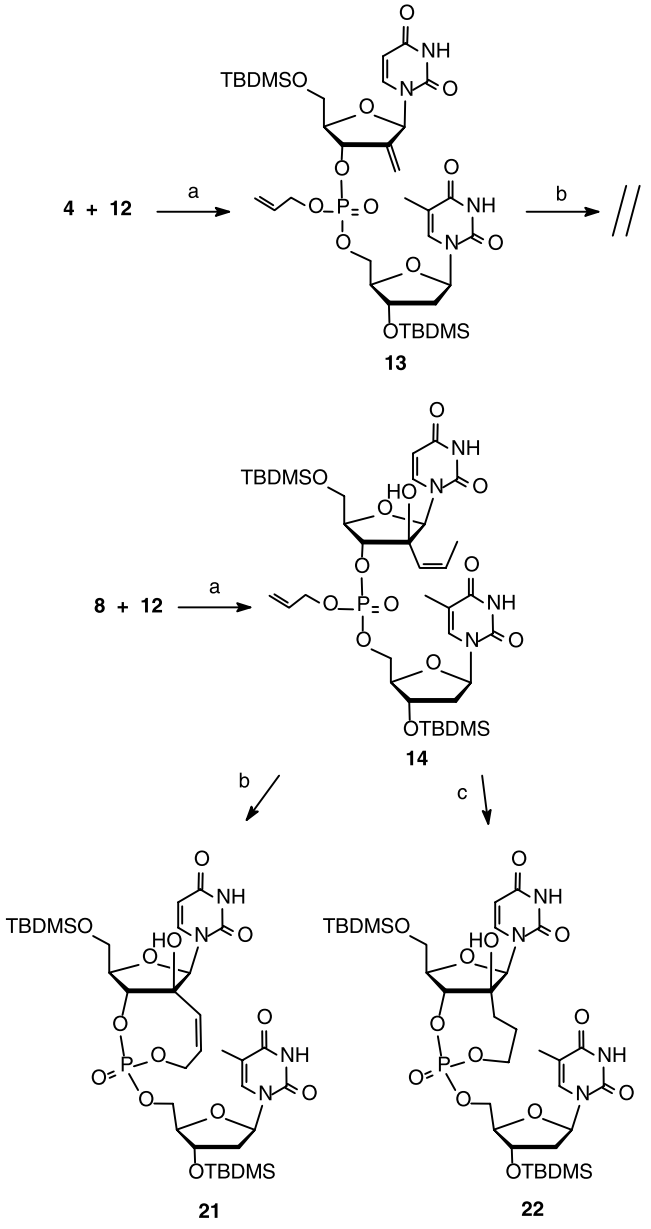
From recently published procedures from our group, the appropriately 5′-protected 2′-deoxy-2′-*C*-allyl and 2′-*O*-allyl derivatives **9**[14] and **10**[9] were also obtained from the same starting material **1** (Scheme 1) via well-known methodologies.[25,26]



Scheme 1. Reagents and conditions: (a) Ref. 16; (b) TBAF, THF, Refs. 16, 17; (c) TBDMSCl, pyridine, 83% (two steps); (d) (i) CrO_3, pyridine, Ac_2O, CH_2Cl_2, 93%; (ii) $CH_3C{\equiv}CMgBr$, $CeCl_3$, THF, 69%; (e) TBAF, THF, 86%; (f) TBDMSCl, imidazole, DMF, 70%; (g) H_2, Lindlar catalyst, quinoline, MeOH, 95%; (h) $((i\text{-}Pr)_2N)_2POCH_2CH{=}CH_2$, 1*H*-tetrazole, CH_3CN, 66%; (i) $((i\text{-}Pr)_2N)_2PCH{=}CH_2$, 4,5-dicyanoimidazole, CH_2Cl_2, 74% (**17**), 71% (**18**). TBDMS=*t*-butyldimethylsilyl.

From the four 5′-protected 2′-olefinic uridine derivatives a series of dinucleotides were prepared as substrates for RCM-reactions. Hence, **4**, **8**, **9** and **10** were all coupled with the allylated 5′-phosphoramidite **12** (obtained from 3′-*O*-TBDMS-thymidine **11**, Scheme 1) to give the four dinucleotides **13–16** in good yields (Schemes 2 and 3). Similarly, two representative vinyl phosphonate dinucleotides

were approached. However, these were realized from 3′-amidites. Thus, **4** and **9** were reacted with bis(diisopropylamino)vinylphosphinate to give the 3′-amidites **17** and **18** in reasonable yields (Scheme 1). These were coupled with 3′-*O*-TBDMS-thymidine **11** to give the two dinucleotides **19** and **20** (Scheme 4).



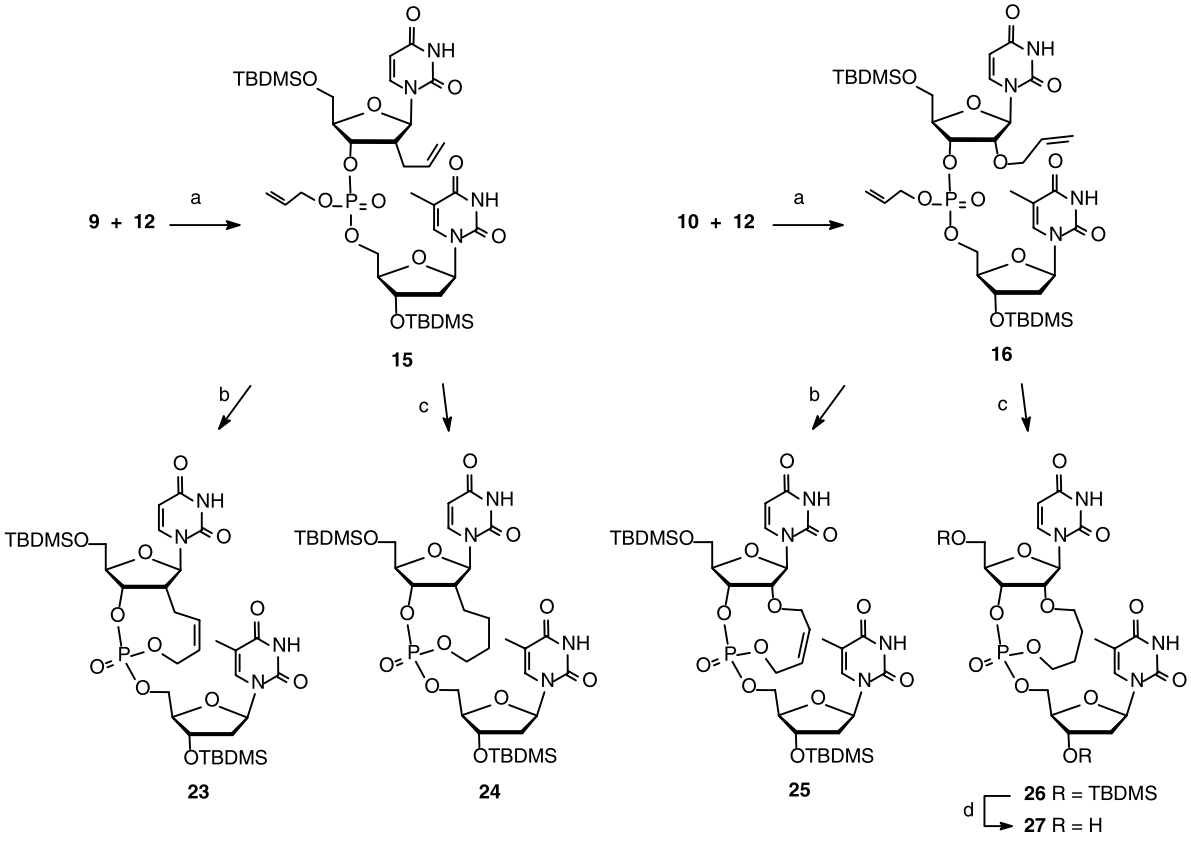
Scheme 2. Reagents and conditions: (a) (i) 1*H*-tetrazole, CH_3CN, (ii) *t*-BuOOH, toluene, CH_3CN, 97% (**13**), 100% (**14**); (b) Grubbs 2nd gen. catalyst **A**, CH_2Cl_2, 26% (**21**); (c) **A**, CH_2Cl_2, then 70 atm H_2, 50 °C, 11%.

The four dinucleotides **13–16** were studied as substrates for RCM-reactions using Grubbs second generation catalyst **A**. Dinucleotide **13**, however, did not afford any product and the starting material was recovered (Scheme 2). The reaction was attempted with varying amounts of **A** in refluxing DCM as well as in a microwave reactor at 100 °C. Also, the Hoveyda–Grubbs catalyst **B** was used under the same conditions but with no ring-closed product formed.

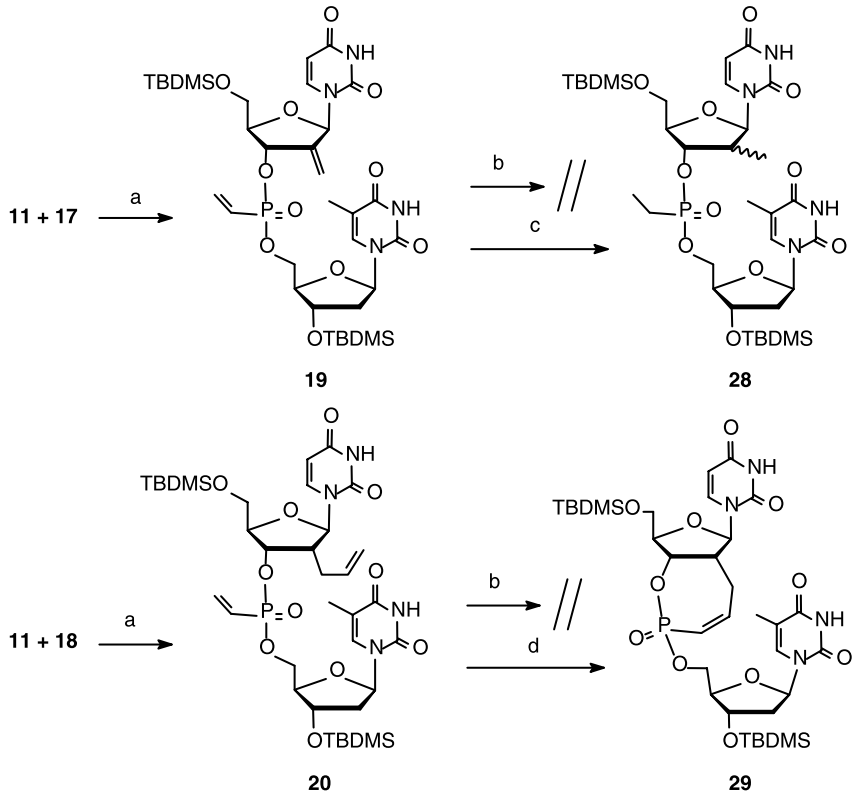
The next substrate **14** was more reactive (Scheme 2). Thus, treatment of **14** with 2×10 mol% of **A** in refluxing DCM for 5 h afforded the product **21** in 26% yield. The product was confirmed by mass spectrometry showing the mass to be decreased by 42 compared to the substrate according to the loss of propylene. ^{31}P NMR showed a 3:2 mixture of phosphorus epimers as also found for the starting material **14**. Hence, the relatively low yield cannot be addressed to different reactivity of the two epimers. The mixture of epimers **21** was separated using simple column chromatography to give the two epimers in 16 and 10% yield, respectively. The two ^1H NMR spectra being very similar demonstrated both the lack of signals from a terminal alkene and the propylene methyl group. From the ^1H NMR spectra of the major of the two epimers, a $^3J_{HC=CH}$ coupling constant of 11.9 Hz was observed confirming the new cyclic alkene to be *Z*-configurated. A tandem RCM-hydrogenation protocol[13] with **14** as the substrate was also performed. Thus, after subjecting the starting material for the RCM-reaction, the reaction mixture was stirred in a 70 atm hydrogen atmosphere in which the catalyst **A** worked as a hydrogenation catalyst. After a chromatographic separation, the two saturated cyclic phosphorus epimers of **22** were isolated in low yields, 8 and 3%, respectively. Nevertheless, the constitution of the products was clearly verified from MS and NMR. In the ^{31}P NMR it was clear that the chemical shifts of the two epimers were more similar indicating the more flexible nature of the saturated cyclic structure **22** compared to the unsaturated counterpart **21**.

The dinucleotide **15** was a much more efficient substrate for an RCM-reaction than **13** and **14** (Scheme 3).[14] Thus, the cyclic product **23** was obtained in a good yield, and so was the saturated product **24**. Thus, after separation, the two phosphorus epimers of **24** were obtained in 48 and 17% yield, respectively. These were desilylated, and the exact phosphorus configurations were established by a combination of advanced NMR and molecular modeling. Finally, the major isomer was incorporated into oligodeoxynucleotides. This part of the study including the discovery of a stabilised artificial three-way junction has been published separately in a recent paper.[14]

The dinucleotide **16** was also efficient as a substrate for an RCM-reaction (Scheme 3). A treatment of **16** with 7 mol% **A** in DCM afforded in a 45% yield the mixture of cyclic dinucleotides **25** in a 3:1 ratio of phosphorus epimers. A similar treatment with **B**, on the other hand, did not afford any product. Again, the product was confirmed by NMR and MS confirming the loss of ethylene. A tandem RCM-hydrogenation afforded the saturated cyclic dinucleotide **26** as a 5:3 mixture of epimers in 62% yield. The starting material was a 3:2 epimeric mixture, and therefore, one of the isomers (the major) seems to react slightly faster than the other. The higher yield of the tandem reaction corresponds to a relatively better reaction of the slower reacting epimer in that case. The mixture of dinucleotides **26** was not separated but deprotected using an acidic treatment to give the epimeric mixture **27** in 59% yield. The stability of the phosphortriester moiety towards basic conditions was examined, and **27** was found to be unchanged after a treatment with saturated ammonia at room temperature for 24 h. This was similar to the findings



Scheme 3. Reagents and conditions: (a) (i) 1*H*-tetrazole, CH_3CN, (ii) *t*-BuOOH, toluene, CH_3CN, 85% (**15**);[14] 95% (**16**), (b) Grubbs 2nd gen. catalyst **A**, CH_2Cl_2, 76% (**23**),[14] 45% (**25**); (c) **A**, CH_2Cl_2, then 70 atm H_2, 50 °C, 65% (**24**),[14] 62% (**26**); (d) 90% TFA (aqueous), 59%.



Scheme 4. Reagents and conditions: (a) 4,5-dicyanoimidazole, CH_3CN, 54% (**19**), 71% (**20**); (b) Grubbs 2nd gen. catalyst **A**, CH_2Cl_2; (c) **A**, CH_2Cl_2, then 70 atm H_2, 50 °C, 57%; (d) **A**, 20% CuCl, CH_2Cl_2, 35%.

for the major isomer of **24**[14] and therefore, the incorporation of this product into oligonucleotides should also be relatively straightforward.

Finally, the possibility of using the vinyl phosphonate moiety as an alternative starting point for RCM-reactions was explored. This has two obvious advantages. First, the potential ring size of the cyclic dinucleotides decreases, and therefore, the conformational strain of the product should increase. Secondly, the base lability of the phosphortriester moiety and the potential troubles with the incorporation of the cyclic dinucleotides into oligonucleotides (as already seen with one of the epimers of **24**)[14] should be avoided. Hence, a phosphonate diester should be significantly less labile. As the first substrate for this study, however, dinucleotide **19** did not afford any product when treated with 5 mol% **A** in neither DCM nor toluene (Scheme 4). The reaction was also attempted under a 70 atm pressure of nitrogen over night followed by a treatment of 70 atm hydrogen in order to perform a tandem RCM-hydrogenation. However, the product isolated in 57% yield displayed a mass increased by 4 Da corresponding to the double hydrogenated product **28**. The NMR spectra indicated a mixture of four diastereomers corresponding to the unselective hydrogenation of the methylene group as well as a pair of phosphorus epimers. These results in combination with the failed attempts of cyclisation of **13** clearly demonstrated the inability of the precatalyst **A** to initiate a catalytic metathesis cycle with a 2′-methylene nucleoside—most probably for steric reasons. On the other hand, when converted to a hydrogen donor, the catalyst is able to induce a hydrogenation of the same position. Another substrate, which is less sterically crowded, **20**, was also studied. However, neither this substrate was active when subjected to 5 mol% **A** in DCM. However, using the known phosphine scavenger CuCl[27] in combination with 5 mol% **A** was also attempted with **20**, and in this case, a cyclised product **29** was obtained in 35% yield as a 3:1 mixture of phosphorus epimers. It has, however, been impossible in our hands to improve this yield by other reaction conditions.

From the present results it is clear that not all the dinucleotide substrates studied were found to be efficient for RCM reactions. The best formed rings have been the larger 9- and 10-membered rings in compounds **23**–**26**. The 2′-*C*-methylene group might be inaccessible for the sterically demanding catalyst. This would explain the complete inactivity of the substrates **13** and **19**. The result that **19** do react with the 'Ru-H_2' intermediate is explained from the lower sterical demand of this intermediate. It is also clear that the vinyl phosphonate group is significantly less reactive compared to the allyl phosphate group. This is of course expected as unsaturated esters are generally less susceptible for metathesis reactions.[1,7] On the other hand, vinyl phosphonates have been used before, also in cross metathesis reactions involving a vinyl 3′-nucleoside phosphonate.[7,28] The lower reactivity was in that case demonstrated by the inability of the substrate to undergo homodimerisation by cross metathesis.[28] Nevertheless, we demonstrated an RCM reaction of vinyl phosphonate **20**.

It could be argued, of course, that a longer range of catalysts and solvents should be included in the study. However, Grubbs second generation catalyst **A** was in all cases tested by us found to be superior to the first generation catalyst ($(Cy_3P)_2$-$RuCl_2CHPh$)[1,2] and the Hoveyda–Grubbs second generation catalyst **B** was attempted and not found to be a good alternative in this study. Concerning solvents, we have attempted toluene in other studies[8,11] but this solvent has been incompatible with our nucleotide substrates. Furthermore, the major subject of this study was to explore the scope of making large rings between a 2′-olefin moiety and the adjacent phosphorus, in other words to find the optimally formed rings.

The most conveniently prepared ring was the nine-membered ring in **23/24**. In other words, the 2′-*C*-allyl-2′-deoxy moiety was the most reactive among the 2′-C connected olefins. The 2′-*O*-allyl moiety was also reactive, and **25/26** were obtained in reasonable yields. This is in contrary to our recent results where dinucleotides consisting of a 2′-*O*-allyluridine moiety with either a 5-allyl-2′-deoxyuridine or a 2′-*O*-allyl-arabinouridine moiety were unreactive towards **A**.[9] Thus, those results must be due to the potential ring-sizes being unfavourable and particular conformational properties of the substrates.[9]

Among the two conveniently formed ring systems, **23/24** and **25/26**, the former was selected for further studies.[14] It was shown that the major of the two phosphorus epimers of **24** after deprotection could be successfully incorporated into oligonucleotides. In these, the cyclic moiety was demonstrated to induce a significant distortion of a duplex but also a slight stabilization of a three-way junction. This is formed between an oligonucletide with the cyclic moiety in the middle of the sequence and a complementary RNA strand with a stem-loop sequence opposite the cyclic moiety.[14] We expect also **27** to be easily incorporated into oligonucleotides. Thus, the stability of **27** against aqueous ammonia was found to be good, and this is crucial if standard procedures for automated oligonucleotide synthesis should be followed. Comparing **26/27** and **24**, completely different conformational behaviour of the resulting oligonucleotides can be expected. Thus, the 2′-oxygen atom in **26/27** should take the nucleoside conformation of the uridine moiety into a *N*-type conformation as usually found for 2′-*O*-alkylated nucleosides due to the *gauche* effect of the 2′-oxygen.[29] However, the ring is larger and perhaps more flexible in **27**. Hence, the ^{1}H NMR data of **27** indicated that none of the two phosphorus epimers seems to be restricted towards an *N*-type conformation. Very different $J_{H1'H2'}$ coupling constants (3.8 and 7.7 Hz, respectively) were found and only the former indicates a slight preference for an *N*-type conformation. For **24**, on the other hand, an *S*-type conformation of the uridine moiety was found[14] as completely expected for a 2′-deoxy-2′-alkylated nucleoside due to the tendency for carbon substituents to prefer a pseudoequatorial orientation. Efforts towards the separation of the phosphorus epimers of **27** and the incorporation of these into oligonucleotides are in progress.

Also the cyclic nucleotide with a phosphonate containing ring **29** might be incorporated into oligonucleotides. Hence, this structure is expected to be more stable compared to the phosphortriester moieties in the other dinucleotides, especially after an eventual hydrogenation. On the other hand, the yield of the ring-closure must be improved.

Finally, the incorporation of **22** can be completed. However, in this case the efficiency of the ring-closure was too low, and we will prefer in the near future to make another ring-system with the same ring-size, for example, from a substrate with a vinyl group instead of a propen-1-yl group and, preferably, without a 2′-hydroxy moiety.

3. Conclusions

Four different cyclic dinucleotides with 2′-C to P connections have been prepared in varying yields. The most efficiently obtained cyclic dinucleotide, containing a 2′-*C*-$(CH_2)_4$O–P connection, was chosen for incorporation into oligonucleotides. In general, dinucleotide substrates with allyl phosphates were much more readily applied than with vinyl phosphonates, and the reactivity of the 2′-C olefins increased by the decrease of sterical demand near the sugar moiety. In the future, the scope of RCM in the preparation of oligonucleotides targeting secondary nucleic acid structures will be further explored.

4. Experimental

4.1. General

All commercial reagents were used as supplied. When necessary, reactions were performed under an atmosphere of nitrogen. An EMRYS™ Creator was used for microwave assisted synthesis. Column chromatography was carried out on glass columns using silica gel 60 (0.040–0.063 mm). NMR spectra were recorded on a Varian Gemini 2000 spectrometer. ^{1}H NMR spectra were recorded at 300 MHz, ^{13}C NMR spectra at 75.5 MHz, and ^{31}P NMR spectra at 121.5 MHz. Values for δ are in ppm relative to tetramethylsilane as internal standard or 85% H_3PO_4 as external standard. Microanalyses were performed at The Microanalytical Laboratory, Department of Chemistry, University of Copenhagen. Assignments of NMR spectra are based on 2D spectra and follow standard nucleoside style; that is, the carbon next to the nucleobase is assigned C-1′. For dinucleotides, the upper (5′) nucleotide is depicted U and the lower nucleoside T.

4.1.1. Preparation of 5′-*O*-*tert*-butyldimethylsilyl-2′-deoxy-2′-methyleneuridine (4). Compound **2**[15] (0.452 g, 0.936 mmol) was dissolved in THF (10 mL) and a 1 M solution of TBAF in THF (3 mL, 3 mmol) was added. The reaction mixture was stirred for 20 min and a mixture of pyridine, methanol and water (5 mL, 3:1:1) was added. A mixture of Dowex 50 W×2 (4 g) in pyridine, methanol and water (25 mL, 3:1:1) was added and the suspension was stirred for 30 min and filtered. The filter was washed with a mixture of pyridine, methanol and water (25 mL, 3:1:1), and the combined filtrates were concentrated under reduced pressure. The residue was purified by dry column chromatography (17% ethanol in ethyl acetate) to give the crude compound **3**. This material (0.337 g) was dissolved in anhydrous pyridine (9 mL) and TBDMSCl (0.146 g, 0.97 mmol) was added. The reaction mixture was stirred for 2 h and an additional amount of TBDMSCl (0.056 g, 0.37 mmol) was added. The reaction mixture was stirred for 6 h, concentrated under reduced pressure and co-evaporated with anhydrous toluene. The residue was dissolved in DCM (50 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (50 mL) and brine (50 mL). The combined aqueous phases were extracted with DCM (50 mL) and the combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by dry column chromatography (1–4% methanol in DCM) to give the product as a white foam (0.248 g, 83%) (Found C, 52.01; H, 7.35; N, 7.42; $C_{16}H_{26}N_2O_5Si\cdot H_2O$ requires C, 51.63; H, 7.48; N, 7.48); R_f 0.19 (5% methanol in DCM); ^{1}H NMR ($CDCl_3$) δ 0.10–0.11 (6H, s, Si$(CH_3)_2$), 0.91 (9H, s, C$(CH_3)_3$), 2.70 (1H, s, OH), 3.87 (1H, m, H-4′), 3.93–4.00 (2H, m, H-5′), 4.76 (1H, m, H-3′), 5.45–5.55 (2H, m, CH=CH_2), 5.69 (1H, d, J=8.1 Hz, H-5), 6.69 (1H, m, H-1′), 7.60 (1H, d, J=8.1 Hz, H-6), 9.33 (1H, s, NH); ^{13}C NMR ($CDCl_3$) δ −5.4, −5.3 (Si$(CH_3)_2$), 18.5 (*C*$(CH_3)_3$), 26.0 (C(*C*$H_3)_3$), 62.0 (C-5′), 70.7 (C-3′), 84.3 (C-4′), 84.8 (C-1′), 102.9 (C-5), 113.8 (C=*C*H_2), 141.0 (C-6), 148.8 (C-2′), 150.9 (C-2), 163.5 (C-4); HiRes MALDI FT-MS *m*/*z* ($M+Na^+$) found/calcd 377.1511/ 377.1503.

4.1.2. Preparation of 1-(2′-*C*-(propyn-1-yl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxandiyl)-β-D-arabino-furanosyl)uracil (5). A mixture of CrO_3 (1.1 g, 12 mmol) and DCM (20 mL) was stirred at 0 °C. Pyridine (1.6 mL) was added dropwise and the mixture was stirred at room temperature. Acetic anhydride (1 mL) and a solution of compound **1** (1.1 g, 2.3 mmol) in DCM (5 mL) were added, and the reaction mixture was stirred for 2 h. Ethyl acetate (50 mL) was added and the mixture was filtered through a layer of silica. The filter was rinsed with more ethyl acetate and the combined filtrates were concentrated under reduced pressure to give the ketone (1.0 g, 93%). $CeCl_3\cdot 7H_2O$ (5.0 g, 13.3 mmol) was dried at 140 °C under reduced pressure for 12 h and added anhydrous THF (15 mL). The mixture was stirred for 2 h at room temperature and then at −78 °C. A 0.5 M solution of $CH_3C{\equiv}CMgBr$ in THF (20 mL, 10 mmol) was added dropwise and the mixture was stirred for 1.5 h. A solution of the ketone (1.0 g, 2.1 mmol) in THF (2.5 mL) was cooled to −78 °C and added dropwise to the stirred solution of Grignard reagent. The mixture was stirred for 9 h and then added acetic acid. The mixture was extracted with ethyl acetate (4×20 mL). The combined organic phases were washed with an aqueous solution of $NaHCO_3$, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography (0–4% methanol in DCM) to give the product as a white foam (0.76 g, 69%); R_f 0.47 (10% methanol in DCM); ^{1}H NMR ($CDCl_3$) δ 1.00–1.10 (28H, s, SiC*H*$(CH_3)_2$), 1.92 (3H, s, C≡CCH_3) 3.05 (1H, s, OH), 3.90–4.20 (4H, m, H-3′, H-4′, H-5′), 5.69 (1H, dd, J=2.0, 8.0 Hz, H-5), 6.02 (1H, s, H-1′), 7.87 (1H, d, J=8.0 Hz, H-6), 8.70 (1H, s, NH); ^{13}C NMR ($CDCl_3$) δ 4.02 (C≡C*C*H_3), 12.5, 12.9, 13.1, 13.5 (Si*C*H$(CH_3)_2$), 16.7, 16.9, 17.0, 17.1, 17.2, 17.4, 17.4, 17.5 (SiCH(*C*$H_3)_2$), 60.0 (C-5′), 73.0 (C-3′), 75.8 (*C*≡CCH$_3$), 76.5 (C≡*C*CH$_3$), 81.4 (C-4′), 85.0 (C-2′), 89.6 (C-1′), 101.8 (C-5), 140.0 (C-6), 151.4 (C-2), 163.2 (C-4); HiRes MALDI FT-MS *m*/*z* ($M+Na^+$) found/calcd 547.2244/547.2266.

4.1.3. Preparation of 1-(2′-*C*-(propyn-1-yl)-arabino-furanosyl)uracil (6). Compound **5** (1.03 g, 1.97 mmol) was dissolved in THF (10 mL) and a solution of TBAF·H_2O

(1.10 g, 3.94 mmol) in THF (4 mL) was added. The solution was stirred for 20 min and added acetic acid (0.2 mL, 4 mmol) and silica gel (4.5 g). The mixture was concentrated under reduced pressure, and the residue purified by column chromatography (0–10% methanol in DCM) to give the product as a white foam (0.575 g, 86%); R_f 0.33 (20% methanol in DCM); ^{1}H NMR (DMSO-d_6) δ 1.83 (3H, s, C≡CCH$_3$) 3.53–3.67 (2H, m, H-5′), 3.70–3.85 (2H, m, H-3′, H-4′), 5.07 (1H, br s, OH), 5.57 (1H, d, J=8.2 Hz, H-5), 5.67 (1H, br s, OH), 6.05 (1H, s, H-1′), 6.23 (1H, br s, OH), 7.68 (1H, d, J=8.2 Hz, H-6), 11.26 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 3.60 (C≡C*C*H$_3$), 60.7 (C-5′), 75.9 (*C*≡CCH$_3$), 77.0 (C-3′), 77.2 (C≡*C*CH$_3$), 83.6 (C-2′), 84.2 (C-4′), 86.9 (C-1′), 100.3 (C-5), 142.4 (C-6), 150.6 (C-2), 163.2 (C-4); HiRes MALDI FT-MS m/z (M+Na$^+$) found/calcd 305.0752/305.0750.

4.1.4. Preparation of 1-(5′-*O*-*tert*-butyldimethylsilyl-2′-*C*-(propyn-1-yl)arabinofuranosyl)uracil (7). Compound **6** (0.565 g, 1.72 mmol) was dissolved in anhydrous DMF (10 mL) and imidazole (0.28 g, 4.0 mmol) was added. A solution of TBDMSCl (0.30 g, 2.0 mmol) in DMF (1.0 mL) was added dropwise, and the reaction mixture was stirred for 1 h. An additional amount of TBDMSCl (0.05 g, 0.33 mmol) dissolved in DMF (0.5 mL) was added and the reaction mixture was stirred for 7 h and quenched by the addition of ethanol (2 mL). Water (30 mL) was added and the mixture was extracted with diethylether (4×10 mL). The combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography (0–10% methanol in DCM) to give the product as a white foam (0.476 g, 70%); R_f 0.35 (9% methanol in DCM); ^{1}H NMR ($CDCl_3$) δ 0.16 (6H, s, Si(CH$_3$)$_2$), 0.94 (9H, s, C(CH$_3$)$_3$), 1.93 (3H, s, C≡CCH$_3$), 2.74 (1H, d, J=2.9 Hz, 3′-OH), 3.86 (1H, m, H-5′), 4.01 (1H, m, H-5′), 4.06 (1H, m, H-3′), 4.14 (1H, m, H-4′), 4.98 (1H, s, 2′-OH), 5.65 (1H, d, J=8.1 Hz, H-5), 6.25 (1H, s, H-1′), 7.76 (1H, d, J=8.1 Hz, H-6), 8.81 (1H, s, NH); ^{13}C NMR ($CDCl_3$) δ −5.5, −5.4 (Si(CH$_3$)$_2$), 18.5 (*C*(CH$_3$)$_3$), 26.0 (C(*C*H$_3$)$_3$), 4.03 (C≡C*C*H$_3$), 63.0 (C-5′), 73.3 (*C*≡CCH$_3$), 77.1 (C≡*C*CH$_3$), 77.4 (C-3′), 83.4 (C-4′), 87.5 (C-1′), 87.8 (C-2′), 101.3 (C-5), 141.5 (C-6), 150.8 (C-2), 163.2 (C-4); HiRes MALDI FT-MS m/z (M+Na$^+$) found/calcd 419.1595/419.1613.

4.1.5. Preparation of 1-(5′-*O*-*tert*-butyldimethylsilyl-2′-*C*-(*cis*-propen-1-yl)-arabinofuranosyl)uracil (8). Lindlar catalyst (0.117 g) was added freshly distilled methanol (6.0 mL) and freshly distilled quinoline (0.46 mL). The mixture was stirred under an atmosphere of hydrogen for 1 h. A solution of compound **7** (0.464 g, 1.17 mmol) in methanol (6.0 mL) was added and the mixture was stirred for 30 min The hydrogen atmosphere was replaced with nitrogen and the mixture was filtered through a layer of Celite. The filtrate was concentrated under reduced pressure and the residue was dissolved in DCM (10 mL) and added silica gel. This mixture was concentrated under reduced pressure and purified by column chromatography (0–10% methanol in DCM) to give the product as a white foam (0.442 g, 95%); R_f 0.40 (9% methanol in DCM); ^{1}H NMR ($CDCl_3$) δ 0.19 (6H, s, Si(CH$_3$)$_2$), 0.96 (9H, s, C(CH$_3$)$_3$), 1.89 (3H, dd, J=1.6, 7.1 Hz, CH=CHC*H*$_3$), 2.24 (1H, d, J=1.4 Hz, 3′-OH), 3.87 (1H, m, H-5′), 4.02 (1H, m, H-5′), 4.22 (1H, m, H-3′), 4.24 (1H, m, H-4′), 4.95 (1H, s, 2′-OH), 5.64 (1H, dd, J=1.8, 8.4 Hz, H-5), 5.69 (1H, m, C*H*=CHCH$_3$), 6.03 (1H, dq, J=7.1, 11.6 Hz, CH=C*H*CH$_3$), 6.24 (1H, s, H-1′), 7.67 (1H, d, J=8.4 Hz, H-6), 8.72 (1H, s, NH); ^{13}C NMR ($CDCl_3$) δ −5.5, −5.4 (Si(CH$_3$)$_2$), 15.2 (CH=CH*C*H$_3$), 18.4 (*C*(CH$_3$)$_3$), 25.8 (C(*C*H$_3$)$_3$), 63.8 (C-5′), 78.6 (C-3′), 81.1 (C-2′), 84.4 (C-4′), 88.1 (C-1′), 101.0 (C-5), 123.8 (CH=*C*HCH$_3$), 136.0 (*C*H=CHCH$_3$), 142.0 (C-6), 150.8 (C-2), 163.1 (C-4); HiRes MALDI FT-MS m/z (M+Na$^+$) found/calcd 421.1753/421.1770.

4.1.6. Preparation of allyloxy(3′-*O*-*tert*-butyldimethylsilylthymidin-5′-yl)diisopropylamino phosphine (12). Compound **11** (0.795 g, 2.23 mmol) was dissolved in anhydrous CH_3CN (4 mL). Allyloxy-bis(diisopropylamino)phosphine (0.97 g, 3.35 mmol) was added, followed by addition of a 0.45 M solution of 1*H*-tetrazole in CH_3CN (7.5 mL, 3.4 mmol) over a period of 5 min. The reaction mixture was stirred for 1 h, filtrated and added DCM (40 mL). The solution was washed with a saturated aqueous solution of $NaHCO_3$ (20 mL) and brine (4 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by dry column chromatography (0–100% ethyl acetate and 0.5% Et_3N in petrol ether) to give the product as an oil with an epimeric mixture (0.800 g, 66%); R_f 0.60 (75% ethyl acetate in petrol ether); ^{1}H NMR ($CDCl_3$) δ 0.09 (6H, br s, Si(CH$_3$)$_2$), 0.90 (9H, br s, C(CH$_3$)$_3$), 1.17–1.28 (12H, m, CH(C*H*$_3$)$_2$), 1.91–1.93 (6H, m, CH$_3$), 2.00–2.14 (1H, m, H-2′), 2.22–2.29 (1H, m, H-2′), 3.54–3.95 (3H, m, C*H*(CH$_3$)$_2$, H-5′), 3.99–4.06 (1H, m, H-3′), 4.11–4.22 (2H, m, CH$_2$OP), 4.42–4.49 (1H, m, H-4′), 5.12–5.31 (2H, m, CH=C*H*$_2$), 5.88–5.97 (1H, m, C*H*=CH$_2$), 6.31–6.38 (1H, m, H-1′), 7.58 (1/2H, s, H-6), 7.75 (1/2H, s, H-6), 8.85 (1H, br s, NH); ^{31}P NMR ($CDCl_3$) δ 148.92, 149.04. ESI FT-MS m/z (2M+Na$^+$) 1109.467.

4.1.7. Preparation of allyl(5′-*O*-*tert*-butyldimethylsilyl-2′-deoxy-2′-methyleneuridin-3′-yl)(3′-*O*-*tert*-butyldimethylsilylthymidin-5′-yl)phosphate (13). Compound **4** (0.050 g, 0.141 mmol) and compound **12** (0.145 g, 0.282 mmol) were dried and co-evaporated twice from anhydrous CH_3CN. The mixture was redissolved in anhydrous CH_3CN (2 mL) and a 0.45 M solution of 1*H*-tetrazole in CH_3CN (0.32 mL, 0.144 mmol) was added over a period of 5 min. The reaction mixture was stirred at room temperature for 90 min. A 3 M solution of *t*-BuOOH in toluene (0.2 mL, 0.60 mmol) was added and the reaction mixture was stirred for 30 min and quenched by the addition of methanol (0.5 mL). The solution was concentrated under reduced pressure and the residue was purified by column chromatography (75–100% ethyl acetate in petrol ether) to give the product as a white foam with an epimeric mixture (0.111 g, 97%); R_f 0.22 (75% ethyl acetate in petrol ether); ^{1}H NMR ($CDCl_3$) δ 0.08–0.10 (12H, m, Si(CH$_3$)$_2$), 0.89–0.90 (18H, m, C(CH$_3$)$_3$), 1.93–1.95 (3H, m, T-CH$_3$), 2.12–2.32 (2H, m, T-H2′), 3.86–3.95 (2H, m, U-H5′), 3.99–4.04 (1H, m, T-H4′), 4.15–4.32 (4H, m, T-H3′, U-H4′, T-H5′), 4.38–4.43 (1H, m, U-H3′), 4.56–4.64 (2H, m, CH$_2$OP), 5.29–5.42 (4H, m, C=CH$_2$, CH=C*H*$_2$), 5.69–5.78 (1H, m, U-H5), 5.84–6.01 (1H, m, C*H*=CH$_2$), 6.28–6.33 (1H, m, T-H1′), 6.76–6.79 (1H, m, U-H1′), 7.38–7.47 (1H, m, T-H6), 7.54–7.62 (1H, m, U-H6),

9.48–9.50 (1H, m, NH), 9.67–9.71 (1H, m, NH); ^{13}C NMR ($CDCl_3$): δ −5.5, −5.4, −4.8, −4.5, 12.6, 18.0, 18.4, 25.6, 25.8, 25.9, 40.8, 62.5, 67.0, 68.9, 69.0, 71.5, 71.5, 76.2, 84.0, 84.1, 84.1, 85.0, 85.1, 85.2, 85.3, 103.3, 111.4, 111.5, 117.3, 119.4, 128.4, 130.2, 133.4, 135.7, 141.1, 144.7, 150.5, 151.0, 163.4, 164.1; ^{31}P NMR ($CDCl_3$) δ −0.12, 0.00; HiRes MALDI FT-MS *m/z* ($M+Na^+$) found/calcd 835.3118/835.3141.

4.1.8. Preparation of allyl(5′-*O*-*tert*-butyldimethylsilyl-2′-(*cis*-propen-1-yl)-2′-arabinouridin-3′-yl)(3′-*O*-*tert*-butyldimethylsilylthymidin-5′-yl)phosphate (14). Compound **8** (0.044 g, 0.11 mmol) was dissolved in anhydrous CH_3CN and a 0.50 M solution of compound **12** in CH_3CN (0.25 mL) followed by a 0.45 M solution of 1*H*-tetrazole in CH_3CN (0.25 mL) was added. The reaction mixture was stirred for 30 min and added another portion of the 0.50 M solution of **12** (0.10 mL). The mixture was stirred for 2 h and added a 3 M solution of *t*-BuOOH in toluene (0.12 mL, 0.4 mmol). The mixture was stirred for 45 min and added ethyl acetate (6 mL). The mixture was washed with brine (5 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography (0–100% ethyl acetate in petrol ether) to give the product as a white foam with an epimeric mixture (0.061 g, 65%); R_f 0.43 (ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 0.07–0.19 (12H, m, $Si(CH_3)_2$), 0.89–0.95 (18H, m, $C(CH_3)_3$), 1.82–1.85 (3H, CH=CHC*H*$_3$), 1.92–1.94 (3H, m, T-CH_3), 2.04–2.32 (2H, m, T-H2′), 3.95–4.45 (7H, m, U-H4′, T-H3′, U-H3′, T-H5′, U-H5′), 4.53–4.65 (2H, m, CH_2OP), 4.74–4.78 (1H, m, T-H4′), 5.11–5.14 (1H, m, OH), 5.26–5.43 (2H, CH=C*H*$_2$), 5.56–5.65 (1H, C*H*=CHCH$_3$), 5.76–5.98 (2H, C*H*=CH$_2$, CH=C*H*CH$_3$), 5.69–5.78 (1H, m, U-H5), 6.17–6.33 (2H, m, T-H1′, U-H1′), 7.31–7.39 (1H, m, T-H6), 7.60–7.64 (1H, m, U-H6), 9.00–9.20 (2H, m, NH); ^{31}P NMR ($CDCl_3$) δ −0.80 (3/5), −0.53 (2/5); HiRes MALDI FT-MS *m/z* ($M+Na^+$) found/calcd 879.3416/879.3404.

4.1.9. Preparation of allyl(2′-*O*-allyl-5′-*O*-*tert*-butyldimethylsilyluridin-3′-yl)(3′-*O*-*tert*-butyldimethylsilylthymidin-5′-yl)phosphate(16). Compound **10**[9] (0.890 g, 2.23 mmol) and compound **12** (1.86 g, 3.42 mmol) were co-evaporated with CH_3CN and redissolved in CH_3CN (37 mL). A 0.45 M solution of 1*H*-tetrazole in CH_3CN (8.0 mL, 3.6 mmol) was added over a period of 10 min and the reaction mixture was stirred for 100 min. A 3 M solution of *t*-BuOOH in toluene (3.7 mL, 11.1 mmol) was added in small portions over 5 min. The reaction mixture was stirred for 145 min and added methanol (2.0 mL). The mixture was concentrated under reduced pressure, and the residue was purified by dry column chromatography (0–100% ethyl acetate in petrol ether) to give the product as a white foam with an epimeric mixture (1.81 g, 95%); R_f 0.41 (75% ethyl acetate in petrol ether); ^{1}H NMR ($CDCl_3$) δ 0.06–0.16 (6H, m, $Si(CH_3)_2$), 0.86–0.93 (18H, m, $C(CH_3)_3$), 1.91–1.95 (3H, m, T-CH_3), 2.09–2.32 (2H, m, T-H2′), 3.75–3.98 (2H, m, U-H5′), 4.00–4.03 (1H, m, T-H4′), 4.08–4.13 (1H, m, U-H2′), 4.16–4.19 (2H, m, 2′-OCH_2), 4.22–4.26 (2H, m, T-H5′), 4.28–4.33 (1H, m, U-H4′), 4.38–4.45 (1H, m, T-H3′), 4.53–4.65 (2H, m, CH_2OP), 4.85–4.93 (1H, m, U-H3′), 5.15–5.40 (4H, m, 2×CH=C*H*$_2$), 5.67–5.73 (1H, m, U-H5), 5.75–6.00 (2H, m, 2×C*H*=CH$_2$), 6.10–6.14 (1H, m, U-H1′), 6.26–6.33 (1H, m, T-H1′), 7.34 (3/5H, d, J=1.1 Hz, T-H6), 7.44 (2/5H, d, J=1.1 Hz, T-H6), 7.82 (2/5H, d, J=8.3 Hz, U-H6), 7.84 (3/5H, d, J=8.3 Hz, U-H6), 9.00–9.24 (2H, m, NH); ^{31}P NMR ($CDCl_3$) δ −0.77 (3/5), −0.61 (2/5); HiRes MALDI FT-MS *m/z* ($M+Na^+$) found/calcd 879.3434/879.3404.

4.1.10. Preparation of vinyl(5′-*O*-*tert*-butyldimethylsilyl-2′-deoxy-2′-methyleneuridin-3′-yl)diisopropylamino phosphinate (17). Compound **4** was dissolved in anhydrous DCM (3 mL) and a 1 M solution of 4,5-dicyanoimidazole in CH_3CN (0.62 mL, 0.62 mmol) was added. A solution of bis(diisopropylamino)vinylphosphinate (0.214 g, 0.828 mmol) in DCM (1.5 mL) was added over 10 min and the reaction mixture was stirred for 2.5 h. An additional amount of bis(diisopropylamino)vinylphosphinate (0.078 g, 0.30 mmol) dissolved in DCM (0.5 mL) was added and the reaction mixture was stirred for 2 h. The reaction mixture was diluted with ethyl acetate (30 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (2×30 mL) and brine (30 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by dry column chromatography (10–40% ethyl acetate and 1% pyridine in petrol ether) to give the product as a colourless oil with an epimeric mixture (0.227 g, 74%); R_f 0.62 (50% ethyl acetate in petrol ether); ^{1}H NMR ($CDCl_3$) δ 0.07–0.12 (6H, m, $Si(CH_3)_2$), 0.89–0.92 (9H, m, $C(CH_3)_3$), 1.03–1.32 (12H, m, $CH(CH_3)_2$), 3.47–3.57 (2H, m, C*H*$(CH_3)_2$), 3.82–4.02 (3H, m, H-5′, H-4′), 4.85–4.97 (1H, m, H-3′), 5.22–5.55 (2H, m, C=C*H*$_2$), 5.60–5.84 (3H, H-5, PCH=C*H*$_2$), 6.15–6.35 (1H, m, PC*H*=CH$_2$), 6.69–6.75 (1H, m, H-1′), 7.55 (1/2H, d, J=8.2 Hz, H-6), 7.74 (1/2H, d, J=8.2 Hz, H-6), 8.55–8.65 (1H, br, NH); ^{31}P NMR ($CDCl_3$) δ 118.64, 120.22.

4.1.11. Preparation of vinyl(5′-*O*-*tert*-butyldimethylsilyl-2′-deoxy-2′-*C*-allyluridine-3′-yl)-*N*,*N*′-diisopropylamino phosphinate (18). Compound **9** (0.100 g, 0.261 mmol) was dissolved in DCM (3 mL) and a 1 M solution of 4,5-dicyanoimidazole in CH_3CN (0.55 mL, 0.55 mmol) was added. Bis(diisopropylamino)vinylphosphinate (0.135 g, 0.522 mmol) was added dropwise over a period of 5 min, and the reaction mixture was stirred for 4 h at room temperature. An additional amount of bis(diisopropylamino)vinylphosphinate (0.135 g, 0.522 mmol) was added and the mixture was stirred for 6 h. An additional amount of the 1 M solution of 4,5-dicyanoimidazole in CH_3CN (0.55 mL, 0.55 mmol) was added and the reaction mixture was stirred at room temperature for 23 h. The reaction mixture was diluted with DCM (40 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (2×20 mL) and brine (30 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by dry column chromatography (0–30% ethyl acetate and 1% pyridine in petrol ether) to give the product as a white foam with an epimeric mixture (0.100 g, 71%); R_f 0.50 (50% ethyl acetate in petrol ether); ^{1}H NMR ($CDCl_3$) δ 0.09–0.12 (6H, m, $Si(CH_3)_2$), 0.91–0.93 (9H, m, $C(CH_3)_3$), 1.04–1.33 (12H, m, $CH(CH_3)_2$), 2.05–2.55 (3H, m, H-2′, 2′–CH_2), 3.41–3.58 (2H, m, C*H*$(CH_3)_2$), 3.78–3.95 (2H, m, H-5′), 4.17–4.22 (1H, m, H-4′), 3.32–4.46 (1H, m, H-3′), 4.95–5.07 (2H, m, CH=C*H*$_2$), 5.60–6.02 (4H, m, H-5, C*H*=CH$_2$, PCH=C*H*$_2$), 6.10–6.55 (2H, m, H-1′, PC*H*=CH$_2$), 7.87 (1/2H, d, J=8.1 Hz, H-6), 7.92 (1/2H, d, J=8.1 Hz, H-6),

8.20–8.35 (1H, m, NH); ^{31}P NMR ($CDCl_3$) δ 111.46, 121.14.

4.1.12. Preparation of vinyl(5′-*O*-*tert*-butyldimethylsilyl-2′-deoxy-2′-*C*-methyleneuridin-3′-yl)(3′-*O*-*tert*-butyldimethylsilylthymidin-5′-yl)phosphonate (19). Compound **17** (0.084 g, 0.17 mmol) and compound **11** (0.068 g, 0.191 mmol) were co-evaporated with DCM (10 mL) and redissolved in anhydrous CH_3CN (8.5 mL). A 1 M solution of 4,5-dicyanoimidazole in CH_3CN (0.17 mL, 0.17 mmol) was added over 5 min and the reaction mixture was stirred for 1 h (resulting phosphonite; R_f 0.40 (75% ethyl acetate in petrol ether)). A 3 M solution of *t*-BuOOH in toluene (0.27 mL, 0.81 mmol) was added over 5 min. The reaction mixture was stirred for 30 min and added methanol (0.5 mL). The mixture was diluted with ethyl acetate (50 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (50 mL) and brine (50 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography (50–100% ethyl acetate in petrol ether) to give the product as a white foam with an epimeric mixture (0.072 g, 54%); R_f 0.18 (75% ethyl acetate in petrol ether); ^{1}H NMR ($CDCl_3$) δ 0.08–0.10 (12H, m, $Si(CH_3)_2$), 0.88–0.92 (18H, m, $C(CH_3)_3$), 1.92–1.94 (3H, m, T-CH_3), 2.07–2.30 (2H, m, H-2′), 3.80–4.45 (6H, m, U-H5′, T-H5′, T-H4′, T-H3′), 4.63–4.69 (1H, m, U-H4′), 5.38–5.65 (2H, m, C=CH_2), 5.66–5.72 (1H, m, H-5), 5.81–5.85 (1H, m, H-3′), 6.00–6.53 (4H, m, PC*H*=CH_2, T-H1′), 6.75–6.77 (1H, m, U-H1′), 7.32–7.35 (1H, m, T-H6), 7.52 (2/5H, d, J=8.0 Hz, H-6), 7.59 (3/5H, d, J=8.0 Hz, H-6), 8.60–8.75 (1H, m, NH), 8.80–8.90 (1H, m, NH); ^{31}P NMR ($CDCl_3$) δ 19.92 (2/5), 20.19 (3/5); HiRes MALDI FT-MS *m*/*z* ($M+Na^+$) found/calcd 805.3035/805.3035.

4.1.13. Preparation of vinyl(5′-*O*-*tert*-butyldimethylsilyl-2′-deoxy-2′-*C*-allyluridin-3′-yl)(3′-*O*-*tert*-butyldimethylsilylthymidin-5′-yl)phosphonate (20). Compound **11** (0.118 g, 0.331 mmol) and compound **18** (0.180, 0.333 mmol) were co-evaporated from CH_3CN (10 mL) and redissolved in CH_3CN (10 mL). A 0.45 M solution of 1*H*-tetrazole (3.7 mL, 1.66 mmol) in CH_3CN was added dropwise over 5 min. The reaction mixture was stirred for 3 h, added methanol (1 mL) and concentrated under reduced pressure. The residue was redissolved in CH_3CN (7 mL) and cooled to 0 °C. A 3 M solution of *t*-BuOOH in toluene (0.55 mL, 1.7 mmol) was added dropwise over 5 min and the reaction mixture was stirred for 2 h at room temperature and concentrated under reduced pressure. The residue was purified by dry column chromatography (10–100% ethyl acetate in petrol ether) to give the product as a white powder with an epimeric mixture (0.061 g, 23%); R_f 0.15 (75% ethyl acetate in petrol ether); ^{1}H NMR (MeOD-d_4) δ 0.12–0.15 (12H, m, $Si(CH_3)_2$), 0.92–0.94 (18H, m, $C(CH_3)_3$), 1.89–1.92 (3H, m, T-CH_3), 2.02–2.50 (5H, T-H2′, U-H2′, 2′-CH_2), 3.73–3.83 (2H, m, U-H5′), 3.85–4.02 (2H, m, T-H5′), 4.06–4.13 (3H, m, T-H4′, U-H4′,T-H3′), 4.36–4.43 (1H, m, U-H3′), 4.90–5.02 (2H, m, CH=CH_2), 5.52–5.73 (2H, m, U-H5, C*H*=CH_2), 5.93–6.01 (1H, m, U-H1′), 6.05–6.40 (4H, m, T-H1′, PC*H*=CH_2), 7.36–7.48 (1H, m, T-H6), 7.65–7.82 (1H, m, U-H6); ^{31}P NMR (MeOD-d_4) δ 19.87, 20.58; HiRes MALDI FT-MS *m*/*z* ($M+Na^+$) found/calcd 833.3354/833.3313.

4.1.14. Preparation of 3*R*- and 3*S*-(1*R*,8*S*,9*R*,11*R*)-3-(3(*S*)-(*tert*-butyldimethylsilyl)oxy-5(*R*)-(thymin-1-yl)-tetrahydrofuran-2(*R*)-yl)methoxy-8-hydroxy-3-oxo-11-(*tert*-butyldimethylsilyl)oxymethyl-9-(thymin-1-yl)-2,4,10-trioxa-3-phosphabicyclo[6.3.0]undec-6(*Z*)-ene (21). Compound **14** (30 mg, 0.035 mmol) and Grubbs second generation catalyst **A** (3 mg, 3.5 μmol) were dissolved in DCM (1.8 mL), and the reaction mixture was refluxed for 2 h. Another portion of **A** (3 mg) dissolved in DCM (0.1 mL) was added and the reaction mixture was stirred for another 4 h. The reaction was purified by column chromatography (0–100% ethyl acetate in petrol ether) to give the product as two separate epimers as white solids as well as recovered starting material (4.3 mg, 14%); Compound **21a** (4.5 mg, 16%) R_f 0.22 (ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 0.05–0.15 (12H, m, $Si(CH_3)_2$), 0.85–0.95 (18H, m, $C(CH_3)_3$), 1.96 (3H, s, T-CH_3), 2.00–2.32 (2H, m, T-H2′), 3.86–4.58 (8H, m, T-H4′, U-H4′, T-H3′, U-H3′, T-H5′, U-H5′), 4.81 (1H, s, OH), 4.87–5.00 (2H, m, POCH_2CH=CH), 5.68 (1H, d, J=8.1 Hz, U-H5), 6.02 (1H, d, J=11.9 Hz, $POCH_2CH$=C*H*), 6.15 (1H, dt, J=7.5, 11.9 Hz, $POCH_2$C*H*=CH), 6.22 (1H, s, U-H1′), 6.28 (1H, m, T-H1′), 7.33 (1H, d, J=0.9 Hz, T-H6), 7.77 (1H, d, J=8.1 Hz, U-H6), 8.50 (1H, br s, NH), 8.78 (1H, br s, NH); ^{31}P NMR ($CDCl_3$) δ −0.12; HiRes MALDI FT-MS *m*/*z* (M+Na) found/calcd 837.2944/837.2942. Compound **21b** (2.8 mg, 10%) R_f 0.12 (Ethyl acetate); ^{31}P NMR ($CDCl_3$) δ −1.55; HiRes MALDI FT-MS *m*/*z* (M+Na) found/calcd 837.2919/837.2942.

4.1.15. Preparation of 3*R*- and 3*S*-(1*R*,8*S*,9*R*,11*R*)-3-(3(*S*)-(*tert*-butyldimethylsilyl)oxy-5(*R*)-(thymin-1-yl)tetrahydrofuran-2(*R*)-yl)methoxy-8-hydroxy-3-oxo-11-(*tert*-butyldimethylsilyl)oxymethyl-9-(thymin-1-yl)-2,4,10-trioxa-3-phosphabicyclo[6.3.0]undecane (22). Compound **14** (22 mg, 0.025 mmol) and Grubbs second generation catalyst **A** (3.8 mg, 4.5 μmol) was dissolved in DCM (1.3 mL), and the reaction mixture was refluxed for 2 h. The reaction mixture was stirred in a 70 atm H_2 atmosphere at 50 °C for 13 h. The reaction was purified by column chromatography (0–100% ethyl acetate in petrol ether) to give the product as two separate epimers as white solids as well as recovered starting material (1.0 mg, 5%); Compound **22a** (0.6 mg, 3%) R_f 0.20 (ethyl acetate); ^{31}P NMR ($CDCl_3$) δ 5.83; HiRes MALDI FT-MS *m*/*z* (M+Na) found/calcd 839.3090/839.3099. Compound **22b** (1.6 mg, 8%) R_f 0.10 (ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 0.05–0.15 (12H, m, $Si(CH_3)_2$), 0.85–0.95 (18H, m, $C(CH_3)_3$), 1.96–1.99 (3H, s, T-CH_3), 1.90–2.40 (6H, m, T-H2′, $POCH_2CH_2CH_2$), 3.76–4.44 (10H, m, T-H4′, U-H4′, T-H3′, U-H3′, T-H5′, U-H5′ $POCH_2CH_2$), 5.02 (1H, s, OH), 5.63 (1H, d, J=8.2 Hz, U-H5), 6.04 (1H, s, U-H1′), 6.24 (1H, m, T-H1′), 7.35 (1H, s, T-H6), 7.67 (1H, d, J=8.2 Hz, U-H6), 8.12 (2H, br s, NH); ^{31}P NMR ($CDCl_3$) δ −0.85; HiRes MALDI FT-MS *m*/*z* (M+Na) found/calcd 839.3070/839.3099.

4.1.16. Preparation of 3*R*- and 3*S*-(1*R*,10*R*,11*R*,13*R*)-3-(3(*S*)-(*tert*-butyldimethylsilyl)oxy-5(*R*)-(thymin-1-yl)tetrahydrofuran-2(*R*)-yl)methoxy-3-oxo-13-(*tert*-butyldimethylsilyl)oxymethyl-11-(thymin-1-yl)-2,4,9,12-tetraoxa-3-phosphabicyclo[8.3.0]tridec-6(*Z*)-ene (25). Compound **16** (0.099 g, 0.116 mmol) was dissolved in DCM (11 mL). Grubbs second generation catalyst **A** (6.8 mg, 8.1 μmol)

was added and the reaction mixture was stirred at reflux for 24 h. The reaction mixture was concentrated under reduced pressure and the residue was purified by dry column chromatography (0–100% ethyl acetate in petrol ether) to give the product as a foam with an epimeric mixture (0.043 g, 45%); R_f 0.33 (ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 0.08–0.13 (12H, m, $Si(CH_3)_2$), 0.91–0.94 (18H, m, $C(CH_3)_3$), 1.91–1.96 (3H, s, T-CH_3), 2.05–2.33 (2H, m, T-H2′), 3.79–3.87 (2H, m, U-H5′), 4.10–4.45 (8H, m, U-H2′, T-H3′, T-H4′, T-H5′, U-H4′, $CH_2CH{=}CH_2$), 4.56–4.64 (2H, m, $CH_2CH{=}CH_2$), 4.87–4.93 (1H, m, U-H3′), 5.26–5.35 (1H, m, CH=C*H*), 5.65–5.69 (1H, m, U-H5), 5.91–5.95 (2H, m, U-H1′, C*H*=CH), 6.28–6.35 (1H, m, T-H1′), 7.27–7.38 (1H, m, T-H6), 7.98–8.06 (1H, m, U-H6), 9.36–9.40 (1H, m, NH), 9.80–9.92 (1H, m, NH); ^{31}P NMR ($CDCl_3$) δ −2.75 (1/4), −1.47 (3/4); HiRes MALDI FT-MS *m/z* ($M+Na^+$) found/calcd 851.3072/851.3091.

4.1.17. Preparation of 3*R*- and 3*S*-(1*R*,10*R*,11*R*,13*R*)-3-(3(*S*)-(*tert*-butyldimethylsilyl)oxy-5(*R*)-(thymin-1-yl)-tetrahydrofuran-2(*R*)-yl)methoxy-3-oxo-13-(*tert*-butyldimethylsilyl)oxymethyl-11-(thymin-1-yl)-2,4,9,12-tetraoxa-3-phosphabicyclo[8.3.0]tridecane (26). Compound **16** (0.666 g, 0.777 mmol) and Grubbs second generation catalyst **A** (32 mg, 0.038 mmol) catalyst were mixed and dissolved in DCM (77 mL). The reaction mixture was stirred at reflux for 26 h and then in a 70 atm H_2-atmosphere at 50 °C for 16 h. The mixture was concentrated under reduced pressure and the residue was purified by column chromatography (50–100% ethyl acetate in petrol ether and then 1–2% methanol in ethyl acetate) to give the product as a foam with an epimeric mixture (0.398 g, 62%); R_f 0.47, 0.53 (10% methanol in ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 0.09–0.18 (12H, m, $Si(CH_3)_2$), 0.83–1.00 (18H, m, $C(CH_3)_3$), 1.53–1.74 (2H, m, OCH_2CH_2), 1.90–2.35 (7H, m, OCH_2CH_2, T-H2′, T-CH_3), 3.80–4.32 (10H, m, U-H5′, U-H2′, T-H4′, T-H5′, U-H4′, T-H3′, OCH_2CH_2), 4.38–4.65 (2H, m, OCH_2CH_2), 4.82–4.90 (1H, m, U-H3′), 5.63–5.71 (1H, m, U-H5), 5.89–5.95 (1H, m, U-H1′), 6.18 (3/8H, m, T-H1′), 6.29 (5/8H, m, T-H1′), 7.25–7.29 (1H, m, T-H6), 7.92 (3/8H, d, J=8.2 Hz, U-H6), 8.00 (5/8H, d, J=8.2 Hz, U-H6), 8.55–8.80 (2H, m, NH); ^{31}P NMR ($CDCl_3$) δ −2.29 (3/8), −1.74 (5/8); HiRes MALDI FT-MS *m/z* ($M+Na^+$) found/calcd 853.3209/853.3247.

4.1.18. Preparation of 3*R*- and 3*S*-(1*R*,10*R*,11*R*,13*R*)-3-(3(*S*)-hydroxy-5(*R*)-(thymin-1-yl)tetrahydrofuran-2(*R*)-yl)methoxy-13-hydroxymethyl-3-oxo-11-(thymin-1-yl)-2,4,9,12-tetraoxa-3-phosphabicyclo[8.3.0]tridecane (27). Compound **26** (0.180 g, 0.217 mmol) was dissolved in a 90% aqueous solution of TFA (3 mL) and stirred at room temperature for 45 min. The mixture was concentrated under reduced pressure and co-evaporated with anhydrous ethanol. The residue was purified by dry column chromatography (0–20% ethanol in ethyl acetate) to give the product as a white powder with an epimeric mixture (0.077 g, 59%). R_f 0.2 (20% ethanol in ethyl acetate); ^{1}H NMR (DMSO-d_6) δ 1.35–1.90 (7H, m, $OCH_2CH_2CH_2$, T-CH_3), 2.08–2.21 (2H, m, T-H2′), 3.49–3.67 (2H, m, U-H5′), 3.69–4.45 (10H, m, T-H3′, T-H4′, T-H5′, U-H4′, U-H2′, 2×OCH_2), 4.70–4.88 (1H, m, U-H-3′), 5.36–5.45 (2H, m, 3′-OH, 5′-OH), 5.67 (3/5H, dd, J=2.0 Hz, J=8.1 Hz, U-H5), 5.71 (2/5H, dd, J=2.0, 8.2 Hz, U-H5), 5.81 (2/5H, d, J=3.8 Hz, U-H1′), 5.90 (3/5H, d, J=7.7 Hz, U-H1′), 6.16–6.21 (1H, m, T-H1′), 7.45 (3/5H, d, J=1.1 Hz, T-H6), 7.50 (2/5H, d, J=1.0 Hz, T-H6), 7.85 (2/5H, d, J=8.1 Hz, U-H6), 7.93 (3/5H, d, J=8.2 Hz, U-H6), 11.31–11.43 (2H, m, NH); ^{31}P NMR (DMSO-d_6) δ −1.39 (2/5), −1.25 (3/5); HiRes MALDI FT-MS *m/z* ($M+Na^+$) found/calcd 625.1490/625.1517.

4.1.19. Preparation of ethyl(5′-*O*-*tert*-butyldimethylsilyl-2′-deoxy-2′-methyluridin-3′-yl)(3′-*O*-*tert*-butyldimethylsilylthymidin-5′-yl)phosphonate (28). Compound **21** (0.022 g, 0.028 mmol) and Grubbs second generation catalyst **A** (2.4 mg, 0.003 mmol) were dissolved in DCM (3 mL). The reaction mixture was stirred in an 70 atm N_2 atmosphere at 50 °C for 16 h and then in an 70 atm H_2 atmosphere at 50 °C for 16 h. The reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography (75–100% ethyl acetate in petrol ether) to give the product as a white foam with an epimeric mixture (12 mg, 57%); R_f 0.07 (75% ethyl acetate in petrol ether). ^{31}P NMR ($CDCl_3$) δ 33.62, 35.42, 35.84, 36.76; MALDI FT-MS *m/z* ($M+Na^+$) 809.

4.1.20. Preparation 3*R*- and 3*S*-(1*R*,7*S*,8*R*,10*R*)-3-(3(*S*)-(*tert*-butyldimethylsilyl)oxy-5(*R*)-(thymin-1-yl)tetrahydrofuran-2(*R*)-yl)methoxy-3-oxo-10-(*tert*-butyldimethylsilyl)oxymethyl-8-(thymin-1-yl)-2,9-dioxa-3-phosphabicyclo[5.3.0]dec-4(*Z*)-ene (29). Compound **20** (0.030 g, 0.037 mmol) was dissolved in DCM (3.7 mL) and Grubbs second generation catalyst **A** (1.6 mg, 0.002 mmol) and CuCl (0.7 mg, 0.007 mmol) were added. The reaction mixture was stirred at reflux for 24 h and concentrated under reduced pressure. The residue was purified by column chromatography (0–4% methanol in ethyl acetate) to give the product as a powder with an epimeric mixture (0.010 g, 35%); R_f 0.52 (10% methanol in ethyl acetate); ^{31}P NMR ($CDCl_3$) δ 19.07 (1/4), 20.23 (3/4) HiRes MALDI FT-MS *m/z* ($M+Na^+$) found/calcd 805.3036/805.3014.

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Synthesis and cation binding properties of new arylazo- and heteroarylazotetrathiacalix[4]arenes

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Abstract—New macrocyclic tetrathiacalix[4]arenes have been synthesized by incorporating arylazo-, thiazoleazo- and β-naphthylazo- units in the tetrathiacalix[4]arene molecular architecture through diazotization and coupling reactions. The new compounds have been characterized by ^{1}H NMR, ^{13}C NMR and FAB-MS spectroscopic analysis. X-ray crystallography for one of the new dyes (**4a**) reveals that the compound is present in the cone conformation. The synthesized macrocycles have been examined for their binding with alkali (Li^+, Na^+, K^+, Cs^+ and Rb^+), alkaline earth (Ca^{2+}, Mg^{2+} and Ba^{2+}) and transition metal cations (Cr^{3+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^+, Hg^{2+}, Pd^{2+} and Pt^{2+}) by UV–visible spectroscopy to reveal selective bathochromic shifts for heavier alkali metal ions (cesium and rubidium) and palladium in a 1:1 and 2:1 stoichiometry respectively. The study has a significant bearing on the development of useful ionic filters and sensor materials.

1. Introduction

Calix[*n*]arenes are now well recognized molecular receptors for chemical and biochemical analysis through principles of molecular recognition. The replacement of the four methylene bridges of *p-tert*-butylcalix[4]arene by sulfide linkages provides tetrathiacalix[4]arenes which are expected to have different chemical behaviour from that of the classical calixarenes first described by Gutsche et al.[1] and a large number of subsequent workers.[2] The difference in chemical and spectral behaviour of calixarenes and tetrathiacalixarenes is primarily due to the possibility of varying oxidation states of the bridging sulfur atoms. The parent tetrathiacalix[4]arene architecture was first achieved by Sone et al. in 1997 via stepwise replacement of methylene linkages of *p-tert*-butylcalix[4]arene by sulfur bridges.[3] In the same year, Kumagai et al. reported a facile synthesis of *p-tert*-butyltetrathiacalix[4]arene in satisfactory yields by the reaction of *p-tert*-butylphenol with elemental sulfur.[4] Considerable efforts have been made thereafter to derivatize *p-tert*-butyltetrathiacalix[4]arenes to achieve molecular scaffolds and novel molecular receptors. Though a small number of tetrathiacalix[4]arenes with substituents at the lower rim are known, information on upper rim derivatization of tetrathiacalix[4]arenes is limited. This is probably due to the ease with which the bridging sulfur atoms can be oxidized to sulphoxide[5] or sulphone[6] derivatives by many of the oxidation catalysts used for derivatization which complicates the outcome of reactions. A few interesting reports on upper rim substitution of tetrathiacalix[*n*]arenes have appeared in the literature recently. For instance, sulphonation,[7] bromination,[8] nitration,[9,10] chloromethylation[11] and organophosphorylation[11] of tetrathiacalix[4]arenes have been reported. Lhotak et al. have reported the synthesis of aminotetrathiacalix[4]-arenes via diazocoupling and reduction[12] of the tetrathiacalix[4]arene analogue. Tetrathiacalix[4]arenes bearing ethynylic groups on the lower rim have also been reported by Parola et al.[13] To the best of our knowledge, very little work seems to have been reported on tetrathiacalix[4]arenes with chromogens at the upper rim, despite the fact that these derivatives are attractive targets for supramolecular assemblages for molecular recognition through visual colour change.[14–18] We describe herein the synthesis and evaluation of new chromogenic tetrathiacalix[4]arenes bearing phenylazo-, thiazoleazo-, pyridylazo- and β-naphthylazo-groups (Table 1) for ionic recognition in the hope to obtain new molecular filters and devices for specific use. While all compounds have been identified by physical and spectroscopic measurements (UV, IR, NMR and FAB MS), structure of one of these chromogenic compounds has been elucidated by X-ray crystallography. The ionic recognition by the synthesized molecular receptors reported in this paper has been investigated by UV–visible spectroscopic methods.

Keywords: Tetrathiacalix[4]arenes; Chromogenic; Diazotization; Ionic recognition.
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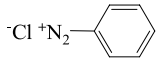
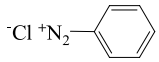
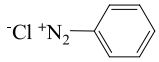
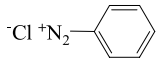
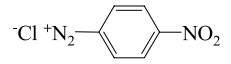
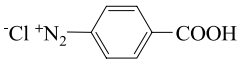
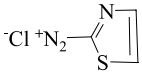
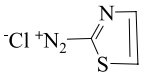
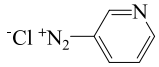
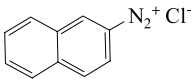
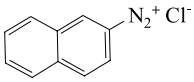
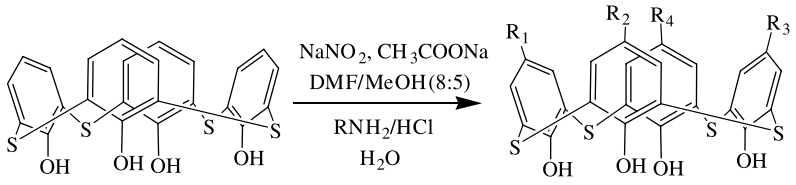
Table 1. Different products of reactions under optimized reaction conditions and their yields

Starting compound	Diazotizing unit	Product no.	Optimized conditions (base/solvent)	% Yields
2		**3a**	DMF:MeOH (8:5)/CH_3COONa	37
2		**3b**	DMF/CH_3COONa	68
2		**3c**	DMF:MeOH (8:5)/CH_3COONa	25
2		**3d**	DMF: MeOH (8:5)/NaOH	74
2		**3e**	DMF/CH_3COONa	71
2		**3f**	DMF/CH_3COONa	77
2		**4a**	DMF:MeOH (8:5)/CH_3COONa	41
2		**4b**	DMF:MeOH (8:5)/CH_3COONa	32
2		**5**	DMF:MeOH (8:5)/CH_3COONa	46
2		**6a**	DMF:MeOH (8:5)/CH_3COONa	47
2		**6b**	DMF:MeOH (8:5)/CH_3COONa	79

2. Results and discussion

2.1. Synthesis and characterization of chromogenic tetrathiacalix[4]arenes

In this work, seven new diazo-coupled tetrathiacalix[4]-arenes were synthesized by a weak base catalyzed coupling reaction of 25,26,27,28-tetrahydroxy-2,8,14,20-tetrathiacalix[4]arene **2** with diazotized solutions of aniline, 2-aminothiazole, 3-aminopyridine and β-naphthylamine (Scheme 1). The parent *p-tert*-butyltetrathiacalix[4]arene and tetrathiacalix[4]arene **2** were obtained by employing literature procedures.[4,19] The coupling reaction was performed at 0–5 °C in a mixed DMF/MeOH (8:5) solvent and sodium acetate as the base[11] followed by separation of the products by column chromatography to yield pure products in reasonable to good yields (Table 1). Detailed ^1H NMR spectral analysis of the synthesized compounds revealed that the phenylazo tetrathiacalix[4]arenes **3a–c** exhibited a singlet for the aromatic protons of the substituted tetrathiacalix[4]arene ring in the range δ 8.15–8.24 while protons on the unsubstituted aromatic tetrathiacalix[4]arene rings gave signals between δ 6.77–7.80. **3d** exhibited a singlet for the aromatic protons of the tetrathiacalix[4]arene ring at δ 8.24. Since sulfur bridges in the present compounds have replaced the methylene bridge



Scheme 1.

3a	$R_1=C_6H_5–N=N–$	$R_2, R_3, R_4=H$
3b	$R_1, R_3=C_6H_5–N=N–$	$R_2, R_4=H$
3c	$R_1, R_2, R_3=C_6H_5–N=N–$	$R_4=H$
3d	$R_1, R_2, R_3, R_4=C_6H_5–N=N–$	—
3e	$R_1, R_2, R_3, R_4=O_2N–C_6H_4–N=N–$	—
3f	$R_1, R_2, R_3, R_4=HOOC–C_6H_4–N=N–$	—
4a	$R_1=NSH_2C_3–N=N–$	$R_2, R_3, R_4=H$
4b	$R_1, R_2=NSH_2C_3–N=N–$	$R_3, R_4=H$
5	$R_1=C_5H_4N–N=N–$	$R_2, R_3, R_4=H$
6a	$R_1=C_{10}H_7–N=N–$	$R_2, R_3, R_4=H$
6b	$R_1, R_3=C_{10}H_7–N=N–$	$R_2, R_4=H$

present in classical calixarenes, exact information regarding the conformation of these chromoionophores could not be obtained from ^{1}H NMR and ^{13}C NMR spectroscopic techniques. A single crystal X-ray of one of the derivatives (**4a**) revealed that the synthesized compound was present in the cone conformation. By analogy and comparison of the NMR spectral data, it appears that the other synthesized tetrathiacalix[4]arenes probably also have the cone conformation but X-ray structure determinations of compounds other than **4a** are yet to be achieved.

It was interesting to note that the solvent used for the coupling reaction has a profound effect on the outcome of the reaction, i.e., the extent of substitution as well as the yield of the final product obtained. For example, when DMF was employed instead of DMF-methanol in the coupling reaction, orange crystals of pure **3b** in far better yields (68%) were obtained in contrast to the yields obtained from the use of DMF-MeOH mixture (43%). In addition, the latter solvent afforded a mixture of **3a**, **3b** and **3c**.

The thiazoleazo coupled tetrathiacalix[4]arenes **4a** and **4b** exhibited a pair of doublets for the protons of the thiazole moiety in the range of δ 7.41 and 8.01 respectively. **4a** and **4b** were identified as cone conformers by a comparative study of their NMR patterns with those of known tetrathiacalix[4]arenes.[20] The pyridylazo substituted tetrathiacalix[4]arene exhibited a singlet at δ 9.06, a pair of doublets at δ 8.00 and 8.60, and a triplet at δ 7.34 for the protons of the 3-pyridylazo moiety and a singlet at δ 8.18 for the protons of the tetrathiacalixaryl aromatic ring bearing the 3-pyridylazo group and two multiplets for the tetrathiacalixaryl protons of the unsubstituted rings at δ 6.75 and 7.64. The characterization of the tetrathiacalix[4]-arenes bearing β-naphthylazo substituents on the upper rim by NMR spectroscopic techniques proved difficult due to the complexity of the spectral data. However, elemental analysis and FAB MS spectroscopic measurements proved useful for identification of compounds **6a** and **6b**.

2.2. Results obtained from X-ray crystallography of 5-[(1-thiazole)azo]-25,26,27,28-tetrahydroxy-2,8,14, 20-tetrathiacalix[4]arene, 4a

Recrystallization of **4a** from chloroform afforded crystals suitable for X-ray diffraction. It was found that **4a** crystallized with chloroform located outside the tetrathiacalixarene cavity with torsion angles ϕ and χ around Ar–S–Ar bonds about S1, S2, S3 and S4 as −89.3(3), 94.7(3), −94.5(3), 91.6(3), −86.6(3), 97.9(3), −97.4(3) and 87.4(3) respectively. The alternate ± sequence observed was consistent with the cone conformation found in parent *p-tert*-butyltetrathiacalix[4]arene[21]and tetrathiacalix[4]-arene.[22] The unit cell of **4a** (Fig. 1) consisted of four molecules, each molecule having one chloroform molecule near the outer periphery of the tetrathiacalix[4]arene cavity. All four aromatic rings A (C1–C6), B (C7–C12), C (C13–C18) and D (C19–C24) were found to be almost planar with angles C2–S1–C24 = 101.62(14), C6–S2–C8 = 103.28(14), C12–S3–C14 = 101.31(15) and C18–S4–C20 = 101.94(15). The connecting sulfur atoms S1, S2, S3 and S4 formed an approximate plane where alternate sulfur atoms lay ±0.053 and ±0.053 Å above and below the plane. The interplanar angles found between this plane and the rings A (C1–C6), B (C7–C12), C (C13–C18) and D (C19–C24) were 57.66, 49.22, 61.91, and 50.97° respectively. The interplanar angles between the rings AC and BD were 60.43 and 79.81° respectively. The dihedral angle between the substituted azothiazole group plane was found to be 8.26°, which corroborated the alignment of the heterocyclic ring with the cone conformation of the tetrathiacalix[4]arene skeleton. The corresponding hydroxyl substituents O1, O2,

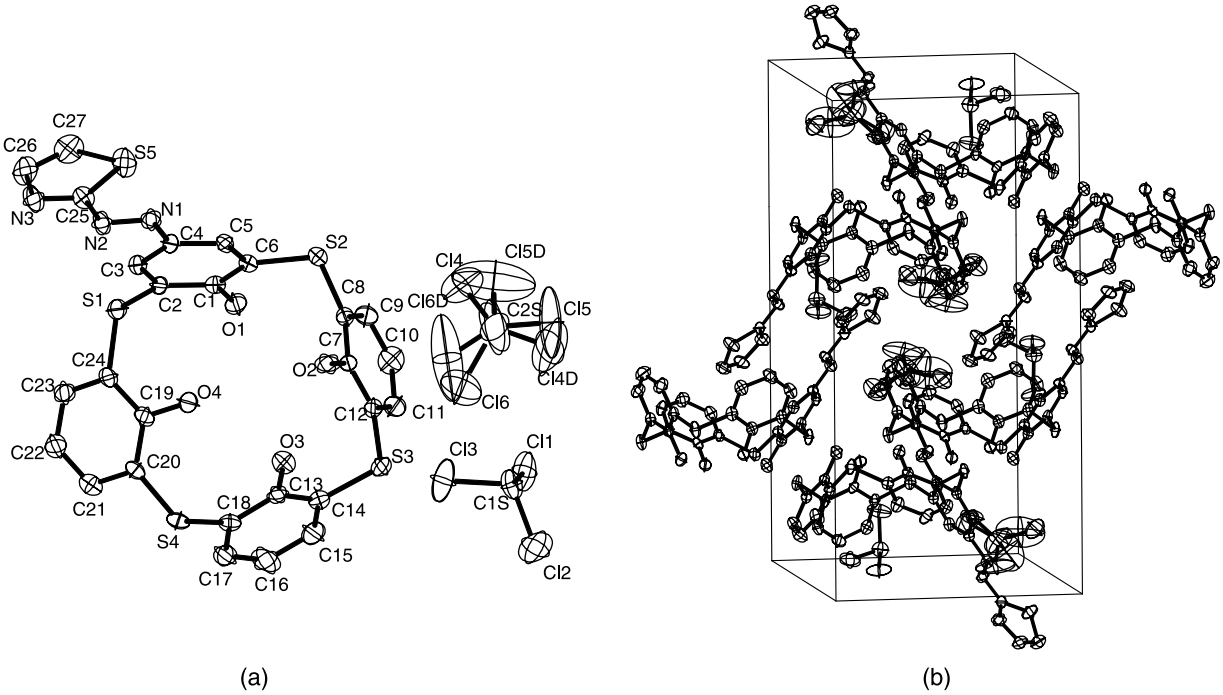


Figure 1. (a) ORTEP diagram of **4a** showing labeling scheme used and (b) contents of a single unit cell.

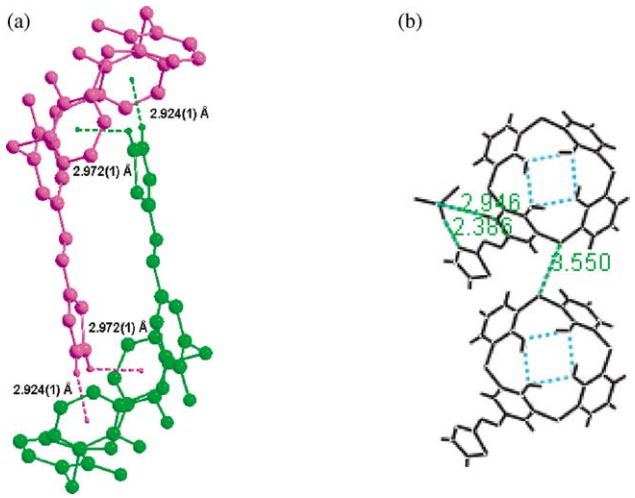


Figure 2. (a) CH–π interaction between two adjacent molecules and (b) H-bonding and S…S interactions in two molecules along *b* axis.

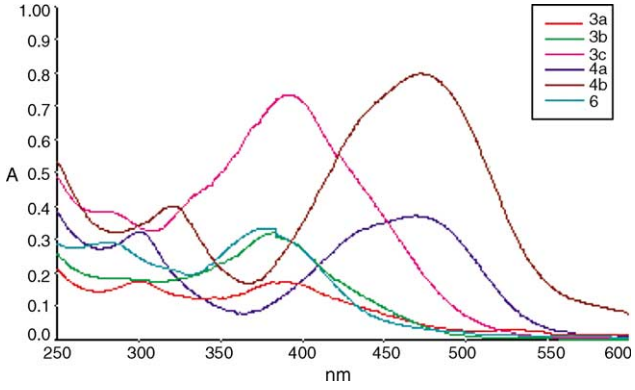


Figure 3. UV–visible spectra showing λ_{max} of chromoionophores **3a–c** and **4a**,**b**.

O3 and O4 were directed inwards the cavity of the calixarene architecture. The average distance between two oxygen atoms was 2.82 Å. Although one cannot exclude the stabilizing role of the solvent, the rather short distance between adjacent oxygen atoms might indicate the existence of an intramolecular H-bond array stabilizing the cone conformation. The average distance between two adjacent sulfur atoms was 5.51 Å which demonstrated that the size of the cavity in tetrathiacalix[4]arene was approximately 0.5 Å bigger than that in classical calix[4]arene. The exocyclic chloroform molecule showed three prominent non bonding interactions between the H of the chloroform molecule and the thiazole moiety attached to the ring A with average CH–π distances of 2.399, 2.473 and 2.944 Å respectively. The CH–π interaction between two adjacent tetrathiacalix[4]arene molecules is given in Fig. 2(a). There was significant S–S interaction between adjacent tetrathiacalix[4]arene molecules with a distance of 3.550 Å (Fig. 2(b)).

2.3. UV–visible spectroscopic studies of synthesized chromoionophores 3a–c and 4a,b.

In order to obtain insight into the metal affinity of the chromogenic tetrathiacalix[4]arenes, the changes in their λ_{max} upon interaction with a variety of hard and soft metal cations was investigated as follows:

2.3.1. Interaction with alkali and alkaline earth metal ions. The affinity of tetrathiacalix[4]arenes **3a–c** and **4a**,**b** for group I (Li^+, Na^+, K^+, Cs^+ and Rb^+) and group II (Ca^{2+}, Mg^{2+} and Ba^{2+}) cations was examined in solution using chloroform/methanol (1:1) as the solvent. Fig. 3 depicts the wavelengths of absorption of the different chromoionophores. The changes in λ_{max} of these chromoionophores upon addition of various cations are listed in Table 2. It has been observed that **3a–c** and **4a**,**b** exhibited

Table 2. $\Delta\lambda_{max}$/nm of the synthesized chromoionophores **3a–c** and **4a**,**b** on addition of 100 equiv. of metal salts using methanol–chloroform (v/v=1/1) as the cosolvent

Metal salts	Blank solutions of ligands[a]							
	3a		**3b**	**3c**	**4a**		**4b**	
	298	389	378	392	301	469	320	473
λ_{max}/nm of chromoionophores after adding metal salts								
Li^+	*nc*	*nc*	*nc*	*nc*	+9	+10	−22	*nc*
Na^+	+21	+14	+19	*s*(329)	+18	+20	*nc*	+13
K^+	+6	+10	+21	*s*(332)	+13	+34	*nc*	+16
Cs^+	+21	+60	+91 (*np* at 320)	+61	+18	+55	+12	+54
Rb^+	+10	+73	+77 (*np* at 318)	+46	+18	+57	+12	+46
Mg^{2+}	+5	*nc*	*ns*	−19	*nc*	*nc*	−22	−43
Ca^{2+}	*nc*	*nc*	*ns*	*nc*	*nc*	−36	−20	*nc*
Ba^{2+}	+33	+12	+20 (*np* at 335)	*nc*	+25	+19	*nc*	*nc*
Sr^{2+}	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*
Cr^{2+}	+51	+66	−39	−43	+90	−22	—	−67
Fe^{2+}	*ns*	*nc*	−41 (*np* at 293)	−14	*nc*	*nc*	−22	*nc*
Co^{2+}	+19	−17	−9	−24	+11	−44	*nc*	−51
Ni^{2+}	+24	*nc*	*s*(327)	−17	+16	−54	*nc*	−57
Cu^{2+}	*nc*	−26	−15	*nc*	*nc*	−46	*nc*	−50
Cd^{2+}	+7	*nc*	*nc*	−20	*nc*	*nc*	−20	−46
Hg^{2+}	+24	*nc*	*nc*	*nc*	+18	*nc*	*nc*	*nc*
Hg^+	+26	*ns*	*nc*	*nc*	+20	−20	*nc*	+17 *s*(554)
Ag^+	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*	*nc*
Pd^{2+}	*nc*	*nc*	*nc*	−45	+20	+148	*nc*	*s*(603)
Pt^{2+}	*nc*	*nc*	*nc*	*nc*	*ns*	*nc*	*nc*	*nc*

[a] Concentration for **3a–c** and **4a**,**b** was 10^{-4} M. + and − indicate bathochromic and hypsochromic shifts respectively. $\lambda_{max}=\lambda_{complex}-\lambda_{max}$ (free host). *nc* denotes no detectable change in λ_{max} upon metal ion complexation. *ns* indicates negligible shifts in λ_{max}. *np* indicates new peak position. *s* indicates shoulder in addition to the main peak.

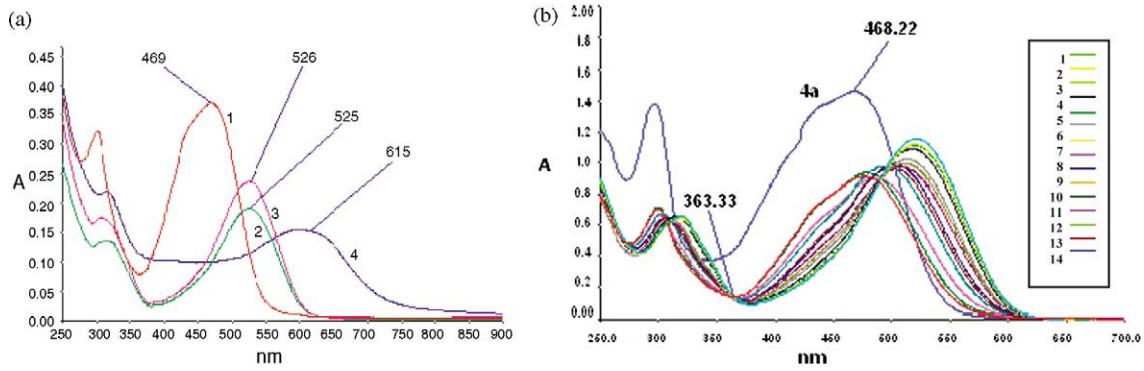


Figure 4. (a) UV–visible spectra of (1)10^{-4} M solution of **4a** and shifts in its λ_{max} upon the addition of 10^{-4} M solutions of (2) Cs_2CO_3, (3) Rb_2CO_3 and (4) K_2PdCl_4 in a Methanol–chloroform (v/v=1/1) cosolvent; (b) changes in the UV–visible spectra of **4a** upon titration by Cs_2CO_3 in a methanol–chloroform (v/v=1/1) cosolvent where the concentration of Cs_2CO_3 (1) 1×10^{-4} (2) 2×10^{-4} (3) 3×10^{-4} (4) 4×10^{-4} (5) 5×10^{-4} (6) 6×10^{-4} (7) 7×10^{-4} (8) 8×10^{-4} (9) 9×10^{-4} (10) 1×10^{-3} (11) 2×10^{-3} (12) 3×10^{-3} (13) 4×10^{-3} (14) 5×10^{-3}.

little or no shifts for the smaller alkali metal ions (Li^+ and Na^+) but significant bathochromic shifts were observed in the case of larger alkali metal ions (Cs^+ and Rb^+) indicating that the availability of the required number of donor oxygen atoms is not the major factor for the interaction of alkali metal cations and the synthesized tetrathiacalix[4]arenes. The cavity size of these azo coupled tetrathiacalix[4]arenes seems to have been affected by somewhat restricted conformational isomerization which fails to provide appropriate binding sites for relatively smaller alkali metal ions. For example, **3b** showed bathochromic shifts of 91 and 77 nm with concomittant appearance of new peaks at 320 and 318 nm respectively in their UV spectra on addition of solutions of Cs_2CO_3 and Rb_2CO_3 in methanol/chloroform. Significant bathochromic shifts in the absorption maximum were accompanied by significant colour changes in the visible region. For instance when UV–visible spectral measurements were carried out for interaction of **4a** with Cs^+ and Rb^+ by addition of Cs_2CO_3 and Rb_2CO_3 in chloroform/methanol to the chloroform–methanol solution of **4a** (Fig. 4(a)), it revealed that absorption of **4a** shifted from 468 to 524 nm with an isobestic point at 365 nm. Quantitative analysis through Job's continuous variation plots indicated that the metal:tetrathiacalix[4]arene interaction was in a 1:1 stoichiometric ratio of **4a** with Cs(I) and Rb(I).

Since significant shifts were not observed in the case of the smaller alkali metal ions and alkaline earth metal cations with the synthesized tetrathiacalix[4]arenes, they seemed to have good potential to be developed into molecular filters for ionic separations.

2.3.2. Interaction with transition metal ions. An investigation into the ionic recognition properties (Table 2) of synthesized chromoionophores with transition metal ions (Cr^{3+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^+, Hg^{2+}, Pd^{2+} and Pt^{2+}) was also investigated but surprisingly significant interaction was not observed despite the fact that sulfur containing molecular receptors should be expected to exhibit pronounced changes on interaction with transition metal ions. A marked bathochromic shift was however observed when a chloroform/methanol (1:4) solution of K_2PdCl_4 was added to **4a** (Fig. 4(a)), which was accompanied by a visual change from yellow to bluish green. This observation revealed a change in the coordination sphere of palladium in the presence of tetrathiacalix[4]arene, **4a**. The absorption intensity of the free tetrathiacalix[4]arene at 468 nm was found to gradually decrease in intensity with the formation of a new absorption band at 617 nm ($\Delta\delta=148$ nm). This observation is consistent with the earlier observation that complexes of palladium with thioethers exhibit a typical blue or green colour due to a d–d transition around 610 nm.[23] Two isobestic points at 363 and 414 nm could be easily discerned from the UV–visible titration spectra of **4a** with K_2PdCl_4 (Fig. 5(a)). The spectral features in the figure are consistent with a 1:2 binding of 4a

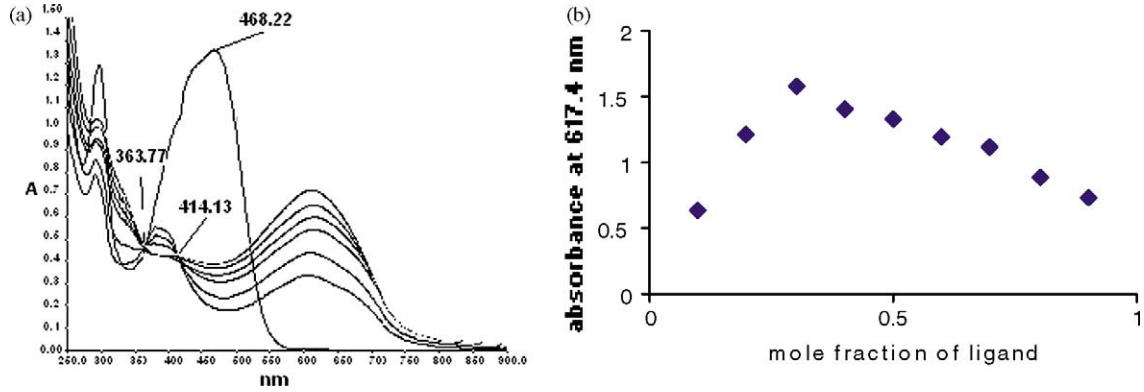


Figure 5. (a) Changes in the UV–visible spectra of 10^{-4} M solution of **4a** upon titration by K_2PdCl_4 in a methanol–chloroform (v/v=1/1) cosolvent where the concentration of K_2PdCl_4 varies from 2 to 8×10^{-4} M and (b) Job plot of 2:1 complex of **4a** and Pd^{2+}.

and Pd^{2+}. Analysis of the molar ratio as well as data from Job's plot experiment (Fig. 5(b)) confirmed a 1:2 binding ratio of **4a** with Pd^{2+}. The coordination of palladium may be visualized through metal–oxygen or through metal–sulfur interactions besides the cavity of the tetrathiacalix[4]arene core although a possible coordination mode through the N=N bonds is not ruled out. Knowledge of the exact coordination mode of palladium would require further investigations. Since negligible or small hypsochromic shifts were observed in the case of other transition metal ions, no quantitative experiments were carried out for these chromoionophores.

3. Experimental

All the reagents used in the study were purchased from Sigma-Aldrich or Merck and were chemically pure. The solvents used were distilled or dried according to the requirement. Column chromatography was performed on silica gel (60–120 mesh) obtained from Merck. Melting points were recorded on an electric melting point apparatus (Toshniwal, India) and are uncorrected. ^{1}H NMR spectra were recorded on a 300 MHz Bruker DPX 300 instrument at room temperature using tetramethylsilane (TMS) as an internal standard. X-ray data was recorded using a Bruker SMART CCD single crystal diffractometer. UV–visible spectra were obtained on a Perkin Elmer (Lambda-3B) recording spectrophotometer. The FAB mass spectra were recorded on a JEOL SX 102/DA-6000 Mass spectrometer/Data System using Argon/Xenon (6 kV, 10 mA) as the FAB gas.

3.1. Preparation of the starting materials

p-tert-Butyltetrathiacalix[4]arene[4] and tetrathia calix[4]-arene[19] **2** were synthesized by methods reported earlier.

3.1.1. Synthesis of 5-[(1-phenyl)azo]-25,26,27,28-tetrahydroxy-2,8,14,20-tetrathiacalix[4]arene (3a) and 5,11,17-*tris*[(1-phenyl)azo]-25,26,27,28-tetrahydroxy-2,8,14,20-tetrathia calix[4]arene (3c). *General procedure*. A solution of phenyl diazonium chloride prepared from aniline (1.396 g, 15.0 mmol), sodium nitrite (0.69 g, 10.0 mmol) and conc. HCl (7 mL) in water (25 mL), was added slowly into a cold (5 °C) solution of tetrathiacalix[4]arene (0.5 g, 1.0 mmol) and sodium acetate trihydrate (2.72 g, 20 mmol) in MeOH-DMF (15 mL, 5:8, v/v) to give an orange suspension. After allowing it to stand for 3 h at room temperature, the suspension was acidified with aqueous HCl (50 mL, 2 M). The resulting red precipitate was filtered and washed with water and methanol to yield a crude product which was purified by column chromatography on silica gel using chloroform to give **3a** (0.22 g, 37%, R_f=0.60) as a bright yellow solid and **3c** (0.20 g, 25%, R_f=0.34) as a red powder.

*Compound **3a***: mp 208 °C. Anal. Calcd for $C_{30}H_{20}N_2O_4S_4$: C, 59.98; H, 3.36; N, 4.66. Found: C, 60.02; H, 3.51; N, 4.58.]. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 9.42 (s, 4H, –OH); 8.22 (s, 2H, tetrathiacalixarene ArH); 7.82 and 7.46 (m, 5H, phenylazo ArH); 7.70 (d, 2H, J=6 Hz, tetrathiacalixarene ArH); 7.63 (d, 4H, J=6 Hz, tetrathiacalixarene ArH); 6.72–6.80 (m, 3H, tetrathiacalixarene ArH). MS-FAB: calcd for $C_{30}H_{20}N_2O_4S_4$: m/z=600.75 [M^+]; found: m/z=601 [M^+, 90%].

*Compound **3c***: Anal. Calcd for $C_{42}H_{28}N_6O_4S_4$: C, 62.36; H, 3.49; N, 10.39. Found: C, 62.52; H, 3.67; N 10.30. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 9.43 (s, 4H, –OH); 8.24 (s, 4H, tetrathiacalixarene ArH); 8.16 (s, 2H, tetrathiacalixarene ArH); 7.77 (m, 6H, phenylazo ArH); 7.65 (d, 2H, J=7.6 Hz, tetrathiacalixarene ArH); 7.39 (m, 9H, phenylazo ArH); 6.76 (t, 1H, J=15.4 Hz, tetrathiacalixarene ArH). MS-FAB: calcd for $C_{42}H_{28}N_6O_4S_4$: m/z=808.97 [M^+]; found: m/z=809[M^+, 42%].

3.1.2. Synthesis of 5,17-bis[(1-phenyl)azo]-25,26,27,28-tetrahydroxy-2,8,14,20-tetrathiacalix[4] arene (3b). A solution of aniline diazonium chloride, which was prepared from aniline (1.396 g, 15.0 mmol), sodium nitrite (0.69 g, 10.0 mmol) and conc. HCl (7 mL) in water (25 mL) was added slowly into a cold (5 °C) solution of tetrathiacalix[4]-arene (0.5 g, 1.0 mmol) and sodium acetate trihydrate (2.72 g, 20 mmol) in DMF (15 mL) to give an orange suspension. After being allowed to stand for 3 h at room temperature, the suspension was acidified with aqueous HCl (50 mL, 2 M). The resulting red precipitate was filtered, washed with water and methanol and dried to yield (0.36 g, 68%) of **3b** as a bright yellow solid. Anal. Calcd for $C_{36}H_{24}N_4O_4S_4$: C, 61.34; H, 3.43; N, 7.95. Found: C, 61.28; H, 3.54; N, 8.01. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 9.37 (s, 4H, –OH); 8.15 (s, 4H, tetrathiacalixarene ArH); 7.75 (m, 4H, phenylazo ArH); 7.66 (d, 4H, J=7.4 Hz, tetrathiacalix-arene ArH); 7.38 (m, 6H, phenylazo ArH); 6.77 (t, 2H, J=15.3 Hz, tetrathiacalixarene ArH). MS-FAB calcd for $C_{36}H_{24}N_4O_4S_4$: m/z=704.86 [M^+]; found: m/z=705 [M^+, 47%].

3.1.3. Synthesis of 5,11,17,23-tetrakis[(1-phenyl)azo]-25,26,27,28-tetrahydroxy-2,8,14,20-tetrathiacalix[4]-arene (3d). A solution of aniline diazonium chloride, which was prepared from aniline (1.396 g, 15.0 mmol), sodium nitrite (0.69 g, 10.0 mmol) and conc. HCl (7 mL) in water (25 mL) was added slowly into a cold (5 °C) solution of tetrathiacalix[4]arene (0.5 g, 1.0 mmol) and sodium hydroxide (0.8 g, 20 mmol) in MeOH-DMF (15 mL, 5:8, v/v) to give a dark red suspension. After being allowed to stand for 3 h at room temperature, the suspension was acidified with aqueous HCl (50 mL, 2 M). The resulting brownish precipitate was filtered and washed with water and methanol to give (0.68 g, 74%) of **3d** as a light brown solid. Anal. Calcd for $C_{48}H_{32}N_8O_4S_4$: C, 63.14; H, 3.53; N, 12.27. Found: C, 63.21; H, 3.67; N, 12.12. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 9.41 (s, 4H, –OH); 8.24 (s, 8H, tetrathiacalix-arene ArH); 7.37–7.77 (m, 20H, phenylazo ArH). MS-FAB calcd for $C_{48}H_{32}N_8O_4S_4$: (m/z)=913.08 [M^+]; found: m/z=913 [M^+, 34%].

3e and **3f** were synthesized according to the literature procedures.[12]

3.1.4. Synthesis of 5-[(1-thiazole)azo]-25,26,27,28-tetrahydroxy-2,8,14,20-tetrathia calix[4]arene (4a) and 5,11-bis[(1-thiazole)azo]-25,26,27,28-tetrahydroxy-2,8,14,20-tetrathiacalix[4]arene (4b). Compounds **4a** and **4b** were

prepared as described above for **3a** using 2-aminothiazole. Column purification using chloroform/ethyl acetate (9:1) gave **4a** (0.25 g, 41%, $R_f=0.68$) as dark red crystals and **4b** (0.23 g, 32%, $R_f=0.31$) as a dark red powder. It should be noted that products formed under these condition do not contain complexed chloroform.

Compound ***4a***: mp >250 °C. Anal. Calcd for $C_{27}H_{17}N_3O_4S_5$: C, 53.36; H, 2.82; N, 6.91. Found: C, 53.25; H, 2.67; N, 7.01. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 9.37 (s, 4H, –OH); 8.34 (s, 2H, tetrathiacalixarene ArH); 8.02 (bs, 1H, thiazole ArH); 7.70 and 6.82 (m, 6H and 3H, tetrathiacalixarene ArH); 7.41 (bs, 1H, thiazole ArH).

Compound ***4b***: mp 170 °C. Anal. Calcd for $C_{30}H_{18}N_6O_4S_6$: C, 50.12; H, 2.52; N, 11.69. Found: C, 50.28; H, 2.34; N, 11.57. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.41 (s, 2H, tetrathiacalixarene ArH); 8.36 (s, 2H, tetrathiacalixarene ArH); 8.01 (bs, 2H, thiazole ArH); 7.70 (d, 2H, $J=7.8$ Hz, tetrathiacalixarene ArH); 7.64 (d, 2H, $J=7.5$ Hz, tetrathiacalixarene ArH); 7.41 (bs, 2H, thiazole ArH); 6.89 (t, 2H, $J=14.8$ Hz, tetrathiacalixarene ArH).

3.1.5. Synthesis of 5-[(3-pyridyl)azo]-25,26,27,28-tetrahydroxy-2,8,14,20-tetrathia calix[4]arene (5). It was prepared as described above for **3a** using 3-aminopyridine. Column purification using chloroform/ethyl acetate (8:2) gave **5** (0.36 g, 59%, $R_f=0.66$) as an orange solid. Anal. Calcd for $C_{29}H_{19}N_3O_4S_4$: C, 57.88; H, 3.18; N, 6.98. Found: C, 57.65; H, 3.20; N, 6.93. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 9.40 (s, 4H, –OH); 8.18 (s, 2H, tetrathiacalixarene ArH); 9.06 (s, 1H, pyridylazo ArH); 7.34 (t, 1H, $J=15$ Hz, pyridylazo ArH); 8.60 (d, 1H, $J=7.7$ Hz, pyridylazo ArH); 8.00 (d, 1H, $J=7.7$ Hz, pyridylazo ArH); 6.75 and 7.64 (m, 9H, tetrathiacalixarene ArH), MS-FAB calcd for $C_{29}H_{19}N_3O_4S_4$: $m/z=601.74$ [M^+]; found: $m/z=602$ [M^+, 88%].

3.1.6. Synthesis of 5-[(β-naphthyl)azo]-25,26,27,28-tetrahydroxy-2,8,14,20-tetrathia calix[4]arene (6a) and 5,17-bis[(1-phenyl)azo]-25,26,27,28-tetrahydroxy-2,8,14,20-tetrathia calix[4]arene (6b). 6a and **6b** were prepared as described above for **3a** using β-naphthylamine. Column purification using chloroform/hexane (1:1) gave **6a** (0.31 g, 47%, $R_f=0.68$) as a dark red solid and **6b** (0.64 g, 79%, $R_f=0.39$) as a maroon coloured powder.

Compound ***6a***: mp 192 °C. Anal. Calcd for $C_{34}H_{22}N_2O_4S_4$: C, 62.75; H, 3.41; N, 4.30. Found: C, 62.66; H, 3.39; N, 4.49. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 9.37 (s, 4H, OH); 8.20 (s, 2H, tetrathiacalixarene ArH); 6.74 and 7.83 (m, 9H, tetrathiacalixarene ArH); 7.45–7.57 and 7.88–8.13 (m, 7H, β-naphthylazo ArH) MS-FAB calcd for $C_{34}H_{22}N_2O_4S_4$: $m/z=650.81$ [M^+]; found: $m/z=651$ [M^+, 50%].

Compound ***6b***: mp 161 °C. Anal. Calcd for $C_{44}H_{28}N_4O_4S_4$: C, 65.65; H, 3.51; N, 6.96. Found: C, 65.81; H, 3.66; N, 6.84. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 9.37 (s, 4H, OH); 8.65 (s, 4H, tetrathiacalixarene ArH); 7.74 (d, 4H, $J=7.7$ Hz, tetrathiacalixarene ArH); 7.38–7.60 and 7.92–8.06 (m, 14H, β-naphthylazo ArH); 6.80 (t, 2H, $J=15.1$ Hz, tetrathiacalixarene ArH). MS-FAB calcd for $C_{44}H_{28}N_4O_4S_4$: $m/z=804.98$ [M^+]; found: $m/z=804$ [M^+, 49%].

3.2. Crystallography

The crystals suitable for single crystal X-ray diffraction were obtained by slow cooling of a warm solution of **4a** in chloroform. Dark red crystals of a 1:1 **4a**. chloroform complex were obtained with molecular formula $C_{29}H_{18}Cl_6N_3O_4S_5$, $M=845.46$, monoclinic, space group P2(1)/n with $a=10.9745(7)$, $b=22.1150(14)$, $c=14.8313(9)$, $\alpha=90.0$, $\beta=90.96(10)$, $\gamma=90.0°$ and $D_c=1.560$ g/cm^3 for $Z=4$. Intensity diffraction data were calculated up to $\theta=26.86°$ by using 2ω step scanning mode with Mo Ka radiation $\lambda=0.71073$ Å) at 273 K. A total of 6693 reflections were calculated and used in structure analysis and refinement. All the non-hydrogen atoms were refined anisotropically using restraints on the bond lengths and thermal parameters. All hydrogen atoms were placed in their geometrical positions and were not refined. The labeling scheme followed is in agreement with that followed in calix[4]arene–solvent host guest complexes.[24,25] The final R index using observed data, refining 455 parameters with no restraints was $R_{all}=0.0769$, $R_{gt}=0.0569$, $wR_{ref}=0.1808$ and $wR_{gt}=0.1677$. Low e.s.d.'s for other atoms suggest that the overall geometry and accuracy of the structure has not been compromised to any significant extent. All the calculations involving structure solution, using SHELXTL-PC performed refinement and graphics. Crystallographic data for the structure have been deposited with Cambridge Crystallographic Database as supplementary publication number CCDC 280307.

3.3. General procedure for UV–visible experiments

Because of the poor solubility of metal salts in chloroform, all of the UV–visible experiments reported in this work were carried out in chloroform–methanol (1:1) unless otherwise specified.

3.3.1. Job's plot experiments. Stock solutions of compound **4a** (10^{-4} M) in chloroform–methanol (1:1) and metal salt solutions Cs_2CO_3 (10^{-4} M), Rb_2CO_3 (10^{-4} M) in chloroform–methanol (1:1) and K_2PdCl_4 (10^{-4} M) in chloroform–methanol (1:4) were prepared. The concentrations of each chloroform–methanol solutions were varied, but their volumes were fixed at 5.0 mL. After the mixture was shaken for 2 min, the UV–visible absorbances at 524 nm for Cs_2CO_3, 526 nm for Rb_2CO_3 and at 617 nm for K_2PdCl_4 were recorded. Assuming that only one complex (ML_n) was formed at equilibrium; the value of 'n' could be calculated from the plot of χ_{max} [mole fraction of the ligand (χ_L) at maximum absorption] by the following relationship, $n=\chi_{max}/1-\chi_{max}$. The value of χ_{max} was noted from the plot of absorbance vs χ_L.

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One-pot synthesis of fluorinated 2-amino-pyrimidine-*N*-oxides. Competing pathways in the four-atom side-chain rearrangements of 1,2,4-oxadiazoles

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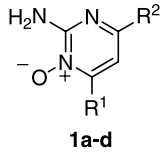
Abstract—Trifluoromethylated 2-amino-pyrimidine *N*-oxides have been synthesized by reaction of the 3-amino-5-methyl-1,2,4-oxadiazole with trifluoromethyl-β-diketones in the presence of perchloric acid, followed by hydrolysis. In this ring-to-ring transformation an initial formation of (unisolated) 1,2,4-oxadiazole-pyrimidinium salts, and subsequent ring-opening at the oxadiazole moiety occurs. Isolation of 2-(hydroxyamino)-pyrimidine from the reaction mixture evidenced the presence of a competing pathway where the N(4) nitrogen of the oxadiazole is involved in the formation of a regioisomeric pyrimidinium salt. The effect of the trifluoromethyl group on the product distribution is discussed. By X-ray analysis, the crystal structure of two different *N*-oxide regioisomers has been unambiguously ascertained.

1. Introduction

In recent years there has been a growing interest for the synthesis of perfluoroalkylated heterocycles as well as for their applications as pharmaceuticals, agrochemicals, and new materials.[1] Among the general methodologies used for such fluorinated targets we can mention the classic *direct fluorination* and *building-block strategy* together with the more recent *ring-rearrangement approach*[2] which has been successfully applied for the synthesis of 1,2,4-[3] and 1,3,4-oxadiazoles,[4] 1,2,4-triazoles,[4b,5] 1,2,4-triazines[6] and quinazolin-4-ones.[7]

In the frame of our researches on the synthesis of fluorinated heterocycles as well as fluorinated analogues of biological molecules, we became interested in the synthesis of functionalized perfluoroalkyl-containing pyrimidine-*N*-oxides. In particular, 2-amino-pyrimidine-*N*-oxides have been widely used as intermediates in the synthesis of other heterocyclic systems[8] as well as herbicides,[9] growth regulators,[9] antidermatitis[10] and hair loss preventing drugs.[11] Since the introduction of a fluorinated moiety generally emphasizes the biological activity, we first looked at the synthesis of 2-amino compounds of the general type **1** (Chart 1), which could, in principle, be obtained through three different approaches: (i) the *N*-oxidation reaction of pre-formed perfluoroalkyl-2-amino-pyrimidines;[8] (ii) ring closure reactions;[8] (iii) a ring-rearrangement of a perfluoroalkylated oxadiazolyl-enamino ketone system.[12]



1a-d

a: $R^1 = R^2 = CF_3$
b: $R^1 = CH_3$; $R^2 = CF_3$
c: $R^1 = CF_3$; $R^2 = CH_3$
d: $R^1 = R^2 = CH_3$

Chart 1

2. Results and discussion

Unlike the well known *N*-oxidation reaction of alkyl-pyrimidines,[8] current literature does not report examples of *N*-oxidation of perfluoroalkylated analogues. In our hands, attempts to obtain **1a** by *N*-oxidation of the 2-amino-4,6-bis-trifluoromethyl-pyrimidine **2a** [either using hydrogen peroxide in acetic acid or *meta*-chloroperbenzoic acid (*m*-CPBA) in chloroform] resulted in a very complex

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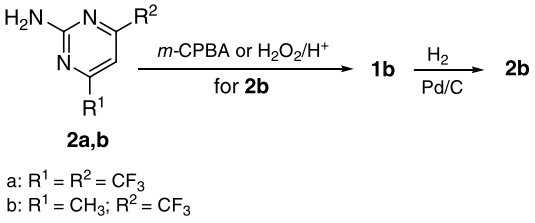
reaction mixture from which no significant product could be isolated. Different results were obtained in the case of the 2-amino-4-trifluoromethyl-6-methyl-pyrimidine **2b** which, by reaction with *m*-CPBA in chloroform, produced high yields of the pyrimidine *N*-oxide **1b** (88%) (Scheme 1). The same **1b** is also obtained (but with lower yields, 47%) by using hydrogen peroxide in acetic acid. X-ray analysis confirmed the position of the *N*-oxide moiety in the proposed structure. Reduction of **1b**, as expected, yielded **2b** (95%). It is noteworthy that in both cases of the oxidation process no trace of the regioisomeric *N*-oxide **1c** was detected. This observation is simply explained considering that the *N*-oxidation reaction engages the more electron-rich nitrogen (i.e. the one far from the trifluoromethyl group).



Scheme 1.

On the basis of the results above, *N*-oxidation reactions were not considered a versatile tool for the obtainment of bis-perfluoroalkylated 2-amino-pyrimidine *N*-oxides. Moreover, the question of synthesizing regioisomeric *N*-oxides such as **1c** was still open. We then looked at the *ring-rearrangement approach* taking the advantage of the reactivity of 3-amino-1,2,4-oxadiazoles. The reactivity of these α-aminoazoles towards β-diketones in the presence of $HClO_4$ to give azolopyrimidinium perchlorates has been studied since 30 years ago and is represented in Scheme 2 by the reaction of 3-amino-5-phenyl-1,2,4-oxadiazole **3** with 2,4-pentandione.[12] Upon hydrolysis, the azolopyrimidinium salt **5** undergoes a nucleophilic attack at the oxadiazole moiety followed by ring-opening to the acylamino-pyrimidine *N*-oxide **6**. A plausible reaction path (also confirmed by a separate experiment)[12a] involves the enaminoketone intermediate species **4** which will undergo a *four-atom side-chain* rearrangement where the N(2) of the oxadiazole ring acts as a nucleophilic, and the carbonyl of the enaminoketone as an electrophilic centre (i.e. a 'reverse' Boulton–Katritzky[13] electronic demand) (Scheme 2).

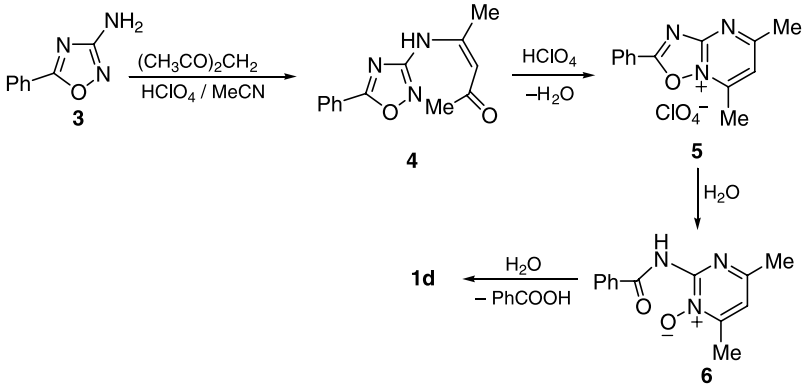
On the basis of our previous results[12] we planned to perform a one-pot reaction of the 3-amino-5-methyl-1,2,4-oxadiazole **7** [the choice of the 5-methyl substitution guarantees an easier hydrolysis step] with bis-trifluoromethyl- or methyl-trifluoromethyl-β-diketones **8a**,**c** in acetonitrile in the presence of perchloric acid followed by hydrolysis with H_2O/HCO_3^-. The total conversion of the starting oxadiazole **7** was monitored before the hydrolytic step.

From the reaction mixture, besides the expected 2-amino-pyrimidine *N*-oxides **1a**,**c**, significant amounts of the starting 3-aminooxadiazole **7** and, more interestingly, of 2-(hydroxyamino)-pyrimidines **14a**,**c** and their acetylated precursors **13a**,**c** were isolated (see Scheme 3 and Table 1).

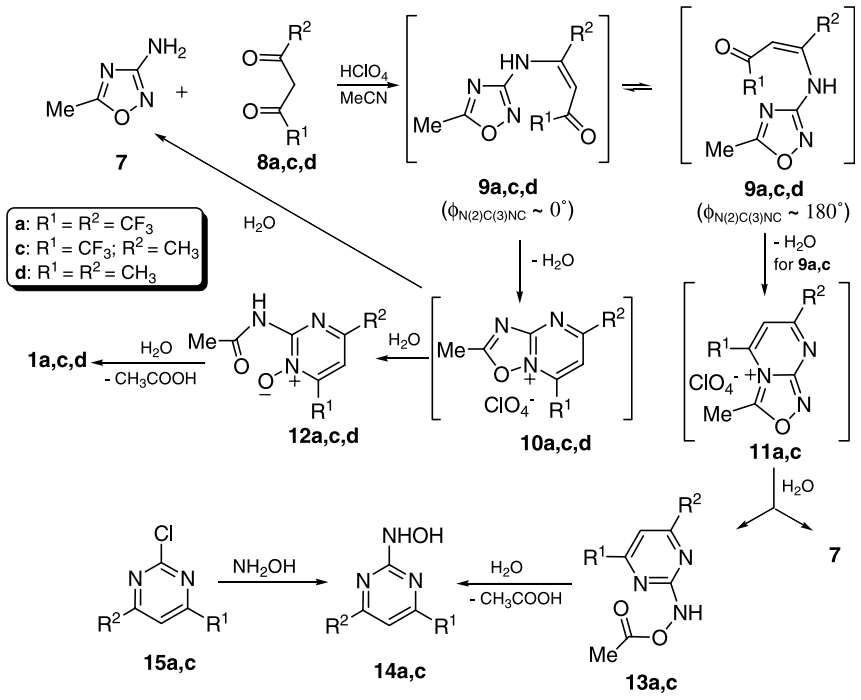
Structure of **1c** was confirmed by means of X-ray analysis; structure of **14a**,**c** has been assigned on the basis of spectroscopic data and confirmed by comparison with authentic samples obtained from the reaction of 2-chloro-pyrimidine **15a**,**c** with hydroxylamine. Of course, a separate experiment demonstrated that compounds **13a**,**c** under the same reaction conditions easily give the deacetylated **14a**,**c** and this justifies the low percentage of isolated **13a**,**c**.

For comparison we then repeated the previously reported[12b] reaction with 2,4-pentandione **8d** under the same conditions obtaining 2-aminopyrimidine *N*-oxide **1d** (92%) and few percent of its acetylated precursor **12d** (6%) (Table 1), while no trace of any 2-(hydroxyamino)-pyrimidine derivative **13d** or **14d** (by comparison with an authentic sample) was observed.

These findings are very interesting from a mechanistic point of view (Scheme 3). First of all, in the reaction with asymmetric diketone **8c** only the *N*-oxide **1c** is obtained, while no trace of its regioisomer **1b** is detected (Figs. 1 and 2). This can be explained by assuming the initial attack of the 3-amino group of the oxadiazole **7** on the less enolized end of the diketone (i.e. the CH_3CO- carbonyl).[14]



Scheme 2.



Scheme 3.

Table 1. Product distribution of reaction of compound **7** with diketones **8a**,**c**,**d**

β-Diketone	Products (Yields %)				
	1	**7**	**12**	**13**	**14**
8a	**1a** (21%)	38%	—	**13a** (2%)	**14a** (39%)
8c	**1c** (50%)	30%	—	**13c** (5%)	**14c** (12%)
8d	**1d** (92%)	—	**12d** (6%)	—	—

The isolation of the starting material after the hydrolysis step points out that the hydrolytic ring-opening of the pyrimidinium salt **10** could involve either the oxadiazole (leading to *N*-oxides **1**) or the pyrimidine ring (leading back to **7**); moreover the presence of perfluoroalkyl groups will increase the hydrolysis on the pyrimidine side (see Table 1). The isolation of 2-(hydroxyamino)-pyrimidines **14a**,**c** points out a new reactivity for the

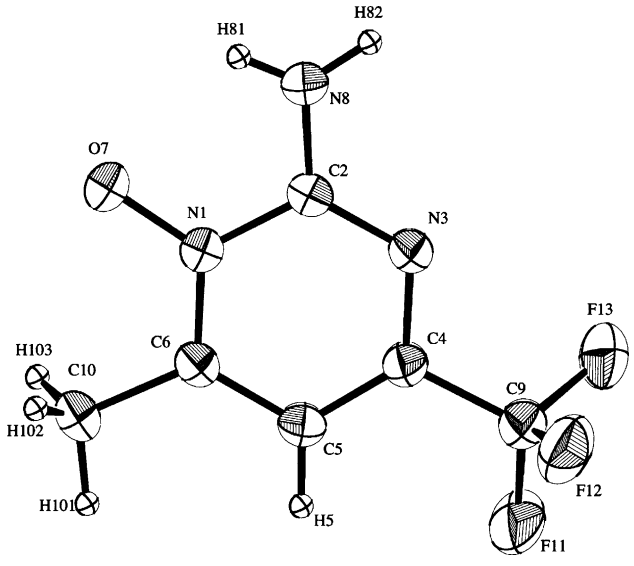


Figure 1. The Ortep drawing of **1b**.

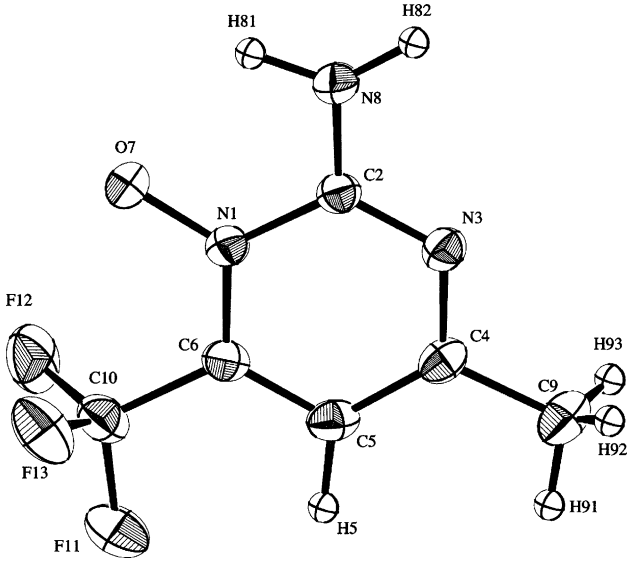


Figure 2. The Ortep drawing of **1c**.

oxadiazolyl-enaminoketone system: this can be explained by a cyclization of the enaminoketone involving the N(4) oxadiazole nitrogen and leading to a regioisomeric pyrimidinium salt **11** which upon hydrolysis leads to **13a**,**c** and then to **14a**,**c**. This pathway is absent in the case of unfluorinated diketones and it becomes a more competitive route by increasing the perfluoroalkyl substitution (see Table 1). This suggests an effect of trifluoromethyl substituent on the conformational stability of the enaminoketone side-chain or on the protonation of either N(2) or N(4) nitrogen.

3. X-ray crystallographic analysis

3.1. Compound 1b

Crystals of **1b** suitable for X-ray analysis were obtained from ethanol.

Due to the occurrence of the intramolecular hydrogen bond N8–H81…O7, the molecules adopt a planar conformation (Fig. 3); in the crystal, the molecules are joined in pairs via intermolecular N–H…N hydrogen bonds [N8…N3 3.052(2) Å, N8–H81…N3 179(2)°, N3 in $-x$, $2-y$, $-z$], giving rise to infinite layers parallel to each other, and all parallel (approximately) to the −202 crystallographic planes.

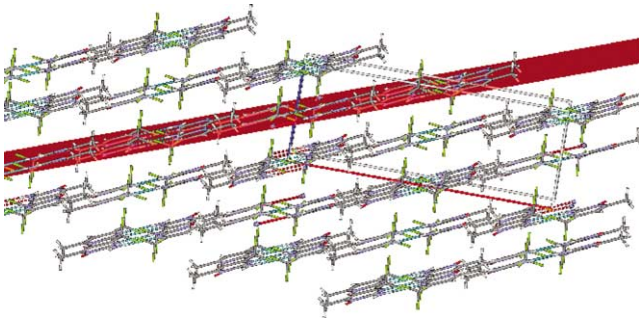


Figure 3. Crystal packing of **1b**.

3.2. Compound 1c

Compound **1c** was crystallized from ethanol, giving well-formed crystals of prismatic shape. Also in the present case the molecule has planar conformation (Fig. 4). In the crystal the molecules are arranged in such a way that each molecule is connected to other two by means of two different intermolecular hydrogen bonds involving the amino group: N8–H81…N3 [N8…N3 3.073(2) Å, N8–H81…N3 144(2)°; N3 in $2-x$, $-y$, $z-\frac{1}{2}$] and N8–H82…O7 [N8…O7 2.895(2) Å, N8–H82…O7 168(2)°; O7 in $2-x$, $-y$, $z+\frac{1}{2}$]. The infinite zig–zag chains so obtained are packed parallel to each other along the **a** direction.

4. Conclusions

The synthesis of perfluoroalkylated 2-amino-pyrimidine *N*-oxides can be achieved by following two different strategies depending on the target substitution pattern. The classic *N*-oxidation will lead to a product where the *N*-oxide moiety is away from the perfluoroalkyl group. The reaction of 3-amino-5-methyl-1,2,4-oxadiazole with perfluoroalkylated β-diketones allows to obtain the other possible *N*-oxide regioisomer in the case of monoperfluoroalkylated pyrimidines and represents a useful 'regio-complementar' alternative to the *N*-oxidation. In the case of bis-perfluoroalkyl pyrimidine *N*-oxides the yields are lower but, at the moment, the *ring-rearrangement approach* represents the only methodology available to obtain these derivatives. Finally, the observation of a new competitive rearrangement of the proposed enamino ketones intermediate engaging the N(4) of the oxadiazole ring in the cyclization process opens the way to new synthetic procedures and to other mechanistic studies.

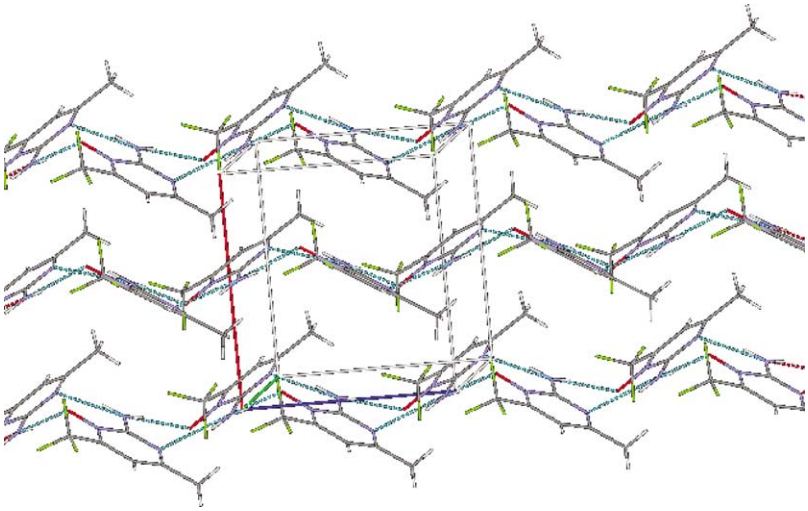


Figure 4. Crystal packing of **1c**.

5. Experimental

5.1. X-ray analysis

After preliminary Laue photographs, the intensity data for compounds **1b** and **1c** were collected by means of a Bruker-Nonius MACH3 diffractometer, equipped with Mo Kα radiation ($\lambda=0.7107$ Å) and graphite monochromator. Cell parameters were obtained by least-squares refinement on diffractometer angles for 25 automatically centred reflections. For both compounds the measured intensities were corrected for Lorentz, polarization and absorption effects. The slight gradual decay observed during the data collection (1b: 5.7%; 1c: 1.5%) was also accounted for.

Direct methods procedures as implemented in SHELXS97[15] were used to solve the structures. Full-matrix least-squares refinements were then accomplished with SHELXL97.[16]

5.1.1. Structure determination and refinement details. 1b. The systematic absences observed (*hkl* with $h+k\neq 2n$ and *h0 l* with $l\neq 2n$) were consistent with the space groups Cc and C2/c. The crystal structure solution was straight in the centrosymmmetric C2/c space group: after some anisotropic least-squares cycles considering the non-hydrogen atoms (all obtained from the first E-map), all the hydrogens were clearly revealed from a difference Fourier map. Their coordinates were then isotropically refined without constraints, apart for the restraints on the two N–H distances (DFIX instruction). One of the fluorine atoms exhibits a large displacement parameter, indicating its tendency to be disordered over two different positions. However, the structure model with the best agreement indexes was that obtained not considering the corresponding split positions. No correlation matrix elements greater than 0.5 were found.

5.1.2. Structure determination and refinement details. 1c. Of the two possible space groups compatible with the observed systematic absences (Pnma and $Pna2_1$), the non-centrosymmetric $Pna2_1$ was confirmed as the correct one in the structure solution step. All the hydrogen atoms were located from a Fourier difference map, after anisotropic refinement of the heavier atoms. The hydrogens of the methyl group were treated as idealized group (AFIX instruction), with the same refinable U_{iso}; the N–H distances were restrained to the same value (DFIX instruction); all the other hydrogen parameters were allowed to refine independently. No correlation matrix elements greater than 0.5 was observed.

5.1.3. Crystal data. 1b. $C_6H_6N_3OF_3$, $M=193.14$, colourless platelet $0.2\times0.2\times0.35$ mm, Monoclinic, C2/c, $Z=8$, $a=19.208(2)$, $b=11.040(1)$, $c=7.323(1)$ Å, $\beta=91.25(1)°$, $V=1552.6(3)$ Å^3, $F(000)=784$, $D_x=1.65$ Mg m^{-3}, μ(Mo Kα)$=0.16$ mm^{-1}

5.1.4. Crystal data. 1c. $C_6H_6N_3OF_3$, $M=193.14$, pale yellow prism $0.2\times0.5\times0.5$ mm, Orthorhombic, $Pna2_1$, $Z=4$, $a=8.418(2)$, $b=11.770(2)$, $c=7.710(1)$ Å, $V=763.9(2)$ Å^3, $F(000)=392$, $D_x=1.68$ Mg m^{-3}, μ(Mo Kα)$=0.17$ mm^{-1}

5.1.5. Data collection conditions and refinement parameters. 1b. ω–θ scans, maximum scan width 1.05°, minimum scan speed 0.9° min^{-1}, $2\theta_{max}=30°$, 4876 total reflections measured, 2264 independent reflections ($R_{equiv}=0.017$), 1464 reflections with $F_o>4\sigma(F_o)$, 143 refined parameters, $R1=0.042$, $wR2=0.128$, $S=1.024$, $\Delta\rho_{min}=-0.24$ e Å^{-3}, $\Delta\rho_{max}=+0.28$ e Å^{-3}.

5.1.6. Data collection conditions and refinement parameters. 1c. ω–θ scans, maximum scan width 1.05°, minimum scan speed 0.9° min^{-1}, $2\theta_{max}=35°$, 3678 total reflections measured, 1779 independent reflections ($R_{equiv}=0.019$), 1306 reflections with $F_o>4\sigma(F_o)$, 132 refined parameters, $R1=0.037$, $wR2=0.097$, $S=0.944$, $\Delta\rho_{min}=-0.18$ e Å^{-3}, $\Delta\rho_{max}=+0.21$ e Å^{-3}.

5.1.7. Crystallographic data. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data centre as supplementary publication numbers CCDC 279693 & 279694. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 IEZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk]

5.2. General methods and materials

Melting points were determined on a REICHART-THERMOVAR hot-stage apparatus and are uncorrected. IR spectra (Nujol) were determined with a Shimadzu FTIR-8300 instrument; ^{1}H NMR spectra were recorded on a BRUKER AC 250 E spectrometer, and GC/MS determinations were carried out on a VARIAN STAR 3400 CX/SATURN 2000 system. Flash chromatography was performed by using silica gel (Merck, 0.040–0.063 mm) and mixtures of ethyl acetate and light petroleum (fraction boiling in the range 40–60 °C) in various ratios. Compound **7** was prepared as reported.[17]

5.3. Reaction of 2-amino-6-methyl-4-trifluoromethyl-pyrimidine 2b with *m*-CPBA

A mixture of 2-amino-6-methyl-4-trifluoromethyl-pyrimidine[14] **2b** (0.18 g, 1 mmol) and *m*-CPBA (0.2 g, 1.15 mmol) in chloroform (5 ml) was stirred at rt for 24 h. The solution was evaporated under reduced pressure and the residue chromatographed giving 2-amino-6-methyl-4-trifluoromethyl-pyrimidine-1-oxide **1b** (0.17 g, 88%). Compound **1b** had mp 161–162 °C (white crystals from EtOH); [Found: C, 37.30; H, 3.20; N, 21.80. $C_6H_6F_3N_3O$ requires C, 37.31; H, 3.13; N, 21.76%]; ν_{max} (Nujol) 3439, 3294, 3234, 1666, 1635 cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6) δ 2.47 (3H, s, CH_3), 7.33 (1H, s, ArH), 8.17 (2H, exch.with D_2O, s, NH_2); GC-MS *m/z* 194 (M+1, 75), 193 (M^+, 100), 177 (8), 176 (12), 149 (19), 82 (19), 69 (8), 43 (18); HRMS (M+1) Found 194,0559. $C_6H_6F_3N_3O$ requires 193.0463.

5.4. Reaction of 2-amino-6-methyl-4-trifluoromethyl-pyrimidine 2b with AcOH and H_2O_2

A solution of 2-amino-6-methyl-4-trifluoromethyl-pyrimidine **2b** (0.18 g, 1 mmol) in acetic acid (2 ml)

was treated with H_2O_2 (30%; 1 ml, in two portions) and stirred at 50–60 °C for 5 h. The mixture was diluted with water. After evaporation under vacuum to remove the solvent and acetic acid, crystallization of the residue gave **1b** (0.09 g, 47%).

5.5. Hydrogenation of 2-amino-6-methyl-4-trifluoromethyl-pyrimidine-1-oxide (1b)

Hydrogenation of compound **1b** (0.19 g, 1 mmol) was carried out in the Parr apparatus (40 psi) for 1 h in EtOH (50 ml), in the presence of palladium-on-charcoal (10%; 0.1 g). The solution was filtered and evaporated under reduced pressure. The residue was crystallized from water giving 2-amino-6-methyl-4-trifluoromethyl-pyrimidine **2b** (0.17 g, 95%) mp 128–129 °C (from water) (lit.[14] 128 °C).

5.6. Reaction of 3-Amino-5-methyl-1,2,4-oxadiazole 7 with fluorinated diketones 8a,c and perchloric acid in acetonitrile. General procedure

To a mixture of 3-amino-5-methyl-1,2,4-oxadiazole **7** (0.5 g, 5 mmol) and the appropriate fluorinated diketone **8a** (1.04 g, 5 mmol) or **8c** (0.77 g, 5 mmol) in acetonitrile (3 ml), perchloric acid (70%, 2 ml) was added. After 24 h at rt, the mixture was diluted with water, neutralized with solid $NaHCO_3$ and then extracted with EtOAc (3×200 ml). The combined organic layers were dried over Na_2SO_4 and evaporated.

5.6.1. Reaction of 3-amino-5-methyl-1,2,4-oxadiazole 7 with 1,1,1,5,5,5-hexafluoro-2,4-pentandione 8a. Chromatography of the residue returned 3-amino-5-methyl-1,2,4-oxadiazole **7** (0.19 g, 38%) and gave: 2-amino-4,6-bis(trifluoromethyl)-pyrimidine-1-oxide **1a** (0.26 g, 21%), 2-(hydroxyamino)-4,6-bis(trifluoromethyl)-pyrimidine **14a** (0.48 g, 39%), *O*-Acetyl-2-(hydroxyamino)-4,6-bis(trifluoromethyl)-pyrimidine **13a** (0.03 g, 2%).

Compound **1a** had mp 137–138 °C (white crystals, from EtOH); [Found: C, 29.00; H, 1.10; N, 16.90. $C_8H_5F_6N_3O$ require C, 29.16; H, 1.22; N, 17.01%]. ν_{max} (Nujol) 3425, 3325, 3132, 1666, 1651 cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6) δ 7.54 (1H, s, ArH), 8.70 (2H, exch. with D_2O, s, NH_2); GC/MS *m/z* 248 (M+1), 247 (58), 231 (5), 69 (10).

Compound **14a** had mp 97–98 °C (yellowish crystals, from EtOH); [Found: C, 29.10; H, 1.20; N, 17.00. $C_6H_5F_6N_3O$ requires C, 29.16; H, 1.22; N, 17.01%], ν_{max} (Nujol) 3328, 3280, 1612, 1595 cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6) δ 7.52 (1H, s, ArH), 9.52 (1H, exch. with D_2O, s, NH), 11.02 (1H, exch. With D_2O, s, OH); GC/MS *m/z* 247 (M^+, 100), 231 (24), 228 (16), 69 (67).

Compound **13a** had mp 89–90 °C (white crystals, from EtOH); [Found: C, 33.10; H, 1.70; N, 14.40. $C_8H_5F_6N_3O_2$ requires C, 33.23; H, 1.74; N, 14.53%]; ν_{max} (Nujol) 3244, 1799 cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6) δ 2.29 (3H, s, $COCH_3$), 7.85 (1H, s, ArH), 12.26 (1H, exch. with D_2O, s, NH); GC/MS *m/z* 290 (M+1, 28), 248 (4), 70 (5), 43 (100).

5.6.2. Reaction of 3-Amino-5-methyl-1,2,4-oxadiazole 7 with 1,1,1-trifluoro-2,4-pentandione 8a. Chromatography of the residue returned 3-amino-5-methyl-1,2,4-oxadiazole **7** (0.15 g, 30%) and gave: 2-amino-4-methyl-6-trifluoromethyl-pyrimidine-1-oxide **1c** (0.48 g, 50%), 2-(hydroxyamino)-6-methyl-4-trifluoromethyl-pyrimidine **14c** (0.12 g, 12%) and *O*-Acetyl-2-(hydroxlamino)-6-methyl-4-trifluoromethyl-pyrimidine **13c** (0.06 g, 5%).

Compound **1c** had mp 214–215 °C (white crystals, from EtOH); [Found: C, 37.20; H, 3.10; N, 21.70. $C_6H_6F_3N_3O$ requires C, 37.31; H, 3.13; N, 21.76%]; ν_{max} (Nujol) 3366, 3298, 3238, 1651, 1632 cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6) δ 2.39 (3H, s, CH_3), 7.13 (1H, s, ArH), 8.06 (2H, exch. with D_2O, s, NH_2); GC/MS *m/z* 193 (M^+, 100), 177 (25), 130 (15), 69 (37), 43 (51); HRMS (M+1) found 194.0537. $C_6H_6F_3N_3O$ 1requires 93.0463,

Compound **14c** had mp 135–137 °C (white crystals, from EtOH); [Found: C, 37.20; H, 3.20; N, 21.70. $C_6H_6F_3N_3O$ requires C, 37.31; H, 3.13; N, 21.76%]; ν_{max} (Nujol) 3298, 3229, 1601 cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6) δ 2.46 (3H, s, CH_3), 7.07 (1H, s, ArH), 9.00 (1H, exch. with D_2O, s, NH), 10.09 (1H, exch. with D_2O, s, OH); GC/MS *m/z* 194 (M^++1, 100), 193 (M^+, 70), 177 (22), 174 (8), 163 (64), 143 (19), 113 (19), 69 (19), 43 (32); HRMS (M+1) found 194.0528. $C_6H_6F_3N_3O$ requires 193.0463.

Compound **13c** had mp 82–83 °C (white crystals, from EtOH); [Found: C, 40.80; H, 3.40; N, 17.90. $C_8H_8F_3N_3O_2$: C, 40.86; H, 3.43; N, 17.87%]; ν_{max} (Nujol) 3186, 1788 cm^{-1}; ^1H NMR (259 MHz, DMSO-d_6) δ 2.46 (3H, s, $COCH_3$), 2.53 (3H, s, CH_3), 7.36 (1H, s, ArH), 11.37 (1H, exch. with D_2O, s, NH); GC/MS *m/z* 236 (M+1, 100), 193 (11), 162 (6), 44 (24).

5.7. Synthesis of 2-chloro-4,6-bistrifluoromethyl-pyrimidine 15a

A mixture of 4,6-bis(trifluoromethyl)-1H-pyrimidin-2-one[18](1.5 g, 6.4 mmol) and $POCl_3$ (4.38 g, 3 ml) was refluxed for 4 h. The mixture was then neutralized with NaOH 8 M and extracted with Et_2O (400 ml). The organic layer was dried over Na_2SO_4 and evaporated at rt Distillation of the residue gave 2-chloro-4,6-bis(trifluoromethyl)-pyrimidine **15a** (0.7 g, 44%) as a colourless oil; [Found: C, 28.70; H, 0.40; N, 11.10. $C_6HClF_6N_2$: C, 28.80; H, 0.40; N, 11.20%]; ^1H NMR (259 MHz, $CDCl_3$) δ 7.51 (1H, s, ArH); MS *m/z* 252 (M+2, 34), 250 (M, 100), 227 (86).

5.8. Synthesis of 2-chloro-6-methyl-4-trifluoromethyl-pyrimidine 15c[19]

A sample of 2-amino-6-methyl-4-trifluoromethyl-pyrimidine **2b**[14] (1.8 g, 10 mmol) was dissolved under good stirring in concentrated hydrochloric acid (5 ml) at 0 °C to give an homogenous solution. Sodium nitrite (1.03 g, 15 mmol) was then added during 30 min at 0 °C. After gas evolution occurred, the solution was stirred for additional 3 h. The solution was then neutralized with NaOH 8 M and extracted with ethyl acetate (3×200 ml). The combined organic layers were dried over Na_2SO_4 and evaporated.

Chromatography of the residue gave 2-chloro-6-methyl-4-trifluoromethyl-pyrimidine **15c**[19] (0.65 g, 33%) as a colourless oil; [Found: C, 36.70; H, 2.10; N, 14.30. $C_6H_4ClF_3N_2$: C, 36.66; H, 2.05; N, 14.25.] ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.48 (3H, s, CH_3), 7.34 (1H, s, Ar); MS *m/z* 198 (M+2, 34), 196 (M, 100), 69 (42).

5.9. Synthesis of fluorinated 2-(hydroxyamino)-pyrimidines 14a,c. General procedure

2-chloro-pyrimidines **15a** (0.25 g, 1 mmol) or **15c** (0.2 g, 1 mmol) were dissolved in Et_2O (5 ml). A filtered solution of hydroxylamine hydrochloride (0.21 g, 3 mmol) and K_2CO_3 (0.42 g, 3 mmol) in Et_2O (5 ml) was then added dropwise and kept under good stirring at rt for 3 h. The solution was then evaporated.

5.9.1. Reaction of 2-chloro-4,6-bis(trifluoromethyl)-pyrimidine 15a with hydroxylamine. Chromatography of the residue gave 2-(hydroxyamino)-4,6-bis(trifluoromethyl)-pyrimidine **14a** (0.07 g, 28%).

5.9.2. Reaction of 2-chloro-6-methyl-4-trifluoromethyl-pyrimidine 15c with hydroxylamine. Chromatography of the residue gave 2-(hydroxyamino)-6-methyl-4-trifluoromethyl-pyrimidine **14c** (0.04 g, 22%).

5.10. Hydrolysis of *O*-Acetyl-2-(hydroxyamino)-pyrimidines 13a,c

To a solution of *O*-Acetyl-2-(hydroxyamino)-pyrimidines **13a** (0.29 g, 1 mmol) or **13c** (0.24 g, 1 mmol) in ethanol (10 mL), hydrochloric acid (0.5 mL) was added and the mixture was refluxed for 8 h. After removal of the solvent, the residue was treated with water, neutralized with solid $NaHCO_3$ and extracted with EtOAc. The combined organic layers were dried over Na_2SO_4 and evaporated. Crystallization of the residue from ethanol gave respectively: 2-(hydroxyamino)-4,6-bis(trifluoromethyl)-pyrimidine **14a** (0.28 g, 98%) or 2-(hydroxyamino)-6-methyl-4-trifluoromethyl-pyrimidine **14c** (0.18 g, 93%).

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Isolation, synthesis and photochemical properties of almazolone, a new indole alkaloid from a red alga of Senegal

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Abstract—An indole alkaloid bearing an oxazolone ring, christened almazolone, has been isolated from a new collection of *Haraldiophyllum* sp. from Dakar (Senegal), as an 88:12 mixture of (*Z*)/(*E*) stereoisomers. The relative ratio could be modified under controlled photochemical and thermal processes. The product (*Z*)-3-indolyl-2-(phenyl-propionylamino)-acrylic acid obtained by oxazolone ring opening has also been observed. Its formation has been confirmed by alkaline hydrolysis of (*Z*)-almazolone available from synthesis, where condensation of indole-3-carboxaldehyde with (3-phenylpropionylamino)-acetic acid represents the key step.

1. Introduction

Some years ago, we reported new alkaloids, such as almazole A–D (**1**–**4**), isolated from a sample of *Haraldiophyllum* sp. collected at low tide at Almadies, north of Dakar (Senegal).[1–3] The structural feature of these compounds is the presence of a rare 2,5-disubstituted oxazole linking indole and *N*,*N*-dimethylphenylalanine moieties (Fig. 1). In particular, almazole C (**3**) was obtained by a biomimetic synthesis which allowed us to assign the absolute configuration.[2] More recently, **1** and **2** have also been prepared via photolysis of *N*-acyl isoxazol-5(2*H*)one.[4]

From a new batch of the same seaweed, collected with the purpose of further investigating the biological properties and the mechanisms whereby such metabolites are biosynthesized, we were quite surprised to realize that almazoles were not present at all in the organic extract of this alga but that a new indole alkaloid, named almazolone (**5**) was present instead. Even if it lacks the characteristic 2,5-disubstituted oxazole and *N*,*N*-dimethylamino moieties of almazoles, its structure is strongly reminiscent of some of the biogenetic features of almazoles.

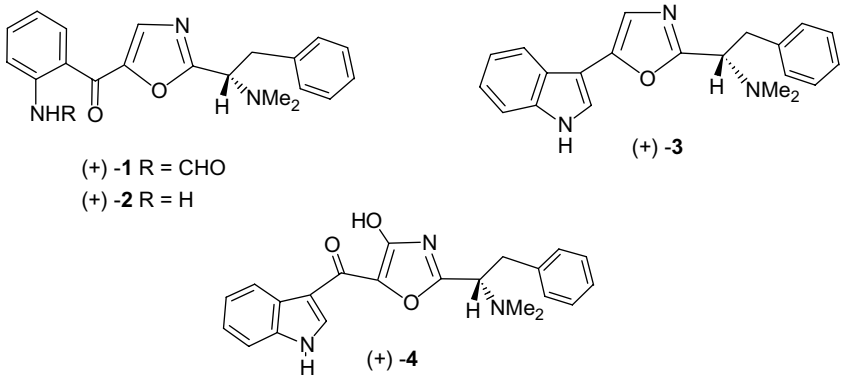

Figure 1. Almazole A–D (**1**–**4**) previously isolated from *Haraldiophyllum* sp.

In our hands almazolone **5** has proved to suffer from both photochemical and thermal processes causing isomerization of the C-8═C-4′ double bond. Besides the structural elucidation of **5**, we report here the results of such photochemical and thermal behaviour. Moreover, a valuable adaptation of already described synthetic methodologies has

Keywords: Marine metabolite; Structure determination; Photochemical isomerization; Alkaloid synthesis; 5(4*H*)-Oxazolone.
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Table 1. NMR data (in $CDCl_3$, 400 MHz, δ in ppm, J in Hz) for almazolone (*Z*)-**5** and (*E*)-**5**

Atom	(*Z*)-**5**		HMBC	(*E*)-**5**	
	δ_H[a]	δ_C		δ_H[a]	δ_C
2	8.45 [8.00](d, 2.8)	132.13(d)	C-7a; C-3; C-3a	9.12[9.11](d 2.8)	132.35(d)
3	—	112.70(s)	—	—	111.9(s)
3a	—	126.94(s)	—	—	127.86(s)
4	7.98 [7.69](m)	119.23(d)	C-7a; C-3; C-6	7.82[7.42] (m)	118.14(d)
5	7.20–7.35(m)	122.05(d)	—	7.20–7.35[7.11](m)	122.05(d)
6	7.20–7.35(m)	123.70(d)	—	7.20–7.35[7.13](m)	123.70(d)
7	7.40 [6.86](m)	111.70(d)	C-7a; C-5; C-3a	7.35[6.86](m)	111.70(d)
7a	—	135.93(s)	—	—	135.47(s)
8	7.61[7.70](s)	125.02(d)	C-2; C-5′; C-3a	7.90[7.97](s)	130.07(d)
2′	—	165.80(s)	—	—	162.21(s)
4′	—	—	—	—	—
5′	—	168.00(s)	—	—	166.12(s)
1″	2.97[2.58](t, 7.6)	31.15(t)	C-3″; C-2′	2.94 [2.58](t, 7.6)	30.88(t)
2″	3.15[2.90](t, 7.6)	31.53(t)	C-2′; C-3″; C-4″	3.12 [2.87](t, 7.6)	31.66(t)
3″	—	140.00(s)	—	—	139.90(s)
4″, 8″	7.20–7.35[7.10](m)	128.66(d)	—	7.20–7.35[7.10](m)	128.66(d)
5″, 7″	7.20–7.35 [7.00](m)	128.35(d)	—	7.20–7.35[7.00](m)	128.30(d)
6″	7.20–7.35 [7.02](m)	126.56(d)	—	7.20–7.35[7.02](m)	126.56(d)
NH	8.80 [6.85](br s)	—	—	8.85[6.88](br s)	—

[a] Values in C_6D_6 reported inside bracket.

been here applied to the highly diastereoselective total synthesis of almazolone.

2. Results and discussion

2.1. Isolation and structure elucidation

The AcOEt extract from the methanolic residue of the alga collected at Almadies, north Dakar, was purified by liquid chromatography (Experimental), to give almazolone (**5**). The composition $C_{20}H_{16}N_2O_2$ was deduced from HR-EIMS data, while NMR data (Table 1) revealed a 3-substituted indole nucleus and a monosubstituted phenyl group. A NMR spectral feature of **5** was the doubling of both proton and carbon signals which could be attributed, with the help of chromatographic analysis, to the presence of two equilibrating (*Z*)/(*E*) stereoisomers in 88:12 ratio (Fig. 2). Actually, this ratio was noted to change depending on the experimental conditions used before the preparation of the NMR samples.

Homo- and heteronuclear correlations of H-8 in the major stereoisomer (sharp singlet at δ_H 7.61 coupled to doublet at δ_C 125.02 ppm) proved essential to establish not only its relative position with respect to indole via its ^3J coupling with C-2 and C-3a, but also the position of the carbonyl group of the oxazolone moiety (δ_C = 168 ppm) via ^3J coupling of the latter with H-8 itself. The same pivotal role was proved to be played, on the opposite side of the molecule, by carbon C-2′(δ_C = 165.80). In fact, its couplings with both benzylic protons 2H-2″ (δ_H = 3.15 t coupled to δ_C = 31.53 ppm) and with homo-benzylic protons 2H-1″ (δ_H = 2.97 t coupled to δ_C = 31.15 ppm) allowed us to define the presence of a –CH_2–CH_2–Ph moiety linked to the oxazolone ring. For the minor isomer the NMR data and correlations from HMBC, HSQC, ^{1}H, ^{1}H-COSY experiments lead to the same conclusions (Table 1).

The stereochemistry at the C-8=C-4′ double bond of compound **5** has been established on the basis of a larger H-8/C-5′ ^{1}H, ^{13}C heteronuclear coupling constant[5] in the (*E*) than in the (*Z*) stereoisomer. Thus, the major isomer shows a H-8/C-5′ coupling constant of 4.9 Hz compatible with the (*Z*) configuration, while the minor isomer shows a coupling constant of 11.1 Hz implying the (*E*) configuration.

Although sharing common biogenetic features with almazole A–D (**1**–**4**), almazolone (**5**) is structurally different. Since almazoles are lacking from this last collection, we can suggest that all these metabolites, including almazolone (**5**), could be synthesized by microorganisms associated with this alga. This hypothesis will be verified by biological and chemical studies on several collections of this alga in our forthcoming investigations.

2.2. Photochemical and thermal conversion

We observed that an initial solution containing the (*Z*)-**5**/(*E*)-**5** isomers in the 88:12 ratio underwent a fast photoisomerization under UV irradiation (350 nm) to give a mixture poorer in the (*Z*) stereoisomer (3:7 after

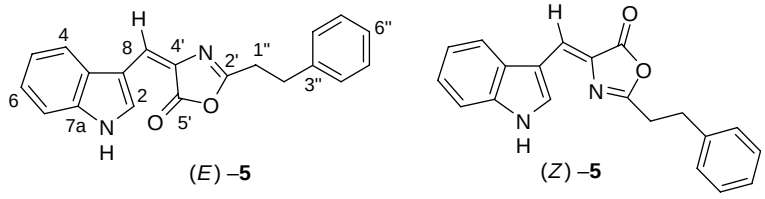


Figure 2. Structure of almazolone (**5**), isolated as a 88:12 mixture of *Z*/*E* isomers.

irradiation for 2 h). The same final ratio was obtained from a solution containing the (*Z*)-**5**/(*E*)-**5** isomers in a different initial ratio (55:45), indicating that the 3:7 ratio should represent the equilibrium position under our photochemical conditions.

On the other hand, we also noticed that in the dark the latter mixture underwent a slow thermal conversion of (*E*) into (*Z*) stereoisomer. When a DMSO-d_6 solution of 28:72 *Z*/*E* isomers was heated at 100 °C for 1 h, the ratio changed to 4:6, while a 55:45 (*Z*)/(*E*) mixture remained practically unchanged when subjected to the same thermal treatment.

All such processes were monitored by ^{1}H NMR analysis following H-2 and H-8 signals in C_6D_6 (Table 1), where they appeared better separated. However, a similar behaviour has been observed in other organic solvents, such as chloroform, benzene, DMSO and 2-propanol.

Actually, when such processes were carried out in DMSO, things became more complicated. In fact, after lengthy storage at room temperature, we noticed the additional appearance of new NMR signals, in particular of two downfield singlets at $\delta_H = 11.56$ and 11.44 ppm, not attributable to any protons of stereoisomeric mixture of almazolone. As will become clearer below, such signals could be attributed to the presence of a new compound deriving from hydrolysis of **5**.

Even though the stereoisomers could be easily separated by CN-HPLC with hexane/*i*-PrOH 90:10 (Experimental), we have never been able to obtain ^{1}H NMR spectra of each single stereoisomer, but only of an equilibrium mixture of *Z*/*E*-**5**. This didn't allow us to obtain quantitative spectrophotometric measurements (i.e., molar extinction coefficients) of single pure stereoisomers. Thus, we can only speculate that the value for the extinction coefficient at 350 nm of (*E*)-**5** would be significantly higher than that of (*Z*)-**5**, as expected for a more extensive transformation of the isomer with stronger light absorption.[5c]

Molecular mechanics calculations (MM2), indicated that the (*Z*)-stereoisomer is just slightly more stable than (*E*), in fair agreement with our experimental observations according to which, after long thermal equilibration, such a mixture showed a similar contribution of the two stereoisomers.

2.3. Synthesis

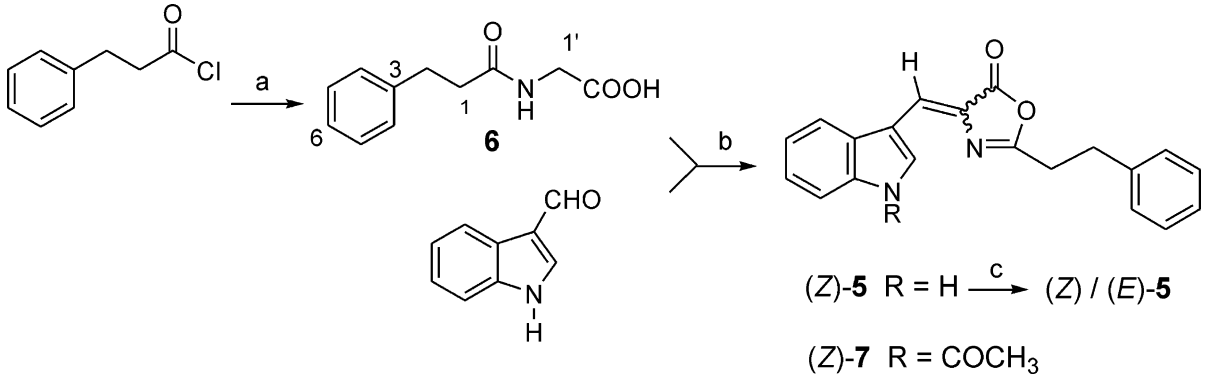
In principle, almazolone (**5**) can be synthesized by the Erlenmeyer condensation of 4-arylidene(alkylidene)-5(4*H*)-oxazolones (azlactone).[6] Although many examples are reported for aromatic aldehydes, the presence of deactivating groups as for indole-3-carboxaldehyde, causes low yields,[7] and affords unwanted *N*-acetyl derivatives due to the presence of acetic anhydride in the reaction condition.[7,8]

The reaction of indole-3-carboxaldehyde with (3-phenylpropionylamino)-acetic acid (**6**), previously prepared by treatment of glycine with the commercial 3-phenylpropionyl chloride and NaOH,[9] was carried out in the presence of acetic anhydride and calcium acetate (Scheme 1). The solvent-free conditions, involving for the first time calcium acetate in the synthesis of azlactones, has recently been reported for substituted benzaldehydes, both under microwave irradiation and thermal conditions.[10] Following the thermal procedure (50 °C for one hour), we were able to obtain only incomplete conversion of reagents to almazolone. The product showed (*Z*) configuration by ^{1}H NMR spectrum on the crude product, but gave equilibration to a 70:30 *Z*/*E*-mixture after chromatography on silica gel. Using a longer reaction time, NMR analysis of the crude residue showed, beside (*Z*)-**5** and (*E*)-**5** in 88:12 ratio, a low relative amount of the *N*-acetyl derivative **7**. Incidentally, the latter was obtained as a major product using sodium acetate under the conditions reported by Nishiyama[11] (Experimental).

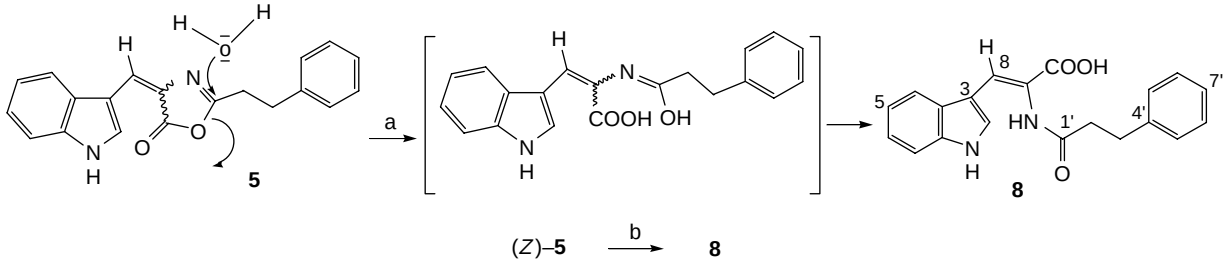
All the spectroscopic data for the synthetic product were in agreement with those obtained for the natural metabolite **5**. Having almazolone available in good amounts by the synthetic methodology described above, we decided to use it for understanding the structure of the degradation product **8** observed during lengthy storage of almazolone in DMSO.

2.4. Hydrolytic behaviour

After standing in DMSO-d_6 for some months, the initial stereoisomeric mixture of *Z*/*E* almazolones was converted in high yield into the ring-opened compound **8**.



Scheme 1. Synthesis of almazolone (**5**). Reagents and conditions: (a) glycine, aq. NaOH, 5 °C, 50 min, aq.HCl; (b) Ac_2O, $Ca(OAc)_2$, 50 °C, for 1 h partial conversion to (*Z*)-**5** (48%), for 3 h, complete conversion to (*Z*)-**5** and (*Z*)-**7**; (c) Si-60 chromatography, *Z*/*E*-**5** in 70:30 ratio.



Scheme 2. Mechanism of opening of almazolone to give compound **8**. Reagents and conditions: (a) on storage for some months in DMSO-d_6 solution with traces of H_2O; (b) aq. Na_2CO_3 solution, reflux, 4 h.

Structure **8** was deduced from MS and NMR data, revealing the composition $C_{20}H_{18}N_2O_3$. NMR signals compared with those of **5** support the presence of a 3-substituted indole nucleus, a $–CH_2–CH_2–Ph$ unit, and the trisubstituted double bond by the singlet at δ_H 7.62 ppm. Additional signals were a singlet at δ_H 9.20 ppm, attributable to the NH–CO group (δ_C 171.3 ppm), whereas the presence of the –COOH group (δ_C = 166.8 ppm) was confirmed by the EI-mass spectra where the base peak derives from the loss of CO_2 (Experimental). The (*Z*) configuration is supported by comparison of NMR data for H-8 (δ_H 7.62 and δ_C 125.5 ppm) with the values obtained for almazolone in DMSO-d_6 (δ_H 7.54 and 7.96 ppm) and in $CDCl_3$ (δ_C 125.02 and 130.07 ppm, for (*Z*)-**5** and (*E*)-**5**, respectively). The configuration of acid **8** was additionally supported by the known alkaline hydrolysis of synthetic (*Z*)-azlactones to afford the corresponding (*Z*)-*N*-acylde-hydroamino acids[11] with retention of configuration,[12] and further confirmed by X-ray crystallographic study.[13]

Such chemical degradation can be explained by assuming the nucleophilic attack of a molecule of water to C-2′ position of **5**, followed by tautomerization (Scheme 2). The origin of the product **8** was confirmed by treating the synthetic (*Z*)-**5** product under alkaline hydrolysis with aqueous Na_2CO_3[11] whereby the same product **8** was again obtained.

3. Conclusion

The novel indole alkaloid bearing a 5(4*H*)-oxazolone unit, almazolone (**5**), was isolated from the Senegalese alga *Haraldiophyllum* sp. and synthesized by condensation of indole-3-carboxaldehyde with the *N*-acyl glycine **6**. The isolated mixture of *Z*/*E* stereoisomers was investigated for its photochemical isomerization and successive thermal re-equilibration, and the double bond configuration was assigned on the basis of a larger H-8/C-5′ heteronuclear coupling constant in the (*E*) than in the (*Z*) stereoisomer. The (*Z*)-isomer of almazolone was subjected to alkaline hydrolysis to give the acid **8** via opening of the oxazolone ring, confirming the origin and the structure of the product observed after long standing of the natural almazolone in a DMSO-d_6 solution containing trace of water.

4. Experimental

4.1. General

All evaporations were carried out at rt at reduced pressure. Calcium acetate and sodium acetate were dried overnight by staying in vacuo in the presence of P_2O_5 before the use. All the other reagents were used without purification. Flash-chromatography (FC) was carried out on Merck Si-60 (15–25 μm), or on reversed-phased Merck LiChroprep RP-18 (15–25 μm).TLC on Merck Kieselgel 60 PF_{254} and Merck RP-18 F_{254}. HPLC separation on 25×1 cm column packed with Merck Lichrosorb CN (7 μm) under UV monitoring at λ 220 nm and solvent flow 5 ml min^{-1}, 7 ml min^{-1} after 9 min. Mp: Kofler hot-stage microscope. UV spectra (λ_{max} in nm) were taken with a Perkin-Elmer Lambda-3 spectrophotometer. NMR spectra were taken with an Avance 400 Bruker spectrometer; ^1H at 400 MHz and ^{13}C at 100 MHz in $CDCl_3$, δ values in ppm rel. to $SiMe_4$ (=0 ppm) in $CDCl_3$ and C_6D_6, and relative to the solvent residual signals in DMSO (δ_H 2.49 ppm), *J* values in Hz. Assignments are from HMBC, HSQC, ^1H, ^1H COSY and NOESY experiments. Electron-impact (EI) mass spectra (*m*/*z*; rel. %) and HR-EI data were taken with a Kratos-MS80 mass spectrometer with home-built computerized acquisition software. ESI-MS data, and tandem fragmentation spectra (MS^n), were taken with a Bruker Esquire-LC™ spectrometer equipped with an electrospray ionization ion source used in negative or positive ion mode as specified, by injection of the sample into the source from a methanolic solution. Molecular mechanics (MM) calculations were carried out by the computer program PCMODEL 7.0, Serena Software, Bloomington, Indiana which uses MMX force field.

4.2. Collection and isolation

The alga collected at low tide in April 1999 at Almadies, north of Dakar, was air dried and then immersed in MeOH for one month, filtered to leave 185 g of dry residue, and concentrated in vacuo. The evaporated residue was added to H_2O (250 ml) and extracted with AcOEt. Evaporation of the organic phase gave a residue (330 mg) that was subjected to RP-18 FC with CH_3CN/H_2O gradient elution from 20:80, on increasing by 5% the CH_3CN content for 16 fractions of 40 ml each. Evaporation of the combined fractions 11–12, furnished a residue (46.5 mg) that was subjected to Si-60 FC with hexane/AcOEt gradient elution, collecting fraction 1 (100:0), fr. 2 (70:30), fr. 3 and 4 (50:50), of 40 ml each. The

combined fractions 1–3 were evaporated and the residue (24 mg) was subjected to CN-HPLC with hexane/*i*-PrOH 90:10, giving (*E*)-**5** (t_R=8 min, 4 mg), (*Z*)-**5** (t_R=12.5 min, 17 mg).

4.2.1. Almazolone (=4-(1*H*-indol-3-ylmethylene)-2-phenethyl-4*H*-oxazol-5-one), 5. Yellow powder. Mp 245–247 °C for a sample of *Z*/*E* isomers=80:20 as evaluated by NMR analysis. λ_{max} (*E*)-**5**: 282; 412 nm; (*Z*)-**5**: 282; 402 nm, (CH_3OH/H_2O 1:1) directly on the eluted fractions from HPLC. NMR data: in Table 1. ESI(−)-MS: 315 ([M-H]$^-$); MS/MS (315): 224. EI-MS (*m*/*z*, %) 317 (15, [M+H]$^+$), 316 (64, M$^{+\cdot}$), 225 (11, [M-CH_2Ph]$^+$), 156 (100), 155 (49), 133 (18), 105 (59, [PhCH_2CH_2]$^+$), 91 (66). HR-EIMS found 316.1211±0.002, calc. for $C_{20}H_{16}N_2O_2$ 316.1208; HR-EIMS found 225.0661±0.004, calc. for $C_{13}H_9N_2O_2$ 225.0664; HRMS found 156.0682±0.001, calc. for $C_{10}H_8N_2$ 156.06875). HR-EIMS found 133.0639±0.002, calc. for C_9H_9O 133.0653.

4.3. Photoisomerization of 5

A 0.03 M solution in C_6D_6 of 12:88 (*E*)/(*Z*) isomeric mixture of almazolone (**5**) irradiated at 350 nm for 2 h in a NMR tube in the dark, gave re-equilibration with values for the (*E*)/(*Z*) ratio of 7:3. The latter mixture gave thermal conversion to 67:33, 54:46 and 37:63 after 24, 48 and 96 h, respectively, at 20 °C. The DMSO-d_6 solution of the same natural isomeric mixture of **5** was irradiated at 350 nm in the dark overnight in a NMR tube, to give a final mixture of 55:45 *Z*/*E* isomers, as deduced by ^{1}H NMR spectra: δ_H 8.40/9.00 ppm (H-2), 8.28/7.98 (H-4), 7.54/7.96 (H-8), 12.20/12.25 (NH) for (*Z*) and (*E*) forms, respectively.

4.4. Synthesis of almazolone

4.4.1. (3-Phenylpropionylamino)-acetic acid (6). Glycine (115 mg, 1.75 mmol) was dissolved in a 4 M aq. NaOH solution (1.15 ml) and phenylpropionyl chloride (0.25 ml, 1.6 mmol) was added in five portions under vigorous stirring cooling at −5 °C over 50 min. The mixture was treated with conc. aq. HCl until pH 1.5, then extracted with AcOEt (3×5 ml). The combined organic phase was dried over anhydrous $MgSO_4$ and evaporated in vacuo, to leave a residue which was purified by RP-FC with H_2O/CH_3CN gradient elution, obtaining pure **6** as white solid (260 mg, 72%).

Data of **6**. Mp 113 °C. ^{1}H NMR ($CDCl_3$) δ_H: 2.57 (t, *J*=7.6 Hz, 2H-1), 2.95 (t, *J*=7.6 Hz, 2H-2), 7.11 and 7.20 (two m, Ph), 4.02 (d, *J*=4.6 Hz, 2H-1′), 6.17 (br s, NH), 10.80 (br s, COOH). ^{13}C NMR ($CDCl_3$, deduced by heterocorrelation experiments) δ_C: 173.64 (CONH), 128.61(C-5, C-7), 128.39 (C-6), 126.32 (C-4, C-8), 140.81 (C-3), 41.75 (C-1′), 36.38 (C-1), 31.21 (C-2). ESI(-)-MS: 206 [M-H]$^-$. MS/MS(206): 162, 74.

4.4.2. Reaction of condensation. Indole-3-carboxaldehyde (47.7 mg, 0.33 mmol), (3-phenylpropionylamino)-acetic **6** (68.2 mg, 0.33 mmol), acetic anhydride (0.09 ml, 0.99 mmol) and dried calcium acetate (33.5 mg) were stirred vigorously at 50 °C for 1 h, then concentrated. The crude mixture contained (*Z*)-**5** and unreacted indole-3-carboxaldehyde as defined by ^{1}H NMR analysis, and when subjected to FC with hexane/AcOEt gradient elution gave almazolone **5** in 70:30 ratio of (*Z*) and (*E*) isomers (49.8 mg, 48%). A complete conversion of indole-3-carboxaldehyde was observed by the reaction for longer reaction time (3 h), but the less polar *N*-acetyl almazolone was also produced. The crude mixture was subjected to FC under the same conditions above, to give *Z*/*E*-**5** (64%) and *Z*/*E*-**7** (30%), each of them approximatively as 60:40 mixtures of isomers. Compound **7** resulted the major product from the reaction with acetic anhydride and sodium acetate at 120 °C for 2 h.

4.4.3. Data for *N*-acetyl almazolone (7). (*Z*)-7 [for (*E*)-7 in bracket, when not superimposable] ^{1}H NMR ($CDCl_3$) δ_H: 8.57 [9.33](s, H-2), 7.78 (m, H-4), 7.43 [7.73] (s, H-8), 7.42 (m, H-7), 7.40–7.20 (m, H-5, H-6, Ph), 3.20 (m, 2H-2″), 2.96 (m, 2H-1″), 2.78 [2.77] (s, CH_3CO). EI-MS (*m*/*z*, %) 359 (11, [M+H]$^+$), 358 (41, M$^{+\cdot}$), 225 (13, [M-PhCH_2]$^+$), 156 (80), 155 (41), 133 (26), 105 (83, [PhCH_2CH_2]$^+$), 91 (100). HR-EIMS found 358.1317±0.002, calc. for $C_{22}H_{18}N_2O_3$ 358.1302.

4.5. Formation of acid 8

4.5.1. Degradation of natural almazolone. Storage in a NMR tube of a 0.036 M solution of **5**, in the dark at 0 °C for some months gave practically pure compound **8**.

4.5.2. (*Z*)-3-(1*H*-Indol-3-yl)-2-(phenyl-propionylamino)-acrylic acid, 8. ^{1}H NMR (DMSO-d_6) δ_H: 7.52 (d, *J*=2.8 Hz, H-2), 7.68 (d, *J*=7.6 Hz, H-4), 7.43 (d, *J*=7.6 Hz, H-7), 7.62 (s, H-8), 2.62 (t, *J*=7.6 Hz, H-2′), 2.88 (t, *J*=7.6 Hz, H-3′), 7.10–7.35 (m, H-5, H-6, H-5′-H-9′), 9.20 (s, NHCO), 11.56 (s, NH). NOE: between 7.43 (H-7) and 11.56 (s, NH). ^{13}C NMR (DMSO-d_6) δ_C: 127.9 (C-2), 109.5 (C-3), 127.2 (C-3a), 117.9 (C-4), 120.6 (C-5), 122.3 (C-6), 112.0 (C-7), 135.9 (C-7a), 125.5 (C-8), 121.6 (C-9), 166.8 (COOH), 171.3 (C-1′), 36.9 (C-2′), 30.7 (C-3′), 141.5 (C-4′), 128.5 (C-5′, C-9′), 128.0 (C-6′, C-8′), 126.4 (C-7′). ESI(+)-MS: 357 ([M+Na]$^+$), 335 ([M+H]$^+$); MS/MS (357): 313 ([M+Na–CO_2]$^+$); MS/MS (335): 291 ([M+H–CO_2]$^+$); ESI(−)-MS: 333 ([M-H]$^-$); MS/MS (333): 289 ([M–H–CO_2]$^-$).

4.5.3. Alkaline hydrolysis of synthetic almazolone. Pure synthetic (*Z*)-**5** (4.8 mg, as more polar isomer from FC) was added to an aqueous 0.2 M Na_2CO_3 solution and refluxed under stirring for 4 h. The resulting solution was acidified with 0.1 M HCl and concentrated in vacuo to give a solid, from which the portion soluble in acetone was recovered and evaporated. ^{1}H NMR in DMSO-d_6 and ESI-MS data were the same as for the acid **8** resulting from degradation of the natural almazolone.

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Exploring unusual antioxidant activity in a benzoic acid derivative: a proposed mechanism for citrinin

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Abstract—A mechanism is proposed for the unusual antioxidant activity in citrinin based on computed O–H bond dissociation enthalpies (BDE). These data suggest that citrinin itself is not the active species, but rather a pair of hydrated Michael addition products consisting of substituted 2,6-dihydroxy benzoic acids. These diastereomers act as radical scavengers via O–H bond dissociation with computed BDE's ranging from 78.9–80.9 kcal/mol for the active groups present. These data represent an unusually facile O–H bond dissociation for a phenol containing a strongly electron withdrawing group. This atypical reactivity arises from an intramolecular network of hydrogen bonds that both stabilize the incipient radical and facilitate extended delocalization through atoms external to the aromatic ring. The additional influence of stereochemistry on BDE is computed to be 2.0 kcal/mol. Data presented are for gas phase molecules, but solvents are unlikely to strongly modify these results since most polar groups are involved in intramolecular hydrogen bonds and thus less available for association with solvent. Citrinin and the Michael addition products are likely too toxic for use as antioxidants in organisms but this study clearly identifies specific reaction sites in the active form, thus guiding rational design of synthetic derivatives with more favorable biocompatibility.

1. Introduction

Antioxidants serve an important role as free radical scavengers in biological systems. In their absence, radicals readily react to modify lipids, proteins, and DNA. This process is amplified by the repeated regeneration of radicals allowing even minor quantities to cause disproportionate damage.[1] The presence of antioxidants alleviates this damage by disrupting the cycle through two distinct mechanisms. The first is hydrogen atom transfer (HAT) in which a weak ArX–H bond in an antioxidant donates a hydrogen to eliminate highly reactive radicals in the following reaction:

$$R\bullet + ArX-H \rightarrow R-H + ArX\bullet \quad (1)$$

The resulting ArX· is much less reactive than a typical radical and effectively stops the regeneration of new radicals. In biological systems phenolic antioxidants (X=oxygen) are most commonly encountered,[2] but X=nitrogen,[3] sulfur[4] and carbon[5] have also been proposed. The second mechanism involves a single electron transfer (SET) from ArXH according to the following reactions:

$$R\bullet + ArXH \rightarrow R^- + ArXH\bullet^+ \quad (2a)$$

$$ARXH\bullet^+ + H_2O \rightarrow ArX\bullet + H_3O^+ \quad (2b)$$

$$R^- + H_3O^+ \rightarrow RH + H_2O \quad (2c)$$

Since the overall result of reaction (2) is identical to (1), clear differentiation of the two processes is challenging. Recently, a theoretical means to differentiate these mechanisms has been proposed.[6,7] According to this method, both bond dissociation enthalpy (BDE) for X–H homolytic cleavage and ionization potential (IP) are computed for a given antioxidant structure and compared to a reference such as phenol. HAT dominates when a compound's IP is no more than 36 kcal/mol below that of phenol and the computed BDE is more than 10 kcal/mol lower than the value for phenol. The SET mechanism is

Keywords: Citrinin; Antioxidant; Mechanism; Bond dissociation enthalpy; Ionization potential; H-atom transfer; Single-electron transfer; Hydrogen bonding.
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predominant in cases where an IP is more than 45 kcal/mol below the phenol reference mark. This approach is relevant since computed BDE's have an accuracy near ±1 kcal/mol using proposed methods.[4b] Calculated IP's are less accurate, but relative IP values, defined here as ΔIP=(IP of test structure−IP of phenol), are of similar accuracy. Application to the major families of antioxidants has demonstrated that the HAT mechanism is dominant.[6]

Hydrogen atom transfer in phenolic antioxidants depends crucially on aromaticity and ring substituents strongly influence this via inductive and spin delocalization effects.[4a,8] Subsequently, substituents that are electron donors such as R, OR, and NR_2 (R=H or alkyl) generally decrease bond dissociation enthalpy and enhance abstraction. Electron withdrawing groups such as COR, COOR, CN, NO_2, CF_3 and SO_2CH_3 increase BDE values and discourage abstraction.[6] Among reference compounds, phenol, with no substituents, has a BDE of ∼88 kcal/mol and is a poor antioxidant while α-tocopherol (Fig. 1) with a BDE of ∼78 kcal/mol is an excellent antioxidant.[9] A variety of both gas phase and solution data are available and it is estimated that the influence of solvent on phenolic O–H BDE is of the magnitude of ∼1.1 kcal/mol,[9] roughly equal to the error in calculated values. However, hydrogen-bonding solvents can have a stronger influence and may differ from gas phase data by as much as ∼6 kcal/mol.[9] Hydrogen bonding typically increases BDE values since cleavage of such bonds involves breaking of both a covalent O–H and a hydrogen bond.[10] Thus, molecules with hydrogen bonding present are usually less efficient antioxidants than those without.

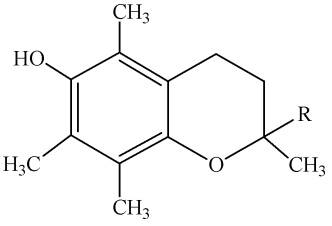
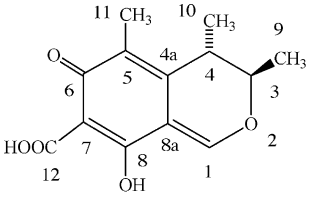
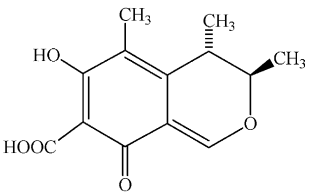


Figure 1. Structure of α-tocopherol (R=$C_{16}H_{33}$) and trolox (R=COOH).

The principles dictating antioxidant activity are therefore considered well understood and rational design of better antioxidants via synthesis appears to be feasible. Recently, however, antioxidant screening of fungal products from our lab identified a product, citrinin, known to exist as *para* tautomer **Ia** and *ortho* tautomer **Ib**,[11] with remarkable activity while containing structural features usually considered fatal to radical scavenging ability. Herein, a mechanism is proposed based on the computational approach.[4b,6] The relevance of the computed results is verified experimentally by measuring activity relative to the water-soluble α-tocopherol derivative trolox (Fig. 1).[12] The unusual activity is proposed to arise from a network of intramolecular hydrogen bonds that strongly stabilize the radical and provide an unusual spin delocalization path. The additional influences of stereochemistry and solvation on activity are also discussed.



Ia



Ib

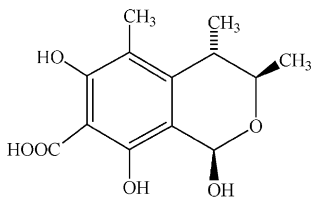
2. Results and discussion

2.1. Isolation and antioxidant activity

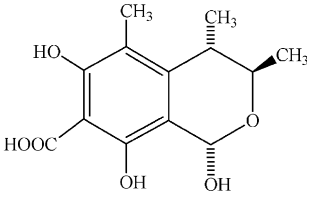
A recent survey of endophytic fungi present in *Ginko biloba* leaves in the Northwest (USA) yielded a bioactive fungus identified as *Penicillium citrinum*. Cultivation of this organism provided an active fraction that gave a single pure compound as described in the Section 4. X-ray crystallography and solution NMR analysis (primarily 2D INADEQUATE) identified the isolated molecule as the *para* tautomer of citrinin, a known product originally isolated in 1931.[13] The NMR analysis provided unambiguous shift assignments that are consistent with those previously reported.[14] Previous work has demonstrated that citrinin has strong antibiotic activity.[15] However, toxicity is also reported,[16] especially toward renal function.[17] Citrinin is also found to inhibit DNA synthesis[16,18] and is carcinogenic.[19] These properties are of particular concern since some fungi that infest grain, dairy and meat products make citrinin.[20] Citrinin is also a contaminant in desirable fungal products such as red fermented rice extract that is used to inhibit cholesterol production.[21] Hence, a present emphasis is the discovery of fungal strains that retain production of useful products while reducing production of citrinin.[22] Despite these undesirable properties, a prior report also indicated 'potent antioxidant' activity in citrinin,[23] thus additional screening was performed to quantify this activity relative to an accepted standard.[24] This analysis demonstrated that citrinin has antioxidant activity 1.24 times greater that trolox. Since aromaticity is usually required for activity, this result is surprising. In order to explain this unusual result, Hamasaki, concluded that 'the carboxylic group may be defined as proton-donor'.[23] However, the relatively large BDE values typically found for H abstractions in COOH groups of 112±3 kcal/mol[25] make this mechanism unlikely. Further mechanistic investigation was thus pursued using the computational approach of DiLabio[4b] and Wright[6] et al.

Two scenarios can be envisioned for the observed activity in citrinin. In the first, the parent structure **Ia** or **Ib** can react by SET or HAT. A second alternative is also available since citrinin is known to undergo Michael addition in aqueous solutions to produce an aromatic hydrate as the primary species present. Two diastereomeric products, **IIa** and **IIb**, are produced by this reaction[14,26] as the only major products and these may be the active species reacting via either SET or HAT. Previous work on citrinin has proposed that both **I** and **II** are present in biological systems with **I** important for

transport across membranes and the hydrate being the primary form present inside the cell and in culture media.[26] However, to date there has been no experimental proof clearly indicating the relevance of a specific form in a controlled test.



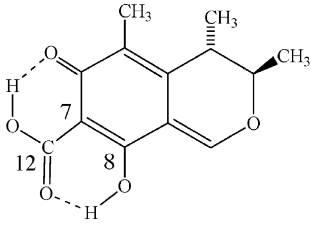
IIa



IIb

2.2. Antioxidant activity predictions in Ia and Ib

The parent structures, **Ia** and **Ib**, were evaluated for SET activity by calculating values for the minimum energy structures. Values of $\Delta IP = -3.1$ and -8.0 kcal/mol were computed for **Ia** and **Ib**, respectively, indicating no reactivity by SET. Conformations evaluated had two intramolecular hydrogen bonds as shown in Figure 2. Four alternate conformations having only one intramolecular hydrogen bond were also evaluated. These energetically less favorable structures have the COOH group rotated 180° together with various OH orientations necessary to create favorable acceptor/donor combinations. These structures have ΔIP values ranging from -13.4 to -18.2 kcal/mol (Table 1). Other orientations of the COOH group were considered energetically improbable since π-conjugation with the aromatic ring would be disrupted. The SET mechanism is thus unfeasible for either tautomer of **I**, regardless of the conformation present.



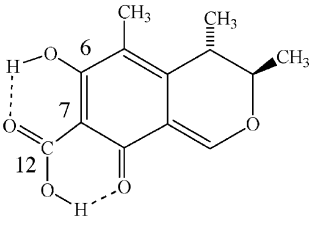


Figure 2. Minimum energy conformations of the citrinin tautomers **Ia** (left) and **Ib**.

Hydrogen abstraction enthalpies in **I** were therefore evaluated at both the COO–H and the O–H groups in separate calculations. The most stable conformation for the parent structure was used and this conformation was retained in the radical as illustrated in Schemes 1 and 2. For the COOH group, BDE values of 110.8 and 111.5 kcal/mol were obtained for **Ia** and **Ib**, respectively, making HAT from the COOH unfeasible. No change in BDE was observed upon 180° rotation about the ·OOC–C7 bond as delocalization of the radical makes both carboxyl oxygens identical. The O–H bonds also exhibited prohibitively high BDE's with values of 112.5 and 108.9 kcal/mol computed for **Ia** and **Ib**, respectively. Hence, it is improbable that either **Ia** or **Ib** are responsible for the observed antioxidant activity.

2.3. Antioxidant activity predictions in the Michael addition product

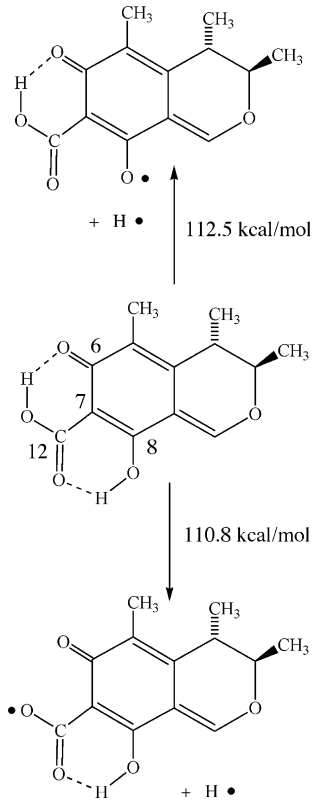
Evaluation of activity in the Michael addition products requires a determination of the minimum energy conformation to ensure accurate results. Accordingly, for both the 1*R* and 1*S* stereoisomers (**IIa** and **IIb**, respectively), 13 structures were evaluated involving all combinations of conformational variations at the COOH and the three OH groups. Structures considered were energy minimized from initial starting points located in approximate local minima and contain as many as three hydrogen bonds and as few as one. The most energetically favorable 1*R* stereoisomer is shown (Fig. 3) and is preferred over the next best structure by 4.0 kcal/mol. The minimum energy 1*S* diastereomer displays the same conformations as the best 1*R* structure and is 2.7 kcal/mol more stable than the next best arrangement. The minimum energy 1*R* structure is 2.1 kcal/mol more stable than the minimum energy 1*S* structure. All ΔIP and BDE values were computed using these minimum energy conformations for both parent and radical.

Single electron transfer was found to be an improbable mechanism for activity in both **IIa** and **IIb** with computed values of $\Delta IP = -18.4$ and -16.2 kcal/mol for the respective isomers. The BDE values were therefore computed for all possible phenolic O–H and COO–H abstractions in both diastereomers. For the 1*S* structure, O–H cleavage was favorable at the C8O–H with a value of 78.9 kcal/mol calculated. In contrast, cleavage of either the COO–H or C6O–H was unfeasible, requiring 92.1 and

Table 1. Ionization potentials relative to phenol (ΔIP) for six energetically probable conformations of the *para* and *ortho* tautomers of citrinin

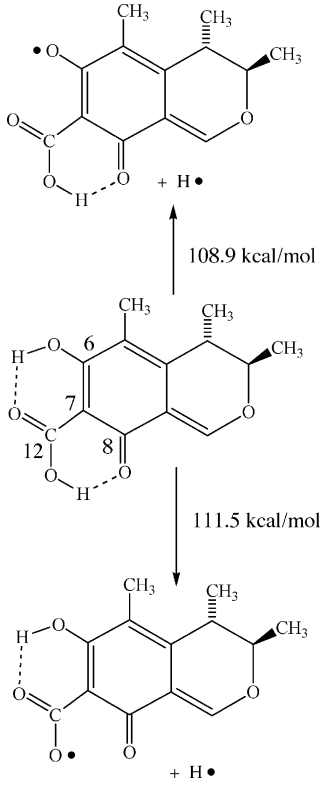
Tautomer	Dihedral angles[a]						ΔIP (Kcal/mol)[b]
	H–O–C6–C7		O–C12–C7–C6		H–O–C8–C7		
	Non radical (°)	Radical (°)	Non radical (°)	Radical (°)	Non radical (°)	Radical (°)	
Ia[c]	—	—	179.9	179.9	0.4	0.5	−3.1
Ia	—	—	15.5	359.8	356.3	0.9	−13.4
Ia	—	—	26.3	0.7	135.9	190.3	−15.1
Ib[c]	0.2	0.3	359.5	359.3	—	—	−8.0
Ib	178.5	180.3	146.4	179.9	—	—	−18.0
Ib	3.2	−4.3	165.9	203.8	—	—	−18.2

[a] Angles are defined such that 0° denotes a structure having the first and last listed atoms eclipsed in a Newman projection placing C6 or C7 toward the front and describing clockwise rotation as positive.
[b] All computed ΔIP values are defined as ΔIP=(IP of test structure−IP of phenol).
[c] Minimum energy structures.

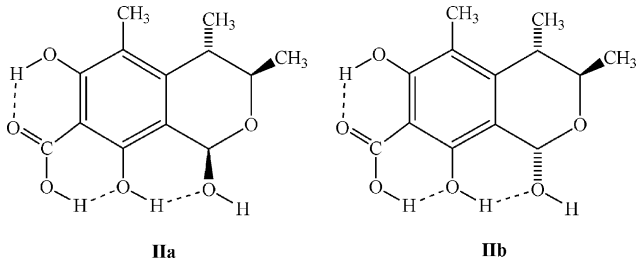


Scheme 1. Computed O–H bond dissociation enthalpy values for **Ia**.

102.2 kcal/mol, respectively (Scheme 3). All radical structures considered were energy minimized after being placed in orientations shown. Treatment of the 1*R* diastereomer gave similar values with a C8O–H BDE of



Scheme 2. Computed O–H bond dissociation enthalpy values for **Ib**.



IIa **IIb**

Figure 3. Minimum energy conformations for the 1*R* (**IIa**) and 1*S* (**IIb**) diastereomeric hydrates of citrinin.

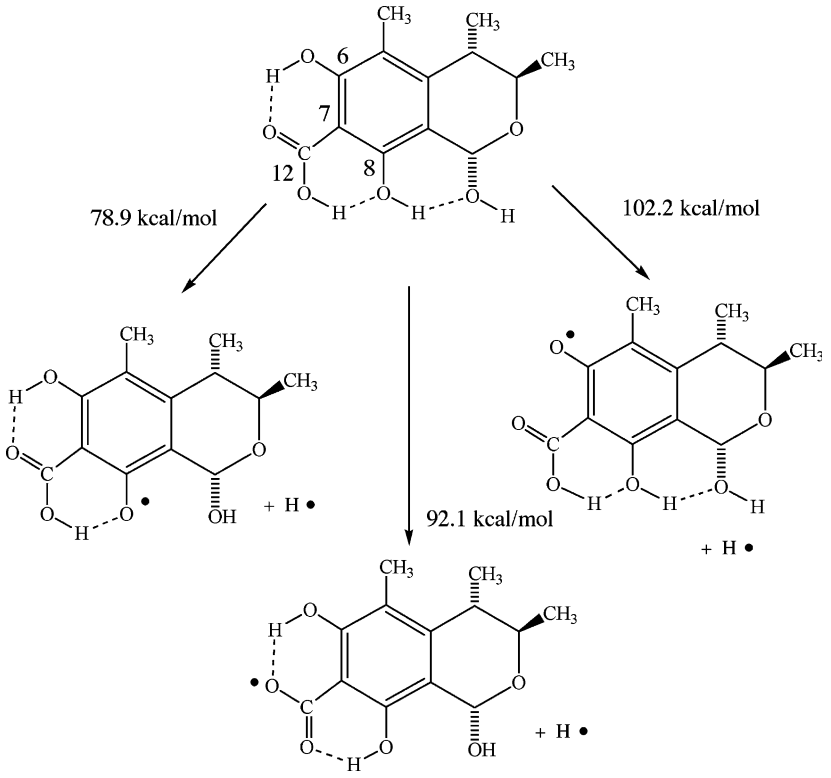
80.9 kcal/mol and COO–H and C6O–H BDE's of 92.8 and 102.5 kcal/mol, respectively (Scheme 4). Based on these data, the antioxidant activity in citrinin appears to arise solely from the Michael addition product as a result of facile C8O–H bond cleavage. However, a significant population of the C6–O· structure is likely present due to a subsequent tautomerization of the C8–O· radical.

The proposed tautomerization between the C8–O· and the C6–O· structures is shown in Scheme 5. Similar hydrogen transfers are known from other work[27] to have a very low energy barrier in cases where the O···O distance is <2.65 Å. In the radical structures considered in Schemes 3 and 4, all relevant O···O distances are <2.60 Å, making such concerted proton transfers energetically favorable. The feasibility of this process is further emphasized by the observation of a closely related process in the parent structure, I.[11,14] In **I**, a concerted intramolecular tautomeric equilibrium is observed to rapidly interconvert **Ia** and **Ib** (Scheme 6), even in the solid state at rt.[14] The relative population of the C6–O· tautomer and it potential contribution to radical scavenging is presently unknown.

Additional resonance stability may potentially arise from radical spin delocalization due to the simultaneous presence of both electron donating and electron withdrawing groups. This so called captodative effect has been demonstrated to enhance radical stability in certain products and may be qualitatively explained by an increase in the number of resonance structures available.[28] Probable contributions from this effect are discussed below.

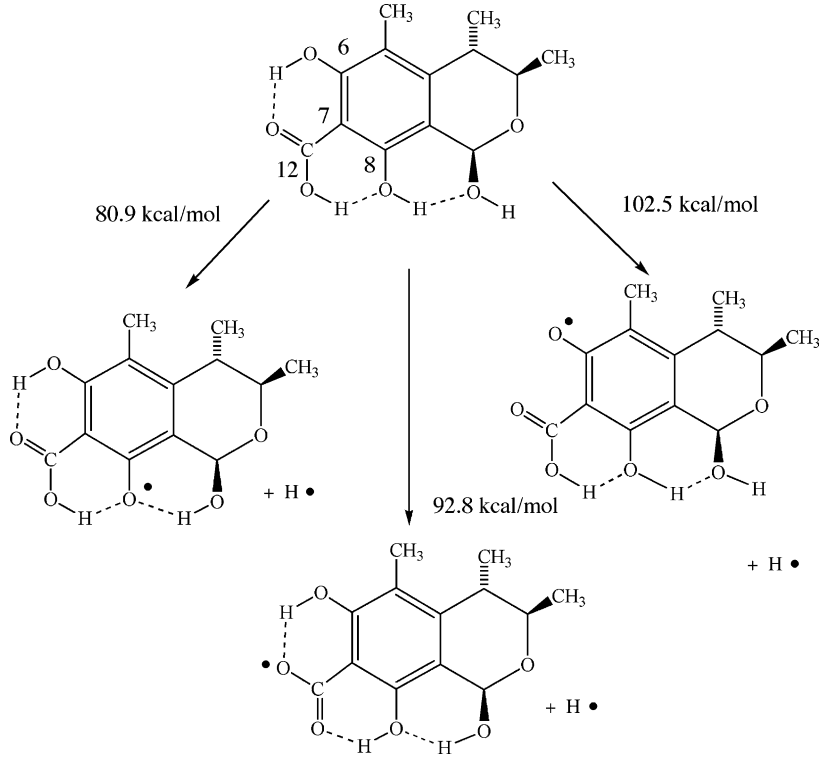
2.4. Evaluating the influences of solvent and hydrogen bonding on activity

Since all results obtained here are for the gas phase, a more complete evaluation of these results must include the probable role of solvent and hydrogen bonding. Assessing the role of solvent in reactivity is critical since it has been established that phenolic OH's involved in intermolecular hydrogen bonds are 'essentially unreactive' to all radicals.[29] However, hydrogens in intramolecular hydrogen bonds remain readily available.[10] This fundamental difference in abstractability appears to arise from two factors. First, hydrogens involved in intramolecular hydrogen bonds make poor donors to hydrogen bond acceptor solvents.[10] Thus, the 'steric protection'[29a] of the phenolic hydrogen by solvent is reduced. Remarkably, this decreased solvent association allows even hydrogens strongly involved in intramolecular hydrogen bonds to react, as illustrated by the activity of

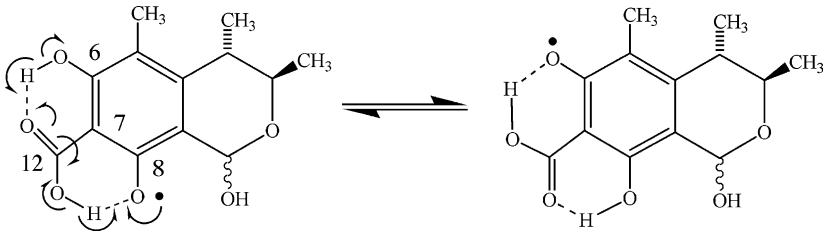


Scheme 3. Computed bond dissociation enthalpy values for all probable abstraction sites in the 1*S* isomer.

2,6-dimethoxy phenol.[10] Nevertheless, reactivity is lower than in comparable non-hydrogen bonded OH's due to a transition state that involves the synchronized rupture of both a covalent O–H bond and a hydrogen bond.[10] Presumably, in the limit of very strong intramolecular hydrogen bonding, abstraction would again become improbable. A second important structural feature in molecules containing multiple OH's is the increase in intramolecular hydrogen bond strength in the radical relative to the same bond in the parent.[30] This factor is important in aromatic



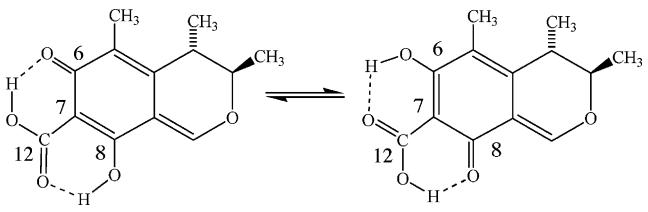
Scheme 4. Computed bond dissociation enthalpy values for all probable abstractions sites in the 1*R* isomer.



Scheme 5. Generation of a C6O radical via a concerted intramolecular tautomerization proposed to occur following abstraction of the C8O–H hydrogen.

diols such as catechol and napthalene diols and allows the free O–H of the pair (i.e., the phenolic hydrogen not acting as the intramolecular donor) to be easily abstracted.[29a,30] Application of these principals to the Michael addition products of citrinin allow for an assessment of the likely role of hydrogen bonding and solvent.

In the non-radical products, **IIa** and **IIb**, both phenolic hydrogens are involved in intramolecular hydrogen bonds leaving them less associated with solvent and thus potentially available for abstraction. However, since any abstraction involves rupture of the intramolecular hydrogen bond as well as the covalent O–H bond,[10] it is necessary to evaluate the strength of hydrogen bonds present. These values may be calculated by rotating each OH hydrogen from its minimum energy position to the closest neighboring minimum in model structures, and noting the enthalpy difference. At C6, this corresponds to rotating the OH hydrogen by 180°. The C6O–H···O=C12 bond is calculated to have a strength of 15.7 kcal/mol in both diastereomers. Although, this value is high for a neutral hydrogen bond, it is consistent with both experimental observation of very strong hydrogen bonds in similar arrangements such the 1-hydroxy-3-keto moiety[31] and computed estimates for 2-hydroxy benzoic acid.[6] Direct computation of the C8O–H···O1 bond strength was not possible since it requires rotation of two groups, that is, the C12O–H and C8O–H, and involves the subsequent formation of a new hydrogen bond between C8O–H··· OC12. An estimate was, therefore, obtained by assessing hydrogen bond strength in a citrinin derivative modified by replacing the COOH with an H. The conformations investigated included one having the C8O–H coplanar with the ring and directed toward O1 and a second with the C8O–H rotated approximately 180°. In these structures, the C8O–H···O1 bond strength was computed to be 5.5 and 4.4 kcal/mol in the 1*R* and 1*S* diastereomers, respectively. These data suggest that abstraction occurs primarily at C8O–H. Cleavage of the C6O–H is less probable due to the high cost of breaking the hydrogen bond. This prediction independently supports our prior conclusion that the C6O–H is unavailable for direct abstraction.



Scheme 6. The tautomeric equilibrium previously observed in **I**.

A comparison of hydrogen bonding strength in the parent versus the radical was performed to see if enhanced bonding in the radical contributes to increased HAT activity at the C8O–H. Evaluation was performed as proposed by Foti et al.[30] and includes evaluations of both the C6OH··· O=C12 and the C12OH···O8 hydrogen bond strengths in separate computations. At both positions, two O–H orientations were considered in both the parent and radical structures. The first orients the O–H hydrogen toward the hydrogen bond acceptor and coplanar with either the aromatic ring (C6OH) or the C=O moiety (C12OH). The second rotates the hydrogens away from the acceptor by 180° to the closest neighboring energy minimum. These computations predict the C12O–H···OC8 bond to be 4.4–5.2 kcal/mol stronger in the 1*R* or 1*S* radical, respectively, than in the parent structure. Surprisingly, the more remote C6O–H···O=C12 bond was also found to be 3.3 kcal/mol stronger in both the 1*S* or 1*R* radical versus the non-radical. Thus, it is the combined effect of hydrogen bonds at C8O and C6O that facilitates O–H bond dissociation. Taken together, the enhanced hydrogen bond strength provides a total stabilization of 7.7–8.5 kcal/mol in the 1*R* and 1*S* radicals, respectively, versus the parent. Similar radical stabilizations of approximately 5–9 kcal/mol were proposed to be important to HAT activity in catechol and napthalene diols.[29a,30]

The results obtained suggest that eliminating certain hydrogen bonds would significantly increase BDE values. These data thus provide an opportunity to evaluate the captodative effect previously described. This effect relies on radical stabilization through generation of additional resonance structures.[28] However, all hydrogen bonding arrangements investigated share a common set of resonance structures for radicals of a given diastereomer. Hence, the captodative effect is anticipated to make relatively minor contributions to the HAT activity in **II**. This conclusion is consistent with the observation that captodative radical stabilizing ability is strongest when both the electron withdrawing and the electron donating groups are directly bonded to the radical center; a condition not met in **II**.[28]

Inspection of structures used to evaluate hydrogen bond strength, suggests that reorientation of C6O–H may result in a steric clash between the OH and the CH_3 at C5. An assessment of the feasibility of this conformation was thus required since such an error could create unreliable computed hydrogen bond strengths. Infrared experimental data is available for 2-methyl phenol[32] and demonstrates

that the steric interaction between the OH and methyl hydrogens is quite small. In this case, the interaction makes less than a 1 kcal/mol contribution to the BDE. Indeed, in solution, nearly 30% of 2-methyl phenol exists with the OH hydrogen oriented directly toward the methyl carbon.[32] More importantly, computations similar to those used here accurately reproduce this result with a computed penalty of 1.06 kcal/mol for orienting the OH hydrogen directly toward the methyl group.[33] In citrinin, we estimate the $OH\cdots CH_3$ interaction to make a 0.65 kcal/mol contribution to the enthalpy based on calculations[30] performed using a 1*R* citrinin molecule modified by the replacing the COOH moiety with an H. Since this steric interaction is present in both radical and parent structures, no corrections to the reported data are required.

2.5. Assessing the influence of stereochemistry on reactivity

The anticipated role of stereochemistry can be understood by recalling that BDE values are primarily a reflection of the energy difference between the parent and the radical produced by hydrogen abstraction. In general, this difference is not required to remain constant for different configurations since diastereomers do not have identical conformational and steric energies. In the present case, computations show that, at the C8 OH, **IIb** is more reactive than **IIa** by 2.0 kcal/mol. No experimental verification of these predictions was obtained due to difficulty in isolating the individual diastereomers as previously noted.[26] Clearly, additional work is needed to establish the general role of stereochemistry in antioxidant activity.

2.6. Evaluating the accuracy of the calculations

Given the complexity of the molecules described above it is important to ask if the stated accuracy of ± 1 kcal/mol in the computations is likely to remain valid. Accurately assessing the error is particularly relevant since the original work provided an estimate for the 'medium level model' (MLM2), utilized here, using only a limited number of small molecules such as ethanol.[4b] Estimating error in larger more complex systems such as substituted phenols is more difficult since the computed data available[6] were obtained using a lower level model (LLM). However, the MLM2 has been found to provide BDE values that are 2.2 times more accurate as those from the LLM.[4b] Thus, the MLM2 error may be estimated to be roughly half that of the LLM. A comparison of 40 LLM computed values from Tables 1 and 3 of Ref. 6 with experimental data[9,34]for substituted phenols gave an error of ± 2.9 kcal/mol. We therefore estimate the MLM2 to have an error of roughly ± 1.5 kcal/mol for larger phenols like those described here. Presently, the only molecules known to contain structural features unsuited to these methods are those with severe steric crowding near the OH such as 2,6-di-*tert*-butyl phenol with an LLM error of 6.29 kcal/mol.[6] It is noteworthy our computed BDE's suggest a reactivity for **II** similar to α-tocopherol and this is experimentally observed via comparison to the closely related molecule trolox. The experimental data therefore support the contention that errors are relatively small.

2.7. Bioactivity in citrinin and rational design of derivatives

The results given here establish for the first time that the Michael addition product is an important species in at least some activities of citrinin. However, the general activity of **II** requires further analysis. As an initial step towards establishing a wider view of activities displayed by citrinin, preliminary fungal, bacterial and insect bioassays were conducted. Strong antifungal activity was observed against *Pythium* and *Scleroderma* using aqueous media likely to contain **II**. Growth was completely inhibited at 2 μg/mL in *Pythium* and 10 μg/mL in *Scleroderma*. Samples of *Rhizoctonia* and *Geotrichum* exhibited slowed growth relative to a control at citrinin at concentrations of 2–10 μg/mL. Antibacterial activity was found against *Staphylococcus aureus* (ATCC 25923) at the 20 and 10 μg levels with zones of inhibition of 1 and 0.5 mm, respectively. No activity was observed against *Escherichia coli*. Citrinin consumption was found to kill tobacco hornworms in 1 day at the 20 μg level using citrinin coated tobacco leaves. Actual hornworm citrinin consumption was significantly less than 20 μg as only a small fraction of the leaf was consumed.

The toxicity reported here, taken together with that previously found[15–19] suggest that citrinin, as produced by the fungus, is unsuitable for use as an antioxidant in organisms. However, this work suggests that the antioxidant activity arises solely from the hydrated Michael addition product and identifies specific reaction sites within the benzoic acid ring. Therefore, synthetic modifications that can reproduce the major features of this ring may simulate the activity found here while reducing or eliminating the toxicity found in **II**. Indeed, the additivity rules of Wright et al.[6] provide strong guidelines for rational design of such derivatives. Our data suggest a major role for the structural segment found at C6, C7, C8, and C12 thus modifications at these positions should retain the hydrogen bonding and spin delocalization features identified here.

3. Conclusions

Work presented supports the conclusion that citrinin acts as a HAT antioxidant only after reaction to form a hydrated Michael addition product. Computed bond dissociation enthalpies for this hydrate suggest that activity arises from the C8 phenolic OH's in both diastereomers with the 1*S* diastereomer predicted to be more active than the 1*R*. The role of intramolecular hydrogen bonding is central to this activity as hydrogen bond strength is significantly enhanced at two positions upon formation of the radical making C8O–H bond cleavage favorable. Thus, activity appears to arise primarily from the presence of the 2,6-dihydroxy benzoic acid moiety. This particular combination of functional groups counteracts the strongly deactivating influence that the COOH group displays when hydrogen bonding is not present. Stereochemical factors are also evaluated here and shown to modify BDE's by 2 kcal/mol. The influence of stereochemistry is thus comparable with many solvent interactions. The relevance of intramolecular hydrogen bonding and stereochemistry on BDE's in more

general cases requires further analysis with a broader range of compounds.

4. Experimental

2D INADEQUATE analysis was performed on a 500 MHz spectrometer using a 5 mm Shigemi microtube susceptibility matched to $CDCl_3$ and designed to position the entire sample within the coil. A sample of 82 mg of citrinin dissolved in $CDCl_3$ was used for analysis. Spectral widths of 25.5 KHz were used in both dimensions together with a pulse delay of 7 s, a ^{13}C 180° pulse of 11.1 s, a temperature of 26 °C and referencing to the central line of $CDCl_3$ at 77.23 ppm. Digital resolutions of 0.32 and 199.3 Hz/point were collected in the acquisition and evolution dimensions, respectively. The acquisition was optimized for analysis of $^1J_{cc}$ values of 55 Hz and data was collected without sample spinning. Analysis of data was performed using the Analyst software package,[35] which established all reported connections at greater than the 99.9% confidence level.

The culture used originated as a quiescent fungus isolated from a *G. biloba* leaf on the University of Portland campus and identified as *P. citrinum* by protocols in Pitt.[36] The fungus is preserved in sterile water at 4 °C in the lab of Hoffmann, University of Portland. The fungus was grown in a solution of potato dextrose broth (24 g/L) containing 6 g/L of sucrose for 2 weeks at 20 °C and 60 rpm shaking. The broth was then filtered and exhaustively extracted with methylene chloride. The extract was dried by rotory evaporation to yield 700–1200 mg of solid. Citrinin was isolated from the crude solid by recrystallization in methanol/water 95:5 (v/v) to yield 140–455 mg of purified product. The resulting yellow crystals decomposed at 172–175 °C. A single crystal grown from $CDCl_3$ gave an X-ray structure identical to that previously reported.[37]

Antioxidant activity was determined using the oxygen radical absorbance capacity (ORAC) assay,[24] employing the high throughput modifications of Huang.[24a] Analyses were performed in 96-well plates, with each well containing 200 μL of fluorescein and 20 μL of citrinin dissolved in DMSO. Citrinin concentrations of 2.28, 0.228 mM, and 22.8 μM were prepared by dilution with 75 mM phosphate buffer (pH 7.4) and evaluated. A sample of 75 μL of the free radical initiator AAPH (2,2′-azobis(2-amidinopropane dihydrochloride) was added to start the reaction (final concentration of 4.8 mM) and fluorescence decay measured every 210 s at excitation and emission wavelengths of 485 and 520 nm, respectively, by a multifluorescent plate reader. To generate a standard curve, a 20 mM solution of the water-soluble α-tocopherol derivative trolox was prepared, then diluted with phosphate buffer into reference solutions of 50, 25, 12.5 and 6.25 μM, which were then analyzed. Final antioxidant activity was expressed relative to the antioxidant activity of trolox in units of 'troxol equivalent antioxidant capacity' (TEAC), with citrinin having a TEAC value of 1.24.

Antifungal activity was evaluated in a 24-well culture cluster plate. Individual wells contained 1 mL of potato dextrose broth plus citrinin dissolved in methanol (2 μg/mL) to create concentrations of 1, 2, 4, 6, 8, 10, 20 μg citrinin/well. Controls were run at 5 and 10 μg/L methanol in 1 mL potato dextrose broth. Plugs of agar (~2 mm diameter) containing live fungus were placed in each well and the plates incubated at 20 °C. Growth was observed at 24, 48, and 72 h and compared to controls.

Activity against tobacco hornworm (*Manduca sexta*) was established using tobacco leaves coated with a 20 μg sample of dissolved citrinin per 1200 mg leaf. Treated leaves were placed on pads of damp cotton in Petri dishes and a weighed tobacco hornworm placed on each leaf. Dishes were place held at 28 °C under constant fluorescent light. Hormworms and leaf consumption were observed daily.

Antibacterial activity was measured against *S. aureus* (ATCC 25923) using the paper disk diffusion method. Samples of 2, 3, 4, 5, 6, 10, and 20 μg of citrinin dissolved in methanol were pipetted onto disks then allowed to air dry. The disks were then placed on a Petri disk containing nutrient agar and covered with a thin layer of bacteria. Methanol (10 μg) and ampicillin (50 μg) were used as positive and negative controls, respectively. Zones of inhibition were measured after incubating at 37 °C for 12–36 h.

Calculation of bond dissociation enthalpies was performed using the medium level model (MLM2) of DiLabio.[4b] The BDE calculations involve computing enthalpy differences for the reaction $ArOH \rightarrow ArO\cdot + H\cdot$ at 298.15 K and 1.0 atmosphere pressure. For the nonlinear molecules studied here, this difference is given by $\Delta H = \Delta E_a + \Delta ZPE + \Delta H_{vib} - 0.49764$ where E_a is the single point electronic energy, ZPE represents the zero point energy and H_{vib} is the vibrational contribution to enthalpy. The -0.49764 term is the exact enthalpy contribution, in hartrees, from $H\cdot$.[6] Molecular geometries and vibrational frequencies for the parent and radical molecules were determined using B3LYP with the 6-31G(d) basis. Factors of 0.9989 and 0.9806 were used to scale the vibrational frequencies and ZPE's, respectively.[4b] All vibrations below 260 cm^{-1} were replaced with the term 1/2(RT) as recommended.[4b] Single point electronic energies were computed at the B3LYP/ 6-311+G(2d,2p) level for ArOH and restricted open-shell (RO) B3LYP/6-311+G(2d,2p) for $ArO\cdot$ radicals. Electronic energies were computed using B3LYP/ 6-31(d) geometry. Full basis sets for all atoms were used in all computations due to uncertainty regarding the influence of a locally dense basis set[38] on the extended hydrogen bonding system present in some structures. Ionization potentials were computed by taking the energy difference between the parent and cationic structures. Geometries and energies were computed at the B3LYP/ 6-31G(d) and B3LYP/6-311+G(2d,2p) levels, respectively. Final reported values are [IP of the model compound − IP of phenol]. All calculations were performed using Gaussian 98 or Gaussian 03 with parallel processing.

Acknowledgements

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enthalpies were provided by the Center for High-Performance Computing at the University of Utah. We acknowledge Dr. Atta M. Arif for collection of X-ray data that allowed for identification of citrinin.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2005.10.066. All structures used to compute reported bond dissociation enthalpies and single electron transfer energies are given as supporting information. Data given include Cartesian coordinates, single point electronic energies, zero-point energies and vibrational enthalpies.

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Revisiting the Corey–Chaykovsky reaction: the solvent effect and the formation of β-hydroxy methylthioethers

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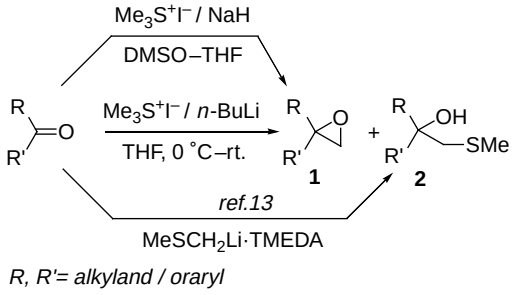
Abstract—The classical Corey–Chaykovsky (CC) reaction of ketones in ethereal solvents (i.e., THF or Et_2O) resulted in the production of a significant amount of β-hydroxy methylthioether **2** along with normal epoxide product **1**. Some interesting and synthetically useful transformations of the CC reaction product of cyclopropyl ketones were also described.

1. Introduction

The classical Corey–Chaykovsky (CC) reaction[1] is a practical and useful transformation (a formal *S*-ylide [1+2] cycloaddition)[2] for the synthesis of epoxides (or cyclopropane derivatives) from the corresponding carbonyl (or activated olefinic) compounds, which is thus widely used in the routine organic synthesis.[3] In connection with an ongoing synthetic program, we recently found that the chemical yields and the distribution of reaction products were quite dependent on the reaction conditions and the type of carbonyl substrates. The CC reactions performed in ethereal solvents (i.e., THF or Et_2O)[3a,4] rather than the traditional DMSO (or a solvent mixture with THF) resulted in the production of a significant amount of β-hydroxy methylthioether **2** along with the normal epoxide product **1** (Scheme 1) in many cases investigated (see examples in Scheme 2 on the next page).[5,6]

β-Hydroxy methylthioethers are useful intermediates or precursors in the preparation of α-hydroxy aldehyde,[7] vinyl[8] or allyl sulfide,[9] epoxide,[10] and olefin.[11] Some direct methods to their syntheses are available in the literatures.[12,13] For example, one such method for the preparation of methylthioethers is carbonyl addition by a TMEDA-complexed (methylthio)methyllithium reagent[13] as shown in Scheme 1. We record herein for the first time the significant solvent effect and the unusual formation of β-hydroxy methylthioether (sulfide) **2** in the classical CC reaction.



Scheme 1. Formation of β-hydroxy methylthioether **2**.

As shown in Table 1, we studied the effects of the solvent and the base of the CC reaction by employing cycloheptanone as a model substrate. Reactions performed in ethereal solvents (i.e., THF or diethyl ether, entries 1 and 2) generally afforded a significant amount of sulfide **2a** along with the normal epoxide product **1a**, while in the original solvent system (DMSO or a solvent mixture with THF (v/v 1:1)) produced solely epoxide **1a** in a good yield (entry 6). There was no observable reaction occurred (entry 3) in nonpolar solvent like toluene, as the sulfonium salt precursor used appeared hardly dissolved in the reaction medium. Interestingly, the base used for the generation of *S*-ylide had a remarkable effect as well. For example, although NaH in THF was incapable of effecting the desired *S*-ylide-transfer reaction at all (entry 5), the use of *tert*-BuOK in THF gave (entry 7) the epoxide product in good yield. Intriguingly, *tert*-BuOK seems more effective than NaH as base for the generation of *S*-ylide . Other organic lithium or sodium bases (entries 8 and 9) were practically equal effective in THF in regard to the product ratio (**1a/2a**). Apparently, the iodide salt of the sulfonium precursor is

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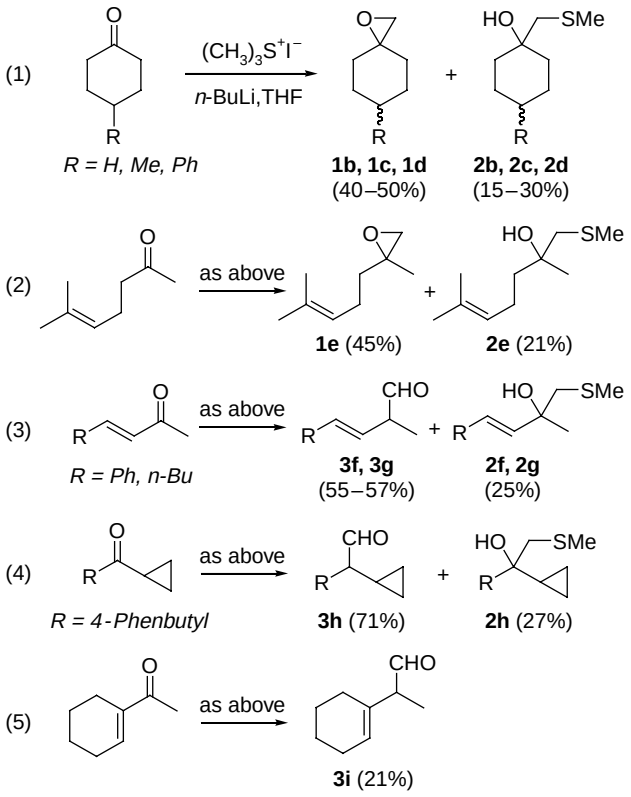


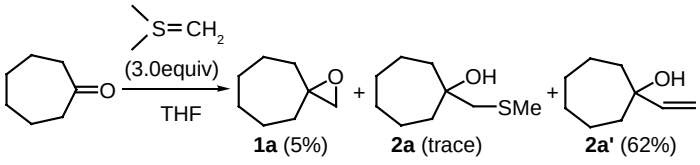
Scheme 2. Formation of sulfide **2** from carbonyl substrates.

more likely to produce a significant amount of sulfide **2a** compared with other counteranion salts (i.e., entries 10[3e] and 11).[14] Although the use of an excess amount of the *S*-ylide in THF suppressed largely the formation of sulfide **2a**, major vinylation product **2a′** was generated along with a small yield of epoxide **1a** (Eq. 1).[15]

We further examined a variety of carbonyl substrates under the *n*-BuLi/THF conditions. As shown in Scheme 2, aliphatic or cyclic alkanones (Eqs. 1 and 2) generally gave a yield of 15–30% for the sulfide product **2**. While cyclopropyl or α,β-unsaturated ketones afforded a significant amount of **2** as well, the corresponding epoxide product **1** initially formed underwent a rapid pinacol-type rearrangement to give an aldehydic product **3** (Eqs. 3 and 4) as the major product during chromatographic purification on silica gel (mild acidic conditions) or by exposing to an activated silica gel (*SiO_2, dried in an oven at 200 °C for 2 h). The CC reaction of hindered α,β-unsaturated ketone (i.e., Eq. 5)[1d] under similar conditions gave a low yield of aldehyde product **3i**.

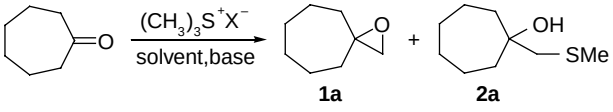
Interestingly, somewhat labile spiro-epoxidation product **5** derived from cyclopropyl ketone **4** was readily converted into the ring-opened product diol **9** or the pinacol-type rearrangement product aldehyde **6**[16] under mild 'aqueous' or 'dry' acidic conditions, respectively, in a good yield, as shown in Scheme 3. The formal regioselective dihydroxylation product **9** from triene **7** can be further transformed into the homoallylic iodo acetate **10** via a stereoselective Julia-type olefination protocol developed recently in our laboratory.[17] Alternatively, the aldehyde product **6** can be prepared from cyclopropyl triene **7** by a highly regioselective bromohydration with NBS in an aqueous THF (to bromohydrin **8**) and the subsequent saponification in methanol in good overall yield.[18] These functionalized acyclic prenylated intermediates (**6**, **9**, and **10**) may be of great value in organic synthesis.

The cyclopropyl sulfide **2h** prepared from the corresponding cyclopropyl ketone can be converted into the halogenated allylsulfide **11** under different mild acidic conditions as shown in Scheme 4. Highly stereoselective production of



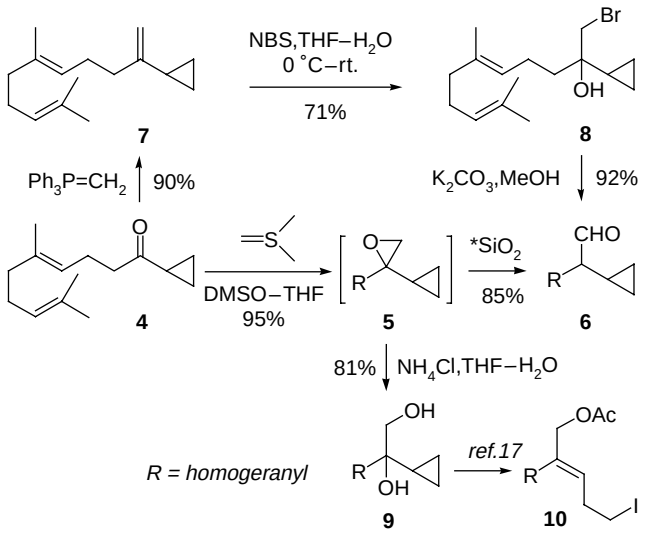
(1)

Table 1. Effects of solvent and base[a]

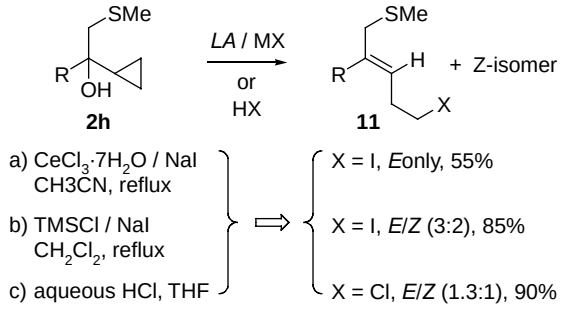


Entry	X	Solvent	Base[b]	**1a** (%)[c]	**2a** (%)[c]
1	I	THF	*n*-BuLi	41	27
2	I	Et_2O	*n*-BuLi	45	29
3	I	Toluene	*n*-BuLi	—	—
4	I	THF–toluene (1/1)	*n*-BuLi	47	25
5	I	THF	NaH	—	—
6	I	DMSO–THF (1/1)	NaH	85	—
7	I	THF	*t*-BuOK	81	—
8	I	THF	LDA	56	25
9	I	THF	$NaN(TMS)_2$	52	21
10	$MeOSO_3$	THF	*n*-BuLi	71	10
11	F_3CSO_3	THF	*n*-BuLi	83	6

[a] Reactions were performed in 1–2 mmol scale.
[b] 1.0 equiv of base was used.
[c] Isolated yield.



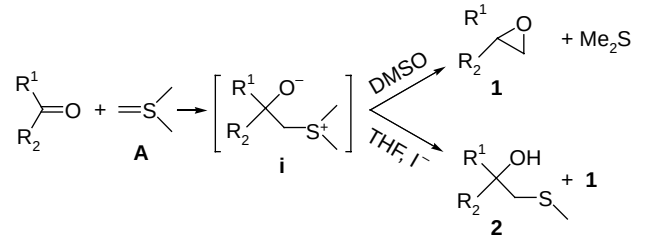
Scheme 3. Homologation of cyclopropyl ketone **4**.



Scheme 4. Synthesis of halogenated allylsulfide.

trisubstituted bifunctional iodo olefin **11** was achieved under the mediation of a mild Lewis acidic reagent system $CeCl_3 \cdot 7H_2O$/NaI as shown.[17]

The distinct solvent effect of THF observed in the classical CC reaction was probably attributed to its strong chelating character for lithium cation (solvation effect) and thus rendered the corresponding iodo anion more nucleophilic to attack effectively the zwitterionic intermediate **i** intermolecularly in a competing pathway leading to the methylthioether **2** in a significant amount (Scheme 5).



Scheme 5.

In short, the methylthioether **2** was identified as a general major side-product in the classical CC reaction when ethereal solvent (i.e., THF or Et_2O) and an organolithium base (i.e., *n*-BuLi) were employed. The presence of DMSO in the reaction medium favored the epoxide formation pathway greatly. Although we were unable to tune the reaction selectively to the formation of the methylthioether **2** favorably over epoxide **1**,[19] this interesting reaction pathway reflected the significant medium effect in nucleophilic reactions in general and the *S*-ylide **A** may be regarded as a synthon of (methylthio)methyl anion equivalent.

2. Experimental

For product purification by flash column chromatography, silica gel (200–300 mesh) and light petroleum ether (bp 30–60 °C) were used. All solvents were purified and dried by standard techniques, and distilled prior to use. All organic extracts were dried over Na_2SO_4, unless otherwise noted. IR spectra were recorded on a Nicolet FT-170SX spectrometer as liquid film. ^1H and ^{13}C NMR spectra were taken on a Varian mercury 300 MHz spectrometer with TMS as an internal standard and $CDCl_3$ as solvent unless otherwise noted. EI-MS spectrum were obtained on HP-5988A GC/MS instrument. HRMS were determined on a Bruker Daltonics APEXII 47e FT-ICR spectrometer. All air and moisture-sensitive reactions were performed in a flame-dried glassware under stream of nitrogen. Other commercially available chemical reagents and solvents were used as received without further purification unless indicated otherwise.

2.1. Typical procedure for ketone homologation under conditions of *n*-BuLi/$(CH_3)_3S^+I^-$/THF

To a stirred slurry of trimethylsulfonium iodide (400 mg, 1.95 mmol) in THF (4.5 mL) was added *n*-BuLi (1.60 M, 1.08 mL) dropwise at 0 °C under Ar. The resulting mixture was stirred for 10 min at 0 °C, to which cycloheptanone (168 mg, 1.5 mmol) in THF (1.5 mL) was added dropwise. The stirring was continued for 0.5 h at 0 °C and then warmed gradually to room temperature over 1 h. The reaction was quenched with water (5 mL) and extracted with Et_2O (2×30 mL). The organic layer was washed with water, brine, and dried over $MgSO_4$. After the solvent was evaporated in vacuo, the crude residue was purified by flash column chromatography on silica gel (PET/AcOEt= 50:1→30:1) to afford 77 mg (41%) of epoxide **1a** and 70 mg (27%) of methylthioether **2a**. *1-Oxa-spiro[2.6]-nonane* (**1a**). R_f=0.68 (PET/AcOEt=8:1); IR (film) ν_{max} 2956, 2924, 2855, 1597, 1456, 1377, 1260, 1072, 1022, 802 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.62 (s, 2H), 1.71–1.45 (m, 12H) ppm; EIMS (*m/z*, %): 126 (M^+, 4.1), 125 (16), 112 (7), 98 (6), 79 (45), 67 (94), 55 (100); HRMS (ESI) *m/z* obsd 127.1120 ($[M+H]^+$, calcd 127.1118 for $C_8H_{15}O$). *1-Methylsulfanylmethyl-cycloheptanol* (**2a**). R_f= 0.22 (PET/AcOEt=7:1); IR (film) ν_{max} 3443, 2922, 2865, 1458, 1435, 1346, 1200, 1116, 1035, 960, 797 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.67 (s, 2H), 2.23 (s, 1H, OH), 2.18 (s, 3H), 1.80–1.28 (m, 12H) ppm; ^{13}C NMR (50 MHz, $CDCl_3$) δ 75.2, 49.1, 40.5 (2C), 29.6 (2C), 22.4 (2C), 18.2 ppm; EIMS (*m/z*, %): 174 (M^+, 2), 113 (26), 95 (33), 62 (100); HRMS (ESI) *m/z* obsd 175.1161 ($[M+H]^+$, calcd 175.1157 for $C_9H_{19}OS$).

2.1.1. 1-Vinyl-cycloheptanol (2a′). R_f=0.21 (PET/AcOEt=7:1); IR (film) ν_{max} 3374, 3085, 2925, 2856, 1640, 1460, 1342, 1272, 1200, 1088, 1031, 995, 917, 846 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.03 (dd, 1H, J=17.4, 10.8 Hz), 5.21 (d, 1H, J=17.4 Hz), 4.99 (d, 1H, J=10.8 Hz), 1.80–1.37 (m, 12H) ppm; ^{13}C NMR (50 MHz, $CDCl_3$) δ 146.6, 110.1, 75.5, 41.1 (2C), 29.4 (2C), 22.1 (2C) ppm; HRMS (ESI) *m/z* obsd 141.1276 ($[M+H]^+$, calcd 141.1274 for $C_9H_{17}O$).

2.1.2. 1-Oxa-spiro[2.5]octane. (Compound **1b**, yield 50%), R_f=0.69 (PET/AcOEt=8:1); IR (film) ν_{max} 2955, 2924, 2855, 1596, 1456, 1377, 1257, 1072, 1022, 802 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.61 (s, 2H), 1.68–1.45 (m, 10H) ppm; HRMS (ESI) *m/z* obsd 113.0958 ($[M+H]^+$, calcd 113.0961 for $C_7H_{13}O$).

2.1.3. 1-Methylsulfanylmethyl-cyclohexanol. (Compound **2b**, yield 30%), R_f=0.20 (PET/AcOEt=7:1); IR (film) ν_{max} 3446, 2930, 2856, 1446, 1349, 1242, 1171, 1147, 1058, 969 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.65 (s, 2H), 2.18 (s, 3H), 1.73–1.17 (m, 10H) ppm; EIMS (*m/z*, %): 160 (M^+, 11), 142 (3), 99 (53), 81 (88), 62 (100); HRMS (ESI) *m/z* obsd 161.0998 ($[M+H]^+$, calcd 161.0995 for $C_8H_{17}OS$).

2.1.4. 6-Methyl-1-oxa-spiro[2.5]octane. (Compound **1c**, yield 40%), R_f=0.60 (PET/AcOEt=8:1); IR (film) ν_{max} 2922, 2852, 1597, 1447, 1261, 1024, 799 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.61 (d, J=1.4 Hz, *trans*-OCH_2), 2.56 (s, *cis*-OCH_2), 1.90–1.66 (m, 4H), 1.55–0.80 (m, 5H), 0.94 (d, J=6.2 Hz, *cis*-CH_3), 0.92 (d, J=6.6 Hz, *trans*-CH_3) ppm; ^{13}C NMR (50 MHz, $CDCl_3$) δ 59.5, 54.8, 53.8, 33.9, 33.2, 32.6, 32.3, 31.4, 31.3, 22.0, 21.5 ppm; HRMS (ESI) *m/z* obsd 127.1120 ($[M+H]^+$, calcd 127.1118 for $C_8H_{15}O$).

2.1.5. *cis*-4-Methyl-1-methylsulfanylmethyl-cyclohexanol. (Compound **2c**, *cis*/*trans* 10:1, total yield 15%), R_f=0.1 (PET/AcOEt=8:1); IR (film) ν_{max} 3449, 2921, 2857, 1449, 1372, 1234, 998 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.75 (s, 2H), 2.41 (s, 1H, OH), 2.18 (s, 3H), 1.80–1.44 (m, 7H), 1.11–0.85 (m, 2H), 0.90 (d, 3H, J=6.4 Hz) ppm; ^{13}C NMR (50 MHz, $CDCl_3$) δ 71.1, 44.3, 36.6, 31.4, 31.3, 21.1, 17.9 ppm; EIMS (*m/z*, %): 174 (M^+, 9.3), 113 (49), 95 (89), 62 (100); HRMS (ESI) *m/z* obsd 175.1156 ($[M+H]^+$, calcd 175.1152 for $C_9H_{19}OS$). *trans-4-Methyl-1-methylsulfanylmethyl-cyclohexanol* (**2c**). R_f=0.12 (PET/AcOEt=8:1); IR (film) ν_{max} 3445, 2923, 2859, 1453, 1089, 1049, 1009 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.59 (s, 2H), 2.16 (s, 3H), 2.05 (s, 1H, OH), 1.71–1.29 (m, 9H), 0.91 (br s, 3H) ppm; EIMS (*m/z*, %): 174 (M^+, 8.4), 113 (35), 95 (64), 62 (100); HRMS (ESI) *m/z* obsd 175.1154 ($[M+H]^+$, calcd 175.1152 for $C_9H_{19}OS$).

2.1.6. 6-Phenyl-1-oxa-spiro[2.5]octane. (Compound **1d**, yield 48%), R_f=0.23 (PET/AcOEt=8:1); IR (film) ν_{max} 3025, 2998, 2935, 2856, 1601, 1491, 1446, 1181, 1097, 979, 911, 753, 699 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 7.35–7.25 (m, 5H), 2.70 (s, 2H), 2.60–2.53 (m, 1H), 2.13–1.38 (m, 8H) ppm; ^{13}C NMR (50 MHz, $CDCl_3$) δ 146.1, 128.4, 126.8, 126.7, 126.2, 126.1, 59.1, 54.9, 53.9, 43.3, 33.8, 33.2, 31.5 ppm; EIMS (*m/z*, %): 188 (M^+, 13), 174 (4), 168 (4.8), 143 (12), 129 (19), 115 (30), 104 (100), 91 (59); HRMS (ESI) *m/z* obsd 189.1276 ($[M+H]^+$, calcd 189.1274 for $C_{13}H_{17}O$).

2.1.7. *cis*-1-Methylsulfanylmethyl-4-phenyl-cyclohexanol. (Compound **2d**, *cis*/*trans* 3.9:1, total yield 26%), R_f=0.04 (PET/AcOEt=8:1); IR (film) ν_{max} 3444, 3059, 3026, 2928, 2859, 1695, 1600, 1494, 1450, 1347, 1198, 1054, 972, 757, 699 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 7.33–7.18 (m, 5H), 2.92 (s, 2H), 2.63 (s, OH), 2.65–2.45 (m, 1H), 2.24 (s, 3H), 2.03–1.29 (m, 8H) ppm; ^{13}C NMR (50 MHz, $CDCl_3$) δ 146.0, 128.3, 126.7, 126.1, 71.4, 60.3, 43.7, 43.2, 37.5, 30.8, 21.0, 17.9, 14.1 ppm; EIMS (*m/z*, %): 236 (M^+, 0.7), 218 (0.11), 174 (13), 157 (10), 129 (7), 91 (39), 77 (9), 62 (100); HRMS (ESI) *m/z* obsd 237.1311 ($[M+H]^+$, calcd 237.1308 for $C_{14}H_{21}OS$). *trans-1-Methylsulfanylmethyl-4-phenyl cyclohexanol* (**2d**). R_f=0.043 (PET/AcOEt=8:1); IR (film) ν_{max} 3530, 3468, 3026, 2928, 2857, 1601, 1493, 1442, 1312, 1211, 1143, 1060, 979, 756, 699 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 7.33–7.21 (m, 5H), 2.69 (s, 2H), 2.52–2.40 (m, 1H), 2.23 (s, 3H), 2.06–1.45 (m, 8H) ppm; EIMS (*m/z*, %): 236 (M^+, 8.3), 218 (1.8), 174 (81), 157 (6.1), 91 (100); HRMS (ESI) *m/z* obsd 237.1310 ($[M+H]^+$, calcd 237.1308 for $C_{14}H_{21}OS$).

2.1.8. 2-Methyl-2-(4-methyl-pent-3-enyl)-oxirane. (Compound **1e**, yield 45%), R_f=0.91 (PET/AcOEt=8:1); IR (film) ν_{max} 3035, 2966, 2923, 2857, 1449, 1384, 1265, 1108, 1072, 901, 796 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.09 (t, 1H, J=7.1 Hz), 2.59 (q, 2H, J=6.4 Hz), 2.07 (q, 2H, J=7.6 Hz), 1.68 (s, 3H), 1.60 (s, 3H), 1.24–1.11 (m, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 131.9, 123.6, 56.8, 53.9, 36.8, 25.6, 23.8, 20.9, 17.6 ppm; EIMS (*m/z*, %): 140 (M^+, 0.04), 125 (0.2), 109 (6.5), 82 (7), 67 (32), 55 (19), 41 (100); HRMS (ESI) *m/z* obsd 141.1277 ($[M+H]^+$, calcd 141.1274 for $C_9H_{17}O$). *2,6-Dimethyl-1-methylsulfanyl-hept-5-en-2-ol* (**2e**, yield 21%). R_f=0.90 (PET/AcOEt=8:1); IR (film) ν_{max} 3446, 2969, 2919, 2858, 1700, 1665, 1442, 1376, 1241, 1150, 1114, 1021, 917, 835 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.11 (t, 1H, J=6.8 Hz), 2.65 (d, 2H, J=4.0 Hz), 2.26 (s, 1H, OH), 2.16 (s, 3H), 2.10–1.99 (m, 2H), 1.68 (s, 3H), 1.61 (s, 3H), 1.58–1.49 (m, 2H), 1.23 (s, 3H) ppm; ^{13}C NMR (50 MHz, $CDCl_3$) δ 131.8, 124.1, 72.6, 47.7, 41.1, 26.1, 25.7, 22.7, 18.1, 17.6 ppm; EIMS (*m/z*, %): 188 (M^+, 0.8), 173 (0.2), 170 (3.2), 155 (1), 26 (18), 109 (67), 69 (100); HRMS (ESI) *m/z* obsd 189.1310 ($[M+H]^+$, calcd 189.1308 for $C_{10}H_{21}OS$).

2.1.9. 2-Methyl-4-phenyl-but-3-enal. (Compound **3f**, yield 57%), R_f=0.51 (PET/AcOEt=6:1); IR (film) ν_{max} 3427, 2974, 2716, 1724, 1639, 1598, 1450, 968, 747, 694 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.66 (d, 1H, J=1.4 Hz), 7.41–7.25 (m, 5H), 6.55 (d, 1H, J=16.0 Hz), 6.16 (dd, 1H, J=16.0, 7.6 Hz), 3.26 (t, 1H, J=7.0 Hz), 1.33 (d, 3H, J=7.0 Hz) ppm; EIMS (*m/z*, %): 160 (M^+, 11), 141 (0.3), 131 (100), 91 (43), 77 (9.3); HRMS (ESI) *m/z* obsd 161.0963 ($[M+H]^+$, calcd 161.0961 for $C_{11}H_{13}O$). *2-Methyl-1-methylsulfanyl-4-phenyl-but-3-en-2-ol* (**2f**, yield 25%). R_f=0.30 (PET/AcOEt=6:1); IR (film) ν_{max} 3443, 3026, 2974, 2918, 1598, 1578, 1494, 1369, 1269, 1067, 970, 749, 694 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.42–7.20 (m, 5H), 6.69 (d, 1H, J=16.0 Hz), 6.25 (d, 1H, J=16.0 Hz), 2.81 (q, 2H, J=13.6 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 136.7, 135.1, 128.5, 128.0, 127.5, 126.4, 72.7, 48.2, 27.6, 18.0 ppm; EIMS (*m/z*, %): 208 (M^+, 2), 147

(100), 129 (34), 91 (7), 77 (11); HRMS (ESI) *m/z* obsd 191.0888 ($[M-H_2O+H]^+$, calcd 191.0889 for $C_{12}H_{15}S$).

2.1.10. 2-Methyl-oct-3-enal. (Compound **3g**, yield 55%), R_f=0.34 (PET/AcOEt=20:1); IR (film) ν_{max} 3440, 3030, 2958, 2927, 2857, 2710, 1728, 1694, 1460, 1301, 1064, 971 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.52 (d, 1H, J=2.7 Hz), 5.80–5.54 (m, 1H), 5.35 (dd, 1H, J=15.4, 8.0 Hz), 2.99 (m, 1H), 2.06–1.95 (m, 2H), 1.35–1.10 (m, 4H), 1.17 (d, 3H, J=6.0 Hz), 0.87 (t, 3H, J=7.0 Hz) ppm; EIMS (*m/z*, %): 140 (M^+, 3.2), 127 (4), 111 (23), 83 (90), 69 (100); HRMS (ESI) *m/z* obsd 141.1276 ($[M+H]^+$, calcd 141.1274 for $C_9H_{17}O$). *2-Methyl-1-methylsulfanyl-oct-3-en-2-ol* (**2g**, yield 25%). R_f=0.25 (PET/AcOEt=7:1); IR (film) ν_{max} 3448, 3022, 2959, 2924, 2858, 1666, 1459, 1372, 1242, 1161, 1110, 1064, 973, 931, 808 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.66 (dt, 1H, J=15.4, 6.5 Hz), 5.45 (d, 1H, J=15.6 Hz), 2.65 (q, 2H, J=13.4 Hz), 2.11 (s, 3H), 2.08–1.95 (m, 2H), 1.40–1.16 (m, 4H), 1.29 (s, 3H), 0.85 (t, 3H, J=7.0 Hz) ppm; ^{13}C NMR (50 MHz, $CDCl_3$) δ 135.2, 129.1, 72.2, 48.2, 31.3, 31.2, 27.4, 22.1, 17.9, 13.9 ppm; EIMS (*m/z*, %): 173 ($[M-15]^+$, 0.14), 170 ($[M-18]^+$, 0.06), 127 (24), 71 (100); HRMS (ESI) *m/z* obsd 189.1319 ($[M+H]^+$, calcd 189.1314 for $C_{10}H_{21}OS$).

2.1.11. 2-Cyclopropyl-6-phenyl-hexanal. (Compound **3h**, yield 71%), R_f=0.36 (PET/AcOEt=8:1); IR (film) ν_{max} 3429, 3025, 2932, 2714, 1723, 1602, 1456, 1023, 909, 734, 699 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.69 (d, 1H, J=2.6 Hz), 7.28–7.16 (m, 5H), 2.62 (t, 2H, J=7.6 Hz), 1.77–1.44 (m, 7H), 0.89–0.72 (m, 1H), 0.61–0.57 (m, 2H), 0.27–0.21 (m, 2H) ppm; EIMS (*m/z*, %): 216 (M^+, 1.2), 183 (0.6), 169 (1.8), 157 (3.5), 91 (100), 77 (9.1); HRMS (ESI) *m/z* obsd 217.1590 ($[M+H]^+$, calcd 217.1587 for $C_{15}H_{21}O$). *2-Cyclopropyl-1-methylsulfanyl-6-phenyl-hexan-2-ol* (**2h**, yield 27%). R_f=0.20 (PET/AcOEt=8:1); IR (film) ν_{max} 3477, 3004, 2934, 2856, 1602, 1495, 1454, 1022, 911, 824, 746, 699 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.32–7.18 (m, 5H), 2.73 (s, 2H), 2.64 (t, 2H, J=7.6 Hz), 2.17 (d, 3H, J=1.4 Hz), 1.73–1.46 (m, 6H), 0.86–0.77 (m, 1H), 0.49–0.31 (m, 4H) ppm; ^{13}C NMR (50 MHz, $CDCl_3$) δ 142.6, 128.4, 128.2, 125.6, 71.3, 47.0, 40.7, 35.9, 32.0, 23.5, 18.8, 17.9, 0.47, 0.24 ppm; EIMS (*m/z*, %): 264 (M^+, 0.27), 249 (0.34), 246 (0.09), 203 (40), 117 (57), 91 (100), 77 (8.6); HRMS (ESI) *m/z* obsd 282.1891 ($[M+NH_4]^+$, calcd 282.1887 for $C_{16}H_{28}ONS$).

2.1.12. 2-Cyclohex-1-enyl-propionaldehyde. (Compound **3i**, yield 21%), R_f=0.29 (PET/AcOEt=20:1); IR (film) ν_{max} 3419, 3037, 2932, 2839, 2714, 1723, 1659, 1610, 1453, 1385, 1140, 1019 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.50 (d, 1H, J=1.8 Hz), 5.60 (m, 1H), 2.92 (q, 1H, J=7.0 Hz), 2.06–1.82 (m, 4H), 1.65–1.45 (m, 4H), 1.17 (d, 3H, J=6.8 Hz) ppm; HRMS (ESI) *m/z* obsd 139.1120 ($[M+H]^+$, calcd 139.1118 for $C_9H_{15}O$).

2.2. Preparation of epoxide 5, aldehyde 6, and diol 9

NaH (600 mg, 15.0 mmol, 60% dispersion in mineral oil, washed three times with *n*-hexane distilled from CaH_2) was placed in round-bottomed flask (50 mL) and DMSO (10 mL, distilled from CaH_2) was introduced under Ar. The resulting mixture was heated with stirring to 70–75 °C. After 20 min, the reaction mixture was diluted with THF (10 mL) and then cooled to 0 °C. A solution of trimethylsulfonium iodide (3.06 g, 15.0 mmol) in DMSO (10 mL) was added over a period of about 5 min. After the addition of the THF (5 mL) solution of ketone **4** (2.2 g, 10.0 mmol) at 0 °C, stirring was continued for 30 min at 0 °C then warmed gradually to room temperature over 1 h. The reaction was quenched by water (10 mL) and extracted with Et_2O (200 mL). The organic layer was washed with water (4×10 mL) brine and dried over $MgSO_4$ and concentrated to give 2.2 g (95%) of the epoxide **5** as pale yellow oil. *2-Cyclopropyl-2-(4,8-dimethyl-nona-3,7-dienyl)-oxirane* (**5**). R_f=0.69 (PET/AcOEt=10:1); IR (film) ν_{max} 3084, 2967, 2921, 2856, 1669, 1447, 1379, 1229, 1149, 1104, 1023, 943, 826 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.15–5.05 (m, 2H), 2.54 (d, 1H, J=5.4 Hz), 2.47 (d, 1H, J=3.9 Hz), 2.20–2.13 (m, 2H), 2.10–1.95 (m, 4H), 1.83–1.73 (m, 2H), 1.67 (s, 3H), 1.62 (s, 3H), 1.59 (s, 3H), 1.18–1.09 (m, 1H), 0.48–0.41 (m, 1H), 0.39–0.27 (m, 2H), 0.21–0.14 (m, 1H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.4, 131.3, 124.2, 123.8, 58.6, 51.6, 39.6, 36.2, 26.6, 25.6, 23.6, 17.6, 15.9, 13.2, 1.8, 0.5 ppm; EIMS (*m/z*, %): 234 (M^+, 0.11), 203 (0.75), 165 (0.76), 123 (4.7), 95 (9.7), 81 (31.7), 69 (100); HRMS (ESI) *m/z* obsd 252.2322 ($[M+NH_4]^+$, calcd 252.2322 for $C_{16}H_{30}ON$).

The crude epoxide **5** was loaded on a short pad of silica gel (activated in an oven at 200 °C for 2 h) eluting with PET–AcOEt (100/1) to give 1.9 g (90%) of the aldehyde **6**. *2-Cyclopropyl-6,10-dimethyl-undeca-5,9-dienal* (**6**). R_f=0.72 (PET/AcOEt=10:1); IR (film) ν_{max} 3080, 2968, 2921, 2856, 2711, 1725, 1448, 1379, 1106, 1022, 822, 668 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.70 (d, 1H, J=2.7 Hz), 5.10–5.06 (m, 2H), 2.11–1.98 (m, 6H), 1.84–1.77 (m, 1H), 1.64 (s, 3H), 1.63–1.49 (m, 2H), 1.60 (s, 3H), 1.59 (s, 3H), 0.76–0.71 (m, 1H), 0.61–0.56 (m, 2H), 0.31–0.18 (m, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 204.5, 136.1, 131.4, 124.2, 123.6, 56.2, 39.7, 29.5, 26.7, 25.7, 25.3, 17.7, 16.0, 10.7, 3.8, 3.0 ppm; EIMS (*m/z*, %): 234 (M^+, 0.1), 205 (0.1), 191 (1), 123 (6), 95 (12), 81 (45), 69 (100); HRMS (ESI) *m/z* obsd 252.2324 ($[M+NH_4]^+$, calcd 252.2322 for $C_{16}H_{30}ON$).

The crude epoxide **5** (1.1 g, 4.7 mmol) was taken up in a mixture of THF (5 mL) and saturated aqueous NH_4Cl (5 mL). After stirring for 8 h at ambient temperature, the reaction mixture was extracted with AcOEt (100 mL). The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated. The crude product was purified by flash chromatography on silica gel (PET/AcOEt=4:1) to give 960 mg (81%) of diol **9** as a white solid. *2-Cyclopropyl-6,10-dimethyl-undeca-5,9-diene-1,2-diol* (**9**). R_f=0.17 (PET/AcOEt=4:1). mp 54–56 °C (recrystallized from EtOH); IR (film) ν_{max} 3407, 3083, 3005, 2966, 2924, 2857, 1667, 1643, 1448, 1379, 1265, 1153, 1105, 1075, 1040, 1022, 914, 875, 823 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.16–5.06 (m, 2H), 3.48 (d, 2H, J=8.7 Hz), 2.55 (t, 1H, OH, J=9.0 Hz), 2.31–1.99 (m, 6H), 1.65 (s, 3H), 1.64–1.53 (m, 2H), 1.60 (s, 3H), 1.58 (s, 3H), 0.82–0.68 (m, 1H), 0.39–0.32 (m, 4H) ppm; ^{13}C NMR (50 MHz, $CDCl_3$) δ 135.5, 131.3, 124.3, 124.2, 72.2, 69.0, 39.6, 37.9, 26.6, 25.6, 22.2, 17.6, 16.2, 15.9, −0.5, −0.9 ppm; EIMS (*m/z*, %): 234 ($[M-H_2O]^+$, 0.4), 221 (0.55), 203 (2.6), 191 (1.2), 165

(1.6), 136 (7.2), 121 (6.6), 95 (14.7), 81 (40), 69 (100); HRMS (ESI) *m/z* obsd 270.2432 ($[M+NH_4]^+$, calcd 270.2428 for $C_{16}H_{32}O_2N$).

2.3. Regioselective bromohydration of triene 7 and alternative preparation of aldehyde 6

To a stirred mixture of triene **7** (1.09 g, 5.0 mmol) in 10 mL of 50% aqueous THF was added NBS (890 mg, 5.0 mmol) portionwise. After stirring for 15 min at ambient temperature, the reaction was quenched with 2 mL of aqueous $NaHCO_3$ (5%) and extracted with Et_2O (2×50 mL). The organic phases were washed with water and brine, dried over $MgSO_4$ and concentrated. The crude residue was purified by flash chromatography on silica gel (PET/AcOEt=6:1) to give 1.05 g (71%) of bromohydrin **8** as a colorless oil. *Bromo-2-cyclopropyl-6,10-dimethyl-undeca-5,9-dien-2-ol* (**8**). R_f=0.70 (PET/AcOEt=10:1); IR (film) ν_{max} 3526, 3085, 3007, 2966, 2920, 2855, 1668, 1442, 1380, 1223, 1107, 1024, 826, 651 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.16 (t, 1H, J=7.2 Hz), 5.09 (t, 1H, J=7.2 Hz), 3.53 (s, 2H), 2.17–1.99 (m, 6H), 1.81–1.63 (m, 2H), 1.67 (s, 3H), 1.63 (s, 3H), 1.60 (s, 3H), 0.97–0.88 (m, 1H), 0.53–0.35 (m, 4H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.9, 131.5, 124.2, 123.7, 70.7, 44.2, 39.6, 39.3, 26.6, 25.7, 22.4, 17.7, 17.1, 16.0, −0.7, −0.02 ppm; EIMS (*m/z*, %): 298 ($[M-18]^+$, 0.05), 296 ($[M-18]^+$, 0.05), 283 (0.03), 273 (0.07), 253 (0.5), 217 (4.2), 123 (26), 105 (13), 93 (22), 81 (31), 69 (100); HRMS (ESI) *m/z* obsd 315.1322 ($[M+H]^+$, calcd 315.1319 for $C_{16}H_{28}OBr$).

A stirred mixture of bromohydrin **8** (445 mg, 1.5 mmol) in methanol (5 mL) at 0 °C was treated with powdered anhydrous K_2CO_3 (280 mg, 2.0 mmol) in one portion. The resulting mixture was stirred for 0.5 h while gradually warmed to room temperature. The reaction mixture was diluted with ether (40 mL) and washed successively with water, brine, and dried. After evaporation of the solvent, the residue was purified by flash chromatography on silica gel eluting with PET–AcOEt (100/1) to give 320 mg (92%) of aldehyde **6**, identical with the sample prepared above.

2.3.1. Acetic acid 2-(3-iodo-propylidene)-6,10-dimethyl-undeca-5,9-dienyl ester (10). The title compound was prepared according to Ref. 17, R_f=0.49 (PET/AcOEt=10:1); IR (film) ν_{max} 2961, 2930, 2863, 1742, 1668, 1447, 1375, 1229, 1024, 963 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.43 (t, 1H, J=6.6 Hz), 5.11–5.09 (m, 2H), 4.51 (s, 2H), 3.13 (t, 2H, J=7.2 Hz), 2.71–2.63 (m, 2H), 2.11–1.95 (m, 8H), 2.08 (s, 3H), 1.71 (s, 3H), 1.61 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 136.2, 136.0, 131.2, 128.0, 124.2, 123.2, 67.8, 39.6, 32.7, 32.6, 28.9, 27.3, 26.6, 25.7, 21.0, 17.7, 4.5 ppm; EIMS (*m/z*, %): 404 (M^+, 2), 344 (2.4), 329 (3.1), 301 (4.5), 217 (6), 137 (28), 95 (30), 81 (66), 69 (100); HRMS (ESI) *m/z* obsd 422.1561 ($[M+NH_4]^+$, calcd 422.1550 for $C_{18}H_{33}O_2IN$).

2.3.2. (8-Iodo-5-methylsulfanylmethyl-oct-5-enyl)-benzene (11). The title compound was prepared according to a typical procedure described in Ref. 17, R_f=0.31 (PET/AcOEt=20:1); IR (film) ν_{max} 3082, 3025, 2929, 2855, 1602, 1453, 1423, 1245, 1167, 1028, 802, 745, 699 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.29–7.20 (m, 5H), 5.20 (t, 1H, J=7.0 Hz), 3.13 (t, 2H, J=7.1 Hz), 3.06 (s, 2H), 2.69–2.60 (m, 4H), 2.27–2.14 (m, 2H), 1.98 (s, 3H), 1.65–1.30 (m, 4H) ppm; ^{13}C NMR (50 MHz, $CDCl_3$) δ 142.4, 137.6, 128.4, 128.3, 127.0, 125.7, 40.7, 35.7, 32.0, 31.3, 28.4, 27.8, 24.8, 5.5 ppm; EIMS (*m/z*, %): 374 (M^+, 0.4), 326 (4.8), 311 (0.2), 247 (25), 157 (15), 117 (45), 91 (100); HRMS (ESI) *m/z* obsd 375.0641 ($[M+H]^+$, calcd 375.0638 for $C_{16}H_{24}SI$).

Acknowledgements

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18. In contrast with this remarkable regioslectivity, diol **9** was prepared from triene **7** by Sharpless dihydroxylation with an AD-mix reagent system in a rather low yield (ca. 10%, poor regioselectivity). Similarly, electrophilic epoxidation of triene **7** with *m*-CPBA also lacks regioselectivity for the production of labile epoxide **5**.
19. This is apparently due to the inherent preference for the oxy-3-*exo*-tet ring closure process. It is evident that the addition of metal (Li, Na, or K) iodide in the reaction mixture (i.e., reaction in Table 1) could not increase the yield of the sulfide formation.

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Synthesis and characterization of a novel electrical and optical-active triads containing fullerene and perylenebisimide units

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Abstract—A new electrical and optical-active triad containing fullerene and perylenebisimide units has been prepared and characterized by UV–vis spectroscopy, fluorescence spectroscopy and voltammetry. For comparison, the triad containing only perylenebisimide units has also been studied. The SEM image indicated that uniform nanofibers of the triad were formed with a diameter of about 50 nm. The thin films of the triad produced steady and prompt photocurrent at irradiation of 20.0 mW cm^{-2} white light.

1. Introduction

Recently, new organic photovoltaic materials have attracted commercial and scientific interest.[1] Molecular dyads or triads composed of an energy or electron donor capable of photoinduced energy or electron transfers to a covalently linked acceptor have become ideal candidates of the materials used in photovoltaic devices.[2] The unique three-dimensional structure of fullerene C_{60} derivatives and the facile electron acceptability of up to six electrons make them good candidates as electron acceptors.[3] The most remarkable property of fullerene C_{60} in electron transfer processes is that it efficiently gives rise to a rapid photoinduced charge separation and a further slow charge recombination[4] due to its facile electron acceptability and small reorganization.[5] Perylene-3,4,9,10-bis(dicarboximide) (PDI), which has excellent chemical, thermal and photochemical stability,[6] is a class of organic semiconductors with a variety of possible applications such as optical switching,[7] dye lasers,[8] and organic solar cells.[9] They posses capability to absorb the light highly efficiently and emit fluorescene with quantum yields near unity.[10] Chromophores based on PDI have been successfully utilized to self-assemble functional materials by π–π stacking.[11] Only very recently, perylene has been used in combination with fullerene as photovoltaic materials[12] and thermally stable dyes.[13]

In the present paper, we report the synthesis and characterization of a novel fullerene derivative covalently linked to perylenebisimide dyes. Two PDI units are attached to the para position of one of the phenyl group of 1,3,5-triphenyl-benzene, and fullerene is attached to the other phenyl group. As mentioned in literature,[11d] this sort of molecules tends to self-assemble into π-stacked dimers. Thus this perylene–fullerene triad may have interesting aggregate structure. Furthermore, Investigation of the derivative and its film provided optical and electrochemical information. The new triad, in which perylene as donor and C_{60} as acceptor, is expected to has potential application in solar cells.

Keywords: Fullerene; Electronic properties; Optical properties; Scanning electron microscopy.
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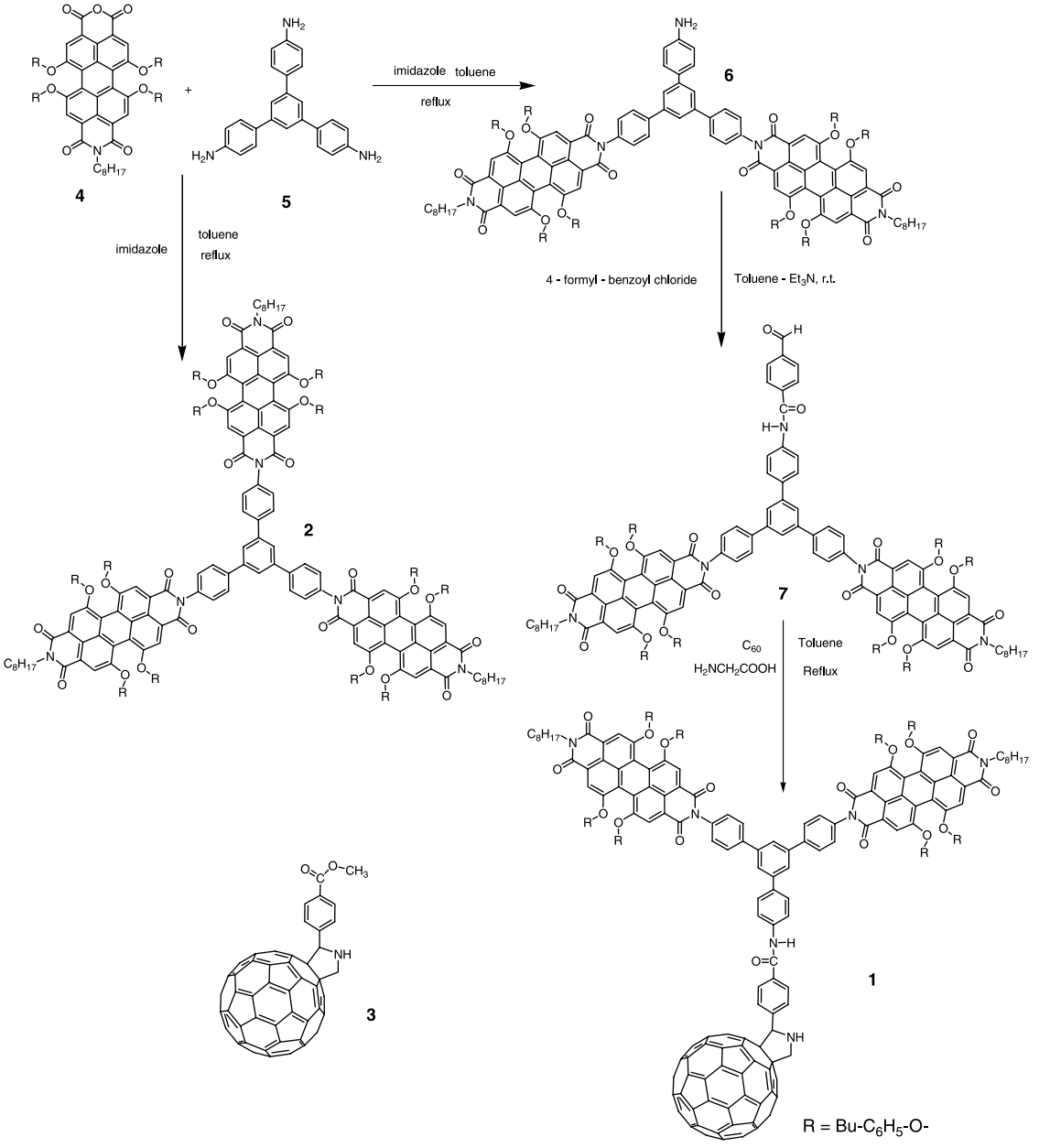
2. Results and discussion

2.1. Synthesis

The synthetic route was summarized in Scheme 1. Perylene tetracarboxylic bisimides are good chromophores with high fluorescence quantum yield.[14] However, the drawback is its intrinsically low solubility. To overcome this problem, we introduced 4-*tert*-butylphenol in the bay region of perylene tetracarboxylic bisimides according to method developed by Seybold et al. and Mullen et al.[15] 1,3,5-Tris(4-amino-phenyl)benzene **5** was prepared by acid-catalyzed condensation of 1-(4-nitro-phenyl)—ethanone and then reduced by hydrated hydrazine according to the general procedure.[16] Compound **5** was condensed with compound **4** to yield **6**.[17] Compound **6** was amidated with 4-formyl-benzoyl chloride to afford **7**. The fullerene–perylene triad **1** was synthesized from glycine and compound **7**, based on the 1,3-dipolar cycloaddition of azomethine ylides to C_{60}.[18] The triads were characterized by ^{1}H NMR, ^{13}C NMR, FT-IR, MS spectra, and elemental analysis.

2.2. Optical properties

The UV–vis spectrum of compound **6** in chloroform solution showed three absorption peaks of perylene at 454, 545, and 587 nm. The absorption spectra of compound **1** and compound **2**, respectively, in this wavelength region were very similar to that of compound **6**. The spectra of compound **1** was a simple combination of the characteristic absorption band of fullerene, and perylene, so it was clear that there was no electronic or energy interaction between the two chromophores in the ground state. The spectra of **1**, **2**, and **6** in toluene showed only a small enhancement of



Scheme 1.

the 0–1 vironic band, (Fig. S1), which indicated there was no clearly cofacial π–π interaction between the perylene units [**6a**]. We presume that this was due to the steric hindrance of 4-butylphenol in the bay region of the perylene tertracarboxylic bisimides (Fig. 1).

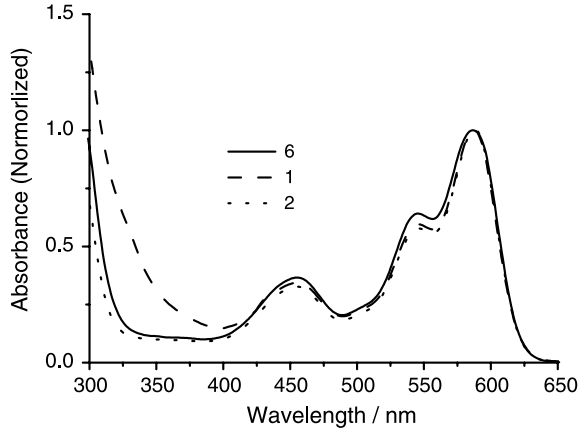


Figure 1. UV–vis absorption spectra of compound **6**, **2**, **1** in $CHCl_3$ [**6**, **2** or **1**]$=2.0\times10^{-5}$ M.

The fluorescent spectra of pure compound **6**, **1**, and **2** showed maximum at 621 nm with a shoulder emission at 675 nm. (Fig. 2) The fluorescence of the triad **1** is strongly quenched compared to that of the triad **2** with the excited wavelength of 546 nm. The fluorescence quantum yield of compound **1** was 11 and 94% for triad **2**, which are obtained by the steady-state comparative method using *N*,*N*′-bis(2,6-diisopropylphenyl)-1,6,7,12-tetraphenoxy-perylene-3,4:9,10-tetracarboxylic acid bisimide as reference ($\Phi_{Fl}=96\%$ in $CHCl_3$).[19] We presume that the quenching was due to the interaction between the perylene and fullerene in excited state. Furthermore, no florescence of C_{60} at 720 nm was detected. Therefore, there is no clear evidence for the existence of singlet–singlet energy transfer from perylene-tetracarboxylic diimide to fullerene within the triad. Such strong quenching of perylenetetracarboxylic diimide components suggested the occurrence of intramolecular photoinduced charge-transfer processes within the triad.

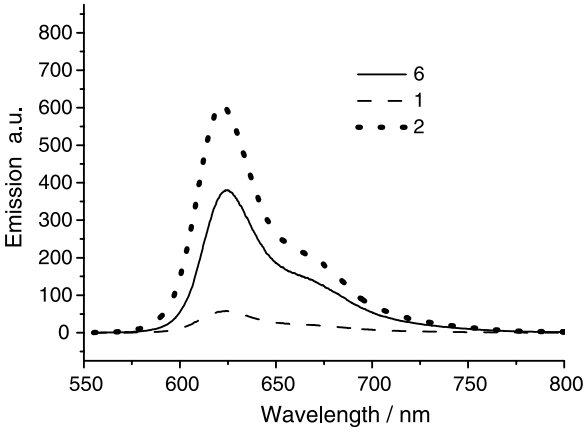


Figure 2. Fluorescence spectra of compound **6**, **2**, and **1** in $CHCl_3$, excitation wavelength is 546 nm; [**6**, **2** or **1**]$=2.0\times10^{-5}$ M.

2.3. Cyclic voltammetry

The redox properties of the **1**, **2**, and **3** were studied by cyclic voltammetry (CV). The experiments were performed at room temperature in dry *o*-dichlorobenzene solutions containing 0.04 M $TBAPF_6$ as a supporting electrolyte. A three-electrode cell consisting of a glassy carbon working electrode, a Pt counter electrode and an Ag wire quasi reference electrode was used. The CV data are summarized in Table 1 (Fig. 3).

Table 1. Electrochemical data for the products

Compound	Potential (V)[a]						
	E_{ox}^{P2}	E_{ox}^{P1}	E_{red}^{F1}	E_{red}^{F2}	E_{red}^{F3}	E_{red}^{P1}	E_{red}^{P2}
2	1.69	1.33				−0.89	−1.03
1	1.73	1.37	−0.79	−1.22	−1.67	−0.94	−1.11
3			−0.82	−1.28	−1.79		

[a] Versus Ag wire. The scan rate was 50 mV s^{-1}.

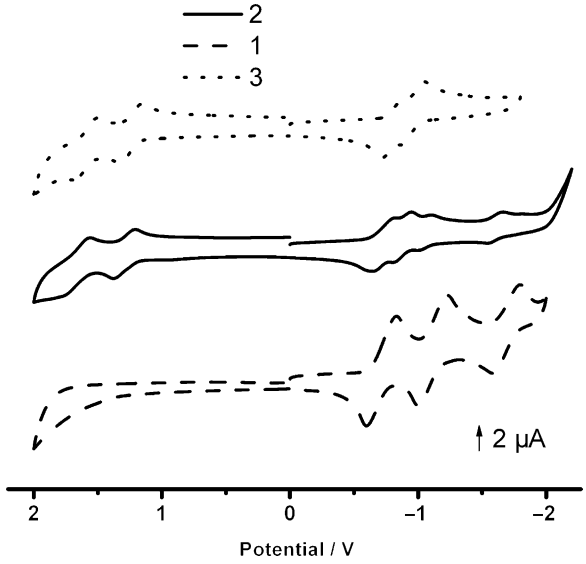


Figure 3. Cyclic voltammograms of **1**, **2**, and **3** in *o*-dichlorobenzene, 0.04 M TBAP (scan rate$=50$ mV s^{-1}).

The CV analysis for triad **1**, showed five reversible waves in the redox part peaked at −0.79, −1.22, −1.67, −0.94, and −1.11 V versus silver wire, which corresponding to the formation of monoanion, dianion, and trianion of fullerene moiety and the formation of monoanion and dianion of perylene unit, respectively. On the other hand, the oxidation waves were peaked at 1.73 and 1.37 V, corresponding to the formation of monocanion and dicanion of perylene, respectively. As shown in Table 1, the reduction potential of fullerene in triad **1** was increased compared to that of the reference compound **3**, which was resulted from the attachment of C_{60} to the 1,3,5-triphenyl-benzene. In the case of triad **2**, the oxidation potential was decreased corresponding to that of triad **1**, which indicated that perylene canion was more stable in **2** than in **1**.

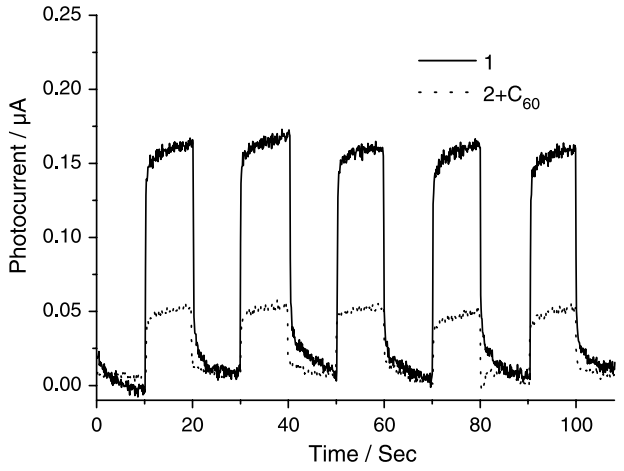


Figure 4. Photocurrent generation of the monolayer films upon the irradiation of 20.0 mW cm^{-2} white light in 0.5 M KCl solution.

2.4. Photocurrent generation

A conventional three-electrode cell was used to measure the photoelectrochemical properties of the monolayer film deposited on indium-tin oxide (ITO) glass. A platinum wire was used 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 all measurements. The ITO glass modified with compound was used as a working electrode.

The thin films of **1** were prepared by immersing the ITO glass into its toluene solution (1 mg/mL) for 2 h to afford films with smooth surface. To investigate the spatial distance effect of the donor and acceptor for the photocurrent generation, the mixture film of compound **2** and C_{60} was prepared by immersing the ITO glass into toluene solution (mixture of 7.7 mg of **2** and 2.3 mg of C_{60} in 10 mL toluene) for 2 h. The absorption intensity of the film of **1** was as much as that of the mixture film of compound **2** and C_{60}, which indicated that the films prepared under the same procedure were comparable. (Fig. S2) The photocurrent of thin film deposited on ITO electrode was measured at 20.0 mW cm^{-2} white light irradiation. For compound **1** a steady and rapid cathodic 164 nA cm^{-2} photocurrent response was produced when the irradiation of the thin film was switched on and off. Importantly, the response to on/off cycling was prompt and reproducible as shown in Figure 4. The photocurrent stability in the systems was rather good during the monitor time. The film fabricated from mixture of compound **2** and C_{60} produced steady cathodic photocurrent response (52 nA/cm^2), however the photocurrent was three fold lower than that of compound **1**. This was due to the fact that when fullerene and perylene are spatially close, electron transfer is much more likely to take place. Electrons transfer from the conduction band of ITO to the compound layer. Upon illumination, the exciton forms, and electron transfer from perylene moiety to C_{60} occurs, forming a charge-separated state (Perylene$^{\cdot+}$–$C_{60}^{\cdot-}$). The $C_{60}^{\cdot-}$ moiety in the charge-separated state gives one electron to the electron carrier such as O_2 of the electrolyte solution, which is thermodynamically possible. And then, the electron flows from ITO through monolayer film to the electrolyte resulting in the observed cathodic photocurrent. No clear photocurrent was produced for the film of the pure traid **2** due to the absence of the electron transfer from **2** to fullerene.

With a change of the excitation of wavelength to the 400–700 nm, a photocurrent action spectrum is obtained (Fig. S2). The incident-photon-to-photocurrent efficiency (IPCE) was obtained through the measure of the generated photocurrent when the electrode was irradiated with monochromatic light, by using the equation:

$$\mathrm{IPCE}(\%) = 100(i_{sc} \times 1240)/(I_{inc}\lambda)$$

where i_{sc} is the short-circuit photocurrent (A cm^{-2}), I_{inc} is the incident light intensity (W cm^{-2}) and λ is the excitation wavelength (nm).[20] The photocurrent action spectrum closely matches the absorption spectrum of the compound **1** modified electrode. (Fig. S2a)

2.5. SEM

The aggregate structure of the traid **1** was also studied by SEM. Figure 5 shows SEM images of a dilute dispersion of **1** in toluene and *p*-xylene (v/v 1:1). The SEM images reveal that the compound assemblies consist of nanofibers structure with a diameter of about 50 nm. We presume that the superstructures of the nanofibers assemblies are constructed by the intermolecular π–π interaction of

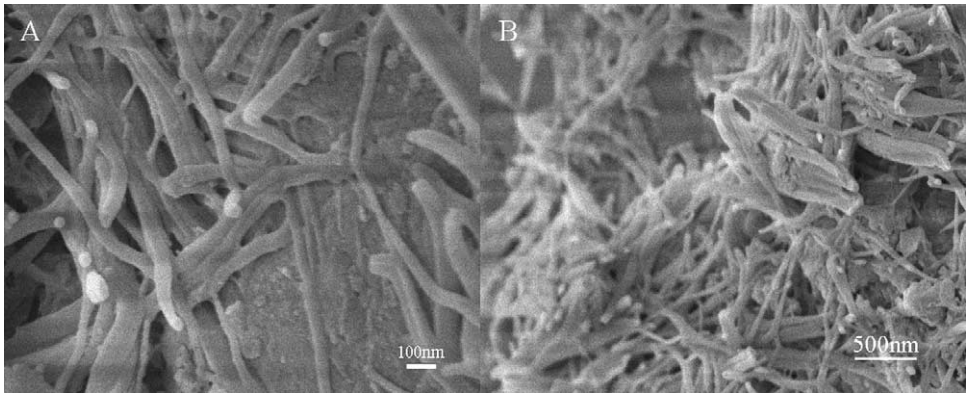


Figure 5. SEM images of compound **1**.

perylene and the additional van der waals attraction of alkyl chains.[11a–e]

3. Conclusion

A new fullerene–perylenebisimide triad have been prepared and characterized by UV–vis spectroscopy, fluorescence spectroscopy, and voltammetry. The SEM image indicated that uniform nanofibers morphology of the triad was formed with a diameter of about 50 nm. The photocurrent generation properites of thin film were measured by a three-electrode cell technique. A steady and prompt photocurrent at irradiation of 20.0 mW cm^{-2} white light was produced by the thin films of the triad, which indicate the electron transfer from perylene to fullerene moiety. This new triad possesses potential application in organic solar cell.

4. Experimental

4.1. Measurements

UV–vis spectra were measured on a Hitachi U-3010 spectrometer. FT-IR spectra were recorded as KBr pellets on a Perkin-Elmer System 2000 spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker ARX100 spectrometer. MALDI-TOF mass spectrometric measurements were performed on Bruker Biflex III MALDI-TOF (both positive and negative ion reflector mode). Cyclic voltammograms (CV) and photocurrent were recorded on CHI660B voltammetric analyzer (CH Instruments, USA). Field emission scanning electron microscopy (FE-SEM) images were taken on a JSM 6700F NT instrument.

4.2. Materials

Unless otherwise stated, reagents were commercially obtained and used without further purification. Compound **4** was synthesized by using a literature procedure.[21] C_{60}–REF **3** was synthesized according to the standard method.[18] The solvents for synthesis were purified before use by the standard methods.

4.2.1. 1,3-Bis[4′-{*N*′-octyl-1,6,7,12-tetrakis-(4-*tert*-butylphenoxy)perylene-3,4:9,10-bis(dicarboxyimide)-*N*-yl}-phenyl-4-yl]-5-(4-aminophenyl)benzene (compound 6). A solution (30 mL) of **4** (52.3 mg, 0.051 mmol), **5** (7.9 mg, 0.023 mmol) and imidazole (1 g, 14.71 mmol) in toluene was refluxed under N_2 for 24 h. The solvent was evaporated, the residue dissolved in dichloromethane and washed with water to remove imidazole. Then the solvent was stripped and the residue was chromatographed on silica gel using dichloromethane–petroleum ether (3/2) as the eluent to afford **6** as red powder (32 mg, 55%). ^1H NMR (300 MHz, $CDCl_3$, TMS): δ=8.24 (s, 8H), 7.79 (d, J=8.3 Hz, 4H), 7.75 (s, 3H), 7.54 (d, J=8.2 Hz, 2H), 7.35 (d, J=8.3 Hz, 4H), 7.22 (d, J=8.1 Hz, 16H), 6.85 (d, J=8.1 Hz, 16H), 6.83 (d, J=8.2 Hz, 2H), 4.10 (t, 4H), 1.74–1.65 (m, 4H), 1.25 (s, 72H), 0.89–0.82 (m, 26H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 162.3, 162.1, 154.7, 151.5, 146.0, 140.4, 133.2, 129.6, 127.6, 126.9, 125.4, 121.3, 121.1, 119.6, 119.1, 118.9, 118.6, 118.5, 118.0, 114.2, 39.4, 33.1, 30.5, 28.4, 26.8, 25.8, 21.3, 12.8. FT-IR (KBr pellet, cm^{-2}): ν 3438, 3042, 2960, 2929, 2851, 1701, 1665, 1589, 1504, 1463, 1432, 1410, 1340, 1312, 1285, 1214, 1174, 1111, 1014, 881, 833, 748, 553, 461. MALDI-TOF MS: *m*/*z*: 2509 $[M+H]^+$. Anal. Calcd for: $C_{168}H_{163}N_5O_{16}$: C, 80.45; H, 6.55; N, 2.79; O, 10.21. Found: C, 80.30; H, 6.27; N, 2.61.

4.2.2. 1,3,5-Tris[4′-{*N*′-octyl-1,6,7,12-tetrakis-(4-*tert*-butylphenoxy)perylene-3,4:9,10-bis(dicarboxyimide)-*N*-yl}phenyl-4-yl]benzene (compound 2). A solution (40 mL) of **4** (72.8 mg, 0.071 mmol), **5** (7.9 mg, 0.023 mmol) and imidazole (1 g, 14.71 mmol) in toluene was refluxed under N_2 for 24 h. The solvent was evaporated, the residue dissolved in dichloromethane and washed with water to remove imidazole. Then the solvent was stripped and the residue was chromatographed on silica gel using dichloromethane–petroleum ester (3/2) as the eluent to afford **2** as red powder (70.0 mg, 85%). ^1H NMR (300 MHz, $CDCl_3$, TMS): δ=8.25 (s, 12H), 7.84 (s, 3H), 7.79 (d, J=7.8 Hz, 6H), 7.35 (d, J=7.8 Hz, 6H), 7.22 (d, J=8.1 Hz, 24H), 6.84 (d, J=8.1 Hz, 24H), 4.10 (t, 6H), 1.74–1.65 (m, 4H), 1.25 (s, 72H), 0.89–0.82 (m, 26H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 162.6, 162.4, 155.0, 151.8, 146.3, 140.7, 140.4, 133.6, 132.0, 128.0, 127.2, 125.6, 121.6, 119.8, 119.4, 119.2, 118.8, 118.3, 39.6, 33.3, 30.7, 28.2, 27.1, 26.1, 21.6, 13.0. FT-IR (KBr pellet, cm^{-2}): ν 3040, 2959, 2926, 2858, 1701, 1665, 1589, 1504, 1462, 1432, 1410, 1340, 1312, 1285, 1214, 1174, 1111, 1014, 881, 833, 748, 553, 461. MALDI-TOF MS: *m*/*z*: 3587 $[M+H]^+$. Anal. Calcd for: $C_{240}H_{234}N_6O_{24}$: C, 80.37; H, 6.56; N, 2.34; O, 10.71. Found: C, 80.10; H, 6.45; N, 2.41.

4.2.3. 1,3-Bis[4′-{*N*′-octyl-1,6,7,12-tetrakis-(4-*tert*-butylphenoxy)perylene-3,4:9,10-bis(dicarboxyimide)-*N*-yl}-phenyl-4-yl]-5-[4′-{(4-formylbenzamide)-*N*-yl}phenyl-4-yl]benzene (compound 7). To a solution of compound **6** (80 mg, 0.032 mmol), and triethylamine (10 μL) in dry CH_2Cl_2 (30 mL), 20 mL of CH_2Cl_2 containing 4-formylbenzoyl chloride[22] (0.032 mmol) was dropwised, then the solution was stirred for 30 min at room temperature under N_2. The solvent was stripped and the residue was chromatographed on silica gel using dichloromethane–methanol (20/1) as the eluent to afford **7** as red powder (30 mg, 35%). ^1H NMR (300 MHz, $CDCl_3$, TMS): δ=10.12 (s, 1H), 8.25 (s, 8H), 8.12 (d, J=8.4 Hz, 2H), 7.97 (d, J=8.4 Hz, 2H), 7.81 (d, J=8.4 Hz, 4H), 7.75 (s, 3H), 7.58 (d, J=8.2 Hz, 2H), 7.36 (d, J=8.3 Hz, 4H), 7.21 (d, J=8.1 Hz, 16H), 6.86 (d, J=8.1 Hz, 16H), 6.83 (d, J=8.2 Hz, 2H), 5.34 (br s, 1H), 4.11 (t, 4H), 1.74–1.65 (m, 4H), 1.26 (s, 72H), 0.89–0.82 (m, 26H). ^{13}C NMR (100 MHz, $CDCl_3$): ^{13}C NMR (100 MHz, $CDCl_3$): δ: 190.0, 169.5, 162.8, 162.6, 155.7, 152.3, 147.5, 141.8, 139.2, 138.3, 134.8, 129.9, 128.4, 126.7, 124.3, 122.3, 124.7, 119.5, 118.1, 118.0, 114.2, 39.5, 33.2, 30.4, 28.9, 26.1, 25.1, 21.9, 13.1. FT-IR (KBr pellet, cm^{-2}): ν 3384, 2957, 2855, 1725, 1700, 1586, 1504, 1434, 1410, 1339, 1284, 1203, 1174, 1108, 1015, 959, 882, 849, 804, 759, 687, 554. MALDI-TOF MS: *m*/*z*: 2641 $[M+H]^+$. Anal. Calcd for: $C_{176}H_{171}N_5O_{18}$: C, 79.94; H, 6.52; N, 2.65; O, 10.89. Found: C, 80.20; H, 6.59; N, 2.71.

4.2.4. Fullerene–perylenebisimide triad 1. Compound **7** (30 mg, 0.011 mmol), glycine (80 mg, 1.1 mmol) and C_{60}

(70 mg, 0.097 mmol) were dissolved in 60 mL of toluene and the solution was refluxed under N_2 for 96 h. After cooling, the solvent was evaporated under reduced pressure. The crude solid was purified on silica gel column using toluene as the eluent (R_f=0.2) to give a black powder (14 mg, 37%). ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ=8.24 (s, 8H), 8.11 (d, J=8.4 Hz, 2H), 7.95 (d, J=8.4 Hz, 2H), 7.80 (d, J=8.4 Hz, 4H), 7.75 (s, 3H), 7.59 (d, J=8.2 Hz, 2H), 7.34 (d, J=8.3 Hz, 4H), 7.22 (d, J=8.1 Hz, 16H), 6.86 (d, J=8.1 Hz, 16H), 6.85 (d, J=8.2 Hz, 2H), 5.80 (s, 1H), 5.05 (d, J=10.2 Hz, 1H), 4.85 (d, J=10.2 Hz, 1H), 4.11 (t, 4H), 1.74–1.65 (m, 4H), 1.26 (s, 72H), 0.89–0.82 (m, 26H). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 163.5, 163.4, 156.1, 156.0, 152.9, 152.8, 147.4, 147.2, 154.2, 146.0, 145.7, 143.0, 142.1, 142.6, 134.7, 129.0, 128.9, 128.2, 126.7, 125.3, 122.6, 122.3, 121.0, 120.3, 120.3, 119.9, 119.5, 39.6, 34.4, 31.9, 28.1, 27.4, 22.7, 21.0, 14.1. FT-IR (KBr pellet, cm^{-2}): ν 3038, 2957, 2858, 1701, 1664, 1589, 1504, 1462, 1432, 1408, 1339, 1311, 1283, 1212, 1172, 1109, 1014, 879, 832, 751, 551, 527. MALDI-TOF MS: m/z: 3375 $[M+H]^+$. Anal. Calcd for: $C_{237}H_{170}N_6O_{17}$: C, 84.37; H, 5.08; N, 2.49; O, 8.06. Found: C, 84.14; H, 5.15; N, 2.51.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2005.10.061.

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5-Alkynyl-2′-deoxyuridines, containing bulky aryl groups: evaluation of structure–anti-HSV-1 activity relationship

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Abstract—Four 5-alkynyl-2′-deoxyuridines containing different bulky substituents and flexible linkers between the triple bond and the aromatic residue have been prepared and tested against HSV-1 in Vero cells. Two nucleosides containing carbonyl groups, 5-(4-benzoylphenoxypropyn-1-yl)-2′-deoxyuridine (**19a**) and 5-(estron-3-yloxypropyn-1-yl)-2′-deoxyuridine (**19c**), showed low cytotoxicity and moderate antiviral activity. The flexible linker appears not to be favorable for antiviral properties of 5-alkynyl-2′deoxyuridines: 5-[(perylen-3-yl)methoxypropyn-1-yl]-2′-deoxyuridine (**19d**) showed considerable cytotoxicity and no antiviral activity in contrast to the active and nontoxic 5-(perylen-3-ylethynyl)-2′-deoxyuridine (**9**), a nucleoside with a rigid triple-bond-connection of the aromatic system to the nucleobase.

1. Introduction

Many antiviral compounds have been found among nucleosides.[1,2] The first antiviral nucleoside, 5-iodo-2′-deoxyuridine, was discovered in 1959.[3] A number of 5-substituted 2′-deoxyuridines was synthesized afterwards,[4,5] including 5-*E*-(2-bromovinyl)-2′-deoxyuridine (BVDU), a compound with remarkable activity against herpes simplex virus (HSV).[6] The first 5-alkynyl-2′-deoxyuridine, the ethynyl derivative **1** (Fig. 1) was prepared nearly three decades ago.[7] Later, various 5-alkynyl-2′-deoxyuridines were synthesized as potential antivirals.[8–12] The lower homologues **1** and **2** showed the highest activity against HSV.[9] The increase in length of the alkyl substituent is not favorable for antiviral properties: the activity decreases in the series **2**>**3**>**4**, and compounds **5** and **6** are completely inactive.[9] It is noteworthy that 5-chloropent-1-ynyl derivative **7** showed considerable activity.[10] Bromo acetylene **8** was also active against HSV, but was inferior in comparison with BVDU. Thus, the synthesis of alkynyl compounds with bulky substituents R seemed to have no prospects in terms of antiviral properties.

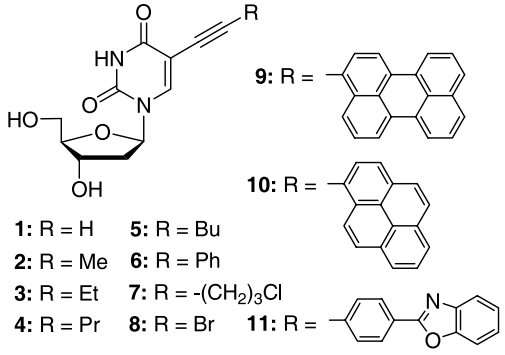

Figure 1. Structures of 5-alkynyl-2′-deoxyuridines studied as anti-HSV compounds.

Recently, we have prepared compounds **9–11** within a fluorescent nucleoside project. Surprisingly, these nucleosides showed pronounced activity against HSV-1, including acyclovir-resistant strains.[13]

The 5-arylethynyl-2′-deoxyuridines **9–11** differ from the inactive nucleoside **6** only by the size and nature of the aryl substituent and therefore constitute a new class of antiviral nucleosides. This finding prompted us to prepare other 5-alkynyl-2′-deoxyuridines with bulky substituents as potential anti-HSV-1 compounds. In the first instance, it was interesting to elucidate how important the direct attachment of a polyaromatic residue to the triple bond is

Keywords: 5-Alkynyl-2′-deoxyuridines; Antiviral nucleosides; Herpes simplex virus.
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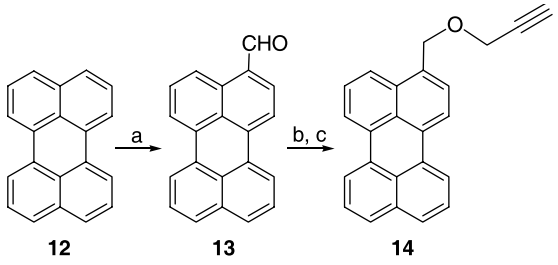
for antiviral activity. To investigate the structure–activity relationship we synthesized and tested nucleosides containing a flexible linker between the triple bond and the aromatic system. The results are reported in this paper.

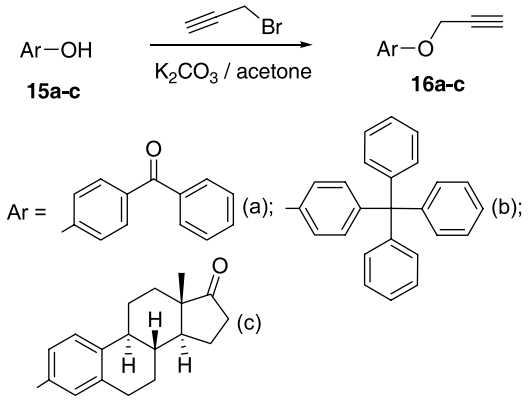
2. Results and discussion

2.1. Synthesis and characterization

The most convenient method for the synthesis of 5-alkynyl-2′-deoxyuridines is Pd/Cu catalyzed Sonogashira coupling of 5-iodonucleosides with alkynes.[12] Therefore, the first step was to prepare the desired acetylenes containing a flexible linker between aromatic residue and ethynyl group. Fluorescent polycyclic aromatic hydrocarbon perylene (**12**) was converted to the acetylene **14** in three steps. The starting hydrocarbon was selectively formylated[14] in the 3 position; the resulting aldehyde **13** was reduced to the alcohol, which was alkylated with propargyl bromide under phase transfer conditions (Scheme 1).



Scheme 1. Synthesis of (perylen-3-ylmethyl)propargyl ether. Reagents and conditions: (a) $Cl_2CHOCH_3/TiCl_4$/1,2-dichlorobenzene; (b) $NaBH_4$/MeOH/THF; (c) $HC{\equiv}CCH_2Br$/50% aq. KOH/dibenzo-18-crown-6/THF.



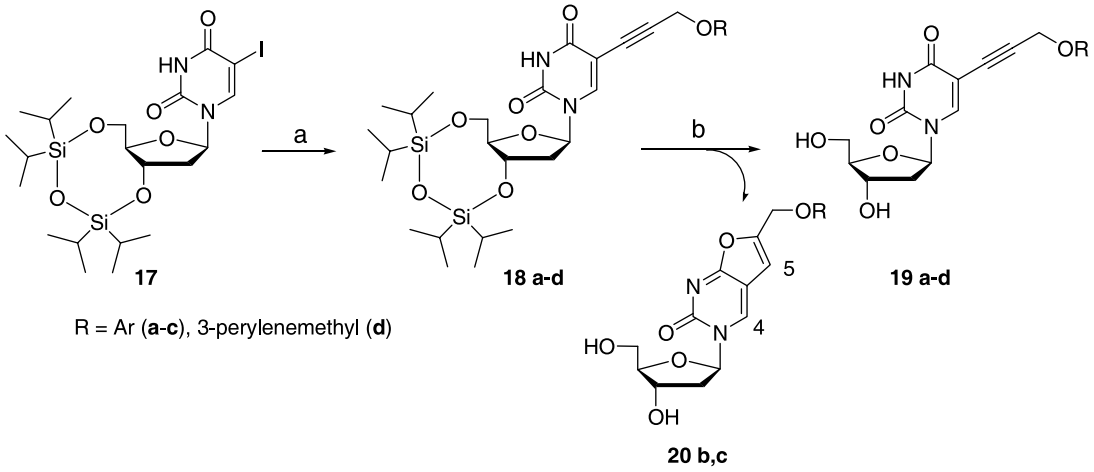
Scheme 2. Preparation of propargyl ethers from 4-hydroxybezophenone, 4-tritylphenol, and estrone.

Other acetylenes **16a–c** were prepared by the alkylation of 4-hydroxybenzophenone (**15a**), 4-tritylphenol (**15b**) and estrone (**15c**) with propargyl bromide in basic conditions (Scheme 2).

An approach to the preparation of 5-alkynyl-2′-deoxyuridines is outlined in Scheme 3. The starting 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-5-iodo-2′-deoxyuridine (**17**) was obtained from 5-iodo-2′-deoxyuridine. The Markiewicz' protection of hydroxyls was chosen to increase solubility and to facilitate the chromatographic purification of coupling products. The Sonogashira coupling was achieved using conditions optimized for nucleosides[15,16] to afford compounds **18a–d**. The silyl protecting group was then removed by treatment of nucleosides **18a–d** with triethylamine trihydrofluoride in THF.[17] Some isomerization was observed in two cases during the reaction: weakly fluorescent byproducts **20b**,**c** were isolated in trace amounts.

Nucleoside **19d** shows bright perylene fluorescence (Fig. 2). In 1,2-dichloroethane, an aprotic and less polar solvent, the excitation and emission maxima of **19d** are 5–8 nm bathochromically shifted as compared to in methanol solution.

This compound, when incorporated into oligonucleotides, could display hybridization sensitive fluorescence changes, because perylene residue after hybridization should be located in the major groove or intercalated between base pairs of DNA duplex, thus increasing the



Scheme 3. Synthesis of 5-alkynylated 2′-deoxyuridines. Reaction conditions and yields: (a) $ROCH_2C{\equiv}CH$ (**16a–c**)/$Pd(PPh_3)_4$/CuI/Et_3N/DMF, 79% (**18a**), 60% (**18b**), 60% (**18c**), 66% (**18d**); (b) $Et_3N{\cdot}3HF$/THF, 77% (**19a**), 77% (**19b**), 90% (**18c**), 89% (**18d**), 2% (**20b**), 7% (**20c**).

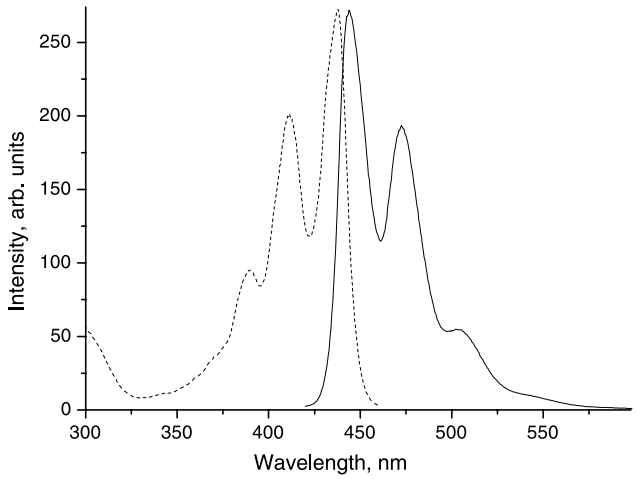


Figure 2. Normalized emission (solid line, λ_{ex} 410 nm) and excitation (dashed line, λ_{em} 480 nm) spectra of perylene nucleoside **19d** in MeOH.

probability of nonradiative quenching of the excited state of the fluorophore. Experiments with oligonucleotides containing **19d** are in progress and results will be reported elsewhere.

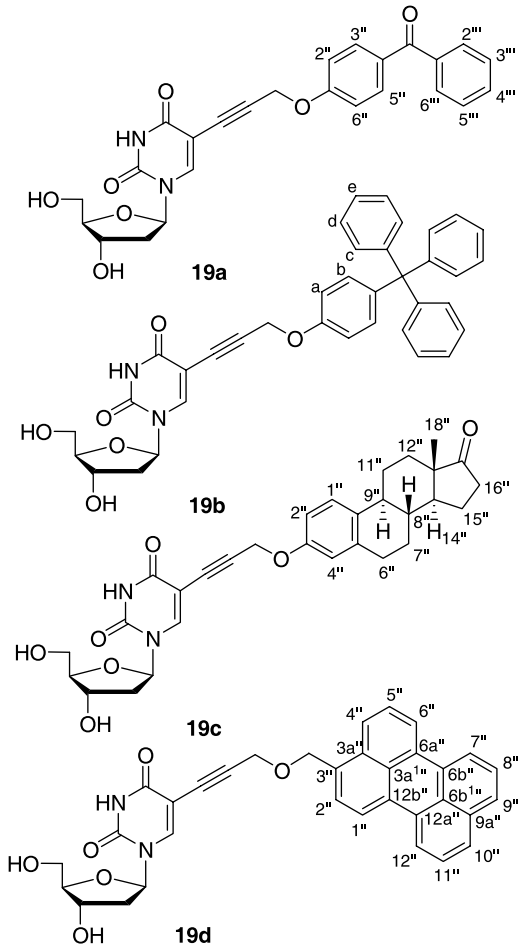


Figure 3. Structures of 5-alkynyl-2′-deoxyuridines tested as anti-HSV-1 compounds.

2.2. Pharmacology

The purified nucleosides **19a–d** (Fig. 3) were tested for cytotoxicity and anti-HSV-1 activity in Vero cells. The results are given in Table 1.

Table 1. Cytotoxic and antiviral activities of 5-alkynyl-2′-deoxyuridines in Vero cell culture (μg/mL)[a]

Compound	CD_{50}	HSV-1		HSV-1/ACV^R		HSV-1/ (ACV+PAA)^R	
		ID_{50}	ID_{95}	ID_{50}	ID_{95}	ID_{50}	ID_{95}
19a	250	31.3	>250	31.3	>250	62.5	>250
19b	15.6	15.6	>15.6	15.6	>15.6	—	—
19c	250	31.3	62.5	62.5	125	62.5	250
19d	62.5	62.5	>62.5	62.5	>62.5	62.5	>62.5
9[b]	250	7.8	15.6	31.2	62.5	—	—
ACV	>400	0.45	0.90	>400	>400	>400	>400

[a] HSV-1—herpes simplex virus type 1, strain L_2; HSV-1/ACV^R—ACV-resistant strain of HSV-1; HSV-1/(ACV+PAA)^R—ACV- and PAA-resistant strain of HSV-1; CD_{50}—cytotoxic dose causing 50% growth inhibition of Vero cells; ID_{50} and ID_{95}—doses inhibiting the cytopathogenic effect of virus by 50 and 95%, respectively.

[b] Data for the compound **9** were taken from a published paper.[13]

Compounds **19b** and **19d** with hydrophobic tetraphenyl and perylene residues show high cytotoxicity. Benzophenone derivative **19a** and estrone derivative **19c**, carrying polar carbonyl groups on 5-modification residues, are much less cytotoxic. Nucleosides **19a**,**c** show moderate but reliable antiviral activity. They also show similar activity against ACV(acyclovir)-resistant and ACV/PAA(phosphonoacetic acid)-resistant strains of HSV-1. The data confirm that their mode of action differs from that of ACV or PAA. A non-nucleoside mechanism, postulated for some lipophilic nucleoside analogs,[18–21] can be assumed for their antiviral action. One can see that the insertion of the three-atom linker CH_2OCH_2 in nucleoside **9** between the triple bond and the perylene, giving nucleoside **19d**, leads to a considerable increase in cytotoxicity and a decrease in antiviral activity. A conclusion can be made, that direct rigid attachment of a bulky aromatic residue to a nucleobase is important for antiviral properties.

To define more exactly how the antiviral activity can be attributed to the bulky aromatic residue, several compounds containing polycyclic residues—quasinucleosides **21a**,**b**[22] and nucleoside-2′-carbamate derivatives **22**,[17] **23**,[23] **24**, **25**[24] (Fig. 4)—were also tested against HSV-1 and showed no detectable activity. This confirms that modification of the 5 position of pyrimidine is essential for biological activity.

3. Conclusions

The obtained results support the suggestion that 5-alkynyl-2′-deoxyuridines with bulky aryl groups belong to a new class of anti-HSV-1 agents with moderate activity and probably non-nucleoside mode of action in the cell. A flexible linker between the aromatic and the nucleobase increases the cytotoxicity and decreases the antiviral activity of the nucleoside. Further investigations to optimize

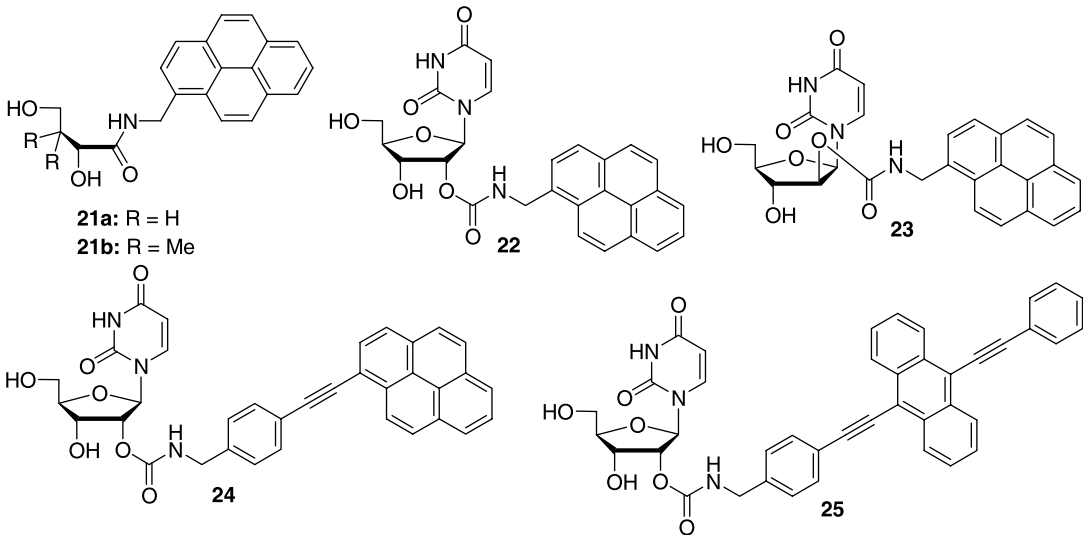


Figure 4. Polycyclic aromatic hydrocarbon derivatives inactive against HSV-1.

the structure of the aromatic substituent to achieve maximum antiviral activity are now in progress.

4. Experimental

4.1. General

^{1}H NMR spectra (500 MHz) and ^{13}C NMR spectra (125.7 MHz) were recorded on a Bruker DRX-500 spectrometer and referenced to $CDCl_3$ (7.25 ppm) and DMSO-d_6 (2.50 ppm for ^{1}H and 39.60 ppm for ^{13}C). ESI-MS analyses in positive ion mode were performed using a Finnigan LCQ Advantage quadrupole ion trap mass spectrometer (4.5 kV, 50 μl/min, N_2, methanol). EI-MS analyses in positive ion mode were performed using a Finnigan Polaris Q ion trap mass spectrometer (the temperature of ion source 150 °C, the energy of ionization 70 eV). EI-TOF HRMS and ESI-TOF HRMS spectra in positive ion mode were using Micromass LCT reflection TOF mass spectrometer. UV spectra were recorded using LKB Ultrospec III spectrophotometer. IR spectra were recorded using Bruker Vector 22 spectrometer. Fluorescence spectra were obtained using a Varian Cary Eclipse Fluorescence Spectrophotometer (excitation and emission slits 5 nm, concentrations 2×10^{-7} M (**19d**) and 1×10^{-5} M (**20b**,**c**)). Melting points were determined using a Boetius heating table and are uncorrected. Analytical thin-layer chromatography was performed on the Kieselgel 60 F_{254} precoated aluminium plates (Merck), spots were visualized under UV light (254 nm). Silica gel column chromatography was performed using Merck Kieselgel 60 0.040–0.063 mm.

4.2. Reagents and solvents

Reagents obtained from commercial suppliers were used as received. Copper (I) iodide, estrone, 5-iodo-2′-deoxyuridine, perylene, propargyl bromide (80% w/w solution in toluene), triethylamine trihydrofluoride, 4-tritylphenol were from Aldrich; 18-crown-6 and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane were from Fluka; 4-hydroxybenzophenone was from Lancaster, triethylamine was from Acros; $TiCl_4$ and $NaBH_4$ were from Reakhim (Russia). Dichloromethyl methyl ether,[14] $Pd(PPh_3)_4$,[25] and compounds **21a**,**b**,[22] **22**,[17] **23**,[23] **24**, **25**[24] were prepared as described. Solvents were from Chimmed (Russia), mainly HPLC grade and used without further purification unless otherwise noted. DCM and pyridine were always used freshly distilled over CaH_2. THF was freshly distilled over powdered $LiAlH_4$ and stored over 4 Å molecular sieves under nitrogen. DMF and 1,2-dichlorobenzene were freshly distilled under reduced pressure.

4.2.1. 3-Formylperylene (13). To a magnetically stirred ice cooled suspension of perylene (500 mg, 1.98 mmol) in 1,2-dichlorobenzene (30 mL) 1,1-dichloromethyl methyl ether (232 μL, 2.57 mmol) (CAUTION: CARCINOGEN) and $TiCl_4$ (326 μL, 2.97 mmol) were subsequently added. The mixture was stirred for 1 h, then allowed to warm to room temperature and poured on ice (100 g) and concd HCl (5 mL). The organic layer was diluted with chloroform (100 mL), washed with 5% HCl (100 mL), water (3×100 mL), dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel in $CHCl_3$ to give the desired compound as orange crystals (398 mg, 72%). R_f 0.51 ($CHCl_3$); mp 236 °C ($CHCl_3$) (mp 236 °C).[26] EI MS: $m/z=280$ $[M]^+$, calcd for $[C_{21}H_{12}O]^+$280. ^{1}H NMR ($CDCl_3$): 10.25 (s, 1H, C*H*O); 9.08 (d, 1H, $J=8.3$ Hz, H-4); 8.24–8.12 (m, 4H, H-1, H-6, H-7, H-12); 7.82 (d, 1H, $J=7.8$ Hz, H-10); 7.76 (d, 1H, $J=7.8$ Hz, H-9); 7.69 (d, 1H, $J=8.0$ Hz, H-2); 7.61 (m, 1H, H-5); 7.48 (app. t, 2H, $J_{7,8}=J_{8,9}=J_{10,11}=J_{11,12}=7.8$ Hz, H-8, H-11).

4.2.2. (Perylen-3-ylmethyl)propargyl ether (14). To the solution of 3-formylperylene (500 mg, 1.79 mmol) in the mixture of THF (50 mL) and methanol (25 mL) sodium borohydride (70 mg, 1.85 mmol) was added. The mixture was stirred for 30 min, diluted with water (50 mL), and pH was adjusted to 1–2 with concd HCl. The intermediate alcohol was extracted with $CHCl_3$ (300 mL), organic layer was washed with water (2×100 mL) and saturated solution of $NaHCO_3$ (100 mL), dried over Na_2SO_4, and evaporated. The residue and 18-crown-6 (50 mg, 0.19 mmol) were

dissolved in THF (50 mL), and 50% aq KOH (50 mL) was added under vigorous stirring. The mixture was stirred for 30 min, and 80% propargylbromide in toluene (1.35 g, 8.5 mmol) was added. The stirring was continued for 2 h, then organic layer was separated, evaporated, the residue was dissolved in chloroform (300 mL), washed with water (3×100 mL), dried over Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography on silica gel in hexane–toluene (1/1 v/v) to give the desired compound as yellow crystals (533 mg, 93%). R_f 0.30 (toluene); mp 168–169 °C (toluene–hexane). EI MS: m/z=320 $[M]^+$, calcd for $[C_{24}H_{16}O]^+$320. EI-TOF HRMS: m/z=320.1207 $[M]^+$, calcd for $[C_{24}H_{16}O]^+$ 320.1196. IR (KBr): 3281 cm^{-1}, ν (≡CH). ^1H NMR (DMSO-d_6): 8.42–8.29 (m, 4H, H-1, H-6, H-7, H-12); 7.95 (d, 1H, J=8.4 Hz, H-4); 7.80 (d, 2H, $J_{8,9}=J_{10,11}$=8.1 Hz, H-9, H-10); 7.60 (m, 1H, H-5); 7.54 (m, 3H, H-2, H-8, H-11); 4.92 (s, 2H, ArCH_2); 4.29 (d, 2H, ^4J=2.3 Hz, CH_2C≡); 3.54 (t, 1H, ^4J=2.3 Hz, ≡CH). ^{13}C NMR (DMSO-d_6): 134.26 (C9a), 133.01 (C3), 132.63 (C3a), 130.87 (C6a), 130.48 (C6b), 130.32 (C12a), 128.20 (C3a1), 128.11, 128.06 (C9, C10), 127.76 (C12b), 127.70 (C6b1), 127.07, 126.95 (3C) (C2, C5, C8, C11), 124.11 (C4), 120.92, 120.87, 120.79 (C6, C7, C12), 120.09 (C1), 80.27 (CH≡C), 77.66 (HC≡), 69.28 (ArCH$_2$), 57.07 (CH$_2$C≡).

4.3. General procedure for the preparation of aryl propargyl ethers (16a–c)

To a solution or a suspension of a corresponding phenol (10.0 mmol) in dry acetone (150 mL), dry K_2CO_3 (14 g, 0.1 mol) and 80% propargylbromide in toluene (2.23 g, 15 mmol) were added, and the mixture was stirred overnight, then filtered, and the solid was washed with acetone. The combined filtrate was evaporated, and the residue was dissolved in $CHCl_3$ (100 mL), filtered, and evaporated. The residue was chromatographed on silica gel in appropriate solvent system.

4.3.1. 4-Propargyloxybenzophenone (16a). The title compound was prepared from 4-hydroxybenzophenone and purified using a 0→3% gradient of EtOAc in toluene (v/v) to give the desired product as colorless crystals (2.22 g, 94%). R_f 0.56 (10% EtOAc in toluene v/v); mp 71–72 °C (ethanol) (mp 72–73 °C).[27] EI MS: m/z=237 $[M+H]^+$, calcd for $[C_{16}H_{12}O_2+H]^+$237. ^1H NMR (DMSO-d_6): 7.76 (d, 2H, J=8.8 Hz, H-2, H-6); 7.70 (m, 2H, H-2′, H-6′); 7.65 (m, 1H, H-4′); 7.55 (m, 2H, H-3′, H-5′); 7.14 (d, 2H, J=8.8 Hz, H-3, H-5); 4.93 (d, 2H, ^4J=2.4 Hz, CH_2); 3.64 (t, 1H, ^4J=2.4 Hz, ≡CH).

4.3.2. 4-(Propargyloxy)tetraphenylmethane (16b). The title compound was prepared from 4-tritylphenol; the reaction time was 48 h; the desired compound was purified by chromatography on silica gel in $CHCl_3$. Yield 2.39 g (64%), colorless crystals. R_f 0.57 (toluene); mp 162–163 °C (hexane–toluene). EI MS: m/z=374 $[M]^+$, calcd for $[C_{28}H_{22}O]^+$374. EI-TOF HRMS: m/z=374.1674 $[M]^+$, calcd for $[C_{28}H_{22}O]^+$374.1665. IR (KBr): 3305 cm^{-1}, ν (≡CH). ^1H NMR (DMSO-d_6): 7.29 (m, 6H, H_d); 7.19 (m, 3H, H_e); 7.13 (m, 6H, H_c); 7.05 (d, 2H, $J_{a,b}$=8.9 Hz, H_b); 6.91 (d, 2H, $J_{a,b}$=8.9 , H_a); 4.75 (d, 2H, ^4J=2.3 Hz, CH_2); 3.55 (t, 1H, ^4J=2.3 Hz, ≡CH). ^{13}C NMR (DMSO-d_6): 155.24 (CO), 146.61 (3C, C_6H_4CC), 139.12 (Ph_3CC), 131.57 (2C, Cb), 130.47 (6C, Cc), 127.70 (6C, Cd), 125.96 (3C, Ce), 113.84 (2C, Ca), 79.32 (C≡CH), 78.24 (≡CH), 63.85 (Ph_3C), 55.39 (CH_2).

4.3.3. O^3-Propargylestrone (16c). The title compound was prepared from estrone (946 mg, 3.50 mmol), 80% propargyl bromide (790 mg, 5.3 mmol) and K_2CO_3 (3.40 g, 24.5 mmol) in dry acetone (50 mL) and chromatographed on silica gel using a 0→5% gradient of EtOAc in toluene (v/v). Yield 868 mg (80%), white solid. R_f 0.46 (10% EtOAc in toluene (v/v)); mp 149–150 °C (hexane) (mp 146–149 °C,[28] 155–158 °C[29]). EI MS: m/z=308 $[M]^+$, calcd for $[C_{21}H_{24}O_2]^+$308. ^1H NMR (DMSO-d_6): 7.19 (d, 1H, $J_{1,2}$=8.6 Hz, H-1); 6.74 (dd, 1H, $^4J_{2,4}$=2.4 Hz, $J_{1,2}$=8.6 Hz, H-2); 6.68 (d, 1H, $^4J_{2,4}$=2.4 Hz, H-4); 4.72 (d, 2H, ^4J=2.2 Hz, CH_2O); 3.51 (t, 1H, ^4J=2.2 Hz, ≡CH); 2.81 (m, 2H, H-6); 2.43 (dd, 1H, ^2J=18.7 Hz, J=8.2 Hz, H-16); 2.33 (m, 1H, H-11); 2.07 (m, 1H, H-9); 1.97 (m, 1H, H-16); 1.95 (m, 2H, H-7, H-15); 1.76 (m, 1H, H-12); 1.51 (m, 3H, H-8, H-14, H-15); 1.36 (m, 3H, H-7, H-11, H-12); 0.83 (s, 3H, H-18).

4.3.4. 3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-5-iodo-2′-deoxyuridine (17). 5-Iodo-2′-deoxyuridine (5.00 g, 14.1 mmol) was suspended in dry pyridine (90 mL) and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (5.07 mL, 15.9 mmol) was added in one portion with stirring and the solution was kept overnight at ambient temperature. The mixture was evaporated, and the residue was diluted with EtOAc (400 mL), the solution was washed with water (2×200 mL), 5% $NaHCO_3$ (100 mL) and water again (100 mL), dried over Na_2SO_4 and evaporated. The residue was chromatographed on silica gel (gradient 0→10% EtOAc in $CHCl_3$ (v/v)) to afford the desired product (7.12 g, 84%) as a white crystalline solid. R_f 0.34 (30% EtOAc in $CHCl_3$ (v/v)); mp 174–176 °C (toluene–hexane) (mp 182 °C).[30] ESI MS: m/z=619.3 $[M+Na]^+$, calcd for $[C_{21}H_{37}IN_2O_6Si_2+Na]^+$619.6. ^1H NMR (DMSO-d_6): 11.67 (s, 1H, NH); 7.94 (s, 1H, H-6); 5.93 (dd, 1H, $J_{1′,2′\alpha}$=7.6 Hz, $J_{1′,2′\beta}$=3.0 Hz, H-1′); 4.53 (m, 1H, H-3′); 4.02 (m, 1H, $^2J_{5′a,5′b}$=12.4 Hz, $J_{4′,5′a}$=4.6 Hz, H-5′a); 3.93 (m, 1H, $^2J_{5′a,5′b}$=12.4 Hz, $J_{4′,5′b}$=3.0 Hz, H-5′b); 3.71 (m, 1H, H-4′); 2.46 (m, 1H, H-2′α); 2.30 (m, 1H, H-2′β); 1.14–0.97 (m, 28H, Pr^i).

4.4. General procedure for the preparation of 3′,5′-*O*-silyl protected 5-alkynylated 2′-deoxyuridines (18a–d)

To a solution of 5-iodo-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-deoxyuridine (1.193 g, 2.00 mmol) and appropriate acetylene (2.5 mmol) in DMF (30 mL) under argon $Pd(PPh_3)_4$ (231 mg, 0.20 mmol), CuI (76 mg, 0.40 mmol) and triethylamine (560 μL, 3.0 mmol) were successively added, and the reaction mixture was stirred for 16 h at room temperature. The mixture was then diluted with EtOAc (200 mL), washed with 3% aq EDTA-$(NH_4)_2$ (4×100 mL) and water (4×100 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was chromatographed on a silica gel column in appropriate solvent.

4.4.1. 3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-5-(4-benzoylphenoxypropyn-1-yl)-2′-deoxyuridine (18a). The title compound was purified in 20% EtOAc in toluene (v/v); white amorphous solid (1.12 g, 79%); R_f 0.30 (30% EtOAc in toluene). ESI MS: m/z=727.6 $[M+Na]^+$, calcd for $[C_{37}H_{48}N_2O_8Si_2+Na]^+$727.9. ESI-TOF HRMS: m/z=705.3116 $[M+H]^+$, 727.2923 $[M+Na]^+$, calcd for $[C_{37}H_{48}N_2O_8Si_2+H]^+$705.3022, $[C_{37}H_{48}N_2O_8Si_2+Na]^+$727.2841. ^{1}H NMR (DMSO-d_6): 11.67 (s, 1H, N*H*); 7.90 (s, 1H, *H*-6); 7.76 (d, 2H, J=8.7 Hz, H-3″, H-5″); 7.69 (m, 2H, H-2‴, H-6‴); 7.65 (m, 1H, H-4‴); 7.55 (m, 2H, H-3‴, H-5‴); 7.16 (d, 2H, J=8.7 Hz, H-2″, H-6″); 5.94 (dd, 1H, $J_{1′,2′\alpha}$=7.3 Hz, $J_{1′,2′\beta}$=2.5 Hz, H-1′); 5.10 (m, 2H, ≡CC*H*$_2$); 4.49 (m, 1H, *H*-3′); 4.04 (m, 1H, $^2J_{5′a,5′b}$=12.7 Hz, $J_{4′,5′a}$=4.2 Hz, H-5′a); 3.91 (m, 1H, $^2J_{5′a,5′b}$=12.7 Hz, $J_{4′,5′b}$=2.9 Hz, H-5′b); 3.72 (m, 1H, H-4′); 2.47 (m, 1H, H-2′α); 2.32 (m, 1H, H-2′β); 1.08–0.94 (m, 28H, Pr^i). ^{13}C NMR (DMSO-d_6): 194.41 (Ph*C*O), 161.57 (C4), 161.09 (C1″), 149.18 (C2), 144.29 (C6), 137.70 (C1‴), 132.20 (C4‴), 132.06 (2C, C3″, 5″), 130.02 (C4″), 129.28 (2C, C2‴, 6‴), 128.49 (2C, C3‴, 5‴), 114.72 (2C, C2″, 6″), 97.15 (C5), 87.24 (CH$_2$C≡*C*), 84.38 (C4′), 84.29 (C1′), 79.96 (CH$_2$*C*≡), 68.80 (C3′), 60.79 (C5′), 56.62 (≡C*C*H$_2$), 38.59 (C2′), 17.37, 17.21 (3C), 17.07, 16.91 (2C), 16.82 (*C*H$_3$), 12.76, 12.47, 12.02, 11.95 (Si*C*).

4.4.2. 3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-5-[4-(triphenylmethyl)phenoxypropyn-1-yl]-2′-deoxyuridine (18b). The title compound was purified using a 0→20% gradient of EtOAc in $CHCl_3$ (v/v); white amorphous solid (1.01 g, 60%); R_f 0.41 (30% EtOAc in $CHCl_3$ (v/v)). ESI MS: m/z=865.8 $[M+Na]^+$, calcd for $[C_{49}H_{58}N_2O_7Si_2+Na]^+$866.2. ESI-TOF HRMS: m/z=843.3909 $[M+H]^+$, calcd for $[C_{49}H_{58}N_2O_7Si_2+H]^+$843.3855. ^{1}H NMR (DMSO-d_6): 11.65 (s, 1H, N*H*); 7.89 (s, 1H, H-6); 7.29 (m, 6H, H$_d$); 7.20 (m, 3H, H$_e$); 7.13 (m, 6H, H$_c$); 7.03 (m, 2H), 6.92 (m, 2H) (H$_a$, H$_b$); 5.94 (dd, 1H, $J_{1′,2′\alpha}$=7.6 Hz, $J_{1′,2′\beta}$=2.8 Hz, H-1′); 4.92 (m, 2H, ArOC*H*$_2$); 4.49 (m, 1H, H-3′); 4.02 (m, 1H, $^2J_{5′a,5′b}$=12.8 Hz, $J_{4′,5′a}$=4.3 Hz, H-5′a); 3.91 (m, 1H, $^2J_{5′a,5′b}$=12.8 Hz, $J_{4′,5′b}$=3.0 Hz, H-5′b); 3.72 (m, 1H, H-4′); 2.46 (m, 1H,[†] H-2′α); 2.31 (m, 1H, H-2′β); 1.07–0.96 (m, 28H, Pr^i). ^{13}C NMR (DMSO-d_6): 161.59 (C4), 155.53 (≡CCH$_2$O*C*), 149.18 (C2), 146.61 (3C, C$_6$H$_4$C*C*), 144.16 (C6), 139.08 (Ph$_3$C*C*), 131.59 (2C, Cb), 130.48 (6C, Cc), 127.68 (6C, Cd), 125.98 (3C, Ce), 113.85 (2C, Ca), 97.33 (C5), 87.88 (CH$_2$C≡*C*), 84.38 (C4′), 84.23 (C1′), 79.44 (CH$_2$*C*≡), 68.85 (C3′), 63.86 (Ph$_3$*C*), 60.84 (C5′), 56.31 (≡C*C*H$_2$), 38.60 (C2′), 17.38, 17.20 (3C), 17.07, 16.91 (2C), 16.82 (*C*H$_3$), 12.76, 12.48, 12.05, 11.96 (Si*C*).

4.4.3. 3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-5-(estron-3-yloxypropyn-1-yl)-2′-deoxyuridine (18c). The title compound was purified using a 0→20% gradient of EtOAc in toluene (v/v); white amorphous solid (934 mg, 60%); R_f 0.25 (30% EtOAc in toluene (v/v)). ESI MS: m/z=799.8 $[M+Na]^+$, calcd for $[C_{42}H_{60}N_2O_8Si_2+Na]^+$800.1. ESI-TOF HRMS: m/z=777.4019 $[M+H]^+$, calcd for $[C_{42}H_{60}N_2O_8Si_2+H]^+$777.3961. ^{1}H NMR (DMSO-d_6): 11.65 (s, 1H, N*H*); 7.88 (s, 1H, H-6); 7.18 (d, 1H, $J_{1″,2″}$=8.6 Hz, H-1″); 6.75 (dd, 1H, $^4J_{2″,4″}$=2.5 Hz, $J_{1″,2″}$=8.6 Hz, H-2″); 6.72 (m, 1H, H-4″); 5.94 (dd, 1H, $J_{1′,2′\alpha}$=7.6 Hz, $J_{1′,2′\beta}$=2.7 Hz, H-1′); 4.88 (m, 2H, ArOC*H*$_2$); 4.50 (m, 1H, H-3′); 4.04 (m, 1H, $^2J_{5′a,5′b}$=12.7 Hz, $J_{4′,5′a}$=4.1 Hz, H-5′a); 3.92 (m, 1H, $^2J_{5′a,5′b}$=12.7 Hz, $J_{4′,5′b}$=2.7 Hz, H-5′b); 3.72 (m, 1H, H-4′); 2.82 (m, 2H, H-6″); 2.48–2.40 (m, 2H, H-2′α, H-16″ (^2J=19.7 Hz, $J_{15″,16″}$=8.5 Hz)); 2.37–2.28 (m, 2H, H-2′β, H-11″); 2.19 (m, 1H, H-9″); 2.07 (m, 1H, H-16″); 1.98 (m, 2H, H-7″, H-15″); 1.76 (m, 1H, H-12″); 1.55 (m, 3H, H-8″, H-14″, H-15″); 1.38 (m, 3H, H-7″, H-11″, H-12″); 1.10–0.94 (m, 28H, Pr^i); 0.83 (s, 3H, H-18″). ^{13}C NMR (DMSO-d_6): 219.58 (C17″), 161.58 (C4), 155.35 (C3″), 149.18 (C2), 144.05 (C6), 137.48 (C5″), 132.47 (C10″), 126.22 (C1″), 114.58 (C4″), 112.38 (C2″), 97.41 (C5), 88.15 (CH$_2$C≡*C*), 84.38, 84.17 (C1′, C4′), 79.18 (CH$_2$*C*≡), 68.91 (C3′), 60.87 (C5′), 56.08 (*C*H$_2$C≡), 49.63 (C14″), 47.34 (C13″), 43.49 (C9″), 38.61 (C2′), 37.85 (C8″), 35.39 (C16″), 31.39 (C12″), 29.20 (C6″), 26.06 (C7″), 25.51 (C11″), 21.17 (C15″), 17.38, 17.23 (3C), 17.07, 16.91 (2C), 16.82 (*C*H$_3$), 13.53 (C18″), 12.77, 12.50, 12.06, 11.96 (Si*C*).

4.4.4. 3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-5-[(perylen-3-yl)methoxypropyn-1-yl]-2′-deoxyuridine (18d). The title compound was purified using a 10→50% gradient of EtOAc in $CHCl_3$ (v/v); yellow amorphous solid (1.044 g, 66%); R_f 0.26 (30% EtOAc in $CHCl_3$ (v/v)). ESI MS: m/z=811.7 $[M+Na]^+$, calcd for $[C_{45}H_{52}N_2O_7Si_2+Na]^+$812.1. ESI-TOF HRMS: m/z=789.3338 $[M+H]^+$, 811.3230 $[M+Na]^+$, calcd for $[C_{45}H_{52}N_2O_7Si_2+H]^+$789.3386, $[C_{45}H_{52}N_2O_7Si_2+Na]^+$811.3205. ^{1}H NMR (DMSO-d_6): 11.68 (s, 1H, N*H*); 8.42–8.30 (m, 4H, H-1″, H-6″, H-7″, H-12″); 7.99 (d, 1H, J=8.2 Hz, H-4″); 7.91 (s, 1H, H-6); 7.80 (d, 2H, $J_{8,9}$=$J_{10,11}$=8.0 Hz, H-9″, H-10″); 7.62–7.52 (m, 4H, H-2″, H-5″, H-8″, H-11″); 5.95 (dd, 1H, $J_{1′,2′\alpha}$=7.4 Hz, $J_{1′,2′\beta}$=2.6 Hz, H-1′); 4.96 (s, 2H, ArC*H*$_2$); 4.49 (m, 1H, H-3′); 4.46 (s, 2H, ≡CC*H*$_2$); 4.04 (m, 1H, $^2J_{5′a,5′b}$=12.7 Hz, $J_{4′,5′a}$=4.3 Hz, H-5′a); 3.90 (m, 1H, $^2J_{5′a,5′b}$=12.7 Hz, $J_{4′,5′b}$=2.7 Hz, H-5′b); 3.71 (m, 1H, H-4′); 2.46 (m, 1H, H-2′α); 2.32 (m, 1H, H-2′β); 1.04–0.93 (m, 28H, Pr^i). ^{13}C NMR (DMSO-d_6): 161.71 (C4), 149.23 (C2), 143.76 (C6), 134.28 (C9a″), 133.11 (C3″), 132.68 (C3a″), 130.88 (C6a″), 130.52 (C6b″), 130.35 (C12a″), 128.83, 128.74, 128.23, 128.06, 127.72 (C3a1″, C6b1″, C9″, C10″, C12b″), 127.10, 126.95 (3C) (C2″, C5″, C8″, C11″), 124.24 (C4″), 120.91 (2C), 120.79 (C6″, C7″, C12″), 120.07 (C1″), 97.69 (C5), 88.95 (CH$_2$C≡*C*), 84.36 (C4′), 84.18 (C1′), 78.98 (CH$_2$*C*≡), 69.25 (C3′), 68.86 (ArCH$_2$), 60.81 (C5′), 57.71 (*C*H$_2$C≡), 38.60 (C2′), 17.33, 17.18 (3C), 17.07, 16.89 (2C), 16.81 (*C*H$_3$), 12.76, 12.45, 12.02, 11.94 (Si*C*).

4.5. General procedure for the preparation of 5-alkynylated 2′-deoxyuridines (19a–d)

To a solution of corresponding 5-alkynyl-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-deoxyuridine (1.00 mmol) in THF (3 mL) in a teflon flask triethylamine trihydrofluoride (0.41 mL, 2.50 mmol) was added in one portion and the mixture was left for 12 h at room temperature, then diluted with hexane (25 mL). The upper layer was removed, and the residue was washed with toluene/hexane mixture, 1:1 (25 mL (v/v)) by decantation, and the residue was dissolved in chloroform (50 mL). The solution was washed

[†] Calculated value; the signal of solvent is also present in the region.

with water (3×50 mL), dried over Na_2SO_4, and evaporated. The residue was chromatographed on a silica gel column in appropriate solvent.

4.5.1. 5-(4-Benzoylphenoxypropyn-1-yl)-2′-deoxyuridine (19a). The title compound was purified using a 0→5% gradient of MeOH in $CHCl_3$ (v/v); colorless crystals (354 mg, 77%); mp 98–100 °C ($CHCl_3$–hexane); R_f 0.43 (12% MeOH in $CHCl_3$ (v/v)). ESI MS: m/z=485.3 $[M+Na]^+$, calcd for $[C_{25}H_{22}N_2O_7+Na]^+$485.4. ESI-TOF HRMS: m/z=485.1411 $[M+Na]^+$, calcd for $[C_{25}H_{22}N_2O_7+Na]^+$485.1319. UV (MeOH): λ_{min}, nm (log ε) 254 (4.29); λ_{max}, nm (log ε) 290 (4.51). ^{1}H NMR (DMSO-d_6): 11.64 (s, 1H, N*H*); 8.30 (s, 1H, H-6); 7.76 (d, 2H, J=8.8 Hz, H-3″,5″); 7.70 (m, 2H, H-2‴,6‴); 7.66 (m, 1H, H-4‴); 7.55 (m, 2H, H-3‴,5‴); 7.17 (d, 2H, J=8.8 Hz, H-2″,6″); 6.09 (app. t, 1H, $J_{1',2'\alpha}=J_{1',2'\beta}$=6.4 Hz, H-1′); 5.22 (d, 1H, $J_{3',OH}$=4.5 Hz, 3′-O*H*); 5.10 (m, 3H, ≡CC*H*$_2$, 5′-O*H*); 4.23 (m, 1H, H-3′); 3.80 (m, 1H, H-4′); 3.66–3.52 (m, 2H, H-5′); 2.14 (m, 2H, H-2′). ^{13}C NMR (DMSO-d_6): 194.50 (Ph*C*O), 161.56 (C4), 161.08 (C1″), 149.46 (C2), 144.82 (C6), 137.73 (C1‴), 132.22 (C4‴), 132.13 (2C, C3″, 5″), 130.05 (C4″), 129.35 (2C, C2‴, 6‴), 128.53 (2C, C3‴, 5‴), 114.80 (2C, C2″, 6″), 97.30 (C5), 87.75 (C4′), 87.08 (CH$_2$C≡*C*), 85.03 (C1′), 80.22 (CH$_2$*C*≡), 70.08 (C3′), 60.96 (C5′), 56.69 (≡C*C*H$_2$), 40.29 (C2′).

4.5.2. 5-[4-(Triphenylmethyl)phenoxypropyn-1-yl]-2′-deoxyuridine (19b). The title compound was purified using a 0→5% gradient of MeOH in $CHCl_3$ (v/v); colorless crystals (464 mg, 77%); mp 114–116 °C ($CHCl_3$–hexane); R_f 0.49 (12% MeOH in $CHCl_3$ (v/v)). ESI MS: m/z=623.4 $[M+Na]^+$, calcd for $[C_{37}H_{32}N_2O_6+Na]^+$623.6. ESI-TOF HRMS: m/z=601.2388 $[M+H]^+$, calcd for $[C_{37}H_{32}N_2O_6+H]^+$601.2333. UV (MeOH): λ_{min}, nm (log ε) 258 (4.02); λ_{max}, nm (log ε) 287 (4.23). ^{1}H NMR (DMSO-d_6): 11.63 (s, 1H, N*H*); 8.27 (s, 1H, H-6); 7.29 (m, 6H, H$_d$); 7.20 (m, 3H, H$_e$); 7.14 (m, 6H, H$_c$); 7.05 (m, 2H), 6.94 (m, 2H) (H$_a$, H$_b$); 6.10 (app. t, 1H, $J_{1',2'\alpha}=J_{1',2'\beta}$= 6.5 Hz, H-1′); 5.22 (d, 1H, $J_{3',OH}$=4.3 Hz, 3′-O*H*); 5.08 (t, 1H, $J_{5',OH}$ =5.1 Hz, 5′-O*H*); 4.94 (s, 2H, ArOC*H*$_2$); 4.23 (m, 1H, H-3′); 3.80 (m, 1H, H-4′); 3.63–3.51 (m, 2H, H-5′); 2.13 (m, 2H, H-2′). ^{13}C NMR (DMSO-d_6): 161.55 (C4), 155.51 (≡CCH$_2$O*C*), 149.46 (C2), 146.64 (3C, C$_6$H$_4$C*C*), 144.63 (C6), 139.12 (Ph$_3$C*C*), 131.63 (2C, Cb), 130.50 (6C, Cc), 127.72 (6C, Cd), 125.98 (3C, Ce), 113.92 (2C, Ca), 97.47 (C5), 87.74 (CH$_2$C≡*C*), 87.71 (C4′), 84.97 (C1′), 79.61 (CH$_2$*C*≡), 70.14 (C3′), 63.88 (Ph$_3$*C*), 60.99 (C5′), 56.37 (≡C*C*H$_2$), 40.23 (C2′). The bicyclic nucleoside, 3-(2-deoxy-β-D-ribofuranosyl)-6-(4-(triphenylmethyl)phenoxymethyl)furo[2,3-*d*]pyrimidin-2-one (**20b**), was isolated as a byproduct (12 mg, 2%), R_f 0.44 (12% MeOH in $CHCl_3$ (v/v)). ESI MS: m/z=623.4 $[M+Na]^+$, calcd for $[C_{37}H_{32}N_2O_6+Na]^+$623.6. ESI-TOF HRMS: m/z=601.2394 $[M+H]^+$, 623.2208 $[M+Na]^+$, calcd for $[C_{37}H_{32}N_2O_6+H]^+$601.2333, $[C_{37}H_{32}N_2O_6+Na]^+$623.2153. Fluorescence, (MeOH): excitation, λ_{max}, nm 337; emission, λ_{max}, nm 401; (1,2-dichloroethane): excitation, λ_{max}, nm 340; emission, λ_{max}, nm 405. ^{1}H NMR (DMSO-d_6): 8.84 (s, 1H, H-4); 7.29 (m, 6H, H$_d$); 7.20 (m, 3H, H$_e$); 7.12 (m, 6H, H$_c$); 7.04 (m, 2H), 6.99 (m, 2H) (H$_a$, H$_b$); 6.90 (s, 1H, H-5); 6.15 (app. t, 1H, $J_{1',2'\alpha}=J_{1',2'\beta}$- =6.1 Hz, H-1′); 5.27 (m, 1H, 3′-O*H* or 5′-O*H*); 5.14–5.08 (m, 3H, ArOC*H*$_2$, 5′-O*H* or 3′-O*H*); 4.23 (m, 1H, H-3′); 3.92 (m, 1H, H-4′); 3.71–3.59 (m, 2H, H-5′); 2.41 (m, 1H); 2.06 (m, 1H) (H-2′).

4.5.3. 5-(Estron-3-yloxypropyn-1-yl)-2′-deoxyuridine (19c). The title compound was purified using a 0→10% gradient of MeOH in $CHCl_3$ (v/v); colorless crystals (481 mg, 90%); mp 127–129 °C ($CHCl_3$–hexane); R_f 0.47 (12% MeOH in $CHCl_3$ (v/v)). ESI MS: m/z=557.7 $[M+Na]^+$, calcd for $[C_{30}H_{34}N_2O_7+Na]^+$557.6. ESI-TOF HRMS: m/z=535.2497 $[M+H]^+$, calcd for $[C_{30}H_{34}N_2O_7+H]^+$535.2439. UV (MeOH): λ_{min}, nm (log ε) 256 (3.92); λ_{max}, nm (log ε) 287 (4.19). ^{1}H NMR (DMSO-d_6): 11.63 (s, 1H, N*H*); 8.24 (s, 1H, H-6); 7.19 (d, 1H, $J_{1'',2''}$=8.5 Hz, H-1″); 6.76 (dd, 1H, $^4J_{2'',4''}$=2.6 Hz, $J_{1'',2''}$=8.5 Hz, H-2″); 6.73 (d, 1H, $^4J_{2'',4''}$=2.6 Hz, H-4″); 6.10 (app. t, 1H, $J_{1',2'\alpha}=J_{1',2'\beta}$=6.4 Hz, H-1′); 5.22 (d, 1H, $J_{3',OH}$=4.3 Hz, 3′-O*H*); 5.09 (t, 1H, $J_{5',OH}$=5.0 Hz, 5′-O*H*); 4.89 (s, 2H, ArOC*H*$_2$); 4.23 (m, 1H, H-3′); 3.80 (m, 1H, H-4′); 3.65–3.53 (m, 2H, H-5′); 2.83 (m, 2H, H-6″); 2.43 (dd, 1H, ^2J=18.9 Hz, $J_{15'',16''}$=8.5 Hz, H-16″); 2.34 (m, 1H, H-11″); 2.19 (m, 1H, H-9″); 2.13 (m, 2H, H-2′); 2.06 (m, 1H, H-16″); 1.95 (m, 2H, H-7″, H-15″); 1.76 (m, 1H, H-12″); 1.61–1.31 (m, 6H, H-7″, H-8″, H-11″, H-12″, H-14″, H-15″); 0.83 (s, 3H, H-18″). ^{13}C NMR (DMSO-d_6): 219.67 (C17″), 161.56 (C4), 155.34 (C3″), 149.46 (C2), 144.47 (C6), 137.56 (C5″), 132.53 (C10″), 126.28 (C1″), 114.65 (C4″), 112.47 (C2″), 97.55 (C5), 88.01 (CH$_2$C≡*C*), 87.75 (C4′), 84.95 (C1′), 79.41 (CH$_2$*C*≡), 70.19 (C3′), 61.03 (C5′), 56.15 (*C*H$_2$C≡), 49.65 (C14″), 47.37 (C13″), 43.51 (C9″), 40.23 (C2′), 37.86 (C8″), 35.43 (C16″), 31.42 (C12″), 29.20 (C6″), 26.08 (C7″), 25.54 (C11″), 21.19 (C15″), 13.57 (C18″). Further elution gave 3-(2-deoxy-β-D-ribofuranosyl)-6-(estron-3-yloxypropyn-1-yl)furo[2,3-*d*]-pyrimidin-2-one (**20c**) as a byproduct (38 mg, 7%), R_f 0.40 (12% MeOH in $CHCl_3$ (v/v)). ESI MS: m/z= 557.3 $[M+Na]^+$, calcd for $[C_{30}H_{34}N_2O_7+Na]^+$557.6. ESI-TOF HRMS: m/z=557.2314 $[M+Na]^+$, calcd for $[C_{30}H_{34}N_2O_7+Na]^+$557.2258. Fluorescence, (MeOH): excitation, λ_{max}, nm 342; emission, λ_{max}, nm 406; (1,2-dichloroethane): excitation, λ_{max}, nm 342; emission, λ_{max}, nm 405. ^{1}H NMR (DMSO-d_6): 8.83 (s, 1H, H-4); 7.19 (d, 1H, $J_{1'',2''}$=8.7 Hz, H-1″); 6.87 (s, 1H, H-5); 6.81 (dd, 1H, $^4J_{2'',4''}$=2.4 Hz, $J_{1'',2''}$=8.7 Hz, H-2″); 6.76 (d, 1H, $^4J_{2'',4''}$= 2.4 Hz, H-4″); 6.15 (m, 1H, H-1′); 5.26 (m, 1H, 3′-O*H* or 5′-O*H*); 5.09 (m, 3H, ArOC*H*$_2$, 5′-O*H* or 3′-O*H*); 4.23 (m, 1H, H-3′); 3.92 (m, 1H, H-4′); 3.71–3.59 (m, 2H, H-5′); 2.82 (m, 2H, H-6″); 2.48–2.32 (m, 3H, H-2′, H-16″(or H-11″)); 2.18 (m, 1H, H-9″); 2.07 (m, 2H, H-11″(or H-16″), H-16″); 1.96 (m, 2H, H-7″, H-15″); 1.75 (m, 1H, H-12″); 1.62–1.32 (m, 6H, H-7″, H-8″, H-11″, H-12″, H-14″, H-15″); 0.82 (s, 3H, H-18″). ^{13}C NMR (DMSO-d_6): 219.65 (C17″), 171.27 (C7a), 155.53 (C3″), 153.78 (C2), 152.06 (C6), 139.24 (C4), 137.67 (C5″), 132.63 (C10″), 126.32 (C1″), 114.80 (C4″), 112.45 (C2″), 105.39 (C4a), 104.84 (C5), 88.30 (C4′), 87.75 (C1′), 69.66 (C3′), 61.61 (ArO*C*H$_2$), 60.81 (C5′), 49.64 (C14″), 47.36 (C13″), 43.49 (C9″), ~40[‡] (C2′), 37.84 (C8″), 35.42 (C16″), 31.40 (C12″), 29.17 (C6″), 26.05 (C7″), 25.51 (C11″), 21.19 (C15″), 13.57 (C18″).

[‡] The signal of solvent is present in the region.

4.5.4. 5-[(Perylen-3-yl)methoxypropyn-1-yl]-2′-deoxyuridine (19d). The title compound was purified using a 0→6% gradient of MeOH in $CHCl_3$ (v/v). Yield 488 mg (89%), orange crystals; mp 182–184 °C ($CHCl_3$–hexane); R_f 0.46 (12% MeOH in $CHCl_3$ (v/v)). ESI MS: m/z=569.3 $[M+Na]^+$, calcd for $[C_{33}H_{26}N_2O_6+Na]^+$569.6. ESI-TOF HRMS: m/z=547.1921 $[M+H]^+$, 569.1775 $[M+Na]^+$, calcd for $[C_{33}H_{26}N_2O_6+H]^+$547.1864, $[C_{33}H_{26}N_2O_6+Na]^+$569.1683. UV (MeOH): λ_{min}, nm (log ε) 236 (4.33), 275 (4.06), 334 (2.95), 397 (4.02), 424 (4.13); λ_{max}, nm (log ε) 253 (4.55), 289 (4.12), 392 (4.05), 412 (4.38), 438 (4.48). Fluorescence, (MeOH): excitation, λ_{min}, nm 395, 422, λ_{max}, nm 390, 412, 438; emission, λ_{min}, nm 461, 498, λ_{max}, nm 444, 473, 502; (1,2-dichloroethane): excitation, λ_{min}, nm 398, 426, λ_{max}, nm 393, 416, 442; emission, λ_{min}, nm 466, 503, λ_{max}, nm 450, 478, 510. ^{1}H NMR (DMSO-d_6): 11.66 (s, 1H, N*H*); 8.43–8.32 (m, 4H, H-1″, H-6″, H-7″, H-12″); 8.32 (s, 1H, H-6); 8.01 (d, 1H, $J_{4'',5''}$=8.2 Hz, H-4″); 7.81 (d, 2H, $J_{8'',9''}=J_{10'',11''}$=8.2 Hz, H-9″, H-10″); 7.65–7.53 (m, 4H, H-2″, H-5″, H-8″, H-11″); 6.13 (app. t, 1H, $J_{1',2'\alpha}=J_{1',2'\beta}$=6.4 Hz, H-1′); 5.23 (m, 1H, 3′-O*H*); 5.12 (m, 1H, 5′-O*H*); 4.92 (s, 2H, ArCH_2); 4.47 (s, 2H, ≡CCH_2); 4.25 (m, 1H, H-3′); 3.81 (m, 1H, H-4′); 3.67–3.55 (m, 2H, H-5′); 2.20–2.10 (m, 2H, H-2′). ^{13}C NMR (DMSO-d_6): 161.69 (C4), 149.51 (C2), 144.12 (C6), 134.29 (C9a″), 133.13 (C3″), 132.73 (C3a″), 130.88 (C6a″), 130.52 (C6b″), 130.36 (C12a″), 128.22, 128.13, 128.08, 128.00, 127.72 (C12b″, C3a1″, C6b1″, C9″, C10″), 127.17, 126.98 (3C) (C2″, C5″, C8″, C11″), 124.34 (C4″), 120.91 (2C), 120.84 (C6″, C7″, C12″), 120.14 (C1″), 97.84 (C5), 88.76 (CH_2C≡*C*), 87.73 (C4′), 84.92 (C1′), 79.23 (CH_2*C*≡), 70.15 (C3′), 69.18 (Ar*C*H_2), 61.01 (C5′), 57.67 (*C*H_2C≡), ~40[§] (C2′).

4.6. Pharmacology

4.6.1. Cells. Vero cells culture (kidney cells from green monkeys) was grown in Eagle's medium (Institute of Poliomyelitis and Viral Encephalitides, Moscow) supplemented with 10% fetal calf serum ('PanEco', Moscow).

4.6.2. Viruses. Herpes simplex virus type 1, strain L_2 (HSV-1) was from the Laboratory of Virus Museum (Ivanovsky Institute of Virology, Moscow, Russia). Acyclovir- and phosphonoacetic acid-resistant strains of HSV-1 were isolated as described elsewhere.[31]

4.6.3. Cytotoxicity assays. Vero cells in 96-well microtiter plates were treated with different concentrations of the experimental drugs (1.4×10^5 cells in 185 μL of the medium per well). Cell cultures were incubated for 72 h. At the indicated time, the cells were colored with Trypan Blue, and the cell number was determined. The 50% cytostatic concentration (CD_{50}) was defined as the compound concentration required to reduce the cell number by 50%.

4.6.4. Antiviral assays. Vero cells were inoculated with various strains of HSV-1 at an input of 0.1 plaque formation units (PFU) per cell and then incubated with medium containing various concentrations of modified nucleosides for 48 h (95–100% virus-inducted cytopathicity in the untreated control). Antiviral activity was expressed as the compound concentration required to reduce virus-induced cytopathicity by 50% (ID_{50}) or 95% (ID_{95}) compared to untreated control.

[§] The signal of solvent is present in the region.

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Uncatalyzed solventless Diels–Alder reaction of 2-amino-3-nitroacrylate: synthesis of new epimeric 2-amino-3-nitro-norbornene- and norbornane-2-carboxylic acids

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Abstract—A highly diastereoselective Diels–Alder reaction between cyclopentadiene and ethyl (*Z*)-2-*N*-Boc-amino-3-nitroacrylate in neat conditions affords the ethyl 2-*t*-butoxycarbonylamino-3-*endo*-nitro-bicyclo[2.2.1]hept-5-ene-2-*exo*-carboxylate: a new constrained carbocyclic amino acid. Catalytic hydrogenation of this cycloadduct gave the corresponding reduced norbornane derivative. A preliminary investigation into the chemistry of these two amino acids was performed. In particular, the epimerization to their corresponding 3-*exo*-nitro compounds by treatment both with acid and base was studied. From this study, valuable information on the *endo*/*exo* process at the C-3 carbon atom, as well as on the stability of the different stereomers, was obtained. The stability is closely related to the presence or the absence of the double bond in the ring and to the substitution pattern. Finally, deprotection of the amino acid function has been performed.

1. Introduction

In a recent review,[1a] we have clearly outlined the importance of conformationally constrained carbocyclic amino acids characterized by methabolic stability and conformational rigidity. The presence of a further substituent on the ring allows for the formation of stereoisomeric derivatives whose interest is related to the attempts to clarify the conformational role of the substituent in bioreceptorial interactions. Furthermore, it is well known that peptides can be conformationally and functionally modulated by modulating the properties of the side chain functional groups, which are known to be important for the peptide activity.[1a,2] The replacement of a natural amino acid of a peptide with a constrained one generally produces a change on its conformation. A variety of carbocyclic amino acid derivatives has been developed in the last two decades;[1,3] however, efforts are still needed to satisfy specific structural and chemical prerequisites. Many examples of carbocyclic amino acids bearing oxygen, amino and sulfur substituents are known but only two examples of this class bearing a nitro-substitution are quoted: 1-amino-2-nitrocyclopentyl-1-carboxylic acid,[4] a metabolite of *Aspergillus wentii*, and 4-nitro-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid,[5] a potent agonist of CNS amino acid receptors. The nitro group is interesting in itself, considering its potential interations with bioreceptors and because of its chemical transformations. For this reason, we included the synthesis of carbocyclic β-nitro amino acids in our program[1b–h] to develop efficient protocols for the synthesis of new constrained β-hetero-substituted amino acids.

Herein we report on the prepration of new epimeric 2-amino-3-nitro-norbornene- and -norbornane-2-carboxylic acids and protected derivatives, characterized by the presence of the 3-*endo*- or 3-*exo*-nitro group and 2-*exo*-carboxylic function, using the Diels–Alder reaction, which offers an easy entry into the assembly of the carbocyclic skeleton with full control of stereochemistry. Since the easy epimerization of C-3 has been a real problem, a systematic study was carried out in order to ascertain the stability of the *endo* and *exo* isomers. It was found that it is strictly dependent on the presence or absence of the double bond in the norbornane skeleton and the functionalization of amino and carboxy groups. Detailed experimental conditions to prepare protected or unprotected amino acids as pure epimer, when stable, or as a mixture of 3-*exo*/*endo* compounds are given.

2. Results and discussion

The new ethyl 2-*N*-(*t*-butoxycarbonyl)-3-nitro-acrylate **3** was prepared and was used in the Diels–Alder reaction.

Keywords: Diels–Alder; 2-Amino-3-nitro-norbornene-2-carboxylic acids; 2-Amino-3-nitro-norbornane-2-carboxylic acids; Epimerization.
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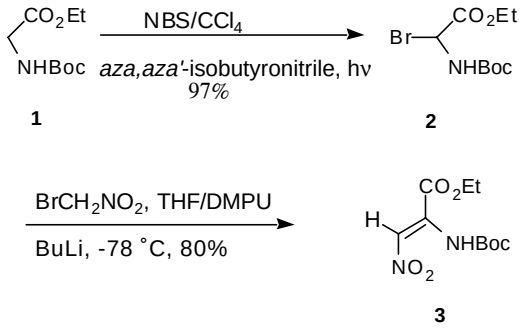
The synthesis of the corresponding 2-benzoylamino derivative is reported in the literature.[6] However, we chose to prepare the *N*-*t*-butoxycarbonyl derivative as it is easier to remove. Unexpectedly, the preparation of **3** was critical and application of the known procedure failed to afford this reagent in acceptable yields. Our protocol consisted of two steps, that is, bromination of **1** to **2**[7] and BuLi mediated condensation with bromonitromethane in a mixed solvent DMPU/THF=1:7. The presence of the co-solvent DMPU gave compound **3** in 77% yield (from **1**) (Scheme 1).



Scheme 1.

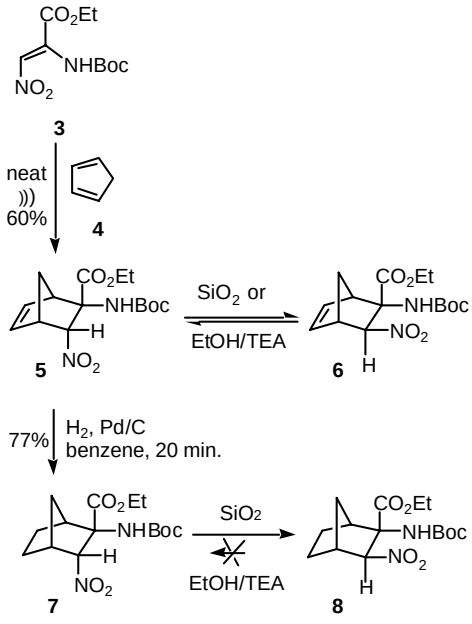
Many examples of Diels–Alder reactions of cyclic dienes and nitro olefins are reported in the literature: the reaction yields and the stereochemical outcome are strictly dependent on the olefin structure and on reaction conditions. Furthermore, it is well known that β-substituted aminoacrylates are scarcely reactive in Diels–Alder reactions.[8] For this reason, significant efforts to optimize this process were devoted using a variety of acyclic dienes[9] or cyclopentadiene **4** under different conditions. In our case, the results of the cycloaddition of **4** to the amminoacrylate **3** are shown in Table 1.

Table 1. Diels–Alder reaction of **3** and **4**

Entry	Catalyst (mol equiv)[a]	Solvent (T °C)[b]	t (h)	Yield %[c]
1	A (0.2)	CH_2Cl_2 (−5)	24	—
2	B (0.2)	CH_2Cl_2 (25)	24	—
3	C (0.2)	$CHCl_3$ (61)	24	—
4	C (0.2)	Toluene (110)	24	—
5	C (0.2)	CH_2Cl_2 (25–40)	24	30
6	C (0.2)	CH_2Cl_2))), (25)	12	—
7	A	Neat[d]	330	20
8	C (0.2)	Neat[d]	96	30
9	C (0.2)	Neat[d]	330	40
10	—	Neat))), (25)[d]	48	48
11	—	Neat))), (25)[e]	48	60

[a] A=$EtAlCl_2$; B=$Yb(OTf)_4 \cdot H_2O$; C=$Mg(ClO_4)_2$.
[b]))): Ultrasounds.
[c] Isolated compound.
[d] **3**:**4**=1:4.
[e] **3**:**4**=1:5.

Our previous experience[1d] on the cycloaddition reaction of β-heterosubstituted-α-amino acrylate derivatives showed that Lewis acids are efficient catalysts. However, in the reaction of **3** with cyclopentadiene **4**, both $EtAlCl_2$ (entry 1), and $Yb(OTf)_4 \cdot H_2O$ (entry 2) were ineffective as catalysts. $Mg(ClO_4)_2$ was inefficient in $CHCl_3$ (entry 3) and toluene (entry 4) but gave the cycloadduct **5** (entry 5) in CH_2Cl_2 although in poor yield (Scheme 2). Surprisingly, when the same reaction conditions were applied in the presence of ultrasound (entry 6), the product of cycloaddition was not observed. Better results were achieved when the reaction was performed in neat conditions, with an excess of cyclopentadiene (1:4) and in the presence of a catalyst. Both $EtAlCl_2$ (entry 7) and $Mg(ClO_4)_2$ at different reaction times (entries 8 and 9) gave the cycloadduct **5**. In this case the yield was unsatisfactory in view of further development. An improvement of yield, accompanied by a decreasing of reaction time, was obtained in absence of the catalyst and solvent and using ultrasound (entry 10). A further improvement involved the same reaction conditions but adding the diene **4** (1:5 ratio) at different time (Section 4; entry 11): compound **5** was obtained in 60% yield. It is to be noted that prolonging the reaction time is not advantageous because the cycloreversion reaction becomes more and more important.



Scheme 2.

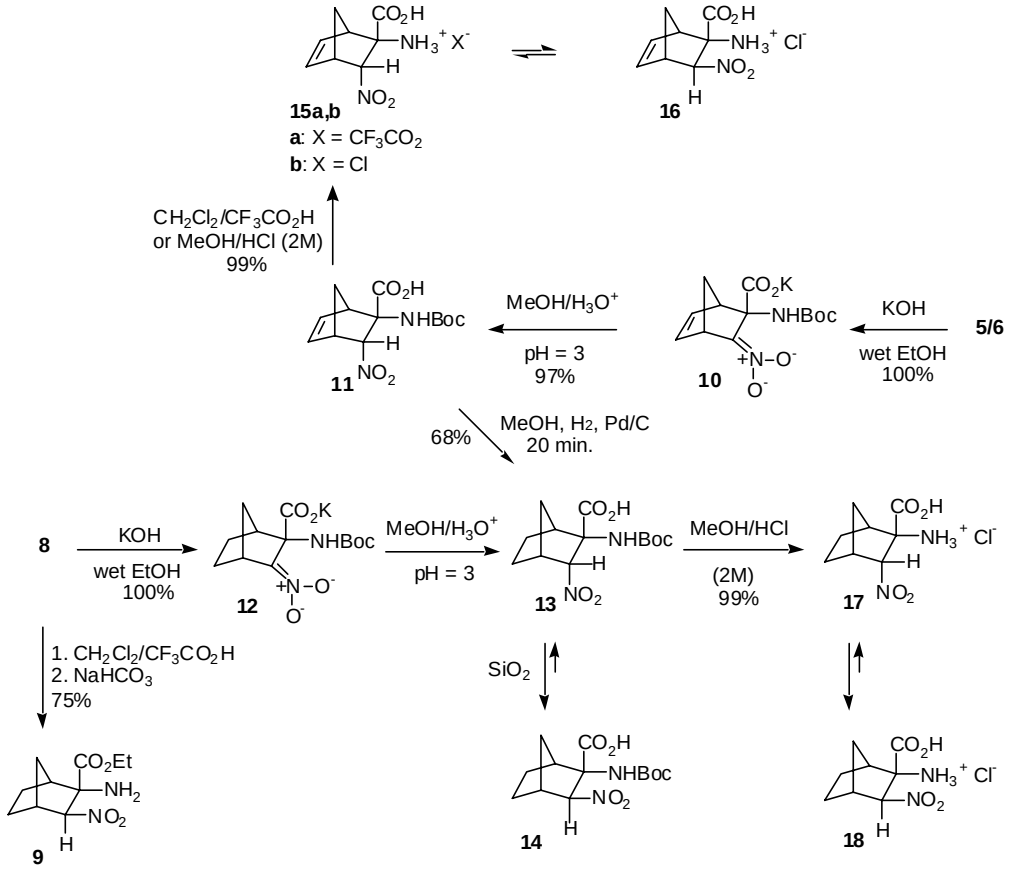
The formation of **5** was highly stereoselective since a single cycloadduct was detected in the ^1H NMR spectrum of the crude reaction mixture, as well as unreacted starting material (30%). This stereochemical result is very uncommon for aminoacrylates, which generally give an *exo*/*endo* mixture in the Diels–Alder reactions.[1d,10] On the other hand, our result is in line with the literature data[11] of the cycloadditions involving nitroalkenes and cyclopentadiene (**4**). It is reported that the *endo*-nitro adduct is generally accompanied by minor amounts of the *exo*-nitro stereomer. It is clear that secondary orbital interactions involving the nitro group play a pivotal role favoring the formation of the stereoisomer **5**.[11b,12] Compound **5** was not stable when purified by column chromatography on silica gel and was partially transformed into its epimer **6** (Scheme 2).

The pure compound **5** was isolated in 40% yield with a mixture of **5** and **6** (20%), which could not be separated (either by column chromatography or by HPLC). The 3-*endo*-nitro compound **5** was stable in the solid state and in

several solvents as shown by ^{1}H NMR in different solvents ($CDCl_3$, CD_3OD, C_6D_6, CD_3CN) recorded at different times, but slowly equilibrated in the presence of silica gel to the epimer **6**. The equilibration was accomplished also using catalytic amounts of a weak base (TEA 0.003 M in CH_2Cl_2, 2 h; **5/6**, 70:30, unchanged with time). Clearly, the ease of the isomerization process of **5** into **6**, via *aci*-nitro tautomer or nitronate ion, depends on the high acidity of the hydrogen atom at the C-3 position. This was demonstrated by a ^{1}H NMR experiment in which **5** was treated with CD_3OD in presence of a catalytic amount of TEA: H-3 was readily exchanged.

Compound **5** was reduced to the norbornane derivative **7** with hydrogen in the presence of Pd/C at atmospheric pressure, in benzene after 20 min. This compound was not stable in solution: in fact, a trace amount of its epimer **8** was already detected in the ^{1}H NMR spectrum of the freshly reduced reaction mixture. During purification by column chromatography, compound **7** was partially transformed into **8**. It was possible to shift further the equilibrium in favor of **8** by crystallization of the mixture of **7** and **8**. This allowed to isolate pure **8** in 77% yield (Scheme 2). In view of this result, we directly reduced the mixture of **5** and **6**. The mixture of the norbornane products **7** and **8** was then recrystallized giving pure **8** in 63% yield. The adduct **8** is the C-3 epimer of **7** and it is stable in solution (CD_3OD, $CDCl_3$). When **8** was treated with catalytic triethylamine, even for a long time, the return epimerization reaction to **7** did not occur.

The preparation of free amino acids was undertaken. Expectedly, deprotection of the amino and carboxy group both in the norbornane and in the norbornene compounds was not easy owing to the known instability of the carbocyclic amino acids bearing a nitro substituent.[4b] This was confirmed by two preliminary experiments. In a first experiment, deprotection of the amino group of **8** was attempted by using trifluoroacetic acid. The corresponding 3-*exo*-nitro compound **9** (Scheme 3) was obtained (the structure of this compound was confirmed by ^{1}H NMR on the crude reaction mixture) but its purification by column chromatography on silica gel gave several unidentified compounds. Similarly, direct achievement of the free amino acid from **5** with aqueous HCl afforded a mixture of unidentified compounds. So, a systematic step-by-step study of the deprotection of the amino and carboxy groups was necessary (Scheme 3). As a final successful protocol in the norbornene compounds, **5** was reacted with potassium hydroxide (3 equiv) in ethanol at reflux. The intermediate nitronate **10** was formed (NMR analyses)[13] which after acidification with 10% HCl in MeOH (pH = 3) and purification on a silica gel column afforded the acid **11** (99%) as a pure compound. The same result was obtained starting from the mixture of **5** and **6**. Compound **11** was isolated as single stereomer. Finally, deprotection of the amino group of compound **11** was done with trifluoroacetic acid in dichloromethane at room temperature for 12 h. 2-Amino-3-nitronorbornene-carboxylic acid **15a** was isolated. Only traces of epimer such as **16** were detected by ^{1}H NMR. Similarly, the deprotection of **11** in MeOH with HCl



Scheme 3.

(2 M) at 25 °C gave the amino acid **15b** (^{1}H MNR analysis). However, the hydrochloride underwent rapid epimerization (1 h) to **16** (**15/16**, 60:40). In the norbornane series ester **8** was transformed with potassium hydroxide into nitronate **12**, which gave the acid **13** when treated with acid. When the acid **13** was chromatographed on silica gel a partial epimerization at C-3 was observed and a mixture of **13** and **14** was obtained (40:60).

The same mixture was obtained when the unsaturated acid **11** was catalytically reduced with hydrogen (Scheme 3). Crude **13** was deprotected with MeOH/HCl and afforded compound **17**, which partially epimerized to **18** in a short time. By prolonged standing of the solventless mixture of **17** and **18** at room temperature, a complete isomerization into the single epimer **18** was observed.

The structure of compounds **5**–**18** was confirmed by NMR analyses (^{1}H- ^{13}C-, NOESY, HETCOR) and the assigned stereochemistry is in agreement with the literature data.[11b,14] The spatial disposition of H-3 for epimers **5** and **6** was demonstrated by NOESY experiments. For **5**, the NOE effect was observed between H-3 (6.14 δ, $J=3.4$ Hz) and the signal at 1.73 δ associated to H-7s; between NH (4.67 δ) and the olefinic H-6 (6.40 δ) and H-1 (3.31 δ). On the basis of this spectroscopic evidence the configuration *exo*-carboxylate, *endo*-nitro was assigned to this adduct. Instead, NOE effect was observed in compound **6** between H-3 (4.24 δ, $J=1.6$ Hz) and H-4 (3.30 δ) and, most of all, between H-3 and the olefinic protons (6.34 δ), thus confirming the *exo* spatial disposition of the nitro group.

The ^{1}H NMR analyses of the nitronates **10** and **12** showed the absence of H-3 and the presence at ^{13}C NMR of a signal associated to C-3 nitronate function (**10**: 131.6; **12**: 131.4 δ).[13] Of relevance, the resonance of H-7s (**10**: 2.60 δ, d, $J=8.4$ Hz; **12**: 2.33 δ, d, $J=9.2$ Hz;) at lower fields.

The NMR spectra of the other compounds are consistent with the proposed structure and the assigned stereochemistry. Some conclusions can be tried from NMR spectra analyses. Diagnostic signals to assign the stereochemistry of C-3 are (i) the H-3, which is at lower field for the *endo* nitro series (H-3 *exo*: 6.14–5.74 δ; H-3 *endo*: 4.60–4.23 δ), (ii) an AB system associated to H-7 protons, which are in the range 1.90–1.50 δ for the *endo* nitro series and an AX system for the *exo* one in which the H-7s proton is at low fields (2.79–2.73 δ) and H-7x resonates in the range 1.90–1.50 δ, (iii) the H-4 is at lower fields with respect to the H-1 in the *endo* series, vice-versa, the H-1 is at low fields in the *exo* one, (iv) the C-3, which resonates in the range 89–85 δ in the *endo* series and at 98–92 δ in the *exo* one.

The above results demonstrate that norbornene and norbornane-3-*endo*-nitro amino acids, both in protected and free form, can undergo equilibration with the more stable 3-*exo* compounds in very mild conditions (i.e. weak base or SiO_2; thermodynamic conditions). A different distribution between the two epimers occurs depending on the presence/absence of (i) the double bond in the ring and (ii) the functionalization at the carboxy or amino groups. The stereochemical results (referring to the configuration of the nitro group on C-3) can be summarized as follows for protected (**5**–**8**), *N*-protected (**11**, **13**, **17**) and non-protected (**15**–**18**) compounds. *Protected compounds*: when the unsaturation is present the *endo*-**5** is stable as solid or in solution, but an equilibrium with *exo*-**6** takes place both in weak base or on silica gel. The equilibrium is in favour of the *endo* isomer. Instead, the saturated *endo*-**7** is not isolable in pure form because it is readily converted into the very stable epimer *exo*-**8**, which does not return to *endo*-**7** when treated with TEA. *N-Deprotected compounds*: independent of the configuration of the starting esters and from the presence or absence of the double bond in the ring, both **5/6** and **8** when hydrolyzed in basic conditions gave the *endo* acids **11** and **13**, respectively, by protonation of the corresponding nitronates **10** and **12**. The unsaturated compound **11** is stable on silica gel or in solution, but the saturated compound **13** equilibrates with the *exo* isomer (**13/14**, 40:60). *Free amino acids*: both unsaturated and saturated acids *endo*-**11** and **13** upon deprotection with 2 M HCl in MeOH afford the *endo* isomers (kinetic compounds) which are then epimerized to the corresponding *exo* products. However, in the case of the unsaturated compound **11** an *endo*/*exo* mixture (60:40) of amino acids **15b/16** is obtained. Instead, the saturated compound **13** is converted quantitatively into the *exo* isomer **18**.

Epimerization studies on norbornene and norbornane nitro derivatives, both unsubstituted or monosubstituted at C-β, are reported in the literature.[11b,c,15] The epimerization of nitro norbornene derivatives on silica gel, through the *aci*-nitro tautomer, was also reported.[11b] It is claimed that the presence of a strong base is usually needed to promote this process via nitronate intermediate and that the protonation of the *exo* face is faster then of the *endo* one. This was explained by a kinetically controlled attack of the protonating species during the quenching process. In a single case, the equilibration of the *endo* compound to the *exo*-nitro epimer was reached with a low base concentration (thermodynamic conditions).[11c]

Our results on the epimerization process fit well with the above findings when two important features are taken into account: (i) the strong acidity of H-3, induced by the presence of electron-withdrawing groups (COOR and NHBoc) linked to C-2 that favors the formation of the nitronate or of the *aci*-nitro tautomer under thermodynamical conditions; (ii) the disubstitution at C-β, that characterizes the present compounds and increases the steric bulk.

It was observed that, both in the unsaturated and saturated series, the *endo* isomers are the kinetic compounds. A different distribution between *endo* and *exo* compounds in the two series was found under equilibration conditions showing that the *endo* isomer is more stable in the unsaturated series, even if both epimers are present at equilibrium. Instead, the epimerization of the saturated *endo* compounds to the *exo* isomers is almost complete. Different theories have been postulated to clarify the *endo*/*exo* equilibria.[11c] Our results point out that steric effects are very relevant in determining the *endo*/*exo* equilibrium (equilibrium governed by entropic values). In fact, in the saturated series, it is to be underlined that spatial proximity between H-5 and H-6 *endo* protons and the nitro group is

evident with respect to the corresponding hygrogen atoms in the unsaturated series. Accordingly, the presence of these hydrogen atoms combined with the presence of the protected or unprotected *endo*-amino group resulted in the decrease of the degrees of freedom of the *endo*-nitro group thus favoring the isomerization of C-3 to the more stable *exo*-nitro epimer.

The stereochemical result of the epimerization of acids **11** and **13** on silica gel is not homogeneous because **11** does not epimerize and **13** gives a 40:60 mixture of *endo*/*exo* isomers. We suggest that, in this case, an *exo* intramolecular protonation of the *aci*-nitro tautomer is probably occurring by assistance of the *exo*-carboxylic acid group on C-2. Finally, when the free amino acid is formed in acid conditions a competition between *exo* and *endo* protonation occurs assisted both by the carboxylic group or by the ammonium group.

3. Conclusion

In conclusion, new epimeric 2-amino-3-nitro-norbornene- and norbornane-2-carboxylic acids and protected derivatives characterized by the presence of the 3-*endo*- or 3-*exo*-nitro group and 2-*exo*-carboxylic function were prepared. The stability of each epimer is strictly dependent on the presence or absence of the double bond on the norbornane skeleton and on the protection of the amino acid functions. Epimerization processes are highly accelerated by the strong acidity of the hydrogen atom on C-α to the nitro group and are governed by steric effects.

4. Experimental

4.1. General

Melting points are uncorrected. IR spectra of the Nujol method were measured using NaCl plates. ^{1}H and ^{13}C NMR were recorded at 200, 500 and 50 MHz, respectively, with $CHCl_3$ as internal standard. Mass spectra were obtained by electron impact ionization at 70 eV fro a Finnigan INCOS 50 or from Finnigan MD 800 instruments using the direct exposure probe (DEP).

Glycine **1** and α-bromoglycine derivative **2**[7] are known compounds.

4.1.1. Ethyl (*Z*)-2-*t*-buthoxycarbonylamino-3-nitro-acrylate (3). Operating under nitrogen atmosphere and stirring a solution of bromonitromethane (11 g, 78.4 mmol) in anhydrous THF (380 mL) and DMPU (52 mL) was cooled at −78 °C. A solution of BuLi (1.6 M in hexane, 50.2 mL, 78.8 mmol) was dropped maintaining the temperature under −65 °C. The solution was then cooled at −78 °C and compound **2** (11 g, 39.2 mmol), dissolved in THF (150 mL), was dropped. The reaction was monitored by ^{1}H NMR and after 4 h the solution was warmed at 25 °C. AcOH (4.8 mL, 100%) was added and the organic layer was washed with a saturated solution of NH_4Cl (270 mL). The aqueous layer was extracted with AcOEt (3×270 mL) and the combined organic layers were first washed with a saturated solution of $NaHCO_3$, then with brine (270 mL). After drying over Na_2SO_4 the solvent was eliminated and the crude reaction mixture was chromatographed on silica gel (cyclohexane/AcOEt, 10:1). Acrylate **3** was obtained in (8.1 g, 80%), as an oil. IR: ν_{max} 3378, 1747, 1724 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.65 (br s, 1H, exch., NH), 6.74 (s, 1H, CH), 4.39 (q, J=7.0 Hz, 2H, OCH_2), 1.56 (s, 9H, CMe_3), 1.37 (t, J=7.0 Hz, 3H, Me); ^{13}C NMR δ 161.5, 150.1, 140.2, 119.9, 84.6, 63.6, 28.1, 14.1; MS (*m*/*z*) APCI: 259 $(M-H)^+$. Anal. Calcd C, 46.15; H, 6.20; N, 10.76. Found: C, 45.97; H, 6.15; N, 10.63.

4.2. General procedure for the Diels–Alder reaction

In a sealed tube, a neat mixture of acrylate **3** (6.93 g, 26.6 mmol) and freshly distilled diene **4** (1.75 g, 26.5 mmol) was sonicated at room temperature for 2 h after which three other crops (26.5 mmol each) of diene were added in a 2 h intervals. After 24 h, a further crop of **4** (26.5 mmol) was added and the reaction was stand under ultrasound for other 24 h. The crude reaction mixture was chromatographed on silica gel (cyclohexane/AcOEt; 15:1) giving a first fraction containing the starting acrylate **3** (2.5 g, 9.6 mmol), a second one containing compound **5** (3.5 g, 40%) and a third one containing a mixture of **5**/**6** (1.75 g, 20%) in a ratio 70:30. (The distribution of **5** and **6** is strongly dependent of the chromatographic process).

4.2.1. Ethyl (1*R,2*S**,3*R**,4*S**)-2-*t*-buthoxycarbonyl-amino-3-nitro-bicyclo[2.2.1]hept-5-ene-2-carboxylate (5).** Mp 127 °C (*i*-Pr_2O); IR: ν_{max} 3412, 1721 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.62–6.60 (m, 1H, H-6), 6.41–6.39 (m, 1H, H-5), 6.14 (d, J=3.4 Hz, 1H, H-3), 4.67 (s, 1H, exch., NH), 4.36–4.25 (m, 2H, OCH_2), 3.46 (br s, 1H, H-4), 3.31 (br s, 1, H, H-1), 1.73, 1.61 (AB system, J=9.8 Hz, 2H, H-7), 1.38 (s, 9H, CMe_3), 1.31 (t, J=7.1 Hz, 3H, Me); ^{13}C NMR δ 171.4, 154.4, 140.6, 132.9, 89.0, 81.3, 69.1, 62.8 52.3, 48.0, 44.3, 28.3, 14.5; MS (*m*/*z*) APCI: 325 $(M-H)^+$. Anal. Calcd C, 55.21; H, 6.79; N, 8.58. Found: C, 55.17; H, 6.82; N, 8.50.

4.2.2. Ethyl (1*R,2*S**,3*S**,4*S**)-2-*t*-buthoxycarbonyl-amino-3-nitro-bicyclo[2.2.1]hept-5-ene-2-carboxylate (6).** (Mixture with **5**). ^{1}H NMR ($CDCl_3$) δ 6.35–6.31 (m, 2H, H-5, H-6), 5.04 (s, 1H, exch., NH), 4.40–4.10 (m, 2H, OCH_2), 4.24 (d, J=1.6 Hz, 1H, H-3), 3.78 (br s, 1H, H-1), 3.30 (br s, 1H, H-4), 2.73, 1.91 (AX system, J=10.3 Hz, 2H, H-7), 1.43 (s, 9H, CMe_3), 1.23 (t, J=6.9 Hz, 3H, Me); ^{13}C NMR δ 169.6, 154.4, 140.6, 135.9, 95.9, 81.2, 70.2, 62.9, 50.1, 48.4, 47.5, 29.6, 14.1.

4.2.3. Epimerization of compound 5 in TEA. Compound **5** (200 mg, 0.66 mmol) was dissolved in EtOH (10 mL) and a catalytic amount of TEA (0.06 mmol) was added. The reaction was left at room temperature for 2 h after which the solvent was eliminated. The residue was taken up with CH_2Cl_2 (15 mL) and washed with HCl (5%, 5 mL) and the organic layer was dried over Na_2SO_4. The crude reaction mixture was analyzed by ^{1}H NMR and HPLC (silica Hipersil column: 250×4.6 mm; CH_2Cl_2/0.5% *i*Pr_2OH; T=30 °C, flow=0.8 ml/min, λ=254) revealing the formation of the mixture **5**/**6** in a 70:30 ratio.

4.2.4. Reduction of 5 or 6. Nitro compound **5** or a mixture of **5/6** (1 g, 3.07 mmol) was dissolved in benzene (125 mL) and Pd/C (10%, 330 mg, 0.307 mmol) was added. The mixture was hydrogenated at room temperature and atmospheric pressure for 20 min. The reaction was monitored by ^{1}H NMR. The catalyst was filtered over Celite and the solvent was removed under vacuum. ^{1}H NMR analysis of the crude reaction mixture from **5** revealed the presence of compound **7** and trace amount of epimer **8**. The purification of the crude reaction mixture by column chromatography on silica gel (cyclohexane/AcOEt, 50:1) gave only the pure epimer **8** (775 mg, 77%). Starting from **5/6** a mixture of **7/8** was detected by ^{1}H NMR analysis. The crystallization of the mixture **7/8** with hexane allowed to obtain pure compound **8** (635 mg, 63%).

4.2.5. Ethyl (1*S,2*S**,3*R**,4*R**)-2-*t*-buthoxycarbonylamino-3-nitro-bicyclo[2.2.1]heptane-2-carboxylate (7).** Crude compound; ^{1}H NMR δ 5.72 (br s, 1H, H-3), 5.41 (br s, 1H, exch., NH), 4.35–4.06 (m, 2H, OCH_2), 2.96 (br s, 1H, H-4), 2.79 (br s, 1H, H-1), 1.90–1.30 (m, 4H, H-5, H-6), 1.86, 1.45 (AB system, J=10.3 Hz, 2H, H-7), 1.44 (s, 9H, CMe_3), 1.27 (t, J=7.0 Hz, 3H, Me); ^{13}C NMR δ 171.9, 155.4, 85.5, 81.1, 68.8, 63.4, 45.7, 42.5, 34.2, 28.1, 28.0, 22.2, 14.1.

4.2.6. Ethyl (1*S,2*S**,3*S**,4*R**)-2-*t*-buthoxycarbonylamino-3-nitro-bicyclo[2.2.1]heptane-2-carboxylate (8).** Mp 152 °C (CH_2Cl_2/iPr_2O); IR ν_{max} 3305, 1748, 1675 cm^{-1}; ^{1}H NMR δ 5.34 (br s, 1H, exch., NH), 4.26 (d, J=2.2 Hz, 1H, H-3), 4.21–4.10 (m, 2H, OCH_2), 3.23 (br s, 1H, H-1), 2.79 (d, J=4.0 Hz, 1H, H-4), 2.68, 1.64 (AX system, J=11.3 Hz, 2H, H-7), 1.90–1.40 (m, 4H, H-5, H-6), 1.46 (s, 9H, CMe_3), 1.24 (t, J=7.0 Hz, 3H, Me); ^{13}C NMR δ 169.2, 155.1, 97.7, 81.07, 70.9, 62.2, 45.0, 41.8, 37.6, 28.2, 26.5, 22.6, 13.7; MS (*m*/*z*) APCI: 327 $(M-H)^+$. Anal. Calcd C, 54.87; H, 7.37; N, 8.53. Found: C, 54.89; H, 7.33; N, 8.56.

4.3. General procedure for the synthesis of nitronate

Compound **5** or a mixture **5/6** or **8** (0.30 mmol) was dissolved in EtOH (2.5 mL) and H_2O (0.25 mL); then KOH (54 mg, 0.90 mmol) was added and the mixture was heated at reflux (**5** and **6**: 12 h; **8**: 36 h). The solvent was removed under vacuum. ^{1}H NMR analyses of the crude reaction mixture revealed the formation of the nitronate **10** or **12** in quantitative yield.

4.3.1. Nitronate (10). ^{1}H NMR (MeOD) δ 6.37–6.33 (m, 1H, H-6), 6.24–6.19 (m, 1H, H-5), 3.73 (br s, 1H, H-4), 3.61 (br s, 1H, H-1), 2.60, 1.57 (AX system, J=8.4 Hz, 2H, H-7), 1.41 1.41 (s, 9H, CMe_3); ^{13}C NMR δ 176.8, 156.1, 138.5, 134.9, 131.6, 78.3, 68.3, 57.1, 49.2, 45.2, 27.8.

4.3.2. Nitronate (12). ^{1}H NMR (MeOD) δ 3.17–3.16 (m, 2H, H-1, H-4), 2.43, 1.30 (AX system, J=9.5 Hz, 2H, H-7), 1.75–1.42 (m, 4H, H-5, H-6), 1.46 (s, 9H, CMe_3); ^{13}C NMR δ 176.4, 156.3, 131.4, 77.9, 68.5, 46.2, 40.3, 34.6, 27.6, 26.8, 23.7.

4.4. General procedure for the protonation of the nitronate

Nitronate **10** or **12** (0.30 mmol) was dissolved in MeOH (2 mL) and the mixture was acidified with HCl 10% (pH=3). After stirring (10 min) the solvent was removed under vacuum. ^{1}H NMR of the crude mixture revealed the presence of the single *endo*-nitro compound **11** or **13**. Starting from **10**, after purification on silica gel (CH_2Cl_2/MeOH, 5:1), pure compound **11** (86 mg, 97%) was obtained. The crude mixture from **12** was (i) suspended in MeOH (2 mL) and the salts were filtered allowing to obtain compound **13** (53 mg, 60%) or (ii) directly purified on silica gel giving a mixture of inseparable compounds **13/14** (40:60, 72 mg, 80%).

4.4.1. (1*R,2*S**,3*R**,4*S**)-2-*t*-Buthoxycarbonylamino-3-nitro-bicyclo[2.2.1]hept-5-ene-2-carboxylic acid (11).** Mp 178 °C (CH_2Cl_2/iPr_2O); IR ν_{max} 3415, 1702, 1675 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.61 (br s, 1H, H-6), 6.38 (br s, 1H, H-5), 6.14 (br s, 1H, H-3), 4.96 (s, 1H, exch., NH), 3.47 (br s, 2H, H-4, H-1), 1.77, 1.67 (AB system, J=9.0 Hz, 2H, H-7), 1.42 (s, 9H, CMe_3); ^{13}C NMR δ 174.5, 155.8, 140.1, 133.2, 88.7, 82.1, 68.6, 52.1, 47.8, 43.9, 28.2; MS (*m*/*z*) APCI: 297 $(M-H)^+$. Anal. Calcd C, 52.34; H, 6.08; N, 9.39. Found: C, 52.30; H, 6.14; N, 9.37.

4.4.2. (1*S,2*S**,3*R**,4*R**)-2-*t*-Buthoxycarbonylamino-3-nitro-bicyclo[2.2.1]heptane-2-carboxylic acid (13).** Impure of inorganic salts. IR ν_{max} 3415, 1702, 1675 cm^{-1}; ^{1}H NMR (MeOD; mixture of conformers) δ 5.87 (br s, 1H, H-3), 2.88 (br s, 1H), 2.63–2.56 (m, 1H), 2.04–1.94 (m, 1H), 1.84–1.78 (m, 1H), 1.75–1.68 (m, 1H), 1.67–1.56 (m, 1H), 1.54–1.30 (m, 11H, CMe_3); ^{13}C NMR (mixture of conformers) δ 174.2, 156.8 (155.1), 85.3 (85.1), 81.4 (79.7), 64.2 (64.0), 45.5 (45.3), 43.3 (43.2), 33.6, 27.2 (26.6), 21.6, 21.1 (20.9); MS (*m*/*z*) APCI: 299 $(M-H)^+$.

4.4.3. (1*S,2*S**,3*S**,4*R**)-2-*t*-Buthoxycarbonylamino-3-nitro-bicyclo[2.2.1]heptane-2-carboxylic acid (14).** (Mixture with **13**). ^{1}H NMR (MeOD) δ 4.60 (br s, 1H, H-3), 3.01 (br s, 1H, H-1), 2.78 (br s, 1H, H-7), 2.60 (br s, 1H, H-4), 1.67–1.30 (m, 5H, H-7, H-5, H-6), 1.46 (s, 9H); ^{13}C NMR δ 173.2, 157.2, 95.6, 79.5, 73.1, 44.9, 42.3, 37.5, 27.8, 26.3, 22.8.

4.4.4. Reduction of 11. Nitro compound **11** (50 mg, 0.17 mmol) was dissolved in MeOH (6 mL) and Pd/C (10%, 13 mg, 0.012 mmol) was added. The mixture was hydrogenated at room temperature and atmospheric pressure for 20 min. The reaction was monitored by TLC (cyclohexane/ethyl acetate 3:1). The catalyst was filtered over Celite and the solvent was removed under vacuum. A mixture of **13/14** (35 mg, 68%) was obtained in a 40:60 ratio.

4.5. General procedure for the deprotection of amino group

Method (a). Operating under nitrogen and stirring, TFA (0.08 mL) was added to a solution of compound **8** or **11** (0.13 mmol) dissolved in anhydrous CH_2Cl_2 (0.8 mL). The mixture was left under stirring over the night. After evaporation of the solvent under vacuum, the deprotected compound was recovered (**15a**: 40 mg, quantitative yield). Starting from **8**, the crude reaction mixture was taken up with $NaHCO_3$ (1.5 mL) and extracted with CH_2Cl_2 (3×2 mL). The organic layer was dried over Na_2SO_4 and the solvent was removed under vacuum giving **9** (20 mg, 75%). The purification of **9** on silica gel gave a mixture of

unidentified compounds. *Method (b)*. To a solution of compound **11** or **13** (0.2 mmol) in MeOH (1 mL), HCl (2 M) was added until the pH = 1. The mixture was stirred for 1 h, after which the solvent was removed under vacuum. Starting from **11** compound **15b** was first obtained but it equilibrated into compound **16** in 1 h (60:40; 46 mg, quantitative yield). Starting from **13**, compound **17** was obtained, which epimerized completely (2 days) into compound **18** (47 mg, quantitative yield).

4.5.1. Ethyl (1*S,2*S**,3*S**,4*R**)-2-amino-3-nitrobicyclo-[2.2.1]heptane-2-carboxylate (9).** Oil. IR ν_{max} 3305, 1748, 1675 cm^{-1}; ^1H NMR δ 4.23 (d, J = 2.2 Hz, 1H), 4.14 (q, J = 7.3 Hz, 2H), 2.85 (d, J = 5.2 Hz, 1H), 2.59 (br s, 1H), 2.10–1.30 (6H), 1.26 (t, J = 7.0 Hz, 3H). Anal. Calcd C, 52.62; H, 7.07; N, 12.27. Found: C, 52.34; H, 6.85; N, 12.00.

4.5.2. (1*R,2*S**,3*R**,4*S**)-2-Amino-3-nitro-bicyclo-[2.2.1]hept-5-ene-2-carboxylic acid trifluoroacetate (15a).** Oil (unstable). IR ν_{max} 3305, 1640, 1583 cm^{-1}; ^1H NMR (D_2O) δ 6.53–6.49 (m, 1H, H-6), 6.35–6.30 (m, 1H, H-5), 5.88 (d, J = 3.3 Hz, 1H, H-3), 3.53 (br s, 1H, H-4), 3.30 (br s, 1H, H-1), 1.85, 1.63 (AB system, J = 9.0 Hz, 2H, H-7); ^{13}C NMR δ 163.0, 140.0, 134.7, 88.8, 68.6, 52.1, 46.9, 43.8.

4.5.3. (1*R,2*S**,3*R**,4*R**)-2-Amino-3-nitro-bicyclo-[2.2.1]hept-5-ene-2-carboxylic acid hydrochloride (15b).** Oil (mixture with **16**). IR ν_{max} 3400, 1580 cm^{-1}; ^1H NMR (CD_3OD) δ 6.69–6.67 (m, 1H, H-6), 6.45–6.44 (m, 1H, H-5), 6.06 (d, J = 3.3 Hz, 1H, H-3), 3.65 (br s, 2H, H-4, H-1), 2.00, 1.71 (AB system, J = 10.3 Hz, 2H, H-7); ^{13}C NMR δ 169.6, 139.9, 134.5, 88.6, 65.0, 52.3, 51.0, 43.8; MS (*m*/*z*) ESI: 197 $(M-H)^+$.

4.5.4. (1*S,2*S**,3*S**,4*R**)-2-Amino-3-nitro-bicyclo[2.2.1]-hept-5-ene-2-carboxylic acid hydrochloride (16).** (Mixture with **15b**). IR ν_{max} 3390, 1585 cm^{-1}; ^1H NMR (CD_3OD) δ 6.66–6.64 (m, 1H, H-6), 6.43–6.41 (m, 1H, H-5), 4.75 (d, J = 2.5 Hz, 1H, H-3), 3.65 (br s, 1H, H-1), 3.42 (br s, 1H, H-4), 2.65, 1.99 (AX system, J = 10.7 Hz, 2H, H-7); ^{13}C NMR δ 168.3, 139.9, 137.0, 91.5, 65.0, 52.3, 51.0, 43.8; MS (*m*/*z*) ESI: 197 $(M-H)^+$.

4.5.5. (1*S,2*S**,3*R**,4*R**)-2-Amino-3-nitro-bicyclo-[2.2.1]heptane-2-carboxylic acid hydrochloride (17).** Oil. IR ν_{max} 3400, 1683, 1605 cm^{-1}; ^1H NMR (CD_3OD) δ 5.73 (d, J = 4.0 Hz, 1H, H-3), 3.08 (br s, 1H, H-4), 2.81 (br s, 1H, H-1), 1.98, 1.72 (AB system, J = 11.2 Hz, 2H, H-7), 1.62–1.30 (m, 4H, H-5, H-6). ^{13}C NMR δ 169.7, 85.1, 61.4, 45.5, 40.6, 33.0, 22.6, 20.0.

4.5.6. (1*S,2*S**,3*S**,4*R**)-2-Amino-3-nitro-bicyclo[2.2.1]-heptane-2-carboxylic acid hydrochloride (18).** Oil. IR ν_{max} 3400, 1680, 1600 cm^{-1}; ^1H NMR (CD_3OD) δ 4.63 (d, J = 1.8 Hz, 1H, H-3), 3.05 (br s, 1H, H-1), 2.68 (br s, 1H, H-4), 2.58, 1.72 (AX system, J = 11.4 Hz, 2H, H-7), 1.62–1.30 (m, 4H, H-5, H-6); ^{13}C NMR δ 169.1, 92.3, 69.3, 46.1, 39.8, 36.5, 24.5, 22.1; MS (*m*/*z*) ESI: 199 $(M-H)^+$. Anal. Calcd C, 48.00; H, 6.04; N, 13.99. Found: C, 47.82; H, 6.10; N, 13.80.

Acknowledgements

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The Mitsunobu reaction in preparing 3-deazapurine carbocyclic nucleosides

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Abstract—The coupling reaction of 4-chloro-1*H*-imidazo[4,5-*c*]pyridine (6-chloro-3-deazapurine, **3**) with several cyclopentyl derivatives under Mitsunubo reaction conditions provides an efficient entry into N-7 and N-9 substituted 3-deazapurine carbocyclic nucleosides of antiviral potential. The versatility of this procedure is illustrated with a new and efficient synthesis of (−)-3-deazaaristeromycin, a formal preparation of 3-deazaneplanocin A, and a route to 3-deaza-5′-homoaristeromycin.

1. Introduction

Nucleoside analogs based on the 3-deazapurine (1*H*-imidazo[4,5-*c*]pyridine) framework have found significant usefulness in antiviral agent design and biochemical investigations.[1,2] The carbocyclic nucleosides[3] 3-deazaristeromycin (**1**)[4] and 3-deazaneplanocin (**2**)[5] have been central to these studies (Fig. 1). In our efforts to further exploit the 3-deazapurine carbocyclic nucleoside platform as a source for new antiviral candidates, it was necessary to seek a more versatile synthetic means to this series that would give access to a number of structural variations. In this regard it was surprising to find that the Mitsunobu reaction,[6] which has been successfully employed to produce traditional carbocyclic nucleosides, had not been investigated in the 3-deazapurine genera. This paper describes the use of the Mitsunobu reaction in the preparation of such derivatives.[7]

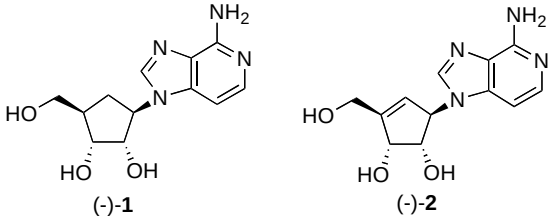
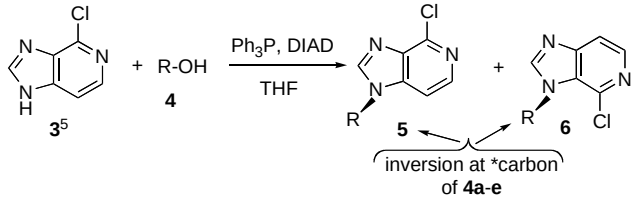
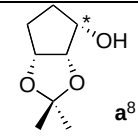
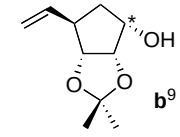
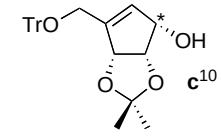
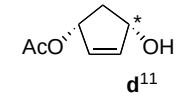
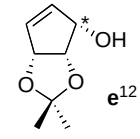


Figure 1.

Table 1. Mitsunubo reaction of 4-chloro-1*H*-imidazo[4,5-*c*]pyridine (3-deaza-6-chloropurine) with substituted cyclopentanols



Entry	R-OH	Products (%)	
		5	**6**
1	 **a**[8]	86	0
2	 **b**[9]	70	0
3	 **c**[10]	42	53
4	 **d**[11]	38	57
5	 **e**[12]	32	43

Keywords: 4-Amino-1*H*-imidazo[4,5-*c*]pyridine; Carbocyclic nucleosides; Aristeromycin; Neplanocin.
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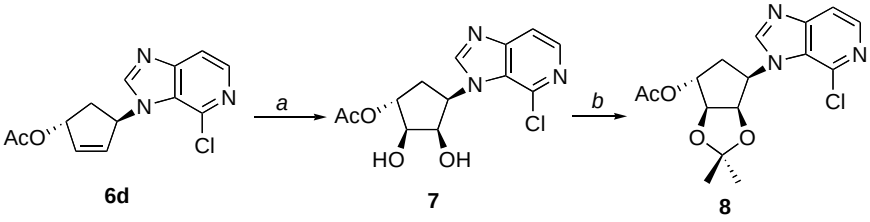


Scheme 1. Reagents: a, OsO_4, NMO, CH_2Cl_2; b, $(MeO)_2CMe_2$, acetone, *p*TSA, 83% (two steps).

2. Chemistry

Following standard Mitsunobu conditions (that is, triphenylphosphine and diisopropyl azodicarboxylate in tetrahydrofuran), the reaction of 4-chloro-1*H*-imidazo-[4,5-*c*]pyridine (**3**)[5] with various cyclopentanols gave the results presented in the Table 1. Thus, reacting **4a** with **3** cleanly gave **5a** as the only isomer. Likewise, compound **4b** provided **5b** as the only regioisomer. The more reactive allylic alcohols **4c–e**, however, yielded the N-1 (purine N-9) products **5c–e** along with the N-3 (purine N-7) isomers **6c–e**, which were the major products.

Structural assignments for the N-1 (N-9) and N-3 (N-7) isomers were possible because the proton on the cyclopentyl carbon bearing the heterocyclic ring in the N-3 product is downfield in the proton NMR spectrum compared to the N-1 product (by correlating the data of Ref. 5 with the data found in this study that is supported by the X-ray structural confirmation of **8**, vide infra). A characteristic carbon-13 NMR peak ($\delta = \sim 106$ ppm) was observed for the carbon (possibly C-2) in the heterocyclic ring of all N-1 products (**5**) while the peak moves to ($\delta = 115$ ppm) in all N-3 products (**6**). Supporting these NMR assignments for N-3 products was the conversion of **6d** to **8** (Scheme 1), whose structure was confirmed by X-ray crystallography (Fig. 2) and whose NMR spectrum fit the diagnostic peaks used for isomer distinction.

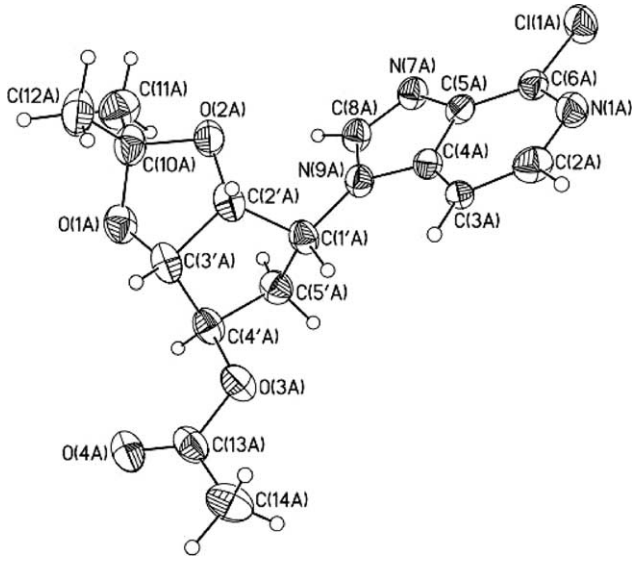
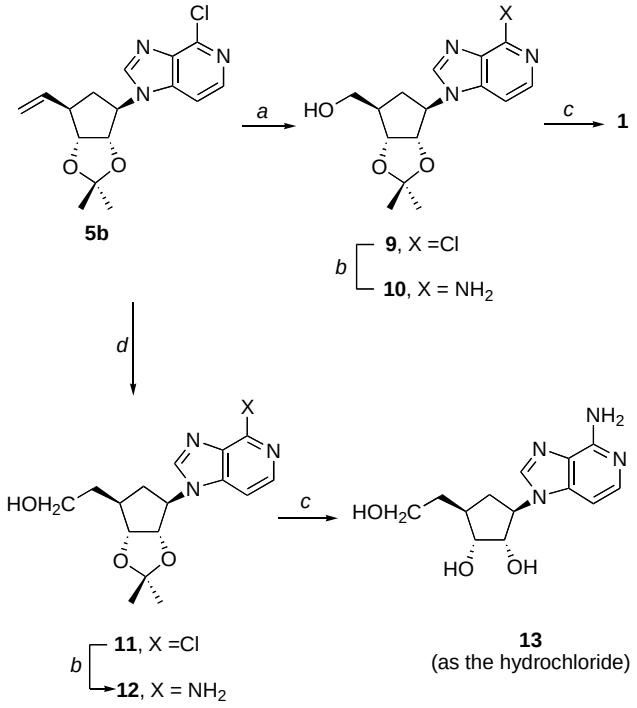


Figure 2. X-ray structure for compound **8**.

Further transformations of the coupled products **5b** and **5c** were sought for additional structural confirmation and to demonstrate extended synthetic versatility by their conversion into two important 3-deazapurine carbocyclic nucleosides, 3-deazaaristeromycin (**1**) and 3-deazaneplanocin (**2**). Whereas compound **2** was obtained from **5c** by modifying a known procedure,[5] manipulation of the vinyl group in **5b** to a hydroxymethyl moiety following our recently reported procedure[9a] furnished (−)-**1** in good overall yield (Scheme 2).[13] Compound **5b** also provided access to 3-deaza-5′-homoaristeromycin (**13**), which is a compound of potentially significant activity toward the orthopox viruses.[14]



Scheme 2. Reagents: a, (i) OsO_4, $NaIO_4$, MeOH; (ii) $NaBH_4$, MeOH, 81%; b, (i) NH_2NH_2, THF; (ii) Ra-Ni, MeOH/H_2O, 75% (two steps for **9**); 80% (two steps) for **12**; c, HCl/MeOH, 89% for **1**; 78% for **13**; d, (i) 9-BBN, THF; (ii) NaOH, H_2O_2, 80% (two steps).

3. Experimental

3.1. General

Melting points were recorded on a Meltemp II melting point apparatus and the values are uncorrected. The combustion analyses were performed at Atlantic Microlab, Norcross, GA. ^1H and ^{13}C NMR spectra were recorded on either a Bruker AC 250 spectrometer (250 MHz for proton and 62.9 MHz for carbon) or a Bruker AV 400 spectrometer (400 MHz for proton and 100 MHz for carbon), referenced to internal tetramethylsilane (TMS) at 0.0 ppm. The X-ray

crystal structure was determined using a Bruker APEX CCD single crystal X-ray diffractometer. The HRMS measurements were obtained using a VG 70S magnetic sector mass spectrometer. The reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm Whatman Diamond silica gel 60-F_{254} precoated plates with visualization by irradiation with a Mineralight UVGL-25 lamp. Column chromatography was performed on Whatman silica, 230–400 mesh and 60 Å using elution with the indicated solvent system. Yields refer to chromatographically and spectroscopically (^{1}H and ^{13}C NMR) homogeneous materials.

3.1.1. (3a*S*,4*S*,6a*R*)-Tetrahydro-2,2-dimethyl-3a*H*-cyclopenta[*d*][1,3]dioxol-4-ol (4a). A mixture of (3a*R*,6a*R*)-2,2-dimethyl-3a*H*-cyclopenta[*d*][1,3]dioxol-4(6a*H*)-one (**i**)[8,9a] (2.0 g, 13.0 mmol), Pd/C and MeOH (30 mL) was shaken under 18 psi of H_2. The reaction was run until TLC monitoring indicated no UV-active starting material was present. After filtration, concentration of the filtrate gave an oily product that was purified by silica gel chromatography (EtOAc/hexanes, 1:2) to provide 1.9 g (93.8%) of (3a*R*,6a*R*)-dihydro-2,2-dimethyl-3a*H*-cyclopenta[*d*][1,3]-dioxol-4(5*H*)-one (**ii**)[8]as a white solid.[9b]

To a stirred solution of ketone **ii** (0.50 g, 3.20 mmol) in MeOH (30 mL) at 0 °C $NaBH_4$ (0.18 g, 4.76 mmol) was added portion wise. After stirring at room temperature for 1 h, the mixture was quenched with H_2O. Most of solvent was removed under reduced pressure and the aqueous layer exacted with CH_2Cl_2; the combined organic layers were washed with H_2O, dried (Na_2SO_4), concentrated, and purified by flash chromatography to give **4a** as colorless oil (0.46 g, 88.8%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.61 (t, J=5.2 Hz, 1H), 4.41 (t, J=5.6 Hz, 1H), 3.84 (m, 1H), 2.40 (d, J=9.8 Hz, 1H), 1.83 (m, 2H), 1.64 (m, 1H), 1.49 (s, 3H), 1.43 (m, 1H), 1.35 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 110.6, 79.6, 78.7, 73.5, 30.3, 27.7, 26.0, 24.3. Anal. Calcd for $C_8H_{14}O_3$: C, 60.74; H, 8.92. Found: C, 60.74; H, 9.17.

3.2. General procedure for the Mitsunobu reaction of 6-chloro-3-deazapurine (4-chloro-1*H*-imidazo [4,5-*c*]pyridine) with cyclopentanols

To a solution of cyclopentanol **4a–e** (10 mmol) and triphenylphosphine (15 mmol) in THF (50 mL) was added 4-chloro-1*H*-imidazo[4,5-*c*]pyridine (**3**) (10 mmol). This suspension was cooled by ice to 0 °C and DIAD (15 mmol) was added dropwise. After completion of the addition, the reaction mixture was warmed to room temperature and stirred at this temperature for 12 h and 50 °C for another 12 h. The solvent was removed under reduced pressure and the residue purified by column chromatography (CH_2Cl_2/EtOAc, 3:1 or CH_2Cl_2/acetone, 4:1) to afford the coupled product.

3.2.1. Compound 5a (86%). White solid, mp 131–133 °C: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, J=5.6 Hz, 1H), 7.94 (s, 1H), 7.47 (d, J=5.6 Hz, 1H), 4.88 (m, 1H), 4.69 (m, 2H), 2.59 (m, 1H), 2.17 (m, 3H), 1.57 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.5, 142.1, 141.8, 140.3, 138.4, 112.2, 106.3, 85.7, 80.5, 63.2, 31.5, 28.6, 26.9, 24.6. HRMS calcd for $C_{14}H_{16}ClN_3O_2$ (M^+) 293.0931, found 293.0936.

3.2.2. Compound 5b (70%). Colorless oil: ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.23 (d, J=5.7 Hz, 1H), 8.04 (s, 1H), 7.60 (d, J=5.7 Hz, 1H), 5.94 (m, 1H), 5.22 (m, 2H), 4.67 (m, 2H), 4.58 (m, 1H), 2.92 (m, 1H), 2.68 (m, 1H), 2.33 (m, 1H), 1.64 (s, 3H), 1.33 (s, 3H). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 143.2, 142.0, 141.8, 140.2, 138.3, 137.0, 117.0, 114.8, 106.6, 84.9, 83.9, 62.3, 47.7, 35.7, 27.5, 25.1. Anal. Calcd for $C_{16}H_{18}ClN_3O_2$: C, 60.09; H, 5.67; N, 13.14. Found: C, 60.06; H, 5.60; N, 12.79.

3.2.3. Compound 5c (42%). White foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (d, J=5.6 Hz, 1H), 7.89 (s, 1H), 7.50 (m, 15H), 7.45 (d, J=5.6 Hz, 1H), 6.16 (m, 1H), 5.48 (m, 1H), 5.22 (d, J=5.8 Hz, 1H), 4.58 (d, J=5.8 Hz, 1H), 4.08 (d, J=15.6 Hz, 1H), 3.92 (d, J=15.6 Hz, 1H), 1.50 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.1, 143.8, 143.4, 142.6, 141.9, 139.7, 138.6, 128.7, 128.2, 127.5, 121.3, 113.3, 105.8, 87.6, 84.8, 83.9, 67.1, 61.5, 27.5, 26.0. Anal. Calcd for $C_{34}H_{30}ClN_3O_3$: C, 72.40; H, 5.36; N, 7.45. Found: C, 72.06; H, 5.67; N, 7.19.

3.2.4. Compound 6c (53%). White foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, J=5.7 Hz, 1H), 7.86 (s, 1H), 7.66 (d, J=5.7 Hz, 1H), 7.53 (m, 15H), 6.30 (m, 1H), 6.19 (m, 1H), 5.17 (d, J=5.4 Hz, 1H), 4.67 (d, J=5.4 Hz, 1H), 4.11 (d, J=15.5 Hz, 1H), 3.93 (d, J=15.5 Hz, 1H), 1.47 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.0, 151.7, 144.6, 143.8, 143.7, 141.6, 134.3, 128.7, 128.2, 127.5, 121.4, 115.2, 112.9, 87.5, 85.1, 83.7, 66.3, 61.4, 27.7, 26.3. Anal. Calcd for $C_{34}H_{30}ClN_3O_3$: C, 72.40; H, 5.36; N, 7.45. Found: C, 72.13; H, 5.60; N, 7.28

3.2.5. Compound 5d (38%). Colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, J=5.8 Hz, 1H), 8.00 (s, 1H), 7.30 (d, J=5.8 Hz, 1H), 6.42 (ddd, J=5.6, 2.7, 2.7 Hz, 1H), 6.26 (m, 1H), 5.95 (m, 1H), 5.78 (m, 1H), 2.60 (ddd, J=14.9, 7.8, 2.6 Hz, 1H), 2.39 (ddd, J=14.9, 7.3, 4.8 Hz, 1H), 2.08 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 143.1, 142.9, 141.4, 139.1, 138.1, 136.4, 134.4, 105.7, 78.0, 61.2, 38.3, 21.0. Anal. Calcd for $C_{13}H_{12}ClN_3O_2$: C, 56.22; H, 4.36; N, 15.13. Found: C, 56.59; H, 4.01; N, 15.36.

3.2.6. Compound 6d (57%). Colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, J=5.5 Hz, 1H), 8.05 (s, 1H), 7.66 (d, J=5.5 Hz, 1H), 6.46 (m, 1H), 6.41 (m, 1H), 6.35 (m, 1H), 5.91 (m, 1H), 2.67 (ddd, J=14.7, 7.8, 3.3 Hz, 1H), 2.39 (ddd, J=14.7, 7.3, 3.8 Hz, 1H), 2.09 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 151.4, 143.8, 141.2, 137.3, 133.6, 133.5, 127.9, 115.0, 77.9, 61.6, 40.7, 21.1. Anal. Calcd for $C_{13}H_{12}ClN_3O_2$: C, 56.22; H, 4.36; N, 15.13. Found: C, 56.13; H, 4.60; N, 14.98.

3.2.7. Compound 5e (32%). White solid, mp 96–98 °C: ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.25 (d, J=5.6 Hz, 1H), 7.90 (s, 1H), 7.50 (d, J=5.6 Hz, 1H), 6.44 (m, 1H), 6.10 (dd, J=5.8, 2.3 Hz, 1H), 5.43 (m, 2H), 4.56 (d, J=5.8 Hz, 1H), 1.52 (s, 3H), 1.36 (s, 3H). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 143.4, 142.3, 141.9, 139.7, 139.0, 138.5, 129.0, 113.0, 105.8, 84.4, 84.2, 67.9, 27.3, 25.6. HRMS calcd for $C_{14}H_{14}ClN_3O_2$ (M^+) 291.0775, found 291.0773.

3.2.8. Compound 6e (43%). Colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, J=5.5 Hz, 1H), 7.92 (s, 1H), 7.52 (d, J=5.5 Hz, 1H), 6.44 (dd, J=5.7, 1.5 Hz, 1H), 6.27 (s, 1H), 6.10 (dd, J=5.7, 1.1 Hz, 1H), 5.38 (dd, J=5.3, 1.3 Hz, 1H), 4.66 (d, J=5.4 Hz, 1H), 1.50 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.0, 144.5, 141.7, 140.0, 134.5, 129.4, 128.4, 115.3, 112.8, 84.7, 84.4, 67.4, 27.8, 26.3. HRMS calcd for $C_{14}H_{14}ClN_3O_2$ (M^+) 291.0775, found 291.0776.

3.2.9. (1*R*,2*R*,3*R*,4*R*)-4-(4-Chloro-3*H*-imidazo[4,5-*c*]-pyridin-3-yl)-2,3-dihydroxycyclopent-1-yl acetate (7). Methylmorpholine-*N*-oxide (2.13 g, 18.2 mmol) was added to a solution of **6d** (2.67 g, 9.64 mmol) in CH_2Cl_2 (50 mL) containing a small amount of H_2O (0.8 mL). After the solution was cooled to 0 °C, a catalytic amount of solid OsO_4 (90 mg, 0.36 mmol) was added and the solution stirred for 12 h at room temperature. The reaction mixture was quenched by addition of sodium bisulfite. The solvent was removed and the residue purified by flash column chromatography (EtOAc) to afford **7** as white solid (2.55 g, 85%), mp 209–210 °C: ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.75 (s, 1H), 8.15 (d, J=5.5 Hz, 1H), 7.73 (d, J=5.5 Hz, 1H), 5.64 (m, 1H), 5.38 (d, J=3.0 Hz, 1H, OH), 5.37 (d, J=4.1 Hz, 1H, OH), 5.07 (m, 1H), 4.16 (m, 1H), 4.08 (m, 1H), 3.00 (m, 1H), 2.16 (m, 1H), 2.09 (s, 3H). ^{13}C NMR (62.9 MHz, DMSO-d_6) δ 171.2, 151.8, 148.8, 141.2, 133.5, 128.8, 115.6, 78.4, 76.8, 73.6, 56.1, 34.3, 21.8. Anal. Calcd for $C_{13}H_{14}ClN_3O_4$: C, 50.09; H, 4.53; N, 13.48. Found: C, 49.84; H, 4.54; N, 13.31.

3.2.10. (3a*R*,4*R*,6*R*,6a*S*)-4-(4-Chloro-3*H*-imidazo[4,5-*c*]-pyridin-3-yl)-tetrahydro-2,2-dimethyl-3a*H*-cyclopenta-[*d*][1,3]dioxol-6-yl acetate (8). To a solution of **7** (3.12 g, 10.0 mmol) and 2,2-dimethoxypropane (15 mL) in dry acetone (20 mL) was added a catalytic amount of *p*-toluenesulfonic acid (50 mg). After the reaction mixture was stirred at room temperature for 12 h, the solvent was removed and the residue dissolved in CH_2Cl_2 (40 mL). This solution was washed with saturated $NaHCO_3$ solution, H_2O and brine. The organic phase was dried ($MgSO_4$) and concentrated. The residue was purified by flash column chromatography (EtOAc/hexanes, 1:2) to afford **8** as a white solid, mp 142–143 °C: ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.29 (s, 1H), 8.23 (d, J=5.5 Hz, 1H), 7.68 (d, J=5.5 Hz, 1H), 5.81 (ddd, J=10.4, 5.5, 5.5 Hz, 1H), 5.17 (d, J=4.4 Hz, 1H), 4.89 (dd, J=4.8, 4.9 Hz, 1H), 4.65 (dd, J=5.4, 1.5 Hz, 1H), 2.69 (ddd, J=13.1, 4.5, 4.5 Hz, 1H), 2.37 (dd, J=13.1, 5.8 Hz, 1H), 2.16 (s, 3H), 1.52 (s, 3H), 1.29 (s, 3H). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 169.9, 151.4, 146.2, 141.3, 133.3, 128.2, 115.4, 112.3, 83.5, 78.5, 74.7, 56.5, 33.2, 26.0, 24.0, 21.2. Anal. Calcd for $C_{16}H_{18}ClN_3O_4$: C, 54.63; H, 5.16; N, 11.94. Found: C, 54.83, H, 5.11, N, 11.85.

3.2.11. ((3a*S*,4*R*,6*R*,6a*R*)-4-(4-Chloro-1*H*-imidazo[4,5-*c*]-pyridin-1-yl)-tetrahydro-2,2-dimethyl-3a*H*-cyclopenta-[*d*][1,3]dioxol-6-yl)methanol (9). To a solution of **5b** (1.00 g, 3.13 mmol) in MeOH (20 mL), H_2O (8 mL) and $NaIO_4$ (1.39 g, 6.48 mmol) were added. After the mixture was cooled to 0 °C, OsO_4 (20 mg) was added. This reaction mixture was stirred at the same temperature for 1 h and then at room temperature for 2 h. The resulting white solid was obtained by filtration and the filtrate cooled to 0 °C. To this $NaBH_4$ (0.80 g, 20 mmol) was added portion wise. After the reaction was stirred at room temperature for 0.5 h, the solvent was removed and the product obtained by short column chromatography (EtOAc/MeOH, 10:1) to give **9** as a white foam (0.82 g, 81%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (s, 1H), 8.20 (d, J=5.6 Hz, 1H), 7.81 (d, J=5.6 Hz, 1H), 4.86 (m, 2H), 4.80 (t, J=6.5 Hz, 1H), 4.57 (dd, J=4.5, 7.0 Hz, 1H), 3.55 (t, J=5.5 Hz, 2H), 2.46 (m, 1H), 2.21 (m, 1H), 2.19 (m, 1H), 1.58 (s, 3H), 1.26 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.3, 142.0, 141.7, 140.9, 138.2, 113.7, 108.2, 84.9, 81.8, 62.9, 62.8, 46.1, 33.7, 28.2, 26.0. Anal. Calcd for $C_{15}H_{18}ClN_3O_3$: C, 55.64; H, 5.60; N, 12.98. Found: C, 55.87; H, 5.67; N, 12.79.

3.2.12. ((3a*S*,4*R*,6*R*,6a*R*)-4-(4-Amino-1*H*-imidazo[4,5-*c*]-pyridin-1-yl)-tetrahydro-2,2-dimethyl-3a*H*-cyclopenta-[*d*][1,3]dioxol-6-yl)methanol (10). A solution of **9** (1.62 g, 5.00 mmol) in hydrazine (15 mL) and MeOH (6 mL) was brought to reflux for 6 h. After cooling to room temperature, the solution was concentrated. The residue was dissolved in MeOH (30 mL) and freshly prepared W2-Raney Ni (prepared from 40 g of alloy) was added to it. The reaction mixture was heated to reflux for 1 h. The hot reaction mixture was filtered and the solid recovered and washed with hot MeOH (3×15 mL). The combined filtrates were evaporated to dryness and the residue purified via column chromatography (EtOAc/MeOH, 10:1) to afford **10** as white solid (1.14 g, 75%), mp 203–205 °C: ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.22 (s, 1H), 7.66 (d, J=5.8 Hz, 1H), 6.87 (d, J=5.8 Hz, 1H), 6.38 (br, 2H), 4.74 (q, J=6.8 Hz, 1H), 4.69 (m, 1H), 4.55 (m, 1H), 3.51 (d, J=4.8 Hz, 2H), 2.39–2.17 (m, 3H), 1.50 (s, 3H), 1.23 (s, 3H). ^{13}C NMR (62.9 MHz, DMSO-d_6) δ 152.4, 140.0, 139.9, 138.1, 126.9, 112.6, 97.3, 84.0, 80.9, 61.8, 61.6, 45.2, 32.9, 27.4, 25.1.[15]

3.2.13. (1*R*,2*S*,3*R*,5*R*)-3-(4-Amino-1*H*-imidazo[4,5-*c*]-pyridin-1-yl)-5-(hydroxymethyl)cyclopentane-1,2-diol ((−)-3-deazaaristeromycin, 1). Compound **10** (304 mg, 1.00 mmol) was dissolved in a mixture of MeOH (5 mL) and 1 N HCl (5 mL) and the resulting solution was stirred at room temperature for 5 h. Basic resin (Amberlite IR 67) was added to neutralize the solution. The mixture was filtered and the filtrate removed under vacuum. The residue was purified by column chromatography (EtOAc/MeOH/NH_4OH, 5:2:1) to afford **1** as a 1 mol HCl salt, white solid (89%), mp >231 °C (dec): ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.63 (s, 1H), 8.50 (br, 2H), 7.74 (d, J=7.0 Hz, 1H), 7.30 (d, J=7.0 Hz, 1H), 4.72 (q, J=9.5 Hz, 1H), 4.14 (dd, J=9.3, 5.4 Hz, 1H), 3.82 (dd, J=5.4, 2.8 Hz, 1H), 3.46 (d, J=5.3 Hz, 2H), 2.31 (dt, J=12.7, 8.8 Hz, 1H), 2.09 (m, 1H), 1.75 (m, 1H). ^{13}C NMR (62.9 MHz, DMSO-d_6) δ 148.9, 143.5, 140.1, 129.0, 126.1, 99.3, 76.1, 72.0, 62.7, 61.0, 45.4, 28.9.[13]

3.2.14. 2-((3a*S*,4*R*,6*R*,6a*R*)-4-(4-Chloro-1*H*-imidazo[4,5-*c*]-pyridin-1-yl)-tetrahydro-2,2-dimethyl-3a*H*-cyclopenta-[*d*][1,3]dioxol-6-yl)ethanol (11). To a solution of **5b** (1.00 g, 3.13 mmol) in THF (20 mL) at 0 °C under N_2 was added 9-BBN-H (0.5 M in THF, 10.0 mL, 5.00 mmol), and the resultant mixture stirred for 3 h. To this, NaOH solution (1 M, 6 mL) followed by H_2O_2 (50% in H_2O, 3 mL) was added and the stirring continued an additional 30 min. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and this

mixture washed with saturated $NaHCO_3$ solution (30 mL). The organic layer was dried ($MgSO_4$), filtered, and the filtrate concentrated in vacuo to give the crude product as a colorless oil, which was purified by flash column chromatography (EtOAc/hexane, 1:4) to afford **11** as a white solid (0.84 g, 80%), mp 190–191 °C: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (s, 1H), 8.17 (d, J=5.6 Hz, 1H), 7.81 (d, J=5.6 Hz, 1H), 4.78 (m, 2H), 4.48 (t, J=5.1 Hz, 1H), 4.44 (dd, J=5.6, 6.6 Hz, 1H), 3.48 (dd, J=11.9, 6.6 Hz, 2H), 2.48 (dd, J=12.1, 6.1 Hz, 1H), 2.13 (m, 2H), 1.73 (ddd, J=13.4, 6.3, 6.3 Hz, 1H), 1.61 (ddd, J=13.6, 6.4, 6.4 Hz, 1H), 1.52 (s, 3H), 1.24 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.4, 141.0, 140.8, 140.0, 137.2, 113.0, 107.3, 84.2, 84.0, 61.6, 59.2, 40.0, 36.2, 35.9, 27.3, 25.1. Anal. Calcd for $C_{16}H_{20}ClN_3O_3$: C, 56.89; H, 5.97; N, 12.44. Found: C, 56.88; H, 6.09; N, 12.29.

3.2.15. 2-((3a*S*,4*R*,6*R*,6a*R*)-4-(4-Amino-1*H*-imidazo[4,5-*c*]-pyridin-1-yl)-tetrahydro-2,2-dimethyl-3a*H*-cyclopenta-[*d*][1,3]dioxol-6-yl)ethanol (12). A solution of **11** (1.69 g, 5.00 mmol) in hydrazine (20 mL) and MeOH (6 mL) was brought to reflux for 6 h. After cooling to room temperature, the solution was concentrated. The residue was dissolved in MeOH (40 mL) and freshly prepared W2-Raney Ni (prepared from 40 g of alloy) was added to it. The reaction mixture was heated to reflux for 2 h and the hot reaction mixture filtered and the solid washed with hot MeOH (3×15 mL). The combined filtrates were evaporated to dryness and the residue purified via column chromatography (EtOAc/MeOH, 10:1) to afford **12** as a white solid (1.29 g, 80%), mp 192–194 °C: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (s, 1H), 7.67 (d, J=5.8 Hz, 1H), 6.89 (d, J=5.8 Hz, 1H), 6.27 (br, 2H), 4.75 (m, 1H), 4.63 (m, 1H), 4.53 (br, 1H), 4.41 (m, 1H), 3.46 (m, 2H), 2.42 (m, 1H), 2.05 (m, 1H), 2.00 (m, 1H), 1.72 (m, 1H), 1.59 (m, 1H), 1.50 (s, 3H), 1.23 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.2, 140.8, 140.7, 139.0, 127.7, 113.8, 98.1, 85.1, 84.9, 62.0, 60.1, 41.0, 37.1, 37.0, 28.2, 26.0. Anal. Calcd for $C_{16}H_{22}N_4O_3$: C, 60.36; H, 6.97; N, 17.60. Found: C, 60.16; H, 7.06; N, 17.49.

3.2.16. (1*R*,2*S*,3*R*,5*R*)-3-(4-Amino-1*H*-imidazo[4,5-*c*]-pyridin-1-yl)-5-(2-hydroxyethyl)cyclopentane-1,2-diol (3-deaza-5′-homoaristeromycin, 13). Compound **12** (0.64 g, 2.01 mmol) was dissolved in a mixture of MeOH (10 mL) and 1 N HCl (10 mL) and the resulting solution was stirred at room temperature for 5 h. Basic resin (Amberlite IR 67) was added for neutralization. The solution was filtered, the filtrate removed under vacuum and the residue purified by column chromatography (EtOAc/MeOH/NH_4OH, 6:2:1) to afford **13**·HCl as a white solid (436 mg, 78%), mp 205–206 °C: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (s, 1H), 7.64 (d, J=5.8 Hz, 1H), 6.81 (d, J=5.8 Hz, 1H), 6.18 (br, 2H), 5.00 (d, J=6.3 Hz, 1H), 4.80 (d, J=4.8 Hz, 1H), 4.48 (m, 2H), 4.14 (q, J=6.3 Hz, 1H), 3.67 (q, J=4.8 Hz, 1H), 3.46 (m, 2H), 2.30 (ddd, J=12.6, 7.8, 7.8 Hz, 1H), 1.96 (m, 1H), 1.76 (ddd, J=13.1, 6.8, 6.8 Hz, 1H), 1.52 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 151.6, 139.4, 139.0, 137.5, 126.1, 96.4, 74.1, 74.0, 60.1, 58.6, 39.5, 36.5, 31.6. Anal. Calcd for $C_{13}H_{19}N_4O_3Cl$: C, 49.60; H, 6.08; N, 17.80. Found: C, 49.40; H, 5.84; N, 17.49.

2.3. X-ray data for compound 8

Crystallographic data (excluding structure factors) for **8** has been deposited with Cambridge Crystallographic Data Centre as supplementary number CCDC 267776. 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].

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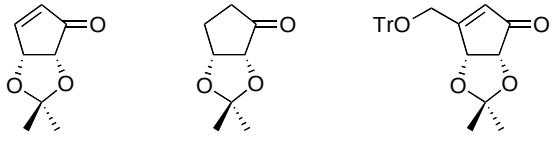

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New cyclic depsipeptides from the green alga *Bryopsis* species; application of a carboxypeptidase hydrolysis reaction to the structure determination

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Abstract—New cyclic depsipeptides, kahalalides P (**1**) and Q (**2**), were isolated from the Hawaiian green alga *Bryopsis* sp. The sequential positions of the DL anti-podal amino acids were determined by a carboxypeptidase hydrolysis reaction. This enzymatic method will be applicable to the structure determination of other non-ribosomal peptides. The absolute chemistry of 3-hydroxy-9-methyldecanoic acid in kahalalides P and Q were determined by the recently introduced convenient Mosher ester procedure.

1. Introduction

Kahalalides, cyclic, and acyclic peptides, have been previously characterized from the sacoglossan mollusk *Elysia rufescens* and its diet, the green alga *Bryopsis* sp.[1–6] Kahalalide F exhibits selective activity against solid tumors and is currently undergoing phase II clinical trials.[7] Kahalalide A exhibits anti-mycobacterium tuberculosis activity.[8,9] Re-investigation of a Hawaiian alga *Bryopsis* sp. extract led to the isolation of two new cyclic depsipeptides kahalalide P (**1**) and kahalalide Q (**2**). We report herein the determination of the absolute stereochemistry of these compounds. A carboxypeptidase hydrolysis reaction was utilized for determining the sequential position of the anti-podal DL amino acids.

Keywords: Marine natural product; Green alga; Depsipeptide; Carboxypeptidase.
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2. Results and discussion

2.1. Isolation of kahalalides P and Q

Bryopsis sp. (1.0 kg wet wt) was collected in Hawaii. The alga was lyophilized and stored at −30 °C. The freeze dried alga was extracted with methanol. After drying, the extract (31 g) was mixed with Celite powder and subjected to low-pressure flash chromatography on an ODS column. The MeOH/H_2O 9:1 fraction was further separated by reverse phase HPLC, which yield kahalalide P (**1**) [5.2 mg (0.0005%)], kahalalide Q (**2**) [1.8 mg (0.0002%)] as well as previously described kahalalides G and F.

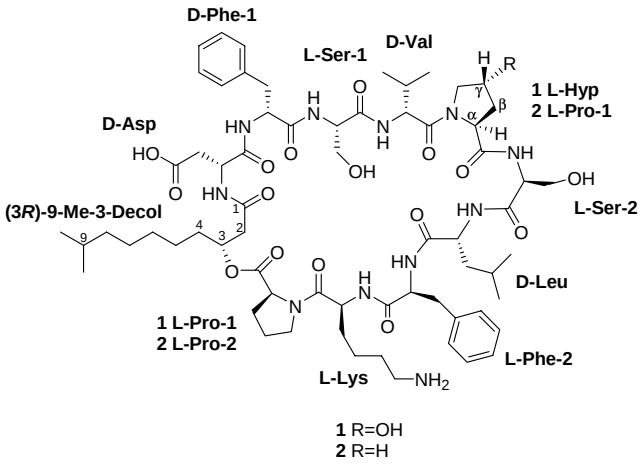
2.2. Structures of kahalalides P and Q

2.2.1. Planar structure of kahalalide P. The molecular formula of kahalalide P (**1**) was established as $C_{66}H_{99}N_{11}O_{17}$ on the basis of the HRFABMS data, *m/z* 1318.7319 $[M+H]^+$(Δ +2.1 mmu). It was corroborated by the ^{13}C NMR spectrum, which displayed signals for 66 carbons. NMR experiments were performed in DMSO-d_6 with addition of 0.05% of TFA, as peak broadening was observed in C_5D_5N and pure DMSO-d_6 solvents. Detailed analysis of the 2D NMR data enabled us to assign all the signals for kahalalide P and revealed a structural framework consisting of peptidal and fatty acid moieties. Examination of the ^1H NMR spectra

suggested **1** was a peptide with aromatic and aliphatic residues. The low field portion of the spectrum showed eight doublets of amide proton signals at 7.4–8.8 ppm, one broad peak (two protons from the NH_2 group of lysine) at 7.55 ppm and two sets of signals of protons of monosubstituted aromatic moieties, each of the five protons were between 7.0–7.4 ppm. Investigation of the 2D NMR data from TOCSY, DQF-COSY, HSQC, and HMBC experiments led to identification of the corresponding ten amino acid residues: Asp, Val, Lys, Leu, 4-*trans*-hydroxy-Pro (Hyp), Pro, two Ser, and two Phe. The Asp spin system could be traced by TOCSY cross peaks between signals 7.47 ppm (NH), 4.54 ppm (Hα), 2.52 and 2.25 ppm (Hβ); Val protons showed correlation in TOCSY spectra between 7.81 ppm (NH), 4.64 ppm (Hα), 1.98 ppm (Hβ) and two methyl doublets at 0.93 and 0.88 ppm; Leu protons were connected from TOCSY cross peaks of NH at 7.74 ppm, Hα at 4.54 ppm, two methylene protons of Hβ at 1.65 and 1.30 ppm, Hγ at 1.47 ppm and two methyl doublets at 0.83 and 0.79 ppm. TOCSY spectrum showed correlation for Ser1 protons at 8.75 ppm (NH), 5.54 ppm (Hα) and 3.58 (2Hβ); and for Ser2 at 8.13 ppm (NH), 4.24 ppm (Hα), 3.71 and 3.56 ppm (2Hβ). Two spin systems, 8.32 ppm (NH), 4.79 ppm (Hα), 2.91 and 2.59 ppm (2Hβ); and 8.67 ppm (NH), 4.99 ppm (Hα) and 2.81 ppm (2Hβ) were assigned to Phe1 and Phe2 residues. Lys residue was traced by TOCSY and COSY cross peaks between NH at 8.41 ppm, Hα at 4.52 ppm, two Hβ protons at 1.68 and 1.38 ppm, two Hγ at 1.20 ppm, two Hδ at 1.50 ppm, two Hε at 2.75 ppm and two NH_2 protons at 7.55 ppm.

The signal of the oxymethyne proton at 4.32 ppm, which correlated with the carbon at 68.2 ppm in the HSQC spectrum was considered as Hγ of the hydroxyproline residue. TOCSY and COSY correlations led to assignment of other hydroxyproline signals: two Hβ protons at 2.11 and 1.88 ppm, Hα at 4.20 ppm and two Hδ protons at 3.77 and 3.55 ppm. Relative stereochemistry of 4-hydroxy-Pro (Hyp) was determined from the NOESY spectrum. The cross peaks Hyp Hα (4.20)/Hyp Hβa (2.11) and Hyp Hγ (4.32)/Hyp Hβb (1.88) appeared stronger than the cross peaks Hyp Hα/Hyp Hβb and Hyp Hγ/Hyp Hβa, indicating that the relative stereochemistry between Hyp Hα and Hγ is trans. This result was further supported by Marfey's analysis. The last amino acid, proline was revealed from TOCSY correlations of Hα at 4.17 ppm, two Hβ at 2.01 and 1.83 ppm, two Hγ at 2.10 and 1.78 ppm and two Hδ protons at 3.56 and 3.13 ppm.

The presence of a 3-hydroxy-fatty acid residue in **1** was indicated by analysis of the NMR and MS data. Sequential COSY correlations were observed between the methylene signals at 2.38, 2.33 ppm (H-2), H-3 at 5.34 ppm, two H-4 protons at 1.58 ppm and methylene signals at 1.24 ppm, and between the signals of the two terminal methyl groups (0.85 ppm, 6H), H-9 (1.48 ppm), two H-8 (1.13 ppm) and methylenes at 1.24 ppm. Exact length of the fatty acid chain was confirmed by HRFAB MS and QTOF MS/MS analysis. Chemical shift of the oxymethyne proton H-3 (5.33 ppm) indicated an acyloxy nature of this bond and cyclic structure of the peptide (the chemical shifts of related protons of the fatty acid residues in the acyclic kahalalides H and J are 3.95 and 4.04 ppm,[3] whereas related protons in the cyclic peptide kahalalides E and K resonate at 5.11 and 5.16 ppm[2,4]).



The sequence of the amino acids was established utilizing NOESY and HMBC experiments and ESI QTOF MS/MS analysis. Sequential HMBC correlations from the NH proton to the neighboring carbonyls were seen between Asp NH/9-Me-3-Decol CO, Leu NH/Ser2 CO, Val NH/Ser1 CO, and Lys NH/Phe2 CO. Other HMBC cross peaks from the NH to the carbonyls were inconclusive due to overlaps in the spectra. Sequential NOESY correlations from the NH proton to the neighboring α proton were seen between 8.67 ppm (Phe2 NH) and 4.536 ppm (Leu Hα), 8.32 ppm (Phe1 NH) and 4.542 ppm (Asp Hα), 8.13 ppm (Ser2 NH) and 4.20 ppm (Hyp Hα), 8.75 ppm (Ser1 NH) and 4.79 ppm (Phe1 Hα), 8.41 ppm (Lys NH) and 4.99 ppm (Phe2 Hα), 7.81 ppm (Val NH) and 5.54 ppm (Ser1 Hα), and 7.74 ppm (Leu NH) and 4.24 ppm (Ser2 Hα). NOESY correlations were also observed between Val Hα at 4.64 ppm and proton Hδ of Hyp at 3.55 ppm, and between Lys Hα at 4.52 ppm and proton Hδ of Pro at 3.13 ppm. The NH proton of the Asp residue at 7.47 ppm showed NOESY peaks with H-2 protons (2.33, 2.38 ppm) and H-3 proton (5.34 ppm) of the 9-Me-3-Decol. However, no correlations were observed between H-3 proton of this fatty acid residue and the proline protons or carbons.

Peptide **1** was subjected to base hydrolysis, which yielded a linear product, **3**. Product **3** was analyzed by nanoelectrospray MS/MS measurement. MS/MS spectrum (Fig. 1), b- and y-type ions as well as several prominent peaks of internal ions clearly confirmed the sequence of amino acids and fatty acid of the acyclic kahalalide P (**3**).

2.2.2. Absolute stereochemistry of kahalalide P. The absolute stereochemistry of amino acids in **1** was determined by Marfey's method,[10] which showed Asp, Val, and Leu to be D, and Ser, Hyp, Pro, and Lys to be L. Both D- and L-Phe enantiomers were present in the peptide.

Enzymatic cleavage by carboxypeptidase to determine the positions of D- and L-Phe in **3** was performed (Fig. 2). Although peptides containing D-amino acid in the second position are resistant to hydrolysis by endopeptidases,[11–13] to the best of our knowledge, this property has never been utilized in the structure elucidation of natural products. The compound (**1**, 0.2 mg) was subjected to base hydrolysis to

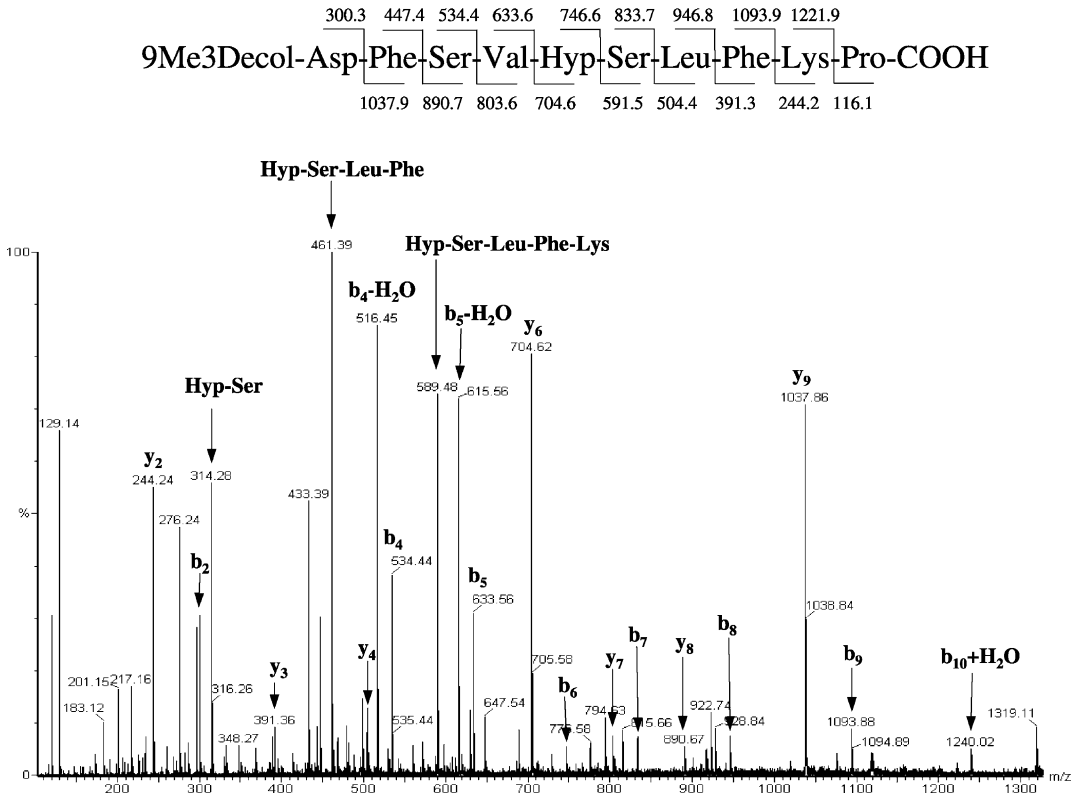


Figure 1. Positive ESI QTOF MS/MS spectrum of the acyclic kahalalide P.

yield the linear peptide **3**. After HPLC purifications, **3** was subjected to enzymatic cleavage with carboxypeptidase P. This enzyme non-specifically releases L-amino acids from the carboxy terminal of proteins and peptides. The reaction was monitored by MALDI TOF and QTOF MS and MS/MS measurements. The reaction completely terminates after two residues, Pro and Lys, are cleaved from the parent peptide, thus indicating a -D-Leu-L-Phe-L-Lys-L-Pro–COOH sequence at the C-terminus of the peptide. Sequencing of **3** with carboxypeptidase Y showed identical results as with carboxypeptidase P. Furthermore, we synthesized two linear model peptides with anti-podal positions of D- and L-Phe, **5a** (H_2N-D-Asp-**D-Phe**-L-Ser-D-Val-L-4-*trans*-hydroxy-Pro-L-Ser-D-Leu-**L-Phe**-L-Lys-L-Pro–COOH) and **5b** (H_2N-D-Asp-**L-Phe**-L-Ser-D-Val-L-4-*trans*-hydroxy-Pro-L-Ser-D-Leu-**D-Phe**-L-Lys-L-Pro–COOH), which were subjected to enzymatic sequencing in the same conditions as **3**. Two C-terminal residues, Pro and Lys, were cleaved off in the reaction with **5a** (mw of final product $[M+H]^+$ 927.27 Da, $[M+Na]^+$ 949.29 Da) similar to the reaction observed with **3**. However, only one C-terminal (Pro) residue was released in the reaction with anti-podal **5b** (mw of final

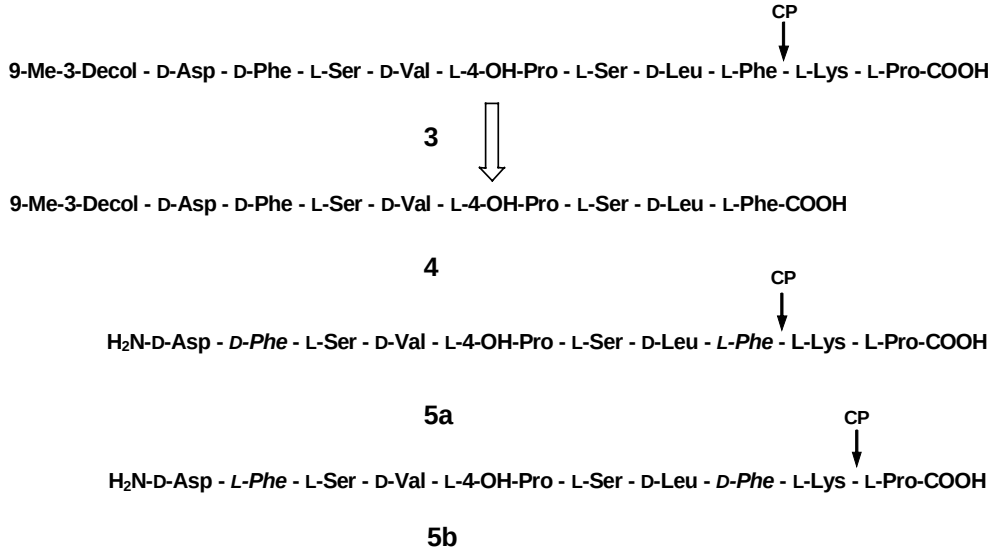
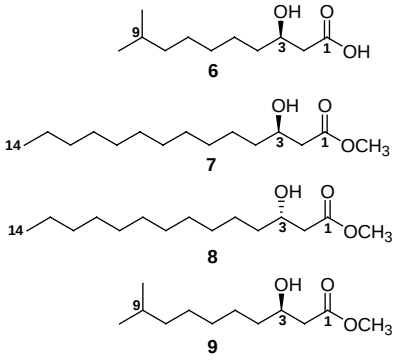


Figure 2. The carboxypeptidase (CP) hydrolysis reaction of acyclic kahalalide P (**3**) and synthesized model compounds.

product $[M+H]^+$ 1055.87 Da, $[M+Na]^+$ 1077.86 Da). These results verify the proposed position of D- and L-Phe in **1**. This enzymatic method will be applicable to the DL determination of anti-podal amino acids in other non-ribosomal peptides, including kahalalides E,[2] J,[3] and O[5] (stereochemistry of these compounds has not been elucidated).



Although the 3-hydroxy-9-methyldecanoic acid (9-Me-3-Decol) moiety in kahalalides E, J, H, and K, and 3-hydroxy-7-methyloctanoic acid moiety in kahalalide D have been reported, the absolute stereochemistries of these fragments were not determined due to the paucity of sample available.[2–4] We applied the Mosher ester procedure[14,15] to determine the absolute stereochemistry of 9-Me-3-Decol (**6**) in **1**. MTPA esters were obtained directly in NMR tubes with deuterated pyridine. The ^{1}H NMR spectra of the products of the reactions were measured without purification following the recently described convenient Mosher ester procedure.[16] We used model compounds, methyl (3*R*)-3-hydroxytetradecanoate (**7**) and methyl (3*R*+3*S*)-3-hydroxytetradecanoate (**7**+**8**, racemic body) to obtain MTPA derivatives. (*R*)- and (*S*)-MTPA esters of these compounds were obtained and the differences in proton chemical shifts between (*S*)-MTPA esters and (*R*)-MTPA esters were measured in C_5D_5N. MTPA esters of **7** have distinctive differences in ^{1}H NMR (Fig. 3). Furthermore, proton spectrum of the MTPA ester of the methyl 3(*R*+*S*)-hydroxytetradecanoate (**7**+**8**, racemic body) was almost the same as the sum of the spectra of (*R*)-MTPA ester of **7** and (*S*)-MTPA ester of **7**.

Two milligram of **1** was subjected to acid hydrolysis and the hydrolyzate was partitioned between $CHCl_3$ and H_2O. The organic layer was evaporated and the free fatty acid obtained was converted to methyl 3-hydroxy-9-methyldecanoate (**9**) with diazomethane. Products of the reaction were divided into two parts, and then converted to MTPA esters directly in NMR tubes as described above.[16] While the spectra of the MTPA derivatives of the fatty acid residue contains signals from impurities, multiplets of two H-2 protons and H-3 proton are clearly visible (Fig. 3). Difference in chemical shift of the protons in the (*S*)-MTPA ester of methyl 3-hydroxy-9-methyldecanoate and (*R*)-MTPA ester of methyl 3-hydroxy-9-methyldecanoate were found to be almost identical to the MTPA esters of **7** (Fig. 3). Thus, the absolute configuration of 9-Me-3-Decol from **1** was proved to be (3*R*) (**6**).

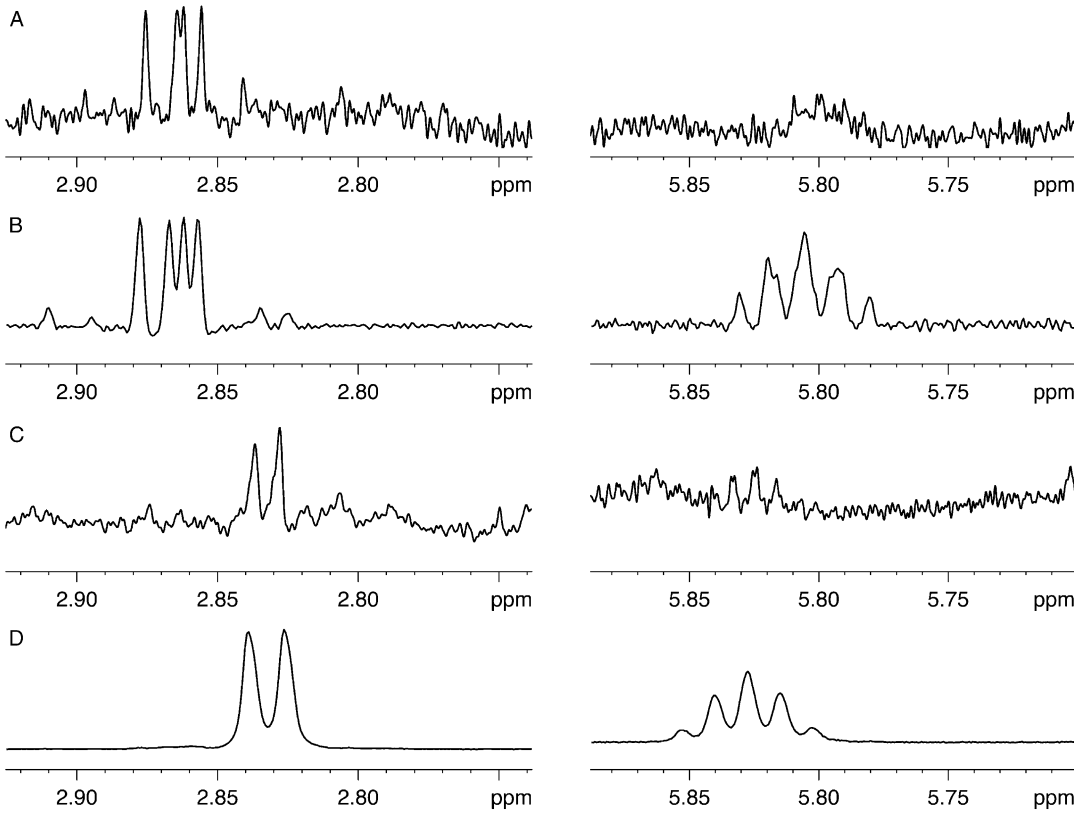


Figure 3. Partial ^{1}H NMR spectra of (*R*)-and (*S*)-MTPA esters of (3*R*)-methyl-3-hydroxytetradecanoate (**7**) and (3*R*)-methyl-3-hydroxy-9-methyldecanoate (**9**). (A) (*R*)-MTPA ester of **9**; (B) (*R*)-MTPA ester of **7**; (C) (*S*)-MTPA ester of **9**; (D) (*S*)-MTPA ester of **7**. (A) and (C) were measured with 750 MHz spectrometer. (B) and (D) were measured with 500 MHz spectrometer.

2.2.3. Structure of kalahalide Q. Kahalalide Q (**2**) showed $[M+H]^+$ ion 1302.7336 ($C_{66}H_{99}N_{11}O_{16}$, Δ −1.3 mmu) from HR FAB MS data, which differ by one oxygen atom from kahalalide P (**1**). ^{1}H HMR showed similar data for all moieties (Table 2) except for the signals of the hydroxyproline residue. Careful analysis of the NMR data showed **2** possessed a proline instead of a hydroxyproline residue. This result was further supported by QTOF MS/MS analysis of **2**, and its linear analog obtained from **2** by base hydrolysis. Determination of absolute stereochemistry of amino acid residues of **2** by Marfey's method showed D- and L-Phe, D-Asp, D-Val, D-Leu, L-Lys, two L-Ser, and two L-Pro. Since the NMR data of the peptides from **1** and **2** are almost indistinguishable, the stereochemistry of the fatty acid residue and positions of the D- and L-Phe in **2** are identical to **1**.

3. Bioactivity

At a concentration of 100 μg/mL, kahalalides P (**1**) and Q (**2**) showed 40 and 30% inhibition of HL-60 cancer cell lines, respectively. At a concentration of 40 μg (using a 8 mm diameter paper disk), both compounds showed no anti-microbial activity toward the marine bacterium *Ruegeria atlantica* TUF-D.[17] No hemolytic activity was observed (0.8% sheep red blood cell suspension assay)[18] with either compound at a concentration of 100 μg/mL.

4. Conclusion

New cyclic depsipeptides, kahalalides P (**1**) and Q (**2**), were isolated from the Hawaiian green alga *Bryopsis* sp. The sequential positions of DL anti-podal amino acids were determined by a carboxypeptidase hydrolysis reaction. This enzymatic method will be applicable to the structure determination of other non-ribosomal peptides. The absolute chemistry of 3-hydroxy-9-methyldecanoic acid in kahalalides P and Q were determined by the recently introduced convenient Mosher ester procedure. Interestingly, the structure of the acyclic kahalalide P (**1**) compared to kahalalides H and J[3] suggest that kahalalide P may possible be an intermediate in the metabolic pathway or end product of these kahalalides. Kahalalide P without the C-terminal Pro and Lys would be kahalalide H and without the C-terminal Pro might be kahalalide J (DL determination of Phe in kahalalide J has not been elucidated).

5. Experimental

5.1. General experimental procedures

Optical rotations were measured on a digital spectropolarimeter JASCO DP-100. ^{1}H and two-dimensional NMR spectra were recorded on Bruker DMX-750 spectrometer; ^{13}C NMR spectra were obtained on Bruker DRX-500 spectrometer. MALDI TOF measurements were performed on Perceptive Biosystem Voyager Elite MALDI TOF mass spectrometer. Nanoflow electrospray ionization time of flight MS/MS and MS were run on Micromass Q-TOF mass spectrometer. HRFAB MS data were obtained on JEOL JMX HX/HX-110A mass spectrometer.

5.2. Isolation

Bryopsis sp. (1.0 kg wet wt) was collected at Kewalo Basin, Oahu, Hawaii (1998). The alga was lyophilized and stored at −30 °C. Freeze dried alga was extracted with methanol. The combined extracts were evaporated to dryness to give 31 g of green powder. The residue was mixed with Celite and purified by two-step ODS flash chromatography (column 2×25 cm) (first elution with 20–50–70–90–100% aqueous MeOH, second with 50–70–80–90–95–100% aqueous MeOH). The MeOH/H_2O 9:1 fraction was further purified by repeated reverse phase HPLC, (Cosmosil 5C_{18}-MS-II, 20×250 mm with aqueous CH_3CN gradient 80–100% and then Cosmosil 5C_{18}-AR-II, 4.6×250 mm with aqueous CH_3CN gradient 60–70%) to yield kahalalide P (5.2 mg, 0.0005%) and kahalalide Q (1.8 mg, 0.0002%) as well as previously described kahalalides G and F.

5.2.1. Kahalalides P (1). White powder; $[\alpha]_D^{25}$ +4.5 (*c* 0.22, MeOH); ^{1}H and ^{13}C NMR data were shown in Table 1; HRFABMS $C_{66}H_{100}N_{11}O_{17}$ as $[M+H]^+$ *m/z* 1318.7319 (Δ +2.1 mmu).

5.2.2. Kahalalides Q (2). White powder; $[\alpha]_D^{25}$ +10.5 (*c* 0.033, MeOH); ^{1}H and ^{13}C NMR data were shown in Table 2; HRFABMS $C_{66}H_{100}N_{11}O_{16}$ as $[M+H]^+$ *m/z* 1302.7336 (Δ −1.3 mmu).

5.3. Acid hydrolysis of kahalalides

Peptides were hydrolyzed with 6 N HCl at 100 °C for 12 h. The hydrolyzate was dried with centrifugation in vacuo.

5.4. Base hydrolysis of kahalalides

Peptides (0.2 mg) were hydrolyzed with 0.1 N NaOH in aqueous methanol (50 μL) at 37 °C for 90 min. After neutralization with 0.1 N HCl, ODS HPLC of the hydrolyzate was performed (Cosmosil 5C18-AR-II, 4.6×250 mm, aqueous CH_3CN 0–60%) to yield the acyclic peptides.

5.5. Marfey analysis of 1 and 2

Part of the acid hydrolyzate was added to a 1% 1-fluoro-2,4-bis(nitrophenyl)-5-L-alanine amide (FDAA) solution in acetone (10 μL) and 1 M $NaHCO_3$ (20 μL). The sample was incubated at 40 °C for 1 h. The reaction mixture was neutralized with 2 N HCl (20 μL) after cooling to room temperature. The mixture was dried with centrifugation in vacuo. The residue was dissolved in DMSO and subjected to HPLC analysis. The FDAA derivatives of standard amino acids were prepared by the same procedure. HPLC analysis was carried out on a Cosmosil 5C_{18}-AR-II, 4.6×250 mm column with two different solvent system (system I: solvent A (0.1 M ammonium acetate, pH 3.0) and solvent B (CH_3CN), samples were eluted as follows: A, 85–55% in 45 min, system II: solvent A (0.1 M ammonium acetate, pH 3.0), 10% and solvent B (CH_3CN), 90%). The retention times (min) of the hydrolyzate FDAA derivatives and the standard derivatives in the solvent system I were as follows: L-Ser (8.00–8.04), D-Asp (10.76–10.81), L-Pro (14.24–14.30), L-Phe (25.72–25.74), D-Val (28.05–28.10),

Table 1. ^{1}H[a] and ^{13}C NMR[b] data for kahalalide P (**1**) in 0.05% TFA/DMSO-d_6

Unit	Position	^{13}C NMR (ppm)[c]	^{1}H NMR (ppm)[d]	Mult	J (Hz)
9Me3Decol	1	168.2	—		
	2	40.4	Hα 2.38	dd	10.1, 14.7
			Hβ 2.33	dd	3.4, 14.7
	3	71.0	5.34	m	
	4	33.6	1.58, 2H	m	
	5	[e]	1.24, 2H	m	
	6	[e]	1.24, 2H	m	
	7	28.8	1.24, 2H	m	
	8	38.3	1.13, 2H	m	
	9	27.3	1.48	m	
	10, 11	22.5, 2C	0.85, 6H	d	6.6
Asp	NH	—	7.47	d	7.7
	α	49.3	4.54	m	
	β	35.0	Hα 2.52	dd	10.6, 16.2
			Hβ 2.25	dd	4.0, 16.2
	C=O	169.3	—		
	COOH	171.9	—		
Phe1	NH	—	8.32	d	8.9
	α	53.9	4.79	dt	9.5, 4.5
	β	39.3	Hα 2.91	dd	4.5, 13.0
			Hβ 2.59	dd	10.2, 13.0
	1′	137.8	—		
	2′, 6′	129.8, 2C	7.37, 2H	d	7.4
	3′, 5′	128.0, 2C	7.29, 2H	t	7.4
	4′	126.3	7.25	t	7.4
	C=O	171.7	—		
Ser1	NH	—	8.75	d	8.3
	α	53.9	5.54	m	
	β	63.7	3.58, 2H	d	5.5
	C=O	169.9	—		—
Val	NH	—	7.81	d	7.1
	α	54.8	4.64	t	7.1
	β	32.0	1.98	m	
	γ	19.5	0.93, 3H	d	6.8
	γ′	17.7	0.88, 3H	d	6.8
	C=O	170.9	—		
Hyp	α	60.5	4.20	t	8.5
	β	37.8	Hα 2.11	m	
			Hβ 1.88	ddd	4.4, 9.4, 13.4
	γ	68.2	4.32	m	
	δ	55.6	Hα 3.77	dd	4.0, 10.5
			Hβ 3.55	m	
	C=O	171.8	—		
Ser2	NH	—	8.13	d	8.5
	α	55.7	4.24	dt	4.0, 8.5
	β	60.9	Hα 3.71	dd	4.0, 11.3
			Hβ 3.56	m	
	C=O	169.0	—		
Leu	NH	—	7.74	d	8.9
	α	50.3	4.54	m	
	β	40.7	Hα 1.65	m	
			Hβ 1.30	m	
	γ	23.8	1.47	m	
	δ	23.2	Hα 0.83, 3H	d	6.7
	δ′	21.8	Hβ 0.79, 3H	d	6.7
	C=O	171.9	—		
Phe2	NH	—	8.67	d	9.0
	α	52.9	4.99	dd	8.0, 9.0
	β	38.3	2.81, 2H	d	8.0
	1″	137.1	—		
	2″, 6″	129.2, 2C	7.14, 2H	d	7.4
	3″, 5″	127.7, 2C	7.10, 2H	t	7.4
	4″	126.1	7.07	t	7.4
	C=O	169.8	—		
Lys	NH	—	8.41	d	8.8
	α	48.8	4.52	m	
	β	31.9	Hα 1.68	m	
			Hβ 1.38	m	
	γ	21.2	1.20, 2H	m	
	δ	26.4	1.50, 2H	m	
	ε	38.7	2.75, 2H	m	
	NH_2	—	7.55, 2H	br s	
	C=O	171.9	—		
Pro	α	58.5	4.17	dd	4.2, 8.5
	β	24.4	Hα 2.01	m	
			Hβ 1.83	m	
	γ	28.7	Hα 2.10	m	
			Hβ 1.78	m	
	δ	46.6	Hα 3.56	m	
			Hβ 3.13	m	
	C=O	171.9	—		

[a] At 750 MHz.
[b] At 188 MHz.
[c] Reference of chemical shift was DMSO-d_6 as 39.5 ppm.
[d] Reference of chemical shift was DMSO-d_6 as 2.49 ppm.
[e] δ ^{13}C 26.6 or 24.8 ppm.

Table 2. ^{1}H[a] and ^{13}C NMR[b] data for kahalalide Q (**2**) in 0.05% TFA/DMSO-d_6

Unit	Position	^{13}C NMR (ppm)[c]	^{1}H NMR (ppm)[d]	Mult	J (Hz)
9Me-3decol	1	168.2	—		
	2	40.0	Hα 2.38	dd	9.8, 14.5
			Hβ 2.33	dd	3.6, 14.5
	3	71.0	5.33	m	
	4	33.6	1.58, 2H	m	
	5	[e]	1.24, 2H	m	
	6	[e]	1.24, 2H	m	
	7	28.7	1.24, 2H	m	
	8	38.3	1.13, 2H	m	
	9	27.4	1.48	m	
	10,11	22.5, 2C	0.85, 6H	d	6.6
Asp	NH	—	7.48	d	7.4
	α	49.2	4.54	m	
	β	35.0	Hα 2.52	m	
			Hβ 2.25	dd	4.0, 16.4
	C=0	169.2	—		
	COOH	[f]	—		
Phe1	NH	—	8.34	d	8.8
	α	53.4	4.78	dt	8.8, 4.5
	β	38.9	Hα 2.92	dd	4.5, 13.0
			Hβ 2.59	dd	11.0, 13.0
	1′	137.8	—		
	2′,6′	129.7, 2C	7.38, 2H	d	7.4
	3′,5′	128.0, 2C	7.29, 2H	t	7.4
	4′	126.3	7.25	t	7.4
	C=O	[f]	—		
Ser1	NH	—	8.77	d	9.0
	α	53.9	5.54	m	
	β	63.7	3.59, 2H	m	
	C=O	169.9	—		—
Val	NH	—	7.82	d	7.0
	α	55.0	4.68	t	7.0
	β	32.1	1.99	m	
	γ	19.0	0.91, 3H	d	6.8
		17.9	0.89, 3H	d	6.8
	C=O	170.7	—		
Pro1	α	61.5	4.13	dd	4.1, 8.6
	β	24.4	1.88, 2H	m	
	γ	29.5	Hα 2.16	m	
			Hβ 1.79	m	
	δ	47.7	Hα 3.72	m	
			Hβ 3.61	m	
	C=O	[f]	—		
Ser2	NH	—	8.08	d	8.5
	α	55.6	4.28	dt	4.0, 8.5
	β	60.9	Hα 3.70	m	
			Hβ 3.56	m	
	C=O	169.0	—		
Leu	NH	—	7.66	d	8.9
	α	50.3	4.54	m	
	β	40.3	Hα 1.66	m	
			Hβ 1.30	m	
	γ	23.8	1.44	m	
	δ	23.2	0.83, 3H	d	6.7
	δ′	21.8	0.79, 3H	d	6.7
	C=O	[f]	—		
Phe2	NH	—	8.66	d	9.1
	α	52.8	5.01	q	9.0
	β	38.6	2.81, 2H	m	
	1″	137.1	—		
	2″,6″	129.2, 2C	7.14, 2H	d	7.4
	3″,5″	127.7, 2C	7.10, 2H	t	7.4
	4″	126.0	7.07	t	7.4
	C=O	169.8	—		
Lys	NH	—	8.43	d	8.3
	α	48.7	4.52	m	
	β	31.9	Hα 1.65	m	
			Hβ 1.38	m	
	γ	21.1	1.19, 2H	m	
	δ	26.4	1.50, 2H	m	
	ε	38.5	2.75, 2H	m	
	NH_2	—	7.53, 2H	br s	
	C=O	169.2	—		
Pro 2	α	58.5	4.18	dd	4.0, 8.5
	β	24.3	Hα 2.01	m	
			Hβ 1.84	m	
	γ	28.7	Hα 2.10	m	
			Hβ 1.77	m	
	δ	46.5	Hα 3.54	m	
			Hβ 3.12	m	
	C=O	[f]	—		

[a] At 750 MHz.
[b] At 188 MHz.
[c] Reference of chemical shift was DMSO-d_6 as 39.5 ppm.
[d] Reference of chemical shift was DMSO-d_6 as 2.49 ppm.
[e] δ ^{13}C 26.6 or 24.8 ppm.
[f] δ ^{13}C 171.9 or 171.8 ppm.

L-Lys (28.97–29.02), D-Phe (31.97–32.03) and D-Leu (33.99–34.04). The configuration of Hyp could not be determined in this system due to poor peak resolution. Further analysis with solvent system II revealed L-Hyp in the samples. (retention time: authentic 4-*trans*-hydroxy-D-Pro 20.85 min, authentic 4-*trans*-hydroxy-L-Pro 22.73 min, the sample 22.90 min).

5.6. Enzymatic hydrolysis

C-terminus of peptide **3** was hydrolyzed with carboxypeptidase P (Takara Bio Inc., Japan) in 30 mmol ammonium acetate buffer (pH 5.0) and carboxypeptidase Y (Oriental Yeast Co., Osaka, Japan) in 30 mmol ammonium acetate buffer (pH 6.0). The carboxy end of peptides **4**, **5** were hydrolyzed with carboxypeptidase P in 30 mmol ammonium acetate buffer (pH 5.0). Each sample (1 nM in 10 μL buffer) was mixed with carboxypeptidase P or carboxypeptidase Y, 1 μL (1 unit), in a capped 0.5 mL polypropylene tube and kept at 37 °C. Reactions were monitored by MALDI MS measurement. Reactions completed approximately in 10 min for **3** and **4** and in 15 min for **5**.

5.7. Synthesis of peptides 5a and 5b

The peptides were synthesized using a FastMOC™ chemistry with a solid-phase peptide synthesizer (Model 433A, Applied Biosystems) and purified by reverse phase HPLC (Cosmosil 5C_{18}-AR-II, 4.6×250 mm) with 70% aqueous CH_3CN.

5.8. Preparation of the (*R*)- and (*S*)-MTPA ester derivatives

(*R*)- and (*S*)-MTPA esters of the compounds were obtained following the reported procedure.[16] Each model compound, methyl (3*R*)-3-hydroxytetradecanoate (**7**) and methyl (3*R*+3*S*)-3-hydroxytetradecanoate (**7**+**8**, racemic body), was divided into two parts, then transferred into a clean NMR tubes and dried under the stream of N_2 gas. Deuterated pyridine (0.6 mL) and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (6 μL) or (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (6 μL) were added to NMR tube immediately under a N_2 gas stream, and then NMR tube was shaken carefully to mix the sample and MTPA chloride evenly. The reaction NMR tubes were permitted to stand at room temperature and were monitored by ^{1}H NMR. The reaction was completed in approximately 4 h. Selected signals of the (*S*)-MTPA ester of **7**: ^{1}H NMR (C_5D_5N, 500 MHz) δ 2.83 (2H, d, J=6.4 Hz, H-2a and H-2b), 5.83 (m, H-3), 1.83 (m, H-4a), 1.75 (m, H-4b) and 1.39 (m, H-5). Selected signals of the (*R*)-MTPA ester of **7**: ^{1}H NMR (C_5D_5N, 500 MHz) δ 2.89 (dd, J=8.5, 16.5 Hz, H-2a), 2.85 (dd, J=5.0, 16.5 Hz, H-2b), 5.81 (m, H-3), 1.70 (2H, m, H-4) and 1.20 (m, H-5, data from TOCSY spectrum).

Proton spectrum of the MTPA ester of the methyl (3*R*+3*S*)-hydroxytetradecanoate (**7**+**8**, racemic body) was almost the same as the sum of the spectra of (*R*)-MTPA ester of **7** and (*S*)-MTPA ester of **7**.

Two milligram of the peptide (**1**) was hydrolyzed by heating the sample in 6 N HCl for 12 h. The hydrolyzate was dried with centrifugation in vacuo. Hydrolyzate was partitioned between $CHCl_3$ and H_2O. Organic layer was evaporated and obtained free fatty acid (**6**) was converted to methyl-3-hydroxy-9-methyldecanoate (**9**) by diazomethane. (*R*)- and (*S*)-MTPA esters of **9** were obtained in the same manner as for the model compounds. Selected signals of the (*S*)-MTPA ester of **9**: ^{1}H NMR (C_5D_5N, 750 MHz) δ 2.83 (2H, d, H-2a and H-2b) and 5.82 (m, H-3). Selected signals of the (*R*)-MTPA ester of **9**: ^{1}H NMR (C_5D_5N, 750 MHz) δ 2.89 (dd, H-2a), 2.85 (dd, H-2b) and 5.80 (m, H-3).

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Efficient esterification of carboxylic acids and phosphonic acids with trialkyl orthoacetate in ionic liquid

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Abstract—An operationally simple, inexpensive, efficient, and environmentally friendly esterification of various carboxylic acids, phosphonic acids, and phosphinic acids with triethyl orthoacetate or trimethyl orthoacetate under neutral conditions in a typical room temperature ionic liquid, 1-butyl-3-methylimidazolium hexafluorophosphate, was successfully carried out to provide the corresponding ethyl esters or methyl esters in high yields.

1. Introduction

Esterification of carboxylic acids is one of the most important functional group conversions in organic synthesis. Therefore, since the Fischer ester synthesis,[1] a number of useful esterification methods catalyzed by Brønsted acids, Lewis acids, ion exchange regins, zeolite, etc. have been reported.[2] Today, environmentally friendly organic synthesis has become much more important and popular, aiming toward green chemistry. Especially, room temperature ionic liquids attract great interest as environmentally friendly reaction media and reaction promotion media for organic synthesis.[3] Thus, these solvents possesses interesting and useful advantages such as negligible vapor pressure, nonflammability, high thermal stability at a wide range of temperature, and easy reusability. These solvents have been successfully used in Friedel-Crafts reaction,[4] hydrogenation,[5] Diels–Alder reactions,[6] Heck, Suzuki, Sonogashira, and olefin metathesis reactions,[7] Michael addition,[8] oxidation,[9] condensation such as Knoevenagel reaction, Fischer esterification, Robinson annulation and related reactions,[10] formation of imines,[11] 1,2-rearrangement,[12] esterification of carboxylic acids and carboxylates,[13] nucleophilic substitution such as the Williamson ether synthesis,[14] etc. Phosphonate esters are also prepared via direct esterification of phosphonic acids with alcohols in the presence of condensing reagents, or the reaction of phosphonochlorides with alcohols, generally.[15]

Here, as a part of our study on environmentally friendly organic synthesis with ionic liquid,[16] we would like to report an operationally simple, inexpensive, efficient, and environmentally friendly esterification of carboxylic acids, phosphonic acids, and phosphinic acids with triethyl orthoacetate or trimethyl orthoacetate under neutral conditions in ionic liquids such as 1-butyl-3-methylimidazolium hexafluorophosphate ([bmim]PF_6), 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim]BF_4), 1-butyl-3-methylimidazolium chloride ([bmim]Cl).

2. Results and discussion

2.1. Esterification of carboxylic acids

It is well known that generally triethyl orthoformate and triethyl orthoacetate can be used for the conversion of carbonyl groups to their acetals and ketals.[17] Additionally, triethyl orthoacetate can be used for the esterification of sulfonic acids[18] and carboxylic acids.[18b] However, generally, esterification of carboxylic acids with triethyl orthoacetate requires high temperature, long reaction time, and excess amount of triethyl orthoacetate. For example, 1-naphthoic acid was converted to the desired ethyl ester in 89% yield with triethyl orthoacetate (3.0 equiv) under refluxing conditions in toluene for 24 h. In our present study, when a mixture of 1-naphthoic acid with triethyl orthoacetate (2.0 equiv) in [bmim]PF_6 (2 mL) was heated at 80 °C for 100 min without any additive, the desired ethyl ester was smoothly obtained in 98% yield (entry 1, Table 1). However, this reaction gave rise to low yields under the same conditions in toluene, DMF, DMSO, and even in

Keywords: Esterification; Carboxylic acid; Phosphonic acid; Phosphinic acid; 1-Butyl-3-methylimidazolium hexafluorophosphate; Triethyl orthoacetate; Trimethyl orthoacetate.
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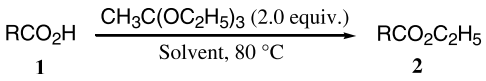
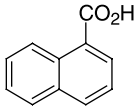
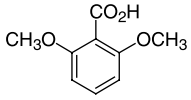
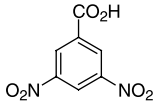
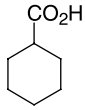
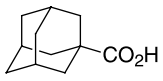
Table 1. Solvent effects in ethylation of carboxylic acids with triethyl orthoacetate

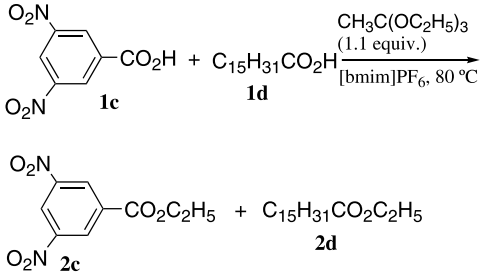


Entry	Carboxylic acid	Time	Yields (%)				
			[bmim]PF_6	Toluene	Neat	DMF	DMSO
1		100 min	98	39	66	23	19 (**2a**)
2		2.5 h	94	19	5	15	23 (**2b**)
3		0.5 h	97	90	98	88	62 (**2c**)
4	$CH_3(CH_2)_{14}CO_2H$	3.5 h[a]	95	66	70	30	28 (**2d**)
5		5 h[a]	94	79	73	39	47 (**2e**)
6		5 h[a]	91	42	22	21	18 (**2f**)

[a] Reaction temperature was 100 °C.

solvent-free conditions. Several examples of esterification with aromatic and aliphatic carboxylic acids by use of our protocol are shown in Table 1, and the formation of the corresponding ethyl esters was much accelerated except for more acidic carboxylic acid such as 3,5-dinitrobenzoic acid (entry 3).[19] Thus, the present system is highly effective, especially for less acidic carboxylic acids. Instead of [bmim]PF_6, other ionic liquids such as [bmim]BF_4, [bmim]Cl were also used as a solvent; however, [bmim]PF_6 showed the most effective reactivity as shown in Table 2 (entries 4–6). Based on these results, other various carboxylic acids were treated with triethyl orthoacetate in [bmim]PF_6, to provide the corresponding ethyl esters in quite good yields as shown in Table 3.

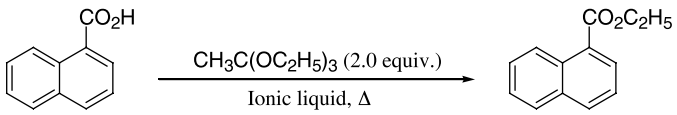
Another interesting advantage of this esterification is the use for sterically hindered carboxylic acids such as 2,4,6-triisopropylbenzoic acid (entry 5), and for amino acid (entry 10) without any racemization as shown in Table 3. Here, ethyl 2,4,6-triisopropylbenzoate cannot be obtained by typical esterification processes such as the Fischer method, DCC method, etc. Moreover, when competitive esterification reaction of 3,5-dinitrobenzoic and palmitic acids was carried out, the corresponding ethyl 3,5-dinitrobenzoate together with recovered palmitic acid was selectively obtained as shown in Eq. 1.



(1)

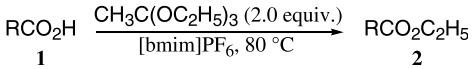
	1c	1d	2c	2d
%	0	79	97	11

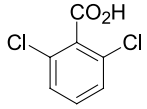
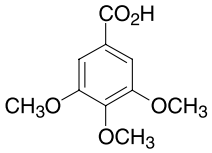
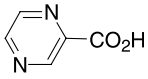
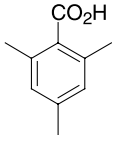
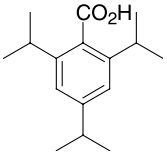
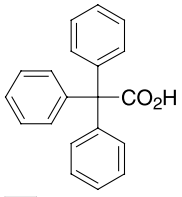
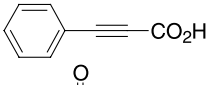
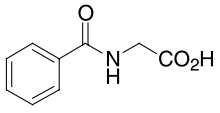
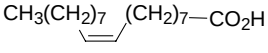
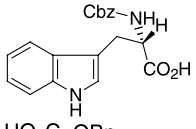
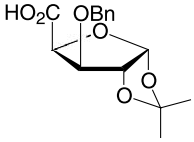
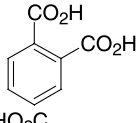
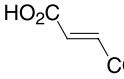
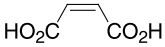
Table 2. Ethylation of 1-naphthoic acid with triethyl orthoacetate in ionic liquids



Entry	Ionic liquid	Time	Temperature (°C)	Yield (%)
1	[bmim]PF_6	24 h	rt	11
2	[bmim]PF_6	15.5 h	40	46
3	[bmim]PF_6	100 min	50	42
4	[bmim]PF_6	100 min	80	98
5	[bmim]BF_4	100 min	80	16
6	[bmim]C1	100 min	80	10

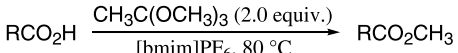
Table 3. Ethylation of various carboxylic acids in [bmim]PF_6

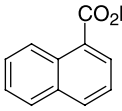
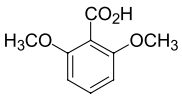
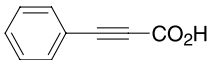


Entry	Carboxylic acid	Time (h)	Yield (%)
1		2	94 (**2g**)
2		4.5	98 (**2h**)
3		3	93 (**2i**)
4		1.5	96 (**2j**)
5		3	97 (**2k**)
6		3	96 (**2l**)
7		0.5	99 (**2m**)
8		3	96 (**2n**)
9		4	90 (**2o**)
10		12	96 (**2p**)[a]
11		2	92 (**2q**)
12		2[b]	99 (**2r**)
13		2.5[b,c]	90 (**2s**)
14		7[b,c]	75 (**2t**)

[a] Optically pure (Dicel Chiralcel OD-H; eluent: hexane/^{i}PrOH=4:1).
[b] TEOA used was 3.0 equiv and yield was for diester.
[c] Reaction temperature was 100 °C.

Table 4. Methylation of carboxylic acids in [bmim]PF_6

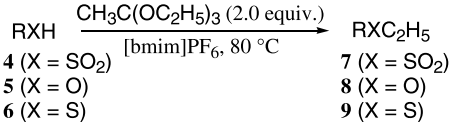


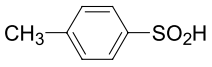
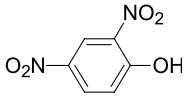
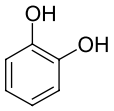
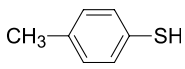
Entry	Carboxylic acids	Time (h)	Yield (%)
1		1.5	96 (**3a**)
2		2	96 (**3b**)
3	$CH_3(CH_2)_{14}CO_2H$	3.5[a]	91 (**3d**)
4		0.5	97 (**3m**)

[a] Reaction temperature was 100 °C.

Trimethyl orthoacetate can be also used for the same esterification reaction of carboxylic acids under the same conditions to form the corresponding methyl esters in high yields as shown in Table 4. Treatment of other protic compounds such as sulfinic acid (**4**), phenols (**5**), and thiol (**6**) with triethyl orthoacetate under the same conditions provided the corresponding ethyl sulfinate (**7**), ethyl ether (**8**), and ethyl thioether (**9**), respectively, in good yields as shown in Table 5. Catechol provided orthoester, not diethyl or monoethyl ether (entry 4).[20]

Table 5. Ethylation of protic compounds in [bmim]PF_6



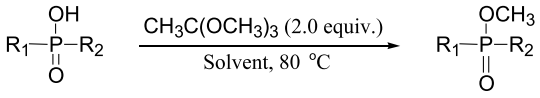
Entry	RXH	Time (h)	Yield (%)
1		0.5	87[a] (**7**)
2		3	88 (**8a**)
3		2	73[b] (**8b**)
4		4	80 (**9**)

[a] Ethyl *p*-touenesulfinate was obtained.
[b] 2-Ethoxy-2-methyl-1,3-benzodioxole was obtained.

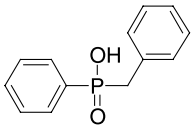
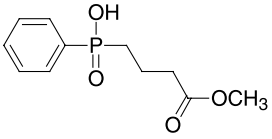
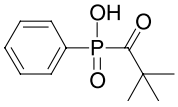
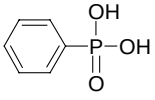
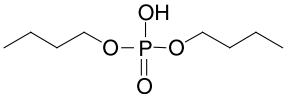
2.2. Esterification of phosphonic acids and phosphinic acids

To date, conversion of phosphonic acids to the corresponding dimethyl phosphonates with trimethyl orthoformate is known;[21] however, it requires high reaction temperature and long reaction time (110 °C, 9 h). In our present study, when a mixture of benzylphenylphosphinic acid (**10A**) with trimethyl orthoacetate (2.0 equiv) in [bmim]PF_6

Table 6. Solvent effect in the methylation of phosphonic and phosphinic acids with trimethyl orthoacetate

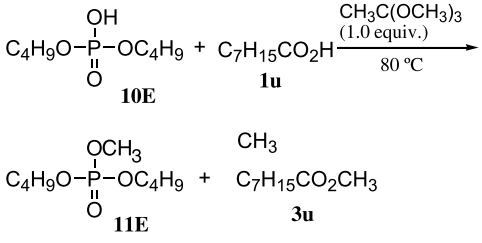


10 **11**

Entry	Phospinic acid	Time (h)	Yields (%)				
			[bmim]PF_6	Toluene	Neat	DMF	DMSO
1		2.5	98	32	70	91	16 (**11A**)
2		1	92	92	93	81	10 (**11B**)
3		1.5	79	71	89	78	40 (**11C**)
4		2[a]	95	78	95	85	13 (**11D**)
5		1	98	70	97	70	29 (**11E**)

[a] This reaction was carried out with 3.5 equiv of TMOA.

(2 mL) was heated at 80 °C for 2.5 h without any additive, the desired methyl ester was smoothly obtained in 98% yield (entry 1, Table 6). Though DMF and solvent-free conditions provided the corresponding methyl ester in good yields, toluene and DMSO showed poor reactivity. Moreover, when phosphinic acids (**10B**), (**10C**), phosphonic acid (**10D**) and dibutyl phosphoric acid (**10E**) were treated with trimethyl orthoacetate, the results indicate that [bmim]PF_6 is the best solvent as shown in Table 6.



(2)

	h / %	**10E**	**1u**	**11E**	**3u**
[bmim]PF_6	1.0	0	93	93	7
Toluene	2.5	23	78	76	21
Solvent free	1.0	61	57	39	35

When triethyl orthoacetate was used instead of trimethyl orthoacetate, ethyl esters were obtained in good yields. However, trimethyl orthoformate showed rather poor reactivity. Unlike [bmim]PF_6, other ionic liquids such as [bmim]BF_4, [bmim]Cl showed poor reactivity again as shown in Table 7. When competitive esterification reaction of dibutylphosphoric acid and octanoic acid was carried out, the selective methylation of phosphoric acid, that is, dibutyl methyl phosphate (93%) and octanoic acid (93%), was observed among [bmim]PF_6, toluene, and solvent-free conditions as shown in Eq. 2.

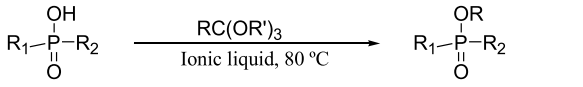
2.3. Recyclic use of 1-butyl-3-methyl imidazolium hexafluorophosphate

Here, room temperature ionic liquid [bmim]PF_6 can be recycled and reused without any loss of chemical yield of esters. Thus, treatment of 1-naphthoic acid (**1a**) and benzylphenylphosphinic acid (**10A**) with triethyl orthoacetate and trimethyl orthoacetate, respectively, in [bmim]PF_6 provided the corresponding ethyl carboxylate and methyl phosphinate in high yields till the fourth regeneration of [bmim]PF_6 as shown in Table 8. After fourth regeneration, [bmim]PF_6 in the esterification of 1-naphthoic acid was almost quantitatively recovered, however, [bmim]PF_6 in the esterification of benzylphenylphosphinic acid was recovered in ~30%. This reason comes from that complete extraction of benzylphenylphosphinate ester with ether from [bmim]PF_6 requires 25 times and therefore [bmim]PF_6 is partly extracted with ether.

3. Conclusion

Thus, an operationally simple, inexpensive, efficient, and environmentally friendly esterification of various carboxylic acids, phosphonic acids, and phosphinic acids with triethyl orthoacetate or trimethyl orthoacetate under

Table 7. Methylation and ethylation of phenylphosphonic acid and benzylphenylphosphonic acid with trialkyl orthoesters in ionic liquids



10

11 (R = CH_3)
12 (R = C_2H_5)

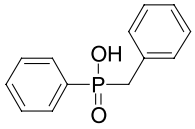
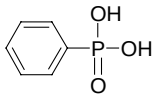
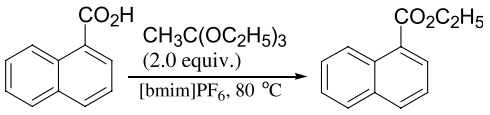
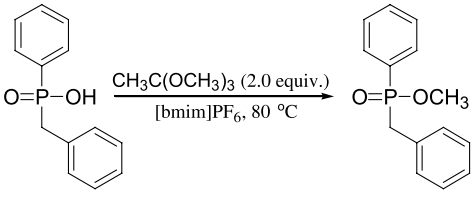
Entry	Substrates	$RC(OR')_{3\ (equiv)}$	Ionic liquid	Time (h)	Yield (%)
1		$CH_3C(OCH_3)_3$ (2.0)	$[bmim]PF_6$	2.5	98 (**11A**)
2		$HC(OCH_3)_3$ (2.0)	$[bmim]PF_6$	2.5	2 (**11A**)
3		$CH_3C(OC_2H_5)_3$ (2.0)	$[bmim]PF_6$	2.5	90 (**12A**)
4		$CH_3C(OCH_3)_3$ (2.0)	$[bmim]BF_4$	2.5	25 (**11A**)
5		$CH_3C(OCH_3)_3$ (2.0)	[bmim]C1	2.5	35 (**11A**)
6		$CH_3C(OCH_3)_3$ (3.5)	$[bmim]PF_6$	2	95 (**11D**)
7		$HC(OCH_3)_3$ (3.5)	$[bmim]PF_6$	2	51 (**11D**)
8		$CH_3C(OC_2H_5)_3$ (3.5)	$[bmim]PF_6$	2	96 (**12D**)
9		$CH_3C(OCH_3)_3$ (3.5)	$[bmim]BF_4$	2	<1 (**11D**)
10		$CH_3C(OCH_3)_3$ (3.5)	[bmim]C1	2	<1 (**11D**)

Table 8. Recycle use of $[bmim]PF_6$



1a **2a**

Regeneration	Time (min)	Yield (%)
0	100	98
1	100	95
2	100	92
3	100	97
4	100	94



10A **11A**

Regeneration	Time (h)	Yield (%)
0	2.5	98
1	2.5	95
2	2.5	94
3	2.5	99
4	2.5	99

neutral conditions in a typical room temperature ionic liquid, 1-butyl-3-methylimidazolium hexafluorophosphate, $[bmim]PF_6$, was successfully carried out to provide the corresponding ethyl esters or methyl esters in high yields. The present reactions can be used for esterification of various kinds of carboxylic acids, phosphonic acids, and phosphinic acids even for sterically hindered ones, and $[bmim]PF_6$, can be recycled.

4. Experimental

4.1. General

^1H and ^{13}C NMR spectra were obtained with JEOL-JNM-LA-400, JEOL-JNM-LA-400s, JEOL-JNM-LA-500, spectrometers. Chemical shifts are expressed in ppm downfield from tetramethylsilane (TMS) in δ units. Mass spectra were recorded on JEOL-HX-110 and JEOL-JMS ATII15 spectrometers. Melting points were determined on Yamato melting points apparatus Model MP-21. Silica Gel 60 (Kanto Kagaku Co.) was used for column chromatography and Wakogel B-5F was used for preparative TLC. Analytical high-performance liquid chromatography (HPLC) was done using a chiral column (4.6 mm×25 cm, Chiralcel OD-H). Optical rotation were measured on a polarimeter.

4.2. Typical procedure for ethylation of carboxylic acid with triethyl orthoacetate in ionic liquid and reuse of ionic liquid

A flask containing 1-butyl-3-methylimidazolium hexafluorophosphate ($[bmim]PF_6$, 2.0 mL) as a solvent was dried under reduced pressure with a vacuum pump for 2 h at 80 °C. Then, 1-naphthoic acid (1.0 mmol) and triethyl orthoacetate (2.0 mmol) were added to the ionic liquid and the obtained mixture was heated at 80 °C under an argon atmosphere. The reaction was monitored by TLC until the starting 1-naphthoic acid disappeared. After 100 min, the mixture was extracted with ether (5×5 mL). The combined ether extract was purified by column chromatography on silica gel (eluent: hexane/EtOAc=9:1) to give pure ethyl 1-naphthoate in 98% yield (bp 120 °C/1 mmHg, lit.[20] 100 °C/0.45 mmHg). After the reaction, ionic liquid was washed with distilled water (5 mL) once and then dried under reduced pressure with a vacuum pump for 2 h at 80 °C, and the ionic liquid was repeatedly used for the same reaction.

4.3. Typical procedure for methylation of carboxylic acid with trimethyl orthoacetate in ionic liquid

A flask containing 1-butyl-3-methylimidazolium hexafluorophosphate ($[bmim]PF_6$, 2.0 mL) as a solvent was dried under reduced pressure with a vacuum pump for 2 h at 80 °C. Then, 1-naphthoic acid (1.0 mmol) and trimethyl orthoacetate (2.0 mmol) were added to the ionic liquid and the obtained mixture was heated at 80 °C under an argon atmosphere. The reaction was monitored by TLC until

the starting 1-naphthoic acid disappeared. After 100 min, the mixture was extracted with ether (5×5 mL). The combined ether extract was purified by column chromatography on silica gel (eluent: hexane/EtOAc=9:1) to give pure methyl 1-naphthoate in 96% yield.

4.4. Competitive reaction of carboxylic acids with triethyl orthoacetate in ionic liquid

A flask containing 1-butyl-3-methylimidazolium hexafluorophosphate ([bmim]PF_6, 2.0 mL) as a solvent was dried under reduced pressure with a vacuum pump for 2 h at 80 °C. Then, 3,5-dinitrobenzoic acid (1.0 mmol), palmitic acid (1.0 mmol), and triethyl orthoacetate (1.1 mmol) were added to the ionic liquid and the obtained mixture was heated at 80 °C under an argon atmosphere. After 30 min, the mixture was extracted with ether (10×5 mL). The combined ether extract was purified by column chromatography on silica gel (eluent: hexane/EtOAc=5:1) to give ethyl 3,5-dinitrobenzoate (97% yield) and ethyl palmitate (11% yield), together with palmitic acid (79% yield).

Compounds **2a**–**2o**, **2r**–**2t**, **3a**–**3d**, **8a**, **8b**, and **9** were identified with commercially available authentic compounds.

4.4.1. Ethyl 1-naphthoate (2a). Colorless oil; ^{1}H NMR ($CDCl_3$) δ=1.47 (3H, t, J=7.2 Hz), 4.48 (2H, q, J=7.2 Hz), 7.52 (2H, m), 7.62 (1H, t, J=7.2 Hz), 7.88 (1H, d, J=8.2 Hz), 8.02 (1H, d, J=8.2 Hz), 8.18 (1H, dd, J=7.2, 1.5 Hz), 8.91 (1H, d, J=8.2 Hz); IR (neat) 2980, 1710, 1510, 1280, 1240, 1200, 1140, 780 cm^{-1}.

4.4.2. Ethyl 2,6-dimethoxybenzoate (2b). Colorless solid; mp 69–70 °C; ^{1}H NMR ($CDCl_3$) δ=1.37 (3H, t, J=7.2 Hz), 3.82 (6H, s), 4.39 (2H, q, J=7.2 Hz), 6.56 (2H, d, J=8.4 Hz), 7.28 (1H, t, J=8.4 Hz); IR (KBr) 2990, 1730, 1590, 1480, 1430, 1290, 1250, 1110, 1075, 780 cm^{-1}.

4.4.3. Ethyl 3,5-dinitrobenzoate (2c). Yellow solid; mp 92–93 °C; ^{1}H NMR ($CDCl_3$) δ=1.48 (3H, t, J=7.2 Hz), 4.53 (2H, q, J=7.2 Hz), 9.18 (2H, d, J=2.1 Hz), 9.23 (1H, t, J=2.1 Hz); IR (KBr) 3000, 1730, 1540, 1350, 1280, 740 cm^{-1}.

4.4.4. Ethyl palmitate (2d). Colorless oil; ^{1}H NMR ($CDCl_3$) δ=0.88 (3H, t, J=6.9 Hz), 1.23–1.34 (27H, m), 1.62 (2H, quin, J=7.5 Hz), 2.29 (2H, t, J=7.5 Hz), 4.12 (2H, q, J=7.2 Hz); IR (neat) 2930, 2850, 1740, 1470, 1180 cm^{-1}.

4.4.5. Ethyl cyclohexanecarboxylate (2e). Colorless oil; ^{1}H NMR ($CDCl_3$) δ=1.17–1.34 (3H, m), 1.25 (3H, t, J=7.2 Hz), 1.43 (2H, qd, J=12.3, 2.7 Hz), 1.64 (1H, m), 1.76 (2H, m), 1.90 (2H, dd, J=12.3, 2.7 Hz), 2.28 (1H, tt, J=11.4, 3.6 Hz), 4.11 (2H, q, J=7.2 Hz); IR (neat) 2930, 2850, 1730, 1450, 1380, 1310, 1250, 1170, 1040 cm^{-1}.

4.4.6. Ethyl 1-adamantanecarboxylate (2f). Colorless oil; ^{1}H NMR ($CDCl_3$) δ=1.24 (3H, t, J=7.2 Hz), 1.71 (6H, t, J=14.5 Hz), 1.88 (6H, d, J=2.9 Hz), 2.01 (3H, s), 4.10 (2H, q, J=7.2 Hz); IR (neat) 2910, 1730, 1450, 1320, 1230, 1180, 1080, 1030, 740 cm^{-1}.

4.4.7. Ethyl 2,6-dichlorobenzoate (2g). Colorless oil; ^{1}H NMR ($CDCl_3$) δ=1.42 (3H, t, J=7.2 Hz), 4.47 (2H, q, J=7.2 Hz), 7.25–7.34 (3H, m); IR (neat) 2980, 1740, 1560, 1440, 1270, 1140, 1060, 800, 780 cm^{-1}.

4.4.8. Ethyl 3,4,5-trimethoxybenzoate (2h). Colorless solid; mp 52 °C; ^{1}H NMR ($CDCl_3$) δ=1.40 (3H, t, J=7.2 Hz), 3.91 (3H, s), 3.92 (6H, s), 4.38 (2H, q, J=7.2 Hz), 7.3 (2H, s); IR (KBr) 2960, 1710, 1590, 1410, 1330, 1230, 1110, 990, 760 cm^{-1}.

4.4.9. Ethyl pyrazinecarboxylate (2i). Colorless solid; mp 48–49 °C; ^{1}H NMR ($CDCl_3$) δ=1.47 (3H, t, J=7.2 Hz), 4.53 (2H, q, J=7.2 Hz), 8.74 (1H, dd, J=2.4, 1.6 Hz), 8.77 (1H, d, J=2.4 Hz), 9.33 (1H, d, J=1.6 Hz); IR (KBr) 2980, 1740, 1370, 1310, 1280, 1160, 1120, 1020, 860, 780 cm^{-1}.

4.4.10. Ethyl 2,4,6-trimethylbenzoate (2j). Colorless oil; ^{1}H NMR ($CDCl_3$) δ=1.38 (3H, t, J=7.2 Hz), 2.28 (3H, s), 2.29 (6H, s), 4.38 (2H, q, J=7.2 Hz), 6.85 (2H, d, J=0.5 Hz); IR (neat) 2980, 1730, 1610, 1270, 1080 cm^{-1}.

4.4.11. Ethyl 2,4,6-triisopropylbenzoate (2k). Colorless oil; ^{1}H NMR ($CDCl_3$) δ=1.24 (18H, m), 1.35 (3H, t, J=7.2 Hz), 2.87 (3H, m), 4.36 (2H, q, J=7.2 Hz), 7.01 (2H, s); IR (neat) 2960, 1730, 1460, 1250, 1080 cm^{-1}.

4.4.12. Ethyl triphenylacetate (2l). Colorless solid; mp 117–118 °C; ^{1}H NMR ($CDCl_3$) δ=1.20 (3H, t, J=7.2 Hz), 4.28 (2H, q, J=7.2 Hz), 7.18 (6H, m), 7.27 (9H, m); IR (KBr) 2980, 1740, 1370, 1310, 1280, 1160, 1120, 1020, 860, 780 cm^{-1}.

4.4.13. Ethyl phenylpropiolate (2m). Colorless oil; ^{1}H NMR ($CDCl_3$) δ=1.36 (3H, t, J=7.2 Hz), 4.31 (2H, q, J=7.2 Hz), 7.38 (2H, t, J=7.4 Hz), 7.45 (1H, tt, J=7.4, 1.7 Hz), 7.59 (2H, d, J=7.4 Hz); IR (neat) 2980, 2210, 1710, 1490, 1370, 1280, 1190, 1020, 760 cm^{-1}.

4.4.14. Ethyl hippurate (2n). Colorless solid; mp 61–62 °C; ^{1}H NMR ($CDCl_3$) δ=1.32 (3H, t, J=7.2 Hz), 4.27 (4H, m), 6.68 (1H, s), 7.45 (2H, t, J=7.4 Hz), 7.53 (1H, t, J=7.4 Hz), 7.82 (2H, d, J=7.4 Hz); IR (KBr) 3340, 1760, 1640, 1530, 1200 cm^{-1}.

4.4.15. Ethyl oleate (2o). Colorless oil; ^{1}H NMR ($CDCl_3$) δ=0.88 (3H, t, J=6.8 Hz), 1.23–1.37 (23H, m), 1.62 (2H, quin, J=7.3 Hz), 2.01 (4H, m), 2.29 (2H, t, J=7.6 Hz), 4.12 (2H, q, J=7.1 Hz), 5.35 (2H, m); IR (neat) 2930, 2850, 1740, 1460, 1180 cm^{-1}.

4.4.16. *N*-Benzyloxycarbonyl-L-tryptophan ethyl ester (2p). Colorless solid; mp 85–86 °C (lit.[22] mp 85–87 °C); ^{1}H NMR ($CDCl_3$) δ=1.19 (3H, t, J=7.2 Hz), 3.31 (2H, d, J=5.4 Hz), 4.12 (2H, m), 4.70 (1H, m), 5.10 (2H, m), 5.32 (1H, d, J=8.0 Hz), 6.94 (1H, d, J=2.0 Hz), 7.08 (1H, t, J=8.0 Hz), 7.18 (1H, t, J=8.0 Hz), 7.33 (6H, m), 7.53 (1H, d, J=8.0 Hz), 8.09 (1H, s, broad); $[\alpha]_D^{21}$ +37.9 (c 1.0, $CHCl_3$); IR (KBr) 3360, 1740, 1710, 1530, 1220 cm^{-1}.

4.4.17. α-D-Xylofuranuronic acid, 1,2-*O*-(1-methylethylidene)-3-*O*-(phenylmethyl)-, ethyl ester (2q). Colorless oil; ^{1}H NMR ($CDCl_3$) δ=1.23 (3H, t, J=7.2 Hz), 1.32

(3H, s), 1.48 (3H, s), 4.14–4.31 (3H, m), 4.53 (2H, d, $J=12.1$ Hz), 4.64 (2H, m), 4.81 (1H, d, $J=3.7$ Hz), 6.09 (1H, d, $J=3.7$ Hz), 7.31 (5H, m); ^{13}C NMR ($CDCl_3$) $\delta=14.01$ (s), 26.19 (s), 26.79 (s), 61.11 (d), 72.08 (d), 79.42 (t), 81.56 (t), 82.65 (t), 105.55 (t), 112.17 (q), 127.50 (t), 127.81 (t), 128.25 (t), 136.81 (q), 167.61 (q); IR (neat) 2990, 2940, 1770, 1730, 1380, 1210, 1120, 1080, 1030 cm^{-1}; HRMS (FAB): Obsd M+H=323.1469, Calcd for $C_{17}H_{23}O_6$ M+H=323.1495.

4.4.18. Diethyl phthalate (2r). Colorless oil; ^{1}H NMR ($CDCl_3$) $\delta=1.37$ (3H, t, $J=7.3$ Hz), 4.37 (2H, q, $J=7.3$ Hz), 7.53 (2H, m), 7.73 (2H, m); IR (neat) 2980, 1730, 1280, 1120, 1070, 750 cm^{-1}.

4.4.19. Diethyl maleate (2s). Colorless oil; ^{1}H NMR ($CDCl_3$) $\delta=1.31$ (6H, t, $J=7.2$ Hz), 4.26 (4H, q, $J=7.2$ Hz), 6.24 (2H, s); IR (neat) 2980, 1730, 1210, 1180, 1030 cm^{-1}.

4.4.20. Diethyl fumarate (2t). Colorless oil; ^{1}H NMR ($CDCl_3$) $\delta=1.32$ (6H, t, $J=7.2$ Hz), 4.26 (4H, q, $J=7.2$ Hz), 6.85 (2H, s); IR (neat) 2980, 1720, 1300, 1260, 1150, 1040 cm^{-1}.

4.4.21. Methyl 1-naphthoate (3a). Colorless oil; ^{1}H NMR ($CDCl_3$) $\delta=4.01$ (3H, s), 7.52 (2H, m), 7.62 (1H, t, $J=8.2$ Hz), 7.89 (1H, d, $J=8.2$ Hz), 8.03 (1H, d, $J=8.2$ Hz), 8.19 (1H, dd, $J=7.2$, 1.5 Hz), 8.91 (1H, d, $J=8.2$ Hz); IR (neat) 2950, 1720, 1510, 1280, 1250, 1200, 1140, 780 cm^{-1}.

4.4.22. Methyl 2,6-dimethoxybenzoate (3b). Colorless solid; mp 88–89 °C; ^{1}H NMR ($CDCl_3$) $\delta=3.82$ (6H, s), 3.91 (3H, s), 6.56 (2H, d, $J=8.3$ Hz), 7.28 (1H, t, $J=8.3$ Hz); IR (KBr) 3000, 1730, 1590, 1480, 1290, 1250, 1110, 1075, 790 cm^{-1}.

4.4.23. Methyl palmitate (3c). Colorless oil; ^{1}H NMR ($CDCl_3$) $\delta=0.88$ (3H, t, $J=6.9$ Hz), 1.23–1.33 (24H, m), 1.62 (2H, quin, $J=7.4$ Hz), 2.30 (2H, t, $J=7.4$ Hz), 3.67 (3H, s); IR (neat) 2920, 2850, 1740, 1460, 1180 cm^{-1}.

4.4.24. Methyl phenylpropiolate (3d). Colorless oil; ^{1}H NMR ($CDCl_3$) $\delta=3.85$ (3H, s), 7.38 (2H, t, $J=7.4$ Hz), 7.45 (1H, tt, $J=7.4$, 1.9 Hz), 7.59 (2H, d, $J=7.4$ Hz); IR (neat) 2220, 1710, 1490, 1430, 1290, 1200, 1170, 760 cm^{-1}.

4.4.25. Ethyl *p*-toluenesulfinate (7). Colorless oil; bp 107 °C/1 mmHg (lit.[23] 75–76 °C/0.1 mmHg); ^{1}H NMR ($CDCl_3$) $\delta=1.28$ (3H, t, $J=7.0$ Hz), 2.43 (3H, s), 3.72 (1H, m), 4.10 (1H, m), 7.34 (2H, d, $J=8.0$ Hz), 7.60 (1H, dt, $J=8.0$, 1.7 Hz); IR (neat) 2980, 1130, 1010, 880, 820, 710 cm^{-1}.

4.4.26. 1-Ethoxy-2,4-dinitrobenzene (8a). Colorless solid; mp 83–84 °C; ^{1}H NMR ($CDCl_3$) $\delta=1.55$ (3H, t, $J=7.0$ Hz), 4.33 (2H, q, $J=7.0$ Hz), 7.19 (1H, d, $J=9.3$ Hz), 8.42 (1H, dd, $J=9.3$, 2.7 Hz), 8.73 (1H, d, $J=2.7$ Hz); IR (KBr) 3120, 1610, 1530, 1350, 1290, 1160, 1020, 740 cm^{-1}.

4.4.27. 2-Ethoxy-2-methyl-1,3-benzodioxole (8b). Colorless oil; ^{1}H NMR ($CDCl_3$) $\delta=1.20$ (3H, t, $J=7.1$ Hz), 1.81 (3H, s), 3.59 (2H, q, $J=7.1$ Hz), 6.82 (4H, m); IR (neat) 2980, 1490, 1260, 1180, 1050, 980, 880, 740 cm^{-1}.

4.4.28. Ethyl *p*-tolyl sulfide (9). Colorless oil; ^{1}H NMR ($CDCl_3$) $\delta=1.28$ (3H, t, $J=7.3$ Hz), 2.32 (3H, s), 2.90 (2H, d, $J=7.3$ Hz), 7.10 (2H, d, $J=8.0$ Hz), 7.25 (2H, d, $J=8.0$ Hz); IR (neat) 2980, 2940, 1490, 1450, 1260, 1090, 810 cm^{-1}.

4.5. Typical procedure for ethylation of phosphonic acid with triethyl orthoacetate in ionic liquid and reuse of ionic liquid

A flask containing 1-butyl-3-methylimidazolium hexafluorophosphate ([bmim]PF_6, 2.0 mL) as a solvent was dried under reduced pressure with a vacuum pump for 2 h at 80 °C. Then, benzylphenylphosphinic acid (1.0 mmol) and trimethyl orthoacetate (2.0 mmol) were added to the ionic liquid and the obtained mixture was heated at 80 °C under an argon atmosphere. The reaction was monitored by TLC until the starting benzylphenylphosphinic acid desappeared. After 2.5 h, the mixture was extracted with Et_2O (25×5 mL). The combined Et_2O extract was purified by column chromatography on silica gel (eluent: hexane/EtOAc, 1:1) to give pure methyl benzylphenylphosphinate in 98% yield.

Compounds **12A**, **11D**, and **12D** were identified with commercially available authentic compounds.

4.5.1. Methyl benzylphenylphosphinate (11A). Colorless solid; mp 80–82 °C (lit.[24] mp 87–93 °C); ^{1}H NMR ($CDCl_3$) $\delta=3.30$ (2H, d, $J=18$ Hz), 3.64 (3H, d, $J=11$ Hz), 7.09 (2H, m), 7.20 (3H, m), 7.41 (2H, m), 7.52 (1H, m), 7.58 (2H, m); ^{31}P NMR ($CDCl_3$, H_3PO_4) $\delta=41.28$ (s); IR (neat) 1439, 1215, 1026, 750, 695 cm^{-1}.

4.5.2. Ethyl benzylphenylphosphinate (12A). Colorless solid; mp 54–55 °C; ^{1}H NMR ($CDCl_3$) $\delta=1.28$ (3H, t, $J=18.1$ Hz), 3.29 (2H, d, $J=11$ Hz), 3.88 (1H, m), 4.07 (1H, m), 7.09 (2H, m), 7.20 (3H, m), 7.40 (2H, m), 7.51 (1H, m), 7.60 (2H, m); ^{31}P NMR ($CDCl_3$, H_3PO_4) $\delta=39.52$ (s); IR (neat) 1438, 1213, 1031, 749, 693 cm^{-1}.

4.5.3. Methyl (2-carbomethoxyethyl)phenylphosphinate (11B). Colorless oil; bp 150 °C/1 mmHg; ^{1}H NMR ($CDCl_3$) $\delta=2.25$ (2H, m), 2.58 (2H, m), 3.64 (3H, d, $J=11$ Hz), 3.63 (3H, s), 7.52 (2H, m), 7.58 (1H, m), 7.79 (2H, m); ^{13}C NMR ($CDCl_3$) $\delta=24.52$ (d), 26.33 (d), 51.11 (s), 51.79 (s), 128.64 (t), 129.10 (q), 131.56 (t), 132.50 (t), 172.35 (q); ^{31}P NMR ($CDCl_3$, H_3PO_4) $\delta=44.12$ (s); IR (neat) 1735, 1439, 1214, 1026, 740, 697 cm^{-1}; HRMS (FAB): Obsd M+H=243.0790, Calcd for $C_{11}H_{16}O_4P$ M+H=243.0786.

4.5.4. Methyl (2,2-dimethyl-1-oxopropyl)phenylphosphinate (11C). Colorless oil; bp 120 °C/1 mmHg; ^{1}H NMR ($CDCl_3$) $\delta=1.29$ (9H, s), 3.77 (3H, d, $J=11$ Hz), 7.46–7.63 (3H, m), 7.84 (2H, m); ^{13}C NMR ($CDCl_3$) $\delta=25.06$ (s), 47.31 (q), 51.11 (s), 51.99 (s), 127.79 (q), 128.61 (t), 132.54 (t), 133.10 (t), 217.29 (q); ^{31}P NMR ($CDCl_3$, H_3PO_4)

δ=20.26 (s); IR (neat) 1716, 1680, 1440, 1230, 1024, 724, 694 cm^{-1}; HRMS (FAB): Obsd M+H=241.0992, Calcd for $C_{12}H_{18}O_3P$ M+H=240.0915.

4.5.5. Dimethyl phenylphosphonate (11D). Colorless oil; ^1H NMR ($CDCl_3$) δ=3.77 (6H, d, J=11 Hz), 7.48 (2H, m), 7.58 (1H, m), 7.81 (2H, m); ^{31}P NMR ($CDCl_3$, H_3PO_4) δ=21.10 (s); IR (neat) 1440, 1249, 1018, 750, 696 cm^{-1}.

4.5.6. Diethyl phenylphosphonate (12D). Colorless oil; ^1H NMR ($CDCl_3$) δ=1.33 (6H, d, J=7.0 Hz), 4.03–4.21 (4H, m), 7.47 (2H, m), 7.56 (1H, m), 7.82 (2H, m); ^{31}P NMR ($CDCl_3$, H_3PO_4) δ=39.52 (s); IR (neat) 1440, 1247, 1017, 748, 696 cm^{-1}.

4.5.7. Dibutyl methyl phosphate (11E). Colorless oil; bp 80 °C/1 mmHg (lit.[25] bp 85–87 °C/2 mmHg); ^1H NMR ($CDCl_3$) δ=0.94 (6H, t, J=7.5 Hz), 1.42 (4H, m), 1.67 (4H, m), 3.76 (3H, d, J=11 Hz), 4.05 (4H, m); ^{31}P NMR ($CDCl_3$, H_3PO_4) δ=−0.22 (s); IR (neat) 1265, 1022 cm^{-1}.

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