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Sugar-Derived Nitrones

Cycloadditions of Sugar-Derived Nitrones Targeting Polyhydroxylated Indolizidines

Daniele Martella,[a] Giampiero D'Adamio,[a] Camilla Parmeggiani*[a,b] Francesca Cardona*[a] Elena Moreno-Clavijo,[c] Inmaculada Robina,[c] and Andrea Goti[a]

Abstract: The 1,3 dipolar cycloaddition reactions of three pentose-derived pyrroline *N*-oxides with mono- and disubstituted alkenes are reported. A strong dependence of the diastereoselectivity of the cycloadditions on the relative configuration of the nitrone stereocentres was observed. Further transformation of the reaction products allowed the synthesis of four new tetrahydroxylated indolizidines. Their activity as glycosidase inhibitors was evaluated against a panel of commercially available glycosidases. The specificity of inhibitory activity against amyloglucosidase and α-mannosidases depends on the configuration at C-2 of the indolizidine moiety.

Introduction

The 1,3-dipolar cycloaddition of nitrones to alkenes is a powerful reaction that allows the synthesis of isoxazolidines with the introduction of up to three new stereocentres.[1] The products can undergo further synthetic transformations to reach various heterocyclic compounds. In particular, 1,3-dipolar cycloadditions of sugar-derived nitrones to alkenes have been widely used to synthesize iminosugar derivatives, such as polyhydroxylated indolizidines and pyrrolizidines (Figure 1).[2]

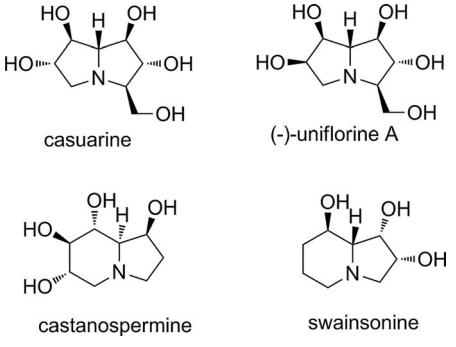


Figure 1. Examples of naturally occurring polyhydroxylated pyrrolizidines and indolizidines.

[a] *Department of Chemistry "Ugo Schiff", University of Florence, Via della Lastruccia 3–13, 50019 Sesto Fiorentino (FI), Italy*
E-mail: francesca.cardona@unifi.it
http://www2.chim.unifi.it/mdswitch.html
[b] *CNR-INO UOS Sesto Fiorentino and LENS, Via N. Carrara 1, 50019 Sesto Fiorentino (FI), Italy*
E-mail: camilla.parmeggiani@unifi.it
[c] *Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, c/ Prof. García González 1, 41012 Sevilla, Spain*
Supporting information and ORCID(s) from the author(s) for this article are available on the WWW under http://dx.doi.org/10.1002/ejoc.201501427.

Their structural resemblance to carbohydrates makes iminosugars excellent inhibitors of glycosidases and glycosyltransferases, and as a result these glycomimetics have the potential to be used against viral infection, cancer, or diabetes.[3] Several naturally occurring iminosugars have been extracted from plants that are well known for their therapeutic properties and are used in folk medicine.[4] However, they are often obtained in impure form and in minute amounts. Consequently, a great deal of research into the development of general strategies for the synthesis of these compounds in a pure form and higher amounts for biological tests has been undertaken during recent years, and many natural iminosugars and their unnatural analogues have been synthesized.[5] Moreover, the development of new synthetic strategies is crucial for ensuring the correct structural assignment of natural samples, and for making available new analogues for structure–activity relationship studies.[6]

We have developed several total syntheses of pyrrolizidine and indolizidine alkaloids using cycloaddition reactions or addition reactions of organometallic derivatives to enantiopure cyclic nitrones.[2,7] In particular, sugar-derived cyclic nitrones[8] are important building blocks for the synthesis of nitrogen-containing compounds. They allow good control of the stereochemistry of the final products; the configurations of the stereocentres of the starting material are retained, and the configurations of the newly created stereocentres derive from the stereoselective cycloaddition reaction.[2,9] In order to understand and rationalize the role of the substituents in the selectivity of the cycloaddition reactions, we studied the reactions of nitrones with different relative stereochemistries with a range of dipolarophiles.

In this paper, we report a complete study of the selectivity in 1,3-dipolar cycloadditions of three nitrones **1**–**3** (Figure 2) derived from D-arabinose, D-xylose, and D-ribose, respectively. The resulting cycloadducts are all suitable for transformation into indolizidine-type iminosugars using simple and versatile procedures. Using 3-buten-1-ol as the dipolarophile, the cycloadducts were transformed into new tetrahydroxyindoliz-

idines. The biological activity of the synthesized iminosugars was evaluated using a panel of 12 commercially available glycosidases.

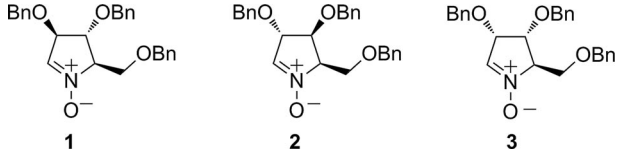
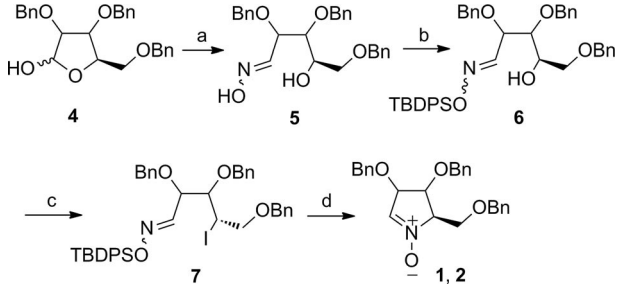


Figure 2. Nitrones derived from D-arabinose **1**, D-xylose **2**, and D-ribose **3**.

Results and Discussion

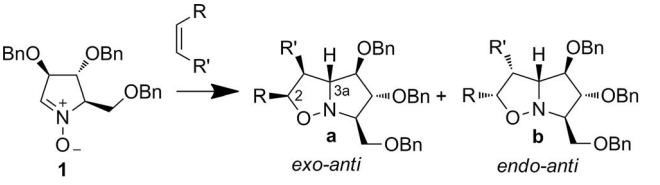
We first synthesized three nitrones **1**–**3** (Figure 2) starting from sugars belonging to the chiral pool. Nitrones **1** and **2** were synthesized starting from the corresponding tribenzylated D-arabinose and D-xylose derivatives, respectively, by a general procedure shown in Scheme 1 (see Supporting Information).[8a]



Scheme 1. Synthesis of nitrones **1** and **2** from tribenzylated D-arabinofuranose and D-xylofuranose. Reaction conditions: a) $NH_2OH{\cdot}HCl$, py, room temp., 24 h; b) TBDPSCl (*tert*-butyldiphenylsilyl chloride), py, room temp., 18 h; c) I_2, PPh_3, ImH (imidazole), toluene, 60 °C, 2 h; d) TBAF (tetrabutylammonium fluoride), THF, room temp., 18 h.

The key feature of this synthetic strategy is the double inversion of configuration at C-4 of the tribenzylated sugars to give nitrones with the same relative configuration as that of the starting materials. Tribenzylated sugar **4** was treated with hydroxylamine hydrochloride to give oxime **5**, which was then selectively protected with *tert*-butyl(chloro)diphenylsilane. Treatment of the secondary alcohol in **6** with iodine and imidazole in the presence of PPh_3 gave **7** with a first inversion of configuration. Then **7** was subjected to TBAF-mediated desilylation, and subsequent spontaneous intramolecular nucleophilic attack of the nitrogen atom onto the iodine-substituted carbon atom gave the cyclic nitrone (Scheme 1).[10] Nitrones **1** and **2** were obtained in 82 and 36 % yields, respectively, from the starting sugars. Nitrone **3** was prepared as previously reported,[8b] using a very similar procedure, and was obtained in 47 % yield from the starting sugar, the tribenzylated D-xylofuranose. The step that most affects the overall yield of the syntheses of the nitrones is the last one; the yield is strongly dependent on the relative configuration of starting material.

We then carried out cycloadditions using D-arabinose-derived nitrone **1** with different dipolarophiles in CH_2Cl_2 at room temperature for 48 h.[11] These reactions install two or three new stereogenic centres, and may, in principle, give four different stereoisomers. In reality, not all of the possible approaches of the reagents are viable, and the diastereomeric ratios depend on the dipolarophile used (Scheme 2).



8: R = $CONMe_2$; R' = H **10**: R = R' = CO_2Me
9: R = CO_2Me; R' = H **11**: R = CO_2Et; R' = $SiPhMe_2$

Scheme 2. Cycloadducts derived from nitrone **1** with different dipolarophiles. The *anti*/*syn* nomenclature refers to the attack of the alkene with respect to the vicinal OBn group of the nitrone.

Table 1 shows the results of the cycloadditions with different dipolarophiles: portions of the reaction products were separated by flash column chromatography and characterized by NMR spectroscopy. The diastereomeric ratios were calculated by integration of signals in the ^1H NMR spectra of the crude reaction products. Details of the stereochemical assignment of the products are reported in the Supporting Information.

Table 1. Yields and ratios for the cycloaddition reactions of nitrone **1** with various dipolarophiles.

Entry	Product	R and R′	Alkene [equiv.]	Yield[a] [%]	Ratio **a**/**b**[b]
1	**8**	R = $CONMe_2$ R′ = H	1.2	85	1:0
2	**9**	R = CO_2Me R′ = H	1.5	98	2.2:1[c,d]
3	**10**	R = R′ = CO_2Me	1	78	1:0
4	**11**	R = CO_2Et R′ = $SiPhMe_2$	2	87	1:0

[a] Total yield of the reaction. [b] Diastereoisomers shown in Scheme 2. [c] A third cycloadduct was detected by NMR spectroscopy, but was not isolated (tentatively assigned as *exo-syn*). [d] A higher 4:1 *exo*/*endo* selectivity was reported with the enantiomer of nitrone **1** for the cycloaddition in THF at room temp. with a higher excess of alkene.[12]

The reactions carried out with monosubstituted alkenes (R′ = H) were completely regioselective, giving only the products with the substituent at C-2, and a high stereoselectivity in favour of the *exo-anti* cycloadducts (**a**). This was the sole product when dimethylacrylamide was used as dipolarophile (**8a**), and the major product when methyl acrylate (**9a**) was used. In this latter case, the *endo-anti* adduct was also formed in noticeable quantities (**9b**). A minor amount of a product derived from a *syn* approach was observed in the crude mixture, but it was not possible to isolate and characterize it. When 1,2-disubstituted alkenes were used (Table 1, entries 3 and 4), the diastereoselectivity was completely in favour of the *exo-anti* adducts. In particular, addition to dimethyl maleate gave *exo-anti* cycloadduct **10a** exclusively, whereas previous reports with malic- and tartaric-acid-derived nitrones describe the formation of at least one more diastereoisomer, namely the *endo-anti*.[13] Moreover, in the related cycloadditions of malic- and tartaric-acid-derived

nitrones to cyclic dipolarophiles, the formation of at least two diastereoisomers in different ratios depending on the nitrone, the dipolarophile, and the reaction temperature was always reported.[14] Our findings highlight how the nitrone used is the primary factor influencing the selectivity of the reaction. Excellent *exo* selectivity was also reported with nitrone **1** in the cycloaddition to copper alkyne derivatives that is proposed to be the first step in the Kinugasa reaction.[15]

With unsymmetrically disubstituted alkene **11** (Table 1, entry 4), two different regioisomers were isolated (i.e., **11a**[7a] and **11′**; Figure 3) in a 7:1 ratio. Adduct **11a** has been used in the total synthesis of casuarine and its derivatives.[7a]

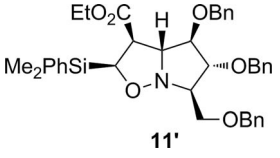


Figure 3. Adduct **11′** derived from an *exo-anti* attack of dipolarophile **11** on nitrone **1**.

The high *exo-anti* selectivity is ascribed to the *trans-trans* relative configuration of the nitrone, which means that the substituents at C-3 and C-5 are located on the same face of the ring. For steric reasons, it is preferable for the dipolarophile to approach from the opposite face (Figure 4); a *syn* adduct was observed only with the less bulky methyl acrylate. Excellent stereoselectivity in favour of the *exo-anti* adduct has previously been observed in the reaction of disubstituted alkenes with a different trisubstituted cyclic nitrone with the same relative configuration as **1**.[16] Our results are consistent with those previously reported for the 1,3-dipolar cycloaddition of cyclic nitrones that showed the *exo* mode of attack to be the preferred one.[17] With this nitrone, the *endo* mode of attack in the *anti* approach is further disfavoured by repulsive interactions with the substituent at C-4. The same nitrone also preferred the *anti* approach with a different dipolarophile, as reported by Vogel and coworkers. They reported the cycloaddition of **1** with allyl alcohol to form the *exo-anti* and *endo-anti* cycloadducts in 3.6:1 ratio.[10a] A worse *exo*/*endo* ratio of 2:1 was reported for the isolated adducts in the cycloaddition to methyl vinyl ketone.[18] This ratio drastically increases when the reaction is carried out with vinyladenine (only the *exo-anti* adduct was recovered) or with vinyluracil or vinylthymine (10:1 in favour of the *exo-anti*).[19] The cycloaddition of nitrone **1** with 3-buten-1-ol, an unactivated dipolarophile, was recently reported by our group; this gave a similar result, with exclusive formation of the *exo-anti* product, even though heating was necessary for the reaction to take place.[7g] There are several other reports of cycloaddition reactions between the same nitrone and different dipolarophiles in which the *exo-anti* adduct was the only isolated product.[20] The high regio- and stereoselectivity associated with nitrone **1** allowed a fourfold reiteration of the cycloaddition on a single calixarene compound bearing four pendant double bonds with the isolation in good yield of a single one of the 70 possible tetraadducts.[21]

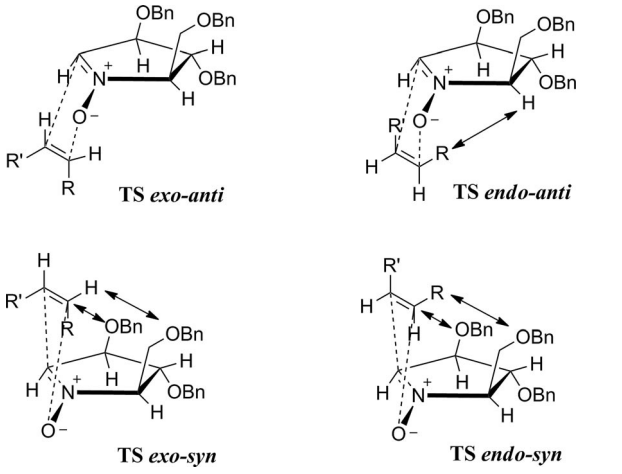
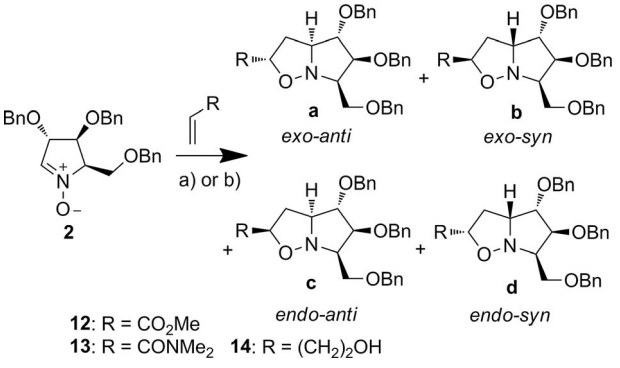


Figure 4. Possible transition states in the cycloaddition with **1**. Arrows show the main steric repulsions in the different approaches.

In order to better understand the effect of the substituents on the stereoselectivity of the 1,3-dipolar cycloaddition, and considering the complete regioselectivity observed for cycloadditions with monosubstituted alkenes, we turned to consider D-xylose- and D-ribose-derived nitrones **2** and **3** in cycloadditions with different monosubstituted alkenes as dipolarophiles.

The cycloaddition reactions of nitrone **2** were carried out in CH_2Cl_2 at room temperature for 48 h (Scheme 3). Only when 3-buten-1-ol was used as dipolarophile was heating at 60 °C in toluene required. The cycloadditions showed lower selectivity than those of nitrone **1**, and both *anti* and *syn* products were recovered in all cases.



Scheme 3. Cycloadducts derived from nitrone **2** with different dipolarophiles. The *anti*/*syn* terminology refers to the attack of the alkene with respect to the vicinal OBn group of the nitrone. Reaction conditions: a) CH_2Cl_2, room temp., 48 h; b) toluene, 60 °C, 72 h.

Table 2 summarizes the results with the different alkenes used. The ratio between the adducts was calculated by integration of signals in the ^1H NMR spectra of the crude reaction mixtures for methyl maleate and dimethyl acrylamide. However, for 3-buten-1-ol, the ratio of the adducts was calculated only at a later stage of the synthesis, since separation of the cycloadducts was not possible and these products were not characterized. For the other adducts, details of the determination of the relative configuration are reported in the Supporting Information.

Table 2. Yields and ratios for the cycloaddition reactions of nitrone **2** with various dipolarophiles.

Entry	Product	R	Alkene [equiv.]	Yield [%][a]	Ratio **a**/**b**/**c**/**d**[b]
1	**12**	CO_2Me	1.5	98	1:1.5:0.1:0.2[c,d]
2	**13**	$CONMe_2$	1.5	85	1:1.4:0: 0.8
3	**14**	$(CH_2)_2OH$	1.5	87	1:1.5:0:0

[a] Total yield of the reaction. [b] Diastereoisomers shown in Scheme 3. [c] **d** was detected by NMR spectroscopy, but was not isolated. [d] The formation of adducts **a**, **b**, and **d** was previously reported in this cycloaddition reaction, but no ratio of diastereoisomers was given.[12]

D-Xylose-derived nitrone **2** prefers an *exo* selectivity. When methyl acrylate was used as the dipolarophile, the *endo* products (i.e., **12c** and **12d**; Table 2, entry 1) were formed only in small quantities (*exo*/*endo* product ratio = 2.5:0.3); only in the case of dimethyl acrylamide was the *endo*/*syn* product isolable in a reasonable amount. But even in this last example, the *exo* derivatives were largely prevalent (*exo*/*endo* products = 3:1).

Unlike what was observed with nitrone **1**, the major products of the cycloaddition reactions of nitrone **2** were the *exo*-*syn* adducts with all the dipolarophiles (**12b**, **13b**, and **14b**; Table 2, entries 1, 2, and 3). Here, the substituents at C-3 and C-5 are placed on opposite faces of the nitrone, and the more hindered face becomes the one bearing the two substituents at C-4 and C-5 (Figure 5). However, the steric hindrance on the two faces is similar, and the *syn*/*anti* selectivity is definitely much lower than the *anti*/*syn* selectivity seen for nitrone **1**. Again, a large preference for *exo* selectivity is observed, with *endo*-*anti* adducts particularly disfavoured as a result of repulsion by the substituents at C-4 and C-5.

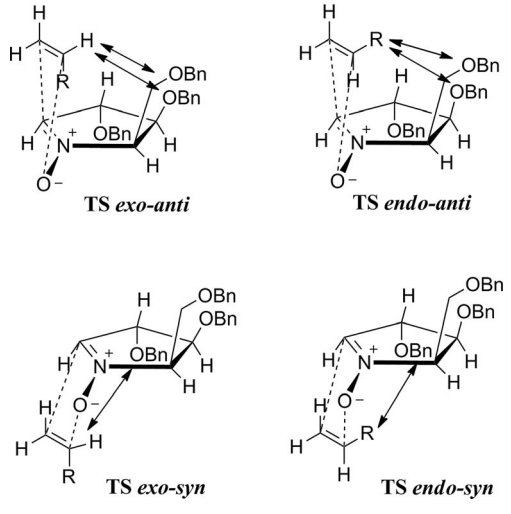
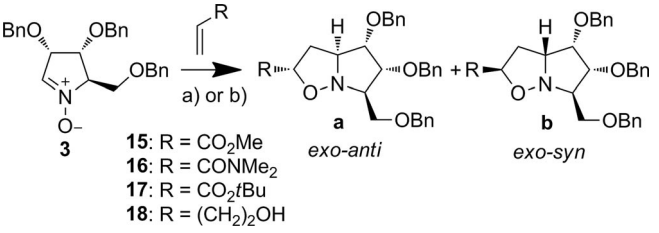


Figure 5. Possible transition states in the cycloaddition reaction with **2**. Arrows show the main steric repulsions in the different approaches.

The reactions with nitrone **3** were carried out under the same conditions: CH_2Cl_2 at room temperature for 48 h, and only when 3-buten-1-ol was used as dipolarophile, at 60 °C in toluene (Scheme 4). The reaction with *tert*-butyl acrylate was reported previously.[7d]



Scheme 4. Cycloadducts derived from nitrone **3** with different dipolarophiles. The *anti*-*syn* terminology refers to the attack of the alkene with respect to the vicinal OBn group of the nitrone. Reaction conditions: a) CH_2Cl_2, room temp., 48 h; b) toluene, 60 °C, 72 h.

Table 3 summarizes the results of the reactions; the ratios between the cycloadducts were calculated as for nitrone **2**. Also in this case, the cycloadducts from 3-buten-1-ol were not characterized, and the ratio was determined at a later stage of the synthesis.

Table 3. Yields and ratios for cycloaddition reactions of nitrone **3** with various dipolarophiles.

Entry	Product	R	Alkene [equiv.]	Yield [%][a]	Ratio **a**/**b**[b]
1	**15**	CO_2Me	1.5	87	1.7:1[c]
2	**16**	$CONMe_2$	1.5	77	2.3:1
3	**17**	CO_2tBu	1.5	88	1.5:1[d]
4	**18**	$(CH_2)_2OH$	1.5	70	1:1.5

[a] Total yield of the reaction. [b] Diastereoisomers shown in Scheme 4. [c] Two other cycloadducts were detected by NMR spectroscopy, but were not isolated. [d] Two other cycloadducts were detected by NMR spectroscopy, but were not isolated.

Also with nitrone **3**, *exo* selectivity is preferred. Only when methyl acrylate and *tert*-butylacrylate were used as dipolarophiles were the *endo* products also formed in very small quantities, and even then they were not characterized (*exo*/*endo* product ratios = 2.7:0.4 and 2.5:0.3, respectively). These adducts were detected in the ^1H NMR spectra of the crude mixtures, but it was not possible to isolate them. A 4:1 ratio of two cycloadducts in favour of the *exo*-*anti* isomer (minor isomer not characterized) was reported for the cycloaddition of nitrone **3** and the disubstituted alkene dimethyl maleate.[22] Since the authors did not characterize the minor isomer, we cannot exclude the formation of an *endo* isomer in that case; however, in the light of our results, an *endo* approach would seem to be rather disfavoured for nitrone **3**. It seems that the preference for *syn* or *anti* approach depends on the dipolarophile used. For acryloyl derivatives, the *anti* approach is favoured, and **15a**, **16a**, **17a** are the major products (a similar selectivity was previously reported for the cycloaddition to methyl vinyl ketone, which gave a 64 % yield of the *exo*-*anti*, and a 32 % yield of the *exo*-*syn* adduct).[18] However, with 3-buten-1-ol, the selectivity is opposite, and *syn* approach is favoured, with **18b** as the major product. However, in this last example, it was not possible to isolate the cycloadducts, and the ratios were calculated on the basis of the products obtained at a later stage of the synthesis. Therefore, we cannot exclude the possibility that the reported ratio is influenced by the subsequent synthetic steps.

In nitrone **3**, the bulkier group, i.e., the group at C-5, is *trans* to both the other two benzyloxy groups, and the difference in steric hindrance between the two faces is relatively small. This results in a very limited *syn*/*anti* selectivity. Conversely, both *endo* approaches are strongly disfavoured by repulsive van der Waals interactions with the substituents on the ring (Figure 6).

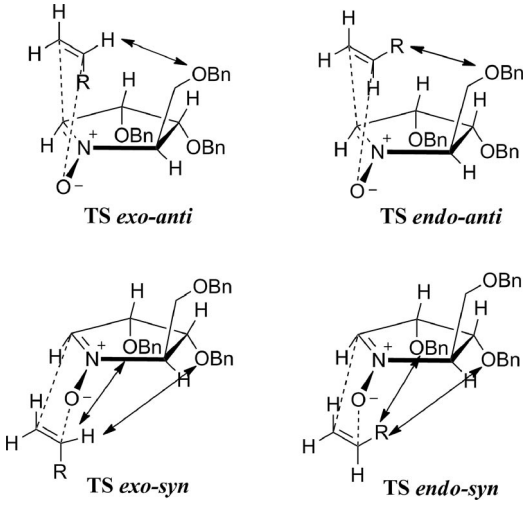


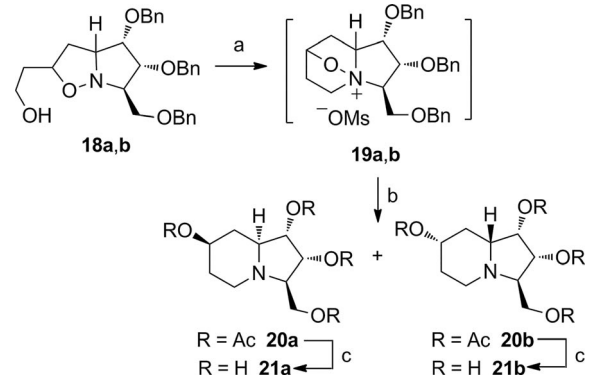
Figure 6. Possible transition states in the cycloaddition with **3**. Arrows show the main steric repulsions in the different approaches.

Adducts derived from *tert*-butyl acrylate (**17a** and **17b**, Table 3) were previously used by us for the synthesis of (+)-hyacinthacine A_1 and some analogues.[7d]

Comparing the diastereoselectivities of the cycloaddition reactions of nitrones **1**–**3** allows us to establish that both a benzyloxy group at C-3 and a benzyloxymethyl group at C-5 effectively shield the attack of dipolarophiles, with the latter group presenting slightly more steric hindrance, at least towards monosubstituted alkenes. Consequently, when these substituents are *cis*, as in nitrone **1**, *anti* facial attack occurs exclusively or to a very large extent. Conversely, when these substituents are *trans*, as in nitrones **2** and **3**, both faces are shielded by one substituent, and both *syn* and *anti* attacks are viable, which results in a low facial selectivity. In this case, the benzyloxy group at C-4 plays a secondary role in determining the preference for attack from the opposite face. Concerning the *exo*/*endo* selectivity, the *exo* preference commonly displayed by cyclic nitrones is amplified for all nitrones **1**–**3** by the presence of the substituents, which make *endo* approaches more greatly disfavoured.

Cycloadditions with 3-buten-1-ol as dipolarophile showed excellent *exo* selectivity with all nitrones **1**–**3**, and the adducts are synthetically useful for the preparation of tetrahydroxyindolizidines. Indolizidines derived from D-*arabino* nitrone **1** have previously been reported, and were shown to have good inhibitory activities against amyloglucosidase from *Aspergillus niger*.[7g] Application of the same synthetic strategy to cycloadducts **14a**,**b** and **18a**,**b** gave four additional diasteromeric indolizidines, as reported below.

Starting from nitrone **3**, separation of the adducts (i.e., **18**) was difficult, and the mixture was directly subjected to the desired transformation (Scheme 5).



Scheme 5. Synthesis of tetrahydroxyindolizidines **21a** and **21b**. Reaction conditions: a) MsCl, NEt_3, dry CH_2Cl_2, room temp., 45 min; b) H_2, Pd/C, MeOH, HCl, room temp., 72 h; then Ac_2O, py, room temp., 18 h; 17 % for **20a**, and 24 % for **20b** over two steps; c) Ambersep 900 OH, MeOH, room temp., 18 h, 100 % for both **21a** and **21b**.

Mesylation of primary alcohols **18** gave intermediate salts **19**, formed by intramolecular S_N2 substitution of the mesylate by the nitrogen atom. Catalytic hydrogenolysis under acidic conditions resulted in N–O bond cleavage and concomitant removal of the benzyl groups, leading directly to the mixture of target compounds **21**. We then treated this mixture with acetic anhydride in pyridine at room temperature for separation and characterization purposes. This led to the two *O*-acetyl derivatives (i.e., **20**), which were separated by flash column chromatography. The structures of **20a** and **20b**, obtained in 17 and 24 % yield, respectively, from **18**, were assigned on the basis of their ^1H NMR, ^{13}C NMR, COSY, HSQC, and 1D NOESY spectra. The structure of **20a**, derived from the *exo-anti* adduct, was assigned on the basis of nOe correlation peaks between 8a-H and 7-H, 5-H_b and 3-H, 8a-H and 5-H_b, and between 8a-H and 3-H (Figure 7).

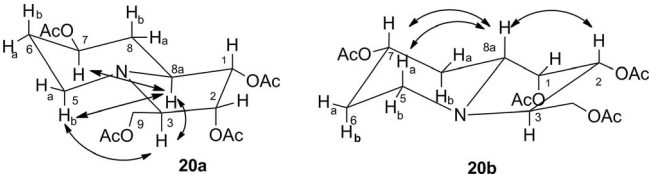


Figure 7. Diagnostic nOe correlation peaks for indolizidines **20a** and **20b**.

The structure of **20b**, derived from the *exo-syn* adduct, was assigned on the basis of nOe correlation peaks between 2-H and 8a-H, 7-H and 8a-H, and between 5-H_a and 8a-H (Figure 7).

Treatment of **20a** and **20b** with the ion-exchange resin Ambersep 900 OH at room temperature in MeOH gave the pure final compounds (i.e., **21a** and **21b**) in quantitative yield.

When the same synthetic strategy was applied to cycloadducts **14a**,**b**, obtained from nitrone **2** and 3-buten-1-ol, this gave products **22a** and **22b** in 35 and 59 % yield, respectively (Figure 8).

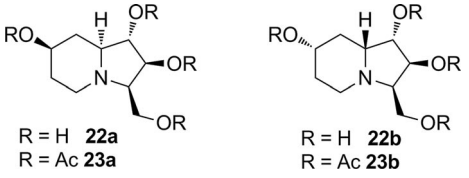


Figure 8. Tetrahydroxyindolizidines **22a** and **22b** and their acetyl derivatives **23a** and **23b**.

The structures of **22a** and **22b** were assigned on the basis of ^{1}H NMR, ^{13}C NMR, COSY, HSQC, and 1D NOESY spectra recorded for their acetyl derivatives (i.e., **23a** and **23b**). In particular, compound **23a**, derived from the *exo-anti* adduct showed nOe correlation peaks between 1-H and 8-H_b, 5-H_b and 7-H, 6-H_a and 7-H, 7-H and 8a-H, 3-H and 5-H_b, and between 3-H and 8a-H (Figure 9).

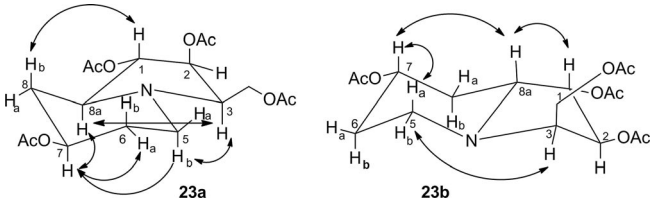


Figure 9. Diagnostic nOe correlation peaks for **23a** and **23b**.

Indolizidine **23b**, derived from the *exo-syn* adduct, showed nOe correlation peaks between 7-H and 8a-H, 1-H and 8a-H, 7-H and 5-H_a, and between 3-H and 5-H_b in its 1D NOESY spectra (Figure 9).

Compounds **21a**, **21b**, **22a**, and **22b** were evaluated using a panel of twelve commercially available glycosidases.[23] The results were compared with those obtained for compound **24**, derived from nitrone **1** (Figure 10).[7g]

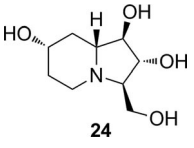


Figure 10. D-Arabinose-derived indolizidine.[7g]

At 1 mM concentration, and under enzyme-optimal pH, none of the compounds inhibited β-galactosidase from *Aspergillus oryzae* or from *Escherichia coli*, α-glucosidase from rice or yeast, α-galactosidase from coffee beans, β-xylosidase from *Aspergillus niger*, or β-*N*-acetylglucosaminidase from Jack beans. Table 4 shows the inhibition results against α-L-fucosidase from bovine kidney, α-mannosidase from jack beans, amyloglucosidase from *Aspergillus niger*, β-glucosidase from almonds, and β-mannosidase from snail.

From the inhibitory activity data for compounds **21a**, **21b**, **22a**, **22b**, and **24** against glycosidases, it can be seen that the relative *trans* configuration at (*R*)C-2/(*R*)C-3 contributes to the inhibition of amyloglucosidase, as **21a**, **21b**, and **24** show good inhibition of this enzyme. The corresponding relative *cis* configuration at (*S*)C-2/(*R*)C-3 diminishes or abolishes the inhibitory activity against this enzyme, as seen for compounds **22a** and **22b**, respectively. The configurations at C-1, C-8a, and C-7 do not seem to be crucial for amyloglucosidase inhibitory activity, since **21a** has the opposite configuration to **21b** at C-8a and C-7, and to **24** (at C-1, C-8a, and C-7), and all three of these compounds are good inhibitors. It is worth noting that on changing the configuration at C-2 as, for example, in going from **21b** to **22b**, a drastic change in the enzymatic inhibitory specificity is observed, from amyloglucosidase to α-mannosidase. An (*S*)C-2 configuration in the latter abolishes amyloglucosidase inhibition, and increases the activity and selectivity against α-mannosidases. Interestingly, compound **22b** also showed 98 % inhibition of a human enzyme, namely human lysosomal α-mannosidase, as determined following a recently reported protocol.[24] This data is in complete agreement with the value obtained against the commercial glycosidase. It is therefore worth noting that inhibition assays against commercially available glycosidases represent a useful tool for a preliminary biological investigation, and usually show data in good agreement with human enzymes.

Table 4. Inhibitory activities of compounds **21a**, **21b**, **22a**, **22b**, and **24** against glycosidases. Percentage inhibition at 1 mM, IC_{50} (in parentheses, μM), and K_i (bold, μM) if measured. Optimal pH, 37 °C.[a,b,c]

	α-L-Fuc-ase	α-Man-ase	AmyloGlc-ase	β-Glc-ase	β-Man-ase
21a	n.i	n.i	96 (13.4) **12.3**	n.i.	n.i.
21b	24	n.i.	96 (5.9) **4.6**	n.i.	n.i.
22a	n.i	n.i	23	n.i.	n.i.
22b	n.i	95 (79)	n.i.	n.i.	n.i.
24	32	n.i.	95 (20.6) **18**	21	15

[a] For conditions of measurements see ref.[23] and the Supporting Information. [b] n.i.: no inhibition at 1 mM concentration of the inhibitor. [c] α-L-Fucase: α-L-fucosidase from bovine kidney; α-Man-ase: α-mannosidase from jack beans; AmyloGlc-ase: Amyloglucosidase from *Aspergillus niger*; β-Glc-ase: β-glucosidase from almonds; β-Man-ase: β-mannosidase from snail.

Conclusions

The reactions studied highlight the differences in the selectivities of 1,3-dipolar cycloadditions of pyrrolidine *N*-oxides derived from different pentoses. These reactions provide two to three new stereogenic centres, and can, in principle, give four different stereoisomers. However, not all the possible reaction pathways are followed, and the observed diastereoisomeric ratios depend on the dipolarophile used and the configuration of the starting nitrone. In particular, D-arabinose-derived nitrone **1** showed the best results in terms of selectivity for *exo-anti* adducts, due to the presence of C-3 and C-5 substituents of the nitrone on the same face of the ring. On the other hand, the major products in the reactions of nitrone **2** were, in all cases, the *exo-syn* adducts. In this case, the substituents at C-3 and C-5 are on different faces of the nitrone; the more hindered face becomes that bearing the two substituents at C-4 and C-5. However, the steric hindrance on the two faces is similar, and the selectivity is definitely lower than that showed by nitrone **1**. In nitrone **3**, the bulkier group, i.e., the one at C-5, is opposite to both the other two bulky groups, and the difference in hindrance between the two faces is limited, but all substituents

strongly disfavour *endo* approaches. With this nitrone, the preference for a *syn* or *anti* approach depends on the dipolarophile used. Cycloadditions with 3-buten-1-ol as the dipolarophile were highly selective for all nitrones, and the resulting adducts were used to obtain different tetrahydroxyindolizidines, which were tested against a panel of twelve commercially available enzymes. Interesting structure–activity relationships can be deduced from the biological data. A remarkable change in specificity from amyloglucosidase to α-mannosidases was observed upon changing the configuration at C-2. This opens the possibility of easily modulating the inhibitory activity.

Experimental Section

General Methods: Commercially sourced reagents were used as received. All reaction mixtures were magnetically stirred. Reactions were monitored by TLC on 0.25 mm silica gel plates (Merck F_{254}), and column chromatography was carried out on Silica Gel 60 (32–63 μm), yields refer to spectroscopically and analytically pure compounds unless otherwise stated. ^{1}H NMR spectra were recorded with a Varian Mercury 400 or a Varian INOVA 400 instrument. ^{13}C NMR spectra were recorded with a Varian Gemini 200 instrument. Infrared spectra were recorded with a Perkin–Elmer Spectrum BX FTIR System spectrophotometer. Electron impact (EI) mass spectra were recorded with a QMD 1000 Carlo Erba instrument by direct inlet; relative intensities are shown in parentheses. Electrospray ionization (ESI) mass spectra were recorded with a Thermo LTQ instrument by direct inlet; relative intensities are shown in parentheses. Elemental analysis was carried out with a Perkin–Elmer 2400 analyser. Optical rotation measurements were carried out with a JASCO DIP-370 polarimeter.

General Procedure for the Cycloaddition Reaction: A solution of nitrone (amount in mmol used is indicated for each cycloadduct) and alkene (in excess as reported in the tables within the text) in CH_2Cl_2 (0.25–0.5 M with respect to the nitrone) was stirred for 3 d at room temp., then the solvent was removed under reduced pressure. Purification by flash column chromatography on silica gel (petroleum ether/EtOAc) provided the pure adducts. In the case of 4-buten-1-ol, toluene was used as solvent, and the mixture was heated at 60 °C for 72 h.

(2*R*,3a*R*,4*R*,5*R*,6*R*)-4,5-Bis(benzyloxy)-6-[(benzyloxy)methyl]-*N*,*N*-dimethylhexahydropyrrolo[1,2-*b*]isoxazole-2-carboxamide (8a): Reaction performed on 1.43 mmol of nitrone **1**. M.p. 72–74 °C. $[\alpha]_D^{20}$ = –52.5 (*c* = 0.86, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40–7.27 (m, 15 H, Ar), 4.85 (dd, *J* = 7.4, 6.6 Hz, 1 H, 2-H), 4.64–4.50 (m, 6 H, Bn), 4.05 (dd, *J* = 6.0, 3.5 Hz, 1 H, 5-H), 4.01 (t, *J* = 3.3 Hz, 1 H, 4-H), 3.90 (ddd, *J* = 9.0, 6.0, 3.1 Hz, 1 H, 3a-H), 3.76 (dd, *J* = 9.9, 5.0 Hz, 1 H, CH_2OBn), 3.65 (dd, *J* = 9.9, 6.3 Hz, 1 H, CH_2OBn), 3.40 (m, 1 H, 6-H), 3.06 (s, 3 H, NMe), 3.01 (ddd, *J* = 12.5, 9.0, 6.5 Hz, 1 H, 3-H_a), 2.95 (s, 3 H, NMe), 2.26 (ddd, *J* = 12.5, 7.5, 6.1 Hz, 1 H, 3-H_b) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 168.0 (s, C=O), 138.2, 137.8, 137.5 (s, Ar), 128.4–127.5 (d, 15 C, Ar), 86.6 (d, C-5), 84.0 (d, C-4), 74.7 (d, C-2), 73.3, 72.3, 71.6 (t, Bn), 70.1 (t, CH_2OBn), 70.0 (d, C-6), 68.5 (d, C-3a), 37.0 (q, NMe), 36.1 (t, C-3), 35.8 (q, NMe) ppm. IR ($CDCl_3$): $\tilde{\nu}$ = 3066, 2930, 2867, 1649, 1497, 1454, 1216, 1100 cm^{-1}. MS (EI): *m*/*z* (%) = 516 (0.2) $[M]^+$, 444 (5), 197 (13), 107 (62), 90 (100), 77 (63), 56 (33). $C_{31}H_{36}N_2O_5$ (516.63): calcd. C 72.07, H 7.02, N 5.42; found C 72.17, H 7.20, N 5.70.

Methyl (2*R*,3a*R*,4*R*,5*R*,6*R*)-4,5-Bis(benzyloxy)-6-[(benzyloxy)methyl]hexahydropyrrolo[1,2-*b*]isoxazole-2-carboxylate (9a): Reaction performed on 0.25 mmol of nitrone **1**. $[\alpha]_D^{24}$ = –44.2 (*c* = 0.96, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37–7.24 (m, 15 H, Ar), 4.67 (dd, *J* = 8.3, 5.8 Hz, 1 H, 2-H), 4.61–4.51 (m, 6 H, Bn), 4.09 (dd, *J* = 5.3, 3.9 Hz, 1 H, 5-H), 3.99 (pseudo t, *J* = 3.9 Hz, 1 H, 4-H), 3.81 (ddd, *J* = 8.3, 5.8, 3.9 Hz, 1 H, 3a-H), 3.76 (s, 3 H, Me), 3.71 (dd, *J* = 9.8, 4.7 Hz, 1 H, CH_2OBn), 3.60 (dd, *J* = 9.8, 6.8 Hz, 1 H, CH_2OBn), 3.46–3.41 (m, 1 H, 6-H), 2.61 (ddd, *J* = 12.7, 8.3, 5.8 Hz, 1 H, 3-H_a), 2.49 (ddd, *J* = 12.7, 8.3, 5.8 Hz, 1 H, 3-H_b) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 171.2 (s, C=O), 138.2, 137.9, 137.6 (s, Ar), 128.4–127.5 (d, 15 C, Ar), 87.1 (d, C-4), 84.7 (d, C-5), 75.2 (d, C-2), 73.4, 72.2, 71.8 (t, 3 C, Bn), 70.8 (d, C-6), 69.9 (t, CH_2OBn), 68.2 (d, C-3a), 52.4 (q, Me), 37.8 (t, C-3) ppm. IR ($CHCl_3$): $\tilde{\nu}$ = 3033, 3007, 2954, 2865, 1739, 1454, 1363 cm^{-1}. MS (ESI): *m*/*z* (%) = 526.33 (100) $[M + Na]^+$. $C_{30}H_{33}NO_6$ (503.59): calcd. C 71.55, H 6.61, N 2.78; found C 71.23, H 6.45, N 2.39.

Methyl (2*S*,3a*R*,4*R*,5*R*,6*R*)-4,5-Bis(benzyloxy)-6-[(benzyloxy)methyl]hexahydropyrrolo[1,2-*b*]isoxazole-2-carboxylate (9b): Reaction performed on 0.25 mmol of nitrone **1**. $[\alpha]_D^{24}$ = –18.5 (*c* = 0.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36–7.23 (m, 15 H, Ar), 4.64–4.50 (m, 7 H, 2-H, 6 Bn), 4.11 (dd, *J* = 6.1, 3.9 Hz, 1 H, 5-H), 4.05 (pseudo t, *J* = 3.9 Hz, 1 H, 4-H), 3.75 (m, 1 H, 3a-H), 3.70 (s, 3 H, Me), 3.68–3.60 (m, 3 H, 6-H, CH_2OBn), 2.71 (dt, *J* = 12.7, 8.3 Hz, 1 H, 3-H_a), 2.45 (dt, *J* = 12.7, 8.1 Hz, 1 H, 3-H_b) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 171.7 (s, C=O), 137.6, 137.3 (s, 3 C, Ar), 128.1–127.1 (d, 15 C, Ar), 85.5 (d, C-4), 84.4 (d, C-5), 77.4 (d, C-2), 72.9, 71.9, 71.6 (t, Bn), 69.4 (d, C-6), 68.9 (t, CH_2OBn), 68.3 (d, C-3a), 52.2 (q, Me), 37.7 (t, C-3) ppm. IR ($CHCl_3$): $\tilde{\nu}$ = 3052, 2986, 2954, 2864, 1746, 1454, 1363 cm^{-1}. MS (ESI): *m*/*z* (%) = 526.17 (100) $[M + Na]^+$. $C_{30}H_{33}NO_6$ (503.59): calcd. C 71.55, H 6.61, N 2.78; found C 71.91, H 6.59, N 2.99.

Dimethyl (2*R*,3*S*,3a*R*,4*R*,5*R*,6*R*)-4,5-Bis(benzyloxy)-6[(benzyloxy)methyl]hexahydropyrrolo[1,2-*b*]isoxazole-2,3-dicarboxylate (10a): Reaction performed on 0.67 mmol of nitrone **1**. $[\alpha]_D^{20}$ = –86.0 (*c* = 0.95, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40–7.25 (m, 15 H, Ar), 4.94 (d, *J* = 8.2 Hz, 1 H, 2-H), 4.66–4.49 (m, 6 H, Bn), 4.28 (d, *J* = 7.6 Hz, 1 H, 3a-H), 4.12 (m, 1 H, 4-H), 4.09–4.08 (m, 1 H, 5-H), 3.80 (s, 3 H, Me), 3.75 (s, 3 H, Me), 3.79–3.74 (m, 2 H, 3-H, CH_2OBn), 3.60 (dd, *J* = 9.7, 7.7 Hz, 1 H, CH_2OBn), 3.48–3.45 (m, 1 H, 6-H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 169.4 (s, C=O), 168.9 (s, C=O), 138.1, 137.5, 137.4 (s, Ar), 128.4–127.5 (d, 15 C, Ar), 85.0, 84.3 (d, 2 C, C-4, C-5), 77.8 (d, C-2), 73.4, 72.1 (t, Bn), 71.9 (d, C-6), 71.6 (t, Bn), 71.4 (d, C-3a), 70.0 (t, CH_2OBn), 54.1 (d, C-3), 52.6 (q, 2 C, Me) ppm. IR ($CDCl_3$): $\tilde{\nu}$ = 3160, 3080, 3030, 3000, 2960, 2920, 2880, 1730, 1625, 1570, 1460, 1380, 1250, 1088 cm^{-1}. MS (EI): *m*/*z* (%) = 561 (0.2), 531 (0.6), 502 (0.3), 113 (8), 91 (100), 65 (3), 77 (3). $C_{32}H_{35}NO_8$ (561.6): calcd. C 68.43, H 6.28, N 2.49; found C 68.00, H 6.42, N 2.34.

Ethyl (2*S*,3*R*,3a*R*,4*R*,5*R*,6*R*)-4,5-Bis(benzyloxy)-6-[(benzyloxy)methyl]-2-[dimethyl(phenyl)silyl]-hexahydropyrrolo-[1,2-*b*]isoxazole-3-carboxylate (11′): Reaction performed on 2.95 mmol of nitrone **1**. $[\alpha]_D^{27}$ = –55.7 (*c* = 1.01, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.60–7.58 (m, 2 H, Ar), 7.39–7.26 (m, 18 H, Ar), 4.70–4.55 (m, 6 H, Bn), 4.09 (m, 2 H, 3a-H, 5-H), 3.94–3.71 (m, 6 H, 3-H, 4-H, CH_2OBn, CH_2CH$_3$), 3.24–3.20 (m, 2 H, 2-H, 6-H), 1.16 (t, *J* = 7.2 Hz, 3 H, CH$_2$CH_3), 0.44 (s, 6 H, SiMe$_2$) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 171.7 (s, C=O), 138.3, 137.8, 137.7, 136.7 (s, Ar), 133.9–127.4 (d, 20 C, Ar), 86.8 (d, C-4), 82.2 (d, C-5), 73.1, 72.4, 71.7 (t, Bn), 71.0 (d, C-3a), 70.0 (t, CH$_2$OBn), 69.5 (d, C-3), 67.5 (d, C-6), 60.8 (t, CH_2CH$_3$), 56.9 (d, C-2), 13.8 (q, CH$_2$CH_3), –3.9, –4.0 (q, 2 C, SiMe$_2$) ppm. IR ($CDCl_3$): $\tilde{\nu}$ = 3067, 3034, 3009, 2865, 1734, 1454, 1365, 1223 cm^{-1}. MS (EI): *m*/*z* (%) = 651 (100), 491 (26), 479 (31), 279 (25), 91 (31), 65 (5), 77 (33). $C_{39}H_{45}NO_6Si$ (651.3): calcd. C 71.86, H 6.96, N 2.15; found C 71.97, H 6.95, N 2.41.

Methyl (2*S*,3a*S*,4*S*,5*S*,6*R*)-4,5-Bis(benzyloxy)-6-[(benzyloxy)methyl]hexahydropyrrolo[1,2-*b*]isoxazole-2-carboxylate (12a): Reaction performed on 0.26 mmol of nitrone **2**. $[\alpha]_D^{24}$ = –8.4 (*c* = 0.7, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.35–7.23 (m, 15 H, Ar), 4.62–4.52 (m, 5 H, 2-H, 4 Bn), 4.61 (d, *J* = 11.7 Hz, 1 H, Bn), 4.35 (d, *J* = 11.7 Hz, 1 H, Bn), 4.26 (dd, *J* = 5.6, 2.6 Hz, 1 H, 5-H), 4.05 (dt, *J* = 8.8, 5.9 Hz, 1 H, 3a-H), 3.97 (dd, *J* = 5.9, 2.6 Hz, 1 H, 4-H), 3.90 (dt, *J* = 7.5, 5.6 Hz, 1 H, 6-H), 3.82–3.80 (m, 1 H, CH_2OBn), 3.78–3.75 (m, 1 H, CH_2OBn), 3.58 (s, 3 H, Me), 2.75 (ddd, *J* = 12.5, 6.8, 5.9 Hz, 1 H, 3-H_a), 2.54 (dt, *J* = 12.5, 8.8 Hz, 1 H, 3-H_b) ppm. ^{13}C NMR (50 MHz, $CDCl_3$, *detected signals*): δ = 137.9, 137.6, 137.1 (s, Ar), 128.1–127.4 (d, 15 C, Ar), 82.0 (d, C-5), 81.3 (d, C-4), 77.8 (d, C-2), 73.1, 72.4, 72.0 (t, Bn), 67.1 (t, CH_2Bn), 67.1 (d, C-6), 65.9 (d, C-3a), 51.8 (q, Me), 33.0 (t, C-3) ppm. IR ($CHCl_3$): $\tilde{\nu}$ = 3011, 2953, 2866, 1748, 1454, 1265 cm^{-1}. MS (ESI): *m/z* (%) = 526.25 (100) $[M + Na]^+$. $C_{30}H_{33}NO_6$ (503.59): calcd. C 71.55, H 6.61, N 2.78; found C 72.01, H 6.32, N 2.95.

Methyl (2*R*,3a*R*,4*S*,5*S*,6*R*)-4,5-Bis(benzyloxy)-6-[(benzyloxy)methyl]hexahydropyrrolo[1,2-*b*]isoxazole-2-carboxylate (12b): Reaction performed on 0.26 mmol of nitrone **2**. $[\alpha]_D^{24}$ = +4.8 (*c* = 0.4, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.30–7.17 (m, 15 H, Ar), 4.60–4.49 (m, 5 H, 2-H, 4 Bn), 4.37 (d, *J* = 12.1 Hz, 1 H, Bn), 4.30 (d, *J* = 11.7 Hz, 1 H, Bn), 4.03–4.01 (m, 1 H, 5-H), 4.00–3.95 (m, 2 H, CH_2OBn), 3.79–3.78 (m, 1 H, 4-H), 3.72–3.68 (m, 2 H, 3a-H, 6-H), 3.67 (s, 3 H, Me), 2.69 (ddd, *J* = 12.5, 6.8, 5.8 Hz, 1 H, 3-H_a), 2.41 (dt, *J* = 12.5, 7.8, 2.5 Hz, 1 H, 3-H_b) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 171.6 (s, C=O), 137.9, 137.6, 137.1 (s, 3 C, Ar), 128.2–127.2 (d, 15 C, Ar), 89.9 (d, C-4), 83.8 (d, C-5), 74.7 (d, C-2), 73.1, 72.2, 71.4 (t, Bn), 71.0, 69.4 (d, C-3a, C-6), 65.1 (t, CH_2OBn), 52.1 (q, Me), 38.1 (t, C-3) ppm. IR ($CHCl_3$): $\tilde{\nu}$ = 3010, 2954, 2865, 1735, 1454, 1363, 1364 cm^{-1}. MS (ESI): *m/z* (%) = 526.25 (100) $[M + Na]^+$. $C_{30}H_{33}NO_6$ (503.59): calcd. C 71.55, H 6.61, N 2.78; found C 71.69, H 6.78, N 2.96.

Methyl (2*R*,3a*S*,4*S*,5*S*,6*R*)-4,5-Bis(benzyloxy)-6-[(benzyloxy)methyl]hexahydropyrrolo[1,2-*b*]isoxazole-2-carboxylate (12c): Reaction performed on 0.26 mmol of nitrone **2**. $[\alpha]_D^{24}$ = –32.9 (*c* = 0.8, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.36–7.23 (m, 15 H, Ar), 4.61–4.49 (m, 6 H, 2-H, 5 Bn), 4.36 (d, *J* = 12.2 Hz, 1 H, Bn), 4.17 (dd, *J* = 4.9, 2.1 Hz, 1 H, 5-H), 4.09 (ddd, *J* = 8.5, 5.9, 2.9 Hz, 1 H, 3a-H), 3.90 (dd, *J* = 5.9, 2.1 Hz, 1 H, 4-H), 3.87–3.85 (m, 1 H, CH_2Bn), 3.78 (dd, *J* = 9.1, 4.7 Hz, 1 H, CH_2Bn), 3.75 (s, 3 H, Me), 3.52 (dt, *J* = 8.9, 4.7 Hz, 1 H, 6-H), 2.72 (ddd, *J* = 12.2, 6.8, 2.9 Hz, 1 H, 3-H_a), 2.35 (dt, *J* = 12.2, 8.5 Hz, 1 H, 3-H_b) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 170.2 (s, C=O), 137.8, 137.5, 137.1 (s, 3 C, Ar), 128.2–127.2 (d, 15 C, Ar), 81.1 (d, C-5), 80.9 (d, C-4), 76.3 (d, C-2), 73.3, 72.4, 71.9, 67.9 (t, CH_2Bn), 67.0 (d, C-6), 66.0 (d, C-3a), 52.2 (q, Me), 33.0 (t, C-3) ppm. IR ($CDCl_3$): $\tilde{\nu}$ = 3032, 2954, 2867, 2248, 1740, 1454, 1363, 1209 cm^{-1}. MS (ESI): *m/z* (%) = 526.25 (100) $[M + Na]^+$. $C_{30}H_{33}NO_6$ (503.59): calcd. C 71.55, H 6.61, N 2.78; found C 71.17, H 6.29, N 2.62.

4,5-Bis(benzyloxy)-6-[(benzyloxy)methyl]-*N*,*N*-dimethylhexahydropyrrolo[1,2-*b*]isoxazole-2-carboxamide (13a or 13d): Reaction performed on 0.28 mmol of nitrone **2**. $[\alpha]_D^{21}$ = +6.92 (*c* = 0.13, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.34–7.22 (m, 15 H, Ar), 4.72 (dd, *J* = 9.3, 7.0 Hz, 1 H, 2-H), 4.56–4.43 (m, 6 H, Bn), 4.27 (dd, *J* = 6.2, 3.5 Hz, 1 H, 5-H), 4.09 (dd, *J* = 6.3, 3.5 Hz, 1 H, 4-H), 4.05–3.99 (m, 1 H, 3a-H), 3.77 (dd, *J* = 8.2, 6.3 Hz, 1 H, CH_2OBn), 3.71–3.65 (m, 1 H, 6-H), 3.65 (dd, *J* = 8.2, 4.7 Hz, 1 H, CH_2OBn), 3.04 (s, 3 H, NMe), 3.04–2.97 (m, 1 H, 3-H_a), 2.92 (s, 3 H, NMe), 2.20 (ddd, *J* = 12.9, 8.2, 7.0 Hz, 1 H, 3-H_b) ppm. ^{13}C NMR (50 MHz, $CDCl_3$, detected signal): δ = 128.1–127.1 (d, 15 C, C-Ar), 82.1 (d, C-5), 81.5 (d, C-4), 77.9 (d, C-2), 73.0, 72.3, 72.1 (t, Bn), 68.0–66.5 (3 C, C-3a, C-6, CH_2OBn), 36.8, 35.7 (q, NMe), 29.4 (t, C-3) ppm. IR ($CDCl_3$): $\tilde{\nu}$ = 3089, 3066, 3032, 2962, 2856, 1649, 1496, 1454, 1308, 1261, 1100, 1074 cm^{-1}. MS (ESI): *m/z* (%) = 539.29 (30) $[M + Na]^+$. $C_{31}H_{36}N_2O_5$ (516.63): calcd. C 72.07, H 5.42, N 7.02; found C 72.23, H 6.61, H 5.31.

(2*R*,3a*R*,4*S*,5*S*,6*R*)-4,5-Bis(benzyloxy)-6-[(benzyloxy)methyl]-*N*,*N*-dimethylhexahydropyrrolo[1,2-*b*]isoxazole-2-carboxamide (13b): Reaction performed on 0.28 mmol of nitrone **2**. M.p. 64–67 °C. $[\alpha]_D^{25}$ = –64.2 (*c* = 0.5, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.34–7.21 (m, 15 H, Ar), 4.66 (dd, *J* = 8.8, 5.9 Hz, 1 H, 2-H), 4.56–4.47 (m, 5 H, Bn), 4.34 (d, *J* = 11.7 Hz, 1 H, Bn), 4.15–3.90 (m, 2 H, 3a-H, 4-H), 3.91 (d, *J* = 9.2 Hz, 1 H, CH_2OBn), 3.88–3.86 (m, 1 H, 5-H), 3.77 (dd, *J* = 9.2, 4.8 Hz, 1 H, CH_2OBn), 3.43 (dt, *J* = 8.8, 4.4 Hz, 1 H, 6-H), 3.00 (s, 3 H, NMe), 2.90 (s, 3 H, NMe), 2.60 (dt, *J* = 12.2, 9.3 Hz, 1 H, 3-H_a), 2.47 (ddd, *J* = 12.2, 5.8, 2.9 Hz, 1 H, 3-H_b) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 168.0 (s, C=O), 138.3, 138.0, 137.6 (s, 3 C, C-Ar), 128.5–127.7 (d, 15 C, C-Ar), 81.1 (d, 2 C, C-4, C-5), 75.4 (d, C-2), 73.5, 72.5, 72.2 (t, C-Bn), 68.2 (t, CH_2OBn), 67.7 (d, C-6), 66.2 (d, C-3a), 37.0, 35.8 (q, NMe), 31.9 (t, C-3) ppm. IR ($CDCl_3$): $\tilde{\nu}$ = 3066, 3030, 3006, 2962, 1711, 1648, 1417, 1363, 1223, 1090, 1027 cm^{-1}. MS (ESI): *m/z* (%) = 539.42 (81) $[M + Na]^+$. $C_{31}H_{36}N_2O_5$ (516.63): calcd. C 72.07, H 5.42, N 7.02; found C 71.69, H 5.22, H 6.64.

Methyl (2*S*,3a*S*,4*S*,5*R*,6*R*)-4,5-Bis(benzyloxy)-6-[(benzyloxy)methyl]hexahydropyrrolo[1,2-*b*]isoxazole-2-carboxylate (15a): Reaction performed on 1.68 mmol of nitrone **3**. $[\alpha]_D^{25}$ = +46.9 (*c* = 0.45, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.38–7.25 (m, 15 H, Ar), 4.66 (d, *J* = 12.2 Hz, 2 H, Bn), 4.61 (d, *J* = 11.7 Hz, 2 H, Bn), 4.54 (d, *J* = 11.7 Hz, 2 H, Bn), 4.44 (dd, *J* = 8.8, 4.4 Hz, 1 H, 2-H), 4.00 (dd, *J* = 7.8, 5.9 Hz, 1 H, 5-H), 3.94 (dd, *J* = 11.7, 8.8 Hz, 1 H, CH_2OBn), 3.85 (t, *J* = 3.4 Hz, 1 H, 3a-H), 3.84–3.79 (m, 1 H, 6-H), 3.79 (dd, *J* = 11.7, 4.4 Hz, 1 H, CH_2OBn), 3.74 (s, 3 H, Me), 3.70 (dd, *J* = 5.9, 3.9 Hz, 1 H, 4-H), 2.85 (ddd, *J* = 12.7, 8.3, 4.4 Hz, 1 H, 3-H_a), 2.30 (ddd, *J* = 12.7, 8.8, 2.9 Hz, 1 H, 3-H_b) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 172.0 (s, C=O), 138.6, 137.9, 137.7 (s, Ar), 128.4–127.3 (d, 15 C, Ar), 81.8 (d, C-4), 79.1 (d, C-5), 74.8 (d, C-2), 73.1, 72.6, 72.2 (t, Bn), 70.5 (d, C-6), 69.8 (d, C-3a), 67.7 (t, CH_2OBn), 52.5 (q, Me), 37.7 (t, C-3) ppm. IR ($CDCl_3$): $\tilde{\nu}$ = 3031, 2954, 2870, 1733, 1496, 1454, 1363, 1215, 1103 cm^{-1}. MS (ESI): *m/z* (%) = 526.33 (100) $[M + Na]^+$. $C_{30}H_{33}NO_6$ (503.59): calcd. C 71.55, H 6.61, N 2.78; found C 71.38, H 6.77, N 2.50.

Methyl (2*R*,3a*R*,4*S*,5*R*,6*R*)-4,5-Bis(benzyloxy)-6-[(benzyloxy)methyl]hexahydropyrrolo[1,2-*b*]isoxazole-2-carboxylate (15b): Reaction performed on 1.68 mmol of nitrone **3**. $[\alpha]_D^{24}$ = –19.6 (*c* = 0.87, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.35–7.25 (m, 15 H, Ar), 4.65 (d, *J* = 11.7 Hz, 2 H, Bn), 4.70 (t, *J* = 7.8 Hz, 1 H, 2-H), 4.58 (d, *J* = 13.2 Hz, 2 H, Bn), 4.55 (d, *J* = 11.7 Hz, 2 H, Bn), 3.99 (dd, *J* = 5.7, 4.7 Hz, 1 H, 4-H), 3.92 (dd, *J* = 5.7, 4.7 Hz, 1 H, 5-H), 3.93–3.88 (m, 1 H, 3a-H), 3.75 (s, 3 H, Me), 3.64 (dd, *J* = 10.1, 4.3 Hz, 1 H, CH_2OBn), 3.59 (dd, *J* = 10.1, 5.1 Hz, 1 H, CH_2OBn), 3.46 (dt, *J* = 5.1, 4.7 Hz, 1 H, 6-H), 2.93 (ddd, *J* = 12.1, 7.4, 4.3 Hz, 1 H, 3-H_a), 2.30 (ddd, *J* = 12.1, 9.0, 8.2 Hz, 1 H, 3-H_b) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 170.7 (s, C=O), 138.2, 137.9, 137.8 (s, Ar), 128.4–127.5 (d, 15 C, Ar), 78.7 (d, C-5), 77.1 (d, C-4), 76.2 (d, C-2), 73.6, 73.1, 72.5 (t, Bn), 70.6 (d, C-6), 69.7 (t, CH_2OBn), 66.0 (d, C-3a), 52.4 (q, Me), 34.4 (t, C-3) ppm. IR ($CDCl_3$): $\tilde{\nu}$ = 3031, 2953, 2866 1742, 1453, 1362, 1212, 1130 cm^{-1}. MS (ESI): *m/z* (%) = 526.33 (100) $[M + Na]^+$. $C_{30}H_{33}NO_6$ (503.59): calcd. C 71.55, H 6.61, N 2.78; found C 71.29, H 6.84, N 3.63.

(2*S*,3a*S*,4*S*,5*R*,6*R*)-4,5-Bis(benzyloxy)-6-[(benzyloxy)methyl]-*N*,*N*-dimethylhexahydropyrrolo[1,2-*b*]isoxazole-2-carboxamide (16a): Reaction performed on 1.44 mmol of nitrone **3**. $[\alpha]_D^{25}$ = +110.9 (*c* = 0.94, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.34–

7.25 (m, 15 H, Ar), 4.63–4.52 (m, 6 H, Bn, 2-H), 4.46 (d, *J* = 11.7 Hz, 1 H, Bn), 3.97 (dd, *J* = 9.5, 5.8 Hz, 1 H, 5-H), 3.97–3.95 (m, 1 H, 3a-H), 3.87 (dd, *J* = 10.7, 7.3 Hz, 1 H, CH_2OBn), 3.80 (dd, *J* = 11.7, 3.9 Hz, 1 H, CH_2OBn), 3.80–3.75 (m, 1 H, 6-H), 3.68 (dd, *J* = 5.8, 2.9 Hz, 1 H, 4-H), 3.19 (ddd, *J* = 12.5, 8.3, 3.9 Hz, 1 H, 3-H_a), 3.02 (s, 3 H, Me), 2.91 (s, 3 H, Me), 1.97 (ddd, *J* = 12.5, 7.8, 4.4 Hz, 1 H, 3-H_b) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 169.3 (s, C=O), 138.3, 137.9, 137.7 (s, Ar), 128.4–127.4 (d, 15 C, Ar), 81.3 (d, C-4), 78.7 (d, C-5), 75.9 (d, C-2), 73.3, 72.6, 71.7 (t, Bn), 69.8 (d, C-3a), 69.2 (d, C-6), 67.8 (t, CH_2OBn), 37.3 (q, Me), 36.8 (t, C-3), 36.5 (q, Me) ppm. IR ($CDCl_3$): $\tilde{\nu}$ = 3450, 2994, 2934, 2869, 1642 1496, 1455, 1402, 1363, 1259, 1028 cm^{-1}. MS (ESI): *m/z* = 539.34 (100) [M + Na]$^+$, 517.36 (34) [M + 1]$^+$. $C_{31}H_{36}N_2O_5$ (516.63): calcd. C 72.07, H 5.42, N 7.02; found C 72.30, H 5.53, H 7.36.

(2*R*,3a*R*,4*S*,5*R*,6*R*)-4,5-Bis(benzyloxy)-6-[(benzyloxy)methyl]-*N*,*N*-dimethylhexahydropyrrolo[1,2-*b*]isoxazole-2-carboxamide (16b): Reaction performed on 1.44 mmol of nitrone **3**. $[\alpha]_D^{25}$ = –11.5 (*c* = 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.34–7.25 (m, 15 H, Ar), 4.76 (dd, *J* = 8.3, 6.8 Hz, 1 H, 2-H), 4.64–4.52 (m, 6 H, Bn), 3.99 (t, *J* = 4.9 Hz, 1 H, 4-H), 3.94 (dd, *J* = 9.3, 3.9 Hz, 1 H, 3a-H), 3.89 (dd, *J* = 6.8, 4.4 Hz, 1 H, 5-H), 3.67 (dd, *J* = 10.2, 4.4 Hz, 1 H, CH_2OBn), 3.62 (dd, *J* = 10.2, 4.9 Hz, 1 H, CH_2OBn), 3.44 (dt, *J* = 5.9, 4.9 Hz, 1 H, 6-H), 2.95 (s, 3 H, NCH_3), 2.91 (s, 3 H, NCH_3), 2.72–2.66 (m, 1 H, 3-H_a), 2.60–2.52 (m, 1 H, 3-H_b) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 167.9 (s, C=O), 138.2, 137.9, 137.8 (s, C-Ar), 128.4–127.5 (d, 15 C, C-Ar), 78.7 (d, C-5), 77.1 (d, C-4), 75.1 (d, C-2), 73.5, 73.4, 72.7 (t, Bn), 69.8 (t, CH_2OBn), 69.6 (d, C-6), 66.0 (d, C-3a), 37.0, 35.9 (q, NMe), 32.9 (t, C-3) ppm. IR ($CDCl_3$): $\tilde{\nu}$ = 3449, 3088, 3008, 2936, 2866, 1951, 1812, 1647, 1496, 1403, 1351, 1309, 1260 cm^{-1}. MS (ESI): *m/z* (%) = 539.42 (100) [M + Na]$^+$. $C_{31}H_{36}N_2O_5$ (516.63): calcd. C 72.07, H 5.42, N 7.02; found C 72.53, H 5.32, H 7.46.

(1*S*,2*R*,3*R*,7*R*,8a*S*)-1,2,7-Acetyloxy-3-(acetyloxymethyl)indolizidine (20a) and (1*S*,2*R*,3*R*,7*S*,8a*R*)-1,2,7-Acetyloxy-3-(acetyloxymethyl)indolizidine (20b): NEt_3 (213 μL, 1.53 mmol) was added to a stirred solution of **18a** and **18b** (500 mg, 1.02 mmol) in dry CH_2Cl_2 (3 mL) under a nitrogen atmosphere. MsCl (87 μL, 1.12 mmol) was then added dropwise at 0 °C. The solution was stirred at room temp. for 45 min. After this time, TLC analysis (EtOAc/petroleum ether, 3:1) showed the disappearance of the starting material. The solvent was removed under reduced pressure.

The residue was dissolved in MeOH (25 mL). The solution was acidified with conc. HCl (5 drops), and hydrogenated in the presence of Pd/C (10 %; 450 mg) for 72 h. The catalyst was then removed by filtration, and washed with MeOH. The filtrate was evaporated to give a mixture of indolizidines **21a** and **21b** (208 mg, 1.02 mmol).

This mixture was stirred in the presence of acetic anhydride (6 mL) and dry pyridine (12 mL) at room temp. for 18 h. The solvent was then removed, and the residue was purified by flash column chromatography (EtOAc/petroleum ether, 3:1, then 2:1) to give pure **20a** (86.5 mg, 0.23 mmol, 24 %) and **20b** (62.4 mg, 0.17 mmol, 17 %), both as yellow oils.

Data for **20a**: $[\alpha]_D^{24}$ = +1.5 (*c* = 0.50, $CHCl_3$). ^{1}H NMR (400 MHz, CD_3OD): δ = 5.16 (dd, *J* = 6.6, 3.5 Hz, 1 H, 2-H), 4.72–4.64 (m, 2 H, 1-H, 7-H), 4.38 (dd, *J* = 11.7, 4.3 Hz, 1 H, CH_2OAc), 4.06 (dd, *J* = 11.7, 3.9 Hz, 1 H, CH_2OAc), 3.19 (ddd, *J* = 13.3, 4.7, 2.7 Hz, 1 H, 5-H_a), 2.70 (dd, *J* = 8.3, 3.9 Hz, 1 H, 3-H), 2.56 (dt, *J* = 9.0, 2.3 Hz, 1 H, 8a-H), 2.24 (td, *J* = 11.7, 2.3 Hz, 1 H, 5-H_b), 2.17 (ddt, *J* = 11.3, 6.6, 2.3 Hz, 1 H, 8-H_a), 2.04–2.01 (4 s, 12 H, CH_3Ac), 1.96 (ddq, *J* = 12.0, 7.7, 2.3 Hz, 1 H, 6-H_a), 1.54 (qd, *J* = 12.0, 4.7 Hz, 1 H, 6-H_b), 1.31 (q, *J* = 11.3 Hz, 1 H, 8-H_b) ppm. ^{13}C NMR (50 MHz, CD_3OD): δ = 171.0, 170.8 (s, Ac), 170.2 (s, 2 C, Ac), 73.1, 72.0 (d, C-1, C-2), 71.0 (d, C-7), 67.3 (d, C-3), 62.8 (d, C-8a), 62.1 (t, CH_2OAc), 47.5 (t, C-5), 34.1 (t, C-8), 30.5 (t, C-6), 19.7, 19.4, 19.2, 19.0 (q, CH_3Ac) ppm. IR ($CHCl_3$): $\tilde{\nu}$ = 2956, 1741, 1370, 1250 cm^{-1}. MS (ESI): *m/z* (%) = 394.25 (100) [M + Na]$^+$, 372.50 (4) [M + 1]$^+$. $C_{17}H_{25}NO_8$ (371.38): calcd. C 54.98, H 6.79, N 3.77; found C 55.47, H 6.80, N 4.06.

Data for **20b**: $[\alpha]_D^{24}$ = –11.5 (*c* = 0.55, $CHCl_3$). ^{1}H NMR (400 MHz, CD_3OD): δ = 5.26–5.20 (m, 2 H, 1-H, 2-H), 4.73 (tt, *J* = 11.5, 4.3 Hz, 1 H, 7-H), 4.26 (dd, *J* = 11.7, 4.3 Hz, 1 H, CH_2OAc), 4.14 (dd, *J* = 11.7, 3.9 Hz, 1 H, CH_2OAc), 3.37–3.28 (m, 2 H, 3-H, 8a-H), 3.11 (ddd, *J* = 13.3, 4.3, 2.3 Hz, 1 H, 5-H_a), 2.75 (dt, *J* = 12.8, 2.7 Hz, 1 H, 5-H_b), 2.06–2.02 (4 s, 12 H, CH_3Ac), 1.84–1.77 (m, 2 H, 6-H_a, 8-H_a), 1.62 (qd, *J* = 12.3, 4.3 Hz, 1 H, 6-H_b), 1.54 (q, *J* = 12.1 Hz, 1 H, 8-H_b) ppm. ^{13}C NMR (50 MHz, CD_3OD): δ = 171.0–170.3 (s, 4 C, Ac), 73.5, 72.0 (d, C-1, C-2), 71.4 (d, C-7), 62.5 (t, CH_2OAc), 62.3 (d, C-3), 59.9 (d, C-8a), 43.3 (t, C-5), 29.3, 27.5 (t, C-8, C-6), 19.8, 19.4, 19.2, 19.0 (q, CH_3Ac) ppm. IR ($CHCl_3$): $\tilde{\nu}$ = 2953, 1741, 1377, 1367, 1248 cm^{-1}. MS (ESI): *m/z* (%) = 394.12 (100) [M + Na]$^+$, 372.53 (11) [M + 1]$^+$. $C_{17}H_{25}NO_8$ (371.38): calcd. C 54.98, H 6.79, N 3.77; found C 55.03, H 6.60, N 3.69.

(1*S*,2*R*,3*R*,7*R*,8a*S*)-1,2,7-Trihydroxy-3-(hydroxymethyl)indolizidine (21a): Compound **20a** (86.5 mg, 0.23 mmol) was dissolved in MeOH (20 mL), and Ambersep 900 OH (300 mg) was added. The mixture was stirred for 18 h, then it was filtered, and the filtrate was evaporated to give pure **21a** (47 mg, 0.23 mmol, quantitative yield) as a viscous yellow oil. $[\alpha]_D^{24}$ = –42 (*c* = 1, MeOH). ^{1}H NMR (400 MHz, D_2O): δ = 3.80 (dd, *J* = 11.7, 4.9 Hz, 1 H, H-2), 3.66–3.47 (m, 4 H, CH_2OH, H-7, H-1), 3.07 (ddd, *J* = 11.2, 4.4, 2.4 Hz, m, 1 H, 5-H_a), 2.24 (q, *J* = 5.4 Hz, 1 H, 3-H), 2.16–2.01 (m, 3 H, 8a-H, 5-H_b, 8a-H), 1.86–1.81 (m, 1 H, 6-H_a), 1.33 (qd, *J* = 12.7, 4.6 Hz, 1 H, 6-H_b), 1.12 (q, *J* = 11.7 Hz, 1 H, 8-H_b) ppm. ^{13}C NMR (50 MHz, D_2O): δ = 72.4 (d, C-1), 72.1 (d, C-3), 71.3 (d, C-2), 68.1 (d, C-7), 65.7 (d, C-8a), 60.9 (t, CH_2OH), 48.8 (t, C-5), 35.9 (t, C-8), 32.8 (t, C-6) ppm. MS (ESI): *m/z* (%) = 242.48 (75) [M + K]$^+$, 204.28 (100) [M + 1]$^+$. $C_9H_{17}NO_4$ (203.24): calcd. C 53.19, H 8.43, N 6.89; found C 52.88, H 8.76, N 6.61.

(1*S*,2*R*,3*R*,7*S*,8a*R*)-1,2,7-Trihydroxy-3-(hydroxymethyl)indolizidine (21b): Compound **20b** (46 mg, 0.12 mmol) was dissolved in MeOH (10 mL), and Ambersep 900 OH (150 mg) was added. The mixture was stirred for 18 h, then it was filtered, and the filtrate was evaporated to give pure **21b** (25 mg, 0.12 mmol, quantitative yield) as a viscous yellow oil. $[\alpha]_D^{24}$ = –20.6 (*c* = 1, MeOH). ^{1}H NMR (400 MHz, D_2O): δ = 3.98–3.93 (m, 2 H, 2-H, 1-H), 3.63–3.56 (m, 2 H, 7-H, CH_2OH), 3.52 (dd, *J* = 11.9, 5.6 Hz, 1 H, CH_2OH), 3.01–2.89 (m, 3 H, 3-H, 5-H_a, 8a-H), 2.58 (td, *J* = 13.1, 3.4 Hz, 1 H, 5-H_b), 1.73–1.67 (m, 1 H, 8-H_a), 1.63 (ddq, *J* = 12.6, 4.3, 2.4 Hz, 1 H, 6-H_a), 1.46–1.38 (m, 1 H, 6-H_b), 1.34 (q, *J* = 12.1 Hz, 1 H, 8-H_b) ppm. ^{13}C NMR (50 MHz, D_2O): δ = 72.9, 70.6 (d, C-1, C-2), 68.5 (d, C-7), 65.8, 61.6 (d, C-3, C-8a), 60.8 (t, CH_2OH), 43.7 (t, C-5), 31.2 (t, C-8), 29.5 (t, C-6) ppm. MS (ESI): *m/z* (%) = 226.12 (100) [M + Na]$^+$. $C_9H_{17}NO_4$ (203.24): calcd. C 53.19, H 8.43, N 6.89; found C 53.44, H 8.82, N 6.51.

(1*S*,2*S*,3*R*,7*R*,8a*S*)-1,2,7-Acetyloxy-3-(acetyloxymethyl)indolizidine (23a) and (1*S*,2*S*,3*R*,7*S*,8a*R*)-1,2,7-Acetyloxy-3-(acetyloxymethyl)indolizidine (23b): NEt_3 (35 μL, 0.26 mmol) was added to a stirred solution of **14a** and **14b** (83 mg, 0.17 mmol) in dry CH_2Cl_2 (2 mL) under a nitrogen atmosphere. MsCl (14 μL, 0.19 mmol) was then added dropwise at 0 °C. The solution was stirred at room temp. for 45 min. After this time, TLC analysis (EtOAc/petroleum ether, 3:1) showed the disappearance of the starting material. The solvent was removed in vacuo.

The residue was dissolved in MeOH (25 mL). The solution was acidified with conc. HCl (5 drops), and hydrogenated in the presence of

Pd/C (10 %; 74 mg) for 72 h. The catalyst was then removed by filtration, and washed with MeOH. The filtrate was evaporated to give a mixture of indolizidines **22a** and **22b** (35 mg, 0.17 mmol).

This mixture was stirred in the presence of acetic anhydride (2 mL) and dry pyridine (4 mL) at room temp. for 18 h. The solvent was then removed, and the residue was purified by flash column chromatography (EtOAc/petroleum ether, 3:1) to give pure **23a** (22 mg, 0.06 mmol, 35 %) and **23b** (37 mg, 0.1 mmol, 59 %), both as yellow oils.

Data for **23a**: $[\alpha]_D^{23} = -13.8$ ($c = 1$, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): $\delta = 5.29$ (dd, $J = 7.4$, 3.9 Hz, 1 H, 2-H), 4.85 (dd, $J = 8.3$, 3.9 Hz, 1 H, 1-H), 4.68 (tt, $J = 11.5$, 4.9 Hz, 1 H, 7-H), 4.17 (dd, $J = 11.5$, 6.6 Hz, 1 H, CH_2OAc), 4.11 (dd, $J = 11.5$, 5.0 Hz, 1 H, CH_2OAc), 3.16 (ddd, $J = 11.2$, 4.4, 2.9 Hz, 1 H, 5-H_a), 2.86 (q, $J = 6.5$ Hz, 1 H, 3-H), 2.25–2.20 (m, 1 H, 8a-H), 2.18–2.15 (m, 1 H, 8-H_a), 2.13–2.09 (m, 1 H, 5-H_b), 2.06–2.00 (4 s, 12 H, CH_3Ac), 1.95 (ddq, $J = 12.7$, 7.1, 2.4 Hz, 1 H, 6-H_a), 1.76 (qd, $J = 12.7$, 4.5 Hz, 1 H, 6-H_b), 1.52 (q, $J = 11.5$ Hz, 8-H_b) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 170.4$, 170.2, 170.0 (s, 4 C, Ac), 80.0 (d, C-1), 76.1 (d, C-2), 70.7 (d, C-7), 64.7 (d, C-8a), 63.2 (d, C-3), 61.4 (t, CH_2OAc), 48.7 (t, C-5), 34.3 (t, C-8), 30.3 (t, C-6), 21.2–20.7 (q, 4 C, CH_3Ac) ppm. IR ($CHCl_3$): $\tilde{\nu} = 2959$, 1739, 1370, 1247 cm^{-1}. MS (ESI): m/z (%) = 394.26 (100) $[M + Na]^+$, 372.35 (55) $[M + 1]^+$. $C_{17}H_{25}NO_8$ (371.38): calcd. C 54.98, H 6.79, N 3.77; found C 55.29, H 6.46, N 4.15.

Data for **23b**: $[\alpha]_D^{23} = -12.1$ ($c = 1$, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): $\delta = 5.38$ (dd, $J = 7.8$, 5.5 Hz, 1 H, 2-H), 5.25 (pseudo t, $J = 5.8$ Hz, 1 H, 1-H), 4.77–4.69 (m, 1 H, 7-H), 4.15 (dd, $J = 11.9$, 4.3 Hz, 1 H, CH_2OAc), 3.99 (dd, $J = 11.9$, 5.0 Hz, 1 H, CH_2OAc), 3.60 (ddd, $J = 8.5$, 4.3, 4.3 Hz, 1 H, 3-H), 3.33 (ddd, $J = 9.7$, 6.8, 2.9 Hz, 1 H, 8a-H), 3.07–3.03 (m, 1 H, 5-H_a), 2.74 (td, $J = 12.7$, 2.5 Hz, 1 H, 5-H_b), 2.07–2.00 (4 s, 12 H, CH_3Ac), 1.81 (dd, $J = 10.2$, 2.0 Hz, 1 H, 6-H_a), 1.75–1.72 (m, 1 H, 8-H_a), 1.56 (ddd, $J = 4.4$ Hz, 1 H, 6-H_b), 1.31–1.23 (m, 1 H, 8-H_b) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 170.4$–170.3 (s, 4 C, Ac), 77.4 (d, C-1), 76.4 (d, C-2), 71.3 (d, C-7), 60.7 (t, CH_2OAc), 58.1 (d, C-8a), 57.9 (d, C-3), 44.6 (t, C-5), 30.2 (t, C-8), 28.3 (t, C-6), 21.8–20.7 (q, 4 C, CH_3Ac) ppm. IR ($CHCl_3$): $\tilde{\nu} = 2952$, 1738, 1369, 1247 cm^{-1}. MS (ESI): m/z (%) = 394.27 (100) $[M + Na]^+$, 372.39 (24) $[M + 1]^+$. $C_{17}H_{25}NO_8$ (371.38): calcd. C 54.98, H 6.79, N 3.77; found C 54.53, H 6.45, N 3.43.

(1*S*,2*S*,3*R*,7*R*,8a*S*)-1,2,7-Trihydroxy-3-(hydroxymethyl)indolizidine (22a): Compound **23a** (22 mg, 0.06 mmol) was dissolved in MeOH (5 mL), and Ambersep 900 OH (100 mg) was added. The mixture was stirred for 18 h, then it was filtered, and the filtrate was evaporated to give pure **22a** (12 mg, 0.06 mmol, quantitative yield) as a viscous yellow oil. $[\alpha]_D^{24} = -31.4$ ($c = 1$, MeOH). ^1H NMR (400 MHz, D_2O): $\delta = 4.07$ (dd, $J = 7.6$, 4.2 Hz, 1 H, 2-H), 3.75 (dd, $J = 11.5$, 7.2 Hz, 1 H, CH_2OH), 3.69–3.60 (m, 2 H, 7-H, CH_2OH), 3.56 (dd, $J = 9.0$, 4.2 Hz, 1 H, 1-H), 3.08 (ddd, $J = 11.5$, 4.4, 2.7 Hz, 1 H, 5-H_a), 2.55 (td, $J = 7.3$, 4.9 Hz, 1 H, 3-H), 2.14 (ddt, $J = 11.7$, 4.7, 2.4 Hz, 1 H, 8-H_a), 2.05 (td, $J = 12.5$, 2.4 Hz, 1 H, 5-H_b), 1.98 (dt, $J = 11.2$, 2.5 Hz, 1 H, 8a-H), 1.89 (ddq, $J = 12.7$, 4.9, 2.4 Hz, 1 H, 6-H_a), 1.39 (qd, $J = 12.7$, 4.4 Hz, 1 H, 6-H_b), 1.19 (q, $J = 11.7$ Hz, 1 H, 8-H_b) ppm. ^{13}C NMR (50 MHz, D_2O): $\delta = 80.1$ (d, C-1), 76.2 (d, C-2), 67.4 (d, C-7), 66.0 (d, C-3), 65.0 (d, C-8a), 57.8 (t, CH_2OH), 48.2 (t, C-5), 35.0 (t, C-6), 31.8 (t, C-8) ppm. MS (ESI): m/z (%) = 226.07 (47) $[M + Na]^+$, 204.09 (100) $[M + 1]^+$. $C_9H_{17}NO_4$ (203.24): calcd. C 53.19, H 8.43, N 6.89; found C 52.77, H 8.82, N 6.56.

(1*S*,2*S*,3*R*,7*S*,8a*R*)-1,2,7-Trihydroxy-3-(hydroxymethyl)indolizidine (22b): Compound **23b** (29 mg, 0.079 mmol) was dissolved in MeOH (5 mL), and Ambersep 900 OH (100 mg) was added. The mixture was stirred for 18 h, then it was filtered, and the filtrate was evaporated to give pure **22b** (16 mg, 0.079 mmol, quantitative yield) as a viscous yellow oil. $[\alpha]_D^{24} = -27.2$ ($c = 0.8$, MeOH). ^1H NMR (400 MHz, CD_3OD): $\delta = 4.23$ (dd, $J = 8.0$, 5.3 Hz, 1 H, 2-H), 4.01 (t, $J = 5.9$ Hz, 1 H, 1-H), 3.74 (dd, $J = 11.5$, 4.9 Hz, 1 H, CH_2OH), 3.64–3.56 (m, 2 H, 7-H, CH_2OH), 3.23 (dt, $J = 8.0$, 4.6 Hz, 1 H, 3-H), 3.11–3.08 (m, 1 H, 5-H_a), 3.07–3.03 (m, 1 H, 8a-H), 2.76 (td, $J = 12.5$, 3.1 Hz, 1 H, 5-H_b), 1.84–1.78 (m, 1 H, 8-H_a), 1.66 (ddq, $J = 12.7$, 4.7, 2.4 Hz, 1 H, 6-H_a), 1.50 (tdd, $J = 12.5$, 11.0, 4.3 Hz, 1 H, 6-H_b), 1.16 (q, $J = 12$ Hz, 1 H, 8-H_b) ppm. ^{13}C NMR (50 MHz, CD_3OD): $\delta = 77.5$ (d, C-1), 76.5 (d, C-2), 67.6 (d, C-7), 60.1 (d, C-3), 59.4 (d, C-8a), 58.8 (t, CH_2OH), 48.9 (t, C-5), 31.5 (t, C-6), 29.3 (t, C-8) ppm. MS (ESI): m/z (%) = 226.07 (100) $[M + Na]^+$, 204.17 (19) $[M + 1]^+$. $C_9H_{17}NO_4$ (203.24): calcd. C 53.19, H 8.43, N 6.89; found C 53.56, H 8.75, N 6.54.

Supporting Information (see footnote on the first page of this article): Experimental details for the synthesis of nitrones **1** and **2**; copies of ^1H and ^{13}C NMR spectra of isolated cycloadducts and final products; details of nOe experiments on intermediates; details of glycosidase inhibition assays.

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[1] P. N. Confalone, E. M. Huie, *Org. React.* **1988**, 1–173.
[2] A. Brandi, F. Cardona, S. Cicchi, F. M. Cordero, A. Goti, *Chem. Eur. J.* **2009**, *15*, 7808–1821, and references cited herein.
[3] P. Compain, O. R. Martin (Eds.), *Iminosugars*, Wiley-VCH, Weinheim, Germany, **2007**.
[4] a) R. J. Nash, P. I. Thomas, R. D. Waigh, G. W. J. Fleet, M. R. Wormald, P. M. de Q. Lilley, D. J. Watkin, *Tetrahedron Lett.* **1994**, *35*, 7849–7852; b) A. A. Watson, G. W. J. Fleet, N. Asano, R. J. Molyneux, R. J. Nash, *Phytochemistry* **2001**, *56*, 265–295; c) T. Yamashita, K. Yasuda, H. Kizu, Y. Kameda, A. A. Watson, R. J. Nash, G. W. J. Fleet, N. Asano, *J. Nat. Prod.* **2002**, *65*, 1875–1881; d) N. Asano, K. Ikeda, M. Kasahara, Y. Arai, H. Kizu, *J. Nat. Prod.* **2004**, *67*, 846–850; e) A. Kato, N. Kato, I. Adachi, J. Hollinshead, G. W. J. Fleet, C. Kuriyama, K. Ikeda, N. Asano, R. J. Nash, *J. Nat. Prod.* **2007**, *70*, 993–997; f) J. P. Saludes, S. C. Lievens, T. F. Molinski, *J. Nat. Prod.* **2007**, *70*, 436–438.
[5] For reviews, see: a) H. Yoda, *Curr. Org. Chem.* **2002**, *6*, 223–243; b) T. Ayad, Y. Genisson, M. Baltas, *Curr. Org. Chem.* **2004**, *8*, 1211–1233; c) S. G. Pyne, M. Tang, *Curr. Org. Chem.* **2005**, *9*, 1393–1418; d) B. G. Davis, *Tetrahedron: Asymmetry* **2009**, *20*, 652–671; e) B. L. Stocker, E. D. Dangerfield, A. L. Win-Mason, G. W. Haslett, M. S. M. Timmer, *Eur. J. Org. Chem.* **2010**, 1615–1637; f) T. Ritthiwigrom, C. W. G. Au, S. G. Pyne, *Curr. Org. Synth.* **2012**, *9*, 583–612; g) R. Lahiri, A. A. Ansari, Y. D. Vankar, *Chem. Soc. Rev.* **2013**, *42*, 5102–5118.
[6] For relevant examples where total synthesis has served to correct previous structural assignments, see: a) T. J. Donohoe, R. E. Thomas, M. D. Cheeseman, C. L. Rigby, G. Bhalay, D. Linney, *Org. Lett.* **2008**, *10*, 3615–3618; b) T. Sengoku, Y. Satoh, M. Takahashi, H. Yoda, *Tetrahedron Lett.* **2009**, *50*, 4937–4940; c) C. W. G. Au, R. J. Nash, S. G. Pyne, *Chem. Com-*

mun. **2010**, *46*, 713; d) W. Zhang, K. Sato, A. Kato, Y.-M. Jia, X.-G. Hu, F. X. Wilson, R. van Well, G. Horne, G. W. J. Fleet, R. J. Nash, C.-Y. Yu, *Org. Lett.* **2011**, *13*, 4414–4417; e) T. Ritthiwigrom, S. G. Pyne, *Org. Lett.* **2008**, *10*, 2769–2771; f) S. E. Denmark, B. Herbert, *J. Org. Chem.* **2000**, *65*, 2887–2896; g) M. R. Wormald, R. J. Nash, P. Hrnicar, J. D. White, R. J. Molyneux, G. W. J. Fleet, *Tetrahedron: Asymmetry* **1998**, *9*, 2549–2558.

[7] a) F. Cardona, C. Parmeggiani, E. Faggi, C. Bonaccini, P. Gratteri, L. Sim, T. M. Gloster, S. Roberts, G. J. Davies, D. R. Rose, A. Goti, *Chem. Eur. J.* **2009**, *15*, 1627–1636; b) C. Parmeggiani, D. Martella, F. Cardona, A. Goti, *J. Nat. Prod.* **2009**, *72*, 2058–2060; c) C. Bonaccini, M. Chioccioli, C. Parmeggiani, F. Cardona, D. Lo Re, G. Soldaini, P. Vogel, C. Bello, A. Goti, P. Gratteri, *Eur. J. Org. Chem.* **2010**, 5574–5585; d) G. D'Adamio, A. Goti, C. Parmeggiani, E. Moreno-Clavijo, I. Robina, F. Cardona, *Eur. J. Org. Chem.* **2011**, 7155–7162; e) F. Cardona, G. Moreno, F. Guarna, P. Vogel, C. Schuetz, P. Merino, A. Goti, *J. Org. Chem.* **2005**, *70*, 6552–6555; f) I. Delso, T. Tejero, A. Goti, P. Merino, *Tetrahedron* **2010**, *66*, 1220–1227; g) D. Martella, F. Cardona, C. Parmeggiani, F. Franco, J. A. Tamayo, I. Robina, E. Moreno-Clavijo, A. J. Moreno-Vargas, A. Goti, *Eur. J. Org. Chem.* **2013**, 4047–4056; h) C. Parmeggiani, F. Cardona, L. Giusti, H.-U. Reissig, A. Goti, *Chem. Eur. J.* **2013**, *19*, 10595–10604.

[8] a) F. Cardona, E. Faggi, M. Liguori, M. Cacciarini, A. Goti, *Tetrahedron Lett.* **2003**, *44*, 2315–2318; b) E. L. Tsou, Y. T. Liang, W. C. Cheng, *Tetrahedron* **2009**, *65*, 93–100; c) W.-B. Wang, M.-H. Huang, Y.-X. Li, P.-X. Rui, X.-G. Hu, W. Zhang, J.-K. Su, Z.-L. Zhang, J.-S. Zhu, W.-H. Xu, X.-Q. Xie, Y.-M. Jia, C.-Y. Yu, *Synlett* **2010**, *3*, 488–492; d) J. Revuelta, S. Cicchi, A. Goti, A. Brandi, *Synthesis* **2007**, 485–504.

[9] L. Fisera, *Top. Heterocycl. Chem.* **2007**, *7*, 287–323.

[10] a) A. T. Carmona, R. H. Wightman, I. Robina, P. Vogel, *Helv. Chim. Acta* **2003**, *86*, 3066–3073; b) S. Desvergnes, S. Py, Y. Vallée, *J. Org. Chem.* **2005**, *70*, 1459–1462.

[11] Some data on the cyloadditions reported in Table 1 were already disclosed in preliminary communications (see ref.[7a,8a]). For this manuscript, reactions were repeated, yields were improved, and all adducts were fully characterized.

[12] G. Podolan, L. Kleščíková, L. Fišera, J. Kožíšek, M. Fronc, *Synlett* **2011**, 1668–1672.

[13] A. Goti, S. Cicchi, M. Cacciarini, F. Cardona, V. Fedi, A. Brandi, *Eur. J. Org. Chem.* **2000**, 3633–3645.

[14] a) D. Socha, M. Jurczak, J. Frelek, A. Klimek, J. Rabiczko, Z. Urbańczyk-Lipkowska, K. Suwińska, M. Chmielewski, F. Cardona, A. Goti, A. Brandi, *Tetrahedron: Asymmetry* **2001**, *12*, 3163–3172; b) S. Stecko, K. Paśniczek, M. Jurczak, Z. Urbańczyk-Lipkowska, M. Chmielewski, *Tetrahedron: Asymmetry* **2006**, *17*, 68–78; c) S. Stecko, K. Paśniczek, M. Jurczak, Z. Urbańczyk-Lipkowska, M. Chmielewski, *Tetrahedron: Asymmetry* **2007**, *18*, 1085–1093.

[15] a) R. K. Khangarot, K. P. Kaliappan, *Eur. J. Org. Chem.* **2011**, 6117–6127; b) M. Maciejko, Stecko, O. Staszewska-Krajewska, M. Jurczak, B. Furman, M. Chmielewski, *Synthesis* **2012**, *44*, 2825–2839.

[16] a) T. Ishikawa, Y. Tajima, M. Fukui, S. Saito, *Angew. Chem. Int. Ed.* **1996**, *35*, 1863–1864; *Angew. Chem.* **1996**, *108*, 1990–1991; b) A. Toyao, O. Tamura, H. Takagi, H. Ishibashi, *Synlett* **2002**, 35–38.

[17] Sk. A. Ali, J. H. Khan, M. I. M. Wazeer, *Tetrahedron* **1988**, *44*, 5911–5920.

[18] P. Gzikis, N. G. Argyropoulos, E. Coutouli-Argyropoulou, *Tetrahedron* **2013**, *69*, 8921–8928.

[19] E. Coutouli-Argyropoulou, C. Xatzis, N. G. Argyropoulos, *Nucleosides Nucleotides Nucleic Acids* **2008**, *27*, 84–100.

[20] a) N. G. Argyropoulos, E. Coutouli-Argyropoulou, P. Gkizis, *ARKIVOC* **2008**, *16*, 223–234; b) J. A. Tamayo, F. Franco, D. Lo Re, F. Sanchez-Cantalejo, *J. Org. Chem.* **2009**, *74*, 5679–5682; c) H.-W. Shih, K.-T. Chen, S.-K. Chen, C.-Y. Huang, T.-J. R. Cheng, C. Ma, C.-H. Wong, W.-C. Cheng, *Org. Biomol. Chem.* **2010**, *8*, 2586–2593.

[21] F. Cardona, G. Isoldi, A. Goti, F. Sansone, A. Casnati, *J. Org. Chem.* **2012**, *77*, 6980–6988.

[22] C. W. Holzapfel, R. Crous, *Heterocycles* **1998**, *48*, 1337–1342.

[23] a) R. Saul, J. P. Chambers, R. J. Molyneux, A. D. Elbein, *Arch. Biochem. Biophys.* **1983**, *221*, 593–597; b) A. Brandi, S. Cicchi, F. M. Cordero, R. Frignoli, A. Goti, S. Picasso, P. Vogel, *J. Org. Chem.* **1995**, *60*, 6806–6812.

[24] C. Parmeggiani, S. Catarzi, C. Matassini, G. D'Adamio, A. Morrone, A. Goti, P. Paoli, F. Cardona, *ChemBioChem* **2015**, *16*, 2054–2064.

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