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A convenient approach toward the synthesis of enantiopure isomers of DMDP and ADMDP

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ABSTRACT

A practical method for the synthesis of five-membered iminocyclitols, pyrrolidine alkaloids bearing multiple hydroxyl substituents, has been developed. All of the eight key intermediates, enantiopure tri-*O*-benzyl cyclic nitrones, are prepared from four cheap, readily available D-aldopentoses. The nucleophilic addition of cyclic nitrones with vinyl magnesium chloride and TMSCN shows high 2,3-trans stereoselectivity. To construct the 2,3-cis configurations, inversion of the C-2 nitrile group is achieved via an elimination–reduction sequence. Using this approach, five isomers of DMDP and six isomers of ADMDP are prepared efficiently. In the biological evaluation, iminocyclitol **27** is a new and potent inhibitor against β-hexosaminidase with an IC_{50} value of 0.2 μM.

1. Introduction

Five-membered iminocyclitols, also called pyrrolidine alkaloids have received a lot of attention recently because of their versatile biological applications and promising therapeutic potentials.[1] For example, 2,5-dihydroxymethyl-3,4-di-hydroxypyrrolidine (DMDP, **4**) is a known glycosidase inhibitor (Fig. 1), which was isolated from plants[4] and has been synthesized.[2,3] In contrast, 1-aminodeoxy-DMDP (ADMDP, **8**) is an unnatural product and can only be obtained by synthesis.[5] A recent research showed that the N-1 modified derivatives of ADMDP significantly enhanced selectivity and potency toward the inhibition of β-glucosidases.[6] Our previous report indicated that ADMDP and its derivatives were selective and potent small molecules for antivirals and osteoarthritis.[7] Interestingly, several synthetic L-iminocyclitols such as L-DMDP (**5**) were found to exhibit interesting or unexpected biological activity.[8]

Although all isomers of DMDP and some isomers of ADMDP have been prepared via different synthetic routes,[2,3,5,8–11] to the best of our knowledge, however, a general approach to cover the preparation of all possible configuration isomers of **4** and **8**, such as L-ADMDP (**9**), has not been completely reported.[3]

The stereogenic centers at C-3, C-4, and C-5 can be obtained by appropriate choice of chiral pool starting material, typically an enantiopure sugar. However, how to efficiently stereocontrol at the 'anomeric center' (C-2) is a synthetic challenge in glycochemistry and glycomimic chemistry (Fig. 2). Fortunately, cyclic nitrones have been shown to perform highly stereoselective 2,3-trans reactions with various nucleophiles and this chemistry may fit our criteria to prepare a diverse range of iminocyclitols.[12,13] Indeed, in our hands the treatment of tri-*O*-benzyl cyclic nitrone **1a** with allylMgBr or vinylMgCl gave single addition product **2a** or **2b** with the trans configuration as the only observable diastereomers in 93 and 90% yields, respectively (entries 1 and 2, Table 1). The reaction with TMSCN gave a mixture of 2,3-trans and 2,3-cis isomers **2c** and **3c** (19:1) in 93% total yield (entry 3, Table 1). Thus, isomers of DMDP and ADMDP with a 2,3-trans configuration such as iminocyclitols **4**–**11** and **14** (Fig. 1) can be potentially prepared using this chemistry. In contrast, new synthetic methods toward isomers,

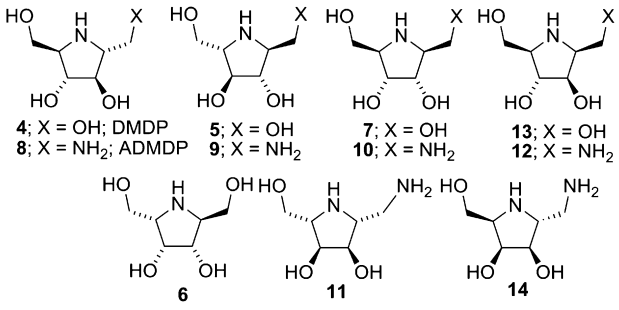

Figure 1. Examples of the prepared iminocyclitols.

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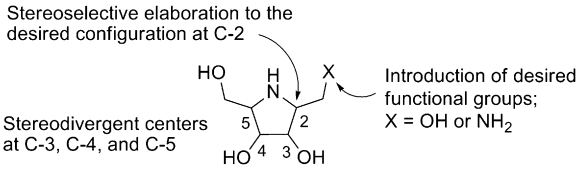
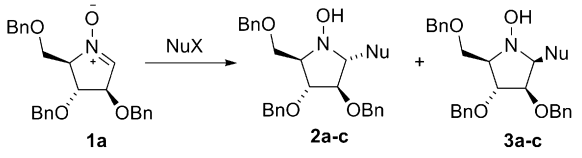


Figure 2. General synthetic criteria for iminocyclitols.

such as **12** and **13**, with a 2,3-cis configuration derived from cyclic nitrones remain to be explored.[14]

Herein, we report a general method for the synthesis of iminocyclitols using chiral cyclic nitrones as key intermediates. This method is exemplified in the synthesis of the 11 stereodiversified polyhydroxylated pyrrolidine alkaloids **4**–**14**. From a structural point of view, several interesting relationships should be noted: (i) iminocyclitols **4**, **8**, and **10**[10] are enantiomers with **5**,[8] **9**, and **11**,[10] respectively; (ii) **12** and **13**[9] are the C-2 epimers of **8** and **4**, respectively; (iii) **6**[3] is the C-4 epimer of **5** and also the C-5 epimer of **7**; (iv) **14** is the C-4 epimer of **8**. Notably, iminocyclitols **9** and **12** are new cores. Besides, the inhibitory activity of selective compounds and their acetylated derivatives against various glycosidases was also studied.

Table 1
Reactions of tri-*O*-benzyl cyclic nitrone **1a** with various nucleophiles



Entry	NuX	Product (trans/cis ratio)[a]	Total yield[b] (%)
1	AllylMgBr	**2a** only	83
2	VinylMgCl	**2b** only	90
3	TMSCN	**2c**/**3c**=19:1	93

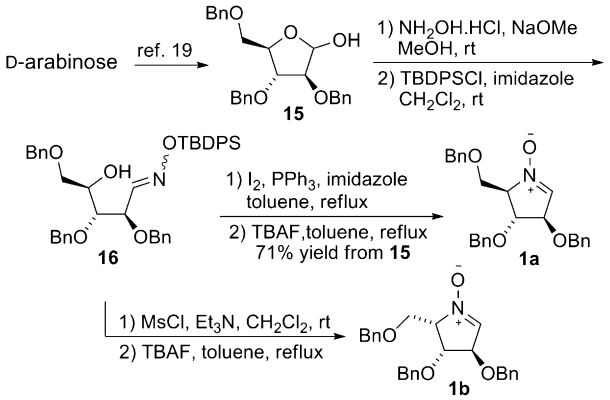
[a] The ratio determined by ^{1}H NMR.
[b] Isolated by column chromatography.

2. Results and discussion

2.1. Chemistry

Following the known method[13,15] with slight modifications to the work-up procedures,[16] nitrone **1a** was obtained in an overall yield of 71%. Likewise, nitrone **1b**, the C-5 epimer of **1a**, was prepared in 54% overall yield from **15** when mesylation was carried out instead of iodination as shown in Scheme 1.[17] Using similar procedures, nitrones **1c**–**h** were prepared from other D-aldopentoses (Fig. 3).[13,15,17,18] The preparation of nitrones **1e**–**g** has not been reported yet. Significantly, due to symmetry operations and nucleophilic substitution (once or twice) at C-5, all eight of the five-membered tri-*O*-benzyl cyclic nitrones were accessible from four cheaper D-aldopentoses instead of more expensive L-sugars.

With nitrones **1a**–**h** in hand, transformations to the target molecules (Fig. 1) could be investigated. For example, the vinyl hydroxylamine **2b** was reduced in the presence of Zn/AcOH[20] to cleave the N–O bond followed by *N*-Boc protection, ozonolysis, and reduction with $NaBH_4$ to furnish alcohol **17** (Scheme 2). After debenzylation and the removal of Boc group, **4** (DMDP) was obtained in an overall yield of 52% from **1a** (Scheme 2). Our synthetic route to **4** is one of the most efficient methods in comparison with the reported protocols.[4] Likewise, iminocyclitols **5** (50%), **6** (46%), **7** (43%), and **13** (43%) were prepared from the corresponding nitrones **1h**, **1d**, **1f**, and **1b** in good yields.



Scheme 1. Preparation of chiral tri-*O*-benzyl cyclic nitrones **1a** and **1b**.

On the other hand, preparation of amine **8** by multi-step transformations from **4** resulted in a low yield. Attempted hydrogenation of **2c** under high pressure (ca. 100 atm) also failed,[14] even in prolonged reaction time (96 h) with freshly prepared catalyst. To overcome this problem, mild reduction conditions using Raney Ni/H_2 (1 atm, rt) in the presence of Boc_2O were developed and both hydroxylamine and nitrile moieties in **2c** were reduced easily to bis-*N*-Boc protected diamine **18** in 4 h. This flexible four-step synthetic route was found to be applicable not only to **8** (75% from **1a**) but also for the direct preparation of **9**, **10**, **11**, and **14** from the corresponding nitrones **1h**, **1c**, **1f**, and **1e** in good overall yields (68–76%).

In this study, we also explored a convenient synthetic route to iminocyclitols with 2,3-cis configuration such as **12** and **13**. The key transformation was the inversion of the C-2 nitrile group via a mild elimination and reduction sequence, differing from the oxidation–reduction sequence described by Goti and co-workers.[14] Mesylation of the hydroxyl group in the **2c**/**3c** mixture followed by elimination in the basic conditions produced iminonitrile **19** in a regioselective manner (Scheme 3). Stereoselective reduction of the sp^2-hybridized carbon at C-2 in **19** was realized to give predominantly the 2,3-cis isomer **20a** due to the steric effect of the benzoxyl group at C-3. In optimal conditions, reduction of **19** with $NaBH_4$ in MeOH at 0 °C for 5 h gave **20a** and the 2,3-trans isomer **20b** in high stereoselectivity (dr >9:1) and good yield (93–95%). After simple separation by column chromatography, an 85% yield of

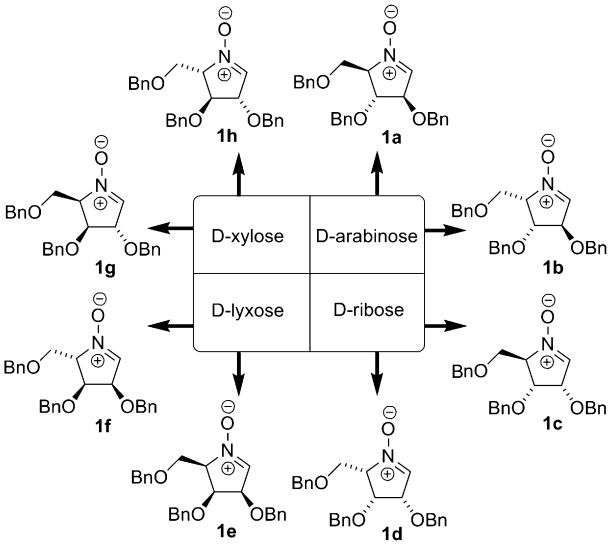
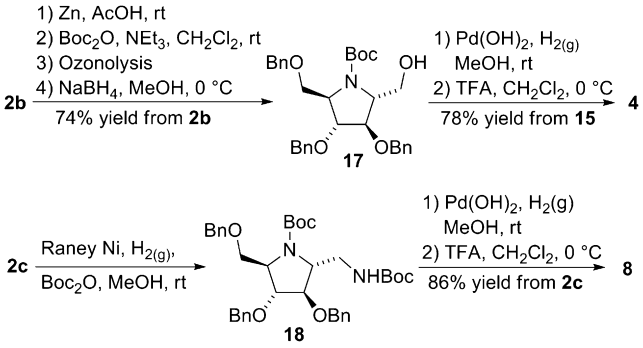
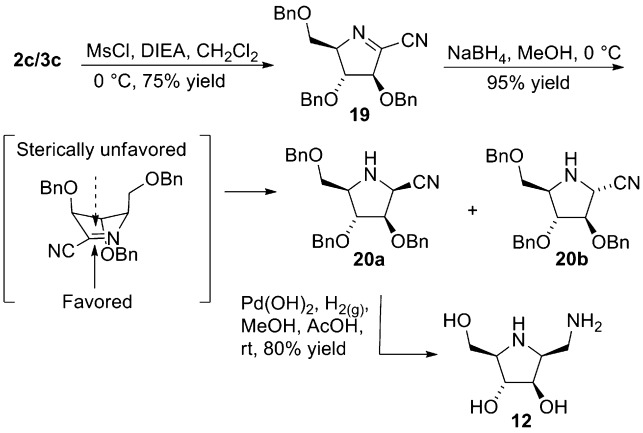


Figure 3. All structures of chiral tri-*O*-benzyl cyclic nitrones **1a**–**h**.

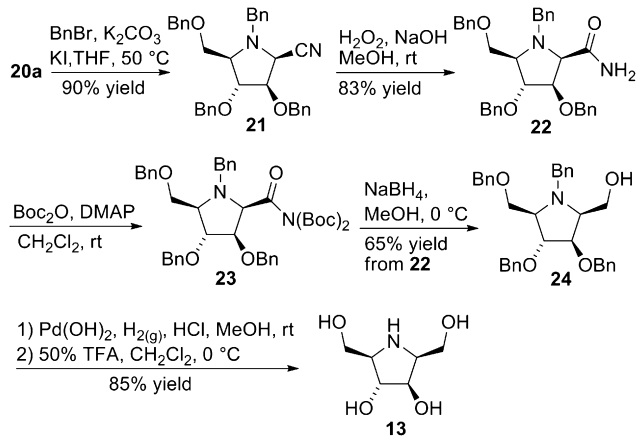


Scheme 2. Synthesis of iminocyclitol **4** and **8** with a 2,3-trans configuration.



Scheme 3. Synthesis of iminocyclitol **12** with a 2,3-cis configuration.

pure **20a** was obtained on a multigram scale. The reaction at a lower temperature (−20 °C) was sluggish, whereas the reaction at elevated temperatures (25 or 50 °C) gave a mixture of **20a**,**b** in inferior diastereoselectivity. As shown in Scheme 3, deprotection of **20a** with concomitant reduction of the nitrile group gave iminocyclitol **12** in 80% yield (51% overall yield for four steps starting from **1a**). Comparing ^{1}H NMR spectra, the C-2 proton in the 2,3-cis compound **12** showed at ca. 3.6 ppm, but in the 2,3-trans isomer **8** at ca. 3.2 ppm. In addition, the two protons at C-2 and C-3 in **12** showed a strong NOE interaction confirming their cis relationship. For the preparation of **13** (Scheme 4), compound **20a** was subjected to N-benzylation, giving the nitrile **21** (90% yield), which was efficiently transformed into the corresponding amide **22** (83% yield) by using 30% hydrogen peroxide under basic conditions (NaOH/MeOH) modified from Katrizky's procedure.[21] Attempted direct hydration of amino-nitrile **20a** without prior N-benzylation gave complicated mixture. Amide **22** was converted to acylimidodicarbonate **23**, which underwent a reductive cleavage with $NaBH_4$ to provide alcohol **24** (65%).[22] After deprotection, iminocyclitol **13** was successfully obtained in 25% overall yield from nitrone **1a**. Compared with the preceding approach shown in Scheme 2, iminocyclitol **13** can also be prepared from nitrone **1b** without inversion of the hydroxymethyl moiety because of the intrinsic 4,5-cis configuration of **1b**. Notably, though the overall yield of **13** derived from **1a** (25%) was poorer than that from **1b** (43%), this general preparation protocol could be useful for the inversion of the hydroxymethyl group at C-2 for other stereoisomer of DMDP.



Scheme 4. Synthesis of iminocyclitol **13** with a 2,3-cis configuration.

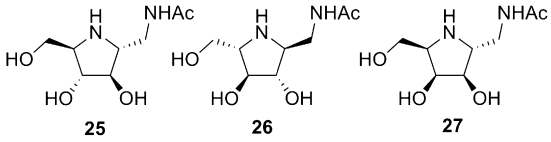


Figure 4. Structures of acetylated iminocyclitols **25**–**27**.

This method allowed us to flexibly construct the 2,3-cis or 2,3-trans configuration with various intrinsic stereogenic centers at C-3, C-4, and C-5 on the pyrrolidine ring. Thus, we are able to efficiently prepare scaffolds of five-membered iminocyclitols and easily modify them for biological study. According to literature reported,[7] ADMDP (**8**) itself is a moderate hexosaminidase inhibitor (IC_{50}=62 μM) but significantly **25** (Fig. 4) bearing an acetamido group at the C-1 position becomes a potent hexosaminidase inhibitor with an IC_{50} value of 0.16 μM. This prompted us to convert iminocyclitols **8**, **9**, and **14** to the corresponding **25**, **26**, and **27** via the selective acetylation.[7] From the structural point of view, **26** and **27** are new compounds; **26** is the L-iminocyclitol and the enantiomer of **25**, and **27** is the C-4 epimer of **25** (Fig. 4).

2.2. Biological evaluation

Glycosidases particularly α-glucosidase (*bacillus*), β-glucosidase (*almonds*), α-mannosidase (*jack beans*), and β-hexosaminidase (*jack beans*, *bovine kidney*, and *human placenta*) were used for test.[7] The IC_{50} values of the selective compounds and their monoacetylated forms were shown in Table 2. ADMDP isomers **9**–**12**, and **14** with a free aminomethyl moiety did not inhibit α- or β-glucosidase, α-mannosidase, β-hexosaminidases expect **14** with a moderate inhibition activity (IC_{50}=32 μM) against α-mannosidase. As expected, **25** was a potent inhibitor of β-hexosaminidases with IC_{50} values ranging from 0.1 to 3 μM.[7] In contrast,

Table 2
Inhibition activities of iminocyclitols against glycosidases

Enzyme	IC_{50} (μM)											
	5	**6**	**7**	**9**	**10**	**11**	**12**	**13**	**14**	**25**	**26**	**27**
α-Glucosidase[a]	32	64	16	—[f]	—	—	—	2.6	—	—	—	—
β-Glucosidase[b]	131	76	93	—	—	—	—	21	—	—	—	—
α-Mannosidase[c]	88	94	52	—	—	—	—	98	32	—	—	144
β-Hexosaminidase[c]	—	—	—	—	—	—	—	220	—	0.1	55	0.2
β-Hexosaminidase[d]	ND[g]	ND	ND	—	—	—	—	ND	—	3	55	3
β-Hexosaminidase[e]	ND	ND	ND	—	—	—	—	ND	—	0.3	27	0.6

[a] From *Bacillus stearothermophilus lyoph.*
[b] From *almonds.*
[c] From *jack beans.*
[d] From *bovine Kidney.*
[e] From *human placenta.*
[f] IC_{50} more than 200 μM.
[g] Not determined.

L-iminocyclitol **26**, the enantiomer of **25**, showed a moderate inhibition activity (IC_{50}=27–55 μM) against β-hexosaminidases. Excitingly, **27** exhibited an inhibitory potency against β-hexosaminidases with IC_{50} values ranging from 0.2 to 3 μM. This observation pointed out that the configuration of the hydroxyl group at the C-4 position does not significantly affect the inhibition activity against β-hexosaminidases. Presumably, chemical modification, such as conjugation with various fragments, at the C-4 position in iminocyclitol **25** or **26** to explore the extra binding pockets is possible to improve the binding affinity or selectivity.

3. Conclusion

We have successfully developed a general and straightforward method to efficiently prepare scaffolds of iminocyclitols and easily modify for biological study. It is the first time to prepare all eight five-membered chiral tri-*O*-benzyl cyclic nitrones **1a**–**h** as key intermediates from D-aldopentoses instead of the more expensive L-sugars. The utility of this methodology was exemplified in the synthesis of 11 natural and unnatural polyhydroxylated pyrrolidines under mild reaction conditions in high regio- and diastereoselectivities. This flexible route allowed us to shorten the tedious synthetic process for several iminocyclitols such as L-DMDP and also diversify the preparation of isomers of DMDP (**4**) and ADMDP (**8**). This is potentially useful in the preparation of their stereoisomers and congeners for systemic biological applications. In the biological study, the new compound **27** is a lead inhibitor (IC_{50}=0.2 μM) against *jack bean* β-hexosaminidase. We are currently using this approach to synthesize and identify potent glycosidase or glycotransferase inhibitors.

4. Experimental section

4.1. General

Ozonolysis was performed by ozone generator (Fischer Tech. OZ 502/10). Mass spectra were measured with a Bruker BioTOF III (ESI-MS). Optical rotations were measured with a Perkin–Elmer Model 341 polarimeter. NMR spectra (^1H at 400 or 600 MHz, ^{13}C at 100 or 150 MHz) were recorded in $CDCl_3$, CD_3OD, or D_2O solvents. Flash column chromatography was carried out using Merck kieselgel Si60 (40–63 μm). All reagents and solvents were purchased from commercial suppliers, and used without further purification. Palladium hydroxide is 20 wt% Pd on carbon, CC refers to column chromatography (silica gel). Concentration refers to rotary evaporation.

4.2. General procedure for the preparation of chiral cyclic nitrones

*4.2.1. (2R,3R,4R)-3,4-Bis(benzyloxy)-2-(benzyloxymethyl)-3,4-dihydro-2H-pyrrole-1-oxide (**1a**)*

2,3,5-Tri-*O*-benzyl-β-D-arabinofuranose (**15**) (5 g, 12 mmol) was reacted with hydroxylamine hydrochloride (6.6 g, 94 mmol) in the presence of sodium methoxide (2.56 g, 47 mmol) in methanol. The mixture was stirred for 5 h and then the solvent was evaporated. The residue was extracted with CH_2Cl_2, washed with H_2O, dried ($MgSO_4$), and concentrated. A mixture of the oxime residue, *tert*-butylchlorodiphenylsilane (TBDPSCl, 4.25 g, 15 mmol), and imidazole (1.21 g, 18 mmol) in CH_2Cl_2 (15 mL) was stirred at rt for 2 h and quenched with water (20 mL). The organic layer was washed with brine, dried ($MgSO_4$), and concentrated to give **16**. A mixture of **16**, triphenylphosphine (9.35 g, 36 mmol), imidazole (2.42 g, 36 mmol), and iodine (6.03 g, 24 mmol) in toluene (30 mL) was refluxed for 1 h, the reaction mixture was filtered, and washed with CH_2Cl_2. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated. The crude intermediate was dissolved in toluene (10 mL) and TBAF (14.2 mL, 1 M in THF) was added. After the reaction was refluxed at 100 °C for 30 min, the mixture was concentrated and purified by CC (33% EA in hexanes, silica gel) to give cyclic nitrone **1a** (3.55 g, 8.5 mmol, 71% from **15**) as a white solid. $[\alpha]_D^{20}$ −40.5 (*c* 0.7, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 3.77 (dd, 1H, *J*=2.9, 10.2 Hz), 4.02 (br, 1H), 4.06 (dd, 1H, *J*=5.0, 10.2 Hz), 4.39 (t, 1H, *J*=2.4, 3.2 Hz), 4.50–4.53 (m, 5H), 4.61 (d, 1H, *J*=12 Hz), 4.66 (br, 1H), 6.90 (s, 1H), 7.27–7.36 (m, 15H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.3, 136.9, 136.7, 132.4, 128.1, 128.0, 127.9, 127.6, 127.5, 127.4, 127.2, 82.3, 79.8, 77.0, 72.9, 71.3, 71.0, 65.6. HRMS calcd for $[C_{26}H_{27}NO_4+H]^+$ 418.2013, found 418.2049.

*4.2.2. (2S,3R,4R)-3,4-Bis(benzyloxy)-2-(benzyloxymethyl)-3,4-dihydro-2H-pyrrole-1-oxide (**1b**)*

Following the same procedure as described for the preparation of **1a** but mesylation was carried out instead of iodination, cyclic nitrone **1b** was obtained in 54% yield from **14** as a colorless oil. $[\alpha]_D^{20}$ −70.5 (*c* 0.5, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 3.82 (dd, 1H, *J*=2.0, 10.1 Hz), 3.98 (dd, 1H, *J*=4.4, 10.1 Hz), 4.15 (br, 1H), 4.36 (dd, 1H, *J*=4.4, 7.6 Hz), 4.50–4.66 (m, 6H), 4.75 (dd, 1H, *J*=1.6, 2.5 Hz), 6.82 (s, 1H), 7.26–7.36 (m, 15H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.8, 137.2, 137.1, 133.3, 128.5, 128.4, 128.2, 128.0, 127.9, 127.8, 127.7, 127.5, 127.4, 83.0, 80.4, 74.0, 73.4, 73.0, 72.3, 64.3. HRMS calcd for $[C_{26}H_{27}NO_4+H]^+$ 418.2013, found 418.2020.

*4.2.3. (2R,3R,4S)-3,4-Bis(benzyloxy)-2-(benzyloxymethyl)-3,4-dihydro-2H-pyrrole-1-oxide (**1c**)*

The reaction was carried out as described above for **1a** starting from 2,3,5-tri-*O*-benzyl-β-D-ribofuranose, derived from D-ribose, to give **1c** (56%) as a colorless oil. $[\alpha]_D^{20}$ +118 (*c* 1.8, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 3.58 (dd, 1H, *J*=2.5, 10.4 Hz), 4.08 (m, 1H), 4.12 (dd, 1H, *J*=2.5, 10.4 Hz), 4.38 (d, 1H, *J*=11.9 Hz), 4.43 (dd, 1H, *J*=4.7, 5.9 Hz), 4.52 (dd, 2H, *J*=7.4, 11.9 Hz), 4.56–4.67 (m, 4H), 6.92 (s, 1H), 7.19 (t, 2H, *J*=1.4 Hz), 7.26–7.32 (m, 13H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.6, 137.4, 137.2, 133.8, 128.7, 128.7, 128.6, 128.34, 128.31, 128.26, 128.0, 127.8, 76.4, 75.4, 74.46, 73.49, 72.5, 72.2, 64.8. HRMS calcd for $[C_{26}H_{27}NO_4+H]^+$ 418.2013, found 418.2002.

*4.2.4. (2S,3R,4S)-3,4-Bis(benzyloxy)-2-(benzyloxymethyl)-3,4-dihydro-2H-pyrrole-1-oxide (**1d**)*

The reaction was carried out as described above for **1b** starting from 2,3,5-tri-*O*-benzyl-β-D-ribofuranose, derived from D-ribose, to give **1d** (34%) as a white solid. $[\alpha]_D^{20}$ +59.6 (*c* 1.8, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 4.07 (dd, 1H, *J*=4.0, 9.8 Hz), 4.12–4.17 (m, 2H), 4.37 (t, 1H, *J*=5.8, 5.9 Hz), 4.54–4.60 (m, 4H), 4.67 (s, 2H), 4.70 (d, 1H, *J*=11.8 Hz), 6.87 (t, 1H, *J*=1.7, 1.8 Hz), 7.24–7.32 (m, 15H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.8, 137.5, 137.3, 133.1, 128.7, 128.6, 128.5, 128.2, 128.1, 127.99, 127.97, 127.8, 76.9, 74.5, 74.1, 73.6, 73.2, 72.6, 66.7. HRMS calcd for $[C_{26}H_{27}NO_4+H]^+$ 418.2013, found 418.2002.

*4.2.5. (2R,3S,4R)-3,4-Bis(benzyloxy)-2-(benzyloxymethyl)-3,4-dihydro-2H-pyrrole-1-oxide (**1e**)*

The reaction was carried out as described above for **1a** starting from 2,3,5-tri-*O*-benzyl-β-D-lyxofuranose, derived from D-lyxose, to give **1e** (12%) as a white solid. $[\alpha]_D^{20}$ −56.9 (*c* 1, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 4.05 (dd, 1H, *J*=4.0, 9.8 Hz), 4.17 (m, 2H), 4.38 (t, 1H, *J*=5.8, 5.9 Hz), 4.53–4.59 (m, 4H), 4.67 (s, 2H), 4.70 (d, 1H, *J*=11.9 Hz), 6.89 (s, 1H), 7.24–7.33 (m, 15H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.8, 137.4, 137.3, 133.5, 128.6, 128.5, 128.4, 128.1, 128.0, 127.9, 127.7, 77.0, 74.6, 74.2, 73.6, 73.2, 72.6, 66.6. HRMS calcd for $[C_{26}H_{27}NO_4+Na]^+$ 440.1832, found 440.1868.

*4.2.6. (2S,3S,4R)-3,4-Bis(benzyloxy)-2-(benzyloxymethyl)-3,4-dihydro-2H-pyrrole-1-oxide (**1f**)*

The reaction was carried out as described above for **1b** starting from 2,3,5-tri-*O*-benzyl-β-D-lyxofuranose, derived from D-lyxose, to

give **1f** (53%) as a colorless oil. $[\alpha]_D^{20}$ −113.8 (*c* 0.8, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 3.59 (dd, 1H, *J*=2.5, 10.4 Hz), 4.09 (m, 1H), 4.12 (dd, 1H, *J*=2.5, 10.4 Hz), 4.38 (d, 1H, *J*=11.9 Hz), 4.43 (dd, 1H, *J*=4.7, 5.9 Hz), 4.52 (dd, 2H, *J*=7.4, 11.9 Hz), 4.57–4.68 (m, 4H), 6.95 (s, 1H), 7.18 (t, 2H, *J*=1.4 Hz), 7.26–7.33 (m, 13H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.5, 137.3, 137.1, 133.9, 128.7, 128.5, 128.4, 128.2, 128.1, 128.09, 128.05, 127.8, 127.6, 76.4, 75.4, 74.5, 73.4, 72.4, 72.1, 64.7. HRMS calcd for $[C_{26}H_{27}NO_4+H]^+$ 418.2013, found 418.2042.

4.2.7. (2R,3S,4S)-3,4-Bis(benzyloxy)-2-(benzyloxymethyl)-3,4-dihydro-2H-pyrrole-1-oxide (**1g**)

The reaction was carried out as described above for **1a** starting from 2,3,5-tri-*O*-benzyl-β-D-xylofuranose, derived from D-xylose, to give **1g** (54%) as a colorless oil. $[\alpha]_D^{20}$ +72.7 (*c* 1, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 3.82 (dd, 1H, *J*=2.0, 10.1 Hz), 4.00 (dd, 1H, *J*=4.4, 10.1 Hz), 4.15 (br, 1H), 4.36 (dd, 1H, *J*=4.4, 7.6 Hz), 4.49–4.76 (m, 6H), 4.77 (dd, 1H, *J*=1.6, 2.5 Hz), 6.84 (s, 1H), 7.28–7.34 (m, 15H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.6, 137.0, 136.9, 133.1, 128.2, 128.1, 128.0, 127.8, 127.7, 127.6, 127.5, 127.2, 82.8, 80.2, 73.8, 73.2, 72.7, 72.0, 64.1. HRMS calcd for $[C_{26}H_{27}NO_4+H]^+$ 418.2013, found 418.2017.

4.2.8. (2S,3S,4S)-3,4-Bis(benzyloxy)-2-(benzyloxymethyl)-3,4-dihydro-2H-pyrrole-1-oxide (**1h**)

The reaction was carried out as described above for **1b** starting from 2,3,5-tri-*O*-benzyl-β-D-xylofuranose, derived from D-xylose, to give **1h** (74%) as a white solid. $[\alpha]_D^{20}$ +37.3 (*c* 1, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 3.77 (d, 1H, *J*=3.9, 10.2 Hz), 4.01 (br, 1H), 4.05 (dd, 1H, *J*=5.0, 10.2 Hz), 4.38 (t, 1H, *J*=2.4, 3.2 Hz), 4.50–4.55 (m, 5H), 4.60 (d, 1H, *J*=12 Hz), 4.65 (br, 1H), 6.89 (s, 1H), 7.24–7.33 (m, 15H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.6, 137.1, 137.0, 132.8, 128.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 82.6, 80.2, 77.4, 73.4, 71.8, 71.5, 66.0. HRMS calcd for $[C_{26}H_{27}NO_4+Na]^+$ 440.1832, found 440.1818.

4.3. Typical procedure for the preparation of DMDP from 1a (1a→2b→17→4)

4.3.1. (2R,3R,4R)-3,4-Bis(benzyloxy)-2(benzyloxymethyl)-5-vinylpyrrolidin-1-ol (**2b**)

Compound **1a** (2 g, 5 mmol) was dissolved in THF (20 mL) and then vinyl magnesium chloride (0.54 g, 6 mmol) was added dropwise at 0 °C under Ar(g). After 15 h, the reaction mixture was quenched with aqueous NH_4Cl solution, extracted with CH_2Cl_2, dried with $MgSO_4$, and concentrated. The crude sample was purified by CC (25% EtOAc in hexanes, silica gel) to give **2b** (1.91 g, 4.3 mmol, 90%) as a brown solid. $[\alpha]_D^{20}$ −19.6 (*c* 0.66, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 3.56 (t, 1H, *J*=4.9 Hz), 3.68 (dd, 1H, *J*=6.0, 9.8 Hz), 3.73 (dd, 1H, *J*=4.9, 9.8 Hz), 3.84 (t, 1H, *J*=8.1 Hz), 3.91 (t, 1H, *J*=5.0 Hz), 3.99 (t, 1H, *J*=3.8 Hz), 4.45–4.56 (m, 6H), 5.31 (q, 2H), 6.02 (m, 1H), 7.23–7.31 (m, 15H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 138.0, 137.9, 137.8, 135.5, 128.1, 128.0, 127.9, 127.7, 127.6, 127.5, 127.4, 127.39, 127.35, 119.0, 85.9, 83.6, 73.0, 72.5, 71.6, 71.4, 69.5, 67.5. HRMS calcd for $[C_{28}H_{31}NO_4+H]^+$ 446.2326, found 446.2344.

4.3.2. (2R,3R,4R,5R)-tert-Butyl-3,4-bis(benzyloxy)-2-(benzyloxymethyl)-5-(hydroxymethyl)-pyrrolidine-1-carboxylate (**17**)

A mixture of **2b** (1.44 g, 3.2 mmol) and zinc powder (0.21 g, 3.2 mmol) in acetic acid was stirred at rt for 8 h. The reaction mixture was filtered through Celite and the filtrate was neutralized by aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2. The organic layers were dried with $MgSO_4$, concentrated, and directly reacted with di-*tert*-butyldicarbonate (0.88 g, 4 mmol) in CH_2Cl_2 (10 mL) in the presence of triethylamine (1 mL) to give a crude Boc-protected amine compound. After ozonolysis and reduction with $NaBH_4$ (0.24 g, 6.3 mmol), the reaction solvent was removed and the residue was extracted with CH_2Cl_2 and water. The organic layers were dried with $MgSO_4$, concentrated, and purified by CC (20% EtOAc in hexanes, silica gel) to give **17** (1.27 g, 2.4 mmol, 74% from **2b**) as a syrup. ^1H NMR (600 MHz, $CDCl_3$, mixture of conformers, major/minor=2:1) (for a major conformer): δ 1.42 (s, 9H), 3.47–3.53 (m, 1H), 3.75 (dd, 1H, *J*=4.1, 8.8 Hz), 3.84–3.90 (m, 3H), 4.05–4.08 (m, 2H), 4.15 (s, 1H), 4.38–4.67 (m, 6H), 7.18–7.35 (m, 15H); (for a minor conformer): δ 1.47 (s, 9H), 3.47–3.53 (m, 1H), 3.72–3.76 (m, 1H), 3.80 (dd, 2H, *J*=4.0, 11.4 Hz), 3.97 (s, 1H), 4.01 (dd, 1H, *J*=4.1, 8.5 Hz), 4.17 (s, 1H), 4.28 (dd, 1H, *J*=3.5, 10.3 Hz), 4.38–4.67 (m, 6H), 7.18–7.35 (m, 15H); ^{13}C NMR (150 MHz, $CDCl_3$, mixture of conformers) δ 155.6, 154.2, 138.7, 138.3, 137.6, 137.5, 137.2, 128.7, 128.6, 128.5, 128.2, 128.1, 128.06, 128.01, 127.9, 127.8, 127.7, 127.6, 85.8, 84.3, 81.9, 80.9, 80.5, 80.4, 73.2, 73.2, 71.5, 71.4, 71.39, 71.32, 68.5, 68.0, 66.6, 65.6, 64.6, 63.6, 63.2, 62.4, 28.6, 28.5. HRMS calcd for $[C_{32}H_{39}NO_6+Na]^+$ 556.2670, found 556.2661.

4.3.3. (2R,3R,4R,5R)-2,5-Bis(hydroxymethyl)pyrrolidine-3,4-diol (**4**, DMDP)

A mixture of **17** (0.3 g, 0.56 mmol) and palladium hydroxide (0.03 g) in MeOH was stirred for overnight under a hydrogen atmosphere. The reaction mixture was filtered through Celite and the filtrate was concentrated. The residue was treated with the solution of CH_2Cl_2/TFA (2 mL/2 mL) and the reaction mixture was stirred at 0 °C for 30 min. The reaction mixture was concentrated and purified by CC (25% aqueous NH_4OH in propanol, silica gel) to give **4** (71.47 mg, 0.44 mmol, 78%) as a syrup (free form). $[\alpha]_D^{20}$ +53.4 (*c* 0.6, H_2O); ^1H NMR (600 MHz, D_2O) δ 3.34 (dd, 2H, *J*=3.4, 5.8 Hz), 3.73 (dd, 2H, *J*=6.1, 12.3 Hz), 3.81 (dd, 2H, *J*=3.8, 12.3 Hz), 3.95 (dd, 2H, *J*=2.2, 5.8 Hz); ^{13}C NMR (150 MHz, D_2O) δ 75.5, 62.1, 59.2. HRMS calcd for $[C_6H_{13}NO_4+H]^+$ 164.0917, found 164.0899.

4.3.4. (2S,3S,4S,5S)-2,5-Bis(hydroxymethyl)pyrrolidine-3,4-diol (**5**, L-DMDP)

The reaction was carried out as described above for **4** starting from **1h** to give **5** (free form, 50% yield from **1h**) as a syrup. $[\alpha]_D^{20}$ −51.0 (*c* 0.2, H_2O); ^1H NMR (600 MHz, D_2O) δ 3.17 (br, 2H), 3.71 (dd, 2H, *J*=6.0, 11.6 Hz), 3.79 (d, 2H, *J*=11.7 Hz), 3.92 (t, 2H, *J*=2.2 Hz); ^{13}C NMR (150 MHz, D_2O) δ 77.1, 61.7, 61.2. HRMS calcd for $[C_6H_{13}NO_4+H]^+$ 164.0917, found 164.0933.

4.3.5. (2S,3R,4S,5S)-2,5-Bis(hydroxymethyl)pyrrolidine-3,4-diol (**6**, L-DALDP)

The reaction was carried out as described above for **4** starting from **1d** to give **6** (free form, 46% yield from **1d**) as a syrup. $[\alpha]_D^{20}$ −29.1 (*c* 1, H_2O); ^1H NMR (600 MHz, D_2O) δ 3.20 (m, 1H), 3.37 (m, 1H), 3.67 (m, 2H), 3.78 (dd, 1H, *J*=3.9, 11.8 Hz), 3.82 (dd, 1H, *J*=6.6, 11.3 Hz), 4.03 (dd, 1H, *J*=4.3, 8.6 Hz), 4.21 (t, 1H, *J*=4.0 Hz); ^{13}C NMR (150 MHz, D_2O) δ 73.2, 71.7, 61.5, 61.4, 60.1, 59.9. HRMS calcd for $[C_6H_{13}NO_4+H]^+$ 164.0917, found 164.0892.

4.3.6. (2S,3S,4R,5R)-2,5-Bis(hydroxymethyl)pyrrolidine-3,4-diol (**7**, DADP)

The reaction was carried out as described above for **4** starting from **1f** to give **7** (free form, 43% yield from **1f**) as a syrup. $[\alpha]_D^{20}$ +39.2 (*c* 2, H_2O); ^1H NMR (600 MHz, D_2O) δ 3.06–3.09 (m, 2H), 3.65 (dd, 2H, *J*=6.2, 11.7 Hz), 3.73 (dd, 2H, *J*=4.2, 11.7 Hz), 3.84–3.87 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 77.5, 61.6, 61.5. HRMS calcd for $[C_6H_{13}NO_4+H]^+$ 164.0917, found 164.0858.

4.3.7. (2S,3R,4R,5R)-2,5-Bis(hydroxymethyl)pyrrolidine-3,4-diol (**13**, DGDP)

The reaction was carried out as described above for **4** starting from **1b** to give **13** (43% from **1b**) as a syrup. $[\alpha]_D^{20}$ +18.1 (*c* 1, H_2O); ^1H NMR (600 MHz, D_2O) δ 3.05 (d, 1H, *J*=5.40 Hz), 3.35 (d,

1H, *J*=5.5 Hz), 3.62–3.67 (m, 2H), 3.72 (dd, 1H, *J*=4.7, 11.6 Hz), 3.77 (dd, 1H, *J*=5.8, 11.5 Hz), 3.85 (dd, 1H, *J*=2.5, 4.4 Hz), 4.08 (t, 1H, *J*=2.5 Hz); ^{13}C NMR (150 MHz, D_2O) δ 78.6, 76.9, 65.1, 61.7, 61.1, 59.6. HRMS calcd for $[C_6H_{13}NO_4+Na]^+$ 186.0737, found 186.0701.

4.4. Typical procedure for the preparation of ADMDP from 1a (1a→2c→8)

*4.4.1. (2R,3R,4R,5R)-3,4-Bis(benzyloxy)-5-(benzyloxymethyl)-1-hydroxypyrrolidine-2-carbonitrile (**2c**, trans isomer) and (2S,3R,4R,5R)-3,4-bis(benzyloxy)-5-(benzyloxymethyl)-1-hydroxypyrrolidine-2-carbonitrile (**3c**, cis isomer)*

A mixture of **1a** (1 g, 2.4 mmol) and trimethylsilyl cyanide (0.76 mL, 5.7 mmol) in dry methanol (10 mL) was stirred at 50 °C for 8 h. The reaction solvent was removed under vacuum and the residue was purified by CC (25% EtOAc in hexanes, silica gel) to give a mixture of **2c**/**3c** (0.99 g, 2.2 mmol, 93%, **2c**/**3c**=19:1 in the ^{1}H NMR spectrum). A mixture of **2c**/**3c** was further separated by CC to give pure **2c** (0.93 g, 2.1 mmol, 88%) as a white solid, pure **3c** (10.6 mg, 0.02 mmol, 1%) as a white solid, and **2c**/**3c** mixture (0.04 g).

Compound **2c**: $[\alpha]_D^{20}$ +8.8 (*c* 1.1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.31 (m, 1H), 3.57 (dd, 1H, *J*=3.5, 10.6 Hz), 3.78 (dd, 1H, *J*=3.2, 10.6 Hz), 4.00 (dd, 1H, *J*=2.3, 6.7 Hz), 4.17 (t, 1H, *J*=2.2 Hz), 4.25 (d, 1H, *J*=1.7 Hz), 4.41–4.43 (m, 3H), 4.49 (dd, 2H, *J*=4.6, 11.9 Hz), 4.58 (d, 1H, *J*=12.0 Hz), 7.26–7.40 (m, 15H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.2, 137.1, 136.3, 128.6, 128.3, 128.1, 128.0, 127.9, 127.8, 127.78, 127.74, 127.69, 115.63, 83.4, 80.9, 73.1, 72.0, 71.9, 69.3, 66.0, 61.2. HRMS calcd for $[C_{27}H_{28}N_2O_4+Na]^+$ 467.1941, found 467.1961.

Compound **3c**: $[\alpha]_D^{20}$ +12.2 (*c* 0.45, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.10 (dd, 1H, *J*=5.3, 10.7 Hz), 3.61 (dd, 1H, *J*=5.6, 10.1 Hz), 3.65 (dd, 1H, *J*=5.2, 10.1 Hz), 3.83 (d, 1H, *J*=5.3 Hz), 4.03 (s, 2H), 4.38 (q, 2H, *J*=11.8 Hz), 4.50–4.56 (m, 3H), 4.65 (d, 1H, *J*=11.9 Hz), 7.18–7.36 (m, 15H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.9, 137.3, 136.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 1163.5, 81.8, 79.9, 73.4, 72.6, 72.0, 71.9, 68.6, 62.2. HRMS calcd for $[C_{27}H_{28}N_2O_4+Na]^+$ 467.1941, found 467.1945.

*4.4.2. (2R,3R,4R,5R)-2-(Aminomethyl)-5-(hydroxymethyl)-pyrrolidine-3,4-diol (**8**, ADMDP)*

A mixture of **2c** (0.64 g, 1.4 mmol), Raney nickel (0.05 g), and di-*tert*-butyldicarbonate (0.6 g, 2.7 mmol) in dry methanol (3 mL) was stirred under a hydrogen atmosphere. After 4 h, palladium hydroxide (0.05 g) was added and the reaction mixture was also stirred under a hydrogen atmosphere for further 10 h. The reaction mixture was filtered through Celite and the filtrate was concentrated. The residue was treated with the solution of CH_2Cl_2/TFA (3 mL/3 mL) and the reaction mixture was stirred at 0 °C for 30 min. The reaction mixture was concentrated and purified by CC (25% aqueous NH_4OH (37%) in propanol, silica gel) to give **8** (0.2 g, 1.2 mmol, 86%) as a white solid. $[\alpha]_D^{20}$ +43.5 (*c* 0.5, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 3.18 (q, 2H), 3.31 (dd, 1H, *J*=4.8, 13.3 Hz), 3.41 (m, 1H), 3.73 (dd, 1H, *J*=6.1, 11.9 Hz), 3.81 (dd, 1H, *J*=3.9, 12.1 Hz), 3.94 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 78.6, 76.4, 62.1, 60.5, 58.0, 41.3. HRMS calcd for $[C_6H_{14}N_2O_3+H]^+$ 163.1077, found 163.1071.

*4.4.3. (2S,3S,4S,5S)-2-(Aminomethyl)-5-(hydroxymethyl)-pyrrolidine-3,4-diol (**9**)*

The reaction was carried out as described above for **8** starting from **1h** to give **9** (76% from **1h**). $[\alpha]_D^{20}$ −41.6 (*c* 0.8, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 3.03 (m, 2H), 3.18 (dd, 1H, *J*=4.3, 12.9 Hz), 3.24 (m, 1H), 3.64 (dd, 1H, *J*=5.7, 11.4 Hz), 3.71 (dd, 1H, *J*=3.4, 11.5 Hz), 3.83 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 79.2, 77.1, 61.7, 61.1, 57.9, 42.0. HRMS calcd for $[C_6H_{14}N_2O_3+H]^+$ 163.1077, found 163.1071.

*4.4.4. (2S,3S,4R,5R)-2-(Aminomethyl)-5-(hydroxymethyl)-pyrrolidine-3,4-diol (**10**)*[10]

The reaction was carried out as described above for **8** starting from **1c** to give **10** (72% from **1c**) as a syrup. $[\alpha]_D^{20}$ −4 (*c* 0.3, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 3.54 (m, 2H), 3.81 (dd, 1H, *J*=4.0, 9.0 Hz), 3.86 (m, 2H), 3.95 (dd, 1H, *J*=3.8, 12.6 Hz), 4.27 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 72.5, 70.2, 65.9, 58.8, 58.4, 38.65. HRMS calcd for $[C_6H_{14}N_2O_3+H]^+$ 163.1077, found 163.1078.

*4.4.5. (2R,3R,4S,5S)-2-(Aminomethyl)-5-(hydroxymethyl)-pyrrolidine-3,4-diol (**11**)*

The reaction was carried out as described above for **8** starting from **1f** to give **11** (68% from **1f**) as a syrup. $[\alpha]_D^{20}$ +3 (*c* 0.2, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 3.55 (m, 2H), 3.83 (dd, 1H, *J*=3.9, 9.00 Hz), 3.88 (m, 2H), 3.96 (dd, 1H, *J*=3.9, 12.7 Hz), 4.29 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 72.5, 70.2, 66.0, 58.8, 58.3, 38.6. HRMS calcd for $[C_6H_{14}N_2O_3+H]^+$ 163.1077, found 163.1074.

*4.4.6. (2R,3R,4S,5S)-2-(Aminomethyl)-5-(hydroxymethyl)-pyrrolidine-3,4-diol (**14**)*

The reaction was carried out as described above for **8** starting from **1e** to give **14** (76% from **1f**) as a syrup. $[\alpha]_D^{20}$ +18 (*c* 0.1, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 3.55 (s, 1H), 3.56 (d, 1H, *J*=3.2 Hz), 3.83 (m, 1H), 3.88 (m, 1H), 3.95 (m, 1H), 4.04 (dd, 1H, *J*=7.3, 17.6 Hz), 4.32 (dd, *J*=5.8, 7.3 Hz), 4.38 (t, 1H, *J*=5.3 Hz); ^{13}C NMR (150 MHz, D_2O) δ 74.1, 69.6, 62.3, 57.6, 57.0, 39.4. HRMS calcd for $[C_6H_{14}N_2O_3+H]^+$ 163.1077, found 163.1083.

4.5. Typical procedure for the inversion of C-2 configuration and the preparation of iminocyclitol 12 (2c/3c→19→20a→12)

*4.5.1. (2R,3R,4R)-3,4-Bis(benzyloxy)-2-(benzyloxymethyl)-3,4-dihydro-2H-pyrrole-5-carbonitrile (**19**)*

A mixture of **2c** and **3c** (1.69 g, 3.8 mmol) was treated with methanesulfonyl chloride (0.36 mL, 4.6 mmol) in CH_2Cl_2 (10 mL) in the presence of triethylamine (1.3 mL, 7.7 mmol) at 0 °C for 3 min. The reaction was quenched with H_2O and extracted with CH_2Cl_2. The organic layers were dried with $MgSO_4$ and concentrated to give **19** (1.21 g, 2.8 mmol, 75%). $[\alpha]_D^{20}$ −4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.65 (dd, 1H, *J*=5.9, 10.0 Hz), 3.77 (dd, 1H, *J*=4.6, 10.0 Hz), 4.22 (t, 1H, *J*=3.7 Hz), 4.38 (dd, 1H, *J*=4.6, 9.1 Hz), 4.54 (dd, 2H, *J*=7.6, 11.8 Hz), 4.58 (s, 2H), 4.69 (s, 1H), 4.71 (d, 1H, *J*=3.1 Hz), 4.78 (d, 1H, *J*=11.6 Hz), 7.30–7.45 (m, 15H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 152.1, 137.8, 137.3, 136.6, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 127.97, 127.94, 127.8, 113.7, 90.2, 84.0, 78.3, 73.5, 73.2, 72.3, 69.7. HRMS calcd for $[C_{27}H_{26}N_2O_3+H]^+$ 427.2016, found 427.2009.

*4.5.2. (2S,3R,4R,5R)-3,4-Bis(benzyloxy)-5-(benzyloxymethyl)-pyrrolidine-2-carbonitrile (**20a**) and (2R,3R,4R,5R)-3,4-bis-(benzyloxy)-5-(benzyloxymethyl)pyrrolidine-2-carbonitrile (**20b**)*

Sodium borohydride (0.28 g, 7.4 mmol) was added to **19** in methanol (8 mL) and the reaction was stirred at 0 °C for 5 h. The solvent was removed and the reaction mixture was extracted with CH_2Cl_2 and brine. The organic layers were dried with $MgSO_4$, concentrated, and purified by CC (25% EtOAc in hexanes, silica gel) to give a mixture of **20a** and **20b** (1.15 g, 2.68 mmol, 95%). Further purification of this mixture gave pure **20a** (1.03 g, 2.4 mmol, 85%) as a colorless syrup and pure **20b** (0.057 g, 0.13 mmol, 5%) as a syrup.

Compound **20a**: $[\alpha]_D^{20}$ −8.7 (*c* 1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.30 (br, 1H), 3.32 (dd, 1H, *J*=5.9, 10.3 Hz), 3.52–3.57 (m, 2H), 3.89 (d, 1H, *J*=3.8 Hz), 4.08 (d, 1H, *J*=6.7 Hz), 4.45–4.53 (m, 4H), 4.61 (q, 2H, *J*=11.9 Hz), 7.22–7.36 (m, 15H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.9, 137.5, 137.0, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1,

128.0, 127.8, 118.0, 83.5, 83.0, 73.3, 72.6, 72.1, 70.7, 62.8, 51.3. HRMS calcd for $[C_{27}H_{28}N_2O_3+H]^+$ 429.2173, found 429.2194.

Compound **20b**: $[\alpha]_D^{20}$ +35.8 (*c* 0.8, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 3.47 (m, 2H), 3.58 (q, 1H, *J*=5.6 Hz), 3.83 (dd, 1H, *J*=3.1, 5.8 Hz), 4.01 (d, 1H, *J*=2.9 Hz), 4.2 (t, 1H, 3.0 Hz), 4.44–4.58 (m, 6H), 7.24–7.36 (m, 15H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.9, 137.7, 136.9, 128.9, 128.7, 128.5, 128.2, 128.1, 128.03, 128.00, 127.9, 119.4, 87.5, 84.3, 73.5, 72.7, 72.4, 68.8, 62.4, 52.0. HRMS calcd for $[C_{27}H_{28}N_2O_3+H]^+$ 429.2173, found 429.2184.

4.5.3. *(2S,3R,4R,5R)-2-(Aminomethyl)-5-(hydroxymethyl)-pyrrolidine-3,4-diol (**12**)*

A mixture of **20a** (0.1 g, 0.23 mmol), palladium hydroxide (0.01 g), and a catalytic amount of acetic acid in methanol (2 mL) was stirred under a hydrogen atmosphere for 48 h. The reaction mixture was filtered through Celite and the filtrate was concentrated. The residue was purified by CC (25% aqueous NH_4OH (37%) in propanol, silica gel) to give **12** (0.03 g, 0.18 mmol, 80%) as a yellow syrup. $[\alpha]_D^{20}$ +14.7 (*c* 0.8, H_2O); ^1H NMR (600 MHz, D_2O) δ 3.09 (dd, 1H, *J*=6.3, 13.1 Hz), 3.13 (q, 1H, *J*=5.5 Hz), 3.24 (dd, 1H, *J*=6.0, 13.1 Hz), 3.59 (q, 1H, *J*=6.1 Hz), 3.64 (dd, 1H, *J*=6.5, 11.6 Hz), 3.74 (dd, 1H, *J*=4.5, 11.6 Hz), 3.90 (dd, 1H, *J*=3.8, 5.3 Hz), 4.20 (dd, 1H, *J*=3.8, 5.8 Hz); ^{13}C NMR (150 MHz, D_2O) δ 77.9, 76.8, 64.0, 62.2, 55.9, 39.2. HRMS calcd for $[C_6H_{14}N_2O+H]^+$ 163.1077, found 163.1074.

4.6. Typical procedure for the inversion of C-2 configuration and the preparation of iminocyclitol 13 (20a→21→22→23→24→13)

4.6.1. *(2S,3R,4R,5R)-1-Benzyl-3,4-bis(benzyloxy)-5-(benzyloxymethyl)pyrrolidine-2-carbonitrile (**21**)*

A mixture of **20a** (0.12 g, 0.28 mmol), benzylbromide (0.04 mL, 0.33 mol), potassium carbonate (0.077 g, 0.56 mmol), and potassium iodide (4 mg, 0.02 mmol) in THF (3 mL) was stirred at 55 °C for overnight. The reaction mixture was filtered through Celite and the filtrate was concentrated. The residue was purified by CC (20% EtOAc in hexanes, silica gel) to give **21** (0.13 g, 0.25 mmol, 90%) as a colorless syrup. $[\alpha]_D^{20}$ −7.7 (*c* 1, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 3.21 (m, 1H), 3.44 (dd, 1H, *J*=5.3, 9.5 Hz), 3.54 (dd, 1H, *J*=8.2, 9.4 Hz), 3.88 (d, 1H, *J*=5.3 Hz), 3.95 (m, 2H), 4.03 (dd, 1H, *J*=2.2, 5.2 Hz), 4.15 (d, 1H, *J*=13.7 Hz), 4.42–4.61 (m, 6H), 7.22–7.40 (m, 20H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.9, 137.4, 136.8, 135.7, 129.5, 128.4, 128.3, 128.29, 128.24, 128.1, 128.0, 127.9, 127.8, 127.7, 127.65, 127.61, 127.58, 127.53, 117.0, 82.9, 81.2, 73.0, 72.2, 71.2, 70.1, 66.0, 56.7, 56.0. HRMS calcd for $[C_{34}H_{34}N_2O_3+H]^+$ 519.2642, found 519.2665.

4.6.2. *(2R,3R,4R,5R)-1-Benzyl-3,4-bis(benzyloxy)-5-(benzyloxymethyl)pyrrolidine-2-carboxamide (**22**)*

A mixture of **21** (0.11 g, 0.22 mmol), aqueous sodium hydroxide (1 N, 3 mL), and aqueous hydrogen peroxide (2 mL, 30%) in MeOH (2 mL) was stirred at rt for 3 h. The reaction mixture was extracted with CH_2Cl_2 (20 mL×3), washed with brine, dried with $MgSO_4$, and concentrated. The residue was purified by CC (20% EtOAc in hexanes, silica gel) to give **22** (0.1 g, 0.18 mmol, 83%) as a colorless syrup. $[\alpha]_D^{20}$ +11.0 (*c* 2, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 3.11 (dd, 1H, *J*=3.7, 9.4 Hz), 3.18 (t, 1H, *J*=3.3 Hz), 3.41 (dd, 1H, *J*=8.0, 9.1 Hz), 3.75 (d, 1H, *J*=13.1 Hz), 3.83 (d, 1H, *J*=6.2 Hz), 3.92 (d, 1H, *J*=13.1 Hz), 4.08 (s, 1H), 4.23 (dd, 1H, *J*=3.2, 5.3 Hz), 4.27 (d, 1H, *J*=11.9 Hz), 4.33 (d, 1H, *J*=11.9 Hz), 4.43 (d, 1H, *J*=11.6 Hz), 4.50 (d, 1H, *J*=12.1 Hz), 4.59 (d, 1H, *J*=12.0 Hz), 4.63 (d, 1H, *J*=11.6 Hz), 5.90 (s, 1H), 7.18–7.35 (m, 20H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.3, 138.2, 138.0, 137.9, 129.5, 128.6, 128.5, 128.45, 128.42, 128.0, 127.8, 127.77, 127.74, 127.69, 83.1, 82.0, 73.0, 72.7, 71.7, 70.7, 69.9, 67.3, 59.6. HRMS calcd for $[C_{34}H_{36}N_2O_4+H]^+$ 537.2748, found 537.2742.

4.6.3. *((2S,3R,4R,5R)-1-Benzyl-3,4-bis(benzyloxy)-5-(benzyloxymethyl)pyrrolidin-2-yl)methanol (**24**)*

A mixture of **22** (0.1 g, 0.18 mmol), di-*tert*-butyldicarbonate (0.16 g, 0.73 mmol), and DMAP (0.1 g, 0.81 mmol) in CH_2Cl_2 (10 mL) was stirred at rt for 1 h. The reaction mixture was filtered through a silica gel pad and concentrated to give a colorless liquid **23**, which was used without further purification. A mixture of **23** and $NaBH_4$ (0.02 g, 0.52 mmol) in methanol was stirred at 0 °C under Ar(g) for 8 h. The solvent was removed and the mixture was extracted with CH_2Cl_2 (10 mL×3). The combined organic layers were dried with $MgSO_4$, concentrated, and purified by CC (25% EtOAc in hexanes, silica gel) to give **24** (63 mg, 0.12 mmol, 65% from **22**) as a colorless syrup. $[\alpha]_D^{20}$ −0.8 (*c* 2, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 3.04 (br, 1H), 3.15 (br, 1H), 3.31 (dd, 1H, *J*=2.5, 9.2 Hz), 3.41 (m, 2H), 3.58 (d, 1H, *J*=10.7 Hz), 3.85 (m, 2H), 4.06 (dd, 1H, *J*=5.8, 6.9 Hz), 4.16 (t, 1H, *J*=5.3 Hz), 4.38 (q, 2H), 4.47–4.56 (m, 3H), 4.64 (d, 1H, *J*=11.8 Hz), 7.24–7.32 (m, 20H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 139.3, 138.6, 138.4, 138.1, 129.2, 128.7, 128.5, 128.0, 127.9, 127.89, 127.81, 127.7, 127.4, 83.7, 82.4, 73.3, 72.4, 72.4, 70.0, 66.8, 64.6, 60.5, 59.0. HRMS calcd for $[C_{34}H_{37}NO_4+H]^+$ 524.2795, found 524.2798.

4.6.4. *(2S,3R,4R,5R)-2,5-Bis(hydroxymethyl)pyrrolidine-3,4-diol (**13**, DGDP)*

A mixture of **24** (63 mg, 0.12 mmol), palladium hydroxide (0.02 g), and a catalytic amount of hydrochloric acid (36% in H_2O, 0.1 mL) in methanol (2 mL) was stirred under a hydrogen atmosphere for 48 h. The reaction solution was filtered through a Celite pad and the filtrate was concentrated. The residue was purified by CC to give **13** (quantitative yield) as a yellowish syrup. The spectra of **13** are consistent with those reported for **1b** (see Section 4.3.7).

4.7. Typical procedure for selective acetylation of iminocyclitol 9

4.7.1. *N-(((2S,3S,4S,5S)-3,4-Dihydroxy-5-(hydroxymethyl)-pyrrolidin-2-yl)methyl)acetamide (**26**)*

A mixture of **9** (10.2 mg, 0.06 mmol) and acetic anhydride (6.1 mg, 0.06 mmol) was stirred at rt for 10 min. The reaction mixture was neutralized with Dowex 550A (OH^-) anion exchange resin. The mixture was filtered and washed with MeOH (2 mL). The filtrate was concentrated and the residue was purified by CC (12% aqueous NH_4OH (37%) in propanol, silica gel) to give **26** (11.8 mg, 92%) as a white solid. $[\alpha]_D^{20}$ −30.5 (*c* 0.5, MeOH); ^1H NMR (600 MHz, D_2O) δ 2.09 (s, 3H), 3.18 (m, 1H), 3.24 (m, 1H), 3.42 (m, 1H), 3.52 (dd, 1H, *J*=4.7, 13.9 Hz), 3.74 (dd, 1H, *J*=6.1, 11.1 Hz), 3.82 (m, 1H), 3.90 (t, 1H, *J*=6.7 Hz), 3.94 (t, 1H, *J*=6.8 Hz); ^{13}C NMR (150 MHz, D_2O) δ 174.6, 79.2, 77.5, 61.7, 61.6, 59.7, 41.6, 21.8. HRMS calcd for $[C_8H_{16}N_2O_4+H]^+$ 205.1183, found 205.1179.

4.7.2. *N-(((2R,3R,4S,5R)-3,4-Dihydroxy-5-(hydroxymethyl)-pyrrolidin-2-yl)methyl)acetamide (**27**)*

Following the procedure for the preparation of **25**, **27** was obtained as a yellow syrup (93%). $[\alpha]_D^{20}$ +18 (*c* 0.1, MeOH); ^1H NMR (600 MHz, D_2O) δ 2.08 (s, 3H), 3.56 (dd, 1H, *J*=7.1, 14.1 Hz), 3.62 (m, 1H), 3.67 (dd, 1H, *J*=3.6, 14.2 Hz), 3.74 (m, 1H), 3.88 (dd, 1H, *J*=8.0, 11.9 Hz), 3.99 (dd, 1H, *J*=5.5, 11.9 Hz), 4.20 (dd, 1H, *J*=3.9, 8.8 Hz), 4.34 (t, 1H, *J*=3.7 Hz); ^{13}C NMR (150 MHz, D_2O) δ 175.7, 73.3, 70.4, 61.4, 60.3, 58.2, 39.2, 21.7. HRMS calcd for $[C_8H_{16}N_2O_4+H]^+$ 205.1183, found 205.1185.

4.8. General procedure for the enzyme assay with various glycosidases

The initial velocities of hydrolysis at rt were measured spectrophotometrically at various concentrations of *p*-nitrophenyl-

glycopyranoside (40 mM, 20 mM, 10 mM, 5 mM, 2.5 mM, 1.25 mM, 0.625 mM, 0 mM) at 405 nm by using multi-detection reader (SpectraMax M5, Molecular Dectce). The data obtained was fitted to the Michaelis–Menten equation by using the GraphPad to determine the K_m values and V_{max} values. The substrate concentrations were used at twofold K_m values for evaluation of the inhibitory effect against various glycosidases. Enzymes at 0.25–1 units per mL were used to provide an ideal progression curve, and inhibitors were tested initially at 500 μM. The compounds that showed activities were selected and further tested at lower concentration to determine IC_{50}. The assays performed in wells of the 96-well microtiter plate contained either sodium phosphate buffer (100 mM, pH 6.8, for α- and β-glucosidase) or sodium citrate buffer (100 mM, pH 6.4, for α-mannosidase).

4.8.1. Kinetic analysis of β-N-acetylglucosaminidase

Incubations were performed in a total volume of 100 μL. Reaction mixture contained citrate sodium buffer solution (100 mM, pH 4.5), various amount of 4-methylumbellifery *N*-acetyl-β-D-glucosaminide, and various amounts of inhibitors with 0.5 mU per well of β-*N*-acetylglucosaminidase. After incubation for 30 min at rt, the reaction was terminated by addition of sodium glycine buffer (0.5 M), pH 10.5. Enzyme activity was measured by the release of 4-methylumbelliferone with an excitation wavelength of 360 nm and an emission wavelength of 460 nm.

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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.2008.10.096.

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