Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 1107–1115

BIOORGANIC & MEDICINAL CHEMISTRY

Design and Synthesis of HIV-1 Protease Inhibitors. Novel Tetrahydrofuran P2/P2′-Groups Interacting with Asp29/30 of the HIV-1 Protease. Determination of Binding from X-ray Crystal Structure of Inhibitor Protease Complex

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Received 2 August 2002; accepted 18 August 2002

Abstract—A series of HIV-1 protease inhibitors having new tetrahydrofuran P2/P2′ groups have been synthesised and tested for protease inhibition and antiviral activity. Six novel 4-aminotetrahydrofuran derivatives were prepared starting from commercially available isopropylidene-α-D-xylofuranose yielding six symmetrical and six unsymmetrical inhibitors. Promising sub nanomolar HIV-1 protease inhibitory activities were obtained. The X-ray crystal structure of the most potent inhibitor (**23,** K_i 0.25 nM) co-crystallised with HIV-1 protease is discussed and the binding compared with inhibitors **1a** and **1b**.

Introduction

Since the discovery of the human immunodeficiency virus (HIV) as the etiologic agent of AIDS, almost two decades ago, several drug targets have been identified from the HIV-1 genome.[1,2] The *pol* gene of HIV-1 encodes for an aspartic protease,[3,4] which was shown to be essential for delivery of structural and functional proteins by proteolytic processing of the *gag*- and *gag-pol* viral gene products.[3,5] Inhibition of this aspartic protease results in the production of non-infectious virions and thus constitutes an ideal drug target.[6–9] Today six protease inhibitors are approved by the FDA for treatment of HIV-1 infection: saquinavir,[10] ritonavir,[11] indinavir,[12,13] nelfinavir,[14] amprenavir,[15] and lopinavir.[16] Due to the rapid turnover of HIV-1 and the high frequency of mutations in the genome, selection of mutant strains conferring clinical resistance to the HAART (Highly Active Anti Retroviral Therapy) regimes is a major concern. Moreover, the high costs of treatment hinder the widespread use of the currently approved HIV-1 protease inhibitors. Not withstanding the major advancements made with current HIV-1 protease inhibitors there is a vast need for improved and cost-effective protease inhibitors.[17,18] We now report on the design, synthesis, and antiviral activity of HIV-1 inhibitors containing a C_2-symmetrical core structure exemplified by lead compounds **1a** and **1b** (Fig. 1). It has been shown[13,19,20] that the conformationally constrained P2/P2′ amino indanol of **1a**[20] and **1b**[21] effectively occupies the lipophilic S2/S2′ pockets of the HIV-1 protease and also forms a hydrogen bond with the Asp 29 backbone of the enzyme. This concept has resulted in numerous very potent inhibitors and in the clinically approved drug indinavir. However, the amino indanol group is susceptible to hCYP450 metabolism, that is, 3A4, which limits oral bioavailability of inhibitors having this group.[22] To overcome the potential liability of aromatic and benzylic CYP450 oxidation and to gain affinity from hydrogen bond formation to both of Asp29 and Asp 30 in the HIV-1 proteases we have designed and synthesised new non-aromatic P2/P2′

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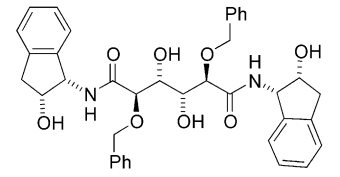
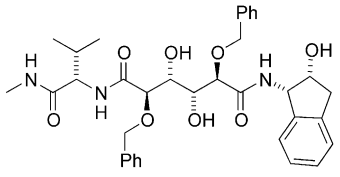
PII: S0968-0896(02)00535-7



1a K_i = 0.6 nM
ED_{50} = 0.1 μM



1b K_i = 0.2 nM
ED_{50} = 0.1 μM

Figure 1. Lead compounds **1a** and **1b** containing the C_2-symmetrical core structure.

tetrahydrofurans and coupled these to the C_2-symmetric L-mannaric P1/P1′ template.[20] From molecular modelling we envisaged that 2-alkoxymethyl-4-aminotetrahydrofurans should form two hydrogen bonds, one to each of the backbones of Asp29/30. Previous work on for example, 'bis THFs'[23] has shown that potent inhibitors can result from utilising these hydrogen bond interactions.

For the synthesis of the 2-alkoxymethyl-4-aminotetrahydrofurans D-xylose was used as starting material. The synthesised aminotetrahydrofurans were coupled to the C_2-symmetric P1/P1′ backbone using either the activated L-mannaric acid **2** or the bislactone **3** (Fig. 2), delivering symmetrical inhibitors.[20]

Previous work has highlighted that in contrast to the synthesis of symmetrical inhibitors, unsymmetrical inhibitors are not readily available using this general-methodology[20] but could be prepared using solid support methodology.[21] We now report on a new solution phase synthesis of the key intermediate monolactone **4** (Fig. 2), from readily available **3**, which provides facile access to these unsymmetrical inhibitors. In summary,

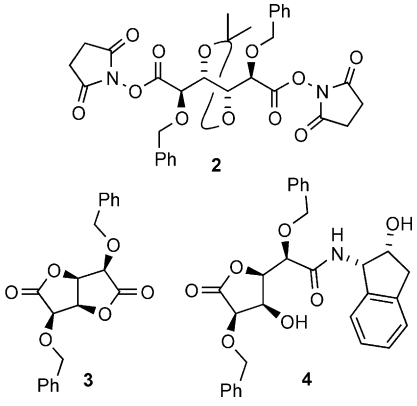


Figure 2. The activated L-mannaric acid, the bislactone and monolactone used for preparation of the symmetrical and unsymmetrical inhibitors.

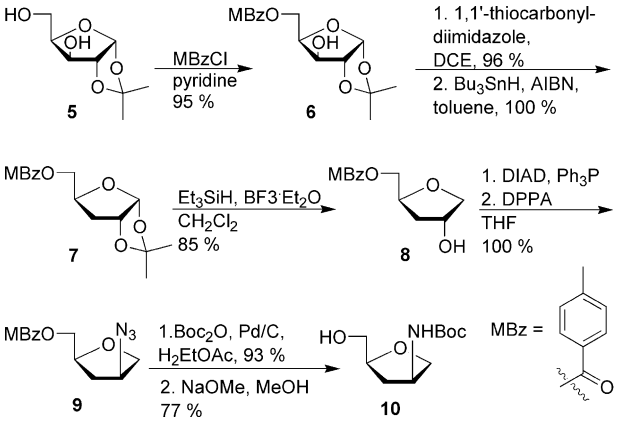
novel aminotetrahydrofurans have been synthesised from which both symmetrical and unsymmetrical protease inhibitors have been prepared and tested for enzyme inhibition and antiviral activity. The most potent compound (**23**) in this new series was co-crystallised with HIV-1 PR and the X-ray structure was determined. The result was compared with the X-ray structures of **1a** and **1b** to support the structure activity relationship.

Results and Discussion

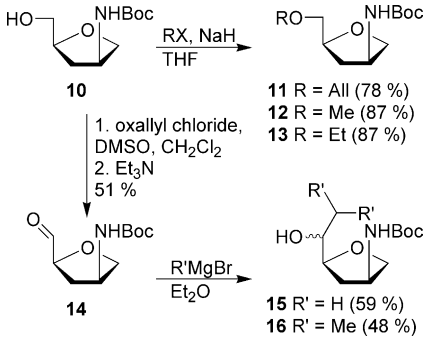
Chemistry

2(*S*)-Hydroxymethylene-4(*S*)-*tert*-butoxycarbonylaminotetrahydrofuran (**10**) was identified as the pivotal intermediate for the synthesis of protected amines **11**–**13**, **15**, and **16**, and a straightforward and short synthesis of **10** was developed (Schemes 1 and 2). Intermediate **9** was prepared from **5** according to Bolon et al.[24] but modified resulting in improvements of the overall yield from 28 to 78%.

The 1,2-*O*-Isopropylidene-α-D-xylofuranose **5** was protected at the primary hydroxyl group giving the toloyl ester **6** as a crystalline solid.[25] Deoxygenation of the 3-hydroxyl group according to Barton–McCombie conditions by reduction of the corresponding 3-*O*-imidazol thiocarbonyl ester using AIBN/Bu_3SnH, gave **7** in almost quantitative yield.[24] Compound **7** was then



Scheme 1.



Scheme 2.

directly converted into the dideoxy compound **8** using Et_3SiH and $BF_3.OEt_2$ at 0 °C in 85% yield.[24,26] Inversion of configuration at C-2 was subsequently achieved employing Mitsunobu conditions,[27] using triphenylphosphine, diisopropyl azodicarboxylate (DIAD) and diphenylphosphonic azide (DPPA) giving the azido derivative **9** in quantitative yield. Reduction of the azide **9** with Pd/C (H_2) in the presence of Boc_2O followed by removal of the toluoate under Zemplén conditions gave **10** in 74% yield.[28] Thus the synthesis of target intermediate tetrahdrofuran **10** could be achieved in an overall yield of 57% from compound **5**.

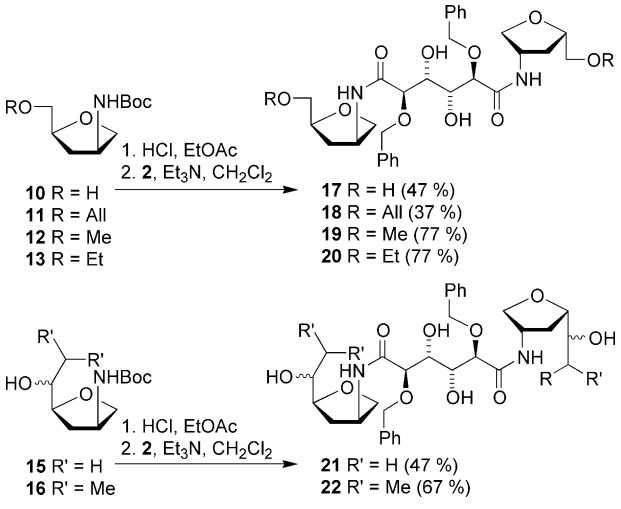
Employing Williamson conditions,[29] the alkylated derivatives **11** (78%), **12** (87%), and **13** (87%) were obtained (Scheme 2). Attempted oxidation of **10** using the Dess–Martin reagent failed to give the corresponding aldehyde **14**. However, Swern oxidation of **10** employing oxalyl chloride in DMSO delivered the aldehyde albeit in a moderate yield, 51%. Grignard alkylations of aldehyde **14** provided derivatives **15** and **16** (Scheme 2).[30] Initially, the Grignard reactions were performed at −60 °C to +10 °C in THF, which only resulted in recovered starting material. However, adding the reagents at 0 °C in Et_2O and allowing the mixture to attain room temperature overnight furnished compounds **15** (59%) and **16** (48%) as diastereomeric mixtures (approximately 1:1), no chelation is observed in this case.[31]

For the preparation of symmetrical inhibitors **17–22** (Scheme 3) the Boc group in compounds **10–13**, **15** and **16** was deprotected using HCl (concd) in EtOAc. The resulting ammonium salts were immediately coupled with disuccinimidylester **2**[20] producing the isopropylidene protected L-mannaric bisamides. After cleavage of the isopropylidene group the symmetrical inhibitors **17** (47%), **18** (37%), **19** (77%),**20** (77%), **21** (62%), and **22** (47%) were obtained.

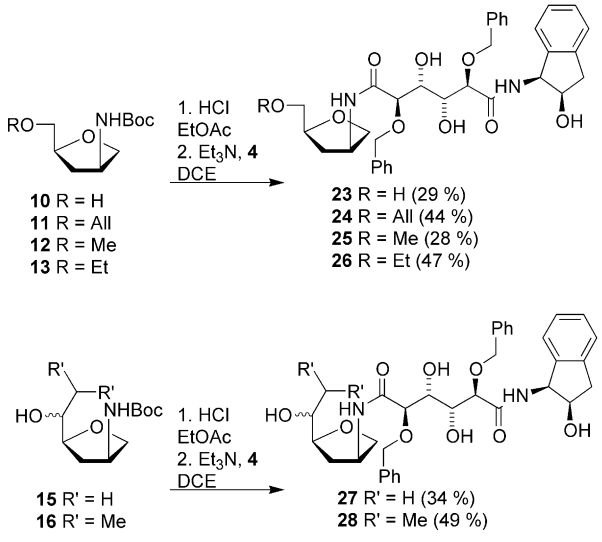
Attempts to couple the amines directly to the benzylated bislactone **3** failed, resulting in β-elimination of **3** and unreacted starting material (TLC examinations). A new methodology was developed for the preparation of monolactone **4** and the subsequent transformation to unsymmetrical inhibitors **23–28**. The mono-opening reaction of bislactone **3** could be achieved with (1*S*, 2*R*)-(−)-1-amino-2-indanol in dichloromethane utilising 2-hydroxypyridine[21] as catalyst resulting in amide **4** (Fig. 1), which was isolated in 40% yield. The major side reactions, β-elimination and aminolysis of the second lactone, was surpressed by selection of the appropriate solvent and catalyst. The ammonium salts of **10–13**, **15** and **16** (Scheme 4) were coupled with the monolactone **4** in refluxing dichloroethane in the presence of triethylamine to give the unsymmetrical products **23** (29%), **24** (34%), **25** (28%), **26** (47%), **27** (44%), and **28** (49%).

Anti-HIV-1 activity and 3-D structure

Notably from examination of Tables 1 and 2 there are some very potent unsymmetrical inhibitors with enzyme inhibition values in the sub nanomolar range; i.e., **23** K_i 0.25 nM, **25** K_i 0.74 nM and **27** K_i 0,54 nM. In contrast to the unsymmetrical inhibitors, the symmetrical inhibitors (**17–22**) are considerable less potent, one explanation being that the lipophilic P2 indanol moiety, for this P1/P1′ core template, gives an excellent fit to the S2 pocket of the enzyme which cannot be achieved for the less lipophilic tetrahydrofurans. This is confirmed by examination of the X-ray crystal structures, vide infra. It is interesting to note that in inhibitor **23**, the modelled hydrogen bonds to Asp29 and Asp30 were observed in the X-ray crystal structure. However, the added affinity from hydrogen bond formation from inhibitor hydroxymethylene group to backbone NH of Asp30 in the HIV-1 PR is offset by not optimally filling of the S2 pocket. In spite of the high potency at the enzyme level, the cellular antiviral activities observed were surprisingly low, even for the most potent unsymmetrical inhibitors. For example compound **23** exhibited an ED_{50} value of 1.1 μM and was consequently considerable less potent than inhibitors **1a** and **1b**, both showing an ED_{50} value of 0.1 μM.

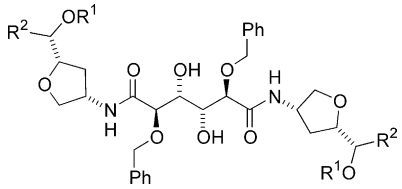


Scheme 3.



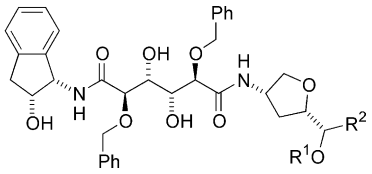
Scheme 4.

Table 1. HIV-1 protease inhibitory activities for compounds **17–22**



Compd	R^1	R^2	K_i (nM)	ED_{50} (μM)
17	H	H	4.80	>10
18	Allyl	H	>5000	>10
19	Me	H	103	>10
20	Et	H	>5000	>10
21	H	Me	208	>10
22	H	*i*-Pr	1590	>10

Table 2. HIV-1 protease inhibitory activities for compounds **23–28**



Comp.	R^1	R^2	K_i (nM)	ED_{50} (μM)
23	H	H	0.25	1.12
24	Allyl	H	11.30	5.45
25	Me	H	0.74	3.10
26	Et	H	3.00	4.60
27	H	Me	0.54	6.10
28	H	*i*-Pr	1.60	3.75

Inhibitor **23** was co-crystallised with HIV-1 PR, and the structure was determined. The binding to the HIV-1 PR was then compared with inhibitors **1a**,[20] and **1b**.[32]

When associated to the active site, all three adopt a similar overall binding conformation. This is clearly apparent upon a structural superimposition of the three, where only minor differences in position of the central diols, P1/P1′ benzyloxy substituents and the P2′ indanol group could be observed (Fig. 3). However, the three different P2 substituents adopt binding modes distinct from each other. Mainly the size and charge distribution and the lining residues of the S2-subsite guide the position of the P2-substituents. The P2 indanol in the symmetric compound **1a** occupies a major part of the S2-subsite volume with optimal vdW contacts to Ala28, Val32, Ile47 and Leu76. In contrast, **1b** and **23** exploit the S2-subsite less effective, due to the smaller P2 substituents, which reduce the number of vdW contacts to the S2 pocket. The reduced number of non-polar contacts of compound **23** is compensated by formation of an additional hydrogen bond from the free hydroxyl group to the backbone nitrogen of Asp30 (3.0 Å) (Fig. 4). Similarly, the valine amide of **1b** is involved in a hydrogen bond to the backbone carbonyl of Gly48 (2.9 Å).

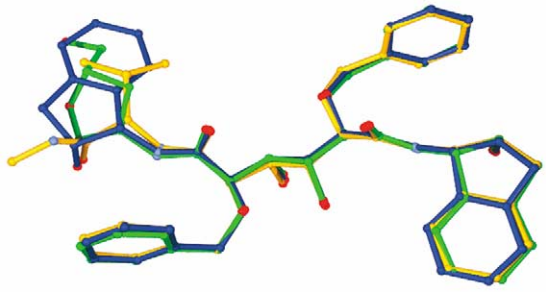


Figure 3. Superimposition of compounds **1a** (blue), **1b** (gold) and **23** (green) bound to the active site of HIV-1 PR. The image was compiled using Swiss-PDB viewer version 3.51[33] and 3-D-rendered with POV-ray version 3.1.[34]

All three compounds form a hydrogen bond with the backbone nitrogen of Asp29 from either the indanol hydroxyl, amide carbonyl or the furan ring oxygen (**1a**: 3.0Å; **1b**: 2.9 Å; **23**: 3.1 Å). Small changes in position of the side chains in the S2-subsite accommodated these substantial differences in size and charge distribution of the P2-substituent in these compounds.

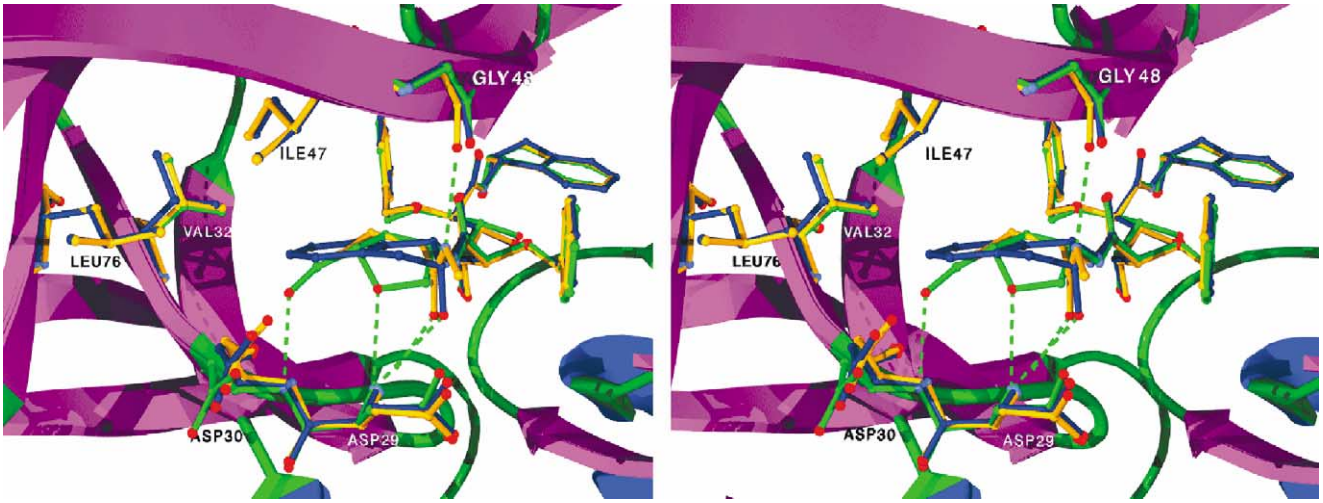


Figure 4. Stereo view of the three compounds **1a** (blue), **1b** (gold) and **23** (green) bound to the S2-subsite of HIV-1 PR. This orientation visualizes the interactions between the different substituents of the P2 arm and key residues in the S2-subsite. The hydrophobic contribution to the binding is reflected in how effective the different substituent are filling out the S2-subsite. The image was compiled using Swiss-PDB viewer version 3.51[33] and 3-D-rendered with POV-ray version 3.1.[34]

Furthermore, all three complexes reveal that one of the hydroxyl group of the central diol points toward the Asp25/125 residues in the active site and is hydrogen bonded to both carboxyl oxygens. The other hydroxyl group points away from the active site but is still hydrogen bonded to one of the Asp residue. This asymmetric interaction pattern of the diol is known for this class of compounds.[20]

Conclusion

In summary, we have developed new tetrahydrofuran P2 analogues exhibiting potent HIV-1 protease inhibiting properties. Examination of the X-ray crystal structure of inhibitor **23** co-crystallized with HIV-1 PR reveals that the hydroxymethyl group forms the predicted hydrogen bond to the backbone NH of Asp 30 accounting for the high potency of this inhibitor and close analogues. In spite of the promising HIV-1 protease inhibition of this series of compounds, the anti-viral activity in cell culture (ED_{50}) was not improved over inhibitor **1a** and **1b** (Fig. 1).[20,21] However, our results strongly support that optimisation of inhibitor potency can be achieved by formation of hydrogen bonds from inhibitor to NH groups of the HIV-1 protease backbone. The X-ray crystal structure of inhibitor **23** will provide the basis towards further optimisation of this new inhibitor series with the aim to fully utilise the S2/S2′ pockets and to achieve high anti-viral activity.

Experimental

Crystallography

The details of the crystallisation and structure determination will be published elsewhere. Briefly, the complex of HIV-1 PR together with **23**, **1a** and **1b** were crystallised in space group $P2_12_12$ and determined to 2.0 Å resolution with *R*-values of 0.19, 0.18 and 0.18 respectively (R_{free} 0.23, 0.20 and 0.21).

HIV-1 protease inhibition

(Tables 1 and 2: column 4) HIV-1 protease was cloned and heterologously expressed in *Escherichia coli*[35] and K_i-values were determined using a fluorometric assay.[36]

In vitro anti-HIV activity

(Tables 1 and 2; column 5) The anti-HIV activity was measured in a HIV cytopathic assay in MT-4 cells where the effect was quantified using vital dye XTT.[37] The 50% inhibitory concentrations (ED_{50}) were calculated from the percent cytoprotection for individual compounds.

General

All glassware was dried over an open flame before use in connection with an inert atmosphere. Concentrations were performed under reduced pressure at $<40\,^{\circ}C$ (bath temperature). Thin layer chromatography was performed using silica gel 60 F-254 plates with detection by UV and charring with 8% sulfuric acid. Silica gel (0.040–0.063 mm) was used for column chromatography. Me_4Si (0.0 ppm) was used as an internal standard in ^1H NMR and Me_4Si or $CDCl_3$ (77.0 ppm) were used in ^{13}C NMR. Optical rotations were measured on a Perkin–Elmer 241 polarimeter. Unless stated otherwise, all materials were obtained from commercial suppliers and used without further purification.

2*R*-*O*-Benzyl-4-[1*R*-*O*-benzyl-2*N*-(2*R*-hydroxy-1*S*-indanyl)]ethylcarbamoyl-3*S*-hydroxy-1,4*R*-γ-lactone (4). (1*S*,2*R*)-(−)-1-amino-2-indanol (0.5 g, 3.36 mmol) and 2-hydroxypyridine (27 mg, 0.28 mmol) were added to a solution of **3** (1.0 g, 2.82 mmol) in CH_2Cl_2 (100 mL) under N_2 and refluxed for 15 h. The solution was concentrated to 20 mL and the residue was purified by silica gel column chromatography (1,2-dichloroethane–MeOH 49:1) to provide compound **4** as a white solid (0.57 g, 1.13 mmol, 40%). $[\alpha]_D^{20}$ −33.1 (*c* 1.09, CH_2Cl_2) ^1H NMR (300 MHz, $CDCl_3$) δ 7.42–7.04 (m, 14H), 5.29 (dd, 1H), 4.91–4.49 (m, 8H), 4.11 (s, 1H), 3.09 (dd, 1H), 2.90 (dd, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.0, 169.8, 140.6, 139.9, 136.5, 136.4 128.7, 128.5, 128.4, 128.4, 127.1, 125.3, 124.3, 79.7, 77.2, 74.4, 74.3, 73.0, 72.7, 68.2, 57.6, 39.4.

3-Deoxy-1,2-*O*-isopropylidene-5-*O*-(4-methylbenzoyl)-α-D-*erythro*-pentofuranose (7). A solution of **6** (20 g, 65 mmol) in dry dichloroethane (400 mL) was brought to reflux. 1,1′-Thiocarbonyldiimidazole (20 g, 112 mmol) was added to the solution and the refluxing was continued for 1.5 h. The solvent was evaporated and the residue was purified by silica gel column chromatography (toluene; toluene–EtOAc 2:1) to provide the thiocarbonyl ester (26.6 g, 62.4 mmol, 96%) as a light yellow solid. $[\alpha]_D^{20}$ −26.1 (*c* 0.56, CH_2Cl_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 184.6, 166.2, 144.4, 137.2, 131.6, 130.0, 129.4, 117.9, 113.0, 105.1, 84.5, 83.0, 76.9, 61.2, 26.8, 26.4, 21.9.

To a refluxing solution of Bu_3SnH (36 mL, 134 mmol) and a catalytic amount of AIBN in toluene (2000 mL), a solution of thiocarbonyl ester (26 g, 62.1 mmol) in toluene (400 mL) was added dropwise over a period of 1 h. The reaction mixture was stirred at reflux for 1 h and then at room temperature overnight. The solvent was evaporated and the residue was purified by silica gel column chromatography (toluene; toluene–EtOAc 6:1) to provide **7** (18.2 g, 62.1 mmol, 100%) as a colourless oil. ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.6, 144.0, 130.0, 129.3, 111.5, 106.0, 80.5, 76.1, 65.4, 35.7, 27.0, 26.4, 21.9.

2(*S*)-(*O*-4-Methylbenzoyl-methyl)-tetrahydrofuran-4(*R*)-ol (8). To a solution of **7** (6.0 g, 20.5 mmol) in CH_2Cl_2 (80 mL) at 0 °C were added Et_3SiH (30 mL, 187 mmol) and $BF_3{\cdot}OEt_2$ (23.6 mL, 186 mmol) and the reaction was stirred at 0 °C for 1 h. The reaction was quenched by addition of ice cold saturated $NaHCO_3/H_2O$ (60 mL). The aqueous layer was extracted (CH_2Cl_2, 3×50 mL)

and the combined organic phase was washed (brine) and dried ($MgSO_4$). Purification by silica gel column chromatography (toluene–EtOAc 1:1) gave **8** (4.1 g, 17.4 mmol, 85%) as a white solid. $[\alpha]_D^{20}$ +18.6 (*c* 0.57, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) δ 7.95–7.92 (d, 2H), 7.27–7.15 (d, 2H), 4.54 (m, 2H), 4.41 (dd, 1H, *J* = 3.5, 11.9), 4.27 (dd, 1H, *J* = 6.2, 11.4 Hz), 4.00 (dd, 1H, *J* = 4.1, 9.8 Hz) 3.82 (dd, 1H, *J* = 1.4, 9.8 Hz), 3.0 (1H, OH), 2.39 (s, 3H), 2.07 (m, 1H), 1.89 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.6, 143.8, 129.7, 129.1, 75.8, 75.7, 72.0, 66.3, 37.7, 21.6. Anal. calcd.for ($C_{13}H_{16}O_4$): C, 66.1; H, 6.8. Found: C, 65.7; H, 6.8.

4(*S*)-Azido-2(*S*)-(*O*-4-methylbenzoyl-methyl)-tetrahydrofuran (9). DIAD (4.2 mL, 21.3) was added dropwise to a cooled (−5 °C) solution of Ph_3P (5.3 g, 20.1 mmol) in THF (80 mL). After 30 min, a solution of **8** (3.9 g, 16.5 mmol) in THF (30 mL) was added. After additional10 min diphenyl phosphorazidate (DPPA; 4.2 mL, 19.5 mmol) was added and the reaction mixture was allowed to warm to room temperature. After stirring over night, the solvent was evaporated and the residue was purified by silica gel column chromatography (toluene; toluene–EtOAc 50:1) to provide **9** (4.3 g, 16.5 mmol, 100%) as a white solid. $[\alpha]_D^{20}$ +33.7 (*c* 1.32, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) δ 7.95–7.92 (d, 2H), 7.24–7.21 (d, 2H), 4.47–4.26 (m, 4H), 4.15 (m, 1H), 3.96 (m, 1H), 3.85 (m, 1H), 2.45–2.32 (m, 4H), 1.89 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.7, 144.0, 130.0, 129.3, 76.8, 72.9, 66.2, 61.1, 34.5, 21.9. Anal. calcd for ($C_{13}H_{15}N_3O_4$): C, 59.8; H, 5.8; N, 16.1. Found: C, 59.7; H, 5.8; N, 15.8.

2(*S*)-Hydroxymethyl-4(*S*)-*tert*-butoxycarbonylamino-tetrahydrofuran (10). A catalytic amount of Pd/C (10%) was added to a solution of **9** (3.6 g, 13.8 mmol) and *tert*-butoxycarbonyl anhydride (Boc_2O; 3.2 g, 14.7 mmol) in EtOAc (150 mL). Hydrogen was added at atmospheric pressure and the reaction mixture was stirred at ambient temperature overnight. The suspension was filtered trough Celite and the solvent was evaporated. The residue was purified by silica gel column chromatography (toluene–EtOAc 6:1) to provide the protected amine (4.3 g, 12.9 mmol, 93%) as a white solid. $[\alpha]_D^{20}$ +8.5 (*c* 0.58, CH_2Cl_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.8, 155.6, 144.0, 129.9, 129.4, 127.3, 79.8, 76.5, 74.1, 66.3, 51.6, 35.5, 28.6, 28.5, 21.9.

To a stirred solution of the protected amine (3.8 g, 11.3 mmol) in MeOH (50 mL), a catalytic amount of sodium was added and the mixture was stirred at ambient temperature. After 6 h Dowex® HCR-W2 (H^+) ion exchange resin was added to neutralise the solution. The resin was filtered off and the solvent was evaporated. The residue was purified by silica gel column chromatography (toluene; toluene–EtOAc 1:1) to provide **10** (1.9 g, 8.7 mmol, 77%) as a colourless syrup, which solidified upon standing. $[\alpha]_D^{20}$ −24.6 (*c* 0.61, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) δ 5.50 (d, 1H, *J* = 7.8 Hz), 4.21 (bs, 1H), 4.04 (m, 1H), 3.82–3.71 (m, 3H), 3.50 (dd, 1H, *J* = 3.6, 11.7), 3.00 (bs, 1H), 2.35 (m, 1H), 1.65 (m, 1H), 1.40 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.7, 78.9, 74.8, 64.1, 51.6, 34.2, 28.6. Anal. calcd for ($C_{10}H_{19}NO_4$): C, 55.3; H, 8.8; N, 6.5. Found: C, 54.7; H, 8.7; N, 6.3.

General method for the preparation of compounds 11–13. Compound **10** (200 mg, 0.92 mmol) in THF (2 mL) was added to a stirred solution of NaH (27 mg, 1.1 mmol) in THF (6 mL) at 0 °C followed by addition of the alkylhalide (9.2 mmol). The reaction was allowed to warm to room temperature. TLC showed completion of the reaction after 0.5–18 h. The reaction was quenched by addition of MeOH and the solvent was evaporated. Purification by silica gel column chromatography (toluene–EtOAc 1:1) gave the target compounds **11**, **12** and **13**.

2(*S*)-Allyloxymethyl-4(*S*)-*tert*-butoxycarbonylamino-tetrahydrofuran (11). The title compound was prepared according to the general procedure, vide supra, using allyl bromide (700 μL, 9.2 mmol) with stirring for 18 h, and was isolated as a colourless syrup in 78% yield (185 mg, 0.72 mmol). $[\alpha]_D^{20}$ −32.5 (*c* 0.97, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) δ 5.92 (m, 1H), 5.74 (bd, 1H), 5.27 (m, 1H), 5.17 (m, 1H), 4.29 (bs, 1H), 4.10 (m, 1H), 4.00 (m, 1H), 3.80 (d, 1H, *J* = 9.7 Hz), 3.71–3.61 (m, 2H), 3.43 (dd, 1H, *J* = 3.3, 10.5 Hz), 2.29 (m, 1H), 1.70 (m, 1H), 1.40 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.4, 134.1, 117.6, 78.8, 77.0, 74.9, 72.6, 72.0, 51.1, 34.2, 28.4.

2(*S*)-Methoxymethyl-4(*S*)-*tert*-butoxycarbonylamino-tetrahydrofuran (12). The title compound was prepared according to the general procedure, vide supra, using MeI (570 μL, 9.2 mmol) with stirring for 30 min, and was isolated as a colourless syrup in 87% yield (185 mg, 0.80 mmol). $[\alpha]_D^{20}$−24.8 (*c* 0.80, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) δ 5.52 (bd, 1H), 4.12 (bs, 1H), 4.10 (m, 1H), 3.72 (d, 1H, *J* = 9.0 Hz), 3.64 (dd, 1H, *J* = 6.0, 10.5 Hz), 3.50 (m, 1H), 3.33 (s, 3H), 3.30 (dd, 1H, *J* = 6.0, 12.0 Hz), 2.23 (m, 1H), 1.58 (m, 1H), 1.37 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.4, 79.0, 77.17, 74.7, 74.4, 59.2, 51.1, 34.2, 28.3.

2(*S*)-Ethoxymethyl-4(*S*)-*tert*-butoxycarbonylamino-tetrahydrofuran (13). The title compound was prepared according to the general procedure, vide supra, using EtI (700 μL, 9.2 mmol) with stirring for 7 h, and was isolated as a colourless syrup in 87% yield (197 mg, 0.81 mmol). $[\alpha]_D^{20}$ −34.0 (*c* 1.33, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) δ 6.01 (bd, 1H), 4.18–4.02 (m, 2H), 3.76 (d, 1H, 10.8), 3.65–3.40 (m, 4H), 3.34 (dd, 1H, *J* = 1.8, 7.5 Hz), 2.22 (m, 1H), 1.68 (m, 1H), 1.35 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.5, 78.7, 77.2, 75.1, 72.4, 67.1, 51.0, 34.1, 28.4, 14.9.

2(*S*)-(1′-Hydroxy)ethyl-4(*S*)-*tert*-butoxycarbonylamino-tetrahydrofuran (15). Oxalyl chloride (550 μL, 6.4 mmol) in CH_2Cl_2 (12 mL) was added to a solution of DMSO (790 μL, 11.1 mmol) in CH_2Cl_2 (5 mL) at −70 °C under argon. After stirring for 5 min, alcohol **10** (1.0 g, 4.6 mmol) dissolved in CH_2Cl_2 (2 mL) was added dropwise. Stirring was continued for an additional 15 min. NEt_3 (3.0 mL, 22.5 mmol) was added and the reaction mixture was stirred during 1.5 h while the reaction mixture slowly was allowed to attain room

temperature. Dilution with H_2O, extraction of the aqueous layer with CH_2Cl_2 (3x), drying ($MgSO_4$) and the solvent was evaporated. Purification by silica gel column chromatography (toluene–EtOAc 1:1) gave the aldehyde **14** (500 mg, 2.32 mmol, 51%) as a colourless oil. $[\alpha]_D^{20}$ −15.1 (*c* 0.97, CH_2Cl_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.2, 155.4, 82.1, 74.5, 51.2, 34.8, 28.6.

The crude aldehyde **14** (140 mg, 0.66 mmol) in diethyl ether (1 mL) was added to a stirred solution of methylmagnesium bromide (3 M in THF, 1 mL, 3 mmol) in diethyl ether (2 mL) at 0 °C. The reaction was stirred at 0 °C for 10 min and 3 h. at room temperatureThe reaction was quenched with saturated NH_4Cl. The aqueous layer was extracted (diethyl ether, 3×50 mL) and the combined organic phase was washed (H_2O) and dried ($MgSO_4$). Purification by silica gel column chromatography (toluene; toluene–EtOAc 1:1) gave **15** (90 mg, 0.39 mmol, 59%) as a diasteroemeric mixture (approx. 1:1). ^{13}C NMR (75 MHz, $CDCl_3$) δ 82.7, 82.4, 74.8, 74.3, 70.2, 67.6, 51.6, 51.2, 35.6, 31.3, 28.6, 19.9, 18.9.

2(*S*)-(1′-Hydroxy-2′-methyl)propyl-4(*S*)-*tert*-butoxycarbonylamino-tetrahydrofuran (16). The crude aldehyde **14** (250 mg, 1.16 mmol) (prepared as described above) in diethyl ether (4 mL) was added to a stirred solution of isopropylmagnesium bromide (2 M in diethyl ether, 5.5 mL, 11 mmol) in diethyl ether (10 mL) at 0 °C. The reaction was stirred at 0 °C for 10 min and then at room temperature overnight. The reaction was quenched with saturated NH_4Cl. The aqueous layer was extracted (diethyl ether, 3×50 mL) and the combined organic phase was washed (H_2O) and dried ($MgSO_4$). Purification by silica gel column chromatography (toluene; toluene–EtOAc 3:1) gave **16** (145 mg, 0.56 mmol, 48%) as a diasteroemeric mixture (approx. 1:1). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.5, 79.8, 78.7, 78.4, 76.8, 74.6, 74.3, 51.4, 35.7, 31.6, 31.3, 28.5, 19.6, 19.5, 18.9, 18.4.

General method for the preparation of compounds 17–22. Compounds **10–13**, **15**, and **16** (2.0 equiv) were dissolved in EtOAc (3 mL) and HCl (concd, 1 mL) and stirred at room temperature until TLC showed completion of the reaction (1 h). The solvent was evaporated. The residue was taken up in toluene (5 mL) and revaporated to give the amine salts. The amine salts were dissolved in CH_2Cl_2 (3 mL) and Et_3N (2.0 equiv) was added followed by addition of disuccinimidylester (1.0 equiv) The reaction mixture was stirred at room temperature for 16 h. The solvent was removed and the crude product purified by column chromatography (CH_2Cl_2; CH_2Cl_2–MeOH 20:1). The purified L-mannaric amides were dissolved in 5 mL of 4% HCl in MeOH, and the reaction mixture was stirred at ambient temperature for 16 h. The mixture was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$, dried, concentrated, and purified by silica gel column chromatography (CH_2Cl_2–MeOH 20:1) to give the title compounds **17–22**.

***N*1,*N*6-Di[2(*S*)-hydroxymethyl-tetrahydrofuran-4(*S*)-yl]-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-*O*-dihydroxyhexanediamide (17).** The title compound was prepared in 47% yield (21 mg, 0.036 mmol) according to general procedure, vide supra, using **10**. $[\alpha]_D^{20}$ −11.3 (*c* 1.15, CH_2Cl_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2, 137.2, 128.7, 128.5, 128.4, 81.1, 79.0, 74.9, 73.8, 71.4, 63.6, 50.2, 33.3. Anal. calcd for ($C_{30}H_{40}N_2O_{10}$): C, 61.2; H, 6.9; N, 4.8. Found: C, 60.8; H, 6.9; N, 4.8.

***N*1,*N*6-Di[2(*S*)-allyloxymethyl-tetrahydrofuran-4(*S*)-yl]-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-*O*-dihydroxyhexanediamide (18).** The title compound was prepared in 37% yield (26 mg, 0.039 mmol) according to general procedure, vide supra, using **11**. $[\alpha]_D^{20}$ −6.24 (*c* 0.85, CH_2Cl_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9, 137.1, 134.6, 128.8, 128.6, 128.5, 118.0, 79.2, 77.7, 76.8, 74.8, 73.9, 72.7, 71.8, 71.1, 49.9, 34.2. Anal. calcd for ($C_{36}H_{48}N_2O_{10}$): C, 64.7; H, 7.2; N, 4.2. Found: C, 64.7; H, 7.4; N, 4.3.

***N*1,*N*6-Di[2(*S*)-methoxymethyl-tetrahydrofuran-4(*S*)-yl]-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-*O*-dihydroxyhexanediamide (19).** The title compound was prepared in 77% yield (26 mg, 0.042 mmol) according to general procedure, vide supra, using **12**. $[\alpha]_D^{20}$ −4.6 (c 0.70, CH_2Cl_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.0, 137.2, 128.8, 128.7, 128.5, 79.2, 77.6, 75.1, 74.7, 74.1, 71.2, 59.5, 49.8, 34.0. Anal. calcd for ($C_{32}H_{44}N_2O_{10}$): C, 62.3; H, 7.2; N, 4.5. Found: C, 62.8; H, 7.5; N, 4.4.

***N*1,*N*6-Di[2(*S*)-ethoxymethyl-tetrahydrofuran-4(*S*)-yl]-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-*O*-dihydroxyhexanediamide (20).** The title compound was prepared in 77% yield (27 mg, 0.042 mmol) according to general procedure, vide supra, using **13**. $[\alpha]_D^{20}$ −4.1 (*c* 0.75, CH_2Cl_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.0, 137.1, 128.8, 128.7, 128.5, 79.2, 77.7, 74.8, 73.9, 72.4, 71.1, 67.2, 49.9, 34.4, 15.1. Anal. calcd for ($C_{34}H_{48}N_2O_{10}$): C, 63.3; H, 7.5; N, 4.3. Found: C, 63.0; H, 7.3; N, 4.6.

***N*1,*N*6-Di[2(*S*)-(1′-hydroxy)ethyl-tetrahydrofuran-4(*S*)-yl]-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-*O*-dihydroxyhexanediamide (21).** The title compound was prepared in 47% yield (32 mg, 0.051 mmol) according to general procedure, vide supra, using **15** as a diasteroemeric mixture (approx. 1:1). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.7, 171.4, 137.1, 128.8–128.4, 82.5, 82.3, 80.5, 80.0, 74.5, 74.3, 74.0, 73.9, 71.5, 71.4, 69.8, 69.8, 67.4, 50.0, 49.7, 35.1, 30.6, 20.1, 19.4 Anal. calcd for ($C_{32}H_{44}N_2O_{10}$): C, 62.3; H, 7.2; N, 4.5. Found: C, 62.4; H, 7.3; N, 4.5.

***N*1,*N*6-Di[2(*S*)-(1′-hydroxy-2′-methyl)propyl-tetrahydrofuran-4(*S*)-yl]-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-*O*-dihydroxyhexanediamide (22).** The title compound was prepared in 62% yield (46 mg, 0.068 mmol) according to general procedure, vide supra, using **16** as a diasteroemeric mixture (approx. 1:1). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.8, 171.5, 137.3, 128.8, 128.7, 128.5, 128.4, 80.3, 79.9, 79.7, 79.5, 78.8, 78.5, 77.0, 74.7, 74.2, 71.5, 71.4, 50.0, 49.8, 35.4, 31.7, 31.6, 30.9, 19.6, 19.5, 19.1, 18.9. Anal. calcd for ($C_{36}H_{52}N_2O_{10}$): C, 64.3; H, 7.8; N, 4.2. Found: C, 64.3; H, 7.9; N, 4.2.

General method for the preparation of compounds 23–28. Compounds **10–13**, **15**, and **18–4** (1.5 equiv) were dissolved in EtOAc (3 mL) and HCl (conc., 1 mL), stirred at room temperature until TLC showed completion

of the reaction (1 h). The solvent was evaporated. The residue was taken up in toluene (5 mL) and revaporated to give the amine salts. The amine salts were dissolved in dichloroethane (8 mL) and Et_3N (1.5 equiv) was added followed by addition of **4** (1.0 equiv). The reaction mixture was stirred at reflux for 16 h. The solvent was removed and the crude product purified by silica gel column chromatography (CH_2Cl_2–MeOH 40:1) to give the title compounds **23**–**28**.

***N*1-[(1*R*,2*S*)-2-Hydroxy-2,3-dihydroxy-1*H*-indenyl]-*N*6-[2(*S*)-hydroxymethyl-tetrahydrofuran-4(*S*)-yl]-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-*O*-dihydroxyhexanediamide (23).** The title compound was prepared in 29% yield (34 mg, 0.055 mmol) according to general procedure, vide supra, using **10**. $[\alpha]D^{20}$ −19.7 (*c* 1.15, CH_2Cl_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.6, 170.3, 140.5, 139.6, 136.6, 136.5, 128.3, 128.2, 128.0, 127.9, 127.8, 126.7, 125.0, 123.8, 80.8, 80.7, 78.3, 74.3, 73.1, 72.3, 71.5, 71.1, 62.9, 57.5, 49.7, 38.9, 32.7. Anal. calcd for ($C_{34}H_{40}N_2O_9$): C, 65.8; H, 6.5; N, 4.5. Found: C, 65.3; H, 6.5; N, 4.5.

***N*1-[(1*R*,2*S*)-2-Hydroxy-2,3-dihydroxy-1*H*-indenyl]-*N*6-[2(*S*)-allyloxymethyl-tetrahydrofuran-4(*S*)-yl]-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-*O*-dihydroxyhexanediamide (24).** The title compound was prepared in 44% yield (40 mg, 0.061 mmol) according to general procedure, vide supra, using **11**. $[\alpha]_D^{20}$ −16.2 (*c* 1.10, CH_2Cl_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2, 171.1, 140.8, 140.1, 136.9, 136.8, 134.5, 128.7–128.3, 127.1, 125.4, 124.1, 117.9, 81.3, 80.5, 77.5, 74.6, 73.7, 72.6, 72.5, 71.5, 57.9, 49.8, 39.6, 33.9. Anal. calcd for ($C_{37}H_{44}N_2O_9$): C, 67.3; H, 6.7; N, 4.2. Found: C, 67.1; H, 6.8; N, 4.3.

***N*1-[(1*R*,2*S*)-2-Hydroxy-2,3-dihydroxy-1*H*-indenyl]-*N*6-[2(*S*)-methoxymethyl-tetrahydrofuran-4(*S*)-yl]-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-*O*-dihydroxyhexanediamide (25).** The title compound was prepared in 28% yield (25 mg, 0.039 mmol) according to general procedure, vide supra, using **12**. $[\alpha]_D^{20}$ −17.3 (*c* 0.85, CH_2Cl_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.0, 170.8, 140.6, 139.8, 136.7, 136.6, 128.5, 128.5, 128.3, 128.2, 128.1, 126.9, 125.2, 123.9, 81.2, 80.5, 76.5, 74.7, 74.2, 73.7, 73.6, 72.4, 71.3, 59.1, 57.7, 49.6, 39.4, 33.4. Anal. calcd for ($C_{35}H_{42}N_2O_9$): C, 66.2; H, 6.7; N, 4.4. Found: C, 66.1; H, 6.7; N, 4.3.

***N*1-[(1*R*,2*S*)-2-Hydroxy-2,3-dihydroxy-1*H*-indenyl]-*N*6-[2(*S*)-ethoxymethyl-tetrahydrofuran-4(*S*)-yl]-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-*O*-dihydroxyhexanediamide (26).** The title compound was prepared in 47% yield (42 mg, 0.065 mmol) according to general procedure, vide supra, using **13**. $[\alpha]_D^{20}$ −13.9 (*c* 1.4, CH_2Cl_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2, 171.2, 140.8, 140.0, 136.9, 136.8, 128.8, 128.7, 128.5, 128.4, 128.4, 128.0, 128.0, 127.2, 125.5, 124.1, 81.5, 80.6, 74.7, 73.9, 73.7, 72.7, 72.0, 71.5, 71.5, 67.2, 57.9, 49.9, 39.6, 34.0, 14.9. Anal. calcd for ($C_{36}H_{44}N_2O_9$): C, 66.7; H, 6.8; N, 4.3. Found: C, 66.9.0; H, 6.9; N, 4.3.

***N*1-[(1*R*,2*S*)-2-Hydroxy-2,3-dihydroxy-1*H*-indenyl]-*N*6-[2(*S*)-(1′-hydroxy)ethyl-tetrahydrofuran-4(*S*)-yl]-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-*O*-dihydroxyhexanediamide (27).** The title compound was prepared in 34% yield (22 mg, 0.035 mmol) according to general procedure, vide supra, using **15** as a diasteroemeric mixture (approx. 1:1). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.3, 172.2, 171.1, 141.0, 140.1, 137.1, 136.9, 128.9–128.5, 127.3, 125.6, 124.3, 82.6, 82.3, 81.4, 81.3, 80.8, 74.4, 74.3, 73.8, 73.8, 72.8, 71.9, 71.6, 69.7, 67.2, 58.0, 50.1, 49.8, 39.5, 35.0, 29.9, 20.1, 19.5. Anal. calcdfor ($C_{35}H_{42}N_2O_9$): C, 66.2; H, 6.7; N, 4.4. Found: C, 66.2; H, 6.8; N, 4.5.

***N*1-[(1*R*,2*S*)-2-Hydroxy-2,3-dihydroxy-1*H*-indenyl]-*N*6-[2(*S*)-(1′-hydroxy-2′-methyl)propyl-tetrahydrofuran-4(*S*)-yl]-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-*O*-dihydroxyhexanediamide (28).** The title compound was prepared in 49% yield (45 mg, 0.068 mmol) according to general procedure, vide supra, using **16** as a diasteroemeric mixture (approx. 1:1). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.1, 170.9, 140.9, 140.0, 137.1, 136.8, 128.7–128.3, 127.1, 125.5, 124.2, 81.3, 80.4, 78.6, 78.4, 74.5–71.5, 58.0, 57.9, 50.0, 49.8, 39.4, 35.2, 31.7, 31.5, 19.5, 19.4, 19.0. Anal. ($C_{37}H_{46}N_2O_9$): C, 67.1; H, 7.2; N, 4.1. Found: C, 67.0; H, 7.1; N, 4.2.

Acknowledgements

We gratefully acknowledge Medivir AB, Huddinge, Sweden for financial support and for the biological testings.

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