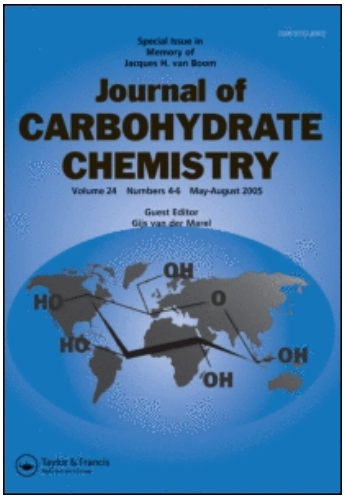

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SYNTHESIS OF C_2 SYMMETRIC POTENTIAL INHIBITORS OF HIV-1 PROTEASE FROM D-MANNITOL

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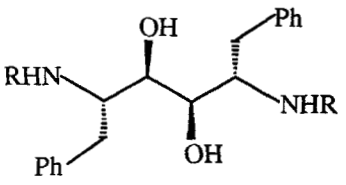
ABSTRACT

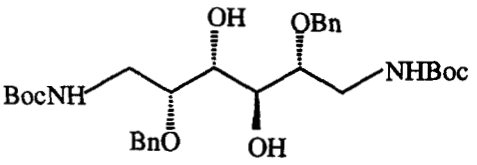
D-Mannitol was used as precursor for the synthesis of acyclic C_2 symmetric potential HIV-1 protease inhibitors. The 1- and 6-hydroxy groups of D-mannitol were substituted by -NHBoc, -NHValZ, -SAr, -SOAr and -SO_2Ar and the 2-and 5-hydroxy groups were benzylated. In some products one of the central hydroxyl groups was either inverted or deoxygenated. Despite a close structural similarity to previously published inhibitors none of the products showed significant inhibitory activity against HIV-1 protease.

INTRODUCTION

In the mid 1980s, it was suggested that HIV encodes a protease[1] and the existence of this protease was subsequently confirmed by mutagenesis experiments.[2] Based on the presence of the Asp-Thr-Gly sequence and weak homology with eukaryotic aspartic proteases such as pepsins, the HIV protease was identified as being a member of the aspartyl protease family.[3] It has been shown that processing of the viral gag and gag-pol polyproteins by HIV-1 protease is essential for viral replication, and inhibition of HIV-1 protease leads to the production of immature, noninfectious viral particles.[4,5] This information has initiated an immense research effort to design, synthesize and develop inhibitors of HIV protease for use in AIDS therapy to arrest the progression of HIV infection.[6,7] Presently several HIV-1 protease inhibitors are being documented in ongoing clinical trials.[8] Structure based drug design efforts towards transition-state-peptidomimetic inhibitors of HIV protease has been greatly aided by access to the crystal structure of dimeric HIV protease apo-enzyme and crystal structures of HIV protease complexed with various inhibitors. In many cases screening of renin inhibitors has provided HIV protease inhibitors that have been used as starting points for synthesis and screening programs.[9] A common feature observed in the crystal structure of almost all acyclic inhibitors of HIV-1 protease is a water molecule that bridges the P_2 and P_2' CO groups of the inhibitor and the Ile 50 and Ile 150 NH groups of the "flaps".[10] Recently a series of potent cyclic urea inhibitors developed by DuPont Merck were designed to incorporate this water molecule in the inhibitor structure.[11] In the dimeric crystal structures of HIV-1 protease the flaps of both monomers are related by crystallographic two-fold symmetry. Abbott Laboratories has explored this symmetry in designing a series of potent C_2 symmetric inhibitors of HIV protease. Prototype leads of this class are represented by the diols **1** and **2**. It has been found that the 3*R*,4*S* configuration of the diol gives the highest potency.[12,13] Several groups have reported on the synthesis of the 3*R*,4*R* isomer (**1** and **2**) starting from D-mannitol.[14-17]

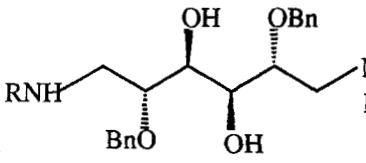
In this paper we describe the synthesis of two novel classes of C_2 symmetric compounds, exemplified by **6** and **16**, utilising readily available D-mannitol as chiral starting material. Inversion of one hydroxyl in **6** gave compound **9**. Compound **9** was predicted, based on molecular modeling, to give a good structural overlap with A-77003 complexed with HIV-1 protease.[13] Work is currently in progress to dock these inhibitors into the active site of HIV-1 protease.

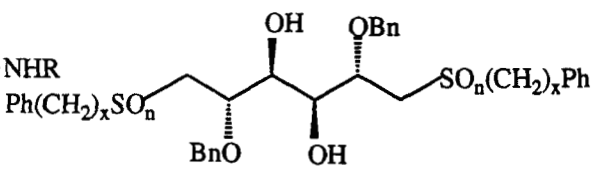




1 R=Boc
2 R=Val-Z

9





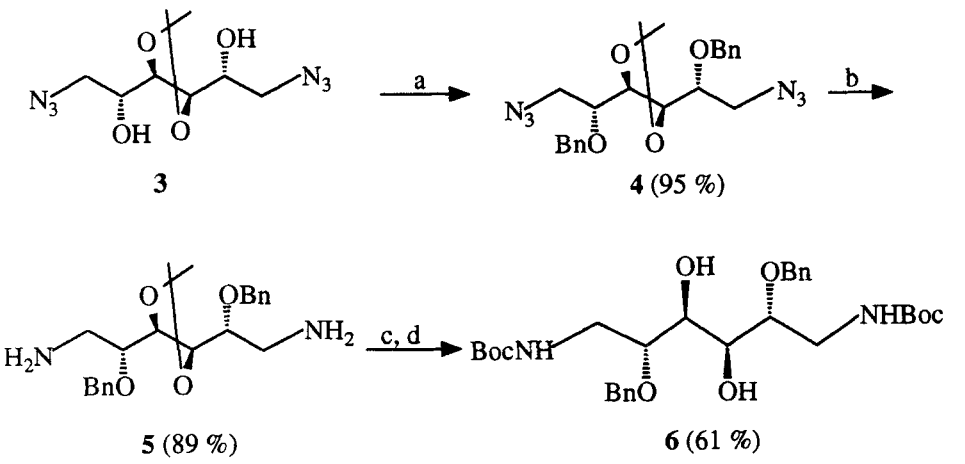
6 R=Boc
11 R=Val-Z

16 n=2; x=0 **19** n=2; x=1
17 n=1; x=0 **20** n=1; x=1

RESULTS AND DISCUSSION

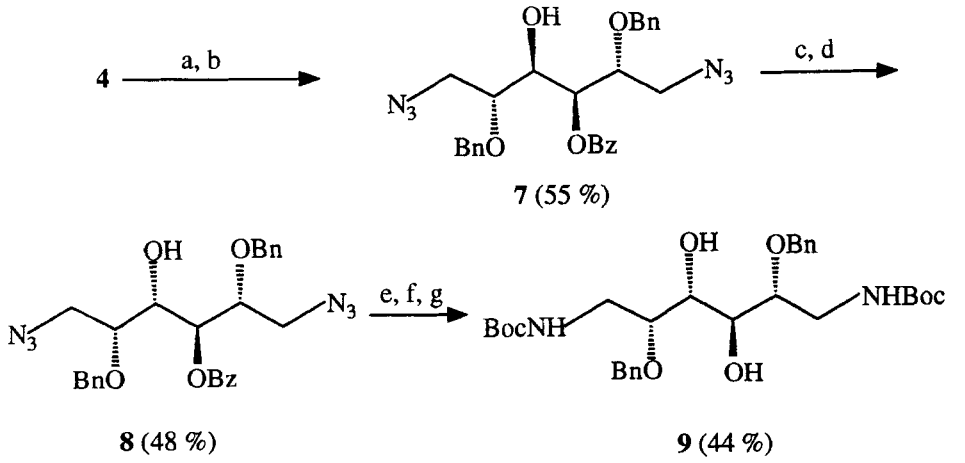
1,6-Diazido-1,6-dideoxy-3,4-*O*-isopropylidene-D-mannitol **3**[18] was benzylated using benzyl bromide-sodium hydride in dry *N,N*-dimethylformamide to give **4** in 95 % yield (Scheme 1). Reduction of the azide groups with triphenylphosphine in dry methanol gave the diamino derivative **5** in 89 % yield. Hydrolysis of the isopropylidene group in 40 % aqueous acetic acid at 90 °C followed by reaction of the resulting diamino diol with di-*tert*-butyl dicarbonate in pyridine-dichloromethane (2:1) gave compound **6** in 61 % yield from **5**.

To synthesize the 3-epimer of **6** the isopropylidene group of **4** was hydrolyzed to give the diol which was monobenzoylated using phase transfer conditions to give **7** in 55 % overall yield (Scheme 2).[19,20] Inversion of the 3-hydroxy group was accomplished using an oxidation reduction procedure. Treatment of **7** with pyridinium chlorochromate in dichloromethane gave the 3-keto compound in 91 % yield. Subsequent reduction with sodium borohydride in dry methanol gave a mixture of the diastereomeric alcohols **7** and **8**. Separation by column chromatography gave pure **8** in 53 % yield and pure **7** in 25 % yield along with additional mixed fractions. Saponification of the benzoyl group,



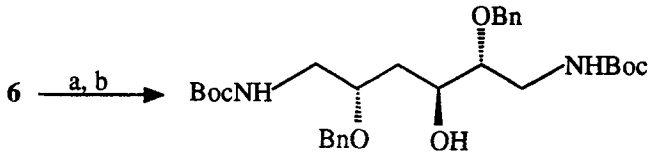
a. BnBr,NaH, DMF; b. PPh_3, MeOH; c. 40 % HOAc in H_2O, 90 °C; d. Boc_2O, pyridine-CH_2Cl_2 (2:1)

Scheme 1



a. 40 % HOAc in H_2O; b. $(Bu)_4NHSO_4$, BzCl, CH_2Cl_2, 5 % NaOH in H_2O; c. PCC, NaOAc, CH_2Cl_2, reflux; d. $NaBH_4$, MeOH; e. 1M NaOMe in MeOH; f. PPh_3, MeOH; g. Boc_2O, CH_2Cl_2.

Scheme 2



10 (40 %)

a. *N,N*-thiocarbonyldiimidazole, THF, reflux; b. Bu_3SnH, AIBN, toluene, reflux.

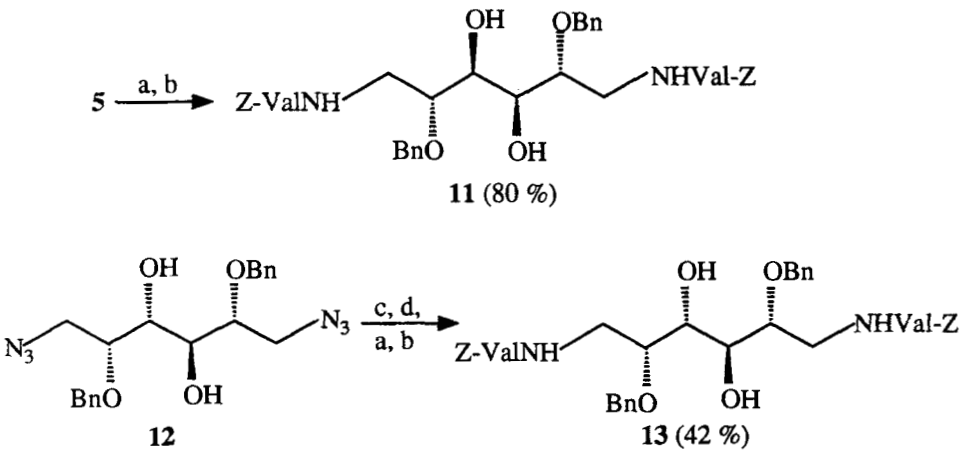
Scheme 3

reduction of the azides to amines followed by reaction of the amines with di-*tert*-butyl dicarbonate gave compound **9** in 44 % yield from **8**.

To synthesize the 3-deoxy analogue compound **6** was reacted with *N,N*-thiocarbonyldiimidazole in dry tetrahydrofuran to give the corresponding cyclic thiocarbonate which was reduced with tributyltin hydride and α,α-azoisobutyronitrile in dry toluene to give compound **10** in 40 % yield (Scheme 3).

To enable comparison with the Abbott compound **2**, the bis-Val-Z compound **11** was synthesized. Thus, **5** was reacted with *N*-benzyloxycarbonyl-L-valine in the precence of 1-isobutoxycarbonyl-2-isobutoxy-1,2-dihydroquinoline in dry dichloromethane to give the bis-Val-Z derivative (Scheme 4).[21] Hydrolysis of the isopropylidene group in 4 % hydrochloric acid in methanol gave compound **11** in 80 % yield from **5**. Compound **12** previously obtained in the synthesis of compound **9** was used for the synthesis of the bisvaline derivative **13** with inverted configuration at one of the central hydroxyls. Protection of the central diol with an isopropylidene ketal, reduction of the azides to amines, coupling with the protected valine derivative and, deprotection of the ketal gave **13** in an overall yield of 42 % from **12**.

For the synthesis of the sulfone and sulfoxide analogues **16** and **17** 1,2:5,6-dianhydro-3,4-*O*-isopropylidene-D-mannitol **14**[18] was opened with sulfur nucleophiles (Scheme 5). Reaction of the diepoxide with thiophenol and potassium carbonate in dry *N,N*-dimethylformamide at 110 °C gave the 1,6-dithiophenyl derivative which was benzylated to give compound **15** in 84 % overall yield. The sulfur atoms were then oxidized either to the sulfones with 3-chloroperbenzoic acid and sodium hydrogen carbonate in dichloromethane or to the diastereomeric sulfoxides using sodium metaperiodate in methanol:water (10:1). Hydrolysis of the isopropylidene ketal gave compounds **16** in 55 % yield and diastereomeric **17** in 36 % yield from **15**. Oxidation to



a. Z-Val-OH, HDQ, CH_2Cl_2; b. 4 % HCl in MeOH; c. 2,2-dimethoxypropane,*p*-TsOH; d. PPh_3, MeOH.

Scheme 4

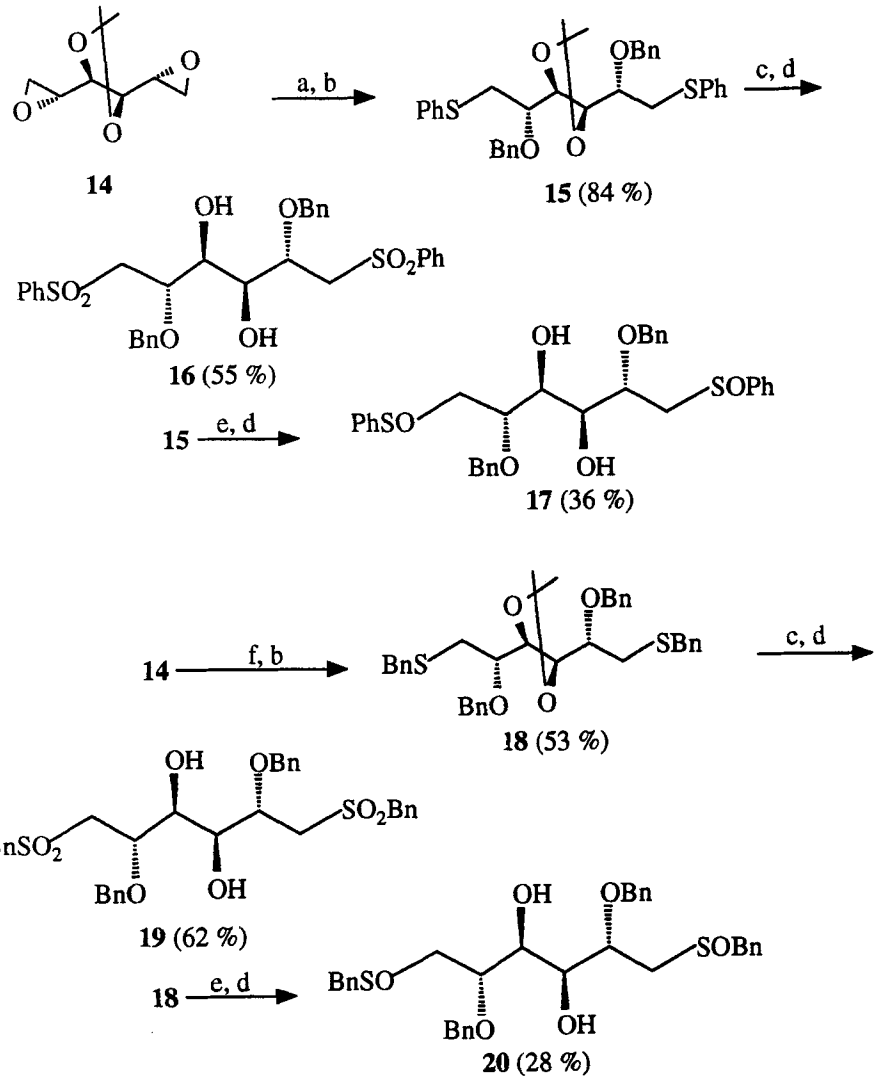
give the diastereomeric sulfoxides was also performed in comparable yield using 3-chloroperbenzoic acid (1.1 equiv. *m*CPBA/sulfur atom). Opening of the diepoxide with benzyl mercaptan and subsequent treatment as described above resulted in compounds **19** and **20** (Scheme 5).

SUMMARY

Further design and synthesis of structurally related compounds is underway in order to find more potent and readily accessible HIV protease inhibitors.

BIOLOGICAL RESULTS

Compounds **6**, **9**, **10**, **11**, **13**, **16**, **17**, **19**, and **20** were tested for inhibition of HIV multiplication in an XTT assay in M 4 cells. Unfortunately no significant activity against HIV-1 protease was observed. Only one of the compounds showed any inhibitory effect against HIV protease. Compound **9** inhibited the enzyme by 25 % at 30



a. PhSH, DMF, K_2CO_3, 110 °C; b. BnBr, NaH, DMF; c. mCPBA, $NaHCO_3$, CH_2Cl_2; d. 4 % HCl in MeOH; e. $NaIO_4$, MeOH:H_2O (10:1); f. BnSH, DMF, K_2CO_3, 110 °C.

Scheme 5

μM concentration. Higher concentrations could not be used due to limited solubility. The other compounds were inactive even at their highest concentration, at least 20 μM.

EXPERIMENTAL

General procedures. Optical rotations were determined using a Perkin-Elmer 141 polarimeter. NMR spectra were recorded using a JEOL FX-100 instrument. Chemical shifts are given in ppm relative to tetramethylsilane. TLC was performed using silica gel plates (F_{254}, Merck) and the spots were detected with UV light and/or by charring with ethanol-sulfuric acid-acetic acid-*p*-anisaldehyde, 90:3:1:2. Column chromatography was performed on silica gel 60 (0.040-0.063 mm, Merck).

Inhibition of HIV replication: The effect of synthesized compounds on the cytopathic effect of HIV on MT4 cells was measured as previously described.[22]

Inhibition of HIV protease: Synthesized compounds were tested as inhibitors of HIV protease using a chromophoric peptide substrate in a continuous spectrophotometric assay as described by Nillroth *et al.*[23]

1,6-Diazido-2,5-di-*O*-benzyl-1,6-dideoxy-3,4-*O*-isopropylidene-D-mannitol (4). Sodium hydride (0.19 g, 7.8 mmol, 80 % dispersion in mineral oil) was added in portions to a stirred ice cooled solution of 1,6-diazido-1,6-dideoxy-3,4-*O*-isopropylidene-D-mannitol **3**[18] (0.63 g, 2.3 mmol) in dry *N,N*-dimethylformamide (5.7 mL). The ice bath was removed and the mixture was stirred at room temperature for 30 min. The mixture was again cooled in an ice bath and benzyl bromide (0.61 mL, 5.1 mmol) was added. Methanol was added after stirring at room temperature for 24 h. The mixture was concentrated and the residue was taken up in dichloromethane, washed with water, dried ($MgSO_4$) and concentrated. Column chromatography (toluene-ethyl acetate, 15:1) gave compound **4** (0.99 g, 95 % yield): $[\alpha]_D$ +2.0° (*c* 0.94, chloroform); ^{13}C NMR ($CDCl_3$): δ 27.1 ($C(CH_3)_2$), 50.8 (C-1, C-6), 72.6, 78.1, 79.1 (OCH_2Ph, C-2, C-3, C-4, C-5), 109.9 ($C(CH_3)_2$), 125.0-128.7, 137.0 (aromatic C).

Anal. Calcd for $C_{23}H_{28}O_4N$: C, 61.0; H, 6.2; N, 18.6. Found: C, 61.2; H, 6.3; N, 18.7.

1,6-Diamino-2,5-di-*O*-benzyl-1,6-dideoxy-3,4-*O*-isopropylidene-D-mannitol (5). Compound **4** (0.77 g, 1.6 mmol) was dissolved in dry methanol (33 mL) and treated with triphenylphosphine (0.89 g, 3.4 mmol). After stirring at room temperature for 20 h the reaction mixture was concentrated to dryness. Purification by column chromatography (CH_2Cl_2-MeOH, 2:1, containing 0.25 % concentrated NH_3) gave compound **5** (0.58 g, 89 % yield): $[\alpha]_D$ +16° (*c* 0.64, chloroform); ^{13}C NMR ($CDCl_3$): δ 27.2 ($C(CH_3)_2$), 41.1

(C-1, C-6), 71.9, 78.4, 81.2 (OCH_2Ph, C-2, C-3, C-4, C-5), 109.4 ($C(CH_3)_2$), 127.5, 128.0, 137.8 (aromatic C).

Anal. Calcd for $C_{23}H_{32}O_4N_2x0.7\ H_2O$: C, 66.9; H, 8.1; N, 6.8. Found: C, 66.9; H, 8.1; N, 6.7.

2,5-Di-*O*-benzyl-1,6-di-[(*tert*-butoxycarbonyl)amino]-1,6-dideoxy-D-mannitol (6). Compound **5** (0.048 g, 0.12 mmol) was treated with acetic acid (40 % solution in water) containing a catalytic amount of concentrated hydrochloric acid at 90 °C. After stirring at 90 °C for 6 h the reaction mixture was concentrated and co-distilled several times with dry toluene to give a crude compound of 1,6-diamino-2,5-di-*O*-benzyl-1,6-dideoxy-D-mannitol (0.040 g, 0.11 mmol, 92 % yield), which was dissolved in a mixture of pyridine-dichloromethane (2:1, 1.5 mL) and cooled to 0 °C. Di-*tert*-butyl dicarbonate (0.047 g, 0.22 mmol) was added and the mixture was stirred at room temperature for 6 h. Evaporation of volatile materials and column chromatography (toluene-ethyl acetate, 1:1) gave compound **6** (0.041 g, 66 % yield): mp 108-109 °C; $[\alpha]_D$ -69° (*c* 1.0, chloroform); ^{13}C NMR ($CDCl_3$): δ 28.1 ($OC(CH_3)_3$), 39.9 (C-1, C-6), 68.2, 72.2, 77.6, 79.6 (C-2, C-3, C-4, C-5, OCH_2Ph, $OC(CH_3)_3$), 127.6, 128.1, 137.9 (aromatic C), 157.1 (C=O).

Anal. Calcd for $C_{30}H_{44}O_8N_2$: C, 64.3; H, 7.9; N, 5.0. Found: C, 64.2; H, 8.0; N, 5.0.

1,6-Diazido-4-*O*-benzoyl-2,5-di-*O*-benzyl-1,6-dideoxy-D-mannitol (7). The isopropylidene ketal in compound **4** was hydrolyzed as described for compound **5**. Purification by column chromatography (toluene-ethyl acetate, 2:1) gave 1,6-diazido-2,5-di-*O*-benzyl-1,6-dideoxy-D-mannitol in 65 % yield. ^{13}C NMR ($CDCl_3$): δ 50.8 (C-1, C-6), 68.8, 72.6, 78.4 (C-2, C-3, C-4, C-5, OCH_2Ph), 127.7, 128.2, 136.9 (aromatic C).

1,6-Diazido-2,5-di-*O*-benzyl-1,6-dideoxy-D-mannitol (0.72 g, 1.7 mmol), tetrabutylammonium hydrogensulphate (0.12 g, 0.35 mmol) and benzoyl chloride (0.22 mL, 1.9 mmol) were dissolved in dichloromethane (43 mL). The mixture was cooled to 0 °C and aqueous sodium hydroxide (3.6 mL of a 5 % solution) was added. After stirring at 0 °C for 1 h the organic layer was separated, washed with water, dried ($MgSO_4$) and concentrated. Purification by column chromatography (toluene-ethyl acetate, 9:1) gave compound **7** (0.76 g, 84 % yield): $[\alpha]_D$ -48° (*c* 0.82, chloroform); ^{13}C NMR ($CDCl_3$): δ 50.1, 51.6 (C-1, C-6), 69.4, 71.0, 72.5, 73.9, 78.9 (C-2, C-3, C-4, C-5, OCH_2Ph), 127.7-129.6, 133.3, 136.5, 136.7 (aromatic C), 165.2 (C=O).

Anal. Calcd for $C_{27}H_{28}O_5N_6$: C, 62.8; H,5.5; N, 16.3. Found: C, 62.9; H, 5.6; N, 16.4.

1,6-Diazido-4-*O*-benzoyl-2,5-di-*O*-benzyl-1,6-dideoxy-D-altritol (8). Compound **7** (0.50 g, 0.97 mmol), sodium acetate (0.081 g, 0.99 mmol) and pyridinium chlorochromate (0.85 g, 3.9 mmol) were dissolved in dichloromethane (24 mL) and heated at reflux temperature. After 6 h diethyl ether (24 mL) was added and the mixture was stirred for 5 min and then put on a silica gel column. The oxidized compound was eluated with ethyl acetate (0.45 g, 91 % yield). The compound (0.45 g, 0.87 mmol) was dissolved in dry methanol (7 mL) and cooled to 0 °C. Sodium borohydride (20 mg, 0.52 mmol) was added and the mixture was stirred for 30 min at room temperature, quenched with water and concentrated. The residue was dissolved in chloroform, washed with water, acetic acid (2 % solution in water), saturated sodium hydrogen carbonate and water, dried ($MgSO_4$) and concentrated. Separation of the diastereomeric alcohols **7** and **8** by column chromatography (toluene-ethyl acetate, 9:1) gave compound **8** (0.24 g, 53 % yield) and compound **7** (0.11g, 25 %) along with additional mixed fractions. **8**: $[\alpha]_D$ -35° (*c* 0.72, chloroform); ^{13}C NMR ($CDCl_3$): δ 51.1, 51.4 (C-1, C-6), 69.9, 71.6, 72.7, 73.7, 76.1, 78.0 (C-2, C-3, C-4, C-5, OCH_2Ph), 127.7-129.5, 133.3, 137.1 (aromatic C), 165.0 (C=O).

Anal. Calcd for $C_{27}H_{28}O_5N_6$: C, 62.8; H, 5.5; N, 16.3. Found: C, 62.7; H, 5.6; N, 16.4.

2,5-Di-*O*-benzyl-1,6-di-[(*tert*-butoxycarbonyl)amino]-1,6-dideoxy-D-altritol (9) Compound **8** (0.13 g, 0.25 mmol) was dissolved in dry methanol (3 mL) and treated with 1M sodium methoxide in methanol (0.3 mL) at room temperature for 6 h. The reaction mixture was concentrated and purification by column chromatography (toluene-ethyl acetate, 6:1) to give 1,6-diazido-2,5-di-*O*-benzyl-1,6-dideoxy-D-altritol (0.096 g, 92 % yield). ^{13}C NMR ($CDCl_3$): δ 49.9, 51.4 (C-1, C-6), 70.4, 71.0, 72.1, 73.2, 78.9 (C-2, C-3, C-4, C-5, OCH_2Ph), 127.9-128.1, 136.9 (aromatic C). Reduction of the azido groups was performed as described for the reduction of compound **4**. The diamino compound was obtained in 62 % yield. Reaction with di-*tert*-butyl dicarbonate in dichloromethane as described for the synthesis of compound **6** gave compound **9** in 77 % yield: $[\alpha]_D$ -10° (*c* 1.0, chloroform); ^{13}C NMR ($CDCl_3$): δ 28.3 ($OC(CH_3)_3$), 39.0, 40.1 (C-1, C-6), 70.0, 71.2, 72.1, 72.5, 76.1, 77.0, 79.2, 79.5 (C-2, C-3, C-4, C-5, OCH_2Ph, $OC(CH_3)_3$), 127.7-128.2, 137.2, 137.6 (aromatic C), 156.1, 156.4 (C=O).

Anal. Calcd for $C_{30}H_{44}O_8N_2$: C, 64.3; H, 7.9; N, 5.0. Found: C, 64.3; H, 7.8; N, 4.9.

2,5-Di-*O*-benzyl-1,6-di-[(*tert*-butoxycarbonyl)amino]-1,6-dideoxy-3-deoxy-D-mannitol (10). Compound **6** (0.41 g, 0.73 mmol) was dissolved in dry tetrahydrofuran (6 mL) and heated at 50 °C while *N,N*-thiocarbonyldiimidazole (0.27 g, 1.5 mmol) was added. The reaction mixture was heated under reflux for 6 h, cooled to room

temperature, filtered and concentrated. Column chromatography (toluene-ethyl acetate, 2:1) gave the thiocarbonate of compound **6** (0.31 g, 70 % yield). ^{13}C NMR ($CDCl_3$): δ 28.3 ($OC(CH_3)_3$), 39.4 (C-1, C-6), 73.0, 76.4, 79.9, 81.9 (C-2, C-3, C-4, C-5, OCH_2Ph, $OC(CH_3)_3$), 128.1, 128.4, 136.5 (aromatic C), 155.5 (C=O), 190.4(C=S).

The thiocarbonate (0.16 g, 0.27 mmol), tributyltin hydride (0.14 mL, 0.54 mmol) and α,α-azoisobutyronitrile (4.1 mg, 25 μmol) in dry toluene (4 mL) were added under nitrogen to refluxing toluene (6 mL) over a period of 15 min. After 2 and 4 h additional portions of Bu_3SnH (72 μL) and AIBN (1.4 mg) were added. After 8 h the temperature was lowered to 40 °C and 10 % aqueous sodium hydroxide solution (3 mL) was added. After stirring at 40 °C for 12 h the organic layer was separated and the aqueous layer was extracted with diethyl ether. The organic layers were combined, washed with water until neutral pH, dried ($MgSO_4$) and concentrated. Purification by column chromatography (toluene-ethyl acetate, 1:1) gave compound **10** (84 mg, 57 % yield): $[\alpha]_D$ -35° (*c* 1.2, chloroform); ^{13}C NMR ($CDCl_3$): δ 28.3 ($OC(CH_3)_3$), 35.1, 39.1, 43.1 (C-1, C-3, C-6), 67.1, 71.5, 71.8, 75.3, 79.0, 79.6, 81.3 (C-2, C-4, C-5, OCH_2Ph, $OC(CH_3)_3$), 127.6-128.7, 137.6, 138.0 (aromatic C), 155.8, 156.8 (C=O).

Anal. Calcd for $C_{30}H_{44}O_7N_2$: C, 66.2; H, 8.1; N, 5.1. Found: C, 66.0; H, 8.1; N, 5.1.

2,5-Di-*O*-benzyl-1,6-di-[*N*-[*N*-[(benzyloxy)carbonyl]-L-valinyl]amino]-1,6-dideoxy-D-mannitol (11). Compound **5** (0.065 g, 0.16 mmol) and *N*-benzyloxycarbonyl-L-valine (0.082 g, 0.32 mmol) were dissolved in dry dichloromethane (1 mL) at room temperature. 1-Isobutoxycarbonyl-2-isobutoxy-1,2-dihydroquinoline (0.093 mL, 0.32 mmol) in dry dichloromethane (0.2 mL) was added and the mixture was stirred at room temperature for 16 h. The reaction mixture was diluted with dichloromethane, washed with 10 % aqueous citric acid, 1 M sodium hydrogen carbonate, and water, dried ($MgSO_4$) and concentrated. Purification by column chromatography (toluene-ethyl acetate, 1:1) gave the protected bisvaline derivative (0.12 g, 86 % yield). The product was treated with 4 % hydrochloric acid in methanol at room temperature for 1 h. Concentration followed by column chromatography (5 % methanol in chloroform) gave compound **11** (0.11 g, 93 % yield): mp 246-248 °C, $[\alpha]_D$ -33° (*c* 0.41, chloroform); ^{13}C NMR (DMSO-d_6): δ 18.4, 19.4 ($CH(CH_3)_2$), 30.4 ($CH(CH_3)_2$), 38.9,(C-1, C-6), 60.4 (C_α), 65.5, 69.1, 71.2, 77.6 (C-2, C-3, C-4, C-5, OCH_2Ph), 127.1-128.2, 137.0, 138.8 (aromatic C), 155.9, 171.9 (NHCOO, NHCO).

Anal. Calcd for $C_{46}H_{58}O_{10}N_4$: C, 66.8; H, 7.1; N, 6.8. Found: C, 66.7; H, 7.1; N, 6.8.

2,5-Di-*O*-benzyl-1,6-di-[*N*-[*N*-[(benzyloxy)carbonyl]-L-valinyl]amino]-1,6-dideoxy-D-altritol (13). 1,6-Diazido-2,5-di-*O*-benzyl-1,6-dideoxy-D-altritol **12** (0.12 g,

0.29 mmol) was treated with 2,2-dimethoxypropane (1 mL) containing a catalytic amount of *p*-toluenesulphonic acid at room temperature for 16 h. The reaction mixture was diluted with dichloromethane, washed with saturated sodium hydrogen carbonate and water, dried ($MgSO_4$) and concentrated. Purification by column chromatography (toluene-ethyl acetate, 9:1) gave 1,6-diazido-2,5-di-*O*-benzyl-1,6-dideoxy-3,4-*O*-isopropylidene-D-altritol (0.13 g, 97 % yield). ^{13}C NMR ($CDCl_3$): δ 25.5, 27.2 ($C(CH_3)_2$), 49.8, 51.6 (C-1, C-6), 70.9, 72.0, 74.8, 75.5, 75.8, 77.6 (C-2, C-3, C-4, C-5, OCH_2Ph), 108.7 ($C(CH_3)_2$), 127.4-128.7, 136.3, 137.8 (aromatic C).

Reduction to the diamino compound was performed in a 68 % yield as described for compound **4**. Coupling with the protected valine derivative and deprotection of the ketal were performed as described for the synthesis of compound **11** in 84 % and 76 % yield respectively. **13**: mp 231-233 °C; $[\alpha]_D$ -8.9° (*c* 0.63, chloroform); ^{13}C-NMR (DMSO-d_6): δ 18.4, 18.5, 19.5 ($CH(CH_3)_2$), 30.3, 30.4 ($CH(CH_3)_2$), 38.9, 40.6 (C-1, C-6), 60.6 (C_α), 65.5, 69.4, 71.0, 72.6, 79.2, 79.4 (C-2, C-3, C-4, C-5, OCH_2Ph), 127.6-128.2, 137.0, 138.9, 139.1 (aromatic C), 156.0, 171.2, 171.5 (NHCOO, NHCO).

Anal. Calcd for $C_{46}H_{58}O_{10}N_4$: C, 66.8; H; 7.1; N, 6.8. Found: C, 66.7; H, 7.2; N, 6.8.

2,5-Di-*O*-benzyl-1,6-dideoxy-3,4-*O*-isopropylidene-1,6-diphenylthio-D-mannitol (15). 1,2:5,6-Dianhydro-3,4-*O*-isopropylidene-D-mannitol **14**[18] (0.54 g, 2.9 mmol), thiophenol (1.5 mL, 15 mmol) and dry potassium carbonate (0.19 g, 1.4 mmol) were stirred in dry *N,N*-dimethylformamide (5 mL) at 110 C for 4 h. The mixture was diluted with toluene, washed with 1 M aqueous sodium hydroxide, dried ($MgSO_4$) and concentrated. Column chromatography (toluene-ethyl acetate, 4:1) gave 1,6-dideoxy-3,4-*O*-isopropylidene-1,6-diphenylthio-D-mannitol (1.1 g, 89 % yield). Benzylation of the hydroxyls as described for the synthesis of compound **4** gave compound **15** in 94 % yield: $[\alpha]_D$ +36° (*c* 0.79, chloroform); ^{13}C NMR ($CDCl_3$): δ 27.1 ($C(CH_3)_2$), 34.9 (C-1, C-6), 72.5, 78.4, 79.0 (C-2, C-3, C-4, C-5, OCH_2Ph), 109.4 ($C(CH_3)_2$), 125.5-128.9, 136.1, 137.4 (aromatic C).

Anal. Calcd for $C_{35}H_{38}O_4S_2$: C, 71.6; H, 6.5; S, 10.9. Found: C, 71.5; H, 6.5; S, 11.0

2,5-Di-*O*-benzyl-1,6-dideoxy-1,6-diphenylsulfonyl-D-mannitol (16). Compound **15** (0.27 g, 0.46 mmol) was dissolved in dichloromethane (4 mL). Sodium hydrogen carbonate (0.34 g, 4.0 mmol) was added and the mixture was cooled in an ice bath (-5 - 0 °C). 3-Chloroperbenzoic acid (0.64 g of a 55 % mixture, stabilized with water and 3-chlorobenzoic acid, 2.0 mmol) was added and the mixture was stirred at room temperature for 18 h. The reaction was quenched by the addition of concentrated aqueous ammonium hydroxide and stirred for 30 min. The organic layer was separated,

washed with concentrated aqueous ammonium hydroxide, dried ($MgSO_4$) and concentrated. Purification by column chromatography (toluene-ethyl acetate, 4:1) gave 2,5-di-*O*-benzyl-1,6-dideoxy-3,4-*O*-isopropylidene-1,6-diphenylsulfonyl-D-mannitol (0.22 g, 73 % yield). Deprotection of the isopropylidene group was performed in a 76 % yield as described for the synthesis of compound **11**. **16**: mp 130-132 °C; $[\alpha]_D$ -9.7° (*c* 0.79, chloroform); ^{13}C NMR ($CDCl_3$): δ 58.0 (C-1, C-6), 69.9, 72.6, 74.6 (C-2, C-3, C-4, C-5, OCH_2Ph), 127.5-128.9, 133.4, 136.8, 139.6 (aromatic C).

Anal. Calcd for $C_{32}H_{34}O_8S_2$: C, 62.9; H, 5.6; S, 10.5. Found: C, 62.8; H, 5.6; S, 10.5.

Diastereomeric mixture of 2,5-Di-*O*-benzyl-1,6-dideoxy-1,6-diphenylsulfinyl-D-mannitol (17). Compound **15** (0.13 g, 0.22 mmol) and sodium metaperiodate (0.10 g, 0.48 mmol) were stirred in methanol-water, 10:1, (2.5 mL) at room temperature for 18 h. Most of the solvent was evaporated and the resulting mixture was extracted several times with chloroform. The combined organic layers were dried ($MgSO_4$) and concentrated. Column chromatography (toluene-ethyl acetate, 1:5) gave a diastereomeric mixture of the sulfoxides (74 mg, 55 % yield). Deprotection of the isopropylidene group as described for the synthesis of compound **11** gave compound **17** in 65 % yield: $[\alpha]_D$ +40° (*c* 1.2, chloroform); ^{13}C NMR ($CDCl_3$): δ 59.7, 59.8, 60.2, 62.0 (C-1, C-6), 70.7, 71.3, 72.0, 72.2, 73.9, 74.7, 75.0, 77.1 (C-2, C-3, C-4, C-5, OCH_2Ph), 123.6, 123.8, 127.6-129.0, 130.8, 137.4, 143.0, 143.4, 143.6 (aromatic C).

Anal. Calcd for $C_{32}H_{34}O_6S_2$: C, 66.4; H, 5.9; S, 11.1. Found: C, 66.2; H, 6.0; S, 10.9.

2,5-Di-*O*-benzyl-1,6-dibenzylthio-1,6-dideoxy-3,4-*O*-isopropylidene-D-mannitol (18). 1,2:5,6-Dianhydro-3,4-*O*-isopropylidene-D-mannitol **14**[18] was reacted with benzyl mercaptan and benzylated as described for the synthesis of compound **15** in 94 % and 56 % yield, respectively. **18**: $[\alpha]_D$ -2.0° (*c* 0.94, chloroform); ^{13}C NMR ($CDCl_3$): δ 27.0 ($C(CH_3)_2$), 31.7, 36.8 (C-1, C-6, SCH_2Ph), 72.0, 79.0 (C-2, C-3, C-4, C-5, OCH_2Ph), 109.1 ($C(CH_3)_2$), 126.4-128.5, 137.5, 137.8 (aromatic C).

Anal. Calcd for $C_{37}H_{42}O_4S_2$: C, 72.3; H, 6.9; S, 10.4. Found: C, 72.1; H, 7.0; S, 10.6

2,5-Di-*O*-benzyl-1,6-dibenzylsulfonyl-1,6-dideoxy-D-mannitol (19). Compound **18** was oxidized and deprotected as described for the synthesis of compound **16** in 79 % and 78 % yield, respectively. **19**: mp 209-211 °C; $[\alpha]_D$ -11° (*c* 0.16, chloroform); ^{13}C NMR (DMSO-d_6): δ 54.3, 59.9 (C-1, C-6, SCH_2Ph), 69.9, 72.1, 75.8 (C-2, C-3, C-4, C-5, OCH_2Ph), 127.6-128.4, 131.1, 137.9 (aromatic C).

Anal. Calcd for $C_{34}H_{38}O_8S_2$: C, 63.9; H, 6.0; S, 10.0. Found: C, 63.8; H, 6.1; S, 10.1

Diastereomeric mixture of 2,5-Di-*O*-benzyl-1,6-dibenzylsulfinyl-1,6-dideoxy-D-mannitol (20). Compound **18** was oxidized and deprotected as described for the synthesis of the diastereomeric compound **17** in 55 % and 50 % yield, respectively. **20**: $[\alpha]_D$ +19° (*c* 1.5, chloroform); ^{13}C NMR ($CDCl_3$): δ 52.8, 53.0, 55.2, 55.3, 58.4, 59.0 (C-1, C-6, SCH_2Ph), 70.6, 70.7, 71.2, 71.4, 72.1, 72.2, 73.8, 74.5, 74.8, 77.0 (C-2, C-3, C-4, C-5, OCH_2Ph), 127.7-129.9, 137.3 (aromatic C).

Anal. Calcd for $C_{34}H_{38}O_6S_2$: C, 67.3; H, 6.3; S, 10.6. Found: C, 67.1; H, 6.4; S, 10.6.

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REFERENCES

1. L. Ratner, W. Haseltine, R. Patarca, K. J. Livak, B. Starcich, S. F. Josephs, E. R. Doran, J. A. Rafalski, E. A. Whitehorn, K. Baumeister, L. Ivanoff, S. R. Petteway, Jr., M. L. Pearson, J. A. Lautenberger, T. S. Papas, J. Ghrayeb, N. T. Chang, R. C. Gallo, and F. Wong-Staal, *Nature*, **313**, 277 (1985).
2. R. A. Kramer, M. D. Schaber, A. M. Skalka, K. Ganguly, F. Wong-Staal, and E. P. Reddy, *Science*, **231**, 1580 (1986).
3. H. Toh, M. Ono, K. Saigo, and T. Miyata, *Nature*, **315**, 691 (1985).
4. N. E. Kohl, E. A. Emini, W. A. S. Chleif, L. J. Davis, J. C. Heimbach, R. A. F.Dixon, E. M. Scolnick, and I. S. Sigal, *Proc. Natl. Acad. Sci. USA*, **85**, 4686 (1988).
5. T. J. McQuade, A. G. Tomasselli, L. Liu, V. Karacostas, B. Moss, T. K. Sawyer, R. L. Heinrikson, and W. G. Tarpley, *Science*, **247**, 454 (1990).
6. A. Wlodawer and J. W. Erickson, *Annu. Rev. Biochem.*, **62**, 543 (1993).
7. J. A. Martin, *Antiviral Res.*, **17**, 265 (1992).
8. S. Redshaw, *Exp. Opin. Invest. Drugs*, **3**, 273 (1994).
9. K. Appelt, *Perspectives in Drug Discovery and Design*, **1**, 23 (1993).
10. J. W. Erickson and S. W. Fesik, *Annu. Rep. Med. Chem.*, **27**, 271 (1992).
11. P. Y. S. Lam, P. K. Jadhav, C. J. Eyermann, C. N. Hodge, Y. Ru, L.T. Bacheler, J. L. Meek, M. J. Otto, M. M. Rayner, Y. N. Wong, C-H. Chang, P. C. Weber, D. A. Jackson, T. R. Sharpe, and S. Erickson-Viitanen, *Science*, **263**, 380 (1994).
12. D. J. Kempf, D. W. Norbeck, L. Codacovi, X. C. Wang, W. E. Kohlbrenner, N. E. Wideburg, D. A. Paul, M. F. Knigge, S. Vasavanonda, A. Craig-Kennard, A. Saldivar, W. Rosenbrook, Jr., J. J. Clement. J. J. Plattner, and J. Erickson, *J. Med. Chem.*, **33**, 2687 (1990).
13. M. V. Hosur, T. N. Bhat, D. J. Kempf, E. T. Baldwin, B. Liu, S. Gulnik, N. E. Wideburg, D. W. Norbeck, K. Appelt, and J. W. Erickson, *J. Am. Chem. Soc.*, **116**, 847 (1994).

14. B. Chenera, J. C. Boehm, and G. B. Dreyer, *Bioorg. & Med. Chem. Lett.*, **1**, 219 (1991).
15. A. K. Ghosh, S. P. McKee, and W. J. Thompson, *Tetrahedron Lett.*, **32**, 5729 (1991).
16. P. K. Jadhav and F. J. Woerner, *Bioorg. & Med. Chem. Lett.*, **2**, 353 (1992).
17. T. Yokomatsu, K. Suemune, and S. Shibuya, *Heterocycles,* **35**, 577 (1993).
18. Y. Le Merrer, A. Duréault, C. Greck, D. Micas-Languin, C. Gravier, and J-C. Depezay, *Heterocycles,* **25**, 541 (1987).
19. P. J. Garegg, T. Iversen, and S. Oscarson, *Carbohydr. Res.*, **53**, C5 (1977).
20. P. J. Garegg, I. Kvarnström, A. Niklasson, G. Niklasson, and S. C. T. Svensson, *J. Carbohydr. Chem.*, **12 (7)**, 933 (1993).
21. M. Bodanszky and A. Bodanszky, *The Practice of Peptide Synthesis*, Springer-Verlag Berlin Heidelberg New York Tokyo 1984, ISBN 3-540-13471-9, ISBN 0-387-13471-9.
22. U. Nillroth, L. Vrang, G. Ahlsén, Y. Besidsky, J. Chattopadhyaya, I. Ugi, and U. H. Danielson, *Antiviral Chemistry and Chemotherapy,* **6**, 50 (1995).
23. U. Nillroth, Y. Besidsky, B. Classon, J. Chattopadhyaya, I. Ugi, and U. H. Danielson, *Drug Design and Discovery* **13**, 43 (1995).