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Impact of the Central Hydroxyl Groups on the Activity of Symmetrical HIV-1 Protease Inhibitors Derived From L-Mannaric Acid

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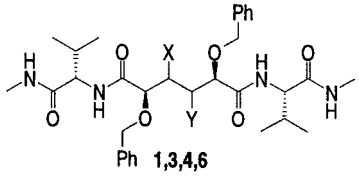
Abstract—The influence of the central hydroxyl groups on the anti-viral activity of symmetrical HIV-1 protease inhibitors derived from L-mannaric acid has been examined. L-Iditol was synthesized and used as a chiral precursor for the synthesis of the corresponding inhibitor with inverted configuration at C-3 and C-4. Key intermediates were 3,4-*O*-isopropylidene-L-iditol and the activated L-idaric acid succinimidyl ester. The configurations of the central hydroxyl groups required for optimal inhibition of the HIV-1 protease were determined to be the C-3*R* and C-4*R*, i.e. the L-manno-configuration. Three C_2-symmetric inhibitors were converted to their thiocarbonates and reduced to provide the corresponding hydroxyethyl transition-state mimics. Deletion of the C-4 hydroxyl group in these inhibitors gave no further improvement in the anti-viral activity. © 2000 Published by Elsevier Science Ltd.

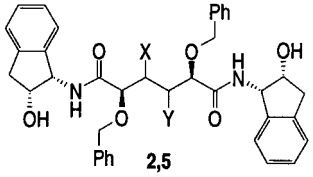
Introduction

The human immunodeficiency virus (HIV) has been identified as the etiologic agent of acquired immunodeficiency syndrome (AIDS).[1–3] The human immunodeficiency virus type 1 (HIV-1) encodes for an aspartic protease which has been shown to be essential for the formation of a mature, infectious virus.[4,5] Numerous reports describing potent protease inhibitors have been disclosed.[6–12] The HIV-1 protease inhibitors on the market are all non-symmetrical. The fact that the HIV-1 protease exists as a C_2-symmetric dimer has stimulated the design and synthesis of C_2-symmetric inhibitors.[13]

We have recently demonstrated that L-mannaric acid is a useful scaffold for the design and synthesis of potent C_2-symmetric HIV-1 protease inhibitors.[14] An attractive feature of these inhibitors is that they are readily available in just three chemical steps starting from commercially available starting materials. Compounds **1** and **2**, in particular, have been shown to be potent HIV-1 protease inhibitors in vitro (Table 1). From examination of the X-ray crystal structure of HIV-1 protease complex with the isoleucine analog of **1** and with **2**, it was evident that the hydroxyl-groups of the central diol do not bind symmetrically to the active site Asp 25/125 residues. One of the hydroxyl groups forms hydrogen bonds with both carboxyls while the other hydroxyl group points away

Table 1. Inhibitory activities of the compounds **1–6**





Compound	X,Y	K_i (nM)	ED_{50} (μM)
1	(*R*)–OH, (*R*)–OH	0.4	1.1
2	(*R*)–OH, (*R*)–OH	0.2	0.11
3	(*S*)–OH, (*S*)–OH	40	24
4	(*R*)–OH, H	2.1	1.2
5	(*R*)–OH, H	1.4	1.4
6	(*S*)–OH, H	530	No activity

Keywords: peptidomimetics; oxidation; hydrolysis; deoxygenation; HIV-1 protease.

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from the active site but still maintains one hydrogen bond to one of the Asp residues.

It has been reported that deletion of one of the hydroxyl groups of symmetric diol based HIV-1 protease inhibitors in some cases enhances anti-HIV activity in cell based assays.[15] The physicochemical background for this observed beneficial effect on anti-HIV activity can be rationalized from reduction of molecular weight, reduction of hydrogen-bond donating and accepting groups and an increase in lipophilicity; factors known to enhance permeability over biological membranes and increase passive absorption.[16,17]

In the present work we report on: (a) the deletion of the hydroxyl at C-4 in inhibitors **1** and **2** to give **4** and **5**, (b) the inversion of the configuration of the diol in **1** to give **3** and (c) the inversion of the configuration at C-3 of **4** to give **6**. These compounds were assayed for anti-HIV-1 protease activity and for in vitro anti-HIV activity (Table 1).

Results and Discussion

Chemistry

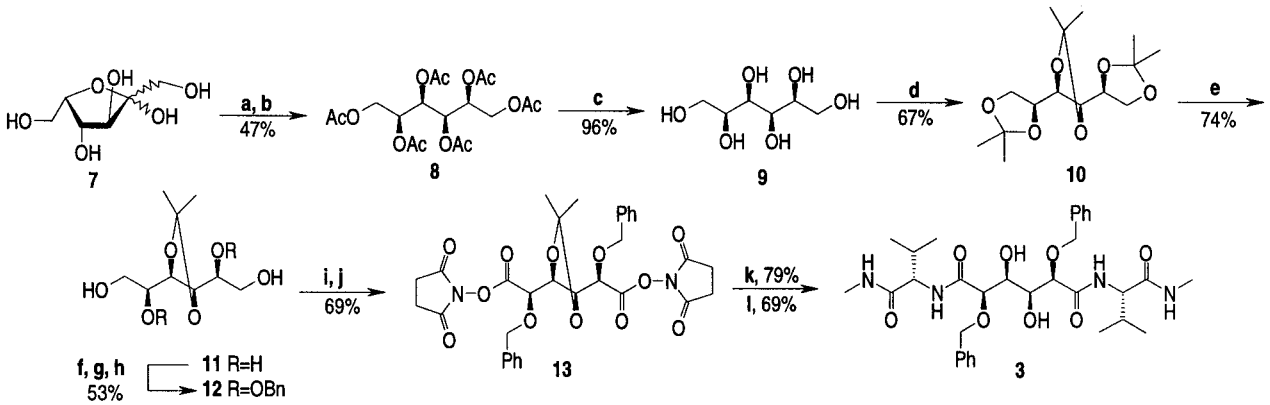
For the synthesis of compound **3**, the C-3, C-4 epimer of **1**, the previously described bis-lactone route[14] could not be applied as the corresponding L-idaro-1,4:3,6-dilactone was not readily accessible. Instead, compound **3** was synthesized from L-iditol (**9**), which in turn was prepared from L-sorbose in a three-step reaction sequence (Scheme 1).[18] Reduction of L-sorbose (**7**) with sodium borohydride in MeOH–water gave a 1:1 mixture of L-iditol and D-glucitol, which after removal of boric acid and without further purification was acetylated using pyridine and acetic anhydride. The crude product mixture was then crystallized from EtOH to give L-iditol hexaacetate (**8**) as white crystals in 47% yield with D-glucitol hexaacetate remaining in solution. De-*O*-acetylation of **8** was accomplished with sodium methoxide in MeOH and pure L-iditol (**9**) was afforded in 96% yield.

Reacting **9** with 2,2-dimethoxypropane, under acidic anhydrous conditions, produced the desired 1,2:3,4:5,6-triacetonide **10** together with the 1,3:2,4:5,6-triacetonide in a 4:1 ratio. Using 3,3-dimethoxypentane gave somewhat better ratio of the desired product.[19] However, this protecting group was associated with selectivity problems in the subsequent hydrolysis step. It was observed that the 1,2:3,4:5,6-triacetonide formed preferentially under kinetically controlled reaction conditions, but without going to complete conversion. The best result was eventually obtained with 2,2-dimethoxypropane and camphorsulphonic acid (CSA) in acetone and with a reaction time of 30 min producing the triacetonoid **10** in 67% yield. Subsequent partial hydrolysis with 70% HOAc at room temperature furnished the monoacetonide **11** in 74% yield.

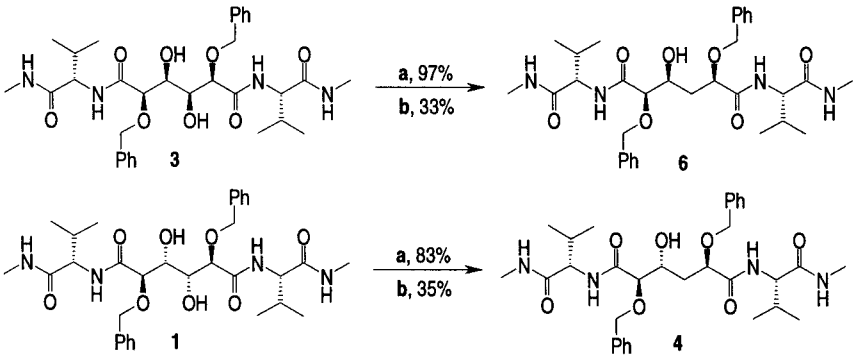
Selective protection of the primary hydroxyl groups employing dimethoxytrityl chloride followed by benzylation of the 2,5-diol with benzyl bromide and sodium hydride in DMF provided 2,5-di-*O*-benzyl-1,6-di-*O*-dimethoxytriphenylmethyl-3,4-*O*-isopropylidene-L-iditol.[20] Detritylation, in the presence of the isopropylidene group, using dichloroacetic acid in CH_2Cl_2 resulted in the 1,6-diol **12** in 53% yield from **11**.[21] Initially the primary diol of **11** was protected as *t*-butyldimethylsilyl ethers, but in the subsequent benzylation step extensive silyl-migration occurred, and therefore this route was not pursued.[14] The diol **12** was oxidized to the corresponding dicarboxylic acid with 2,2,6,6-tetramethylpiperidine 1-oxyl radical (TEMPO)–sodium hypochlorite in a buffered solution.[22,23] The very unstable dicarboxylic acid was immediately reacted with *N*,*N*′-disuccinylimidyl carbonate (DSC) in pyridine to give, after purification, the crystalline activated diacid **13** in 69% yield from **12**.[24]

Coupling of **13** with L-valine methylamide in CH_2Cl_2–THF (2:1) gave the desired product in 79% yield. Attempted selective cleavage of the 3,4-*O*-isopropylidene with a variety of acids was unsuccessful and gave complex product mixtures, whereas 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in CH_3CN–water (5:1) eventually delivered the 3,4-diol **3** in 69% yield.[25]

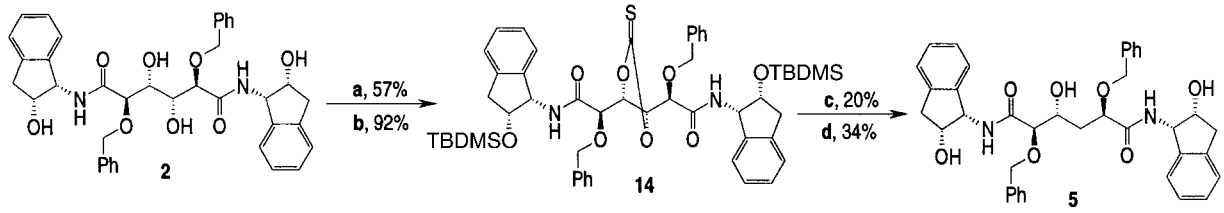
The deoxygenated compound **6** was prepared by converting the diol **3** to its corresponding thiocarbonate using *N*,*N*′-thiocarbonyl diimidazole in THF (97% yield), followed by a reduction with 2,2′-azobisisobutyronitrile (AIBN) and tributyltin hydride to give the desired product **6** in 33% yield (Scheme 2). Compound **4** was prepared from



Scheme 1. (a) $NaBH_4$, MeOH, H_2O. (b) Ac_2O, pyridine. (c) NaOMe, MeOH. (d) 2,2-dimethoxypropane, CSA, acetone. (e) 70% HOAc. (f) DMTrCl, pyridine. (g) NaH, BnBr, DMF, 0°C. (h) dichloroacetic acid, CH_2Cl_2. (i) TEMPO, NaOCl, KBr, Bu_4NBr, $NaHCO_3$, NaCl, CH_2Cl_2, H_2O, 0°C. (j) *N*,*N*′-disuccinimidyl carbonate, CH_3CN, pyridine. (k) L-valine methylamide, CH_2Cl_2, THF. (l) DDQ, CH_3CN, H_2O, 75°C.



Scheme 2. (a) *N*,*N*′-Thiocarbonyl diimidazole, THF, reflux. (b) Bu_3SnH, AIBN, toluene, reflux.



Scheme 3. (a) TBDMSOTf, lutidine, CH_2Cl_2, 0°C. (b) Thiocarbonyl diimidazole, THF, reflux. (c) Bu_3SnH, AIBN, toluene, reflux. (d) H^+ Dowex, MeOH.

compound **1**, according to the same procedure, to give the thiocarbonate in 83% yield and the deoxygenated product **4** in 35% yield.

For the deletion of the C-4 hydroxyl in compound **2** the indanol hydroxyls were silylated using *t*-butyldimethylsilyl triflate and lutidine in CH_2Cl_2 to deliver 57% of the disilylated product, which subsequently was converted to the thiocarbonate **15** in 92% yield (Scheme 3). Reduction gave *N*1,*N*6-di(2*R*-*t*-butyldimethylsilyloxy-1*S*-indanyl)-2*R*,5*R*-dibenzyloxy-3*R*-hydroxy hexanediamide in 20% yield. Desilylation using H^+-Dowex in MeOH gave **5** in 34% yield. The major by-products from the deoxygenation reactions resulted from desulfurization and from rearrangement of the thiocarbonates.[26]

HIV-1 protease inhibition. (Table 1; column 3) HIV-1 protease was cloned and heterologously expressed in *Escherichia coli*[27] and K_i-values were determined using a fluorometric assay.[28]

In vitro anti HIV activity. (Table 1; column 4) The anti HIV activity was measured in a HIV cytopathic assay in MT-4 cells where the effect was quantified using vital dye XTT.[29] The 50% inhibitory concentrations (ED_{50}) were calculated from the percent cytoprotection for individual compounds.

Conclusion

Deletion of one of the hydroxyl groups in **1**, which was accomplished smoothly by reduction of the corresponding cyclic thiocarbonate gave the less potent HIV-1 protease inhibitor **4** with K_i=2.1 nM as compared to K_i=0.4 nM for **1**. Notably however, both compounds exhibit similar antiviral activity, ED_{50}=1.2 μM for **4** compared to ED_{50}=1.1 μM for **1**. This result suggests that the inhibitor **4** has superior cell membrane permeability compared to **1**, but that this advantage is offset by the lower HIV-1 protease inhibitory activity.

The moderately active compound **3**, with the C-3, C-4 hydroxyl groups inverted with respect to inhibitor **1**, was readily obtained from L-sorbose via L-iditol (Scheme 1). Deoxygenation of one of the hydroxyl groups in **3** proceeded in moderate yield via the cyclic thiocarbonate and gave the inactive compound **6**.

Deletion of one of the central hydroxyl groups in inhibitor **2** (K_i=0.2 nM), via the cyclic thiocarbonate, resulted in the less potent HIV-1 protease inhibitor **5** (K_i=1.4 nM). Notably, a dramatic decrease in anti-viral activity was observed, going from ED_{50}=0.11 μM for **2** to ED_{50}=1.4 μM for **5**, reflecting decreased potency (HIV-1 protease inhibition) but notably also significantly reduced efficacy for cell membrane permeability.

It appears that, for this series of inhibitors, both hydroxyls contribute to the efficacy in the binding of the inhibitor to the enzyme. Although increased cell membrane permeability is indicative for inhibitor **4** this effect is offset by lower enzyme inhibition potency. Further studies are under way to explore the potential for increased in vitro anti-viral activity for these new types of HIV-1 protease inhibitors.

Experimental

General procedures

All solvents were distilled prior to use. Thin layer chromatography was performed using silica gel 60 f-254 (Merck) plates with detection by UV, charring with 8% sulfuric acid, ninhydrin or ammonium-molybdat–cerium(IV)sulfate–10%

sulfuric acid (100 g: 2 g: 2 L). Column chromatography was performed on silica gel (Matrix Silica Si 60A, 35–70 μm, Amicon). Organic phases were dried over anhydrous sodium sulfate. Concentrations were performed under reduced pressure. Optical rotations were recorded on a Perkin Elmer 241 polarimeter. NMR spectra were recorded on a JEOL GSX-270 instrument, shifts are given in ppm downfield from tetramethylsilane in $CDCl_3$, acetone-D_6 and CD_3OD, and from acetone (δ_C: 31.04) in D_2O. Accurate mass measurement was recorded on a Finnigan MAT 900S instrument, using electrospray.

L-Iditol hexaacetate (8). To a stirred solution of L-sorbose (**7**) (15.1 g, 83.3 mmol) in MeOH (200 mL) and water (10 mL) at ambient temperature was added sodium borohydride (6.3 g, 166.6 mmol) in portions during 30 min. After 1.5 h acetic acid (10 mL) was added. The mixture was concentrated to give a semi-crystalline syrup, which was dissolved in MeOH (400 mL) and acetic acid (40 mL) and concentrated again to give a white solid (hexitol and NaOAc). To a stirred suspension of this compound in pyridine (230 mL) was added acetic anhydride (240 mL) dropwise during 1 h. The mixture was left at 50°C overnight. After addition of water (15 mL), the mixture was concentrated followed by co-evaporation with added toluene. The residue was dissolved in $CHCl_3$, washed with water (4×), saturated aqueous $NaHCO_3$ (1×), brine (1×), aqueous 10% $CuSO_4$ (3×) and water (3×). The organic layer was dried and concentrated to give a light brown solid. The crude product was recrystallized twice from EtOH (500 mL) to give white crystals of the L-iditol hexaacetate **8** (17.1 g, 39.4 mmol, 47%) and the D-glucitol hexaacetate remaining in solution. NMR ($CDCl_3$): δ_H: 2.06 (6H, s), 2.10 (6H, s), 2.11 (6H, s), 4.04 (2H, dd, J=5.9 and 12.1 Hz), 4.31 (2H, dd, J=4.4 and 12.1 Hz), 5.23–5.32 (2H, m), 5.37 (2H, dd, J=1.2 and 3.3 Hz); δ_C: 20.4, 20.5, 61.5, 68.5, 69.0, 169.5, 169.7, 170.1, mp 119–120°C, $[\alpha]_D$=−22.7 (c 1.27, $CHCl_3$). Anal. Calcd for $C_{18}H_{26}O_{12}$: C, 49.77; H, 6.03. Found: C, 49.67; H, 5.94.

L-Iditol (9). To a stirred solution of compound **8** (17.1 g, 39.4 mmol) in MeOH (300 mL) was added NaOMe (1 M, 15 mL). After 7 h at ambient temperature TLC indicated complete reaction and the mixture was neutralized with H^+ Dowex, filtered, concentrated and dried to give L-iditol (**9**) (6.9 g, 37.9 mmol, 96%) as a colorless syrup which solidified after three weeks. NMR (D_2O): δ_H: 3.6–3.77 (6H, m), 3.82–3.88 (2H, m); δ_C: 63.5, 71.9, 72.5. mp 74–76°C, $[\alpha]_D$=−4.3 (c 1.02, H_2O). Anal. Calcd for $C_6H_{14}O_6$: C, 39.56; H, 7.75. Found: C, 39.32; H, 7.69. Literature values:[18] mp 74.5–75°C, $[\alpha]_D$=−3.44 (c 10.0, H_2O).

1,2-3,4-5,6-Tri-*O*-isopropylidene-L-iditol (10). To a stirred solution of L-iditol (**9**) (9.09 g, 49.9 mmol) in dry acetone (300 mL) were added 2,2-dimethoxypropane (50 mL, 407 mmol) and camphorsulfonic acid (CSA) (30 g, 129 mmol). After 30 min at ambient temperature the mixture was neutralized with $NaHCO_3$, stirred for an additional 10 min, filtered and concentrated. The residue was dissolved in EtOAc, washed with water (2×), dried, concentrated and purified by column chromatography (toluene–EtOAc 12:1) to give the triacetonid **10** (10.1 g, 33.4 mmol, 67%) as a light yellow syrup. NMR ($CDCl_3$): δ_H: 1.87 (6H, s), 1.94 (12H, s), 3.88 (2H, t J=8.1 Hz), 4.01–4.07 (4H, m), 4.08–4.16 (2H, m); δ_C: 25.5, 26.2, 27.1, 65.7, 74.9, 76.8, 109.7 and 110.0 $[\alpha]_D$=+0.062 (c 1.04, $CHCl_3$). Anal. Calcd for $C_{15}H_{26}O_6$: C, 59.58; H, 8.67. Found: C, 58.98; H, 8.61.

3,4-*O*-Isopropylidene-L-iditol (11). A solution of compound **10** (10.1 g, 33.4 mmol) in 70% acetic acid (200 mL) was stirred at ambient temperature for 5 h. The solution was concentrated and the remaining acetic acid removed by co-evaporation with added toluene. The residue was purified by column chromatography ($CHCl_3$–MeOH 3:1) to give the monoacetonide **11** (5.51 g, 24.7 mmol, 74%) as a pale pink solid. NMR (acetone-d_6): δ_H: 1.40 (6H, s), 3.67 (6H, s), 4.20 (2H, s), 4.82 (4H, s); δ_C: 27.2, 64.8, 71.4, 78.0, 110.2. $[\alpha]_D$=+35.9 (c 0.91, MeOH). Anal. Calcd for $C_9H_{18}O_6$: C, 48.64; H, 8.16. Found: C, 48.45; H, 8.15. Remaining diacetonide was recovered in 17% yield (1.50 g, 5.72 mmol).

2,5-Di-*O*-benzyl-3,4-*O*-isopropylidene-L-iditol (12). To a stirred solution of compound **16** (4.3 g, 19.3 mmol) in dry pyridine (100 mL) under argon atmosphere was added dimethoxytrityl chloride (15.6 g, 46.0 mmol). After 1 h at ambient temperature TLC indicated complete reaction and MeOH (10 mL) was added. The solution was concentrated and co-evaporated with added toluene. The residue was dissolved in $CHCl_3$, washed with saturated aqueous $NaHCO_3$ (2×), dried, concentrated and roughly purified by quick flash chromatography (toluene–EtOAc 5:1) to give 1,6-di-*O*-dimethoxytriphenylmethyl-3,4-*O*-isopropylidene-L-iditol (15.8 g, 19.1 mmol) as a yellow syrup, which was used directly in the next step. NMR ($CDCl_3$): δ_H: 1.35 (6H, s), 3.22 (4H, d, J=6.7 Hz), 3.60 (2H, br), 3.63 (12H, s), 3.71 (2H, s), 4.14 (2H, s), 6.76 (8H, d, J=9.1 Hz), 7.06–7.50 (18H, m); δ_C: 27.1, 55.1, 65.4, 69.0, 77.2, 86.3, 109.4, 113.1, 126.8, 127.8, 128.2, 130.1, 136.0, 138.6, 144.9, 158.5. $[\alpha]_D$=+15.4 (c 1.38, $CHCl_3$). To a cold (−3°C) and stirred suspension of NaH (1.90 g, 79.2 mmol) in dry DMF (35 mL) under argon atmosphere, were added simultaneously during 1 h the tritylated product from above (15.8 g) in DMF (70 mL) and benzyl bromide (5.50 mL, 46.2 mmol) in DMF (70 mL). The reaction was allowed to reach room temperature and then stirred overnight before the addition of MeOH (20 mL). Toluene was added and the mixture was washed with brine (1×), saturated aqueous $NaHCO_3$ (1×) and water (1×). The organic layer was dried, concentrated and purified roughly by flash chromatograhy (toluene–EtOAc 15:1) to give 2,5-di-*O*-benzyl-1,6-di-*O*-dimethyoxytriphenylmethyl-3,4-*O*-isopropylidene-L-iditol (15.6 g, 15.5 mmol) as a light yellow syrup. NMR ($CDCl_3$): δ_H: 1.31 (6H, s), 3.32 (4H, d, J=6.8 Hz), 3.67 (12H, s), 3.75 (2H, s), 4.20 (2H, s), 4.63 (4H, dd, J=11.7 and 51.7 Hz), 6.72 (8H, d, J=9.1 Hz), 7.05–7.48 (18H, m); δ_C: 27.0, 55.1, 64.0, 73.1, 76.9, 77.4, 86.4, 109.0, 113.1, 126.7, 127.4, 127.7, 128.2, 130.2, 136.2, 138.6, 144.9, 158.4. $[\alpha]_D$=+21.7 (c 1.25, $CHCl_3$). To a stirred solution of the benzylated product (15.6 g) in CH_2Cl_2 (170 mL) was added dichloroacetic acid (7 mL), turning the solution bright red. After 2 h at ambient temperature the mixture was washed with saturated aqueous $NaHCO_3$ (3×), dried, concentrated and purified by column chormatography

(toluene–EtOAc 1:1) to give the diol **12** (4.06 g, 10.2 mmol) as a colorless syrup in 53% yield from **11**. NMR ($CDCl_3$): δ_H: 3.02 (2H, br), 3.43 (2H, br), 3.70 (4H, ddd, J=4.7, 11.8 and 25.4 Hz), 4.22 (2H, s), 4.61 (4H, dd, J=11.8 and 35.2 Hz), 7.15–7.35 (10H, m); δ_C: 26.9, 61.9, 72.5, 77.0 77.6, 109.5, 127.9, 128.0, 128.5, 138.0. $[\alpha]_D$=+19.0 (*c* 1.07, $CHCl_3$). Anal. Calcd for $C_{23}H_{30}O_6$: C, 68.64; H, 7.51. Found: C, 68.43; H, 7.45.

***N*1,*N*6-Di-succinimidyl-2*R*,5*R*-dibenzyloxy-3*S*,4*S*-dihydroxy-3,4-*O*-isopropylidenehexanediester (13).** To a stirred solution of **12** (1.84 g, 4.57 mmol) in CH_2Cl_2 (90 mL) were added 2,2,6,6-tetramethylpiperidine 1-oxyl radical (TEMPO) (36 mg, 0.23 mmol), potassium bromide (104 mg, 0.87 mmol), tetrabutylammonium bromide (163 mg, 0.51 mmol) and saturated aqueous $NaHCO_3$ (20 mL). The mixture was cooled to 0°C and a solution of NaOCl (42 mL, 1.2 M, 50.4 mmol), saturated aqueous $NaHCO_3$ (10 mL) and brine (20 mL) was added over a period of 1.5 h. After 30 min at 0°C, the organic layer was separated and extracted with saturated aqueous $NaHCO_3$ (3×) and water (3×). To the combined water phase was added EtOH (50 mL) and solution was stirred for 30 min. Then EtOAc (100 mL) was added and the mixture was brought from pH 9 to 2 by adding H^+-Dowex. The Dowex was filtered, the phases separated, and the water phase was extracted with EtOAc (4×). The combined organic layer was dried and concentrated under reduced pressure without warming to give 2,5-di-*O*-benzyl-3,4-*O*-isopropylidene-L-idaric acid (1.40 g, 3.25 mmol) as a light yellow syrup, which had to be used without further purification due to instability. NMR (CD_3OD–acetone-d_6 2:1): δ_H: 1.32 (6H, s), 3.98 (2H, s), 4.59 (2H, s), 4.63 (4H, dd, J=12.6 and 103.9 Hz), 7.22–7.44 (10H, m); δ_C: 27.1, 73.7, 77.4, 78.0, 111.0, 128.8, 129.1, 129.3, 138.6, 172.7. To a stirred solution of the crude diacid (1.40 g) in dry CH_3CN (20 mL) under argon were added pyridine (1.58 mL, 19.5 mmol) and *N*,*N*′-disuccinylimidyl carbonate (DSC) (3.30 g, 12.9 mmol). After 15 h at ambient temperature EtOAc was added and the mixture was washed with water (3×) and brine (1×), dried, concentrated and purified by column chromatography ($CHCl_3$–MeOH 20:1). The activated diacid **13** was isolated (1.88 g, 3.17 mmol) as a colorless syrup in 69% yield from the diol **12**. NMR ($CDCl_3$): δ_H: 1.42 (6H, s), 2.85 (8H, s), 4.46 (2H, d, J=1.5 Hz), 4.65 (2H, dd, J=1.5 and 2.4 Hz), 4.68 (4H, dd, J=11.5 and 99.6 Hz), 7.26–7.34 (10H, m); δ_C: 25.6, 26.6, 73.1, 75.5, 75.9, 111.7, 128.3, 128.4, 128.6, 136.1, 165.7, 168.5. $[\alpha]_D$=+100.2 (*c* 0.88, $CHCl_3$). Anal. Calcd for $C_{31}H_{32}N_2O_{10}$: C, 62.83; H, 5.44; N, 4.73. Found: C, 62.74; H, 5.35; N, 4.51.

***N*1,*N*6-Di[2-methyl-1*S*-(methylcarbamoyl)propyl]-2*R*,5*R*-dibenzyloxy-3*S*,4*S*-dihydroxy hexanediamide (3).** To a stirred solution of the activated diacid **13** (974 mg, 1.64 mmol) in a 2:1 mixture of CH_2Cl_2–THF (7 mL) under argon was added L-valine methylamide (560 g, 4.30 mmol). After 18 h at ambient temperature CH_2Cl_2 was added and the mixture was washed with saturated aqueous NH_4Cl (1×) and water (1×). The organic layer was dried, concentrated and purified by column chromatography ($CHCl_3$–MeOH 20:1) to give *N*1,*N*6-di[2-methyl-1*S*-(methylcarbamoyl)propyl]-2*R*,5*R*-dibenzyloxy-3*S*,4*S*-dihydroxy-3,4-*O*-iso-propylidenehexanediamide (844 mg, 1.29 mmol, 79%) as a white solid. NMR ($CDCl_3$): δ_H: 0.81 (6H, d, J=7.0 Hz), 0.95 (6H, d, J=7.0 Hz), 1.44 (6H, s), 2.48–2.58 (2H, m), 2.75 (6H, d, J=4.8 Hz), 3.70 (2H, s), 4.39 (2H, dd, J=4.1 and 9.5 Hz), 4.54 (2H, s), 4.59 (4H, dd, J=11.5 and 52.2 Hz), 6.81 (2H, d, J=4.8 Hz), 6.92 (2H, d, J=9.5 Hz), 7.29–7.41 (10H, m); δ_C: 16.7, 19.7, 26.2 27.2, 28.9 57.8, 73.7, 77.1, 77.7, 110.1, 128.2, 128.4, 128.7, 129.0, 135.7, 169.8, 170.9. To a stirred solution of coupled compound (775 mg, 1.18 mmol) in a 5:1 mixture of CH_3CN–H_2O (30 mL) was added, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (30 mg, 0.13 mmol). After two and four days at 75°C additional portions of DDQ (2×60 mg) were added and after six days the reaction was concentrated. The residue was dissolved in EtOAc, washed with water (2×), filtered through a pad of charcoal–celite–Na_2SO_4 and purified by column chromatography ($CHCl_3$–MeOH 20:1) to give the diol **3** (501 mg, 0.81 mmol, 69%) as a white solid. NMR ($CDCl_3$): δ_H: 0.83 (6H, d, J=7.0 Hz), 0.94 (6H, d, J=7.0 Hz), 2.50–2.68 (2H, m), 2.70 (6H, d, J=4.8 Hz), 3.72 (2H, br), 4.05 (2H, d, J=4.5 Hz), 4.25 (2H, d, H=4.6 Hz), 4.31 (2H, dd, J=4.1 and 9.5 Hz), 4.65 (4H, d, J=3.5 Hz), 7.05 (2H, d, J=9.5 Hz), 7.24 (2H, d, J=4.8 Hz), 7.29–7.48 (10H, m); δ_C: 16.7, 19.7 26.2, 28.6, 57.7, 69.6, 73.7, 76.6, 128.2, 128.9, 129.0, 135.9, 170.8, 172.3. $[\alpha]_D$=−22.9 (c, 1.4, $CHCl_3$). Anal. Calcd for $C_{32}H_{46}N_4O_8$: C, 62.52; H, 7.54; N, 9.11. Found: C, 62.41; H, 7.39; N, 8.98.

***N*1,*N*6-Di[2-methyl-1*S*-(methylcarbamoyl)propyl]-2*R*,5*R*-dibenzyloxy-3*S*-hydroxyhexanediamide (6).** To a stirred solution of **3** (58 mg, 94 μmol) in dry THF (5 mL) under an argon atmosphere at 45°C was added *N*,*N*′-thiocarbonyl diimidazole (40 mg, 0.22 mmol). The mixture was refluxed for 30 h, concentrated and purified on column chromatography ($CHCl_3$–MeOH 20:1) to give N_1,N_6-di[2-methyl-1*S*-(methylcarbamoyl) propyl]-2*R*,5*R*-dibenzyloxy-3*S*,4*S*-dihydroxy-3,4-*O*-thiocarbonylhexanediamide (60 mg, 91 μmol, 97%) as a white solid. NMR ($CDCl_3$): δ_H: 0.80 (6H, d, J=7.0 Hz), 088 (6H, d, J=7.0 Hz) 2.25–2.46 (2H, m), 2.86 (6H, d, J=4.8 Hz), 4.07 (2H, s), 4.28 (2H, dd, J=4.3 and 8.9 Hz), 4.65 (4H, d, J=10.1 Hz), 5.13 (2H, s), 6.49 (2H, d, J=4.8 Hz), 7.06 (2H, d, J=8.9 Hz), 7.28–7.47 (10H, m); δ_C: 17.2, 19.5, 26.4, 29.7, 58.3, 75.6, 78.0, 83.4, 128.3 128.9, 129.1, 135.2, 167.7, 170.6, 189.6. A suspension of the thiocharbonate (60 mg, 91 μmol), tributyltin hydride (50 μL, 0.19 mmol) and 2,2′-azobisisobutyronitrile (AIBN) (15 mg, 91 μmol) in dry toluene (4 mL) was added dropwise to refluxing toluene (2 mL)over a period of 20 min. The mixture was refluxed for 45 min, allowed to cool and concentrated. The residue was dissolved in CH_3CN and washed with hexane (2×). The CH_3CN layer was concentrated and purified by column chromatography (toluene–acetone 1:1) to give **6** (18 mg, 30 μmol, 33%) as a white solid. NMR ($CDCl_3$): δ_H: 0.82 (3H, d, J=7.0 Hz), 0.87 (3H, d, J=7.0 Hz), 0.95 (6H, d, J=7.0 Hz) 1.86–1.98 (2H, m) 2.30–2.48 (2H, m) 2.73 (3H, d, J=4.9 Hz), 2.76 (3H, d, J=4.9 Hz), 3.86 (1H, d, J=3.1 Hz), 4.10 (1H, dd, J=3.1 and 8.7 Hz), 4.21–4.32 (3H, m), 4.62 (4H, dd, J=13.4 and 33.7 Hz), 6.53 (1H, d, J=4.8 Hz), 6.68 (1H, d, J=4.8 Hz), 7.07 (2H, d, J=9.1 Hz), 7.25–7.47 (10H, m); δ_C: 17.3, 17.4, 19.6, 26.3, 29.5, 34.5, 58.0, 58.1, 68.5, 72.7, 73.7, 76.8, 81.1, 127.9, 128.3, 128.5, 128.6, 128.8, 136.2, 136.5, 171.1, 171.4, 171.6, 172.5. $[\alpha_D]$=+6.1 (*c* 0.7, $CHCl_3$).

Anal. Calcd for $C_{32}H_{46}N_4O_7$: C, 64.19; H, 7.74; N, 9.36. Found: C, 63.95; H, 7.48; N, 9.20.

***N*1,*N*6-Di[2-methyl-1*S*(methylcarbamoyl)propyl]-2*R*,5*R*-dibenzyloxy-3*R*-hydroxyhexanediamide (4).** To a stirred solution of **1** (144 mg, 0.23 mmol) in dry THF (8 mL) under argon atmosphere at 45°C was added *N*,*N*′-thiocarbonyl diimidazole (104 mg, 0.58 mmol). The mixture was refluxed for 17 h, concentrated and purified on column chromatography ($CHCl_3$–MeOH 20:1) to give N_1,N_6-di[2-methyl-1*S*-(methylcarbamoyl) propyl]-2*R*,5*R*-dibenzyloxy-3*R*,4*R*-dihydroxy-3,4-*O*-thiocarbonylhexanediamide (125 mg, 0.19 mmol, 83%) as a white solid. NMR ($CDCl_3$): δ_H: 0.72 (6H, d, *J*=7.5 Hz), 0.88 (6H, d, *J*=8.0 Hz) 2.15–2.30 (2H, m), 2.76 (6H, d, *J*=4.8 Hz), 4.04 (2H, dd, *J*=8.8 and 9.9 Hz), 4.40 (2H, s), 4.75 (4H, dd, *J*=11.7 and 77.4 Hz), 5.33 (2H, s), 6.87 (2H, d, *J*=4.8 Hz), 7.16 (2H, d, *J*=8.8 Hz), 7.32–7.41 (10H, m); δ_C: 17.4, 19.4, 26.3, 30.2, 58.4, 75.8, 77.7, 82.4, 128.9, 129.1, 135.3, 167.0, 170.6, 190.7. To a refluxing solution of the thiocarbonate (125 mg, 0.19 mmol) in dry toluene (4 mL) under argon atmosphere was added over a period of 10 min a solution of tributyltin hydride (153 μL, 0.57 mmol) and AIBN (47 mg, 0.28 mmol) in dry toluene (3 mL). The mixture was refluxed for 20 h, allowed to cool and concentrated. The residue was dissolved in CH_3CN and washed with hexane (2×). The CH_3CN layer was concentrated and purified by column chromatography ($CHCl_3$–MeOH 20:1) to give **4** (40 mg, 67 μmol, 35%) as a white solid. NMR ($CDCl_3$): δ_H: 0.83–0.94 (12H, m), 1.90–2.36 (4H, m), 2.67 (3H, d, *J*=4.8 Hz), 2.74 (3H, d, *J*=4.8 Hz), 3.89 (1H, d, *J*=3.5 Hz), 4.11 (1H, dd, *J*=5.0 and 7.0 Hz), 4.19–4.29 (3H, m) 4.58 (2H, s), 4.62 (2H, d, *J*=2.8 Hz), 6.50 (1H, d, *J*=4.8 Hz), 6.75 (1H, d, *J*=4.8 Hz), 6.96 (1H, d, *J*=9.0 Hz), 7.20 (1H, d, *J*=9.0 Hz), 7.25–7.37 (10H, m); δ_C: 17.5, 18.0, 19.4, 19.6, 26.1, 26.3, 29.5, 30.6, 35.5, 58.2, 58.3, 70.2, 73.0, 73.3, 77.2, 83.0, 127.9, 128.0, 128.4, 128.6, 128.7, 136.7, 170.5, 171.3, 171.4, 173.1. $[\alpha_D]$=+1.8 (*c* 0.8, $CHCl_3$). Anal. Calcd for $C_{32}H_{46}N_4O_7$: C, 64.19; H, 7.74; N, 9.36. Found: C, 64.00; H, 7.50; N, 9.15.

***N*1,*N*6-Di[2*R*-*t*-butyldimethylsilyloxy-1*S*-indanyl)-2*R*,5*R*-dibenzyloxy-3*R*,4*R*-dihydroxy-3*R*, 4*R*-dihydroxy-3,4-*O*-thiocarbonylhexanediamide (14).** To a stirred solution of **2** (400 mg, 620 mmol) in CH_2Cl_2 (3 mL) at 0°C under argon atmosphere were added lutidine (142 μL, 1.22 mmol) and *t*-butyldimethylsilyl triflate (296 μL, 1.29 mmol). After 4 h 1 M NaOH (0.5 mL) was added and the mixture was washed with 1 M HCl (1×) and brine (1×). The organic layer was dried, concentrated and purified by column chromatography ($CHCl_3$–MeOH 80:1) to give *N*1,*N*6-di(2*R*-*t*-butyldimethylsilyloxy-1*S*-indanyl)-2*R*,5*R*-dibenzyloxy-3*R*,4*R*-dihydroxyhexanediamide (313 mg, 355 μmol, 57%) as a light yellow syrup. NMR ($CDCl_3$): δ_H: −0.04 (12H, s), 0.64 (18H, s), 2.95 (4H, dd, *J*=15.4 and 68.6 Hz), 3.88 (2H, br), 4.02 (2H, d, *J*=7.7 Hz) 4.28 (2H, d, *J*=7.7 Hz), 4.48–4.62 (2H, m), 4.68 (4H, dd, *J*=12.8 and 27.4 Hz), 5.31 (2H, dd, *J*=5.1 and 8.6 Hz), 6.80–7.36 (18H, m), 7.52 (2H, d, *J*=8.6 Hz); δ_C: −4.8, 17.9, 25.7, 40.6, 56.3, 71.1, 74.0, 77.2, 124.5, 124.8, 126.9, 127.9, 128.2, 128.5, 136.7, 139.8, 140.9, 176.2. The monosilylated compound was isolated in 24% yield (116 mg) and starting material was isolated in 7% yield (28 mg). To a stirred solution of the disilylated compound (922 mg, 1.05 mmol) in dry dichloroethane (21 mL) was added *N*,*N*′-thiocarbonyl diimidazole (598 mg, 3.36 mmol). The mixture was refluxed for 22 h, concentrated and purified on column chromatography (toluene–EtOAc 30:1) to give **14** (887 mg, 0.961 mmol, 92%) as a white solid. NMR ($CDCl_3$): δ_H: 0.10 (6H, s), 0.13 (6H, s), 0.75 (18H, s), 2.82 (2H, d, *J*=15.4 Hz), 3.07 (2H, dd, *J*=4.6 and 15.4 Hz), 4.43 (2H, s), 4.72 (2H, dd, *J*=10.8 and 45.3 Hz), 4.73–4.80 (2H, m) 5.17 (2H, dd, *J*=4.6 and 8.3 Hz), 5.40 (2H, s), 7.05–7.30 (18H, m), 7.60 (2H, d, *J*=8.3 Hz); δ_C: −4.8, −4.9, 17.8, 25.6, 40.5, 56.9, 74.3, 75.2, 78.2, 82.7, 124.6, 124.8, 126.6, 128.6, 128.8, 129.0, 135.6, 140.0, 141.5, 165.7, 192.0. Anal. Calcd for $C_{51}H_{66}N_2O_8SSi_2$: C, 66.34; H, 7.20; N, 3.03. Found: C, 66.35; H, 7.28; N, 3.01.

***N*1,*N*6-Di[2*R*-hydroxy-1*S*-indanyl)-2*R*,5*R*-dibenzyloxy-3*R*-hydroxyhexanediamide (5).** To a refluxing solution of **14** (794 mg, 0.86 mmol) in dry toluene (84 mL) under argon atmosphere were added over a period of 20 min a solution of tributyltin hydride (0.70 mL, 2.60 mmol) and AIBN (284 ng, 1.73 mmol) in dry toluene. The mixture was refluxed for 20 h and additional tributyltin hydride (0.23 mL) and AIBN (71 mg) was added. After 4 h further reflux the mixture was concentrated, dissolved in toluene and washed with 2.5 M NaOH (1×) and water (3×), dried and concentrated. The residue was purified by column chromatography (toluene–EtOAc 3:1) to give *N*1,*N*6-di(2*R*-*t*-butyldimethylsilyloxy-1*S*-indanyl)-2*R*,5*R*-dibenzyloxy-3*R*-hydroxyhexanediamide (150 mg, 0.17 mmol, 20%) as a light yellow syrup. NMR ($CDCl_3$): δ_H: 0.05 (6H, s), 0.08 (6H, s), 0.76 (18H, s), 1.98–2.25 (1H, m), 2.87 (2H, d, *J*=15.5 Hz), 3.10 (2H, dd, *J*=3.9 and 15.5 Hz), 4.02 (1H, d, *J*=5.8 Hz), 4.20–4.35 (2H, m), 4.55–4.76 (6H, m), 5.37 (2H, dd, *J*=7.8 and 13.6 Hz), 7.15–7.38 (18H, m), 7.52 (2H, d, *J*=7.8 Hz); δ_C: −4.7, −4.8, 18.0, 25.7, 36.4, 40.5, 40.6, 56.3, 56.4, 69.8, 73.3, 74.0, 74.5, 82.0, 124.5, 124.7, 124.8, 126.9, 127.0, 127.1, 127.7, 127.9, 128.0, 128.2, 128.3, 128.4, 128.5, 136.7, 139.7, 141.2, 141.3, 171.3, 173.4. To a stirred solution of the deoxygenated compound (150 mg, 0.17 mmol), in MeOH (6 mL) was added H^+-Dowex. After three days at ambient temperature the mixture was filtered, concentrated purified by column chromatography ($CHCl_3$–MeOH 20:1), followed by recrystallization from MeOH to give **5** (38 mg, 60 μmol, 34%) as white crystals. NMR ($CDCl_3$–MeOD): δ_H: 2.01–2.10 (1H, m), 2.86 (2H, d, *J*=16.5 Hz), 3.10 (2H, dd, *J*=4.5 and 16.5 Hz), 4.05 (1H, d, *J*=3.5 Hz), 4.17–4.25 (2H, m), 4.54–4.63 (6H, m), 5.20–5.32 (2H, m), 7.08–7.28 (20H, m); δ_C: 35.9, 39.8, 39.9, 57.2, 57.3, 69.2, 72.5, 72.7, 73.4, 74.0, 77.6, 84.0, 124.1, 124.2, 125.5, 127.2, 128.3, 128.4, 128.5, 128.8, 137.1, 140.2, 140.4, 140.6, 140.7, 171.4, 174.4. mp 110–113°C, $[\alpha]_D$=+18.2 (*c* 0.38, $CHCl_3$–MeOH, 1:1). Due to instability of this compound elemental analysis did not give satisfying results. HRMS Calcd for $C_{38}H_{40}N_2O_7$: 636.2836. Found 636.2844.

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