Design and Synthesis of New Potent C_2-Symmetric HIV-1 Protease Inhibitors. Use of L-Mannaric Acid as a Peptidomimetic Scaffold

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A study on the use of derivatized carbohydrates as C_2-symmetric HIV-1 protease inhibitors has been undertaken. L-Mannaric acid (**6**) was bis-*O*-benzylated at C-2 and C-5 and subsequently coupled with amino acids and amines to give C_2-symmetric products based on C-terminal duplication. Potent HIV protease inhibitors, **28** $K_i = 0.4$ nM and **43** $K_i = 0.2$ nM, have been discovered, and two synthetic methodologies have been developed, one whereby these inhibitors can be prepared in just three chemical steps from commercially available materials. A remarkable increase in potency going from $IC_{50} = 5000$ nM (**23**) to $IC_{50} = 15$ nM (**28**) was observed upon exchanging −COOMe for −CONHMe in the inhibitor, resulting in the net addition of one hydrogen bond interaction between each of the two −NH− groups and the HIV protease backbone (Gly 48/148). The X-ray crystal structures of **43** and of **48** have been determined (Figures 5 and 6), revealing the binding mode of these inhibitors which will aid further design.

Introduction

The human immunodeficiency virus (HIV) has been identified as the etiologic agent of acquired immunodeficiency syndrome (AIDS).[1] The *pol* gene of the human immunodeficiency virus type 1 (HIV-1) encodes for the aspartic protease which mediates proteolytic processing of the *gag* and the *gag-pol* viral gene products liberating functional enzymes and structural proteins which are essential for the formation of the mature, infectious virus.[2] Inactivation of the aspartic protease leads to the formation of noninfectious virions.[3] As a result the HIV-1 protease has become one of the major targets for therapeutic intervention in AIDS and in HIV infection. Recently four protease inhibitors, saquinavir (**1**), ritonavir (**2**), indinavir (**3**), and nelfinavir (**4**) were approved for clinical use by the FDA (Figure 1). Despite the clinical efficacy the benefits from long-term treatment with these agents remains to be demonstrated. Selection of a drug resistant mutant virus is likely to occur after prolonged treatment reducing efficacy of therapy. The high cost of synthesis is today a barrier to the widespread use of the currently approved protease inhibitors, notably in less developed countries. It is thus important that new improved protease inhibitors accessible at low costs are developed.[4]

Numerous reports describing potent protease inhibitors have been disclosed.[5] On the basis of X-ray crystal structures it has been shown that the HIV-1 protease exists as a C_2-symmetric dimer. Recognizing that both the N- and C-terminal of a substrate or inhibitor binds into identical subsites of the HIV protease and that duplication of either the N- or the C-terminal produces a C_2-symmetric compound has led to the design and synthesis of new inhibitors.[5] A successful exploration of the N-terminal duplication concept was demonstrated for the (3*R*,4*R*)-diaminodiol **5** and for a related series of compounds.[6]

We have recently explored D-mannitol as a linear peptidomimetic scaffold[7] and have shown that D-mannitol based C_2-symmetric protease inhibitors of the general structure **6** have antiviral activities similar to the diaminodiol inhibitors **5** (Figure 2).[8] Cyclic protease inhibitors derived from D-mannitol and L-mannitol have also been recently disclosed by us and others.[9]

Inhibitor Design. The present work describes the use of carbohydrates in the design and synthesis of structurally new C_2-symmetric protease inhibitors incorporating C-terminal duplication (Figure 3). From molecular modeling L-mannaric acid (**7**) was selected as a C_2-symmetric backbone where *O*-benzylation of the hydroxyls at C-2 and C-5 of **7** and subsequent coupling with amino acids or amines gives inhibitors of the general structure **8** (Figure 3). While inhibitors based on N-terminal duplication have been extensively studied, there are fewer reports on C_2-symmetric inhibitors based on C-terminal duplication.[10] The absolute stereochemistry of the P1/P1′ substituents and of the central diol varies from one class of inhibitors to another, cf. A-75925[6] (**9**) and DMP 323,[11] (**10**) (Figure 4). We have used computer-assisted molecular modeling to build and conformationally refine potential inhibitors in extended

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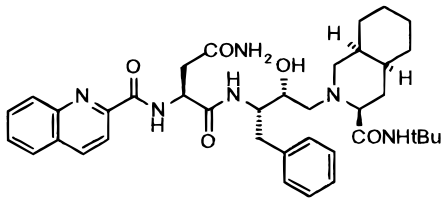
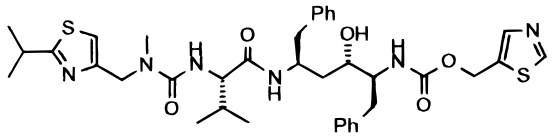


1
Saquinavir



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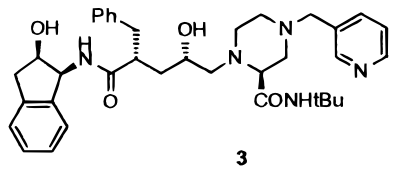
Ritonavir



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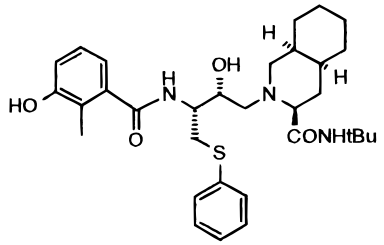
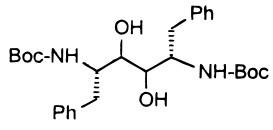
Indinavir

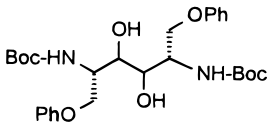


4
Nelfinavir

Figure 1. FDA-approved HIV-1 protease inhibitors.

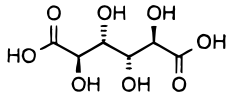


5 (3R,4R)

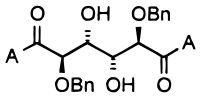


6

Figure 2.



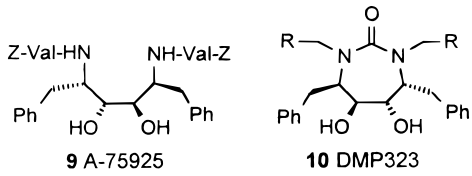
7 L-Mannaric acid



8 (A = amine)

Figure 3.

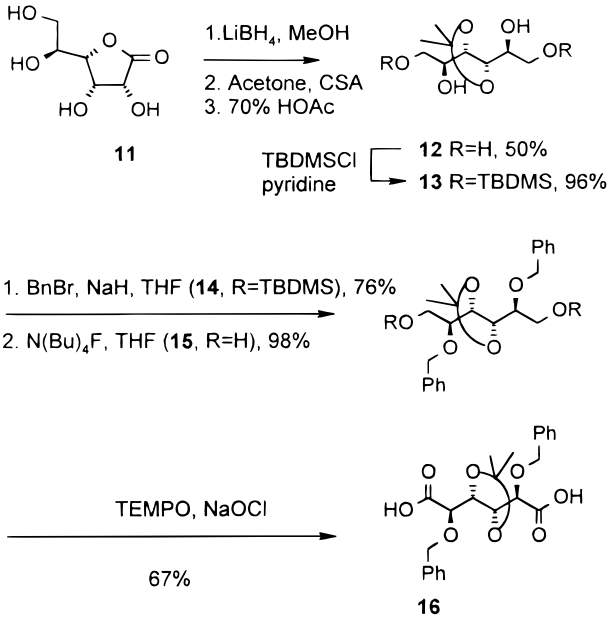
conformations[12] and to match these with X-ray crystal structures.[13] This analysis indicated that inhibitors derived from substituted L-mannaric acid would give a good fit with respect to (a) the spacial direction of the P1/P1′ substituents (benzyloxy), (b) the carbonyl oxygens of **8** hydrogen bonding to the structural water, and (c) the stereochemistry of the central hydroxyl groups forming hydrogen bonds to the carboxylic acid residues of Asp 25 and Asp 125. The amino acids or amines providing the P2/P2′ substituents were modeled extending into the lipophilic S2/S2′ pockets of the HIV-1 protease and/or hydrogen bonding to the inhibitor backbone. It is noteworthy that with the present inhibitor design strategy diverse P1 and P2 substituents in **8** are synthetically readily available making optimization of potential inhibitors feasible.



9 A-75925 **10** DMP323

Figure 4.

Scheme 1. Synthesis of Protected L-Mannaric Acid

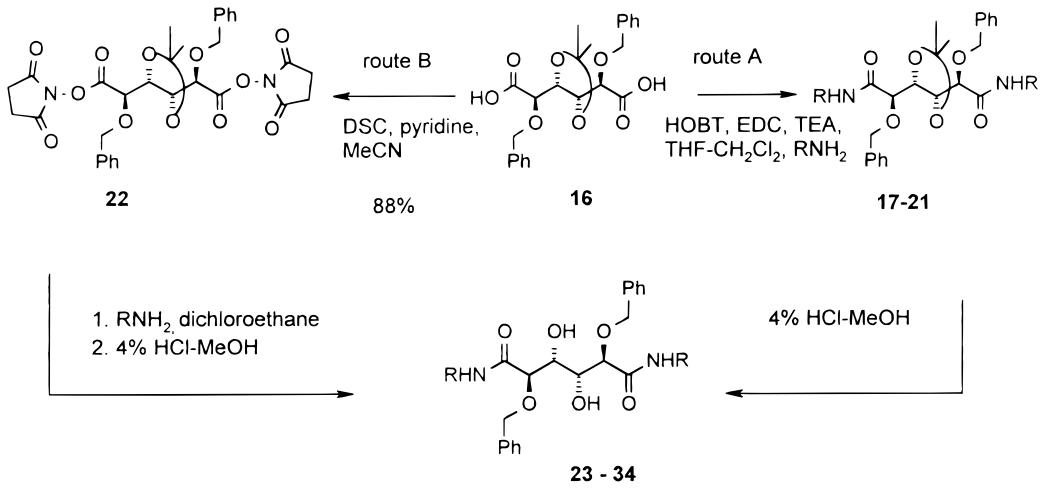


Results and Discussion

Chemistry. For the synthesis of inhibitors of the general structure **8** two synthetic routes were developed. In the first route (synthetic route 1) a suitably protected 2,5-di-*O*-benzylated L-mannaric acid was prepared and coupled to a set of amines and amino acid derivatives. In the second synthetic route developed (synthetic route 2), which is substantially shorter, 2,5-di-*O*-benzyl-L-mannaro-1,4:3,6-di-γ-lactone (**36**) was prepared and subsequently in one step reacted with various amines and amino acid derivatives giving bisamides **8** by nucleophilic ring opening of the bislactone **36**.

Synthetic Route 1. (Schemes 1 and 2) The monoacetonide **12** was prepared using a modified procedure as described for the corresponding D-mannitol acetonide.[14] Reduction of L-mannonic γ-lactone (**11**) with lithium borohydride in dry MeOH gave L-mannitol which, after azeotropic removal of the boric acid and without further purification, was protected using 2,2-dimethoxypropane in acetone containing camphorsulfonic acid to give the triacetonide in 78% overall yield. Partial hydrolysis with aqueous HOAc (70%) provided the monoacetonide **12** in 64% yield after recrystallization. Subsequent selective protection of the primary hydroxyls with *tert*-butyldimethylsilyl chloride in pyridine, containing *p*-(dimethylamino)pyridine, afforded

Scheme 2. Synthesis of L-Mannaric Diamides



compound **13** in 96% yield.[15] Benzylation of the 2,5-diol using benzyl bromide, sodium hydride, and a catalytic amount of tetrabutylammonium iodide in THF gave **14** in 76% yield. Desilylation of **14** using tetrabutylammonium fluoride in THF delivered compound **15** in 98% yield. For the oxidation of primary hydroxyls to the corresponding dicarboxylic acid, the majority of published methods gives lactones when applied to 1,4-, 1,5-, or 1,6-diols. Attempted oxidation of compound **15** with Jones oxidation[16] resulted in a complex product mixture, and attempts to oxidize the 1,6-diol using PDC and acetic acid anhydride[17] produced a slightly more polar product which decomposed upon workup. The reagent system 2,2,6,6-tetramethylpiperidine 1-oxyl radical (TEMPO)−NaOCl has previously been employed for the direct conversion of alcohols to carboxylic acids and for the preparation of lactones from 1,4- and 1,5-diols.[18] However with this reagent system, it is reported that oxidation of 1,6-diols to the corresponding lactone results in the formation of polymerization products.[18] Notably, by using 0.05 molar equivalents of TEMPO and an excess of NaOCl at 0 °C, compound **15** was oxidized directly to the dicarboxylic acid **16** in 67% yield (Scheme 1). The successful implementation of this oxidation procedure seems to rely upon the presence of the cyclic 3,4-*O*-isopropylidene group which effectively prevents intramolecular side reactions. In the absence of the cyclic protection groups the dicarboxylic acid could not be isolated.

For introducing the amino acids and amines (Scheme 2) corresponding to the P2/P2′ substituents of the inhibitor, compound **16** was dissolved in CH_2Cl_2−THF and condensed with the corresponding amino acid derivatives with HOBT-EDC. The condensation products, compounds **17**−**21**, were isolated in yields ranging from 34% to 73%. The 3,4-*O*-isopropylidene group was cleaved off with 4% HCl in MeOH, giving the corresponding diol products **23**−**27** in approximately 70% yield (Table 1). Compounds **28**−**31** were prepared from **16** (Scheme 2, route A) in the same manner as that for the preparation of **23**−**27,** but the intermediate monoacetonide was not isolated in this case. Valin methylamide, used in the preparation of **28**, was synthesized through amidation of Z-Val-OSu in dry THF followed by hydrogenolysis to furnish the methyl amide in 78% overall yield.[19]

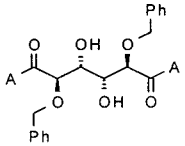
In an alternative coupling procedure L-mannaric acid **16** was activated with *N*,*N*-disuccinimidyl carbonate[20] to give the corresponding activated bissuccinimidyl ester **22** in 88% yield, which conveniently could be stored prior to use. This protocol avoids repeating the activation step before coupling with the appropriate amines (Scheme 2, route B). Other synthetic routes[21] examined to prepare the active ester **22** gave lower yields, and attempts to prepare the corresponding pentafluorophenyl diester were not successful.[22] Coupling of **22** with three hydroxy anilines in CH_2Cl_2 followed by cleavage of the isopropylidene group with 4% HCl in MeOH delivered the diamide products **32**−**34** in 27−38% overall yield.

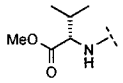
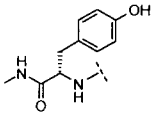
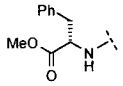
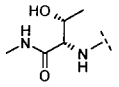
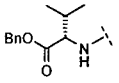
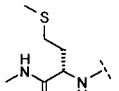
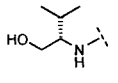
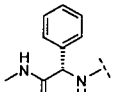
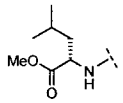
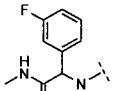
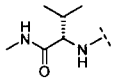
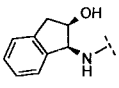
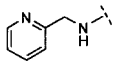
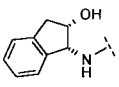
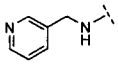
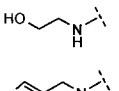
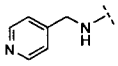
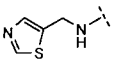
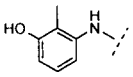
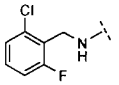
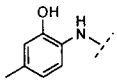
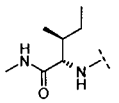
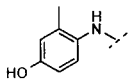
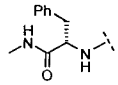
The cleavage step of the 3,4-*O*-isopropylidene groups in the synthesis of compounds **23**−**34** was very sensitive to the reaction conditions and other cleavage conditions examined, i.e., using TFA−H_2O mixtures or PTS in dioxane−H_2O gave complex product mixtures. Alternatively the hydrolysis was successfully executed with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in 9:1 CH_3CN−H_2O.[23]

Synthetic Route 2. (Scheme 3) Oxidation of L-mannonic γ-lactone (**11**) with aqueous nitric acid provided, after workup, L-mannaro-1,4:3,6-di-γ-lactone (**35**) in 60% yield.[24] Benzylation of bislactone **35** was performed using benzyltrichloroacetimidate[25] with a catalytic amount of trifluoromethanesulfonic acid in dry dioxane furnishing the dibenzylated product **36** in 72% yield. Numerous other solvents besides dioxane were examined, but either yields were low or compound **35** could not be dissolved in the solvent. Benzylation procedures employing basic conditions including benzylation with benzylbromide and silveroxide were also examined, but all failed to give the desired product in reasonable yields probably attributed due to the base lability of **35**.

Nucleophilic ring opening of dilactone **36** with amines gave the bisamide derivatives **28**, **37**−**48** in 22−76% yields (Scheme 3). Best yields were obtained in CH_2Cl_2 at reflux while moderate yields were obtained in chloroform, CH_3CN, dioxane, or THF. Low yields were obtained in dimethylformamide and in ethanol. The major side product of the reaction results from β-elimination of **36** to give **49** (Scheme 4). The formation of

Table 1. Structures, Yields, Methods of Preparation, and HIV-1 Protease Inhibititory Activity of L-Mannaric Diamides with 2*R*,3*R*,4*R*,5*R* Configuration



A-	Cmpd. no.	Yield %	Method[a]	IC_{50} (μM)[b]	K_i (nM)[c]	A-	Cmpd. no.	Yield %	Method[a]	IC_{50} (μM)[b]	K_i (nM)[c]
	**17**[d]	72%	I				**38**	60%	IV	nd	5000
	23	69%	I	5	nd						
	**18**[d]	70%	I				**39**	61%	IV	nd	500-1500
	24	70%	I	ni	nd						
	**19**[d]	71%	I				**40**	73%	IV	nd	660
	25	71%	I	ni	nd						
	**20**[d]	61%	I				**41**	31%	IV	nd	2.3
	26	74%	I	ni	nd						
	**21**[d]	73%	I				**42**	20%	IV	nd	81
	27	73%	I	ni	nd						
	**28**	88%	II	0.015	0.4		**43**	35%	IV	nd	0.2
	28	70%	IV								
	**29**	29%	II	ni	nd		**44**	22%	IV	ni	nd
	**30**	30%	II	ni	nd		**45**	49%	IV	ni	nd
	**31**	21%	II	ni	nd		**46**	65%	IV	nd	7100
	**32**	25%	III	ni	nd		**47**	59%	IV	nd	2000
	**33**	32%	III	ni	nd		**48**	46%	IV	nd	0.9
	**34**	38%	III	ni	nd						
	**37**	76%	IV	nd	140						

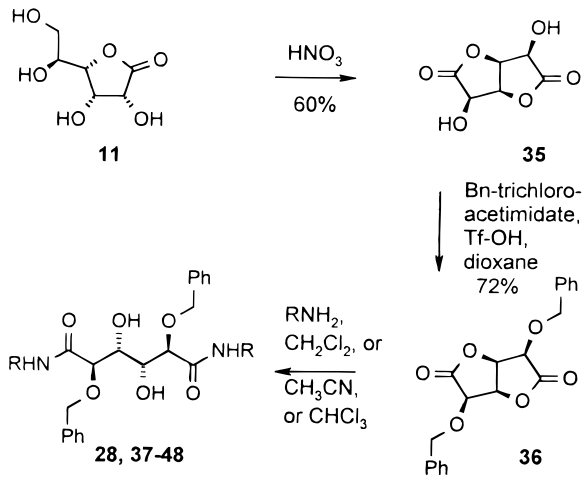
[a] Method I: see Scheme 2, route A. Method II: see Scheme 2, route A (overall yields from **16**). Method III: see Scheme 2, route B (yields from **22**). Method IV: see Scheme 3 (amine opening of bislactone **36**). [b] ni = no inhibition at 10 μM. [c] nd = not determined. [d] 3,4-*O*-isoprypylidene protected coupling product.

49 is favored in polar solvents and in reactions involving basic amines. This interesting elimination product **49** can be produced in high yield (95%) by reacting the bislactone **36** with 1 M NaOH in dioxane (Scheme 4).

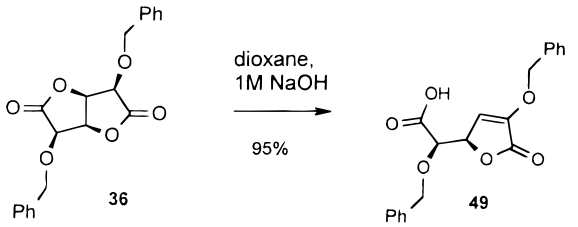
A notable feature of the bislactone **36** is that the reaction rate for opening of the first lactone ring is considerably slower than the rate for opening of the second lactone ring. Thus only a minor amount of the monolactone product was traced during the reaction or was isolated from the product mixture when an excess of amine is used.

HIV-1 Protease Inhibition. (Table 1) HIV-1 protease was cloned and heterologously expressed in *Escherichia coli* as described elsewhere.[26] The inhibitory effect of the synthesized compounds was initially determined with purified HIV-1 protease in a standardized spectrophotometric assay.[27] The results are presented as IC_{50} values, i.e., the concentration of inhibitor result-

Scheme 3. Synthesis of L-Mannaric Acid Diamides



Scheme 4. A High Yielding Ring Opening Elimination Product



ing in 50% inhibition in this assay. For subsequent compounds and those that exhibited significant inhibitory effects, K_i values were determined by a more sensitive fluorometric assay.[28]

Structure−Activity Relationship and X-ray Crystallographic Data. For compounds **23**−**25** and **27** each having lipophilic P2/P2′ amino acid methyl ester side chains, only compound **23** showed any enzyme inhibitory activity. A dramatic increase in inhibitory activity (IC_{50}) going from 5000 nM to 15 nM was obtained when the methyl ester of compound **23** was changed to the corresponding *N*-methyl amide (**28**). Thus duplication of one additional hydrogen bond between the C_2-symmetric inhibitor and the HIV protease backbone results in this substantial increase in inhibitory activity.

To explore hydrophilic interactions to the enzyme backbone or to groups lining the hydrophobic S2/S2′ pockets, a number of amines each containing hydrogen bond donating or accepting functionalities were selected and coupled to give compounds **26**, **29**−**34**, and **45**−**47**. However, these compounds exhibited only weak or no HIV-1 protease inhibitory activity. Efforts were then turned to optimizing the P2/P2′ side chains of the methyl amide **28**. Compounds **37**−**42** and **48** were prepared where enzyme inhibition data indicated that only lipophilic substituents at this position produced active inhibitors, K_i (nM) = 660, 140, 81, 2.3, and 0.9 for inhibitors **40** (Met), **37** (Phe), **42** (m-F-phenylGly), **41** (phenylGly), and **48** (Ile) where compounds **28** and **48** were the most potent in this series.

The X-ray crystal structure of HIV-1 protease complexed with the isoleucine inhibitor **48** (Figure 5) was determined.[29] It reveals that one of the hydroxyls of the central diol points toward the active site Asp 25/125 residues 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 residues. This difference in usage of the hydroxyls results in an asymmetric positioning of the inhibitor in the center of the protease. The P1/P1′ benzyloxy groups are positioned in the S1/S1′ pockets. The 1,6-dicarbonyl oxygens of the L-mannaric acid backbone and the amide nitrogens of the residues Ile 50/150 coordinate a structural water molecule in a distorted tetrahedral arrangement. The carbonyl oxygen of Ile (Figure 5) binds to the backbone amide nitrogens of Asp 29/129 in the S3/S3′ site, and the nitrogen of the *N*-methyl amide binds to the Gly 48/148 carbonyl. The aliphatic Ile side chains are placed in the S2/S2′ pockets in close packing distances to Val 32/132. From this structural information it is apparent that the S2/S2′ pockets of the Ile inhibitor are not optimally filled up and that conformationally restricted side chains might induce improved fit to the S2/S2′ pockets.

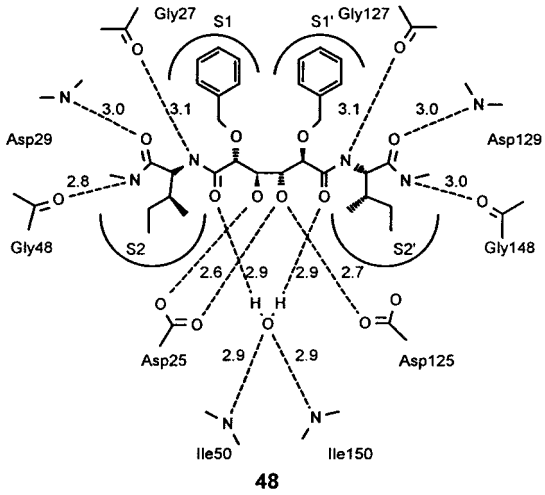
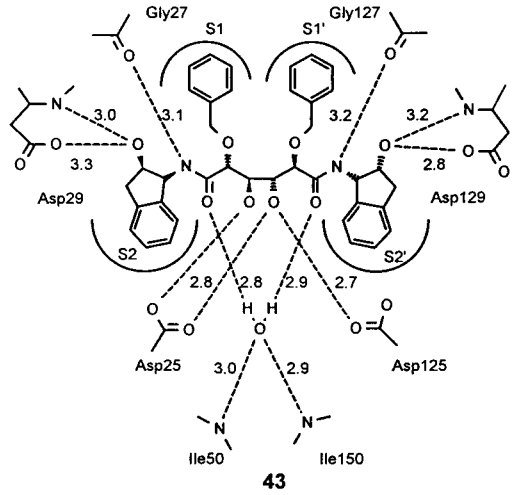


Figure 5. Schematic drawing showing the hydrogen bonds between the HIV-1 protease and the C_2-symmetric inhibitor **48**.

1(*S*)-Amino-2(*R*)-hydroxyindan has successfully been incorporated in HIV-1 protease inhibitors by, for example, researchers at Merck (Figure 1, indinavir). As deduced from molecular modeling experiments, 1(*S*)-amino-2(*R*)-hydroxyindan excellently fit with respect to filling the S2/S2′ subsite pockets and with respect to hydrogen bonding between the hydroxyl group and the protease backbone. Compound **43,** having the 1(*S*)-amino-2(*R*)-hydroxyindan moiety, was thus synthesized and found to be twice as active as compound **28** (K_i 0.2 nM). As expected the corresponding enantiomer of 1(*S*)-amino-2(*R*)-hydroxyindan (**44**) exhibited no enzyme inhibition activity.

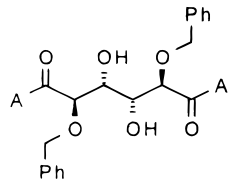
The X-ray crystal structure of HIV-1 protease complexed with inhibitor **43** (Figure 6) was determined, and reveled that both inhibitors **43** and **48** bind similarly.[29] The hydroxyl group of 1(*S*)-amino-2(*R*)-hydroxyindan in inhibitor **43** binds to the backbone amide nitrogens of Asp 29/129 in the S3/S3′ site. The aromatic portion of the 1(*S*)-amino-2(*R*)-hydroxyindan groups in inhibitor **43** are positioned such that they truly form the intended expansion of the Ile groups, nicely filling out the S2/S2′ pockets.

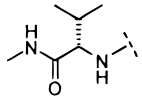
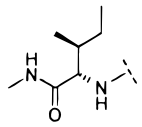
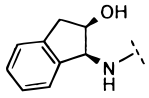


43

Figure 6. Schematic drawing showing the hydrogen bonds between the HIV-1 protease and the C_2-symmetric inhibitor **43**.

Table 2. In Vitro Anti-HIV-1 Activity[30]



A-	Cmpd. no.	ED_{50} (μg/mL)
Ritonavir	**2**	0.05
Indinavir	**3**	0.04
	**28**	0.9
	**48**	1.0
	**43**	0.06

In Vitro Anti-HIV Activity. (Table 2) The anti-HIV activity was assayed by an HIV cytopathic assay in MT-4 cells where the effect was quantified using vital dye XTT.[30] The 50% inhibitory concentrations (ED_{50}) were calculated from the percent cytoprotection for individual compounds.

As can be seen in Table 2, compound **43** has an anti-HIV-1 activity in cell culture which is comparable to that of ritonavir and indenavir whereas compounds **28** and **48** are more than 10 times less active in the assay.

Conclusion

Application of the concept of C-terminal duplication with a carbohydrate, L-mannaric acid, as a new template for a peptidomimetic enabled the design of a number of potent HIV-1 protease inhibitors. One of the compounds, **43,** also demonstrated promising anti-HIV activity in cell culture. Two synthetic routes leading up to these inhibitors have been developed, one of which gives access to these inhibitors in just three chemical steps from commercially available starting materials.

The structure−activity relationships for these C_2-symmetric inhibitors have been rationalized with the aid of X-ray crystal structures. An IC_{50} going from 5000 nM to 15 nM was observed upon changing −OMe (**23**) into −NHMe (**28**), resulting in the net addition of one more hydrogen bond interaction from each NHMe group to the enzyme backbone. By sacrificing this important hydrogen bonding interaction but by optimizing binding to the S2/S2′ subsite pockets, an even more potent inhibitor was discovered (**43**). Further studies of this class of compounds are currently under way.

Experimental Section

Thin layer chromatography was performed using silica gel 60 F-254 (Merck) plates with detection by UV and/or charring with 8% sulfuric acid. Column chromatography was performed on silica gel (Matrix Silica Si 60A, 35−70 μm, Amicon) eluting with CH_3Cl−MeOH (gradient 5−20% MeOH) if not otherwise stated. Organic phases were dried over anhydrous sodium sulfate or magnesium sulfate. Concentrations were performed under reduced pressure. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. Infrared spectra were recorded on a Perkin-Elmer 1600 series FTIR instrument. NMR spectra were recorded on a JEOL GSX-270 instrument; ^{13}C NMR 67 MHz and ^1H NMR 270 MHz. Chemical shifts are reported in ppm (δ) downfield from tetramethylsilane in $CDCl_3$, unless otherwise stated.

Accurate mass measurements were recorded on a JEOL SX 102 mass spectrometer/MS-MP7000 data system.

3,4-*O*-Isopropylidene-L-mannitol (12). To a stirred and cooled (0 °C) solution of L-mannonic γ-lactone (20.0 g, 112 mmol) in dry MeOH (700 mL) was added lithium borohydride (4.80 g, 225 mmol) in four portions. The reaction mixture was stirred at room temperature for 30 min, cooled to 0 °C, and acidified with HOAc. After the mixture was stirred for 15 min, it was concentrated until dryness and the residue dissolved in MeOH (200 mL). Another portion of HOAc and MeOH was added and the acidic solution was again concentrated. Drying in a vacuum gave L-mannitol as a white foam which was suspended in dry acetone (600 mL) to which 2,2-dimethoxypropane (100 mL, 821 mmol) and camphorsulfonic acid (60.0 g, 260 mmol) were added. The reaction mixture was stirred overnight and concentrated. EtOAc (300 mL) was added, and the organic layer was washed with saturated aqueous $NaCO_3$ (300 mL), dried, and concentrated. Purification by column chromatography (toluene:EtOAc, 10:1) gave the triacetonide as a white solid (26.5 g, 87.6 mmol, 78%).

The triacetonide (25.1 g, 83.0 mmol) was dissolved in 70% HOAc (500 mL) and stirred at 40 °C for 1.5 h. The solution was concentrated, the residue extracted with acetone (L-mannitol remains as a solid residue), and the extract concentrated. The remaining traces of HOAc were removed by coevaporation with added toluene. The solid residue was recrystallized from warm acetone to give compound **12** (11.7 g, 53 mmol, 64%) as white crystals. ^1H NMR was in agreement with that previously reported[9b] (patent WO 93/07128).

1,6-Di-*O*-*tert*-butyldimethylsilyl-3,4-*O*-isopropylidene-L-mannitol (13). *tert*-Butyldimethylsilyl chloride (814 mg, 5.4 mmol) was added to a stirred solution of compound **12** (566 mg, 2.57 mmol) in pyridine (10 mL) containing (dimethylamino)pyridine (7 mg, 0.05 mmol). The mixture was stirred at 40 °C for 1.5 h and then concentrated to dryness by coevaporation with added toluene. The residue was dissolved in CH_2Cl_2 (30 mL) and washed with saturated aqueous $NaCO_3$,

dried, concentrated, and purified by column chromatography (toluene:EtOAc, 10:1) to give **13** as a clear oil (1.10 g, 2.47 mmol, 96%). ^{13}C NMR ($CDCl_3$): δ 5.2, 18.5, 26.0, 27.1, 64.6, 79.6, 109.4. Anal. ($C_{21}H_{46}O_6Si_2$) C, H.

2,5-Di-*O*-benzyl-1,6-di-*O*-*tert*-butyldimethylsilyl-3,4-*O*-isopropylidene-L-mannitol (14). To a cold (0 °C), stirred suspension of NaH (290 mg, 12.1 mmol) in dry THF (30 mL) under an atmosphere of argon was added **13** (2.27 g, 5.06 mmol) over 10 min. The ice bath was removed, and the resulting tan solution was stirred at room temperature for 15 min. Tetrabutylammonium iodide (15 mg) and benzyl bromide (1.32 mL, 11.1 mmol) were added, and the reaction mixture was stirred at room temperature for 15 h. H_2O (3 mL) was added, and the resulting mixture was concentrated. The residue was taken up in Et_2O (40 mL) and washed with H_2O (3 × 40 mL). The combined organic extracts were dried, concentrated, and purified by column chromatography (light petroleum ether−EtOAc, 17:1) to give compound **14** as a clear oil (2.43 g, 3.85 mmol, 76%). ^{13}C NMR ($CDCl_3$): δ 5.4, 18.2, 25.9, 27.2, 63.8, 73.1, 78.3, 81.1, 109.5, 127.7, 128.1, 138.8. Anal. ($C_{35}H_{58}O_6Si_2$) H,C.

2,5-Di-*O*-benzyl-3,4-*O*-isopropylidene-L-mannitol (15). Compound **14** (2.10 g, 3.3 mmol) was dissolved in THF (15 mL), a solution of tetrabutylammonium fluoride in THF (7.2 mL, 1.1 M) was added, and the reaction mixture was stirred at room temperature for 1 h. Most of the solvent was removed under reduced pressure, and the residue was flashed through a short column of silica gel with CH_3CN as eluent, concentrated, and purified by column chromatography (light petroleum ether−EtOAc, 1:1) to give compound **15** as a clear oil (1.29 mg, 3.2 mmol, 98%) which solidified upon standing. ^{13}C NMR ($CDCl_3$): δ 27.3, 61.4, 80.0, 110.1, 128.1, 128.7, 137.9. Anal. ($C_{23}H_{30}O_6$) C, H.

2,5-Di-*O*-benzyl-3,4-*O*-isopropylidene-L-mannaric acid (16). To a stirred solution of compound **15** (1.080 g, 2.68 mmol) in CH_2Cl_2 (36 mL) was added TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy, free radical) (22 mg, 0.14 mmol) and a solution of saturated aqueous $NaCO_3$ (11 mL), KBr (59 mg, 0.50 mmol), and tetrabutylammonium bromide (90 mg, 0.28 mmol). The mixture was cooled to 0 °C, and a solution of sodium hypochlorite (17.4 mL, 1.2 M), saturated aqueous $NaCO_3$ (5.9 mL), and brine (11.8 mL) was added over 45 min. After the mixture was stirred at 0 °C for an additional 45 min, the organic layer was separated and washed with H_2O (3 × 20 mL). The combined aqueous phases were acidified with 4 M HCl, extracted with EtOAc (3 × 20 mL), dried, and concentrated to give compound **16** as a white solid (775 mg, 1.80 mmol, 67%). ^{13}C NMR (DMSO-d_6): δ 27.0, 71.7, 78.4, 79.1, 110.0, 127.6, 128.3, 137.5, 170.6. A small portion was recrystallized from EtOAc and light petroleum ether to give the dicarboxylic acid as white crystals which were subjected to elementary analysis. Anal. ($C_{23}H_{26}O_8$) C, H.

Procedure for Preparation of Compounds 17−21. Method I. Compound **16** (200−300 mg, 0.46−0.70 mmol), amino acid derivative (2.2 equiv, 1.01−1.54 mmol), and 1-hydroxybenzotriazole hydrate (3.0 equiv, 186−284 mg, 1.38−2.10 mol) were dissolved in 6−8 mL CH_2Cl_2−THF (2:1) under an atmosphere of argon. The pH of the stirred reaction mixture was adjusted to 7.5 by dropwise addition of triethylamine. The temperature was lowered to 0 °C, and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.4 equiv, 211−322 mg, 0.96−1.68 mmol) was added in one portion. After 1 h, the temperature was raised to room temperature, and the reaction mixture was stirred for 2−4 h, depending on the amino acid derivative used. CH_2Cl_2 (20 mL) was added, and the organic layer was washed with saturated aqueous $NaCO_3$. The organic layer was separated, dried, and concentrated. Column chromatography (toluene−EtOAc, 3:1−4:1) furnished compounds **17**−**21**. Details about yields and amino acid derivatives used are listed in Table 1.

***N*1,*N*6-Di[(1*S*)-2-methyl-1-(methoxycarbonyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxy-3,4-*O*-isopropylidenehexanediamide (17).** ^{13}C NMR ($CDCl_3$): δ 17.8, 19.0, 27.0, 31.2, 52.0, 56.7, 73.9, 77.6, 79.4, 110.4, 128.2, 128.3, 136.8, 169.0, 171.8. Anal. ($C_{35}H_{48}N_2O_{10}\cdot 1H_2O$) C, H, N.

***N*1,*N*6-Di[(1*S*)-2-phenyl-1-(methoxycarbonyl)ethyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxy-3,4-*O*-isopropylidenehexanediamide (18).** ^{13}C NMR ($CDCl_3$): δ 27.0, 37.9, 52.1, 52.6, 73.6, 78.1, 79.8, 110.6, 128.4, 128.5, 128.7, 129.2, 135.6, 136.5, 169.0, 171.6. Anal. ($C_{43}H_{48}N_2O_{10}\cdot 0.5H_2O$) C, H, N.

***N*1,*N*6-Di[(1*S*)-2-methyl-1-(benzyloxycarbonyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxy-3,4-*O*-isopropylidenehexanediamide (19).** ^{13}C NMR ($CDCl_3$): δ 17.67, 19.0, 27.0, 31.3, 56.7, 66.9, 73.9, 77.5, 79.5, 110,4, 128.2, 128.3, 128.6, 129.5, 135.5, 136.9, 169.1, 171.2. Anal. ($C_{47}H_{56}N_3O_{10}\cdot 0.5H_2O$) C, H, N.

***N*1,*N*6-Di[(1*S*)-2-methyl-1-(hydroxymethyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxy-3,4-*O*-isopropylidenehexanediamide (20).** Compound **20** was directly subjected to deprotection. ^{13}C NMR ($CDCl_3$): δ 18.2, 19.4, 27.3, 30.2, 56.9, 63.3, 75.1, 77.5, 79.3, 110.4, 128.3, 128.8, 129.0, 136.7, 169.6.

***N*1,*N*6-Di[(1*S*)-3-methyl-1-(methoxycarbonyl)butyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxy-3,4-*O*-isopropylidenehexanediamide (21).** Compound **21** was directly subjected to deprotection. ^{13}C NMR ($CDCl_3$): δ 21.9, 23.0, 24.9, 27.1, 41.7, 50.3, 52.3, 74.0, 77.8, 79.6, 110,5; 128.4, 128.7, 129.1, 136.9, 169.2, 173.0.

***N*1,*N*6-Disuccinimidyl-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (22).** A mixture of compound **16** (1.01 g, 2.35 mmol), *N*,*N*-disuccinimidyl carbonate (2.41 g, 9.4 mmol) and pyridine (1.15 mL, 14.1 mmol) in CH3CN (15 mL) was stirred at ambient temperature overnight. The mixture was concentrated and the residue dissolved in EtOAc. The organic phase was washed with H2O, dried, and concentrated, and the residue was purified by column chromathograpy to give **22** (1.29 g, 2.07 mmol, 88%) as a white foam. A small portion was crystallized from 2-propanol. [α]D = +53.8° (c = 0.935, $CHCl_3$). ^{1}H NMR (CDCl3): δ 1.4 (s, 6H), 2.8 (s, 8H), 4.4−4.65 (m, 6H), 4.85 (d, 2H), 7.2−7.4 (m, 10H). ^{13}C NMR (CDCl3): δ 25.6, 27.0, 73.0, 77.0, 77.8, 112.2, 128.2, 128.5, 136.1, 165.5, 168.4. Anal. (C31H32N2O12) C, H, N.

Procedure for Preparation of Compounds 23−27. Method I. Compounds **17**−**21** were dissolved in MeOH (15 mL) acidified with HCl (4%, w:w) and stirred at room temperature for 2 h. The clear solution was concentrated and purified by column chromatography (toluene−EtOAc, 1:1; CH_2Cl_2−MeOH, 95:5 for compound **26**) to give compounds **23**−**27** in 70−74% yield (see Table 1).

***N*1,*N*6-Di[(1*S*)-2-methyl-1-(methoxycarbonyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (23).** ^{13}C NMR ($CDCl_3$): δ 17.6, 19.0, 31.3, 52.1, 56.6, 71.6, 74.2, 78.0, 128.3, 128.5, 136.4, 171.7, 172.1. $[\alpha]_D$ = +20.53° (c = 1.13, $CHCl_3$). Anal. ($C_{32}H_{44}N_2O_{10}\cdot 0.5H_2O$) C, H, N.

***N*1,*N*6-Di[(1*S*)-2-phenyl-1-(methoxycarbonyl)ethyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (24).** $[\alpha]_D$ = +44.21° (c = 1.07, $CHCl_3$). ^{13}C NMR ($CDCl_3$): δ 37.7, 52.6, 53.0, 70.9, 74.0, 79.1, 128.3, 128.7, 128.8, 129.2, 135.8, 136.8, 171.5, 172.3. Anal. ($C_{40}H_{44}N_2O_{10}\cdot 1H_2O$) C, H, N.

***N*1,*N*6-Di[(1*S*)-2-methyl-1-(benzyloxycarbonyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (25).** $[\alpha]_D$ = +16.11° (c = 0.95, $CHCl_3$). ^{13}C NMR ($CDCl_3$): δ 17.5, 18.9, 31.0, 56.9, 67.0, 70.8, 74.0, 78.4, 128.4, 128.6, 128.7, 129.6, 135.3, 136.8, 171.0, 172.6. Anal. ($C_{44}H_{52}N_2O_{10}\cdot 1H_2O$) C, H, N.

***N*1,*N*6-Di[(1*S*)-2-methyl-1-(hydroxymethyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (26).** ^{13}C NMR ($CDCl_3$): δ 18.9, 19.6, 29.0, 56.9, 62.9, 71.8, 73.2, 81.1, 128.1, 128.3, 128.6, 136.8, 171.7. Anal. ($C_{30}H_{44}N_2O_8\cdot 1H_2O$) C, H, N.

***N*1,*N*6-Di[(1*S*)-3-methyl-1-(methoxycarbonyl)butyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (27).** ^{13}C NMR ($CDCl_3$): δ 21.7, 23.0, 24.9, 27.1, 41.7,

50.4, 52.3, 70.9, 74.0, 78.8, 128.2, 128.3, 128.7, 136.9, 172.4, 172.7. Anal. ($C_{34}H_{48}N_2O_{10}$) C, H, N.

Valine Methylamide. To a cooled (−15 °C) solution of Z-Val-OSu (348 mg, 1 mmol) in dry THF (5 mL) was added methylamine (2 mmol, 2 M solution in THF). The reaction mixture was brought to room temperature and stirred for 4 h. The *N*-hydroxysuccinimide was filtered off and the residue concentrated, dissolved in EtOAc (25 mL), washed with $NaHCO_3$ (2 × 15 mL), dried, and concentrated to give the crude methylamide which was crystallized from EtOAc. The product was dissolved in EtOAc and hydrogenated over Pd/C for 6 h. The catalyst was removed and the residue concentrated to give the desired methylamide in 78% yield (102 mg, 0.78 mmol). Spectral data were in agreement with those previously reported.[19]

***N*1,*N*6-Di[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (28).** Compound **16** (181 mg, 0.42 mmol), valine methylamide (121 mg, 0.93 mmol), and 1-hydroxybenzotriazole hydrate (170 mg, 1.26 mmol) were dissolved in 6 mL of CH_2Cl_2−THF (2:1) under an atmosphere of argon. The pH of the stirred reaction mixture was adjusted to 7.2 by dropwise addition of triethylamine. The temperature was lowered to 0 °C, and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (191 mg, 1.0 mmol) was added in one portion. After 1 h, the temperature was raised to room temperature and the reaction mixture was stirred 4 h. CH_2Cl_2 (20 mL) was added, and the organic layer was washed with saturated aqueous $NaCO_3$. The organic layer was separated, dried, and concentrated. The residue was dissolved in MeOH (15 mL) acidified with HCl (4%, w:w) and stirred at room temperature for 2 h. The clear solution was concentrated and purified by column chromatography (toluene−EtOAc, 1:1) to give **28** in 88% yield. $[\alpha]_D = -6.27°$ ($c = 1.03$, $CHCl_3$). ^{13}C NMR ($CDCl_3$−CD_3OD, 7:3): δ 17.5, 19.6, 26.0, 30.2, 58.5, 72.1, 73.2, 80.5, 128.4, 128.6, 137.2, 172.1, 172.4. Anal. ($C_{32}H_{46}N_4O_8 \cdot 1H_2O$) C, H, N.

General Method for the Preparation of Compounds 29−31. Method II. The l-mannaric acid **16** (1.0 equiv), the amine (2.2 equiv), and HOBT (3.0 equiv) were dissolved in 2:1 CH_2Cl_2−THF with stirring under argon. The pH was adjusted to 7.5 by dropwise addition of triethylamine, and the temperature was adjusted to 0 °C. EDC (2.4 equiv) was added, and stirring was continued for 1 h at 0 °C and then for 16 h at ambient temperature. The reaction mixture was diluted with CH_2Cl_2 (2× volume), washed with saturated aqueous $NaHCO_3$, dried, and concentrated, and the residue was purified by column chromatography. The purified l-mannaric amides were dissolved in 5 mL of 4% HCl in MeOH, and the reaction mixture was stirred at ambient temperature until TLC indicated complete reaction. The mixture was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$, dried, concentrated, and purified by column chromatography to give the target compounds **29**−**31**.

***N*1,*N*6-Di(2-pyridylmethyl)-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (29).** The title compound was prepared in 29% yield (50 mg, 0.088 mmol) according to method II, using 2-aminomethyl pyridine $[\alpha]_D = +21.7°$ ($c = 1.34$, $CHCl_3$). ^{13}C NMR ($CDCl_3$): δ 43.9, 71.3, 73.7, 79.5, 121.9, 122.4, 128.1, 128.3, 128.5, 137.0, 148.9, 156.1, 172.4. Anal. ($C_{32}H_{34}N_4O_6 \cdot 0.5H_2O$) C, H, N.

***N*1,*N*6-Di(3-pyridylmethyl)-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4- dihydroxyhexanediamide (30).** The title compound was prepared in 30% yield **30** (62 mg, 0.109 mmol) according to method II, using 3-aminomethyl pyridine. $[\alpha]_D = +49.2°$ ($c = 1.34$, $CHCl_3$). ^{13}C NMR (CD_3OD): δ 40.5, 40.6, 71.2, 73.3, 80.3, 124.2, 128.4, 128.7, 134.7, 136.4, 137.1, 148.0, 148.5, 172.6, 172.7. Anal. ($C_{32}H_{34}N_4O_6$) C, H, N.

***N*1,*N*6-Di(4-pyridylmethyl)-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (31).** The title compound was prepared in 21% (94 mg, 0.165 mmol) according to method II, using 4-aminomethyl pyridine. $[\alpha]_D = +31.4°$ ($c = 0.36$, MeOH). ^{13}C NMR ($CDCl_3$): δ 41.8, 71.5, 73.8, 79.8, 122.2, 128.3, 128.5, 128.7, 136.6, 147.2, 149.6, 172.3. Anal. ($C_{32}H_{34}N_4O_6 \cdot 0.5H_2O$) C, H, N.

General Method for the Preparation of Compounds 32−34. Method III. The succinimidyl diester **22** (1.0 equiv) and the amine (2.5 equiv) were dissolved in dichloroethane. The mixture was stirred for 16 h under argon at 65 °C. After concentration the mixture was dissolved in CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$, dried, and concentrated, and the residue was subjected to column chromathograpy. The purified l-mannaric amides were dissolved in 5 mL of 4% HCl in MeOH, and the reaction mixture was stirred at ambient temperature until TLC indicated complete reaction. The mixture was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$ (2×), dried, concentrated, and purified by column chromatography to give title compounds **32**−**34**.

***N*1,*N*6-Di(3-hydroxy-2-methylphenyl)-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (32).** The title compound was prepared in 25% yield (40 mg, 0.067 mmol) according to method III, using 3-amino-*o*-cresol. $[\alpha]_D = +37.8°$ ($c = 0.54$, MeOH). ^{13}C NMR (CD_3OD and $CDCl_3$): δ 10.0, 71.5, 73.9, 80.4, 112.8, 115.4, 118.0, 126.6, 128.6, 128.9, 135.8, 137.1, 155.7, 170.8. HRMS calcd for $C_{34}H_{36}N_2O_8$ 600.2472, found 600.2508.

***N*1,*N*6-Di(2-hydroxy-4-methylphenyl)-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (33).** The title compound was prepared in 32% yield (75 mg, 0.13 mmol) according to method III, using 6-amino-*m*-cresol. $[\alpha]_D = +46.2°$ ($c = 0.75$, MeOH). ^{13}C NMR (CD_3OD): δ 21.2, 72.4, 74.1, 81.7, 116.9, 121.0, 122.4, 124.2, 129.0, 129.4, 129.5, 136.4, 138.6, 148.9, 171.7. Anal. ($C_{34}H_{36}N_2O_8$) C, H, N.

***N*1,*N*6-Di(4-hydroxy-2-methylphenyl)-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (34).** The title compound was prepared in 38% yield (96 mg, 0.16 mmol) according to method III, using 4-amino-3-methylfenol. $[\alpha]_D = +30.3°$ ($c = 0.75$, DMSO). ^{13}C NMR (DMSO-d_6): δ 18.0, 70.0, 71.5, 80.2, 112.6, 116.6, 127.2, 127.5, 127.6, 127.8, 128.2, 134.1, 138.0, 155.1, 169.9. Anal. ($C_{34}H_{36}N_2O_8$) C, H, N.

L-Mannaro-1,4:3:6-di-γ-lactone (35). L-Mannonic γ-lactone (**11**) (1.0 equiv, 3.0 g, 17.0 mmol), HNO_3 (9 mL, concentrated), and H_2O (1.7 mL) were heated to 85 °C under stirring with the evolution of nitrous gases. The reaction mixture was refluxed for 16 h and concentrated twice with addition of H_2O (30 mL). The residue was lyophilized from H_2O (40 mL). The resulting white solid was suspended in ethanol (5 mL) and diethyl ether (30 mL). After filtration and drying **35** was obtained as a white solid (1.76 g, 10.1 mmol, 60%). $[\alpha]_D = -158.81°$ ($c = 1.18$, MeOH). ^{1}H NMR (DMSO-d_6): δ 4.75 (d, 2H), 5.1 (d, 2H), 6.4 (broad s, 2H). ^{13}C NMR (DMSO-d_6): δ 69.2, 75.9, 174.3. IR (KBr): 1799.4 cm^{-1}. Anal. ($C_6H_6O_6$) C, H.

2,5-Di-*O*-benzyl-L-mannaro-1,4:3,6-di-γ-lactone (36). To a stirred solution of L-mannaro-1,4:6,3-di-γ-lactone (300 mg, 1.72 mmol) and benzyl-2,2,2-trichloroacetimidate (0.96 mL, 5.17 mmol) in dry dioxane was added triflormethanesulfonic acid (240 μL) dropwise under nitrogen. Within 1−2 h the color changed to red-brown. After an additional 30 min the reaction mixture was filtered through 2 cm of $NaCO_3$ on 2 cm of silica in a glass filter-funnel and subsequently evaporated under reduced pressure. Warm diethyl ether was added to the crude product, the mixture was stirred for 1 min, and the diethyl ether was thereafter decanted. Recrystalization from CH_2Cl_2 gave white crystals of 2,5-di-*O*-benzyl-L-mannaro-1,4:3,6-di-γ-lactone (439.5 mg, 72%). $[\alpha]_D = -125.58°$ ($c = 1.04$, CH_2Cl_2). IR (KBr) 1785 cm^{-1}. Mp 184−186 °C. ^{13}C NMR (DMSO-d_6): δ 72.04, 74.39, 74.82, 127.97, 128.04, 128.4, 136.92, 171.76. Anal. ($C_{20}H_{18}O_6$) C, H.

General Method for the Preparation of Compounds 28, 37−40. Method IV. Bislactone **36** (40 mg, 0.11 mmol) was dissolved in CH_2Cl_2 (4 mL). Six equivalents of the amino acid derivative was added to the solution, and the mixture was stirred at reflux for 16 h. The solvent was removed under reduced pressure and the crude product purified by silica gel chromatography.

***N*1,*N*6-Di[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (28).** The title compound was prepared using valine

methylamide[19] according to method IV using CH_2Cl_2−MeOH (20: 1) as eluent to give **28** (48.6 mg, 70%).

***N*1,*N*6-Di[(1*S*)-2-phenyl-1-(methylcarbamoyl)ethyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (37).** The title compound was prepared using phenylalanine methylamide[31] according to method IV using CH_2Cl_2−MeOH (20:1) as eluent to give product **37** (60.9 mg, 76%). $[\alpha]_D = +5.59°$ ($c = 1.02$, $CHCl_3$); IR (KBr): 3324, 1650, 1525 cm^{-1}. ^{13}C NMR ($CDCl_3$): δ 26.20, 37.02, 54.03, 72.83, 73.67, 81.87, 127.07, 127.81, 128.42, 128.65, 128.78, 129.00, 136.38, 134.98, 170.00, 172.06. Anal. ($C_{40}H_{46}N_4O_8$) C, H, N.

***N*1,*N*6-Di[(1*S*)-2-(*p*-hydroxy)phenyl-1-(methylcarbamoyl)ethyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (38).** The title compound was prepared using tyrosine methylamide[31] according to method IV using CH_2Cl_2−MeOH (9:1) as eluent to give product **38** (60.0 mg, 60%). $[\alpha]_D = +7.13°$ ($c = 0.87$, EtOH). IR (KBr): 3320, 1651, 1514 cm^{-1}. ^{13}C NMR (CD_3OD): δ 26.13, 37.07, 55.32, 72.86, 72.93, 81.13, 116.14, 128.83, 129.15, 130.93, 138.32, 157.18, 172.92, 173.59. Anal. ($C_{40}H_{46}N_4O_{10}$) C, H, N.

***N*1,*N*6-Di[(1*S*,2*R*)-2-hydroxy-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (39).** The title compound was prepared according to method IV using threonine methylamide using CH_2Cl_2−MeOH (9:1) to give product **39** (42.9 mg, 61%). $[\alpha]_D = -3.24°$ ($c = 1.02$, MeOH). IR (KBr): 3393, 1651, 1518 cm^{-1}. ^{13}C NMR (CD_3OD): δ 20.59, 26.18, 59.53, 67.09, 73.13, 81.02, 128.90, 129.08, 129.24, 138.39, 172.69, 173.21. Anal. ($C_{30}H_{42}N_4O_{10}$) C, H, N.

***N*1,*N*6-Di[(1*S*)-3-thiomethyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (40).** The title compound was prepared using methionine methylamide[32] according to method IV using CH_2Cl_2−MeOH (20:1) as eluent to give product **40** (55.0 mg, 72%). $[\alpha]_D = -13.84°$ ($c = 1.51$, MeOH). IR (KBr): 3303, 1636, 1541 cm^{-1}. ^{13}C NMR (CD_3OD): δ 15.37, 26.58, 31.42, 32.21, 53.73, 72.72, 73.64, 81.55, 129.16, 129.41, 129.60, 138.86, 174.02, 174.22. Anal. ($C_{32}H_{46}N_4S_2O_8$) C, H, N.

General Method for the Preparation of Compounds 41−48. Method IV. A mixture of the amine (2.2−6 equiv) in specified solvent was heated at 40−70 °C with stirring. The benzylated bislactone **36** (1.0 equiv) was added, and the mixture was stirred for 1−16 h at elevated temperature. The solvent was evaporated, and the crude product was purified by silica gel column chromatography and/or crystallization to give the target compounds **41**−**48**.

L-Phenylglycine Methyl Amide. Z-PhGly-OSu (1 equiv, 2.01 g, 5.26 mmol) was suspended in THF (20 mL) under argon at −10 °C. Methylamine (1.6 equiv, 4.2 mL as a 2.0 M solution in THF, 8.4 mmol) was added dropwise during 20 min. Stirring was continued at −10 °C for 2 h and at room temperature for 1.5 h. The mixture was filtered through Celite, concentrated, and partitioned between $CHCl_3$, H_2O, and NH_4Cl, dried, and concentrated. Crystallization from EtOAc gave Z-L-phenylglycine methyl amide (649 mg, 2.18 mmol, 42%) as a white solid. ^{13}C NMR (CD_3OD and $CDCl_3$): δ 26.3, 26.5, 58.7, 67.2, 127.2, 128.0, 128.3, 128.5, 128.6, 129.0, 136.2, 138.1, 156.3, 171.3.

***Z*-L-Phenylglycine Methyl Amide** (1.0 equiv, 607 mg, 2.03 mmol) was dissolved in EtOAc (12 mL). Pd/C (44 mg) was added, and the suspension was stirred under hydrogen for 21 h, filtered through Celite, and concentrated to give L-phenylglycine methyl amide (274 mg, 1.67 mmol, 77%) as a yellow oil which was used in the next step without further purification. ^{13}C NMR (CD_3OD): δ 26.4, 59.8, 128.2, 129.3, 129.8, 140.9, 174.9.

***N*1,*N*6-Di[(1*S*)-1-phenyl-1-(methylcarbamoyl)methyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (41).** The title compound was prepared in 31% yield (89 mg, 0.130 mmol) according to method IV, using l-phenylglycine methyl amide (274 mg, 1.67 mmol) in CH_3CN (1 mL) at 65 °C for 14 h. $[\alpha]_D = +75.7°$ ($c = 0.39$, CH_3OH). ^{13}C NMR (CD_3OD and $CDCl_3$): δ 26.3, 26.4, 57.1, 71.8, 73.4, 80.3, 127.4, 128.3, 128.4, 128.5, 128.6, 129.0, 136.5, 137.2, 170.3, 171.3. Anal. Calcd for $C_{38}H_{42}N_4O_8$: C, 66.85; H, 6.20; N, 8.21. Found: C, 66.74; H, 6.34; N, 8.12.

***N*1,*N*6-Di[1-(3-fluorophenyl)-1-(methylcarbamoyl)methyl]- (2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (42).** The title compound was prepared in 20% yield (49 mg, 0.068 mmol) according to method IV, using 3-fluorophenylglycine methyl amide (220 mg, 1.21 mmol) in CH_3CN (1 mL) at 70 °C for 16 h. ^{13}C NMR (CD_3OD and $CDCl_3$): δ 26.5, 57.4, 57.6, 71.7, 71.9, 72.2, 73.6, 73.7, 80.2, 80.3, 80.9, 115.0, 115.2, 115.4, 115.5, 115.7, 116.0, 124.0, 124.3, 128.7, 128.9, 129.2, 131.2, 131.3, 138.1, 140.9, 162.1, 165.7, 171.7, 172.8, 173.0. Anal. ($C_{38}H_{40}N_4O_8F_2$) C, H, N.

***N*1,*N*6-Di[(2*R*)-hydroxy-1(*S*)-indanyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (43).** The title compound was prepared according to method IV, using (1*S*,2*R*)-(−)-*cis*-1-amino-2-indanol (0.915 g, 6.2 mmol) in $CHCl_3$ (12 mL) at 45 °C for 4. The reaction mixture was concentrated and partitioned between $CHCl_3$ and saturated aqueous NH_4Cl and H_2O. The organic layer was separated, dried ($MgSO_4$), and concentrated. Crystallization from MeOH gave **43** (0.65 g, 0.996 mmol, 35%). $[\alpha]_D = -20.7°$ ($c = 0.68$, $CHCl_3$). ^{13}C NMR ($CDCl_3$): δ 39.2, 57.8, 71.6, 72.5, 73.5, 81.5, 124.0, 125.3, 127.0, 128.2, 128.3, 128.6, 136.7, 139.8, 140.8, 171.6. Anal. ($C_{38}H_{40}N_2O_8$) C, H, N.

***N*1,*N*6-Di[(2*R*)-hydroxy-1(*S*)-indanyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (44).** The title compound was prepared in 22% yield (60 mg, 0.092 mmol) according to method IV using (1*R*,2*S*)-(+)-*cis*-1-amino-2-indanol (370 mg, 2.5 mmol) in CH_3CN (1 mL) at 70 °C for 16 h. $[\alpha]_D = +3.2°$ ($c = 0.53$, CH_3OH). ^{13}C NMR (CD_3OD and $CDCl_3$): δ 40.1, 57.9, 71.6, 73.1, 73.5, 80.4, 124.8, 125.6, 127.5, 128.6, 128.8, 128.9, 137.6, 140.9, 173.2. Anal. ($C_{38}H_{40}N_2O_8$) C, H, N.

***N*1,*N*6-Di(2-hydroxyethyl)-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (45).** The title compound was prepared in 49% yield (105 mg, 0.296 mmol) according to method IV using 2-hydroxyethylamine (43 μL, 0.78 mmol) in $CHCl_3$ (2 mL) at 40 °C for 1 h. $[\alpha]_D = +64.0°$ ($c = 0.69$, $CHCl_3$). ^{13}C NMR (CD_3OD): δ 42.5, 61.4, 71.6, 73.6, 81.2, 128.8, 129.2, 138.5, 174.0. Anal. ($C_{24}H_{32}N_2O_8$) C, H, N.

5-Aminomethyl Thiazole. 5-Hydroxymethyl thiazole (1.0 equiv, 315 mg, 2.74 mmol), triphenyl phosphine (1.4 equiv, 995 mg, 3.79 mmol), and LiN_3 (6.3 equiv, 855 mg, 17.5 mmol) were suspended in DMF (15 mL). CBr_4 (1.4 equiv, 1.25 g, 3.78 mmol) was added, and the reaction mixture was stirred at room temperature for 23 h after which MeOH (3 mL) was added. The mixture was partitioned between toluene and H_2O (4×), dried, and concentrated. The residue was purified using column chromathograpy eluting with toluene−EtOAc (5:1) to give 5-azidomethyl thiazole (297 mg, 2.12 mmol, 77%) as a yellow viscous oil. ^1H NMR ($CDCl_3$): δ 4.58, 7.74, 8.84. ^{13}C NMR ($CDCl_3$): δ 46.3, 132.4, 142.7, 154.3. Anal. ($C_4H_4N_4S$) C, H, N. 5-Azidomethyl thiazole (1.0 equiv, 229 mg, 1.63 mmol) was dissolved in ethanol (20 mL), and Pd/C (0.2 g) was added. The suspension was stirred under hydrogen atmosphere for 20 h, filtered through Celite, and concentrated to give 5-aminomethyl thiazole (138 mg, 1.21 mmol, 74%) as a yellow syrup which was used without further purification. ^1H NMR (CD_3OD): δ 4.04, 4.71, 7.76, 8.89. ^{13}C NMR (CD_3OD): δ 38.5, 140.9, 141.7, 154.6.

***N*1,*N*6-Di(5-thiazolylmethyl)-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (46).** The title compound was prepared in 65% yield (106 mg, 0.182 mmol) according to method IV using 5-aminomethyl thiazole (138 mg, 1.21 mmol) in CH_3CN (1 mL) at 70 °C for 1 h. $[\alpha]_D = +29.0°$ ($c = 0.38$, CH_3OH). ^{13}C NMR (CD_3OD and $CDCl_3$): δ 35.5, 71.3, 73.4, 80.6, 128.6, 128.9, 129.0, 137.7, 137.8, 141.6, 155.0, 173.4. Anal. ($C_{28}H_{30}N_4O_6S_2$) C, H, N.

***N*1,*N*6-Di(2-chloro-6-fluorobenzyl)-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (47).** The title compound was prepared in 59% yield (114 mg, 0.169 mmol) according to method IV using 2-chloro-6-fluorobenzylamine (282 mg, 1.77 mmol) in CH_3CN (1 mL) at 70 °C for 21 h. $[\alpha]_D = +15.6°$ ($c = 0.93$, $CHCl_3$). ^{13}C NMR (CD_3OD and

$CDCl_3$): δ 34.8, 71.0, 73.7, 80.0, 114.5, 114.9, 123.3, 123.6, 125.8, 125.9, 128.5, 128.6, 128.8, 130.3, 130.4, 135.9, 137.2, 160.2, 163.9, 172.3, 172.4. Anal. ($C_{34}H_{32}N_2O_6Cl_2F_2$) C, H, N.

***N*1,*N*6-Di[(1*S*,2*S*)-2-methyl-1-(methylcarbamoyl)butyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy)-3,4-dihydroxyhexanediamide (48).** Prepared using isolucine methylamide[33] according to method IV using chloroform−methanol (20:1) as eluent to give (34.4 mg, 46%). $[\alpha]_D = -6.8°$ ($c = 0.50$, $CHCl_3$). IR (KBr): 3324, 1650, 1541 cm^{-1}. ^{13}C (CD_3OD): δ 11.67, 16.07, 25.13, 26.34, 37.20, 58.46, 72.26, 73.39, 80.84, 128.72, 129.13, 137.72, 172.68, 173.12. Anal. ($C_{34}H_{50}N_4O_8$) C, H, N.

(5*R*)-5-[(*R*)-(Benzyloxy)-(hydroxycarbonyl)methyl]-2-benzyloxy-2(5*H*)-furanone (49). Bislactone **36** (40 mg, 0.113 mmol) was dissolved in dioxane (6 mL). NaOH (1 M, 2 mL) was added, and the reaction mixture was stirred for 5 min. The solvent was removed under reduced pressure, and the crude product was dissolved in saturated $NaCO_3$ (20 mL). The solution was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phase was dried with $MgSO_4$, filtered, and concentrated under reduced pressure to give product **49** (38 mg, 95%). $[\alpha]_D = -16.8°$ ($c = 1.06$, MeOH). IR (KBr): 3428.7, 1781.3, 1651.0, 1590.2 cm^{-1}. ^1H NMR (CD_3OD): δ 4.43 (d, $J = 3.9$, 1H), 4.49 (d, $J = 11.6$, 1H), 4.74 (d, $J = 11.6$, 1H), 4.95 (s, 2H), 5.32 (dd, $J = 2.3$, $J = 3.8$, 1H), 6.31 (d, $J = 2.3$, 1H), 7.32 (m, 10H). ^{13}C NMR (CD_3OD): δ 73.74, 74.19, 78.27, 79.60, 115.75, 128.72, 128.95, 129.19, 129.37, 136.43, 138.15, 147.53, 169.02, 171.75. HRMS calcd for $C_{20}H_{18}O_6$ 355.1182, found 355.1170.

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