Solid phase assisted synthesis of HIV-1 protease inhibitors. Expedient entry to unsymmetrical substitution of a C_2 symmetric template

Karin Oscarsson, Björn Classon, Ingemar Kvarnström, Anders Hallberg, and Bertil Samuelsson

Abstract: A solid phase synthesis has been developed leading up to unsymmetrical HIV-1 protease inhibitors that are not readily available by conventional solution phase chemistry (**18a–g)**. To prepare these compounds the hydroxyl group of (1*S*,2*R*)-(–)-*cis*-1-phthalimido-2-indanol (**3**) was coupled to a Merrifield resin via a dihydropyrane linker. Cleavage of the phthalimido protecting group and reaction of the liberated amine with the bis-activated symmetrical diacid **15** resulted in the resin bound amide **16**. Coupling of **16** with amino acids and amines followed by hydrolysis produced the desired unsymmetrical products **18a–g** from which potent HIV-1 protease inhibitors were identified, e.g., **18e** (k_i = 0.1 nM), **18a** (k_i = 0.2 nM) and **18c** (k_i = 2 nM).

Key words: HIV, inhibitor, protease, solid phase.

Résumé : On a mis au point une synthèse en phase solide qui permet d'obtenir des inhibiteurs non symétriques de protéase HIV-1 (**18a–g**) qu'il est difficile d'obtenir par la chimie conventionnelle en solution. Dans cette synthèse, on couple le groupement hydroxyle du (1*S*,2*R*)-(–)-*cis*-1-phtalimido-indan-2-ol (**3**) à une résine de Merrifield à l'aide d'une liaison avec un dihydropyrane. Après clivage du groupe phtalimido protecteur et réaction de l'amine libérée avec le diacide symétrique **15** doublement activé, on obtient l'amide **16** lié à la résine. Le couplage de **16** avec des acides aminés ou avec des amines suivi d'une hydrolyse permet d'obtenir les produits non symétriques désirés **18a–g** à partir desquels il a été possible d'identifier de puissants inhibiteurs de protéase de l'HIV-1, par exemple **18e** (k_i = 0,1 nM), **18a** (k_i = 0,2 nM) et **18c** (k_i = 2 nM).

Mots clés : HIV, inhibiteur, protéase, phase solide.

[Traduit par la Rédaction]

Introduction

The human immunodeficiency virus (HIV) has been identified as the etiologic agent of AIDS (1, 2). The HIV-1 protease is a member of the aspartic protease family of enzymes (3–5), producing essential structural and functional viral proteins by proteolytic processing of the *gag*- and *gag-pol* viral gene products (6, 7). Inhibition of the HIV-1 PR have been shown to lead to the inactivation of HIV-1 (4, 8–10). Notably, protease inhibitors have shown clinical efficacy and there are presently five protease inhibitors approved by the FDA for the treatment of HIV-1 infection: saquinavir, ritonavir, indinavir, nelfinavir, and amprenavir (11, 12). Whereas these inhibitors effectively reduce the plasma viral load in infected individuals (11), the rapid turnover of HIV-1 and the high frequency of mutations in the HIV genome eventually results in the selection of mutant strains and in development of clinical resistance (5, 13–15). Moreover, the high cost of therapy precludes the widespread use of the currently approved HIV-1 protease inhibitors. Not withstanding the progress achieved to date there is thus a pressing need for the development of new and cost-effective HIV-1 protease inhibitors.

We recently reported on the design, synthesis, and antiviral activity of new carbohydrate based C_2-symmetrical protease inhibitors e.g., compounds **1** and **2** (Table 1) (16) which are readily prepared in three steps from commercially available starting materials. However, to prepare unsymmetrical inhibitors based on the C_2-symmetric core template (Fig. 1), a new synthetic procedure had to be developed, as treatment

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Dedicated to Professor Stephen Hanessian in recognition of his outstanding contribution to the art and logic of organic synthesis.

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Scheme 1. Synthesis of protected (1*S*,2*R*)-(–)-*cis*-1-amino-2-indanol.

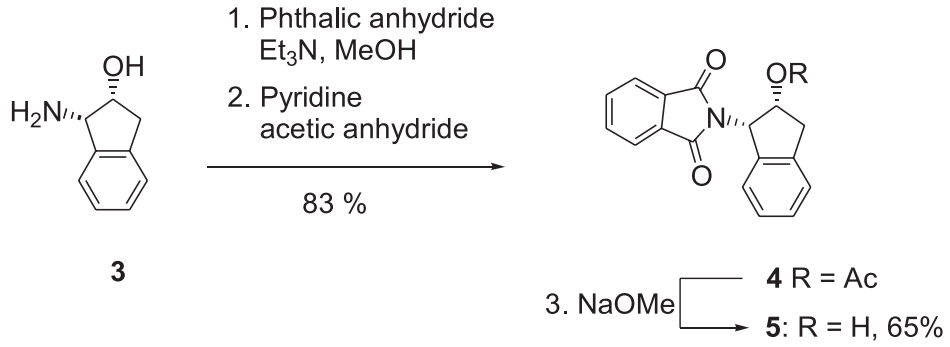
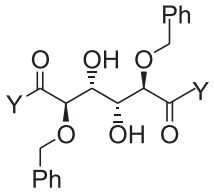
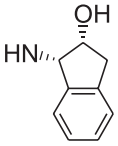
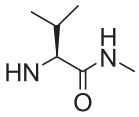


Table 1. C_2-symmetrical protease inhibitors.



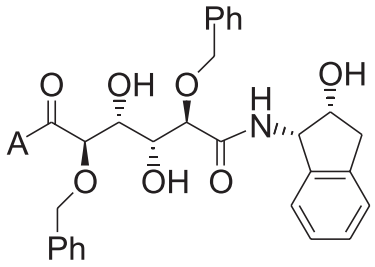
Y	Cmpd. no.	K_i [a] (nM)	ED_{50} [b] (μM)
	**1**	0.4	0.1
	**2**	0.6	1

[a] Standard error 20 %

[b] ED_{50} for reference substances tested in the same assay: ritnonavir (ED_{50}, 0.06 μM), indinavir (ED_{50}, 0.06 μM), saquinavir (ED_{50}, 0.01 μM), nelfinavir (ED_{50}, 0.04 μM).

of either the bislactone (**10**) or the activated L-mannaric acid (**15**) with amines at various conditions preferentially gives the symmetrical products with only minor amounts of the monosubstituted product being present in the reaction mixture at any given time. We now report on the use of solid phase synthesis to prepare unsymmetrical protease inhibitors based on the previously disclosed C_2-symmetric core template (16). The amino indanol **3** has been shown by others (17–19) and us (16) to be an excellent *P*2/*P*2′ ligand for the *S*2/*S*2′ pockets of the HIV-1 protease. Thus in the present work the amino indanol *P*2 residue (**3**) has been kept constant while the *P*2′ residue has been varied using parallel synthesis (Fig. 1). The protected amino indanol **5** was coupled to a THP linker (20) on a Merrifield resin (21), followed by deprotection of the phthalimido group liberating

Figure 1. Unsymmetrical inhibitors, A = Amines.



the free amino function which was reacted with the bis-activated L-mannaric acid (**15**) resulting in the resin bound monoactivated amide **16**. Coupling of **16** with a selection of amines, followed by hydrolysis gave the desired products **18a**–**g** and **1**. Not withstanding this selectivity, a minor amount of the symmetrical inhibitor **1** was formed from "in-land" cross- coupling as evidenced by analysis of the products formed in the reaction. Notably the present solid phase chemistry methodology opens up for further developments of this class of compounds using combinatorial chemistry (22, 23). The compounds produced were screened for HIV-1 protease inhibition and for anti HIV-1 activity in a cell culture assay.

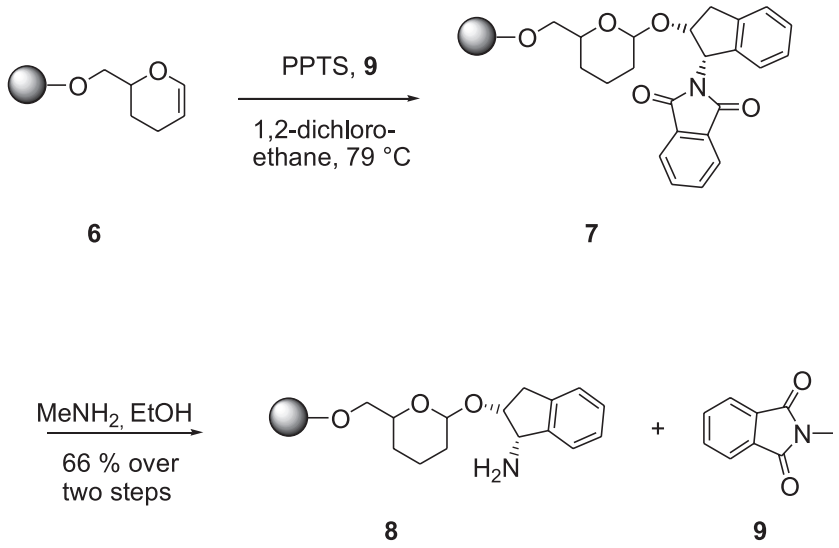
Results and discussion

Chemistry

The phthalimido derivative **5** was prepared in two steps by reacting compound **3** with phthalic anhydride in the presence of triethylamine in methanol followed by pyridine – acetic anhydride to give **4** in 83% yield. Deacetylation with sodium methoxide in methanol–dichloromethane gave **5** in 65% yield (Scheme 1). The protected amino indanol **5** was coupled to the solid support 3,4-dihydro-2*H*-2-ylmethoxymethyl polystyrene (**6**) in 1,2-dichloroethane using pyridinium *p*-toluenesulfonate (PPTS) (24) as a catalyst to give **7** (Scheme 2) (22). The phthalimido protecting-group was thereafter cleaved off using methylamine in ethanol for 16 h to give the free amine **8** and the liberated *N*-methyl phthalimide (**9**) in solution. The loading onto the solid support was calculated to be 66% based on the recovery of **9**.

Initial attempts to react the bislactone (**10**) (16) (lactone ring opening) with the free amino group on the solid support **8** failed to give satisfactory results due to unexpected low reactivity under standard reaction conditions (dichloromethane, reflux). Instead an alternative route was investigated

Scheme 2. Coupling of protected (1*S*,2*R*)-(–)-*cis*-1-amino-2-indanol to Merrifield resin.



employing the bissuccinimidyl ester (**15**) (16) for which a new and improved synthetic route was developed. Benzylated bislactone (**10**) (16) was thus reacted with methylamine catalyzed by 2-hydroxypyridine to give bisamide (**11**) in 87% yield (Scheme 3). Use of 2-hydroxypyridine (25) as a catalyst for this reaction gave the highest yields due to suppression of a β-elimination side reaction. The diol in compound **11** was protected using 2,2-dimethoxypropane in acetone containing camphorsulfonic acid to give **12** in 85% yield (16). The corresponding dicarboxylic acid (**14**) was prepared in essentially quantitative yield by reacting compound **12** with dinitrogen tetraoxide (26) and anhydrous sodium acetate in dichloromethane (27, 28) to provide the bis-[*N*-nitrosoamide] (**13**), which was hydrolyzed with lithium hydroxide and hydrogen peroxide (29, 30) to furnish the dicarboxylic acid (**14**). Activation of **14** with *N*,*N*-disuccinimidyl carbonate delivered the corresponding activated bissuccinimidyl ester (**15**) in 95% yield (16).

The amino indanol on the solid support (**8**), was reacted with compound **15** to give the monoamide **16** (Scheme 4) and subsequent coupling of **16** with a series of amines (A = amine, Table 2) gave the unsymmetrical products **17a**–**g** as well as the symmetrical product **17h** linked to solid support. Hydrolysis with 2.3 M HCl in methanol produced compounds **18a**–**g** together with the symmetrical inhibitor **1**. The yields of pure products (**18a**–**g**) over the three steps, based on the loading of the solid support, ranged from 17–47%. Approximately 4% yield of symmetrical inhibitor **1** isolated by chromatography, was formed in each reaction (Table 2).

HIV-1 protease inhibition

(Table 1 and 2) HIV-1 protease was cloned and heterologously expressed in *Escherichia coli* (31). K_i-values were determined in a fluorometric assay (32).

In vitro anti-HIV activity

(Table 1 and 2) The anti-HIV activity was assayed by a HIV cytopathic assay in MT-4 cells where the effects were quantified using vital dye XTT (33). The 50% inhibitory concentrations (ED_{50}) were calculated from the percent cytoprotection for individual compounds.

Structure activity relationship

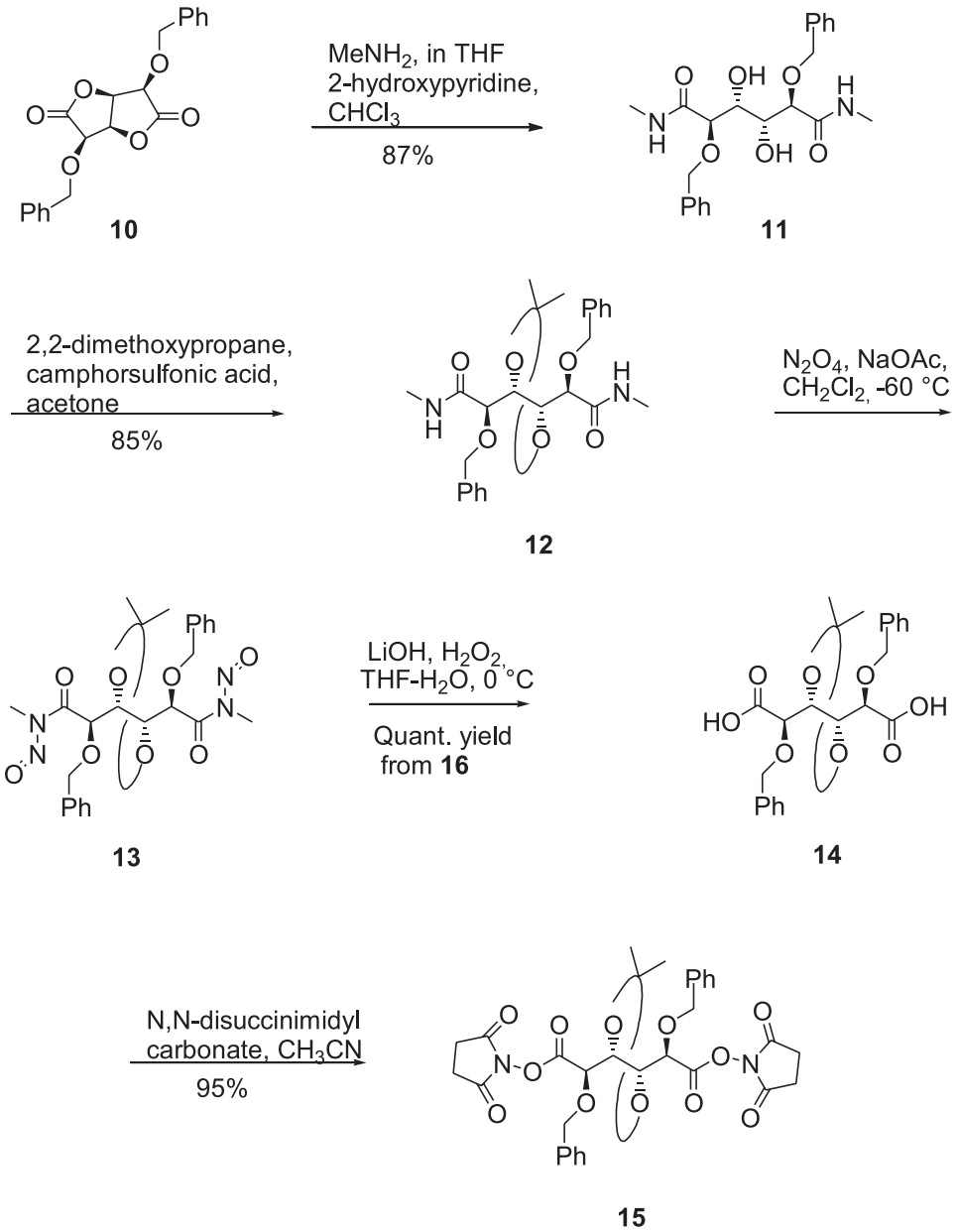
From Table 2 it is evident that unsymmetrical L-mannaric amides of simple structures can be potent HIV-1 protease inhibitors. The potency of inhibitor **18e** (K_i = 0.1 nM) is somewhat surprising. The *P*2′ benzyl group of inhibitor **18e** can be viewed as a truncated *P*2′ amino indanol of **1** lacking the conformational rigidity of the amino indanol and lacking the hydrogen bond of the corresponding amino indanol hydroxyl group to the Asp 29 of the HIV-1 protease (16). Indeed, the X-ray crystal structure of **18e** complexed with HIV-1 protease shows that the phenyl group is superimposed onto the aryl part of the corresponding amino indanol in inhibitor **1**.[2] However, substitution of the benzyl *P*2′ residue with hydrogen bond accepting or electron donating groups as in the inhibitors **18f** and **18g** leads to less active compounds. These results correlate well with earlier observations, that aromatic substitutions lead to less active inhibitors (15).

Inhibitor **18a** has similar potency (k_i = 0.2 nM) as the potent corresponding symmetrical inhibitors **1** (k_i = 0.4 nM) and **2** (k_i = 0.6 nM) (16). Inhibitors **18b**, **18c**, and **18d** are *P*2′ truncated simplified analogues of **18a**. Both **18b** and **18d** are considerably less potent, while the methyl amide **18c** (k_i = 2 nM) is notably potent. This inhibitor effectively lacks a *P*2′ residue and the high potency presumably results from an energetically more favorable hydrogen bond interaction between the methyl amide carboxyl oxygen and the structural water that is tetra- coordinated to the inhibitor carbonyls and the Ile 50/150 residues of the HIV-1 protease.

In spite of the promising HIV-1 protease inhibition activities obtained for some of these inhibitors, the antiviral activities in cell culture (ED_{50}) were not improved over inhibitor **1** although inhibitor **18a** was equipotent (Table 2). Further studies are necessary to rationalize the SAR for antiviral activity in cell culture for this class of compounds (34, 35).

[2] Unpublished results.

Scheme 3. Synthesis of bissuccinimidyl ester.



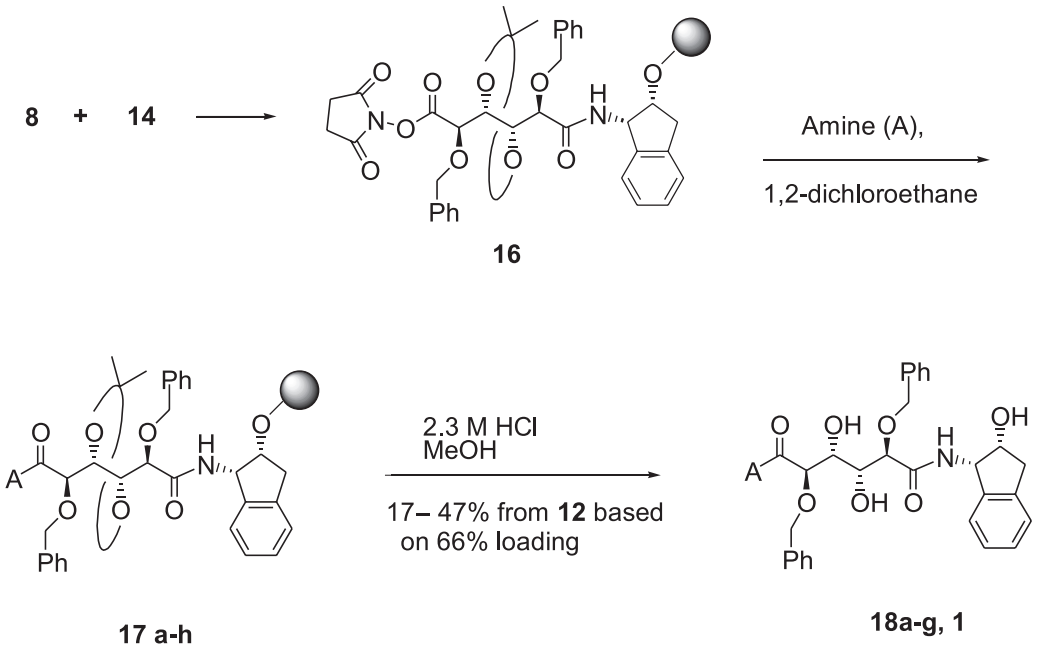
Experimental section

Thin layer chromatography was performed using silica gel 60 F-254 (Merck) plates with detection by UV and (or) charring with 8% sulphuric acid or by charring with ammonium molybdate (100 g):Cer(IV)sulfate (2 g):sulfuric acid (10%, 2 L). Column chromatography was performed on silica gel (Matrix Silica Si 60A, 35–70m, Amicon). Organic phases were dried over anhydrous sodium sulfate or magnesium sulphate. Concentrations were performed under reduced pressure. NMR spectra were recorded on a JEOL GSX-270 instrument (^{13}C NMR 67 MHz and ^{1}H 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 Finnigan MAT 900S Electrospray. Mass spectra were recorded on a Hewlett–Packard 1100MSD.

(1*S*,2*R*)-(–)-*cis*-1-Phthalimido-2-*O*-acetoxy (4)

A mixture of **3** (3.0 g, 20 mmol), phthalic anhydride (3.6 g, 24.3 mmol), and triethylamine (2.8 mL, 20.0 mmol) in MeOH (50 mL) was stirred at room temperature for 70 min under an argon atmosphere. After concentration the oily residue was dissolved in pyridine (40 mL) and acetic anhydride (20 mL) and stirred at room temperature for 16 h. The mixture was worked-up by adding CH_2Cl_2 (100 mL) extracted with cold 10% HCl (2 × 40 mL). The combined aqueous phases were washed with CH_2Cl_2 (2 × 50 mL) and the organic layer was washed with saturated $NaHCO_3$ (2 × 100 mL) and saturated NaCl (2 × 100 mL). The combined aqueous phases were washed with CH_2Cl_2 (2 × 75 mL), dried and concentrated. The crude product was purified using column chromatography (toluene – ethyl acetate, 15:1) to give **4** (5.3 g 83%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.86 (s, 3H, 1 CH_3), 3.46–3.49 (t, 2H, 1 CH_2), 5.64–5.72 (q,

Scheme 4. Synthesis of L-mannaric diamides.



1H, 1 CH), 5.96–5.99 (d, 1H, 1 CH), 7.20–7.32 (m, 4H, Ar), 7.71–7.86 (m, 4H, Ar). ^{13}C NMR (67MHz, $CDCl_3$) δ: 20.3 (CH_3), 37.7 (CH_2), 54.4 (*C*H-N), 72.6 (*C*H-O), 123.0, 124.3, 124.5, 126.9, 128.7, 131.4, 133.8, 136.4, and 140.6 (aromatic C), 167.4 and 168.9 (C=O). MS (API-ES) calcd. for 321.10; found: 321.1.

(1*S*,2*R*)-(–)-*cis*-1-Phthalimido-2-indanol (5)

To a stirred solution of **4** (5.3 g, 16 mmol) in MeOH (250 mL) and CH_2Cl_2 (100 mL), sodium methoxide in MeOH (0.1 M) was added dropwise until pH reached 7.5. After 5 h Dowex® HCR-W2 (H^+) ion exchange resin was added to neutralize the solution. The product was purified using column chromatography (toluene – ethyl acetate, 5:1). Concentration gave pure **5** (2.98 g, 65%) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 2.84 (s, 1H, 1 OH), 3.20–3.38 (m, 2H, 1 CH_2), 4.75–4.77 (q, 1H, CHOH), 5.77–5.80 (d, 1H, CHN), 7.09–7.33 (m, 4H, Ar), 7.67–7.83 (m, 4H, Ar). ^{13}C NMR (67MHz, $CDCl_3$) δ: 41.2 (CH_2), 57.7 (*C*H-N), 74.0 (*C*H-OH), 123.4, 124.2, 125.5, 126.9, 128.5, 131.8, 134.1, 137.1, and 140.2 (aromatic C), 169.0 (C=O). Anal. calcd. ($C_{17}H_{13}NO_3$): C 73.11, H 4.69, N 5.02; found: C 72.98, H 4.55, N 4.98.

(1*S*,2*R*)-(–)-*cis*-1-Phthalimido-2-indanol on solid support (7)

Dry Merrifield resin with a dihydropyran linker (**6**) (400 mg, 0.51 mmol/g, 0.20 mmol linker) was swollen in dry 1,2-dichloroethane (5.5 mL) under an argon atmosphere. Anhydrous PPTS (100 mg, 0.40 mmol) and **5** (200 mg, 0.72 mmol) were added and the mixture was heated to 79°C. After 16 h the mixture was cooled to room temperature and the solid support was rinsed with CH_2Cl_2 (20 mL) and MeOH (20 mL) to give **7**. The filtrate was concentrated and the solid residue was purified using column chromatography (toluene – ethyl acetate, 5:1) to recover unreacted **3** (104 mg, 0.47 mmol). All the solid phase was used in the subsequent step.

(1*S*,2*R*)-(–)-*cis*-1-Amino-2-indanol on solid support (8)

Methylamine in ethanol (4.5 mL, 33%) was added to the solid phase from the previous step (**7**) and stirred for 16 h. The solid phase was rinsed with CH_2Cl_2 (10 mL), MeOH (10 mL), and then $CHCl_3$ (10 mL) to give **8**. All the solid phase will be used in the subsequent step.

The combined organic phases were concentrated and the formed *N*-methylphthalimide (**9**) was purified using column chromatography (chloroform–methanol, 20:1) which gave pure **9** as a white solid (21 mg, loading 66%). ^{1}H NMR (270 MHz, $CDCl_3$) δ: 2.94 (s, 3H, 1 CH_3), 7.01–7.67 (m, 4H, Ar). ^{13}C NMR (67MHz, $CDCl_3$) δ: 26.8 (CH_3), 125.7, 128.3, 129.0, 130.1, 134.6, and 140.7 (aromatic C), 170.0 (C=O).

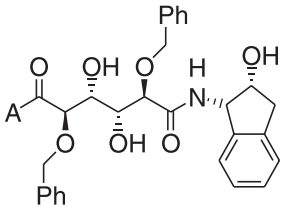
***N*1,*N*6-Dimethyl-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-dihydroxy hexanediamide (11)**

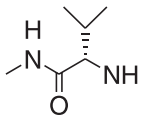
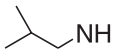
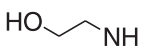
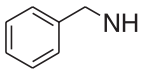
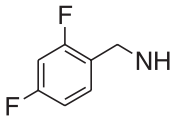
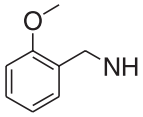
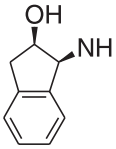
A mixture of benzylated bislactone (**10**) (7.64 g, 21.6 mmol), 2-hydroxypyridine (2.06 g, 21.7 mmol), and methylamine in THF (2 M, 23 mL, 46 mmol) in $CHCl_3$ (60 mL) was stirred at ambient temperature for 15 min. The organic phase was washed with saturated $NaHCO_3$ (3x), dried, concentrated, and the residue purified by column chromatography ($CHCl_3$–MeOH, 20:1) to give pure **11** (7.83 g, 18.8 mmol, 87%) as a white solid. ^{13}C NMR ($CDCl_3$) δ: 25.8 (2 *C*H_3-NH), 71.1, 73.8, 79.6 (2 O*C*H_2Ph, C2, C3, C4, and C5), 128.1, 128.2, 128.6, 136.7 (aromatic C), 172.7 (2 C=O). MS (API-ES) calcd. for 416.1; found: 416.1.

***N*1,*N*6-Dimethyl-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-dihydroxy-3,4-*O*-isopropylidenehexanediamide (12)**

Dimethoxypropane (15 mL, 123 mmol) and camphorsulfonic acid (2.66 g, 11.5 mmol) were added to a stirred mixture of **11** (6.0 g, 14.4 mmol) in dry acetone (70 mL). The precipitated product was filtered off after 15 min to give pure **12**. The solution was concentrated, CH_2Cl_2 was added

Table 2. Structure, yields, and HIV-1 protease inhibitory acitivity and in vitro antiHIV-1 acitvity of unsymmetrical L-mannaric diamides with 2*R*, 3*R*, 4*R*, 5*R* configuration.



A	Cmpd. no.	Yield (%)[a]	K_i[b] (nM)	ED_{50}[c] (μM)
	**18 a**	23	0.2	0.1
	**18 b**	28	70	> 100
CH_3NH	**18 c**	35	2	2
	**18 d**	26	200	60
	**18 e**	21	0.1	3
	**18 f**	17	20	>100
	**18 g**	47	2	4
	**1**	17	0.4	0.1

[a] from **12** based on 66% loading

[b] Standard error 20 %

[c] ED_{50} for reference substances tested in the same assay: ritnonavir (ED_{50}, 0.06 μM), indinavir (ED_{50}, 0.06 μM), saquinavir (ED_{50}, 0.01 μM), nelfinavir (ED_{50}, 0.04 μM).

to the residue, and the organic phase was washed with saturated $NaHCO_3$ (3x), dried and concentrated. The solid was washed with a small amount of dry, cold acetone to give pure **12** (5.58 g, 12.2 mmol, 85%). ^{13}C NMR ($CDCl_3$) δ: 25.6, 26.9 (4 *C*H_3), 73.5, 77.5, 79.3 (2 O*C*H_2Ph, C2, C3, C4, and C5), 110.0 (O-*C*-O), 128.0, 128.1, 128.5, 136.8 (aromatic C), 169.3 (C=O). Anal. calcd. ($C_{25}H_{32}N_2O_6$): C 65.77, H 7.06. N 6.14; found: C 65.65, H 6.83, N 6.01.

***N*1,*N*6-Dimethyl-*N*1,*N*6-dinitroso-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-dihydroxy-3,4-*O*-isopropylidenehexanediamide (13)**

To produced N_2O_4, $Pb(NO_3)_2$ (20 g, 60 mmol) was heated over an open flame and the gas was led down into CH_2Cl_2 at –10°C. The solution was cooled to –60°C under an argon atmosphere and anhydrous sodium acetate (4.2 g, 51.2 mmol) was added followed by **12** (3.25 g, 7.12 mmol). The –60°C

bath was replaced with an ice salt bath after 30 min and the mixture was stirred at –10°C until the substrate was consumed (1 h). The solution was poured into ice and water and the phases were separated. The organic phase was washed with ice cold 5% sodium bicarbonate. The organic phase was washed with ice cold 5% sodium bicarbonate, dried briefly at 0°C, and concentrated to give **13** as a yellow oil. The nitrosoamide solution was never allowed to warm above 0°C. All the product was used immediately in the next step.

2,5-Di-*O*-benzyl-3,4-*O*-isopropylidene-L-mannaric acid (14)

Hydrogen peroxide (12.5 g 30%) and LiOH (1.26 g, 52.7 mmol) were added to a stirred solution of **13** from the previous step in THF–H_2O (3:1) (100 mL) at 0°C. The mixture was stirred for 20 min at 0°C. The reaction was quenched with Na_2SO_3 (15 mL, 1.5 M). The THF was evaporated and the water phase was acidified with Dowex® HCR-W2 (H^+) ion exchange resin, and extracted with EtOAc. The phases were separated and the organic phase was dried and concentrated to give **14** (3.05 g, 7.08 mmol, 100%) as a white solid. ^{13}C NMR (CD_3OD) δ: 27.5 (2 CH_3), 73.6, 80.0, 80.3 (2 OCH_2Ph, C2, C3, C4, and C5), 112 (O-*C*-O), 129.0, 129.3, 129.4, 138.5 (aromatic C), 173.1 (C=O).

***N*1-[(2*R*)-Hydroxy-1(*S*)indanyl]-*N*6-succinimidyl-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-dihydroxy-3,4-*O*-isopropylidenehexanediamide on solid support (16)**

The solid phase from the previous step (**8**) was swollen in dry 1,2-dichloroethane (4.5 mL) for 40 min under an argon atmosphere. The bissuccinimidyl diester (**15**) (900 mg, 1.44 mmol) was added and the mixture was stirred for 16 h under an argon atmosphere at 60°C. The solid phase was rinsed with $CHCl_3$ (20 mL), THF (10 mL), and then 1,2-dichloroethane (10 mL) to give **16**. All the solid phase was used in the subsequent step.

General procedure for preparation of compounds 17a–h

The solid phase from the previous step (**16**) was swollen in dry 1,2-dichloroethane (4 mL) for 40 min under an argon atmosphere. The amine (2.0 mmol) was added and the mixture was stirred for 16 h under argon at 60°C. The solid phase was rinsed with $CHCl_3$ (20 mL), THF (10 mL), and then 1,2-dichloroethane (10 mL) to give **17a–h**. All the solid phase was used in the subsequent step.

***N*1-[(1*R*,2*S*)-2-Hydroxy-2,3-dihydro-1*H*-indenyl]-*N*6-(isobutyl)-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-dihydroxy-3,4-*O*-isopropylidenehexanediamide on solid support (17a)**

The solid phase **16** was reacted according to the general procedure, vide supra, using the amine isobutylamine (200 μL, 2.01 mmol) to give **17a**.

***N*1-[(1*R*,2S)-2-Hydroxy-2,3-dihydro-1*H*-indenyl]-*N*6-(methyl)-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-dihydroxy-3,4-*O*-isopropylidenehexanediamide on solid support (17b)**

The solid phase **16** was reacted according to the general procedure, vide supra, using the amine methylamine in THF (2 mL, 2 M) to give **17b**.

***N*1-[(1*R*,2*S*)-2-Hydroxy-2,3-dihydro-1*H*-indenyl]-*N*6-(2-hydroxyethyl)-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-dihydroxy-3,4-*O*-isopropylidenehexanediamide on solid support (17c)**

The solid phase **16** was reacted according to the general procedure, vide supra, using the amine ethanolamine (120 μL, 2 mmol) to give **17c**.

***N*1-[(1*R*,2*S*)-2-Hydroxy-2,3-dihydro-1*H*-indenyl]-*N*6-[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-dihydroxy-3,4-*O*-isopropylidenehexanediamide on solid support (17d)**

The solid phase **16** was reacted according to the general procedure, vide supra, using the amine valine methylamide (195 mg, 1.5 mmol) to give **17d**.

***N*1-[(1*R*,2*S*)-2-Hydroxy-2,3-dihydro-1*H*-indenyl]-*N*6-(2,4-difluorobenzyl)-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-dihydroxy-3,4-*O*-isopropylidenehexanediamide on solid support (17e)**

The solid phase **16** was reacted according to the general procedure, vide supra, using the amine 2,4-difluorobenzylamine (240 μL, 2.02 mmol) to give **17e**.

***N*1-[(1*R*,2*S*)-2-Hydroxy-2,3-dihydro-1*H*-indenyl]-*N*6-(benzyl)-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-dihydroxy-3,4-*O*-isopropylidenehexanediamide on solid support (17f)**

The solid phase **16** was reacted according to the general procedure, vide supra, using the amine benzylamine (220 μL, 2.01 mmol) to give **17f**.

***N*1-[(1*R*,2*S*)-2-Hydroxy-2,3-dihydro-1*H*-indenyl]-*N*6-(2-methoxybenzyl)-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-dihydroxy-3,4-*O*-isopropylidenehexanediamide on solid support (17h)**

The solid phase **16** was reacted according to the general procedure, vide supra, using the amine 2-methoxybenzylamine (250 μL, 1.92 mmol) to give **17h**.

***N*1,*N*6-Di[(2*R*)-hydroxy-1(*S*)-indenyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-di(benzyloxy-3,4-dihydroxy-3,4-dihydroxyhexanediamide on solid support (17g)**

The solid phase **16** was reacted according to the general procedure, vide supra, using the amine (1*S*,2*R*)-(–)-cis-1-amino-2-indanol (301 mg, 2.03 mmol) to give **17g**.

General procedure for the preparation of compounds 18a–h and 1

2.3 M HCl in methanol (5.5 mL) was added to the solid phase from the previous step (**17a–h**). After stirring for 4 h at room temperature under an argon atmosphere, the solid phase was rinsed with $CHCl_3$ (20 mL) and MeOH (10 mL). The combined organic layer was diluted by 20 mL $CHCl_3$ and extracted with saturated $NaHCO_3$ (2 × 30 mL). The combined aqueous phases were washed with $CHCl_3$ (2 × 40 mL). The combined organic layer was dried, concentrated, and purified by column chromatography to give **18a–h** and **1**.

***N*1-[(1*R*,2*S*)-2-Hydroxy-2,3-dihydro-1*H*-indenyl]-*N*6-(isobutyl)-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-*O*-isopropylidenehexanediamide (18a)**

The solid phase **17a** was reacted according to the general procedure, vide supra, to give pure **18a** (21 mg, 0.036 mmol, 28% based on loading) after purification with column chromatography (chloroform–methanol, 40:1). ^{13}C NMR ($CDCl_3$) δ: 20.0 (2 CH_3), 28.4 (CH), 39.3 (*C*H_2-Ar), 46.4 (*C*H_2-NH), 58.0 (*C*H-NH), 71.6, 71.9, 72.6, 73.6, 74.1, 80.5, 81.4 (*C*H-OH, 2 O*C*H_2Ph, C2, C3, C4, and C5), 124.0, 125.4, 127.0, 128.2, 128.3, 128.5, 128.7, 128.8, 136.7, 139.8, 140.8 (aromatic C), 171.5, 172.3 (2 C=O). HRMS calcd. for 576.2835; found 576.2829.

***N*1-[(1*R*,2*S*)-2-Hydroxy-2,3-dihydro-1*H*-indenyl]-*N*6-(methyl)-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-*O*-isopropylidenehexanediamide (18b)**

The solid phase **17b** was reacted according to the general procedure, vide supra, to give pure **18b** (24 mg, 0.045 mmol, 35% based on loading) after purification with column chromatography (chloroform–methanol, 40:1). ^{13}C NMR ($CDCl_3$) δ: 25.9 (CH_3), 39.3 (*C*H_2-Ar), 58.0 (*C*H-NH), 71.5, 71.9, 72.6, 73.6, 74.0, 80.7, 81.5 (*C*H-OH, 2 O*C*H_2Ph, C2, C3, C4, and C5), 124, 125.4, 127.1, 128.2, 128.4, 128.4, 128.7, 136.6, 139.7, 140.8 (aromatic C), 172.0, 172.3, (2 C=O). HRMS calcd. for 534.2366, found 534.2359.

***N*1-(1*R*,2*S*)-2-Hydroxy-2,3-dihydro-1*H*-indenyl]-*N*6-(2-hydroxyethyl)-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-*O*-dihydroxyhexanediamide (18c)**

The solid phase **17c** was reacted according to the general procedure, vide supra, to give pure **18c** (19 mg, 0.034 mmol, 26% based on loading) after purification with column chromatography (chloroform–methanol, 20:1). ^{13}C NMR ($CDCl_3$) δ: 39.3 (*C*H_2-Ar), 41.9 (*C*H_2-NH), 57.6 (*C*H-NH), 61.1 (*C*H_2-OH), 71.5, 71.6, 72.5, 73.2, 73.3 (*C*H-OH, 2 O*C*H_2Ph, C2, C3, C4, and C5), 124.0, 125.3, 127.0, 128.1, 128.2, 128.3, 128.6, 136.6, 136.8, 139.8, 140.7 (aromatic C),171.6 (C=O). HRMS calcd. for 564.2472; found 564.2460.

***N*1-[(1*R*,2*S*)-2-Hydroxy-2,3-dihydro-1*H*-indenyl]-*N*6-[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-*O*-isopropylidenehexanediamide (18d)**

The solid phase **17d** was reacted according to the general procedure, vide supra, to give pure **18d** (17 mg, 0.029 mmol, 23% based on loading) after purification with column chromatography (chloroform–methanol, 40:1). ^{13}C NMR ($CDCl_3$) δ: 17.1, 19.6 (*C*H_3-CH); 26.1 (*C*H_3-NH), 29.1 (CH), 39.3 (*C*H_2-Ar); 58.0, 58.4 (*C*H-NH and O=C-*C*H-NH), 72.2, 72.4, 72.8, 73.4, 81.3, 81.8 (*C*H-OH, 2 O*C*H_2Ph, C2, C3, C4, and C5), 123.9, 125.4, 127.0, 128.1, 128.2, 128.4, 128.5, 128.7, 128.7, 136.4, 139.6, 140.8 (aromatic C), 170.9, 172.0 (2 C=O). HRMS calcd. for 610.2679; found 610.2673.

***N*1-[(1*R*,2*S*)-2-Hydroxy-2,3-dihydro-1*H*-indenyl]-*N*6-(2,4-difluorobenzyl)-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-*O*-isopropylidenehexanediamide (18e)**

The solid phase **17e** was reacted according to the general procedure, vide supra, to give pure **18e** (14 mg, 0.020 mmol 17% based on loading) after purification with column chromatography (chloroform–methanol, 40:1). ^{13}C NMR ($CDCl_3$) δ: 36.6 (*C*H_2-NH), 39.3 (*C*H_2-Ar), 58.0 (*C*H-NH), 71.6, 71.8, 72.5, 73.6, 74.0, 80.4, 81.3 (*C*H-OH, 2 O*C*H_2Ph, C2, C3, C4, and C5), 103.9, 111.6, 124.0, 125.4, 127.1, 128.2, 128.3, 128.5, 128.7, 130.9, 136.5, 136.6, 139.7, 140.8 (aromatic C), 171.6, 172.3 (2 C=O). HRMS calcd. for 646.2491; found 646.2493.

***N*1-[(1*R*,2*S*)-2-Hydroxy-2,3-dihydro-1*H*-indenyl]-*N*6-(benzyl)-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-*O*-isopropylidenehexanediamide (18f)**

The solid phase **17f** was reacted according to the general procedure, vide supra, to give pure **18f** (17 mg, 0.028 mmol, 21% based on loading) after purification with column chromatography (chloroform–methanol, 40:1). ^{13}C NMR ($CDCl_3$) δ: 39.3 (*C*H_2-Ar), 43.1 (*C*H_2-NH), 57.9 (*C*H-NH), 71.7, 71.8, 72.5, 73.6, 74.0, 80.4, 81.4 (*C*H-OH, 2 O*C*H_2Ph, C2, C3, C4, and C5), 124.1, 125.4, 127.1, 127.6, 128.2, 128.4, 128.7, 130.9, 136.5, 137.5, 139.7, 140.8 (aromatic C), 171 5, 172.3 (2 C=O). HRMS calcd. for 633.3050; found 633.3056.

***N*1-[(1*R*,2*S*)-2-Hydroxy-2,3-dihydro-1*H*-indenyl]-*N*6-(2-methoxybenzyl)-(2*R*,3*R*,4*R*,5*R*)-2,5-dibenzyloxy-3,4-*O*-isopropylidenehexanediamide (18g)**

The solid phase **17g** was reacted according to the general procedure, vide supra, to give pure **18g** (38 mg, 0.06 mmol, 47% based on loading) after purification with column chromatography (chloroform–methanol, 40:1). ^{13}C NMR ($CDCl_3$) δ: 39.2, 39.2 (*C*H_2-Ar and *C*H_2-NH), 55.1 (*C*H_3-O), 57.9 (*C*H-NH), 71.4, 71.8, 72.5, 73.4, 73.8, 80.4, 81.1 (*C*H-OH, 2 O*C*H_2Ph, C2, C3, C4, and C5), 110.2, 120.6, 124.0, 125.3, 125.5, 126.9, 128.1, 128.2, 128.5, 129.0, 129.5, 136.7, 136.7, 139.9, 140.7, 157.5 (aromatic C), 171.0, 172.2 (2 C=O). HRMS calcd. for 640.2785; found 640.2783.

***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 (1)**

The solid phase **17h** was reacted according to the general procedure, vide supra, to give pure **1** (14 mg, 0.021 mmol, 17% based on loading) after purification with column chromatography (chloroform–methanol, 40:1). ^{13}C NMR ($CDCl_3$) δ: 39.1 (2 *C*H_2-Ar), 57.7 (2 *C*H-NH), 71.6, 72.5, 73.4, 81.3 (*C*H-OH, 2 O*C*H_2Ph, C2, C3, C4, and C5), 124.0, 125.3, 126.9, 128.1, 128.2, 128.6, 136.7, 139.8, 140.8 (aromatic C), 171.5 (C=O).

Conclusions

A solid phase parallel synthesis of unsymmetrical inhibitors has been developed which will aid further development and combinatorial amplification. A number of new potent inhibitors were discovered.

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