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New Anti-HIV Derivatives: Synthesis and Antiviral Evaluation

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Abstract—A small focused library of 18 compounds incorporating the motif 1,3-(*N*,*N*′-dibenzyl)diamino-2-propanol has been synthesized, using adapted synthetic methodologies. These series of compounds were evaluated for their in vitro anti-HIV activity on infected MT_4 cells (*syncytium* formation observation). Some of the new synthesized compounds show potent anti-HIV activities. EC_{50} values for compounds (**31**, **40**, **34**, **37** and **46**) range from 0.1 to 1 μM. In order to determine at which level these new derivatives interfere with the HIV replicative cycle, inhibition assays on recombinant HIV protease and HIV integrase have been performed. None of the compounds were found active on these two enzymatic targets. Experiments are in progress in order to identify their biological target within the HIV replicative cycle.

Introduction

Progress in HIV biology has provided detailed knowledge of molecular events in the replicative cycle of HIV-1. Current understanding of these molecular events in the HIV life cycle proposes seven steps: viral entry, reverse transcription, integration, gene expression, gene assembly, budding and maturation. In theory every stage in the viral life cycle could serve as a potential target for designing anti-HIV agents and therapies.[1] Currently a quite large number of drugs are known to act as: virus adsorption inhibitors,[2,3] virus-cell fusion inhibitors,[4] virus fusion uncoating inhibitors,[5] reverse transcriptase inhibitors,[6] integrase inhibitors,[7] Tat and Rev inhibitors,[8] protease inhibitors,[9] myristoylation and glycosylation inhibitors.[10] What is remarkable among these drugs is the large chemical diversity of their molecular structure. Compounds as diverse as polymers, peptides, pseudopeptides, modified nucleosides, bis polyazamacrocycles, oligonucleotides and polyheterocycles have been found to be active in vitro on various HIV infected culture cells and some of them are clinically used for anti-HIV therapy.[11] Usually the design of HIV compounds is based on the knowledge of well-defined targets such as HIV protease, reverse transcriptase or chemokine receptors, which allows the design of tailor-made molecules. Another approach to discover new anti-HIV candidates would be to randomly screen collections of compounds through a primary screening test, which translates any cell protection against HIV cell infection, whatever the viral step at which the inhibitor acts in the course of the infection. Observation of MT_4 *syncytium* formation[12] constitutes a suitable test to screen the inhibitory activity of numerous compounds, since whatever the step of the replicative cycle they target, the appearance of *syncytia* will be delayed. This approach offers strengths and weaknesses. This method overcomes the problem that a compound could be very active on an enzymic in vitro test (antiprotease or anti-RT) but found inactive on infected cell cultures because the compound cannot penetrate the cell membrane. Moreover this approach allows the discovery of totally new chemical structures as potential anti-HIV compounds. The weakness of this test is that it gives no information on the mechanism of action of these new primary analogues, and consequently no information on the biological target.

It has been shown[13] that compounds incorporating the symmetrical motif 1,3-(*N*,*N*′-dibenzyl)diamino-2-propanol elicited anti-HIV activities. Moreover, another reason to employ this motif was that in a random screening *syncytium* formation assay a compound from our library incorporating the motif 1,3-(*N*,*N*′-dibenzyl)diamino-2-propanol slightly protected HIV infected MT_4 cells against *syncytium* formation. Furthermore the reason which strengthened the use of this symmetrical structural

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motif in the design of anti-HIV derivatives was the results reported by Kemp et al. which show that compounds incorporating this motif allow anti-HIV activity. In order to explore more deeply the anti-HIV properties of this type of compounds, we have synthesized a small focused library based on this structural motif, substituted at the two end positions by various mono- or polyaminated substituted chains as indicated in Figure 1. It is known that aminated chains allow a reduction in the peptidic character of bioactive molecules by replacing peptidic residues by alkyl diamine without impairing the biological activity of the resulting derivatives.[14] Our research approach is to discover new lead compounds for potential anti-HIV agents and to modify these compounds to find still more potent anti-HIV agents. The present paper describes the synthesis and the antiviral evaluation of a new family of compounds, using observation of *syncytium* formation as screening test.

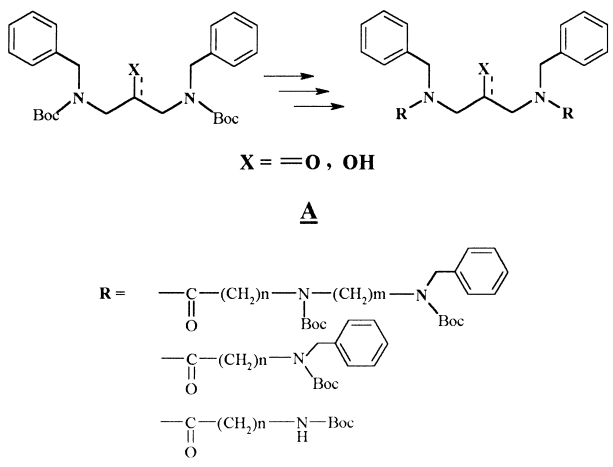
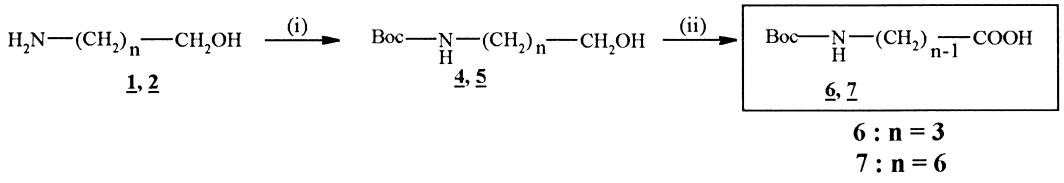


Figure 1. General structure of the synthesized compounds.

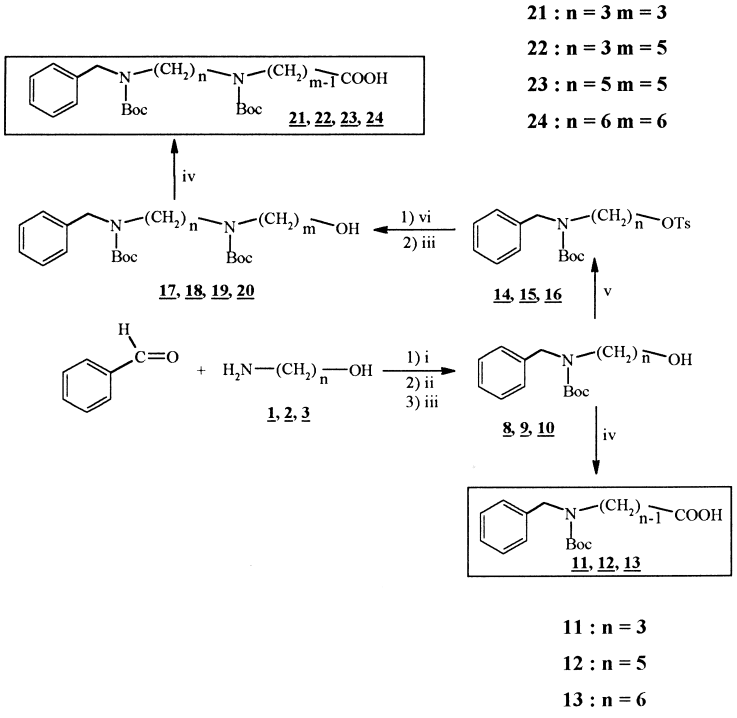
Results and Discussion

Chemistry

As shown in Schemes 1, 2 and 3, the synthesis of polyaminated inhibitors required the preliminary preparation of specific synthons. The *N*-Boc and *N*-benzyl-*N*-Boc protected carboxylic acid synthons **6**, **7**, **11**, **12**, **13** and



Scheme 1. (i) Boc_2O, CH_2Cl_2, 0 °C; (ii) PDC, DMF, rt.



Scheme 2. (i) Na_2SO_4, CH_2Cl_2, rt; (ii) $NaBH_4$, EtOH, 0 °C; (iii) Boc_2O, CH_2Cl_2, 0 °C; (iv) PDC, DMF, rt; (v) TsCl, Et_2O/Et_3N, rt; (vi) various ω-aminoalcohols, K_2CO_3, CH_3CN, rt.

21, **22**, **23**, **24** were prepared from commercial ω-amino alcohols **1**, **2** according to a synthetic route shown in Schemes 1 and 2. After Boc protection, the resulting alcohols **4**, **5** and **8**, **9**, **10** were oxidized into their corresponding carboxylic acids **6**, **7** and **11**, **12**, **13** using PDC in dry DMF (Schemes 1 and 2).

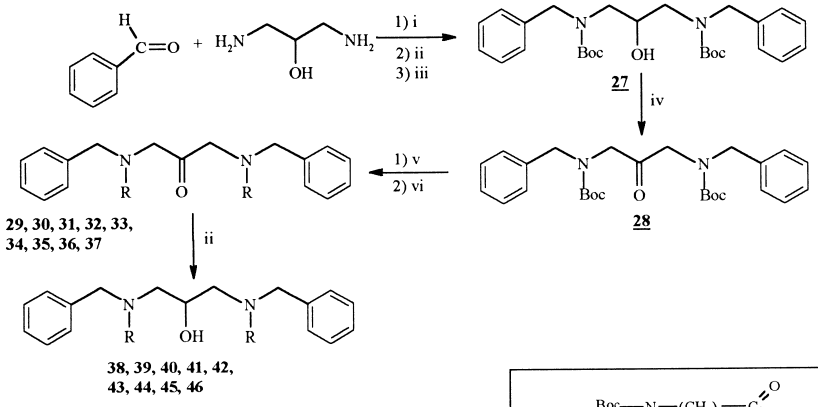
The fully protected ketone **28** synthon was obtained from the following sequences: condensation of benzaldehyde on 2-hydroxy-1,3-diaminopropane, followed by a subsequent reduction with $NaBH_4$ and Boc protection of the resulting amine, led to the hydroxy derivative **27**, which was oxidized into the corresponding ketone **28** using Swern oxidation conditions[15] (Scheme 3).

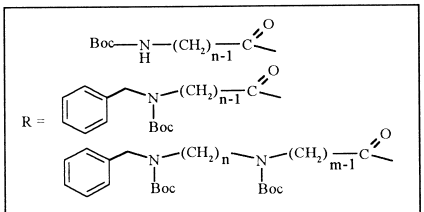
Protected diaminoalkyl carboxylic acids **21**, **22**, **23**, **24** were prepared in 7 steps from ω-amino alcohols **1**, **2**, **3** according to a standard route summarized in Scheme 2.

N-Boc protected amino alcohols **8**, **9**, **10** were first tosylated in order to obtain the corresponding derivatives **14**, **15**, **16**. These were then condensed on various ω-amino alcohols. After a protection step of the *N*-diaminoalkyl derivatives, the resulting compounds **17**, **18**, **19**, **20** were oxidized into the final carboxylic synthons **21**, **22**, **23**, **24** using PDC as catalyst in dry DMF.

All the obtained carboxylic acid intermediates **6**, **7**, **11**, **12**, **13**, **21**, **22**, **23** and **24** were coupled using BOP reagent to the deprotected ketone synthon **28**. Using $NaBH_4$ in ethanol (Scheme 3) the resulting analogues **29, 30, 31, 32, 33, 34, 35, 36, 37** were then reduced into the corresponding alcohol derivatives **38, 39, 40, 41, 42, 43, 44, 45, 46**.

Both series of ketones (**29, 30, 31, 32, 33, 34, 35, 36, 37**) and alcohols (**38, 39, 40, 41, 42, 43, 44, 45, 46**) were then submitted to anti-HIV screening.

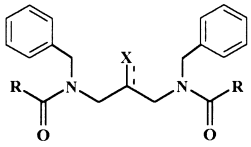


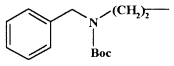
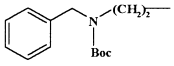
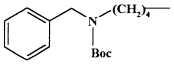
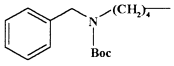
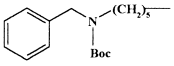
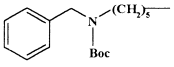
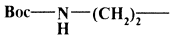
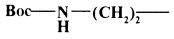
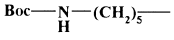
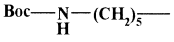
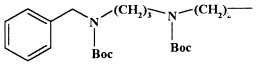
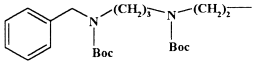
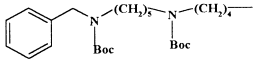
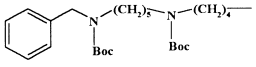
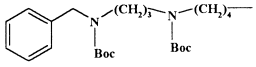
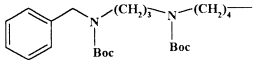
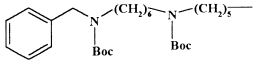


Compounds	n	m
29	2	-
30	4	-
31	5	-
32	2	-
33	5	-
34	3	2
35	5	4
36	3	4
37	6	5
38	2	-
39	4	-
40	5	-
41	2	-
42	5	-
43	3	2
44	5	4
45	3	4
46	6	5

Scheme 3. (i) Na_2SO_4, CH_2Cl_2, rt; (ii) $NaBH_4$, EtOH, 0 °C; (iii) Boc_2O, CH_2Cl_2, 0 °C; (iv) DMSO, TFAA, Et_3N, CH_2Cl_2, −60 °C; (v) TFA, CH_2Cl_2, rt; (vi) BOP, RCOOH, Et_3N, CH_2Cl_2, rt.

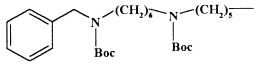
Table 1. HIV activities of the new derivatives on MT_4 HIV infected cells



No.	R	X	EC_{50}[a]	CC_{50}[b]	SI[c]	Log P[d]
29		=O	1	> 1	> 1	8.28 ± 0.77
38		-OH	Inactive	10	—	7.82 ± 0.72
30		=O	1	> 50	> 50	9.13 ± 0.75
39		-OH	Inactive	Toxic	—	9.13 ± 0.75
31		=O	0.1–1	> 50	50–500	9.58 ± 0.74
40		-OH	0.1–1	50	50–500	9.12 ± 0.70
32		=O	1	10	10	4.94 ± 0.63
41		-OH	Inactive	10	—	4.48 ± 0.57
33		=O	NT[e]	NT[e]	—	6.43 ± 0.59
42		-OH	Inactive	10	—	5.97 ± 0.52
34		=O	0.1–1	50	50–500	10.75 ± 0.92
43		-OH	10	> 50	> 5	10.29 ± 0.88
35		=O	Inactive	—	—	13.16 ± 0.90
44		-OH	NT[e]	NT[e]	—	12.70 ± 0.86
36		=O	10–50	50	1–50	11.57 ± 0.91
45		-OH	NT[e]	NT[e]	—	11.11 ± 0.87
37		=O	0.1	50	500	14.15 ± 0.90

(continued on next page)

Table 1 (*continued*)

No.	R	X	EC_{50}[a]	CC_{50}[b]	SI[c]	Log P[d]
46		-OH	0.1–1	10	10–100	13.69 ± 0.85

[a]EC_{50} was defined as the concentration of the compound at μM concentrations that inhibited 50% of the viral replication.
[b]CC_{50} was defined as the concentration of the compound at μM concentrations that reduced the viability of the cells to 50% compared to untreated cells.
[c]SI: Selective index: $SI = CC_{50}/EC_{50}$.
[d]Log P: partition coefficient (octanol–water) calculated from ACD software.
[e]NT: not tested.

Virology

***Syncytium* formation.** The newly synthesized analogues were evaluated for their inhibitory effects on HIV replication in MT_4 cell cultures (Table 1). Under the assay conditions, some of the new compounds elicited potent anti-HIV activities, with EC_{50} values ranging from 0.1 to 10 μM (**29, 30, 31, 32, 34, 37, 40, 43, 46**). It can also be observed that cytotoxicity values (CC_{50}) of the new analogues are relatively high. Their Selective Index values range from 1 to 50 (Table 1). This observation suggests that the analogues permeated cell membranes quite easily. One explanation could be the relatively high lipophilicity of these derivatives. Indeed log P values of the studied compounds (Table 1) calculated using ACD Software (Advanced Chemistry Development, Inc) range from 4 to 14. Derivatives like **31, 34**, **37**, **40**, **46** whose EC_{50} ranged between 0.1 and 1 μM could represent interesting hits which could be optimized, since we have found that, in a similar assay (MT_4 *syncytium* formation), the anti-RT inhibitor 3TC and the antiprotease Saquinavir elicited EC_{50} values of 0.1 and 0.05 μM respectively. Saquinavir was the most potent compound under our testing conditions.

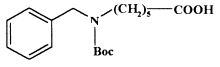
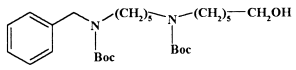
From the results reported in Table 1, several comments can be made:

1. The presence of an hydroxyl group or a ketone at the 2 position of the diamino propyl motif (Fig. 1) led to active anti-HIV compounds.
2. As far as cytotoxicity of the new analogues is concerned, it can be observed that some of the active compounds are relatively cytotoxic on infected MT_4 cells (**30**) while other compounds (**31**, **34**, **36**, **37, 40**) show selectivity index values (SI) ranging from 50 to 500. This observation is quite intriguing since the coefficient partition values (log P) calculated for the whole compounds are quite high (log P values ranging from 4 to 14).

From the results reported in Table 2, the fact that compounds **13, 19** are found to be inactive on HIV-replication in MT_4 cells suggests that both moieties polyaminated or mono- and 1,3-diamino-2-propanol or ketone represented in Figure 1 are required for anti-HIV activity. If we make the assumption that these analogues cross the cell membranes by non-facilitated diffusion, the rather high lipophilicity should confer them a similar diffusion rate into the cells. Such observations have been reported by Agrawal et al.[16] in the case of various lipophilic AZT prodrugs. It can be confirmed that as shown in Table 2, the structural motif 2-hydroxy- or oxo-1,3-[N_1,N_3′-dibenzyl]diaminopropane **A** (Fig. 1) is required for HIV activity since polyaminated carboxylic chains alone (compounds **13**, **19**) do not show any anti-HIV activity.

Antiprotease assay. Both series of derivatives ketones (**29, 30, 31, 32, 33, 34, 35, 36, 37**) and alcohols (**38, 39, 40, 41, 42, 43, 44, 45, 46**) were also screened for their HIV-1 protease inhibitory activity according to a classical HIV protease assay[17] described in the Experimental. We found that both series of compounds tested at 10 μM do not demonstrate any significant HIV-1 protease inhibitory activity. In identical assay conditions, the HIV-1 protease inhibitor Saquinavir[18] elicited enzyme inhibition in the nanomolar range ($IC_{50} = 5$ nM). These results clearly indicate that under these assay conditions, the new synthesized derivatives do not interfere at the HIV protease level. These results are in good agreement with the HIV protease molecular modelling predictions, since none of the derivatives studied presented favourable fitting energies (ΔE_c) within the HIV protease active-site. However, these results should be examined cautiously since it has been suggested that HIV protease inhibitors not only interfered with the virus maturation in the late phase of the HIV replication cycle but can also interfere

Table 2. Anti-HIV activities of compounds **5d**, **7h** and **8h**

No.	Compound	EC_{50}[a]	CC_{50}[b]	Log P[c]
13		Inactive	50	3.24 ± 0.56
19		Inactive	50	5.09 ± 0.62

[a]EC_{50} was defined as the concentration of the compound at μM concentrations that inhibited 50% of the viral replication.
[b]CC_{50} was defined as the concentration of the compound at μM concentrations that reduced the viability of the cells to 50% compared to untreated cells.
[c]Log P: partition coefficient (octanol–water) calculated from ACD software.

in an early phase of the cycle, before the formation of the provirus. This result strengthened the argument that these derivatives are probably not transition state HIV protease inhibitors. Indeed very often HIV protease inhibitors are characterized by an hydroxyethylamine moiety which mimics the hydrolysis transition state.

Integrase inhibition assay. The most active compounds (**13b**, **15c**, **16c**, **21d**, **27g**, **28g**) were also tested as possible integrase inhibitors according to a screening protocol described by Pommier et al.[7] None of these compounds were found to be integrase inhibitors.

Conclusion

Through this study it can be observed that some of the new analogues bearing a ketone or an hydroxyl function (**11a**, **13b**, **15c**, **16c**, **17a**, **21d**, **22d**, **25e**, **27g**, **28g**) elicited potent in vitro anti-HIV activity on infected MT_4 cells. Enzymatic inhibition studies on recombinant HIV protease and on integrase reveal that these new series of compounds appear to act neither at the HIV protease level of the HIV replication cycle nor at the integrase level. One way to determine at what stage of the HIV replicative cycle these compounds interfere would be to perform time-of-addition experiments, whereby the new derivatives are added at different times after infection. However it seems that this series of compounds do not have specific anti-HIV activities.

This result suggests that both approaches for the discovery of new anti-HIV drugs are complementary. The rational approach, allowing tailor-made molecules to address to well-defined targets is well adapted for optimization process, while random screening using a multiple target screening model (*syncytium* formation) appears to be suitable for new anti-HIV drug discovery.

Experimental

Chemistry

Nuclear magnetic resonance spectra were recorded with a Bruker AC-250 (^{1}H NMR); chemical shifts are expressed as δ units (part per million) downfield from TMS. Fast atom bombardment mass spectral analyses were obtained by Dr. Astier (Laboratoire de Mesures Physiques-RMN, USTL, Montpellier, France) on a Jeol DX-100 using a cesium ion source and glycerol: thioglycerol (1:1) (GT) or *m*-nitrobenzyl alcohol (NOBA) as matrix. Mass calibration was performed using cesium iodide. Thin layer chromatography (TLC) and preparative layer chromatography (PLC) were performed using silica gel plates 0.2, 1 or 2 mm thick ($60F_{254}$ Merck). Preparative flash column chromatography was carried out on silica gel (230–240 mesh, G60 Merck). Analytical HPLC was performed on a Waters 600E instrument with M991 photodiode detector using the following conditions: 4.6×150 mm Column (Waters Spherisorb S5 ODS2, 5 μM).

All reagents were of commercial quality (Aldrich Company) from freshly opened containers.

The described compounds were obtained using the following procedures.

General procedure for ω-*N*-tert-butyloxycarbonylamino carboxylic acids 6, 7. To a solution of ω-amino alcohol **1, 2** (1 equiv) in CH_2Cl_2 (40 mL) cooled to 0 °C, Boc_2O was added slowly (1.1 equiv). The reaction was followed by TLC. After 3 h, the solvent was removed and the crude product (1 equiv) obtained was directly dissolved in dry DMF (52.5 mL). PDC was added (3.5 equiv) and the solution was stirred overnight under nitrogen. The reaction was stopped by adding 10 volumes (525 mL) of a solution: H_2O:Brine (1:1). The solution was extracted with Et_2O (2×200 mL). The organic phase was washed with Brine solution (2×150 mL), 1N NaOH solution (2×200 mL). The aqueous layer was acidified by 1N HCl solution (2×200 mL), then extracted with CH_2Cl_2 (2×150 mL). The organic layer was dried over Na_2SO_4 and evaporated.

3-*N*-*tert*-Butyloxycarbonylamino propanoic acid (6) (yield: 48%). ^{1}H NMR ($CDCl_3$): δ 1.45 (11H, m, Boc, CH_2-$\underline{CH_2}$-CH_2); 2.46 (2H, t, $\underline{CH_2}COOH$); 2.91 (2H, m, $\underline{CH_2}N$); 4.63 (1H, m, $N\underline{H}$).

6-*N*-*tert*-Butyloxycarbonylamino hexanoic acid (7) (yield: 43%). ^{1}H NMR ($CDCl_3$): δ 1.46 (13H, m, Boc, CH_2-($\underline{CH_2}$)$_2$-CH_2); 2.47 (2H, t, $\underline{CH_2}COOH$); 2.92 (2H, m, $\underline{CH_2}N$); 4.68 (1H, m, $N\underline{H}$).

General procedure for ω-*N*-[*N*-(*tert*-butyloxycarbonyl)-benzylamino]-carboxylic acids 11, 12, 13. To a solution of ω-amino alcohol **1, 2, 3** (1 equiv) in CH_2Cl_2 (150 mL), benzaldehyde (1.1 equiv) and Na_2SO_4 (1 equiv) were added at room temperature. The solution was stirred overnight under nitrogen. Na_2SO_4 was filtered and the solvent evapored under vacuum. The crude product **8, 9, 10** was directly used without further purification. To a solution of the previous product **8, 9, 10** (1 equiv) dissolved in EtOH (15 mL) and cooled to 0 °C, $NaBH_4$ was added slowly (1.5 equiv). After complete reaction (2 h), the reaction was quenched by adding H_2O (10 mL). The solvent was removed. The residue was dissolved in EtOAc (40 mL) and washed with 5% $NaHCO_3$ solution (1×20 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated. The crude product was directly used for the next step without purification.

To a solution of the previous product (1 equiv) in CH_2Cl_2 (70 mL), Boc_2O was added slowly at 0 °C (2.5 equiv). Once the reaction was complete (3 h), the solvent was evaporated and the crude Boc residue was isolated in very good yield. 3.7 mmol of the previous residue were directly dissolved in dry DMF (9.8 mL) before PDC was added (3.5 equiv). After 3 h, the reaction was stopped by adding 10 volumes (98 mL) of a solution: H_2O:Brine (1:1) and the solution was extracted with Et_2O (3×50 mL). The organic phase was consecutively washed with Brine solution (2×30 mL) and 1N NaOH solution (2×30 mL). The aqueous layer was acidified by addition of 1N HCl solution (1×30 mL), then extracted

with CH_2Cl_2 (3×30 mL). The organic layer was dried over Na_2SO_4 before evaporation.

3-*N*-[*N*(*tert*-Butyloxycarbonyl)benzylamino]-propanoic acid (11) (yield: 44%). ^{1}H NMR ($CDCl_3$): δ 1.36 and 2.31 (11H, m, CH_2, Boc), 2.75 (2H, t, CH_2COOH), 3.05 (2H, s, CH_2N), 4.25 (2H, s, CH_2Ph), 7.05 (5H, m, Ph).

4-*N*-[*N*(*tert*-Butyloxycarbonyl)benzylamino]-pentanoic acid (12) (yield: 46%). ^{1}H NMR ($CDCl_3$): δ 1.30 and 2.34 (13H, m, 2 CH_2, Boc), 2.70 (2H, t, CH_2COOH), 3.05 (2H, s, CH_2N), 4.25 (2H, s, CH_2Ph), 7.05 (5H, m, Ph).

6-*N*-[*N*(*tert*-Butyloxycarbonyl)benzylamino]-hexanoic acid (13) (yield: 42%). ^{1}H NMR ($CDCl_3$): δ 1.32 and 2.32 (17H, m, 4 CH_2, Boc), 2.70 (2H, t, CH_2COOH), 3.05 (2H, s, CH_2N), 4.25 (2H, s, CH_2Ph), 7.05 (5H, m, Ph).

General procedure for ω-[benzyl(tert-butoxycarbonyl)-amino]-ω-alkyl-1-benzenesulfonate 14, 15, 16. To a solution of ω-amino alcohol **1, 2, 3** (1 equiv) in Et_2O:Et_3N (1:1), tosyl chloride was added to the solution (1.1 equiv). After 3 h, the solvent was evaporated. The product dissolved in EtOAc (1×20 mL) was washed with 5% citric acid solution (1×15 mL), H_2O (1×15 mL), 5% $NaHCO_3$ solution (1×15 mL), then the organic layer was dried over Na_2SO_4, filtered and evaporated. The crude product (1.74 g) was purified by flash chromatography with EtOAc:Hexane as eluent (3:7) to give pure compounds **14, 15, 16**.

3-[Benzyl(*tert*-butoxycarbonyl)amino]propyl-3-propyl-1-benzenesulfonate (14) (yield: 50%). ^{1}H NMR ($CDCl_3$): δ 1.61 (11H, m, CO-CH_2-CH_2-CH_2-OTs, Boc); 2.62 (3H, m, CH_3-Ph); 3.20 (2H, m, CH_2Ph); 3.74 (2H, t, CH_2OTs); 4.91 (2H, s, CH_2Ph) 7.66 (9H, m, Ph, CH_3Ph).

4-[Benzyl(*tert*-butoxycarbonyl)amino]pentyl-4-pentyl-1-benzenesulfonate (15) (yield: 48%). ^{1}H NMR ($CDCl_3$): δ 1.62 (15H, m, CO-CH_2-$(CH_2)_3$-CH_2-OTs, Boc); 2.63 (3H, m, CH_3-Ph); 3.22 (2H, m, CH_2Ph); 3.71 (2H, t, CH_2OTs); 4.94 (2H, s, CH_2Ph) 7.67 (9H, m, Ph, CH_3Ph).

6-[Benzyl(*tert*-butoxycarbonyl)amino]hexyl-6-hexyl-1-benzenesulfonate (16) (yield: 53%). ^{1}H NMR ($CDCl_3$): δ 1.61 (17H, m, CO-CH_2-$(CH_2)_4$-CH_2-OTs, Boc); 2.62 (3H, m, CH_3-Ph); 3.21 (2H, m, CH_2Ph); 3.70 (2H, t, CH_2OTs); 4.94 (2H, s, CH_2Ph) 7.62 (9H, m, Ph, CH_3Ph).

General procedure for *tert*-butyl-*N*-benzyl-*N*-[ω-*N*-*tert*-butyl(benzylamino)]alkyl-*N*-(ω-hydroxyalkyl)carbamates 17, 18, 19, 20. To a solution of product **14, 15, 16** (1 equiv) in CH_3CN (20 mL), ω-amino alcohol **1, 2, 3** (1.2 equiv) and K_2CO_3 (3 equiv) were added. After 5 days at room temperature, K_2CO_3 was filtered off and the solvent evaporated. The remaining crude product was directly used for the next step without purification.

To a solution of the previous product (1 equiv) in CH_2Cl_2 (15 mL) cooled to 0 °C, Boc_2O was added (1.2 equiv). After 2 h, the solvent was evaporated and the compound was purified by flash chromatography with EtOAc:Hexane as eluent (5:5). The pure products **17, 18, 19, 20** were isolated in very good yield.

***tert*-Butyl-*N*-benzyl-*N*-[3-*N*-*tert*-butyl(benzylamino)]propyl-*N*-(3-hydroxypropyl)carbamate (17) (yield: 90%).** ^{1}H NMR ($CDCl_3$): δ 1.20 (13H, m, Boc, 2 CH_2); 2.95 (2H, m, NCH_2); 3.44 (2H, t, CH_2OH); 4.24 (2H, s, $PhCH_2$); 7.10 (5H, m, Ph).

***tert*-Butyl-*N*-benzyl-*N*-[4-*N*-*tert*-butyl(benzylamino)]propyl-*N*-(4-hydroxypentyl)carbamate (18) (yield: 94%).** ^{1}H NMR ($CDCl_3$): δ 1.22 (17H, m, Boc, 4 CH_2); 2.96 (2H, m, NCH_2); 3.45 (2H, t, CH_2OH); 4.25 (2H, s, $PhCH_2$); 7.11 (5H, m, Ph).

***tert*-Butyl-*N*-benzyl-*N*-[5-*N*-*tert*-butyl(benzylamino)]pentyl-*N*-(5-hydroxypentyl)carbamate (19) (yield: 89%).** ^{1}H NMR ($CDCl_3$): δ 1.20 (19H, m, Boc, 5 CH_2); 2.95 (2H, m, NCH_2); 3.48 (2H, t, CH_2OH); 4.27 (2H, s, $PhCH_2$); 7.12 (5H, m, Ph).

***tert*-Butyl-*N*-benzyl-*N*-[6-*N*-*tert*-butyl(benzylamino)]hexyl-*N*-(6-hydroxyhexyl)carbamate (20) (yield: 88%).** ^{1}H NMR ($CDCl_3$): δ 1.21 (23H, m, Boc, 7 CH_2); 2.92 (2H, m, NCH_2); 3.46 (2H, t, CH_2OH); 4.22 (2H, s, $PhCH_2$); 7.10 (5H, m, Ph).

General procedure for ω-[ω-*N*-*tert*-butyl(benzylamino) alkyl](*tert*-butoxycarbonyl)amino] carboxylic acids 21, 22, 23, 24. PDC (3.5 equiv) was added to a solution of **17, 18, 19, 20** (1 equiv) in dry DMF (3.6 mL). The solution was stirred overnight under nitrogen and the solvent was evaporated. After 3 h, the reaction was stopped by adding 10 volumes (40 mL of a (1:1) H_2O:Brine solution). The solution was extracted with Et_2O (3×15 mL) and the organic layer was washed with Brine solution (2×10 mL), 1N NaOH solution (2×10 mL). The aqueous layer was acidified by addition of a solution of 1N HCl (1×10 mL), then extracted with CH_2Cl_2 (2×15 mL), dried over Na_2SO_4, and evaporated. The pure compounds **21, 22, 23, 24** were isolated in good yield.

3-[3-*N*-*tert*-Butyl(benzylamino)propyl](*tert*-butoxycarbonyl)amino]propanoic acid (21) (yield: 75%). ^{1}H NMR ($CDCl_3$): δ 1.22 (20H, m, Boc, CH_2); 2.10 (2H, t, CH_2COOH); 2.98 (2H, s, NCH_2); 4.22 (2H, m, CH_2Ph); 7.16 (5H, m, Ph).

4-[4-*N*-*tert*-Butyl(benzylamino)propyl](*tert*-butoxycarbonyl)amino]pentanoic acid (22) (yield: 72%). ^{1}H NMR ($CDCl_3$): δ 1.20 (24H, m, Boc, 3 CH_2); 2.12 (2H, t, CH_2COOH); 2.94 (2H, s, NCH_2); 4.23 (2H, m, CH_2Ph); 7.14 (5H, m, Ph).

6-[6-*N*-*tert*-Butyl(benzylamino)pentyl](*tert*-butoxycarbonyl)amino]pentanoic acid (23) (yield : 70%). ^{1}H NMR ($CDCl_3$): δ 1.22 (28H, m, Boc, 5 CH_2); 2.10 (2H, t, CH_2COOH); 2.96 (2H, s, NCH_2); 4.24 (2H, m, CH_2Ph); 7.15 (5H, m, Ph).

6-[6-*N*-*tert*-Butyl(benzylamino)hexyl](*tert*-butoxycarbonyl) amino]hexanoic acid (24) (yield: 69%). ^{1}H NMR ($CDCl_3$): δ 1.22 (32H, m, Boc, 7 CH_2); 2.14 (2H, t, CH_2COOH); 2.94 (2H, s, NCH_2); 4.25 (2H, m, CH_2Ph); 7.19 (5H, m, Ph).

1,3-di[*N*-(*N′*-*tert*-Butyloxycarbonyl)benzylamino]-propan-2-ol (27). To a solution of 1,3-diaminopropan-2-ol (1 equiv: 33.30 mmol, 3 g) in CH_2Cl_2 (100 mL), Na_2SO_4 (2 equiv, 66.60 mmol, 9.50 g) then slowly benzaldehyde were added (2 equiv, 66.60 mmol, 6.80 mL). The reaction was stirred overnight under nitrogen. After filtration of Na_2SO_4, the solvent was evaporated. The crude product was purified by recrystallization in hot hexane (250 mL). White crystals of diimine product were isolated after filtration (3.31 g, 20 mmol, 60% yield).

The diimine (1 equiv: 19 mmol, 5.08 g) was dissolved in EtOH (100 mL), cooled to 0 °C and $NaBH_4$ was added slowly (2.5 equiv: 48 mmol, 1.80 g). The reaction was quenched by adding H_2O (48 mL). The solvent was removed and the residue dissolved in EtOAc (40 mL) was washed with 5% $NaHCO_3$ solution (1×10 mL). The organic layer was washed with 1N HCl solution (2×10 mL). Then, the aqueous layer was basified with 1N NaOH solution (2×10 mL) and extracted with CH_2Cl_2 (2×20 mL). The resulting organic layer was dried over Na_2SO_4, filtered and evaporated. The pure product (3.67 g, 13.70 mmol) was isolated in 72% yield.

To a solution of 1,3-di(*N*-benzylamino)-2-propanol (1 equiv: 3.70 mmol, 1 g) in CH_2Cl_2 (1×20 mL) cooled to 0°C, Boc_2O was added slowly (2.2 equiv: 8.15 mmol, 17.80 g). After 30 min, the solvent was evaporated. The crude product was purified by flash chromatography with EtOAc:Hexane (1:4) as eluent. The pure product **27** (1.55 g, 3.30 mmol) was isolated in 89% yield. ^{1}H NMR ($CDCl_3$): δ 2.61 (4H, m, 2 $\underline{CH_2}$), 3.83 (3H, 2s, $\underline{CH_2}$Ph and CHOH), 7.32 (10H, m, Ph).

1,3-di-*N*-[*N′*-(*tert*-Butyloxycarbonyl)benzylamino]acetone (28). A solution of DMSO (2 equiv: 7.40 mmol, 530 μL) in dry CH_2Cl_2 (15 mL) was cooled to −60 °C under nitrogen before trifluoroacetic anhydride was added (1.5 equiv: 5.60 mmol, 795 μL). After 10 min, the compound **27** (1 equiv: 3.70 mmol, 1.73 g) was added to the solution. After 45 min, Et_3N (4.4 equiv: 16.30 mmol, 2.30 mL) was added. The reaction was quenched with H_2O (1×10 mL). The organic phase was washed with H_2O (2×15 mL), dried over Na_2SO_4, filtered and evaporated. The crude product (2.08 g) was purified by flash chromatography with EtOAc:Hexane: (15:85) as eluent. Pure compound **28**: (1.10 g, 2.85 mmoles, 64% yield). ^{1}H NMR ($CDCl_3$): δ 1.31 (18H, 2s, 2Boc); 3.79 (4H, q, $BocNCH_2CO$); 4.36 (4H, s, $\underline{CH_2}$Ph); 7.15 (10H, m, Ph).

General procedure for 1,3-di[*N*-[*N′*-(*N*-benzyl-*N′*-*tert*-butyloxycarbonyl)-ω-amino alkanoyl benzylamino]-propan-2-one 29, 30, 31, 32, 33, 34, 35, 36, 37. Trifluoroacetic acid was added (30 equiv) to a solution of compound **28** (1 equiv) in CH_2Cl_2 (2 mL). After 20 min, the solvent was evaporated. The dried crude remaining product was dissolved in CH_2Cl_2 (2 mL) before the addition of BOP (2.4 equiv) compound **6, 7, 11, 12, 13**, **21, 22, 23** or **24** (2.4 equiv) and Et_3N (12 equiv). After 2 h, the solvent was removed. The residue dissolved in EtOAc (3 mL) was washed with 5% $NaHCO_3$ solution (2×4 mL), then Brine solution (1×4 mL), and 5% citric acid solution (2×4 mL). The organic phase was dried (Na_2SO_4), filtered and evaporated. The crude product (330 mg) was purified by flash chromatography with EtOAc:hexane (5:5) as eluent to give pure compounds **29, 30, 31, 32, 33, 34, 35, 36, 37**.

1,3-di[*N*-[*N′*-(*N*-Benzyl-*N′*-*tert*-butyloxycarbonyl)-3-amino propanoyl benzylamino]-propan-2-one (29) (yield: 85%). ^{1}H NMR ($CDCl_3$): δ 1.30 (30H, m, $COCH_2$-$\underline{CH_2}$-CH_2, Boc); 2.22 (4H, t, $\underline{CH_2}CON$); 3.01 (4H, m, $CH_2\underline{CH_2}N$); 4.05 (4H, m, $NCH_2\underline{CO}$); 4.20 (4H, m, $PhCH_2\underline{NCO}$); 4.41 (4H, m, $PhCH_2\underline{NBocCH_2}$); 7.10 (20H, m, Ph). Mass spectrum (NOBA, FAB$^+$): 833 $(M+H)^+$. R_f = 0.5 in EtOAc:hexane (2:3).

1,3-di[*N*-[*N′*-(*N*-Benzyl-*N′*-*tert*-butyloxycarbonyl)-4-amino pentanoyl benzylamino]-propan-2-one (30) (yield: 87%). ^{1}H NMR ($CDCl_3$): δ 1.31 (30H, m, $COCH_2$-$(\underline{CH_2})_2$-CH_2, Boc); 2.20 (4H, t, $\underline{CH_2}CON$); 3.08 (4H, m, $CH_2\underline{CH_2}N$); 4.10 (4H, m, $NCH_2\underline{CO}$); 4.23 (4H, m, $PhCH_2\underline{NCO}$); 4.44 (4H, m, $PhCH_2\underline{NBocCH_2}$); 7.16 (20H, m, Ph). Mass Spectrum (NOBA, FAB$^+$): 847 $(M+H)^+$. R_f = 0.4 (EtOAc:Hexane: 2:3).

1,3-di[*N*-[*N′*-(*N*-Benzyl-*N′*-*tert*-butyloxycarbonyl)-3-amino hexanoyl benzylamino]-propan-2-one (31) (yield: 85%). ^{1}H NMR ($CDCl_3$): δ 1.33 (30H, m, CO-CH_2-$(\underline{CH_2})_4$-CH_2, Boc); 2.21 (4H, t, $\underline{CH_2}CON$); 3.30 (4H, m, $CH_2\underline{CH_2}N$); 4.00 (4H, m, $NCH_2\underline{CO}$); 4.21 (4H, m, $PhCH_2\underline{NCO}$); 4.40 (4H, m, $PhCH_2\underline{NBocCH_2}$); 7.11 (20H, m, Ph). Mass spectrum (NOBA, FAB$^+$): 861 $(M+H)^+$. R_f = 0.5 (EtOAc:Hexane: 2:3).

1,3-di[*N′*-(*tert*-Butyloxycarbonyl)-3-amino propanoyl benzylamino]-propan-2-one (32) (yield: 89%). ^{1}H NMR ($CDCl_3$): δ 1.43 (22H, m, 2Boc, $2\underline{CH_2}$); 2.61 (4H, m, $\underline{CH_2}CON$); 3.50 (4H, m, $BocNH\underline{CH_2}$); 4.01 (4H, m, $\underline{CH_2}CO\underline{CH_2}$); 4.61 (4H, m, $PhCH_2$); 7.30 (10H, m, Ph). Mass spectrum (NOBA, FAB$^+$): 611 $(M+H)^+$. R_f = 0.35 (EtOAc:Hexane: 1:1).

1,3-di[*N′*-(*tert*-Butyloxycarbonyl)-6-amino hexanoyl benzylamino]-propan-2-one (33) (yield: 88%). ^{1}H NMR ($CDCl_3$): δ 1.43 (30H, m, 2Boc, $6\underline{CH_2}$); 2.63 (4H, m, $\underline{CH_2}CON$); 3.55 (4H, m, $BocNH\underline{CH_2}$); 4.02 (4H, m, $\underline{CH_2}CO\underline{CH_2}$); 4.65 (4H, m, $PhCH_2$); 7.36 (10H, m, Ph). Mass spectrum (NOBA, FAB$^+$): 695 $(M+H)^+$ R_f = 0.4 (EtOAc:Hexane: 9:1).

1,3-*N*-{*N′*[*N″*(*N*-Benzyl-N′-*tert*-Butyloxycarbonyl)-3-propyl]-*N′*-*tert*-Butyloxycarbonyl-3-amino propanol benzylamino]-propan-2-one (34) (yield: 85%). ^{1}H NMR ($CDCl_3$): δ ;1.42 (40H, m, 4Boc, $2\underline{CH_2}$); 2.41 (4H, 2t, $CO\underline{CH_2}$); 3.11 (12H, m, $PhCH_2BocN\underline{CH_2}$, $CH_2NBoc\underline{CH_2}$); 4.10 (4H, s, $CH_2CO\underline{CH_2}$); 4.45 (4H, s, $PhCH_2BocN\underline{CH_2}$); 4.66 (4H, s, $PhCH_2BocN\underline{CH_2}$); 7.30 (20H, m, Ph). Mass spectrum (NOBA, FAB$^+$): 1105 $(M+H)^+$. R_f = 0.4 (EtOAc: Hexane: 1:1).

1,3-*N*-{*N′*[*N″*(*N*-Benzyl-*N′*-*tert*-butyloxycarbonyl)-5-pentyl]-*N′*-*tert*-butyloxycarbonyl-5-amino pentanol benzylamino]-propan-2-one (35) (yield: 83%). ^{1}H NMR ($CDCl_3$): δ 1.40 (56H, m, 4Boc, $10\underline{CH_2}$); 2.44 (4H, 2t, $CO\underline{CH_2}$); 3.11 (12H, m, $PhCH_2BocN\underline{CH_2}$, $CH_2NBoc\underline{CH_2}$); 4.12

(4H, s, $\underline{CH_2}CO\underline{CH_2}$); 4.45 (4H, s, $PhCH_2BocN\underline{CH_2}$); 4.60 (4H, s, $PhCH_2BocN\underline{CH_2}$); 7.30 (20H, m, Ph). Mass spectrum (NOBA, FAB^+) 1219 $(M+H)^+$. $R_f=0.4$ (EtOAc:Hexane: 2:3).

1,3-*N*-{*N*′[*N*″(*N*-Benzyl-*N*′-*tert*-butyloxycarbonyl)-3-propyl]-*N*′-*tert*-butyloxycarbonyl-5-amino pentanol benzylamino]-propan-2-one (36) (yield: 81%). ^1H NMR ($CDCl_3$): δ 1.47 (56H, m, 4Boc, $10\underline{CH_2}$); 2.44 (4H, 2t, $CO\underline{CH_2}$); 3.12 (12H, m, $PhCH_2BocN\underline{CH_2}$, $CH_2NBoc\underline{CH_2}$); 4.10 (4H, s, $CH_2CO\underline{CH_2}$); 4.43 (4H, s, $PhCH_2BocN\underline{CH_2}$); 4.65 (4H, s, $PhCH_2BocN\underline{CH_2}$); 7.36 (20H, m, Ph). Mass spectrum (NOBA, FAB^+): 1163 $(M+H)^+$. $R_f=0.4$ (EtOAc:Hexane: 2:3).

1,3-*N*-{*N*′[*N*″(*N*-Benzyl-*N*′-*tert*-butyloxycarbonyl)-6-hexyl]-*N*′-*tert*-butyloxycarbonyl-6-amino hexanol benzylamino]-propan-2-one (37) (yield: 82%). ^1H NMR ($CDCl_3$): δ 1.42 (68H, m, 4Boc, $16\underline{CH_2}$); 2.40 (4H, 2t, $CO\underline{CH_2}$); 3.15 (12H, m, $PhCH_2BocN\underline{CH_2}$, $\underline{CH_2}NBoc\underline{CH_2}$); 4.12 (4H, s, $\underline{CH_2}CO\underline{CH_2}$); 4.46 (4H, s, $PhCH_2BocN\underline{CH_2}$); 4.69 (4H, s, $PhCH_2BocN\underline{CH_2}$); 7.37 (20H, m, Ph). Mass spectrum (NOBA, FAB^+): 1261 $(M+H)^+$. $R_f=0.4$ (EtOAc:Hexane: 4:1).

General procedure for 1,3-di[*N*-[*N*′-(*N*-Benzyl-*N*′-*tert*-butyloxycarbonyl-ω-amino alkanoyl benzylamino]-propan-2-ol 38, 39, 40, 41, 42, 43, 44, 45, 46. To a solution of the previous products **29, 30, 31, 32, 33, 34, 35, 36, 37** (1 equiv) in EtOH (5 mL) cooled to 0 °C, $NaBH_4$ was added (1.2 equiv). After 10 min, the solvent was evaporated. The product dissolved in EtOAc (6 mL) was washed in brine solution (2×4 mL), dried over Na_2SO_4, filtered and evaporated. The crude product was purified by PLC with EtOAc:hexane as eluent: (2:3) to give pure compounds **38, 39, 40, 41, 42, 43, 44, 45, 46**.

1,3-di[*N*-[*N*′-(*N*-Benzyl-*N*′-tert-butyloxycarbonyl)-3-amino]propanoyl benzylamino]-propan-2-ol (38) (yield: 63%). ^1H NMR ($CDCl_3$): δ 1.30 (18H, 2s, Boc); 2.13 (4H, t, $\underline{CH_2}CON$); 2.96 (4H, m, $CH_2\underline{CH_2}N$); 3.51 (1H, m, $\underline{CH}OH$); 4.05 (4H, m, $N\underline{CH_2}CO$); 4.22 (4H, m, $Ph\underline{CH_2}NCO$); 4.41 (4H, m, $Ph\underline{CH_2}NBocCH_2$); 7.14 (20H, m, Ph). Mass spectrum (NOBA, FAB^+): 835 $(M+H)^+$. $R_f=0.4$ (EtOAc:Hexane: 1:1).

1,3-di[*N*-[*N*′-(*N*-Benzyl-*N*′-*tert*-butyloxycarbonyl)-4-amino]pentanoyl benzylamino]-propan-2-ol (39) (yield: 62%). ^1H NMR ($CDCl_3$): δ 1.32 (26H, m, $CO\text{-}CH_2\text{-}(\underline{CH_2})_2\text{-}CH_2$, Boc); 2.14 (4H, t, $\underline{CH_2}CON$); 2.90 (4H, m, $\underline{CH_2}CH_2N$); 3.53 (1H, m, $\underline{CH}OH$); 4.02 (4H, m, $N\underline{CH_2}CO$); 4.25 (4H, m, $Ph\underline{CH_2}NCO$); 4.41 (4H, m, $Ph\underline{CH_2}NBocCH_2$); 7.17 (20H, m, Ph). Mass spectrum (NOBA, FAB^+): 849 $(M+H)^+$. $R_f=0.35$ (EtOAc:Hexane: 9:1).

1,3-di[*N*-[*N*′-(*N*-Benzyl-*N*′-*tert*-butyloxycarbonyl)-6-amino]hexanoyl benzylamino]- propan-2-ol (40) (yield: 61%). ^1H NMR ($CDCl_3$): δ 1.33 (34H, m, $CO\text{-}CH_2\text{-}(\underline{CH_2})_4\text{-}CH_2$, Boc); 2.10 (4H, t, $\underline{CH_2}CON$); 2.98 (4H, m, $\underline{CH_2}\,\underline{CH_2}N$); 3.54 (1H, m, $\underline{CH}OH$); 4.06 (4H, m, $N\underline{CH_2}CO$); 4.24 (4H, m, $Ph\underline{CH_2}NCO$); 4.40 (4H, m, $Ph\underline{CH_2}NBocCH_2$); 7.14 (20H, m, Ph). Mass spectrum (NOBA, FAB^+) : 863 $(M+H)^+$. $R_f=0.4$ (EtOAc:Hexane: 2:3).

1,3-di[*N*′-(*tert*-Butyloxycarbonyl)-3-amino propanoil benzylamino]-propan-2-ol (41) (yield: 64%). ^1H NMR ($CDCl_3$): δ 1.43 (22H, m, 2Boc, $2\underline{CH_2}$); 2.62 (4H, m, $\underline{CH_2}CON$); 2.98 (4H, m, $BocNH\underline{CH_2}$); 3.17 (4H, m, $\underline{CH_2}CHO\ H\underline{CH_2}$); 3.50 (1H, m, $\underline{CH}OH$); 4.64 (4H, m, $Ph\underline{CH_2}$); 7.31 (10H, m, Ph). Mass spectrum (NOBA, FAB^+) : 613 $(M+H)^+$. $R_f=0.4$ (EtOAc:Hexane: 1:1).

1,3-di[*N*′-(*tert*-Butyloxycarbonyl)-6-amino hexanoyl benzylamino]-propan-2-ol (42) (yield: 65%). ^1H NMR ($CDCl_3$): δ 1.43 (30H, m, 2Boc, $6\underline{CH_2}$); 2.64 (4H, m, $\underline{CH_2}CON$); 2.89 (4H, m, $BocNH\underline{CH_2}$); 3.10 (4H, m, $\underline{CH_2}CHOH\underline{CH_2}$); 3.51 (1H, m, $\underline{CH}OH$); 4.67 (4H, m, $Ph\underline{CH_2}$); 7.30 (10H, m, Ph). Mass spectrum (NOBA, FAB^+): 697 $(M+H)^+$. $R_f=0.5$ (EtOAc:Hexane: 3:1).

1,3-*N*-{*N*′[*N*″(*N*-Benzyl-*N*′-*tert*-butyloxycarbonyl)-3-propyl]-*N*′-*tert*-butyloxycarbonyl-3-amino propanol benzylamino]-propan-2-ol (43) (yield: 60%). ^1H NMR ($CDCl_3$): δ 1.40 (40H, m, 4Boc, $2\underline{CH_2}$); 2.42 (4H, 2t, $CO\underline{CH_2}$); 3.14 (12H, m, $PhCH_2BocN\underline{CH_2}$, $\underline{CH_2}NBoc\ \underline{CH_2}$); 3.28 (4H, m, $\underline{CH_2}CHOH\underline{CH_2}$); 3.55 (1H, m, $\underline{CH}OH$); 4.41 (4H, s, $Ph\underline{CH_2}BocN\underline{CH_2}$); 4.64 (4H, s, $PhCH_2Boc\ N\underline{CH_2}$); 7.37 (20H, m, Ph). Mass spectrum (NOBA, FAB^+): 1107 $(M+H)^+$. $R_f=0.4$ (EtOAc:Hexane: 2:3).

1,3-*N*-{*N*′[*N*″(*N*-Benzyl-*N*′-*tert*-butyloxycarbonyl)-5-pentyl]-*N*′-*tert*-butyloxycarbonyl-5-amino pentanol benzylamino]-propan-2-ol (44) (yield: 66%). ^1H NMR ($CDCl_3$): δ 1.44 (46H, m, 4Boc, $10\underline{CH_2}$); 2.42 (4H, 2t, $CO\underline{CH_2}$); 3.11 (12H, m, $Ph\underline{CH_2}BocN\underline{CH_2}$, $\underline{CH_2}NBoc\underline{CH_2}$); 3.22 (4H, m, $\underline{CH_2}CHOH\underline{CH_2}$); 3.55 (1H, m, $\underline{CH}OH$); 4.43 (4H, s, $Ph\underline{CH_2}BocN\underline{CH_2}$); 4.6 7(4H, s, $PhCH_2Boc\ N\underline{CH_2}$); 7.35 (20H, m, Ph). Mass spectrum (NOBA, FAB^+): 1221 $(M+H)^+$. $R_f=0.4$ (EtOAc:Hexane: 2:3).

1,3-*N*-{*N*′[*N*″(*N*-Benzyl-*N*′-*tert*-butyloxycarbonyl)-3-propyl]-*N*′-*tert*-butyloxycarbonyl-5-amino pentanol benzylamino]-propan-2-ol (45) (yield: 62%). ^1H NMR ($CDCl_3$): δ 1.44 (46H, m, 4Boc, $5\underline{CH_2}$); 2.45 (4H, 2t, $CO\underline{CH_2}$); 3.12 (12H, m, $PhCH_2BocN\underline{CH_2}$, $CH_2NBoc\underline{CH_2}$); 3.27 (4H, m, $\underline{CH_2}CHOH\underline{CH_2}$); 3.56 (1H, m, $\underline{CH}OH$); 4.43 (4H, s, $Ph\underline{CH_2}BocN\underline{CH_2}$); 4.67 (4H, s, $PhCH_2Boc\ N\underline{CH_2}$); 7.35 (20H, m, Ph). Mass spectrum (NOBA, FAB^+): 1165 $(M+H)^+$. $R_f=0.4$ (EtOAc:Hexane: 1:1).

1,3-*N*-{*N*′[*N*″(*N*-Benzyl-*N*′-*tert*-butyloxycarbonyl)-6-hexyl]-*N*′-*tert*-butyloxycarbonyl-6-amino hexanol benzylamino]-propan-2-ol (46) (yield: 65%). ^1H NMR ($CDCl_3$): δ 1.42 (56H, m, 64 Boc, $14\underline{CH_2}$); 2.42 (4H, 2t, $CO\underline{CH_2}$); 3.13 (12H, m, $PhCH_2BocN\underline{CH_2}$, $\underline{CH_2}NBoc\underline{CH_2}$); 3.21 (4H, m, $\underline{CH_2}CHOH\underline{CH_2}$); 3.53 (1H, m, $\underline{CH}OH$); 4.44 (4H, s, $Ph\underline{CH_2}BocN\underline{CH_2}$); 4.68 (4H, s, $PhCH_2BocN\underline{CH_2}$); 7.32 (20H, m, Ph). Mass spectrum (NOBA, FAB^+): 1263 $(M+H)^+$. $R_f=0.5$ (EtOAc:Hexane: 1:1).

Virology

Anti-HIV activity was monitored by the efficiency of drug compounds to inhibit *syncytium* formation after HIV-infection of MT_4, as already described.[19] Briefly, 3×10^5 MT_4 cells were first pre-incubated with 100 μL of various concentrations of drug compounds dissolved in

DMSO or in H_2O then diluted in phosphate buffer saline solution for one hour at 37 °C. Then 100 μL of an appropriate virus dilution was added to the mixture and incubated for another hour at 37 °C. After three washes, cells were resuspended in culture medium in the presence or absence of the drug compounds. Cultures were then continued for 7 days at 37 °C, under 5% CO_2 atmosphere and culture medium replaced at day 3 post-infection (supplemented or not with drug compounds). Each culture condition was carried out in duplicate. The appearance of *syncytia* was followed each day with an inverted optical microscope. Typically, the virus dilution used in this assay (multiplicity of infection of 0.1 TCID50/cell) allowed *syncytium* formation at day 5 post infection. The inhibitory concentration of drug compounds was expressed as the concentration that caused 50% inhibition of *syncytium* formation (EC_{50}) without direct toxicity for the cells. Cytotoxic concentration (CC_{50}) of drug compounds was monitored on growth of non-infected cells by trypan blue exclusion assay and corresponded to the concentration required to cause 50% of cell death.

HIV-1 protease inhibition assay. The HPLC based assay for inhibition of HIV-1 protease was performed by using cloned HIV-1 protease and HIV-1 protease substrate (H-Hys-Lys-Ala-Arg-Val-Leu-pnitro-Phe-Glu-Ala-Ser-NH_2, BACHEM S.A.). Potential inhibitor was solubilized in DMSO at various concentrations. Fifteen microlitres of this solution were diluted in 100 μL of buffer (sodium acetate 50 nM pH 4.9, sodium chloride 200 nM, dithiothreitol 5 nM and glycerol 10%, V/V) to obtain a $[I]_{buffered}$ concentration. 40 μL of $[I]_{buffered}$ were mixed with 15 μL of the HIV-1 substrate, then with 25 cdmL of rec-HIV-1 protease. The mixture was incubated at 37 °C during 20 minutes and quenched by 10 μL of a 10% TFA solution. Twenty microlitres of the pre-incubated assay was injected in reverse phase HPLC (Waters C_{18}) with CH_3CN:H_2O (5:95 to 100:0) in 30 min. Inhibition was determined by relative percent of the peak areas corresponding to the HIV-1 protease substrate cleavage.

Ten micromolar was the optimal concentration at which the new analogues were tested.

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