Anti-HIV-1 activity of a tripodal receptor that recognizes mannose oligomers

Eva Rivero-Buceta,[a] Paula Carrero,[a,b] Elena Casanova,[a,c] Elisa G. Doyagüez,[d] Andrés Madrona,[a] Ernesto Quesada,[a] María Jesús Peréz-Pérez,[a] Raquel Mateos,[e] Laura Bravo,[e] Sam Noppen,[f] Sandra Liekens,[f] Jan Balzarini,[f] María José Camarasa[a] and Ana San-Félix*[a]

[a] *Instituto de Química Médica (IQM-CSIC), Juan de la Cierva 3, 28006 Madrid*
[b] *ABG Patentes, Avenida de Burgos 16D, 28036 Madrid*
[c] *Euroquímica S.A., Crta. Yeles, Km 2 Illescas (Toledo)*
[d] *Centro de Química Orgánica "Lora-Tamayo" (CSIC), Juan de la Cierva 3, 28006 Madrid*
[e] *Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN-CSIC), Jose Antonio Novais 10, 28040 Madrid*
[f] *Rega Institute for Medical Research, KU Leuven, B-3000 Leuven, Belgium*

Abstract

The glycoprotein gp120 of the HIV-1 viral envelope has a high content in mannose residues, particularly α-1,2-mannose oligomers. Compounds that interact with these high-mannose type glycans may disturb the interaction between gp120 and its (co)receptors and are considered potential anti-HIV agents. Previously, we demonstrated that a tripodal receptor (**1**), with a central scaffold of 1,3,5-triethylbenzene substituted with three 2,3,4-trihydroxybenzoyl groups, recognizes selectively α-1,2-mannose polysaccharides. Here we present additional studies to determine the anti HIV-1 activity and binding capacity towards gp120 of **1** and similar structural analogues. Our studies indicate that compound **1** shows anti-HIV-1 activity in the low micromolar range and has pronounced gp120 binding capacity.

Keywords: Antiviral agents, AIDS, HIV, polyphenols

Corresponding author:

Instituto de Química Médica (CSIC), Juan de la Cierva 3, 28006 Madrid

Telephone: 34 91 2587617; Fax: 34 91 5644853

E-mail: anarosa@iqm.csic.es

1. Introduction

Highly active antiretroviral therapy (HAART) has significantly contributed to reduce the morbidity and mortality caused by human inmunodeficiency virus (HIV), the retrovirus responsible for the transmission and development of AIDS [1]. However, issues such as long-term toxicities, adverse drug-drug interactions, and the emergence and transmission of drug-resistant viral strains represent a serious problem to a successful long-term treatment [2]. There is, therefore, a need to

develop novel therapeutic approaches and drugs for the efficient treatment of HIV-1 [3].

HIV is an enveloped virus that contains the glycoproteins gp120 and gp41 on its surface. These envelope glycoproteins interact with the CD4 receptor on the T cell surface. Glycoprotein gp120 is of particular importance during viral fusion and entry, as it serves as the first point of contact with the host cell. This glycoprotein is the main target for neutralizing antibodies that appear during natural infection [4]. The HIV gp120 glycoprotein is extensively glycosylated, so that approximately 50% of its molecular weight is due to a dense carbohydrate array. Interestingly gp120 carbohydrates contain an unusually high amount of mannose residues, in particular α-1,2-mannose, α-1,3-mannose and α-1,6-mannose oligomers [5].

A number of plant lectins, in particular those with specificity for mannose oligomers, display potent inhibitory activity against several viruses, including HIV [5]. These plant lectins exert their antiviral action by strongly binding to the carbohydrates of gp120, thereby compromising the required conformational changes in gp120/gp41 for optimal interaction with the (co)-receptors and fusion with the target cell membrane. However, the macromolecular and peptidic nature of these plant lectins precludes their use as anti-HIV agents due to the lack of the appropriate pharmacokinetic properties. Based on the anti HIV activity of these plant lectins a novel therapeutic concept to fight against HIV infection has been proposed [5]. According to this proposal agents that interact with the high-mannose type glycans of the HIV-1 gp120 may disturb the interaction between gp120 and its (co)receptors and, as consecuence, show anti-HIV activity.

Very recently [6] our group described the synthesis of two tripodal receptors, **1** and **2,** with a triethylbenzene central scaffold substituted respectively with three 2,3,4-trihydroxybenzoyl or its isomeric 3,4,5-trihydroxybenzoyl (galloyl) moieties (Figure 1). Molecular Modelling and NMR studies showed that only compound **1** has a unique conformation that facilitates the recognition of α(1→2)-linked mannose polysaccharides that mimic the mannose composition of the glycans that are abundantly present on the HIV envelope glycoprotein gp120.

In this work compound **1** and similar structural analogues have been synthesized and tested against HIV in cell culture. Also the ability of these compounds to bind to gp120 has been determined by SPR experiments.

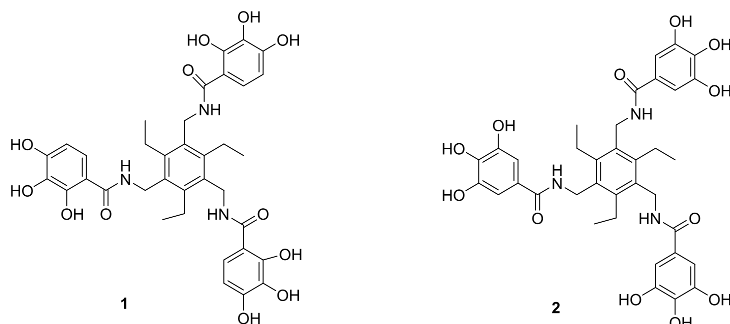


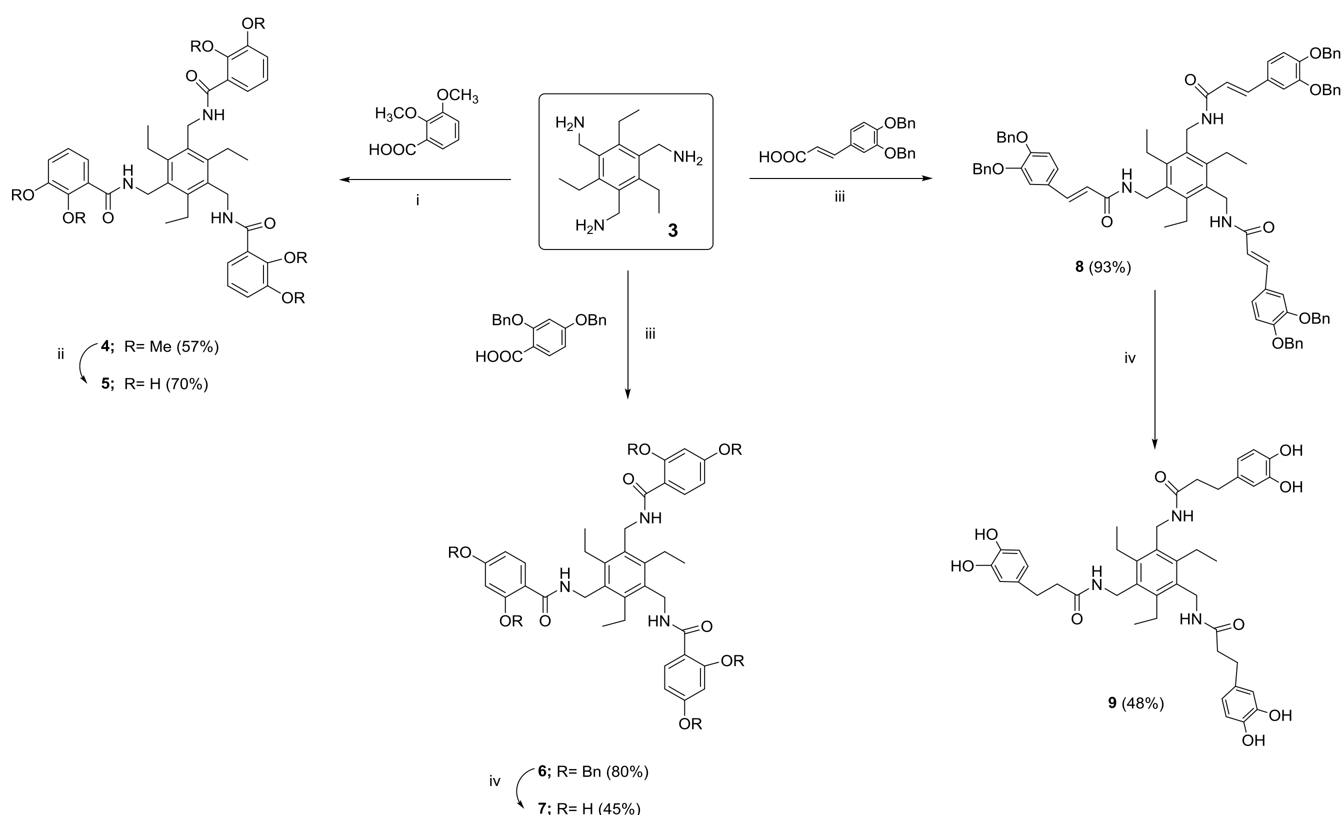
Fig. 1

2. Results and discussion

2.1. Chemical results

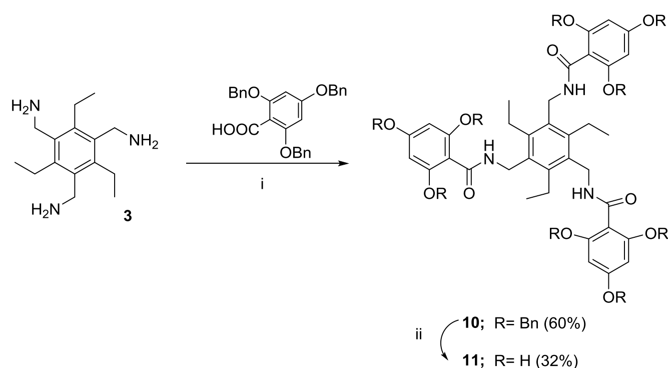
To investigate the importance of the number and position of the phenolic OHs, compounds **5** and **7** with two contiguous and non-contiguous OHs respectively, were prepared (Scheme 1). These compounds were synthesized by reaction of 1,3,5-tris(aminomethyl)-2,4,6-triethylbenzene (**3**) [7], with the respective OMe or OBn-protected dihydroxybenzoic acids. Reactions were performed in the presence of PyBOP/triethylamine or HATU/DIPEA as coupling reagents. These reactions allowed the synthesis of the OMe (**4**) or OBn (**6**) protected derivatives in good yields (57 % and 80 % respectively). Removal of the methyl groups in **4**, using boron tribromide gave **5** (70 %) [8]. On the other hand, hydrogenolysis of **6** (H_2, 10% Pd/C) gave the corresponding phenol deprotected derivative **7** (45 %)**.**

Compound **9**, bearing three dihydroxyphenylethyl moieties, as in hydroxytyrosol, a well-known polyphenol of natural origin [9], was also prepared (Scheme 1). For the synthesis of this compound the OBn protected derivative of caffeic acid [10] was used. Reaction of this compound with trisamine **3** in the presence of HATU/DIPEA afforded compound **8** in 93% yield that after simultaneous deprotection and reduction of the unsaturated double bond by catalytic hydrogenation afforded compound **9** in 48% in yield.



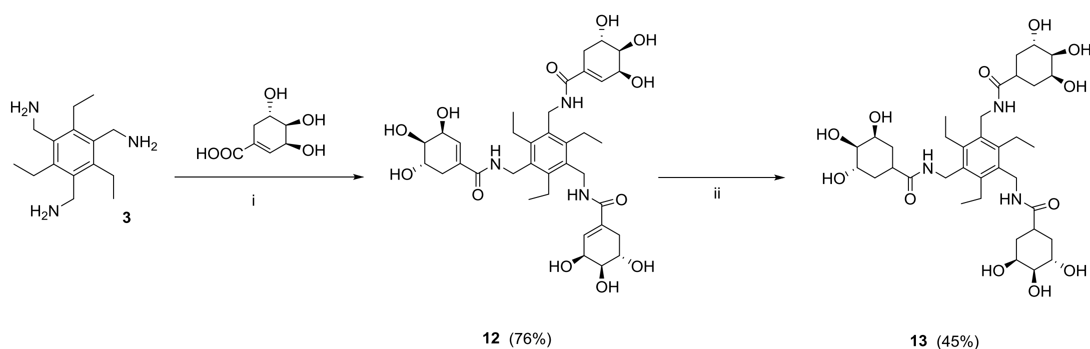
Scheme 1

Next, compound **11,** with three non-contiguous OHs, was prepared by reaction of **3** [7] with the corresponding benzyl protected 2,4,6-trihydroxybenzoic acid [11] in the presence of HATU/DIPEA, followed by hydrogenolysis of the corresponding benzyl protected compound **10** (Scheme 2).



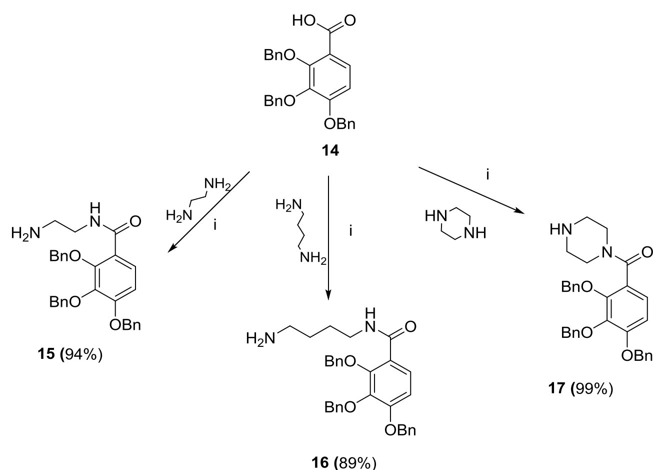
Scheme 2

Shikimic acid and its corresponding fully saturated cyclohexyl derivative were also incorporated to the central scaffold (Scheme 3). The shikimic acid derivative **12** was prepared in 76 % yield by treatment of the trisamine **3** [7] with the commercially available shikimic acid in the presence of HATU/DIPEA. Catalytic hydrogenation of **12** rendered the fully saturated derivative **13** in 45 % yield.



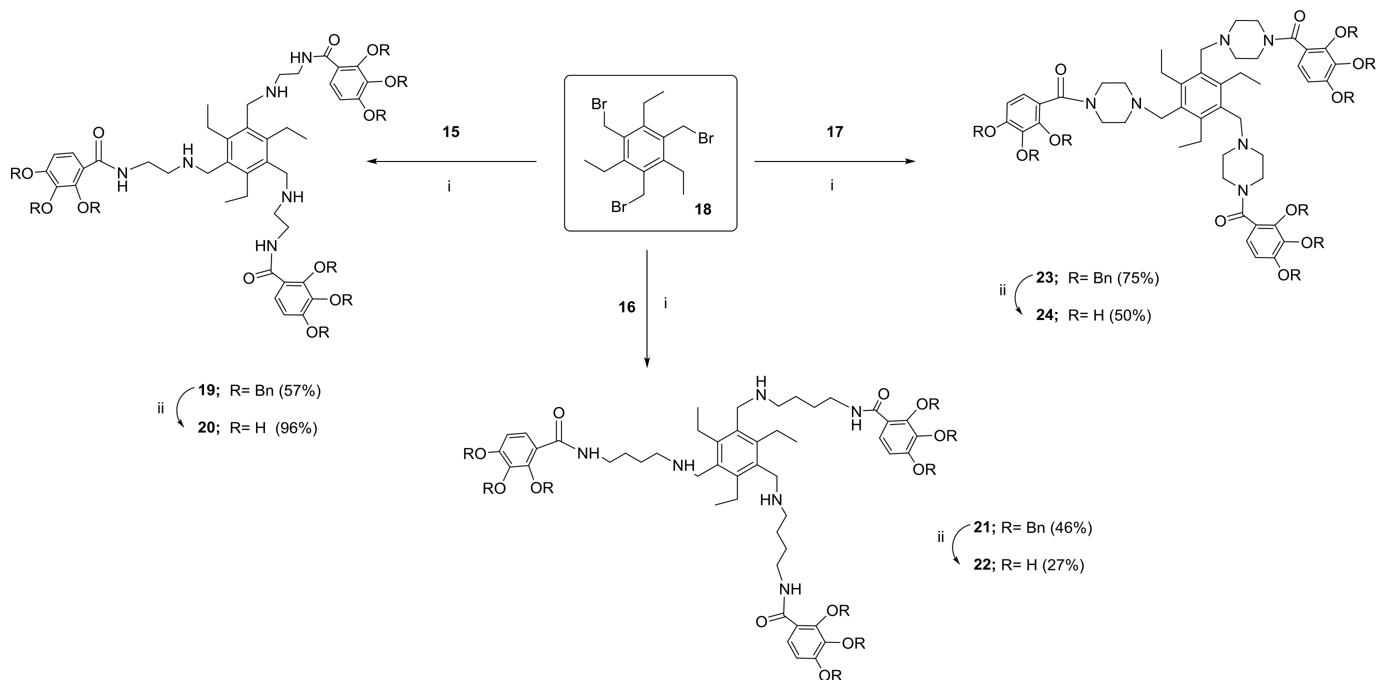
Scheme 3

Next, compounds **20, 22** and **24** with the three phenolic groups connected to the central scaffold by spacers of different lengths were prepared. With this purpose compounds **15**, **16** and **17** (Scheme 4) were firstly synthesized. These compounds were prepared in excellent yields (94%, 89% and 99% respectively) by reaction of 2,3,4-tribenzyloxybenzoic acid (**14)** [12] with excess of ethylenediamine, tetramethylene diamine or piperazine in the presence of PyBOP and trietylamine.



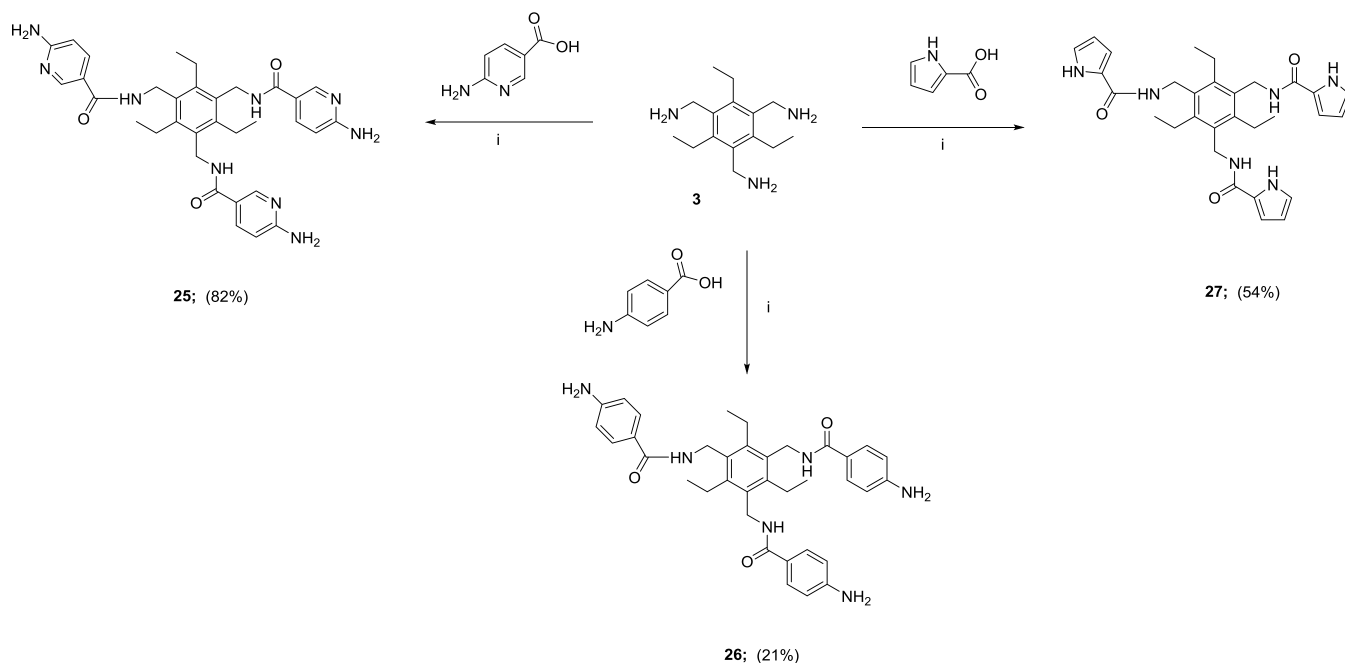
Scheme 4

Treatment of **15**-**17** with the 1,3,5-tris(bromomethyl)-2,4,6-trietylbenzene intermediate **18** [7] in the presence of triethylamine afforded **19** (57%), **21** (46%) and **23** (75%) (Scheme 5). Hydrogenolysis of **19**, **21** and **23** in the presence of 10% Pd/C gave the corresponding phenol-deprotected derivatives **20** (96 %)**,** **22** (27 %) and **24** (50 %).



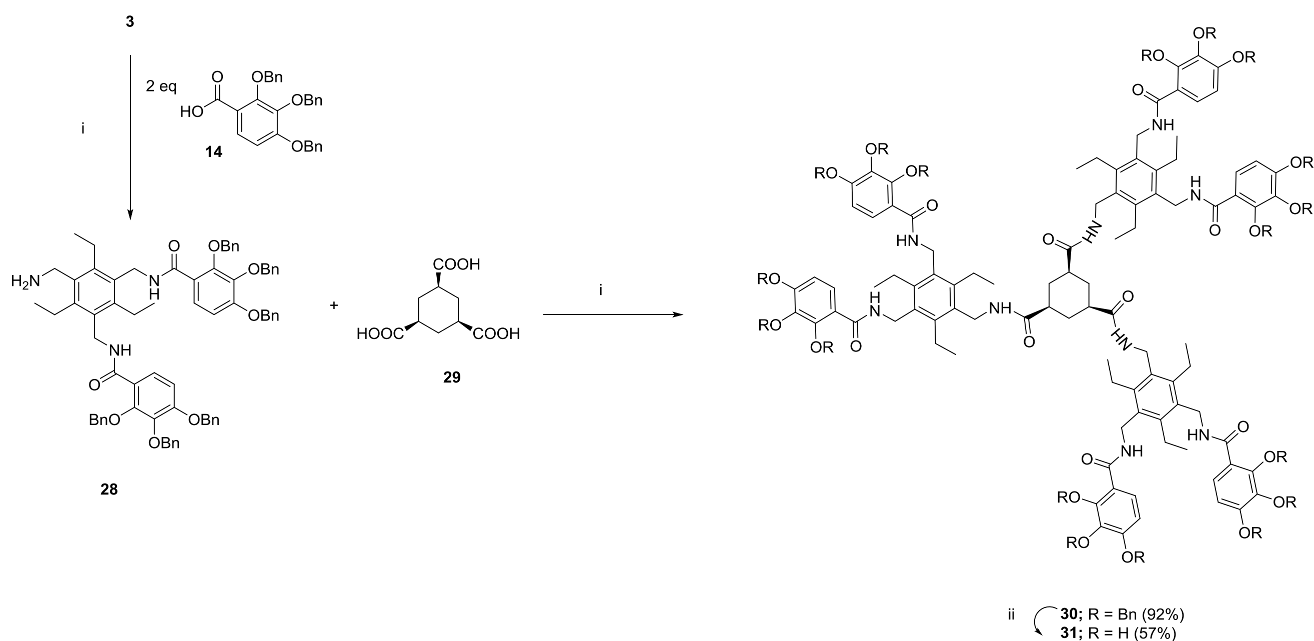
Scheme 5

Next, aminopyridine, as carbohydrate recognition moiety [13], aniline and pyrrole, were incorporated as substituents of the triethylbenzene scaffold. The corresponding compounds **25** (82%), **26** (21%) and **27**(54%) [14] were prepared in good to moderate yields by coupling of **3** [7], with the corresponding carboxylic acid in the presence of PyBOP/Et_3N (Scheme 6).



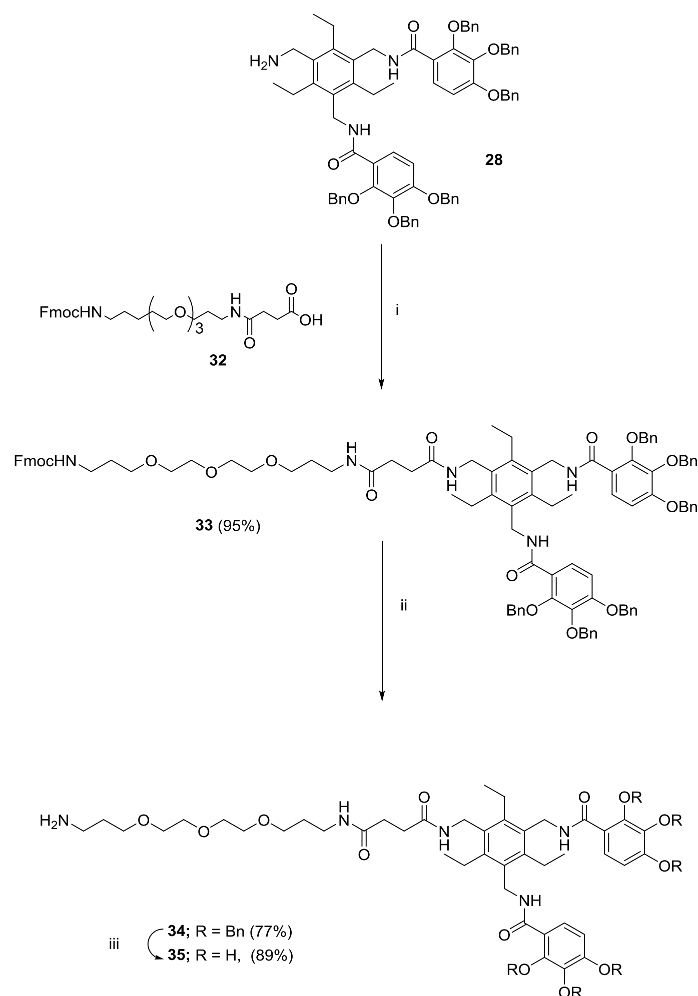
Scheme 6

Next, we prepared compound **31** with six phenolic entities around the central scaffold (Scheme 7). In this case the disubstituted amine **28** was used as starting compound and *cis,cis*-1,3,5-ciclohexane carboxylic acid (**29**) as central core. The disubstituted amine **28** was prepared by reaction of **3** [7] with two equivalents of **14** [12]. Starting from **28** and following the coupling/deprotection procedure, compound **30** (92%) and its corresponding deprotected derivative **31** (57%) were obtained.



Scheme 7

Finally, and in order to perform SPR experiments, compound **35,** an analogue of **1** containing a linker (**32**) with a terminal amino (NH_2) group suitable for its covalent attachment to the SPR sensorchip, was synthesized (Scheme 8). Linker **32** was prepared, from commercially available 4,7,10-trioxa-1,13-tridecanediamine by reaction with succinic anhydride and subsequent amine protection with Fmoc *N*-hydroxysuccinimide ester (Fmoc-OSu) [15]. Reaction of the disubstituted derivative **28** with linker **32** [15], in the presence of PyBOP and trimethylamine, gave compound **33** (95%). Subsequent removal of the Fmoc protecting group using 20% of piperidine in DMF at room temperature gave compound **34** in 77% yield that after removal of OBn afforded **35** in 89% yield.



Scheme 8

2.2. Antiviral activity

The compounds synthesized were evaluated as potential inhibitors of the replication of human immunodeficiency virus (HIV-1 and HIV-2) in human CD_4^+ T-lymphocyte CEM cell cultures and the results are given in Table 1.

As reference, Pradimicin-A (PRM-A), a non-peptidic antibiotic of natural origin that shows anti-HIV activity by binding to the high-mannose type glycans of the HIV-1 gp120, was included.

As shown in table 1, the conformationally-constrained tripodal receptor **1**, is the only compound able to inhibit the replication of HIV-1 with a clear safety profile. This result highlights the importance of the unique structural features present in **1** (three 2,3,4-trihydroxybenzoyl groups directly attached to a trietylbenzene scaffold through amide linkers) for anti-HIV activity.

It should be emphasized that the activity showed by **1** (6.3 ± 1.0 μM), in the low micromolar range, is very similar to that of Pradimicin A (3.4 ± 1.3 μM). However, Pradimicin A is a structurally complex molecule difficult to synthesize and obtained by fermentation. By contrast, **1** is a molecule that is easy to prepare by conventional synthetic procedures.

Table 1.

Compound	EC_{50} (μM)[a]		CC_{50} (μM)[b]
	HIV-1	HIV-2	
1	6.3 ± 1.0	41 ± 14	144 ± 15
2	>10	>10	49 ± 2.6
5	>2	>2	5.0 ± 0.14
7	>4	>4	28 ± 1.4
9	>10	>10	22 ± 0.71
11	>50	>50	112 ± 4.2
12	>250	>250	>250
13	>250	>250	>250
20	>10	>10	117 ± 3.5
22	>10	>10	25 ± 9.9
24	>10	>10	36 ± 25
25	>250	>250	>250
26	>250	>250	>250
27	>10*	>10*	>10*
31	>10	>10	25 ± 2.1
PRM-A	3.4 ± 1.3		>50

Data are the mean ± S.D. of at least 2 to 4 independent experiments.
[a] 50% Effective concentration, or the compound concentration required to inhibit HIV-induced cytopathicity by 50%.
[b] 50% Cytostatic concentration, or the compound concentration required to inhibit CEM cell proliferation by 50%.
*Compound precipitation (due to insolubility) was detected at higher compound concentration

2.3. SPR experiments

Surface Plasmon Resonance (SPR) was used to study in real time the interactions of **1** with the mannose oligomers of the glycoprotein gp120. First, the glycoprotein gp120 was attached to the sensor chip surface ("direct experiment"). As shown in Figure 2, a remarkable binding signal was observed for **1**. The binding amplitude was comparable or even somewhat stronger than that of the reference compound, Pradimicin-S (PRM-S), a soluble derivative of PRM-A (Figure 2). The remarkable binding signal suggests that there are pronounced interactions between **1** and the gp120 glycoprotein. Moreover, whereas the on-rate (association of **1** to gp120) is in the same order of magnitude as Pradimicin-S, the off-rate (dissociation of **1** from gp120) proved much slower.

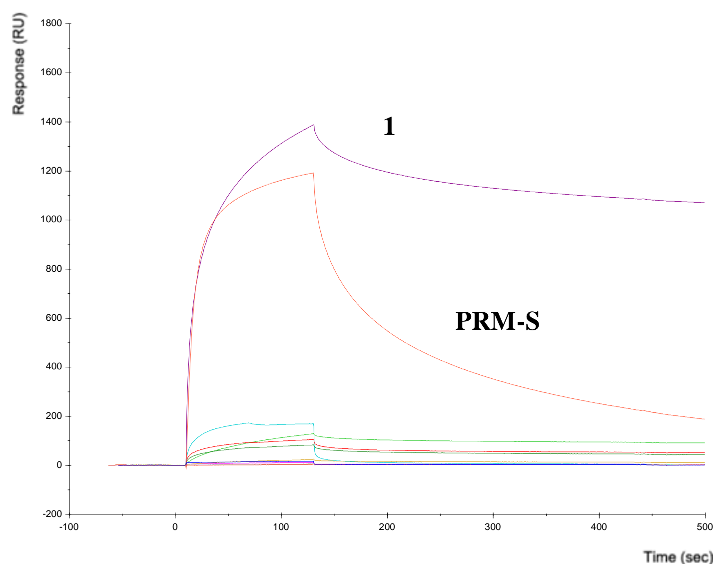


Fig. 2.

In a reverse experiment, compound **35,** the analogue of **1** containing a linker with a terminal amino (NH_2) group suitable for its covalent attachment to the SPR sensorchip, was attached to the SPR sensor chip surface through the terminal amino group of its flexible linker and glycoprotein gp120 was then injected (Figure 3).

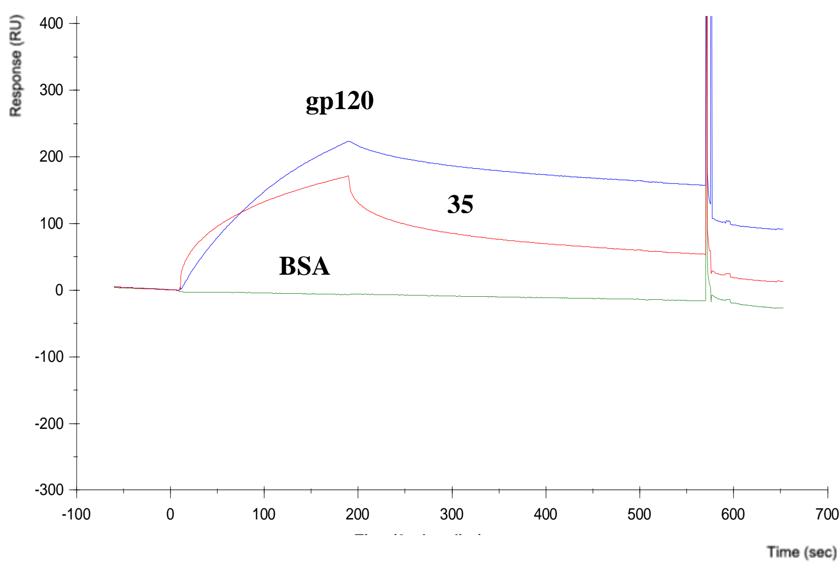


Fig.3.

As in the direct binding experiment, where gp120 was bound to the sensorchip, it was observed that the off-rate (dissociation) was quite slow compared with the on-rate.

In addition bovine serum albumin (BSA), (as negative non-glycosylated protein control) was injected across the sensor chip surface (green curve) but no binding of BSA to compound **35** was detected (Figure 3). These results add further

evidence to the fact that **35,** and by extension **1,** is able to interact in a rather specific manner with the HIV glycoprotein gp120. Moreover, it was observed that **35** also binds to itself (red curve) (self-aggregation). This result supports the markedly pronounced accumulation of molecules on the chip surface observed in the direct binding experiment, where gp120 was bound to the sensorchip.

The association and dissociation of compound **1** to the gp120-bound sensorchip was also studied in the presence of two different mannose trimers mann(α-1,2)$_3$ and mann(α-1,3-1,6)$_3$ at two different concentrations, 100 μM and 400 μM (Fig. 4). Interestingly, both mannose trimers were able to decrease the interaction of compound **1** with gp120, being mann(α-1,3/1,6)$_3$ more efficient than mann(α-1,2)$_3$ in the preventive binding of compound **1** to gp120. These findings suggest an interaction of compound **1** with these mannose glycans and by extension to those that are present on gp120.

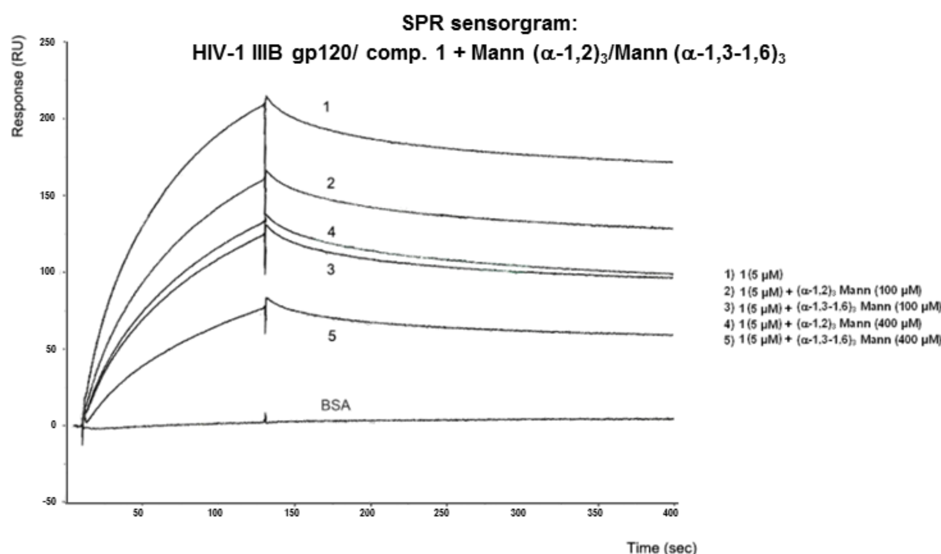


Fig. 4.

Although compound **1** clearly shows mannose-specific gp120 binding (demonstrated in our SPR experiments) it cannot be excluded that other targets of the HIV life cycle could also be targeted by this compound, among them the virus-encoded integrase (IN) enzyme. In this respect it has been reported that several polyphenols, such as caffeic acid phenyl ester (CAPE), dicaffeoyl quinic acid (DCQA) and dicaffeoyl tartaric acid exhibit IN inhibitory activity and antiviral activity [16]. Although the structure of the latter compounds differs from that of **1**, in which quinic or tartaric acids and caffeoyl moieties are not present, it still cannot be excluded that IN inhibition may also play a certain role in the eventual antiviral activity of this compound.

It is currently even not known whether compound **1** is taken-up by the HIV-infected cells, which is not needed for inhibition of viral entry but a prerequisite to interact with the viral IN. Further studies are therefore required to clarify this issue and to unambiguously reveal which is the major antiviral target of **1.**

3. Conclusions

Previous studies of our group showed that the tripodal receptor **1,** with a central scaffold of 1,3,5-triethylbenzene substituted with three 2,3,4-trihydroxybenzoyl groups, is able to recognize α-1,2-mannose polysaccharides similar to those that are present in the envelope glycoprotein of HIV. Based on this result we decided to determine the anti-HIV activity of **1** and several analogues and the results are described in this work. Only compound **1** markedly inhibits the replication of HIV-1.

"Direct" or "reverse" SPR binding experiments showed the specific interaction of **1** with the HIV glycoprotein gp120. The interaction of compound **1** with the glycoprotein gp120 was also studied in the presence of two different mannose trimers similar to those present on gp120. Our results suggest a preferential interaction of compound **1** with these mannose trimers and by extension to those present on gp120.

It can be concluded that the particular structural features of **1**, containing three 2,3,4-trihydroxybenzoyl residues directly attached to a central triethylbenzene scaffold through amide linkers, are important for their anti-HIV activity and ability to specifically interact with the HIV glycoprotein gp120.

4. Materials and methods

4.1. Synthesis

4.1.1. General method for OBn deprotection

A solution of the corresponding OBn protected derivative in THF/methanol (1:1) (20 mL) containing 30 wt% of Pd/C (10%) was hydrogenated at 30 ºC overnight under atmospheric pressure using a balloon filled with hydrogen gas and a glass flask as the reaction vessel. The Pd/C was filtered through Whatman® filter paper 42, washed with methanol and the solvent was removed under reduced pressure to give the crude product which was then purified as mentioned for each case.

*4.1.2. 1,3,5-Tris(2,4-dibenzyloxybenzamidomethyl)-2,4,6-triethylbenzene (**6**)*

To a solution of trisamine **3** [7] (100 mg, 0.4 mmol) in DMF (20 mL), 2,4-dibenzyloxybenzoic acid (550 mg, 1.6 mmol) [17], HATU (608 mg, 1.6 mmol) and DIPEA (279 µL, 1.6 mmol) were added. The mixture was stirred at room temperature overnight and then evaporated to dryness. The residue was dissolved in dichloromethane (50 mL) and washed successively with an aqueous solutions of citric acid (10%) (3 × 20 mL), saturated $NaHCO_3$ (3 × 20 mL) and brine (3 × 20 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The residue was purified on a Biotage HPFC system (High Performance Flash Chromatography) using hexane:ethyl acetate (1:1) as eluent to afford 381 mg (80%) of **6** as an amorphous solid. ^{1}H-NMR (300 MHz, $CDCl_3$) *δ:* 1.00 (t, J = 7.3 Hz, 9H, CH_3CH_2), 2.44 (m, 6H, CH_2CH_3), 4.53 (s, 6H, CH_2NH), 4.84 (s, 6H, CH_2Ar), 5.13 (s, 6H, CH_2Ar), 6.60 (d, J = 2.3 Hz, 3H, Ar), 6.75 (dd, J = 2.3 Hz y J = 6.5 Hz, 3H, Ar), 7.02 (m, 15H, Ar), 7.45 (m, 18H, Ar), 8.29 (s, 3H, NH).

*4.1.3. 1,3,5-Tris(2,4-dihydroxybenzamidomethyl)-2,4,6-triethylbenzene (**7**)*

Following the general deprotection procedure, the OBn derivative **6** (381 mg, 0.32 mmol) gave a crude product which was then purified on a Biotage HPFC system (High Performance Flash Chromatography) on reverse phase using

water:acetonitrile (1:1) as eluent to afford 119 mg (45%) of **7** as a white solid m.p. 301-303 ºC. MS (ES+): *m/z* 680.3 $(M+Na)^+$, *m/z* 658.3 $(M+H)^+$. ^{1}H-RMN (500 MHz, DMSO-d_6) *δ:* 1.11 (t, *J* = 7.3 Hz, 9H, CH_3CH_2), 2.78 (m, 6H, CH_2CH_3), 4.54 (s, 6H, CH_2NH), 6.23 (m, 3H, Ar), 7.76 (d, *J* = 8.7 Hz, 3H, Ar), 8.40 (s, 3H, NHCO), 9.96 (br s, 3H, *p*-OH), 12.43 (br s, 3H, *o*-OH).^{13}C-NMR (125 MHz, DMSO-d_6) *δ:* 21.31 (CH_3), 27.86 (CH_2), 42.51 (CH_2), 107.82 (CH), 112.16 (C), 112.67 (C), 135.39 (CH), 137.12 (CH), 148.99 (CH), 166.55 (C), 167.18 (C),173.17 (C=O). HPLC [gradient: A:B, 10-100% of A in 10 min]: 7.80 min. Anal. $C_{36}H_{39}N_3O_9$: C, 65.74; H, 5.98; N, 6.39. Found: C, 65,68; H, 6.08; N, 6.14.

*4.1.4. 1,3,5-Tris-(N-3,4-dibenzyloxyphenylpropionylaminomethyl)-2,4,6-triethylbenzene (**8**)*

To a solution of the OBn protected caffeic acid [10] (721 mg, 2 mmol), in DMF (20 mL), trisamine **3** [7] (100 mg, 0.4 mmol), HATU (760 mg, 2 mmol) and DIPEA (348 µL, 2 mmol) was added. The mixture was treated as described for **6** to afford a residue that was triturated with acetone/ether to yield 476 mg (93%) of **8** as a yellow solid; m.p. 208-210 ºC**.** ^{1}H-NMR (300 MHz, DMSO) *δ:* 1.11 (m, 9H, CH_3CH_2), 2.73 (m, 6H, CH_2CH_3), 4.43 (s, 6H, CH_2NH), 5.11 (s, 6H, CH_2Ar), 5.14 (s, 6H, CH_2Ar), 6.57 (d, *J* = 15.7 Hz, 3H, CH=CH), 7.05 (s, 3H, Ar), 7.21-7.43 (m, 45H, CH=CH, Ar), 7.94 (s, 3H, NH). Anal. Calcd for: $C_{84}H_{81}N_3O_9$: C, 79.03; H, 6.40; N, 3.29. Found: C, 78,91; H, 6.51; N, 3.53.

*4.1.5. 1,3,5-Tris-(N-3,4-dihydroxyphenylpropionylaminomethyl)-2,4,6-triethylbenzene (**9**)*

Following the general deprotection procedure, the OBn caffeoyl derivative **8** (476 mg, 0.37 mmol) gave a crude product which was then triturated with hexane to afford 143 mg (48%) of **9** as a white solid; m.p. 180-182 ºC. HRMS (ES+): *m/z* calculated for $C_{42}H_{25}N_3O_9^+$ $(M+H)^+$ 742.3704; found 742.3657. ^{1}H-NMR (300 MHz, CD_3OD) *δ:* 1.05 (m, 9H, CH_3CH_2), 2.41 (m, 6H,CH_2CH_2), 2.52 (m, 6H,CH_2CH_3), 2.76 (t, *J* = 7.2 Hz, 6H, CH_2CH_2), 4.31 (s, 6H, CH_2NH), 6.51 (m, 3H, Ar), 6.62 (m, 6H, Ar). ^{13}C-NMR (75 MHz, CD_3OD) *δ:* 16.85 (CH_3), 24.13 (CH_2), 32.78 (CH_2), 39.36 (CH_2), 39.42 (CH_2), 116.76 (CH), 117.05 (CH), 121.06 (C), 133.02 (C), 134.02 (C), 145.03 (C), 145.66 (C), 146.60 (C), 175.49 (C=O). HPLC [gradient: A:B, 10-100% of A in 10 min]: 5.64 min. Anal. Calcd for $C_{42}H_{51}N_3O_9$: C, 68.56; H, 6.16; N, 5.71. Found: C, 68,39; H, 6.39; N, 5.61.

*4.1.6. 1,3,5-Tris(2,4,6-tribenzyloxybenzamidomethyl)-2,4,6-triethylbenzene (**10**)*

To a solution of trisamine **3** [7] (50 mg, 0.2 mmol) in DMF (20 mL), 2,4,6-tribenzyloxybenzoic acid [11] (440 mg, 1 mmol), HATU (274 mg, 1 mmol) and DIPEA (126 µL, 1 mmol) were added. The mixture was stirred at room temperature overnight and then evaporated to dryness. The residue was dissolved in dichloromethane (50 mL) and washed successively with aqueous solutions of citric acid (10%) (3 × 20 mL), saturated $NaHCO_3$ (3 × 20 mL), and brine (3 × 20 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was purified on a Biotage HPFC system (High Performance Flash Chromatography) using hexane:ethyl acetate (1:1) as eluent to afford 184 mg (60%) of **10** as an amorphous solid. ^{1}H-NMR (300 MHz, $CDCl_3$) *δ:* 0.94 (t, *J* = 7.3 Hz, 9H, CH_3CH_2), 2.46 (q, *J* = 7.5 Hz, 6H, CH_2CH_3), 4.49 (s, 6H, CH_2NH), 4.91 (m, 12H, CH_2Ar), 4.95 (m, 6H, CH_2Ar), 6.13 (s, 6H, Ar), 7.23 (m, 30H, Ar), 7.34 (m, 15H, Ar).

4.1.7. *1,3,5-Tris(2,4,6-trihydroxybenzamidomethyl)-2,4,6-triethylbenzene (**11**)*

Following the general deprotection procedure, the OBn derivative **10** (184 mg, 0.12 mmol) gave a crude product which was then triturated with ether to afford 24 mg (32%) of **11** as a white solid; m.p. 301-303 ºC. MS (ES+): *m/z* 706.82 $(M+H)^+$.

^{1}H-NMR (300 MHz, DMSO-d_6) *δ:* 1.26 (m, 9H, CH_3CH_2), 2.88 (m, 6H,CH_2CH_3), 4.68 (m, 6H, CH_2NH), 5.49 (m, 3H, Ar), 5.80 (m, 3H, Ar), 8.55 (s, 3H, NHCO), 9.95 (s, 3H, *p*-OH), 12.55 (s, 3H, *o*-OH). ^{13}C-NMR (75 MHz, CD_3OD) *δ:* 17.07 (CH_3), 24.25 (CH_2), 38.78 (CH_2), 97.23 (CH), 134.05 (C), 145.90 (C), 163.40 (C), 163.84 (C), 171.86 (C=O). HPLC [gradient: A:B, 10-100% of A in 10 min]: 6.66 min. Anal. Calcd for $C_{36}H_{39}N_3O_{12}$: C, 61.27; H, 5.57; N, 5.95. Found: C, 61,11; H, 5.8; N, 5.86.

*4.1.8. 1,3,5-Tris-((3S,4R,5S)-3,4,5-trihydroxycyclohex-1-en-1-yl-carbonylaminomethyl)-2,4,6-triethylbenzene (**12**)*

To a solution of trisamine **3** [7] (50 mg, 0.2 mmol) in DMF (20 mL), shikimic acid (122 mg, 0.7 mmol), HATU (266 mg, 0.7 mmol) and DIPEA (105 µL, 0.6 mmol) were added. The mixture was stirred at room temperature overnight and then evaporated to dryness. The residue was dissolved in isobutanol (50 mL) and washed with brine (3 × 20 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was triturated with ether to yield 109 mg (76%) of **12** as a white solid; m.p. 242-244 ºC. MS (ES+): *m/z* 740.36 $(M+Na)^+$; *m/z* 718.43 $(M+H)^+$. ^{1}H-NMR (300 MHz, CD_3OD) *δ:* 1.17 (t, 9H, *J* = 7.4 Hz, CH_3CH_2), 2.15 (dd, 3H, *J* = 18.0 Hz, 6.0 Hz, CH_{2A}), 2.75 (m, 9H, CH_{2B} and CH_2CH_3), 3.62 (dd, 3H, *J* = 7.5 Hz, 4.2 Hz, *CH*OH), 3.96 (m, 3H, *CH*OH), 4,30 (m, 3H, *CH*OH) 4.51 (s, 6H, CH_2NH), 6.28 (m, 3H, CH=CH).^{13}C-NMR (75 MHz, CD_3OD) *δ:* 16.09 (CH_3), 23.38 (CH_2), 32.01 (CH_2), 39.10 (CH_2), 66.97 (CH), 68.19 (CH), 72.80 (CH),133.00 (CH), 133,19 (C), 134.68 (C), 146.07 (C), 170.96 (C=O). HPLC [gradient: A:B, 2-30% of A in 5 min]: 4.93 min. Anal Calcd for $C_{36}H_{51}N_3O_{12}$: C, 60.24; H, 7.16; N, 5.85. Found: C, 60,37; H, 6.87; N, 5.45.

*4.1.9. 1,3,5-Tris-((3S,4R,5S)-3,4,5-trihydroxycyclohexylcarbonylaminomethyl)-2,4,6-triethylbenzene (**13**)*

To a solution of the unsaturated derivative **12** (69 mg, 0.1 mmol) in THF/methanol (1:1) was added 30 wt% of Pd/C (10%) and the mixture was hydrogenated at 30 ºC and 2.9 atm (42 psi) during 1 h. The Pd/C was filtered through Whatman® filter paper 42, washed with methanol and the solvent was removed under reduced pressure. The residue was triturated with methanol/ether (1:1) to give 31 mg (45%) of **13** as a white solid; m.p. 285-287 ºC. HRMS (ES+): *m/z* calculated for $C_{36}H_{58}N_3O_{12}^+$ $(M+H)^+$ 724.4020; found 724.4002; *m/z* calculated for $C_{36}H_{57}N_3NaO_{12}^+$ $(M+Na)^+$ 746.3840; found 746.3805; *m/z* calculated for $C_{36}H_{57}KN_3O_{12}^+$ $(M+K)^+$ 762.3579; found 762.3511.724.4002 $(M+H)^+$; *m/z* 746.3805 $(M+Na)^+$; *m/z* 762.3511 $(M+K)^+$. ^{1}H-NMR (300 MHz, CD_3OD) *δ:* 1.20 (m, 9H, CH_3CH_2), 1.85-2.10 (m, 12H, CH_A, $CH_{B'}$, CH_B), 2.72-2.78 (m, 9H, CH_2CH_3, *CH*CH_2), 3.97 (m, 3H, *CH*OH), 4.16 (m, 3H, *CH*OH), 4.24 (m, 3H, *CH*OH), 4.50 (s, 6H, CH_2NH), 7.52 (s, 3H, NH). HPLC [gradient: A:B, 10-100% of A in 10 min]: 4.04 min. Anal. $C_{36}H_{57}N_3O_{12}$: C, 59.73; H, 7.94; N, 5.81. Hallado: C, 59,99; H, 7.88; N, 5.51

*4.1.10. N-(2-aminoethyl)-2,3,4-tris(benzyloxy)benzamide (**15**)*

To a solution of **14** [12] (750 mg, 1.70 mmol) in dichloromethane (8.5 mL), PyBOP (1.15 g, 2.21 mmol) and triethylamine were added (307 µl, 2.21 mmol). The reaction mixture was stirred at room temperature for 2 h and then a solution of ethylenediamine (433 µl, 6.38 mmol) in dichloromethane (5 mL) was added. The reaction mixture was stirred at room temperature for 3 h, diluted with dichloromethane (25 mL) and washed with brine (2×20 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness. The residue was purified by CCTLC using a gradient of dichloromethane:methanol (10:1) to dichloromethane:methanol:ammonia (10:1:0.2) as eluent to yield 482 mg (94%) of **15** as a yellow oil. MS (ES+): *m/z* 483 $(M+H)^+$. ^{1}H-NMR (400 MHz, $CDCl_3$) *δ:* 2.85 (br s, 2H, CH_2NH_2), 3.35 (m, 2H, CH_2NH),

5.06 (s, 2H, CH_2Ar), 5.15 (s, 2H, CH_2Ar), 5.16 (s, 2H, CH_2Ar), 6.87 (d, J = 9.0 Hz, 1H, Ar), 7.28-7.46 (m, 15H, Ar), 7.84 (d, J = 8.9 Hz, 1H, Ar), 8.22 (t, J = 5.4 Hz, 1H, NH). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 41.2 (CH_2), 42.2 (CH_2), 70.6 (CH_2), 75.4 (CH_2), 76.8 (CH_2), 108.8 (CH), 119.4 (C), 126.6 (CH), 127.3-126.6 (CH), 135.9 (C), 136.1 (C), 136.9 (C), 140.8 (C), 151.5 (C), 155.4 (C), 165.0 (C=O).

*4.1.11. N-(4-aminobutyl)-2,3,4-tris(benzyloxy)benzamide (**16**)*

In a procedure analogous to that described for compound **15**, a solution of **14** (198 mg, 0.45 mmol) in dichloromethane (6.7 mL) was treated with a solution of butane-1,4-diamine (270 µl, 2.67 mmol) in dichloromethane (2.2 mL) to yield 205 mg (89%) of **16** as a colorless oil. MS (ES+): *m/z* 512 (M+ H)$^+$. ^{1}H-NMR (300 MHz,$CDCl_3$) δ: 1.29 (m, 4H, CH_2CH_2), 1.92 (br s, 2H, NH_2), 2.63 (t, J = 6.4 Hz, 2H, CH_2NH_2), 3.25 (m, 2H, CH_2NH), 5.07 (s, 2H, CH_2Ar), 5.14 (s, 2H, CH_2Ar), 5.16 (s, 2H, CH_2Ar), 6.88 (d, J = 9.0 Hz, 1H, Ar), 7.31-7.45 (m, 15H, Ar), 7.90-7.94 (m, 2H, Ar, NH). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 26.6 (CH_2), 30.4 (CH_2), 39.3 (CH_2), 41.5 (CH_2), 70.8 (CH_2), 75.7 (CH_2), 77.2 (CH_2), 109.1 (CH), 119.5 (C), 126.8 (CH), 127.5, 128.2 (CH), 128.4 (CH), 128.6-128.7 (CH), 136.2 (C), 136.4 (C), 137.1 (C), 141.0 (C), 151.7 (C), 155.6 (C), 164.8 (C=O).

*4.1.12. Piperazin-1-yl-(2,3,4-tris(benzyloxy)phenyl)methanone (**17**)*

In a procedure analogous to that described for compound **15**, a solution of **14** (198 mg, 0.45 mmol) in dichloromethane (6.7 mL) was treated with a solution of piperazine (235 mg, 2.67 mmol) in dichloromethane (2.2 mL) to yield a residue that was purified by CCTLC using dichloromethane:methanol (20:1) as eluent to afford 225 mg (99%) of **17** as a yellow oil. MS (ES+): *m/z* 509 (M+H)$^+$. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.67 (m, 3H, CH_2CH_2), 2.86 (m, 1H, CH_2CH_2), 3.12 (m, 2H, CH_2CH_2), 3.72 (m, 2H, CH_2CH_2), 4.88 (d, J = 10.6 Hz, 1H, CH_2Ar), 5.04-5.20 (m, 5H, CH_2Ar), 6.81 (d, J = 8.5 Hz, 1H, Ar), 6.97 (d, J = 8.6 Hz, 1H, Ar), 7.24-7.48 (m, 15H, Ar). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 42.6 (CH_2), 45.4 (CH_2), 45.9 (CH_2), 47.9 (CH_2), 71.0 (CH_2), 75.2 (CH_2), 76.3 (CH_2), 109.8 (CH), 122.7 (C), 124.3 (CH), 127.4 (CH), 128.0-128.8 (CH), 136.5 (C), 137.1 (C), 137.3 (C), 141.2 (C), 149.5 (C), 154.0 (C), 167.4 (C=O).

*4.1.13. 1,3,5-Tris(2,3,4-tribenzyloxybenzamidoethylaminomethyl)-2,4,6-triethylbenzene (**19**)*

To a solution of the tris bromo derivative **18** [7] (50 mg, 0.11 mmol) in dichloromethane (1 mL) the aminoethyl derivative **15** (231 mg, 0.47 mmol) and Et_3N (65 µl, 0.47 mmol) were added. The reaction mixture was stirred at 30 ºC overnight, diluted with dichloromethane (15 mL) and washed with brine (3×15 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness. The residue was purified by CCTLC using dichloromethane:methanol/ammonia (15:1:0.4) as eluent to yield 106 mg (57%) of **19** as a colorless oil. MS (ES+): *m/z* 1647 (M+H)$^+$; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.14 (t, J = 7.2 Hz, 9H, CH_3CH_2), 2.63-2.69 (m, 12H, CH_2CH_3, CH_2CH_2), 3.36 (m, 6H, CH_2CH_2), 3.61 (s, 6H, CH_2NH), 5.04 (s, 6H, CH_2Ar), 5.09 (s, 6H, CH_2Ar), 5.14 (s, 6H, CH_2Ar), 6.86 (dd, J = 9.2, 0.5 Hz, 3H, Ar), 7.22-7.41 (m, 45H, Ar), 7.88 (dd, J = 8.9, 0.7 Hz, 3H, Ar), 8.01 (t, J = 5.2 Hz, 3H, NH). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 17.0 (CH_3), 22.6 (CH_2), 39.5 (CH_2), 47.5 (CH_2), 49.4 (CH_2), 70.8 (CH_2), 75.6 (CH_2), 109.1 (CH), 119.9 (C), 126.7 (CH), 127.5 (CH), 128.1-128.8 (CH), 134.0 (C), 136.2 (C), 137.1 (C), 141.1 (C), 142.1 (C), 151.7 (C), 155.6 (C), 164.9 (C=O).

*4.1.14. 1,3,5-Tris(2,3,4-trihydroxybenzamidoethylaminomethyl)-2,4,6-triethylbenzene (**20**)*

Following the general deprotection procedure, the OBn derivative **19** (119 mg, 0.23 mmol) gave 45 mg (96%) of **20** as a white solid; m.p. 164-166 ºC. MS (ES+): m/z 835 $(M+1)^+$. ^{1}H-NMR (400 MHz, CD_3OD) *δ*: 1.19 (t, *J* = 7.3 Hz, 9H, CH_3CH_2), 2.91 (q, *J* = 7.3 Hz, 6H, CH_2CH_3), 3.50 (m, 6H, CH_2CH_2), 3.81 (m, 6H, CH_2CH_2), 4.22 (s, 6H, CH_2NH), 6.39 (d, *J* = 8.8 Hz, 3H, Ar), 7.22 (d, *J* = 8.8 Hz, 3H, Ar). ^{13}C-NMR (100 MHz, CD_3OD) *δ*: 16.6 (CH_3), 25.7 (CH_2), 37.5 (CH_2), 43.3 (CH_2), 50.2 (CH_2), 108.2 (C), 108.4 (CH), 120.0 (CH), 128.9 (C), 134.1 (C), 149.4 (C), 151.4 (C), 150.7 (C), 173.2 (C=O). Anal. Calcd for $C_{42}H_{54}N_6O_{12}$: C, 60.42; H, 6.52; N, 10.07. Found: C, 60.21; H, 6.75; N, 9.94.

*4.1.15. 1,3,5-Tris(2,3,4-tribenzyloxybenzamidobutylaminomethyl)-2,4,6-triethylbenzene (**21**)*

To a solution of the tris bromo derivative **18** [7] (29 mg, 0.07 mmol) in dichloromethane (0.5 mL), the aminobutyl derivative **16** (133 mg, 0.26 mmol) and triethylamine were added (36 µl, 0.47 mmol). The reaction mixture was stirred at room temperature for 2 h, diluted with dichloromethane (15 mL) and washed with brine (3×15 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by CCTLC using dichloromethane:methanol (10:1) as eluent to yield 51 mg (46%) of **21** as a yellow oil. MS (ES+): *m/z* 866 $(1/2M+1)^+$; ^{1}H-NMR (400 MHz, $CDCl_3$) *δ*: 1.15 (t, *J* = 6.2 Hz, 9H, CH_3CH_2), 1.37 (m, 6H, CH_2), 1.64 (m, 6H, CH_2), 2.88 (m, CH_2CH_3, CH_2NH), 3.20 (m, 6H, CH_2NH), 3.93 (br s, 6H, CH_2NH), 5.05 (s, 6H, CH_2Ar), 5.12 (s, 6H, CH_2Ar), 5.14 (s, 6H, CH_2Ar), 6.86 (d, *J* = 7.8 Hz, 3H, Ar), 7.26-7.42 (m, 45H, Ar), 7.86 (d, *J* = 7.8 Hz, 3H, Ar), 7.94 (m, 3H, NH). ^{13}C-NMR (75 MHz, $CDCl_3$) *δ*: 17.1 (CH_3), 24.2 (CH_2), 25.0 (CH_2), 27.2 (CH_2), 39.1 (CH_2), 46.63 (CH_2), 49.5 (CH_2), 71.09 (CH_2), 75.91 (CH_2), 77.59 (CH_2), 109.1 (CH), 119.7 (CH), 127.8 (CH), 128.4 (CH) 128.6 (CH), 128.8 (CH), 128.8 (CH), 128.9 (CH), 129.0 (CH), 129.1 (CH), 136.4 (C), 137.3 (C), 141.3 (C), 152.0 (C), 156.0 (C), 165.3 (C=O).

*4.1.16. 1,3,5-Tris(2,3,4-trihydroxybenzamidobutylaminomethyl)-2,4,6-triethylbenzene (**22**)*

Following the general deprotection procedure, the OBn derivative **21** (50 mg, 0.023 mmol) gave a crude product which was then purified on a Biotage HPFC system (High Performance Flash Chromatography) on reverse phase using water:acetonitrile (100:0 to 70:30) as eluent to afford 7 mg (27%) of **22** as a white amorphous solid. MS (ES+): m/z 920 $(M+H)^+$. ^{1}H-NMR (300 MH, CD_3OD) *δ:* 1.18 (t, *J* = 7.5 Hz, 9H, CH_3CH_2), 1.72 (m, 6H, CH_2CH_2), 1.85 (m, 6H, CH_2CH_2), 2.87 (q, *J* = 7.3 Hz, 6H, CH_2CH_3), 3.32 (m, 6H, CH_2NH), 3.40 (m, 6H, CH_2NH), 3.48 (m, 6H, $NHCH_2CH_2$), 4.32 (s, 6H, CH_2NH), 6.35 (d, *J* = 8.8 Hz, 3H, Ar), 7.09 (d, *J* = 8.8 Hz, 3H, Ar). ^{13}C-NMR (100 MHz, CD_3OD) *δ:* 16.3 (CH_3), 24.1 (CH_2), 25.3 (CH_2), 27.7 (CH_2), 38.9 (CH_2), 45.9 (CH_2), 108.0 (C), 108.6 (CH), 119.1 (CH), 128.8 (C), 134.0 (C), 149.3 (C), 150.9 (C), 151.4 (C), 172.2 (C=O). Anal. Calcd for $C_{48}H_{66}N_6O_{12}$: C, 66.73; H, 7.24; N, 9.14. Found: C, 66.97; H, 7.16; N, 9.08.

*4.1.17. 1,3,5-Tris[(4-(2,3,4-tribenzyloxyphenylcarbonyl)piperazin-1-yl)methyl]-2,4,6-triethylbenzene (**23**)*

To a solution of the tris bromo derivative **18** [7] (43 mg, 0.10 mmol) in dichloromethane (1 mL), the piperazinyl derivative **17** (198 mg, 0.39 mmol) and Et_3N were added (54 µl, 0.39 mmol). The reaction mixture was stirred at room temperature overnight, diluted with dichloromethane (15 mL) and washed with brine (3×15 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by CCTLC using dichloromethane:methanol (30:1) to yield 127 mg (75%) of **23** as a yellow oil. MS (ES+): *m/z* 1725 $(M + 1)^+$, 863 $(1/2M + 1)^+$; ^{1}H-RMN (400 MHz, Acetone-d_6) *δ:* 1.09 (t, *J* = 6.9 Hz, 9H, CH_3CH_2), 2.27 (m, 6H, CH_2CH_3), 2.33 (m, 3H, CH_2CH_2), 2.46 (m, 3H, CH_2CH_2),

2.90 (m, 6H, CH_2CH_2), 3.01 (m, 6H, CH_2CH_2), 3.47 (m, 9H, CH_2CH_2, CH_2NH), 3.70 (m, 3H, CH_2CH_2), 4.90 (m, 3H, CH_2Ar), 5.08-5.19 (m 15H, CH_2Ar), 6.88-6.93 (m, 6H, Ar), 7.20-7.24 (m, 9H, H-Ar), 7.35-7.41 (m, 30H, Ar), 7.53-7.55 (m, 6H, Ar). ^{13}C-NMR (100 MHz, Acetone-d_6) δ: 17.0 (CH_3), 22.3 (CH_2), 42.4 (CH_2), 47.8 (CH_2), 52.9 (CH_2), 53.2 (CH_2), 55.7 (CH_2), 71.5 (CH_2), 75.5 (CH_2), 76.5 (CH_2), 110.5 (CH), 123.2 (C), 125.9 (CH), 128.6-129.6 (CH), 131.9 (C), 138.0 (C), 138.4 (C), 138.6 (C), 141.9 (C), 145.4 (C), 150.3 (C), 154.6 (C), 167.2 (C=O).

*4.1.18. 1,3,5-Tris[(4-(2,3,4-trihydroxyphenylcarbonyl)piperazin-1-yl)methyl]-2,4,6-triethylbenzene (**24**)*

Following the deprotection procedure, the OBn derivative **23** (122 mg, 0.07 mmol) gave a crude product which was then purified on a Biotage HPFC system (High Performance Flash Chromatography) on reverse phase using water:acetonitrile (100:0 to 80:20) as eluent to afford 32 mg (50%) of **24** as a white solid; m.p >350 ºC. MS (ES+): m/z 914 $(M + 1)^+$. ^{1}H-NMR (400 MHz, Acetone-d$_6$) δ 1.10 (t, J = 7.2 Hz, 9H, CH_3CH_2), 3.17 (m, 6H, CH_2CH_3), 3.45 (br s, 12H, CH_2CH_2), 3.88 (br s, 12H, CH_2CH_2), 4.57 (s, 6H, CH_2NH), 6.41 (d, J = 8.5 Hz, 3H, Ar), 6.70 (d, J = 8.5 Hz, 3H, Ar). ^{13}C-NMR (100 MHz, Acetone-d$_6$) δ: 16.4 (CH_3), 24.7 (CH_2), 52.7 (CH_2), 54.9 (CH_2), 99.87 (CH), 107.5 (C), 112.5 (C), 120.2 (CH), 133.5 (C), 146.2 (C), 148.7 (C), 170.3 (C=O). Anal. Calcd for $C_{48}H_{60}N_6O_{12}$: C, 63.14; H, 6.62; N, 9.20. Found: C, 63.37; H, 6.60; N, 9.48.

*4.1.19. 1,3,5-Tris(6-aminopyridinyl-3-carbonylaminomethyl)-2,4,6-triethylbenzene (**25**)*

To a solution of 6-aminoniconitic acid (111 mg, 0.8 mmol) in DMF (20 mL), PyBOP (424 mg, 0.8 mmol) was added. After 5 minutes **3** [7] (50 mg, 0.2 mmol) and triethylamine (102 µL, 0.8 mmol) were added. The reaction mixture was stirred at room temperature for 2 h, and volatiles were removed. The residue was dissolved in isobutanol (20 mL) and washed with brine (3×20 mL). The organic phase was dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was triturated with ether and methanol to afford 99.94 mg (82%) of **25** as a white solid; m.p. 267-269 ºC. HRMS (ES+): *m/z* calculated for $C_{33}H_{39}N_9O_3$ 609.3176; found 609.3176; ^{1}H-NMR (300 MHz, DMSO) δ: 1.09 (m, 9H, CH_3CH_2), 2.81 (m, 6H, CH_2CH_3), 4.50 (s, 6H, CH_2NH), 6.41 (m, 3H, Ar), 7.84 (m, 3H, Ar), 8,07 (br s, 6H, NH_2-Ar), 8,40 (s, 3H, Ar).^{13}C-NMR (75 MHz, DMSO) δ: 15.90 (CH_3), 22.36 (CH_2), 38.35 (CH_2), 106.21 (CH), 117.52 (C), 131.81 (C), 136.01 (C), 143.22 (CH), 148.32 (CH),161.00 (C),164.71 (C=O). HPLC [gradient: A:B, 10-100% de A in 10 min]: 4.39 min.

*4.1.20. 1,3,5-Tris(4-aminobenzamidomethyl)-2,4,6-triethylbenzene (**26**)*

In a procedure analogous to that described for compound **25**, a solution of **3** [7], (50 mg, 0.2 mmol), 4-aminobenzoic acid (110 mg, 0.8 mmol), PyBOP (424 mg, 0.8 mmol) and Et_3N (102 µL, 0.8 mmol) in DMF (20 mL), gave a residue that was dissolved in isobutanol (20 mL) and washed with brine (3×20 mL). The organic phase was dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by trituration with ether and methanol to afford 25 mg (21%) of **26** as a white solid; m.p. >350 ºC. HRMS (ES+): *m/z* calculated for $C_{36}H_{42}N_6O_3$ 606.3318; found 606.3330; ^{1}H-RMN (300 MHz, DMSO-d_6) δ: 1.10 (t, J = 7.4 Hz, 9H, CH_3CH_2), 2.77 (m, 6H, CH_2CH_3), 4.49 (s, 6H, CH_2NH), 5.23 (br s, 6H, NH_2), 6.48 (d, J = 8.7 Hz, 6H, Ar), 7.38 (br s, 3H, NH), 7.53 (d, J = 8.7 Hz, 6H, Ar). ^{13}C-RMN (75 MHz, DMSO-d_6) δ: 16.91 (CH_3), 23.34 (CH_2), 38.45 (CH_2), 113.18 (CH), 121.80 (CH), 129.49 (C), 132.75 (C), 144.11 (C), 151.99 (C), 166.97 (C=O). HPLC [gradient: A:B, 10-100% of A in 10 min]: 5.51 min.

*4.1.21. 1-Aminomethyl-3,5-bis(2,3,4-tribenzyloxybenzamidomethyl)-2,4,6-triethylbenzene (**28**)*

To a solution of 2,3,4-tribenzyloxybenzoic acid **14** (200 mg, 0.45 mmol) [12] in dichloromethane (3 mL) PyBOP (263 mg, 0.50 mmol) and Et_3N (70 µL, 0.50 mmol) were added. The mixture was stirred at room temperature for 1 h, and then added dropwise to a suspension of trisamine **3** (63 mg, 0.25 mmol) [7] in dichloromethane (1 mL). The mixture was stirred at room temperature for 2 h. Dichloromethane (50 mL) was added and the reaction mixture was washed with brine (20 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness. The residue was purified by CCTLC using dichloromethane/methanol (20:1) as eluent to afford 114 mg (41%) of **28** as a pale yellow oil. MS (ES+): *m/z:* 1094 $(M+H)^+$; ^{1}H-NMR (300 MHz, $CDCl_3$) *δ:* 0.96-1.28 (m, 9H, $\mathit{CH_3}CH_2$), 2.44 (m, 2H, $CH_3\mathit{CH_2}$), 2.66 (m, 4H, $CH_3\mathit{CH_2}$), 3.93 (m, 2H, $\mathit{CH_2}NH_2$), 4.52-4.60 (m, 4H, $\mathit{CH_2}NH$), 4.83-4.94 (m, 8H, CH_2Ar), 5.14 (m, 4H, CH_2Ar), 6.78-7.40 (m, 32, Ar), 7.85 (m, 2H, NH), 7.94 (m, 2H, Ar).

*4.1.22. Cis-N^1,N^3,N^5-tris[3,5-bis(2,3,4-tribenzyloxybenzamidomethyl)-1-methyl-2,4,6-triethylphenyl]-1,3,5-cyclohexanetricarboxamide (**30**)*

To a solution of *cis*-1,3,5-cyclohexanetricarboxylic acid **29** (10.1 mg, 0.05 mmol) in dichloromethane (0,5 mL), PyBOP (78 mg, 0.15 mmol) was added. After 5 minutes **28** (137 mg, 0.15 mmol), and triethylamine (33 µL, 0.24 mmol) were added. The reaction mixture was stirred at room temperature for 2 h. Dichloromethane (50 mL) was added and the reaction mixture was washed with brine (20 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness. The residue was purified by CCTLC using dichloromethane/methanol (20:1) as eluent to afford 69 mg (92%) of **30** as a colorless oil. ^{1}H-RMN (400 MHz, $CDCl_3$) *δ:* 1.08 (m, 27H, $\mathit{CH_3}CH_2$), 1.34 (m, 3H, $\mathit{CH_2}CH$), 1.72 (m, 3H, $\mathit{CH_2}CH$), 2.49-2.58 (m, 18H, $CH_3\mathit{CH_2}$), 3.15 (m, 3H, $\mathit{CH}CH_2$), 4.30 (br s, 6H, $\mathit{CH_2}NH$), 4.62 (br s, 12H, $\mathit{CH_2}NH$), 4.89-4.92 (m, 24H, $\mathit{CH_2}Ar$), 5.15 (s, 12H, $\mathit{CH_2}Ar$), 6.81-7.52 (m, 96H, Ar), 7.89 (m, 6H, NH), 7.97 (m, 6H, Ar). ^{13}C-RMN (100 MHz, $CDCl_3$) *δ:* 16.3 (CH_3), 16.4 (CH_3), 22.9 (CH_2), 37.8 (CH_2), 38.3 (CH_2), 43.5 (CH_2), 69.7 (CH), 70.8 (CH_2), 75.5 (CH_2), 76.4 (CH_2),109.3 (CH), 119.4 (C), 126.8 (CH), 127.5 (CH), 128.0-128.5 (CH), 131.3 (C), 132.6 (C), 135.9 (C), 136.0 (C), 136.8 (C), 141.0 (C), 143.8 (C), 144.3 (C), 151.4 (C), 155.8 (C), 164.4 (C=O), 173.0 (C=O).

*4.1.23. Cis-N^1,N^3,N^5-tris[3,5-bis(2,3,4-trihydroxybenzamidomethyl)-1-methyl-2,4,6-triethylphenyl]-1,3,5-cyclohexanetricarboxamide (**31**).*

Following the deprotection procedure, the OBn derivative **30** (147 mg, 0.042 mmol) gave a residue that was triturated with dichloromethane/methanol to give 43.4 mg (57 %) of **31** as a brownish amorphous solid. HRMS (ES+): m/z calculated for $C_{96}H_{111}N_9O_{27}$ 1821.7589; found 1821.7681. ^{1}H-NMR (400 MHz, CD_3OD) *δ:* 1.14 (t, 18H, J = 7.5 Hz, $\mathit{CH_3}CH_2$), 1.19 (t, 9H, J = 7.5 Hz, $\mathit{CH_3}CH_2$), 1.59-1.73 (m, 3H, $\mathit{CH_2}CH$), 1.76-1.85 (m, 3H, $\mathit{CH_2}CH$), 2.21-2.30 (m, 3H, $\mathit{CH}CH_2$), 2.76 (q, 12H, J = 7.5 Hz, $CH_2\mathit{CH_3}$), 2.88 (q, 6H, J = 7.5 Hz, $CH_2\mathit{CH_3}$), 4.40 (s, 6H, $\mathit{CH_2}NH$), 4.64 (s, 12H, $\mathit{CH_2}NH$), 6.34 (d, 6H, J = 9.8 Hz, Ar), 7.19 (d, 6H, J = 9.8 Hz, Ar). ^{13}C-NMR (100 MHz, CD_3OD) *δ:* 15.44 (CH_3), 15.53 (CH_3), 22.67 (CH_2), 22.89 (CH_2), 31.42 (CH_2), 37.87 (CH_2), 43.36 (CH), 107.95 (CH), 109.52 (C), 120.22 (CH), 133.08 (C), 133.94 (C), 145.63 (C), 150.63 (C), 150.78 (C), 170.73 (C=O), 177.04 (C=O). HPLC [gradient: A:B, 10-100% of A in 10 min]: 7.29 min.

*4.1.24. 1-N-(Fmoc-18-amino-4-oxo-9,12,15-trioxa-5-aminooctadecanoil)aminomethyl-3,5-bis(2,3,4-tribenzyloxybenzamidomethyl)-2,4,6-triethylbenzene (**33**)*

To a solution of Fmoc-18-amino-4-oxo-9,12,15-trioxa-5-aminooctadecanoic acid (**32**) (232 mg, 0.43 mmol) [12] in dichloromethane (2 mL), PyBOP (222 mg, 0.43 mmol) was added. After 5 minutes **28** (233 mg, 0.21 mmol) and triethylamine Et_3N (60 µL, 0.43 mmol) were added. The reaction mixture was stirred at room temperature for 1 h, diluted with dichloromethane (15 mL) and washed with brine (3 x 15 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness. The residue was purified by CCTLC using dichloromethane/methanol, (20:1) as eluent to afford 326 mg (95%) of **33** as a pale yellow amorphous solid. HRMS (ES+): *m/z:* 1620 $(M+H)^+$. ^{1}H-RMN (400 MHz, $CDCl_3$) *δ:* 1.05 (t, *J* = 7.1 Hz, 3H, CH_3CH_2), 1.13 (t, *J* = 7.2 Hz, 6H, CH_3CH_2), 1.67-1.78 (m, 4H, CH_2CH_2), 2.23 (m, 2H, CH_2CH_2), 2.38-2.50 (m, 4H, CH_3CH_2, CH_2CH_2), 2.66 (m, 4H, CH_3CH_2), 3.35 (m, 4H, CH_2CH_2), 3.48-3.63 (m, 12H, CH_2CH_2), 4.21 (m, 2H, CH_2NH), 4.32-4.41 (m, 3H, CH_2CH, $CHCH_2$), 4.60 (m, 4H, CH_2NH), 4.88 (s, 4H, CH_2Ar), 4.91 (s, 4H, CH_2Ar), 5.14 (s, 4H, CH_2Ar), 6.89 (m, 6H, Ar), 7.05 (m, 4H, Ar), 7.14-7.41 (m, 34H, Ar), 7.59 (m, 2H, Ar), 7.75 (m, 2H, Ar), 7.89 (m, 2H, NH), 7.96 (d, *J* = 9 Hz, 2H, H-Ar). ^{13}C-NMR (100 MHz, $CDCl_3$) *δ:* 16.6 (CH_3), 23.1 (CH_2), 24.6 (CH_2), 26.5 (CH_2), 26.6 (CH_2), 29.0 (CH_2), 29.6 (CH_2), 31.5 (CH_2), 38.1 (CH_2), 46.7 (CH_2), 46.8 (CH_2), 47.5 (CH_2), 66.6 (CH_2), 7034 (CH_2), 70.7 (CH_2), 71.0 (CH_2), 75.8 (CH_2), 76.1 (CH_2), 109.5 (CH), 120.2 (CH), 125.3 (CH), 127.1 (CH), 127.2 (CH), 127.8 (CH), 127.9 (CH), 128.5 (CH), 128.8 (C), 136.1 (C), 144.2 (C), 151.7 (C), 156.1 (C=O), 164.7 (C=O), 190.7 (C=O).

*4.1.25. 1-N-(18-amino-4-oxo-9,12,15-trioxa-5-aminooctadecanoyl)aminomethyl-3,5-bis(2,3,4-tribenzyloxybenzamidomethyl)-2,4,6-triethylbenzene (**34**)*

A solution of **33** (325 mg, 0.20 mmol) in dichloromethane (2 mL) was treated with piperidine (0.5 mL). The reaction mixture was stirred at room temperature for 1 h, and the volatiles were removed. The residue was purified by CCTLC using dichloromethane/methanol, (10:1) as eluent to afford 217 mg (77%) of **34** as a pale yellow amorphous solid. MS (ES+): *m/z:* 1397 $(M+H)^+$; ^{1}H-NMR (400 MHz, DMSO-d_6) *δ:* 0.92-1.03 (m, 9H, CH_3CH_2), 1.57 (m, 2H, CH_2CH_2), 1.77 (m, 2H, CH_2CH_2), 2.25 (m, 4H, CH_2CH_2), 2.50-2.59 (m, 2H, CH_2NH_2), 2.81 (m, 2H, CH_3CH_2), 3.03 (m, 4H, CH_3CH_2), 3.37-3.52 (m, 14H, CH_2CH_2), 4.21 (m, 2H, CH_2NH), 4.45 (m, 4H, CH_2NH), 4.89 (s, 4H, CH_2Ar), 4.91 (s, 4H, CH_2Ar), 5.19 (s, 4H, CH_2Ar), 7.03-7.57 (m, 34H, Ar), 7.89 (m, 4H, NH).

*4.1.26. 1-N-(18-amino-4-oxo-9,12,15-trioxa-5-aminooctadecanoyl)aminomethyl-3,5-bis(2,3,4-trihydroxybenzamidomethyl)-2,4,6-triethylbenzene (**35**)*

Following the deprotection procedure, the OBn derivative **34** afforded a residue that was triturated with methanol/ether to give 70 mg (89%) of **35** as a pale yellow amorphous solid. MS (ES+): *m/z* 856 $(M+H)^+$. ^{1}H-NMR (400 MHz, CD_3OD) *δ:* 1.18-1.23 (m, 9H, CH_3CH_2), 1.71 (m, 2H, CH_2CH_2), 1.91 (m, 2H, CH_2CH_2), 2.47 (s, 4H, CH_2CH_2), 2.81 (q, *J* = 7.6 Hz , 4H, CH_2CH_3), 2.90 (q, *J* = 7.4 Hz, 2H, CH_2CH_3), 3.08 (t, *J* = 6.4 Hz, 2H, CH_2CH_2), 3.20 (m, *J* = 7.0 Hz, 2H, CH_2CH_2), 3.46 (t, *J* = 6.1 Hz, 2H, CH_2CH_2), 3.55 (m, 2H, CH_2CH_2), 3.60-3.65 (m, 8H, CH_2CH_2), 4.45 (m, 2H, CH_2NH), 4.66 (s, 4H, CH_2NH), 6.34 (d, *J* = 8.8 Hz, 2H, Ar), 7.21 (d, *J* = 8.8 Hz, 2H, Ar). ^{13}C-NMR (100 MHz, CD_3OD) *δ:* 16.6 (CH_3), 16.7 (CH_3), 23.9 (CH_2), 24.1 (CH_2), 28.0 (CH_2), 30.4 (CH_2), 32.0 (CH_2), 32.2 (CH_2), 37.8 (CH_2), 38.9 (CH_2), 39.0 (CH_2), 40.2 (CH_2), 69.7 (CH_2), 70.4 (CH_2), 71.0 (CH_2), 71.3 (CH_2), 108.0 (C), 109.5 (CH), 120.2 (CH), 133.0 (C), 133.9 (C), 145.6 (C), 145.7 (C), 150.6 (C), 150.8 (C), 170.7 (C=O), 174.2 (C=O), 174.6 (C=O).

Supporting information

General chemistry and biological procedures as well as copies of representative ^{1}H and ^{13}C NMR spectra are included.

Acknowledgments

The Spanish MICINN/MINECO (Project: SAF 2012-39760-C02-01, co-financed by the FEDER programme); Plan Nacional de Cooperación Público-Privada. Subprograma INNPACTO (IPT-2012-0213-060000, co-financed by the FEDER programme) and the Comunidad de Madrid (BIPEDD2-CM-S2010/BMD-2457) are acknowledged for financial support. The Spanish MICINN /MINECCO is also acknowledged for a grant to E. Rivero-Buceta. We thank Leentje Persoons, Frieda De Meyer, Leen Ingels, Stijn Delmotte, Katrien Geerts, and Inge Vliegen for excellent technical assistance. Financial support of KU Leuven (GOA 10/14; PF 10/18) and the FWO (G-0528.12N) was provided for the antiviral experiments.

References

[1] R.J. Pomerantz and D.L. Horn, Twenty years of therapy for HIV-1 infection., *Nat. Med.,* **9**, 7, 2003, 867-873.

[2] P.A. Volberding and S. G. Deeks, Antiretroviral therapy and management of HIV infection., *The Lancet*, **376**, 9734, 2010, 49-62.

[3] C. Flexner, HIV drug development: the next 25 years., *Nat. Rev. Drug. Discov.,* **6**, 12, 2007, 959-966.

[4] (a) S. Olofsson and J.E. Hansen, Host cell glycosylation of viral glycoproteins-a battlefield for host defence and viral resistance., *Scan. J. Infect. Dis.,* **30**, 1998, 435-440. (b) B. Losman, A. Bolmstedt, K. Schonning, A. Bjorndal, C. Westin, E.M. Fenyo and S. Oloffson. Protection of neutralization epitopes in the V3 loop of oligomeric human immunodeficiency virus type 1 glycoprotein 120 by N-linked oligosaccharides in the V1 region., *AIDS Res. Hum.Retrov.* **17**, 2001, 1067-1076.

[5] J. Balzarini, Targeting the glycans of gp120: A novel approach aimed at the Achilles heel of HIV., *Lancet Infect. Dis*., **5**, 2005, 726-731.

[6] P. Carrero, A. Ardá, M. Álvarez, E.G. Doyagüez, E. Rivero-Buceta, E. Quesada, A. Prieto, D. Solís, M.J. Camarasa, M.J. Pérez-Pérez, J. Jiménez-Barbero and A. San-Félix, Differential recognition of mannose-based polysaccharides by tripodal receptors based on a triethylbenzene scaffold substituted with trihydroxybenzoyl moieties., *Eur. J. Org. Chem.*, 2013, 65-76.

[7] (a) C. Walsdorff, W. Saak and S. Pohl, Synthesis of 1,3,5-tris(bromomethyl)-2,4,6-triethylbenzene – a versatile precursor to predisposed ligands., *J. Chem. Res* (M), 1996, 1601-1609. (b) A. Metzger, V. Lynch and E. Anslyn, Ein synthetischer Citrat-selektiver Rezeptor., *Angew. Chem.,* **109**, 1997, 911-914. (c) A. Metzger, V. Lynch and E. Anslyn, Synthetic Receptor Selective for Citrate., *Angew. Chem Ed. Engl.,* **36**, 1997, 862-864. (d) K. J. Wallace, J. Robert, E. Anslyn, J. Morey, K. V. Kilway and J. Siegel, Preparation of 1,3,5-Tris(aminomethyl)-2,4,6-triethylbenzene from Two Versatile 1,3,5-Tri(halosubstituted) 2,4,6-Triethylbenzene Derivatives., *Synthesis*, **12**, 2005, 2080-2083.

[8] (a) T. Stack, Z. Hou, and K.N. Raymond, Rational reduction of the conformational space of a siderophore analog through nonbonded interactions: the role of entropy in enterobactin., *J. Am. Chem. Soc.*, **115**, 1993, 6466-6467. (b) Z. Hou,

T.D.P. Stack, C.J. Sunderland and K.N. Raymond, Enhanced iron(III) chelation through ligand predisposition: syntheses, structures and stability of tris-catecholate enterobactin analogs., *Inorg. Chim. Acta*, **263**, 1997, 341-355.

[9] (a) O. Benavente-García, J. Castillo, J. Lorente, A. Ortuño and J.A. del Río, Antioxidant activity of phenolics extracted from Olea europaea L. leaves., *Food Chemistry*, **68**, 2000, 457-462. (b) M.H. Gordon, F. Paiva-Martins and M. Almeida, Antioxidant activity of hydroxytyrosol acetate compared with that of other olive oil polyphenols., *J. Agric. Food, Chem.,* **49**, 2001, 2480-2485. (c) H. Chimi, J. Cilalrd and M. Rahmani, Peroxyl and hydroxyl radical scavenging activity of some natural phenolic antioxidants. *J. Am. Oil Chem. Soc.* 68, 1991, 307-312. (d) A. Saija, D. Trambetta, A. Tomaino, R. Lo Cascio, P. Princi, N. Uccella, F. Bonina and F. Castelli, '*In vitro'* evaluation of the antioxidant activity and biomembrane interaction of the plant phenols oleuropein and hydroxytyrosol. *International J. of Pharmaceutics*, **166**, 2, 1998, 123-133. (e) J.G. Fernández-Bolaños, O. López, M.A. López-García and A. Marset, Biological Properties of Hydroxytyrosol and Its Derivatives. *www.intechopen.com/download/pdf/27043*.

[10] J. Kavitha, G. Rajasekhar, G.V. Subbaraju and G.N. Ramesh, Synthesis of vinyl caffeate, an antioxidant from Perilla frutescens Britton var. crispa (Thunb.). *Indian J. Chem*., 38B, 1999, 1280-1281.

[11] (a) E.G. Sundholm, Synthesis of 2,2',4,4',6,6'-Pentahydroxy-6-methyl benzophenone. *Acta Chemica Scandinavica series B*, **28**, 1974, 1102-1103. (b) E.G. Sundholm, Isomerisation of benzophenones and direct observation of intermediates in the aromatic acylation of phloroglucinol derivatives with trifluoroacetic anhydride. *Tetrahedron* **33** (9), 1977, 991–994.

[12] N. Takashi, K. Kumiko, A. Akiko, M. Kenji and T. Koichiro, JP8143525 (A), 1996-06-04.

[13] a) H. Cavga and M. Mazik, Molecular Recognition of N-Acetylneuraminic Acid with Acyclic Benzimidazolium-and Aminopyridine/guanidinium-Based Receptors., *J. Org. Chem.,* **72**, 2007, 831-838. b) A. Buthe, and M. Mazik, Highly effective receptors showing di- vs.monosaccharide preference. *Org. Biomol. Chem.,* **6**, 2008, 1558-1568. c) M. Kuschel and M. Mazik, Amide, Amino, Hydroxy and Aminopyridine Groups as Building Blocks for Carbohydrate Receptors., *Eur. J. Org. Chem.,* **9**, 2008, 1517-1526. d) M. Kuschel and M. Mazik, Highly Effective Acyclic Carbohydrate Receptors Consisting of Aminopyridine, Imidazole, and Indole Recognition Units. *Chem. Eur. J.,* **14**, 2008, 2405-2419. e) A. Buthe and M. Mazik, Recognition properties of receptors based on dimesitylmethane-derived core: Di- vs.monosaccharide preference., *Org. Biomol. Chem.,* **7,** 2009, 2063-2071.

[14] G.V. Zyryanov, M.A. Palacios and P. Anzenbacher, Rational Design of a Fluorescence-Turn-On Sensor Array for Phosphates in Blood Serum., *Angew. Chem. Int. Ed.* **46**, 2007, 7849–7852.

[15] (a) Z.G. Zhao, J.S. Im, K.S. Lam and D.F. Lake, Site-Specific Modification of a Single-Chain Antibody Using a Novel Glyoxylyl-Based Labeling Reagent. *Bioconjugate Chem.,***10**, 1999, 424-430. (b) J.J. Reina, O. S. Maldonado, G. Tabarini, F. Fieschi and J. Rojo, Mannose Glycoconjugates Functionalized at Positions 1 and 6. Binding Analysis to DC-SIGN Using Biosensors. *Bioconjugate Chem.* **18**, 2007, 963-969.

[16] (a) S.Yu and G. Zhao, Development of polyphenols as HIV-1 integrase inhibitors: a summary and perspective, *Curr. Med. Chem.*, **32**, 2012, 5536-5561. (b) C.O.R. Junior, S.C. Verde, C.A.M. Rezende, W. Caneschi, M.R. C. Couri, B.R. McDougall, W.E. Robinson and M.V. de Almeida, Synthesis and HIV-1 inhibitory activities of dicaffeoyl and digalloyl esters of quinic acid derivatives, *Curr. Med. Chem.*, **20**, 2013, 724-733. (c) W.E. Robinson Jr, M. Cordeiro, S. Abbel-Malek, Q. Jia, S.A. Chow, M.G. Reinecke and W.M. Mitchell, Dicaffeoylquinic acid inhibitors of human

immunodeficiency virus integrase: inhibition of the core catalytic domain of human immunodeficiency virus integrase, *Mol. Pharmacol.* **50**, 1996, 846-855.

[17] B.A. McKittrick, R.T. Scannell and R. Stevenson, Natural Benzofurans: Synthesis of the Arylbenzofuran Constituents of Sophora tomentosa., *J. Chem. Soc., Perkin Trans. I,* 1982, 3017-3020.

LIST OF CAPTIONS

Fig. 1. Structure of **1** and **2**

Scheme 1. Synthesis of compounds **5, 7** and **9.** *Reagent and conditions:* (i) pyBOP, Et_3N (ii) BBr_3, CH_2Cl_2 (iii) HATU, DIPEA (iv) H_2, Pd/C

Scheme 2. Synthesis of compound **11.** *Reagent and conditions:* (i) HATU, DIPEA (ii) H_2, Pd/C

Scheme 3. Synthesis of compounds **12** and **13.** *Reagent and conditions:* (i) HATU, DIPEA (ii) H_2, Pd/C

Scheme 4. Synthesis of compounds **15-17.** *Reagent and conditions:* (i) pyBOP, Et_3N

Scheme 5. Synthesis of compounds **20, 22** and **24.** *Reagent and conditions:* (i) Et_3N (ii) H_2, Pd/C

Scheme 6. Synthesis of compounds **25-27.** *Reagent and conditions:* (i) pyBOP, Et_3N

Scheme 7. Synthesis of compound **31.** *Reagent and conditions:* (i) pyBOP, Et_3N (ii) H_2, Pd/C

Scheme 8. Synthesis of compound **35.** *Reagent and conditions:* (i) pyBOP, Et_3N (ii) piperidine, CH_2Cl_2 (iii) H_2, Pd/C

Fig. 2. “Direct” SPR experiment

Fig. 3. “Reverse” SPR experiment

Fig. 4. “Direct” SPR experiment in the presence of mannose trimers

Table 1. Inhibitory effects of test compounds on HIV-1 and HIV-2 replication in CEM cell culture