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A new structural theme in C_2-symmetric HIV-1 protease inhibitors: *ortho*-Substituted P1/P1′ side chains

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Abstract—In this report, the rapid syntheses of 24 novel C_2-symmetric HIV-1 protease inhibitors are described. Two *ortho*-iodobenzyloxy containing C-terminal duplicated inhibitors served as starting materials for microwave-enhanced palladium(0)-catalyzed carbon–carbon bond forming reactions (Suzuki, Sonogashira, Heck, and Negishi). Highly potent inhibitors equipped with *ortho*-functionalized P1/P1′ side chains as the structural theme were identified. Computational efforts were applied to study the binding mode of this class of inhibitors and to establish structure–activity relationships. The overall orientation of the inhibitors in the active site was reproduced by docking which suggested three possible conformations of the P1/P1′ groups of which two seem more plausible.

1. Introduction

The HIV-1 protease is an essential enzyme for HIV-1 replication and constitutes an important target in the treatment of HIV/AIDS.[1–3] Efficient combination therapies using inhibitors of the reverse transcriptase and protease enzymes have led many to reevaluate HIV-1 infections from a terminal condition to a chronic-but-manageable disease in the developed world. Unfortunately, the emergence of drug resistant viral strains and severe treatment-related adverse effects limit the benefits of current anti-HIV/AIDS drugs for many patients.[4] Furthermore, less than one in ten patients infected with HIV-1 in low- and middle-income countries have access to proper treatments. These important shortcomings highlight the need for new, cost effective anti-HIV/AIDS drugs with unique properties.[5]

We are presently engaged in a program aiming for the development of simple and inexpensive carbohydrate-based HIV-1 protease inhibitors.[6] In the project, we have employed the stereochemistry of L-mannitol for the straightforward synthesis of linear C_2-symmetric, C-terminal duplicated inhibitors.[6] The carbohydrate-based scaffold in these inhibitors, containing a central dihydroxyethylene transition-state isoster unit, has been utilized in several studies with the intent to optimize the binding of the P1/P1′ and P2/P2′ side chains to the C_2-symmetric HIV-1 protease.[7,8] In particular, palladium(0)-catalyzed coupling chemistry has proven to be effective in modifying halide functionalized benzyl ether P1/P1′ side chains.[9,10] Guidance in the design of new inhibitors with novel substituents has come from crystal structures of HIV-1 protease coupled with different inhibitors.[1,6,9]

In our systematic mapping of the P1/P1′ groups interacting with S1/S1′ sites, we recently published $Mo(CO)_6$-mediated aminocarbonylations in order to investigate the effects of *ortho*-functionalizations on the activities of these types of inhibitors in an expedient way.[11] It was initially speculated that bulky *ortho*-substituents might collide with the protease surface. However, to our surprise we found that compounds with large anilide substituents in the *ortho*-position of the P1/P1′ benzyloxy group showed nanomolar inhibition (Fig. 1).[11] Nevertheless, because of the presence of a

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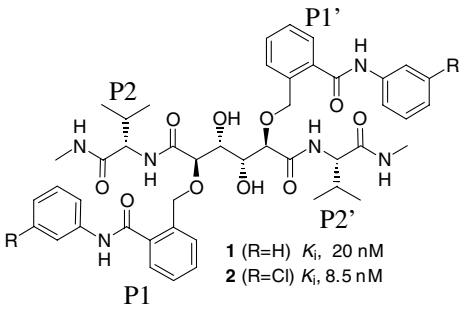


Figure 1.

large number of amide bonds, these inhibitors were rather unattractive from a drug development perspective.

The aim of the present study was to further evaluate the concept of *ortho*-substitution in the P1/P1′ positions by replacing the amide functionality with vinyl, alkynyl, acyl or aryl substituents while achieving similar or higher potencies. We envisioned the use of microwave-assisted chemoselective carbon–carbon bond forming reactions to introduce the alternative substituents.[12] In order to further reduce the peptide character it was decided to also exploit the well-known *N*-(1*S*,2*R*)-2-hydroxy-1-indanyl moiety as a P2/P2′ group (e.g., like in indinavir[13]).

Here, we report the rapid preparation and biological evaluation of 24 novel HIV-1 protease inhibitors. The low nanomolar activity values of the inhibitors with these rather large P1/P1′ *ortho*-groups lead us to further investigate the nature of their binding by employing computational techniques. As a result, docking studies were performed in an attempt to identify the possible binding mode of these inhibitors and a CoMFA model was derived based on the proposed binding conformation.

2. Results and discussion

2.1. Chemistry

The synthesis of C_2-symmetrical aryl palladium precursor **3** was carried out according to the previously published strategy.[6,9,11] With aryl iodo substrate **3** in hand, an abundance of possibilities to explore palladium(0)-catalyzed carbon–carbon bond forming reactions in order to produce new HIV-1 protease inhibitors were recognized.[14] From a synthetic perspective, the major goal was to identify robust conditions for decoration of non-protected precursor **3** without undesired water elimination of the diol. A second goal was to keep reaction times to a minimum, preferably 30 min or shorter under non-inert conditions. Specific coupling reactants were selected according to commercial availability and in order to achieve some degree of diversity in the size and flexibility of the P1/P1′-modified inhibitors. In addition, the styrene and benzofuran type of *ortho*-substituents might be viewed as isosteres to the active *ortho*-anilides in Figure 1.

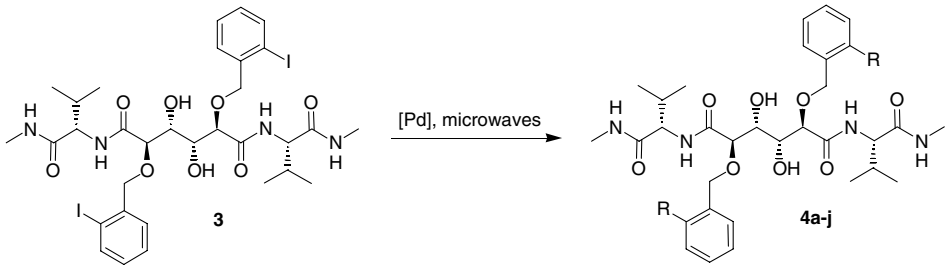
The introduction of a non-functionalized vinyl group by substitution of an aryl halide can be achieved by different palladium-catalyzed methods, the most common perhaps being the Stille cross-coupling with tributyl(vinyl)tin or the Heck reaction using ethene under pressure.[14] The usage of toxic tin reagents or the need for a reactive gas are obvious drawbacks of these strategies. A more attractive alternative would be to use a vinylboronic acid derivative in a Suzuki reaction. Free, monomeric vinylboronic acid is relatively unstable but the bench stable derivative 2,4,6-trivinylcyclotriboroxane–pyridine complex has been described as a useful coupling partner.[15] This commercially available reagent was utilized in a double Suzuki coupling to produce vinyl derivative **4a** in 57% yield after only 15 min of irradiation. The swift and straightforward Suzuki-microwave methodology was also applied to produce **4e** (65%), **4f** (44%) and **4j** (19%) from the corresponding boronic acids and parent **3**. Offsetting the advantages of the Suzuki reaction are some detractions. With non-gaseous olefins and acetylenes, direct Heck- and Sonogashira reactions are performed in high atom-efficiency avoiding the usage of organometallic reactants. The difficulty in introducing benzylic groups by the Suzuki methodology is another drawback. Therefore, continued modifications of the P1/P1′ side chains were conducted with complementary reaction protocols.

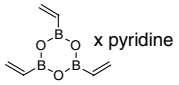
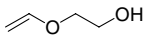
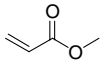
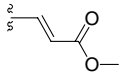
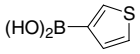
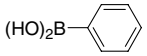
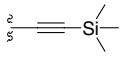
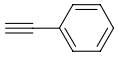
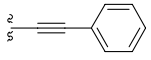
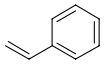
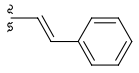
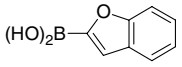
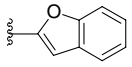
The acetylene units of **4b** were incorporated by a procedure where **3** was initially reacted with trimethylsilylacetylene in a Sonogashira coupling followed by basic removal of the silyl group to give **4b** in 37% two-step yield. In another run, the TMS–acetylene containing inhibitor **4g** was isolated (36%). The methyl ketone derivative **4c** was obtained by a two-step sequence. First, a regioselective internal Heck coupling of 2-hydroxyethyl vinyl ether with **3** afforded the 1,3-dioxolane protected ketone.[16] After an acidic deprotection of the 1,3-dioxolane, methyl ketone **4c** could be isolated in an excellent 85% overall yield from **3**. Reactions of methyl acrylate and styrene with **3** under standard Heck conditions produced methyl cinnamate derivative **4d** and stilbene derivative **4i** in 55% and 65% yield, respectively. Last in the series, phenylacetylene and **3** were coupled via Sonogashira chemistry to give **4h** in a satisfying 93% yield (Table 1).

The aminoindanol moiety present in indinavir was used as P2/P2′ group in a second series of inhibitors (Table 2). The same synthetic route as for **3** was exploited but in this case the bis-lactone **6**[11] was stirred with (1*S*,2*R*)-1-amino-2-indanol in 1,2-dichloroethane at 50 °C for 4 h, conveniently affording bis-aryl-iodide **7** in 55% isolated yield and in high purity (Scheme 1).

In an initial attempt to evaluate the potential of indanolamine containing inhibitors originating from **7**, compound **8** was synthesized by aminocarbonylation[17,18] of **7** with aniline as the nucleophile and $Mo(CO)_6$ as carbon monoxide source (Scheme 2).[19,20] Rewardingly, *ortho*-anilide **8** proved to be an equipotent inhibitor to valine containing **1** (**1**, K_i = 20 nM; **8**, K_i = 18 nM). This

Table 1. Synthesis and biological evaluation of inhibitors **4a–k**



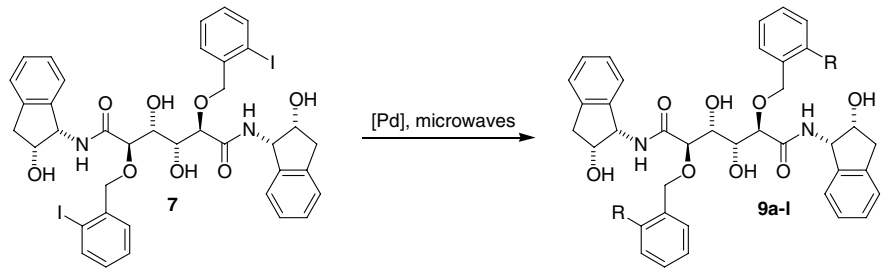
Compound	Reactant	Time (min)	Temp (°C)	R	Isolated yield (%)	K_i (nm)
3	—	—	—		—	4.3
4a	 x pyridine	15	100		57[a]	0.41
4b		15	120		36[b,c]	17
4c		30	100		85[d]	1100
4d		5	120		55[e]	760
4e		30	120		65[a]	290
4f		30	120		44[a]	1900
4g		15	120		37[b]	860
4h		15	100		93[b]	88
4i		5	120		65[e]	120
4j		30	120		19[a]	1600
4k	—	—	—		—	0.4[f]

[a] $Pd(OAc)_2$, $[(t\text{-}Bu)_3PH]BF_4$, K_2CO_3, DME, and H_2O.
[b] $Pd(PPh_3)_2Cl_2$, CuI, Et_2NH, and CH_3CN.
[c] Then K_2CO_3 (satd) in MeOH, rt, 2 h.
[d] $Pd(OAc)_2$, DPPP, TlOAc, $(i\text{-}Pr)_2EtN$, DMF, and H_2O.
[e] $Pd_2(OAc)_2(P(o\text{-}tol)_3)_2$, $(i\text{-}Pr)_2EtN$, DMF, and H_2O.
[f] Data from Ref. 6.

result encouraged us to proceed with the second series as planned (Scheme 2).

The identical protection group free strategy of Suzuki, Heck, and Sonogashira couplings was used for the synthesis of the aminoindanol series of inhibitors. Generally similar yields were obtained for these compounds (Table 2) compared to those in Table 1. Further, a Negishi coupling of **7** with benzylzinc bromide delivered **9g** in a disappointing 25% yield but without detected epimerization or water elimination. To prepare **9h**, 2-bromothiazole was first reacted with the 2,4,6-trivinylcyclotriboroxane–pyridine complex in a Suzuki reaction. After a quick workup, the formed 2-vinylthiazole was subsequently coupled with **7** under Heck conditions to produce **9h** in 62% isolated yield.

Table 2. Synthesis and biological evaluation of inhibitors **9a–m**

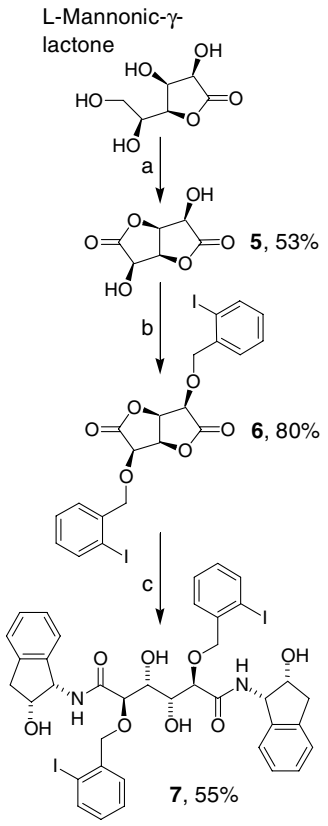


Compound	Reactant	Time (min)	Temp (°C)	R	Isolated yield (%)	K_i (nm)
7	—	—	—	—I		33
9a	x pyridine	15	100		51[a]	14
9b	—Si(Me)$_3$	15	120		53[b,c]	13
9c	OH	30	100		90[d]	190
9d		5	120		57[e]	660
9e	(HO)$_2$B—	30	120		53[a]	100
9f	(HO)$_2$B—	30	120		29[a]	450
9g	BrZn—	5	100		25[f]	14
9h		10	120		62[e]	220
9i	—Si(Me)$_3$	15	120	—Si(Me)$_3$	44[b]	21
9j		15	100		72[b]	200
9k		5	120		63[e]	4.1
9l	(HO)$_2$B—	30	120		45[a]	220
9m	—	—	—	—H		0.2[g]

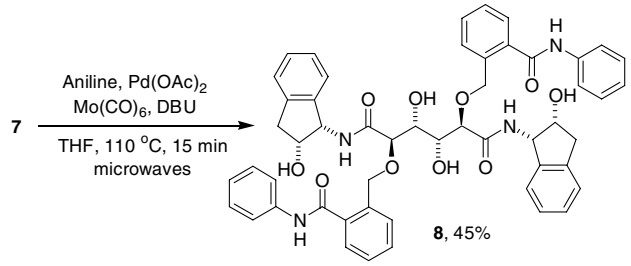
[a] Pd(OAc)$_2$, [(*t*-Bu)$_3$PH]BF$_4$, K_2CO_3, DME, and H_2O.
[b] Pd(PPh$_3$)$_2$Cl$_2$, CuI, Et$_2$NH, and CH_3CN.
[c] Then K_2CO_3 (satd) in MeOH, rt, 4 h.
[d] Pd(OAc)$_2$, DPPP, TlOAc, (*i*-Pr)$_2$EtN, DMF, and H_2O.
[e] Pd$_2$(OAc)$_2$(P(*o*-tol)$_3$)$_2$, (*i*-Pr)$_2$EtN, DMF, and H_2O.
[f] Pd(OAc)$_2$, [(*t*-Bu)$_3$PH]BF$_4$, and THF.
[g] Data from Ref. 6.

2.2. Biological results

The synthesized compounds were tested for their ability to inhibit HIV-1 protease in an enzyme-based assay and inhibition constants (K_i-values) were determined (Tables 1 and 2). In the case of P2 valine methylamides, the small *ortho*-substituents on the P1/P1′ benzyloxy groups gave the most potent inhibitors in this series with activities



Scheme 1. Reagents and conditions: (a) HNO_3, 90 °C, 5 h; (b) *o*-iodobenzyl-2,2,2-trichloroacetimidate, $BF_3 \cdot Et_2O$, rt, 18 h; (c) (1*S*,2*R*)-1-amino-indan-2-ol, 50 °C, 4 h.



Scheme 2. Synthesis of **8** by aminocarbonylation of **7** with aniline.

of 4.3, 0.41, and 17 nM for **3**, **4a** and **4b**, respectively. The P1/P1′ ketone **4c**, gave a great reduction in the potency (K_i = 1100 nM). The incorporation of aromatic substituents was not well tolerated as can be seen from the decrease in their potencies, with the biphenyl derivative being the least active (1900 nM, **4f**). Table 2 shows the P2/P2′ aminoindanol series where small lipophilic *ortho*-substitutions of the P1/P1′ benzyloxy side chains (**7**, **9a**, and **9b**) yielded inhibitors with low nanomolar activities. Polar acyclic substitutions in **9c** and **9d** provided decreased activities of 190 nM and 660 nM. When comparing **9f** (450 nM) with **9g** (14 nM), it was clear that the introduction of a flexible one-carbon linker between the two phenyl rings was highly advantageous. Exchanging the phenyl in **9f** to a thiophene moiety furnished improved potency (100 nM, **9e**). Methyl cinnamate **9d** and vinyl thiazole **9h** resulted in lower activity compared to stilbene derivative **9k** (4.1 nM), which also was the most active compound in the second series. The TMS-ethynyl derivative **9i** was more potent than diphenylacetylene **9j**. The benzofuran derivative **9l** (220 nM) exhibited similar activity to the vinyl thiazole **9h** (220 nM). In order to rationalize the structure–activity relationships and to elucidate the binding mode of especially the *ortho*-functionalized aminoindanols, docking studies along with 3D QSAR CoMFA were employed.

2.3. Computational studies

2.3.1. Molecular modeling. Three-dimensional structure building and all modeling was performed using the SYBYL program package, version 6.9[21] and FLO96.[22] The FLO+ module in FLO96, (also called QXP, Quick eXPlore), was used to dock the molecules into the active site of the HIV-1 protease. FLO96 search algorithms were derived from the method of Monte Carlo perturbation using a modified version of the AMBER force field. The molecular modeling is discussed in detail in Section 4.

2.3.2. Protein structure and docking results. We chose PDB[23] entry 1EC0, resolved at 1.79 Å, as a starting point for our docking studies since the co-crystallized P1/P1′ *ortho*-fluorobenzyloxy inhibitor in this X-ray crystal structure most closely resembled the compounds under investigation.[24] After minimization of the protein, the resulting structure had a heavy atom rmsd of 0.26 Å compared to the heavy atoms of the starting structure. A low rmsd suggested no significant change from the original crystal structure.

2.3.3. Common structural features of the inhibitor complexes. Most of the docked inhibitors were bound to the enzyme active site in an extended conformation. Thus, when they were superimposed upon one another, their functional elements aligned quite well. The dihedral angles in the backbone of all the inhibitors were maintained and the contacts between the backbone atoms of all the inhibitors and the protease were consistent. The hydrogen bonds were typically seen between the backbone atoms of the enzyme and the inhibitor. It has previously been shown that C_2-symmetric inhibitors bind to the protease in an asymmetric fashion.[25] We experienced similar trends with the investigated class of inhibitors **4** and **9** where one of the hydroxyls of the diol was positioned between the D25/25′ carboxyls of the protease, within hydrogen bonding distance to at least one carboxylate oxygen of each aspartate. Due to the lack of large P1/P1′ *ortho*-substituted inhibitors reported in the literature, focus was applied on detailing the binding of these groups in the S1/S1′ pockets. Although the docked backbone along with the P1/P1′ benzyloxy groups of these inhibitors showed interactions reported by Unge's group earlier,[9,24] the various *ortho*-substituents on these benzyloxy groups could be found positioned in three different areas of the enzyme. When studying the different docking conformations it was seen that all inhibitors could place their *ortho*-substituents in these areas with similar energies making it difficult to select the most probable binding mode for

the two series of inhibitors. Interestingly, upon closer visual inspection it was revealed that two of these observed conformations corresponded to the binding mode of co-crystallized inhibitors reported earlier[8,27] and is discussed below.

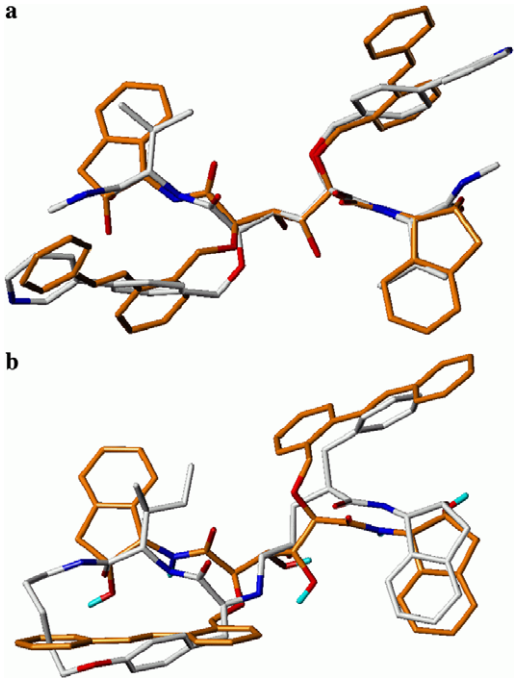


Figure 2. (a) Compound **9k** in conformation A over the symmetric P1/P1′ pyridine substituted inhibitor co-crystallized with HIV-1 protease (PDB entry: 1EC2). (b) Inhibitor **9k** in conformation B over the macrocylic inhibitor co-crystallized with HIV-1 protease (PDB entry: 1D4K). Carbon atoms of **9k** are colored orange, all other atoms are colored according to Sybyl atom types. Only polar hydrogens are displayed for clarity reasons.

The potent stilbene derivative **9k** was used as a representative compound to describe the observed binding modes. In conformation A, the terminal phenyl rings could be seen occupying the space between F53/53′ and P81/81′ in a fashion similar to the *para*-substituted pyridines in the P1 and P1′ positions of 1EC2[8,9] (Fig. 2). The binding mode in conformation B was consistent with the X-ray structure of the macrocyclic tripeptide analogs found in PDB entry, 1D4K[26] (Fig. 2). In the third unprecedented binding mode, the phenyl ring was observed lying in the grove formed by the hydrophobic side chains of R8/8′, L10/10′, L23/23′, and V82/82′. Figure 3I shows the various conformations for the *ortho*-substituted stilbene analog **9k** and Figure 3II illustrates the corresponding hydrogen bonding network for conformation A, including structural waters.

To find out if any quantitative correlation existed between these structures and their biological activities, we performed a CoMFA analysis. The inhibitors with conformation A have a more close structural resemblance to the corresponding co-crystallized ligand (BEJ in 1EC2),[8,9] as a result we decided to work with conformation A. PLS analysis with LOO cross-validation yielded a three-component model with a reasonable q^2 of 0.422 and a standard error of prediction of 0.86. The non-cross-validated PLS analysis gave a conventional r^2 of 0.83. The steric and electrostatic contributions amounted to 48% and 52%, respectively. These analyses suggested a substantial correlation between the structures and their respective biological activities. Although these CoMFA analyses pertain only to

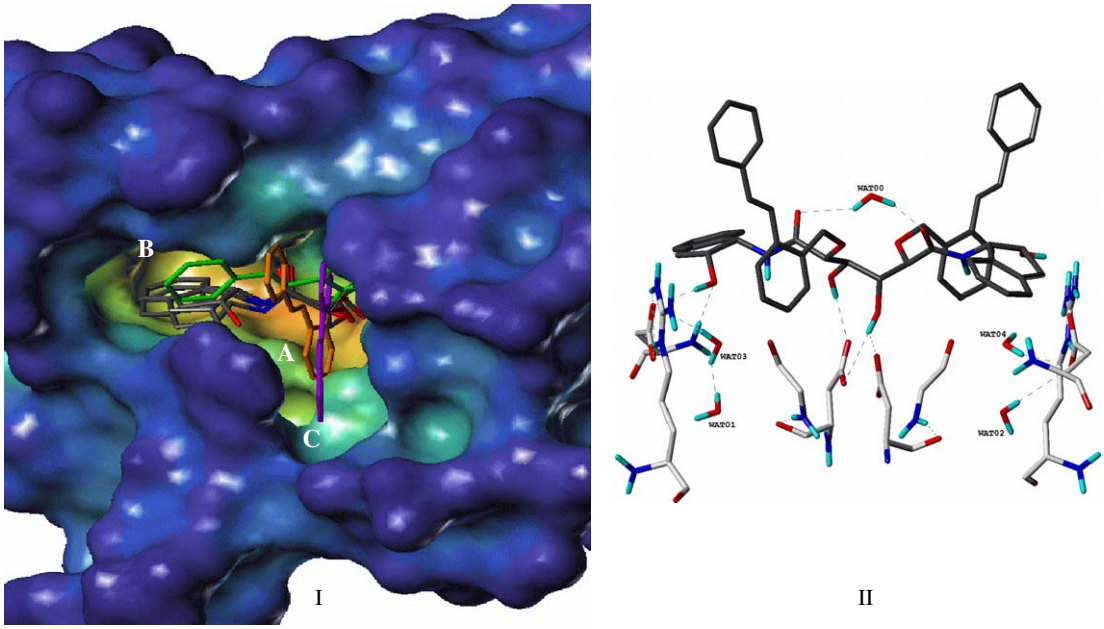


Figure 3. (I) Connolly surface color-coded with cavity depth is shown with the three probable binding regions for the P1 and P1′ substituents which are colored in green (conformation A), orange (conformation B) and purple (conformation C). (II) Hydrogen bonding network in conformation A between representative compound **9k** and the protease with structural waters. The carbon atoms of the inhibitor are colored dark grey while all other atoms are colored according to Sybyl atom types. Only polar hydrogens are displayed for clarity reasons

conformation A, we believe that any of the modes discussed above could be a plausible binding mode for these inhibitors.

3. Conclusions

In this investigation, the synthesis, biological evaluation and docking of 24 novel HIV-1 protease inhibitors were presented. Knowledge gained from a previous study using carbonylation methodology guided the design of this new set of more drug-like compounds. Two series of microwave-accelerated Suzuki, Heck, Sonogashira, and Negishi reactions were performed to rapidly modify the aryl iodide core structure and it was demonstrated that potent inhibitors containing *ortho*-substituted P1/P1′ side chains could be isolated. In general, small lipophilic 1-2 carbon-tethered aromatic *ortho*-substitutions of the P1/P1′ benzyloxy side chains provided inhibitors of high potency as compared to sterically demanding biaryl analogs. Docking studies were found to be in good agreement with earlier reported interactions in similar compounds co-crystallized with the protein. The *ortho*-substituents in P1/P1′ showed three plausible binding modes of which two coincided with the conformations of known crystal structures in the PDB. The alignment obtained from the docking studies enabled us to derive a 3D QSAR model for the present set of inhibitors.

4. Experimental

4.1. General information

^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury-400 spectrometer or a Jeol JNM-EX400 spectrometer at 400 and 100.5 MHz, respectively. Chemical shifts were referenced directly to tetramethylsilane (solvent mixtures) or indirectly via the residual solvent signal (pure solvents). All microwave-assisted synthesis was carried out in a Smith/Emrys™ Synthesizer single-mode microwave cavity producing controlled irradiation at 2450 MHz (Biotage AB, Uppsala, Sweden). Thin-layer chromatography was performed on silica gel 60 F-254 plates (E. Merck) and visualized with UV light and ninhydrin. Flash column chromatography was performed using silica gel 60 (0.040–0.063 mm) (E. Merck). RP-LC–MS analysis of reaction mixtures and pure products were performed using a Gilson HPLC system with a Chromolith SpeedROD RP-18e column (50 × 4.6 mm) and a Finnigan AQA quadropole mass spectrometer using a 4 mL/min CH_3CN/H_2O gradient (0.05% HCOOH) and detection by UV (DAD) and MS (ESI+). Preparative RP-LC–MS purifications were performed by UV-triggered fraction collection with a similar Gilson–Finnigan AQA system and a Zorbax SB C8 column (150 × 21.2 mm), using a 15 mL/min CH_3CN/H_2O gradient (0.05% HCOOH) with UV (214, 255 nm) and MS (ESI+) detection. Exact molecular masses were determined on Micromass Q-Tof2 mass spectrometer equipped with an electrospray ion source.

4.2. HIV protease inhibition

The HIV-1 protease was cloned and heterologously expressed in *Escherichia coli* and purified as described elsewhere.[27] The K_i values for the synthesized compounds were determined from two individual measurements by a fluorometric assay.[28] Assay variability was checked by inclusion a known inhibitor and the standard deviation for the enzyme assays was ±50% of the mean.

4.3. Chemistry

4.3.1. General procedure for the Suzuki couplings. Syntheses of 4a, 4e, 4f, 4j, 9a, 9e, 9f, and 9l. A mixture of **3** or **7** (0.050 mmol), boronic acid (0.30 mmol), $Pd(OAc)_2$ (1.1 mg, 0.0050 mmol), $[(t\text{-}Bu)_3PH]BF_4$ (3.0 mg, 0.010 mmol) and K_2CO_3 (41.5 mg, 0.30 mmol), H_2O (0.30 mL) and 1,2-dimethoxyethane (1.0 mL) in a 2.0 mL microwave vial was irradiated to 100 °C for 15 min. The organic layer of the reaction mixture was filtered through Celite and the solvent evaporated under reduced pressure. The residue was purified as indicated below.

4.3.2. General procedure for the Sonogashira couplings. Syntheses of 4g, 4h, 9i, and 9j. A mixture of **3** or **7** (0.050 mmol), alkyne (0.20 mmol), $Pd(PPh_3)_2Cl_2$ (1.8 mg, 0.0025 mmol), CuI (1.9 mg, 0.010 mmol), diethylamine (0.50 mL), and acetonitrile (0.50 mL) in a 2.0 mL microwave vial was irradiated at the indicated temperature for 15 min. The reaction mixture was filtered through Celite and the solvent evaporated under reduced pressure. The residue was purified as indicated below.

4.3.3. *N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-vinylbenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (4a). The 2,4,6-trivinylcyclotriboroxane–pyridine complex (24.1 mg, 0.10 mmol) was used as a boronic acid equivalent. The residue was purified by silica gel flash chromatography (3–5% MeOH in $CHCl_3$) to give **4a** as a colorless solid in 57% yield (19.1 mg). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ 1:1): δ 7.57 (dd, J = 7.7, 1.7 Hz, 2H), 7.39–7.23 (m, 6H), 7.08 (dd, J = 17.4, 11.0 Hz, 2H), 5.72 (dd, J = 17.4, 1.5 Hz, 2H), 5.35 (dd, J = 11.0, 1.5 Hz, 2H), 4.78 (d, J = 11.5 Hz, 2H), 4.69 (d, J = 11.5 Hz, 2H), 4.26 (d, J = 5.7 Hz, 2H), 4.20 (AA′ part of AA′BB′, 2H), 4.10 (BB′ part of AA′BB′, 2H), 2.74 (s, 6H), 2.29–2.17 (m, 2H), 0.94 (d, J = 6.9 Hz, 6H), 0.86 (d, J = 6.9 Hz, 6H). ^{13}C NMR (100.5 MHz, $CDCl_3/CD_3OD$ 1:1): δ 172.8, 172.4, 137.9, 134.39, 134.37, 130.3, 129.3, 128.3, 126.4, 116.8, 80.6, 72.1, 71.1, 58.9, 30.7, 26.1, 19.6, 17.7. Anal. Calcd for $C_{36}H_{50}N_4O_8$+0.3H_2O: C, 64.32; H, 7.59; N, 8.33. Found: C, 63.92; H, 7.49; N, 8.17.

4.3.4. *N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-ethynylbenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (4b). Sonogashira coupling at 120 °C with trimethylsilylacetylene. To the crude product was added 2 mL of a saturated solution of K_2CO_3 in MeOH and the mixture was stirred at room temperature for 2 h. The solvent was evaporated and the residue was purified by silica gel flash chromatogra-

phy (5% MeOH in $CHCl_3$) to give 12.0 mg of **4b** (36%, light yellow solid). ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD 1:1): δ 7.59–7.24 (m, 4H), 7.39 (dt, J = 7.6, 1.8 Hz, 2H), 7.31 (dt, J = 7.6, 1.7 Hz, 2H), 4.88 (d, J = 12.0 Hz, 2H), 4.80 (d, J = 12.0 Hz, 2H), 4.28–4.21 (m, 4H), 4.18–4.15 (m, 2H), 3.59 (s, 2H), 2.75 (s, 6H), 2.27–2.16 (m, 2H), 0.94 (d, J = 6.9 Hz, 6H), 0.87 (d, J = 6.9 Hz, 6H). ^{13}C NMR (100.5 MHz, $CDCl_3$/CD_3OD 1:1): δ 173.0, 172.5, 139.8, 133.5, 129.6, 129.2, 128.6, 122.2, 83.3, 81,7, 81.0, 72.2, 71.5, 59.0, 30.9, 26.2, 19.6, 17.9. Anal. Calcd for $C_{36}H_{46}N_4O_8$+0.25H_2O: C, 64.80; H, 7.02; N, 8.40. Found: C, 64.7; H, 7.0; N, 8.1.

4.3.5. *N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-acetylbenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (4c). A 2.0 mL microwave vial was charged with **3** (43.3 mg, 0.050 mmol), $Pd(OAc)_2$ (2.2 mg, 0.010 mmol), 1,3-bis(diphenylphosphino)propane (dppp) (4.4 mg, 0.010 mmol), 2-hydroxyethyl vinyl ether (0.045 mL, 0.50 mmol), TlOAc (28.1 mg, 0.10 mmol), (i-Pr)$_2$EtN (0.035 mL, 0.20 mmol) and 1.0 mL of DMF/H_2O (9:1). The vial was capped with a Teflon septum and irradiated with microwaves to 100 °C for 30 min. The dioxolane was deprotected by addition of 2 mL of 0.5 M HCl in MeCN/H_2O (1:1). After stirring for 30 min at room temperature, the reaction mixture was poured onto 40 mL of 2 M HCl (aq) and extracted with 3× 40 mL CH_2Cl_2. The combined organic layers were washed with brine (40 mL), dried with $MgSO_4$ and evaporated to dryness. The residue was purified by silica gel flash chromatography (2–4% MeOH in $CHCl_3$) to give 29.5 mg of **4c** (85%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD 1:1): δ 7.87 (dd, J = 7.8, 1.6 Hz, 2H), 7.72 (dd, J = 7.8, 1.5 Hz, 2H), 7.58 (dd, J = 7.6, 1.5 Hz, 2H), 7.46 (dd, J = 7.6, 1.5 Hz, 2H), 4.96 (d, J = 13.2 Hz, 2H), 4.90 (d, J = 13.2 Hz, 2H), 4.30–4.25 (m, 2H), 4.21 (AA′ part of AA′BB′, 2H), 4.12 (BB′ part of, J = 7.5, 1.9 Hz, 2H), 7.36–7.30 (m, 6H), 7.27–7.22 (m, 4H), 7.05 (d, J = 16.2, 2H), 4.84 (d, J = 11.8 Hz, 2H), 4.76 (d, J = 11.8 Hz, 2H), 4.24–4.14 (m, 6H), 2.68 (s, 6H), 2.21–2.10 (m, 2H), 0.86 (d, J = 6.8 Hz, 6H), 0.79 (d, J = 6.8 Hz, 6H). ^{13}C NMR (100.5 MHz, $CDCl_3$, CD_3OD 1:1): δ 172.8, 172.3, 137.9, 137.5, 134.7, 131.9, 130.5, 129.4, 129.2, 128.4, 128.1, 127.3, 126.4, 125.6, 80.5, 72.3, 71.0, 58.8, 30.4, 26.1, 19.6, 17.5. Anal. Calcd for $C_{48}H_{58}N_4O_8$+0.5H_2O: C, 69.63; H, 7.18; N, 6.77. Found: C, 69.6; H, 7.1; N, 6.7.

4.3.6. *N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-((*E*)-2-methoxycarbonylvinyl)benzyloxy)-3,4-dihydroxyhexane-1,6-diamide (4d). A 2.0 mL microwave vial was charged with **3** (43.3 mg, 0.050 mmol), Herrmann's catalyst (*trans*-di-(μ-acetato)-bis[*o*-(di-*o*-tolylphosphino)benzyl]dipalladium(II)) (2.4 mg, 0.0025 mmol), methyl acrylate (0.045 mL, 0.50 mmol), (i-Pr)$_2$EtN (0.035 mL, 0.20 mmol), and 1.0 mL DMF/H_2O (9:1). The vial was capped with a Teflon septum and irradiated with microwaves to 120 °C for 5 min. The reaction mixture was filtered through Celite and the solvent removed under reduced pressure. The residue was purified by preparative RP-LC–MS (20–70% MeCN in H_2O, 25 min gradient) to give **4d** in 55% yield (21.5 mg, colorless solid). ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD 1:1): δ 8.04 (d, J = 15.9 Hz, 2H), 7.67–7.63 (m, 2H), 7.47–7.36 (m, 6H), 6.42 (d, J = 15.9 Hz, 2H), 4.86 (d, J = 11.7 Hz, 2H), 4.68 (d, J = 11.7 Hz, 2H), 4.27–4.20 (m, 4H), 4.17–4.14 (m, 2H), 3.75 (s, 6H), 2.74 (s, 6H), 2.28–2.18 (m, 2H), 0.94 (d, J = 6.9 Hz, 6H), 0.87 (d, J = 6.9 Hz, 6H). ^{13}C NMR (100.5 MHz, $CDCl_3$/CD_3OD 1:1): δ 172.9, 172.3, 168.5, 142.5, 136.6, 134.2, 130.84, 130.75, 129.6, 127.4, 119.9, 80.9, 71.8, 70.8, 58.9, 52.2, 30.5, 26.2, 19.7, 17.7. Anal. Calcd for $C_{40}H_{54}N_4O_{12}$+0.5H_2O: C, 60.67; H, 7.00; N, 7.08. Found: C, 60.5; H, 6.9; N, 7.0.

4.3.7. *N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-thiophen-3-ylbenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (4e). Suzuki coupling with 3-thienylboronic acid. The residue was purified by preparative RP-LC–MS (30-90% MeCN in H_2O, 25 min gradient) to give **4e** in 65% yield (25.3 mg, colorless solid). ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD 1:1): δ 7.58–7.55 (m, 2H), 7.41–7.34 (m, 10H), 7.18 (dd, J = 4.8, 1.7 Hz, 2H), 4.62 (s, 4H), 4.24 (d, J = 5.6 Hz, 2H), 4.12 (AA′ part of AA′BB′, 2H), 4.02 (BB′ part of AA′BB′, 2H), 2.72 (s, 6H), 2.28-2.16 (m, 2H), 0.89 (d, J = 6.9 Hz, 6H), 0.82 (d, J = 6.9 Hz, 6H). ^{13}C NMR (100.5 MHz, $CDCl_3$, CD_3OD 1:1): δ 172.9, 172.4, 141.2, 137.3, 134.9, 130.5, 130.3, 129.4, 128.8, 128.1, 126.1, 123.8, 81.3, 72.2, 71.5, 58.9, 30.7, 26.2, 19.7, 17.7. Anal. Calcd for $C_{40}H_{50}N_4O_8S_2$+0.5H_2O: C, 60.97; H, 6.52; N, 7.11. Found: C, 60.95; H, 6.54; N, 6.98.

4.3.8. *N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-phenylbenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (4f). Suzuki coupling with phenylboronic acid. The residue was purified by preparative RP-LC–MS (30–90% MeCN in H_2O, 25 min gradient) to give **4f** in 44% yield (16.9 mg, colorless solid). ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD 1:1): δ 7.62–7.58 (m, 2H), 7.41–7.27 (m, 18H), 4.58 (s, 4H), 4.21 (d, J = 5.6 Hz, 2H), 4.01–3.96 (m, 4H), 2.70 (s, 6H), 2.26–2.15 (m, 2H), 0.86 (d, J = 6.8 Hz, 6H), 0.79 (d, J = 6.8 Hz, 6H). ^{13}C NMR (100.5 MHz, $CDCl_3$/CD_3OD 1:1): δ 172.8, 172.4, 142.5, 141.1, 134.8, 130.6, 129.7, 129.6, 128.8, 128.6, 128.1, 127.9, 81.5, 72.4, 71.2, 58.8, 30.6, 26.1, 19.7, 17.7. Anal. Calcd for $C_{44}H_{54}N_4O_8$: C, 68.91; H, 7.10; N, 7.31. Found: C, 68.91; H, 7.10; N, 7.20.

4.3.9. *N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-trimethylsilanylethynylbenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (4g). Sonogashira coupling at 120 °C with trimethylsilylacetylene. The residue was purified by preparative RP-LC–MS (30–100% MeCN in H_2O, 25 min gradient) to give **4g** in 37% yield (14.9 mg, yellow solid). ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD 1:1): δ 7.81 (q, 4.7 Hz, 2H), 7.55 (dd, J = 7.7, 1.7 Hz, 2H), 7.47 (dd, J = 7.6, 1.7 Hz, 2H), 7.37 (dt, J = 7.6, 1.6 Hz, 2H), 7.28 (dt, J = 7.6, 1.6 Hz, 2H), 4.89 (d, J = 12.6 Hz, 2H), 4.83 (d, J = 12.6 Hz, 2H), 4.31–4.25 (m, 4H), 4.19–4.16 (m, 2H), 2.73 (s, 6H), 2.32–2.21 (m, 2H), 0.92 (d, J = 6.9 Hz, 6H), 0.83 (d, J = 6.9 Hz, 6H), 0.25 (s, 18H). ^{13}C NMR (100.5 MHz, $CDCl_3$/CD_3OD 1:1): δ 172.8, 172.3, 139.6, 133.1, 129.4, 128.3, 128.2, 122.3, 102.9, 99.9, 81.6, 72.6, 71.4, 58.7, 30.4, 26.1, 19.7, 17.6, 0.1. Anal. Calcd

for $C_{42}H_{62}N_4O_8Si_2$: C, 62.50; H, 7.74; N, 6.94. Found: C, 62.2; H, 7.8; N, 6.8.

4.3.10. *N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-phenylethynylbenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (4h). Sonogashira coupling at 100 °C with phenylacetylene. The residue was purified by preparative RP-LC–MS (30-90% MeCN in H_2O, 25 min gradient) to give **4h** in 93% yield (37.8 mg, colorless solid). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ 1:1): δ 7.57–7.50 (m, 8H), 7.38–7.28 (m, 10H), 4.95 (d, J = 12.3 Hz, 2H), 4.90 (d, J = 12.3 Hz, 2H), 4.29–4.26 (m, 2H), 4.23-4.19 (m, 4H), 2.69 (s, 6H), 2.24–2.13 (m, 2H), 0.87 (d, J = 6.9 Hz, 6H), 0.81 (d, J = 6.9 Hz, 6H). ^{13}C NMR (100.5 MHz, $CDCl_3/CD_3OD$ 1:1): δ 172.8, 172.3, 139.1, 132.8, 132.1, 129.2, 129.1, 128.9, 128.8, 128.6, 123.5, 122.8, 94.8, 87.2, 81.3, 72.6, 71.5, 58.8, 30.5, 26.1, 19.6, 17.5. Anal. Calcd for $C_{48}H_{54}N_4O_8$: C, 70.74; H, 6.68; N, 6.87. Found: C, 70.6; H, 6.8; N, 6.8.

4.3.11. *N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-((*E*)-styryl)benzyloxy)-3,4-dihydroxyhexane-1,6-diamide (4i). A 2.0 mL microwave vial was charged with **3** (43.3 mg, 0.050 mmol), Herrmann's catalyst (*trans*-di-(μ-acetato)-bis[*o*-(di-*o*-tolylphosphino)benzyl]dipalladium(II)) (2.4 mg, 0.0025 mmol), styrene (0.055 mL, 0.50 mmol), (i-Pr)$_2$EtN (0.035 mL, 0.20 mmol) and 1.0 mL of DMF/H_2O (9:1). The vial was capped with a Teflon septum and irradiated with microwaves to 120 °C for 5 min. The reaction mixture was filtered through Celite and the solvent removed under reduced pressure. The residue was purified by silica gel flash chromatography (0–3% MeOH in $CHCl_3$) to give **4i** in 65% yield (26.8 mg, colorless solid). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ 1:1): δ 7.66 (dd, J = 7.8, 1.8 Hz, 2H), 7.57–7.52 (m, 4H), 7.46 (d, J = 16.2, 2H), 7.39 (dd, J = 7.5, 1.9 Hz, 2H), 7.36–7.30 (m, 6H), 7.27–7.22 (m, 4H), 7.05 (d, J = 16.2, 2H), 4.84 (d, J = 11.8 Hz, 2H), 4.76 (d, J = 11.8 Hz, 2H), 4.24–4.14 (m, 6H), 2.68 (s, 6H), 2.21–2.10 (m, 2H), 0.86 (d, J = 6.8 Hz, 6H), 0.79 (d, J = 6.8 Hz, 6H). ^{13}C NMR (100.5 MHz, $CDCl_3/CD_3OD$ 1:1): δ 172.8, 172.3, 137.9, 137.5, 134.7, 131.9, 130.5, 129.4, 129.2, 128.4, 128.1, 127.3, 126.4, 125.6, 80.5, 72.3, 71.0, 58.8, 30.4, 26.1, 19.6, 17.5. Anal. Calcd for $C_{48}H_{58}N_4O_8$+0.5H_2O: C, 69.63; H, 7.18; N, 6.77. Found: C, 69.6; H, 7.1; N, 6.7.

4.3.12. *N*1,*N*6-Bis[(1*S*)-2-methyl-1-(methylcarbamoyl)propyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-benzofuran-2-ylbenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (4j). Suzuki coupling with 2-benzofuranylboronic acid. Product purified by preparative RP-LC–MS (30–90% MeCN in H_2O, 25 min gradient) to give 8.0 mg of **4j** (19%, colorless solid). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ 1:1): δ 7.87 (dd, J = 7.6, 1.8 Hz, 2H), 7.76 (q, J = 4.7 Hz, 2H), 7.64–7.59 (m, 4H), 7.52 (d, J = 8.1 Hz, 2H), 7.47–7.37 (m, 4H), 7.29 (dt, J = 7.3, 1.6 Hz, 2H), 7.23 (dt, J = 7.3, 1.3 Hz, 2H), 7.10 (d, J = 1.1 Hz, 2H), 4.97 (d, J = 12.1 Hz, 2H), 4.90 (d, J = 12.1 Hz, 2H), 4.31 (AA′ of AA′XX′, 2H), 4.22 (d, J = 5.4 Hz, 2H), 4.17 (XX′ of AA′XX′, 2H), 2.69 (s, 6H), 2.22–2.11 (m, 2H), 0.79 (d, J = 6.8 Hz, 6H), 0.71 (d, J = 6.8 Hz, 6H). ^{13}C NMR (100.5 MHz, $CDCl_3/CD_3OD$ 1:1): δ 172.7, 172.2, 155.4, 154.8, 134.7, 130.5, 130.3, 129.6, 129.3, 129.1, 129.0, 125.1, 123.5, 121.7, 111.5, 106.2, 80.9, 72.4, 71.4, 58.7, 30.3, 26.1, 19.5, 17.4. Anal. Calcd for $C_{48}H_{54}N_4O_{10}$: C, 68.07; H, 6.43; N, 6.61. Found: C, 67.83; H, 6.52; N, 6.46.

4.3.13. *N*1,*N*6-Bis[(1*S*,2*R*)-2-hydroxy-1-indanyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-iodobenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (7). The bis-lactone **6** (2.00 g, 3.30 mmol) was dissolved in 200 mL of 1,2-dichloroethane. (1*S*,2*R*)-1-Amino-2-indanol (2.00 g, 13.4 mmol) was added to the solution and the reaction mixture was stirred at 50 °C for 5 h. The solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (5% MeOH in $CHCl_3$) to give **7** (1.63 g, 55%) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (dd, J = 7.9, 1.2 Hz, 2H), 7.44–7.37 (m, 4H), 7.31 (ddd, J = 7.9, 7.6, 1.2 Hz, 2H), 7.26–7.18 (m, 6H), 6.99 (ddd, J = 7.9, 7.4, 1.7 Hz, 2H), 5.31 (dd, J = 8.8, 5.2 Hz, 2H), 4.74 (d, J = 11.8 Hz, 2H), 4.70 (d, J = 11.8 Hz, 2H), 4.63 (app dt, J = 5.4, 2.5 Hz, 2H), 4.34 (AA′ part of AA′BB′, 2H), 4.26 (BB′ part of AA′BB′, 2H), 3.08 (dd, J = 16.6, 5.6 Hz, 2H), 2.88 (dd, J = 16.6, 2.5 Hz, 2H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 171.4, 141.0, 139.8, 139.7, 139.2, 130.2, 128.7, 128.5, 127.2, 125.5, 124.5, 99.0, 82.3, 72.7, 71.5, 58.0, 39.4. Anal. Calcd for $C_{38}H_{38}I_2N_2O_8$+H_2O: C, 49.47; H, 4.37; N, 3.04. Found C, 49.3; H, 4.6; N, 3.1.

4.3.14. *N*1,*N*6-Bis[(1*S*,2*R*)-2-hydroxy-1-indanyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-phenylcarbamoylbenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (8). A 2.0 mL microwave vial was charged with **7** (45.2 mg, 0.050 mmol), $Pd(OAc)_2$ (2.2 mg, 0.01 mmol), $Mo(CO)_6$ (26.4 mg, 0.10 mmol), aniline (91 μL, 1.0 mmol), DBU (0.75 mL, 0.50 mmol) and dry THF (1.5 mL). The vial was immediately capped with a Teflon septum and irradiated with microwaves to 110 °C for 15 min. After cooling, the reaction mixture was filtered and the solvent was removed under reduced pressure. The residue was purified by preparative RP-LC–MS (20-70% MeCN in H_2O, 25 min gradient) to produce **8** in 45% (20.1 mg) yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22 (s, 2H), 7.58-7.06 (m, 28H), 5.25 (dd, J = 8.6, 5.2 Hz, 2H), 4.90 (d, J = 11.6 Hz, 2H), 4.76 (d, J = 11.6 Hz, 2H), 4.53 (app dt, J = 5.4, 2.2 Hz, 2H), 4.09 (AA′ part of AA′BB′, 2H), 4.05 (BB′ part of AA′BB′, 2H), 3.08 (dd, J = 16.6, 5.6 Hz, 2H), 2.89 (dd, J = 16.6, 2.2 Hz, 2H). ^{13}C NMR (100.5 MHz, $CDCl_3/CD_3OD$ 1:1): δ 172.9, 169.6, 141.0, 140.9, 138.9, 137.2, 135.6, 131.1, 130.7, 129.4, 128.9, 128.6, 128.4, 127.5, 125.7, 125.2, 124.9, 121.4, 81.1, 73.4, 71.5, 71.2, 58.1, 40.1. HRMS (M+1): Calcd: 891.3605. Found: 891.3607.

4.3.15. *N*1,*N*6-Bis[(1*S*,2*R*)-2-hydroxy-1-indanyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-vinylbenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (9a). The 2,4,6-trivinylcyclotriboroxane–pyridine complex (24.1 mg, 0.10 mmol) was used as a boronic acid equivalent. The residue was purified by silica gel flash chromatography (3–5% MeOH in $CHCl_3$) to give **9a** as a colorless solid in 51% yield (17.8 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50 (dd, J = 7.7, 1.6 Hz, 2H), 7.34–7.18 (m, 14H), 7.11 (dd, J = 7.3,

1.2 Hz, 2H), 6.99 (dd, J = 17.4, 11.0 Hz, 2H), 5.59 (dd, J = 17.4, 1.5 Hz, 2H), 5.28 (dd, J = 8.7, 5.1 Hz, 2H), 5.23 (dd, J = 11.0, 1.5 Hz, 2H), 4.81 (d, J = 11.4 Hz, 2H), 4.73 (d, J = 11.4 Hz, 2H), 4.65 (app dt, J = 5.4, 2.3 Hz, 2H), 4.23 (AA′ part of AA′BB′, 2H), 4.19 (BB′ part of AA′BB′, 2H), 3.10 (dd, J = 16.6, 5.6 Hz, 2H), 2.90 (dd, J = 16.6, 2.4 Hz, 2H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 171.6, 141.1, 139.7, 137.4, 133.8, 133.6, 130.1, 129.1, 128.5, 128.1, 127.1, 126.3, 125.5, 124.2, 117.0, 82.1, 72.5, 72.1, 71.3, 58.1, 39.5. Anal. Calcd for $C_{42}H_{44}N_2O_8$+H_2O: C, 69.79; H, 6.41; N, 3.88. Found: C, 69.93; H, 6.34; N, 3.81.

4.3.16. *N*1,*N*6-Bis[(1*S*,2*R*)-2-hydroxy-1-indanyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-ethynylbenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (9b). Sonogashira coupling at 120 °C with trimethylsilylacetylene. To the crude product was added 2 mL of a saturated solution of K_2CO_3 in MeOH and the mixture was stirred at room temperature for 2 h. The solvent was evaporated and the residue was purified by silica gel flash chromatography (5% MeOH in $CHCl_3$) to give 18.6 mg of **9b** (53%, light yellow solid). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49 (dd, J = 7.4, 1.8 Hz, 2H), 7.41 (dd, J = 7.5, 1.8 Hz, 2H), 7.36–7.14 (m, 14H), 5.33 (ddd, J = 8.7, 5.1, 1.2 Hz, 2H), 4.90 (d, J = 11.5 Hz, 2H), 4.84 (d, J = 11.5 Hz, 2H), 4.70 (app dt, J = 5.4, 2.2 Hz, 2H), 4.31 (AA′ part of AA′BB′, 2H), 4.26 (BB′ part of AA′BB′, 2H), 3.13 (dd, J = 16.5, 5.6 Hz, 2H), 3.08(s, 2H), 2.95 (dd, J = 16.5, 2.2 Hz, 2H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 171.7, 141.1, 139.8, 138.8, 133.3, 129.4, 129.2, 128.6, 128.5, 127.1, 125.5, 124.4, 121.8, 82.8, 82.4, 81.6, 72.6, 72.5, 71.4, 58.1, 39.5. Anal. Calcd for $C_{42}H_{40}N_2O_8$: C, 71.98; H, 5.75; N, 4.00. Found: C, 71.8; H, 5.9; N, 3.9.

4.3.17. *N*1,*N*6-Bis[(1*S*,2*R*)-2-hydroxy-1-indanyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-acetylbenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (9c). A 2.0 mL microwave vial was charged with **7** (45.2 mg, 0.050 mmol), $Pd(OAc)_2$ (2.2 mg, 0.010 mmol), 1,3-bis(diphenylphosphino)propane (dppp) (4.4 mg, 0.010 mmol), 2-hydroxyethyl vinyl ether (0.045 mL, 0.50 mmol), TlOAc (28.1 mg, 0.10 mmol), (i-Pr)$_2$EtN (0.035 mL, 0.20 mmol) and 1.0 mL of DMF/H_2O (9:1). The vial was capped with a Teflon septum and irradiated with microwaves to 100 °C for 30 min. The dioxolane was deprotected by addition of 2 mL of 0.5 M HCl in MeCN/H_2O (1:1). After stirring for 30 min at room temperature, the reaction mixture was poured onto 40 mL of 2 M HCl (aq) and extracted with 2× 40 mL CH_2Cl_2. The combined organic layers were washed with brine (40 mL), dried with $MgSO_4$ and evaporated to dryness. The residue was purified by silica gel flash chromatography (3–5% MeOH in $CHCl_3$) to give 33.2 mg of **9c** (90%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (dd, J = 7.7, 1.5 Hz, 2H), 7.62 (dd, J = 7.7, 1.5 Hz, 2H), 7.53–7.46 (m, 4H), 7.40 (dt, J = 7.6, 1.4 Hz, 2H), 7.26–7.13 (m, 8H), 5.33 (dd, J = 8.6, 5.1 Hz, 2H), 5.03 (d, J = 12.6 Hz, 2H), 4.97 (d, J = 12.6 Hz, 2H), 4.75–4.69 (m, 2H), 4.33 (AA′ part of AA′BB′, 2H), 4.27 (BB′ part of AA′XX′, 2H), 3.14 (dd, J = 16.6, 5.4 Hz, 2H), 2.98 (dd, J = 16.6, 2.2 Hz, 2H), 2.55 (s, 6H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 201.7, 171.9, 141.1, 139.9, 137.5, 136.6, 132.6, 130.1, 129.8, 128.4, 128.1, 127.1, 125.5, 124.2, 83.3, 72.6, 72.4, 71.2, 58.3, 39.6, 29.2. Anal. Calcd for $C_{42}H_{44}N_2O_{10}$: C, 68.46; H, 6.02; N, 3.80. Found C, 68.21; H, 6.16; N, 3.56.

4.3.18. *N*1,*N*6-Bis[(1*S*,2*R*)-2-hydroxy-1-indanyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-((*E*)-2-methoxycarbonylvinyl)benzyloxy)-3,4-dihydroxyhexane-1,6-diamide (9d). A 2.0 mL microwave vial was charged with **5** (45.2 mg, 0.050 mmol), Herrmann's catalyst (2.4 mg, 0.0025 mmol), methyl acrylate (0.045 mL, 0.50 mmol), (i-Pr)$_2$EtN (0.035 mL, 0.20 mmol) and 1.0 mL of DMF/H_2O (9:1). The vial was capped with a Teflon septum and irradiated with microwaves to 120 °C for 5 min. The reaction mixture was filtered through Celite and the solvent removed under reduced pressure. The residue was purified by silica gel flash chromatography (3–5% MeOH in $CHCl_3$) to give **9d** in 57% yield (23.8 mg, colorless solid). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.01 (d, J = 15.9 Hz, 2H), 7.60–7.55 (m, 2H), 7.39–7.30 (m, 8H), 7.23 (dd, J = 5.0, 1.2 Hz, 4H), 7.20–7.08 (m, 2H), 6.27 (d, J = 15.9 Hz, 2H), 5.33 (ddd, J = 8.7, 5.1, 1.2 Hz, 2H), 4.97 (d, J = 11.4 Hz, 2H), 4.73 (d, J = 11.4 Hz, 2H), 4.70–4.64 (m, 2H), 4.28 (m, 4H), 3.55 (s, 6H), 3.12 (dd, J = 16.5, 5.5 Hz, 2H), 2.94 (dd, J = 16.5, 2.1 Hz, 2H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 171.7, 167.7, 141.9, 141.0, 140.0, 135.9, 134.0, 130.6, 130.4, 129.2, 128.4, 127.04, 127.01, 125.4, 124.2, 119.7, 81.6, 72.7, 71.7, 71.1, 58.0, 51.9, 39.6. Anal. Calcd for $C_{46}H_{48}N_2O_{12}$: C, 67.30; H, 5.89; N, 3.41. Found: C, 67.2; H, 6.2; N, 3.4.

4.3.19. *N*1,*N*6-Bis[(1*S*,2*R*)-2-hydroxy-1-indanyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-(3-thiophenyl)benzyloxy)-3,4-dihydroxyhexane-1,6-diamide (9e). Suzuki coupling with 3-thienylboronic acid. Product purified by preparative RP-LC–MS to give 21.7 mg of **9e** (53%, colorless solid). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46 (d, J = 7.1, 2H), 7.35–7.16 (m, 18H), 7.13 (dd, J = 4.9, 1.6 Hz, 2H), 7.03 (d, J = 7.4 Hz, 2H), 5.27 (dd, J = 8.8, 5.0 Hz, 2H), 4.72–4.65 (m, 4H), 4.64–4.58 (m, 2H), 4.12 (m, 4H), 3.06 (dd, J = 16.6, 5.5 Hz, 2H), 2.86 (dd, J = 16.6, 2.3 Hz, 2H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 171.8, 141.1, 140.7, 139.7, 137.0, 134.2, 130.0, 128.9, 128.7, 128.5, 127.9, 127.2, 125.9, 125.5, 124.1, 123.4, 82.8, 72.5, 71.9, 71.6, 58.2, 39.4. Anal. Calcd for $C_{46}H_{44}N_2O_8S_2$+H_2O: C, 66.17; H 5.55; N, 3,35. Found: C, 65,96; H, 5,61; N, 3,31.

4.3.20. *N*1,*N*6-Bis[(1*S*,2*R*)-2-hydroxy-1-indanyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-phenylbenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (9f). Suzuki coupling with phenylboronic acid. Product purified by preparative RP-LC–MS to give 11.8 mg of **9f** (29%, colorless solid). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52–7.47 (m, 2H), 7.39–7.23 (m, 20H), 7.21–7.14 (m, 4H), 6.97 (d, J = 7.4 Hz, 2H), 5.25 (dd, J = 8.7, 5.0 Hz, 2H), 4.69–4.58 (m, 6H), 4.05 (AA′ part of AA′XX′, 2H), 4.01 (AA′ part of AA′XX′, 2H), 3.08 (dd, J = 16.6, 5.4 Hz, 2H), 2.91 (dd, J = 16.6, 2.0 Hz, 2H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 171.8, 142.1, 141.1, 140.6, 139.7, 134.0, 130.4, 129.5, 129.2, 128.5, 127.9, 127.6, 127.2, 125.5, 124.0, 83.1, 72.4,

71.7, 71.5, 58.2, 39.5. Anal. Calcd for $C_{50}H_{48}N_2O_8$+-H_2O: C, 72.97; H, 6.12; N, 3.40. Found: C, 73.0; H, 6.1; N, 3.3.

4.3.21. *N*1,*N*6-Bis[(1*S*,2*R*)-2-hydroxy-1-indanyl]-(2*R*,3*R*, 4*R*,5*R*)-2,5-bis(2-benzylbenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (9g). To an oven-dried 2.0 mL microwave vial was added **7** (45.2 mg, 0.050 mmol), $Pd(OAc)_2$, (1.1 mg, 0.0050 mmol) and $[(t\text{-Bu})_3PH]BF_4$ (3.0 mg, 0.010 mmol). The vial was capped with a septum and evacuated and backfilled with N_2 three times. Benzylzinc bromide (0.5 M in THF, 1.0 mL, 0.50 mmol) was added by syringe and the resulting mixture was irradiated with microwaves to 100 °C for 15 min. The reaction mixture was poured onto 50 mL of cold 2 M HCl (aq) and extracted with 2× 50 mL CH_2Cl_2. The combined organic layers were washed with brine and dried with $MgSO_4$ and the solvent removed under reduced pressure. The residue (containing starting material, mono- and bis-coupled product) was purified by RP-LC–MS to give 10.4 mg of **9g** (25%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33 (dd, J = 7.4, 1.7 Hz, 2H), 7.30–7.13 (m, 20H), 7.09–7.04 (m, 6H), 5.29 (ddd, J = 8.7, 5.0, 1.4 Hz, 2H), 4.69–4.63 (m, 6H), 4.09–4.04 (m, 8H), 3.11 (dd, J = 16.6, 5.4 Hz, 2H), 2.93 (dd, J = 16.6, 2.0 Hz, 2H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 171.9, 141.1, 140.3, 139.7, 139.4, 134.9, 131.0, 129.8, 129.0, 128.8, 128.7, 128.6, 127.2, 127.0, 126.4, 125.6, 124.0, 82.6, 72.5, 71.8, 71.7, 58.3, 39.5, 38.6. Anal. Calcd for $C_{52}H_{52}N_2O_8$+2H_2O: C, 71.87; H, 6.50; N, 3.22. Found: C, 71.78; H, 6.29; N, 3.06.

4.3.22. *N*1,*N*6-Bis[(1*S*,2*R*)-2-hydroxy-1-indanyl]-(2*R*,3*R*, 4*R*,5*R*)-2,5-bis(2-((*E*)-2-thiazol-2-yl-vinyl)-benzyloxy)-3,4-dihydroxyhexane-1,6-diamide (9h). A mixture of 2,4,6-trivinylcyclotriboroxane–pyridine complex (0.120 g, 0.5 mmol), 2-bromothiazole (0.045 mL, 0.5 mmol), $Pd(OAc)_2$ (5.6 mg, 0.025mmol), $[(t\text{-Bu})_3PH]BF_4$ (14.5 mg, 0.050 mmol), K_2CO_3 (138 mg, 1.0 mmol), 0.3 mL H_2O and 1 mL DME was heated by microwaves to 100 C for 15 min in a 2 mL process vial. The reaction mixture was loaded onto a 2 cm silica pad, which was subsequently washed with $CHCl_3$. The solvent was evaporated and the crude 2-vinylthiazole was dissolved in 2 mL of MeCN/H_2O (10:1) and the mixture was transferred to a process vial loaded with **7** (45.3 mg, 0.050 mmol), (i-Pr)$_2$EtN (0.052 mL, 0.30 mmol) and Herrmann's catalyst (4.7 mg, 0.0050 mmol) and heated for 10 min at 120 C. The reaction mixture was filtered through Celite and the solvent removed under reduced pressure. The residue was purified by silica gel flash chromatography (2–5% MeOH in $CHCl_3$) to give **9h** in 62% yield (26.8 mg, colorless solid). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.84 (d, J = 16.0 Hz, 2H), 7.68–7.64 (m, 2H), 7.62 (d, J = 7.7 Hz, 2H), 7.39–7.12 (m, 14H), 7.09 (d, J = 16.0 Hz, 2H), 5.32 (dd, J = 8.6, 4.9 Hz, 2H), 4.94 (d, J = 11.0 Hz, 2H), 4.71 (d, J = 11.0 Hz, 2H), 4.67–4.61 (m, 2H), 4.38 (AA′ part of AA′BB′, 2H), 4.32 (BB′ part of AA′BB′, 2H), 3.12 (dd, J = 16.7, 5.4 Hz, 2H), 2.92 (dd, J = 16.7, 2.1 Hz, 2H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 171.8, 166.9, 143.5, 140.9, 140.1, 135.5, 135.0, 131.1, 130.9, 129.2, 129.0, 128.3, 127.1, 126.2, 125.4, 124.3, 121.9, 118.9, 81.4, 72.7, 71.8, 70.8, 58.2, 39.6. HRMS (M+1): Calcd: 871.2835. Found: 871.2833.

4.3.23. *N*1,*N*6-Bis[(1*S*,2*R*)-2-hydroxy-1-indanyl]-(2*R*,3*R*, 4*R*,5*R*)-2,5-bis(2-trimethylsilanylethynylbenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (9i). Sonogashira coupling at 120 °C with trimethylsilylacetylene. Product purified by silica gel flash chromatography (1–2% MeOH in $CHCl_3$) to give **9i** in 44% yield (18.7 mg, yellow solid). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49 (dd, J = 7.3, 1.9 Hz, 2H), 7.42 (dd, J = 7.3, 2.0 Hz, 2H), 7.34–7.11 (m, 14H), 5.26 (ddd, J = 8.3, 5.0, 1.4 Hz, 2H), 4.95 (d, J = 12.1 Hz, 2H), 4.87 (d, J = 12.1 Hz, 2H), 4.74 (app dt, J = 5.2, 1.7 Hz, 2H), 4.32-4.28 (m, 4H), 3.14 (dd, J = 16.6, 5.4 Hz, 2H), 2.98 (dd, J = 16.5, 1.9 Hz, 2H), 0.24 (s, 18H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 171.6, 141.2, 139.6, 138.8, 133.0, 129.1, 128.54, 128.47, 128.3, 127.1, 125.5, 124.0, 122.5, 102.6, 100.0, 83.8, 72.7, 72.4, 71.4, 58.6, 39.7, 0.1. Anal. Calcd for $C_{48}H_{56}N_2O_8$-Si_2+0.5H_2O: C, 67.50; H, 6.73; N, 3.28. Found: C, 67.2; H, 6.7; N, 3.2.

4.3.24. *N*1,*N*6-Bis[(1*S*,2*R*)-2-hydroxy-1-indanyl]-(2*R*,3*R*, 4*R*,5*R*)-2,5-bis(2-phenylethynylbenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (9j). Sonogashira coupling at 100 °C with phenylacetylene. Product purified by silica gel flash chromatography (0–2% MeOH in $CHCl_3$) to give **9j** in 72% yield (30.6 mg, colorless solid). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55–7.52 (m, 2H), 7.50–7.47 (m, 4H), 7.44–7.41 (m, 2H), 7.38–7.28 (m, 12H), 7.22–7.19 (m, 4H), 7.09–7.07 (m, 4H), 5.24 (dd, J = 8.5, 4.9 Hz, 2H), 4.98 (d, J = 12.0 Hz, 2H), 4.91 (d, J = 12.0 Hz, 2H), 4.66 (app dt, J = 5.2, 2.1 Hz, 2H), 4.33 (AA′ part of AA′BB′, 2H), 4.28 (BB′ part of AA′BB′, 2H), 3.08 (dd, J = 16.6, 5.5 Hz, 2H), 2.89 (dd, J = 16.6, 2.1 Hz, 2H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 171.8, 141.0, 139.6, 138.2, 132.6, 131.7, 128.9, 128.84, 128.76, 128.6, 128.43, 128.42, 127.1, 125.4, 124.1, 122.8, 122.7, 94.5, 87.0, 82.7, 72.5, 72.4, 71.5, 58.3, 39.5. Anal. Calcd for $C_{54}H_{48}N_2O_8$: C, 76.04; H, 5.67; N, 3.28. Found: C, 75.8; H, 5.8; N, 3.3.

4.3.25. *N*1,*N*6-Bis[(1*S*,2*R*)-2-hydroxy-1-indanyl]-(2*R*,3*R*, 4*R*,5*R*)-2,5-bis(2-((*E*)-styryl)benzyloxy)-3,4-dihydroxyhexane-1,6-diamide (9k). A 2.0 mL microwave vial was charged with **7** (45.2 mg, 0.050 mmol), Herrmann's catalyst (2.4 mg, 0.0025 mmol), styrene (0.055 mL, 0.50 mmol), (i-Pr)$_2$EtN (0.035 mL, 0.20 mmol) and 1.0 mL of DMF/H_2O (9:1). The vial was capped with a Teflon septum and irradiated with microwaves to 120 °C for 5 min. The reaction mixture was filtered through Celite and the solvent removed under reduced pressure. The residue was purified by silica gel flash chromatography (2–5% MeOH in $CHCl_3$) to give **9k** in 63% yield (27.0 mg, colorless solid). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 (dd, J = 7.7, 1.6 Hz, 2H), 7.47–7.43 (m, 2H), 7.40 (d, J = 16.1 Hz, 2H), 7.36–7.18 (m, 20H), 7.10–6.98 (m, 4H), 6.89 (d, J = 16.1 Hz, 2H), 5.22 (dd, J = 8.6, 5.0 Hz, 2H), 4.88 (d, J = 11.7 Hz, 2H), 4.72 (d, J = 11.7 Hz, 2H), 4.53 (app dt, J = 5.3, 2.0 Hz, 2H), 4.25 (AA′ part of AA′BB′, 2H), 4.22 (BB′ part of AA′BB′, 2H), 3.02 (dd, J = 16.6, 5.5 Hz, 2H), 2.79 (dd, J = 16.6, 2.1 Hz, 2H).

^{13}C NMR (100.5 MHz, $CDCl_3$): δ 171.6, 140.8, 139.8, 137.2, 137.0, 133.8, 131.6, 130.3, 129.1, 128.9, 128.6, 128.4, 128.1, 127.8, 127.1, 126.9, 126.1, 125.4, 125.0, 124.1, 81.2, 72.6, 71.8, 71.3, 58.0, 39.5. Anal. Calcd for $C_{54}H_{52}N_2O_8 + \frac{2}{3}H_2O$: C, 74.63; H, 6.19; N, 3.22. Found C 74.6, H 6.2, N 3.3.

4.3.26. *N*1,*N*6-Bis[(1*S*,2*R*)-2-hydroxy-1-indanyl]-(2*R*,3*R*,4*R*,5*R*)-2,5-bis(2-benzofuran-2-ylbenzyloxy)-3,4-dihydroxyhexane-1,6-diamide (9l). Suzuki coupling with 2-benzofuranylboronic acid. Product purified by preparative RP-LC–MS to give 19.9 mg of **9l** (45%, colorless solid). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (dd, J = 7.7, 1.7 Hz, 2H), 7.56 (dd, J = 7.6, 1.8 Hz, 2H), 7.52–7.48 (m, 4H), 7.40 (dt, J = 7.6, 1.8 Hz, 2H), 7.33 (dt, J = 7.5, 1.8 Hz, 2H), 7.29–7.13 (m, 10H), 7.03–6.97 (m, 4H), 6.90 (d, J = 7.5 Hz, 2H), 5.21 (dd, J = 8.6, 5.1 Hz, 2H), 5.00 (d, J = 12.0 Hz, 2H), 4.91 (d, J = 12.0 Hz, 2H), 4.61–4.56 (m, 2H), 4.29–4.25 (m, 4H), 3.02 (dd, J = 16.5, 5.5 Hz, 2H), 2.79 (dd, J = 16.5, 2.3 Hz, 2H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 171.7, 154.9, 154.6, 140.9, 139.5, 133.8, 130.1, 129.13, 129.12, 129.0, 128.9, 128.4, 127.1, 125.4, 124.8, 124.0, 123.2, 121.4, 111.4, 105.8, 82.2, 72.5, 72.2, 71.6, 58.2, 39.4. Anal. Calcd for $C_{54}H_{48}N_2O_{10} + \frac{1}{2}H_2O$: C, 72.55; H, 5.52; N, 3.13. C, 72.50; H, 5.69; N, 3.25.

4.4. Computational studies

4.4.1. Preparation of ligands, protein coordinates, and definition of active sites. The structures were prepared in MOL2 format using the sketcher module of SYBYL6.9 and were then superimposed on the inhibitor *N*,*N*-[2,5-*O*-di-2-fluoro-benzyl-glucaryl]-di-[1-amino-indan-2-ol] (BED) co-crystallized with HIV1PR[24] (PDB entry 1EC0). The Tripos force field was used for the energy calculations, and a dielectric constant of 4.0 was employed. Partial charges were calculated by the method of Gasteiger and Huckel. The minimization was run until they converged to a maximum derivative of 0.05 kcal/mol/Å, and the final coordinates were stored in database. Care was taken to remove the non-polar hydrogen atoms before docking the inhibitors.

Reference protein coordinates used for docking were taken from the X-ray structure of HIV1PR in complex with BED (PDB entry: 1EC0) resolved at 1.79 Å. All the crystallization water molecules along with the native ligand were removed from the HIV1PR, except the structural waters referred to in this article as WAT00, WAT01, WAT02, WAT03, and WAT04 (Fig. 3). Only polar hydrogen atoms were added in a smart way using standard QXP geometries. Protonation states were assumed to be those most common at pH 7, that is, lysines, arginines, aspartates, and glutamates were considered in the ionized form except for D25/25′ which were kept protonated and neutral. Partial charges were calculated using bond dipole moments. Despite the hydrogen optimization, there still exist a few structural discrepancies in assigning the side chains of an X-ray structure resolved at around 2.0 Å, so it was decided to refine the crystal structure by restrained minimization. Here, the hydrogens were allowed to move freely while the rest of the protein was restrained by a force constant of 100 kJ/mol/Å^2. A 1000-step modified Polak–Ribiere conjugate gradient minimization with modified AMBER force field was carried out. A solvation model was not used; however, important structural waters were included. Once the minimization was complete, the active site was defined as the collection of amino acids enclosed within a 12.0 Å radius sphere centered on the bound ligand, which was placed in the cavity after the protein minimization. The protein now comprised of 128 amino acids and five structurally important water molecules.

4.4.2. Protein conformational flexibility and docking methodology. NMR studies have previously confirmed that the protease flaps are indeed flexible, and suggest that this flexibility may be important for inhibitor binding.[29] As a consequence, we choose to dock the ligands by taking into account this protein flexibility. Flap residues, I47/47′, G48/48′, G49/49′, I50/50′, and G51/51′, along with the residues constituting the flexible 80 s loop, T80/80′, P81/81′, V82/82′, N83/83′, I84/84′, and other critical residues, R8/8′, G27/27′, A28/28′, and D29/29′, were tethered. They were allowed to move freely up to 0.5 Å and then a quadratic 20 kJ/mol/Å^2 penalty was imposed.

The ligands were docked into the active site by Fulldock+, which is a thorough near systematic ligand docking module with contact potency scoring. Here, we carried out repeated independent docking of each ligand to get energy and geometry convergence of best docking modes followed by merging of these results and a local Monte Carlo docking search on the pooled ensemble. A total of 10 runs of systematic docking were performed and the top 40 solutions for each ligand were saved. Each of these 40 solutions was then subjected to local Monte Carlo perturbation cycles and was minimized by 300 steps of PRCG method. The conformations were randomly generated in such a way that each new conformation generated differed from the previous one by a similarity distance of 1.0 Å and had an energy window of not more than 10.0 kJ/mol/Å. The maximum movement of an atom in any single time step was limited to 0.1 Å and hydrogen vibrations were damped by assigning an atomic weight of 10.

4.4.3. Data set and 3D QSAR studies. The data set comprised of 24 docked conformations of the inhibitors, which were used to generate the CoMFA alignment. The CoMFA fields were generated using default settings as implemented in Sybyl 7.0. A 3D cubic lattice was created with a grid spacing of 2 Å and extending 4 Å units beyond the docked inhibitors in all directions. An sp^3 carbon with +1 charge was used as probe atom to produce steric and electrostatic field energies. Cut-offs for both steric and electrostatic fields were set to 30 kcal/mol, employing a distance dependent dielectric ($1/r^2$) for calculating electrostatics and a 6–12 Lennard–Jones potential for sterics. The CoMFA descriptor fields were used as independent variables in the PLS (partial least squares)[30] regression analysis with measured pK_i ($-\log K_i$) values as the dependent variable. A LOO

(leave-one-out) validation, and a maximum of six components were used for all cross-validation calculations to determine the q^2 (cross-validated correlation coefficient), the PRESS value (standard error of prediction) and the r^2 (non-validated correlation coefficient).

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