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ORIGINAL RESEARCH

Synthesis and NMR elucidation of pentacycloundecane-derived hydroxy acid peptides as potential anti-HIV-1 agents

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Abstract The synthesis and NMR elucidation of eight novel peptides incorporating the pentacycloundecane (PCU)-derived hydroxy acid are reported. The PCU cage amino acids were synthesized as racemates and the incorporation of the PCU-derived hydroxy acid with natural (*S*)-amino acids produced inseparable diastereomeric peptides. A series of overlapping signals from the cage and that of the peptide side chain was observed in the ^{1}H- and ^{13}C-NMR spectra, complicating the elucidation thereof. Two-dimensional NMR techniques proved to be a very useful tool in overcoming these difficulties. These compounds are potential HIV protease inhibitors.

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Introduction

Polycyclic cage compounds have been studied extensively over the past few decades [1–24]. South African authors have contributed considerably toward utilizing NMR spectroscopy as a tool to determine the structures of these cage compounds, several of which have found application in medicinal chemistry [10, 15, 19, 24–45]. The assignments of ^{1}H spectra of the cage compounds becomes more challenging due to broad unresolved overlapping resonances as a result of through-space effects, geminal and vicinal proton–proton coupling, and long-range proton–proton interactions. Our research group has extensively used two-dimensional (2D) NMR spectroscopic techniques to study the structure of the cage skeleton in relation to its side "arms" [19, 24, 37–45]. As a part of our ongoing research program, the synthesis of eight pentacycloundecane (PCU)-derived hydroxy acid peptides (**1–8**) was attempted (Fig. 1).

The use of cage compounds in drug design has found several applications in the pharmaceutical drug discovery research [11, 15, 16, 19–24, 44–48]. Cage-based compounds have been reported to improve drug lipophilicity; it also serves as a transport medium in facilitating drug delivery through cell membranes such as the blood–brain barrier (BBB) and the central nervous system (CNS) [2, 4, 23, 49]. The cage compounds increase the duration of drug action by retarding the action of drug metabolism [13, 50].

In this study, the PCU hydroxy acid [33] incorporated into short peptides was used to mimic an irreversible non-scissile ether-bridged type isostere as potential HIV-1

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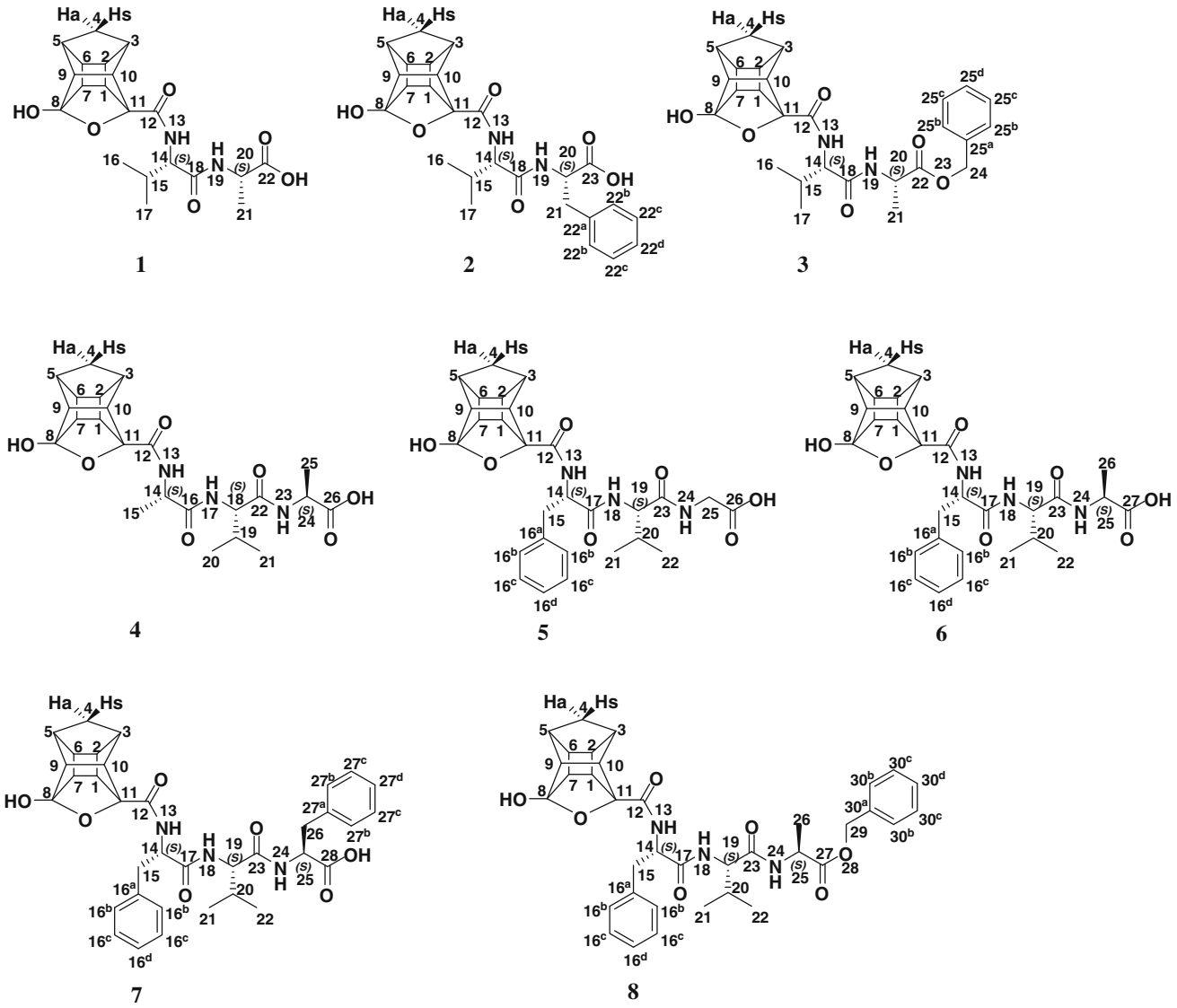


Fig. 1 Structures of compounds **1-8**

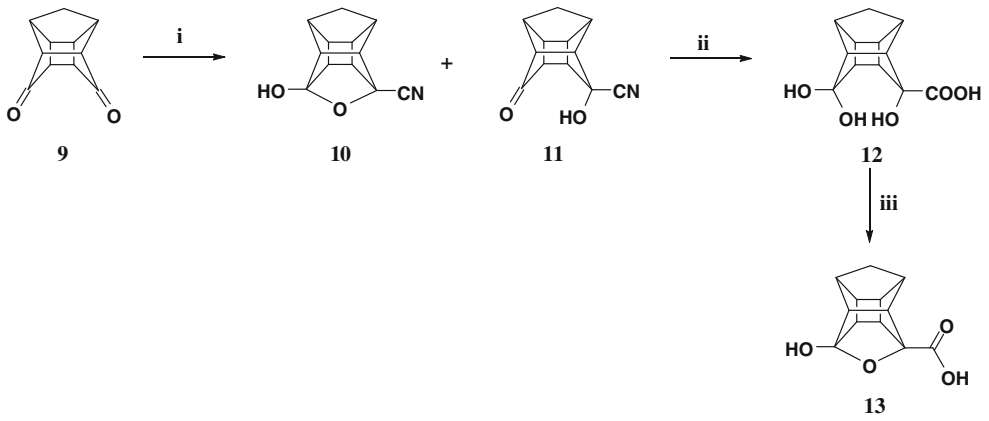
protease inhibitors analogs to a non-cage ether reported by Ghosh et al. [51]. It was shown that the cage ether bridge (C_9–C_8–O–C_{11}–C_{10}) is virtually non-hydrolyzable under conditions that were harsher than physiological conditions [33]. Compounds **1**, **5**, and **6** (Fig. 1) gave promising in vitro activity against HIV-1 wild-type C-SA protease enzyme with IC_{50} values ranging from 0.6 to 2 μm [22].

Synthesis

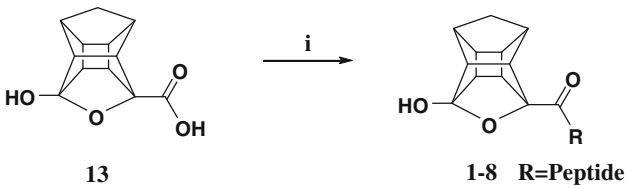
The PCU peptide derivatives **1–8** were synthesized from the cage ether **13**, with Cookson's dione **9** [3, 7, 52] as the starting material. The latter can be easily obtained from the photocyclization of the Diels–Alder adduct of cyclopentadiene and *p*-benzoquinone. Treatment of the dione **9** with aqueous sodium cyanide stirred in a mixture of water and acetic acid resulted in a mixture of cyanohydrins (**10** and **11**) [33]. This cyanohydrin mixture, when treated with concentrated hydrochloric acid, gave the tri-hydroxy carboxylic acid **12** [33]. The resulting tri-hydroxy carboxylic acid **12** was refluxed in 1,4-dioxane to give the desired hydroxy acid **13** as a racemate. These reactions are outlined in Scheme 1.

Compounds **1–8** were obtained by coupling the corresponding short peptides to **13** in the presence of coupling reagents HATU, DIPEA in DMF at ambient temperatures as shown in Scheme 2. It should be noted that the resulting cage peptides are mixtures of two diastereomers as the PCU hydroxy cage acid was synthesized as a racemate.

The products **1–8** (Fig. 1) were isolated and purified as described in the "Experimental" section. It was not



Scheme 1 Synthesis of 5-hydroxy-4- oxahexacyclo[5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$.$0^{8,11}$]dodecane -3-carboxylic acid **13**; *i* NaCN, CH_3COOH, H_2O, 0 °C; *ii* conc. HCl, reflux; *iii* 1,4-dioxane, reflux



Scheme 2 Synthesis of 5-hydroxy-4-oxahexacyclo[5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$.$0^{8,11}$]dodecane peptide analogs. *i* HATU, DIPEA, DMF, rt

possible to separate the diastereomeric mixtures by preparative HPLC. The correctness of the cage peptides were confirmed with standard NMR and MS techniques. The synthesized cage peptides were obtained in high purity (>98 %) which was determined with HPLC. These synthesized compounds **1–8** are polar and lipophilic in nature with CLog*P* values −0.76, 1.08, 1.17, −1.03, 0.46, 0.81, 2.65, and 2.74, respectively.

Results and discussion

The PCU cage structures show complex overlapping of the protons as the PCU cage derivatives (**1–8**) are diastereomeric, asymmetric, and non-meso compounds which further complicates the structural elucidation of these compounds. Due to the diastereomeric nature of these compounds, all the ^{13}C NMR signals of these compounds (**1–8**) are split. It is known from the literature that the proton NMR spectrum of PCU cage compounds usually show a pair of doublets (geminal protons) with an AB spin resonance at approximately 1.5 and 1.8 ppm, having a coupling constant of about $\approx$10 Hz. The assignments for all PCU cage compounds **1–8** are presented in Tables 1 and 2.

The geminal bridge methylene proton (H-4a) for the PCU hydroxy peptide **1** register as a doublet (AB system) at 1.47 ppm (H-4a) with a coupling constant of 10.4 Hz, but in the case of H-4s a pair of doublets is observed resonating at 1.82 and 1.84 ppm with a *J* value of 6.0 Hz. Similar observations are recorded for compounds **5** and **6**, which can perhaps be attributed to a side-chain conformational effect. For compound **3**, the H-4s signal at 1.83 ppm (J = 9.4 Hz) appears as a triplet due to coalescence of the pair of doublets. In PCU cage compounds, proton H-4a (H-4s) normally exhibits COSY and NOESY/ROESY interactions with H-3/5 and H-2/6 (H-3/5 and H-9/10), respectively, which has proven to be a convenient handle in solving the NMR spectra of these compounds. A similar interaction is also observed between H-2/6 and H-1/7. HMBC, NOESY, and ROESY techniques are useful methods to determine the position of the "arms"/substituents in relation to the cage moiety especially when the cage is asymmetric.

In particular, both H-4a (1.47 ppm) and H-4s (1.83 ppm) of **1** display COSY correlations to the resonances at 2.52 and 2.46 ppm. These resonances were assigned to H-3/H-5. As the molecule is asymmetric, the specific assignment is still pending. In addition, the H-4a resonance shows a NOESY correlation to 2.66 and 2.68 ppm (H-2/H-6) and the H-4s resonance to 2.52 and 2.71 ppm (H-9/H-10). In order to distinguish between the proton resonances of H-9 and H-10, a HMBC correlation between carbonyl resonance C-12 (170.5 ppm) and the protons resonance at 2.71 ppm indicated that this resonance is associated to H-10. The position of H-10 was further confirmed as it displayed COSY correlation to a proton signal at 2.52 ppm, which was assigned to H-9. The carbonyl resonance of C-12 also displays a HMBC correlation to H-10 and H-1 (2.75 ppm). The latter in turn showed a COSY correlation to H-2 (2.66 ppm). A COSY correlation between the resonance at 2.52 ppm (also H-9) and the signal of H-10 as well as H-2 was observed, indicating that the proton signal of H-3 and H-9 are overlapping. This was further confirmed by the HMBC spectrum, wherein the carbon peaks of C-3 (46.1 ppm) and C-9

Table 1 ^{1}H NMR data for PCU hydroxy peptides **1–8**

Atom	1 $\delta^{1}H^{B,C}$	2 $\delta^{1}H^{A,C}$	3 $\delta^{1}H^{B,C}$	4 $\delta^{1}H^{A,C}$	5 $\delta^{1}H^{B,C}$	6 $\delta^{1}H^{B,C}$	7 $\delta^{1}H^{A,C}$	8 $\delta^{1}H^{A,C}$
1	2.75	2.77	2.72	2.76	2.73	2.73	2.71	2.72
2	2.66	2.65	2.58	2.63	2.59	2.59	2.57	2.58
3	2.52	2.51	2.45	2.47	2.44	2.46	2.43	2.45
4a	1.47 d, J = 10.4 Hz	1.47 d, J = 10.1 Hz	1.47 d, J = 9.3 Hz	1.48 d, J = 10.2 Hz	1.44 d, J = 10.2 Hz	1.45 d, J = 10.1 Hz	1.44 d, J = 10.2 Hz	1.44 d, J = 10.1 Hz
4s	1.82/1.84 d, J = 6.0 Hz, H-4 s/4sb^{D}	1.83 d, J = 10.1 Hz	1.82 t, J = 9.3 Hz, H-4 s^{E}	1.83 d, J = 10.2 Hz	1.79/1.80 d, J = 6.0 Hz, H-4 s/4sb^{D}	1.79/1.80 d, J = 6.1 Hz, H-4 s/4sb^{D}	1.78 d,J = 10.2 Hz	1.79 d, J = 10.1 Hz
5	2.46	2.49	2.44	2.47	2.45	2.45	2.43	2.44
6	2.68	2.66	2.64	2.67	2.65	2.67	2.66	2.64
7	2.48	2.51	2.47	2.50	2.44	2.42	2.46	2.45
8	–	–	–	–	–	–	–	–
9	2.52	2.55	2.49	2.51	2.47	2.48	2.45	2.50
10	2.71	2.69	2.66	2.68	2.65	2.65	2.66	2.64
11	–	–	–	–	–	–	–	–
12	–	–	–	–	–	–	–	–
13	6.91/6.93 d, J = 3.1 Hz, NH-13a/13b^{D}	6.95 d, J = 9.3 Hz	6.88/6.90 d, J = 5.7 Hz, NH-13a/13b^{D}	7.48/7.50 d, J = 3.2 Hz, NH-13a/13b^{D}	7.40 t, J = 9.0 Hz, NH-13^{E}	7.44 t, J = 9.0 Hz, NH-13^{E}	7.38/7.41 d, J = 5.5 Hz, NH-13a/13b^{D}	7.44/7.46 d, J = 6.3 Hz, NH-13a/13b^{D}
14	4.25	4.16	4.24	4.37	4.62	4.60	4.61	4.60
15	1.97	1.92	1.93	1.22	2.99	2.99	2.90/2.98	2.97
16/16^{b-d}	0.79/0.81 d, J = 6.7 Hz, H-16a/16b^{F}	0.73-0.77	0.74/0.77 d, J = 6.7 Hz, H-16a/16b^{F}	–	7.08–7.23	7.11–7.24	7.08–7.16	7.10–7.22
17	0.86/0.88 d, J = 6.7 Hz, H-17a/17b^{F}	0.73-0.77	0.82/0.84 d, J = 6.7 Hz, H-17a/17b^{F}	7.81/7.87 d, J = 9.0 Hz, NH-17a/17b^{D}	–	–	–	–
18	–	–	–	4.21	8.01/8.05 d, J = 9.0 Hz, NH-18a/18b^{D}	7.97 d/8.02, J = 9.0 Hz, NH-18a/18b^{D}	7.93/7.98 d, J = 9.1 Hz, NH-18a/18b^{D}	8.01/8.06 d, J = 9.0 Hz, NH-18a/18b^{D}
19	8.44 t, J = 8.0 Hz, NH-19^{E}	8.40 t, J = 7.1 Hz, NH-19^{E}	8.60/8.61 d, J = 6.8 Hz, NH-19a/19b^{D}	1.94	4.24	4.23	4.25	4.23
20	4.19 q, J = 7.4 Hz	4.44	4.33	0.82/0.84 d, J = 3.9 Hz, H-20a/20b^{F}	1.97	1.96	1.96	1.92
21	1.26/1.27 d, J = 5.2 Hz, H-21a/21b^{F}	2.85/3.07	1.30/1.31 d, J = 5.2 Hz, H-21a/21b^{F}	0.87/0.88 d, J = 3.4 Hz, H-21a/21b^{F}	0.87	0.86 t, J = 6.0 Hz, H-21^{E}	0.83	0.83
22/22^{b-d}	–	7.17-7.27	–	–	0.87	0.89/0.90 d, J = 4.6 Hz, H-22a/22b^{F}	0.83	0.83
23	–	–	–	8.25/8.27 d, J = 3.4 Hz, NH-23a/23b^{D}	–	–	–	–
24	–	–	5.10 d, J = 1.3 Hz	4.21	8.36	8.36 t, J = 6.3 Hz, H-24^{E}	8.33 d, J = 7.2 Hz	8.55/8.57 d, J = 4.6 Hz, NH-24a/24b^{D}
25/25^{b-d}	–	–	7.30-7.37	1.25	3.78	4.20	4.45	4.35
26	–	–	–	–	–	1.29 d, J = 7.2 Hz	2.92/3.05	1.32 d, J = 7.2 Hz
27/27^{b-d}	–		–	–	–	–	7.17-7.25	–
28	–		–	–	–	–	–	–

Table 1 continued

Atom	1 $\delta^1H^{B,C}$	2 $\delta^1H^{A,C}$	3 $\delta^1H^{B,C}$	4 $\delta^1H^{A,C}$	5 $\delta^1H^{B,C}$	6 $\delta^1H^{B,C}$	7 $\delta^1H^{A,C}$	8 $\delta^1H^{A,C}$
29	–		–	–	–	–	–	5.11
30/30[b−d]	–		–	–	–	–	–	7.33–7.37

[A] 400 MHz

[B] 600 MHz

[C] Solvent $(CD_3)_2SO$

[D] Two separate resonances potentially due to a side-chain conformational effect

[E] Due to coalescence of the pair of doublets, the signal appears as a triplet

[F] The methyl protons appear as a pair of doublets

(56.2 ppm) displayed correlation to the same proton signal resonating at 2.52 ppm (H-3). Since now the position of H-3 (2.52 ppm) is confirmed, therefore, the resonance at 2.46 ppm was attributed to H-5. In addition to the COSY correlation between H-1 and H-2, a further correlation between H-1 and 2.48 ppm allowed this resonance to be assigned to H-7. In the ^{13}C NMR spectrum, the quaternary carbon signals appearing at 118.2 and 89.75 ppm were assigned to C-8 and C-11 based on their deshielding effect and chemical environment. All the remaining cage carbon signals were obtained from the HSQC spectrum.

The next section describes the NMR correlations for the rest of the peptide sequence attached to the PCU cage of compound **1**. The methyl (alanine) and isopropyl methyl (valine) carbons register at 17.1, 17.8, and 19.1 ppm and were identified from the APT ^{13}C NMR spectrum. The carbon signal at 17.1 ppm exhibits HSQC correlations to the ^1H NMR signals appearing as a pair doublets at 1.26 and 1.27 ppm ($J = 5.20$ Hz), similarly the isopropyl methyl carbon signals 17.8 and 19.1 ppm display correlations to two pairs of doublets at 0.79/0.81 and 0.86/0.88 ppm with a coupling constant approximately $\approx$6.7 Hz. The isopropyl methyl groups are also split due to the asymmetric nature of the environment close by. Hence, these side-chain methyl protons were assigned to H-21 (1.26/1.27 ppm), H-16 (0.79/0.81 ppm), and H-17 (0.86/0.88 ppm), respectively. The H-15 methine signal at 1.97 ppm was identified by a COSY correlation to the isopropyl methyl protons H-16 and H-17. This H-15 resonance in turn shows a COSY interaction with the chiral H-14 methine proton signal appearing as a multiplet at 4.25 ppm. The chiral H-14 proton further displays COSY correlation to H-13 (NH) a doublet signal resonating at 6.91 ppm ($J = 4.1$ Hz). For this resonance, a pair of doublets is seen because of the diastereomeric nature of the compound, which is further validated by the APT ^{13}C NMR spectrum where the carbonyl carbon (C-12) is registered as a split signal. The H-15 resonance shows a HMBC correlation to a carbonyl carbon registered at 170.2 ppm. This was then assigned to C-18.

The H-21 methyl signal shows a correlation with the chiral methine proton H-20 appearing as a quartet signal at 4.19 ppm ($J = 7.40$ Hz). H-20 further shows a COSY correlation to a triplet proton signal at 8.44 ppm (H-19, $J = 8.04$ Hz). This pair of doublets coalesces into a triplet signal at 8.44 ppm. All the compounds **1**–**8** give perfect pairs of doublets for the corresponding NH proton signals. From literature, it is known that NH proton signals in linear peptides usually appear as doublets [53]. As these PCU cage peptides are a mixture of diastereomers, the amide protons appear as a pair of doublets. The remaining carbonyl peak registers at 173.8 ppm in the ^{13}C NMR spectrum and was assigned to C-22. This was further confirmed by HMBC spectra wherein H-21 displays correlation to the terminal carbonyl carbon C-22. All other assignments were further verified utilizing HSQC and HMBC spectra. ROESY correlations were observed between H-13 of the peptide chain and the PCU cage protons (H-1, H-2 and H-10). Further to this, the isopropyl methyl protons H-16/H-17 displayed ROESY correlations to H-1, H-4a, and H-4s.

In NOESY experiments, the nuclear Overhauser effect (NOE) depends on the correlation time τ_c of the compound and the observation frequency ω. As the NOE changes sign at $\omega\tau_c \approx 1.12$, leading to little or no NOE observations for medium-sized compounds (MW $\approx$1,000 g mol^{-1}) [54]. This results in poor correlation signals and also prevents the extraction of distance information from the NOESY spectra. In order to overcome this problem, ROESY experiments were carried out for compounds 1, 4, 5, 6, and 7 to look for interactions between closely related protons. As ROESY spectrum yields negative cross-peaks (corresponding to positive Overhauser enhancements) for all values of $\omega\tau_c$ [55–57]. For the remainder of the compounds **2**–**8**, the same procedure was used for the elucidation of the cage signals. This discussion for the remaining compounds will be omitted for the sake of brevity. For the peptide side chains, only differences to previous compounds will be highlighted. The assignments for compound **1** are summarized in Tables 1 and 2.

Table 2 ^{13}C NMR chemical shifts of compounds **1–8**

Atom	1 $\delta^{13}C^{B,C}$	2 $\delta^{13}C^{A,C}$	3 $\delta^{13}C^{B,C}$	4 $\delta^{13}C^{A,C}$	5 $\delta^{13}C^{B,C}$	6 $\delta^{13}C^{B,C}$	7 $\delta^{13}C^{A,C}$	8 $\delta^{13}C^{A,C}$
1	48.9 (CH)	48.7 (CH)	48.2 (CH)	48.4 (CH)	48.1 (CH)	48.2 (CH)	48.1 (CH)	48.2 (CH)
2	42.2 (CH)	42.1 (CH)	42.2 (CH)	42.2 (CH)	42.1 (CH)	42.1 (CH)	42.0 (CH)	42.1 (CH)
3	46.1 (CH)	46.0 (CH)	46.2 (CH)	46.1 (CH)	46.2 (CH)	46.1 (CH)	46.0 (CH)	46.1 (CH)
4a	42.8 (CH_2)	42.8 (CH_2)	42.8 (CH_2)	42.8 (CH_2)	42.8 (CH_2)	42.8 (CH_2)	42.8 (CH_2)	42.8 (CH_2)
4s	42.8 (CH_2)	42.8 (CH_2)	42.8 (CH_2)	42.8 (CH_2)	42.8 (CH_2)	42.8 (CH_2)	42.8 (CH_2)	42.8 (CH_2)
5	42.7 (CH)	42.7 (CH)	42.8 (CH)	42.7 (CH)	42.7 (CH)	42.7 (CH)	42.7 (CH)	42.8 (CH)
6	41.1 (CH)	40.9 (CH)	41.0 (CH)	41.0 (CH)	40.9 (CH)	40.9 (CH)	41.0 (CH)	41.0 (CH)
7	46.3 (CH)	46.2 (CH)	46.4 (CH)	46.3 (CH)	46.2 (CH)	46.3 (CH)	46.3 (CH)	46.4 (CH)
8	118.2 (C)	118.1 (C)	118.2 (C)	118.2 (C)	118.1 (C)	118.2 (C)	118.1 (C)	118.2 (C)
9	56.2 (CH)	56.4 (CH)	56.2 (CH)	56.2 (CH)	56.1 (CH)	56.1 (CH)	56.1 (CH)	56.2 (CH)
10	58.6 (CH)	58.5 (CH)	57.9 (CH)	58.2 (CH)	57.9 (CH)	57.9 (CH)	58.2 (CH)	57.9 (CH)
11	89.8 (C)	89.6 (C)	89.7 (C)	89.5 (C)	89.6 (C)	89.5 (C)	89.5 (C)	89.6 (C)
12	170.5 (C)	170.4 (C)	170.5 (C)	170.3 (C)	170.5 (C)	170.4 (C)	170.7 (C)	170.5 (C)
13	–	–	–	–	–	–	–	–
14	56.4 (CH)	56.5 (CH)	55.9 (CH)	47.8 (CH)	52.9 (CH)	53.1 (CH)	52.9 (CH)	53.1 (CH)
15	31.5 (CH)	31.4 (CH)	31.5 (CH)	18.6 (CH_3)	37.3 (CH_2)	37.4 (CH_2)	37.4 (CH_2)	37.3 (CH_2)
16/16[a]	17.8/19.1 (CH_3)	17.8/19.0 (CH_3)	17.7/19.0 (CH_3)	171.8 (C)	137.3 (C)	137.3 (C)	137.4 (C)	137.4 (C)
16[b]	–	–	–	–	129.3 (CH)	129.2 (CH)	129.2 (CH)	129.2 (CH)
16[c]	–	–	–	–	127.9 (CH)	127.9 (CH)	128.1 (CH)	128.0 (CH)
16[d]	–	–	–	–	126.2 (CH)	126.2 (CH)	126.3 (CH)	126.2 (CH)
17	17.8/19.1 (CH_3)	17.8/19.0 (CH_3)	17.7/19.0 (CH_3)	–	170.3 (C)	170.2 (C)	170.3 (C)	170.3 (C)
18	170.1 (C)	170.4 (C)	170.4	56.8 (CH)	–	–	–	–
19	–	–	–	31.1 (CH)	57.2 (CH)	57.0 (CH)	57.0 (CH)	56.9 (CH)
20	47.2 (CH)	53.3 (CH)	47.6 (CH)	17.9/19.1 (CH_3)	30.9 (CH)	31.1 (CH)	31.1 (CH)	31.1 (CH)
21	17.1 (CH_3)	36.6 (CH_2)	16.7 (CH_3)	17.9/19.1 (CH_3)	18.0/19.0 (CH_3)	18.1/19.0 (CH_3)	17.9/19.1 (CH_3)	18.1/19.0 (CH_3)
22/22[a]	173.9 (C)	137.4 (C)	172.1 (C)	170.3 (C)	18.0/19.0 (CH_3)	18.1/19.0 (CH_3)	17.9/19.1 (CH_3)	18.1/19.0 (CH_3)
22[b]	–	128.9 (CH)	–	–	–	–	–	–
22[c]	–	128.1 (CH)	–	–	–	–	–	–
22[d]	–	126.3 (CH)	–	–	–	–	–	–
23	–	172.7 (C)	–	–	171.0 (C)	170.5 (C)	170.5 (C)	170.6 (C)
24	–	–	66.0 (CH_2)	47.4 (CH)	–	–	–	–
25/25[a]	–	–	138.8 (C)	17.0 (CH_3)	40.5 (CH_2)	47.4 (CH)	53.4 (CH)	47.6 (CH)
25[b]	–	–	127.8 (CH)	–	–	–	–	–
25[c]	–	–	128.0 (CH)	–	–	–	–	–
25[d]	–	–	128.4 (CH)	–	–	–	–	–
26	–	–	–	173.9 (C)	171.1 (C)	16.9 (CH_3)	36.5 (CH_2)	16.7 (CH_3)
27/27[a]	–	–	–	–	–	173.9 (C)	137.4 (C)	172.2 (C)
27[b]	–	–	–	–	–	–	128.9 (CH)	–
27[c]	–	–	–	–	–	–	127.8 (CH)	–
27[d]	–	–	–	–	–	–	126.2 (CH)	–
28	–	–	–	–	–	–	172.7 (C)	–
29	–	–	–	–	–	–	–	65.9 (CH_2)
30[a]	–	–	–	–	–	–	–	135.9 (C)

Table 2 continued

Atom	1 $\delta^{13}C^{B,C}$	2 $\delta^{13}C^{A,C}$	3 $\delta^{13}C^{B,C}$	4 $\delta^{13}C^{A,C}$	5 $\delta^{13}C^{B,C}$	6 $\delta^{13}C^{B,C}$	7 $\delta^{13}C^{A,C}$	8 $\delta^{13}C^{A,C}$
30[b]	–	–	–	–	–	–	–	127.8 (CH)
30[c]	–	–	–	–	–	–	–	128.4 (CH)
30[d]	–	–	–	–	–	–	–	127.9 (CH)

[A] 100 MHz for ^{13}C

[B] 150 MHz for ^{13}C

[C] Solvent $(CD_3)_2SO$

For compound **2**, the aromatic protons in the ^{1}H NMR spectra are not well resolved, but the carbons for the aromatic region in the ^{13}C NMR spectra are very well separated. In the HMBC spectrum, the chiral methine proton H-20 (4.44 ppm) displays a correlation to the quaternary carbon C-22[a] (137.4 ppm) of the phenyl ring and the diastereomeric methylene protons H-21[a]/H-21[b] (2.85/3.07 ppm) show correlations to the aromatic carbon C-22[b] (128.9 ppm). Carbon signals for C-22[c] (128.1 ppm) and C-22[d] (126.3 ppm) were assigned [58, 59] based on the intensity of the peaks.

The carbonyl signal at 170.4 ppm for compound **2** shows HMBC correlations to various proton signals (H-20, H-19, H-15, H-14, H-13, H-10, and H-1), which confirms the overlapping of the carbonyl carbon signals of C-18 and C-12. The methodology to elucidate the remaining signals of the peptide side chain was similar to that described for compound **1**. NOESY correlations were observed between H-13 and H-1. The aromatic protons (H-22) displayed through space correlations to H-9 and H-6 of the cage protons. The assignments of the peaks were further confirmed by HMBC and HSQC spectra. These assignments are presented in Tables 1 and 2.

In the ^{1}H NMR spectra of compound **3**, a doublet (diastereomeric) methylene proton signal (H-24) resonating at 5.10 ppm ($J = 1.38$ Hz) and integrating to two protons shows HMBC correlation to C-22 (172.1 ppm). H-24 further shows HMBC correlations to a quaternary and aromatic carbon signals registered at 138.8 and 127.8 ppm, which were assigned to C-25[a] and C-25[b], respectively. These assignments were further confirmed from the ^{13}C-APT spectra. Based on the intensity of the peaks [58, 59], the carbon signals registered at 128.0 and 128.4 ppm were assigned to C-25[c] and C-25[d]. Through-space interactions were observed in the NOESY spectrum between the PCU cage protons (H-4a, H-1, H-2, and H-10) and the methyl protons of the peptide chain (H-16 and H-17). The ^{1}H- and ^{13}C-NMR assignments are shown in Tables 1 and 2.

For compound **4**, no ROESY correlations were observed between the PCU cage protons and the peptide chain. In ROESY spectrum of compound **5**, the aromatic protons H-16 displayed through space correlation with the PCU cage protons H-5, H-7, and H-9, whereas in compound **6** H-13 displays ROESY correlations to the proton signals H-3, H-10, H-21/22, and H-26. For compound **7**, the aromatic protons H-16 and H-27 display ROESY correlations to the isopropyl methyl protons H-21/22. Similarly, in compound **8**, the isopropyl protons H-21/22 display NOESY correlations to the aromatic protons H-30 and the cage proton H-1. The ^{1}H- and ^{13}C-NMR peak assignments of compounds **4**–**8** are given in Tables 1 and 2.

Conclusion

The synthesis and complete NMR elucidation of eight novel PCU cage peptides were successfully carried out. The synthesis was achieved utilizing known protocols from the literature. The NOESY and ROESY experiments showed interesting through space correlations between the cage side chain and the PCU cage protons. Compounds **1–8** are all non-separable diastereomers and are unsymmetrical in nature, which complicated the assignments of PCU cage protons. However, 2D NMR spectroscopy proved to be a crucial tool for effective structural elucidation of these compounds in spite of major overlapping of proton signals. All the ^{13}C carbon signals for these compounds are split. Incorporation of the PCU hydroxy cage acid **13** into the peptides as potential HIV-1 protease inhibitors was successful.

Experimental

Analysis was performed on an Agilent 1100 HPLC (Waters Xbridge C18 150 mm × 4.6 mm × 5 µs) coupled to a UV detector (215 nm) and an Agilent VL ion trap mass spectrophotometer in the positive mode. Semi-preparative HPLC was carried out on a Shimadzu 8A instrument (Ace C18 150 mm × 21.2 mm × 5 µs) with a UV/Vis detector (215 nm) and automated fraction collector. A two-buffer system was employed, utilizing formic acid as the ion-pairing agent. Buffer A consisted of 0.1 % formic acid/H_2O (v/v) and buffer B consisted of 0.1 % formic acid/

acetonitrile (v/v). High-resolution electron spray ionization mass spectroscopic (HR-ESI-MS) analysis was performed on a Bruker MicroTOF QII mass spectrometer in positive mode with an internal calibration. Microwave couplings were conducted on a Discovery CEM Liberty microwave peptide synthesizer. The coupling conditions of the peptide synthesizer were adapted from literature [19, 20]. Optical rotations were measured at room temperature in dry methanol using a Perkin Elmer Polarimeter-Model 341. All IR spectra were recorded on a Perkin Elmer Spectrum 100 instrument with a universal ATR attachment.

The ^{1}H- and ^{13}C-NMR data for compounds **2**, **4**, **7**, and **8** were recorded on a Bruker AVANCE III 400 MHz spectrometer and compounds **1**, **3**, **5**, and **6** were analyzed using a Bruker AVANCE III 600 MHz when higher sensitivity was required. The chemical shifts were referenced to the solvent peak $\delta = 2.50$ ppm (^{1}H) and 39.9 ppm (^{13}C) for $(CD_3)_2SO$ at room temperature. The ^{1}H-NMR spectra for compounds **1**, **3**, **5**, and **6** were recorded at a transmitter frequency of 600.100 MHz, with a spectral width of 12,335.526 Hz; acquisition time of 1.328 s, pulse width of 13.57 μs (**1** and **6**) and 13.75 μs (**3** and **5**); relaxation delay of 1.0 s and scans of 8 and 16 for compounds **1** and **3**, **5**, **6**, respectively. For compounds **2**, **4**, **7**, and **8**, the ^{1}H-NMR spectra were recorded at a transmitter frequency of 400.220 MHz, with a spectral width of 8223.685 Hz; acquisition time of 1.992 s (**2**, **7**, and **8**) and 3.98 s (**4**); pulse width of 10.00 μs; relaxation delay of 1.0 s and scans of 16.

The ^{13}C-NMR spectra for compounds **1**, **3**, **5**, and **6** were recorded at 150.910 MHz, with a spectral width of 36,057.69 Hz; acquisition time of 0.908 s; pulse width of 9.00 μs; relaxation delay of 2 s and scans of 8160, 1600, 4800 (**7**, and **8**), respectively. All the previous data were obtained from Bruker AVANCE III 600 MHz instrument. For compounds **2**, **4**, **7**, and **8**, the ^{13}C-NMR spectra were recorded at 100.645 MHz, with a spectral width of 24,038.461 Hz; acquisition time of 1.363 s; pulse width of 8.40 μs; relaxation delay of 2 s and scans of 1024 (**2**, **4** and **8**), 512 (7), respectively, on AVANCE III 400 MHz NMR instrument.

All 2D experimental data obtained on the Bruker AVANCE III 600 MHz for compounds **1**, **3**, **5**, and **6** were as follows: 90° pulse width of 10.00 μs (**3**), 13.75 μs (**3** and **5**) and 13.57 μs (**6**), respectively, for all spectra. Spectral widths for ^{1}H spectra **1**, **3**, **5**, and **6** are 12,335.526 Hz (COSY), 72,11.539 Hz (ROESY), 7,075.472 Hz (for **3**, NOESY), 12,335.526 Hz (HSQC), 9,615.385 Hz (HMBC). For compounds **2**, **4**, **7**, and **8** all 2D experimental data obtained on Bruker AVANCE III 400 MHz were as follows: 90° pulse width of 10.00 μs for all spectra. The ^{1}H spectral widths for all the 2D experiments (COSY, ROESY, HSQC, and HMBC) are 5813.954 Hz (**2**), 5952.381 Hz (**4**), 5747.126 Hz (**7**), and 3703.704 Hz (**8**), respectively. The spectral widths for the ^{13}C spectra are 12335.526, 33333.332 Hz (HSQC and HMBC) for **1**, **3**, **5**, and **6**; 5813.954, 16666.666 (HSQC), 5813.954, 27932.961 Hz (HMBC) for **2**; 5952.381, 16666.666 (HSQC); 5952.381, 27932.961 Hz (HMBC) for **4**; 5747.126; 16666.666 Hz (HSQC), 5747.126, 27932.961 Hz (HMBC) for **7**; and 3703.704 Hz, 16666.666 Hz (HSQC), 3703.704 Hz, 27932.961 Hz (HMBC) for **8**. The number of data points per spectrum in F2 are 2048 (COSY, NOESY/ROESY for **1–8**), 2048 (HSQC for **1**, **3**, **5**, and **6**), 1024 (HSQC for **2**, **4**, **7**, and **8**), and 4096 (HMBC for **1–8**), whereas the number of time-incremented spectra in F1 were 128 (COSY), 256 (NOESY for **2–4** and **8**), 512 (ROESY for **5–7** and **1**), 128 (HMBC), and 256 (HSQC) for compounds **1–8**; relaxation delay was 3.0 s (COSY and HMBC for **1**, **3**, **5**, and **6**), 1.5 s (COSY for **2**, **4**, **7**, and **8**), 1.5 s (ROESY for **1** and **5–7**), 1.5 s (HSQC and HMBC for **2**, **4**, **7**, and **8**), 2.0 s (NOESY for **2–4** and **8**) and 1.0 s (HSQC for **1**, **3**, **5**, and **6**). The spectra acquired in phase-sensitive mode for all compounds are NOESY/ROESY and HSQC; the spectra for all compounds were acquired in absolute value mode. Gradients were used for COSY, HSQC, and HMBC spectra of all compounds. All NMR spectra are available as Supplementary Material. All the amino acids, resins, and coupling reagents are commercially available and were purchased from GL Biochem (Shanghai) Ltd. Analytical grade solvents for synthesis were procured from Sigma-Aldrich (South Africa).

General procedure for the synthesis of 5-hydroxy-4-oxahexacyclododecane-3-carboxylic acid, compound **13**

Compound **13** was synthesized according to the previously reported method [33].

General procedure for manual SPPS loading of first amino acid to the 2-chlorotrityl chloride resin [19, 20]

Activated 2-chlorotrityl chloride resin (1 g, 1.33 mmol) was swelled in dry DCM (10 mL) for 10 min in a sintered glass reaction vessel. Fmoc-amino acid-OH (3.99 mmol) in DCM (10 mL) and *N*,*N*-diisopropylethylamine (DIPEA) (6.65 mmol) were added to the resin and were mixed for 2 h with a stream of nitrogen bubbles. The solvent was removed by filtration and the resin was washed with DCM (3 × 10 mL). A few resin beads (~5 mg) were removed from the reaction vessel and dried under vacuum for an hour. A solution of 20 % piperidine in DMF was added to the dried resin beads (1 mg) and left to stand for 20 min. General washing procedure for manual SPPS: After each step of SPPS, the resin was washed with DCM (3 × 10 mL), DMF (3 × 10 mL), and DCM (3 × 10 mL).

General procedure for the synthesis of peptides using microwave power

Stock solutions of amino acids (0.2 mM), DIPEA (1 mM), and *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluoro-phosphate (HBTU) (2 mM) were prepared and added into their appropriate reaction vessels on the peptide synthesizer. All peptides were synthesized on a 0.5 mmol scale using microwave power (see Table 1 of the Supplementary information). The standard coupling method for arginine (Arg) [60] was used for attachment of the second amino acid to the resin (1 g) preloaded with the first amino acid. The standard coupling method was used for subsequent reactions and for Fmoc deprotection [19, 20, 61]. The washing procedures were similar to those used for manual SPPS. Final cleavage from the resin was performed manually. The resin-bound peptide was washed with DCM (3 × 10 mL) in a reaction vessel while nitrogen was bubbled through the solution. A cleavage mixture of 0.5 % (v/v) TFA and 95 % (v/v) DCM was added to the dried resin while nitrogen was bubbled through the solution for 10 min. The resin was washed three times with the cleavage mixture and the cleaved peptide in solution was separated from the resin by filtration and collected in a flask containing water (100 mL). The filtrate was extracted several times with DCM to remove the peptide from the water layer. DCM was removed under reduced pressure using a Teflon pump at 40 °C and affording the peptide as a white powder.

General procedure for coupling of peptides to 5-hydroxy-4-oxahexacyclododecane-3-carboxylic acid to yield compounds **1**–**8**

The cleaved peptide (1.2 eq.) was dissolved in DCM (3 mL) followed by the addition of 5-hydroxy-4-oxahexacyclododecane-3-carboxylic acid [33] (1 equiv), HATU (2.5 equiv) in DMF (7 mL), and DIPEA (3 equiv) as a base. The mixture was left to stir at room temperature for 24 h. The product was evaporated to dryness under vacuum using a Teflon pump attached to the Buchi rotary evaporator, which was maintained at 40 °C. A cleavage mixture (10 mL) of 95 % (v/v) TFA and 5 % (v/v) DCM was added to the coupled peptide and stirred for 24 h at room temperature to remove the *N*-Boc protecting group. The TFA was removed from the mixture by bubbling air through the peptide and the remaining DCM was removed under vacuum at 30 °C. The product was obtained as yellow oil which was purified by preparative HPLC [buffer solution A: 0.1 % formic acid/H_2O (v/v) and buffer solution B: 0.1 % formic acid/acetonitrile (v/v)].

Compound ***1***

A colorless oil (76 %). Retention time: 14.13 min (HPLC), $[\alpha]_D^{20} = -20.00$ (*c* 0.20 in MeOD); IR (neat): 3288.6, 2970.5, 1726.1, 1638.0, 1529.2, 1454.7, 1326.9, 1292.2, 1204.7, 1137.1, 992.5, 950.2, 906.4, 868.8, 641.7, 521.5 cm^{-1}; HR-ESI-MS (*m/z*): calculated for $C_{20}H_{26}N_2NaO_6$ ($[M+Na]^{23+}$) 413.1683 found 413.1687. The NMR data for **1** are presented in Tables 1 and 2.

Compound ***2***

A white solid (62 %). Retention time: 15.23 min (HPLC), melting point: 129–130 °C, $[\alpha]_D^{20} = -11.76$ (ca. 0.17 in MeOD); IR (neat): 3300.5, 2967.2, 2868.8, 1725.5, 1640.5, 1527.3, 1455.4, 1326.0, 1292.2, 1204.9, 1138.1, 1105.6, 991.9, 949.5, 905.6, 868.6, 740.6, 698.9, 521.6, 495.4 cm^{-1}; HR-ESI-MS (*m/z*): calculated for $C_{26}H_{30}N_2O_6$ ($[M+H]^+$) 467.2177 found 467.2156. The NMR data for **2** are listed in Tables 1 and 2.

Compound ***4***

A pale white solid (48 %). Retention time: 14.28 min (HPLC), melting point: 159–160 °C, $[\alpha]_D^{20} = -41.67$ (*c* 0.12 in MeOD); IR (neat): 3293.0, 2969.8, 1725.6, 1639.4, 1527.3, 1454.4, 1330.9, 1292.1, 1206.3, 1138.7, 993.7, 951.8, 905.6, 868.9, 789.5, 641.5, 521.7 cm^{-1}; HR-ESI-MS (*m/z*): calculated for $C_{23}H_{31}N_3O_7$ ($[M+H]^+$) 462.2235 found 462.2269. The NMR data for **4** are presented in Tables 1 and 2.

Compound ***5***

A pale yellow oil (54 %). Retention time: 15.28 min (HPLC), $[\alpha]_D^{20} = -27.27$ (*c* 0.22 in MeOD); IR (neat): 3288.5, 2965.7, 1728.2, 1638.4, 1524.0, 1327.6, 1206.3, 1138.62, 949.5, 905.3, 699.0 cm^{-1}; HR-ESI-MS (*m/z*): calculated for $C_{28}H_{33}N_3NaO_7$ ($[M+Na]^{23+}$) 546.2110 found 546.2190. The NMR data for **5** are listed in Tables 1 and 2.

Compound ***6***

A pale yellow oil (42 %). Retention time: 15.35 min (HPLC), $[\alpha]_D^{20} = -23.81$ (*c* 0.21 in MeOD); IR (neat): 3277.7, 2966.7, 1721.5, 1637.6, 1528.4, 1454.6, 1350.3, 1226.6, 1141.49, 911.2, 743.8, 698.9 cm^{-1}; HR-ESI-MS (*m/z*): calculated for $C_{29}H_{35}N_3NaO_7$ ($[M+Na]^{23+}$) 560.2367 found 560.2351. The NMR data for **5** are given in Tables 1 and 2.

Compound 7

A yellow oil (42 %). Retention time: 15.62 min (HPLC), $[\alpha]_D^{20} = -33.33$ (ca. 0.21 in MeOD); IR (neat): 3287.8, 2964.4, 1714.9, 1640.5, 1524.1, 1327.2, 1205.2, 1138.4, 737.7, 697.0 cm^{-1}. HR-ESI-MS (*m/z*): calculated for $C_{35}H_{39}N_3NaO_7$ ($[M+Na]^{23+}$) 636.2719 found 636.2680. The NMR data are presented in Tables 1 and 2.

General procedure [62] for the synthesis of benzyl-protected 5-hydroxy-4-oxahexacyclododecane peptides, **3** and **8**

5-Hydroxy-4-oxahexacyclododecane peptide (1 eq) and benzylbromide (2.5 eq) were dissolved in DMF (8 mL). Cesium carbonate (2 eq) was added to the solution at ambient temperature. After stirring for 2 h, saturated aqueous sodium bicarbonate (100 mL) was added to the solution and extracted with ethyl acetate (3 × 100 mL). The combined organic phases were washed with 5 % aqueous citrate and saturated aqueous sodium chloride (100 mL) and dried over sodium sulfate. The solvent was evaporated, and the crude peptide was precipitated in cold ether and dried under reduced pressure. This crude product was further purified by preparative HPLC [buffer solution A: 0.1 % formic acid/H_2O (v/v) and buffer solution B: 0.1 % formic acid/acetonitrile (v/v)].

Compound 3

A pale yellow oil (51 %). Retention time: 15.90 min (HPLC), $[\alpha]_D^{20} = -13.33$ (*c* 0.21 in MeOD); IR (neat): 3286.7, 2966.8, 1742.8, 1643.5, 1524.4, 1455.5, 1347.2, 1203.2, 1160.4, 1138.8, 1052.4, 991.3, 949.3, 904.7, 738.0, 697.3, 640.9 cm^{-1}; HR-ESI-MS (*m/z*): calculated for $C_{27}H_{32}N_2NaO_6$ ($[M+Na]^{23+}$) 503.2153 found 503.2150. The NMR data for **3** are listed in Tables 1 and 2.

Compound 8

A yellow oil (46 %). Retention time: 15.68 min (HPLC), $[\alpha]_D^{20} = -19.05$ (*c* 0.21 in MeOD); IR (neat): 3289.0, 2965.3, 1720.5, 1641.6, 1523.6, 1326.3, 1206.3, 1138.5, 906.4, 698.1 cm^{-1}; HR-ESI-MS (*m/z*): calculated for $C_{36}H_{41}N_3NaO_7$ ($[M + Na]^{23+}$) 650.2837 found 650.2828. The NMR data for **8** are listed in Tables 1 and 2.

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