S. Afr. J. Chem., 2012, **65**, 108–114,
<http://journals.sabinet.co.za/sajchem/>.

Synthesis and NMR Elucidation of Novel Pentacycloundecane-Derived Peptides

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Received 14 December 2012, revised 24 January 2012, accepted 10 February 2012.

ABSTRACT

Herein we report the synthesis and NMR elucidation of five novel pentacycloundecane (PCU)-derived short peptides as potential HIV protease inhibitors. ^{1}H and ^{13}C spectral analysis show major overlapping of methine resonance of the PCU 'cage' thereby making it extremely difficult to assign the NMR signals. Attachment of short peptides to the cage at position C-8/C-11 results in conformational differences of the peptide side chains due to diastereomeric interactions between the cage skeleton and the chiral side chains. The use of two-dimensional NMR techniques proved to be highly effective in the elucidation of such systems.

KEYWORDS

^{1}H NMR, ^{13}C NMR, 2D NMR, PCU diol diaminoacid, HIV protease inhibitors.

1. Introduction

The chemistry of polycyclic 'cage' compounds such as adamantane,[1–8] pentacycloundecane (PCU),[9,16] trishomocubane[11,17,34] have been extensively studied by organic chemists over the years. A number of South African scientists have focused particularly on the chemistry and applications of PCU and trishomocubane polycyclic compounds.[8,10,14–16,17,18,24,25,27–29,30,35–49,50–52] Our research group has reported NMR studies in a bid to understand how the 'cage' skeleton interacts/relates to its side 'arms'.[15,16,35–63] As a part of ongoing research in this field, the NMR assignments of five PCU derivatives (**1**–**5**) are reported (Fig. 1). Compounds **1** and **2** have previously been reported but have never been fully characterized. Compounds **3–5** are novel PCU derivatives.

The unique features of the cage molecules such as their rigidity and long range proton–proton interactions result in broad overlapping resonance and geminal/vicinal proton–proton couplings. This further complicates the structural elucidation of these compounds. Several authors have commented on the challenges encountered with the NMR elucidation of cage compounds,[38,41,64–66] but the availability of 2D NMR techniques has helped to overcome these former difficulties.

The incorporation of the cage moiety in medicinal compounds introduces a variety of advantages in terms of activity and pharmacology.[4,11] We have recently reported a family of PCU lactam peptides as potential South African human immunodeficiency virus type 1 subtype C protease (CSA-HIV-1 PR) inhibitors, which showed promising *in vitro* inhibition activity (IC_{50} = 0.78 μM) against the CSA-HIV-1 protease enzyme[15,16]. An extension of this study led to the design of compounds **1**–**5** (Fig. 1). The diols in the cage molecule are envisaged to interact with the catalytic ASP25/ASP25′ residues in the active site of the HIV PR enzyme. It could also serve as a transition state mimic, due to the structural resemblance to a dihydroxyethylene type isostere.[67,68]

2. Synthesis

Cookson's dione **6** [69] is reacted with freshly prepared allyl magnesium bromide (Grignard reaction) to obtain the *endo*-8, *endo*-11 diol **7**.[46,70] Ozonolysis of the diol **7** followed by an oxidative workup yielded the PCU diol dicarboxylic acid **1** in good yields[63] (Scheme 1).

Enantiomerically pure *tert*-butyl amino acid esters (**12** and **13**), were synthesized by reacting amino acids (**8** and **9**) with *tert*-butyl acetate (**11**) and $HClO_4$.[71] 2-Phenylglycine (**10**) was reacted with thionyl chloride and methanol to yield the corresponding enantiomerically pure methyl ester **14** (Scheme 2).[72]

Compound **1** in dry DCM was reacted with *tert*-butyl amino acid esters **12** and **13** or the methyl ester **14** in the presence of the coupling reagent HATU (2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate methanaminium) and DIPEA (*N,N*-diisopropylethylamine) as a base to give the corresponding compounds **5**, **15**, **16** and **17** (Scheme 3). Deprotection of PCU diol *tert*-butyl amino acid esters **15** and **16** was carried out using TFA (trifluoroacetic acid) in DCM (1:1) to yield the corresponding enantiopure cage amino acids **2** and **3** while the deprotection of compound **17** was achieved using aqueous KOH in methanol to yield enantiopure compound **4** (Scheme 3).

3. Results and Discussion

Compound **1** is mesomeric while compounds **2–5** are optically pure (carbons C-8/C-11 have opposite chiralities). It is known from literature, that the H-4 cage proton signals appear as a pair of doublets (geminal protons) one each for H-4a and H-4s with an AB spin resonances at approximately 1.5 and 1.8 ppm (J ~10 Hz), respectively.

For compound **1**, the ^{1}H NMR spectra exhibits the geminal PCU bridge methylene protons resonances of H-4a and H-4s at 1.02 and 1.49 ppm with a coupling constant of 10.4 Hz. A correlation was observed in the COSY and NOESY spectra for H-4a and H-4s with a signal at 2.36 ppm which was assigned to H-3/H-5. These protons also show COSY correlations with two other methine protons (H-2/H-6 and H-9/H-10). The H-2/H-6 (2.47 ppm) signal correlates with that of H-1/H-7 (2.58 ppm) while the other signal (H-9/H-10) exhibits no further correla-

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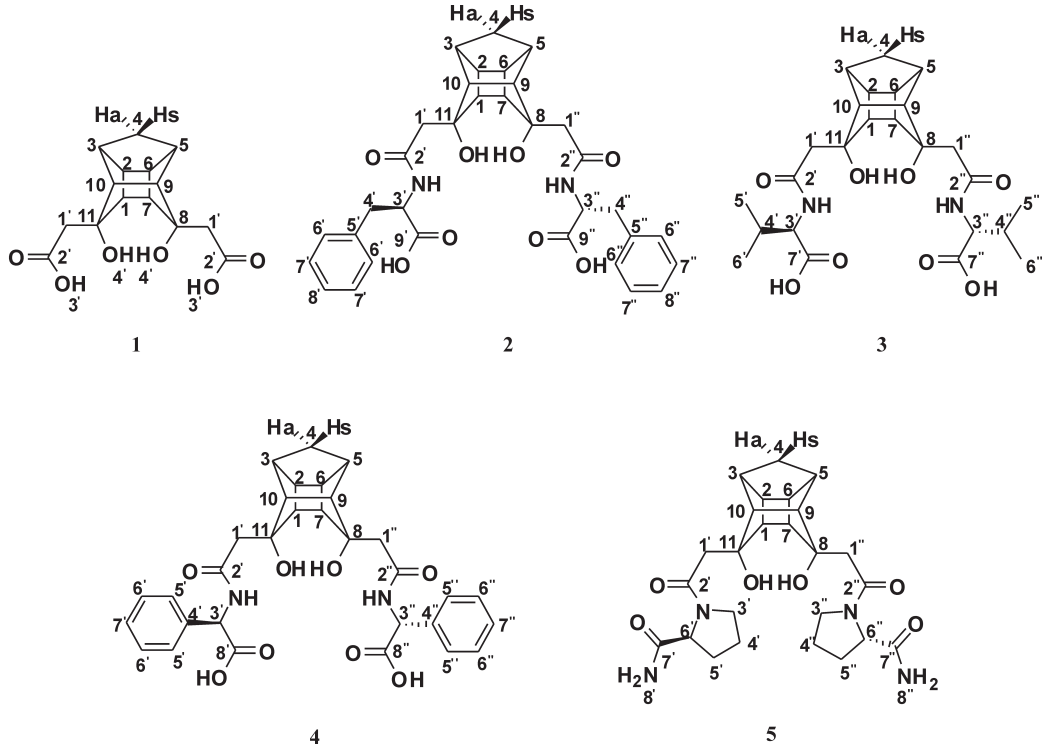
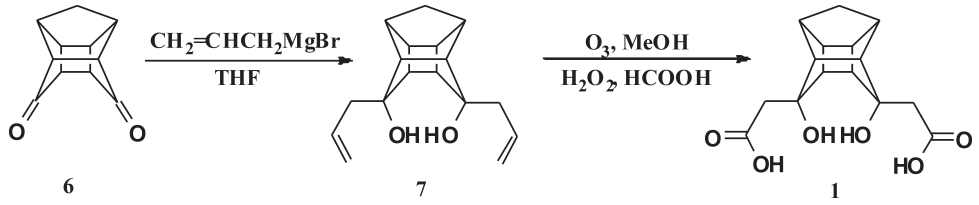


Figure 1 Structure of compounds **1**–**5**.



Scheme 1
Synthesis of PCU cage diol dicarboxylic acid (**1**).

tions. The NOESY spectrum also shows correlation of H-4a with H-2/H-6, while H-4s correlates with H-9/H-10. The HMBC and NOESY techniques are vital tools to determine how the side chains interact with the cage. The HMBC spectrum shows correlation of the C-9/C-10 peak with H-1′ at 2.23 ppm and H-4a at 1.02 ppm. The quaternary carbon signal of C-8/C-11 (76.4 ppm) and the carbonyl carbon C-2′ (172.3 ppm) also show HMBC correlations to H-1′ (2.23 ppm), further confirming these assignments. Further verifications were carried out using the HSQC spectrum. The assignments for compound **1** are presented in Table 1.

A similar methodology was used to assign the NMR signals of the cage protons and carbons for the remaining compounds. Details of the subsequent NMR assignments of the PCU skeleton will be omitted in further discussions for the sake of brevity.

All carbon atom signals for compound **2** appear as pairs. It is impossible to distinguish between split carbon and proton signals. The two quaternary carbons registering at 77.2 and 77.8 ppm were assigned to C-8/C-11 or C-11/C-8. The C 8 and C-11 signals exhibits a HMBC correlation with a pair of doublets registering at 2.24 and 2.34 ppm (J = 15 Hz) which were assigned to H-1′/H-1″. The H-1′/H-1″ protons are diastereotopic due to the neighbouring chiral carbon (C-8 and C-11). H-1′/H-1″ displays HMBC correlation to two overlapping C-2′/C2″ carbonyl carbons at 173.1–173.9 ppm. The two multiplets at 4.73 and 4.81 ppm, both integrating to one proton, were assigned to H-3′/H-3″. H-3′/H-3″ display both COSY and NOESY correlations with two methylene signals (multiplets) registering at 2.96 and 3.27 ppm (H-4′/H-4″). These two methylene protons H-4′/H-4″ are also diastereotopic. H-3′/H-3″ and H-4′/H-4″ display HMBC correlations with a carbonyl carbon at 173.1–173.9 ppm, which were assigned to C-9′/C-9″ (C-2′/C-2″ and C-9′/C-9″ are overlapping). H-3′/H-3″ show a HMBC correlation to two quaternary carbons registering at 137.2 and 137.5 ppm, which were assigned to C-5′/C-5″. H-4′/H-4″ show

Table 1 NMR data for compound **1** in $(CD_3)_2SO$.

Atom	Compound **1**		
	δ^1H [a,b]	J/Hz	$\delta^{13}C$ [a,b]
1/7	2.58	–	42.2
2/6	2.47	–	38.8
3/5	2.36	–	43.8
4a	1.02	10.4	33.4
4s	1.49	10.4	33.4
8/11	–	–	76.4
9/10	2.30	–	49.9
1′	2.23	–	44.1
2′	–	–	172.3
3′ (OH)	11.92	–	–
4′ (OH)	7.16	–	–

[a] Solvent $(CD_3)_2SO$.
[b] 400 MHz for ^1H and 100 MHz for ^{13}C.

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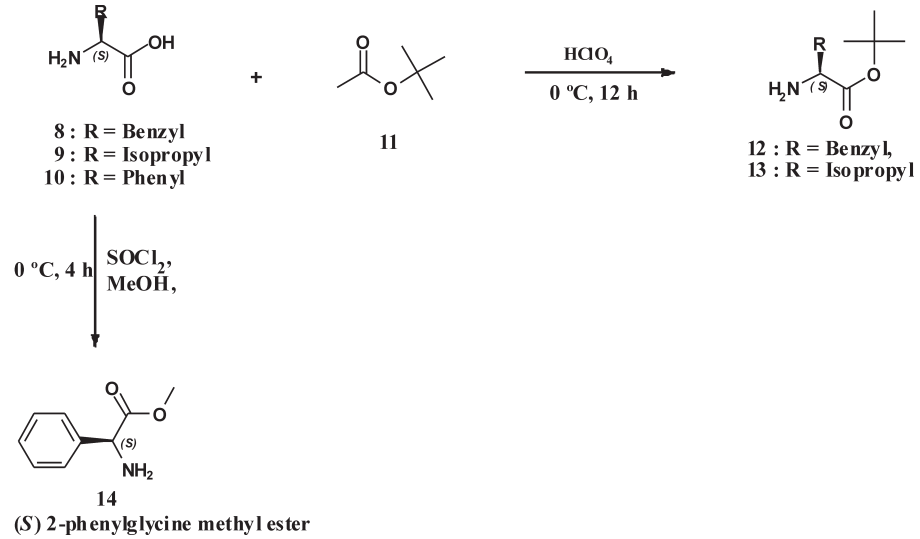
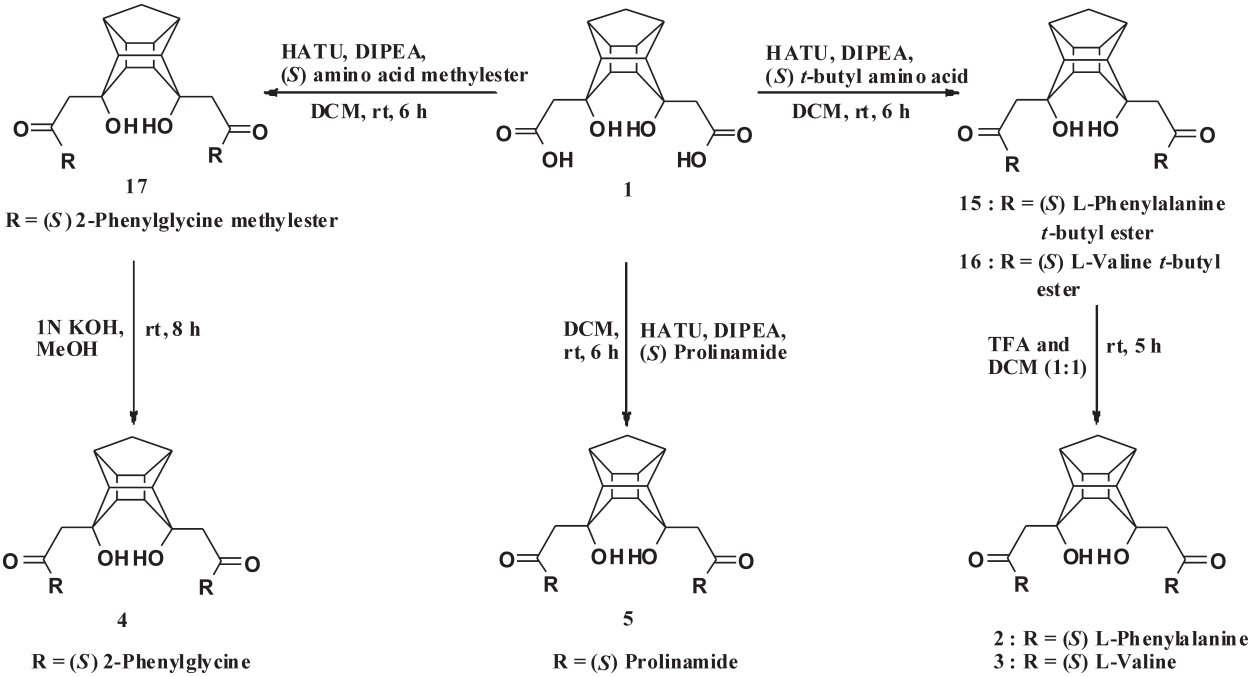


Scheme 2
Synthesis of enantiomerically pure *tert*-butyl amino acid esters **12** and **13** and amino acid methyl ester **14**.

HMBC correlations to aromatic carbons registered at 128.8 and 128.9 ppm (C-6′/C-6″). Carbon signals for C7′ (129.5 ppm) and C8′/C8″ (127.1/127.3) were assigned[73,74] based on the intensity of the peaks. Peculiarly, C7′ (129.5 ppm) did not display any splitting of the aromatic carbon signal.

We previously reported the X-ray crystal structure of compound **2** (Fig. 2).[75] It is evident from the crystal structure that a conformational difference of the 'arms' of the cage exists, with one arm pointing towards the back of the cage while the other is positioned in front of the cage. We have previously reported several cases where the same observation was made from NMR experiments in solution.[15,16,53–55,57,59] From the NOESY spectrum H-4s, H-9 and H-10 display weak correlations with the aromatic protons. These assignments are presented in Table 2.

The ^{1}H NMR spectrum of compound **3** shows a muliplet (theoretically it should be a pair of doublets) for the chiral protons H-3′/H-3″ registering at 4.36 ppm (J = 4.8 Hz) which integrate for two protons. These chiral methine protons exhibit a COSY correlation to a multiplet registering at 2.19 ppm, which was assigned to the adjacent proton H4′. This methylene proton (H4′ at 2.19 ppm) displays both NOESY and COSY interactions with two methyl protons at 0.94, 1.00 and a chiral proton registered at 4.36 ppm (C-3′/C-3″). Based on these correlations, the two methyl protons were assigned to H-5′/H-5″ and H-6′/H-6″. The C-3′/C-3″ peaks (58.6/58.7 ppm) also show HMBC correlations to H-4′, H-5′ and H-6′. Overlapping carbonyl signals in the region of 174.3–174.6 ppm (C-2′ and/or C-7′) show HMBC correlations to H-3′/H-3″, H-4′ and H-1′/H-1″, hence the carbonyl carbons could not be distinguished. Splitting of some carbon signals are observed. This has been previously reported for similar compounds and it was attributed to conformational effects by the side arms.[55–57,59] In the case of compound **3** splitting of carbons for atom H-4′ was not observed. These assignments are presented in Table 2.



Scheme 3
Synthesis of PCU derivatives **2–5**.

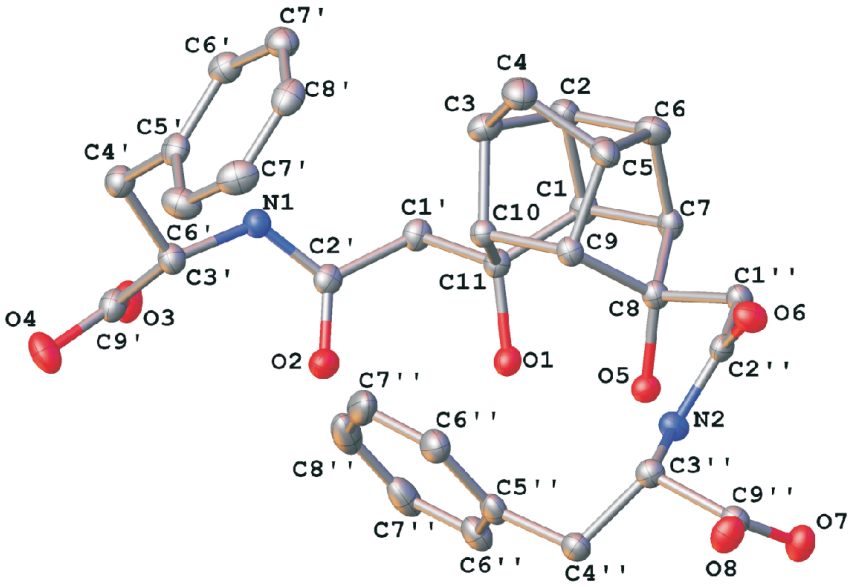


Figure 2 Crystal structure of compound **2** [75]. Hydrogens atoms are ommitted for clarity.

The ^{13}C NMR spectrum of compound **4** shows two overlapping carbonyl peaks registering in the region of 173.8 ppm. These overlapping signals were assigned to C-2′ and C-8′. Interestingly the majority of the side chain atoms were not split as for compounds **2** and **3**.

C-8′ displays a HMBC correlation to a methine proton registering at 5.43 ppm; this methine proton further exhibits a HMBC correlation to a quaternary carbon at 138.8 ppm and a phenyl carbon registering at 128.7 ppm. The methine proton was assigned to H-3′ (5.43 ppm) while the quaternary carbon and the phenyl carbon were assigned to C-4′ and C-5′ respectively. H-3′ also displayed NOESY interaction with the phenyl protons at regions 7.30–7.43 ppm. The remaining phenyl carbons registering at 129.4 and 129.8 ppm were assigned to C-6′ and C-7′ based on the integration of the peaks.[73] C-2′ (173.8 ppm) and C-8/11 (a chiral carbon, 78.7 ppm) both display HMBC correlations to overlapping proton signals in the region of 2.17 and 2.24 ppm. The HSQC spectrum shows that these two signals correlate to one carbon (45.3 ppm) suggesting that they are doublets (this observation could not be made exclusively from the ^{1}H spectrum due to the overlapping in this region). These two signals were assigned to H-1′ and H1″. All remaining carbons and protons were assigned using the HSQC spectrum. These assignments are presented in Table 3.

The ^{13}C NMR spectrum of compound **5** shows two carbonyl signals at 170.6 and 173.8 ppm. The side chains did not reveal split carbon signals. These signals are due to the C-2′/C-2″ or C-7′/C-7″ carbonyl carbons. Only the signal at 173.8 ppm shows HMBC correlation to the H-8′/H-8″ protons at 6.92 and 7.08 ppm, respectively; the carbonyl signal at 173.8 ppm was therefore assigned to C-7′ and C-7″. The signal at 170.6 ppm was assigned to C-2′ and C-2″. The carbonyl carbon (C-7′/ C-7″) signal also shows correlation to the chiral methine proton at 4.16 ppm, which was assigned to H-6′ and H-6″. The chiral methine proton (H-6′/H-6″) shows COSY correlation to H-5′/H-5″ at 1.99 pm. This was further confirmed from the HMBC spectrum since the signal of the carbonyl carbon C-7′/C-7″ shows correlation to H5′/H-5″. H5′/H-5″ displays both COSY and NOESY correla-

Table 2 NMR data for PCU derivatives **2** and **3** in (CD_3) OD.

	Compound **2**				Compound **3**		
Atom [c]	δ^{1}H [a,b]	J/Hz	δ^{13}C [a,b]	Atom [c]	δ^{1}H [a,b]	J/Hz	δ^{13}C [a,b]
1/7	2.09/2.11		44.5/44.8	1/7	2.55		44.3
2/6	2.48		39.4/39.7	2/6	2.62		40.6/40.7
3/5	2.26/2.30		43.2–43.8	3/5	2.49/2.55		45.7
4a	1.04	10.6	33.9	4a	1.18	10.8	34.8
4s	1.39	10.5	33.9	4s	1.60	10.7	34.8
8/11	–		77.2/77.8	8/11	–		78.5/78.6
9/10	1.67/1.89	8.8	49.1/49.4	9/10	2.33/2.40		50.7/50.9
1′/1″	2.24/2.34	15	43.2/44.8	1′/1″	2.33/2.47		45.2/45.3
2′/2″/9′/9″	–		173.1–173.9	2′/7′	–		174.3/174.6
3′/3″	4.73/4.81	4.6	53.8/53.9	3′/3″	4.36	4.8	58.6/58.7
4′/4″	2.96/3.27	6.5	37.5/37.6	4′	2.19	4.6	31.7
5′/5″	–		137.2/137.5	5′/5″/6′/6″	0.94–1.00		18.0/18.2/19.6/19.7
6′/6″	7.14–7.32		128.8/128.9				
7′	7.14–7.32		129.5		–		
8′/8″	7.14–7.32		127.1/127.3				

[a] Solvent CD_3OD.
[b] 400 MHz for ^{1}H and 100 MHz for ^{13}C. Compound **2**: 600 MHz for ^{1}H and 125 MHz for ^{13}C.
[c] It was not possible to distinguish between the atoms on the left and right side of the cage and side arms.

Table 3 NMR data for PCU derivatives **4** in (CD_3) OD and **5** in $(CD_3)_2SO$.

	Compound **4**				Compound **5**		
Atom	$\delta^1H^{b,c}$	*J*/Hz	$\delta^{13}C^{b,c}$	Atom	$\delta^1H^{a,c}$	*J*/Hz	$\delta^{13}C^{a,c}$
1/7	2.37		45.7	1/7	2.67		42.0
2/6	2.51		40.6	2/6	2.52		38.5
3/5	2.43		44.4	3/5	2.42		43.9
4a	1.10	10.8	34.8	4a	1.04	10.6	33.5
4s	1.50	10.7	34.8	4s	1.48	10.5	33.5
8/11			78.7	8/11	–		77.8
9/10	2.15		50.8	9/10	2.10		50.3
1′/1″	2.17/2.24		45.3	1′/1″	2.04/2.37		41.3/42.2
2′/8′			173.8	2′	–		170.6
3′	5.43		58.2	3′	3.50		47.3
4′			138.8	4′	1.81		24.0
5′	7.30–7.43		128.7	5′	1.99		29.3
6′	7.30–7.43		129.4	6′	4.16	5.59	59.3
7′	7.30–7.43		129.8	7′	–		173.8
				8′/8″	6.92/7.08		–

[a] Solvent $(CD_3)_2SO$.
[b] Solvent $(CD_3)OD$.
[c] 400 MHz for ^1H and 100 MHz for ^{13}C.

tions to a signal at 1.81 ppm, which was assigned to H-4′ and H-4″. The methylene protons registered at 3.50 ppm showed COSY correlation to H-4′/H-4″ which was then assigned to H-3′ and H-3″. In addition, the C-2′/C-2″ signal shows a HMBC correlation to H-3′/H-3″. The carbonyl carbon C-2′/C-2″ and the quaternary carbons C-8/C-11 display HMBC correlations to methylene protons at 2.04 and 2.37 ppm which were assigned to H-1′ and H-1″. HMBC and HSQC spectra were further used to confirm the assigned proton and carbon signals. The signal assignments for compounds **4** and **5** are presented in Table 3.

4. Conclusion

The full NMR elucidation of several novel PCU cage derivatives was successfully achieved. Considerable overlapping of proton and carbon signals was observed, but this was overcome by using 2D NMR techniques. This technique proved to be a vital and convenient tool in elucidating PCU cage short peptides derivatives. These compounds are currently being evaluated as potential HIV protease inhibitors. It is unclear why some of the compounds give split side chain signals and others not.

5. Experimental

IR spectra were obtained from a Perkin Elmer Spectrum 100 instrument with an attenuated total reflectance attachment. High Resolution Electron Spray Ionization Mass Spectroscopic analysis was performed for all compounds on a Bruker MicroTOF QII mass spectrometer in positive mode with the exception of compound **1** for which data were collected in the negative mode. All MS samples were analysed with an internal calibration. Melting point analysis (uncorrected) was performed on a Stuart Scientific digital melting point apparatus SMP3. Tetrahydrofuran (THF) was freshly distilled using sodium wire/benzophenone under nitrogen (N_2) atmosphere. Dichloromethane (DCM) was dried using phosphorus pentoxide as drying agent. The NMR data were recorded on Bruker AVANCE III 400 and 600 MHz instruments; the chemical shifts were referenced to the solvent peak, namely δ = 7.24 ppm for $CDCl_3$, δ = 2.50 ppm for $(CD_3)_2SO$, and δ = 3.34 ppm for CD_3OD at ambient temperature. Analytical analysis was performed on an Agilent 1100 HPLC (Waters Xbridge C18 150 mm × 4.6 mm × 5 microns) 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 microns) with a UV/VIS detector (215 nm) and an 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). All the enantiopure (*S*) amino acids and coupling reagents were purchased from GL Biochem (Shanghai) Ltd. Analytical grade solvents and reagents such as allyl magnesium bromide, *tert*-butyl acetate, DIPEA, prolinamide for synthesis were procured from Sigma-Aldrich (South Africa).

5.1. Synthesis of *exo*-8-*exo*-11-diallylcarboxilic acid pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-*endo*-8-*endo*-11-diol (1)

A solution of diene **7** [48,70] (15.0 g, 58.0 mmol) in dry methanol (100 mL) was cooled in an external dry ice-isopropanol bath (–78 °C). Ozone was bubbled into the reaction mixture until a blue-purple colour persisted indicating the presence of excess ozone in the system and hence the completion of the reaction. The excess ozone gas was flushed from the reaction vessel with a stream of nitrogen and the solvent removed *in vacuo*. Formic acid (100 mL) was added to the ozonide and the mixture was cooled in a ice bath with stirring. Hydrogen peroxide (150 mL, 30 %) was then added drop-wise to the stirred cooled reaction mixture. The reaction was left to attain ambient temperature for 1 h and then gently refluxed for 12 h, the resulting mixture was concentrated in *vacuo* to yield crude product. The crude product was purified *via* column chromatography on silica gel using CH_3COOH:MeOH:EtOAc: Hexane (1:2:20:77, R_f = 0.3, 92 % yield) to obtain compound **1** as a viscous pale yellow oil, which solidified on standing at room temperature to give a white solid. Melting point: 155–157 °C, IR ν_{max}: 3102, 2985, 2864, 1698, 1639, 1451, 1292, 1280, 1181, 1165, 1120, 1068, 1045, 923, 862, 799, 736, 652, 575 and 477 cm^{-1}. ^1H NMR [$(CD_3)_2SO$, 400 MHz]: δ_H 1.02 (AB, J_{AB} = 10.4 H_Z, 1H), 1.49 (AB, J_{AB} = 10.4 H_Z, 1H), 2.23 (s, 3H), 2.26 (s, 2H), 2.37 (s, 2H), 2.48–2.50 (m, 3H), 2.57–2.59 (m, 2H), 7.16 (s, 2H), 11.92 (s, 2H). ^{13}C NMR [$(CD_3)_2SO$, 100 MHz]: δ_C 33.4 (CH_2), 38.8 (CH), 42.2 (CH), 43.8 (CH), 44.0 ($^{-}CH_2$), 49.9 (CH), 76.4 (C), 172.3 (C). HR ESI *m*/*z*: calculated for $C_{15}H_{18}O_6$ [M-H] –: 293.1031 found 293.1030.

5.2. General procedure for the synthesis of *exo*-8-*exo*-11-di(*S*)-amino acid pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-*endo*-8-*endo*-11-diol (**2**, **3** and **4**)

A solution of compound **1** (1.0 eq., 0.5 g) in dry DCM (15 mL) was stirred at room temperature for 5 min. To this mixture was added *tert*-butyl amino acid esters/(*S*)-amino acid methyl esters (4.0 eq). The reaction mixture was cooled in an ice water bath and stirred for 5 min. This was followed by the addition of HATU (5.0 eq., 3.24 g) and DIPEA (8.0 eq., 1.76 g, 2.4 mL, a base). The solution was then slowly brought to room temperature and stirred for 6 h. The crude reaction mixture was washed with water (100 mL) and then with of 10 % HCl (100 mL). The organic layer was dried over anhydrous Na_2SO_4 and filtered. The crude product was evaporated at 40 °C to dryness under vacuum to obtain a thick yellow oil. The crude *tert*-butyl esters of the PCU cage diol diaminoacids (**15** and **16**, ≈ 0.8–1.0 g) were further dissolved in 8 mL of 1:1 (v/v) DCM and TFA solvent mixture and stirred overnight. TFA was removed from the mixture by bubbling air through the peptide and the remaining DCM was removed under vacuum at 30 °C, to obtain crude PCU cage diol diamino acids (**2** and **3**) as pale yellow oils.

The methyl esters of PCU cage diol di-(*S*)-amino acids (**17**, ≈ 0.9 g) were dissolved in methanol (3 mL) and excess of 1N KOH (5 mL) and stirred overnight. Glacial acetic acid was added until the mixture was neutral and then diluted with chloroform. The organic solution was washed once with water and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give a crude oily compound **4**. These crude compounds were then purified by using preparative HPLC to give enantiomerically pure PCU cage diol diamino acids (**2**, **3** and **4**).

5.3. Data for *exo*-8-*exo*-11-diphenyl-(*S*)-alanine-pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-*endo*-8-*endo*-11-diol (**2**)

A white solid (67 %). Melting point: 231–232 °C, $[\alpha]^{20}_D$: +14.29 (*c* 0.14 in MeOH). IR ν_{max}: 3261, 2957, 1758, 1638, 1522, 1191, 758, 701 and 489 cm^{-1}. ^{1}H NMR [CD_3OD, 400 MHz]: δ_H 1.04 (1H, d, *J* = 10.6 Hz), 1.39 (1H, d, *J* = 10.5 Hz), 1.67 (1H, d, *J* = 8.8 Hz), 1.90 (1H, d, *J* = 8.8 Hz), 2.09–2.34 (8H, m), 2.45–2.47 (2H, m), 2.91–3.01 (2H, m), 3.24–3.31 (2H, m), 4.69–4.73 (1H, q, *J* = 4.7 Hz), 4.77–4.81 (1H, q, , *J* = 4.6 Hz), 7.13–7.17 (1H, m), 7.22–7.32 (9H, m). ^{13}C NMR [CD_3OD, 100 MHz]: δ_C 33.9 (CH_2), 37.5 (CH_2), 37.6 (CH_2), 39.4 (CH), 39.7 (CH), 43.2 (CH, CH_2), 43.8 (CH), 43.8 (CH), 44.5 (CH), 44.8 (CH, CH_2) 49.1 (CH), 49.4 (CH), 53.8 (CH), 53.9 (CH), 77.2 (C), 77.8 (C), 127.1 (CH), 127.3 (CH), 128.8 (CH), 128.9 (CH), 129.5 (CH), 137.2 (C), 137.5 (C), 173.1 (C), 173.9 (C). HR ESI *m/z*: calculated for $C_{33}H_{36}N_2O_8$ [M+H] $^+$: 589.2544 found 589.2541.

5.4. Data for *exo*-8-*exo*-11-di-(*S*)-valinepenta-cyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-*endo*-8-*endo*-11-diol (**3**)

A colourless oil (72 %). $[\alpha]^{20}_D$: +8.69 (*c* 0.23 in MeOH). IR ν_{max}: 2960, 2929, 1721, 1621, 1537, 1464, 1392, 1259, 1209, 1064, 986 and 544 cm^{-1}. ^{1}H NMR [CD_3OD, 400 MHz]: δ_H 1.18 (1H, d, *J* = 10.8 Hz), 1.60 (1H, d, *J* = 10.7 Hz), 0.94–1.00 (12H, m), 2.19 (2H, q, *J* = 4.6 Hz), 2.20–2.62 (12H, m), 4.36 (2H, t, 4.8). ^{13}C NMR [CD_3OD, 100 MHz]: δ_C 34.8 (CH), 40.6 (CH), 40.7 (CH), 44.3 (CH), 45.2 (CH_2), 45.3 (CH_2), 45.7 (CH), 50.7 (CH), 50.9 (CH), 78.5 (C), 78.6 (C), 174.3 (C), 174.6 (C). HR ESI *m/z*: calculated for $C_{25}H_{36}N_2O_8$ [M+H]$^+$: 493.2531 found 493.2544.

5.5. Data for *exo*-8-*exo*-11-di-(*S*)-2-phenylglycinepenta-cyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-*endo*-8-*endo*-11-diol (**4**)

A colourless oil (58 %). $[\alpha]^{20}_D$: –30.00 (*c* 0.1 in MeOH). IR ν_{max}: 3060, 2994, 2863, 1723, 1629, 1524, 1452, 1256, 1179, 1133, 1063, 722, 696, 596 and 513 cm^{-1}. ^{1}H NMR [CD_3OD, 400 MHz]: δ_H 1.10 (1H, d, *J* = 10.8 Hz), 1.50 (1H, d, *J* = 10.7 Hz), 2.15–2.58 (12H, m) 5.43 (2H, s), 7.30–7.43 (10H, m). ^{13}C NMR [CD_3OD, 100 MHz]: δ_C 34.8 (CH_2), 40.6 (CH), 44.4 (CH), 45.3 (CH_2), 45.7 (CH), 50.8 (CH), 58.2 (CH), 78.7 (C), 128.7 (CH), 129.4 (CH), 129.8 (CH), 173.8 (C). HR ESI *m/z*: calculated for $C_{31}H_{32}N_2O_8$ [M+H] $^+$: 561.2213 found 561.2231.

5.6. Synthesis of *exo*-8-*exo*-11-di-(*S*)-prolinamidepenta-cyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-*endo*-8-*endo*-11-diol (**5**)

A solution of compound **1** (1.0 eq., 0.5 g) in dry DCM (15 mL) was stirred at room temperature for 5 min. To this mixture was added enantiopure (*S*)-prolinamide (4.0 eq., 0.8 g) whilst it was cooled in an ice water bath with stirring for 5 min. To the above cooled mixture was added HATU (5.0 eq., 3.24 g) followed by DIPEA (8.0 eq., 1.76 g, 2.4 mL). This reaction mixture was then slowly brought to room temperature and stirred for 6 h. The crude reaction mixtures was diluted with DCM and filtered. The crude product was evaporated to dryness at 40 °C under vacuum using a Teflon pump to obtain a thick yellow oil. The product was purified with preparative HPLC to give enantiopure PCU cage diol diprolinamide as a pale yellow solid (82 %). Melting point: 83–85 °C. $[\alpha]^{20}_D$: –37.50 (*c* 0.16 in MeOH). IR ν_{max}: 3179, 2957, 2872, 1669, 1603, 1448, 1417, 1288, 1191, 1168, 1071, 987, 908, 653, 539 and 421 cm^{-1}. ^{1}H NMR [$(CD_3)_2SO$, 400 MHz]: δ_H 1.04 (1H, t, *J* = 10.6 Hz), 1.48 (1H, t, *J* = 10.5 Hz), 1.74–2.72 (18H, m), 3.50–3.68 (2H, m), 4.16–4.22 (2H, m), 6.92–6.95 (2H, m), 7.08–7.22 (2H, m). ^{13}C NMR [$(CD_3)_2SO$, 100 MHz]: δ_C 24.03 (CH_2), 29.3 (CH_2), 33.5 (CH_2), 38.5 (CH), 41.3 (CH_2), 42.0 (CH), 42.2 (CH_2) 43.9 (CH), 47.3 (CH_2), 50.3 (CH), 59.3 (CH), 77.8 (C), 170.6 (C), 173.8 (C). HR ESI *m/z*: calculated for $C_{25}H_{34}N_4O_6$ [M+H]$^+$: 487.2542 found 487.2551.

6. Supporting Information

All the spectra mentioned in the text are available as supporting information.

Acknowledgements

This research was supported by NRF (SA) TG (GUN: 66319), HGK (GUN: 69728), HGK and PIA (SA-Sweden bilateral grant), Aspen Pharmacare and University of KwaZulu-Natal for financial support. The authors thank Dr Katja Petzold for assistance with the EASY-ROESY experiments. We also thank Mr. Dilip Jagjivan (UKZN, South Africa) for assistance with NMR experiments.

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