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ORIGINAL PAPER

# The Synthesis and Crystal Structure of Cbz-[(1*R*,2*S*)-ACPC]$_3$-OH: A Tripeptide Derived from the β-Amino Acid (1*R*,2*S*)-Cispentacin

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**Abstract** Crystals of the tripeptide Cbz-[(1*R*,2*S*)-ACPC]$_3$-OH **4**, derived from the β-amino acid (1*R*,2*S*)-2-aminocyclopentanecarboxylic acid, were synthesised and studied by X-ray diffraction. Tripeptide **4** crystallizes in the orthorhombic space group P $2_12_12_1$ with cell parameters of $a = 7.8957(1)$ Å, $b = 12.9289(2)$ Å, $c = 24.7850(4)$ Å, $V = 2530.12(6)$ Å$^3$ and $Z = 4$. Adjacent molecules are linked by a series of N–H···O=C and O–H···O=C hydrogen bonds to form a double tape-like arrangement.

**Keywords** β-Peptide · β-Sheet · Cispentacin

## Introduction

β-Peptides [1–19] are known to offer improved bioavailability and therapeutic lifetimes when compared to their α-amino acid derived analogues [20–24], whilst still displaying potent biological activity [25–27]. As such, there has been intense interest in the secondary structural characteristics of oligomers of the cyclic β-amino acids cispentacin and transpentacin, the diastereoisomers of 2-aminocyclopentanecarboxylic acid (ACPC) [28–31]. For example, Fülöp et al. have reported that pentamer **3**, derived from (1*R*,2*S*)-ACPC, forms a non-polar strand and adopts a sheet-like structure in solution [32]. We have also reported crystallographic analyses of dimers **1** and **2** (Fig. 1), derived from (1*R*,2*S*)-ACPC and (1*S*,2*R*,3*S*)-3-Me-ACPC, respectively, which form β-sheet like structures in the solid state resulting from arrays of N–H···O=C hydrogen bonds [33]. Herein we report the synthesis and crystal structure of Cbz-[(1*R*,2*S*)-ACPC]$_3$-OH **4**, a tripeptide derived from (1*R*,2*S*)-ACPC.

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## Experimental

### Synthesis

All reagents were used as supplied without prior purification. Elemental analyses were recorded by the microanalysis service of the London Metropolitan University, UK. Melting points were recorded on a Gallenkamp Hot Stage apparatus and are uncorrected. IR spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer as KBr discs. Selected characteristic peaks are reported in cm$^{-1}$. NMR spectra were recorded on Bruker Avance spectrometers in the deuterated solvent stated. Spectra were recorded at rt. The field was locked by external referencing to the relevant deuteron resonance. Low-resolution mass spectra were recorded on either a VG MassLab 20–250 or a Micromass Platform 1 spectrometer. Accurate mass measurements were run on a Micromass GCT instrument fitted with a Scientific Glass Instruments BPX5 column (15 m × 0.25 mm) using amyl acetate as a lock mass. In each case, product distributions and diastereoisomeric ratios were determined by integration of the 400 MHz $^1$H NMR spectra of the crude reaction mixtures and isolated products.

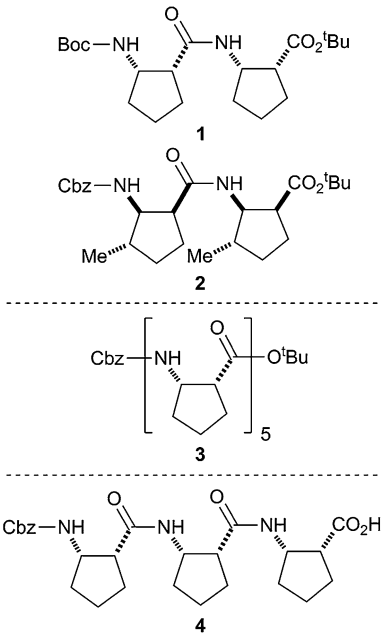



**Fig. 1** Dimer **1**, trimer **4** and pentamer **3** [derived from (1*R*,2*S*)-ACPC] and dimer **2** [derived from (1*S*,2*R*,3*S*)-3-Me-ACPC]

H-[(1*R*,2*S*)-ACPC]$_2$-O$^t$Bu **6**

$Pd(OH)_2$/C (25 % by wt, 488 mg) was added to a stirred, degassed solution of **5** (1.95 g, 4.50 mmol, >99:1 dr) [33] in MeOH/AcOH (40:1 mL). The resultant suspension was stirred under $H_2$ (1 atm) for 16 h. The reaction mixture was then filtered through Celite® (eluent MeOH) and concentrated *in vacuo*. The residue was dissolved in $CH_2Cl_2$ (50 mL) and the resultant solution was washed with satd aq $NaHCO_3$ (3 × 50 mL) and brine (50 mL), then dried over $MgSO_4$ and concentrated *in vacuo* to give **6** as a white solid (1.24 g, 92 %, >99:1 dr); mp 74 °C; $[\alpha]_D^{22}$–42.7 (*c* 1.0 in $CHCl_3$); $\nu_{max}$ (KBr) 3319 (br, N–H), 1699 (br, C=O); $\delta_H$ (400 MHz, $CDCl_3$) 1.44 (9H, s, C*Me*$_3$), 1.46–2.03 (14H, m, 2 × C(3)*H*$_2$, 2 × C(4)*H*$_2$, 2 × C(5)*H*$_2$, N*H*$_2$), 2.45–2.54 (1H, m, C(1)*H*), 2.83–2.91 (1H, m, C(1)*H*), 3.46–3.56 (1H, m, C(2)*H*), 4.47–4.56 (1H, m, C(2)*H*), 7.05 (1H, d, *J* 7.2 N*H*); $\delta_C$ (100 MHz, $CDCl_3$) 22.1, 22.2, 26.8, 28.0, 32.5, 35.1 (2 × *C*(3), 2 × *C*(4), 2 × *C*(5)), 28.1 (C*Me*$_3$), 47.5, 50.9 (2 × *C*(1)), 51.9, 54.7 (2 × *C*(2)), 80.7 (*C*Me$_3$), 173.2, 174.0 (N*C*O, *C*O$_2^t$Bu); *m*/*z* (ESI$^+$) 297 ($[M+H]^+$, 100 %); HRMS (ESI$^+$) $C_{16}H_{29}N_2O_3^+$ ($[M+H]^+$) requires 297.2173; found 297.2173.

Cbz-[(1*R*,2*S*)-ACPC]$_3$-O$^t$Bu **7**

A stirred solution of **6** (415 mg, 1.40 mmol, >99:1 dr) in $CHCl_3$ (10 mL) was successively treated with $Et_3N$ (1.00 mL, 7.00 mmol), HOBt (227 mg, 1.68 mmol), **8** (368 mg, 1.40 mmol, >99:1 dr) [34] and EDC·HCl (322 mg, 1.68 mmol) under anhydrous conditions. After 16 h, the reaction mixture was washed with 1.0 M aq HCl (20 mL), satd aq $NaHCO_3$ (20 mL) and brine (20 mL), then dried over $MgSO_4$ and concentrated *in vacuo*. Purification via recrystallisation ($CHCl_3$/heptane) gave **7** as a white solid (443 mg, 58 %, >99:1 dr); $C_{30}H_{43}N_3O_6$ requires C, 66.5; H, 8.0; N, 7.8 %; found C, 66.5; H, 8.1; N, 7.7 %; mp 144–146 °C ($CHCl_3$/heptane); $[\alpha]_D^{23}$–85.0 (*c* 1.0 in $CHCl_3$); $\nu_{max}$ (KBr) 3327 (N–H), 1714, 1694, 1650, 1542 (C=O); $\delta_H$ (400 MHz, $CDCl_3$) 1.37–2.02 (18H, m, 3 × C(3)*H*$_2$, 3 × C(4)*H*$_2$, 3 × C(5)*H*$_2$), 1.43 (9H, s, C*Me*$_3$), 2.67 (1H, obsc m, C(1)*H*), 2.73 (1H, obsc m, C(1)*H*), 2.80–2.90 (1H, m, C(1)*H*), 4.17 (1H, app t, *J* 7.5, C(2)*H*), 4.38 (1H, obsc m, C(2)*H*), 4.41 (1H, obsc m, C(2)*H*), 5.04 (1H, d, *J* 12.3, C*H*$_A$H$_B$Ph), 5.10 (1H, d, *J* 12.3, CH$_A$*H*$_B$Ph), 5.50 (1H, d, *J* 7.9, N*H*), 6.26 (1H, d, *J* 8.5, N*H*), 6.43 (1H, d, *J* 7.9, N*H*), 7.26–7.40 (5H, m, *Ph*); $\delta_C$ (100 MHz, $CDCl_3$) 22.2, 22.7, 22.8, 28.1, 28.3, 28.7, 32.4, 32.8, 32.9 (3 × *C*(3), 3 × *C*(4), 3 × *C*(5)), 28.1 (C*Me*$_3$), 47.3, 47.7, 47.7 (3 × *C*(1)), 52.0, 52.5, 54.5 (3 × *C*(2)), 66.5 (*C*H$_2$Ph), 80.9 (*C*Me$_3$), 128.0, 128.4 (*o*,*m*,*p*-*Ph*), 136.6 (*i*-*Ph*), 156.1 (N*C*O, carbamate), 173.2, 173.3, 173.7 (2 × N*C*O, amide; *C*O$_2^t$Bu); *m*/*z* (ESI$^+$) 542 ($[M+H]^+$, 100 %); HRMS (ESI$^+$) $C_{30}H_{43}N_3NaO_6^+$ ($[M+Na]^+$) requires 564.3044; found 564.3045.

Cbz-[(1*R*,2*S*)-ACPC]$_3$-OH **4**

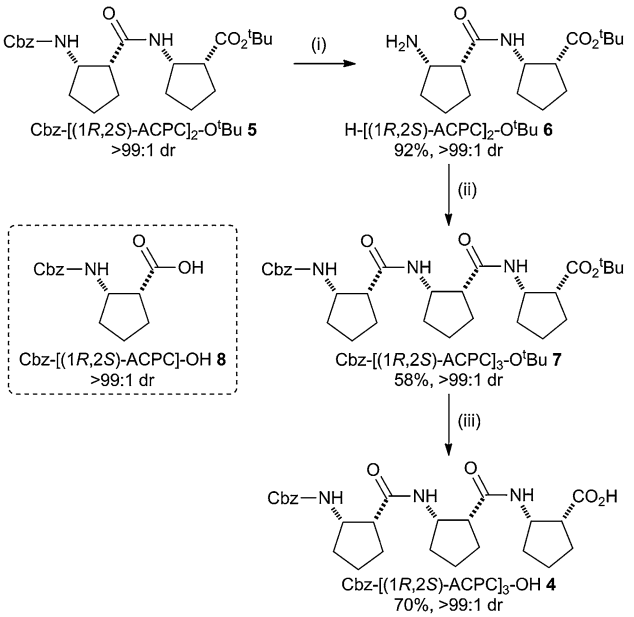
TFA (0.70 mL) was added to a stirred solution of **7** (250 mg, 0.46 mmol, >99:1 dr) in $CH_2Cl_2$ (2.10 mL) at 0 °C. The resultant mixture was allowed to warm to rt and stirred for 3 h before being concentrated *in vacuo* and dried under high vacuum. Purification via recrystallisation ($CHCl_3$/heptane) gave **4** as a white solid (157 mg, 70 %, >99:1 dr); mp 170–172 °C ($CHCl_3$/heptane); $[\alpha]_D^{20}$–101.7 (*c* 0.6 in MeOH); $\nu_{max}$ (KBr) 3340 (br, N–H, O–H), 1701, 1662, 1542 (C=O); $\delta_H$ (400 MHz, $CDCl_3$, 26 mM) 1.39–2.11 (18H, m, 3 × C(3)*H*$_2$, 3 × C(4)*H*$_2$, 3 × C(5)*H*$_2$), 2.70–2.90 (2H, m, 2 × C(1)*H*), 2.98–3.10 (1H, m, C(1)*H*), 4.22 (1H, obsc m, C(2)*H*), 4.33 (1H, obsc m, C(2)*H*), 4.46 (1H, obsc m, C(2)*H*), 5.04 (1H, d, *J* 12.4, C*H*$_A$H$_B$Ph), 5.11 (1H, d, *J* 12.4, CH$_A$*H*$_B$Ph), 5.42 (1H, d, *J* 8.6, N*H*), 6.61 (1H, d, *J* 8.1, N*H*), 6.89 (1H, d, *J* 8.3, N*H*), 7.22–7.39 (5H, m, *Ph*); $\delta_C$ (100 MHz, MeOH-$d_4$) 22.2, 22.9, 27.9, 28.2, 28.5, 31.6, 32.4, 32.9 (3 × *C*(3), 3 × *C*(4), 3 × *C*(5)), 46.1, 47.7, 47.8 (3 × *C*(1)), 52.0, 52.7, 54.6 (3 × *C*(2)), 66.7 (*C*H$_2$Ph), 128.0, 128.1, 128.5 (*o*,*m*,*p*-*Ph*), 136.4 (*i*-*Ph*), 156.2 (N*C*O, carbamate), 173.6, 173.9, 176.8 (2 × N*C*O, amide; *C*O$_2$H); *m*/*z* (ESI$^+$) 508 ($[M+Na]^+$, 100 %); HRMS (ESI$^+$) $C_{26}H_{36}N_3O_6^+$ ($[M+H]^+$) requires 486.2599; found 486.2598.

**Table 1** Crystallographic data and refinement details for tripeptide **4**

| Compound | **4** |
|---|---|
| Empirical formula | $C_{26}H_{35}N_3O_6$ |
| Formula weight | 485.58 |
| Crystal habit, colour | Plate, colourless |
| Crystal system | Orthorhombic |
| Space group | $P2_12_12_1$ |
| *a* (Å) | 7.8957(1) |
| *b* (Å) | 12.9289(2) |
| *c* (Å) | 24.7850(4) |
| α (°) | 90 |
| β (°) | 90 |
| γ (°) | 90 |
| Volume (Å$^3$) | 2530.12(6) |
| *Z* | 4 |
| Density (calc. g cm$^{-3}$) | 1.275 |
| Temperature (K) | 190 |
| Radiation type/λ (Å) | Mo Kα/0.71073 |
| Absorption coeff. (mm$^{-1}$) | 0.091 |
| *F*(000) | 1,040 |
| Crystal size (mm) | 0.05 × 0.20 × 0.20 |
| Reflections measured | 5,776 |
| Independent reflections | 3,277 |
| Observed reflns. [$I > 2.0\sigma(I)$] | 2,139 |
| Number of parameters | 316 |
| Goodness-of-fit on $F^2$ | 0.9978 |
| Final *R* indices [$I > 2.0\sigma(I)$] | $R_1 = 0.042$, $wR_2 = 0.094$ |
| *R* indices [all data] | $R_1 = 0.073$, $wR_2 = 0.103$ |
| $\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å$^{-3}$) | 0.37, –0.34 |
| CCDC deposition no. | 977339 |

## Single Crystal X-ray Diffraction

Single crystal diffraction data for tripeptide **4** were collected using a Nonius κ-CCD diffractometer (Mo-Kα radiation, λ = 0.71073 Å) at 150(2) K with an Oxford Cryosystems Cryostream $N_2$ open-flow cooling device [35]. Raw frame data were processed using the DENZO-SMN package [36], including unit cell parameter refinement and inter-frame scaling (which were carried out using SCALEPACK within DENZO-SMN). The structure was solved using SIR92 [37] and refinement was carried out using full-matrix least-squares within the CRYSTALS suite [38], on $F^2$. All non-hydrogen atoms were refined with anisotropic displacement parameters. The positions and isotropic displacement parameters of hydrogen atoms were refined using restraints prior to inclusion into the model with riding constraints [39]. In the absence of a significant anomalous signal, the Friedel pairs were merged for the final refinement.




**Scheme 1** *Reagents and conditions:* (i) $Pd(OH)_2/C$, $H_2$ (1 atm), MeOH/AcOH (40:1), 16 h; (ii) **8**, EDC·HCl, HOBt, $NEt_3$, $CHCl_3$, rt, 16 h; (iii) TFA/$CH_2Cl_2$ (1:3), 0 °C to rt, 3 h

Selected structural details for tripeptide **4** are included in Table 1 and full crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 977339. Copies of these data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

## Results and Discussion

Tripeptide **4** was prepared in accordance with our previously optimised procedures [30, 31]: first, hydrogenolytic *N*-deprotection of dipeptide **5** [33] in the presence of Pearlman's catalyst [$Pd(OH)_2/C$] under an atmosphere of $H_2$ gave dimer amine **6** in 92 % isolated yield. Coupling of **6** with monomer acid **8** (>99:1 dr) [34] was achieved with HOBt/EDC·HCl to give trimer **7** as a single diastereoisomer (>99:1 dr) in 58 % yield after purification of the crude reaction mixture via recrystallisation ($CHCl_3$/heptane). Subsequent treatment of trimer **7** with TFA gave Cbz-[(1*R*,2*S*)-ACPC]$_3$-OH **4** in 70 % yield and >99:1 dr (Scheme 1). Recrystallisation of **4** from $CHCl_3$/heptane gave colourless plates which were subjected to X-ray diffraction analysis.

The molecular structure of **4** is illustrated below (Fig. 2). Adjacent molecules are linked by a series of intermolecular N–H···O=C and O–H···O=C hydrogen bonds to form a double tape-like arrangement in the crystal

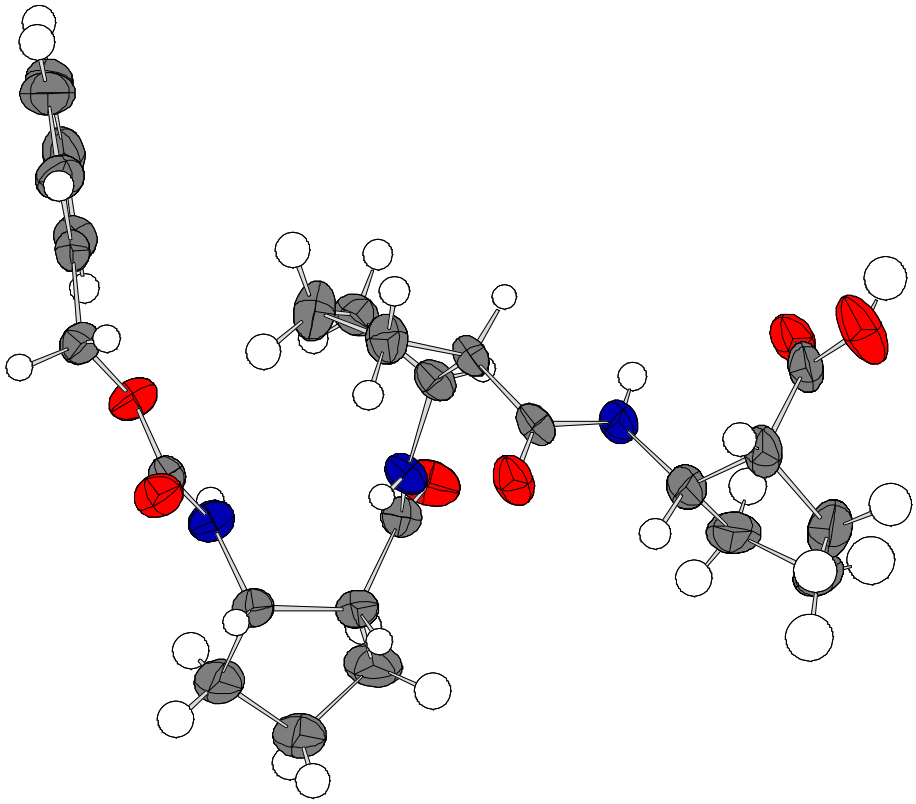


**Fig. 2** Molecular structure of **4**, showing the displacement ellipsoids drawn at the 50 % probability level

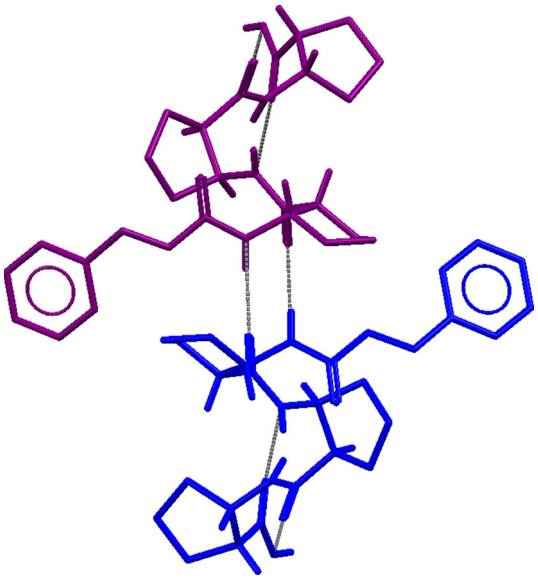


**Fig. 3** H-bonding within the crystal structure of **4**, viewed along the *a* axis; selected H atoms are omitted for clarity. Hydrogen bonding interactions are shown as grey dotted lines, forming R2,2(14) intermolecular rings [40] that connect molecules together to give hydrogen bonded peptide chains [N–H···O=2.998(4), 3.240(4), 2.869(4) Å; O–H···O=2.579(4) Å]

structure consisting of R2,2(10) and R2,2(14) intermolecular rings (Figs. 3 and 4) [40]. Whilst it was not possible to produce crystals of the corresponding tetramers or pentamers which were suitable for X-ray crystallographic analyses, a combination of IR, $^{1}$H NMR and CD spectroscopic analyses confirmed that these oligomers display traits indicative of *intermolecular* hydrogen bonding [32] and are therefore consistent with a similar conformation in solution.

## Conclusion

In conclusion, the crystal structure of the β-peptide Cbz-[(1*R*,2*S*)-ACPC]$_3$-OH, which is derived from (1*R*,2*S*)-cispentacin, was studied by X-ray diffraction. Adjacent molecules are linked by a series of N–H···O=C and O–H···O=C hydrogen bonds to form a double tape-like arrangement in the solid state.

## Supporting Information

Full crystallographic data for **4** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 977339. Copies of these data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

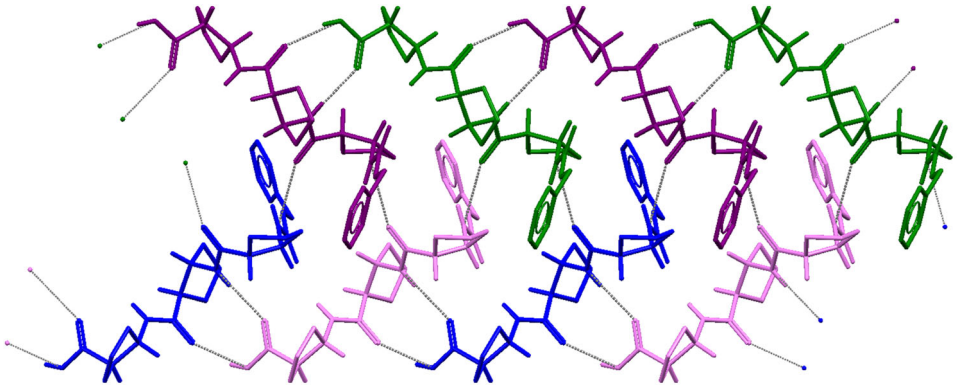


**Fig. 4** H-bonding within the crystal structure of **4**, viewed perpendicular to the *a* axis; selected H atoms are omitted for clarity. Hydrogen bonding interactions are shown as grey dotted lines, forming R2,2(10) intermolecular rings [40] that connect together to give hydrogen bonded peptide chains [N–H···O=2.998(4), 3.240(4), 2.869(4) Å; O–H···O=2.579(4) Å]

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