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C^{α}-Hydroxymethyl Methionine: Synthesis, Optical Resolution and Crystal Structure of its (+)-N^{α}-Benzoyl Derivative

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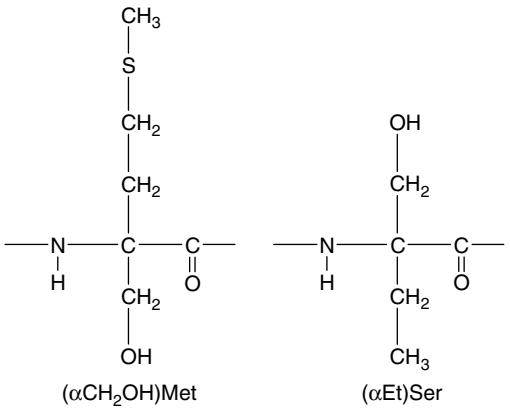
Abstract: (*R*, *S*)-Methionine was transformed into C^{α}-hydroxymethyl methionine by a route involving C^{α}-hydroxymethylation of 2-phenyl-4-methylthioethyl-5-oxo-4,5-dihydro-1,3-oxazole. The absolute configuration of (–)-C^{α}-hydroxymethyl methionine was elucidated to be (*S*) by chemical correlation with (*S*) (–)-C^{α}-ethyl serine. Absolute structure determination (by single crystal X-ray diffraction) on N^{α}-benzoyl-C^{α}-hydroxymethyl methionine confirmed the (*R*)-configuration for the (+)-enantiomer. In addition, the X-ray diffraction analysis showed that the $C^{\alpha,\alpha}$-disubstituted glycyl residue adopts the fully extended (C_5) conformation. Copyright © 2001 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: absolute configuration; α-amino acid synthesis; $C^{\alpha,\alpha}$-dialkylated α-amino acid; conformational analysis; enantiomeric resolution; C^{α}-hydroxymethyl methionine; X-ray diffraction

INTRODUCTION

C^{α}-Hydroxymethyl methionine, HmMet, belongs to the family of $C^{\alpha,\alpha}$-disubstituted glycines, which are useful replacements for protein amino acids because of their strong secondary structure-promoting property and the increased proteolytic stability of the resulting peptides [1,2]. The great current interest in this family of amino acids led us to develop a general and efficient method for the preparation of C^{α}-hydroxymethylated α-amino acids *via* selective C^{α}-hydroxymethylation of protein amino acids [3]. This procedure was subsequently exploited by other groups [4]. Crystal structures of some of the N^{α}-Bz derivatives were investigated in order to assess the absolute configuration at the α-carbon atom in the C^{α}-hydroxymethylated analogues of cysteine [5], tyrosine [6], aspartic acid [7,8] and ornithine [9]. It is interesting to note that the fully extended



Abbreviations: Ac, acetyl; Bz, benzoyl; (αEt)Ser, C^{α}-ethyl serine; HmMet, C^{α}-hydroxymethyl methionine.

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(C_5) conformation [10,11], characterized by an $i \rightarrow i$ intramolecular N–H ... O=C H-bond, is that generally adopted by the C^{α}-hydroxymethylated α-amino acid residues.

Here we describe the synthesis, optical resolution and absolute configuration (*via* X-ray diffraction analysis of the N^{α}-benzoyl derivative) of HmMet, a $C^{\alpha,\alpha}$-disubstituted glycine never studied so far.

MATERIALS AND METHODS

Peptide Synthesis and Characterization

All solvents were purified by conventional methods. Evaporations were carried out under reduced pressure. Melting points were determined on a capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 250 MHz on a Bruker model DPX-250. Splitting patterns are abbreviated as follows: (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet. The optical rotations were measured on a Horiba high-speed automatic polarimeter at 589 nm. For the thin-layer chromatography runs 250 mm silica gel GF protected uniplates (Analtech) were used with the following solvent systems: (A) $CHCl_3$–MeOH–AcOH 90 : 10 : 2, (B) Bu^nOH–AcOH–EtOAc–H_2O 1 : 1 : 1 : 1, (C) EtOAc–hexane 1 : 1, (D) Bu^nOH–AcOH–H_2O–pyridine 15 : 3 : 3 : 10. The chromatograms were visualized with chlorine followed by starch/KI or with ninhydrin. HPLC was performed on a LDC Analytical instrument using a Vydac C_{18} (0.46 × 25 cm) column: flow rate 1.0 ml/min, detection at 220 nm, and eluants (A) 0.05% trifluoroacetic acid in water and (B) 0.038% trifluoroacetic acid in acetonitrile–H_2O 90:10 with a gradient application.

N^{α}-Benzoyl-(*R,S*)-methionine, Bz-(*R,S*)-Met-OH (1)

This compound was prepared according to the general procedure of the Schotten–Baumann reaction. From 29.8 g (200 mmol) of (*R, S*)-methionine and 25.4 ml (220 mmol) of Bz-Cl, 48.8 g (96%) of **1** were obtained. Mp 145°–147 °C; R_f(A) = 0.25, R_f(B) = 0.80. ^{1}H NMR ($CDCl_3$)δ: 2.11(s, 3H), 2.15–2.40 (m, 2H), 2.69 (t, 2H), 4.92 (q, 1H), 7.50 (m, 3H), 7.81 (m, 2H).

(*R,S*)-5-Benzoylamino-5-methylthioethyl-4-oxo-1,3-dioxane (3) (3,12)

N^{α}-Benzoyl-(*R, S*)-methionine **(1)** (48.8 g, 192 mmol) in 240 ml of $(Ac)_2O$ was heated on a steam bath until a clear solution formed. The solution was cooled and evaporated to dryness. The residue was re-evaporated twice with toluene. Crude 2-phenyl-4-methylthioethyl-5-oxo-4,5-dihydro-1,3-oxazole **(2)** was dissolved in pyridine (25 ml) and 96 ml of aqueous (35%) formaldehyde were added. The mixture was stirred overnight at room temperature and diluted with water. The precipitate was collected and recrystallized from EtOAc–hexane. Yield: 51 g (88%). Mp 95°–97 °C; R_f(A) = 0.85, R_f(C) = 0.30; HPLC purity 100%, t_r = 7.89 min with a linear gradient (40–80%B) over 25 min. ^{1}H NMR ($CDCl_3$)δ: 2.13 (s, 3H), 2.34 (t, 2H), 2.66–2.83 (m, 2H), 4.13,4.27 (2H, AB system, J = 8 Hz), 5.46, 5.69 (2H, AB system, J = 5.4 Hz), 7.49 (m, 3H), 7.91 (m, 2H), 8.28 (broad s, 1H).

N^{α}-Benzoyl-(*R,S*)-C^{α}-hydroxymethyl methionine, Bz-(*R,S*)-HmMet-OH (4) (3,12)

A solution of **3** (50 g, 169 mmol) in 2 N NaOH was stirred for 4 h at room temperature and then acidified with 2 N HCl. The aqueous layer was extracted with EtOAc. After evaporation the crude product was recrystallized from EtOAc–hexane. Yield 35 g (71%). Mp 130°–132 °C; R_f(A) = 0.50, R_f(B) = 0.85; HPLC purity 99%, t_r = 9.12 min with a linear gradient (20–70%B) over 25 min. ^{1}H NMR (DMSO-d_6)δ: 2.01 (s, 3H), 2.17–2.75 (m, 4H), 2.79 (s, 2H), 7.49 (m, 3H), 7.83 (m, 2H).

Resolution of N^{α}-benzoyl-(*R,S*)-C^{α}-hydroxymethyl methionine, Bz-(*R,S*)-HmMet-OH (4)

To a solution of N^{α}-benzoyl-(*R, S*)-C^{α}-hydroxymethyl methionine **(4)** (21.1 g, 70.9 mmol) in EtOH (200 ml), quinine (26.84 g, 70.9 mmol) was added. The product was separated by fractional crystallization from EtOH into the diastereoisomeric salts A and B. After five runs, the less soluble salt A (32.3 g, 68%) had a constant melting point of 204°–205 °C and a constant $[\alpha]_D^{20} = -38.0°$ (c = 1, MeOH). Salt A was suspended in 2 N HCl and shaken. The mixture was extracted with EtOAc (3 × 30 ml). Evaporation of the dried extract resulted in the formation of crystalline (+)-N^{α}-benzoyl-C^{α}-hydroxymethyl methionine (**4a**). Yield 6.9 g (66%, based on the quantity of fraction A). Mp 146°–148 °C; $[\alpha]_D^{20} = +13.8°$ (c = 0.5, MeOH). The same procedure applied to the more soluble diastereoisomeric salt B yielded (–)-N^{α}-benzoyl-C^{α}-hydroxymethyl methionine (**4b**) (6.32 g, 60%). Mp 146°–148 °C and $[\alpha]_D^{20} = -13.1°$ (c = 1, MeOH).

Hydrogenolysis of (+)- and (−)-N$^{\alpha}$-benzoyl-C$^{\alpha}$-hydroxymethyl methionine, Bz-HmMet-OH (4a, 4b)

The procedure is based on the Mozingo's method of desulfuration [13]. A mixture of (−)-N^{α}-benzoyl-C^{α}-hydroxymethyl methionine (**4b**) (1 g, 3.35 mmol), Raney Ni ready to use (5 g) and EtOH (50 ml) was refluxed for 14 h. The Ni catalyst was removed by filtration and washed on the filter with 1 N NaOH. The combined filtrates were acidified with 2 N HCl and extracted with EtOAc (3 × 20 ml). Evaporation of the dried extract followed by crystallization of the residue from EtOH–hexane afforded 0.6 g (71%) of (−)-N^{α}-benzoyl-C^{α}-ethyl serine (**6b**). Mp 163°–165 °C; R_f(A) = 0.65, R_f(D) = 0.75; $[\alpha]_D^{20}$ = −9.5° (c = 1, MeOH). ^{1}H NMR ($CDCl_3$)δ: 0.92 (t, 3H), 1.89 (q, 2H), 3.64, 3.95 (2H, system AB, J = 11.5 Hz), 7.25–7.72 (m, 5H). The same procedure applied to (+)-N^{α}-benzoyl-C^{α}-hydroxymethyl methionine (**4a**) yielded (+)-N^{α}-benzoyl-C^{α}-ethyl serine (**6a**). Mp 163°–165 °C; $[\alpha]_D^{20}$ = +10.1° (c = 1, MeOH).

Hydrolysis of (+)- and (−)-N$^{\alpha}$-benzoyl-C$^{\alpha}$-hydroxymethyl methionine, Bz-HmMet-OH (4a, 4b)

A suspension of (−)-N^{α}-benzoyl-C^{α}-hydroxymethyl methionine (**4b**) (5 g, 17 mmol) in 5N HCl (20 ml) was refluxed for 5 h. The resulting insoluble benzoic acid was filtered off. The solution was evaporated to dryness to give a solid residue. The crude amino acid was dissolved in water and passed through an ion-exchange column (Amberlite IR-120, H^+-form). The column was washed with water until the eluate became neutral, and then eluted with 2 N NH_3 until the ninhydrin test became negative. The eluates were combined and evaporated to dryness. Crystallization of the solid residue from H_2O–EtOH afforded 2.5 g (82%) of (−)-C^{α}-hydroxymethyl methionine (**5b**) of enantiomeric purity 99% (determined by using the Marfey's reagent [14]). Mp 224°–226 °C; $[\alpha]_D^{20}$ = −10.5° (c = 1, 5N HCl), R_f(B) = 0.55, R_f(D) = 0.65. ^{1}H NMR (D_2O) δ: 2.09 (s, 3H), 1.64–2.72 (m, 4H), 3.47,3.73 (system AB, 2H). The same procedure applied to (+)-N^{α}-benzoyl-C^{α}-hydroxymethyl methionine (**4a**) gave (+)-C^{α}-hydroxymethyl methionine (**5a**), which had 99% enantiomeric purity. Mp 224°–226 °C; $[\alpha]_D^{20}$ = +11.0° (c = 1, 5N HCl).

Hydrolysis of (+)- and (−)-N$^{\alpha}$-benzoyl-C$^{\alpha}$-ethyl serine, Bz-(αEt)Ser-OH (6a, 6b)

A suspension of (−)-N^{α}-benzoyl-C^{α}-ethyl serine (**6b**) (0.5 g, 1.98 mmol) in 5N HCl (5 ml) was refluxed for 5 h. The resulting insoluble benzoic acid was filtered off. The filtrate was evaporated to dryness to give a solid residue. The crude amino acid was dissolved in water and passed through an ion-exchange column (Amberlite IR-120, H^+-form). The column was washed with water until the eluate became neutral and then eluted with 2 N NH_3 until the ninhydrin test became negative. The eluates were combined and evaporated to dryness. Crystallization of the solid residue from H_2–OEtOH afforded 0.28 g (89%) of (−)-C^{α}-ethyl serine (**7b**). Mp 264°–265 °C; $[\alpha]_D^{20}$ = −3.0° (c = 1, 5N HCl) [lit. [15] $[\alpha]_D^{20}$ = −3.3° (c = 1, 5N HCl)]; R_f(B) = 0.40, R_f(D) = 0.50. ^{1}H NMR (1 N DCl/D_2O; DSS) δ: 0.90 (t, 3H), 1.85 (q, 2H), 3.60, 3.90 (2H, system AB, J = 12 Hz). The same procedure applied to (+)-N^{α}-benzoyl-C^{α}-ethyl serine **(6a)** gave (+)-C^{α}-ethyl serine **(7a)**. Mp 224°–226 °C; $[\alpha]_D^{20}$ = +3.1° (c = 1, 5N HCl).

X-Ray Diffraction

A sample of (+)-Bz-HmMet-OH was crystallized from a methanol–water mixture by slow evaporation. Large crystals formed within a few days. From one of these a plate of approximate dimensions 0.40 × 0.40 × 0.10 mm was cut and mounted on the tip of a glass capillary. Data collection was performed on a Philips PW1100 four-circle diffractometer, using graphite-monochromated CuKα radiation, in the following hkl range: h from −10 to 10, k from −11 to 11, l from −17 to 17. Intensities were corrected for Lorentz and polarization effects. Symmetry equivalents, but not Bijovet pairs, were averaged. Three standard reflections, monitored every 50 reflections, did not show any significant intensity variation during the entire data collection. Crystallographic data are listed in Table 1.

The structure was solved by direct methods of the SHELXS97 program [16], and refined by full-matrix least squares on F^2, using all data, by application of the SHELXL97 program [17], with all non-H atoms anisotropic. The positions of the H-atoms linked to the O1G and OT atoms were recovered from a difference Fourier map, while the remaining H-atoms were calculated at idealized positions. All H-atoms were refined as riding with U_{iso} set equal to 1.2 (or 1.5 for the OH and CH_3 groups) times the U_{eq} of the parent atom.

Table 1 Crystallographic Data and Structure Refinement for (+)-Bz-(*R*)-HmMet-OH

Empirical formula	$C_{13}H_{17}NO_4S$
Formula weight (a.m.u.)	283.3
Temperature (K)	293(2)
Wavelength (λ, Å)	1.54178
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
a (Å)	9.442(2)
b (Å)	10.115(3)
c (Å)	15.421(4)
V (Å^3)	1473(1)
Z (molecules/unit cell)	4
Density (calc.) (g/cm^3)	1.278
Absorption coefficient (mm^{-1})	2.047
F(000)	600
Crystal size (mm)	0.40 × 0.40 × 0.10
Scan mode	$\theta - 2\theta$
θ range for data collection (°)	5.49 to 60.00
Limiting indices	$-10 \le h \le 10$, $-11 \le k \le 11$, $-17 \le l \le 17$
Collected reflections	2733
Independent reflections	2185 [$R_{int} = 0.0377$]
Observed reflections	2090 [$F \ge 4.0\sigma(F)$]
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	2185/0/174
Goodness-of-fit (on F^2)	0.944
Final R indices [$F \ge 4.0\sigma(F)$]	$R_1 = 0.0422$, $wR_2 = 0.1172$
R indices (all data)	$R_1 = 0.0436$, $wR_2 = 0.1190$
Absolute structure parameter	−0.018(1)
Largest diff. peak/hole (e· Å^{-3})	0.459/−0.414

The absolute structure determination was carried out by full-matrix refinement of the Flack parameter [18–20] on the entire data set (all possible 452 h,k,l and $-h$, $-k$, $-l$ pairs within the θ range of data collection) by use of the TWIN and BASF commands of the SHELXL97 program.

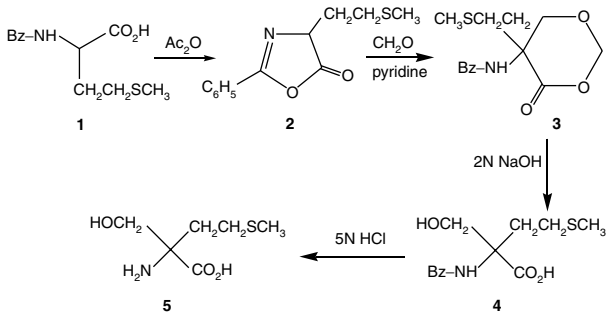
Tables of final atomic positional parameters, anisotropic displacement parameters for non-hydrogen atoms, bond distances and bond angles for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk.

RESULTS AND DISCUSSION

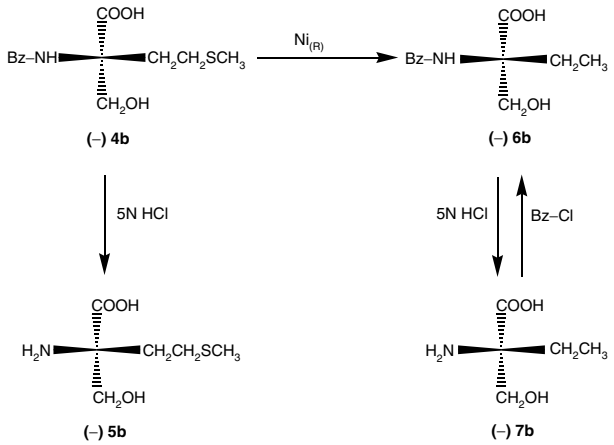
Chemistry

HmMet was readily prepared as a racemic product by a general procedure developed in our laboratory [3], involving selective C^{α}-hydroxymethylation of the 5(4*H*)-oxazolone derived from (*R*, *S*)-Met. Dehydration of Bz-(*R*, *S*)-Met-OH (**1**) to the corresponding 5(4*H*)-oxazolone (**2**), followed by treatment with aqueous formaldehyde in the presence of pyridine, yielded 5-benzoylamino-5-methylthioethyl-4-oxo-1,3-dioxane (**3**) (Scheme 1). Ring opening of lactone **3** under controlled alkaline conditions (2 N aqueous NaOH, room temperature) resulted in the formation of Bz-HmMet-OH (**4**) [12] with an overall yield of 58% for the four step synthesis. Resolution of **4** into enantiomers was achieved by fractional crystallization of the diastereoisomeric salts formed with (−) quinine. By this procedure both enantiomers of Bz-HmMet-OH could be isolated. They were hydrolysed under acidic conditions to afford free (+) and (−) H-HmMet-OH (**5a**, **5b**) of 99% enantiomeric purity, as determined *via* HPLC using the Marfey's [*N*-(2,4-dinitro-5-fluorophenyl)-alanine amide] reagent [14].

It is known that the enzymatic approach to the absolute configuration of $C^{\alpha,\alpha}$-disubstituted glycines may lead to contradictory conclusions [21]. For that reason a chemical correlation was undertaken between the optically active (−)-Bz-HmMet-OH (**4b**) and (−)-C^{α}-ethyl serine, H-(αEt)Ser-OH (**7b**), the absolute (*S*)-configuration of which had been already established [15]. The correlation strategy (Scheme 2), previously applied in the



Scheme 1



Scheme 2

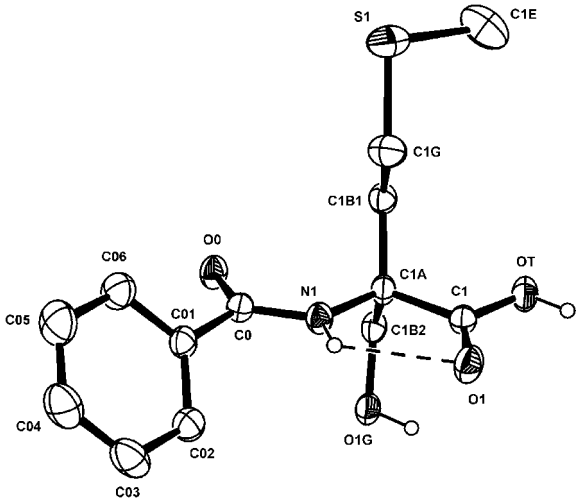


Figure 1 ORTEP [36] drawing of (+)-Bz-(*R*)-HmMet-OH with numbering of the atoms. Non H-atoms represented as displacement ellipsoids are plotted at the 30% probability level, while H-atoms bound to heteroatoms are shown as small spheres of arbitrary radius. The intramolecular H-bond is represented by a dashed line.

case of C^{α}-hydroxymethyl cysteine [22], involves hydrogenolytic desulfuration [13] of (–)-Bz-HmMet-OH (**4b**) to (–)-Bz-(αEt)Ser-OH (**6b**) followed by hydrolysis to (*S*)(–)-H-(αEt)Ser-OH (**7b**). Correlation between (–)-H-HmMet-OH (**5b**) and (–)-Bz-HmMet-OH (**4b**) is evident from the hydrolytic reaction **4b** → **5b**.

The same sequence of reactions applied to (+)-Bz-HmMet-OH (**4a**) afforded (*R*)-(+)- H-(αEt)Ser-OH (**7a**) and (+)-H-HmMet-OH (**5a**). This result proves the (*R*)-configuration for the (+)-enantiomer of H-HmMet-OH.

X-Ray Diffraction Analysis

We determined the absolute configuration and the crystal-state molecular structure of (+)-Bz-HmMet-OH by X-ray diffraction analysis. The molecular structure with the atomic numbering scheme is shown in Figure 1.

The absolute configuration was determined by use of the Flack parameter [18–20]. The trial solution having the best combined figure of merit, as obtained by direct methods, gave a model corresponding to the (*R*) configuration for the α-carbon of the HmMet residue. After refinement the final *R* indices were $R_1 = 0.0422$ [on $F \geq 4\sigma(F)$] and $wR_2 = 0.1190$ (on F^2, all data), the goodness of fit on F^2 was 0.944, and the Flack parameter converged to −0.018(1). Then, the structure with the reversed signs for all positional parameters was similarly refined. This inverse model [(*S*)-configuration] led to $R_1 = 0.0514$ [on $F \geq 4\sigma(F)$], $wR_2 = 0.1464$ (on F^2, all data), goodness of fit on $F^2 = 1.166$, and Flack parameter = 1.018(1). A comparison of these data clearly shows that the model with the inverse (*S*)-configuration has to be rejected. A sound basis for such a conclusion is provided by the contribution of the sulfur atom to the inversion-distinguishing power and by the presence in the dataset of all possible *hkl* and *-h-k-l* Bijovet pairs within the θ range of data collection.

The carboxylic acid moiety is found in the -C(=O)-OH form, the C1-O1 and C1-OT bond distances being significantly different, 1.204(3) and 1.321(3) Å, respectively. The value of the N1-C1A-C1 bond angle, 105.2(2)°, is remarkably compressed compared with the regular tetrahedral value (109.5°) [23,24]. This is a first indication of the presence of an intramolecular interaction between the N–H and (carboxylic acid) C=O groups forming the fully-extended, pentagonal (C_5) conformation [10,11]. The occurrence of this structure is corroborated by the following additional observations: (i) The value of the torsion angle N1-C1A-C1-O1, 4.9(3)°, indicates an approximate *synperiplanar* arrangement for the N–H and (carboxylic acid) C=O groups [25], an uncommon observation for N^{α}-acylated α-amino acids [26]. (ii) The secondary amide torsion angle ω_0 [27] is in the usual *trans* conformation, −178.6(2)° [26,28]. (iii) The HmMet residue is fully extended (falling in the region E of the φ, ψ space [29]) with $\varphi_1 = -178.5(2)^{\circ}$ and $'\psi_1' = -176.1(2)^{\circ}$. (iv) The N1 ... O1 [2.615(3) Å] and N1-H ... O1 (2.18 Å) intramolecular distances and the N1-H ... O1 angle (111°) are compatible with

the onset of an intramolecularly H-bonded [30–32] pentagonal structure.

The angle between normals to the phenyl and the pentagonal pseudo-ring planes is 45.4(1)° [26]. The $\chi_1^{1'}$ (N1-C1A-C1B2-O1G) torsion angle of the 'Ser-like' side chain is −51.7(3)°, while the corresponding angle for the Met side chain, $\chi_1{}^1$ (N1-C1A-C1B1-C1G) is 53.4(3)° [33]. The $\chi_1{}^2$ (C1A-C1B1-C1G-S1) and $\chi_1{}^3$ (C1B1-C1G-S1-C1E) torsion angles of the Met side chain have values of 179.2(2) and −68.3(3)°, respectively [33].

In the unit cell the (+)-Bz-HmMet-OH molecules are packed through intermolecular (carboxylic acid) OT-H ... O1G (alcohol) $(-x-1,\ y+1/2,\ -z+3/2)$ and O1G-H ... O0═C0 (amide) $(-x-1,\ y+1/2,\ -z+3/2)$ H-bonds, with the OT ... O1G and O1G ... O0 separations [2.638(2) and 2.728(2) Å, respectively] within the range expected for such H-bonds [34,35], forming rows along the *b*-direction.

CONCLUSIONS

In this work we have expanded the scope of our ongoing research on C^{α}-hydroxymethylated, chiral α-amino acids [3,5–9,12,22] by synthesizing the (*S*)- and (*R*)-enantiomers of HmMet. The absolute configuration of the N^{α}-benzoylated derivative of the (+)-enantiomer was established by X-ray diffraction analysis to be (*R*). In the crystal state Bz-HmMet-OH adopts the fully extended (C_5) conformation. The authors foresee a bright future for this new sub-class of conformationally restricted, C^{α}-tetrasubstituted α-amino acids [1,2].

Acknowledgements

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