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α–Hydroxymethylserine as a Peptide Building Block: Synthetic and Structural Aspects

MARCIN STASIAK[1], WOJCIECH M. WOLF[2] AND MIROSLAW T. LEPLAWY[1]

[1]Institute of Organic Chemistry, Technical University, Łódź, Poland
[2]Institute of General and Ecological Chemistry, Technical University, Łódź, Poland

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Abstract: A synthetic methodology has been developed for peptide bond formation with α-hydroxmethylserine as the carboxyl or amino component and also for the preparation of homo-sequences. The key intermediate, O,O-protected α-hydroxymethylserine in the form of an isopropylidene derivative, is easily accessible and represents the first example of a heterocyclic $C^{\alpha,\alpha}$-disubstituted amino acid containing an 1,3-dioxane ring. The use of this intermediate facilitates protection of the sterically hindered amino and carboxyl groups and is advantageous for the coupling and deprotection steps. X-ray structure determination of Z-HmS(Ipr)–Ala–OMe revealed that the two crystallographically independent molecules present in the asymmetric unit adopt an S-shaped conformation. In the one molecule the achiral HmS(Ipr) residue has the torsion angle values ($\phi = 61.4°$, $\psi = 40.8°$) in the left-handed helical region of the Ramachandran map, while in the second molecule the negative torsion angles ($\phi = -60.1°$, ψ=–44.4°) are associated with the right-handed helix. © 1998 European Peptide Society and John Wiley & Sons, Ltd.

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INTRODUCTION

Research interest in peptides containing α-hydroxymethylserine (HmS) is motivated by the following findings and considerations. α-Hydroxymethylserine is contained as the N-terminal residue in the closely related dehydropeptide antibiotics antrimycins [1] and cirratiomycins [2], combining tuberculostatic activity with low toxicity. A natural, β-substituted derivative of α-hydroxymethylserine, designated as ISP-1 and found to be identical to the previously isolated myriocin, is extensively studied as a potent immunosuppressant [3]. As a member of the family of $C^{\alpha,\alpha}$-dialkyl amino acids [4], α-hydroxymethylserine should generate specific constraints on the conformational freedom of the peptide chain. This point is relevant to the potential usefulness of this residue in the studies on the structure–activity relationships of bioactive peptides. One can expect that this amino acid would furnish peptides resistant to enzymatic attack and with an enhanced hydrophilicity advantageous for an increase of bioaccessibility. The presence of the side-chain OH functional groups can contribute to binding and offers the possibility of further modification. α-Hydroxymethylserine was found to be a much more efficient chelating ligand for transition metals than serine [5].

Abbreviations: Ac_6c, 1-aminocyclohexaneacarboxylic acid; AcOEt, Ethyl acetate; DCM, dichloromethane; DIEA, Diisopropylethylamine; HmS, α-hydroxymethylserine; Ipr, *O,O*-isopropylidene; MeA, C^{α}-methylalanine (or Aib); MOM, methoxymethyl; OPfp, pentafluorophenyloxy; TBTU, *O*-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium tetrafluoroborate; TosOH, 4-toluene-sulphonic acid; Z, benzyloxycarbonyl.

Address for correspondence: Prof. M. T. Leplawy, Insitute of Organic Chemistry, Technical University, 90–924, Łódź, Poland.

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In spite of its potential as a peptide building block, α-hydroxymethylserine remains neglected by peptide chemists. Only few examples of the incorporation of this residue into peptide chain have been reported so far. In the total synthesis of the heptapeptide antibiotic antrimycin D_v, accomplished by Schmidt and Riedl [6], the N-terminal α-hydroxymethylserine was attached to the hexapeptide intermediate in the from of Boc-HmS(*O,O*-benzylidene)-OPfp derivative, obtained in a six-step preparation from the commercially available tris(hydroxymethyl)nitromethane. In another synthesis of this antibiotic reported by Nakamura *et al.* [7], the α-hydroxymethylserine derivative Boc-HmS$(MOM)_2$-OH was coupled with H-Ala-OBzl in the presence of diphenylphosphoryl azide and the resulting dipeptide was used for the final (2 + 5) coupling. We have published a preliminary investigation of routes to peptides containing a single HmS residue [8]. It was pointed out that steric hindrance and the presence of the hydroxyl groups pose a problem, particularly in the synthesis of peptides with a C-terminal HmS. These complications have now been mastered and we are able to report on an improved methodology for peptide bond formation with HmS as the carboxyl or amino component and also for the synthesis of homo-sequences. We also describe here the results of an X-ray diffraction analysis of the dipeptide Z-HmS(Ipr)-Ala-OMe, containing a N-terminal α-hydroxymethylserine bearing an acetal-type *O,O*-isopropylidene protecting group.

MATERIALS AND METHODS

Synthesis

Evaporations were performed on a rotavapour under reduced pressure. Melting points were determined on a Gallenkamp capillary melting point apparatus and are uncorrected. Optical rotations were measured on a Horiba polarimeter in a 1 dm cell (1 ml) at 589 nm (Na D line) at room temperature. TLC was carried out on 250 μm silica gel GF precoated uniplates (Analtech) with the following solvent systems: **I** 2-propanol/25% aq. NH_3 7:3; **II** $CHCl_3$/MeOH/AcOH 80 : 20 : 3; **III** $CHCl_3$/MeOH 1 : 1; **IV** $CHCl_3$/MeOH 10 : 1; **V** $CHCl_3$/MeOH/AcOH 20 : 1 : 1; **VI** AcOEt/hexane 1 : 1; **VII** AcOEt/heptane 1 : 1; **VIII** AcOEt/heptane 1 : 2. Spots were visualized by UV light, chlorine followed by starch/KI spray or by spraying with ninhydrin. HPLC was performed on a Thermoseparation instrument using a C_{18} Vydac (0.46 × 25 cm) column thermostated at 36 °C, flow rate 1 ml/min, detection at 220 nm. The following solvent system (with gradient change over 25 min) was used: **A** 0.05% TFA in water; **B** 0.038% TFA in acetonitrile/water 90 : 10. ^{1}H-NMR spectra were obtained on a 250 MHz instrument (Bruker Avance DPX) with TMS as internal standard. Chemical shifts are in p.p.m. FAB mass spectra were recorded on a APO Electron (Ukraine) Model MI 12001E mass spectrometer equipped with a FAB ion source. For flash chromatography, columns packed with silica gel 60 were used. α-Hydroxymethylserine was synthesized according to the modified procedure reported elsewhere [8].

TosOH × H-HmS(Ipr)-OH (**1**), ($C_{14}H_{21}NO_7S$, M=347.4). To a stirred solution of TosOH monohydrate (5.71 g, 30 mmol) in acetone (50 ml) were added dimethoxypropane (10 ml, 80 mmol) and α-hydroxymethylserine (1.35 g, 10 mmol). After 10 min, the solid material dissolved and then the product begun to precipitate. The reaction mixture was left overnight at room temperature. The product was filtered off, washed with acetone and ether, and then dried. Yield 2.87 g (83%), m.p. 170–171 °C, TLC: 0.50 (I). ^{1}H-NMR (DMSO-d_6): 1.40 (s, 3H, Ipr); 1.44 (s, 3H, Ipr); 2.28 (s, 3H, CH_3 TosOH); 3.80 (d, 2H, J_{AX}=12.8 Hz, H_β HmS); 4.26 (d, 2H, J_{AX}=12.8 Hz, H_β HmS); 7.10 (d, 2H, J_{AX}=8 Hz, TosOH arom.); 7.46 (d, 2H, J_{AX}=8 Hz, TosOH arom.); 8.60 (b, 3H, NH_3) FAB-MS (*m/z*). 176 $(MH)^+$. Calculated for $C_7H_{13}NO_4$, 175.

Z-HmS(Ipr)-m→OH (**2**), ($C_{15}H_{19}NO_6$,M=309.3). To compound **1** (3.01 g, 8.66 mmol) suspended in dioxane (40ml) was added a saturated $NaHCO_3$ solution (70 ml). Benzyl chloroformate (90%, 5.5 ml, 35 mmol) in dioxane (30 ml) was added dropwise with stirring and the mixture was kept overnight at room temperature. After 24 h, dioxane was evaporated and the excess of benzyl chloroformate was extracted with ether. The aqueous layer was acidified with $NaHSO_4$ and extracted with AcOEt. The extract was dried over anhydrous $MgSO_4$ and evaporated. The residue was crystallized from hot AcOEt/hexane. Yield 2.088 g (78%), m.p. 165–167 °C (dec.), TLC: 0.78 (III), 0.48 (V). HPLC: purity 100%, rt 16.048 min (20–50% B). ^{1}H-NMR ($CDCl_3$): 1.47 (s, 3H, Ipr); 1.50 (s, 3H, Ipr); 2.75 (b, 1H, COOH); 4.13 (d, 2H, J_{AB}=12 Hz, H_β HmS); 4.20 (d, 12H, J_{AB}=12 Hz, H_β HmS); 5.14 (s,2H, Z CH_2); 5.79 (bs, 1H, NH); 7.35 (bs, 5H, Z arom.).

Z-HmS(Ipr)-OMe **(3)** ($C_{16}H_{21}NO_6$, M=323.3). To a solution of compound **2** (0.99 g, 3.2 mmol) in methanol (40 ml), a freshly distilled ethereal solution of diazomethane was added dropwise with stirring at room temperature. When the solution became yellow, the addition was stopped and solvents were evaporated. The residue was taken up in AcOEt and washed with solution of $NaHCO_3$, $NaHSO_4$ and finally with brine. The organic layer was dried over anhydrous $MgSO_4$ and evaporated. The residue was crystallized from AcOEt/hexane. Yield 0.900 g (87%), m.p. 77–78 °C, TLC: 0.74 (IV); 0.58 (VI). HPLC: purity 100%, rt 14.892 min (30–60% B). ^{1}H-NMR ($CDCl_3$): 1.42 (s, 3H, Ipr); 1.48 (s, 3H, Ipr); 3.76 (s, 3H, OCH_3); 4.08 (d, 2H, J_{AB}=12 Hz, H_β HmS); 4.22 (d, 2H, J_{AB}=12 Hz, H_β HmS); 5.11 (s, 2H, Z CH_2); 5.73 (bs, 1H, NH); 7.37 (bs, 5H, Z arom.). FAB-MS (m/z): 324 $(MH)^+$.

TosOH × H-HmS(Ipr)-OMe **(4)** ($C_{15}H_{23}NO_7S$, M= 361.4). *Method A.* To a stirred suspension of compound **1** (2.584 g, 7.44 mmol) in dioxane (50 ml) a freshly distilled ethereal diazomethane solution was added dropwise with stirring. When the solution became clear and yellow the addition was stopped. The solvents were evaporated and the residue was crystallized from MeOH/ether. Yield 0.784 g (29%), m.p. 148–149 °C, TLC: 0.71 (III). ^{1}H-NMR ($CDCl_3$): 1.43 (s, 3H, Ipr); 1.50 (s, 3H,Ipr); 2.34 (s, 3H, TosOH CH_3); 3.72 (s, 3H, OCH_3); 4.19 (d, 2H, J_{AB}=12.6 Hz, H_β HmS); 4. 31 (dH, 2h, J_{AB}=12.6 Hz, H_β HmS); 7.12 (d,2H, J_{AX}=8 Hz, TosOH arom.); 7.74 (d, 2H, J_{AX}=8 Hz, TosOH arom.); 8.73 (b, 3H, NH_3).

Method B. Compound **3** (0.972 g, 3 mmol) in MeOH (20ml) was hydrogenated in the presence of 10% Pd/C catalyst (100 mg) at a pressure of 3–4 kg/cm^2 on a Parr apparatus for 2 h. After evaporation of the filtered solution the residual oil was dissolved in ether (20 ml), cooled to 0 °C and treated dropwise with a precooled ethereal solution of TosOH monohydrate (0.573 g in 15 ml, 3 mmol) with stirring. The precipitated salt **4** was filtered off, washed with ether and dried. Yield: 0.970 g (90%). Analytical data (m.p., TLC, NMR) were in agreement with values given for method A.

Z-HmS-OMe **(5)** ($C_{12}H_{17}NO_6$, M=283.3). *Method A.* Compound **3** (0.234 g, 1 mmol) was dissolved in methanol (2 ml) and 2N HCl (2 ml) was added. The starting material precipitated and then, in the course of reaction, dissolved. After 10 min, TLC revealed complete O,O-deprotection. The mixture was neutralized with $NaHCO_3$ and after evaporation of methanol, the solution was diluted with water and extracted with AcOEt (three times). The extract was dried and evaporated to furnish a semi-solid residue which was crystallized from AcOEt/hexane. Yield 0.257 g (90%), m.p. 65–67 °C, TLC: 0.71 (III), 0.41 (IV). HPLC: purity 99%, rt 7.780 min (20–50% B), rt 14.466 min (10-40% B). ^{1}H-NMR ($CDCl_3$): 2.90 (b, 2H, OH); 3.79 (s, 3H, OCH_3); 3.92 (D, 2h, J_{AB}=11.5 Hz, H_β HmS); 4.03 (d, 2H, J_{AB}=Hz, H_β HmS); 5.13 (s, 2H, Z CH_2); 5.90 (bs, 1H, NH); 7.37 (bs, 5H, Z arom.). *Method B.* Compound 3 (0.162 g, 0.5 mmol) was treated with a TFA/water mixture (9 : 1, 2 ml) for 15 min at room temperature. The reaction mixture was diluted with AcOEt, washed with saturated $NaHCO_3$, water, brine, and then dried and evaporated. The crude **5** was recrystallized from AcOEt/hexane. Yield 0.123 g (87%). For the data see method A.

Alkaline Hydrolysis of Z-HmS(Ipr)-OMe. To a stirred solution of compound **3** (0.324 g, 1 mmol) in dioxane (4 ml) 2N NaOH (0.55 ml, 1.1 eq.) was added dropwise. After 6 h (TLC monitoring) dioxane was evaporated, the solution was diluted with water and washed with AcOEt. The extract was dried, evaporated and the residue was crystallized from hot AcOEt/hexane affording analytically pure Z-HmS(Ipr)-OH. Yield 0.248 g (80%). For the data see compound 2.

Alkaline Hydrolysis of Z-HmS-OMe and Z-HmS-OH. Compound **5** (0.284 g, 1 mmol) dissolved in dioxane (4 ml) was treated with 2N NaOH (0.55 ml, 1.1 eq.). The progress of hydrolysis was monitored by TLC and HPLC. The disappearance of the starting material was observed in less than 1 h. HPLC revealed the presence of benzyl alcohol (the UV-active product of Z-group cleavage); TLC showed the ninhydrin positive compounds H-HmS-OMe and HmS sodium salt. No desired product Z-HmS-OH was obtained owing to the cleavage of the Z-group. Under the same alkaline conditions, Z-HmS-OH (compound reported previously [8]) was hydrolysed in 1 h to the sodium salt of HmS and benzyl alcohol.

General Procedure for Coupling. Z-HmS(Ipr)-OH (**2**) (0.619 g, 2 mmol), salt of amino acid ester (2 mmol), TBTU (0.706 g, 2.2 mmol) and DIEA (1.05 ml, 6 mmol) were stirred in DCM (10 ml) for 24 h. After solvent evaporation, the residue was taken up in

AcOEt, washed with a 1N $NaHSO_4$ solution, a saturated $NaHCO_3$ solution, brine, dried and evaporated. The crude peptide was purified by crystallization or column chromatography. In the synthesis of tripeptides, the dipeptide acids Z-HmS(Ipr)-AA-OH (0.5 mmol), obtained from alkaline hydrolysis of the corresponding esters, were reacted with TosOH × H-HmS(Ipr)OMe (**4**) (0.181 g, 0.5 mmol), TBTU (0.177 g, 0.55 mmol) and DIEA (0.258 ml, 1.5 mmol) in DCM (2 ml) for 24 h. The crude tripeptides were obtained after the identical work-up as for the dipeptides.

Z-HmS(Ipr)-Gly-OMe (**6**) ($C_{18}H_{24}N_2O_7$, M=380.4). HCl × H-Gly-OMe was used, coupling on 3 mmol scale. Crystallized from AcOEt/hexane. Yield 1.004 g (88%), m.p. 127–129 °C, TLC: 0.78 (IV). HPLC: purity 99% rt 9.776 min (30–60% B). ^{1}H-NMR ($CDCl_3$): 1.58–1.62 (2s, 6H, Ipr); 3.82 (s, 3H, OCH_3); 3.93 (d, 2H, J_{AX}=12.5 Hz, H_β HmS); 4.14 (d, 2H, J=5 Hz, H_αGly); 4.66 (d, 2H, J_{AX}=12.5 Hz, H_β HmS); 5.14 (s, 2H Z CH_2); 6.06 (bs, 1H, NH HmS); 7.39 (bs, 5H, Z arom.); 7.85 (b, 1H, NH Gly).

Z-HmS(Ipr)-Ala-OMe (**7**) ($C_{19}H_{26}N_2O_7$, M=394.4). HCl × H-Ala-OMe was used. Crystallized from ether/hexane. Yield 9.677 g (86%), m.p. 90–92 °C, $[\alpha]_D$=-35.2 ° (C=0.25, MeOH), TLC: 0.80 (IV), 0.47 (VI). HPLC: purity 99%, rt 13.660 min (30–60% B). ^{1}H-NMR ($CDCl_3$): 1.44 (d, 3H, J=7 Hz, H_β Ala); 1.53, 1.63 (2s, 6H, Ipr); 3.76 (s, 3H, OCH_3); 3.82–3.90 (m, 2H, H_β HmS); 4.52–4.63 (m, 3H, H_β HmS and H_α Ala); 5.09 (s, 2H, Z CH_2); 6.03 (bs, 1H, NH HmS); 7.35 (bs, 5H, Z arom.); 7.84 (b, 1H, NH Ala).

Z-HmS(Ipr)-Ala-OBzl (**8**) ($C_{25}H_{30}N_2O_7$, M=470.5). TosOH × H-Ala-OBzl was used, coupling on 0.5 mmol scale. The crude product was purified by flash chromatography (AcOEt/heptane 1 : 1) to furnish TLC homogeneous viscous oil. Yield 0.183 g (78%), $[\alpha]_D$=−33.0 ° (c=0.3, MeOH), TLC: 0.84 (IV), 0.55 (VII). HPLC: purity 95.2%, rt 15.481 min (40–70% B). ^{1}HNMR ($CDCl_3$): 1.44 (d, 3H, J=7 Hz, H_β Ala); 1.50, 1.62 (2s, 6H, Ipr); 3.81–3.89, 4.20–4.24, 4.56–4.64 (m, 5H, H_β HmS and Ala); 5.09 (s, 2H, CH_2); 5.15 (d, 1H, J_{AB}=12.5 Hz, CH Bzl); 5.22 (d, 1H, J_{AB}=12.5 Hz, CHBzl); 6.01 (bs, 1H, NH HmS); 7.36 (bs, 10H, Z and Bzl arom.); 7.84 (bd, 1H, J=5 Hz, NH Ala).

Z-HmS(Ipr)-MeA-OMe (**9**) ($C_{20}H_{28}N_2O_7$, M=408.4). HCl × H-MeA-OMe was used. Crystallized from AcOEt/hexane. Yield 0.650 g (80%), m.p. 108–110 °C, TLC: 0.81 (IV), 0.50 (VII). HPLC: purity 100% rt 14.694 min (30–60% B). ^{1}H-NMR ($CDCl_3$): 1.50 (s, 6H H_β MeA); 1.57, 1.64 (2s, 6H, Ipr); 3.74 (s, 3H, OCH_3); 3.82 (d, 2H, J_{AX}=10 Hz, H_β HmS); 5.09 (s, 2H, Z CH_2); 6.07 (bs, 1H, NH HmS); 7.36 (bs, 5H, Z arom.); 7.81 (bs, 1H, NH MeA).

Z-HmS(Ipr)-HmS(Ipr)-OMe (**10**) ($C_{23}H_{32}N_2O_9$, M=480.5). The amino component H-HmS(Ipr)-OMe was obtained by catalytic hydrogenation of Z-HmS(Ipr)-OMe as described for compound **4**, method B. Coupling on 5 mmol scale in the presence of 10 mmoles of DIEA. The crude dipeptide was purified by flash chromatography (AcOEt/heptane 1 : 2), and crystallized from AcOEt/heptane. Yield 1.537 g (64%), m.p. 136–139 °C, TLC: 0.82 (IV), 0.47 (VI), 0.17 (VIII). HPLC: purity 100%, rt 17.980 min (30–60% B), rt 12.289 min (40–70% B). ^{1}H-NMR ($CDCl_3$): 1.43; 1.48; 1.53; 1.60 (4s, 12H, Ipr groups); 3.73 (s, 3H, OCH_3); 3.83 (d, 2H, J_{AX}=12.5 Hz, H_β HmS-2); 3.97 (d, 2H, J_{AB}=12.5 Hz, H_β HmS-1); 4.22 (d, 2H, J_{AB}=12.5 Hz, H_β HmS-1); 4.71 (d, 2H, J_{AX}=12.5 Hz, H_β HmS-2); 5.09 (s, 2H, Z CH_2); 6.15 (bs, 1H, NH HmS-1); 7.35 (bs, 5H, Z arom.); 8.17 (bs, 1H, NH, HmS-2).

Z-HmS(Ipr)-Ala-HmS(Ipr)-OMe (**11**) ($C_{26}H_{37}N_3O_{10}$, M=551.6). Obtained from Z-HmS(Ipr)-Ala-OH (**14**). The crude product was crystallized from AcOEt/hexane. Yield 0.143 g (52%), m.p. 186–188 °C, $[\alpha]_D$=−2.0 ° (c=0.35, MeOH), TLC: 0.73 (IV), 0.50 (VI), HPLC: purity 96% rt 9.620 min (40–70% B). ^{1}H-NMR ($CDCl_3$): 1.37–1.76 (group of s, 15H, Ipr groups and H_β Ala); 3.70 (s, 3H, OCH_3); 3.88–3.97, 4.11–4.20, 4.46–4.62 (m, 9H, H_β HmS-1 HmS-3 and H_α Ala); 5.08 (s, 2H, Z CH_2); 5.96 (bs, 1H, NH HmS-1); 7.18 (bs, 1H, NH HmS-3); 7.34 (bs, 5H, Z arom.); 7.57 (bd, 1H, J=7.5 Hz, NH Ala). FAB-MS (m/z): 552 $(MH)^+$, 590 $(MK)^+$.

Z-HmS(Ipr)-MeA-HmS(Ipr)-OMe (**12**) ($C_{27}H_{39}N_3O_{10}$, M=565.6). Synthesized from Z-HmS(Ipr)-MeA-OH (**15**). The crude product was chromatographed (AcOEt/heptane 1 : 2) to furnish a TLC homogeneous product as a viscous oil. Yield 0.129 g (46%), TLC: 0.83 (IV), 0.64 (VI), 0.39 (VII), 0.20 (VIII). HPLC: purity 93% rt 10.977 min (40–70% B), rt 17.575 min (30–60% B). ^{1}H-NMR

($CDCl_3$): 1.45–1.60 (group of s, 18H, Ipr groups and H_β MeA); 3.69 (s, 3H, OCH_3); 3.90 (d, 2H, J_{AX}=12.5 Hz, H_β HmS-3); 4.05 (d, 2H, J_{AB}= 11.5 Hz, H_β HmS-1); 4.23 (d, 2H, J_{AB}=11.5 Hz, H_β HmS – 1);4.52 (d, 2H, J_{AX}=12.5 Hz, H_β HmS-3); 5.09 (s, 2H, Z CH_2); 5.71 (bs, 1H, NH HmS-1); 6.02 (bs, 1H, NH HmS-3); 7.35 (bs, 5H Z arom.); 7.70 (bs, 1H, NH MeA). FAB-MS (*m/z):* 588 $(MNa)^+$, 604 $(MK)^+$.

Z-HmS(Ipr)-HmS(Ipr)-HmS(Ipr)-OMe **(13)** ($C_{30}H_{43}N_3O_{12}$, *M*=637.7). Prepared from Z-HmS(Ipr)-HmS(Ipr)-OH (**16**). Crystallized from AcOEt/hexane. Yield 0.134 g (42%), m.p. 143–145 °C, TLC: 0.82 (IV), 0.40 (VI), 0.35 (VII). HPLC: purity 100%, rt 16.593 min (40–70% B). H-NMR ($CDCl_3$): 1.4–1.60 (group of s, 18H, Ipr groups); 3.72 (s, 3H, OCH_3); 3.96–4.51 (m, 12H, H_β HmS-1 HmS-2 HmS-3); 5.11 (s, 2H, Z CH_2); 5.97 (bs, 1H, NH HmS-1); 7.35 (bs, 5H, Z arom.); 7.84 (bs, 1H, NH HmS-3); 7.96 (bs, 1H, HmS-2). FAB-MS (*m/z*): 638 $(MH)^+$, 660 $(MNa)^+$.

Deprotection of Peptide Methyl Esters

Z-HmS(Ipr)-Ala-OH **(14)** ($C_{18}H_{24}N_2O_7$, *M*=380.4). To a stirred solution of compound **7** (0.395 g, 1 mmol) in dioxane (2 ml) 2N NaOH (0.55 ml, 1.1 eq.) was added dropwise. Stirring was continued for 6 h. After the usual work-up (see alkaline hydrolysis of Z-HmS(Ipr)-OMe), the crude dipeptide was purified by flash chromatography ($CHCl_3$/MeOH/AcOH 20 : 1 : 1) and crystallized from AcOEt/hexane. Yield 0.233 g (61%), m.p. 120–122 °C, $[\alpha]_D$=−22.6° (*c*=0.35, MeOH), TLC: 0.59 (II), 0.37 (V), HPLC: purity 99.5%, rt 9.856 min (30–60% B), rt 16.560 min (20–50% B). ^{1}H-NMR (DMSO-d_6): 1.26 (d, 3H, *J*=7 Hz, H_β Ala); 1.36 (s, 3H, Ipr); 1.39 (s, 3H, Ipr) 3.93–4.12 (m, 4H, H_β HmS); 4.26 (q, 1H, *J*=7 Hz, Hα Ala) 5.04 (s, 2H, Z CH_2) 7.37 (bs, 5H, Z arom.); 7.47 (bs, 1H, NH HmS); 7.81 (bd, 1H, J=7 Hz, NH Ala); 12.75 (b, 1H, COOH).

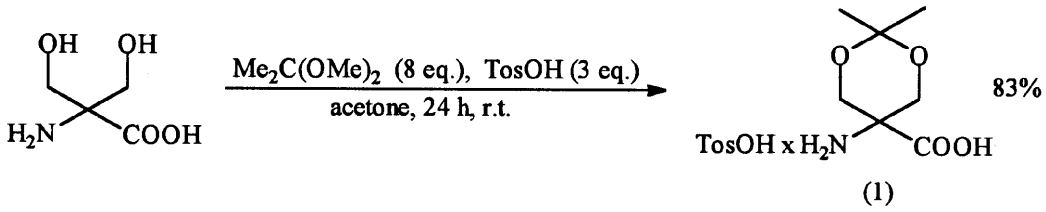
Z-HmS(Ipr)-MeA-OH **(15)** ($C_{19}H_{26}N_2O_7$, *M*=394.4). To a stirred solution of compound **9** (0.613 g, 1.5 mmol) in MeOH (4 ml) 2N NaOH (1.5 ml, 2 eq.) was added dropwise. Stirring was continued for 4 h. After the usual work-up, the crude dipeptide was crystallized from AcOEt/hexane. Yield 0.473 g (80%), m.p. 143–144 °C, TLC: 0.83 (II), 0.78 (III), 0.55 (V). HPLC: purity 95%, rt 13.468 min (25–55% B). ^{1}H-NMR (DMSO-d_6): 1.37 (bs, 12H, Ipr and H_β MeA); 3.93 (d, 2H, J_{AB}=12 Hz, H_β HmS); 4.06 (d, 2H, J_{AB}=12 Hz, H_β HmS); 5.05 (s, 2H, Z CH_2); 7.35 (bs, 6H, Z arom. and NH HmS); 7.69 (s, 1H, NH MeA); 12.53 (b, 1H, COOH).

Z-Hms(Ipr)-HmS(Ipr)-OH **(16)** ($C_{22}H_{30}N_2O_6$, *M*= 466.5). To a stirred solution of compound **10** (0.481 g, 1 mmol) in dioxane (6 ml) 2N NaOH (0.55 ml, 1.1 eq.) was added dropwise. Stirring was continued for 6 h. After the usual work-up, the crude dipeptide was crystallized from hot AcOEt/MeOH/hexane. Yield 0.334 g (72%), m.p. 187–188 °C (dec.), TLC: 0.80 (II), 0.53 (V), HPLC: purity 98%, rt 13.295 min (30–60% B). ^{1}HNMR (DMSO-d_6): 1.36, 1.38 (2s, 12H, Ipr groups); 3.69–4.12 (m, 8H, H_β HmS-1 and HmS-2); 5.04 (s, 2H, z CH_2); 7.37 (bs, 5H, Z arom.); 7.46 (bs, 1H, NH HmS-1); 7.90 (s, 1H, NH HmS-2); 12.78 (b, 1H, COOH), FAB-MS (*m/z*): 467 $(MH)^+$.

Removal of the Isopropylidene Protecting Group

Z-HmS-HmS-OMe **(17)** ($C_{17}H_{24}N_2O_9$, *M*=400.4). Dipeptide **10** (0.171 g, 0.35 mmol) was treated with a TFA/water mixture (9 : 1, 4 ml) for 15 min. The solution was evaporated and the residue was crystallized from $CHCl_3$/MeOH/hexane. Yield 0.122 g (87%), m.p. 127–128 °C, TLC: 0.14 (IV), 0.65 (III). HPLC: purity 99.6%, rt 6.704 min (20–50% B). rt 11.676 min (10–40% B), rt 21.198 min (0–30% B). ^{1}H-NMR (DMSO-d_6): 3.58 (s, 3H, OCH_3); 3.64–3.80 (m, 8H, H_β HmS-1 and HmS-2); 4.61 (bt, 2H, *J*=6 Hz, OH); 4.98 (t, 2H, *J*=6 Hz); 5.00 (s, 2H, Z CH_2); 6.85 (bs, 1H, NH HmS-1); 7.35 (s, 5H, Z arom.); 7.80 (s, 1H, NH HmS-2). FAB-MS (*m/z):* 423 (MNa^+), 439 (MK^+).

Z-HmS-HmS-HmS-OMe **(18)** ($C_{21}H_{31}N_3O_{12}$, *M*= 517.5). Obtained from tripeptide **13** (0.160 g, 0.25 mmol) by the same procedure as dipeptide **17**. Yield 0.120 g (93%), m.p. 146–148 °C, TLC: 0.05 (IV), 0.71 (III), HPLC: purity 100%, rt 9.893 min (10–40% B), rt 18.130 min (0–30% B). ^{1}H-NMR (DMSO-d_6) : 3.55(s, 3H, OCH_3); 3.58–3.79 (m, 12H, H_β HmS-1, HmS-2 and HmS-3), 4.39 (b, OH); 4.86 (b, OH); 5.02 (s, 2H, Z CH_2); 7.30 (bs, 1H, NH); 7.36 (s, 5H, Z arom.); 7.46 (bs, 1H, NH); 7.69 (s, 1H, NH). FAB-MS (*m/z*): 518 (MH^+), 540 (MNa^+), 556 (MK^+).



(1)

Figure 1 The O,O-protection of α-hydroxymethylserine.

Crystal Structure

$C_{19}H_{26}N_2O_7$; M_r=394.4; crystal system orthorhombic; space group, $p2_12_12_1$; cell dimensions, a=9.370(1), b=19.961(1), c=21.471(2) Å, V=4015.8(6) Å^3; Z=8; D_m=1.29 (by flotation in aqueous KI solution), D_x=1.305 Mgm^{-3}; room temperature; λ(CuKα)=1.54178 Å; μ=0.837 mm^{-1}; F(000)=1680; refinement on F^2 converged at the standard R=0.043 (calculated for 4431 reflections with $F_0 > 4_\sigma(F_0)$). R=0.056 (calculated for all 5281 unique reflections), wR_2=0.146, and goodness of fit S=1.061.

Colourless crystals of an elongated prismatic shape were grown by slow evaporation from an ethyl acetate/hexane 1:2 mixture. Three specimens cut from the best looking prisms were examined using the standard X-ray Laue technique. The best diffracting crystal of approximate dimensions 0.5 × 0.3 × 0.2 mm was mounted on the Kuma Diffraction KM4 automated diffractometer and used for data collection. The graphite-monochromated CuKα radiation (λ=1.54178 Å) was applied. Accurate cell dimensions were determined by the least-squares refinement from the angular settings of 90 reflections located within $9 < \Theta < 27°$. Data were collected at room temperature using the $\Theta/2\Theta$ scan mode up to a maximum of 2Θ=160.0°(h, 0→11; k, 0→25; l, −2→27). Three orientation and intensity controlling reflections [(2,2,3), (1,2,5), (1,4,2)], monitored every 100 measured reflections, showed no significant variation during data collection. The total number of 5383 measured X-ray intensities was further reduced to the 5281 unique reflections [R_{int}=0.0542]. An empirical absorption correction based on all measured ψ scans of six reflections was applied; μ=3.01 mm^{-1}; maximum and minimum transmission factors were 0.438 and 0.387, respectively. The coordinates of all non-hydrogen atoms were found using the direct methods [9]. Structure was refined on F^2 by the full-matrix lest-squares, all unique data except the seven suspicious intensities were included in the refinement. Positional parameters of all hydrogen atoms were determined from the difference Fourier map calculated after three cycles of the anisotropic refinement. All positional parameters as well as all temperature factors accompanied by the isotropic extinction parameter were freely allowed to refine. An overall isotropic extinction parameter was refined to 0.013(4). Weights were applied according to the formula $1/[\sigma^2(F_0)^2 + (0.096\ P)^2 + 0.59P]$, where P={max[$(F_0)^2$, 0] + 2(F_0)} / 3. The maximum shift/e.s.d. ratio in the final cycle of refinement was 0.041 for the y coordinate of H392 atom. Highest peak in the final difference Fourier map was 0.23, the deepest minimum −0.24 eÅ^{-3}. Atomic scattering factors were as in the SHELXL-93 [10]. SHELXL-93 and SHELXTL-PC [11] suites of programs were used for structure calculations. Analyses of the structure was performed with PARST [12] and INSIGHT [13].

RESULTS AND DISCUSSION

Peptide Synthesis

In our earlier investigation [8] of routes to peptides of α-hydroxymethylserine, syntheses were performed using the minimal protection strategy, i.e. with unprotected side-chain hydroxyl groups. Difficulties were encountered in the preparation of both amino- and carboxy-protected derivatives. Z-HmS-OH and H-HmS-OMe could be obtained in relatively low yields (41% and 35% respectively). As attempts to optimize these reactions failed, it became evident that for practical syntheses of peptides derived from HmS, an expedient access to O,O-protected derivative is crucial. While the presence of two hydroxyl groups in the β,β-positions diminishes the value of customary O-protection methods used in peptide work, it favours the acetal protection common in carbohydrate chemistry, which in the case of α-hydroxymethylserine would lead to formation of the 1,3-dioxane ring. A preliminary communication [8] from our laboratory has shown that the α-hydroxymethylseryl residue present in the dipeptide Z-HmS-Ser(Bzl)-OMe could be transformed into *O,O*-isopropylidene protected derivative. Now, the intro-

duction of this protecting group into α-hydroxymethylserine itself was re-examined. After optimization, conditions (Figure 1) were found enabling efficient protection of both OH groups by transformation of α-hydroxymethylserine into 5-amino-2,2-dimethyl-1,3-dioxane-5-carboxylic acid (**1**) [14].

This method is simple to carry out and isolation of the pure *O,O*-isopropylidene derivative **1** precipitating from the reaction mixture in high yield (83%) is straightforward. Because α-hydroxymethylserine side chains are tied up in a 1,3-dioxane ring, severe steric hindrance typical for acyclic $C^{\alpha,\alpha}$-dialkyl amino acids is partly reduced and reactions with HmS(Ipr) proceed more satisfactorily than with unprotected HmS. A good example is the urethane-type N^{α}-protection. The Z-HmS(Ipr)-OH derivative (**2**) was obtained in 78% yield, in contrast to the low (41%), hardly reproducible yield of the three day preparation of Z-HmS-OH[8]. The C-protection is represented by the preparation of the methyl ester H-HmS(Ipr)-OMe (**4**). The indirect route involving methylation of Z-HmS(Ipr)-OH (**2**) with diazomethane, followed by hydrogenolytic cleavage of the Z-group, has been found to be more efficient (overall yield 78%) than the direct methylation of TosOH × H-HmS(Ipr)-OH (1), affording the methyl ester in only 29% yield. In the past [**8**], our attempted reactions of *O*-unprotected Z-HmS-OH with diazomethane led to a complex mixture of overmethylated products even at −30 °C.

The Z/Ipr/OMe combination satisfies requirements for complete orthogonality of the protecting groups. Catalytic hydrogenolysis of Z-HmS(Ipr)-OMe (**3**) under non-acidic conditions in the presence of palladium resulted in the selective cleavage of Z-group. The *O,O*-isopropylidene protection survives catalytic hydrogenolytic conditions, but it can be removed selectively and rapidly under mild acidic conditions. Treatment of Z-HmS(Ipr)-OMe (**3**) with a 2N HCl/methanol (1 : 1) mixture or TFA/water (9 : 1) for 10–15 min afforded Z-HmS-OMe (**5**) in 90% yield. Other examples (Table 1) demonstrate the *O,O*-deprotection of the homo-dipeptide **10** and the homo-tripeptide **13**, yielding Z-$(HmS)_2$-OMe (**17**) and Z-$(HmS)_3$-OMe (**18**), respectively. Alkaline hydrolysis of a peptide methyl ester containing an *O,O*-protected HmS residue proceeds satisfactorily, as documented by preparation of the dipeptide acids **14–16** (Table 1) and hydrolysis of Z-HmS(Ipr)OMe (**3**). In contrast, the derivatives of *O,O*-unprotected α-hydroxymethylserine Z-HmS-OMe and Z-HmS-OH were degraded under the same alkaline conditions to benzyl alcohol and free amino acid in less than 1 h. The accelerated and unexpected cleavage of the Z-group can be explained in terms of the effect of the neighbouring OH groups and therefore the protection of the hydroxyl groups is critical for the survival of the Z-group under alkaline conditions. The exception is the dipeptide Z-HmS(Ipr)-Gly-OMe, which like other Z-AA-Gly-OR peptides is degraded by aqueous alkali to hydantoin and benzyl alcohol [15].

Coupling and deprotection experiments are summarized in the Table1. TBTU was used as the condensing agent in all peptide bond forming reactions. The results presented show that the Z/*O,O*-isopropylidene derivative of HmS can be efficiently coupled to a proteinogenous amino acid. The yields of coupling with H-HmS(Ipr)-OMe as an amino component were less satisfactory, albeit acceptable. This is not an unexpected result. Actually, in the common coupling reactions, steric hindrance at the amino group of this amino acid is expected to be more severe than that at the carboxyl group, as

Table 1 α-Hydroxymethylserine Peptides from Coupling and After Deprotection

Peptides from coupling[a]	Yield (%)	After deprotection	Yield (%)
Z-HmS(Ipr)-Gly-OMe (**6**)	88	Degraded to hydantoin	
Z-HmS(Ipr)-Ala-OMe (**7**)	86	Z-HmS(Ipr)-Ala-OH (**14**)[c]	61
Z-HmS(Ipr)-Ala-OBzl (**8**)	78		
Z-HmS(Ipr)-MeA-OMe (**9**)	80	Z-HmS(Ipr)-MeA-OH (**15**)[d]	80
Z-HmS(Ipr)-HmS(Ipr)-OMe(**10**)	64	Z-HmS(Ipr)-HmS(Ipr)-OH (**16**)[c]	72
		Z-HmS-HmS-OMe (**17**)[e]	87
Z-HmS(Ipr)-Ala-HmS(Ipr)-OMe (**11**)[b]	52		
Z-HmS(Ipr)-MeA-HmS(Ipr)-OMe (**12**)[b]	46		
Z-HmS(Ipr)-HmS(Ipr)-HmS(Ipr)-OMe (**13**)[b]	42	Z-HmS-HmS-HmS-OMe (**18**)[e]	93

Reaction conditions: (**a**) TBTU (1.1 mol eq.), DIEA (2–3 mol eq.), DCM, 24 h at room temperature; (**b**) 2+1 coupling; (**c**) 2N NaOH (1.1 mol eq.), dioxane, 6 h room temperature; (**d**) 2N NaOH (2 mol eq.), methanol, 4 h room temperature; (**e**) 90% aqueous TFA, 15 min room temperature.

found for derivatives of α-methylalanine [16], the prototype of sterically hindered $C^{\alpha,\alpha}$-dialkyl amino acids.

X-Ray Structure of Z-HmS(Ipr)-Ala-OMe (7)

A view of the two crystallographically independent molecules present in the asymmetric unit with atom labelling is given in Figure 2. The final atomic coordinates are listed in Table 2. The torsion angles defining the peptide chain conformation are given in Table 3. The dipeptide molecule has one chiral centre. In both crystallographically independent molecules the centre of chirality (C(16) or C(36)) has the (S) configuration, as expected from the synthetic procedure. Bond lengths and bond angles (data available from the authors on request) are close to the values commonly encountered in related compounds [17].

Both molecules adopt an S-shaped conformation which is not stabilized by any intramolecular hydrogen bond. In one molecule the ϕ, ψ torsion angles of the a chiral HmS(Ipr) residue are in the left-handed helical region of the Ramachandran map, while in the second molecule they are found in the right-handed helical region. The conformational preferences of the six-membered heterocyclic HmS(Ipr) residue, as described by the ϕ and ψ torsion angles, are closely similar to those of the alicyclic Ac_6c residue, which is known to adopt the ϕ and ψ torsion angles in the helical region of the Ramachandran map [4]. The conformation of the Ala residue (semi-extended) is influenced by the fact that the carbonyl oxygen atoms O(5) and O(6) in the first molecule, and O(25) and O(26) in the second molecule, come close to the collision region (the inter-atomic distances are 3.218(4) Å and 3.131(4) Å respectively). The resulting steric interactions inflict a large deviation from planarity ($|\Delta\omega = 10$–$15°$) to the central peptide bond. Interestingly, the ϕ_1 and ϕ_2 torsion angles in each molecule have the values with the opposite sign.

The important feature influencing the crystal packing arrangement is the presence of the two crystallographically independent molecules in the asymmetric unit. The two molecules are related to each other by the *pseudo* glide *a* plane perpendicular to the crystallographic *b* axis, the main difference being the location of the hydrogen atom attached to the chiral centre. As a consequence, all the respective ϕ and ψ torsion angles in the two molecules have values with opposite signs. In particular, the L-Ala residue in one of the molecules is forced to adopt the unusual positive ϕ value [54.3(3)°]. The superposition of the two independent molecules, as determined by the least-squares fitting of the common fragments, is presented in Figure 3.

The N-terminal Z-group adopts the sterically favoured *trans* conformation [18] with the central torsion angles C(1)-C(7)-O(1)-C(8) and C(21)-C(27)-O(21)-C(28) equal to −169.0(3)° and 161.7(3)°, respectively. The phenyl rings are located too far from each other to suggest any type of stacking interaction.

Both 1,3-dioxane rings, which are present in the two crystallographically independent molecules, exist in a distorted chair conformation slightly flattened at the C(9) and C(29) atoms. The amino substituent occupies the axial position. The main *pseudo*-symmetry elements are two-fold axes perpendicular to the O(3)-C(11) and O(23)-C(30) bonds, accompanied by mirror planes passing through the C(9) and C(34) atoms, in the first and second molecules respectively. The endocyclic torsion angles and the three lowest asymmetry parameters [19] are summarized in Table 4.

The environment of the molecules in the crystal lattice with the hydrogen bond network is shown in Figure 4. All hydrogen atoms bonded to nitrogens are involved in intermolecular hydrogen bonds with carbonyl oxygen atoms (Table 5). The hydrogen bonds form two infinite chains, which extend along the *a* axis. The packing mode approximately resembles the *Pbac* space group symmetry, which is the centrosymmetric equivalent of the real space group $P2_12_12_1$. However, as pointed out earlier, all peptide molecules have the same configuration at the Ala residue. Conflict between packing requirements and chirality of molecules forces the N(2)–H(2)·····O(25) hydrogen bond to be much longer than the other hydrogen bonds (the unusual N(2)·····O(25) distance is 3.273(3) Å]. In the crystal the peptide molecules are oriented in such a way that their longest axis is approximately perpendicular to the *a*-axis.

CONCLUSIONS

α-Hydroxymethylserine offers a useful addition to the arsenal of peptide chemists whether their major concern is the stereochemistry of a peptide chain, enzymatic resistance or interesting biological effects. Efficient incorporation of this $C^{\alpha,\alpha}$-disubstituted amino acid into the peptide chain can be achieved

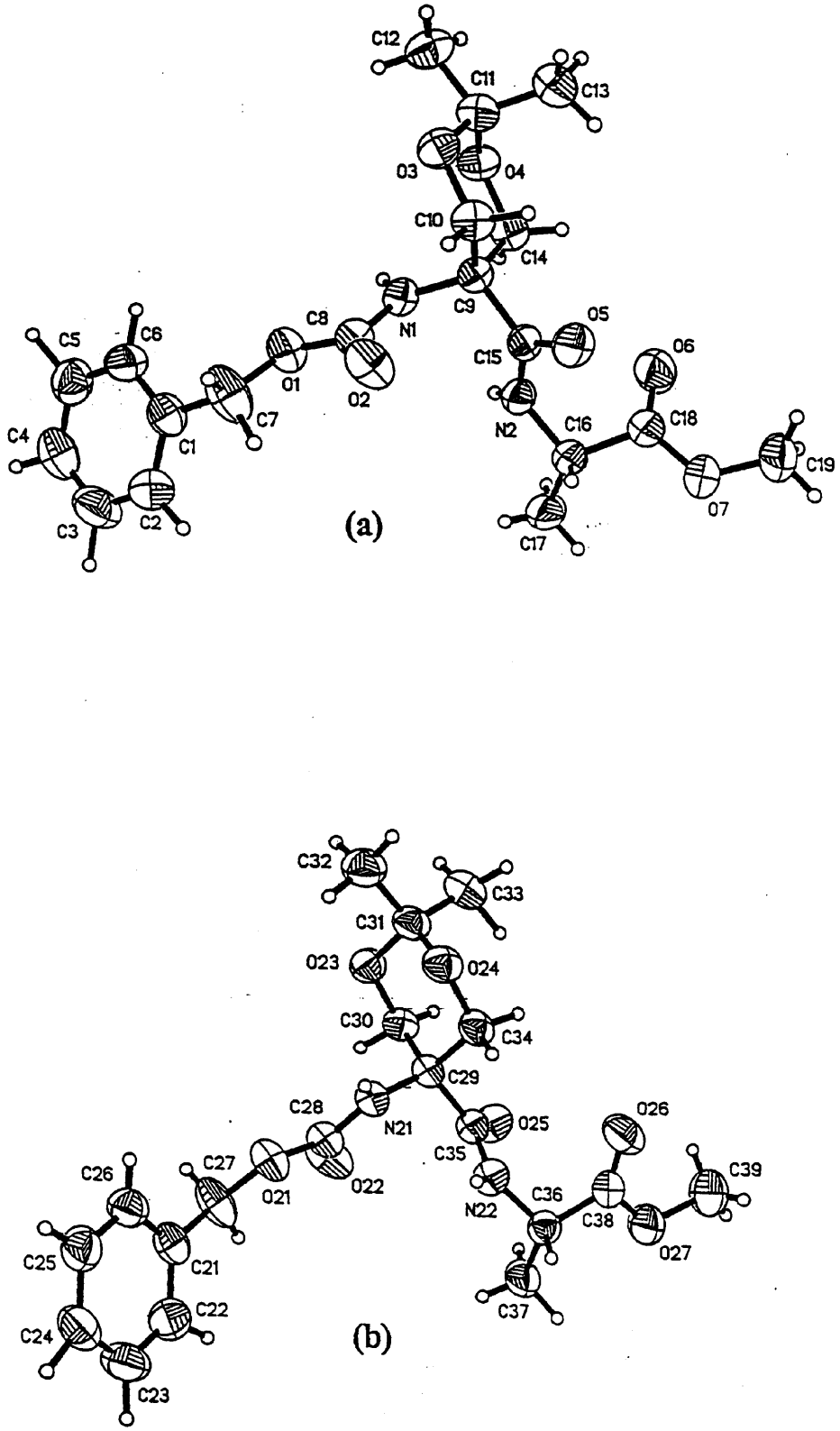


Figure 2 View of the two crystallographically independent molecules (a) and (b) of Z-HmS(Ipr)-Ala-OMe with atom numbering. Thermal ellipsoids are drawn at the 50% probability level.

Table 2 Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Coefficients ($Å^2 \times 10^3$) U_{eq}, Defined as one-third of the Trace of the Orthogonalized U_{ij} Tensor for Z-HmS(Ipr)-Ala-OMe

	x	y	*z*	u_{eq}		*x*	y	*z*	u_{eq}
N(1)	775(2)	10 221(1)	2 007(1)	41(1)	N(21)	5 787(2)	9 738(1)	2 015(1)	39(1)
N(2)	12(3)	9 959(1)	3 249(1)	47(1)	N(22)	5 330(3)	10 057(1)	3 255(1)	46(1)
O(1)	910(2)	9 305(1)	1 419(1)	52(1)	O(21)	5 793(2)	10 612(1)	1 375(1)	50(1)
O(2)	−1 219(2)	9 622(1)	1 806(1)	65(1)	O(22)	3 765(2)	10 332(1)	1 876(1)	63(1)
O(3)	−285(2)	11 524(1)	1 523(1)	49(1)	O(23)	4 734(2)	8 434(1)	1 623(1)	50(1)
O(4)	1 730(2)	11 634(1)	2 137(1)	50(1)	O(24)	6 830(2)	8 364(1)	2 195(1)	52(1)
O(5)	−1 870(2)	10 641(1)	3 118(1)	56(1)	O(25)	3 322(2)	9 436(1)	3 218(1)	60(1)
O(6)	307(3)	10 775(1)	4 270(1)	77(1)	O(26)	5 592(4)	9 233(1)	4 272(1)	81(1)
O(7)	−1 304(3)	10 174(1)	4 787(1)	72(1)	O(27)	4 222(3)	9 925(1)	4 833(1)	73(1)
C(1)	1 251(3)	8 268(2)	870(2)	54(1)	C(21)	6 016(3)	11 648(1)	820(1)	51(1)
C(2)	1 215(5)	7 609(2)	1 065(2)	71(1)	C(22)	5 895(4)	12 311(2)	1 009(2)	64(1)
C(3)	2 170(6)	7 146(2)	830(2)	81(1)	C(23)	6 806(5)	12 790(2)	777(2)	74(1)
C(4)	3 150(5)	7 338(2)	398(2)	75(1)	C(24)	7 835(4)	12 618(2)	345(2)	65(1)
C(5)	3 201(4)	7 995(2)	198(2)	71(1)	C(25)	7 944(4)	11 968(2)	162(2)	60(1)
C(6)	2 263(4)	8 452(2)	441(2)	61(1)	C(26)	7 051(4)	11 486(2)	394(2)	55(1)
C(7)	182(4)	8 759(2)	1 116(3)	75(1)	C(27)	4 998(3)	11 138(2)	1 066(2)	67(1)
C(8)	55(2)	9 715(2)	1 751(1)	44(1)	C(28)	5 017(2)	10 232(1)	1 767(1)	42(1)
C(9)	33(3)	10 732(1)	2 366(1)	39(1)	C(29)	5 124(3)	9 252(1)	2 424(1)	39(1)
C(10)	−1 028(3)	11 133(1)	1 972(2)	46(1)	C(30)	4 030(3)	8 822(1)	2 085(1)	46(1)
C(11)	685(3)	11 989(1)	1 781(2)	51(1)	C(31)	5 780(3)	7 992(1)	1 853(2)	51(1)
C(12)	1 500(5)	12 276(2)	1 238(2)	67(1)	C(32)	6 570(5)	7 726(2)	1 299(2)	66(1)
C(13)	−43(5)	12 528(2)	2 155(2)	68(1)	C(33)	5 127(5)	7 431(2)	2 235(2)	66(1)
C(14)	1 149(3)	11 227(1)	2 616(1)	45(1)	C(34)	6 282(3)	8 775(1)	2 668(1)	47(1)
C(15)	−706(3)	10 429(1)	2 934(1)	42(1)	C(35)	4 483(3)	9 958(1)	2 999(1)	43(1)
C(16)	−494(3)	9 729(1)	3 843(1)	47(1)	C(36)	5 096(3)	10 330(1)	3 875(1)	46(1)
C(17)	422(6)	9 145(2)	4.070(2)	74(1)	C(37)	3 871(4)	10 829(2)	3 899(2)	61(1)
C(18)	−425(3)	10 288(1)	4 318(1)	49(1)	C(38)	4 995(4)	9 759(2)	4 340(1)	54(1)
C(19)	−1 256(8)	10 647(2)	5 300(2)	90(1)	C(39)	4 132(11)	9 415(3)	5 313(2)	103(2)

using the easily accessible O,O-protected α-hydroxymethylserine in the from of the *O,O*-isopropylidene derivative. Because α-hydroxymethylserine sidechains are tied up in a 1,3-dioxane ring, severe steric hindrance typical for acyclic $C^{\alpha\alpha}$-dialkyl amino acids is partly reduced and as a consequence the N-protection, C-protection and coupling reactions proceed more satisfactorily than with O,O-unprotected α-hydroxymethylserine. In particular, an easy access to the highly hindered homo-sequences of this amino acid is noteworthy. The Z/Ipr/OMe combination satisfies requirements for complete orthogonality of the protecting groups. X-ray structure determination of Z-HmS(Ipr)-Ala-OMe revealed that the two crystallogrpahically independent molecules present in the asymmetric unit adopt an S-shaped conformation. In one molecule the achiral HmS(Ipr) residue has the positive torsion angle values in the left-handed helical region of the

Table 3 Main–chain Torsion Angles (°) Defining the Peptide Conformation of the Two Crystallographically Independent Molecules (a) (First Entry) and (b) (Second Entry) of the Z–HmS(Ipr)–Ala–OMe

ω_o	O(1)–C(8)–N(1)–C(9)	177.9(2)
	O(21)–C(28)–N(21)–C(29)	−176.8(2)
ϕ_1	C(8)–N(1)–C(9)–C(15)	61.4(3)
	C(28–N(21)–C(29)–C(35)	−60.1(3)
ψ_1	N(1)–C(9)–C(15)–N(2)	40.8(3)
	N(21)–C(29)–C(35)–N(22)	−44.4(3)
ω_1	C(9)–C(15)–N(2)–C(16)	169.6(2)
	C(29)–C(35)–N(22)–C(36)	−165.2(2)
ϕ_2	C(15)–N(2)–C(16)–C(18)	−65.6(4)
	C(35)–N(22)–C(36)–C(38)	54.3(3)
ψ_2	N(2)–C(16)–C(18)–O(7)	158.3(3)
	N(22)–C(36)–C(38)–O(27)	−153.5(3)
ω_2	C(16)–C(18)–O(7)–C(19)	175.1(4)
	C(36)–C(38)–O(27)–C(39)	−177.2(5)

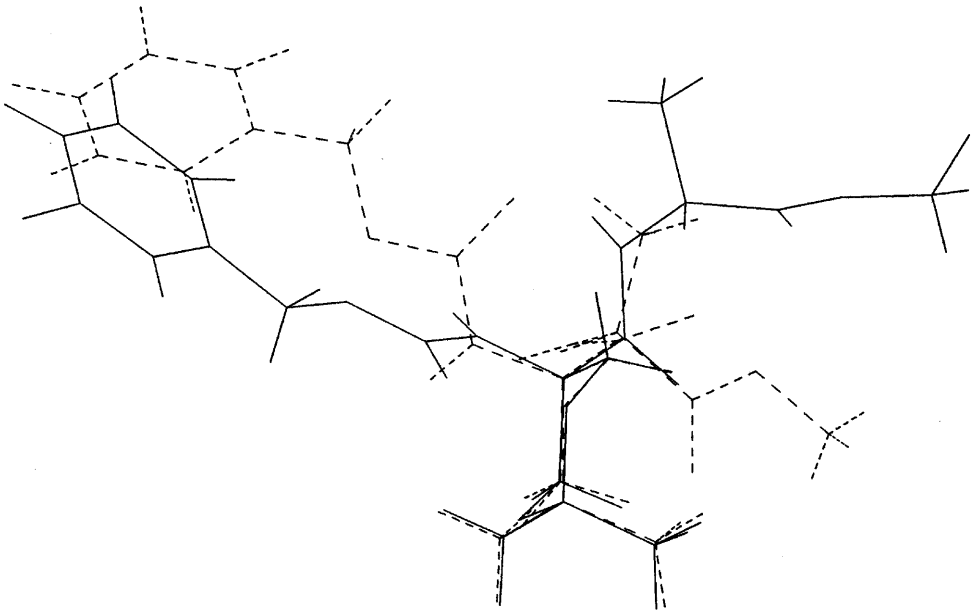


Figure 3 Superposition of the two crystallographically independent molecules of Z-HmS(Ipr)-Ala-OMe. Only distances between the respective endocyclic dioxane ring atoms were minimized. Molecules (a) and (b) are indicated by the full and dashed lines, respectively.

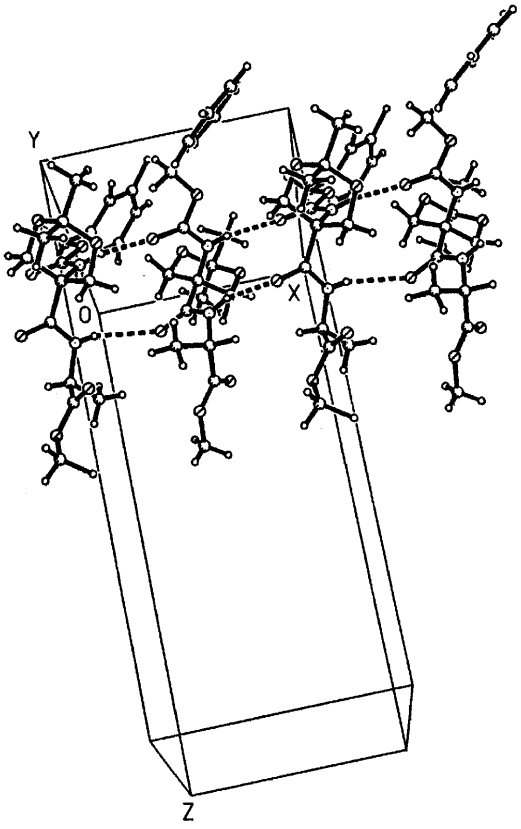


Figure 4 Packing diagram of the molecules of Z-HmS(Ipr)-Ala-OMe with hydrogen bonds indicated as dashed lines.

Table 4 Endocyclic 1,3–Dioxane Torsion Angles (°) for Each Independent Molecule (a) (Upper Section) and (b) (Lower Section) of the Z–HmS(Ipr)–Ala–OMe Accompanied by the Three Lowest Asymmetry Parameters

O–(3)–C(11)–O(4)–C(14)	−56.9(3)
C(11)–O(4)–C(14)–C(9)	54.8(3)
O(4)–C(14)–C(9)–C(10)	−50.5(3)
C(14)–C(9)–C(10)–O(3)	52.1(3)
C(9)–C(10)–O(3)–C(11)	−59.8(3)
C(10)–O(3)–C(11)–O(4)	60.0(3)
$\Delta C_2^{C(11)-O(3)} = 2.80(6)$ $\Delta C_s^{C(9)} = 3.52(6)$ $\Delta C_s^{C(14)} = 3.72(6)$	
O(23)–C(31)–O(24)–C(34)	53.6(3)
C(31)–O(24)–C(34)–C(29)	−53.8(3)
O(24)–C(34)–C(29)–C(30)	52.7(3)
C(34–C(29)–C(30)–O(23)	−54.7(3)
C(29)–C(30)–O(23)–C(31)	60.6(3)
C(30)–O(23)–C(31)–O(24)	−57.5(3)
$\Delta C_2^{C(30)-O(23)} = 2.08(6)$ $\Delta C_s^{C(34)} = 2.00(6)$ $\Delta C_s^{C(30)} = 4.39(6)$	

Table 5 Intermolecular Hydrogen Bond Parameters for Z–HmS(Ipr)–Ala–OMe

D–H...A	D–H	H...A	D...A	D–H...A	Symmetry code
N(1)–H(1)···O(22)	0.89(3)	1.95(3)	2.824(3)	168.6(2.8)	*x,y,z*
N(2)–H(2)···O(25)	0.77(5)	2.53(5)	3.273(3)	164.7(4.7)	*x,y,z*
N(21)–H(3)···O(2)	0.81(4)	2.05(4)	2.850(3)	167.3(3.6)	$1+x,y,z$
N(22)–H(4)···O(5)	0.84(3)	2.10(3)	2.886(3)	154.8(2.6)	$1+x,y,z$

Distances and angles are in (Å) and (°), respectively.

Ramachandran map, while in the second molecule the negative torsion angles are associated with the right-handed helix.

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