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Conformationally Constrained Amino Acids: a Concise Route to a Methionine Analogue

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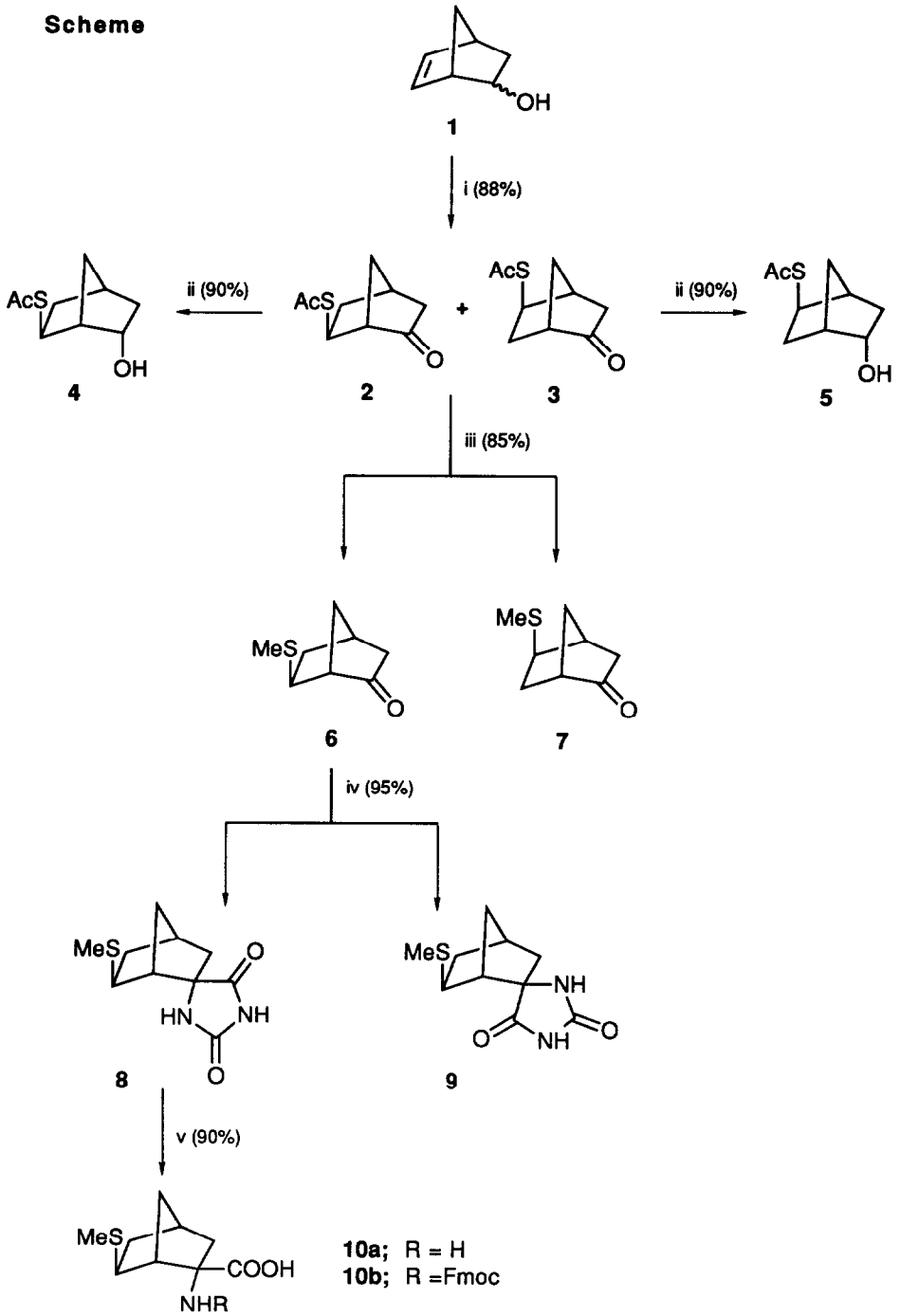
Abstract: A facile and concise synthesis of (±)-2-*endo*-amino-6-*exo*-(methylthio)[2.2.1]heptane-2-*exo*-carboxylic acid starting from the commercially available 5-norbornen-2-ol is decribed.

A central goal in peptide and protein research is the development of rational approaches to the design of peptide and protein ligands with specific physical, chemical and biological properties. In this field conformational constraints play an important role and various systematic approaches have been proven such as the use of cyclic amino acid analogues to constrain amino acid residues to particular conformational states and numerous examples now exist which illustrate its potential.[1] Moreover, the development of potential therapeutic agents based on the structure of peptides has stimulated a great interest in the synthesis of unusual and unnatural amino acids.[2-6] When incorporated into the peptide of interest, these surrogates may infer conformational constraint as well as increasing biostability making them non-peptidic and suitable for potential use as drug candidates.[7]

On the basis of these considerations, the synthesis of a conformationally restricted methionine analogue is important because L-methionine is an essential sulfur amino acid whose critical roles in cellular function relate, in part, to its biochemical conversion to S-adenosylmethionine and subsequent participation in methylation processes and polyamine biosynthesis. These pathways of S-adenosylmethionine metabolism have become attractive targets for the development of chemotherapeutics agents.[8,9] Recently Glass reported the synthesis of 2-*exo*-amino-6-*endo*-(methylthio)-bicyclo[2.2.1]heptane-2-*endo*-carboxylic acid which was utilized in anodic oxidation[10] and in radical decarboxylation .[11]

Our work describe a facile and concise synthesis of the racemic 2-*endo*-amino-6-*exo*-(methylthio)bicyclo[2.2.1]heptane-2-*exo*-carboxylic acid starting from the commercially available 5-norbornen-2-ol **1** with the aim of inserting this conformationally restricted methionine analogue in various peptidic sequence. The reaction of norbornenol **1** (Scheme) with thiolacetic acid in benzene and subsequent oxidation afforded a 1:1 mixture of the regioisomeric 6-*exo* and 5-*exo*- acetylthio derivatives **2** and **3** (88% yield). In

Scheme



Scheme *Reagents and conditions* : i) 1. AcSH, C_6H_6, AIBN, Δ; 2. Jones' reagent, 0° C; ii) $NaBH_4$, MeOH , 0° C; iii) NaOMe, MeI; iv) KCN, $(NH_4)_2CO_3$; v) $Ba(OH)_2$, 120 °C.

order to assign the structure, compounds **2** and **3** were separated by flash chromatography and reduced with sodium borohydride to the corresponding 2-*endo*-alcohols **4** and **5**. Comparing the NMR spectra of the ketone **2** with that of the alcohol **4**, the down shield shift of the 6-*endo* proton from δ 3.61 (compound **2**) to δ 4.08 (compound **4**) caused by the oxygen atom in close proximity to the proton is observed.[12] On the other hand, the chemical shift of the 5H-*endo* proton is not influenced from the OH function and, moreover, the NMR spectrum of the alcohol **5** show a long range W coupling (J = 1.5 Hz) betweeen the 6-*exo* and the 2-*exo* protons.

The 1:1 mixture of the acetylthio derivatives **2** and **3** were subsequently treated with methyl iodide in sodium methoxide/methanol to afford, in practically quantitative yield, the methylthio derivatives **6** and **7** which were more easily separated by chromatography. The 6-*exo*-(methylthio)heptanone **6** was converted to the amino acid **10a** through a modification of the Bucherer reaction.[13] Treating the ketone with potassium cyanide and ammonium carbonate at 80 °C a mixture of the epimeric hydantoins **8** and **9** were obtained in 95% yield (molar ratio 8:2 from NMR spectrum). Separation of the pure hydantoin **8** (64%) was achieved by preparative HPLC. The structure of this epimer was assigned on the basis of a positive ^{1}H nuclear Overhauser effect (in $CDCl_3$) between the 6H-*endo* at δ 3.00 and 2NH-*endo* at δ 6.65 of hydantoin moiety. This effect is obviously not present in compound **9**. Moreover, the chemical shift of 6H-*endo* of **9** (at δ 3.62) respect to the 6H-*endo* of **8** (at δ 3.00) due to the long-range deshielding effect of the 2CO-*endo* of hydantoin **9** confirms further the structure of **8**. This assigment is indirectly supported also by previous works: i) the hydantoin 6-methylthio epimer was obtained by Glass;[13] ii) the isomer obtained with the Bucherer-Lieb procedure is mainly with the carboxyl group *exo* while the Strecker procedure gave the amino group *exo*.[14] Hydrolysis of hydantoin **8** with barium hydroxide at 120 °C afforded the 2-*endo*-amino-2-*exo*-carboxylic acid **10a**[15] (90% yield) which was fully characterized as *N*-(9-fluorenyl-methoxycarbonyl) derivative **10b** by reaction with *N*-(9-fluorenylmethoxycarbo-nyloxy)succinimide.

In conclusion, this is a simple methodology which permits, starting from the commercially available norborneol, to achieve the synthesis of the methionine analogue amino acid **10a** in only four step and with overall yield 32%. Furthermore, the N-Fmoc derivative **10b** has been utilized in the solid phase synthesis of deltorphin A analogue (i.e; H-Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2). This is an heptapeptide with opioid activity and high affinity and selectivity for the δ receptors. The methionine analogue **10a** has been inserted in 2 or 6 positions or in both positions of the peptide. Binding affinities data will be published elsewere.[16]

EXPERIMENTAL

6-*exo*- 2 and 5-*exo*-(Acetylthio)bicyclo[2.2.1]heptan-2-one 3. A solution of a mixture of *endo-exo* 5-norbornen-2-ol (Aldrich) (7 g, 63.5 mmol), thiolacetic acid (7.2 g, 95.2 mmol) and azo-bisisobutyronitrile (AIBN) (0.4 g) in benzene (200 ml) was refluxed for 4 h. After allowing to cool to r.t., the reaction mixture was treated with saturated $NaHCO_3$ (100 ml), the organic layer was separated, washed with H_2O, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give 11.2 g of a colourless oil. ^{1}H NMR spectrum showed a quantitative conversion to four acetylthio 2-norbornanols that were used without further purification. To a solution of this crude mixture in acetone (250 ml) Jones' reagent was added dropwise at 0° C until the yellow colour of the reagent persisted. The reaction mixture was stirred for 3 h at r.t., concentrated *in vacuo*, treated with saturated $NaHCO_3$ (30 ml) and extracted with CH_2Cl_2 (4 x 50 ml). The combined extracts were washed with H_2O, dried over anhydrous $MgSO_4$ and concentrated to give a

sticky oil which was chromatographed (silica gel, petroleum ether/diethyl ether 3:2) to afford a mixture of the ketones **2** and **3** (9.7 g, 88%, molar ratio 1:1 from ^{1}H NMR and GC). This mixture was used for the following reaction but the compounds **2** and **3** were separated by flash column chromatography (silica gel, petroleum ether/diethyl ether 4:1) and fully characterized after their transformation in the corresponding *endo*-norbornanols **4** and **5** with $NaBH_4$.

6-exo-(Acetylthio)bicyclo[2.2.1]heptan-2-one **2** showed the following: oil ; IR (film) 2950, 1740, 1675 cm^{-1}; ^{1}H NMP (300 MHz, $CDCl_3$) δ 1.68-1.81 (m, 3 H), 2-2.2 (m, 3 H), 2.30 (s, 3 H), 2.58 (s, 1 H), 2.75 (br s, 1 H), 3.61 (dd, 1 H, *J* = 4.4 and 8.3 Hz, 6H-*endo*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 30.06, 35.30, 35.87, 36.43, 39.27, 44.10, 55.91, 194.62, 214.04. Anal. calcd for $C_9H_{12}O_2S$: C, 58.66; H, 6.56. Found: C, 58.73; H, 6.51.

5-exo-(Acetylthio)bicyclo[2.2.1]heptan-2-one **3** : oil ; IR (film) 2950, 1740, 1670 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.65-1.80 (m, 3 H), 2-2.2 (m, 3 H), 2.32 (s, 3 H), 2.62 (m, 2 H), 3.56 (dd, 1 H, *J* = 4.5 and 8.9 Hz, 5H-*endo*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 30.01, 31.48, 35.19, 42.30, 42.54, 43.99, 49.27, 195.40, 215.42. Anal. calcd for $C_9H_{12}O_2S$: C, 58.66; H, 6.56. Found: C, 58.70; H, 6.59.

The reduction of **2** and **3** (0.1 g in 5 ml of MeOH) with $NaBH_4$ (0.025 g) at 0 °C afforded, after usual work up (water and extraction with diethyl ether), the corresponding crude *endo*-alcohols **4** and **5** (90% yield) which were analyzed without further purification.

6-exo-(Acetylthio)bicyclo[2.2.1]heptan-2-endo-ol **4:** ^{1}H NMR (300 MHz, $CDCl_3$ + D_2O) δ 0.85-0.95 (m, 1 H), 1.3-1.5 (m, 3 H), 1.85-2.0 (m, 3 H), 2.25 (m, 1H) 2.27 (s, 3 H), 4.08 (ddd, 1 H, *J* = 1.6, 4.9, and 8.8 Hz, 6H-*endo*), 4.28 (dt, 1H, *J* = 3.9 and 10.1 Hz, CHOH).

5-exo-(Acetylthio)bicyclo[2.2.1]heptan-2-endo-ol **5:** ^{1}H NMR (300 MHz, $CDCl_3$ + D_2O) δ 1.0-1.1 (m, 1 H), 1.15-1.5 (m,4 H), 2.0-2.2 (m, 2 H), 2.27 (s, 3 H), 2.52 (m, 1 H), 3.45 (ddd, 1H, J = 1.8, 4.1, and 9.0 Hz, 5H-*endo*), 4.20 (dtd, 1 H, *J* = 1.5, 4.1, and 10.2 Hz, CHOH).

6-*exo*- 6 and 5-*exo*-(Methylthio)bicyclo[2.2.1]heptan-2-one 7. To a solution of the S-acetylthio compounds **2** and **3** (5 g, 27.2 mmol) in methanol (100 ml), degased with argon and cooled at 0 °C, a freshly prepared and degased solution of NaOMe (1.5 g, 27 mmol) in MeOH (25 ml) was added dropwise. After stirring the reaction mixture for 30 min at 0 °C, methyl iodide (9.2 g, 65 mmol) was added dropwise and stirring was continued for further 10 min at 0 °C and 2 h at room temperature. The solvent was evaporated *in vacuo* and the residue was slurried in CH_2Cl_2 (100 ml) and the solid NaI was filtered off. The filtrate was concentrated under vacuum and chromatographed (silica gel, petroleum ether/diethyl ether 9:1) to give 6-*exo*-(methylthio)ketone **6** (1.9 g, 45%) and the 5-*exo* derivative **7** (1.7 g, 40%).

Compound **6** showed the following: oil ; IR (film) 1740 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (m, 1 H), 1.72 (m, 1 H), 1.84 (dd, 1 H, *J* = 3.9 and 17.5 Hz), 1.92-2.08 (m, 3 H), 2.12 (s, 3 H), 2.63 (s, 1 H), 2.69 (br s, 1 H), 2.89 (ddd, 1 H, *J* = 1.7, 4.6 and 8.4 Hz). Anal. calcd for $C_8H_{12}OS$: C, 61.50; H, 7.74. Found: C, 61.54; H, 7.81.

Compound **7**: oil; IR (film) 1740 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.55 (m, 1 H), 1.70 (m, 1 H), 1.83 (dd, 1 H,*J* = 3.9 and 17.8 Hz), 1.96-2.07 (m, 2 H), 2.10-2.23 (m, 1 H), 2.15 (s, 3 H), 2.59 (dd, 1 H, *J* = 1 and 4.8 Hz), 2.65 (br s, 1 H), 2.82 (ddd, 1 H, *J* = 1.7, 4.5 and 8.2 Hz). Anal. calcd for $C_8H_{12}OS$: C, 61.50; H, 7.74. Found: C, 61.46; H, 7.69.

Hydantoins 8 and 9. To a solution of $(NH_4)_2CO_3$ (5.3 g, 56 mmol), 1 M aqueous KCN solution (13 ml, 13 mmol) and H_2O (20 ml) a solution of 6-*exo* derivative **6** (1.5 g, 9 mmol) in MeOH (10 ml) was added.

A resulting cloudy solution was degassed and sealed in a high pressure flask and then heated with stirring at 80 °C. After 1 day the reaction mixture was cooled and extracted with EtOAc (3 X 150 ml). The organic phase was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to afford a solid residue (1.93 g, 95%) which from the ^1H NMR spectrum showed the presence of a mixture of the hydantoins **8** and **9** (molar ratio 8:2 from the multiplets of the 6H-*endo* protons at δ 3.00 for **8** and at δ 3.62 for **9**). Pure hydantoin **8** (1.3 g, 64%) was obtained after purification by preparative HPLC with a water Delta Prep 3000 using a Delt Pak C18 column (30 mm X 30 cm, 15μm, spherical). The gradient was made up of two solvents: A, 10% acetonitrile in water; B, 60% acetonitrile in water, both containing 0.1% trifluoroacetic acid. The gradient program used was as follows: linear gradient from 100% to 50% A in 25 min at flow rate of 30 ml/min. Homogeneity of the purified product was accessed by analytical HPLC, performed on a Bruker liquid chromatograph LC21-C instrument using a Vydac C18 218 TP 5415 column (175 X 4.5 mm, 5 μm particle size). The gradient program used was identical to that preparative purification at a flow rate of 1 ml/min and monitored at 220 nm (tr = 10.1)

Hydantoin **8** showed the following: m. p. 243-246 °C; IR (nujol) 1755, 1710 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 1.37 (m, 2 H), 1.78 (m, 1 H), 1.96 (ddd, 1 H, J = 2.4, 8.5 and 13.2 Hz), 2.12 (s, 3 H, SCH_3), 2.17 (ddd, 1 H, J = 2.9, 4.5 and 13.2 Hz), 2.27 (m, 1 H), 2.37 (br s, 2 H), 3.12 (ddd, 1 H, J = 1.7, 4.2 and 8.4 Hz, 6H-*endo*); ^{13}C NMR (75 MHz, CD_3OD) δ 15.66, 35.19, 37.37, 38.42, 41.66, 44.30, 52.70, 68.56, 159.38, 188.14. Selected ^1H NMR data in $CDCl_3$: δ 2.10 (s, 3 H, SCH_3), 3.00 (m, 1 H, 6H-*endo*), 6.65 (s, 1 H, 2-NH-*endo*), 8.27 (s, 1 H, NH). Anal. calcd for $C_{10}H_{14}N_2O_2S$: C, 53.07; H, 6.23; N, 12.38. Found: C, 53.00; H, 6.27; N, 12.31.

Hydantoin **9**: m.p. 205-208 °C; IR (nujol) 1750, 1715 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 1.26-1.36 (m, 1 H), 1.69 (m, 1 H), 1.78-1.96 (m, 4 H), 2.08 (s, 3 H, SCH_3), 2.36 (s, 1 H), 2.44 (br s, 1 H), 3.64 (ddd, 1 H, J = 1.6, 4.1 and 8.1 Hz, 6H-*endo*); ^{13}C NMR (75 MHz, CD_3OD) δ 15.59, 37.23, 38.02, 38.26, 41.01, 43.87, 54.37, 68.85, 158.80, 179.72. Selected ^1H NMR data in $CDCl_3$: δ 2.08 (s, 3 H, SCH_3), 3.62 (m, 1 H, 6H-*endo*), 6.62 (s, 1 H, 2-NH-*exo*), 8.32 (s, 1 H, NH). Anal. calcd for $C_{10}H_{14}N_2O_2S$: C, 53.07; H, 6.23; N, 12.38. Found: C, 53.12; H, 6.28; N, 12.42.

Amino Acid 10a. A mixture of hydantoin **8** (0.5 g , 2.2 mmol) and $Ba(OH)_2$ (1.88 g, 11 mmol) in H_2O (65 ml) was heated in an autoclave at about 120 °C overnight. The reaction mixture was then diluted with H_2O to about 200 ml and boiled. Dry ice was added in small pieces and with great caution until no more turbidity was noticeable. The mixture was filtered and lyophilized. The white solid residue (0.58 g) was dissolved in the minimum amount of deionized H_2O (50 ml) and the pH of the solution was adjusted to about 6.5 by the addition of dilute aqueous H_2SO_4. The resulting white precipitate was removed and the aqueous phase lyophilized to afford pure amino acid **10a** (0.4 g, 90 %): m.p. 235-238 °C dec.; IR (KBr) 3450, 3280, 1650, 1360 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.18 (dd, 1H, J = 2.9 and 13.2 Hz), 1.29, (m, 1 H), 1.62 (d, 1 H, J = 11.3 Hz), 1.75-1.9 (m, 2 H), 2.00 (s, 3 H), 2.15-2.28 (m, 2 H), 2.42 (s, 1 H), 2.85 (m, 1 H). Anal. calcd for $C_9H_{15}NO_2S$: C, 53.70; H, 7.51; N, 6.95. Found: C, 53.61; H, 7.59; N, 6.89.

***N*-Protected Amino Acid 10b.** An ice-cold solution of *N*-(9-fluorenylmethoxycarbonyloxy)-succinimide (Fmoc-OSu) (0.37 g, 1.1 mmol) in acetone (80 ml) was added to a solution of the amino acid **10a** (0.22 g, 1.1 mmol) in 0.22 M sodium hydrogen carbonate (5 ml, 1.1 mmol) cooled at 0 °C. The reaction mixture was stirred in an ice-bath keeping the pH at 8-9 with 5% sodium hydrogen carbonate. After 7 h the

organic solvent was removed *in vacuo* and the aqueous solution was first extracted with ethyl acetate (4 X 5 ml) and then acidified to pH 2 with solid $KHSO_4$. The resulting precipitate was collected by filtration, washed with ice-cold water, dried *in vacuo* over P_2O_5 and precipitated from diethyl ether with light petroleum ether (0.41 g, 89%). Purification of the *N*-protected amino acid **10b** was achieved by preparative HPLC (see above for hydantoin) (purification yield 82%). The homogeneity of the purified product was assessed by TLC and analytical HPLC, performed on a Bruker liquid chromatograph LC21-C instrument using a Vydac C18 218 TP 5415 column (175 X 4.5 mm, 5 μm particle size). The gradient program used was identical to that preparative purification at a flow rate of 1 ml/min and monitored at 220 nm (tr = 12.4 min). The *N*-protected amino acid (R = Fmoc) **10b** showed the following: m.p. 127-130 °C; ^1H NMR (300 MHz, CD_3OD) δ 1.20 (m,1 H), 1.48 (m, 1 H), 1.83 (m, 1 H), 2.0 (s, 3 H), 2.11-2.28 (m, 2 H), 2.98 (br s, 1 H), 3.03 (m, 1 H), 4.23 (m, 1 H), 4.33 (br d, 2 H), 7.38 (m, 4 H), 7.70 (t, 2 H, J = 7.3 Hz), 7.82 (d, 2 H, J = 7.3 Hz). Anal. calcd for $C_{24}H_{25}O_4NS$: C, 68.07; H, 5.95; N, 3.31. Found: C, 68.21; H, 5.88; N, 3.29.

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