Synthesis of (S)-β-Cyclooctatetraenylalanine

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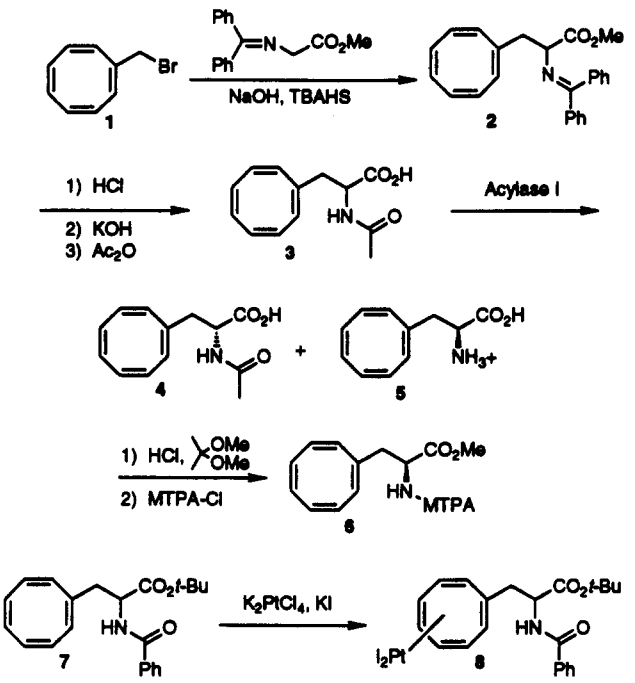
As the recognition of the biological activity of peptides and proteins has burgeoned, the potential of such molecules as drugs has drawn increasing attention.[1] For most applications, however, peptides composed exclusively of the genetically-coded amino acids have serious disadvantages regarding oral activity, serum degradation, and pharmacokinetics (both clearance and passage of the blood-brain barrier). Consequently, there has been a great deal of interest in developing unnatural amino acid analogues that can be incorporated into bioactive peptides for the purpose of overcoming these disadvantages.[2] With new techniques in molecular biology, it may be possible to generate more effective therapeutics or elucidate structure-activity relationships via incorporation of unnatural residues into proteins.[3]

The properties of cyclooctatetraenes[4] suggest they may confer desirable characteristics to peptides. They undergo conformational changes with barriers comparable to those for amide rotation,[5] potentially providing both structural limitation and flexibility. They are potent excited-state quenchers[6] and easily accept electrons.[7] They may thus be useful for studies of light- and redox-activated processes. Finally, cyclooctatetraenes are able to bind transition metals and actinides.[8] For these reasons, cyclooctatetraene amino acids have been targeted for synthesis. This report concerns the preparations of the simplest member of the class, β-cyclooctatetraenylalanine (COT-Ala, 5), in enantiomerically-pure form.

The method of Whitesides was utilized for the synthesis of optically active COT-Ala.[9] Thus, (bromomethyl)-cyclooctatetraene (1) was prepared from (methoxycarbonyl)cyclooctatetraene by a literature route.[10] It was then treated with the benzophenone imine of methyl glycinate in the presence of a phase transfer catalyst and NaOH in a two-phase system. The alkylation product 2, obtained



in 82% yield on a 5-g scale, is an oil. Hydrolysis of the imine and ester and conversion of the amine to the acetamide provides 3. This material is subjected to kinetic resolution with Acylase I to give 4 in 50% yield ($[\alpha]_D$ +3.04°) and (S)-5 in 43% yield. The latter is obtained by ion-exchange chromatography and crystallization ($[\alpha]_D$ -87.2°). The absolute configurations of these materials are assigned on the basis of the widely studied stereoselectivity of the enzyme. In order to determine the enantiomeric excess of 5, it was converted to its methyl ester with the procedure of Rachele[11] and treated with the acyl chloride of Mosher's acid.[12] The product MTPA-amide methyl ester 6 was analyzed by capillary gas chromatography and shown by comparison with a racemic ester amide and by coinjection to be of >99% ee. The remaining (R)-acetamide 4 is racemized in 90% yield by heating in Ac_2O/AcOH at 100 °C for 16 h to permit full throughput of material to the desired L-amino acid. It is worthy of mention that initial attempts to prepare optically-active 5 using the asymmetric alkylation catalyst of O'Donnell[13] proceeded in good yield but provided racemic product. One explanation for this observation is that the alkylation proceeds by an electron-transfer process. The easily-produced cyclooctatetraene radical anion could fragment to cyclooctatetraenylmethyl radical that subsequently couples with the enolate radical. If verified, this mechanism would suggest caution concerning use of the O'Donnell procedure with easily reduced alkyl halides.

A number of technological and scientific advances rely on the specific binding of peptides and proteins to metal

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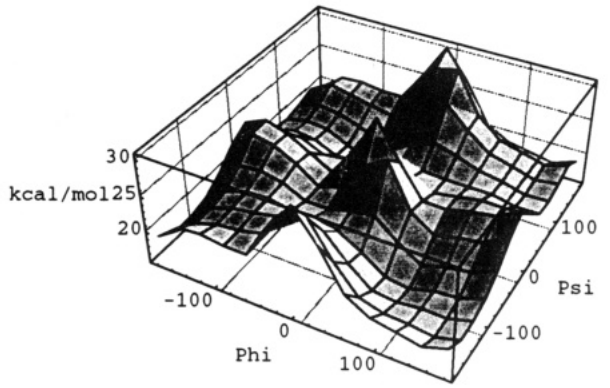

Figure 1.

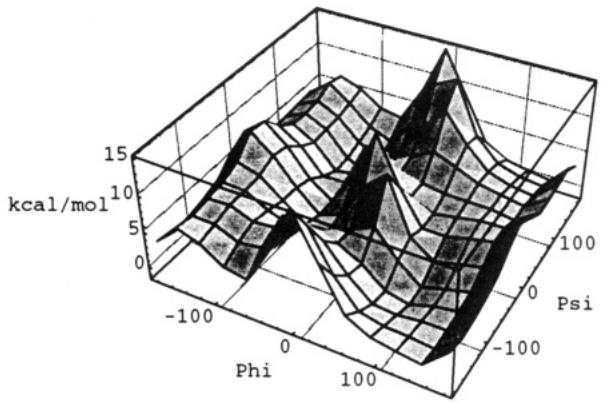

Figure 2.

ions.[14] The metal complexing capability of COT-alanine was therefore demonstrated by treatment of amide **7** with potassium tetrachloroplatinate and potassium iodide.[15] Complex **8** is crystallized from the solution. It was characterized by spectroscopic techniques and elemental analysis.

To evaluate the changes in peptides and proteins that will be engendered by the incorporation of COT-Ala, its conformational energy surface has been studied by molecular mechanics calculations using the MMX force field. Its Ramachandran plot (Figure 1) is quite similar to that of phenylalanine (Figure 2), suggesting that it will prove a suitable substitution for this and related residues.

In summary, we have prepared cyclooctatetraenylalanine derivatives that are intended for incorporation into bioactive peptides and proteins. The syntheses and properties of the latter will be reported separately.

Experimental Section

Methyl *N*-(Diphenylmethylidene)-β-cyclooctatetraenylalanine (2). The glycine methyl ester Schiff's base (5.00 g, 19.7 mmol), bromide **1** (4.70 g, 22.0 mmol), and tetrabutylammonium hydrogen sulfate (6.80 g, 20.0 mmol) were dissolved in 50 mL of dichloromethane, and 50 mL of 10% aqueous sodium hydroxide was added. The resulting two-phase mixture was stirred at room temperature for 24 h. The organic layer was separated and concentrated. The residue was taken up in ether (150 mL) and washed with water (to remove the residual quaternary salt) followed by brine and dried over Na_2SO_4. The ether was removed to give a crude product which was flash chromatographed over silica gel (50 g) eluting with 5% ethyl acetate–petroleum ether and recrystallized from hexane to afford pure alkylation product **2** (5.97 g) in 82% yield: mp 62 °C; IR (Nujol) 3003, 1741, 1620, 1172, cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.7–7.2 (10 H, m), 5.8–5.4 (7 H, m), 4.18 (1 H, dd, J = 8.0, 5.6), 3.71 (3 H, s), 2.73 (1 H, dd, J = 13.3, 5.2), 2.58 (1 H, dd, J = 13.2, 8.1). Anal. Calcd for $C_{25}H_{23}NO_2$: C, 81.27; H, 6.27; N, 3.79. Found: C, 81.31, H, 6.22, N, 3.81.

***N*-Acetyl-β-cyclooctatetraenylalanine (3).** Schiff's base methyl ester **2** (5.00 g, 13.5 mmol) was dissolved in 10 mL of THF, and 50 mL of 5% hydrochloric acid was added. The mixture was stirred at room temperature for 1 h. Saturated $NaHCO_3$ was added slowly, and the mixture was extracted with ethyl acetate (3 × 100 mL). The combined organic extract was washed with water and brine and dried. The crude product product obtained after removal of the solvent was flash chromatographed over silica gel (15 g) eluting with ethyl acetate to afford the pure methyl ester (2.38 g) in 86% yield: IR (thin film) 3378, 3000, 1737, 1195 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.9–5.6 (7 H, m), 3.71 (3 H, s), 3.54 (1 H, t, J = 4.4), 2.52 (1 H, dd, J = 12.8, 4.4), 2.3 (1 H, m).

The above methyl ester (2.0 g, 9.7 mmol) was dissolved in 50 mL of methanol, and sodium hydroxide (0.8 g, 20 mmol) was added and stirred at room temperature for 5 h. Methanol was removed and the remaining solid was dissolved in 12 mL of water. Sufficient sodium hydroxide (0.8 g) was added to make a 4 N solution (containing 3 equiv of base) that was cooled to −5 °C. Acetyl chloride (785 mg, 10 mmol) was added dropwise, and stirring was continued for 1 h. The mixture was acidified to pH 1.5 with concentrated hydrochloric acid and extracted with ethyl acetate (3 × 100 mL). The combined extract was washed with water and brine and dried over Na_2SO_4. The crude product obtained after removal of the solvent was recrystallized from ethyl acetate–petroleum ether to afford acetamide **3** (1.7 g) in 63% yield: mp 161.5 °C; IR (Nujol) 3337, 3007, 1717, 1613, 1557 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.0–5.4 (7 H, m), 4.4 (1 H, m), 2.7–2.5 (2 H, m), 2.07 (3 H, s). Anal. Calcd for $C_{13}H_{15}NO_3$: C, 66.94; H, 6.48; N, 6.00. Found: C, 66.91; H, 6.51; N, 6.09.

Kinetic Resolution of 3. To distilled water were added racemic acetamide **3** (1.0 g, 4.3 mmol) and sufficient potassium hydroxide to make the final solution pH 7.5–8.0. The concentration of acetamide was brought to 0.5 M by the addition of distilled water. To this solution was added 1 mM $CoCl_2$ followed by 76 mg of Acylase I (*Aspergillus*, activity 0.47 units/mg) and stirred for 24 h at 40 °C while maintaining the pH of the reaction at approximately 7.5 with periodic addition of base. The mixture was brought to pH 5.0 with concentrated hydrochloric acid, heated to 60 °C with Norit (100 mg) for 15 min, and filtered. The filtrate was acidified to pH 1.5 with concd HCl and extracted with ethyl acetate (2 × 100 mL). The aqueous layer was purified on a Dowex-50(H^+) column eluting with 1 N ammonia to afford pure amino acid **5** (374 mg) in 43% yield: mp 175.4 °C (methanol); ^1H NMR (D_2O) δ 5.8–5.5 (7 H, m), 3.08 (1 H, m), 2.27 (1 H, m), 2.05 (1 H, m). The ethyl acetate extract was dried over Na_2SO_4 and concentrated to give a crude product which was recrystallized from ethyl acetate–petroleum ether to obtain pure acetamide **4** (500 mg) in 50% yield, mp 162.5 °C.

Derivatization and Determination of Enantiomeric Excess of 5. Amino acid **5** (50 mg, 0.25 mmol) was dissolved in 3 mL of 2,2-dimethoxypropane, and 0.5 mL of anhydrous methanol was added, followed by 2 drops of concentrated HCl. The mixture was refluxed for 1 h and then stirred at room temperature for 15 h. The solvent was removed, and the crude product obtained was taken up in 3 mL of carbon tetrachloride and 0.6 mL of pyridine. To this solution was added Mosher's acid chloride (65 mg, 0.26 mmol). After 12 h at room temperature, the reaction mixture was diluted with ether and washed with dilute HCl, water, $NaHCO_3$, and brine and dried over Na_2SO_4. The crude MTPA-amide methyl ester was used as such for the determination of the diastereomeric ratio using capillary GC. The racemic MTPA-amide methyl ester was prepared using the same procedure and used as a standard.

Racemization of 4. Acetamide **4** (500 mg, 2.04 mmol) was dissolved in 5 mL of acetic acid, and 0.25 mL of acetic anhydride was added. This mixture was heated at 100 °C for 15 h. The

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mixture was concentrated under reduced pressure, and the crude material was recrystallized from ethyl acetate–petroleum ether to afford racemic product (450 mg) in 90% yield.

[*N*-Benzoyl-β-cyclooctatetraenylalanine *tert*-butyl ester]platinum Diiodide (8). Potassium tetrachloroplatinate (100 mg, 0.24 mmol) was dissolved in 1 mL of water at 60 °C, potassium iodide (150 mg, 0.9 mmol) was added and the mixture was cooled. *N*-Benzoyl-β-cyclooctatetraenylalanine *tert*-butyl ester (80 mg, 0.24 mmol) was then added with vigorous stirring. A yellow precipitate immediately separated. After standing for 24 h at room temperature, the solution was filtered and the residue was washed with water, dried, and recrystallized from chloroform: mp 168.3 °C; IR (Nujol) 3269, 2912, 1730, 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.77 (2 H, m), 7.55 (1 H, m), 7.47 (2 H, m), 6.63 (1 H, m) 6.16 (3 H, m), 5.60 (3 H, m), 4.74 (1 H, dd, J = 7.0, 5.4), 2.99 (1 H, dd, J = 14.8, 5.4), 2.67 (1 H, dd, J = 14.8, 7.0), 1.56 (9 H, s). Anal. Calcd for $C_{22}H_{25}I_2NO_3Pt$: C, 33.01; H, 3.14; N, 1.75. Found: C, 33.96; H, 3.21; N, 1.85.