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TETRAHEDRON: *ASYMMETRY*

Synthesis of novel chiral amino acids possessing a porphyrin moiety

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Abstract

Phenylalanine derivatives bearing a porphyrin moiety at the *para*-position were prepared in an enantiomerically pure form. The synthetic nonnatural aromatic amino acid reacted with amines and acids to give novel functionalized peptides without loss of the enantiomeric purity. © 1999 Elsevier Science Ltd. All rights reserved.

Unusual amino acids are useful for the development of artificial proteins as well as elucidation of the structure and function of naturally occurring proteins.[1,2] Many unusual amino acids have been reported[3] and some of them are commercially available. Porphyrins and their related compounds are important as cofactors of proteins, for example, in respiratory and photosynthetic apparatus. Several amino acids bearing a porphyrin moiety at the side chain are described in the literature[4,5] and all of them utilize the functional groups in the side chain of natural amino acids for the linkage: the porphyrin moiety was covalently bonded with the amino acids through amido, ester or ether bonds. These bonds might react with some reagents to give decomposed products and/or to induce removal of the porphyrin moiety from peptide chains. Moreover, the functional linkages would interact with some other functional groups in peptides, for example, by hydrogen bonding, to affect the molecular conformations. To overcome these problems, we designed a direct introduction of a porphyrin unit into peptide chains without any use of the above functional groups. During preparation of this communication, Hyslop, Therien and their colleagues[6] reported an α-amino acid linked with a porphyrin moiety through a C=C bond, although the chirality at the α-position was not confirmed. Here we report the synthesis of chiral phenylalanine derivatives directly substituted with a porphyrin moiety at the *para*-position.

N-Acetyl-(*p*-hydroxymethyl)phenylalanine (D,L-**2**) was prepared from methyl *p*-toluate according to the reported procedure.[7] Methylation of the carboxylic acid and oxidation of the hydroxymethyl group in D,L-**2** gave *N*,*C*-protected (*p*-formyl)phenylalanine D,L-**3** in 70% yield (see Scheme 1). BF_3-catalyzed condensation of D,L-**3** (1 equiv.) and 3,5-di-*t*-butylbenzaldehyde (3 equiv.) with pyrrole (4 equiv.) in

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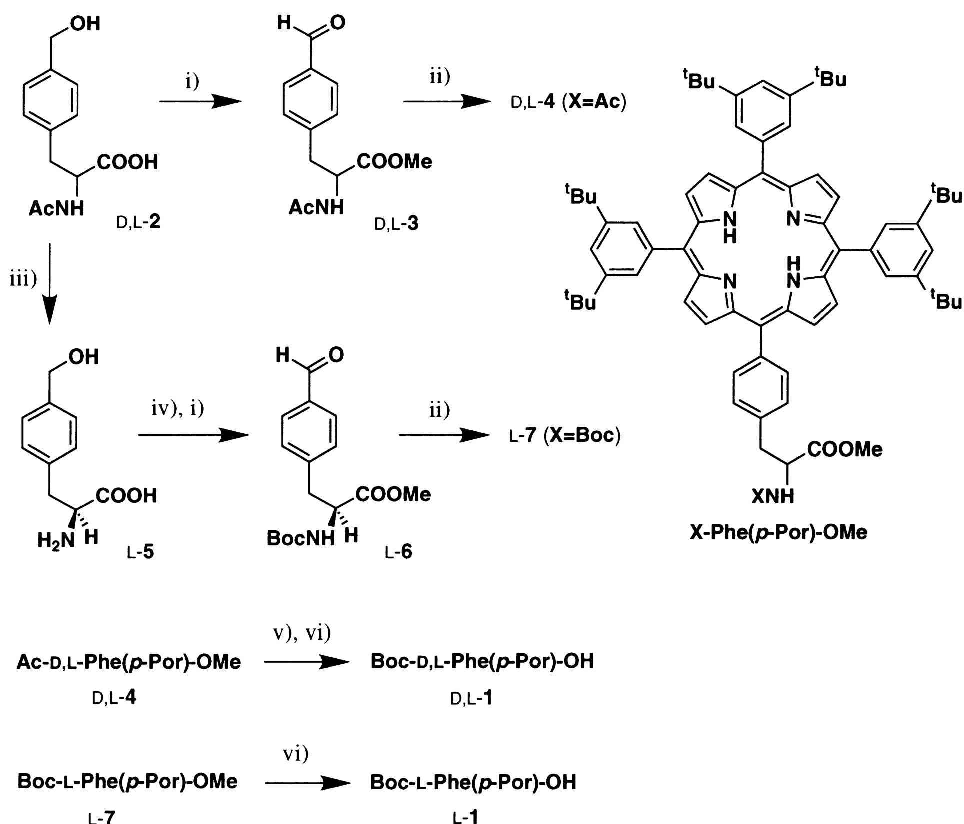
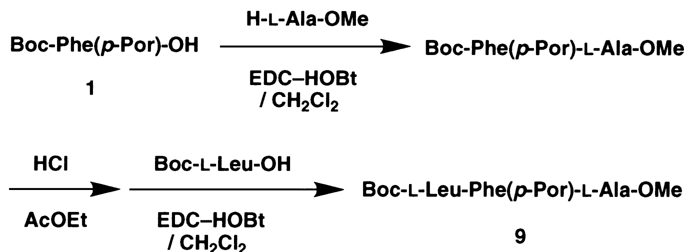
chloroform and successive oxidation by *p*-chloranil (Lindsey's method[8]) gave symmetrical 5,10,15,20-terakis(3,5-di-*t*-butylphenyl)porphyrin (**8**, 16%) and the desired *N*-acetyl-(*p*-porphyrinyl)phenylalanine methyl ester D,L-**4** (Ac–Phe(*p*-Por)–OMe, 12%) which were separated by flash column chromatography on silica gel (0–5%, Et_2O–CH_2Cl_2). Boc protection of the imino group in D,L-**4** followed by LiOH hydrolysis of acetyl amido and methyl ester groups[9] afforded *N*-*t*-butoxycarbonyl-(*p*-porphyrinyl)phenylalanine D,L-**1** (48%). In spite of the presence of the large π-conjugated macrocycle in the molecule, both amino and carboxylic acid groups in the synthetic Phe(*p*-Por) are reactive for several reagents. Using conventional methods for solution phase peptide synthesis, a diastereomeric mixture of tripeptide, Boc–L-Leu–D,L-Phe(*p*-Por)–L-Ala–OMe (**9**) was prepared as shown in Scheme 2. Yields of the coupling and deprotection steps were moderate, >80–90%. Boc–Phe(*p*-Por)–OH (**1**) was highly soluble in ordinary organic solvents except in the case of alcohols, including methanol, because of the presence of six sterically bulky *t*-butyl groups in the molecule:[4] the solubilities of **1** were >0.1 M in both dichloromethane and 1-methyl-2-pyrrolidinone. Therefore, **1** can be used for the preparation of various oligopeptides in the solid phase as well as in solution. Visible and fluorescence spectra of **1** were identical to those of **8** in dilute solutions (ca. 10^{-6} M), e.g., hexane, dichloromethane and tetrahydrofuran, indicating that the porphyrin moiety did not interact with functional groups of the phenylalanine part intramolecularly.



Scheme 1. Synthesis of *N*-*t*-butoxycarbonyl-(*p*-porphyrinyl)phenylalanine (Boc–Phe(*p*-Por)–OH, **1**). (i) CH_2N_2/Et_2O; $C_5H_5NH^+ClCrO_3^-$(=PCC)/CH_2Cl_2; (ii) pyrrole (4 equiv.), 3,5-$^t Bu_2C_6H_3CHO$ (3 equiv.), $BF_3\cdot Et_2O/CHCl_3$; *p*-chloranil; (iii) acylase/H_2O (pH=8); (iv) Boc_2O/1,4-dioxane, H_2O, aq 1 M NaOH; (v) Boc_2O, (*p*-Me_2N)C_5H_4N(=DMAP)/THF; (vi) aq 2 M LiOH



Scheme 2. Synthesis of tripeptide **9**. EDC=1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, HOBt=1-hydroxybenzotriazole

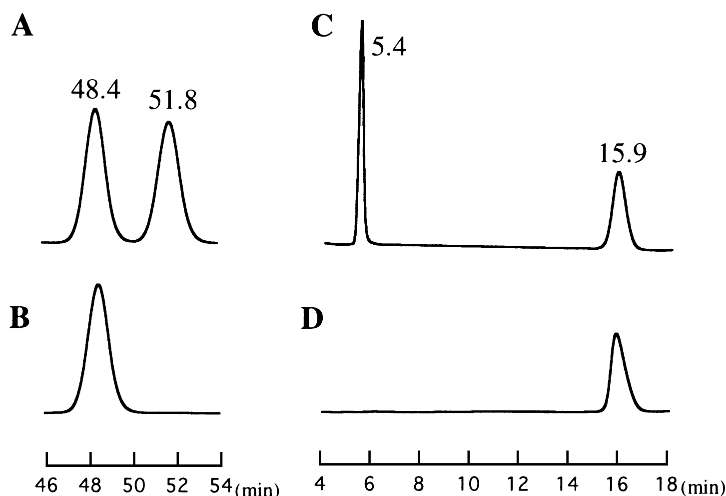


Figure 1. High performance liquid chromatograms of D,L-**1** (A, separation ratio (R_s)=1.8) and L-**1** (B) by a chiral-phase column (SUMICHIRAL OA-4500, 4.6 ∅×250 mm, hexane:CH_2ClCH_2Cl:MeOH:CF_3COOH=93:5:2:0.2, 1.0 ml/min) and of D,L-**5** (C, R_s=14) and L-**5** (D) by a chiral-phase column (SUMICHIRAL OA-6100, 4.6 ∅×150 mm, 2 mM $CuSO_4$ in H_2O:CH_3CN=95:5, 1.0 ml/min)

Racemic D,L-**1** was fully separated by chiral-phase HPLC (see Fig. 1A). Although each enantiomer of **1** was obtained pure after the HPLC separation, the amount of the isolated enantiomers was small and the absolute configuration could not be determined. Hence, an alternative route was used to obtain L-**1** as follows (see also Scheme 1). Racemic **2** was treated with acylase[2] to give enantiomerically pure L-(*p*-hydroxymethyl)phenylalanine (L-**5**, 40%): the remaining *N*-acetyl compound **2** (55%) was D-rich. The enantiomeric excess of the kinetically resolved L-**5** was confirmed by analysis of the chiral HPLC (Fig. 1C, D). The unnatural L-α-amino acid L-**5** was protected by Boc_2O and CH_2N_2 and oxidized by PCC to give **6** (53%).[10] Similarly with the synthesis of D,L-**4** (vide supra) **6** was converted to the corresponding porphyrin **7** (6%) without deprotection of the Boc group. After hydrolysis of **7** by treatment of LiOH, the resulting **1** (76%) was analyzed by chiral HPLC (Fig. 1B), which showed that the sample was enantiomerically pure; the rapidly moving band (48.4 min) was attributed to L-**1** and the second band (51.8 min) to D-**1**.[11] Reactions of enantiomerically pure L-**1** (vide supra) afforded a diastereomerically pure tripeptide **9** from the ^{1}H NMR analysis. These results indicate that no racemization occurred during the several synthetic procedures described above.

In conclusion, enantiomerically pure amino acid–porphyrin dyad, L-**1** was easily prepared and should be useful in the construction of novel functionalizing peptides including electron/energy transfer systems.

Acknowledgements

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11. L-**1**: dark purple solid; vis (CH_2Cl_2), λ_{max}=648 (0.01), 592 (0.01), 551 (0.02), 517 (0.03), 420 nm (1.00); fluorescence (CH_2Cl_2, λ_{exc}=420 nm) λ_{em}=653 (1.0), 718 nm (0.5); ^{1}H NMR (20% CD_3OD–$CDCl_3$) δ=8.71 (8H, br, β-H of pyrrole), 7.97 (2H, d, J=8 Hz, 2,6-H of *meso*-phenylene), 7.89 (6H, br-s, 2,6-H of *meso*-phenyl), 7.61 (3H, m, 4-H of *meso*-phenyl), 7.46 (2H, d, J=8 Hz, 3,5-H of *meso*-phenylene), 4.41 (1H, br-t, α-H of Phe), 3.41 (2H, m, β-H of Phe), 1.34, 1.32 (18H+36H, s, 3,5-^{t}Bu of *meso*-phenyl), 1.29 (9H, s, ^{t}Bu of Boc). MS (FAB) found: *m/z* 1137, calcd for $C_{76}H_{91}N_5O_4$: M^+, 1137.