Solid-phase synthesis has been widely applied for the preparation of peptides. Fluorenylmethoxycarbonyl (Fmoc) amino acids have recently come into general use, since removal of the protective groups and cleavage of the peptides from a solid support can be easily achieved by treating with moderately strong acid such as trifluoroacetic acid (TFA). 1

The title compound (1) has been designed as a useful peptide-resin linker for the synthesis of peptide-amides, which can be readily cleaved from the solid-phase with TFA, after completing the peptide synthesis.

We found, however, the published synthesis of 1 of I to be unsatisfactory, and describe here some important modifications to the procedure for a much improved reaction yield of I. The modified synthesis is shown in the Scheme. In the first step, nitrobenzene proved to be a good solvent for the Friedel Crafts reaction, although subsequent removal of the solvent was effected by steam distillation. O-Alkylation with 2 by ethyl chloroacetate and subsequent hydrolysis of ester 3 gave 4 in a better yield than by the direct coupling reaction with chloroacetic acid which has been described in the literature. 1-3

In the fourth step, carboxylic acid 4 was easily converted to oxime 5 with excess hydroxylamine hydrochloride in triethylamine. The 1H- and 13C-NMR spectra show that the resulting product was a mixture of the two possible syn and anti isomers, which could not be separated by high-performance liquid chromatography (HPLC) under the conditions shown in the experimental section. One of the isomers was obtained by repeated recrystallization, but the stereochmometry of the isomers has not yet been elucidated.

Hydrogenation of 5 over platinum oxide was much better than reduction with zinc that is shown in ref. 1-3. During the reduction of the oxime, the formation of hydroxylamine as an intermediate was observed. The final step follows the method described in the literature. 1-3 the overall yield of final product I being 36% through these six steps.

Experimental

Chemicals. As the standard specimen, I was purchased from Nova Biochem, Switzerland. The solvents for NMR analysis were from CEA, France, while the other chemicals were of reagent grade from Nacalai Tesque, Kyoto.

Measurements. Melting points (mp) are uncorrected. NMR spectra were taken on a JEOL JNM EX-270 spectrometer (270 MHz for protons, 67.5 MHz for 13C) in the solvent shown for each synthesis with tetramethylsilane (TMS) as an internal standard. HPLC analysis was carried out in a TSK gel ODS-120A column (4.6 x 250 mm, Tosoh, Tokyo), with methanol-0.05 mM phosphoric acid (1:1) as the eluant and detection at 270 nm.

Synthesis

4-((Oxoy-2,4-dimethoxybenzyl)phenoxyacet) (2). A mixture of 4-hydroxybenzoic acid (125 g, 0.9 mol), 1,3-dimethoxynbenzene (117 g, 0.85 mol), phosphorl chloride (154 g, 1.0 mol), and zinc chloride (136 g, 1.0 mol) was gently heated at 80°C for 3 h in nitrobenzene (1.5 kg). The reaction mixture was made alkaline with 4 N sodium hydroxide (1.5 liters), and the mixture was applied to steam distillation to remove the nitrobenzene and unreacted 1,3-dimethoxybenzene. The filtrated alkaline layer was acidified with acetic acid to precipitate 2, which was recrystallized from 50% ethanol in a yield of 120 g (64%), mp 128-139°C. Anal. Found: C, 69.64; H, 5.54%. Calcd for C13H16O7: C, 69.75; H, 5.46%. 1H-NMR, in CDCl3, δ ppm from TMS: 3.68 (s, 3H), 3.86 (s, 3H), 6.48-6.55 (m, 2H), 6.86-6.90 (m, 2H), 7.30-7.36 (m, 1H), 7.68-7.75 (m, 2H). 13C-NMR, δ ppm: 55.4, 55.5, 58.7, 104.4, 115.1, 120.0, 130.0, 131.3, 132.4, 135.9, 161.9, 162.7, 194.5. IR, cm⁻¹, KBr pellet: 3333, 3013, 2964, 2840, 1633, 1604, 1573, 1507.

Ethy 4-((Oxoy-2,4-dimethoxybenzyl)phenoxyacet (3). A mixture of 2 (90 g, 0.34 mol), ethyl chloroacetate (60 g, 0.50 mol), anhydrous potassium carbonate (60 g, 0.45 mol) and potassium iodide (5 g) in ethanol (2.0 liters) was refluxed for 3 days. The reaction mixture was poured into water (1.5 liters) to precipitate 3, which was recrystallized from ethanol-water in a yield of 103 g (88%), mp 82-83°C. Anal. Found: C, 66.09; H, 6.01%. Calcd for C24H28O11: C, 66.27; H, 5.85%. 1H-NMR, in CDCl3, δ ppm: 1.26 (t, J=8.0, 3H), 3.68 (s, 3H), 3.84 (s, 3H), 4.24 (q, J=8.0, 2H), 4.65 (s, 2H), 6.64-6.54 (m, 2H), 6.85-6.92 (m, 2H), 7.19-7.35 (m, 1H), 7.71-7.78 (m, 2H). 13C-NMR, δ ppm: 14.1, 55.4, 55.5, 61.5, 65.2, 98.8, 104.5, 113.9, 121.7, 131.7, 132.1, 132.4, 159.2, 161.2, 163.0, 168.3, 194.2.

4-((Oxoy-2,4-dimethoxybenzyl)phenoxyacet (4). A solution of 3 (100 g, 0.32 mol) in ethanol (500 ml) and 2 N sodium hydroxide (200 ml) was refluxed for 16 h. The resulting mixture was acidified with hydrochloric acid to precipitate 4, which was recrystallized from methanol in a yield of 100 g (91%), mp 158-159°C. Anal. Found: C, 64.17; H, 5.00%. Calcd for C24H26O10: C, 64.55; H, 5.10%. 1H-NMR, in CDCl3, DMSO (1:1), δ ppm: 3.68 (s, 3H), 3.85 (s, 3H), 4.74 (s, 2H), 6.58-6.63 (m, 2H), 6.94-6.97 (m, 2H), 7.19-7.25 (m, 1H), 7.62-7.65 (m, 2H). 13C-NMR, δ ppm: 53.5, 53.6, 62.6, 93.6, 103.2, 112.1, 119.4, 128.7, 129.3, 112.9, 156.5, 159.4, 160.6, 167.7, 191.2. IR, cm⁻¹, KBr pellet: 3400, 2984, 1756, 1603, 1506, 1461.
4-[(Oximino-2,4-dimethoxybenzyl)phenoxyacetic acid (5)]. A mixture of 4 (90 g, 0.28 mol), hydroxylamine hydrochloride (70 g, 1.0 mol) in methanol (700 ml), water (200 ml), and triethylamine (300 ml) was refluxed for 3 days. The mixture was concentrated under reduced pressure to half its original volume, and acidified with 1N acetic acid to yield a crystalline precipitate of 5, which was shown to be a mixture of its isomers (27:73) by the NMR spectra. The product was dissolved in 1N sodium hydroxide, and the solution was neutralized with acetic acid to precipitate the first crystalline crop (20 g). The filtrate acidified with acetic acid gave the second crop after standing for several days (61 g). The first crystal showed mainly one of the isomers, the second crop being a mixture of isomers in a total yield of 81 g (85%). After repeated recrystallization of the first crop, the mp was 179—180°C. Anal. Found: C, 61.65; H, 5.25; N, 4.29%. Caled. for C_{17}H_{20}NO_6: C, 61.63; H, 5.17; N, 4.23%. ^1H-NMR, in DMSO, δ ppm: 3.64 (s, 3H), 3.81 (s, 3H), 4.66 (s, 2H), 6.56—6.63 (m, 2H), 6.84 (d, J = 8.9, 2H), 6.92 (d, J = 8.2, 1H), 7.29 (d, J = 7.0, 2H), 10.85 (s, 1H). 13C-NMR, δ ppm: 55.2, 55.4, 64.4, 95.4, 104.9, 114.1, 115.3, 127.3, 129.7, 130.0, 152.4, 157.3, 158.0, 160.7, 170.0. IR, cm⁻¹, KBr pellet: 3450, 3100—2400, 1730, 1615, 1520. For the isomers mixture, ^1H-NMR, in DMSO, δ ppm: 3.30 (br. s), 3.52 (s), 3.65 (s), 3.80 (s), 3.81 (s), 4.66 (s), 4.68 (s), 6.50—6.65 (m, 2H), 6.80—6.93 (m, 2H), 7.14 (d, J = 7.0), 7.27 (d, J = 8.2), 7.43 (d, J = 8.2), 8.10 (s, 1H). 13C-NMR, δ ppm: 55.2, 55.3, 55.5, 64.4, 95.4, 98.5, 104.6, 104.9, 113.3, 114.0, 115.3, 118.8, 127.2, 129.7, 130.0, 130.6, 131.3, 152.4, 157.2, 157.3, 158.0, 158.5, 160.6, 160.8, 170.0.

4-[(RS)-1-Amino-(2,4-dimethoxybenzyl)phenoxyacetic acid (6)]. To a stirred mixture of oxime 5 (10 g, 30 mmol) and platinum oxide (0.5 g) in acetic acid (100 ml), hydrogen was bubbled for two weeks at ambient temperature under atmospheric pressure. The solution, after checking the completion of hydrogenation by HPLC analysis, was filtered to remove the catalyst, and the filtrate was concentrated under reduced pressure to a volume of 20 ml. The residue was diluted with water to precipitate 6, which was recrystallized from methanol-water (1:1) in a yield of 9.7 g, 98%, mp 215—217°C (decomposed). Anal. Found: C, 64.07; H, 6.10; N, 4.63%. Caled. for C_{17}H_{20}NO_6: C, 64.34; H, 6.04%. ^1H-NMR, in D_2O-NaOD, δ ppm: 3.11 (s, 3H), 3.41 (s, 3H), 4.07 (s, 2H), 4.80 (s, 1H), 5.15—6.18 (m, 2H), 5.60 (d, J = 8.1, 2H), 6.78—6.86 (m, 3H). 13C-NMR, δ ppm: 54.8, 57.7 (for 2C of OCH₃), 69.1, 101.2, 107.2, 116.5, 128.4, 130.0, 130.2, 139.6, 158.7, 159.6, 161.4, 179.0. IR, cm⁻¹, KBr pellet: 3480, 3200, 3050, 2960, 2860, 2630, 1660, 1620, 1520, 1410.

4-[(RS)-1-[(9H-Fluoren-9-yl)methoxycarbonylamino-(2,4-dimethoxybenzyl)phenoxyacetic acid (1)]. A mixture of 6 (0.37 g, 1.0 mmol), (9H-Fluoren-9-yl)methoxycarbonylsucinimide (Fmoc-OSu, 0.40 g, 1.2 mmol) and potassium carbonate (69 mg, 0.5 mmol) in dimethoxiane-water (2:1, 50 ml) was stirred for 3 hr. The solution was neutralized with acetic acid to pH 6.0 and concentrated under reduced pressure to half its original volume. A crystalline precipitate was collected, washed with water, and recrystallized from acetone-water (1:1) in a yield of 0.45 g (84%), mp 178—179°C. Anal. Found: C, 71.08; H, 5.59; N, 2.86%. Caled. for C_{35}H_{24}NO_6: C, 71.23; H, 5.42; N, 2.60%. ^1H-NMR, in DMSO, δ ppm: 3.34 (br. s, 1H), 3.71 (s, 3H), 3.73 (s, 3H), 4.17—4.30 (m, 3H), 4.62 (s, 2H), 6.45 (d, J = 8.9, 1H), 6.50—6.53 (m, 2H), 6.81 (d, J = 8.6, 2H), 7.07 (d, J = 8.3, 2H), 7.24—7.37 (m, 3H), 7.40—7.43 (m, 2H), 7.72 (d, J = 8.3, 2H), 7.88 (d, J = 7.6, 2H), 8.15 (d, J = 9.0, 1H). 13C-NMR, δ ppm: 47.0, 51.3, 55.5, 55.9, 64.7, 65.8, 105.0, 114.3, 120.4, 123.3, 125.6, 125.7, 127.4, 127.9, 128.4, 135.7, 141.0, 144.1, 155.8, 156.8, 157.2, 159.9, 170.5. IR, cm⁻¹, KBr pellet: 3500, 3100, 3000, 1760, 1660, 1620, 1520.

References
4) The spectra agree completely with those of a commercial product (Nova Biochem, Switzerland).