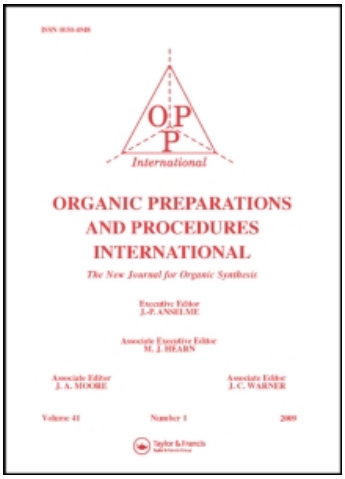

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PREPARATION OF N-t-BUTYLOXYCARBONYL-O$^{\omega}$-9-FLUORENYLMETHYL ESTERS OF ASPARTIC AND GLUTAMIC ACIDS

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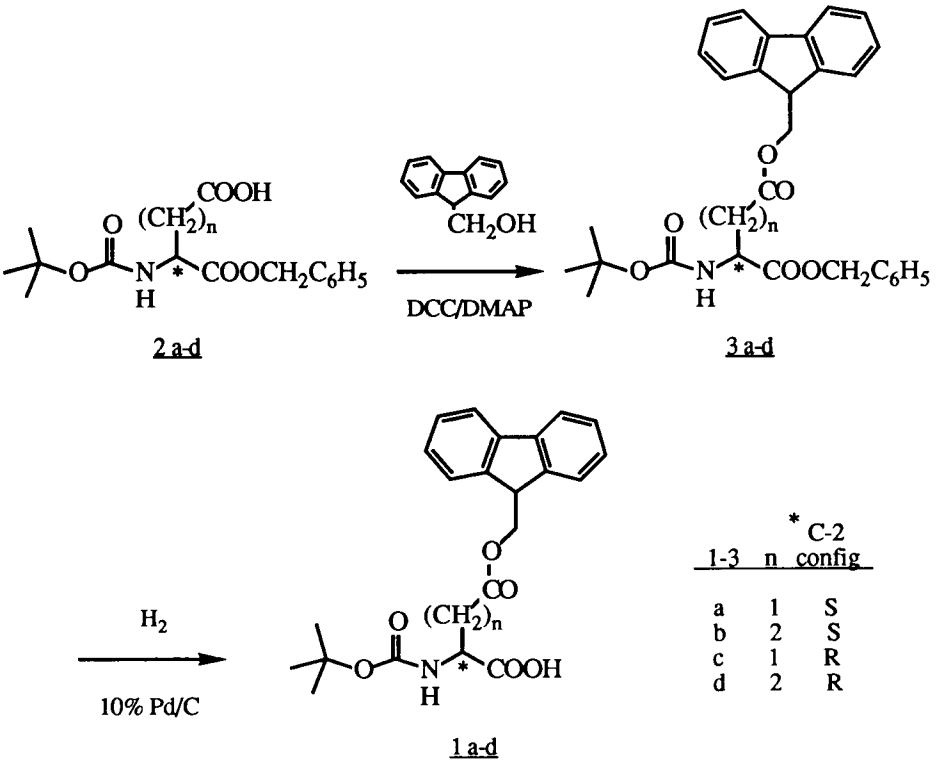
The recent expansive growth of biotechnology has brought about a renewed interest in peptides as potential therapeutic agents. Because most small peptides are conformationally flexible in solution, a substantial research effort is ongoing to develop methods to restrict conformational mobility while retaining biological activity. One particularly useful approach is the introduction of large ring structures into natural acyclic peptides. The construction of covalent linkages (i.e. disulfides or amides) between side chain functional groups of a biologically important peptide has the potential to enhance biological potency, receptor selectivity and enzymatic stability.[1-3]

A number of groups[4-6] have reported successful syntheses of cyclic peptides using modified Merrifield solid phase methodology.[7] Formation of the peptide backbone amide bonds as well as intramolecular lactamization was performed while the peptide remained attached to the polymeric support. In more complex peptides, where selectivity between similar amino acid residues is necessary, a scheme incorporating a triply orthogonal protection strategy would be desirable. The difference in chemistries between the common t-butyl, benzyl and 9-fluorenylmethyl (Fm) groups[8] appeared to satisfy these requirements. The weak acid and strong acid labilities of t-butyl and benzyl, respectively, complement the base lability of the Fm group.[9-11]

We now report a convenient procedure for the preparation of the side chain Fm esters of Boc-aspartic and Boc-glutamic acids (1a-d). Initial attempts at esterification of the side-chain carboxyl of N-Boc-O$^{\alpha}$-benzyl aspartic acid (Boc-Asp-O$^{\alpha}$-Bzl) led to only

limited success. Treatment of a mixture of 2a and 9-fluorenylmethanol[12] with N,N'dicyclohexylcarbodiimide/l-hydroxybenzotriazole (DCC/HOBt) in DMF or with carbonyldiimidazole in DMF gave poor yields of 3a.[13] However, excellent results were obtained using a procedure of Tam et al.[14] The commercially available N-Boc-O$^{\alpha}$-benzyl esters of L and D aspartic and glutamic acids (2a-d) were esterified with DCC and 9-fluorenylmethanol in methylene chloride using 4-dimethylaminopyridine (DMAP) as a catalyst (Scheme 1).

Scheme I



The diesters 3a-d were isolated in high yield as crystalline solids. Selective reduction of the O$^{\alpha}$-benzyl ester function was accomplished by hydrogenation for 1.5-3.5 hrs in MeOH or EtOH with 10% Pd/C catalyst to yield the final products (1a-d) as crystalline solids. Contrary to previous reports,[15-17] the Fm esters were essentially stable to hydrogenation under these conditions. Trace amounts of Boc-diacid contaminants were

detected by TLC if the reduction was allowed to proceed past the optimum time for debenzylation, i.e. 6-8 hrs. Compounds 1a-d as isolated were of high optical purity and no significant amounts of racemization were detected.

The utility of O$^{\omega}$-Fm esters of aspartic and glutamic acids in an orthogonal strategy with Boc-benzyl protection has been demonstrated in several syntheses.[18-20] The Fm esters, in combination with Fmoc protected amine functions, were incorporated into appropriately designed peptides. Both Fm and Fmoc groups, which remained intact during treatment with trifluoroacetic acid (which is required at the beginning of each cycle for deprotection of the Boc group), were removed by brief treatment with 20% piperidine/DMF. Specific lactam formation was then performed to generate side-chain to side-chain cyclic peptides.

EXPERIMENTAL SECTION

Melting points were determined on a Thomas-Hoover Unimelt melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on either a Varian XL-200 or XL-400 FT-NMR spectrometer, at 200 or 400 MHz, respectively. Thin layer chromatography (TLC) was performed on precoated TLC plates (silica gel 60, F-254, 0.2 mm thick) from E. Merck and Co. Optical rotations were obtained on a Perkin-Elmer Model 241 polarimeter at 589 nm. DCC (Fluka), DMAP (Aldrich) and all solvents were used without further purification. The protected amino acids 2a-d were obtained from Chemical Dynamics Corp. and fully characterized (NMR, TLC, [α], and MP) before use.

N-t-butyloxycarbonyl-O$^{\alpha}$-benzyl-O$^{\beta}$-9-fluorenylmethyl-L-aspartate (3a). To a chilled (ice bath) solution of N-t-butyloxycarbonyl-O$^{\alpha}$-benzyl-L-aspartate (8.31 g, 25.7 mmol) and 9-fluorenylmethanol (4.80 g, 24.5 mmol) in 150 mL CH_2Cl_2 was added 4-(dimethylamino)pyridine (30 mg, 0.24 mmol) with stirring. N,N'-dicyclohexylcarbodiimide (5.31 g, 25.7 mmol) was added in portions over 10 minutes and the resulting mixture was stirred for 1 hr with continued cooling. The precipitated N,N'-dicyclohexylurea was removed by filtration and the filtrate diluted with 300 mL CH_2Cl_2. This solution was extracted with 2 x 40 mL 10% citric acid, 1 x 40 mL H_2O, 2 x 40 mL 2.5% $NaHCO_3$, 2 x 40 mL H_2O, dried over $MgSO_4$, filtered, and concentrated to an oily solid (12.34 g). Recrystallization from methanol/ether/pet. ether (1:3:6)

yielded 10.11 g (82%) of 3a, mp 74-76°C; NMR ($CDCl_3$): δ 1.44 (s, 9H), 2.92 (dd, 1H, J = 17 Hz, 5 Hz), 3.13 (dd, 1H, J = 17 Hz, 4 Hz), 4.15 (t, 1H, J = 7 Hz), 4.36 (d, 2H, J = 7 Hz), 4.64 (m, 1H), 5.12 (s, 2H), 5.46 (br d, 1H, J = 9 Hz), 7.30 (s, 5H), 7.46-7.24 (m, 4H), 7.56 (d, 2H, J = 7 Hz), 7.78 (d, 2H, J = 8 Hz); $[\alpha]_D$: -11.67° (c 1, methanol).

Anal. Calcd. for $C_{30}H_{31}NO_6$: C, 71.84; H, 6.23; N, 2.79.

Found: C, 71.67; H, 6.20;N, 2.94.

N-t-butyloxycarbonyl-O$^{\beta}$-9-fluorenylmethyl-L-aspartate (1a). A solution of 5.5 g (10.9 mmol) of 3a in 70 mL methanol was hydrogenated over 300 mg 10% Pd/C for 1.25 hours at room temperature with an initial pressure of 40 psi. The catalyst was filtered off and the filtrate was concentrated. The residual oil was dissolved in 200 mL ether and extracted with 1 x 40 mL 2.5% $NaHCO_3$, 1 x 40 mL H_2O, 1 x 40 mL 2.5% $NaHCO_3$, and 1 x 40 mL H_2O. The aqueous layers were combined and acidified to pH 2 with 10% citric acid. The resultant precipitated mixture was extracted with 4 x 40 mL ether. The combined ether extracts were dried over $MgSO_4$, filtered, and concentrated to a white foam (3.96 g). Recrystallization from methanol/ether yielded 3.56 g (79%) of 1a: mp 141-142°C; NMR ($CDCl_3$) δ 1.46 (s, 9H), 2.91 (dd, 1H, J = 17 Hz, 4 Hz), 3.14 (dd, 1H, J = 17 Hz, 4 Hz), 4.22 (t, 1H, J = 7 Hz), 4.42 (m, 2H), 4.64 (m, 1H), 5.47 (d, 1H, J = 8 Hz), 7.5-7.3 (m, 4H), 7.58 (d, 2H, 8 Hz), 7.78 (d, 2H, J = 8 Hz), 9.52 (br, 1H); $[\alpha]_D$: +5.52° (c 1, methanol).

Anal. Calcd. for $C_{23}H_{25}NO_6$: C, 67.14; H, 6.12; N, 3.40.

Found: C, 67.02; H, 6.21; N, 3.38.

N-t-butyloxycarbonyl-O$^{\alpha}$-benzyl-O$^{\gamma}$-9-fluorenylmethyl-L-glutamate(3b). N-t-butyloxycarbonyl-O$^{\alpha}$-benzyl-L-glutamate (9.0 g, 26.6 mmol) and 9-fluorenylmethanol (4.98 g, 25.3 mmol) were dissolved in 200 mL CH_2Cl_2. The stirred solution was chilled in an ice bath and to it was added 4-(dimethylamino)pyridine (31 mg, 0.25 mmol). N,N'-dicyclohexylcarbodiimide (5.50 g, 26.6 mmol) was added and the resulting mixture was stirred with cooling for 4 hours. Precipitated N,N'-dicyclohexylurea was filtered off

and the filtrate was diluted with 250 mL CH_2Cl_2. This solution was extracted with 2 x 50 mL 10% citric acid, 2 x 50 mL H_2O, 2 x 50 mL 2.5% $NaHCO_3$, and 2 x 50 mL H_2O, dried over $MgSO_4$, filtered, and concentrated to an off white solid (14.62 g). Recrystallization from methanol/ether/pet. ether (3:4:6) yielded 11.7 g (89.7%) of 3b: mp 102-103°C; NMR ($CDCl_3$) δ 1.43 (s, 9H), 1.98 (m, 1H), 2.20 (m, 1H), 2.45 (m, 2H), 4.21 (t, 1H, J = 7 Hz), 4.39 (m, 1H), 4.40 (d, 2H, J = 7 Hz), 5.12 (br m, 1H), 5.18 (s, 2H), 7.36 (s, 5H), 7.46-7.26 (m, 4H), 7.59 (d, 2H, J = 8 Hz), 7.78 (d, 2H, J = 7 Hz); $[\alpha]_D$: -15.21° (c 1, methanol).

Anal. Calcd. for $C_{31}H_{33}NO_6$: C, 72.21; H, 6.45; N, 2.72.

Found: C, 71.99; H, 6.29; N, 2.72.

N-t-butyloxycarbonyl-O$^{\gamma}$-9-fluorenylmethyl-L-glutamate (1b). 5.0 g (9.69 mmol) of 3b was hydrogenated in 300 mL methanol containing 350 mg 10% Pd/C catalyst for 3.5 hours at room temperature at 40 psi. The methanol was filtered and concentrated to an oily residue. The residue was redissolved in 150 mL ether and extracted with 75 mL 5% citric acid. The aqueous layer was back-extracted with 2 x 30 mL ether. The combined ether layers were dried over $MgSO_4$, filtered, and concentrated to a white foam (4.2g). Recrystallization from ether/pet. ether yielded 3.75 g (91%) of 1b: mp 130-131°C; NMR ($CDCl_3$) δ 1.44 (s, 9H), 2.20 (m, 1H), 2.44 (m, 1H), 2.55 (m, 2H), 4.22 (t, 1H, J = 7 Hz), 4.4 (m, 1H), 4.42 (d, 2H, J = 7 Hz), 5.19 (br d, 1H, J = 8 Hz), 7.5-7.3 (m, 4H), 7.62 (d, 2H, J = 8 Hz), 7.79 (d, 2H, J = 7 Hz); $[\alpha]_D$: + 9.33° (c 1, EtOAc).

Anal. Calcd. for $C_{24}H_{27}NO_6$: C, 67.75; H, 6.40; N, 3.29.

Found: C, 67.72; H, 6.37; N,3.50.

N-t-butyloxycarbonyl-O-benzyl-O$^{\beta}$-9-fluorenylmethyl-D-aspartate (3c). N-t-butyloxycarbonyl-O$^{\alpha}$-benzyl-D-aspartate (4.85 g, 15.0 mmol) and 9-fluorenylmethanol (2.80 g, 14.29 mmol) were dissolved in 92 mL CH_2Cl_2. The stirred solution was chilled in an ice-bath and to it was added 4-(N,N'-dimethylamino)-pyridine (17.5 mg, 0.143 mmol). N,N'-dicyclohexylcarbodiimide (3.10 g, 15.0 mmol) was added

in portions over 10 minutes and the resulting mixture was stirred for 1 hour with continued cooling. The precipitated N,N'-dicyclohexylurea was collected and the filtrate diluted with 182 mL CH_2Cl_2. This solution was extracted with 2 x 24 mL 10% citric acid, 1 x 24 mL H_2O, 2 x 24 mL 2.5% $NaHCO_3$, 2 x 24 mL H_2O, dried over $MgSO_4$, filtered, and concentrated to an oily solid. Recrystallization of the oily solid from methanol/ether/pet. ether (1:2:4) yielded 6.45 g (90.0%) of product with mp 70-73°C; NMR ($CDCl_3$) δ 1.44 (s, 9H), 2.91 (dd, 1H, J = 17 Hz, 6 Hz), 3.13 (dd, 1H, J = 18 Hz, 6 Hz), 4.15 (t, 1H, J = 8 Hz), 4.36 (d, 2H, J = 8 Hz), 4.64 (m, 1H), 5.13 (s, 2H), 5.46 (br d, 1H, J = 10 Hz), 7.30 (s, 5H), 7.31 (s, 2H), 7.41 (t, 2H), 7.56 (m, 2H), 7.77 (d, 2H, J = 8 Hz); $[\alpha]_D$: + 9.71° (c 1, methanol).

Anal. Calcd. for $C_{30}H_{31}NO_6$; C, 71.84; H, 6.23; N, 2.79.

Found: C, 71.62; H, 6.33; N, 3.05.

N-t-butyloxycarbonyl-O$^{\beta}$-9-fluorenylmethyl-D-aspartate (1c). 4.60 g (9.17 mmol) of **3c** was hydrogenated in 150 mL methanol containing 300 mg 10% Pd/C catalyst for 1.5 hours at room temperature using a Vibro-mixer with continuous flow of hydrogen. The catalyst was filtered off and the filtrate was concentrated. The residual oil was recrystallized from ether/pet. ether (40:80 mL) to give white crystals 3.14 g (83.3%) of product with mp 137-138°C; NMR ($CDCl_3$) δ 1.45 (s, 9H), 2.90 (dd, 1H, J = 16 Hz, 5 Hz), 3.12 (dd, 1H, J = 17 Hz, 4 Hz), 4.20 (t, 1H, J = 7 Hz), 4.41 (m, 2H), 4.61 (m, 1H), 5.48 (d, J = 7 Hz), 7.4-7.3 (m, 4H), 7.56 (d, 2H, J = 7 Hz), 7.75 (d, 2H, J = 7 Hz), 9.52 (br, 1H); $[\alpha]_D$: -2.76 (c 1, methanol).

Anal. Calcd. for $C_{23}H_{25}NO_6$; C, 67.14; H, 6.12; N, 3.40.

Found: C, 66.74; H, 6.27; N, 3.62.

N-t-butyloxycarbonyl-O$^{\alpha}$-benzyl-O$^{\gamma}$-9-fluorenylmethyl-D-glutamate(3d). N-t-butyloxycarbonyl-O$^{\alpha}$-benzyl-D-glutamate (5.44 g, 16.1 mmol) and 9-fluorenylmethanol (3.01 g, 15.3 mmol) were dissolved in 100 mL CH_2Cl_2 and chilled in an ice-bath. 4-(N,N'-dimethylamino)-pyridine (19 mg, 0.15 mmol) was added followed by addition of 3.32 g (16.1 mmol) N,N'dicyclohexylcarbodiimide in one portion. After stirring for 7 hrs

with cooling, the precipitated urea was filtered off. The filtrate was diluted with 75 mL CH_2Cl_2, washed with 2 x 25 mL 10% citric acid, 2 x 25 mL H_2O, 2 x 25 mL 2.5% $NaHCO_3$, 3 x 30 mL H_2O, dried over $MgSO_4$, filtered and concentrated to a white solid. Recrystallization from methanol/ether/pet. ether (1:5:7) yielded 7.52 g (95.3%) of fine needles: mp 104.5-105.5; NMR ($CDCl_3$) δ 1.42 (s, 9H), 1.95 (m, 1H), 2.20 (m, 1H), 2.45 (m, 2H), 4.19 (t, 1H, J = 7 Hz), 4.36 (d, 2H, J = 7Hz), 4.40 (m, 1H), 5.11 (bd, 1H, J = 8 Hz), 5.17 (dd, 2H, J = 15 Hz, 10 Hz), 7.35 (s, 5H), 7.38 (m, 4H), 7.56 (d, 2H, J = 7 Hz), 7.75 (d, 2H, J = 8 Hz); $[\alpha]_D$: + 15.43° (c 1, methanol).

Anal. Calcd. for $C_{31}H_{33}NO_6$: C, 72.21; H, 6.45; N, 2.72

Found: C, 72.23; H, 6.35; N, 2.82

N-t-butyloxycarbonyl-O$^{\gamma}$-9-fluorenylmethyl-D-glutamate (1d). 5.0 g (9.69 mmol) of 3d was hydrogenated in 250 mL ethanol containing 350 mg 10% Pd/C for 3 hours at room temperature with an initial pressure of 40 psi. The catalyst was filtered off and the filtrate was concentrated to an oil. The residue was taken up in 150 mL ether and washed with 75 mL 5% citric acid. The aqueous layer was back-extracted with 2 x 30 mL ether. The combined ether layers were dried over $MgSO_4$, filtered, and concentrated to an oily foam. Recrystallization from CH_2Cl_2/pet. ether yielded 3.33 g (80.8%) of fine needles of 1d: mp 129.5-130.5°C; NMR ($CDCl_3$) δ 1.44 (s, 9H), 2.01 (m, 1H), 2.24 (m, 1H), 2.53 (m, 2H), 4.20 (t, 1H, J = 7 Hz), 4.3 (m, 1H), 4.39 (d, 2H, J = 7 Hz), 5.18 (bd, 1H, J = 9 Hz), 7.31 (dd, 2H, J = 7.5 Hz), 7.39 (dd, 2H, J = 7 Hz, 7 Hz), 7.58 (d, 2H, J = 7.5 Hz), 7.75 (d, 2H, J = 7 Hz); $[\alpha]_D$: -8.19° (c 1, EtOAc).

Anal. Calcd. for $C_{24}H_{27}NO_6$: C, 67.75; H, 6.40; N, 3.29

Found: C, 67.46; H, 6.35; N, 3.40

REFERENCES

1. P. W. Schiller, T. M.-D. Nguyen, C. Lemieux and L. A. Maziak, "Peptides: Structure and Function. Proc. Ninth Amer. Pept. Symp.," p. 483, C. M. Deber, V. J. Hruby and K.D. Kopple, Eds, Pierce, Rockford, IL, 1985.
2. J. Rivier, J. Varga, J. Porter, M. Perrin, Y. Haas, A. Corrigan, C. Rivier, W. Vale, S. Struthers and A. Hagler, "Peptides: Structure and Function. Proc. Ninth Amer. Pept. Symp.", ibid., p. 541.
3. W. L. Cody, M. Mahoney, J. J. Knittel, V. J. Hruby, A. M. Castrucci and M. E. Hadley, J. Med. Chem., 28, 583 (1985).
4. M. Lebl and V. J. Hruby, Tetrahedron Lett., 25, 2067 (1984).
5. P. W. Schiller, T. Nguyen and J. Miller, Int. J. Pept. Prot. Res., 25, 171 (1985).
6. O. Ploux, G. Chassaing and A. Marquet, ibid., 29, 162 (1987).
7. R. B. Merrifield, J. Am. Chem. Soc., 85, 2149 (1963).
8. L . A. Carpino and G. Y. Han, J. Org. Chem., 37, 3404 (1972); ibid., 38, 4218 (1973).
9. M. A. Bednarek and M. Bodanszky, Int. J. Pept. Prot. Res., 21, 196 (1983).
10. H. Kessler and R. Siegmeier, Tetrahedron Lett., 24, 281 (1983).
11. A. Kozhich, A. Ostrovsky and V. Pozdnev, "Peptides 1986: Proc. Nineteenth Eur. Pept. Symp.", p. 75, D. Theodoropoulos, Ed., Walter de Gruyter & Co., Berlin, 1987.
12. L. A. Carpino, J. Org. Chem., 45, 4250 (1980).
13. Attempted transesterification of Boc-Glu(OPCP)-OBzl with 4 and imidazole in toluene (Ref. 9) also failed to yield favorable results.
14. J. P. Tam, T.-W. Wong, M. W. Rieman, F.-S. Tjoeng and R. B. Merrifield, Tetrahedron Lett., 4033 (1979). See also Ref. 10.
15. J. Martinez, J. C. Tolle and M. Bodanszky, J. Org. Chem., 44, 3596 (1979).
16. E. Atherton, C. Bury, R. Sheppard and B. J. Williams, Tetrahedron Lett., 3041 (1979).
17. R. Geiger and W. König, in "The Peptides: Analysis, Synthesis, Biology." Vol. 3, p. 24, E. Gross and J. Meienhofer, Eds., Academic Press, New York, 1981.
18. D. R. Bolin, I.-I. Sytwu, J. M. Cottrell, R. J. Garippa, C. C. Brooks and M. O'Donnell, "Peptides: Chemistry and Biology, Proc. Tenth Amer. Pept. Symp." p. 441, G. Marshall, Ed., Escom, Leiden, 1988.
19. A. M. Felix, C.-T. Wang, E. Heimer, A. Fournier, D. R. Bolin, M. Ahmad, T. Lambros, T. Mowles and L. Miller, "Peptides: Chemistry and Biology, Proc. Tenth Amer. Pept. Symp." ibid., p. 465.
20. A. M. Felix, C.-T. Wang, E. P. Heimer and A. Fournier, Int. J. Pept. Prot. Res., 31, 231(1988).

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