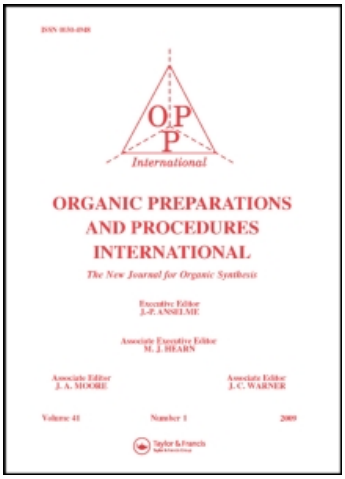

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A PRACTICAL METHOD FOR THE SYNTHESIS OF N-FLUORENYLMETHOXYCARBONYL DIAMINES

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and C-5'), 117.50 (C-5), 116.65 (C-4'), 104.00 (C-4), 102.08 (C-2).
Anal. Calcd for $C_{10}H_{10}N_2$: C, 75.92; H, 6.37; N, 17.71. Found: C, 75.76; H, 6.34; N, 17.26

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A PRACTICAL METHOD FOR THE SYNTHESIS OF N-FLUORENYLMETHOXYCARBONYL DIAMINES

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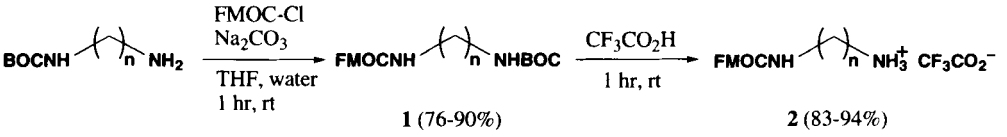
Monoprotected alkyldiamines are extremely important homobifunctional reagents needed as spacers in many applications in biotechnology. Such linkers are used to attach complex drugs to proteins for the development of antibodies, to link antigens and antibodies to solid supports for use in affinity chromatography, and for the regioselective attachment of drugs to monoclonal antibodies for site specific delivery.[1,2] Desirable monoprotected diamine linkers would allow connection of costly biological and chemical moieties *via* carboxylic acid groups in a controlled, predictable fashion and in high yield. The choice of groups useful for amine protection is rather limited, since the conditions required for *de*protection denature proteins such as monoclonal antibodies and enzymes. Complex antigens (macrocyclic lactones, glycosides, quassinoids, etc.) also contain numerous structural features which render them sensitive to reduction and hydrolysis under acidic or basic conditions. The fluorenylmethoxycarbonyl (FMOC) group, however, is cleavable under a variety of mild conditions[3,4] and is thus an excellent choice for such applications. The synthesis of stable mono-fluorenylmethoxycarbonyl diamines has not been previously described. We report here a simple, practical synthesis of mono-fluorenylmethoxycarbonyl diamines.

The N-*tert*-butoxycarbonyl diamine[5] was first protected by acylation with fluorenylmethyl chloroformate in aqueous tetrahydrofuran in the presence of sodium carbonate as an acid scavenger[6]

to yield the diprotected diamines **1a-e** as solids, which are easily crystallized from ethyl acetate or



1 (76-90%) **2** (83-94%)

a) n = 2 b) n = 3 c) n = 4 d) n = 5 e) n = 6

ethyl acetate-hexane mixtures. Removal of the *tert*-butoxycarbonyl group with neat trifluoroacetic acid,[7] followed by crystallization of the salts from dichloromethane or dichloromethane-hexane mixtures, provided stable, easily isolated mono-fluorenylmethoxycarbonyl diamine trifluoroacetates **2a-e** in high yield.

In conclusion, we have developed a simple, practical synthesis of mono-fluorenylmethoxycarbonyl protected diamine trifluoroacetates as stable, crystalline solids in high yields. We believe that these linkers will find widespread application in bioconjugation chemistry.

EXPERIMENTAL SECTION

All reagents were purchased from Aldrich and used without further purification. N-BOC diamines were prepared as described in the literature; acceptable analytical data and comparable yields were obtained.[5] Solvents used were of HPLC grade and used without further purification. ^1H NMR and ^{13}C NMR were recorded at 300 and 75 MHz, respectively, on a Varian Gemini spectrometer. Chemical ionization mass spectra were recorded on a Finnegan MAT SSQ700. Melting points are uncorrected and were taken on an Electrothermal capillary melting point apparatus. Elemental analyses were performed by Robertson Microlit Laboratories, Inc., Madison, NJ.

Representative Experimental Procedure. N-Fluorenylmethoxycarbonyl-N'-*tert*-butoxycarbonylbutanediamine (1c).- N-*tert*-Butoxycarbonylbutanediamine (2.8 g, 16 mmol) was dissolved in tetrahydrofuran (32 mL) and added to a solution of sodium carbonate (5.2 g, 49 mmol) in water (32 mL) in a flask equipped with magnetic stirring. Fluorenylmethyl chloroformate (4.6 g, 18 mmol) was added. After stirring for 12 hrs, the reaction was diluted with water (60 mL) and extracted with ethyl acetate (3 x 60 mL). The combined extracts were washed with water (2 x 20 mL), dried ($MgSO_4$), and the solvents evaporated to give a colorless solid. Crystallization from ethyl acetate-hexane gave 5.2 g (79%) of **1c** as colorless crystals, mp. 143-144°. ^1H NMR ($CDCl_3$): δ 1.45 (s, 9H), 1.45-1.59 (m, 4H), 3.12-3.26 (m, 4H), 4.21 (t, 1H, J = 6.8 Hz), 4.40 (d, 2H, J = 6.8 Hz), 4.62 (br, 1H), 4.97 (br, 1H), 7.31 (t, 2H, J = 7.6 Hz), 7.40 (t, 2H, J = 7.4 Hz), 7.61 (d, 2H, J = 7.6 Hz), 7.76 (d, 2H, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$): δ 156.5, 144.0, 141.4, 127.7, 127.1, 125.1, 120.0, 66.5, 47.3, 40.7, 40.2, 28.5, 27.4, 26.0; MS (DCI/NH_3): 411 $(M + H)^+$, 428 $(M + NH_4)^+$.

Anal. Calcd for $C_{24}H_{30}N_2O_4$: C, 70.22; H, 7.37; N, 6.82. Found C, 69.92; H, 7.60; N, 6.57

N-Fluorenylmethoxycarbonyl-N'-*tert*-butoxycarbonylethanediamine (1a): 76% yield; mp. 154-155°. ^1H NMR ($CDCl_3$): δ 1.45 (s, 9H), 3.21-3.35 (m, 4H), 4.21 (t, 1H, J = 6.7 Hz), 4.41 (d, 2H, J =

6.6 Hz), 4.81(br, 1H), 5.22 (br, 1H), 7.32 (t, 2H, J = 7.3 Hz), 7.41 (t, 2H, J = 7.4 Hz), 7.60 (d, 2H, J = 7.3 Hz), 7.77 (d, 2H, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$): δ 156.5, 144.0, 141.4, 127.7, 127.2, 127.1, 125.1, 120.0, 66.7, 47.3, 40.7, 37.7, 37.1, 32.8; MS (DCI/NH_3) 383 $(M + H)^+$, 400 $(M + NH_4)^+$.

Anal. Calcd for $C_{22}H_{26}N_2O_4$: C, 69.09; H, 6.85; N, 7.32. Found C, 68.98; H, 6.72; N, 7.18

N-Fluorenylmethoxycarbonyl-N'-*tert*-butoxycarbonylpropanediamine (1b): 92% yield; mp. 127-128°. ^{1}H NMR ($CDCl_3$): δ 1.44 (s, 9H), 1.62-1.65 (m, 2H), 3.17-3.26 (m, 4H), 4.22 (t, 1H, J = 7 Hz), 4.40 (d, 2H, J = 7 Hz), 4.82 (br, 1H), 5.35 (br, 1H), 7.32 (t, 2H, J = 7.5Hz), 7.41 (t, 2H, J = 7.1 Hz), 7.61 (d, 2H, J = 7.4 Hz), 7.77 (d, 2H, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$): δ 156.5, 144.0, 141.4, 127.7, 127.2, 127.1, 125.1, 120.0, 66.7, 47.3, 40.7, 37.7, 37.1, 30.6, 28.5; MS (DCI/NH_3) 397 $(M + H)^+$, 414 $(M + NH_4)^+$.

Anal. Calcd for $C_{23}H_{28}N_2O_4$: C, 69.68; H, 7.12; N, 7.07. Found C, 69.55; H, 7.00; N, 6.82

N-Fluorenylmethoxycarbonyl-N'-*tert*-butoxycarbonylpentanediamine (1d): 83% yield; mp. 121-122°. ^{1}H NMR ($CDCl_3$): δ 1.44 (s, 9H), 1.33-1.40 (m, 6H), 3.08-3.20 (m, 4H), 4.21 (t, 1H, J = 6.9 Hz), 4.40 (d, 2H, J = 6.9 Hz), 4.58 (br, 1H), 4.88 (br, 1H), 7.31 (t, 2H, J = 7.4 Hz), 7.41 (t, 2H,J = 7.6 Hz), 7.60 (d, 2H, J = 7.5 Hz), 7.77 (d, 2H, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$): δ 156.5, 144.0, 141.4, 127.7, 127.1, 125.1, 120.0, 66.7, 47.3, 40.9, 40.4, 29.8, 29.6, 28.5, 23.8; MS (DCI/NH_3) 425 $(M + H)^+$, 442 $(M + NH_4)^+$.

Anal. Calcd for $C_{25}H_{32}N_2O_4$: C, 70.73; H, 7.60; N, 6.60. Found C, 70.50; H, 7.89; N, 6.37

N-Fluorenylmethoxycarbonyl-N'-*tert*-butoxycarbonylhexanediamine (1e): 90% yield; mp. 128-129°. ^{1}H NMR ($CDCl_3$): δ 1.44 (s, 9H), 1.24-1.40 (m, 8H), 3.08-3.22 (m, 4H), 4.22 (t, 1H, J = 6.8 Hz,), 4.40 (d, 2H, J = 6.4 Hz), 4.56 (s, 1H), 4.89 (br, 1H), 7.32 (t, 2H, J = 7.4 Hz), 7.40 (t, 2H, J = 7.3 Hz), 7.60 (d, 2H, J = 7.5 Hz), 7.76 (d, 2H, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$): δ 156.5, 144.0, 141.4, 127.7, 127.1, 125.1, 120.0, 66.5, 47.3, 40.9, 40.4, 30.1, 29.9, 28.6, 28.4, 26.3; MS (DCI/NH_3) 439 $(M + H)^+$, 456 $(M + NH_4)^+$.

Anal. Calcd for $C_{26}H_{34}N_2O_4$: C, 71.21; H, 7.81; N, 6.39. Found C, 71.13; H, 7.94; N, 6.23

Representative Experimental Procedure. N-Fluorenylmethoxycarbonylbutanediamine trifluoroacetate (2c):- To magnetically stirred trifluoroacetic acid (1.0 mL) was added **1c** (500 mg, 1.22 mmol). After stirring for 1 hr, the trifluoroacetic acid was evaporated, and the resulting solid was crystallized from dichloromethane-hexane to yield 490 mg (94%) of **2c** as colorless crystals, mp. 120-122°. ^{1}H NMR (CD_3OD): δ 1.52-1.67 (m, 4H), 2.92 (t, 2H, J = 7.3 Hz), 3.14 (t, 2H, J = 7.3 Hz), 4.21 (t, 1H, J = 6.4 Hz), 4.36 (d, 2H, J = 6.5 Hz), 7.30 (t, 2H, J = 7.4 Hz), 7.39 (t, 2H, J = 7.4 Hz),7.63 (d, 2H, J = 7.1 Hz), 7.79 (d, 2H, J = 7.5 Hz); ^{13}C NMR (CD_3OD): δ 147.9, 145.2, 131.3, 130.7, 128.7, 123.6, 123.5, 70.1, 43.4, 43.3, 42.9, 30.4; MS (DCI/NH_3): 311 $(M + H)^+$.

Anal. Calcd for $C_{21}H_{23}F_3N_2O_{4:C}$, 59.43; H, 5.46; N, 6.60. Found C, 59.42; H, 5.61; N, 6.30

N-Fluorenylmethoxycarbonylethanediamine trifluoroacetate (2a): 91% yield; mp. 137-138°. ^{1}H NMR (CD_3OD): δ 3.01 (t, 2H, J = 6 Hz), 3.37 (t, 2H, J = 6 Hz,), 4.21 (t, 1H, J = 6.4 Hz), 4.41 (d, 2H, J = 6.4 Hz), 7.31 (t, 2H, J = 7.3 Hz,), 7.39 (t, 2H, J = 7.2 Hz), 7.64 (d, 2H, J = 7.0 Hz), 7.79 (d, 2H, J = 7.4 Hz); ^{13}C NMR (CD_3OD): δ 147.8, 145.2, 131.4, 130.7, 128.6, 123.5, 70.6, 43.7, 42.0; MS

(DCI/NH_3): 283 $(M + H)^+$.

Anal. Calcd for $C_{19}H_{19}F_3N_2O_4$: C, 57.58; H, 4.83; N, 7.07. Found C, 57.33; H, 4.66; N, 6.89

N-Fluorenylmethoxycarbonylpropanediamine trifluoroacetate (2b): 91% yield; mp. 142-143°. ^1H NMR (CD_3OD): δ 1.77-1.82 (m, 2H), 2.89 (t, 2H, J = 7.6 Hz,), 3.19 (t, 2H, J = 6.5 Hz), 4.20 (t, 1H, J = 6.2 Hz), 4.41 (d, 2H, J = 6.6 Hz), 7.30 (t, 2H, J = 7.4 Hz), 7.41 (t, 2H, J = 7.1 Hz), 7.63 (d, 2H, J = 7.3 Hz), 7.79 (d, 2H, J = 7.3 Hz); ^{13}C NMR (CD_3OD): δ 147.8, 145.2, 131.4, 130.7, 128.6, 123.5, 70.2, 42.3, 41.9, 40.8; MS (DCI/NH_3): 297 $(M + H)^+$.

Anal. Calcd for $C_{20}H_{21}F_3N_2O_4$: C, 58.43; H, 5.16; N, 6.83. Found C, 58.05; H, 5.12; N, 6.74

N-Fluorenylmethoxycarbonylpentanediamine trifluoroacetate (2d): 86% yield; mp. 97-99°. ^1H NMR (CD_3OD): δ 1.35-1.70 (m, 6H), 2.89 (t, 2H, J = 7.6 Hz,), 3.11 (t, 2H, J = 6.7 Hz), 4.19 (t, 1H, J = 6.9 Hz,), 4.35 (d, 2H, J = 6.7 Hz), 7.30 (t, 2H, J = 7.6 Hz), 7.38 (t, 2H, J = 7.4 Hz), 7.63 (d, 2H, J = 7.3 Hz), 7.79 (d, 2H, J = 7.3 Hz); ^{13}C NMR (CD_3OD): δ 147.8, 145.2, 131.4, 130.7, 128.6, 123.6, 123.5, 70.1, 43.8, 43.2, 32.9, 30.7, 27.1; MS (DCI/NH_3): 311 $(M + H)^+$.

Anal. Calcd for $C_{22}H_{25}F_3N_2O_4$: C, 60.27; H, 5.75; N, 6.39. Found C, 59.93; H, 5.60; N, 6.31

N-Fluorenylmethoxycarbonylhexanediamine trifluoroacetate (2e): 90% yield; mp. 96-97°. ^1H NMR (CD_3OD): δ 1.27-1.66 (m, 8H,), 2.89 (t, 2H, J = 7.5 Hz,), 3.10 (t, 2H, J = 7.0 Hz), 4.18 (t, 1H, J = 6.7 Hz), 4.33 (d, 2H, J = 6.8 Hz), 7.29 (t, 2H, J = 7.3 Hz,), 7.38 (t, 2H, J = 7.5 Hz,),7.63 (d, 2H, J = 7.3 Hz), 7.78 (d, 2H, J = 7.4 Hz); ^{13}C NMR (CD_3OD): δ 147.8, 145.3, 131.4, 130.7, 128.6, 123.5, 70.6, 41.4, 40.6, 30.7, 28.5, 27.2, 27.0; MS (DCI/NH_3): 339 $(M + H)^+$.

Anal. Calcd for $C_{23}H_{27}F_3N_2O_4$: C, 61.05; H, 6.01; N, 6.19. Found: C, 60.81; H, 6.18; N, 6.16

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