An Improved Synthesis of Fmoc-*N*-Methyl- α -Amino Acids

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Supporting information

Experimental Details

Synthesis of Fmoc-oxazolidinones

S2-S3

Synthesis of Fmoc-*N*-methyl amino acids

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References

S5

Copies of HPLC traces with UV/VIS (254 nm), universal evaporative light scattering (ELSD), and electrospray mass spectra (ESI) detection; and ESI spectra (positive mode) of compounds **9a-11a**, **1b-5b**, and **8b-11b**.

S6-S17

Experimental Details

Experimental Section

Fmoc-protected α -amino acids and other reagents were purchased from commercial sources. Flash column chromatography was performed employing silica gel 60 (35-70 μ m). Thin-layer chromatography (TLC) was performed using silica-plates (0.20 mm silica gel 60 with fluorescent indicator UV₂₅₄).

Melting point determination was done with a melting point apparatus. Infrared spectra were recorded on an FT-IR spectrometer. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a 400 MHz spectrometer. Chemical shifts (δ) in ppm are reported using residual chloroform as internal reference (^1H δ 7.26, ^{13}C δ 77.0), and coupling constants (J) in Hz. High pressure liquid chromatography (HPLC) coupled to MS and universal evaporative light-scattering detection (ELSD) was done on a system consisting of a pump, autosampler, and an UV/VIS detector, coupled in series with a mass spectrometer (electrospray in the positive mode) and an ELSD. The reverse phase HPLC analyses were done using a C18 column (3 $\times$ 3.0*150 mm) with acetonitrile-water (both containing 0.1 % formic acid) as mobile phase (Gradient: 5-95 % acetonitrile in 6 minutes + 6 minutes at 95%, flow 1.0 ml/min).

General procedure for synthesis of oxazolidinones from Fmoc amino acids:¹

The Fmoc amino acid (5 mmol), paraformaldehyde (1 g) and *p*-toluenesulphonic acid (100 mg) were suspended in toluene (100 ml). The mixture was refluxed in a Dean-Stark setup until no more starting material could be detected by TLC (97.5:2:0.5 CHCl_3 :MeOH:AcOH). The solution was cooled, washed with saturated NaHCO_3 and dried over anhydrous MgSO_4 . Concentration in vacuo gave the crude product which in most cases solidified upon standing. In some cases, purification via flash chromatography was required (silica gel, 3:7 ethylacetate:pentane).

Fmoc-protected oxazolidinones **1a-6a**, **8a** and **10a** were synthesized and characterized as described in ref. 1 and 2.

(S)-4-tert-Butoxymethyl-5-oxo-oxazolidine-3-carboxylic acid 9H-fluoren-9-ylmethyl ester (7a). White solid; 92%; mp 124.5-125.5°C. $[\alpha]_D^{25} +95$. IR (KBr): 1803, 1716 cm^{-1} . ^1H NMR (400 MHz, CDCl_3): 1.08 (s, 9H); 3.07-4.04 (br m, 3H); 4.27 (t, $J = 5.7$ Hz, 1H); 4.69 (br s, 2H); 5.12 (m, 1H); 5.40 (s, 1H); 7.34 (t, $J = 7.3$ Hz, 2H); 7.43 (t, $J = 7.3$ Hz, 2H); 7.55 (t, $J = 7.3$ Hz, 2H); 7.79 (d, $J = 7.9$ Hz, 2H). ^{13}C NMR (100 MHz, CDCl_3): 27.4 (q), 47.3 (d), 56.7 (d), 60.5 (t), 67.5 (t), 73.8 (s), 78.9 (t), 120.3 (d), 124.7 (d), 127.4 (d), 128.1 (d), 141.6 (s), 143.7 (s), 152.4 (s), 171.5 (s). Retention time on HPLC 9.3 minutes; MS (ESI) m/z 396.0 $[\text{M}+\text{H}]^+$.

(R)-4-Allyloxycarbonylmethyl-5-oxo-oxazolidine-3-carboxylic acid 9H-fluoren-9-ylmethyl ester (9a). Colourless oil; yield 94%; $[\alpha]_D^{25} -97.02$. IR (KBr): 3068, 1800, 1731, 1713 cm^{-1} . ^1H NMR (400 MHz, CDCl_3): 2.24-3.36 (br m, 2H); 4.24 (t, $J = 5.4$, 2H); 4.46-4.80 (m, 4H); 5.11-5.50 (m, 4H); 5.83-5.95 (m, 1H); 7.34 (t, $J = 7.6$ Hz, 2H); 7.43 (t, $J = 7.1$ Hz, 2H); 7.54 (d, $J = 7.1$ Hz, 2H); 7.79 (d, $J = 7.1$ Hz, 2H). ^{13}C NMR (100 MHz, CDCl_3): 34.4 (t), 47.4 (d), 51.6 (d), 66.0 (t), 67.6 (t), 78.4 (t), 119.0 (t), 120.3 (d), 120.3 (d), 124.8 (d), 127.4 (d), 128.2 (d), 131.7 (d), 141.6 (s), 143.6 (s), 143.6 (s), 152.7 (s), 169.6 (s), 171.6 (s). Retention time on HPLC 8.9 minutes; MS (ESI) m/z 408.0 $[\text{M}+\text{H}]^+$.

(S)-4-(4-Benzoyloxycarbonylamino-butyl)-5-oxo-oxazolidine-3-carboxylic acid 9H-fluoren-9-ylmethyl ester (11a). Colourless oil; yield 55%; $[\alpha]_D^{25} +72.26$. IR (KBr): 3358, 1799, 1714 cm^{-1} . ^1H NMR (400 MHz, CDCl_3): 0.80-1.89 (br m, 6H); 3.13 (br s, 2H); 4.23 (t, $J = 5.2$ Hz, 2H); 4.69 (m, 3H); 5.03 (d, $J = 4$ Hz, 1H); 5.12 (s, 2H); 5.30 (s, 1H); 7.29-7.46 (m, 9H); 7.55 (d, $J = 7.7$ Hz, 2H); 7.79 (d, $J = 7.7$ Hz, 2H). ^{13}C NMR (100 MHz, CDCl_3): 21.5 (t), 29.6 (t), 30.2 (t), 40.9 (t), 47.5 (d), 54.8 (d), 66.8 (t), 77.8 (t), 120.3 (d), 124.7 (d), 127.4 (d), 128.2 (d), 128.2 (d), 128.7 (d), 136.9 (s), 141.6 (s), 141.7 (s), 143.5 (s), 143.6 (s), 156.5 (s), 172.1 (s). Retention time on HPLC 9.0 minutes; MS (ESI) m/z 515.1 $[\text{M}+\text{H}]^+$.

General procedure for synthesis of *N*-methylated Fmoc amino acids from oxazolidinones:

To a solution of the oxazolidinone (1 equiv) and Lewis acid (2 equiv) in dry DCM (20 ml / 1 mmol oxazolidinone) was added triethylsilane (2 equiv). The reaction was stirred at ambient temperature until TLC (1:3 Ethylacetate:hexane) showed the absence of starting material. An additional amount of DCM was added (twice the reaction volume) and the organic phase was washed with 1M HCl. The organic phase was dried over anhydrous magnesium sulphate and concentrated in vacuo. The crude product was purified via column chromatography on silica gel (ethylacetate and hexane).

For larger scale syntheses, Fmoc-protected *N*-methyl amino acids of sufficient purity (>95 %) for solid-phase peptide synthesis can be obtained by extraction: The crude product was taken up in aqueous sodium carbonate solution. The aqueous layer was extracted with diethyl ether to remove non-acidic impurities, acidified with hydrochloric acid, and the product extracted with DCM and evaporated.

Fmoc-*N*-methylated amino acids **1b-5b**, **8b** and **10b** were characterized, and found to be identical to, the authentic samples reported in references 1 and 2.

HPLC chromatograms with UV/VIS (254 nm), universal evaporative light scattering (ELSD), and MS-detection, showing the purity; and electrospray (positive mode) showing the identity, of these samples are reproduced at the end of this document.

(R)-2-[(9H-Fluoren-9-ylmethoxycarbonyl)-methyl-amino]-succinic acid 4-allyl ester (9b). Colourless oil; yield 92 %; $[\alpha]_D^{25} +46.35$. IR (KBr): 1735, 1707 cm^{-1} . ^1H NMR (400 MHz, CDCl_3): 2.72-3.30 (m, 5H), 4.16-4.38 (m, 1H), 4.39-4.96 (m, 5H), 5.18-5.40 (m, 2H), 5.80-6.00 (m, 1H), 7.20-7.88 (m, 8H), 9.52 (s, 1H, D_2O exchangeable). ^{13}C NMR (100 MHz, CDCl_3): 34.2 (q), 34.6 (t), 47.4 (d), 58.0 (d), 65.8 (t), 68.1 (t), 118.7 (t), 120.1 (d), 124.9 (d), 125.2 (d), 127.3 (d), 127.9 (d), 132.0 (d), 141.6 (s), 144.0 (s), 156.7 (s), 170.5 (s), 174.8 (s). Retention time on HPLC 9.3 minutes; MS (ES) m/z 410.1 $[\text{M}+\text{H}]^+$.

(S)-6-Benzyloxycarbonylamino-2-[(9H-fluoren-9-ylmethoxycarbonyl)-methyl-amino]-hexanoic acid (11b). Colourless oil, 80%; $[\alpha]_D^{25} -12.54$. IR (KBr): 3331, 1700 cm^{-1} . ^1H NMR (400 MHz, CDCl_3): 1.10-2.18 (m, 6H); 2.89 (s, 3H); 3.23 (s, 2H); 4.16-4.69 (m, 4H); 4.73-5.04 (m, 1H); 5.15 (br s, 2H); 7.23-7.52 (m, 4H); 7.62 (br s, 2H); 7.79 (br s, 2H); 10.55 (br s, 1H). ^{13}C NMR (100 MHz, CDCl_3): 23.4 (t), 28.3 (t), 29.5 (t), 30.8 (q), 41.0 (t), 47.6 (d), 58.6 (d), 66.9 (t), 68.1 (t), 120.2 (d), 125.2 (d), 127.3 (d), 127.9 (d), 128.3 (d), 128.7 (d), 141.6 (s), 144.2 (s), 157.4 (s), 175.7 (s). Retention time on HPLC 9.7 minutes; MS (ES) m/z 517.2 $[\text{M}+\text{H}]^+$.

References;

- Freidinger, R. M.; Hinkle, J. S.; Perlow, D. S.; Arison, B. H. *J. Org. Chem.* **1983**, 48, 77.
- Holladay, M. W.; Bennett, M. J.; Tufano, M. D.; Lin, C. W.; Asin, K. E.; et al. *J. Med. Chem.*; 35; 16; **1992**; 2919.

HPLC chromatograms and ESI-MS spectra

Wed May 18 2005 21:50:57

Results Report

Page 1

SQ_Asp(OAl)lactone#894 RT: 8.93 AV: 1 NL: 1.78E5
T: (0.0) + c ESI sid=30.00 Full ms [82.00-915.00]

Wed Apr 27 2005 19:30:38

Results Report

Page

SQ_Lys(cbz)lactone#705 RT: 9.07 AV: 1 NL: 1.40E5
T: (0,0) + c ESI sid=30.00 Full ms [82.00-915.00]

1b

Wed May 18 2005 21:03:45

Results Report

Page 1

Data File: d:\data\2005\14-20\20050518.003\050518.gdt
 Date acquired: Wed May 18 2005 20:14:01
 Control Method: C:\GILSON\UNIPONT\NEW30LMW.GCT
 Sample name: NmetAla

3Q_NmetAla_050518200655#703 RT: 9.05 AV: 1 NL: 2.49E5
 -: (0,0) + c ESI sid=30.00 Full ms [82.00-915.00]

2b

Wed May 18 2005 21:04:16

Results Report

Page 1

Data File: d\data\2005\14-20\20050518.003\050518.gdt
Date acquired: Wed May 18 2005 19:06:30
Control Method: C:\GILSON\N\POINTNEW30LMW.GCT
Sample name: NmetGly

3Q_NmetGly#686 RT: 8.83 AV: 1 NL: 1.63E5
T: (0,0) + c ESI sid=30.00 Full ms [82.00-915.00]

3b

4b

ed May 16 2005 21:04:29

Results Report

Page 1

Data File: d:\data\2005\14-20\20\050518\003\050518.gdt
Date acquired: Wed May 16 2005 16:48:28
Control Method: C:\GILSON\UNIPONT\NEW30LMW.GCT
Sample name: NmethylLeu

2 NmethylLeu#759 RT: 9.77 AV: 1 NL: 2.52E5
(0,0) + c ESI sid=30.00 Full ms [82.00-915.00]

5b

Wed May 18 2005 21:05:29

Results Report

Page 1

Data File: c:\data\2005\14-20\20050518\003\050518.gdt
Date acquired: Wed May 18 2005 17:24:09
Control Method: C:\GILSON\UNIPONT\NEW30LMV.GCT
Sample name: Valine

SQ_Valine#736 RT: 9.47 AV: 1 NL: 2.56E5
T: {0,0} + c ESI sid=30.00 Full ms { 82.00-915.00}

Wed May 18 2005 22:44:39

Results Report

Page 1

Data File: d:\data\2005\14-20\20050518.003\050518.gdt
 Date acquired: Wed May 18 2005 22:25:47
 Control Method: C:\GILSON\UNPOINT\NEW30LMW.GCT
 Sample name: Asp(benzyl)2

SQ_Asp(benzyl)2_050518222841#748 RT: 9.63 AV: 1 NL: 2.45E5
 T: [0,0] + c ESI sid=30.00 Full ms [82.00-915.00]

9b

Wed May 18 2005 21:28:04

Results Report

Page 1

Data File: d:\data\2005\14-05\20\050518.003\050518.gdt
 Date acquired: Wed May 18 2005 20:58:10
 Control Method: C:\GILSON\UNIPONTNEW30LMW.GCT
 Sample name: Nmethylasp

Q: Nmethylasp#725 RT: 9.33 AV: 1 NL: 2.34E5
 : [0.0] + c ESI sid=30.00 Full ms [82.00-915.00]

10b

Thu May 19 2005 18:50:48

Results Report

Page 1

Data File: d:\data\2005\14-20\20\050519\005\060519.gdt
 Date acquired: Thu May 19 2005 18:30:16
 Control Method: C:\OILSON\NIPONTNEW30\LMW.GCT
 Sample name: FmocNmethSer(bz)

SO_FmocNmethSer(bz)#750 RT: 9.65 AV: 1 NL: 2.50E5
 T: {0.0} + c ESI sid=30.00 Full ms [82.00-915.00]

11b

Wed May 18 2005 20:59:36

Results Report

Page 1

Data File: d:\data\2005\14-20\20050518.003\050518.gdt
Date acquired: Wed May 18 2005 20:40:06
Control Method: C:\GILSON\UNIPONT\NEW30LMW.GCT
Sample name: Nmethyllys

Q: Nmethyllys#751 RT: 9.66 AV: 1 NL: 2.10E5
: (0,0) + c ESI sid=30.00 Full ms [82.00-915.00]