

## Supplementary Information

### Peptide coupling using recyclable bicyclic benziodazolone

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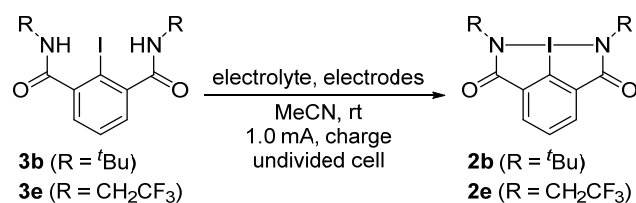
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## 1. Optimization of Reaction Conditions

**Table S1.** Evaluation of iodine reagents, additives and solvents for the peptide coupling.

entry	1 or 2	R <sub>3</sub> P (eq.)	Solvent	(°C)	(h)	Dipeptide <sup>a</sup> (%)	3 <sup>a</sup> (%)
1	<b>2a</b> (X = N <sup>i</sup> Pr, Y = H)	Ph <sub>3</sub> P (1.0)	toluene	rt	24	37	ND
2	<b>2a</b>	Ph <sub>3</sub> P (1.0)	DCM	rt	24	64	ND
3	<b>2a</b>	Ph <sub>3</sub> P (1.0)	DCE	rt	24	84	ND
4	<b>2a</b>	Ph <sub>3</sub> P (1.0)	MeNO <sub>2</sub>	rt	24	78	ND
5	<b>2a</b>	Ph <sub>3</sub> P (1.0)	MeCN	rt	24	74	ND
6	<b>2a</b>	Ph <sub>3</sub> P (1.0)	THF	rt	24	51	ND
7	<b>2a</b>	Ph <sub>3</sub> P (1.0)	DMF	rt	24	15	ND
8	<b>2a</b>	Ph <sub>3</sub> P (1.0)	DMSO	rt	24	trace	ND
9	<b>2a</b>	(4-MeOC <sub>6</sub> H <sub>4</sub> ) <sub>3</sub> P (1.0)	DCE	rt	24	90	ND
10	<b>2a</b>	(EtO) <sub>3</sub> P (1.0)	DCE	rt	24	73	ND
11	<b>2a</b>	(PhO) <sub>3</sub> P (1.0)	DCE	rt	24	68	ND
12	<b>2a</b>	DPPE (1.0)	DCE	rt	24	60	ND
13	<b>2a</b>	DPPB (1.0)	DCE	rt	24	36	ND
14	<b>2a</b>	none	DCE	rt	24	0	ND
15	<b>2a</b>	none	DCE	60	24	0	ND
16	<b>2b</b> (X = N <sup>i</sup> Bu, Y = H)	Ph <sub>3</sub> P (1.0)	DCE	rt	24	76	ND
17	<b>2c</b> (X = NCy, Y = H)	Ph <sub>3</sub> P (1.0)	DCE	rt	24	81	ND
18	<b>2d</b> (X = NMe, Y = H)	Ph <sub>3</sub> P (1.0)	DCE	rt	24	74	ND
19	<b>2d</b>	Ph <sub>3</sub> P (1.0)	MeCN	rt	24	84	ND
20	<b>2e</b> (X = NCH <sub>2</sub> CF <sub>3</sub> , Y = H)	Ph <sub>3</sub> P (1.0)	DCE	rt	24	26	ND
21	<b>2e</b>	Ph <sub>3</sub> P (1.0)	MeCN	rt	24	90	ND
22	<b>2e</b>	Ph <sub>3</sub> P (1.4)	MeCN	25	24	97	83
23	<b>2a</b>	Ph <sub>3</sub> P (1.2)	DCE	25	24	74	ND
24	<b>2a</b>	Ph <sub>3</sub> P (1.2)	MeCN	25	24	93	71
25	<b>2a</b>	Ph <sub>3</sub> P (1.2)	MeCN	40	5	quant.	48
26	<b>1a</b> (X = O, Y = H)	Ph <sub>3</sub> P (1.2)	MeCN	25	24	52	-
27	<b>1a</b>	Ph <sub>3</sub> P (1.2)	MeCN	40	5	30	-
28	<b>1b</b> (X = O, Y = <i>p</i> -BTFP)	Ph <sub>3</sub> P (1.2)	MeCN	25	24	45	-
29	PhI(OAc) <sub>2</sub>	Ph <sub>3</sub> P (1.2)	MeCN	25	24	43	-
30	IBA-OBz	Ph <sub>3</sub> P (1.2)	MeCN	25	24	75	-
31	IBAm( <sup>i</sup> Pr)-mCBA	Ph <sub>3</sub> P (1.2)	MeCN	25	24	62	-

DCM = dichloromethane, DCE = 1,2-dichloroethane, THF = tetrahydrofuran, DMF = *N,N*-dimethylformamide, DMSO = Dimethyl sulfoxide, DPPE = 1,2-Bis(diphenylphosphino)ethane, DPPB = 1,4-Bis(diphenylphosphino)butane, Cy = cyclohexyl. BTFP = 3,5-(CF<sub>3</sub>)<sub>2</sub>C<sub>6</sub>H<sub>3</sub>. IBA-OBz = 3-oxo-1 $\lambda^3$ -benzo[*d*][1,2]iodaoxol-1(3*H*)-yl benzoate. IBAm(<sup>i</sup>Pr)-mCBA = 2-isopropyl-3-oxo-2,3-dihydro-1*H*-1 $\lambda^3$ -benzo[*d*][1,2]iodazol-1-yl 3-chlorobenzoate. ND = Not determined.  
<sup>a</sup> Isolated yields.

**Table S2.** Evaluation of electrolyte, electrodes and others for the electrochemical synthesis of iodine(III) reagents.

entry	anode	cathode	electrolyte	charge (F/mol)	MeCN (mL)	<b>2</b>	Yield <sup>a</sup> (%)
1	carbon felt	Pt	LiClO <sub>4</sub> (0.2 M)	2.2	2.5	<b>2b</b>	18
2	carbon felt	carbon felt	LiClO <sub>4</sub> (0.2 M)	4.4	2.5	<b>2b</b>	0
3	Pt	Pt	LiClO <sub>4</sub> (0.2 M)	2.2	2.5	<b>2b</b>	0
4	carbon felt	Pt	TBAPF <sub>6</sub> (0.2 M)	3.0	2.5	<b>2b</b>	0
5	carbon felt	Pt	LiBr (0.1 M)	2.0	2.5	<b>2b</b>	0
6	graphite	Pt	LiClO <sub>4</sub> (0.1 M)	2.0	5.0	<b>2b</b>	42
7	graphite	Pt	TBAClO <sub>4</sub> (0.1 M)	2.0	5.0	<b>2b</b>	72
8	graphite	Pt	TBAClO <sub>4</sub> (0.1 M)	2.8	5.0	<b>2b</b>	86
9	graphite	Pt	TBAPF <sub>6</sub> (0.1 M)	2.8	5.0	<b>2b</b>	39
10 <sup>b</sup>	graphite	Pt	TBAClO <sub>4</sub> (0.1 M)	2.8	5.0	<b>2b</b>	95
11	graphite	Pt	TBAClO <sub>4</sub> (0.1 M)	2.2	5.0	<b>2e</b>	83

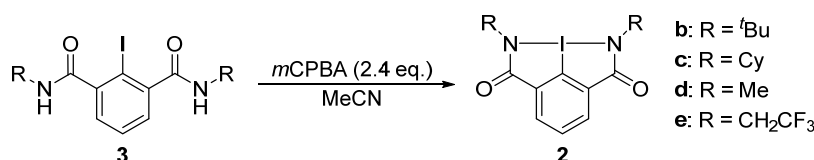
TBA = tetra-*n*-butylammonium. <sup>a</sup> Determined by <sup>1</sup>H NMR using CH<sub>2</sub>Br<sub>2</sub> as an internal standard. <sup>b</sup> 2.2 V CPE (Constant Potential Electrolysis) instead of 1.0 mA CCE (Constant Current Electrolysis).

## 2. General Information

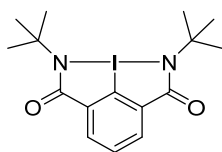
All reactions were carried out under an argon atmosphere. According to procedures reported in the literatures, bicyclic benziodoxolone **1a**<sup>1a</sup> and benziodazolone **2a**<sup>1b</sup> were prepared. *N*- and *C*-Protected amino acids other than those bound with hydrophobic tags and 2-iodoisophthalic acid are commercially available. All solvents were purchased as the “anhydrous” and used without further purification. For the thin-layer chromatography (TLC) analysis, Merck precoated TLC plates (silica gel 60 F<sub>254</sub>) were used. Column chromatography was performed on silica gel 60N (63-200 μm, neutral, Kanto Kagaku Co., Ltd.). Preparative thin layer chromatography (PTLC) was performed on Wakogel® B-5F (FUJIFILM Wako Pure Chemical Corp.). Medium pressure liquid chromatography (MPLC) was carried out with YAMAZEN EPCLC-Wprep 2XY.

<sup>1</sup>H and <sup>13</sup>C NMR spectra were measured at 500 (400 or 600) and 125 (100 or 150) MHz in CDCl<sub>3</sub>, DMSO-*d*<sub>6</sub> or D<sub>2</sub>O and the chemical shifts are given in ppm using CHCl<sub>3</sub> (7.26 ppm) in CDCl<sub>3</sub>, DMSO (2.50 ppm) in DMSO-*d*<sub>6</sub> or DOH (4.75 ppm) in D<sub>2</sub>O for <sup>1</sup>H NMR and CDCl<sub>3</sub> (77.0 ppm), DMSO-*d*<sub>6</sub> (39.51 ppm) or acetone (Me, 32.97 ppm) for <sup>13</sup>C NMR as an internal standard, respectively. Splitting patterns of an apparent multiplet associated with an averaged coupling constant were designed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broadened). Mass spectra and HRMS were recorded on double-focusing magnetic sector by FAB or ESI methods.

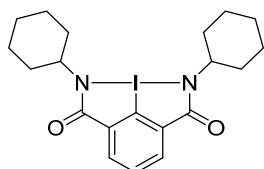
## 3. Preparation and Characterization of Bicyclic Benziodazolones 2b-2e



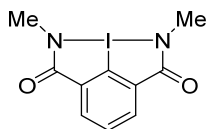
According to procedures reported in the literatures,<sup>1b</sup> to a solution of 2-iodoisophthalamide **3b-3e** (1.0-1.5 mmol) in MeCN (10-15 mL) was added *m*-chloroperbenzoic acid (*m*CPBA, 2.4 eq.). After being stirred overnight at room temperature, the reaction was quenched with sat. NaHCO<sub>3</sub> aq. and extracted with AcOEt. The organic layer was dried over MgSO<sub>4</sub> and concentrated in vacuo to dryness. The residue was washed with Et<sub>2</sub>O to give benziodazolone **2b-2e**.



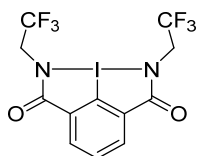
**2,3-Di-*tert*-butyl-2aλ<sup>3</sup>-ioda-2,3-diazacyclopenta[hi]indene-1,4(2H,3H)-dione (2b):** 80% (320 mg from **3b** 402 mg). Yellow solid. Mp 167-169 °C. IR (KBr)  $\nu$  cm<sup>-1</sup>: 2963, 1632, 1601, 1346. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 8.26 (d, *J* = 7.5 Hz, 2H), 7.87 (t, *J* = 7.5 Hz, 1H), 1.62 (s, 18H). <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 163.1, 133.1, 132.6, 130.9, 110.7, 57.0, 30.0. HRMS (ESI) *m/z* calcd. for C<sub>16</sub>H<sub>22</sub>IN<sub>2</sub>O<sub>2</sub><sup>+</sup> [M + H]<sup>+</sup> 401.0720, found 401.0724.



**2,3-Dicyclohexyl-2aλ<sup>3</sup>-ioda-2,3-diazacyclopenta[hi]indene-1,4(2H,3H)-dione (2c):** 90% (407 mg from **3c** 454 mg). White solid. Mp 219 °C (decomp.). IR (KBr)  $\nu$  cm<sup>-1</sup>: 2929, 2853, 1632, 1590, 1361. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 8.29 (d, *J* = 7.5 Hz, 2H), 7.87 (t, *J* = 7.5 Hz, 1H), 4.01 (tt, *J* = 11.5, 4.0, Hz, 2H), 2.13 (d, *J* = 10.3 Hz, 4H), 1.85 (d, *J* = 13.8 Hz, 4H), 1.73 (d, *J* = 13.2 Hz, 2H), 1.52-1.40 (m, 4H), 1.39-1.30 (m, 4H), 1.27-1.15 (m, 2H). <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 162.6, 132.6, 132.2, 131.0, 113.4, 53.8, 35.6, 25.7, 25.4. HRMS (ESI): *m/z* calcd. for C<sub>20</sub>H<sub>26</sub>IN<sub>2</sub>O<sub>2</sub><sup>+</sup> [M+H]<sup>+</sup> 453.1033, found 453.1041.



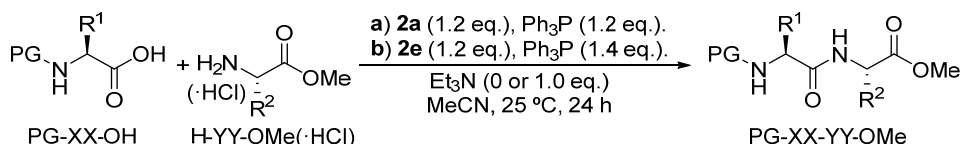
**2,3-Dimethyl-2aλ<sup>3</sup>-ioda-2,3-diazacyclopenta[hi]indene-1,4(2H,3H)-dione (2d):** 82% (337 mg from **3d** 413 mg). White solid. Mp 224-226 °C. IR (KBr)  $\nu$  cm<sup>-1</sup>: 2925, 1631, 1595, 1370. <sup>1</sup>H-NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 8.14 (d, *J* = 6.3 Hz, 2H), 7.91 (t, *J* = 6.3 Hz, 1H), 3.05 (s, 6H). <sup>13</sup>C-NMR (125 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 164.3, 132.2, 131.5, 129.9, 114.5, 29.3. HRMS (ESI): *m/z* calcd. for C<sub>10</sub>H<sub>10</sub>IN<sub>2</sub>O<sub>2</sub><sup>+</sup> [M+H]<sup>+</sup> 316.9781, found 316.9783.



**2,3-Bis(2,2,2-trifluoroethyl)-2aλ<sup>3</sup>-ioda-2,3-diazacyclopenta[hi]indene-1,4(2H,3H)-dione (2e):** 90% (610 mg from **3e** 681 mg). White solid. Mp 238-240 °C. IR (KBr)  $\nu$  cm<sup>-1</sup>: 2944, 1630, 1603, 1373, 1160. <sup>1</sup>H-NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 8.27 (d, *J* = 7.5 Hz, 2H), 7.98 (t, *J* = 7.5 Hz, 1H), 4.40 (q, *J* = 9.9 Hz, 4H). <sup>13</sup>C-NMR (125 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 165.1, 132.6, 131.6, 130.6, 125.1 (q, *J* = 280.3 Hz), 114.8, 42.6 (q, *J* = 32.8 Hz). HRMS (ESI): *m/z* calcd. for C<sub>12</sub>H<sub>8</sub>F<sub>6</sub>IN<sub>2</sub>O<sub>2</sub><sup>+</sup> [M+H]<sup>+</sup> 452.9529, found 452.9536.

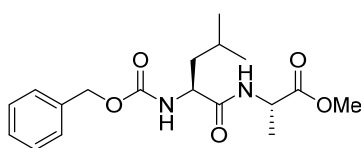
<sup>1</sup> (a) J. Tian, W.-C. Gao, D.-M. Zhou and C. Zhang, *Org. Lett.*, 2012, **14**, 3020–3023; (b) A. Yoshimura, M. T. Shea, C. L. Makitalo, M. E. Jarvi, G. T. Rohde, A. Saito, M. S. Yusubov, V. V. Zhdankin, *Beilstein J. Org. Chem.*, 2018, **14**, 1016–1020.

#### 4. Preparation and Characterization of *N*-Protected Dipeptide Methyl Esters

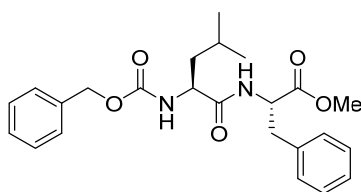


**The case using hydrochloric acid salts (Method a or b):** To a solution of **2a** (89.3 mg, 0.24 mmol, Method a) or **2e** (108.5 mg, 0.24 mmol, Method b) in MeCN (5.0 mL), Et<sub>3</sub>N (27.8  $\mu$ L, 0.2 mmol), *N*-protected amino acid (0.2 mmol), amino acid methyl ester hydrochloride (0.2 mmol) and Ph<sub>3</sub>P (Method a: 63.0 mg, 0.24 mmol. Method b: 73.4 mg, 0.28 mmol. Entries 15-17 and 19: (4-MeOC<sub>6</sub>H<sub>4</sub>)<sub>3</sub>P, 84.6 mg, 0.24 mmol.) were added successively. After being stirred at 25 °C (40 °C for entry 16) for 24 h, the reaction mixture was quenched with sat. NaHCO<sub>3</sub> aq. and extracted with AcOEt. The organic layer was washed with sat. NH<sub>4</sub>Cl aq. and dried over MgSO<sub>4</sub>. After concentration of the filtrate in vacuo to dryness, the residue was washed with Et<sub>2</sub>O to give **3a** or **3e**. The remaining solution was concentrated in vacuo to dryness and then the residue was purified by silica gel chromatography (hexane:AcOEt = 2:1 to 4:3) to afford the desired dipeptide.

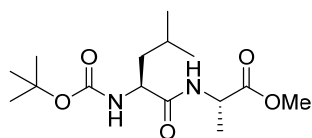
**The case not using hydrochloric acid salts (Method c, entries 6 and 10):** To a solution of **2a** (89.3 mg, 0.24 mmol) in MeCN (5.0 mL), *N*-protected amino acid (0.2 mmol), amino acid methyl ester (0.2 mmol) and Ph<sub>3</sub>P (63.0 mg, 0.24 mmol) were added successively. After being stirred at 25 °C for 24 h, the reaction mixture was quenched with 1.0 M HCl aq. and extracted with DCM. The organic layer was washed with sat. NH<sub>4</sub>Cl aq. and dried over MgSO<sub>4</sub>. After concentration of the filtrate in vacuo to dryness, the residue was washed with Et<sub>2</sub>O to give **3a**. The remaining solution was concentrated in vacuo to dryness and then the residue was purified by silica gel chromatography (hexane:AcOEt = 2:1 to 4:3) to afford the desired dipeptide.



**Methyl [(benzyloxy)carbonyl]-*L*-leucyl-*L*-alaninate (Cbz-Leu-Ala-OMe):** 93% (Method a, 65.2 mg); 97% (Method b, 68.0 mg). White solid. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 7.38-7.26 (m, 5H), 7.07 (br.s, 1H), 5.66 (br.s, 1H), 5.09 (d, *J* = 12.1 Hz, 1H), 5.05 (d, *J* = 12.1 Hz, 1H), 4.52 (dq, *J* = 7.3, 7.3 Hz, 1H), 4.37-4.23 (m, 1H), 3.71 (s, 3H), 1.75-1.47 (m, 3H), 1.34 (d, *J* = 7.3 Hz, 3H), 0.91 (d, *J* = 6.4 Hz, 3H), 0.89 (d, *J* = 6.0 Hz, 3H). <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 173.0, 172.4, 156.3, 136.1, 128.4, 128.1, 127.7, 66.9, 53.3, 52.3, 48.0, 41.4, 24.5, 22.8, 21.8, 17.8. The <sup>1</sup>H and <sup>13</sup>C NMR spectra of the product were identical to those reported in the literature.<sup>2</sup>



**Methyl [(benzyloxy)carbonyl]-*L*-leucyl-*L*-phenylalaninate (Cbz-Leu-Phe-OMe):** 93% (Method b, 79.7 mg). White solid. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 7.70-7.28 (m, 5H), 7.28-7.19 (m, 3H), 7.08 (d, *J* = 6.9 Hz, 2H), 6.49 (br.s, 1H), 5.16 (br.d, *J* = 5.2, 1H), 5.11 (d, *J* = 12.3 Hz, 1H), 5.08 (d, *J* = 12.3 Hz, 1H), 4.85 (ddd, *J* = 7.5, 5.7, 5.7 Hz, 1H), 4.18 (ddd, *J* = 8.6, 8.6, 4.6 Hz, 1H), 3.71 (s, 3H), 3.14 (dd, *J* = 13.8, 5.7 Hz, 1H), 3.07 (dd, *J* = 13.8, 5.7 Hz, 1H), 1.70-1.56 (m, 2H), 1.53-1.40 (m, 1H), 0.90 (d, *J* = 5.7 Hz, 6H). <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 171.7, 171.6, 156.0, 136.1, 135.6, 129.2, 128.5, 128.2, 128.0, 127.1, 67.0, 53.4, 53.1, 52.3, 41.2, 37.8, 24.6, 22.8, 21.9 (note that two carbon peaks overlap with each other and the two methyl groups of the isobutyl group behave as non-equivalent functional groups). The <sup>1</sup>H and <sup>13</sup>C NMR spectra of the product were identical to those reported in the literature.<sup>3</sup>

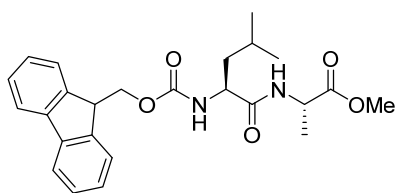


**Methyl (*tert*-butoxycarbonyl)-*L*-leucyl-*L*-alaninate (Boc-Leu-Ala-OMe):** 87% (Method a, 54.8 mg). White solid. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 6.78 (br.d, *J* = 5.7 Hz, 1H), 5.02 (d, *J* = 7.5 Hz, 1H), 4.56 (dq, *J* = 7.5, 6.8 Hz, 1H), 4.19-3.91 (m, 1H), 3.72 (s, 3H), 1.74-1.58 (m, 2H), 1.52-1.46 (m, 1H), 1.42 (s, 9H), 1.37 (d, *J* = 6.8 Hz, 3H), 0.92 (d, *J* = 5.7 Hz, 3H), 0.91 (d, *J* = 6.3 Hz, 3H). <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 173.1, 172.2, 155.7, 80.0, 52.9, 52.4, 47.9, 41.2, 28.2, 24.6, 22.9, 21.9, 18.1 (note that the two methyl groups of the isobutyl group behave as non-equivalent functional groups). The <sup>1</sup>H and <sup>13</sup>C NMR spectra of the product were identical to those reported in the literature.<sup>4</sup>

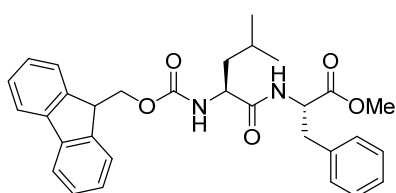
<sup>2</sup> W.-C. Gao and C. Zhang, *Tetrahedron Lett.*, 2014, **55**, 2687–2690.

<sup>3</sup> P. Gibbons, E. Verissimo, V. Barton, G. L. Nixon, R. K. Amewu, J. Chadwick, P. A. Stocks, G. A. Biagini, A. Srivastava, P. J. Rosenthal, J. Gut, R. C. Guedes, R. Moreira, R. Sharma, N. Berry, M. Lurdes, S. Cristiano, A. E. Shone, S. A. Ward and P. M. O'Neill, *J. Med. Chem.*, 2010, **53**, 8202–8206.

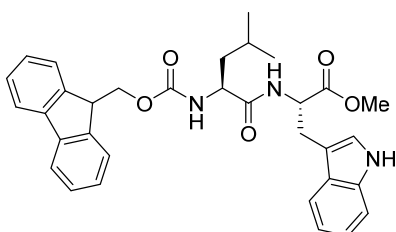
<sup>4</sup> C. Zhang, S. S. Liu, B. Sun and J. Tian, *Org. Lett.*, 2015, **17**, 4106–4109.



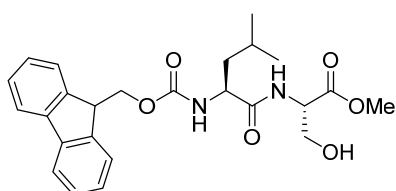
**Methyl ((9H-fluoren-9-yl)methoxy)carbonyl-L-leucyl-L-alaninate (Fmoc-Leu-Ala-OMe):** 84% (Method a, 73.9 mg). White solid.  $^1\text{H-NMR}$  (600 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 7.76 (d,  $J = 7.6$  Hz, 2H), 7.58 (d,  $J = 6.9$  Hz, 2H), 7.40 (dd,  $J = 7.6, 7.6$  Hz, 2H), 7.31 (dd,  $J = 7.6, 6.9$  Hz, 2H), 6.46 (br.d,  $J = 6.2$  Hz, 1H), 5.22 (br.d,  $J = 8.3$  Hz, 1H), 4.57 (ddd,  $J = 7.6, 6.9, 6.2$  Hz, 1H), 4.47–4.35 (m, 2H), 4.26–4.16 (m, 2H), 3.75 (s, 3H), 1.73–1.63 (m, 2H), 1.60–1.49 (m, 1H), 1.40 (d,  $J = 6.9$  Hz, 3H), 1.00–0.91 (m, 6H).  $^{13}\text{C-NMR}$  (150 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 173.1, 171.7, 156.2, 143.8, 143.7, 141.3, 127.7, 127.1, 125.0, 119.99, 119.97, 67.0, 53.4, 52.5, 48.1, 47.2, 41.6, 24.6, 22.9, 22.0, 18.3 (note that two carbon peaks overlap with each other, and the two methyl groups of the isobutyl group and four carbons of fluorenyl group behave as non-equivalent functional groups). The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of the product were identical to those reported in the literature.<sup>5</sup>



**Methyl ((9H-fluoren-9-yl)methoxy)carbonyl-L-leucyl-L-phenylalaninate (Fmoc-Leu-Phe-OMe):** 92% (Method a, 94.5 mg). White solid.  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 7.77 (d,  $J = 7.5$  Hz, 2H), 7.59 (d,  $J = 7.5$  Hz, 2H), 7.40 (dd,  $J = 7.5, 7.5$  Hz, 2H), 7.31 (dd,  $J = 7.5, 7.5$  Hz, 2H), 7.22 (dd,  $J = 7.5$  Hz, 2H), 7.17 (t,  $J = 7.5$  Hz, 1H), 7.08 (d,  $J = 7.5$  Hz, 2H), 6.54 (br.d,  $J = 7.5$  Hz, 1H), 5.27 (br.d,  $J = 8.6$  Hz, 1H), 4.85 (ddd,  $J = 7.5, 5.7, 5.7$  Hz, 1H), 4.44 (dd,  $J = 10.3, 7.5$  Hz, 1H), 4.35 (dd,  $J = 10.3, 7.5$  Hz, 1H), 4.26–4.14 (m, 2H), 3.71 (s, 3H), 3.15 (dd,  $J = 13.8, 5.7$  Hz, 1H), 3.08 (dd,  $J = 13.8, 5.7$  Hz, 1H), 1.70–1.57 (m, 2H), 1.56–1.44 (m, 1H), 0.93 (d,  $J = 5.2$  Hz, 3H), 0.91 (d,  $J = 4.6$  Hz, 3H).  $^{13}\text{C-NMR}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 171.8, 171.6, 156.0, 143.74, 143.69, 141.2, 135.5, 129.2, 128.5, 127.7, 127.1, 127.0, 125.1, 125.0, 120.0, 67.0, 53.3, 53.1, 52.3, 47.1, 41.3, 37.8, 24.6, 22.8, 21.9 (note that the two methyl groups of the isobutyl group and four carbons of fluorenyl group behave as non-equivalent functional groups). The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of the product were identical to those reported in the literature.<sup>6</sup>



**Methyl ((9H-fluoren-9-yl)methoxy)carbonyl-L-leucyl-L-tryptophanate (Fmoc-Leu-Trp-OMe):** 75% (Method b, 83 mg). White solid.  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 8.17 (br.s, 1H), 7.77 (dd,  $J = 7.5, 2.3$  Hz, 2H), 7.56 (d,  $J = 7.5$  Hz, 1H), 7.53–7.47 (m, 2H), 7.44–7.36 (m, 2H), 7.30 (dd,  $J = 7.5, 7.5$  Hz, 2H), 7.25 (d,  $J = 6.9$  Hz, 1H), 7.12 (dd,  $J = 7.5, 6.9$  Hz, 1H), 7.06 (dd,  $J = 7.5, 7.5$  Hz, 1H), 6.92 (br.s, 1H), 6.79 (br.d,  $J = 8.0$  Hz, 1H), 5.31 (br.d,  $J = 8.6$  Hz, 1H), 4.92 (ddd,  $J = 8.0, 5.7, 5.2$  Hz, 1H), 4.35–4.29 (m, 2H), 4.16–4.12 (m, 2H), 3.64 (s, 3H), 3.33–3.25 (m, 2H), 1.68–1.55 (m, 2H), 1.52–1.42 (m, 1H), 0.88 (d,  $J = 5.2$  Hz, 3H), 0.86 (d,  $J = 4.6$  Hz, 3H).  $^{13}\text{C-NMR}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 172.2, 172.0, 156.1, 143.8, 143.6, 141.2, 135.9, 127.7, 127.4, 127.0, 125.1, 125.0, 123.2, 122.1, 119.9, 119.5, 118.3, 111.3, 109.3, 66.9, 53.3, 52.8, 52.3, 47.0, 41.5, 27.5, 24.6, 22.9, 21.8 (note that the two methyl groups of the isobutyl group and four carbons of fluorenyl group behave as non-equivalent functional groups). The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of the product were identical to those reported in the literature.<sup>7</sup>



**Methyl ((9H-fluoren-9-yl)methoxy)carbonyl-L-leucyl-L-serinate (Fmoc-Leu-Ser-OMe):** 72% (Method b, 65.7 mg). White solid. Mp 172–174 °C. IR (KBr)  $\nu$   $\text{cm}^{-1}$ : 3443, 3300, 3064, 2953, 1694, 1653, 1536, 1257.  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 7.75 (d,  $J = 7.5$  Hz, 2H), 7.56 (dd,  $J = 7.5, 4.0$  Hz, 2H), 7.38 (dd,  $J = 7.5, 4.0$  Hz, 2H), 7.28 (dd,  $J = 7.5, 7.5$  Hz, 2H), 7.11 (br.d,  $J = 7.5$  Hz, 1H), 5.50 (br.d,  $J = 8.0$  Hz, 1H), 4.72–4.58 (m, 1H), 4.40 (dd,  $J = 10.3, 6.9$  Hz, 1H), 4.34 (dd,  $J = 10.3, 6.9$  Hz, 1H), 4.31–4.23 (m, 1H), 4.18 (dd,  $J = 6.9, 6.9$  Hz, 1H), 3.94–3.89 (m, 2H), 3.75 (s, 3H), 2.54 (br s, 1H), 1.75–1.62 (m, 2H), 1.62–1.52 (m, 1H), 0.96 (d,  $J = 6.3$  Hz, 3H), 0.94 (d,  $J = 8.0$  Hz, 3H).  $^{13}\text{C-NMR}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 172.6, 170.7, 156.5, 143.7, 143.5, 141.2, 127.7, 127.0, 125.0, 120.0, 67.2, 62.7, 54.7, 53.7, 52.7, 47.0, 41.4, 24.6, 22.9, 22.0

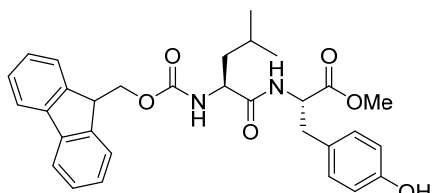
<sup>5</sup> D. Dev, N. B. Palakurthy, K. Thalluri, J. Chandra and B. Mandal, *J. Org. Chem.*, 2014, **79**, 5420–5431.

<sup>6</sup> M. L. Di Gioia, A. Leggio, A. Le Pera, A. Liguori, F. Perri and C. Siciliano, *Eur. J. Org. Chem.*, 2004, 4437–4441.

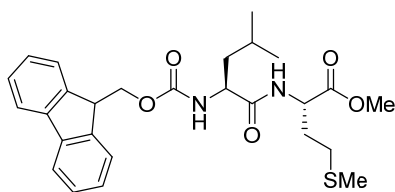
<sup>7</sup> R. E. Cozett, G. A. Venter, M. R. Gokada and R. Hunter, *Org. Biomol. Chem.*, 2016, **14**, 10914–10925.

(note that the two methyl groups of the isobutyl group and two carbons of fluorenyl group behave as non-equivalent functional groups). HRMS (ESI)  $m/z$  calcd. for  $C_{25}H_{31}N_2O_6^+ [M + H]^+$  455.2177, found 455.2176.

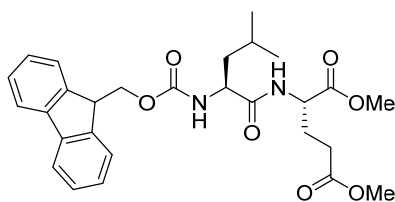
**Methyl {(9H-fluoren-9-yl)methoxy}carbonyl-L-leucyl-L-tyrosinate (Fmoc-Leu-Tyr-OMe):** 97% (Method **b**, 103.0 mg); 96% (Method **c**, 102.4 mg). White solid. Mp 118-120 °C. IR (KBr)  $\nu$   $cm^{-1}$ : 3306, 3066, 2955, 2870, 1667, 1516, 1249.  $^1H$ -NMR (500 MHz,  $CDCl_3$ )  $\delta$  ppm: 7.75 (d,  $J = 7.5$  Hz, 2H), 7.57 (d,  $J = 7.5$  Hz, 2H), 7.38 (ddd,  $J = 7.5, 7.5, 2.3$  Hz, 2H), 7.27 (dd,  $J = 7.5, 7.5$  Hz, 2H), 6.89 (d,  $J = 8.3$  Hz, 2H), 6.77 (br.d,  $J = 8.0$  Hz, 1H), 6.63 (d,  $J = 8.3$  Hz, 2H), 5.43 (br.d,  $J = 9.2$  Hz, 1H), 4.84 (ddd,  $J = 7.5, 5.7, 5.7$  Hz, 1H), 4.41 (dd,  $J = 10.3, 7.5$  Hz, 1H), 4.30 (dd,  $J = 10.3, 6.9$  Hz, 1H), 4.27-4.20 (m, 1H), 4.18 (dd,  $J = 7.5, 6.9$  Hz, 1H), 3.69 (s, 3H), 3.04 (dd,  $J = 14.0, 5.7$  Hz, 1H), 2.97 (dd,  $J = 14.0, 5.7$  Hz, 1H), 1.70-1.35 (m, 3H), 0.89 (d,  $J = 6.3$  Hz, 3H), 0.87 (d,  $J = 8.0$  Hz, 3H).  $^{13}C$ -NMR (125 MHz,  $CDCl_3$ )  $\delta$  ppm: 172.1, 171.8, 156.3, 155.3, 143.7, 143.6, 141.24, 141.20, 130.3, 127.7, 127.0, 126.8, 125.1, 125.0, 120.0, 115.5, 67.2, 53.33, 53.29, 52.4, 47.0, 41.2, 37.0, 24.6, 22.8, 21.9 (note that the two methyl groups of the isobutyl group and six carbons of fluorenyl group behave as non-equivalent functional groups). HRMS (ESI)  $m/z$  calcd. for  $C_{31}H_{35}N_2O_6^+ [M + H]^+$  531.2490, found 531.2495.



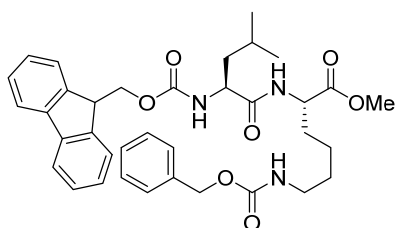
**Methyl {(9H-fluoren-9-yl)methoxy}carbonyl-L-leucyl-L-methioninate (Fmoc-Leu-Met-OMe):** 82% (Method **b**, 81.8 mg). White solid. Mp 139 °C. IR (KBr)  $\nu$   $cm^{-1}$ : 3293, 3064, 2954, 1746, 1659, 1538, 1285.  $^1H$ -NMR (500 MHz,  $CDCl_3$ )  $\delta$  ppm: 7.76 (d,  $J = 7.5$  Hz, 2H), 7.58 (dd,  $J = 7.5, 2.3$  Hz, 2H), 7.40 (dd,  $J = 7.5, 7.5$  Hz, 2H), 7.31 (dd,  $J = 7.5, 7.5$  Hz, 2H), 6.71 (br.d,  $J = 7.5$  Hz, 1H), 5.27 (br.d,  $J = 8.0$  Hz, 1H), 4.70 (ddd,  $J = 7.5, 7.5, 5.2$  Hz, 1H), 4.43 (dd,  $J = 10.3, 6.9$  Hz, 1H), 4.37 (dd,  $J = 10.3, 6.9$  Hz, 1H), 4.26-4.17 (m, 2H), 3.75 (s, 3H), 2.49 (dd,  $J = 7.5, 7.5$  Hz, 2H), 2.22-2.12 (m, 1H), 2.05 (s, 3H), 2.03-1.93 (m, 1H), 1.73-1.62 (m, 2H), 1.61-1.50 (m, 1H), 0.96 (d,  $J = 5.7$  Hz, 3H), 0.94 (d,  $J = 5.2$  Hz, 3H).  $^{13}C$ -NMR (125 MHz,  $CDCl_3$ )  $\delta$  ppm: 172.1, 172.0, 156.1, 143.8, 143.6, 141.3, 127.7, 127.0, 125.0, 119.97, 119.95, 67.0, 53.4, 52.5, 51.5, 47.1, 41.4, 31.4, 29.8, 24.6, 22.9, 22.0, 15.4 (note that the two methyl groups of the isobutyl group and four carbons of fluorenyl group behave as non-equivalent functional groups). HRMS (ESI)  $m/z$  calcd. for  $C_{27}H_{34}N_2NaO_5S^+ [M + Na]^+$  521.2081, found 521.2080.



**Dimethyl {(9H-fluoren-9-yl)methoxy}carbonyl-L-leucyl-L-glutamate (Fmoc-Leu-Glu(OMe)-OMe):** 70% (Method **b**, 71.8 mg). White solid. Mp 115-116 °C. IR (KBr)  $\nu$   $cm^{-1}$ : 3308, 3065, 2954, 1740, 1691, 1657, 1537, 1266.  $^1H$ -NMR (500 MHz,  $CDCl_3$ )  $\delta$  ppm: 7.76 (d,  $J = 7.5$  Hz, 2H), 7.59 (dd,  $J = 7.5, 2.3$  Hz, 2H), 7.39 (dd,  $J = 7.5, 7.5$  Hz, 2H), 7.31 (dd,  $J = 7.5, 7.5$  Hz, 2H), 6.75 (br.d,  $J = 7.5$  Hz, 1H), 5.29 (br.d,  $J = 8.0$  Hz, 1H), 4.60 (ddd,  $J = 7.5, 7.5, 5.2$  Hz, 1H), 4.49-4.34 (m, 2H), 4.30-4.17 (m, 2H), 3.74 (s, 3H), 3.60 (s, 3H), 2.47-2.30 (m, 2H), 2.27-2.14 (m, 1H), 2.06-1.93 (m, 1H), 1.74-1.60 (m, 2H), 1.59-1.48 (m, 1H), 0.95 (s, 6H).  $^{13}C$ -NMR (125 MHz,  $CDCl_3$ )  $\delta$  ppm: 173.2, 172.2, 171.9, 156.1, 143.9, 143.7, 141.27, 141.25, 127.7, 127.0, 125.0, 120.0, 67.1, 53.4, 52.5, 51.8, 51.6, 47.1, 41.5, 29.9, 27.0, 24.6, 22.9, 22.0 (note that the two methyl groups of the isobutyl group and four carbons of fluorenyl group behave as non-equivalent functional groups). HRMS (ESI)  $m/z$  calcd. for  $C_{28}H_{35}N_2O_7^+ [M + H]^+$  511.2439, found 511.2455.

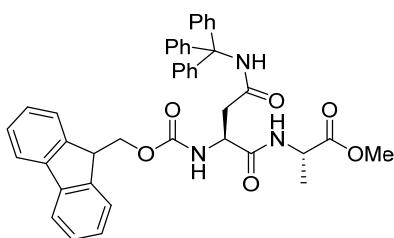


**Methyl  $N^2$ -{(9H-fluoren-9-yl)methoxy}carbonyl-L-leucyl- $N^6$ -[(benzyloxy)carbonyl]-L-lysinate (Fmoc-Leu-Lys(Cbz)-OMe):** 70% (Method **b**, 88.5 mg). White solid. Mp 108-109 °C. IR (KBr)  $\nu$   $cm^{-1}$ : 3303, 3065, 2953, 2868, 1695, 1537, 1261.  $^1H$ -NMR (500 MHz,  $CDCl_3$ )  $\delta$  ppm: 7.75 (d,  $J = 7.5$  Hz, 2H), 7.62-7.51 (m, 2H), 7.39 (dd,  $J = 7.5, 7.5$  Hz, 2H), 7.35-7.21 (m, 7H), 6.88 (br.d,  $J = 7.5$  Hz, 1H), 5.49 (br.d,  $J = 8.6$  Hz, 1H), 5.33 (br s, 1H), 5.03 (d,  $J = 12.0$  Hz, 1H), 4.99 (d,  $J = 12.0$  Hz, 1H), 4.54 (ddd,  $J = 8.0, 7.5, 4.0$  Hz, 1H), 4.45 (dd,  $J = 10.3, 7.5$  Hz, 1H), 4.33-4.26 (m, 2H), 4.18 (dd,  $J = 6.5, 6.5$  Hz, 1H), 3.71 (s, 3H), 3.26-3.14 (m, 1H), 3.12-3.00 (m, 1H), 1.88-1.74 (m, 1H),

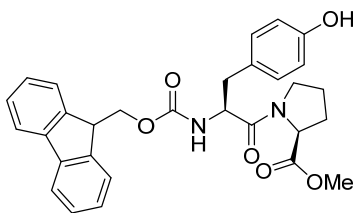


1.72-1.56 (m, 3H), 1.55-1.38 (m, 3H), 1.37-1.18 (m, 2H), 0.91 (s, 6H).  $^{13}\text{C}$ -NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 172.5, 172.4, 156.5, 156.3, 143.8, 143.6, 141.23, 141.20, 136.5, 128.4, 128.1, 128.0, 127.7, 127.05, 127.03, 125.03, 124.96, 120.0, 119.9, 66.9, 66.6, 53.3, 52.3, 52.0, 47.1, 41.4, 40.2, 31.5, 29.1, 24.5, 22.9, 22.2, 21.8 (note that the two methyl groups of the isobutyl group and ten carbons of fluorenyl group behave as non-equivalent functional groups). HRMS (ESI)  $m/z$  calcd. for  $\text{C}_{36}\text{H}_{43}\text{N}_3\text{NaO}_7^+ [\text{M} + \text{Na}]^+$  652.2993, found 652.2994.

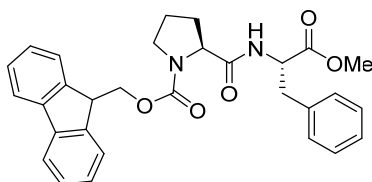
**Methyl  $N^2$ -{[(9H-fluoren-9-yl)methoxy]carbonyl}- $N^4$ -trityl-L-asparaginyl-L-alaninate (Fmoc-Asn(NHTr)-Ala-OMe):** 88% (Method b, 120.0 mg). White solid.  $^1\text{H}$ -NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 7.77 (d,  $J = 7.5$  Hz, 2H), 7.59 (d,  $J = 6.9$  Hz, 2H), 7.41 (dd,  $J = 7.5, 6.9$  Hz, 2H), 7.35-7.24 (m, 12H), 7.24-7.15 (m, 6H), 7.04 (br.s, 1H), 6.52 (br.d,  $J = 6.9$  Hz, 1H), 4.61 (br.s, 1H), 4.52-4.43 (m, 1H), 4.41-4.35 (m, 2H), 4.20 (dd,  $J = 6.9, 6.9$  Hz, 1H), 3.72 (s, 3H), 3.13 (d,  $J = 15.2$  Hz, 1H), 2.69 (dd,  $J = 15.2, 5.2$  Hz, 1H), 1.32 (d,  $J = 6.9$  Hz, 3H).  $^{13}\text{C}$ -NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 172.8, 170.6, 170.5, 156.1, 144.1, 143.7, 143.6, 141.18, 141.16, 128.6, 127.9, 127.6, 127.05, 127.01, 125.1, 119.9, 70.8, 67.2, 52.3, 51.1, 48.3, 47.0, 38.3, 17.6 (note that four carbons of fluorenyl group behave as non-equivalent functional groups). HRMS (ESI)  $m/z$  calcd. for  $\text{C}_{42}\text{H}_{39}\text{N}_3\text{NaO}_6^+ [\text{M} + \text{Na}]^+$  704.2731, found 704.2730. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of the product were identical to those reported in the literature.<sup>8</sup>



**Methyl {[(9H-fluoren-9-yl)methoxy]carbonyl}-L-tyrosyl-L-prolinate (Fmoc-Tyr-Pro-OMe):** 80% (Method b, 81.9 mg). White solid. Mp 148 °C. IR (KBr)  $\nu$   $\text{cm}^{-1}$ : 3251, 3067, 2953, 1746, 1702, 1636, 1515, 1267.  $^1\text{H}$ -NMR (500 MHz,  $\text{CDCl}_3$ , 3:1 mixture of rotamers)  $\delta$  ppm: 7.80-7.70 (m, 2H), 7.60 (d,  $J = 7.5$  Hz, 0.5H), 7.56 (dd,  $J = 7.5, 1.2$  Hz, 1.5H), 7.43-7.35 (m, 2H), 7.34-7.27 (m, 2H), 7.11 (d,  $J = 8.0$  Hz, 1.5H), 7.06-7.01 (m, 0.5H), 6.74-6.67 (m, 2H), 5.77-5.71 (m, 0.25H), 5.67 (br.d,  $J = 9.2$  Hz, 0.75H), 4.78-4.56 (m, 1H), 4.52 (dd,  $J = 8.0, 4.0$  Hz, 0.75H), 4.49-4.40 (m, 0.25H), 4.38-4.25 (m, 2H), 4.24-4.13 (m, 1H), 3.74 (s, 2.25H), 3.72-3.58 (m, 1H), 3.65 (s, 0.75H), 3.41-3.29 (m, 1H), 3.06 (dd,  $J = 14.3, 6.3$  Hz, 0.75H), 2.98 (dd,  $J = 13.2, 5.2$  Hz, 0.25H), 2.92 (dd,  $J = 14.3, 5.7$  Hz, 0.75H), 2.87 (dd,  $J = 13.2, 9.7$  Hz, 0.25H), 2.26-2.07 (m, 1H), 2.07-1.83 (m, 3H).  $^{13}\text{C}$ -NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 172.3, 170.6, 155.8, 155.2, 143.8, 143.7, 141.2, 130.9, 130.5, 127.7, 127.1, 125.23, 125.18, 119.9, 115.4, 67.2, 59.0, 53.7, 52.3, 47.1, 47.0, 37.8, 29.0, 24.9 (note that four carbons of fluorenyl group behave as non-equivalent functional groups). HRMS (ESI)  $m/z$  calcd. for  $\text{C}_{30}\text{H}_{30}\text{N}_2\text{NaO}_6^+ [\text{M} + \text{H}]^+$  537.1996, found 537.2001.

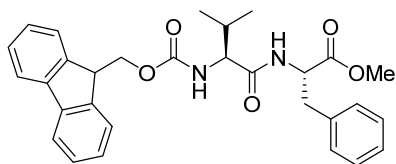


**Methyl {[(9H-fluoren-9-yl)methoxy]carbonyl}-L-prolyl-L-phenylalaninate (Fmoc-Pro-Phe-OMe):** 88% (Method b, 87.8 mg). White solid.  $^1\text{H}$ -NMR (500 MHz,  $\text{CDCl}_3$ , 3:2 mixture of rotamers)  $\delta$  ppm: 7.88-7.71 (m, 2H), 7.67-7.57 (m, 1.2H), 7.57-7.47 (m, 0.8H), 7.47-7.37 (m, 2H), 7.37-7.27 (m, 2H), 7.26-6.88 (m, 5.6H), 6.34 (br.s, 0.4H), 5.01-4.77 (m, 1H), 4.58-4.09 (m, 4H), 3.72 (s, 1.8H), 3.54 (s, 1.2H), 3.52-3.32 (m, 2H), 3.19 (dd,  $J = 13.7, 5.2$  Hz, 1H), 3.01 (dd,  $J = 13.7, 6.9$  Hz, 1H), 2.47-2.24 (m, 0.6H), 2.23-1.99 (m, 0.8H), 1.97-1.77 (m, 2.2H), 1.72-1.55 (m, 0.4H).  $^{13}\text{C}$ -NMR (125 MHz,  $\text{CDCl}_3$ , 3:2 mixture of rotamers)  $\delta$  ppm: 171.7 (major), 171.5 (minor), 171.4 (minor), 171.0 (major), 156.0 (major), 155.0 (minor), 144.0 (minor), 143.8 (major), 141.2, 135.9 (major), 135.7 (minor), 129.2, 128.5 (minor), 128.3 (major), 127.7, 127.0, 126.8, 125.1 (major), 125.0 (minor), 120.0, 68.0 (minor), 67.7 (major), 60.8 (minor), 60.1 (major), 53.1 (major), 52.6 (minor), 52.3, 47.4 (minor), 47.1, 46.8 (major), 37.8, 30.9 (minor), 27.8 (major), 24.5 (major), 23.3 (minor). HRMS (ESI)  $m/z$  calcd. for  $\text{C}_{30}\text{H}_{31}\text{N}_2\text{O}_5^+ [\text{M} + \text{H}]^+$  499.2227, found 499.2235. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of the product were identical to those reported in the literature.<sup>8</sup>

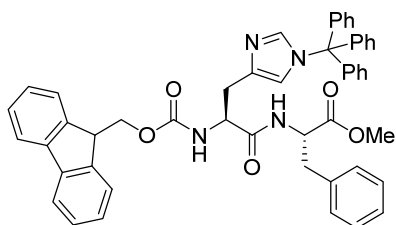


<sup>8</sup> J. K. Zbigniew, K. Beata, K. Justyna, S. Giuseppina, C. Mario, R. Paolo, B. Michal, L. G. Marek, and M. P. Anna, *J. Am. Chem. Soc.*, 2005, **127**, 16912–16920.

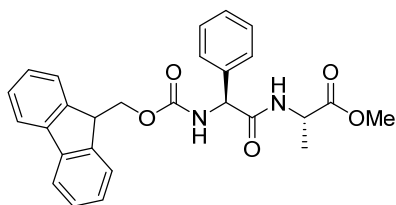




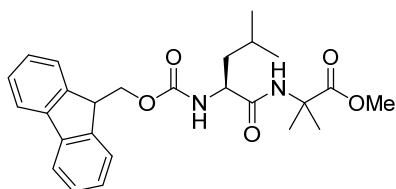
**Methyl [(9H-fluoren-9-yl)methoxy]carbonyl-L-valyl-L-phenylalaninate (Fmoc-Val-Phe-OMe):** 80% (Method **b**, 80.3 mg). White solid.  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 7.77 (d,  $J = 7.5$  Hz, 2H), 7.60 (d,  $J = 7.5$  Hz, 2H), 7.40 (dd,  $J = 7.5, 7.5$  Hz, 2H), 7.32 (dd,  $J = 7.5, 7.5$  Hz, 2H), 7.24 (dd,  $J = 7.5, 7.5$  Hz, 2H), 7.19 (t,  $J = 7.5$  Hz, 1H), 7.08 (d,  $J = 7.5$  Hz, 2H), 6.27 (br.d,  $J = 8.0$  Hz, 1H), 5.36 (br.d,  $J = 8.6$  Hz, 1H), 4.89 (ddd,  $J = 8.0, 5.7, 5.7$  Hz, 1H), 4.44 (dd,  $J = 10.9, 6.9$  Hz, 1H), 4.33 (dd,  $J = 10.9, 6.9$  Hz, 1H), 4.22 (dd,  $J = 6.9, 6.9$  Hz, 1H), 4.00 (dd,  $J = 8.6, 6.3$  Hz, 1H), 3.71 (s, 3H), 3.14 (dd,  $J = 13.8, 5.7$  Hz, 1H), 3.08 (dd,  $J = 13.8, 5.7$  Hz, 1H), 2.16-1.95 (m, 1H), 0.94 (d,  $J = 6.9$  Hz, 3H), 0.90 (d,  $J = 6.9$  Hz, 3H).  $^{13}\text{C-NMR}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 171.6, 170.8, 156.2, 143.83, 143.79, 141.3, 135.5, 129.2, 128.6, 127.7, 127.2, 127.1, 125.12, 125.05, 120.0, 67.1, 60.2, 53.1, 52.3, 47.1, 37.9, 31.2, 19.0, 17.7 (note that the two methyl groups of the isopropyl group and four carbons of fluorenyl group behave as non-equivalent functional groups). The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of the product were identical to those reported in the literature.<sup>9</sup>



**Methyl  $N^\alpha$ -[(9H-fluoren-9-yl)methoxy]carbonyl- $N^\epsilon$ -trityl-L-histidyl-L-phenylalaninate (Fmoc-Phg-Ala-OMe):** 84% (Method **b**, 131.9 mg). Colorless amorphous. IR (KBr)  $\nu$   $\text{cm}^{-1}$ : 3296, 3061, 2950, 1724, 1670, 1531, 1216.  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ , 85:15 mixture of rotamers)  $\delta$  ppm: 8.20 (br.s, 1H), 7.75 (m, 2H), 7.65 (br.s, 1H), 7.58 (m, 2H), 7.43-7.35 (m, 2H), 7.35-7.27 (m, 10H), 7.25-7.01 (m, 12H), 6.70 (br.s, 1H), 6.59 (br.d,  $J = 6.30$  Hz, 0.85H), 6.45 (br.d,  $J = 7.5$  Hz, 0.15H), 4.77 (ddd,  $J = 6.9, 6.9, 6.9$  Hz, 1H), 4.67 (br.s, 1H), 4.37-4.27 (m, 1H), 4.27-4.08 (m, 2H), 3.63 (s, 2.55H), 3.59 (s, 0.45H), 3.35-2.86 (m, 4H).  $^{13}\text{C-NMR}$  (125 MHz,  $\text{CDCl}_3$ , major rotamer)  $\delta$  ppm: 171.6, 170.6, 156.1, 143.9, 143.7, 141.2, 141.0, 136.5, 136.2, 134.8, 129.6, 129.3, 128.5, 128.4, 128.3, 127.9, 127.64, 127.62, 127.1, 127.0, 126.9, 125.3, 125.1, 119.9, 67.2, 54.5, 54.1, 52.1, 46.9, 37.6, 30.6 (note that two carbon peaks overlap with each other and four carbons of fluorenyl group behave as non-equivalent functional groups). HRMS (ESI)  $m/z$  calcd. for  $\text{C}_{50}\text{H}_{45}\text{N}_4\text{O}_5^+$  [ $\text{M} + \text{H}$ ]<sup>+</sup> 781.3384, found 781.3383.



**Methyl [(9H-fluoren-9-yl)methoxy]carbonyl-L-phenylglycyl-L-alaninate (Fmoc-Phg-Ala-OMe):** 72% (Method **b**, 66.4 mg). White solid. Mp 188-190 °C. IR (KBr)  $\nu$   $\text{cm}^{-1}$ : 3313, 3064, 2953, 1738, 1688, 1650, 1536, 1254.  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 7.75 (d,  $J = 7.5$  Hz, 2H), 7.67-7.24 (m, 11H), 6.31 (d,  $J = 6.3$  Hz, 1H), 6.17 (d,  $J = 5.7$  Hz, 1H), 5.27 (d,  $J = 6.3$  Hz, 1H), 4.62-4.47 (m, 1H), 4.36 (d,  $J = 6.9$  Hz, 2H), 4.20 (t,  $J = 6.9$  Hz, 1H), 3.67 (s, 3H), 1.41 (d,  $J = 6.9$  Hz, 3H).  $^{13}\text{C-NMR}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 172.7, 169.3, 155.6, 143.8, 143.7, 141.2, 137.5, 129.1, 128.6, 127.7, 127.3, 127.0, 125.1, 119.9, 67.1, 58.7, 52.5, 48.4, 47.0, 18.2 (note that two carbons of fluorenyl group behave as non-equivalent functional groups). HRMS (ESI)  $m/z$  calcd. for  $\text{C}_{27}\text{H}_{27}\text{N}_2\text{O}_5^+$  [ $\text{M} + \text{H}$ ]<sup>+</sup> 459.1914, found 459.1919.

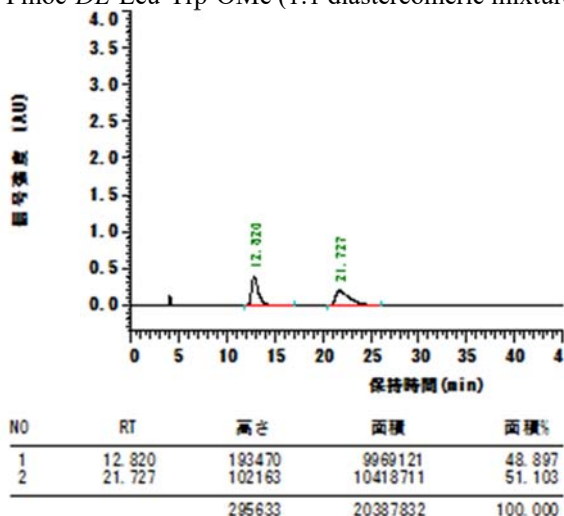


**Methyl (S)-2-[2-([(9H-fluoren-9-yl)methoxy]amino)-4-methylpentanamido]-2-methylpropanoate (Fmoc-Leu-Aib-OMe):** 92% (Method **b**, 83.2 mg). White solid. Mp 95-96 °C. IR (KBr)  $\nu$   $\text{cm}^{-1}$ : 3306, 3065, 2954, 2871, 1743, 1692, 1660, 1540, 1280.  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 7.76 (d,  $J = 7.5$  Hz, 2H), 7.58 (d,  $J = 7.5$  Hz, 2H), 7.39 (dd,  $J = 7.5, 7.5$  Hz, 2H), 7.30 (dd,  $J = 7.5, 7.5$  Hz, 2H), 6.68 (br.s, 1H), 5.33 (br.d,  $J = 8.5$  Hz, 1H), 4.48-4.34 (m, 2H), 4.21 (dd,  $J = 6.9, 6.9$  Hz, 1H), 4.19-4.12 (m, 1H), 3.70 (s, 3H), 1.72-1.59 (m, 2H), 1.57-1.41 (m, 1H), 1.53 (s, 6H), 0.95 (d,  $J = 5.2$  Hz, 3H), 0.93 (d,  $J = 4.0$  Hz, 3H).  $^{13}\text{C-NMR}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm: 174.7, 171.4, 156.2, 143.73, 143.65, 141.3, 127.7, 127.0, 125.0, 120.0, 119.9, 67.0, 56.5, 53.3, 52.6, 47.1, 41.3, 24.7, 24.60, 24.57, 22.9, 22.0 (note that the four methyl groups and four carbons of fluorenyl group behave as non-equivalent functional groups). HRMS (ESI)  $m/z$  calcd. for  $\text{C}_{26}\text{H}_{32}\text{N}_2\text{NaO}_6^+$  [ $\text{M} + \text{Na}$ ]<sup>+</sup> 475.2203, found 475.2214.

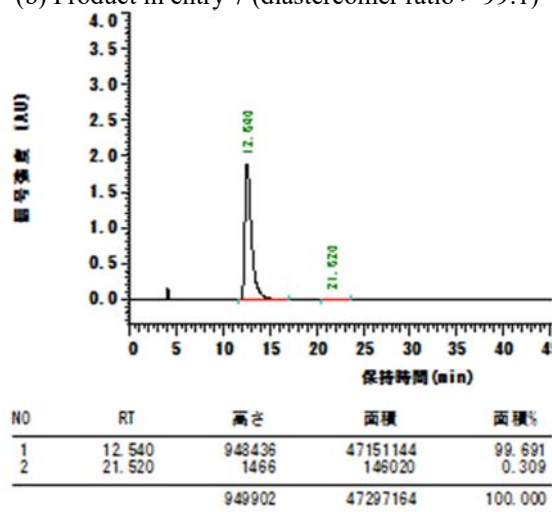
<sup>9</sup> V. Erapalapati, U. A. Hale and N. Madhavan, *Tetrahedron Lett.*, 2019, **60**, 151311.

## 5. Chiral HPLC Analysis of *N*-Protected Dipeptide Methyl Esters

(a) Fmoc-*DL*-Leu-Trp-OMe (1:1 diastereomeric mixtures)

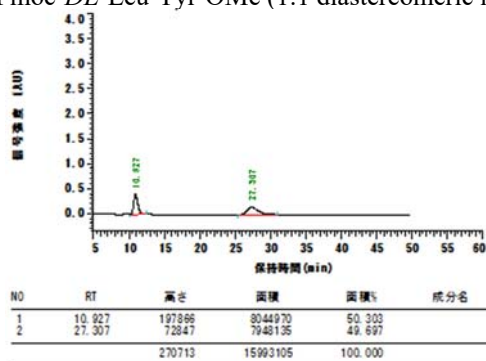


(b) Product in entry 7 (diastereomer ratio > 99:1)

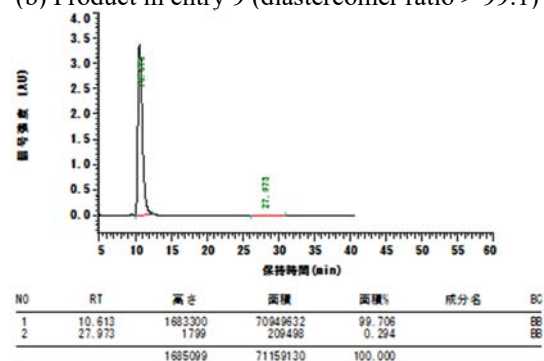


**Figure S1.** Chiral HPLC analysis of Fmoc-Leu-Trp-OMe (entry 7). CHIRALPAK IA (4.6 mmφ × 250 mL), *n*-Hexane/2-propanol = 5/1, flow rate 1.0 mL/min, detection at 254 nm.

(a) Fmoc-*DL*-Leu-Tyr-OMe (1:1 diastereomeric mixtures)

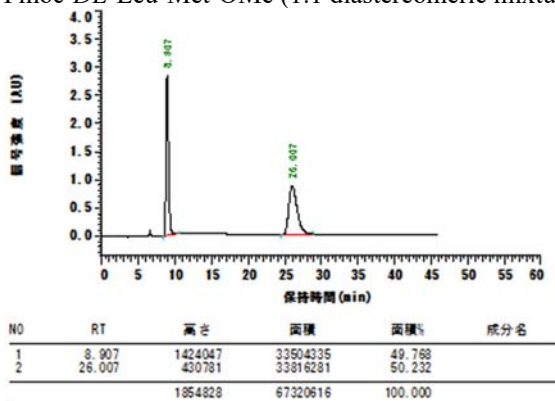


(b) Product in entry 9 (diastereomer ratio > 99:1)

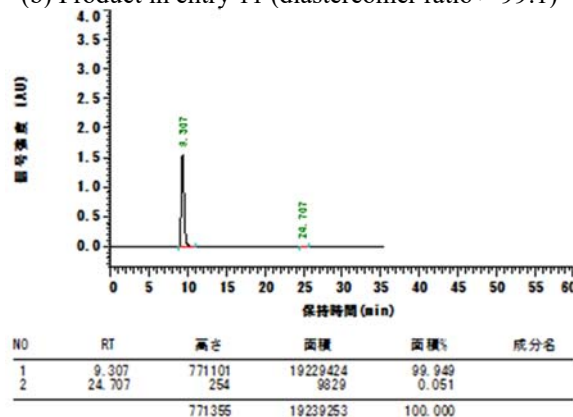


**Figure S2.** Chiral HPLC analysis of Fmoc-Leu-Tyr-OMe (entry 9). CHIRALPAK IA (4.6 mmφ × 250 mL), *n*-Hexane/2-propanol = 5/1, flow rate 1.0 mL/min, detection at 254 nm.

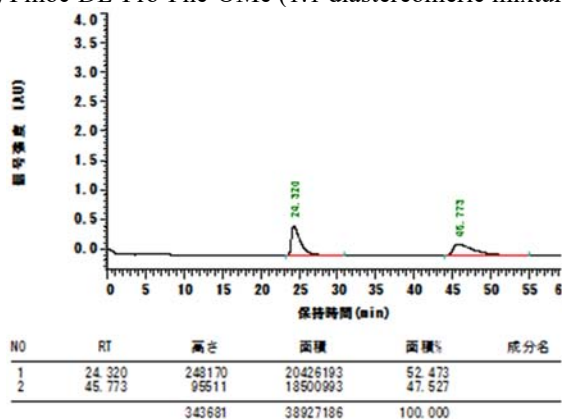
(a) Fmoc-*DL*-Leu-Met-OMe (1:1 diastereomeric mixtures)



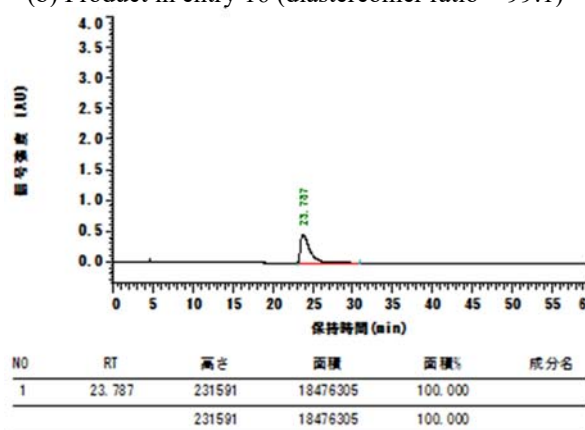
(b) Product in entry 11 (diastereomer ratio > 99:1)



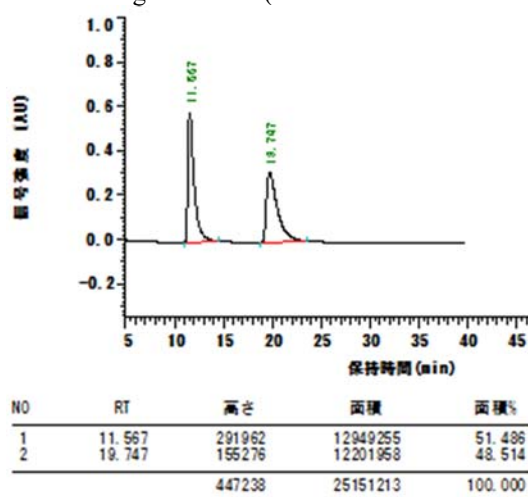
**Figure S3.** Chiral HPLC analysis of Fmoc-Leu-Tyr-OMe (entry 11). CHIRALPAK IC (4.6 mmφ × 250 mL), *n*-Hexane/2-propanol = 5/1, flow rate 1.0 mL/min, detection at 254 nm

(a) Fmoc-*DL*-Pro-Phe-OMe (1:1 diastereomeric mixtures)

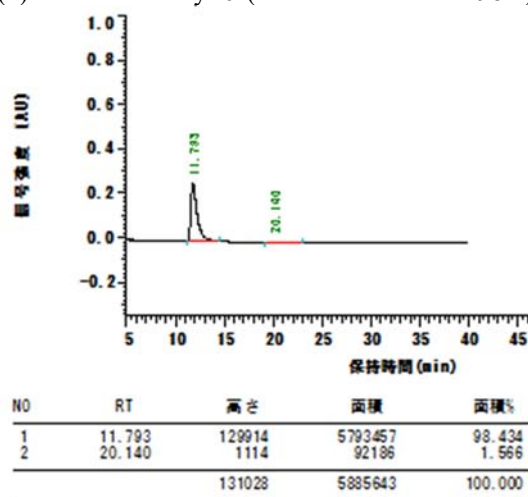
(b) Product in entry 16 (diastereomer ratio &gt; 99:1)



**Figure S4.** Chiral HPLC analysis of Fmoc-Pro-Phe-OMe (entry 16). CHIRALPAK IB (4.6 mm $\phi$   $\times$  250 mmL), *n*-Hexane/2-propanol = 5/1, flow rate 1.0 mL/min, detection at 254 nm

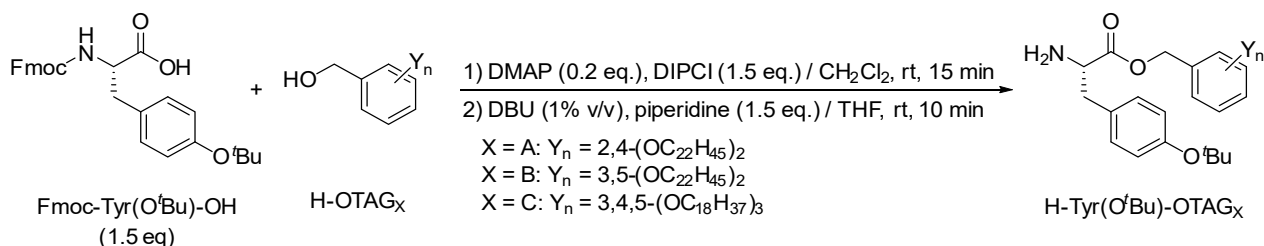
(a) Fmoc-*DL*-Phg-Ala-OMe (1:1 diastereomeric mixtures)

(b) Product in entry 19 (diastereomer ratio &gt; 98:2)

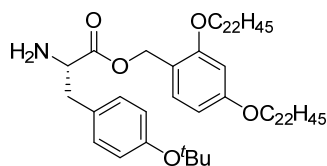


**Figure S5.** Chiral HPLC analysis of Fmoc-Phg-Ala-OMe (entry 19). CHIRALPAK IB (4.6 mm $\phi$   $\times$  250 mmL), *n*-Hexane/2-propanol = 5/1, flow rate 1.0 mL/min, detection at 254 nm

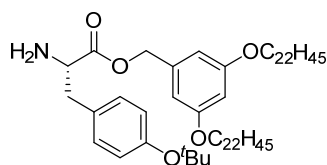
## 6. Preparation and Characterization of Tag-Protected Amino Acid Esters



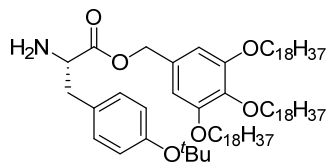
- To a solution of H-OTAG<sub>X</sub> (X = A or B, 379 mg, 0.50 mmol; X = C, 457 mg, 0.50 mmol) in DCM (10 mL), H-Tyr(O<sup>t</sup>Bu)-OH (345 mg, 0.75 mmol), DMAP (12.2mg, 0.10 mmol) and DIPCI (94.7 mg, 0.75 mmol) were added. After being stirred at room temperature for 15 min, the reaction mixture was concentrated in vacuo to dryness. The residue was washed with MeCN to give Fmoc-Tyr(O<sup>t</sup>Bu)-OTAG<sub>X</sub> as white solid quantitatively.
- To a solution of Fmoc-Tyr(O<sup>t</sup>Bu)-OTAG<sub>X</sub> (0.50 mmol) in THF (10 mL), piperidine (74.2  $\mu$ L, 0.75 mmol) and DBU (100  $\mu$ L, 0.67 mmol) were added. After being stirred at room temperature for 10 min, the reaction mixture was neutralized with 6 M HCl aq., and then concentrated in vacuo to dryness. The residue was washed with MeCN to give H-Tyr(O<sup>t</sup>Bu)-OTAG<sub>X</sub> as white solid quantitatively.



**2,4-Bis(docosyloxy)benzyl (S)-2-amino-3-(4-(tert-butoxy)phenyl)propanoate (H-Tyr(OtBu)-OTAG<sub>A</sub>):** Mp 44-45 °C. IR (KBr)  $\nu$  cm<sup>-1</sup>: 2917, 2849, 1735, 1615, 1588, 1506, 1468, 1176, 1162. <sup>1</sup>H-NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 7.17 (d, *J* = 8.3 Hz, 1H), 7.04 (d, *J* = 8.6 Hz, 2H), 6.87 (d, *J* = 8.6 Hz, 2H), 6.45 (d, *J* = 2.1 Hz, 1H), 6.42 (dd, *J* = 8.3, 2.1 Hz, 1H), 5.14 (s, 2H), 3.98-3.91 (m, 4H), 3.72 (dd, *J* = 7.6, 5.5 Hz, 1H), 3.05 (dd, *J* = 13.8, 5.5 Hz, 1H), 2.85 (dd, *J* = 13.8, 7.6 Hz, 1H), 1.81-1.74 (m, 4H), 1.49-1.41 (m, 4H), 1.38-1.21 (m, 72H), 1.32 (s, 9H), 0.88 (t, *J* = 7.2 Hz, 6H). <sup>13</sup>C-NMR (150 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 174.8, 160.9, 158.5, 154.1, 131.7, 131.4, 129.8, 124.1, 116.2, 104.5, 99.6, 78.2, 68.09, 68.06, 62.4, 55.6, 39.9, 31.9, 29.7, 29.59, 29.56, 29.4, 29.3, 29.24, 29.17, 28.8, 26.0, 22.7, 14.1 (note that 32 peaks of the docosyl groups overlap with other carbons). HRMS (ESI) *m/z* calcd. for C<sub>64</sub>H<sub>113</sub>NNaO<sub>5</sub><sup>+</sup> [M + Na]<sup>+</sup> 998.8511, found 998.8509. IR (KBr)  $\nu$  cm<sup>-1</sup>: 3306, 3066, 2955, 2870, 1667, 1516, 1249.

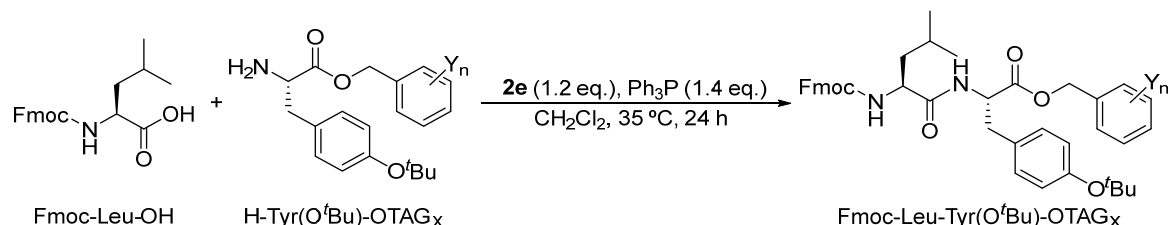


**3,5-Bis(docosyloxy)benzyl (S)-2-amino-3-(4-(tert-butoxy)phenyl)propanoate (H-Tyr(OtBu)-OTAG<sub>B</sub>):** Mp 47-48 °C. IR (KBr)  $\nu$  cm<sup>-1</sup>: 2915, 2848, 1738, 1599, 1507, 1466, 1237, 1162. <sup>1</sup>H-NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 7.04 (d, *J* = 7.6 Hz, 2H), 6.88 (d, *J* = 7.6 Hz, 2H), 6.44 (s, 2H), 6.40 (s, 1H), 5.04 (d, *J* = 12.4 Hz, 1H), 5.01 (d, *J* = 12.4 Hz, 1H), 3.91 (t, *J* = 5.5 Hz, 4H), 3.80-3.71 (m, 1H), 3.17-3.10 (m, 2H), 3.05 (dd, *J* = 13.8, 6.2 Hz, 1H), 2.87 (dd, *J* = 13.8, 7.6 Hz, 1H), 2.01-1.96 (m, 2H), 1.93-1.84 (m, 2H), 1.79-1.70 (m, 4H), 1.46-1.38 (m, 4H), 1.33-1.22 (m, 68H), 1.30 (m, 9H), 0.87 (t, *J* = 6.5 Hz, 6H). <sup>13</sup>C-NMR (150 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 174.5, 160.4, 154.3, 137.4, 131.6, 129.7, 124.2, 106.6, 101.1, 78.2, 68.1, 66.8, 55.7, 44.5, 40.1, 31.9, 29.7, 29.59, 29.56, 29.38, 29.33, 29.2, 28.8, 26.0, 22.7, 22.5, 22.4, 14.1 (18 peaks of the docosyl groups overlap with other carbons). HRMS (ESI) *m/z* calcd. for C<sub>64</sub>H<sub>113</sub>NNaO<sub>5</sub><sup>+</sup> [M + Na]<sup>+</sup> 998.8511, found 998.8501.

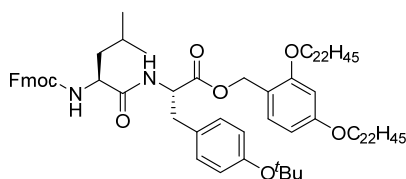


**3,4,5-Tris(octadecyloxy)benzyl (S)-2-amino-3-(4-(tert-butoxy)phenyl)propanoate (H-Tyr(OtBu)-OTAG<sub>C</sub>):** Mp 49-50 °C. IR (KBr)  $\nu$  cm<sup>-1</sup>: 2915, 2847, 1740, 1599, 1506, 1468, 1235, 1161. <sup>1</sup>H-NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 7.02 (d, *J* = 8.9 Hz, 2H), 6.88 (d, *J* = 8.9 Hz, 2H), 6.51 (s, 2H), 5.03 (d, *J* = 11.7 Hz, 1H), 4.99 (d, *J* = 11.7 Hz, 1H), 3.94 (t, *J* = 6.9 Hz, 4H), 3.93 (t, *J* = 6.2 Hz, 2H), 3.75 (dd, *J* = 7.6, 4.8 Hz, 1H), 3.17-3.10 (m, 2H), 3.04 (dd, *J* = 13.1, 4.8 Hz, 1H), 2.85 (dd, *J* = 13.1, 7.6 Hz, 1H), 1.94-1.86 (m, 2H), 1.83-1.70 (m, 7H), 1.69-1.63 (m, 1H), 1.50-1.41 (m, 6H), 1.36-1.23 (m, 80H), 1.31 (s, 9H), 0.87 (t, *J* = 6.9 Hz, 9H). <sup>13</sup>C-NMR (150 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 174.8, 154.3, 153.2, 138.3, 131.6, 130.4, 129.7, 124.2, 107.2, 78.3, 73.4, 69.1, 67.1, 55.8, 44.5, 40.2, 31.9, 30.3, 29.7, 29.63, 29.59, 29.42, 29.39, 29.34, 28.8, 26.1, 22.7, 22.5, 22.4, 14.1 (note that 32 peaks of the octadecyl groups overlap with other carbons). HRMS (ESI) *m/z* calcd. for C<sub>74</sub>H<sub>133</sub>NNaO<sub>6</sub><sup>+</sup> [M + Na]<sup>+</sup> 1155.0025, found 1155.0044.

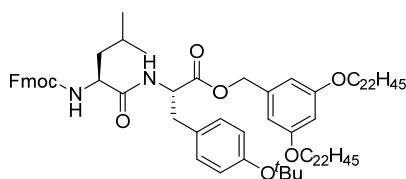
## 7. Preparation and Characterization of Tag-Protected Dipeptides



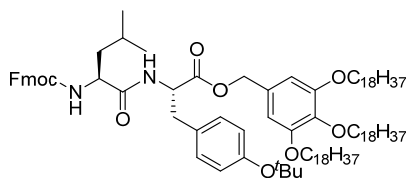
To a solution of **2e** (108.5 mg, 0.24 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5.0 mL), Fmoc-Leu-OH (70.7 mg, 0.2 mmol), H-Tyr(OtBu)-OTAG<sub>X</sub> (0.2 mmol) and Ph<sub>3</sub>P (73.4 mg, 0.28 mmol) were added successively. After being stirred at 35 °C for 24 h, the white precipitate was filtered out and washed with DCM to give **3e** (93.7-103.5 mg, 86-95%). The filtrate was concentrated to dryness in vacuo and the resulting residue was washed with MeCN to give Fmoc-Leu-Tyr(OtBu)-OTAG<sub>X</sub>.



**2,4-Bis(docosan-1-yloxy)benzyl** (*S*)-2-[(*S*)-2-((9H-fluoren-9-yl)methoxy)carbonyl]amino)-4-methylpentanamido]-3-[4-(*tert*-butoxy)phenyl]propanoate (**Fmoc-Leu-Tyr(O<sup>t</sup>Bu)-OTAGA**): 92% (241.4 mg). White solid. Mp 79-80 °C. IR (KBr)  $\nu$  cm<sup>-1</sup>: 2917, 2849, 1740, 1651, 1590, 1507, 1468, 1234, 1163. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 7.77 (d, *J* = 6.9 Hz, 2H), 7.60 (d, *J* = 6.9 Hz, 2H), 7.40 (dd, *J* = 6.9, 6.9 Hz, 2H), 7.32 (dd, *J* = 6.9, 6.9 Hz, 2H), 7.15 (d, *J* = 8.0 Hz, 1H), 6.90 (d, *J* = 8.0 Hz, 2H), 6.79 (d, *J* = 8.0 Hz, 2H), 6.45 (s, 1H), 6.42 (d, *J* = 8.0 Hz, 1H), 6.33 (d, *J* = 8.0 Hz, 1H), 5.31-5.00 (m, 3H), 4.92-4.76 (m, 1H), 4.42 (dd, *J* = 9.7, 7.5 Hz, 1H), 4.37 (dd, *J* = 9.7, 6.9 Hz, 1H), 4.29-4.11 (m, 2H), 4.01-3.90 (m, 4H), 3.18-2.90 (m, 2H), 1.87-1.40 (m, 12H), 1.39-1.19 (m, 80H), 0.95-0.85 (m, 12H). <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 171.5, 171.1, 161.0, 158.5, 156.0, 154.3, 143.8, 143.7, 141.3, 131.7, 130.3, 129.8, 127.7, 127.0, 125.1, 124.0, 119.9, 115.6, 104.4, 99.6, 78.2, 68.13, 68.07, 67.0, 63.0, 53.4, 53.1, 47.1, 41.6, 36.9, 31.9, 29.7, 29.6, 29.40, 29.35, 29.3, 29.2, 28.8, 26.0, 24.6, 22.9, 22.7, 21.9, 14.1 (note that the two methyl groups of the isobutyl group and two carbons of fluorenyl group behave as non-equivalent functional groups and 31 peaks of the docosyl groups overlap with other carbons). HRMS (ESI) *m/z* calcd. for C<sub>85</sub>H<sub>134</sub>N<sub>2</sub>NaO<sub>8</sub><sup>+</sup> [M + Na]<sup>+</sup> 1334.0032, found 1334.0058.

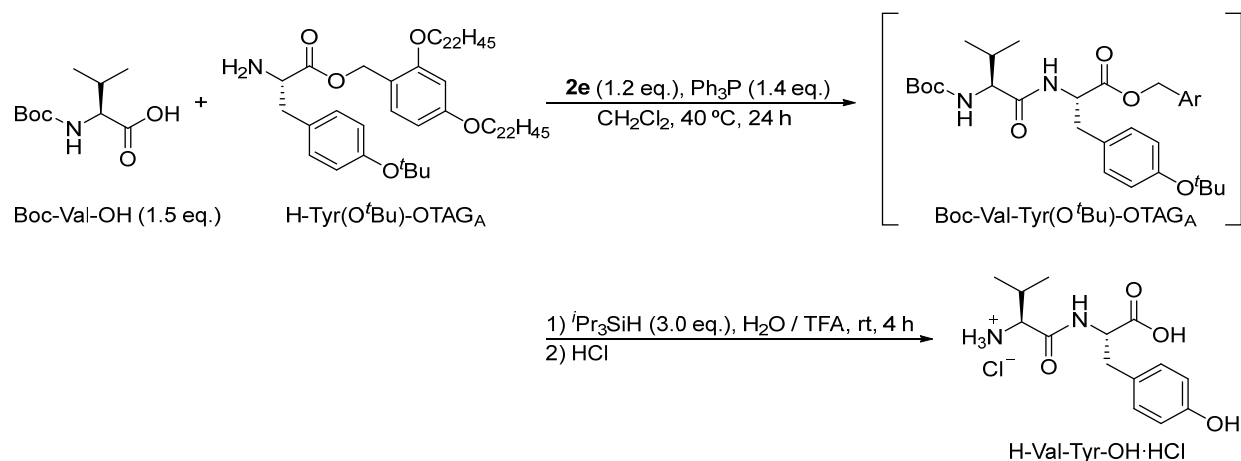


**3,5-Bis(docosan-1-yloxy)benzyl** (*S*)-2-[(*S*)-2-((9H-fluoren-9-yl)methoxy)carbonyl]amino)-4-methylpentanamido]-3-[4-(*tert*-butoxy)phenyl]propanoate (**Fmoc-Leu-Tyr(O<sup>t</sup>Bu)-OTAGB**): 94% (246.7 mg). White solid. Mp 65-66 °C. IR (KBr)  $\nu$  cm<sup>-1</sup>: 2918, 2846, 1694, 1656, 1600, 1509, 1468, 1241, 1172. <sup>1</sup>H-NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 7.76 (d, *J* = 6.9 Hz, 2H), 7.60 (d, *J* = 6.9 Hz, 2H), 7.40 (dd, *J* = 6.9, 6.9 Hz, 2H), 7.31 (dd, *J* = 6.9, 6.9 Hz, 2H), 6.92 (d, *J* = 8.3 Hz, 2H), 6.82 (d, *J* = 8.3 Hz, 2H), 6.44 (s, 2H), 6.42 (s, 1H), 6.42-6.33 (m, 1H), 5.22-5.09 (m, 1H), 5.05 (d, *J* = 12.4 Hz, 1H), 5.02 (d, *J* = 12.4 Hz, 1H), 4.91-4.79 (m, 1H), 4.43 (dd, *J* = 10.3, 7.6 Hz, 1H), 4.37 (dd, *J* = 10.3, 6.9 Hz, 1H), 4.22 (dd, *J* = 6.9, 6.2 Hz, 1H), 4.19-4.11 (m, 1H), 3.91 (t, *J* = 6.2 Hz, 4H), 3.11 (dd, *J* = 13.8, 5.5 Hz, 1H), 3.06 (dd, *J* = 13.8, 4.8 Hz, 1H), 1.80-1.73 (m, 4H), 1.66-1.58 (m, 2H), 1.50-1.41 (m, 5H), 1.39-1.22 (m, 79H), 0.92-0.68 (m, 12H). <sup>13</sup>C-NMR (150 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 171.7, 171.0, 160.5, 156.0, 154.5, 143.9, 143.7, 141.3, 136.9, 130.2, 129.8, 127.7, 127.1, 125.1, 124.1, 119.9, 106.8, 101.3, 78.3, 68.1, 67.3, 67.0, 53.4, 53.2, 47.2, 41.5, 37.1, 31.9, 29.7, 29.64, 29.62, 29.59, 29.42, 29.35, 29.3, 28.8, 26.1, 24.6, 22.8, 22.7, 22.0, 14.1 (note that the two methyl groups of the isobutyl group and two carbons of fluorenyl group behave as non-equivalent functional groups and 22 peaks of the docosyl groups overlap with other carbons). HRMS (ESI) *m/z* calcd. for C<sub>85</sub>H<sub>134</sub>N<sub>2</sub>NaO<sub>8</sub><sup>+</sup> [M + Na]<sup>+</sup> 1334.0032, found 1334.0059.



**3,4,5-Tris(octadecyloxy)benzyl** (*S*)-2-[(*S*)-2-((9H-fluoren-9-yl)methoxy)carbonyl]amino)-4-methylpentanamido]-3-[4-(*tert*-butoxy)phenyl]propanoate (**Fmoc-Leu-Tyr(O<sup>t</sup>Bu)-OTAGC**): 92% (270.2 mg). White solid. Mp 84-85 °C. IR (KBr)  $\nu$  cm<sup>-1</sup>: 2918, 2850, 1743, 1642, 1614, 1589, 1509, 1469, 1253, 1177. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 7.76 (d, *J* = 6.9 Hz, 2H), 7.60 (d, *J* = 6.9 Hz, 2H), 7.40 (dd, *J* = 6.9, 6.9 Hz, 2H), 7.31 (dd, *J* = 6.9, 6.9 Hz, 2H), 6.89 (d, *J* = 8.0 Hz, 2H), 6.81 (d, *J* = 8.0 Hz, 2H), 6.51 (s, 2H), 6.41 (br.d, *J* = 6.9 Hz, 1H), 5.17 (br.d, *J* = 8.0 Hz, 1H), 5.05 (d, *J* = 11.7 Hz, 1H), 4.99 (d, *J* = 11.7 Hz, 1H), 4.90-4.77 (m, 1H), 4.43 (dd, *J* = 10.3, 6.9 Hz, 1H), 4.37 (dd, *J* = 10.3, 6.9 Hz, 1H), 4.23 (dd, *J* = 6.9, 6.9 Hz, 1H), 4.20-4.13 (m, 1H), 4.04-3.85 (m, 6H), 3.16-3.01 (m, 2H), 1.86-1.41 (m, 18H), 1.33-1.22 (m, 90H), 0.93-0.85 (m, 15H). <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 171.6, 171.1, 156.0, 154.5, 153.2, 143.8, 143.7, 141.3, 138.3, 130.1, 129.84, 129.77, 127.7, 127.0, 125.0, 124.1, 120.0, 119.9, 107.2, 78.3, 73.4, 69.1, 67.7, 67.0, 53.3, 53.1, 47.1, 41.4, 37.1, 31.9, 30.3, 29.7, 29.6, 29.5, 29.4, 29.3, 28.8, 26.1, 24.6, 22.8, 22.7, 21.9, 14.1 (note that the two methyl groups of the isobutyl group and four carbons of fluorenyl group behave as non-equivalent functional groups and 32 peaks of the octadecyl groups overlap with other carbons). HRMS (ESI) *m/z* calcd. for C<sub>95</sub>H<sub>154</sub>N<sub>2</sub>NaO<sub>9</sub><sup>+</sup> [M + Na]<sup>+</sup> 1490.1457, found 1490.1434.

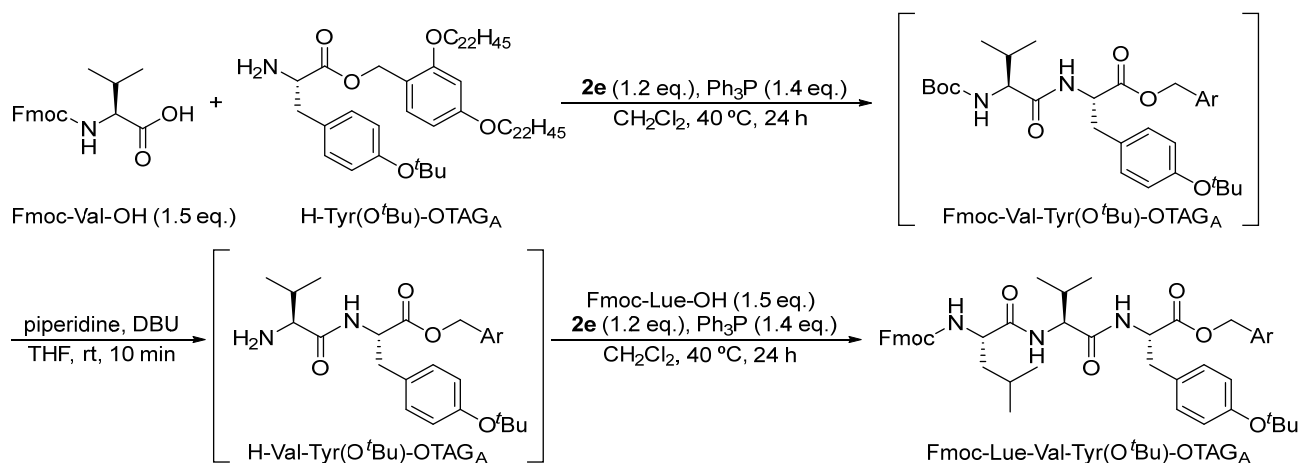
## 8. Preparation and Characterization of L-Valyl-L-Tyrosine



To a solution of **2e** (108.5 mg, 0.24 mmol) in  $\text{CH}_2\text{Cl}_2$  (5.0 mL), Boc-Val-OH (65.2 mg, 0.3 mmol), H-Tyr(O<sup>t</sup>Bu)-OTAG<sub>A</sub> (198.0 mg, 0.2 mmol) and Ph<sub>3</sub>P (73.4 mg, 0.28 mmol) were added successively. After being stirred at 40 °C for 24 h, the white precipitate was filtered out and washed with DCM to give **3e** (106.0 mg, 97%). And then the filtrate was concentrated to dryness in vacuo. The resulting residue was washed with MeCN and added to the solution of triisopropylsilane (125 μL, 0.6 mmol) and H<sub>2</sub>O (125 μL) in trifluoroacetic acid (TFA, 4.75 mL). After being stirred at room temperature for 4 h, the reaction solution was concentrated to dryness in vacuo. The hydrochloric acid solution of the resulting residue was filtered and concentrated to dryness in vacuo to give H-Val-Tyr-OH (60.3 mg, 95%) as a whit solid.

**(S)-1-[(S)-1-Carboxy-2-(4-hydroxyphenyl)ethyl]amino}-3-methyl-1-oxobutan-2-aminium chloride (H-Val-Tyr-OH·HCl):** <sup>1</sup>H-NMR (600 MHz, D<sub>2</sub>O) δ ppm: 7.10 (d, *J* = 8.9 Hz, 2H), 6.78 (d, *J* = 8.9 Hz, 2H), 4.58 (dd, *J* = 8.3, 6.2 Hz, 1H), 3.72 (d, *J* = 6.9 Hz, 1H), 3.08 (dd, *J* = 14.4, 6.2 Hz, 1H), 2.95 (dd, *J* = 14.4, 8.3 Hz, 1H), 2.13 (d septet, *J* = 6.9, 6.9 Hz, 1H), 0.93 (d, *J* = 6.9 Hz, 3H), 0.91 (d, *J* = 6.9 Hz, 3H). <sup>13</sup>C-NMR (150 MHz, D<sub>2</sub>O) δ ppm: 177.4, 172.1, 157.5, 133.5, 131.1, 118.5, 61.2, 57.5, 38.4, 33.2, 20.5, 19.6. The <sup>13</sup>C NMR spectra of the product were identical to those reported in the literature.<sup>10</sup>

## 9. Preparation and Characterization of Tag-Protected Tripeptides



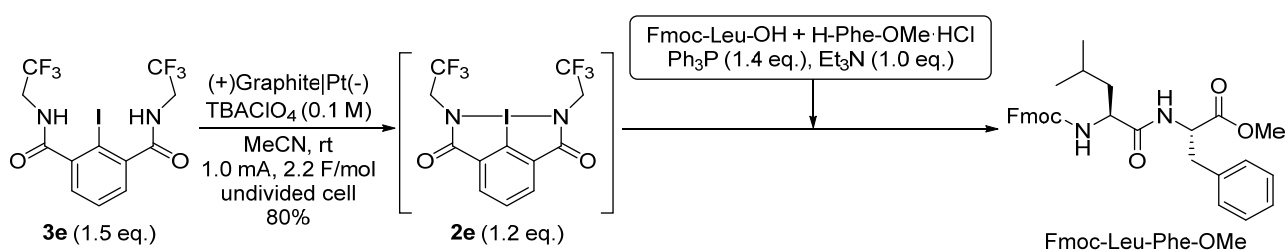
To a solution of **2e** (108.5 mg, 0.24 mmol) in  $\text{CH}_2\text{Cl}_2$  (5.0 mL), Fmoc-Val-OH (101.8 mg, 0.3 mmol), H-Tyr(O<sup>t</sup>Bu)-OTAG<sub>A</sub> (198.0 mg, 0.2 mmol) and Ph<sub>3</sub>P (73.4 mg, 0.28 mmol) were added successively. After being stirred at 40 °C for 24 h, the white precipitate was filtered out and washed with DCM to give **3e** (99.2 mg, 91%). And then the filtrate was concentrated to dryness in vacuo. The resulting residue was washed with MeCN and dissolved in THF (28 mL). To the solution, piperidine (278 μL, 2.79 mmol) and DBU (278 μL, 1.85 mmol) were added at room temperature. After being stirred at same temperature for 10 min, the reaction solution was neutralized with 6M HCl to pH 7.0. and then MeCN was added until white solids precipitate. The remaining solids were washed with MeCN and added to a solution of **2e** (89.3 mg, 0.24 mmol) and Fmoc-Lue-OH (106.0 mg, 0.3 mmol) in  $\text{CH}_2\text{Cl}_2$  (5.0 mL). And then, after the addition of Ph<sub>3</sub>P (73.4 mg, 0.28 mmol), the reaction mixture was stirred at 40 °C for 24 h. The white precipitate was filtered out and washed with DCM to give **3e** (108.0

<sup>10</sup> <sup>13</sup>C-NMR spectral data for H-Val-Tyr-OH were obtained from the National Institute of Advanced Industrial Science and Technology (Japan).

mg, 99%). The filtrate was concentrated to dryness in vacuo and the resulting residue was washed with MeCN to give Fmoc-Leu-Tyr(OtBu)-OTAG<sub>x</sub> (244.2 mg, 87%) as a whit solid.

**2,4-Bis(docosan-1-yloxy)benzyl (5*S*,8*S*,11*S*)-11-[4-(*tert*-butoxy)benzyl]-1-(9*H*-fluoren-9-yl)-5-isobutyl-8-isopropyl-3,6,9-trioxo-2-oxa-4,7,10-triazadodecan-12-oate (Fmoc-Leu-Val-Tyr(O*t*Bu)-OTAG<sub>A</sub>):** <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ ppm: 7.76 (d, *J* = 6.9 Hz, 2H), 7.58 (d, *J* = 6.4 Hz, 2H), 7.39 (dd, *J* = 6.9, 6.9 Hz, 2H), 7.30 (dd, *J* = 6.9, 6.4 Hz, 2H), 7.14 (d, *J* = 8.2 Hz, 1H), 6.89 (d, *J* = 6.9 Hz, 2H), 6.81 (d, *J* = 6.9 Hz, 2H), 6.54 (br.s, 1H), 6.44 (s, 1H), 6.42 (d, *J* = 6.9 Hz, 1H), 6.20 (br.s, 1H), 5.37-5.20 (m, 1H), 5.17 (d, *J* = 11.4 Hz, 1H), 5.10 (d, *J* = 11.4 Hz, 1H), 4.95-4.76 (m, 1H), 4.52-4.34 (m, 2H), 4.30-4.13 (m, 3H), 4.04-3.83 (m, 4H), 3.16-2.95 (m, 2H), 2.12-1.98 (m, 1H), 1.86-1.72 (m, 4H), 1.70-1.59 (m, 4H), 1.50-1.20 (m, 84H), 1.03-0.78 (m, 18H). <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>) δ ppm: 172.3, 171.1, 170.4, 160.9, 158.4, 156.2, 154.3, 143.9, 143.7, 141.2, 131.5, 129.7, 129.4, 127.6, 127.0, 125.1, 124.0, 119.9, 115.7, 104.4, 99.7, 99.5, 78.1, 68.1, 68.0, 66.9, 62.8, 61.8, 58.3, 53.6, 53.1, 47.1, 41.4, 37.1, 31.9, 29.7, 29.3, 28.8, 26.0, 24.7, 23.0, 22.6, 22.0, 18.9, 18.1, 14.1 (note that the two methyl groups of the isobutyl group, two methyl groups of the isopropyl group and two carbons of fluorenyl group behave as non-equivalent functional groups and 35 peaks of the docosyl groups overlap with other carbons). HRMS (ESI) *m/z* calcd. for C<sub>90</sub>H<sub>143</sub>N<sub>3</sub>NaO<sub>9</sub><sup>+</sup> [*M* + Na]<sup>+</sup> 1433.0716, found 1433.0716.

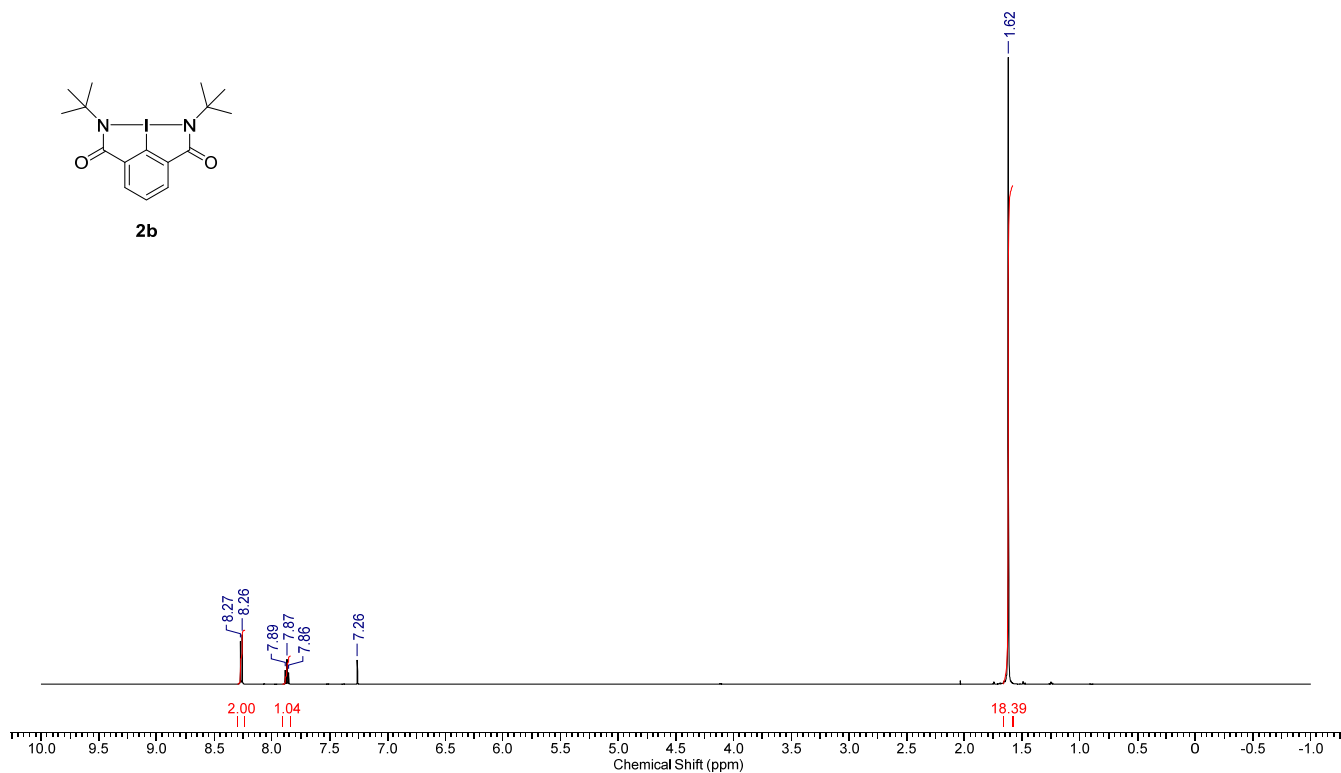
## 10. Peptide Coupling using in situ Electrochemically Generated Iodine(III) Reagent



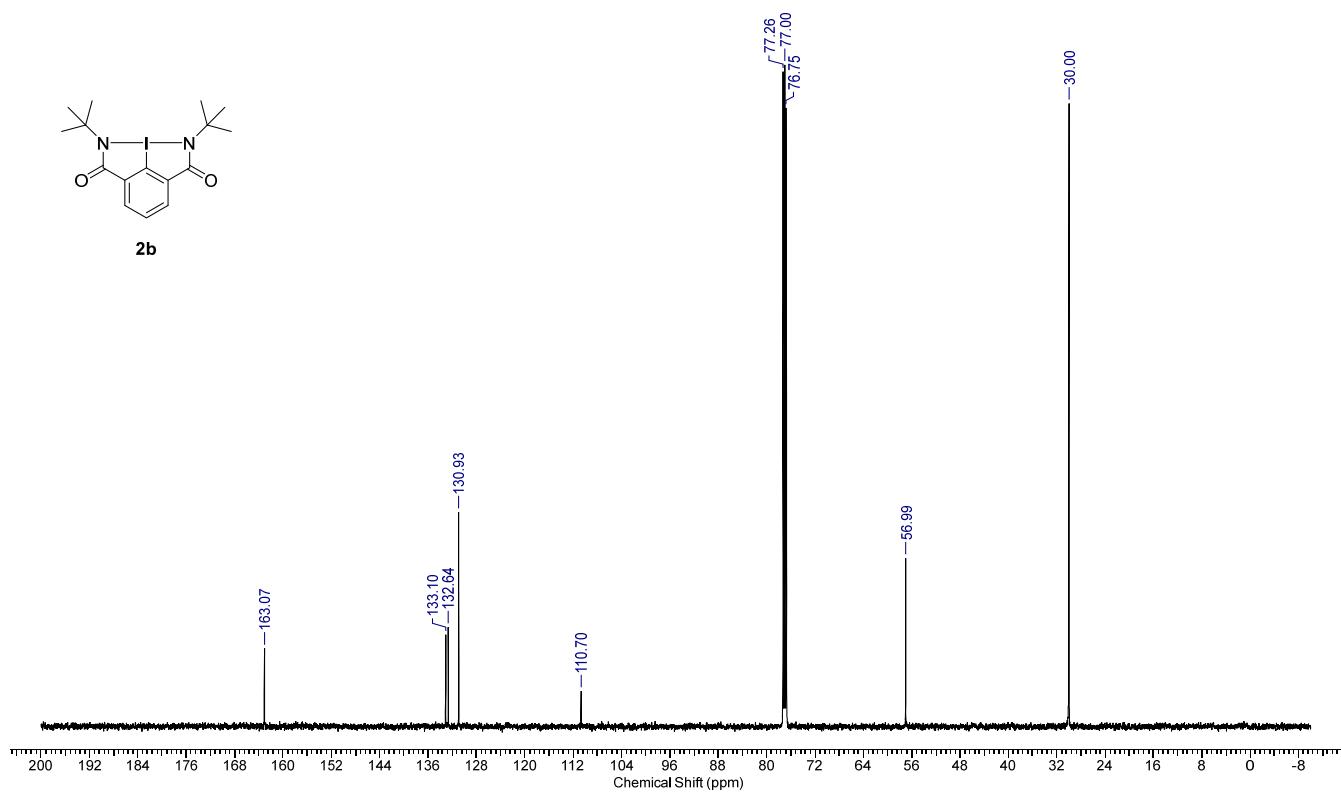
To a solvent of **3e** (90.8 mg, 0.2 mmol) in MeCN (5.0 mL), tetrabutylammonium perchlorate (170.9 mg, 0.5 mmol) was added. The solution was electrolyzed at constant current (1.0 mA/cm<sup>2</sup>) using graphite (anode) and platinum (cathode) electrodes at room temperature. After 2.2 F/mol of electricity based on **3e** was passed, Et<sub>3</sub>N (18.1 μL, 0.13 mmol), Fmoc-Leu-OH (46.0 mg, 0.13 mmol), H-Phe-OMe·HCl (28.0 mg, 0.13 mmol) and Ph<sub>3</sub>P (47.7 mg, 0.18 mmol) were added successively. The resulting reaction mixture was stirred at 25 °C for 24 h, quenched with sat. NaHCO<sub>3</sub> aq. and extracted with AcOEt. The organic layer was washed with sat. NH<sub>4</sub>Cl aq. and dried over MgSO<sub>4</sub>. After concentration of the filtrate in vacuo to dryness, the residue was washed with Et<sub>2</sub>O to give **3e** (68.1 mg, 75%). The remaining solution was concentrated in vacuo to dryness and then the residue was purified by silica gel chromatography (hexane:AcOEt = 2:1) to afford Fmoc-Leu-Phe-OMe (44.2 mg, 66%).

## 11. $^1\text{H}$ and $^{13}\text{C}$ NMR Spectra of 2b-2e and Dipeptides

$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ) of **2b**

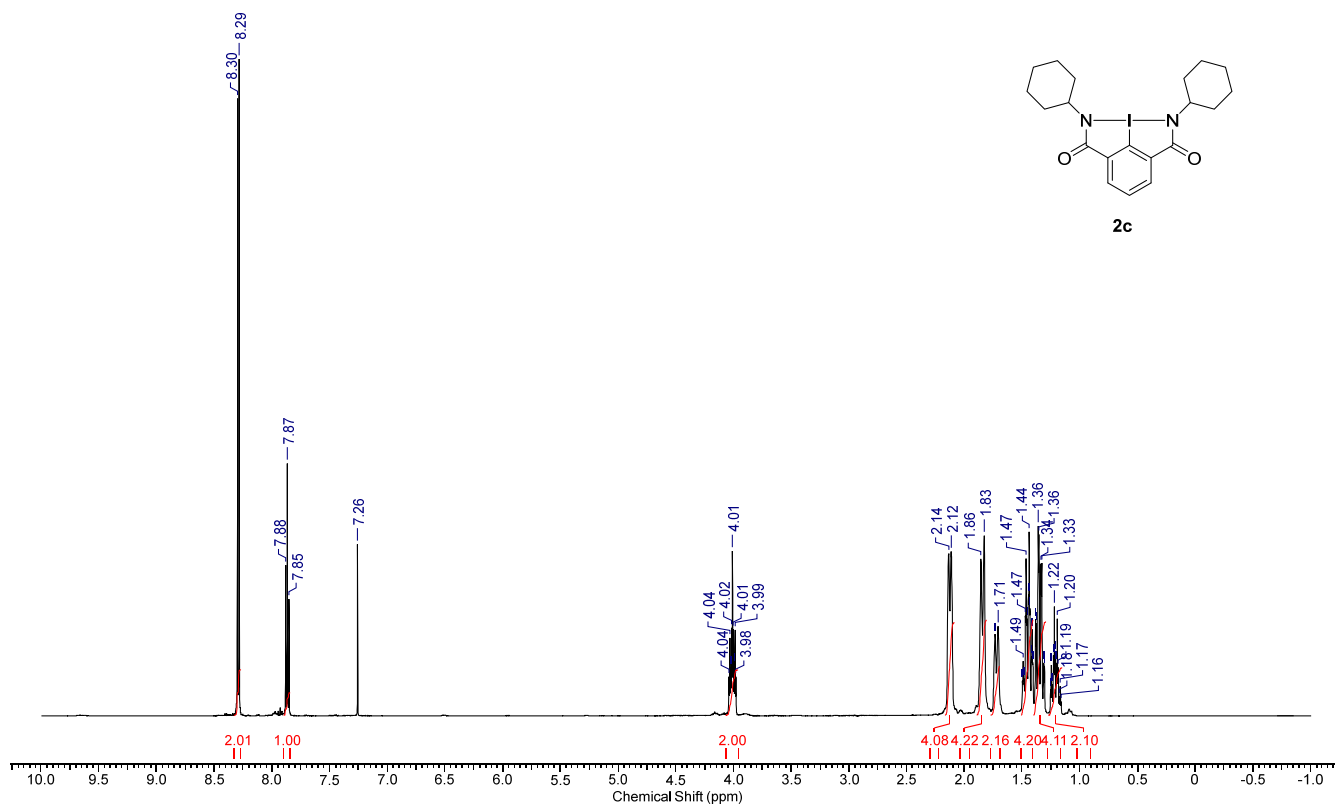


$^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ) of **2b**

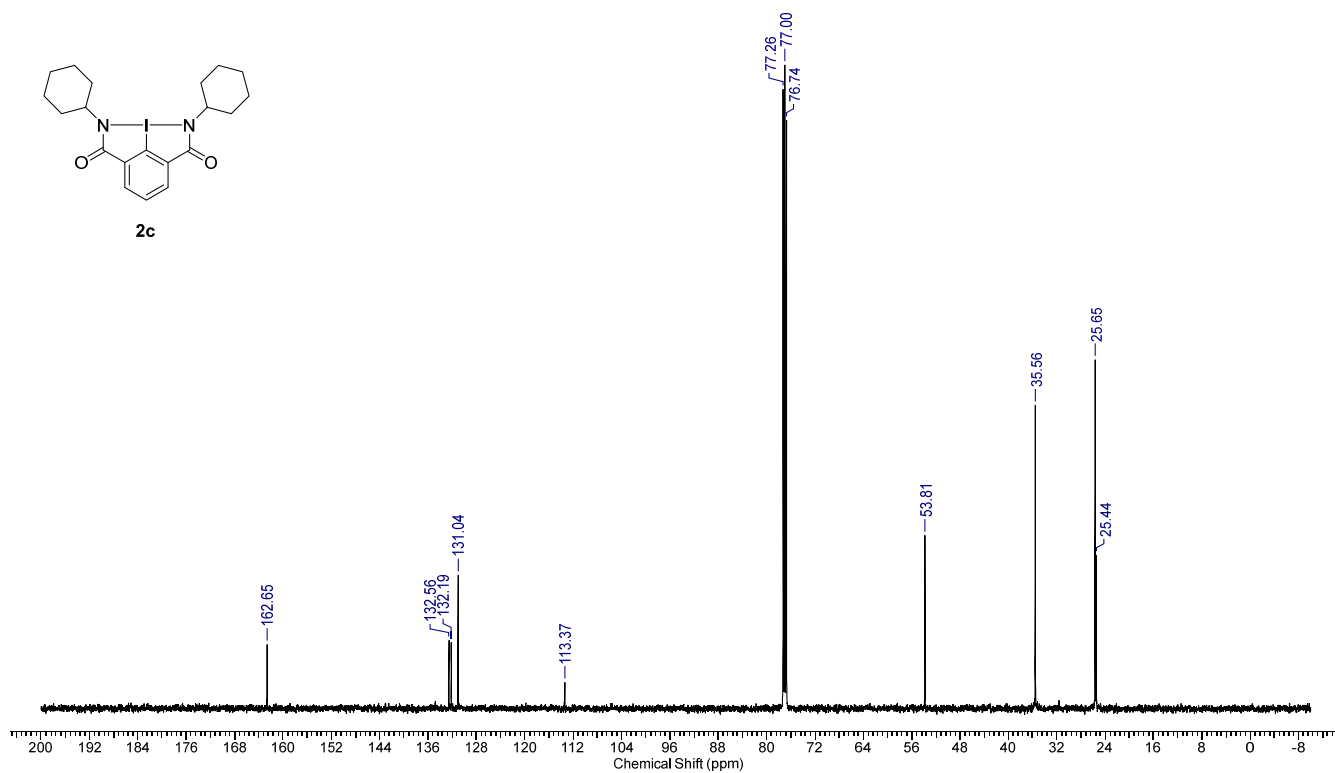




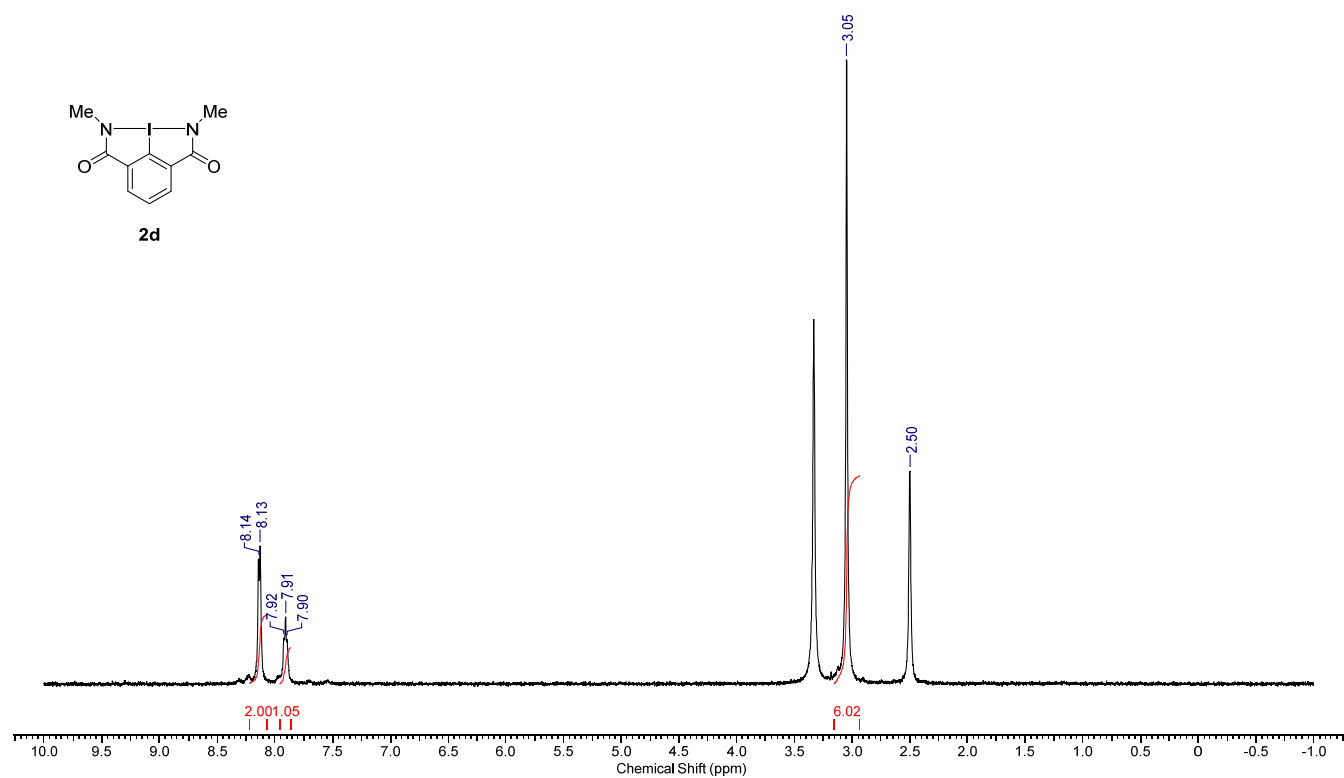
<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) of **2c**



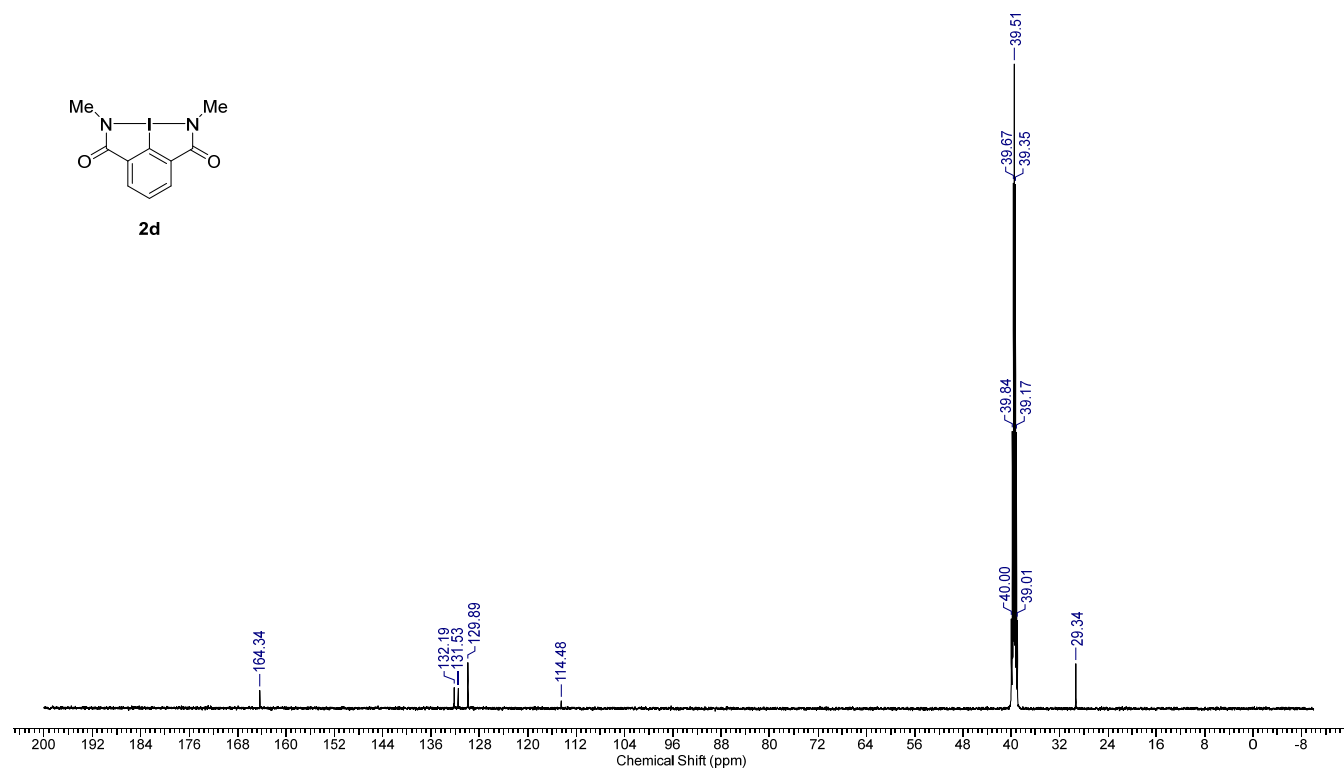
<sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) of **2c**



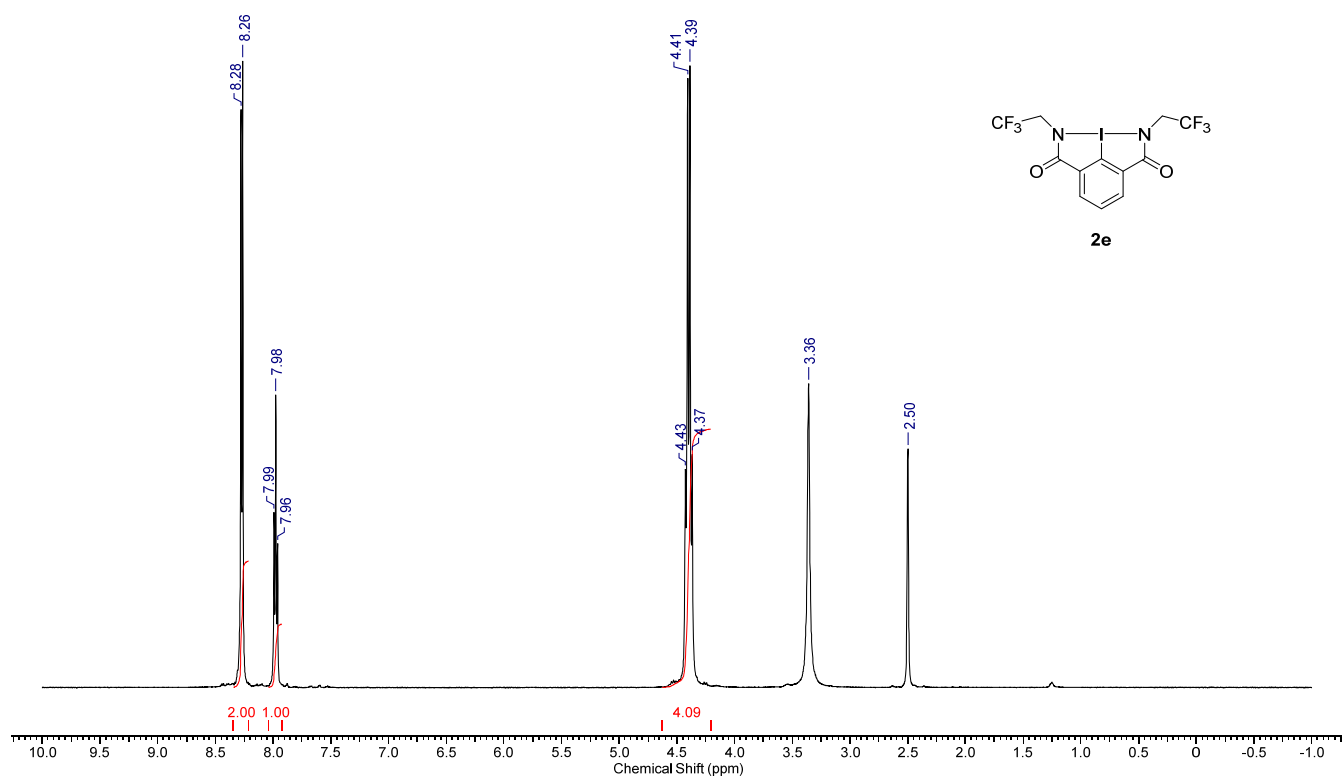
<sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) of **2d**



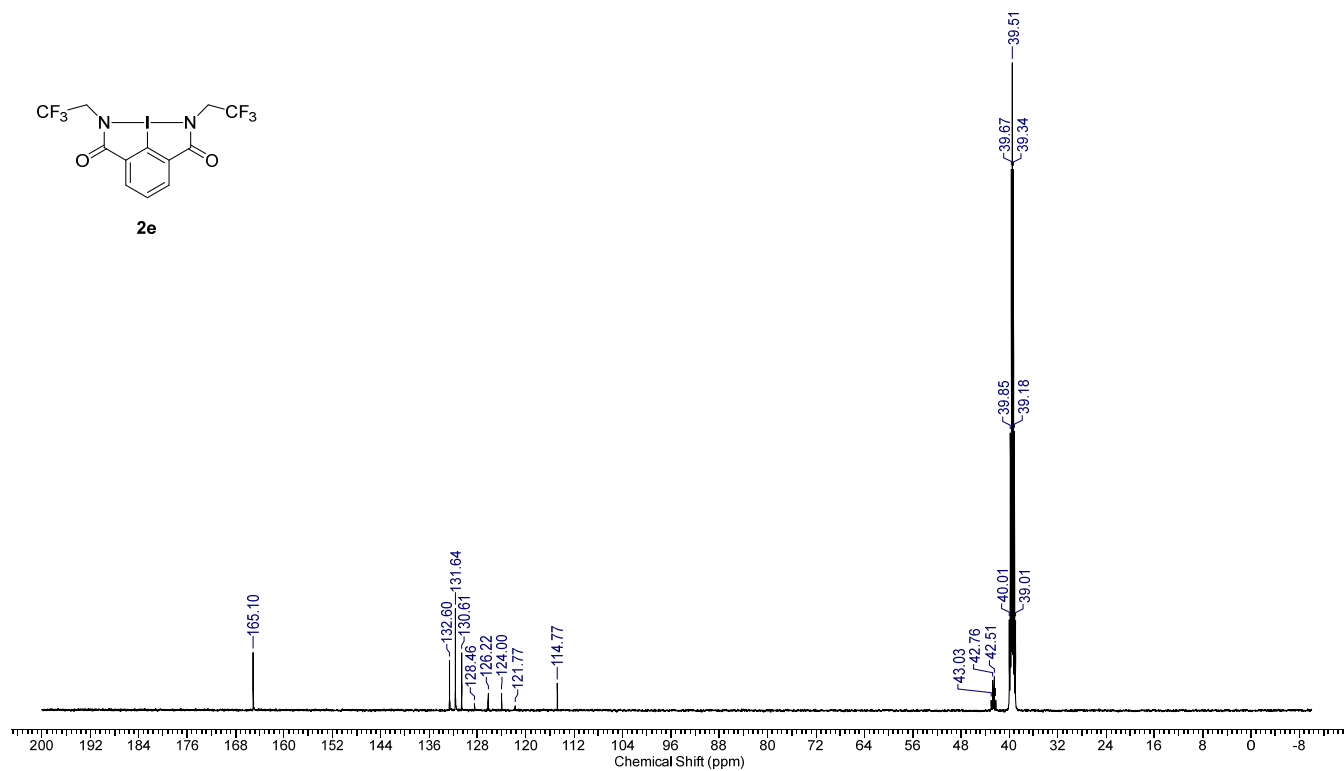
<sup>13</sup>C NMR (125 MHz, DMSO-*d*<sub>6</sub>) of **2d**



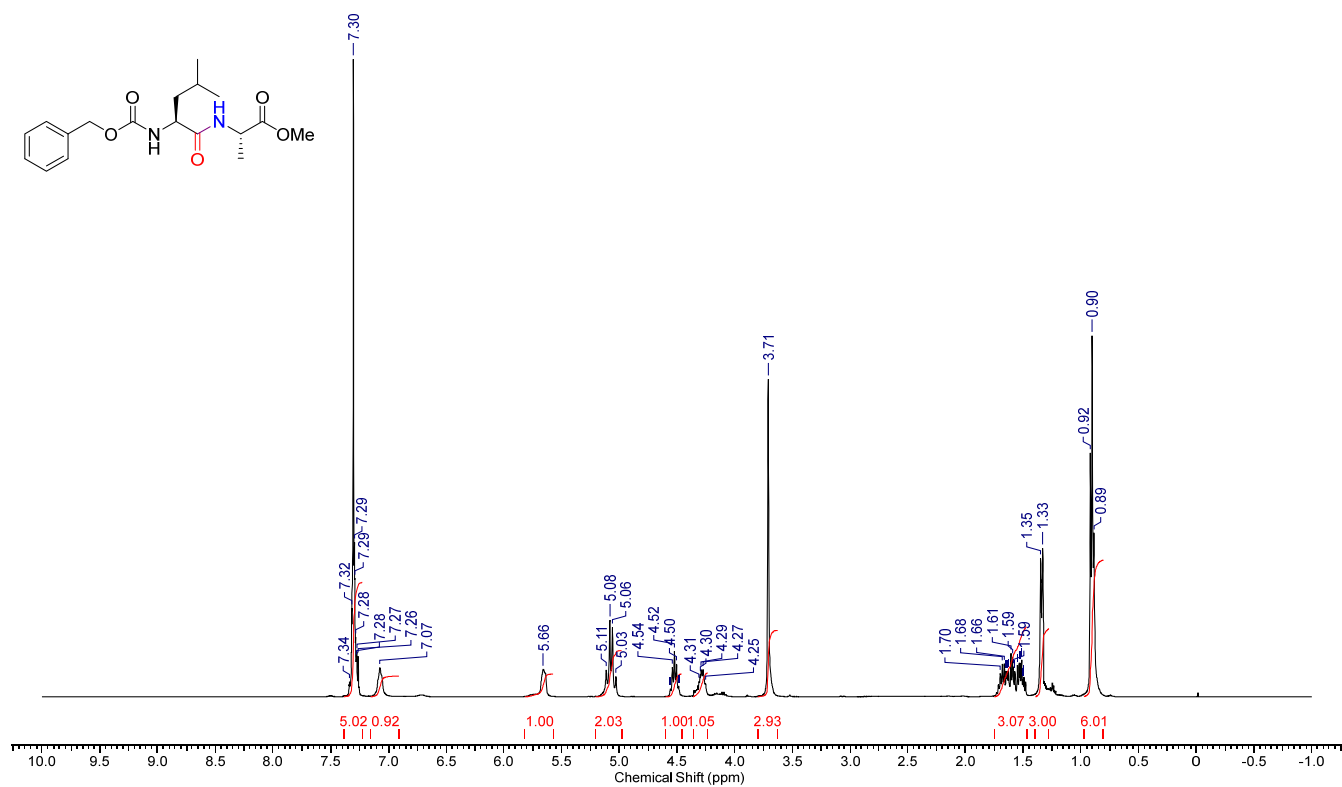
$^1\text{H}$  NMR (500 MHz,  $\text{DMSO-}d_6$ ) of **2e**



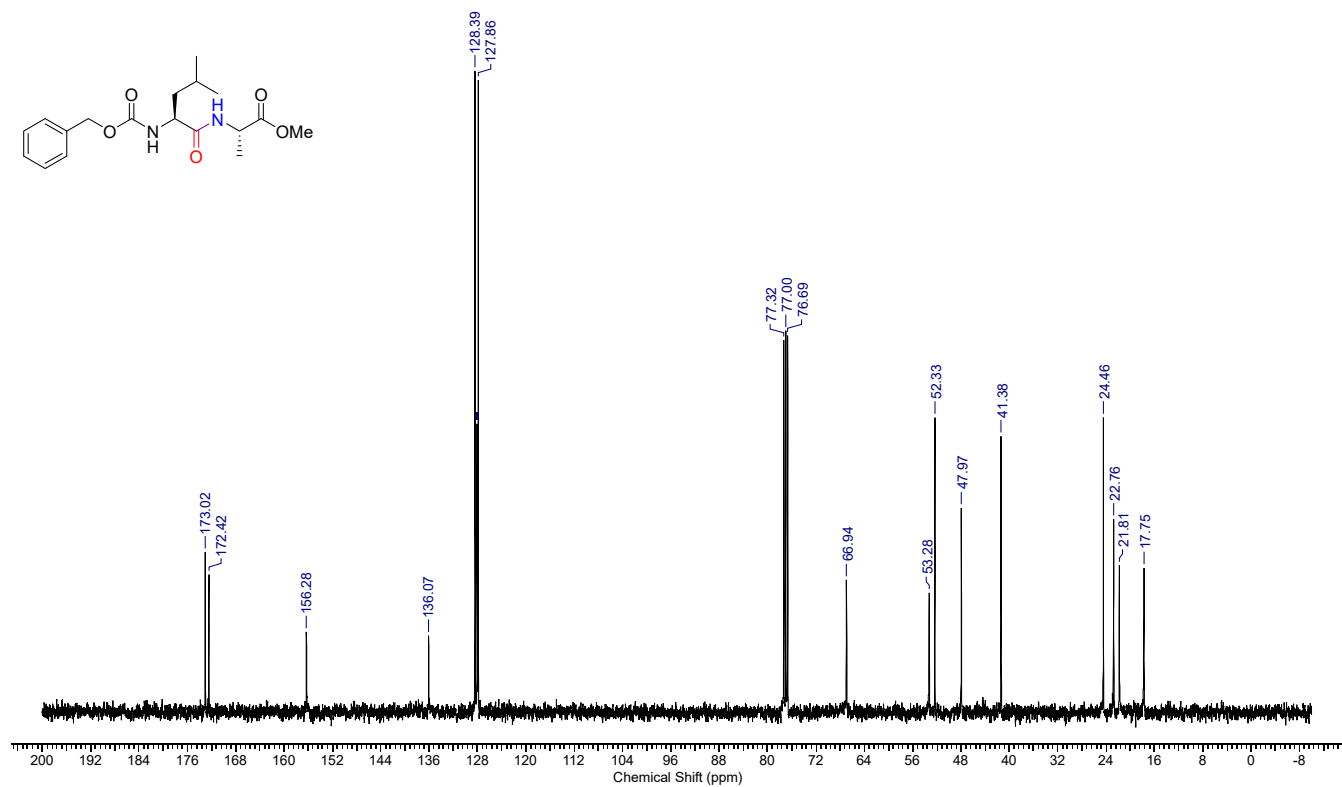
$^{13}\text{C}$  NMR (125 MHz,  $\text{DMSO-}d_6$ ) of **2e**



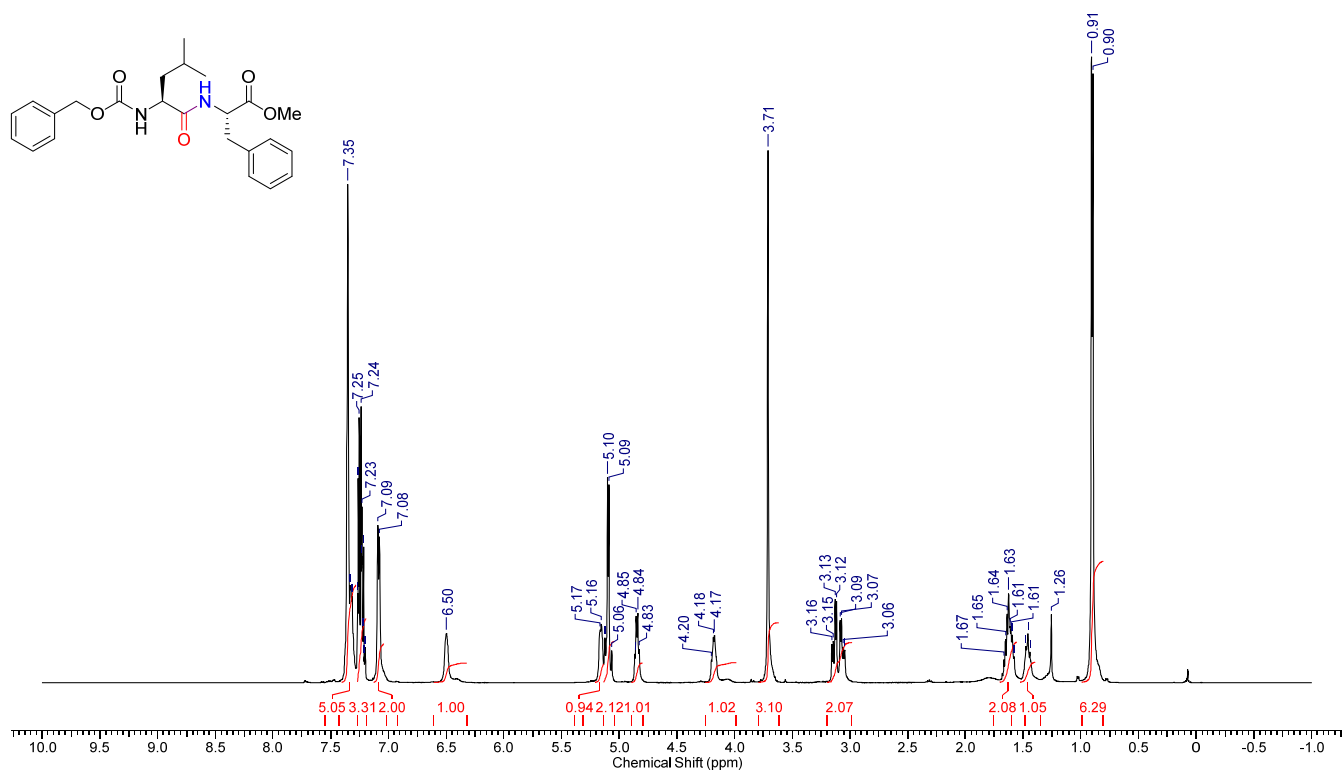
<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) of Cbz-Leu-Ala-OMe



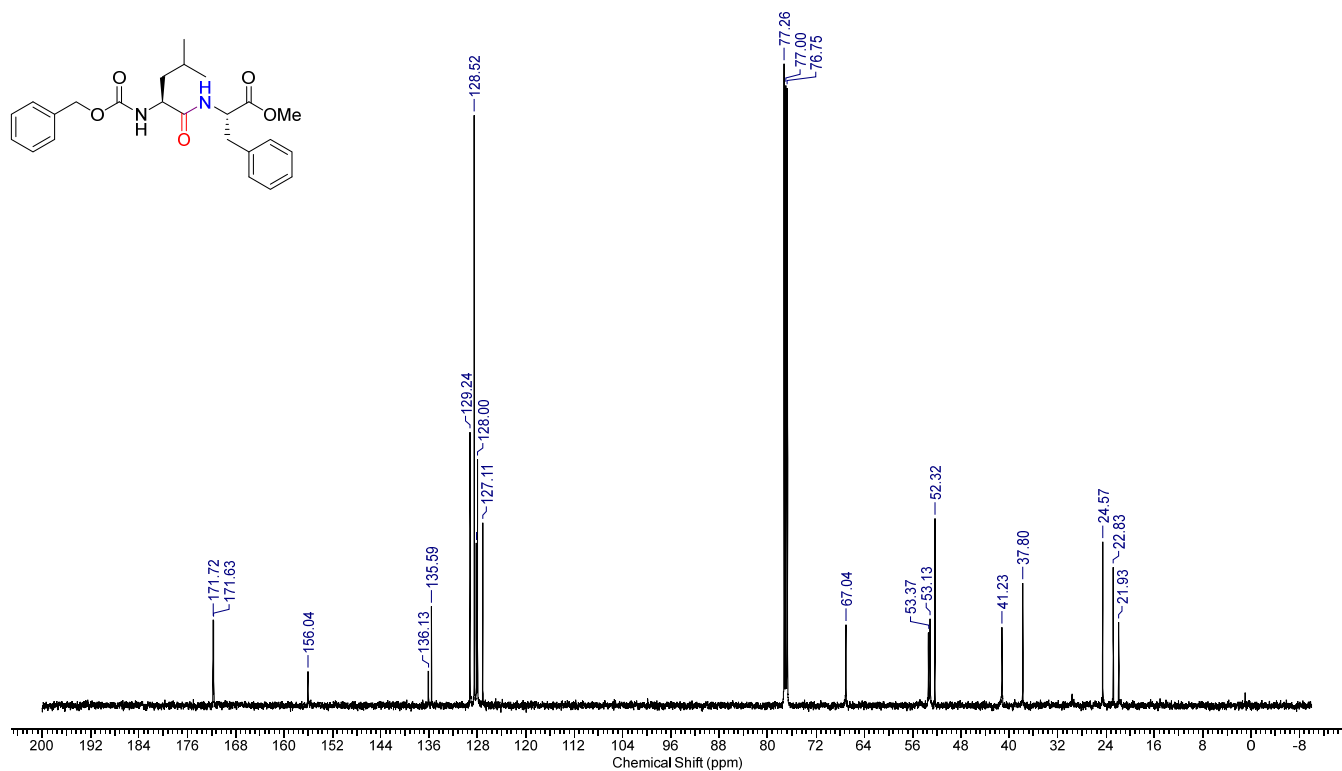
<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) of Cbz-Leu-Ala-OMe



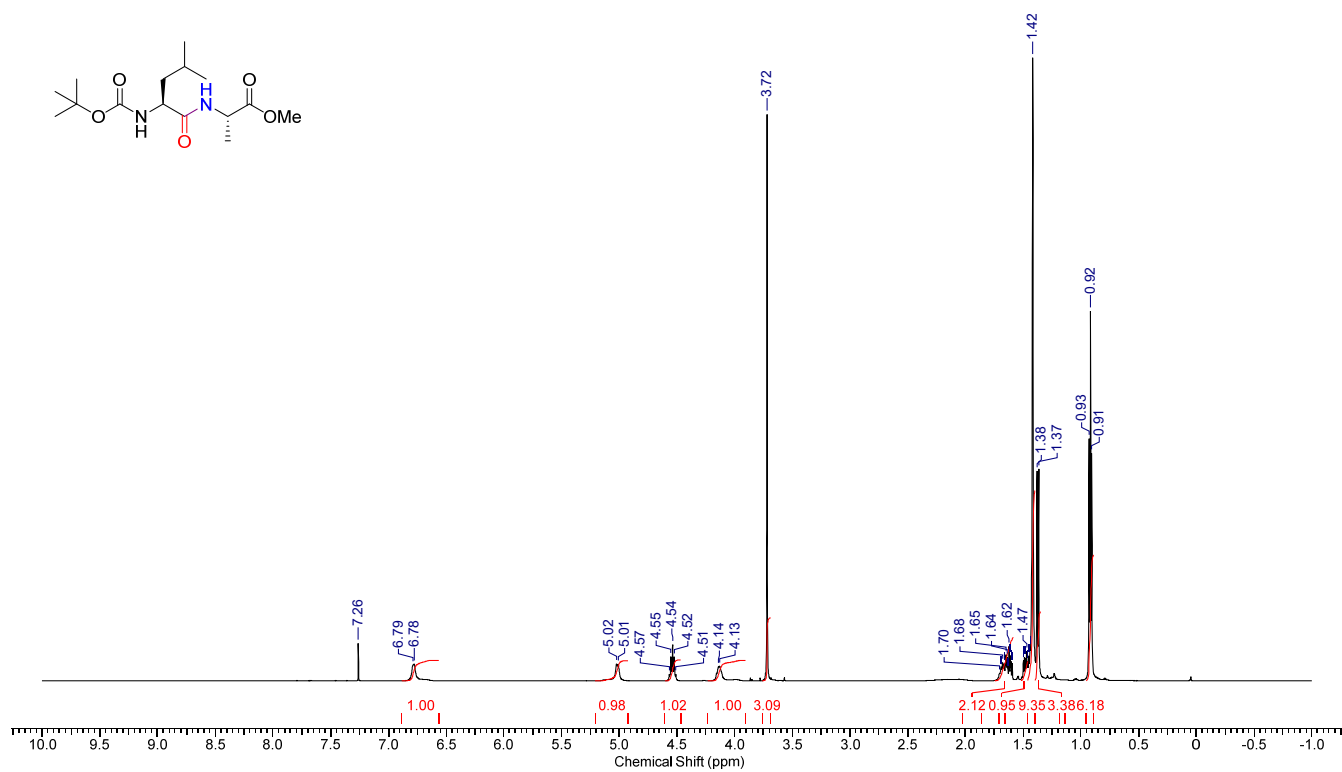
<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) of Cbz-Leu-Phe-OMe



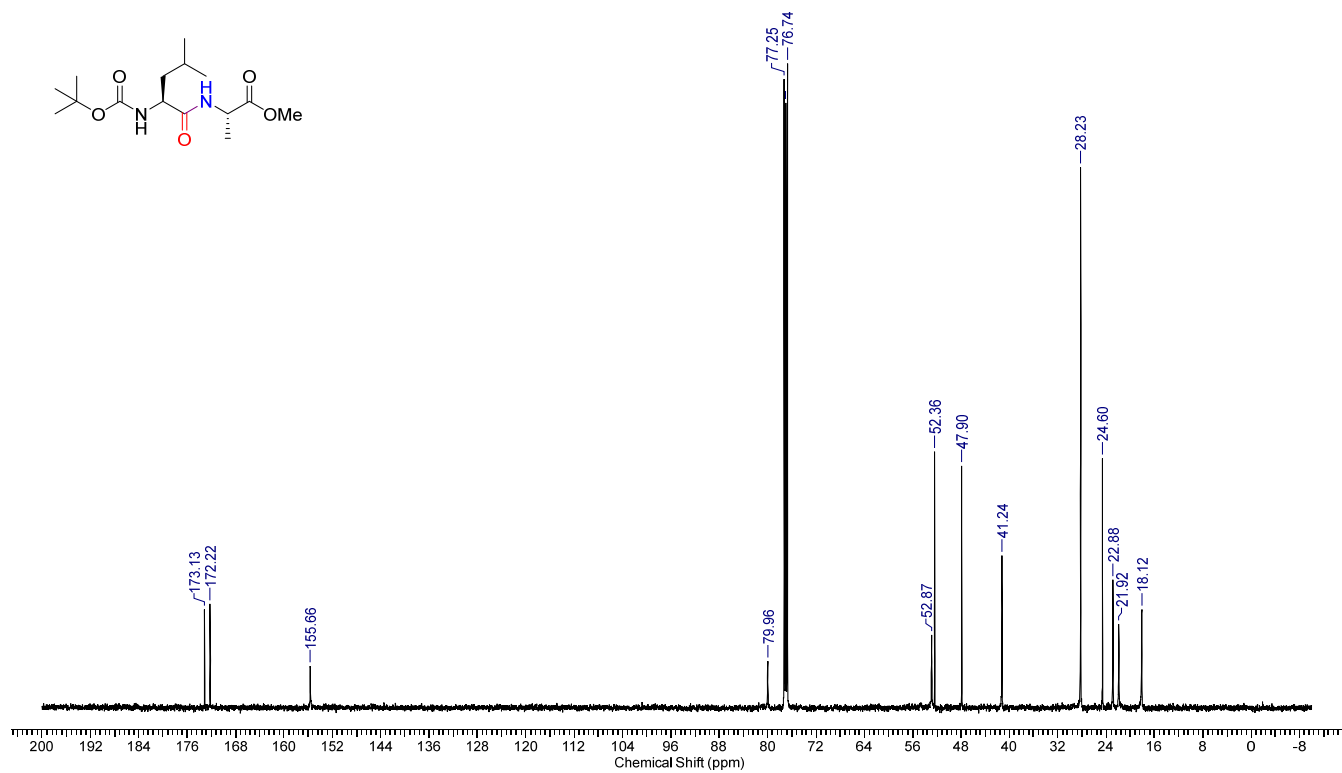
<sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) of Cbz-Leu-Phe-OMe



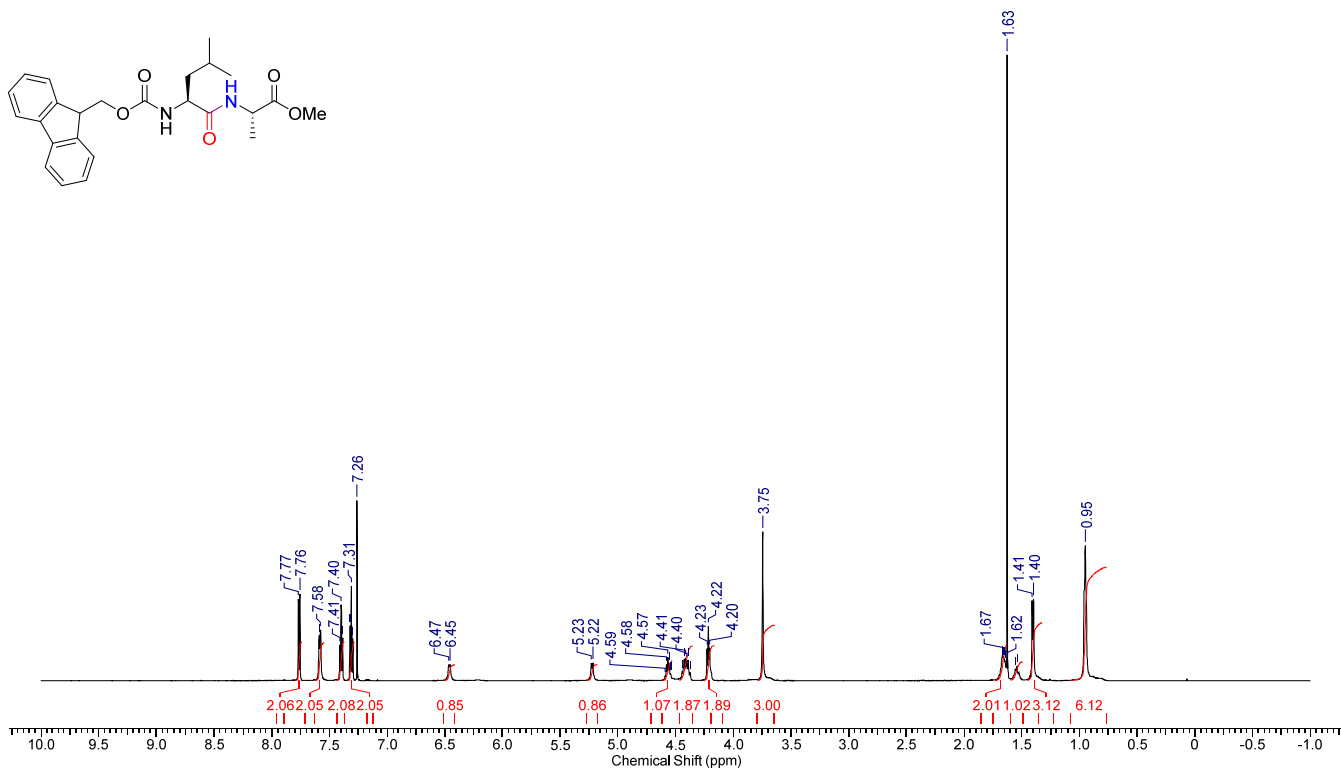
<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) of Boc-Leu-Ala-OMe



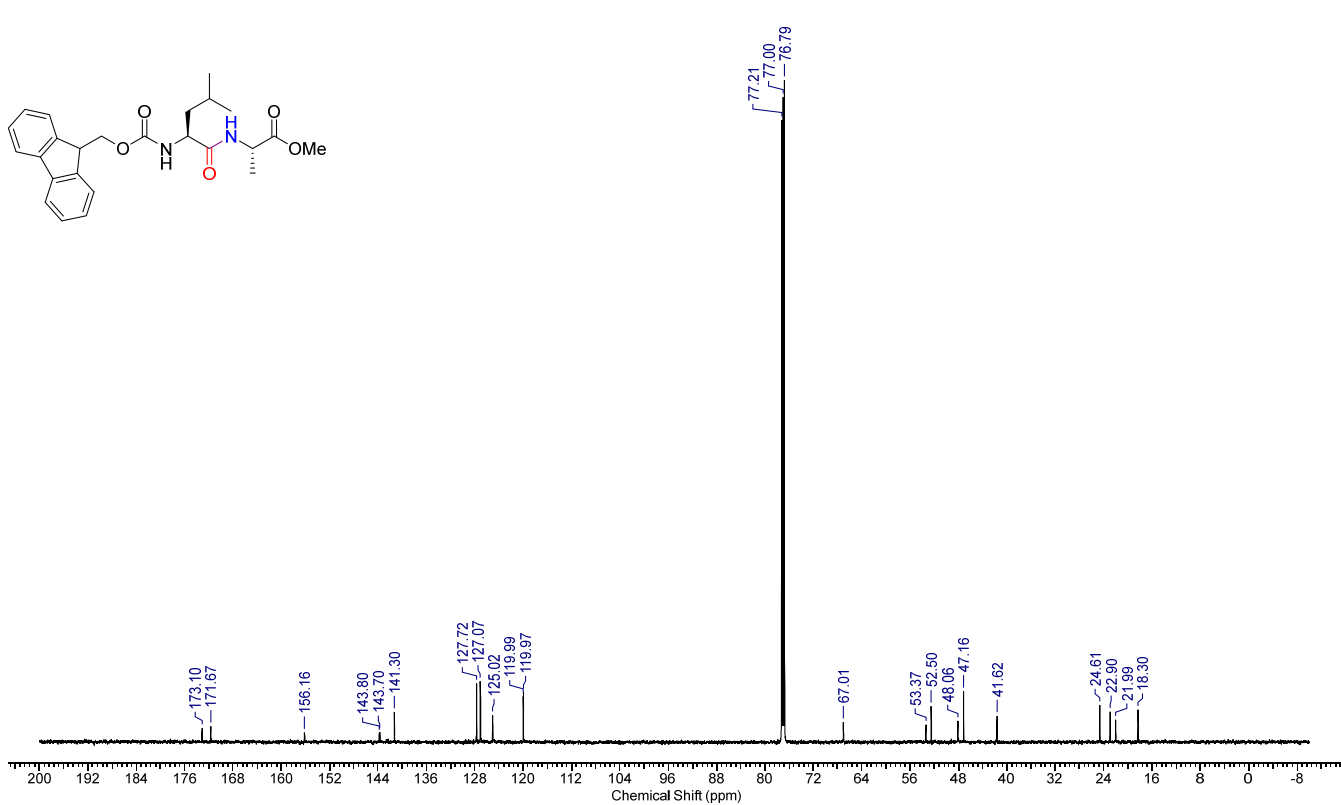
<sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) of Boc-Leu-Ala-OMe



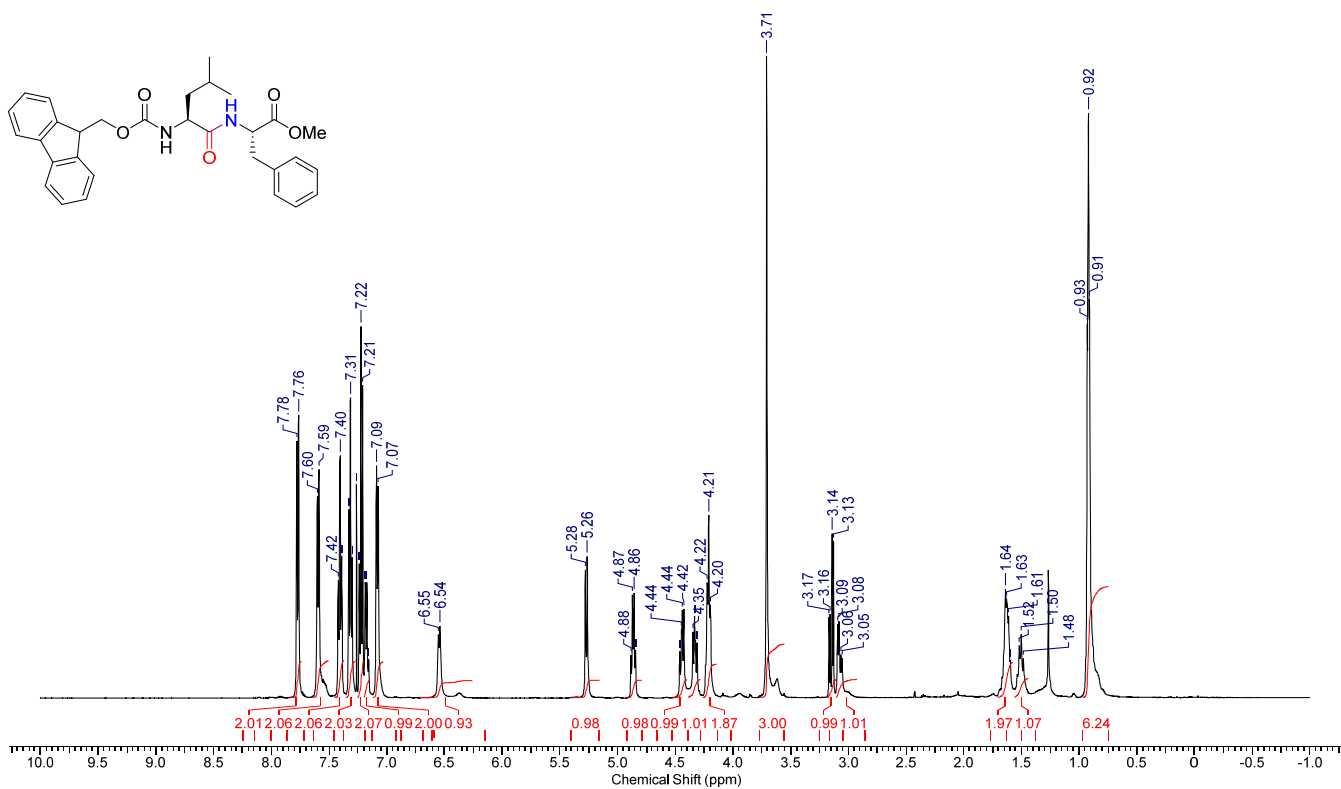
<sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) of Fmoc-Leu-Ala-OMe



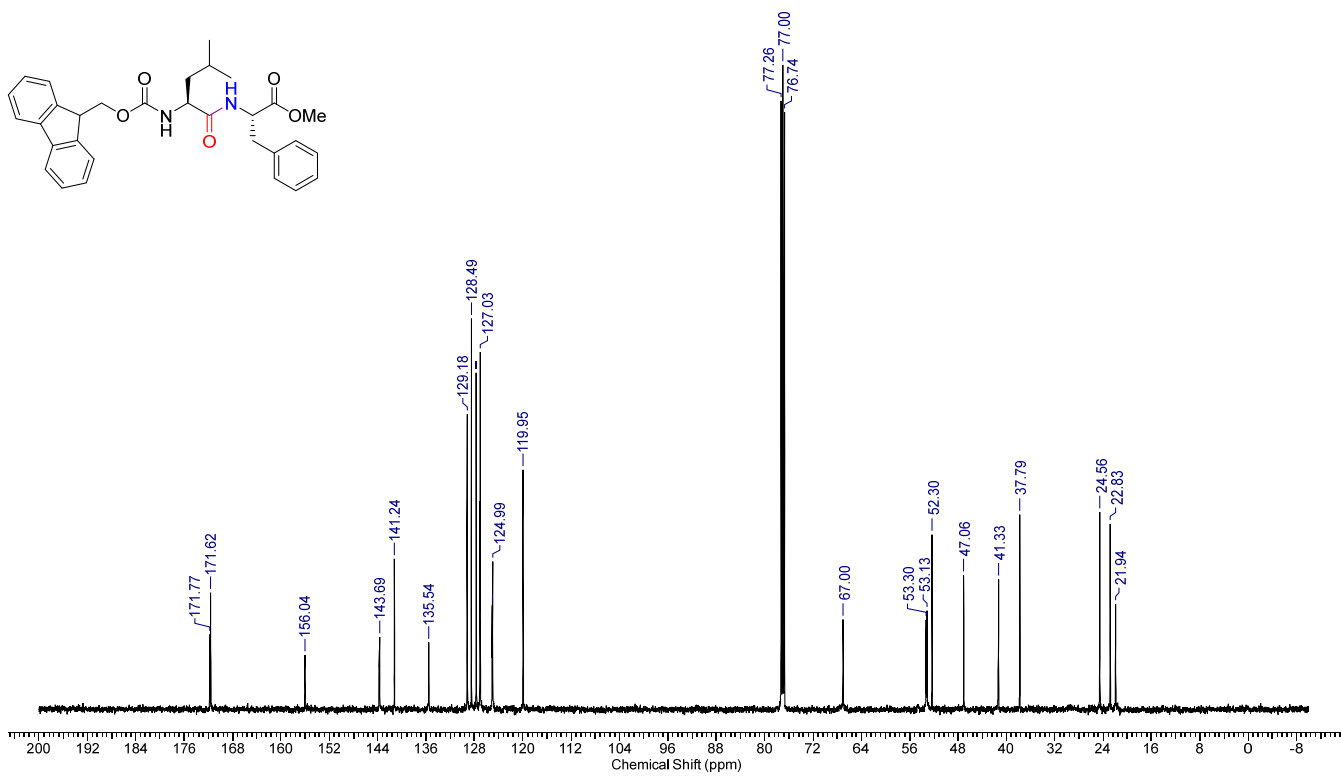
<sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) of Fmoc-Leu-Ala-OMe



<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) of Fmoc-Leu-Phe-OMe

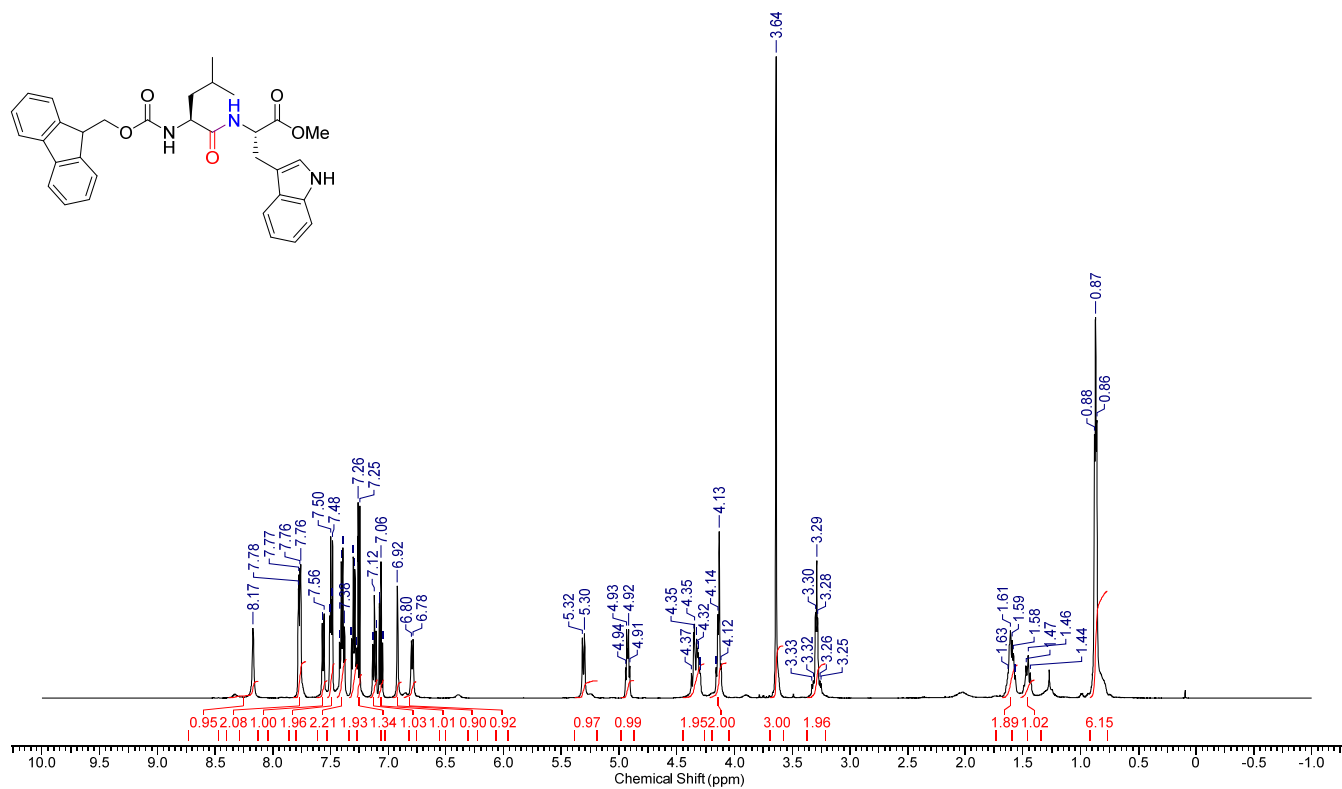


<sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) of Fmoc-Leu-Phe-OMe

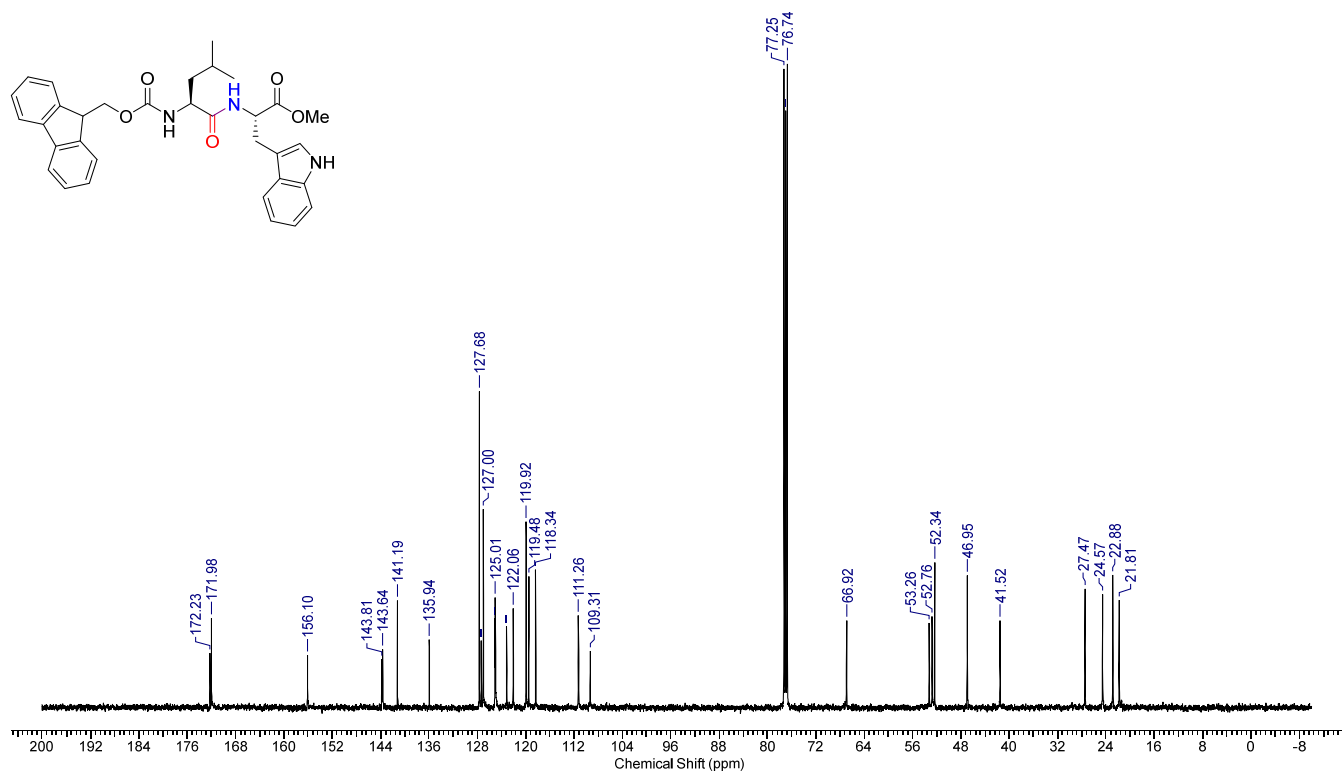




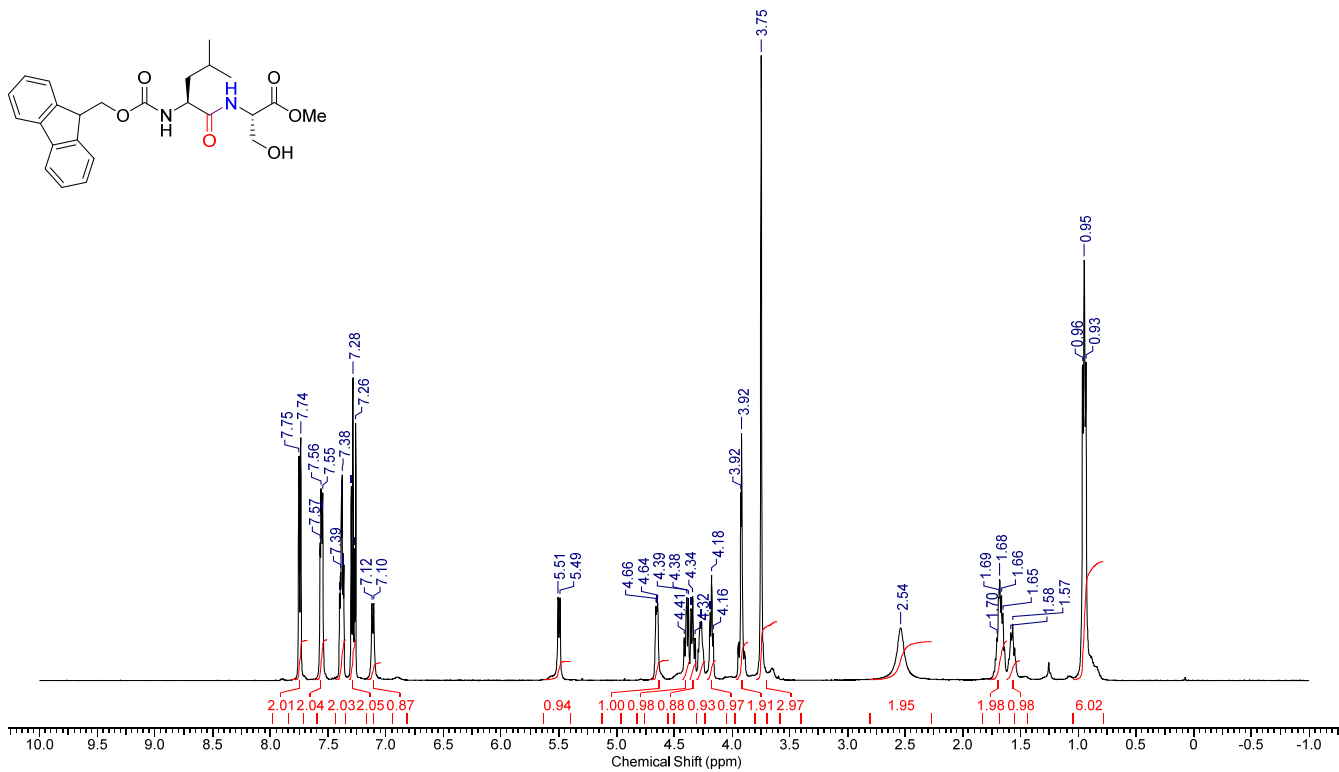
<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) of Fmoc-Leu-Trp-OMe



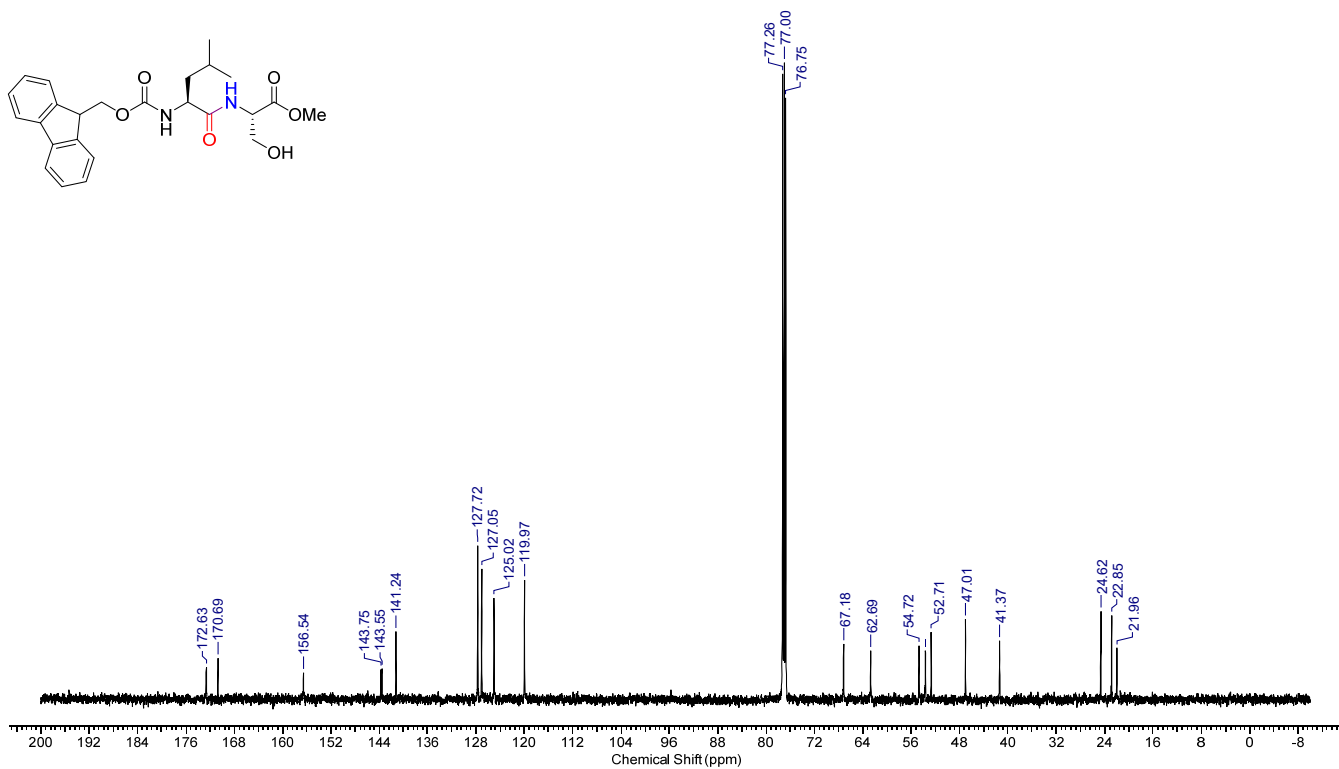
<sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) of Fmoc-Leu-Trp-OMe



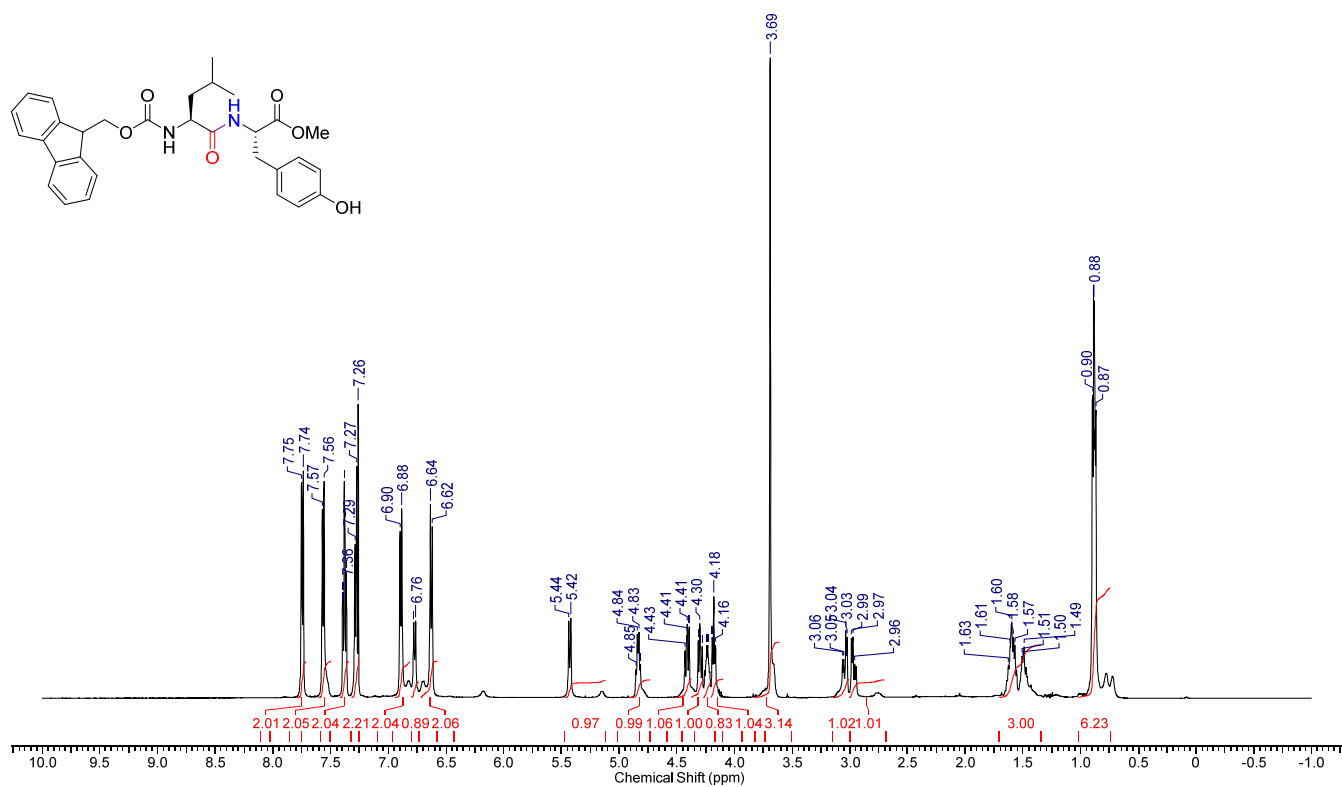
<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) of Fmoc-Leu-Ser-OMe



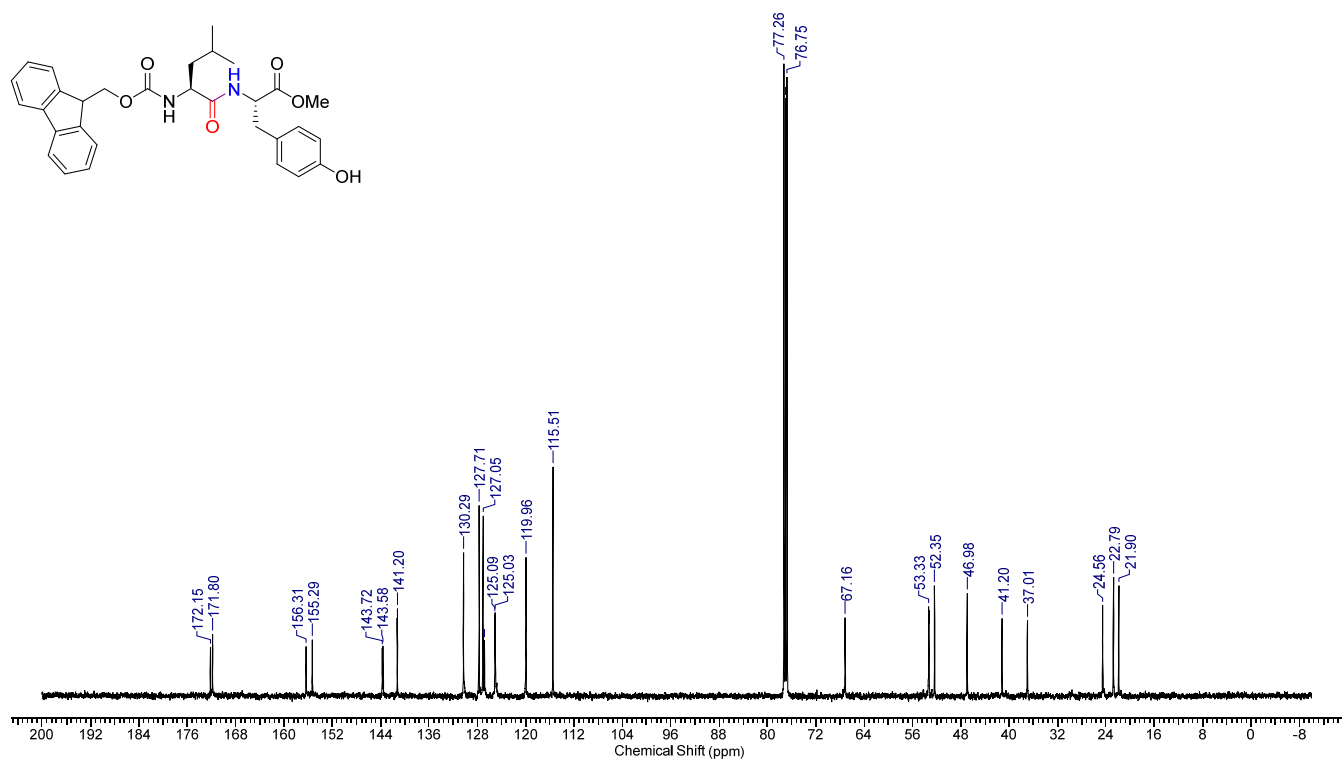
<sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) of Fmoc-Leu-Ser-OMe



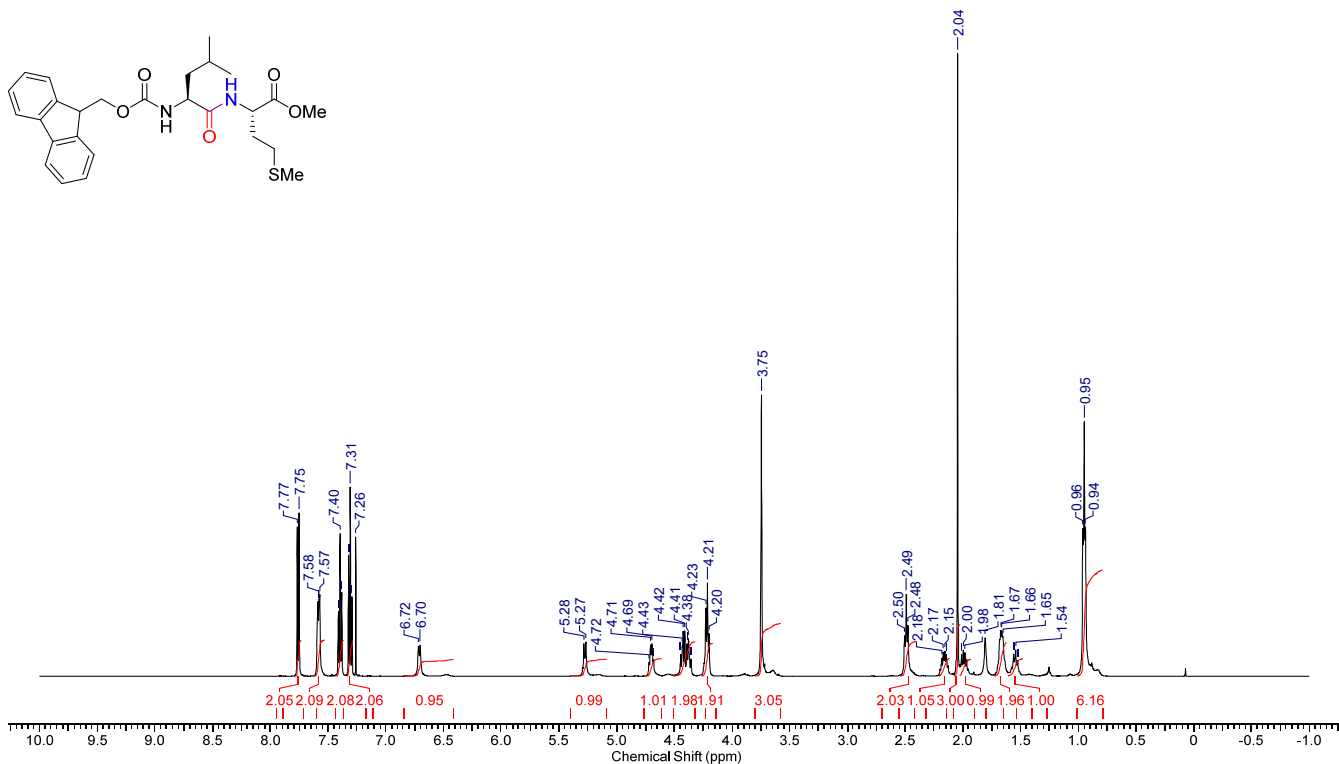
<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) of Fmoc-Leu-Tyr-OMe



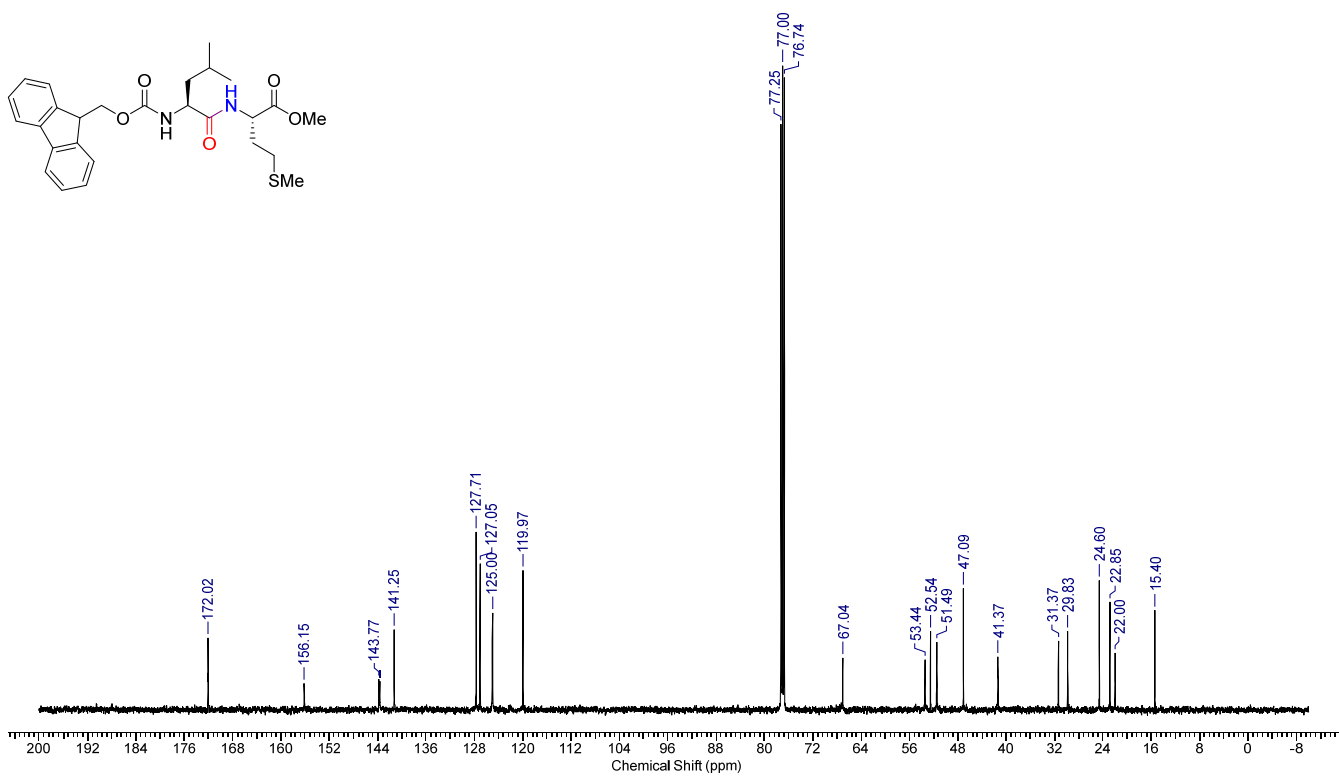
<sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) of Fmoc-Leu-Tyr-OMe



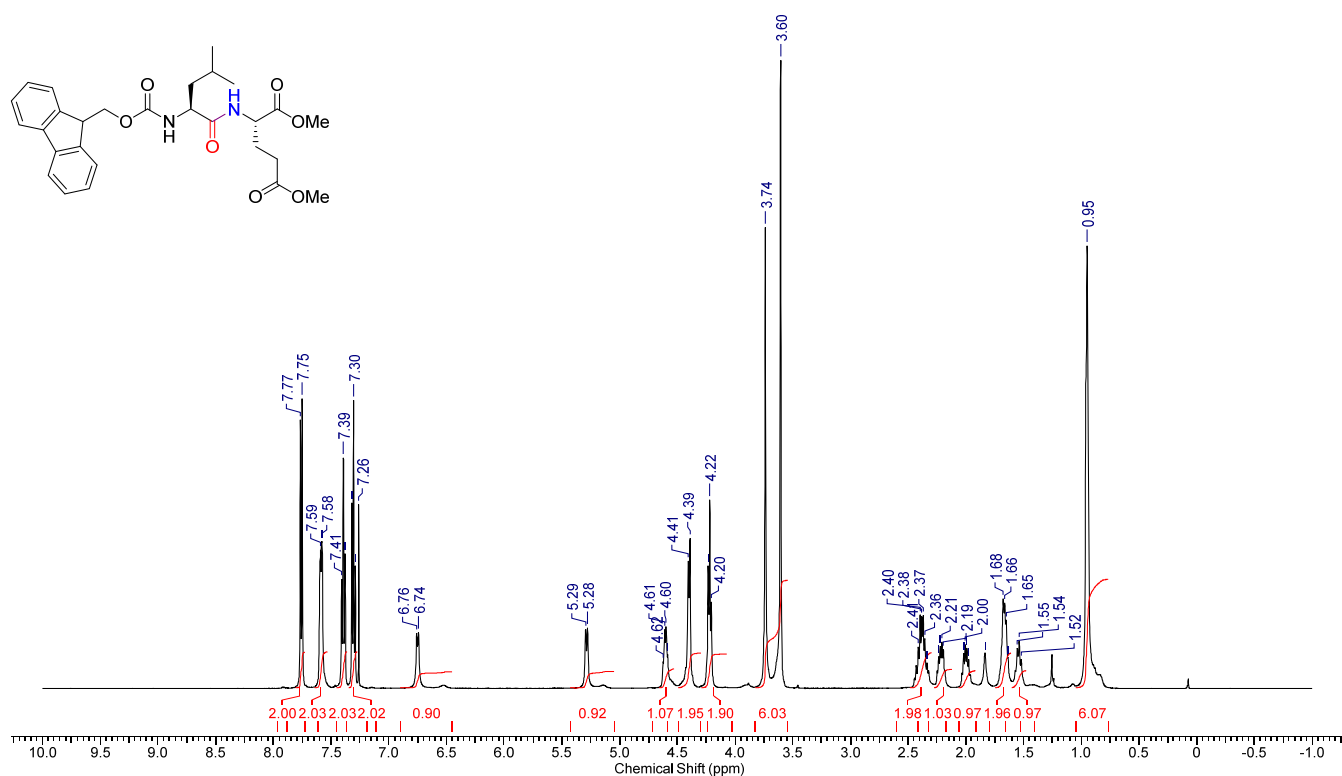
<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) of Fmoc-Leu-Met-OMe



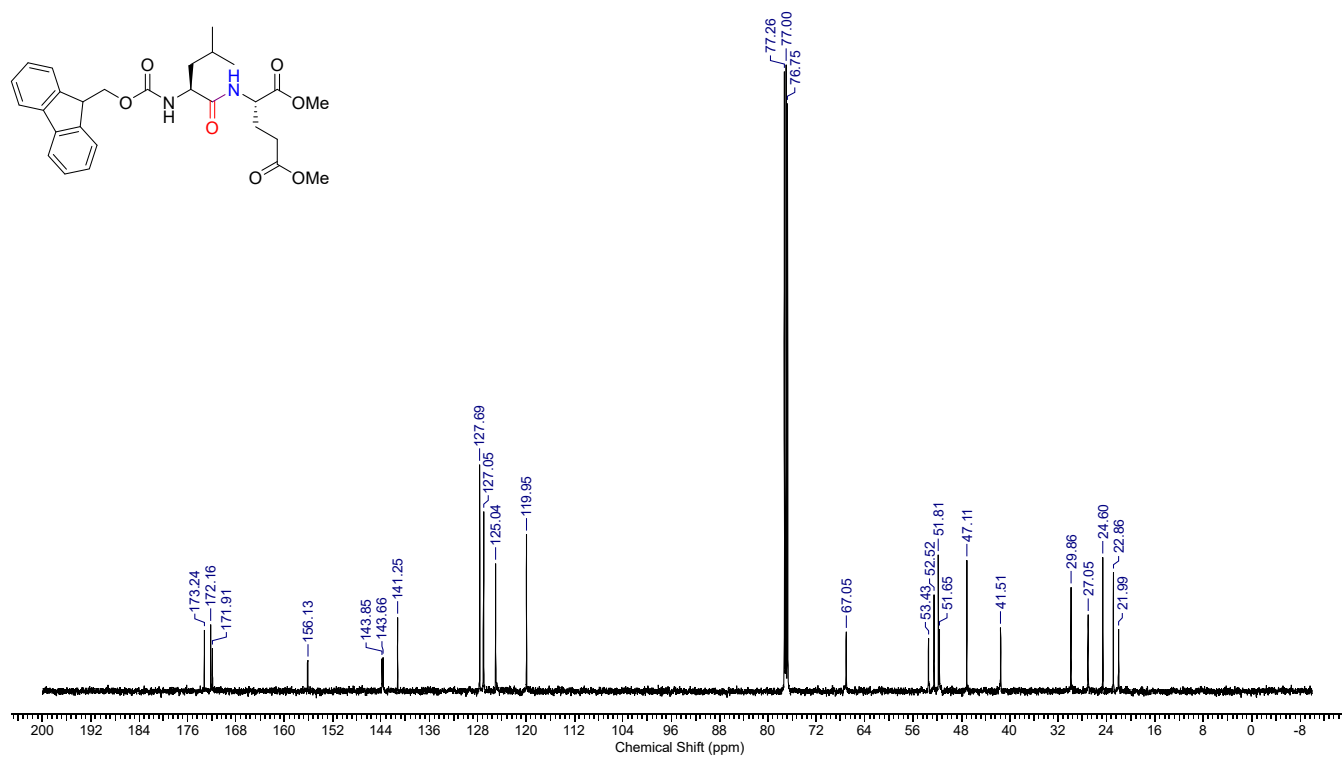
<sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) of Fmoc-Leu-Met-OMe



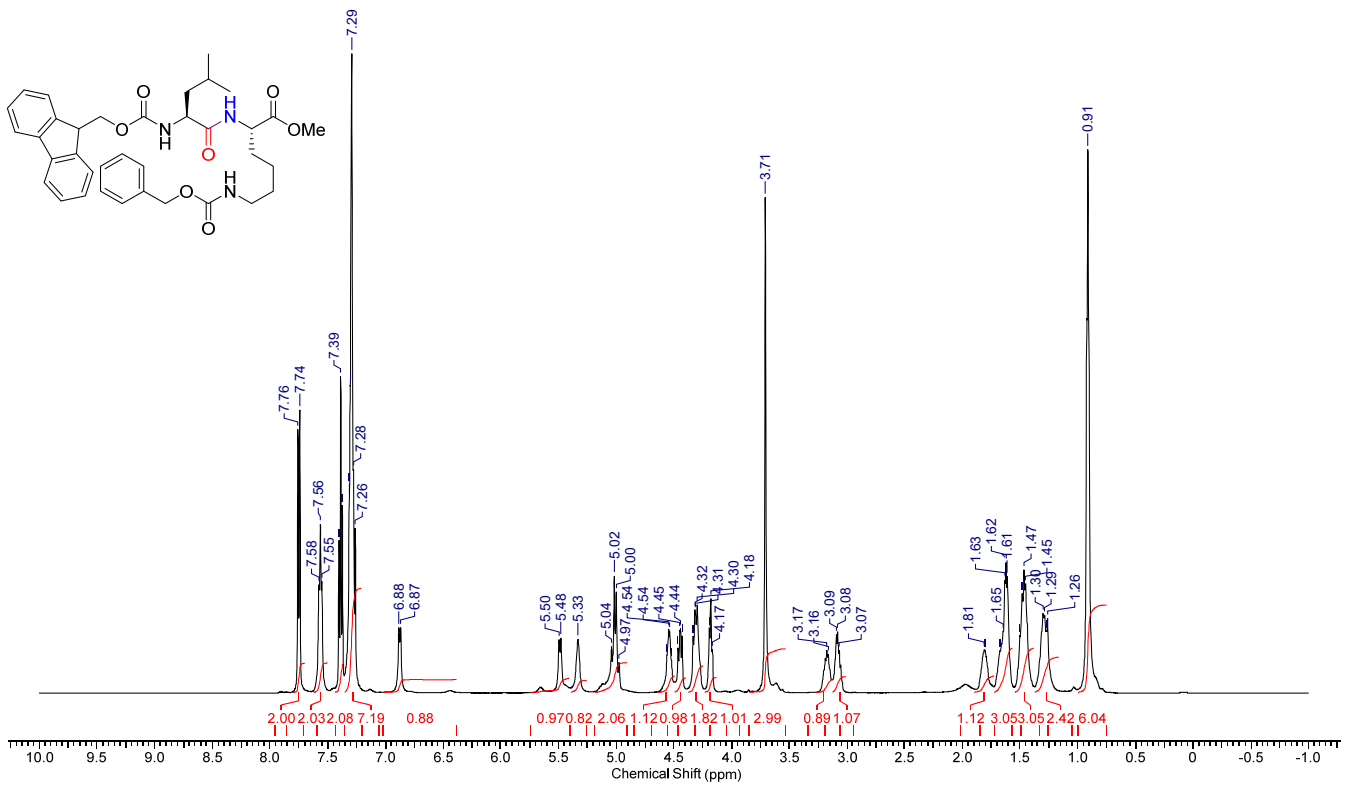
<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) of Fmoc-Leu-Glu(OMe)-OMe



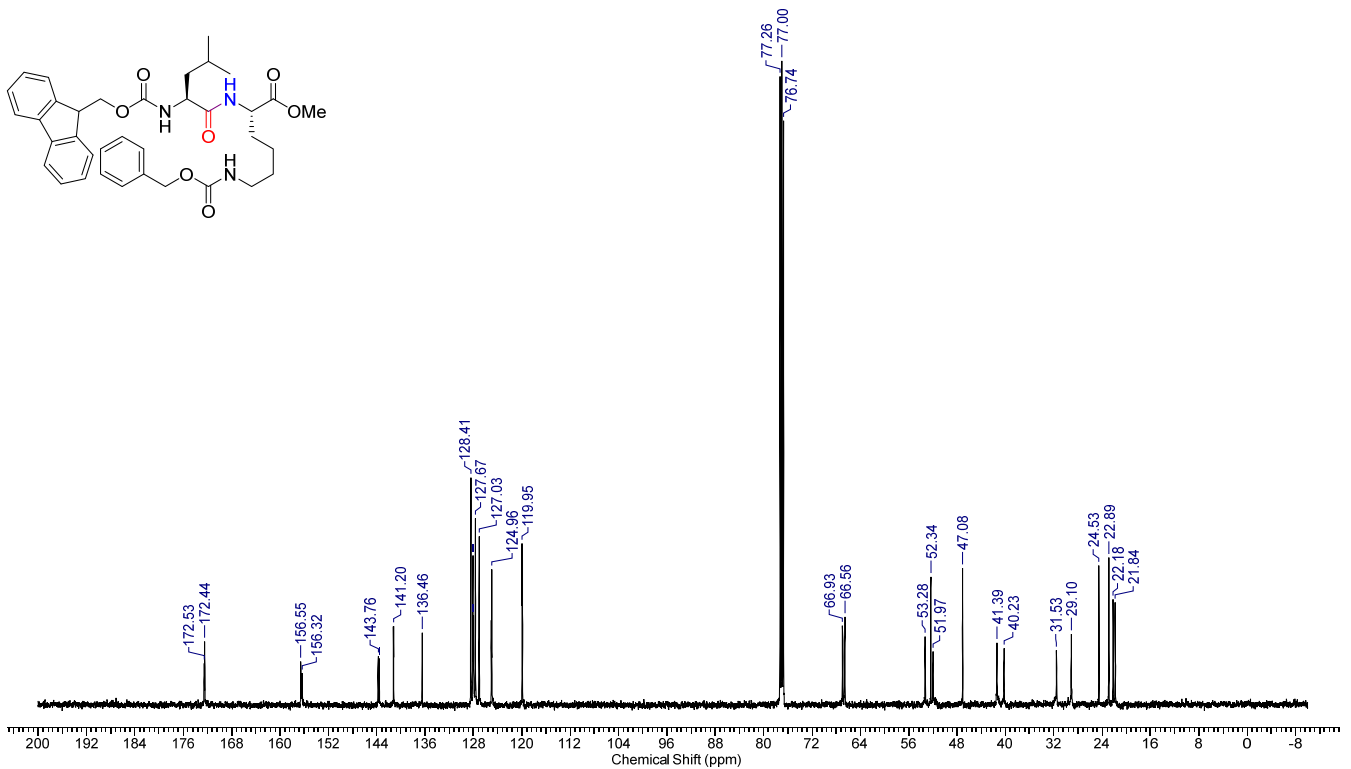
<sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) of Fmoc-Leu-Glu(OMe)-OMe



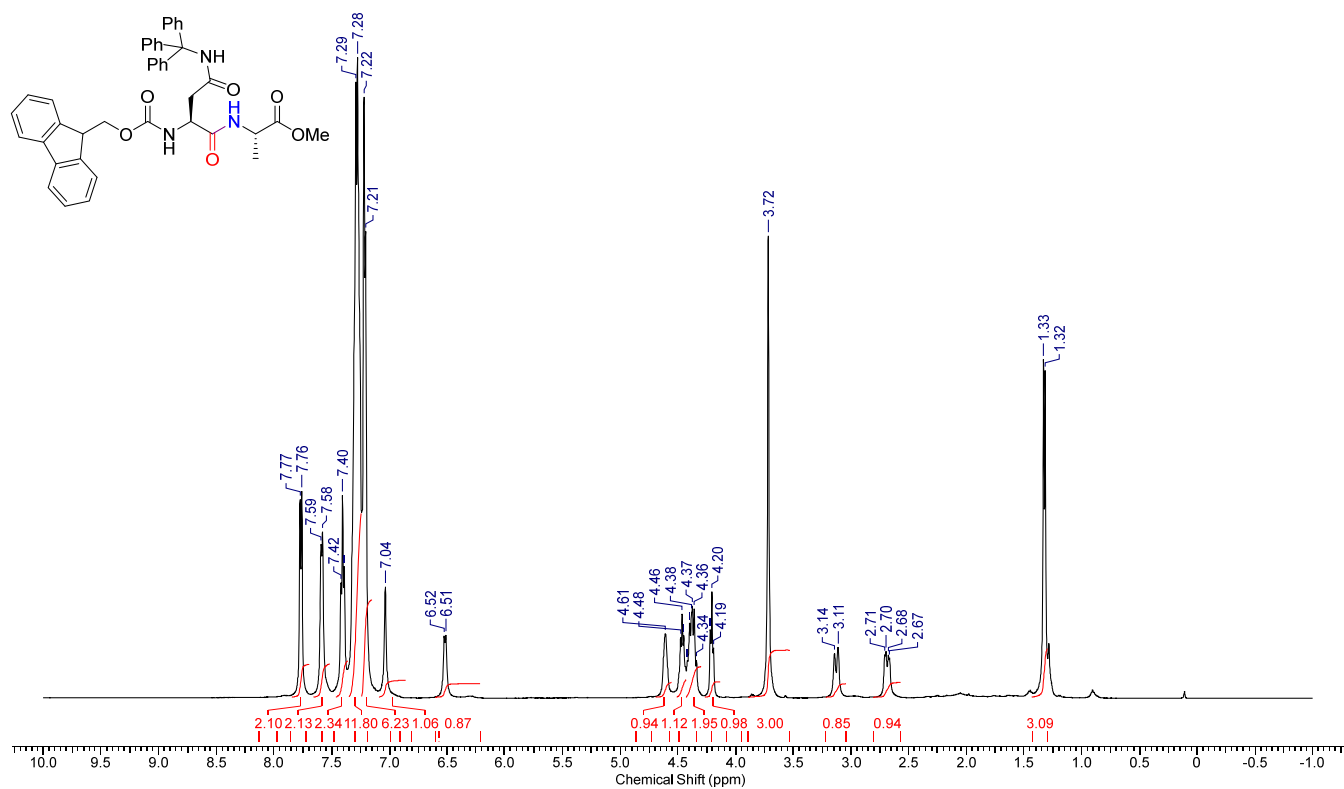
<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) of Fmoc-Leu-Lys(Cbz)-OMe



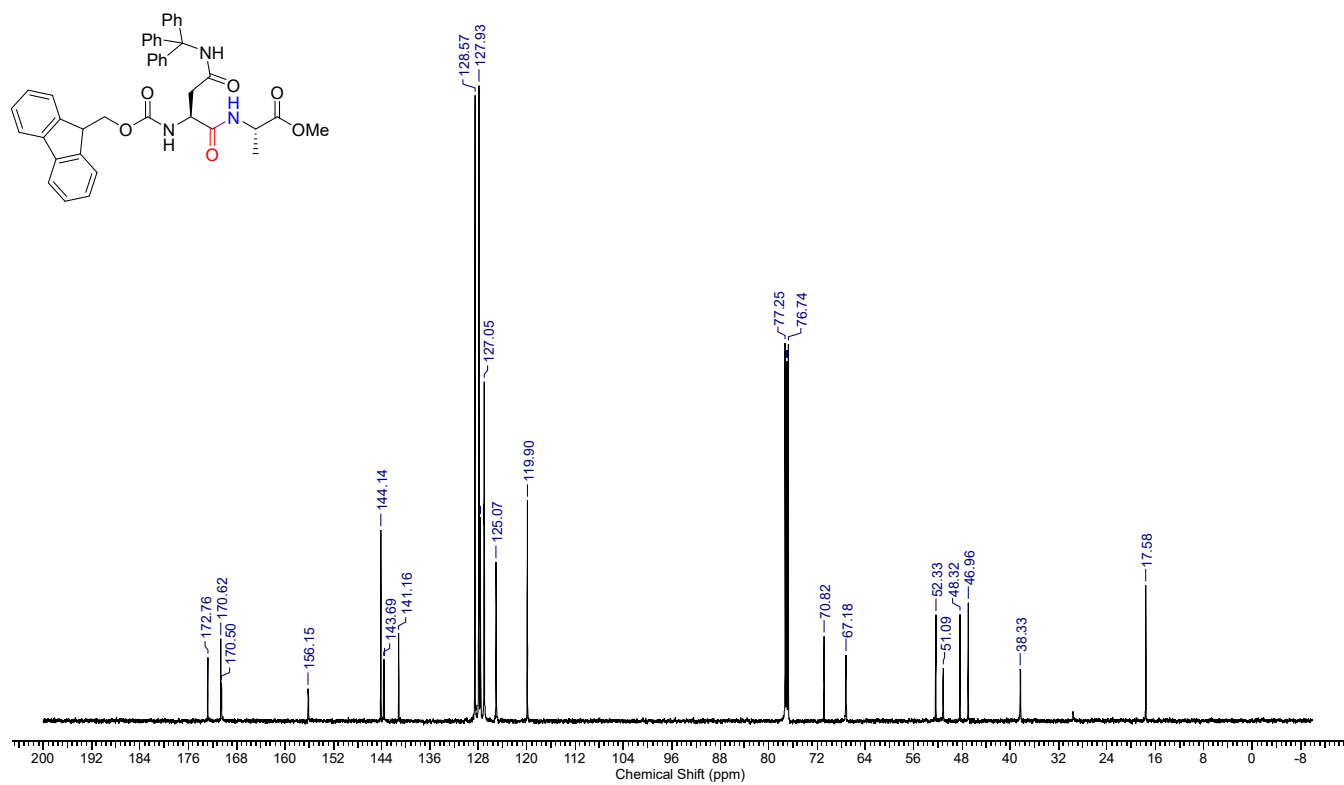
<sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) of Fmoc-Leu-Lys(Cbz)-OMe



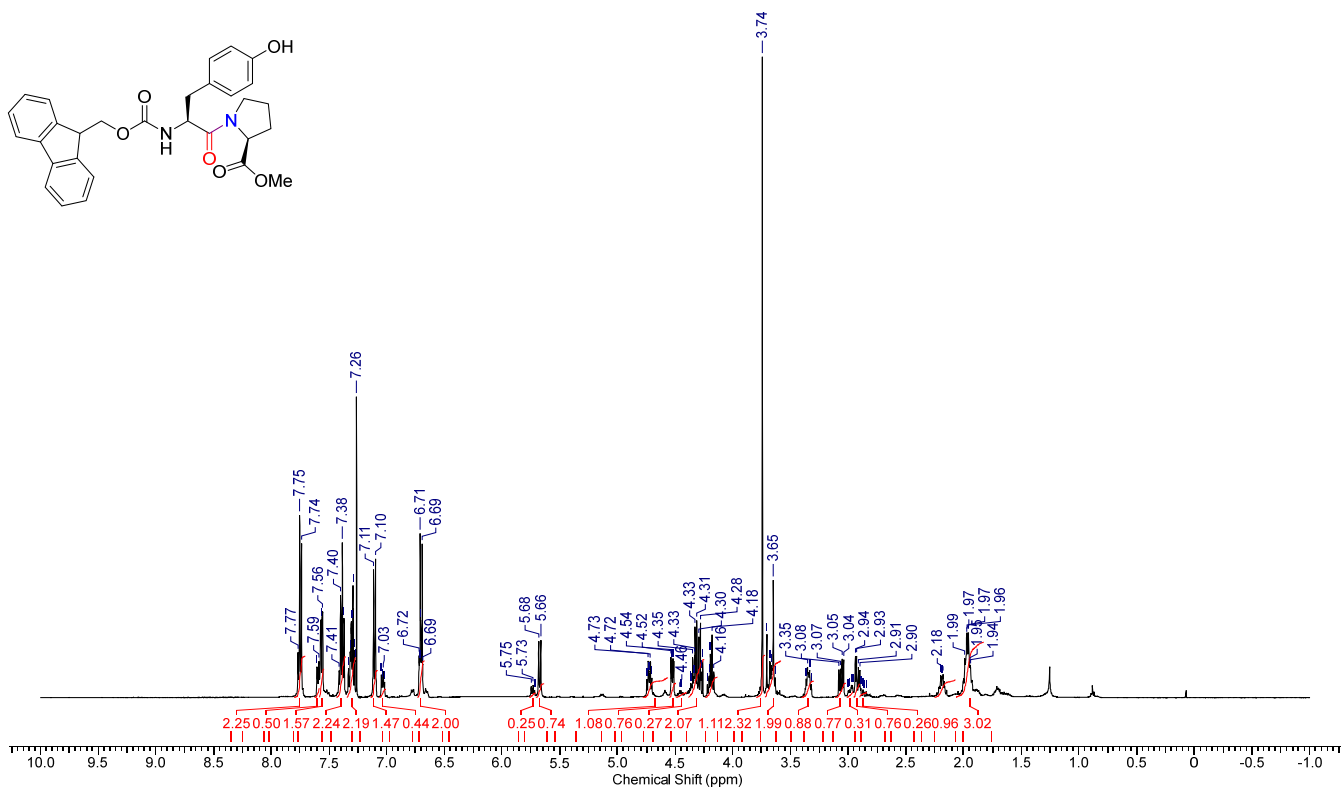
<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) of Fmoc-Asn(NHTr)-Ala-OMe



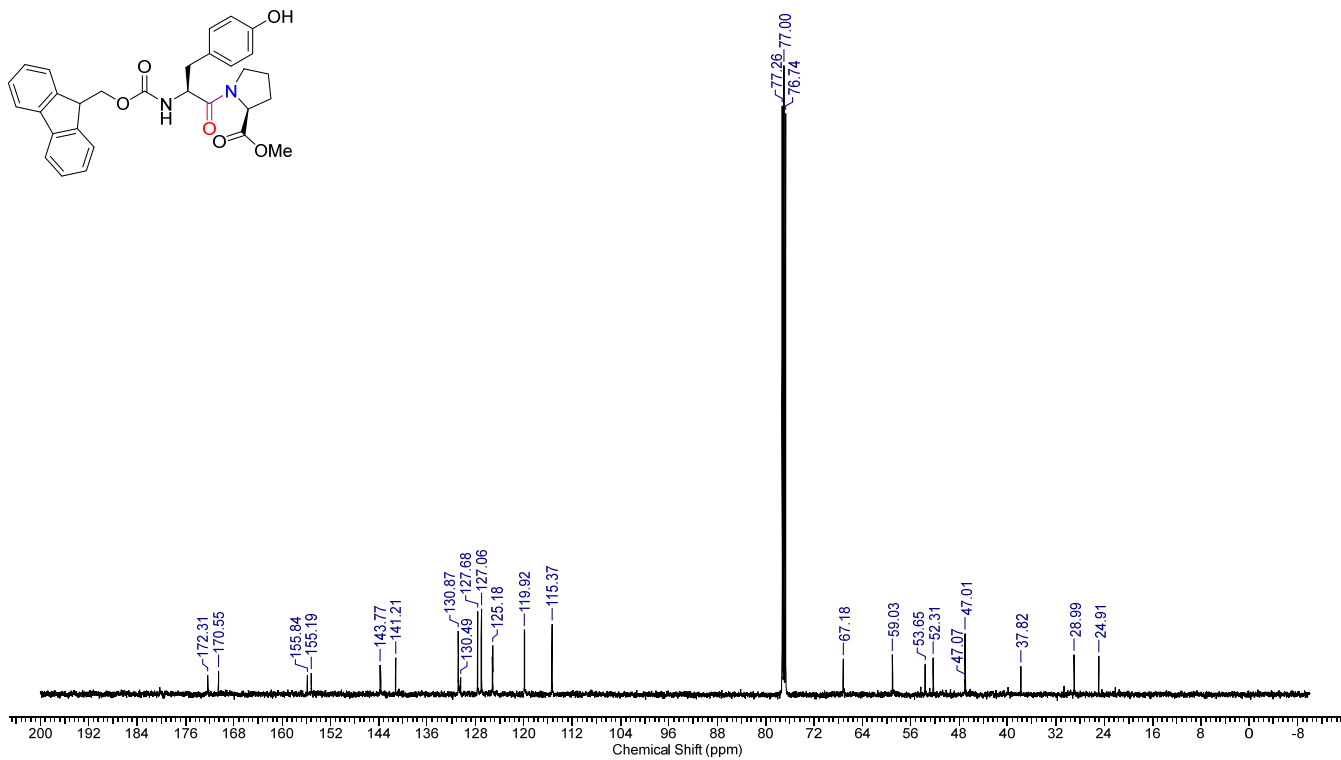
<sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) of Fmoc-Asn(NHTr)-Ala-OMe



<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) of Fmoc-Tyr-Pro-OMe

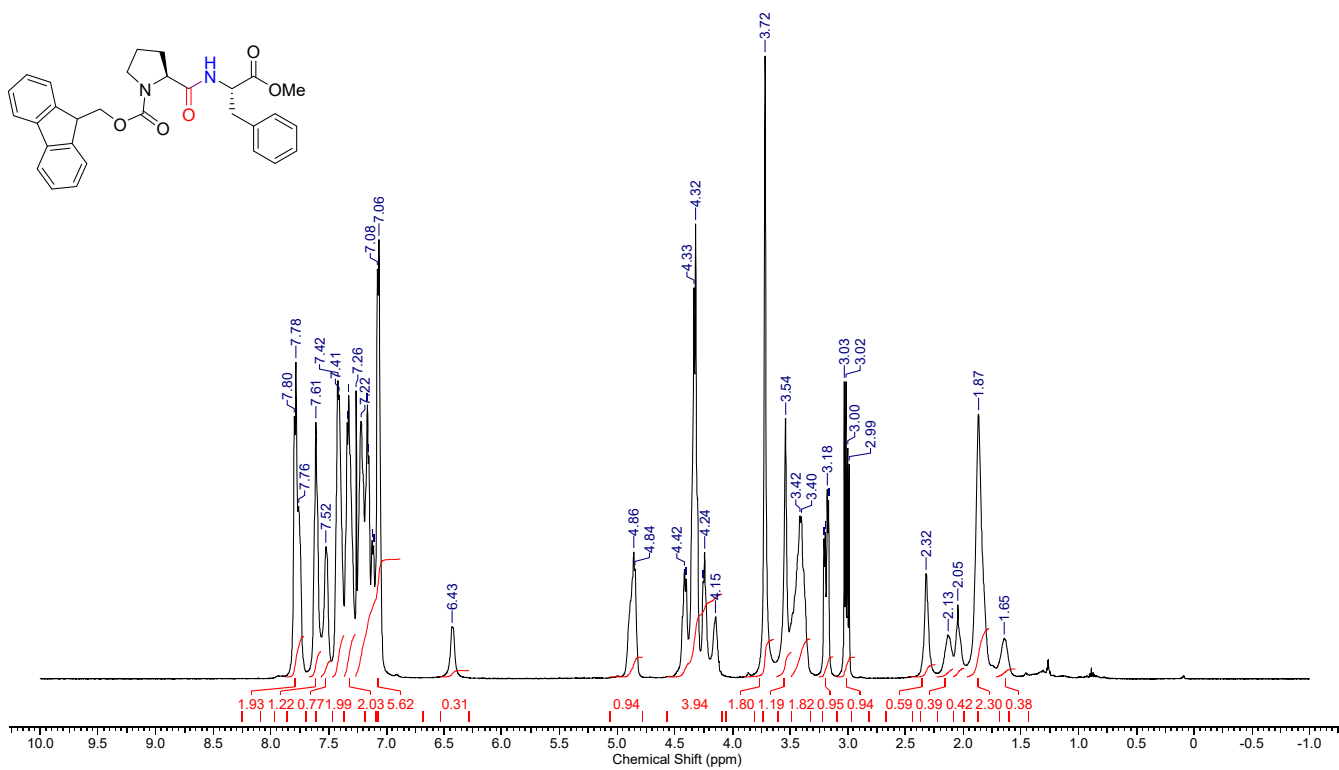


<sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) of Fmoc-Tyr-Pro-OMe

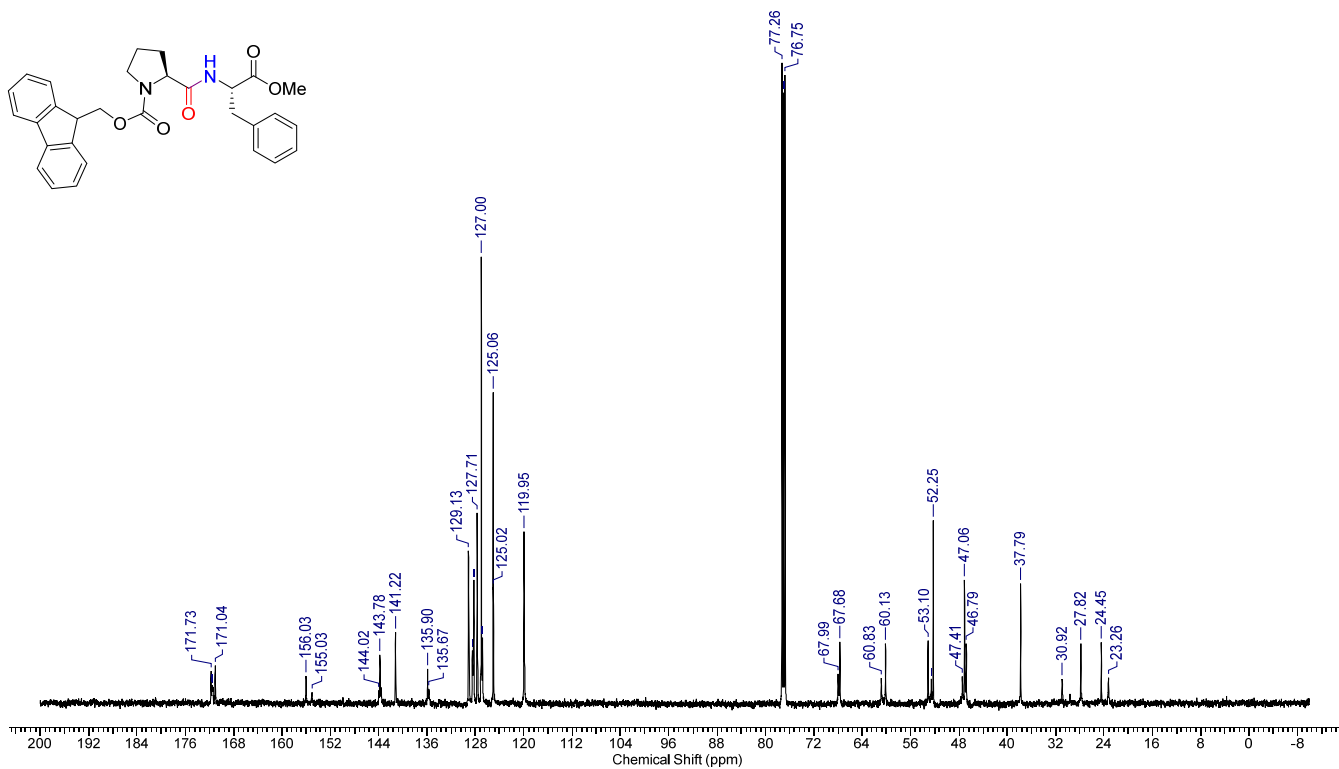




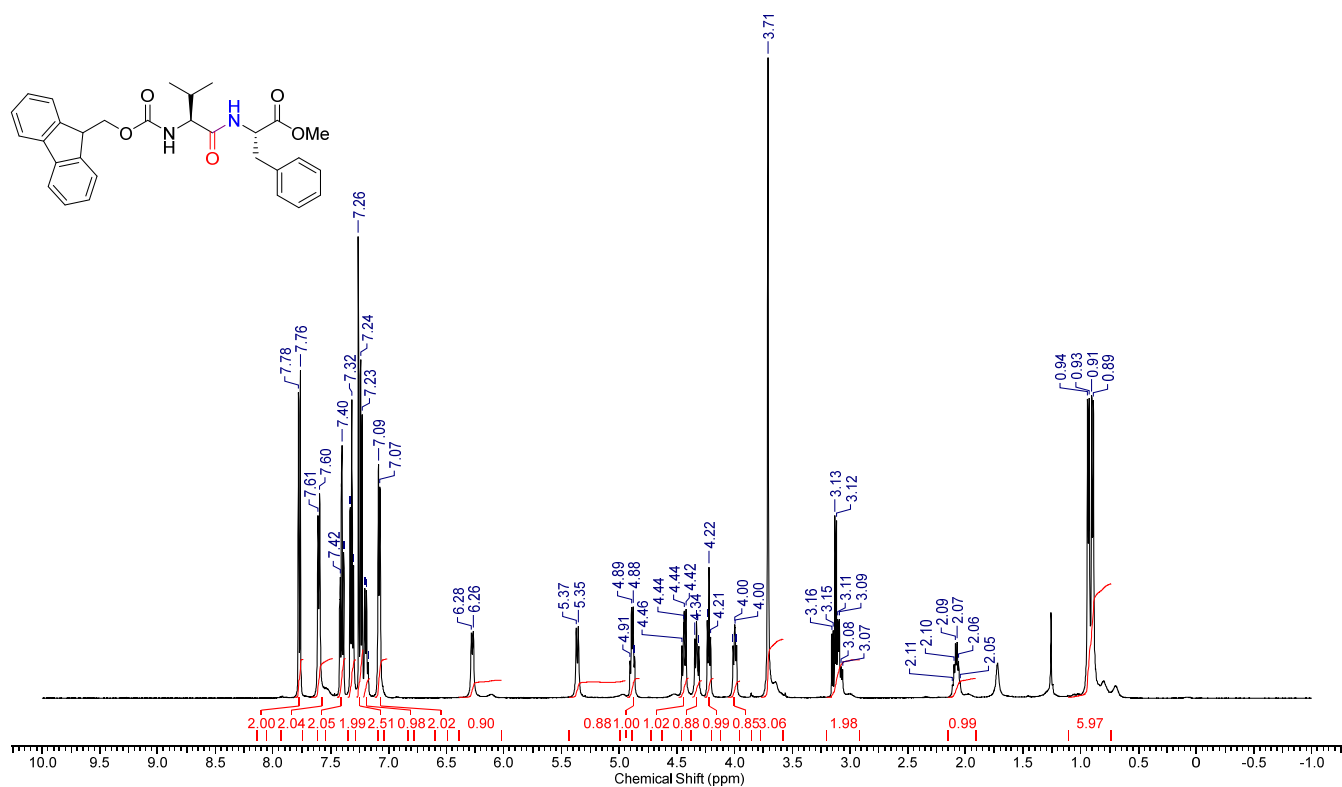
<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) of Fmoc-Pro-Phe-OMe



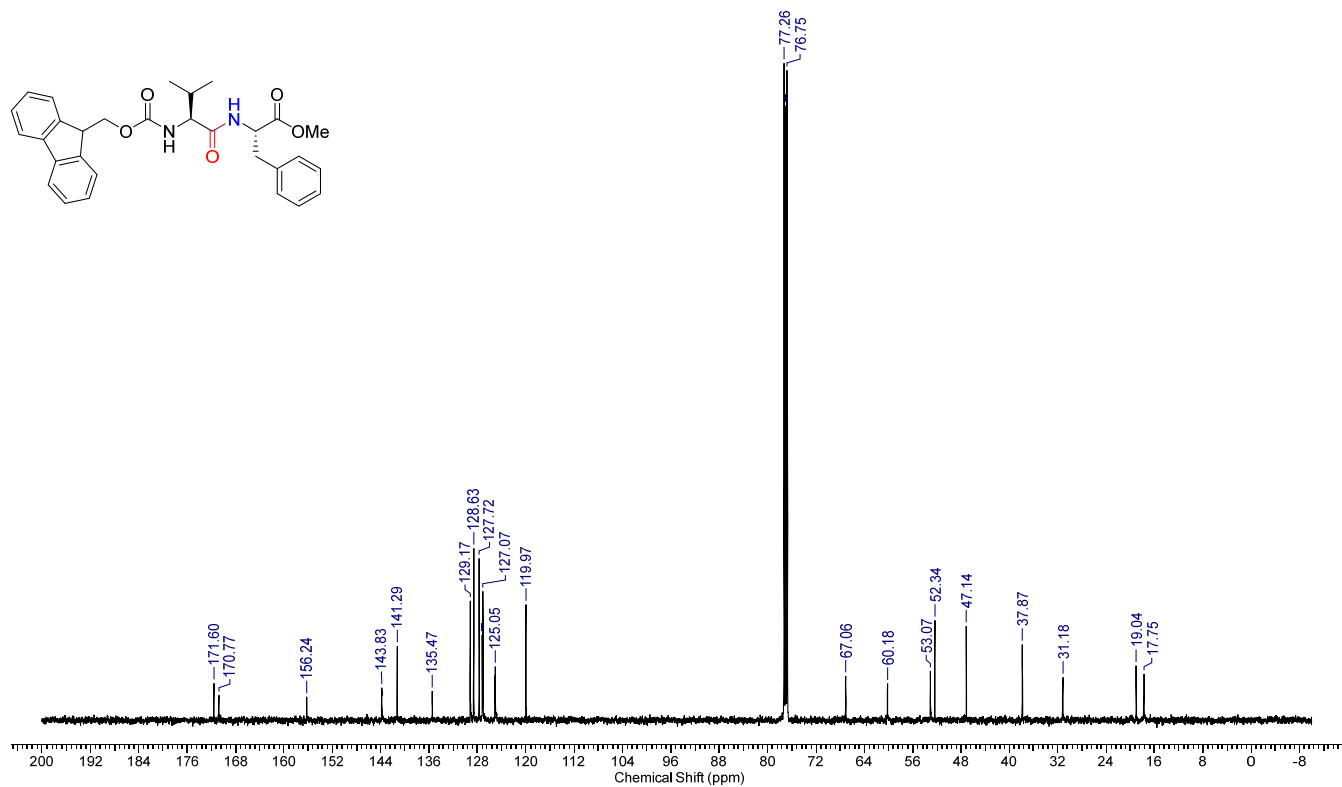
<sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) of Fmoc-Pro-Phe-OMe



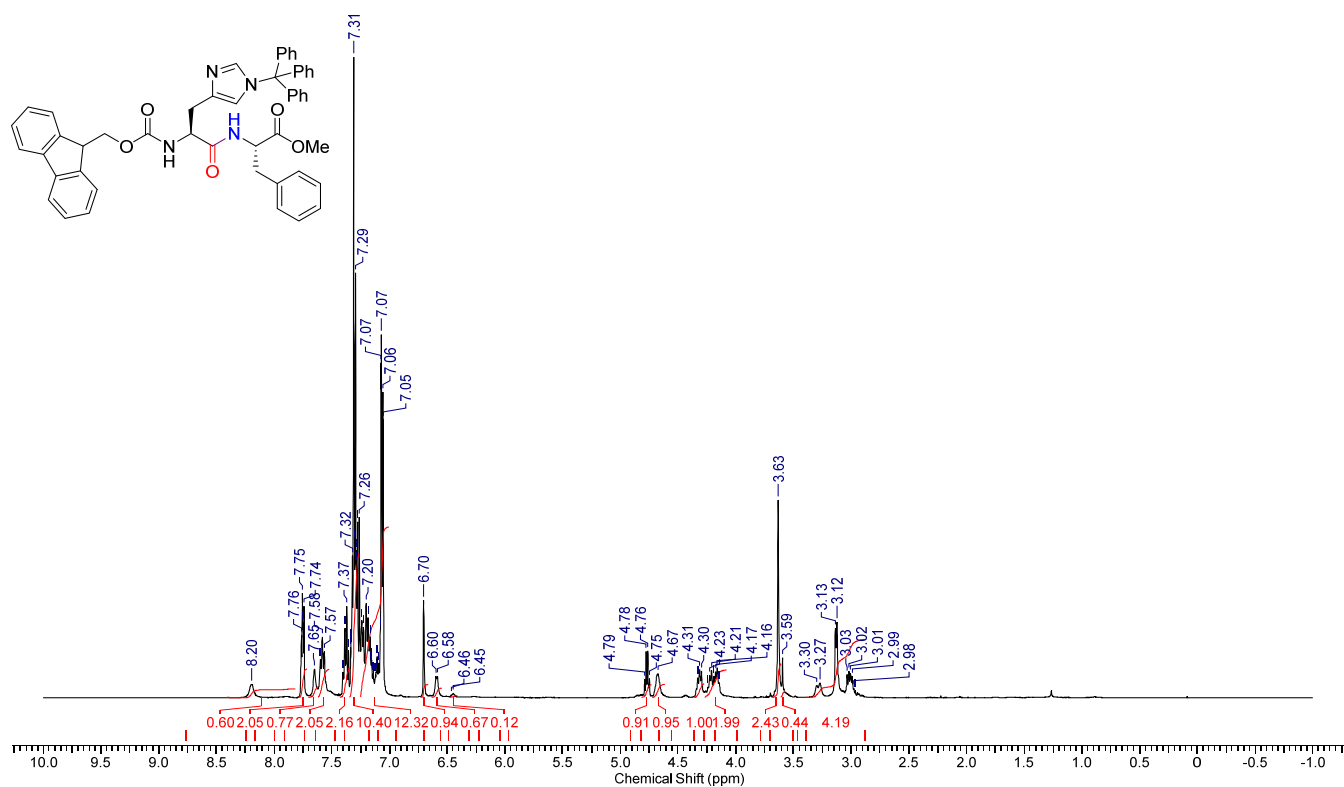
<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) of Fmoc-Val-Phe-OMe



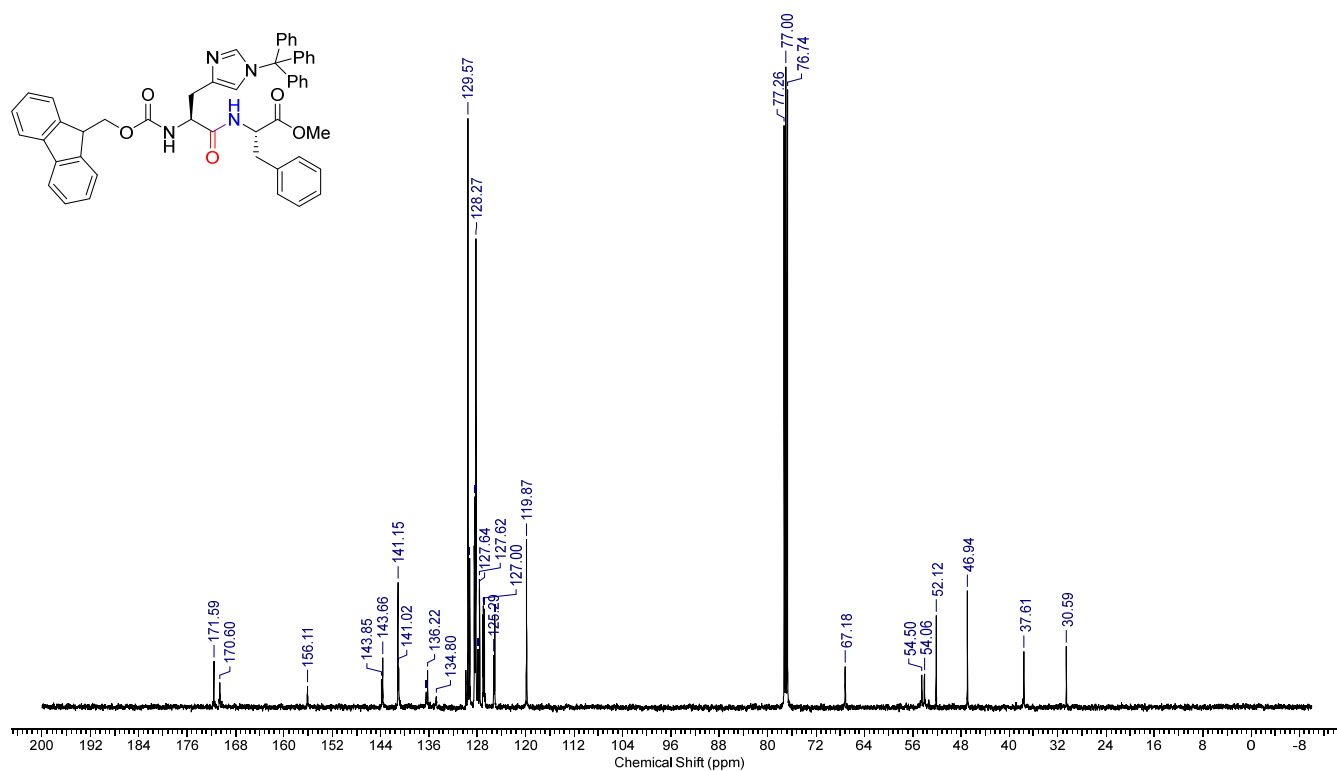
<sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) of Fmoc-Val-Phe-OMe



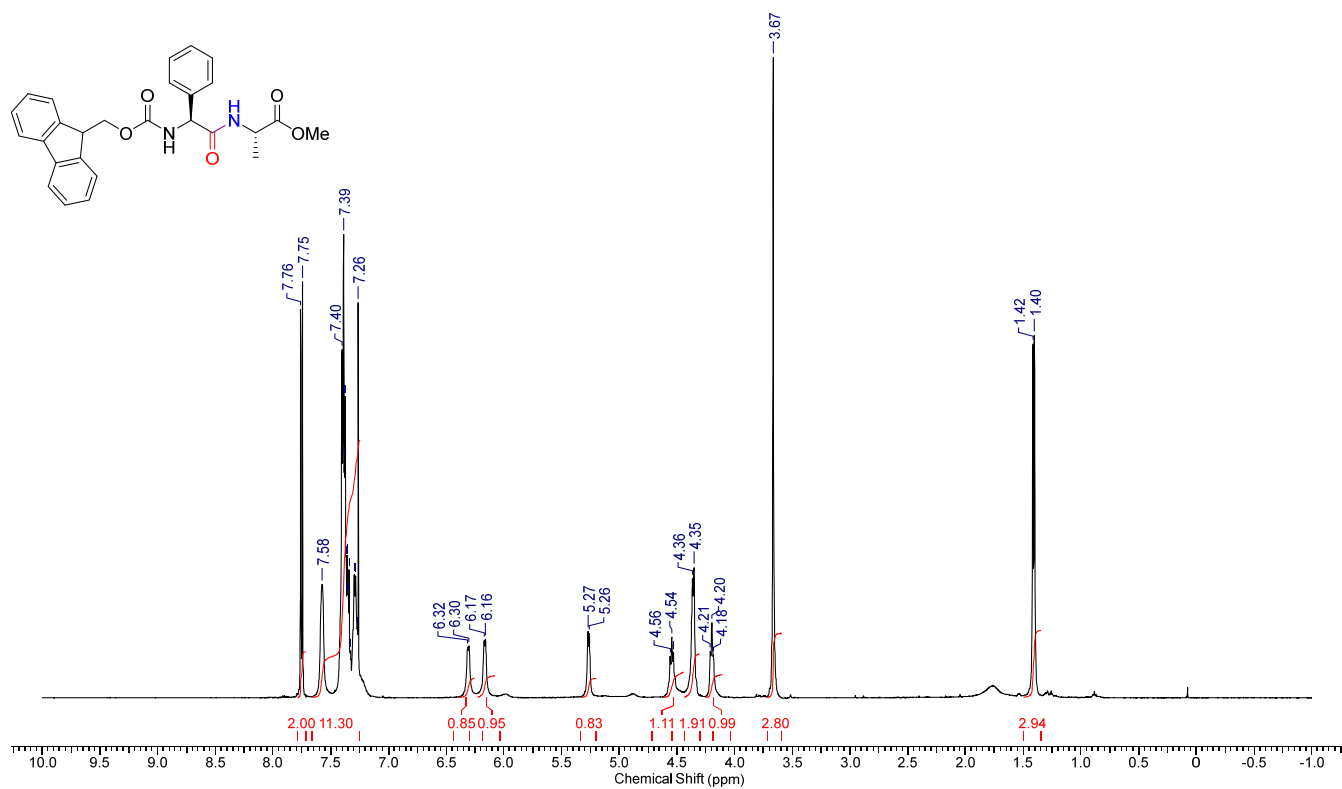
<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) of Fmoc-His(Trt)-Phe-OMe



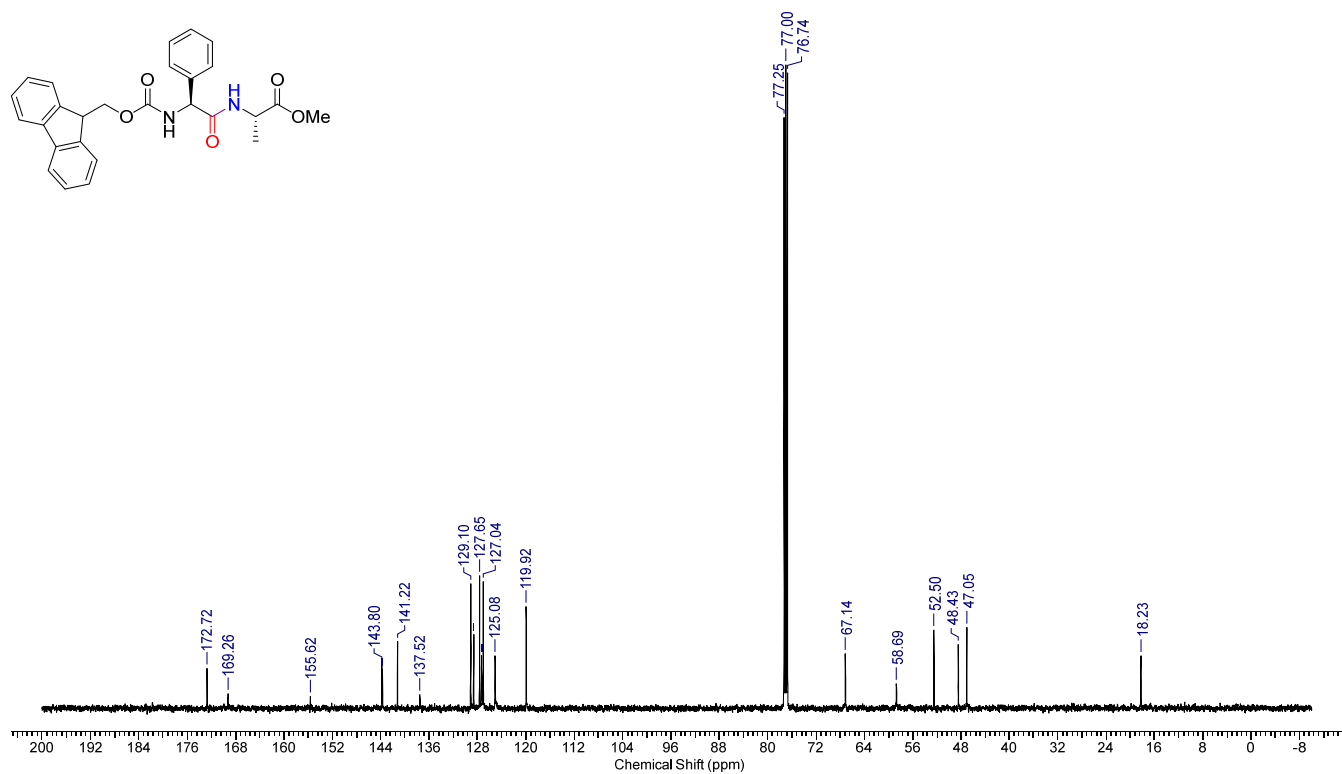
<sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) of Fmoc-His(Trt)-Phe-OMe



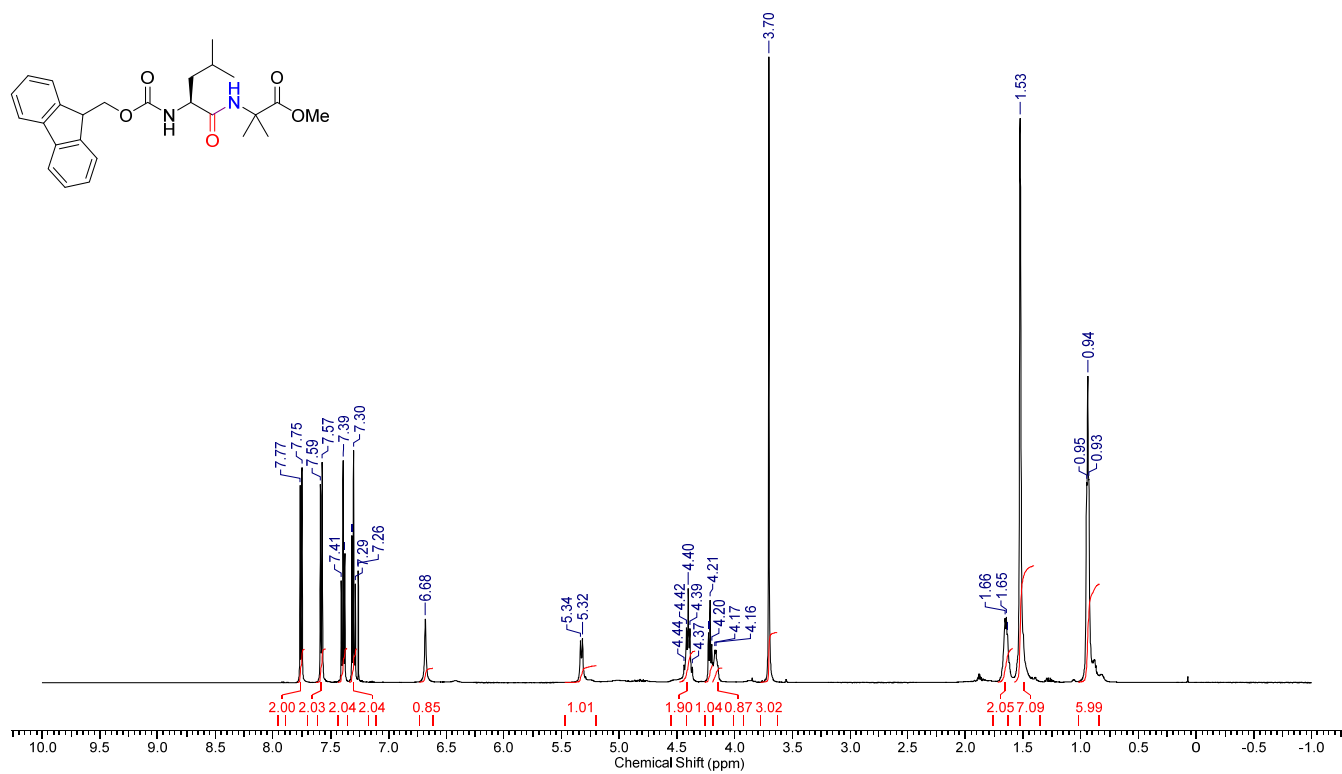
<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) of Fmoc-Phg-Ala-OMe



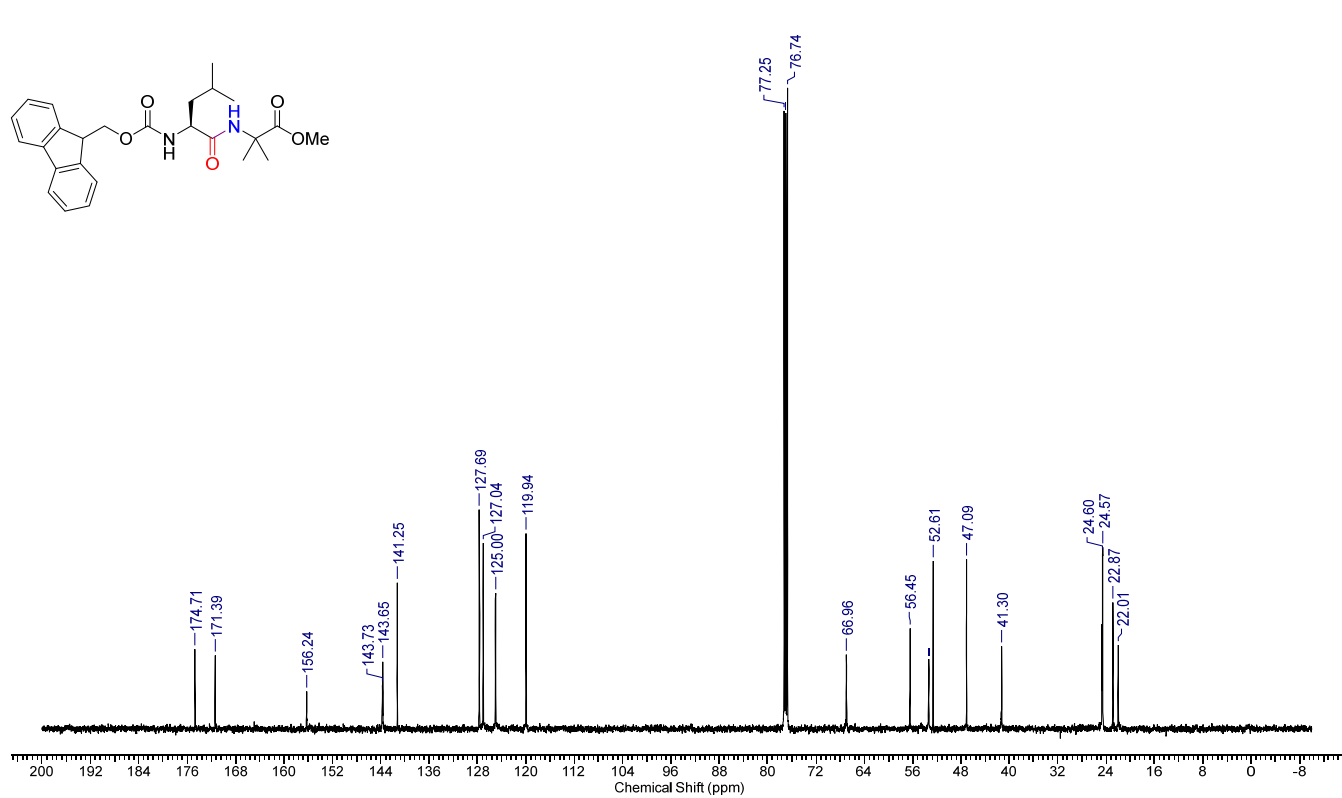
<sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) of Fmoc-Phg-Ala-OMe



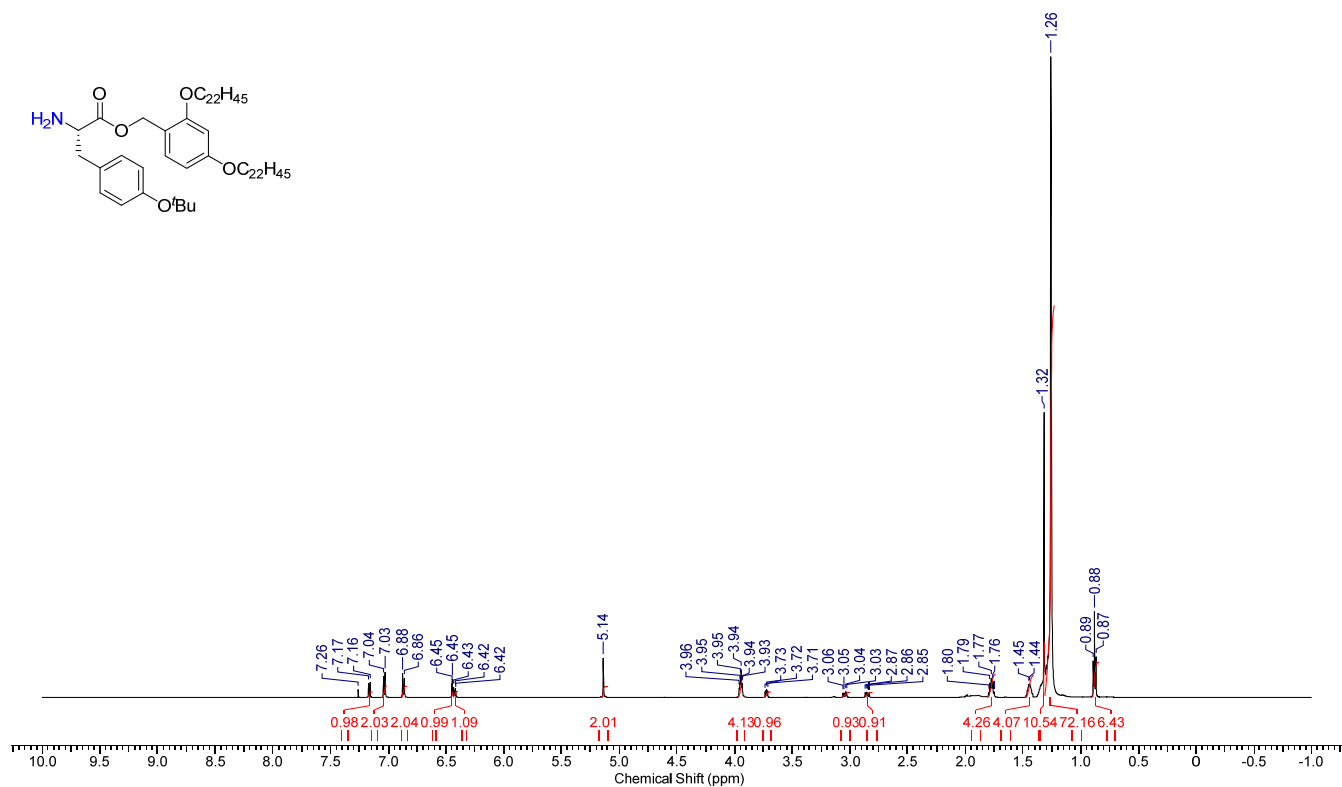
<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) of Fmoc-Leu-Aib-OMe



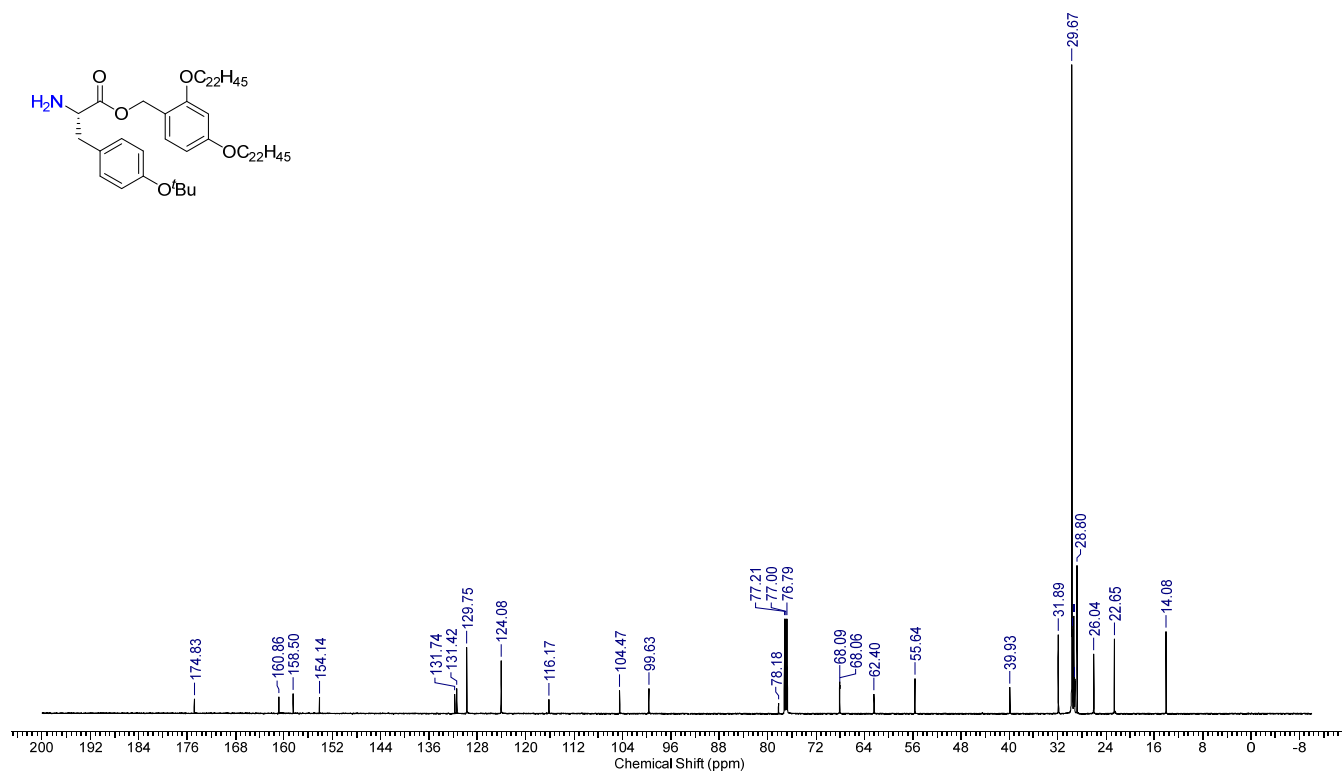
<sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) of Fmoc-Leu-Aib-OMe



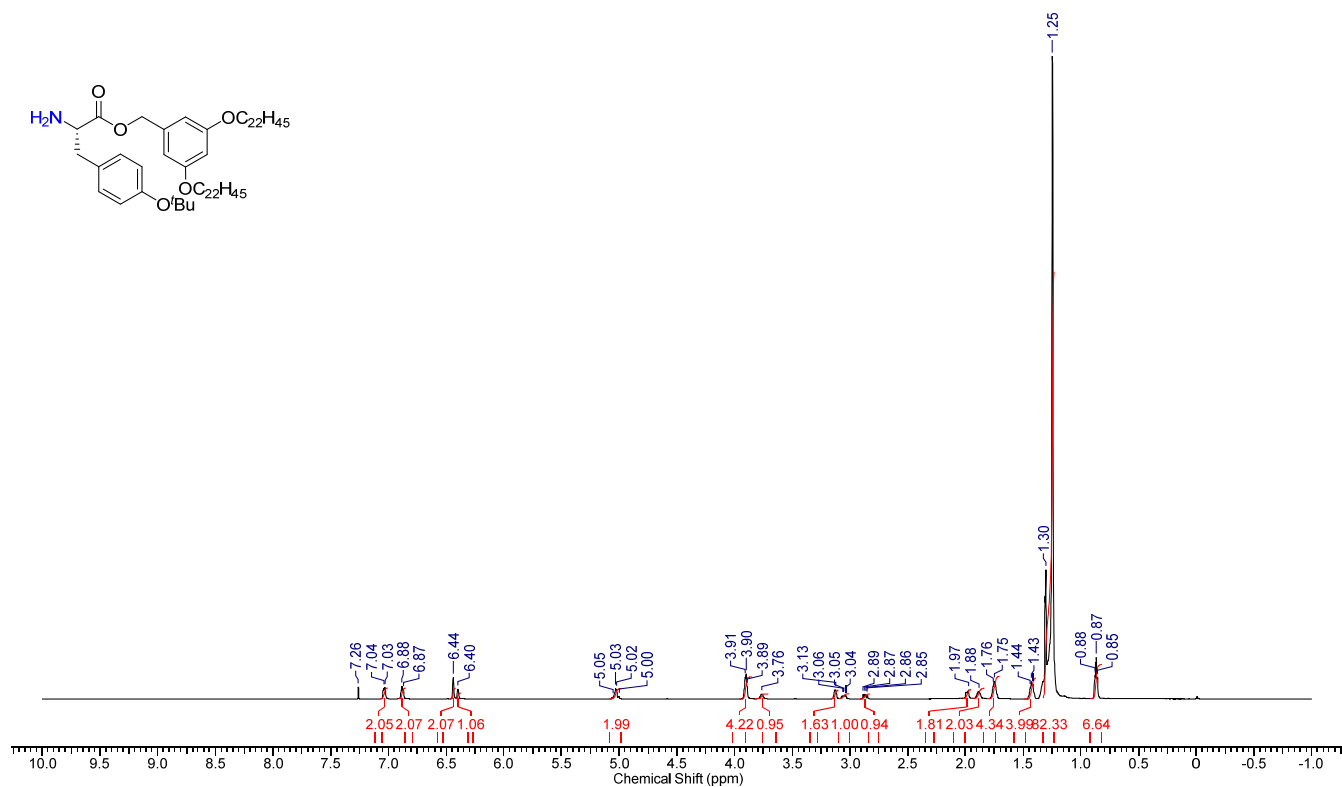
$^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ ) of H-Tyr(O<sup>t</sup>Bu)-OTAG<sub>A</sub>



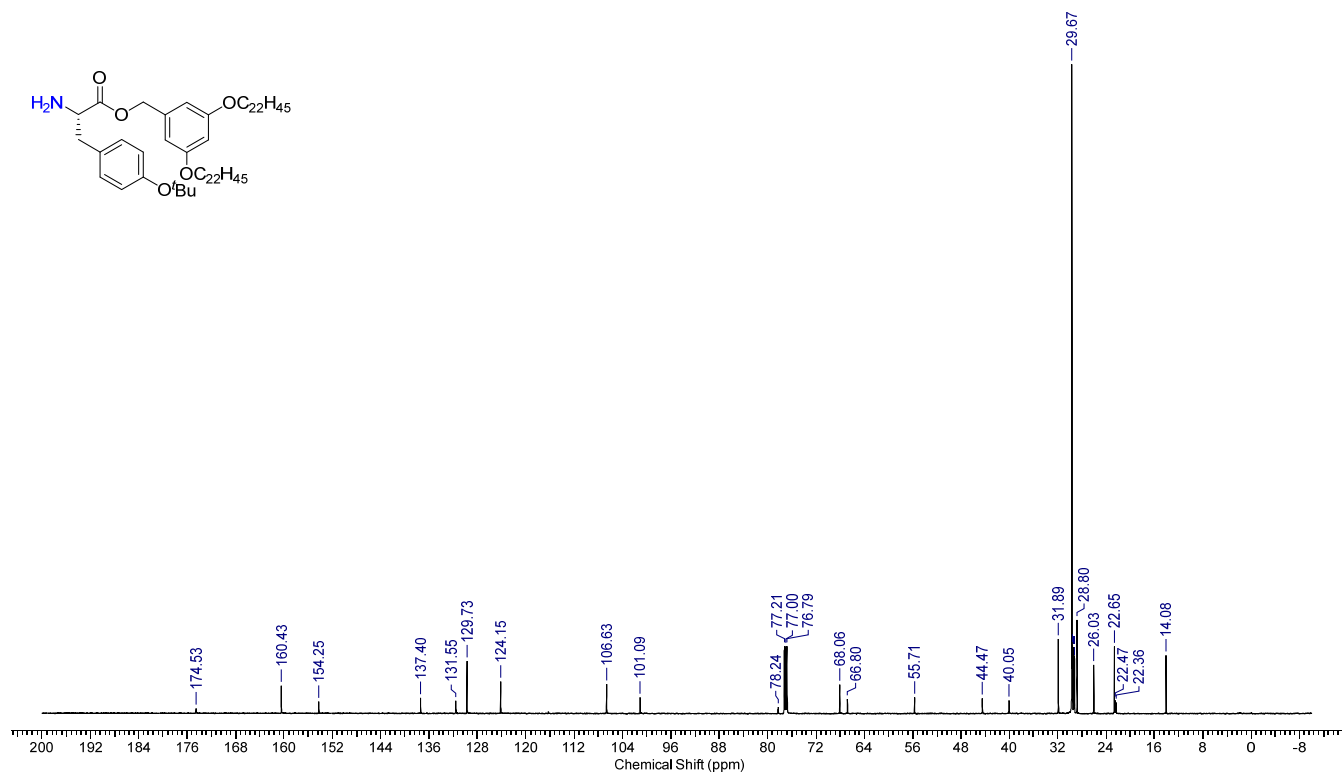
$^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ) of H-Tyr(O<sup>t</sup>Bu)-OTAG<sub>A</sub>



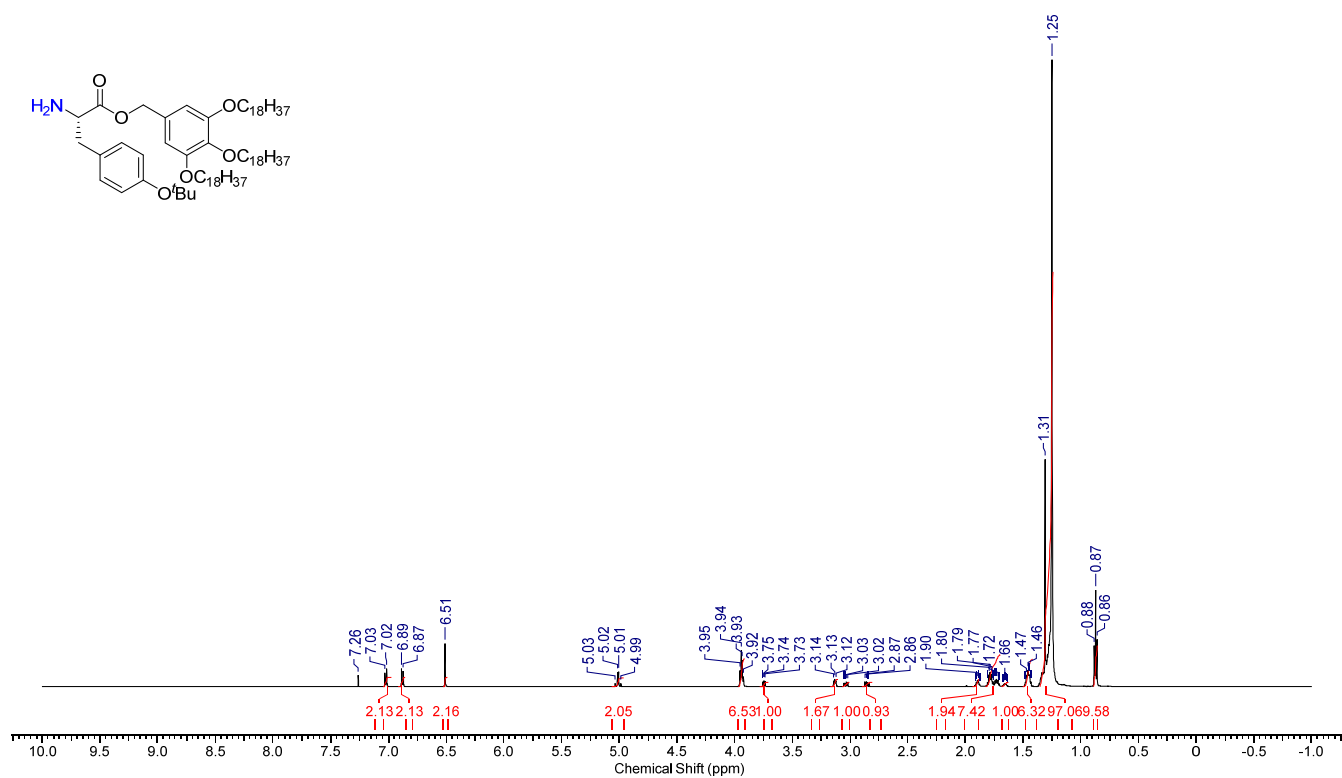
$^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ ) of H-Tyr(O<sup>t</sup>Bu)-OTAG<sub>B</sub>



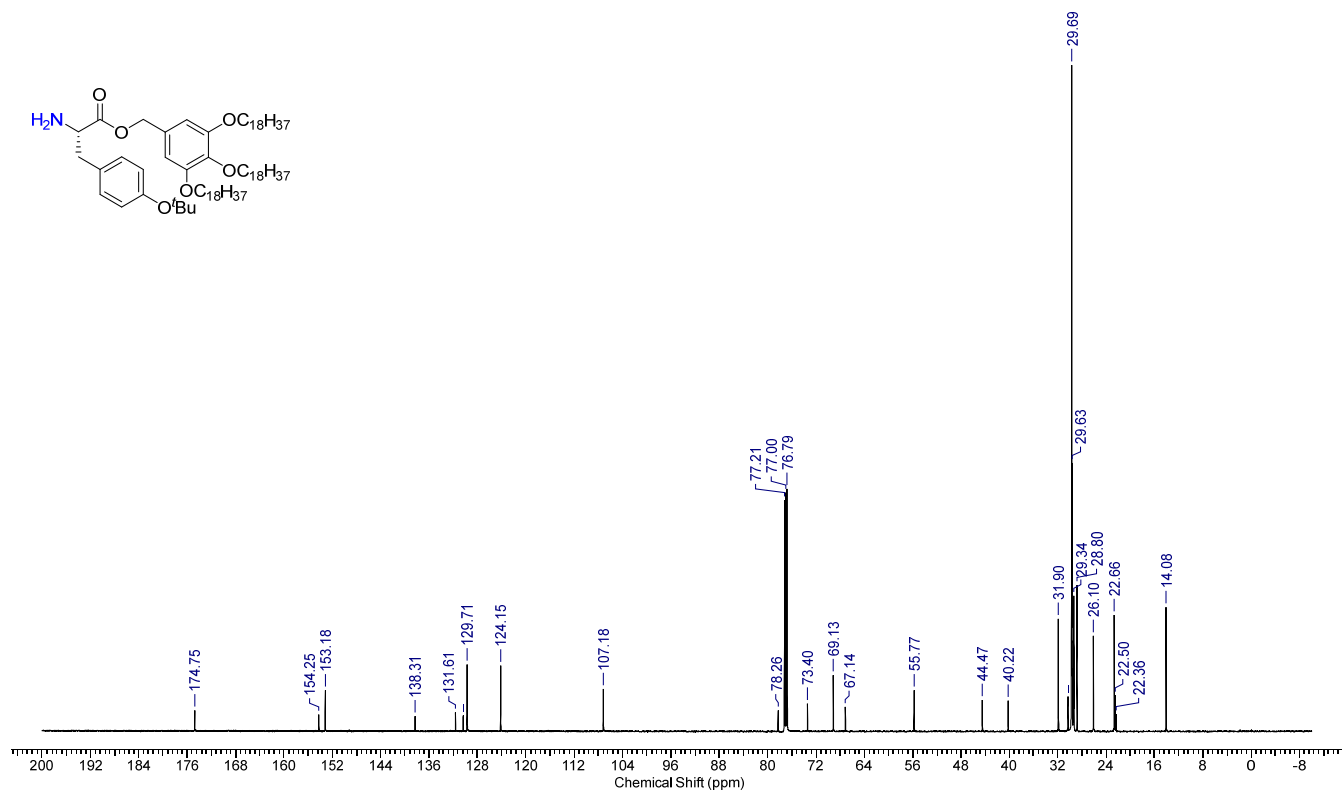
$^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ) of H-Tyr(O<sup>t</sup>Bu)-OTAG<sub>B</sub>



$^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ ) of H-Tyr(O<sup>t</sup>Bu)-OTAG<sub>C</sub>

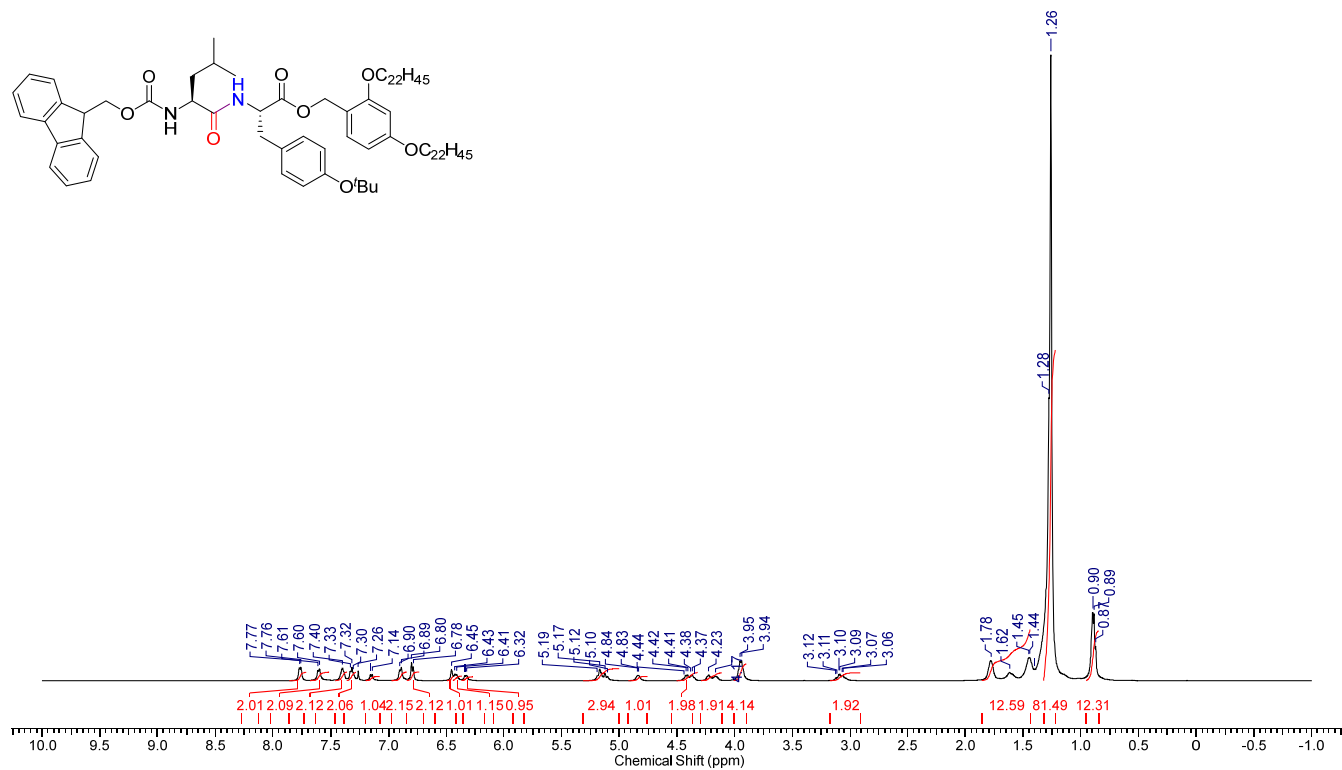


$^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ) of H-Tyr(O<sup>t</sup>Bu)-OTAG<sub>C</sub>

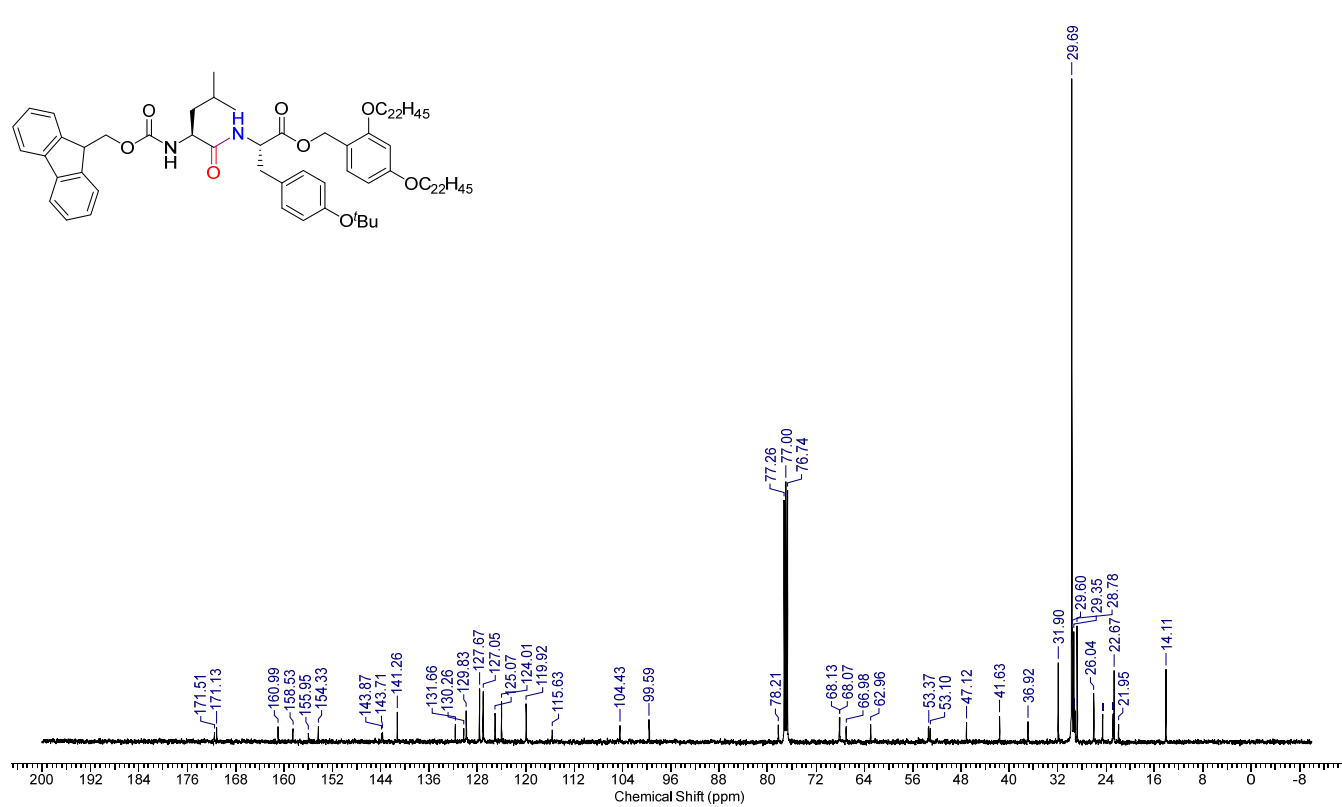




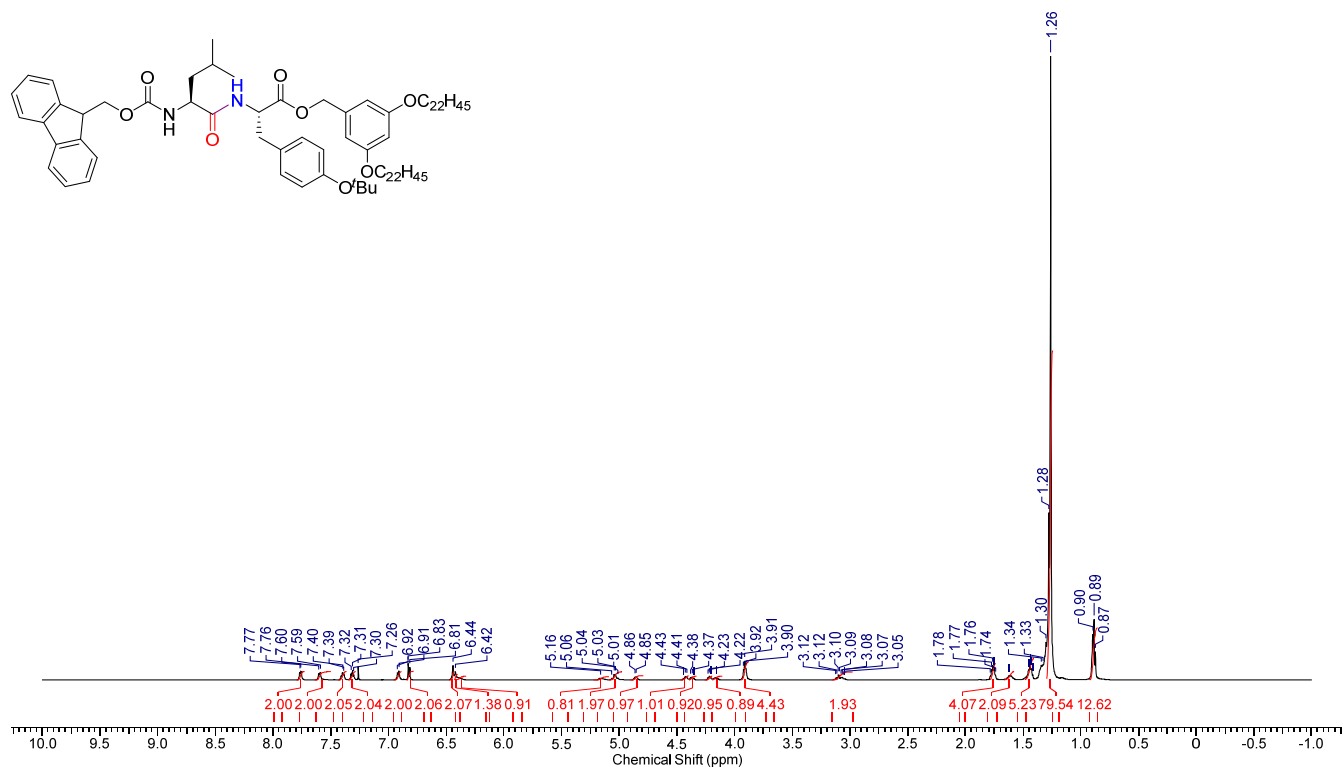
$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ) of Fmoc-Leu-Tyr(O<sup>t</sup>Bu)-OTAG<sub>A</sub>



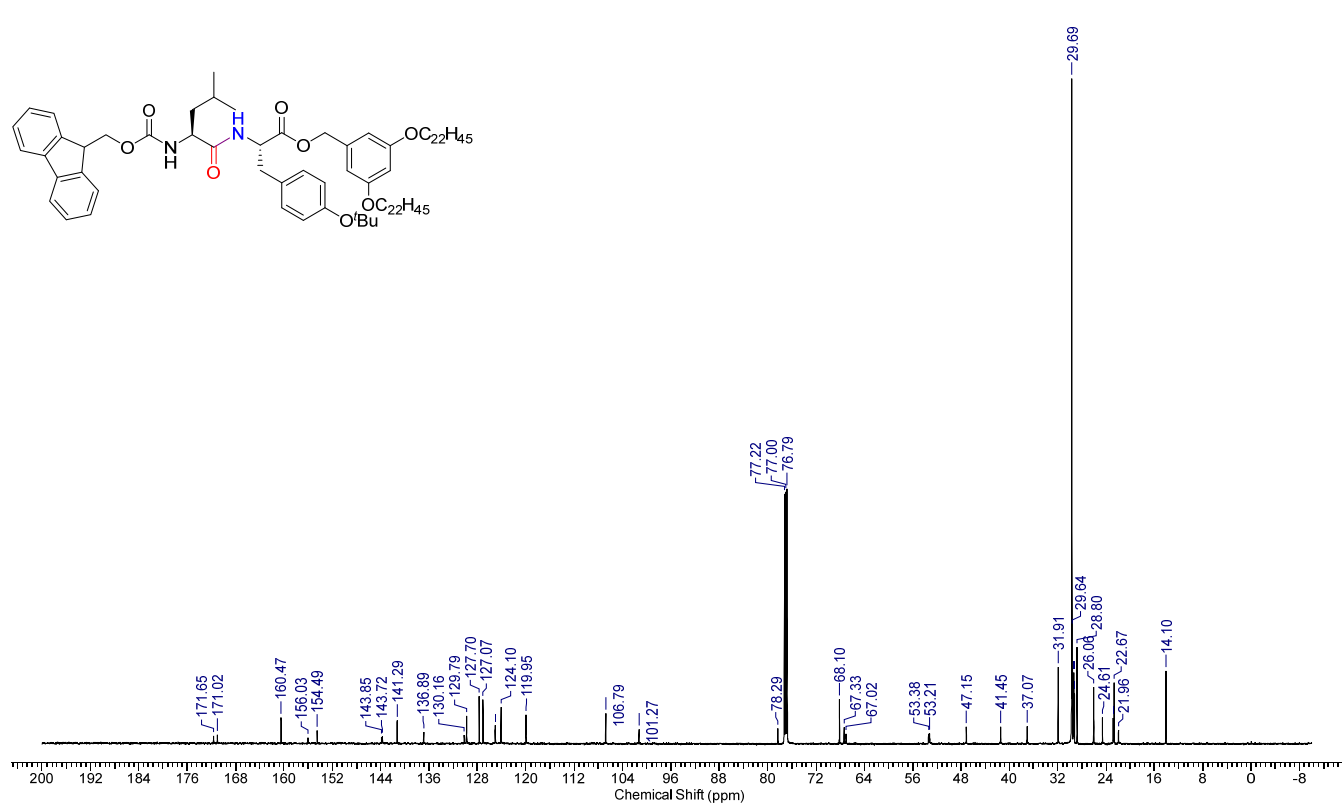
$^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ) of Fmoc-Leu-Tyr(O<sup>t</sup>Bu)-OTAG<sub>A</sub>



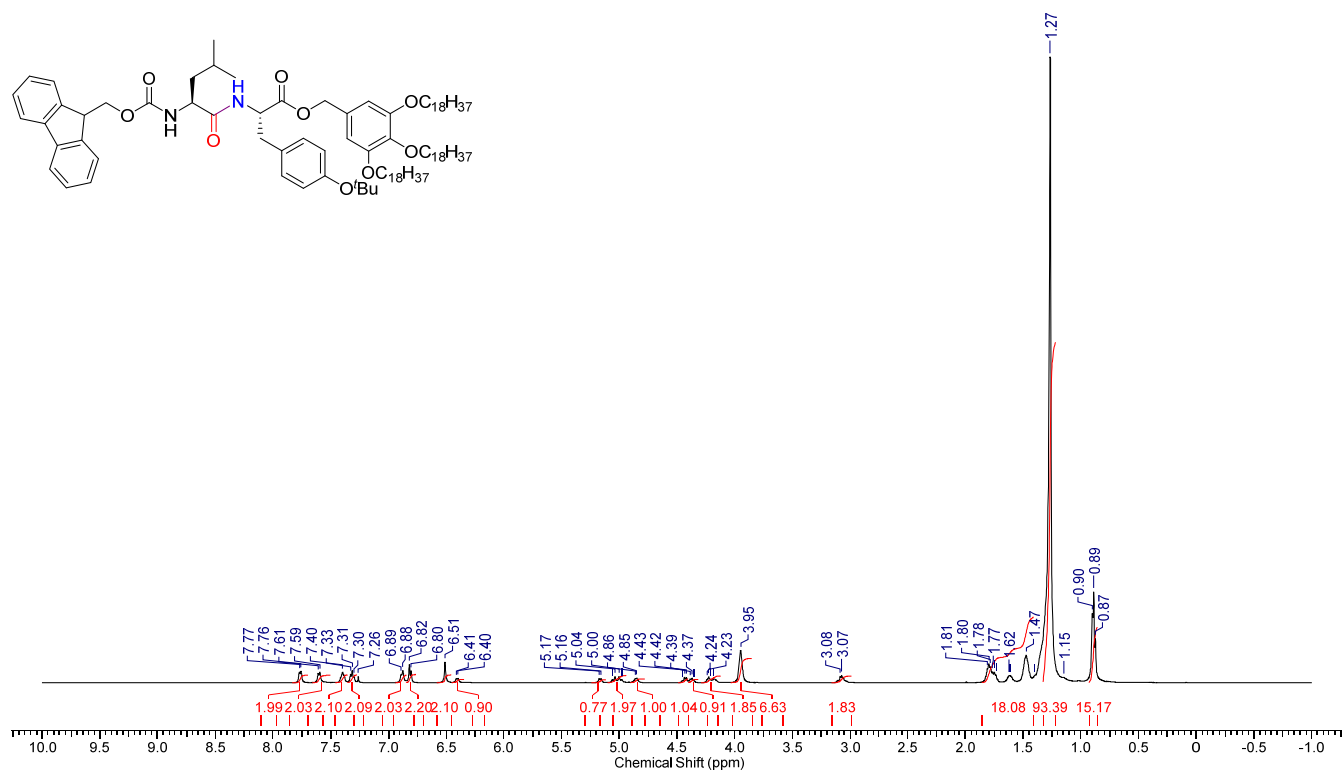
<sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) of Fmoc-Leu-Tyr(O'Bu)-OTAG<sub>B</sub>



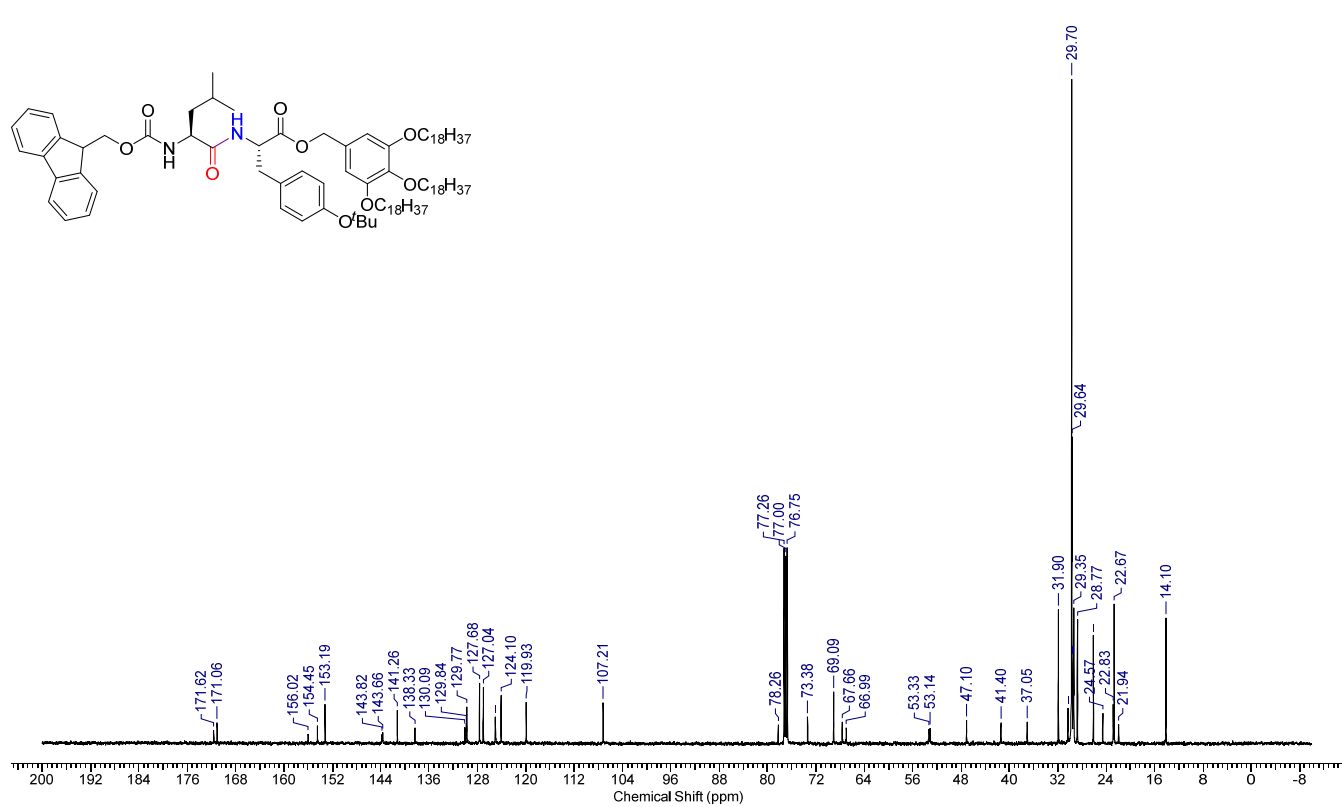
<sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) of Fmoc-Leu-Tyr(O'Bu)-OTAG<sub>B</sub>



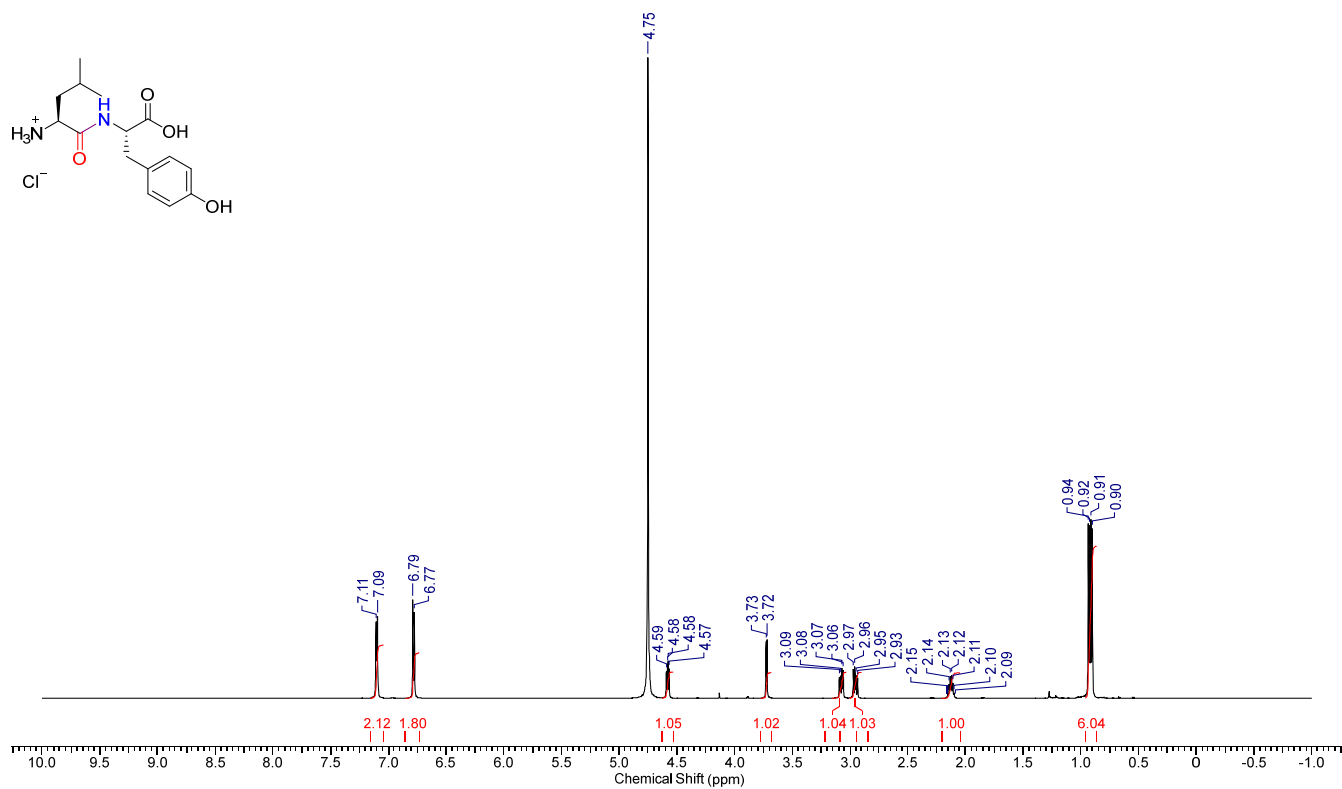
$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ) of Fmoc-Leu-Tyr(O'Bu)-OTAG<sub>C</sub>



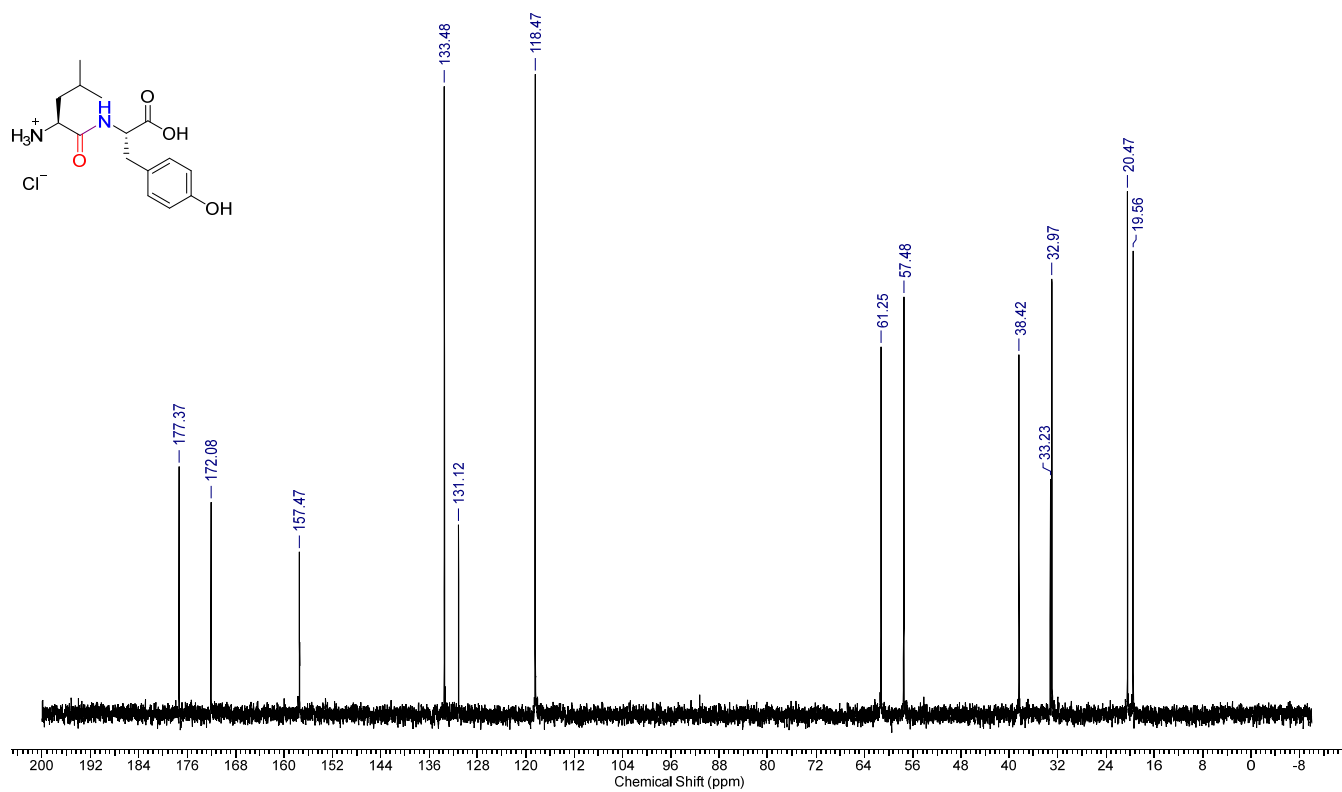
$^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ) of Fmoc-Leu-Tyr(O'Bu)-OTAG<sub>C</sub>



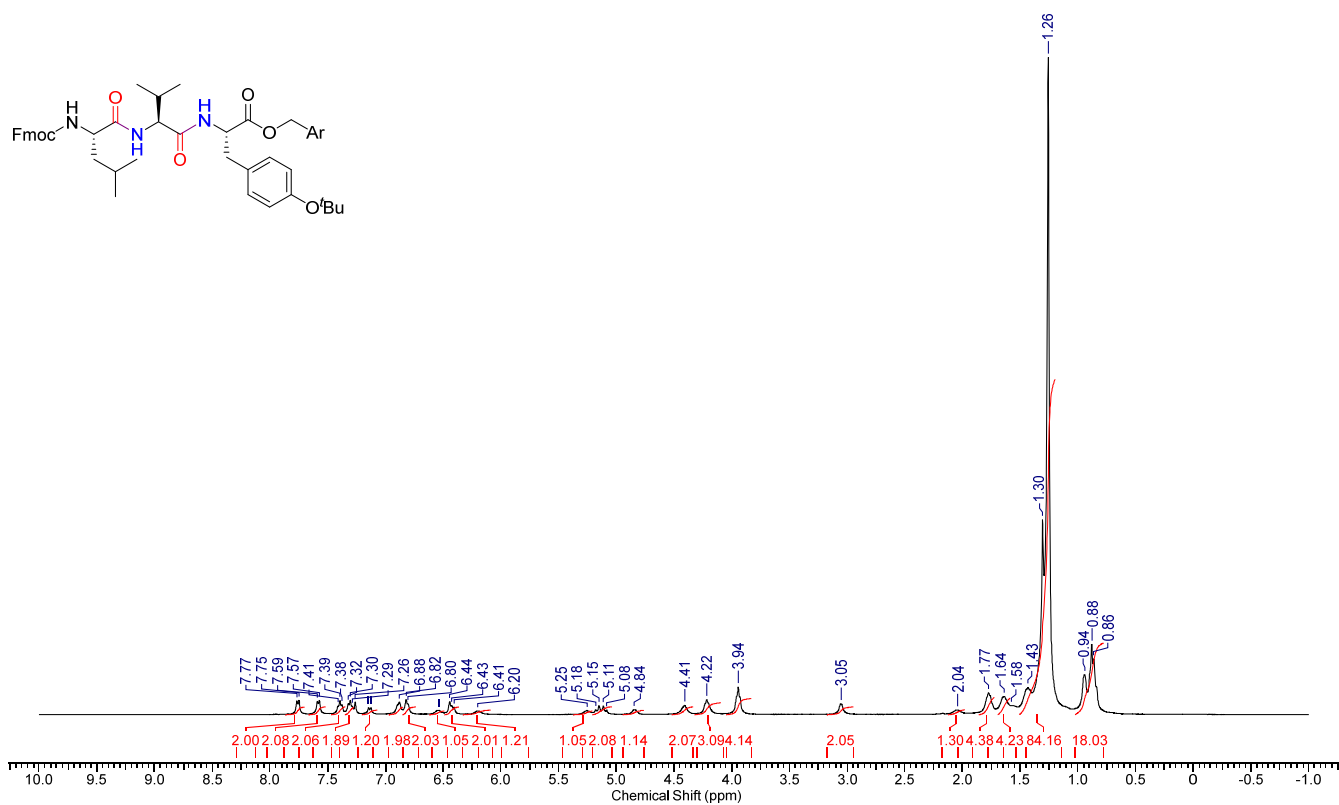
$^1\text{H}$  NMR (600 MHz,  $\text{D}_2\text{O}$ ) of H-Val-Tyr-OH·HCl



$^{13}\text{C}$  NMR (150 MHz,  $\text{D}_2\text{O}$ ; acetone [Me, 32.97] as an internal standard) of H-Val-Tyr-OH·HCl



$^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ ) of Fmoc-Lue-Val-Tyr(O<sup>t</sup>Bu)-OTAG<sub>A</sub>



$^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ) of Fmoc-Lue-Val-Tyr(O<sup>t</sup>Bu)-OTAG<sub>A</sub>

