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# Supporting Information



69451 Weinheim, Germany

# Inhibition of HIV-1 Fusion by Hydrogen-Bond Surrogate Based α-Helices

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**General.** Commercial-grade reagents and solvents were used without further purification except as indicated. $CH_2Cl_2$, THF, and DMF were dried prior to use by percolation through anhydrous $Al_2O_3$ as described by Grubbs and coworkers.[1] Dichloroethane was distilled from sodium before use in the metathesis reaction. All reactions were stirred magnetically; moisture-sensitive reactions were performed under argon in flame-dried glassware. Flash chromatography with silica gel was performed following the conditions described by Still and coworkers.[2] Reverse-phase HPLC experiments were conducted with 4.6 x 150 mm (analytical scale) or 21.4 x 150 mm (preparative scale) Waters $C_{18}$ reverse phase columns using a Beckman Coulter HPLC equipped with a System Gold 168 Diode array detector. The typical flow rates for analytical and preparative HPLC were 1 mL/min and 8 mL/min, respectively. In all cases, 0.1% aqueous trifluoroacetic acid and acetonitrile buffers were used. Proton NMR spectra of monomer were obtained on a Bruker AV-400 (400 MHz). Carbon NMR spectra were obtained on a Bruker (100.5 MHz) spectrometer. Proton chemical shifts are reported as d values relative to tetramethylsilane (TMS, 0.00 ppm) or to the particular solvent used in the experiment ($CDCl_3$: 7.26 ppm, ACN-d3: 1.94 ppm, $D_2O$: 4.75 ppm). Carbon chemical shifts are reported as d values relative to TMS (0.00 ppm) or to the particular solvent used in the experiment ($CDCl_3$: 77.0 ppm). Data is reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, br = broad), coupling constant, and integration. High-resolution mass spectra (HRMS) were obtained on a LC/MSD TOF (Agilent

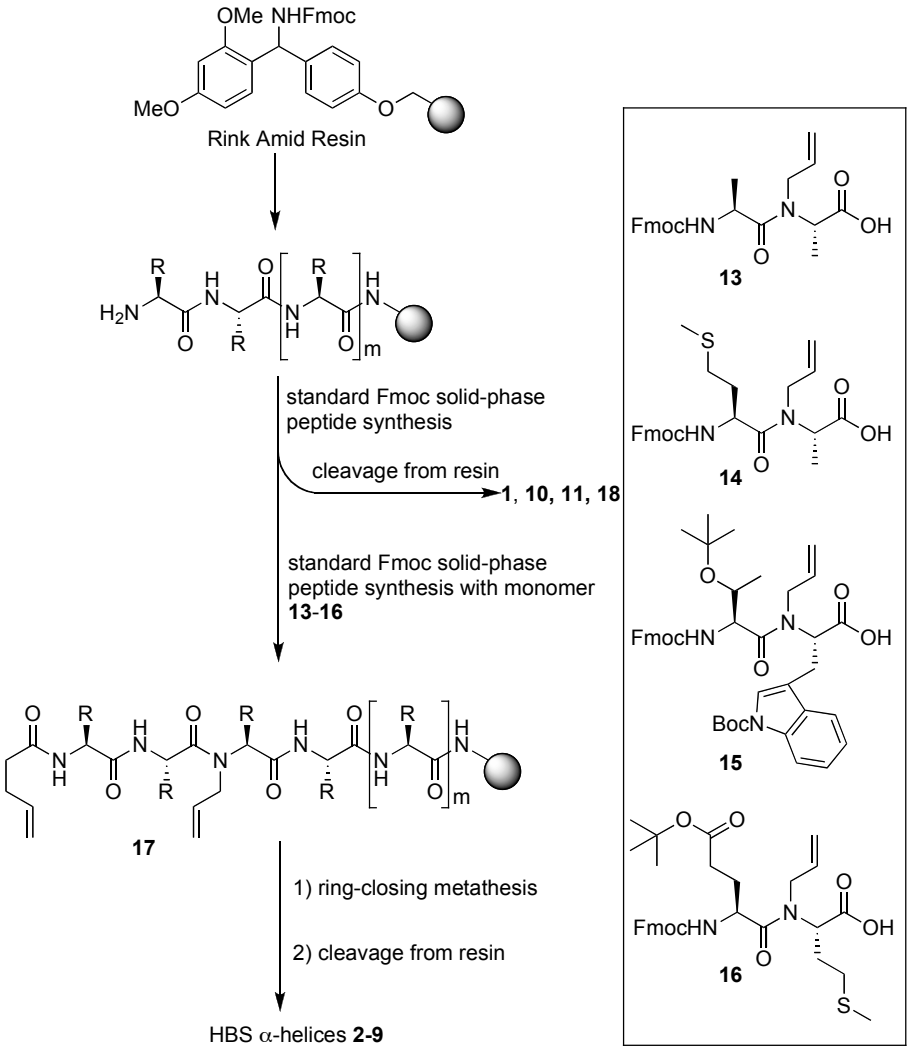
1. Pangborn, A., Giardello, M. A., Grubbs, R. H., Rosen, R. K., Timmers, F. J. *Organometallics* **1996**, *15*, 1518-1520.

2. Still,W., Kahn, M. , Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923-2925.

Technologies). LCMS data was obtained on an Agilent 1100 series LC/MSD (XCT) electrospray trap. The microwave reactions were performed in the CEM Discover single-mode reactor with controlled power, temperature, and time settings.

## Synthesis and characterization of peptides 1-12

**Scheme S1.** Synthesis of peptides **1-10**

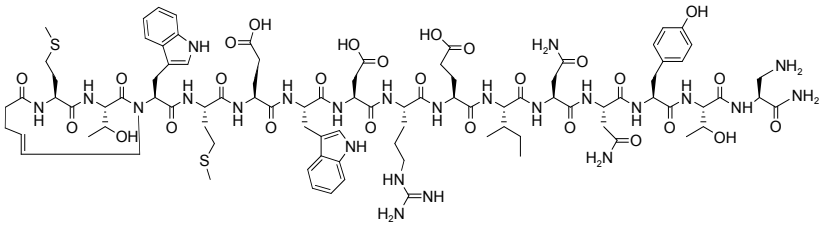


**Peptide 1-10**.[3] Peptides **1, 10** and **11** and resin-bound bis-olefins (**17**) were synthesized by conventional Fmoc solid phase chemistry on Rink amide HMBA resin (NovaBiochem), 0.05–0.15 mmol scale, with appropriate substitutions of *N*(allyl)-dipeptides **13-16** and 4-pentenoic acid. In each coupling step, Fmoc group was removed by treatment with 20% piperidine in NMP (2×20 min). The next Fmoc amino acid (4 equiv) in the sequence was activated with HBTU (3.6 equiv) in 5% DIPEA/NMP solution for 15 minutes, added to the resin bearing the free amine. The resulting mixture was shaken for 60 minutes. The coupling efficiency for each step was monitored by ninhydrin test. After the peptide was assembled on the resin, the resin were thoroughly washed with DMF, methanol and dichloromethane respectively, and dried under vacuum overnight.

The microwave-assisted ring-closing metathesis reactions on resin-bound bis-olefins (**17**) were performed as described with the Hoveyda-Grubbs catalyst (0.15 equiv) in dichloroethane.[3] The reaction mixture was irradiated with the following settings: 250 W maximum power, 120 ˚C, ramp time 5 min, hold time 10 min. Resin bound peptides were cleaved from the resin by treatment with the cleavage cocktail ($CF_3CO_2H$:$H_2O$:triisopropylsilane, 95:2.5:2.5) for 1.5 hour, and purified by reversed-phase HPLC to afford HBS α-helices **2-9**.

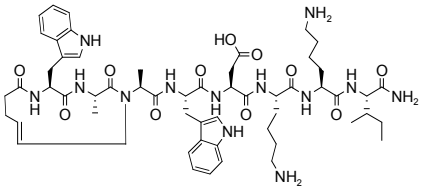
Peptide **1**: AcMTWMEWDERINNYT-$NH_2$

HBS α-helix **2**:

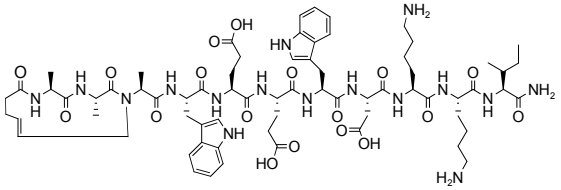


3. Chapman, R. N., Arora, P. S. *Org*. *Lett*. **2006**, *8*, 5825-5828.

HBS α-helix **3**:



HBS α-helix **4**:



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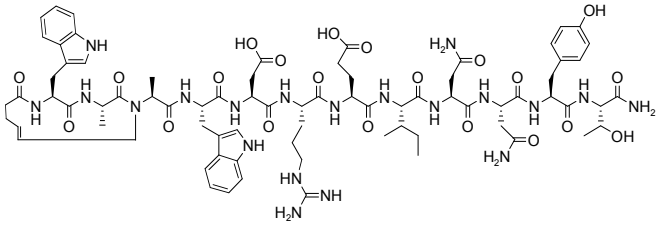
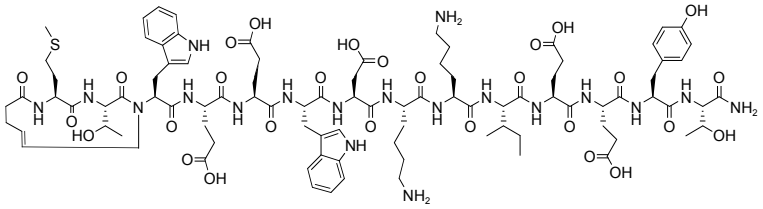
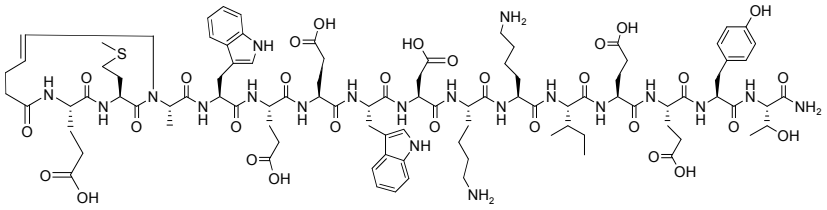


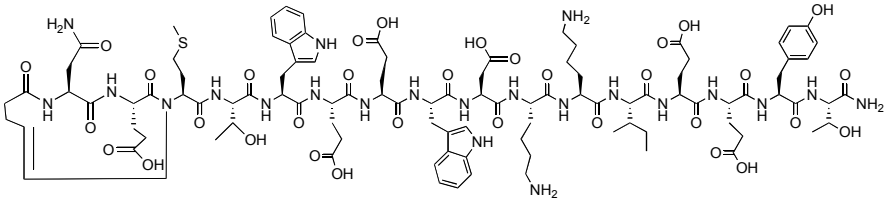
HBS α-helix **6**:



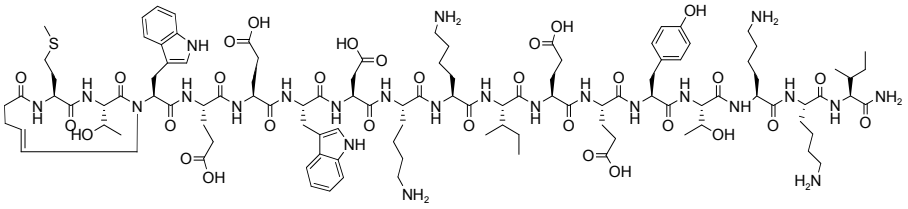
HBS α-helix **7**:



HBS α-helix **8**:



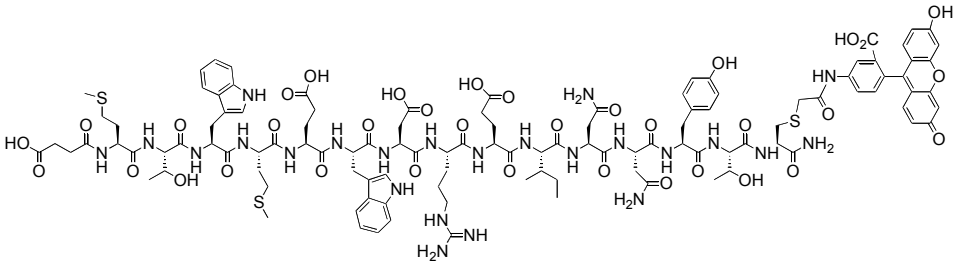
HBS α-helix **9**:



Peptide **10**: AcMTWEEWDKKIEEYTKKI-$NH_2$

Peptide IZN17 **11**: AcIKKEIEAIKKEQEAIKKKIEAIEKLLQLTVWGIKQLQARIL-$NH_2$.

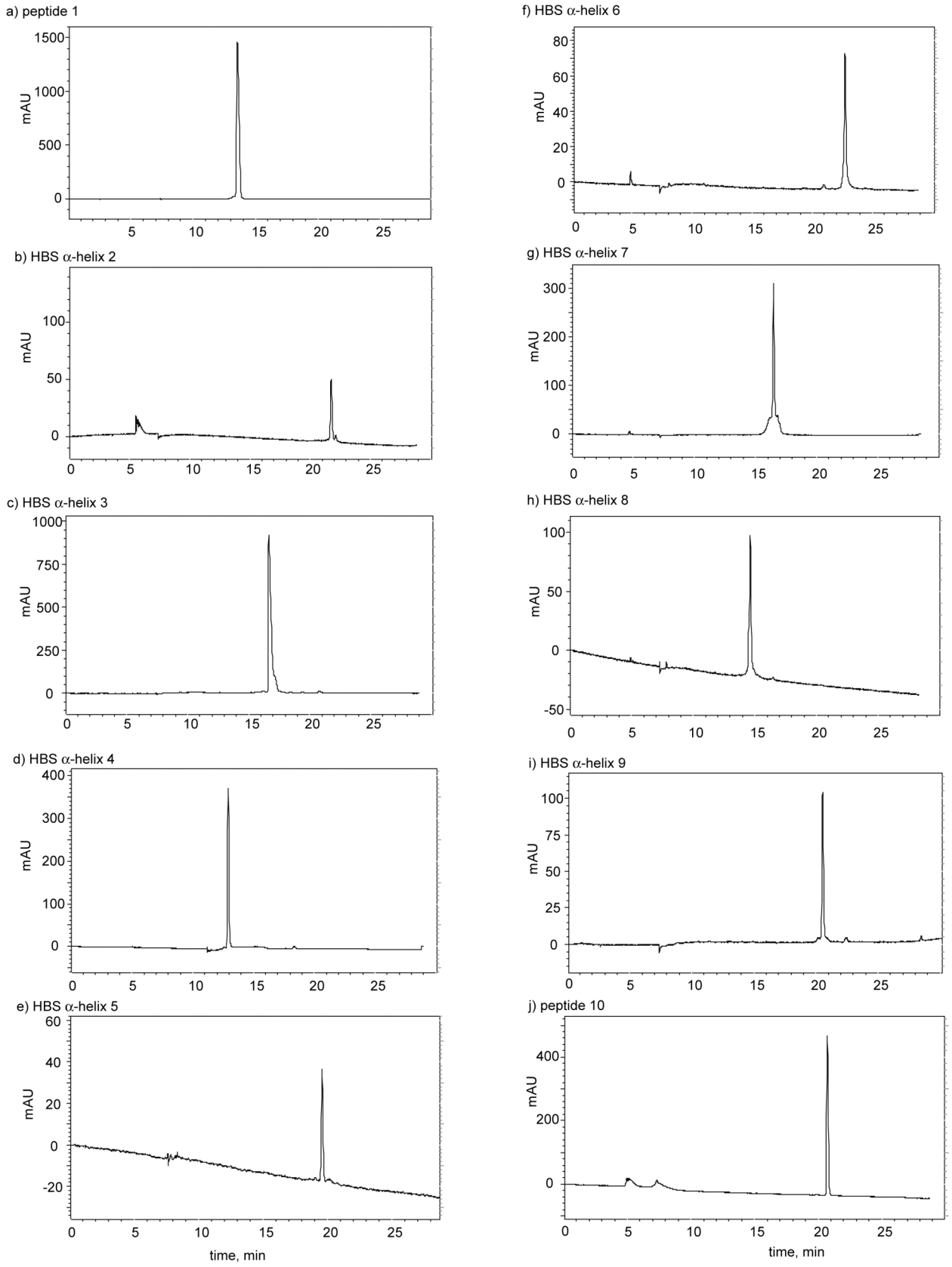
Peptide **12**: Suc-MTWMEWDERINNYTC$^{Flu}$-$NH_2$



**12**

**Table S1**. Mass spectrometry results. LCMS data was obtained on an Agilent 1100 series LC/MSD (XCT) electrospray trap.

| peptide | Expected $[M+H]^+$ | Found $[M+H]^+$ |
|---|---|---|
| **1** | 1929.8 | 1929.6 |
| **2** | 2068.0 | 2068.6 |
| **3** | 1110.6 | 1111.1 |
| **4** | 1439.7 | 1440.5 |
| **5** | 1631.8 | 1632.2 |
| **6** | 1981.0 | 1981.8 |
| **7** | 2080.0 | 2080.0 |
| **8** | 2224.0 | 2225.0 |
| **9** | 2350.2 | 2351.2 |
| **10** | 2298.2 | 2299.0 |
| **11** | 2478.0 | 2478.6 |
| **12** | 4855.9 | 4854.7 |


a) peptide 1
b) HBS α-helix 2
c) HBS α-helix 3
d) HBS α-helix 4
e) HBS α-helix 5
f) HBS α-helix 6
g) HBS α-helix 7
h) HBS α-helix 8
i) HBS α-helix 9
j) peptide 10
mAU
time, min

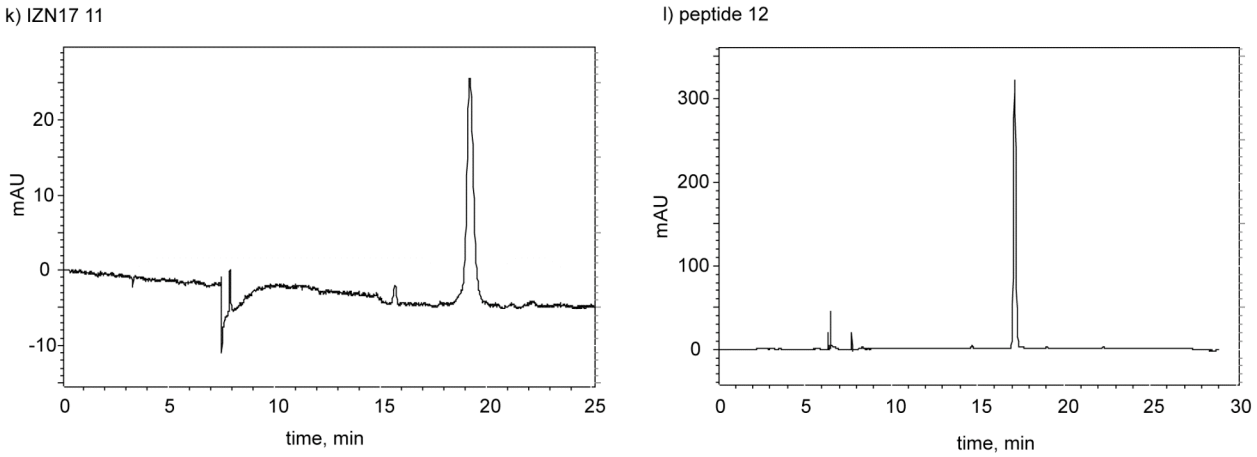
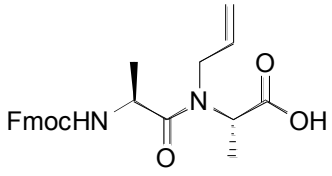



**Figure S1**. Analytical HPLC plot for peptides. HPLC conditions: $C_{18}$ reversed-phase column. 5% B to 15% B in 3 min, 15% B to 35% B in 20 min, 35% B to 100% B in 7 min; A: 0.1% aqueous TFA, B: acetonitrile; flow rate: 1.0 mL/min; monitored at 275 nm.

## Synthesis and characterization of *N*-allyl-dipeptides 13-16

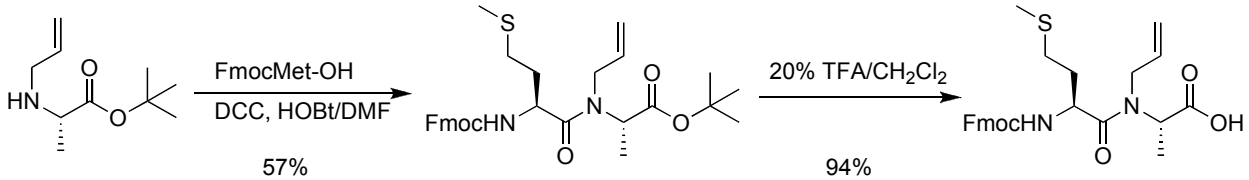
**FmocAla-*N*(allyl)Ala-OH (13).** The synthesis and characterization of this *N*-allyldipeptide have been previously described.[4]




13

**FmocMet-*N*(allyl)Ala-O*H* (14)**

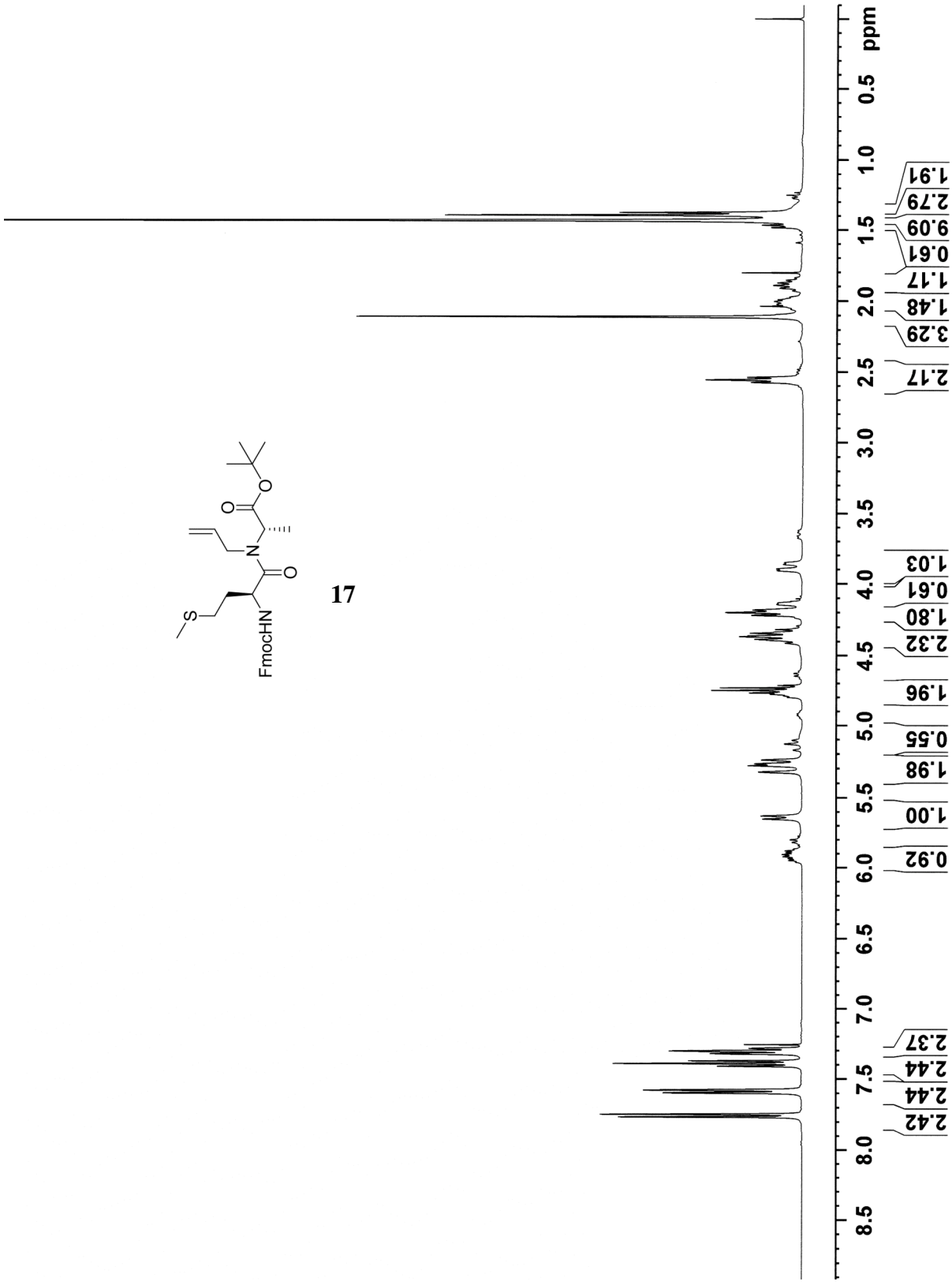
**Scheme S2.** Synthesis of FmocMet-*N*(allyl)Ala-OH (**14**)




**FmocMet-*N*(allyl)Ala-O*t*Bu (17).** A solution of *N*, *N*-dicyclohexylcarbodiimide (DCC, 2.62 g, 12.7 mmol), 1-hydroxybenzotriazole (HOBt, 1.72 g, 12.7 mmol), FmocMet-OH (4.71 g, 12.7 mmol) and 40 mL of DMF was stirred for 15 min. *N*-Allyl-alanine-*t*-butyl ester (1.81 g, 9.77 mmol) was then added to the flask and the reaction mixture was stirred at 50 ˚C. After 12 h, the reaction mixture was poured into 40 mL of water and extracted with ether (3 × 40 mL). The

4. Chapman, R. N., Dimartino, G., Arora, P. S. *J. Am Chem. Soc*. **2004**, *126*, 12252-12253.

combined ether layers were washed with water (3 × 40 mL) and dried with anhydrous magnesium sulfate, filtered and concentrated by rotary evaporation. The residue was purified with flash chromatography (95:5, dichloromethane: ethyl acetate) to afford 3.00 g of FmocMet-*N*(allyl)AlaO-*t*Bu **17** (57%) as a yellow oil: $^{1}$H NMR (400 MHz, $CDCl_3$, major rotamer) δ 7.76 (d, *J* = 7.50 Hz, 2H), 7.59 (d, *J* = 7.50 Hz, 2H), 7.39 (t, *J* = 7.50 Hz, 2H), 7.30 (t, *J* = 7.50 Hz, 2H), 5.95-5.86 (m, 1H), 5.65 (d, *J* = 8.8 Hz, 1H), 5.30（d, *J* = 17.5 Hz, 1 H）, 5.25 (d, *J* = 10.5 Hz, 1 H) , 4.80-4.70 (m, 2 H), 4.36 (h, *J* = 10.3 Hz, 3H), 4.20 (d, *J* = 7.2 Hz, 1 H), 4.18-4.10 (dd, AB pattern, *J* = 17.5, 5.0 Hz, 1H), 3.90-3.85 (dd, AB pattern, *J* = 17.5, 5.0 Hz, 1H), 2.55 (t, *J* = 7.1 Hz, 2 H), 2.10 (s, 3 H), 2.00 (m, 1 H), 1.90 (m, 1H), 1.45 (s, 9 H), 1.39 (s, 3H), 1.37 (d, *J* = 7.9 Hz, 2 H); $^{13}$C NMR (100 MHz, $CDCl_3$, major rotamer) δ 172.20, 170.51, 155.91, 143.90, 141.30, 133.85, 127.71, 127.06, 125.15, 120.00, 117.83, 81.61, 66.99, 54.46, 50.51, 48.46, 47.19, 45.96, 33.58, 29.97, 28.00, 15.74, 14.68; ESIMS *m/z* for $C_{30}H_{39}N_2O_5$ $[M+H]^+$, calcd 539.2501, found 539.2580.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2.0
1.5
1.0
0.5
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2.44
2.44
2.37
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1.00
1.98
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1.96
2.32
1.80
0.61
1.03
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3.29
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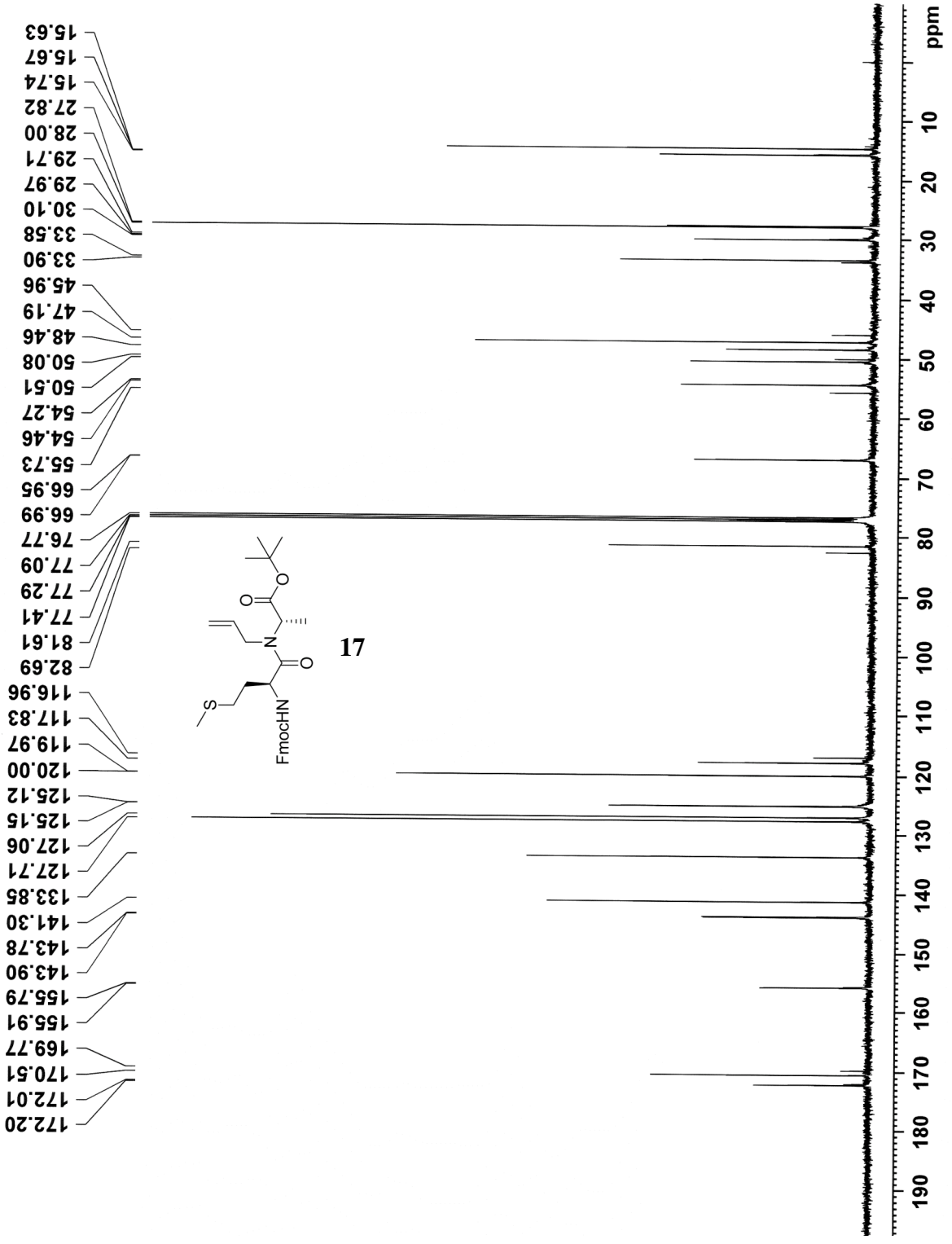
9.09
2.79
1.91
FmocHN
S

**17**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54.46
55.73
66.95
66.99
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77.09
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77.41
81.61
82.69
116.96
117.83
119.97
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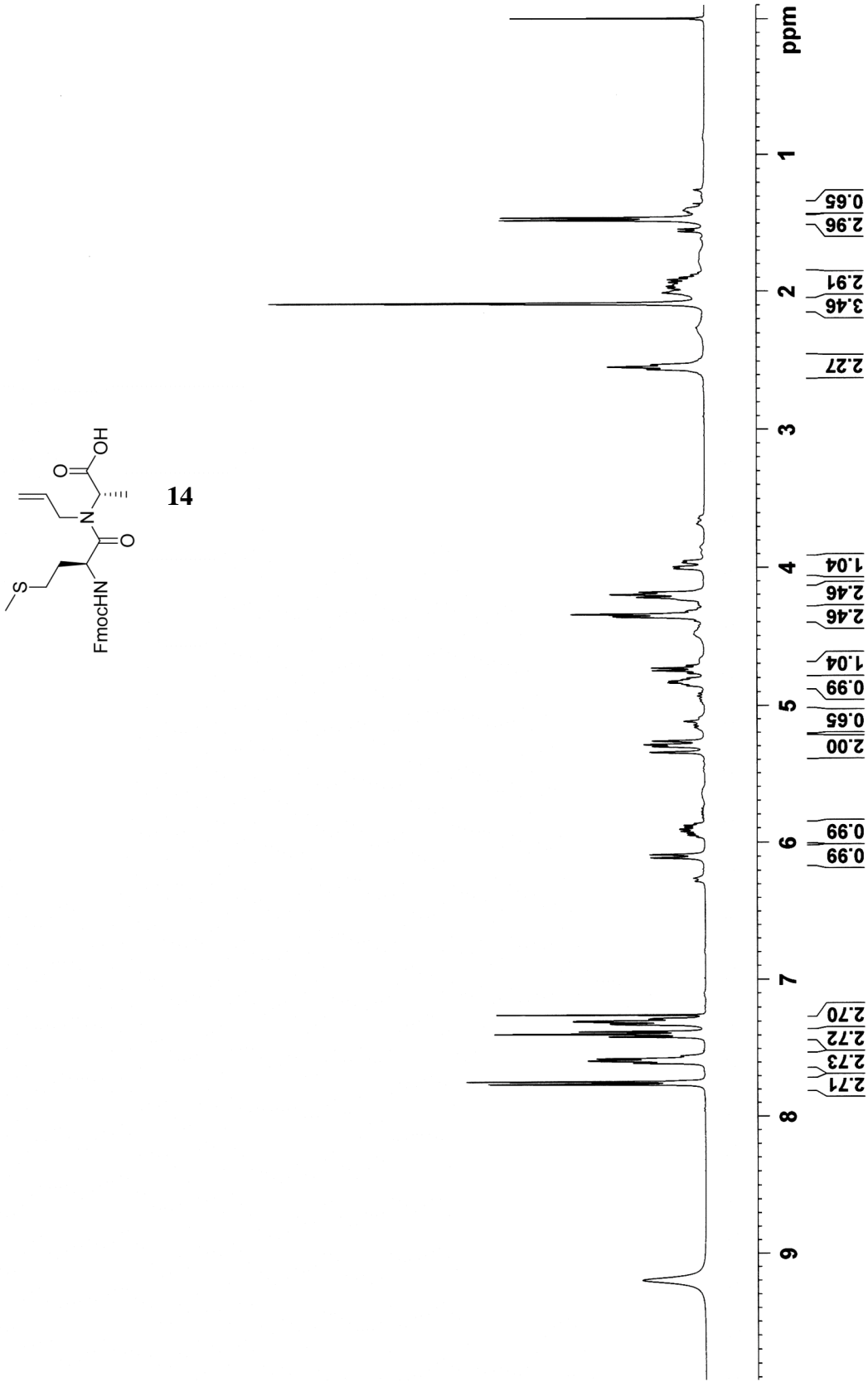
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
FmocHN
17

**FmocMet-*N* (allyl)-Ala-OH (14).**A solution of FmocMet-*N*(allyl)Ala-O*t*Bu (2.90g, 5.86mmol), 80 mL of dichloromethane, and 20 mL of trifluoroacetic acid (TFA) was stirred for 4 h, and then concentrated under vacuum. The residue was redissolved in 80 mL of dichloromethane and washed with water (3 × 40 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography (1:1, hexane: ethyl acetate) to afford 2.60 g of **14** (94%) as a white foam. $^{1}$H NMR (400 MHz, $CDCl_3$, major rotamer) δ 7.75 (d, *J* = 7.50 Hz, 2H), 7.59 (d, *t*= 7.00 Hz, 2H), 7.39 (t, *J* = 7.50 Hz, 2H), 7.30 (t, *J* = 7.50 Hz, 2H), 6.32(d, *J* = 8.9 Hz, 1 H ), 5.95-5.86 (m, 1H), 5.32 (d, *J* = 17.2 Hz, 1 H), 5.26 (d, *J* = 10.2 Hz, 1 H), 4.80 (m, 1 H), 4.70 (q, *J* = 7.4 Hz, 1 H), 4.35 (d, *J* = 5.9 Hz, 2 H), 4.20 (m, 2 H), 3.95 (dd, AB mode, *J* = 17.8, 5.0 Hz, 1 H), 2.55 (t, *J* = 7.0 Hz, 2 H), 2.10 (s, 3 H), 2.00-1.87 (m, 3 H), 1.45 (d, *J* = 7.3 Hz, 3 H); $^{13}$C NMR (100 MHz, $CDCl_3$, major rotamer) δ 175.90, 173.78, 156.78, 143.78, 141.30, 133.02, 127.79, 127.11, 125.21, 120.02, 118.25, 67.69, 55.51, 50.55, 49.08, 47.06, 32.97, 30.04, 16.02, 14.36; HRMS *m/z* for $C_{26}H_{31}N_2O_5$ $[M+H]^+$, calcd 483.1875, found 483.1954.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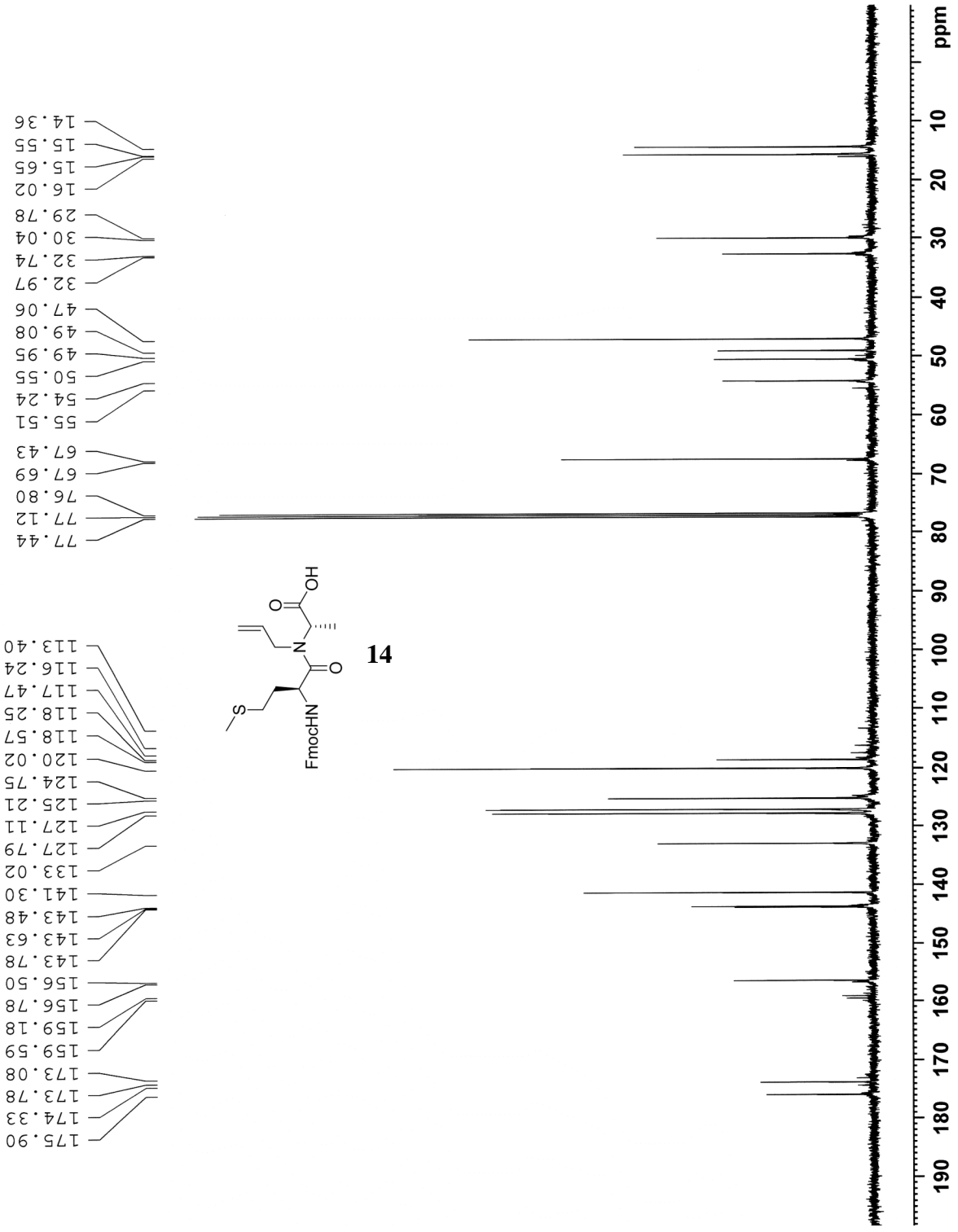
2.71

**14**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77.12
77.44
113.40
116.24
117.47
118.25
118.57
120.02
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125.21
127.11
127.79
133.02
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143.48
143.63
143.78
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156.78
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159.59
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173.78
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N
O
S
FmocHN
14
ppm
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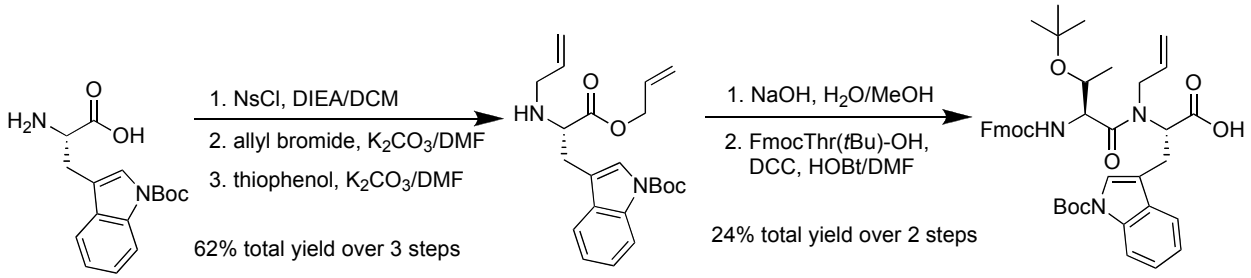
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190

**FmocThr(O*t*Bu)-*N*(allyl)Trp(Boc)-OH (15)**

**Scheme S3.** Synthesis of FmocThr(O*t*Bu)-*N*(allyl)Trp(Boc)-OH (**15**)



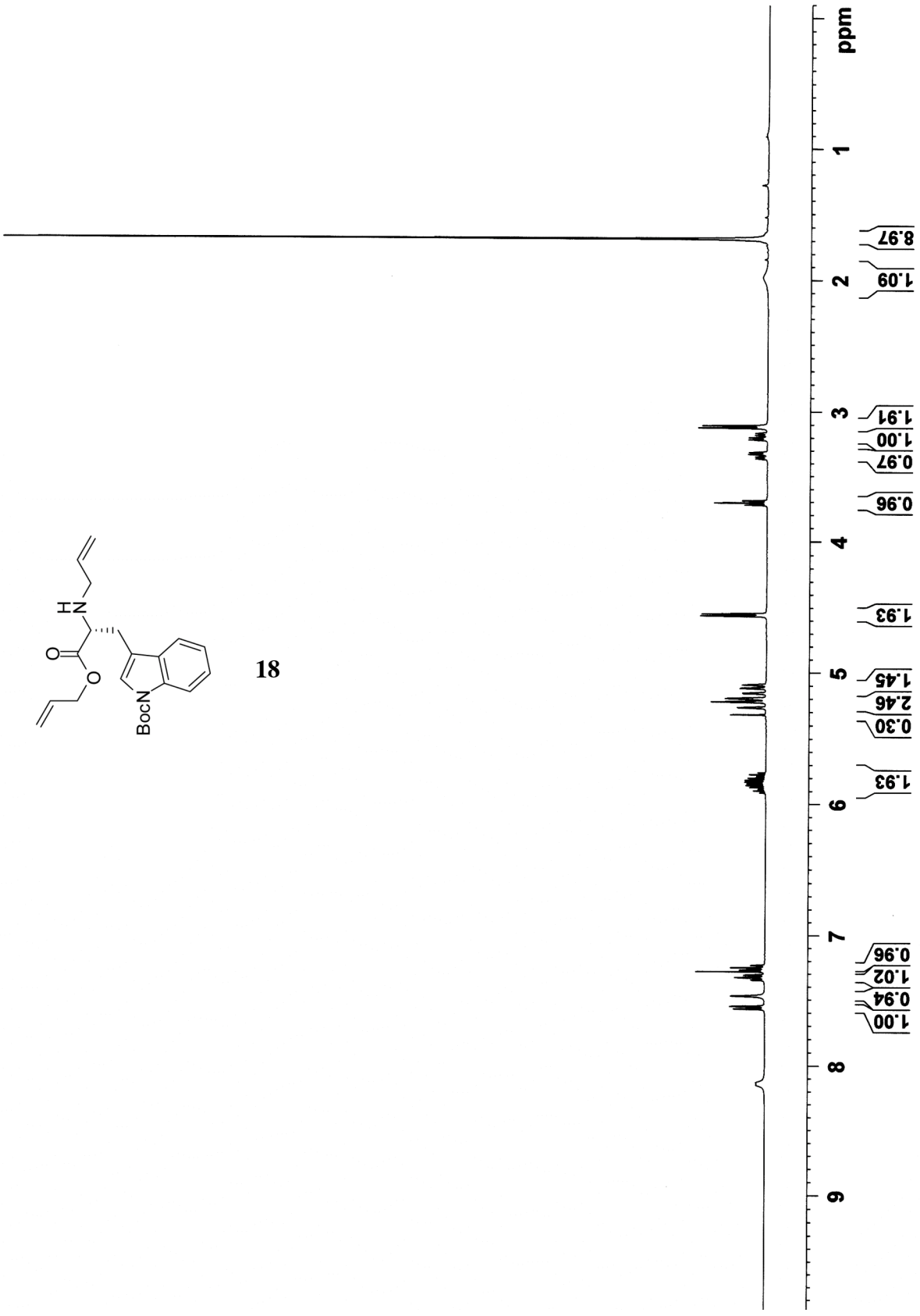

***N*(Allyl)Trp(Boc)-Allyl Ester (18).** A solution of Trp(Boc)-OH (3.78 g, 12.43 mmol), *o*-nitrobenzenesulfonyl chloride ( 3.18 g, 14.11 mmol), 15 ml of saturated sodium carbonate aqueous solution and 20 mL of dioxane was stirred overnight. The pH of the solution was adjusted with 0.5 M HCl aqueous solution to 7.0. The solution was concentrated and extracted with ether (3 × 50 mL). The combined organic layers were washed with water, dried (magnesium sulfate) and concentrated to afford 5.48 g of *N* (*o*-Ns)-Trp(Boc)-OH (90%) as a yellow solid.

A solution of *N*(*o*-Ns)-Trp(Boc)-OH (2.00 g, 4.08 mmol), potassium carbonate (2.00 g, 14.30 mmol), ally bromide (1.48g, 12.25 mmol) in 50 mL of DMF was stirred for 8 h and poured in to 50 mL of water. The aqueous layer was extracted by ether (3 × 30 mL). The combined organic layers was washed with water, dried (magnesium sulfate) and concentrated to afford 2.00g of *N*(*o*-Ns)-*N*(allyl)-Trp(Boc)- allyl ester (86%) as a pale yellow solid.

Thiophenol (0.48 g, 4.22 mmol) was added to a solution of *N*(*o*-Ns)-*N*(allyl)-Trp(Boc)-allyl ester (2.00 g, 3.51 mmol) and $K_2CO_3$ (1.46 g, 10.54 mmol) in 40 mL of dry DMF under nitrogen. The reaction mixture was stirred for 1.5 h and then poured into water (40 mL). The aqueous layer

was extracted with ether (3 x 40 mL). The combined organic layers were concentrated and the residue was purified by flash chromatography (EtOAc:Hexane, 15: 85) to afford 1.29 g of *N*(allyl)-Trp(Boc)-allyl ester **18** (80%) as a pale yellow solid.

$^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, *J* = 6.7 Hz, 1 H), 7.56 (d, *J* = 7.7 Hz, 1H), 7.48 (s, 1 H), 7.33 (t, *J* = 8.1 Hz, 1 H), 7.25 (t, *J* = 7.4 Hz, 2 H), 5.75-5.90 (m, 2 H), 5.30 (d, *J* = 17.5 Hz, 1 H), 5.24 (d, *J* = 17.5 Hz, 1 H), 5.18 (d, *J* = 10.3 Hz, 1 H), 5.10 (d, *J* = 10.3 Hz, 1 H), 4.56 (d, *J* = 5.8 Hz, 1 H), 3.70 (t, *J* = 6.7 Hz, 1 H), 3.37-3.30 (dd, AB mode, *J* = 13.9, 5.8 Hz,1 H), 3.22-3.16 (dd, AB mode, *J* = 13.9, 5.8 Hz,1 H), 3.11 (d, *J* = 6.6 Hz, 2 H), 1.69 (s, 9 H).; $^{13}$C NMR (100 MHz, $CDCl_3$) δ 174.18, 149.64, 135.93, 135.42, 131.76, 130.47, 124.43, 124.08, 122.45, 118.96, 118.73, 116.71, 115.94, 115.26, 83.52, 65.47, 60.57, 50.68, 29.10, 28.23; ESIMS *m/z* for $C_{22}H_{29}N_2O_4$ $[M+H]^+$, calcd 385.2, found 385.2.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2
3
4
5
6
7
8
9
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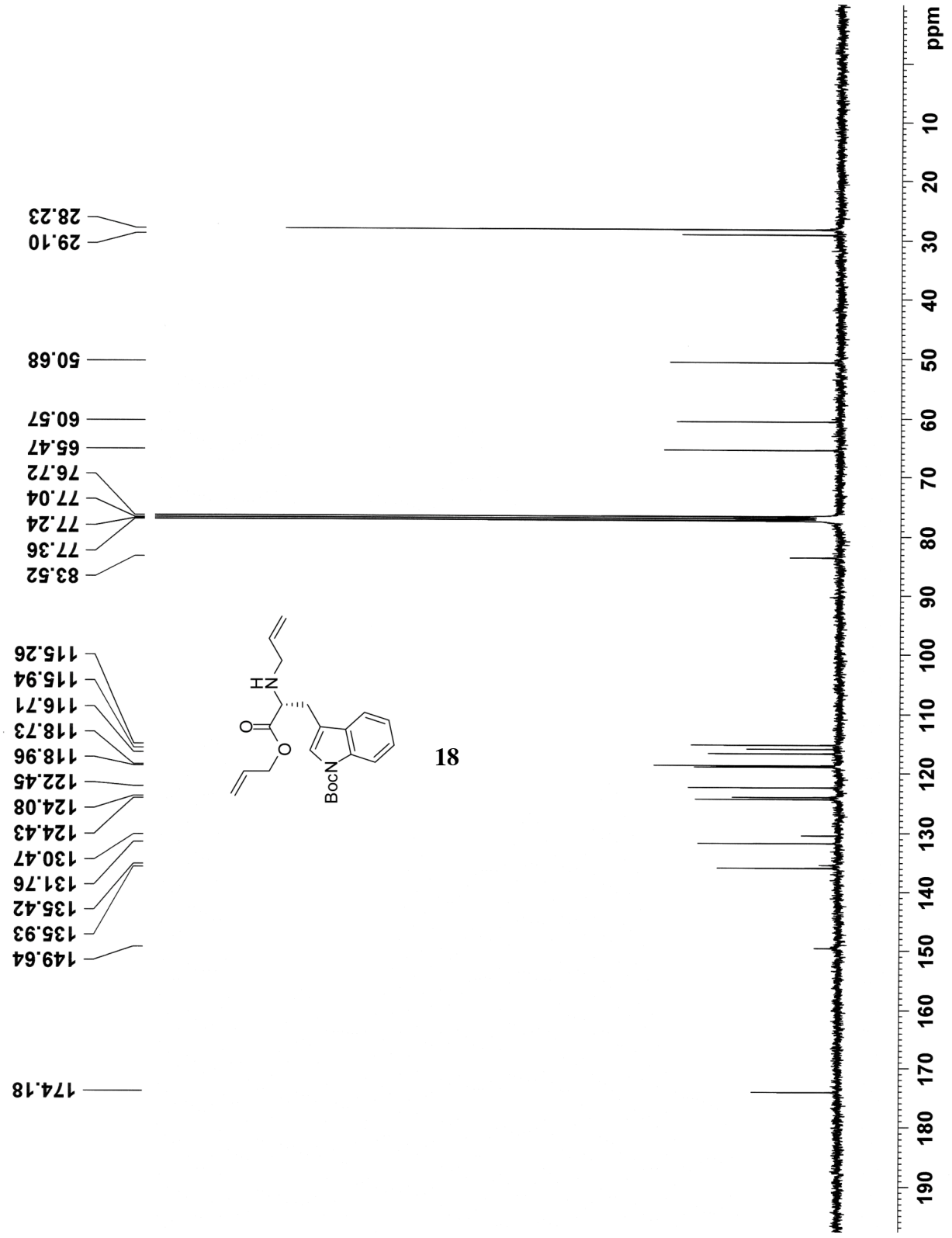
O
BocN

**18**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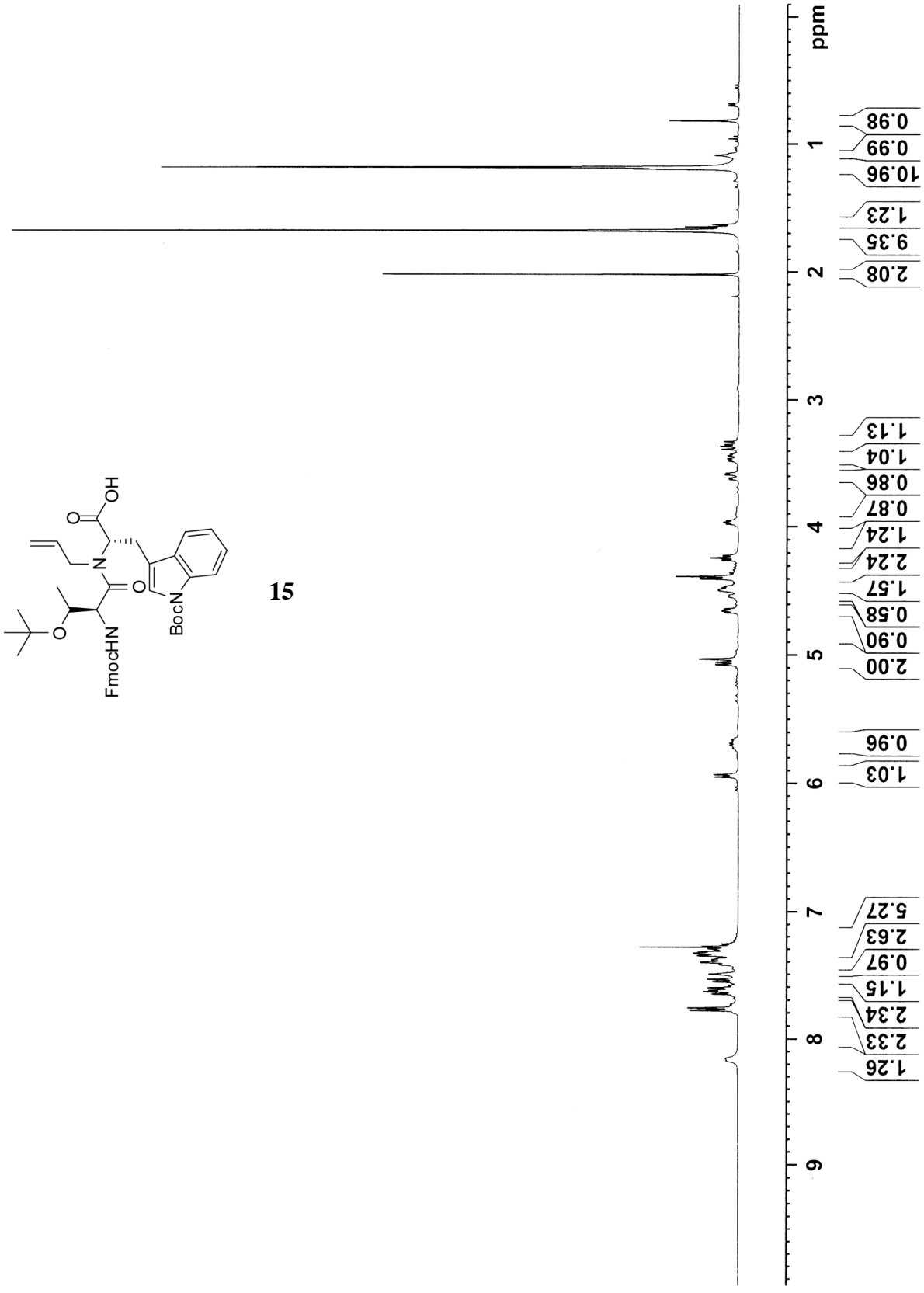
170
180
190
BocN
H
N
O
O

**18**

**FmocThr(O*t*Bu)-*N*(allyl)-Trp(Boc)-OH (15)** A solution of *N*(allyl)-Trp(Boc)-allyl ester **18** (0.97 g, 2.53 mmol), sodium hydroxide (0.15 g, 3.80 mmol) in 1 mL of water and 3 mL of methanol was stirred for 4 h. The pH of the solution was adjusted with 0.5 M HCl aqueous solution to 7.0. The solution was concentrated and dried in vacuum.

A solution of DCC (0.68 g, 3.29 mmol), HOBt (0.44 g, 3.29 mmol), FmocThr(O*t*Bu)-OH (1.31 g, 3.29 mmol) and 15 mL of DMF was stirred for 15 min, then added to the above flask. The reaction mixture was stirred at 50 ˚C. After 12 h, the reaction mixture was poured into 15 mL of water and extracted with ether (3 × 30 mL). The combined ether layers were washed with water (3 × 30 mL) and dried with anhydrous magnesium sulfate, filtered and concentrated. The residue was purified with flash chromatography (DCM:MeOH, 98:2) to afford 0.27 g of FmocThr(O*t*Bu)-*N*(allyl)-Trp(Boc)-OH **15** (24% over two steps) as a white foam. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, *J* = 6.7 Hz, 1 H), 7.80 (d, *J* = 7.7 Hz, 2 H), 7.66 (d, *J* = 7.6 Hz, 1 H), 7.61 (d, *J* = 7.6 Hz, 1 H), 7.56 (d, *J* = 7.7 Hz, 1 H), 7.48 (s, 1 H), 7.40 (m, 2 H), 7.35-7.20 (m, 4 H), 5.95 (d, *J* = 7.4 Hz, 1H) ,5.80-5.60 (m, 1H), 5.08 (d, *J* = 17.5 Hz, 1H), 5.05 (d, *J* = 9.8 Hz, 1H), 4.65 (dd, *J* = 13.3, 5.8 Hz, 1H), 4.50-4.44 (m, 2 H), 4.40 (d, *J* = 7.5 Hz, 2 H), 4.24 (t, *J* = 7.0 Hz, 1 H), 3.98 (t, *J* = 6.9 Hz, 1 H), 3.60 (dd, AB mode, *J* =13.4, 5.8 Hz, 1 H), 3.45 (dd, AB mode, 13.4, 5.8 Hz, 1 H), 3.35 (dd, 12.5, 6.5 Hz, 1 H), 1.78 (s, 9 H), 1.65 (d, *J* = 7.4 Hz, 3 H), 1.20 (s, 9 H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 176.30, 172.32, 157.17, 150.63, 144.03, 143.71, 141.33, 135.53, 132.78, 130.04, 127.69, 127.05, 125.27, 124.90, 124.52, 122.62, 119.59, 118.95, 118.65, 116.02, 115.76, 83.57, 74.85, 68.80, 66.98, 59.74, 56.40, 52.84, 47.21, 28.22, 25.42, 18.39; HRMS *m/z* for $C_{42}H_{50}N_3O_8$ $[M+H]^+$, calcd 724.3520, found 724.3598.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FmocHN
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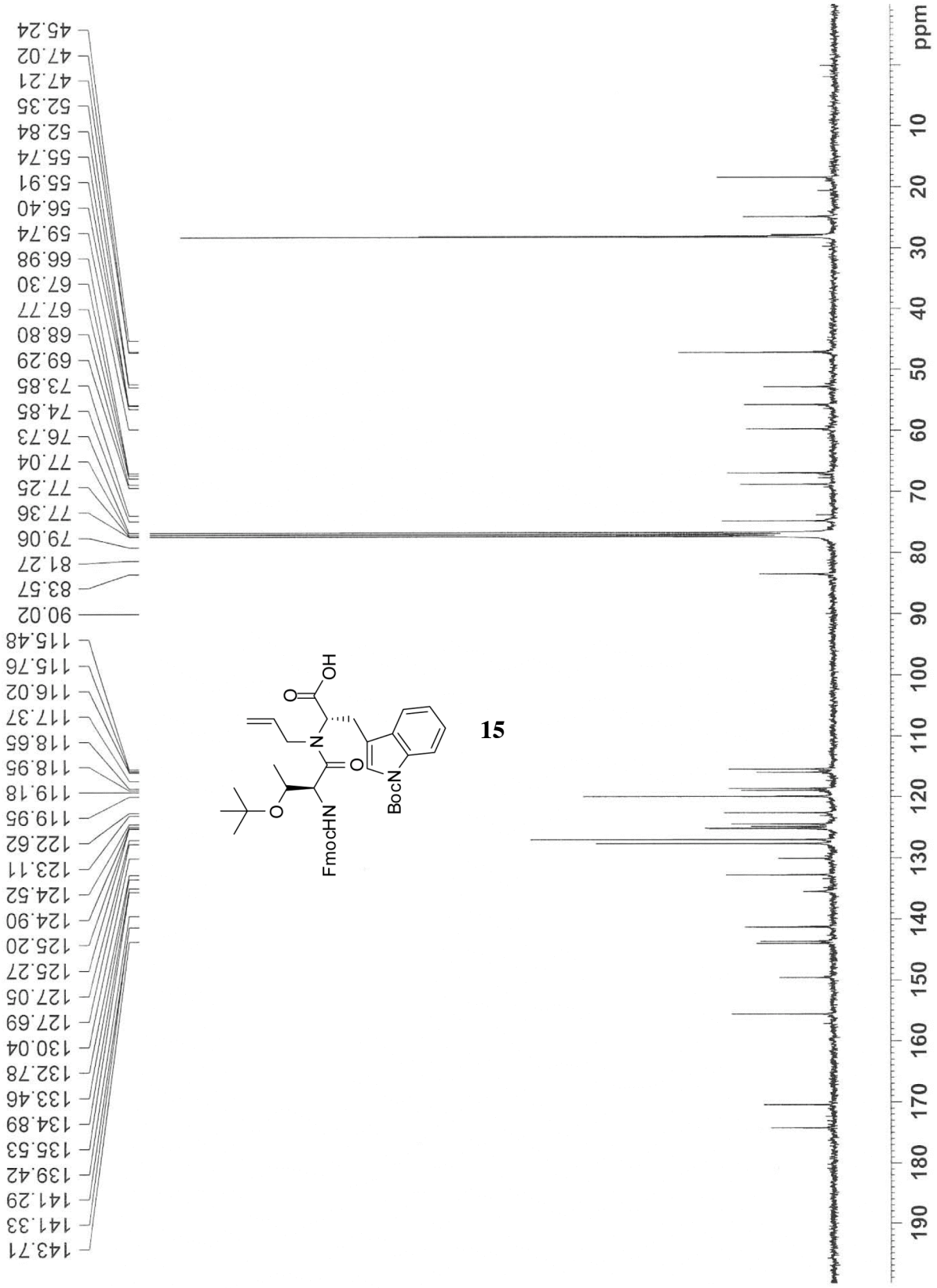
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115.76
116.02
117.37
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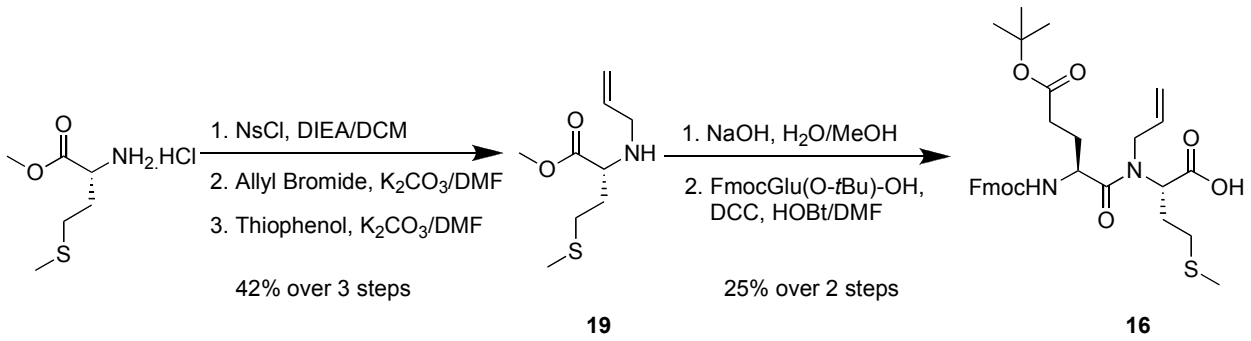
160
170
180
190
OH
O
N
O
O
BocN
FmocHN
O

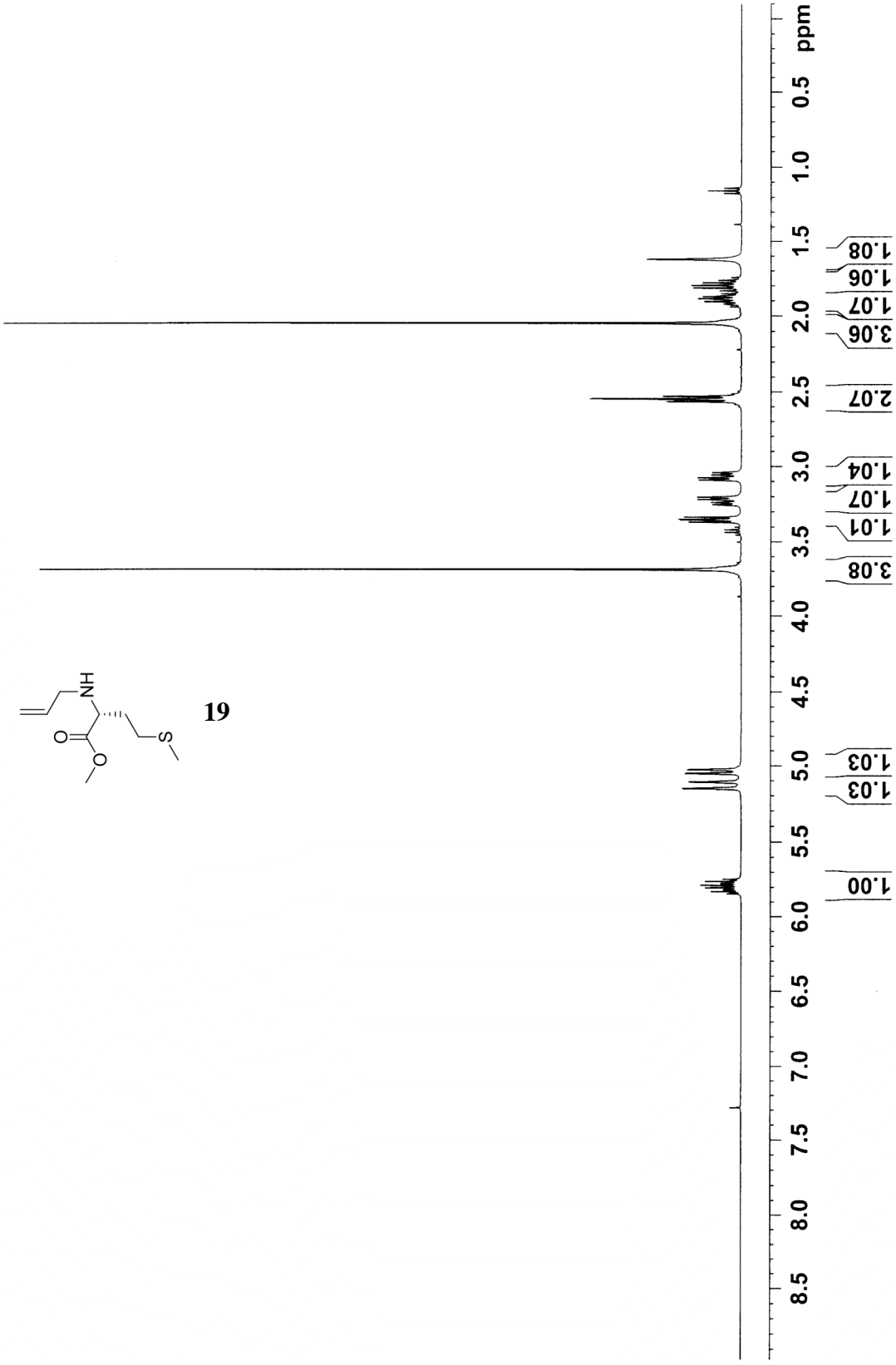
**15**

**FmocGlu(O*t*Bu)-*N*(allyl)Met-OH (16)**

**Scheme S4.** Synthesis of FmocGlu(O*t*Bu)-*N*(allyl)Met-OH (**16**)




***N*(Allyl)-Met-OMe (19)** *N*(Allyl)-Met-OMe was synthesized from methionine methyl ester, following a procedure similar to that described above for *N*(allyl)-Trp(Boc)-allyl ester (**18**), in 42% overall yield (Scheme S4). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 5.85-5.75 (m, 1 H), 5.13 (d, $J$ = 17.1 Hz, 1 H), 5.04 (d, $J$ = 10.2 Hz, 1 H), 3.70 (s, 3 H), 3.35 (dd, $J$ = 7.7, 2.1 Hz, 1 H ), 3.25-3.20 (dd, AB pattern, $J$ = 14.0, 5.0 Hz, 1 H), 3.10-3.04 (dd, AB pattern, $J$ = 14.0, 5.0 Hz, 1 H), 2.55 (t, $J$ = 7.6, 2 H), 2.05 (s, 3 H), 1.89 (m, 1 H), 1.80 (m, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) ẟ 175.48, 136.28, 116.27, 59.33, 51.74, 50.66, 32.83, 30.47, 15.33; HRMS *m/z* for $C_{96}H_{18}NO_2S$ $[M+H]^+$, calcd 204.0980, found 204.1058.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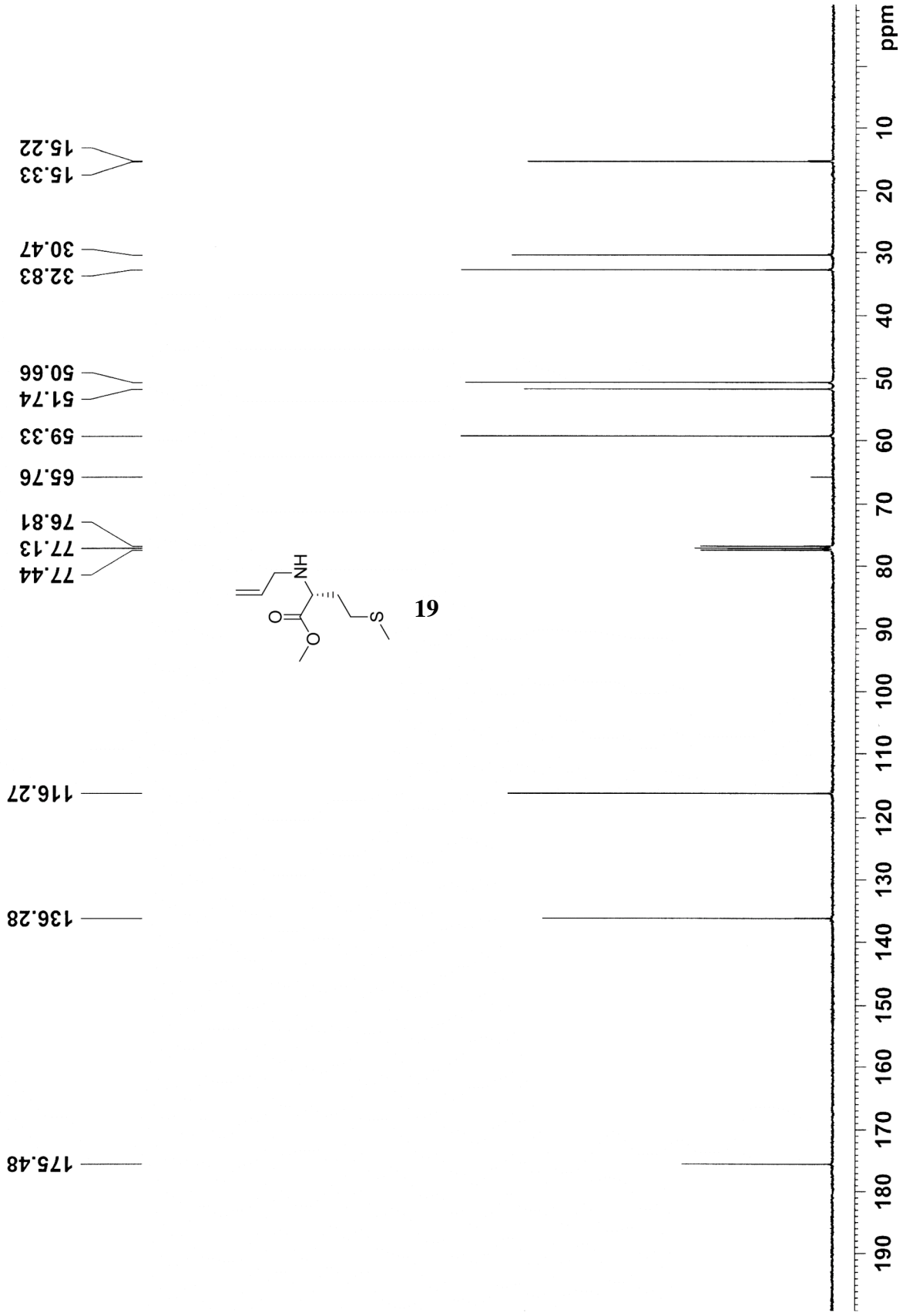
O
S

**19**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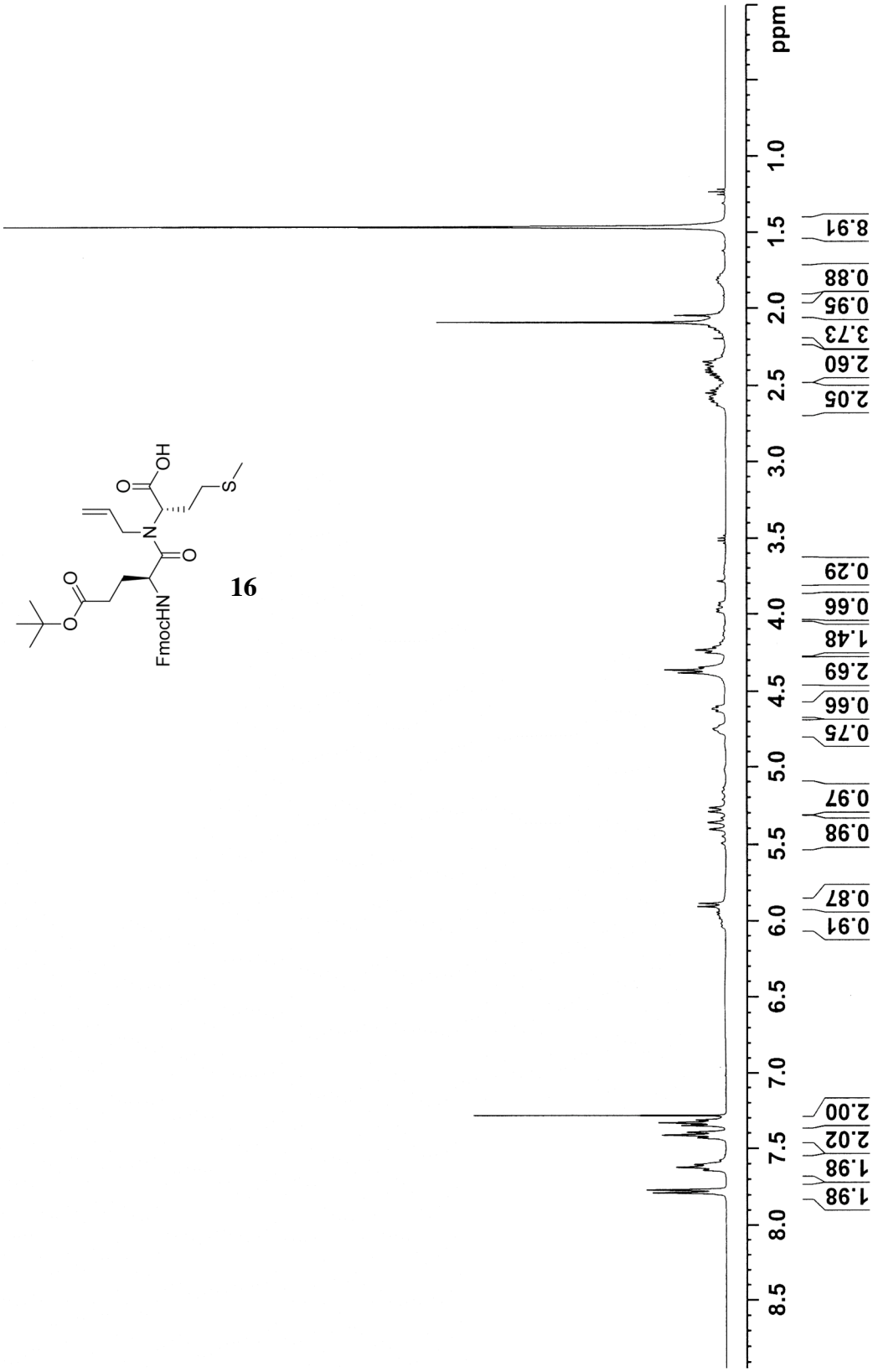
30.47
32.83
50.66
51.74
59.33
65.76
76.81
77.13
77.44
116.27
136.28
175.48
19

**FmocGlu(O*t*Bu)-*N*(allyl)-Met-OH (16)** Fmoc-Glu(O*t*Bu)-N(allyl)-Met-OH **16** was synthesized similar to FmocThr(O*t*Bu)-*N*(allyl)-Trp(Boc)-OH **15** from *N*(allyl)-Met-OMe **19** in 25% yield over 2 steps. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.6 Hz, 2 H), 7.62 (t, *J* = 7.2 Hz, 2 H), 7.41 (t, *J* = 7.2 Hz, 2 H), 7.35 (t, *J* = 7.8 Hz, 2 H), 6.02-5.92 (m, 1 H), 5.90 (d, *J* = 8.8 Hz, 1 H), 5.39 (d, *J* = 17.7 Hz, 1 H), 5.29 (d, *J* = 10.2 Hz, 1 H), 4.75 (t, *J* = 8.7 Hz, 1 H), 4.61 (t, *J* = 8.7 Hz, 1 H), 4.38 (d, *J* = 7.3, 2 H), 4.35 (m, 1 H), 4.22 (m, 2 H), 4.00-3.90 (dd AB mode, *J* = 16.8, 6.3 Hz, 1 H), 2.59 (m, 2 H), 2.39 (m, 3 H), 2.10 (s, 3 H), 2.15 - 2.00 (m, 2 H), 1.80 (m, 1 H), 1.47 (s, 9 H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 174.43, 174.03, 173.29, 172.36, 156.23, 144.00, 143.80, 141.30, 133.09, 132.63, 127.70, 127.11, 125.29, 119.96, 119.13, 80.90, 67.13, 57.85, 50.91, 50.69, 47.13, 30.97, 28.19, 15.25; HRMS *m/z* for $C_{32}H_{41}N_2O_7S$ $[M+H]^+$, calcd 597.2556, found 597.2634.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2.02
1.98
2.00
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0.98
0.97
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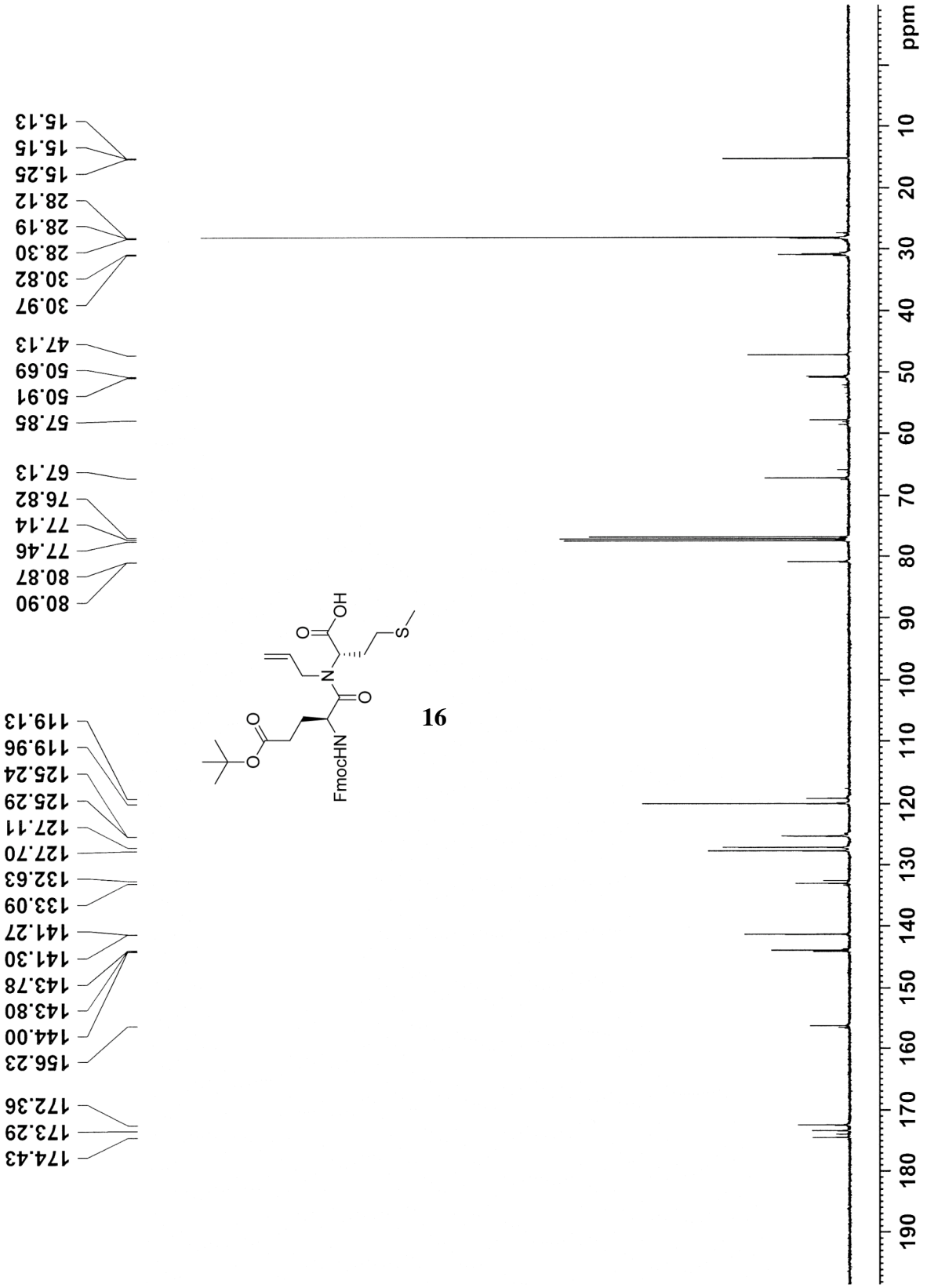
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50
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80
90
100
110
120
130
140
150
160
170
180
190

**Circular dichroism spectra of peptides 1-10.**

CD spectra were recorded on AVIV 202SF CD spectrometer equipped with a temperature controller using 1 mm length cells and a scan speed of 5 nm/min. The spectra were averaged over 10 scans with the baseline subtracted from analogous conditions as that for the samples. The samples were prepared in 0.1x phosphate buffered saline (13.7 mM NaCl, 1 mM phosphate, 0.27 mM KCl, pH 7.4), containing 10% trifluoroethanol, with the final peptide concentration of 50 μM - 100 μM. The concentrations of unfolded peptides were determined by the UV absorption of tyrosine residue at 276 nm in 6.0 M guanidinium hydrochloride aqueous solution. The helix content of each peptide was determined from the mean residue CD at 222 nm, $[\theta]_{222}$ (deg $cm^2$ $dmol^{-1}$) corrected for the number of amino acids. Percent helicity was calculated from the ratio $[\theta]_{222}/[\theta]_{max}$, where $[\theta]_{max} = (-44000 + 250T)(1 - k/n)$, with k = 4.0 and n = number of residues. For details on $\theta_{max}$ calculations for HBS helices, see: D. Wang, K. Chen, J. L. Kulp, III, P. S. Arora, *J. Am. Chem. Soc*. **2006**, *128*, 9248-9256.

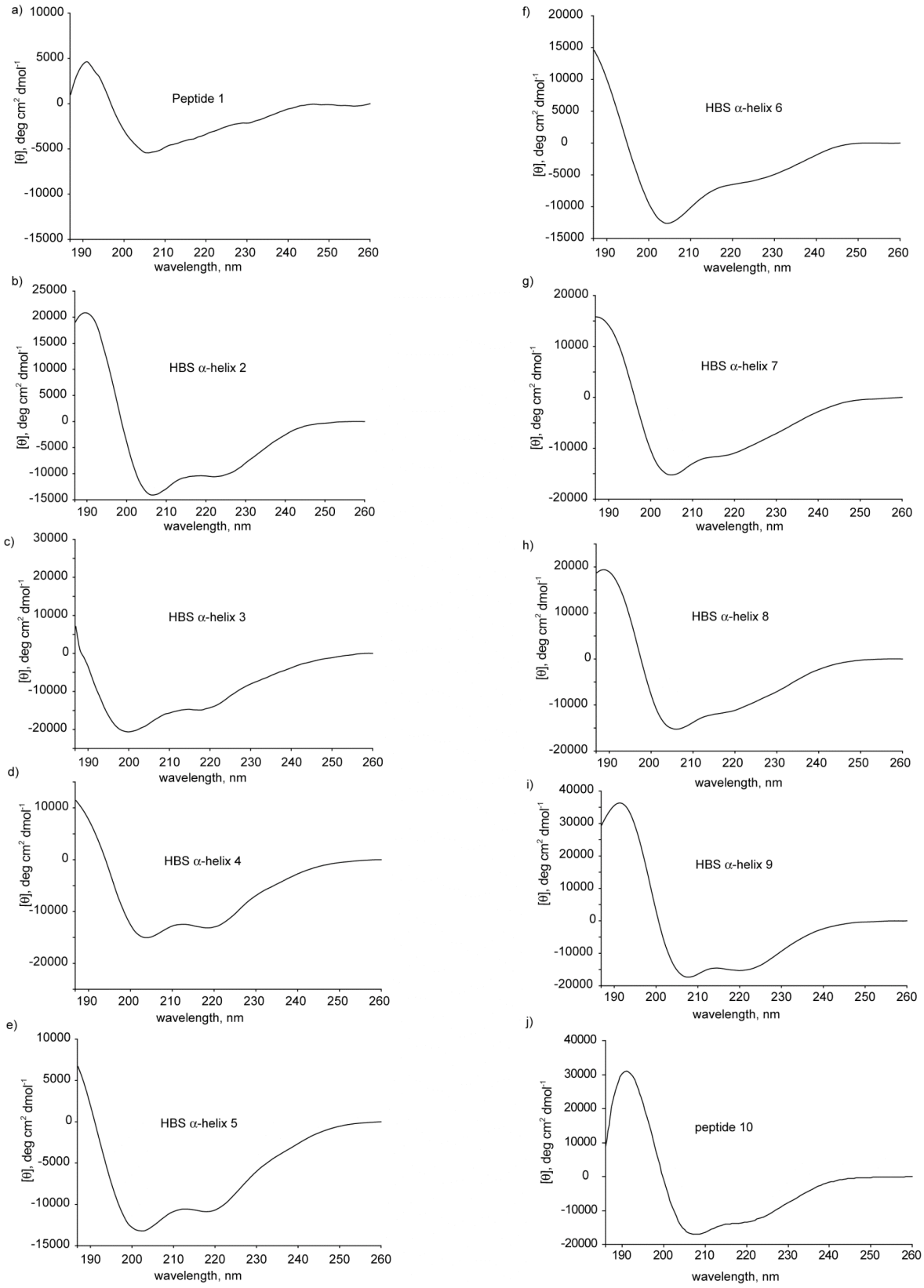


**Figure S1**. CD spectra of peptides in 10% TFE/PBS buffer.

**IZN17 Binding Assay**[5]

The relative affinity of each peptide for **IZN17** (**11**) was determined using fluorescence polarization-based competitive binding assay with fluorescein-labeled peptide **12**. The anisotropy experiments were performed with a DTX 880 Multimode Detector (Beckman) at 25 °C, with excitation and emission wavelengths of 485 and 525 nm, respectively. All samples were prepared in 96 well plates in 1x phosphate buffered saline (137 mM NaCl, 10 mM phosphate, 2.7 mM KCl, pH 7.4) with 0.1% pluronic F-68 (Sigma). The binding affinity ($K_D$) values reported for each peptide are the averages of 3~5 individual measurements, and were determined by fitting the experimental data to a sigmoidal dose-response nonlinear regression model on GraphPad Prism 4.0.

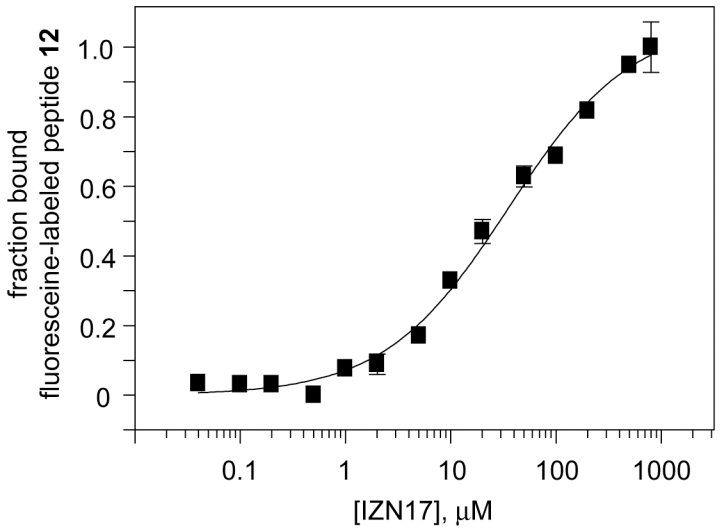


**Figure S3**. Saturation binding curve of fluorescein-labeled peptide **12** with **IZN17** in PBS buffer at 25 °C.

Prior to the competition experiments, the affinity of fluorescein-labeled peptide **12** for **IZN17** (**11**) was determined by monitoring polarization of the fluorescent probe upon addition of

5. (a) D. M. Eckert, P. S. Kim, *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 11187-11192. (b) O. M. Stephens, S. Kim, B. D. Welch, M. E. Hodsdon, M. S. Kay, A. Schepartz, *J. Am. Chem. Soc.* **2005**, *127*, 13126-13127.

**IZN17**. Addition of an increasing concentration (0 nM to 800 μM) of **IZN17** to a 15 nM solution of fluorescein-labeled peptide **12** in PBS buffer at 25 °C afforded a saturation binding curve (Figure S3). The $IC_{50}$ value obtained from this binding curve was used with equation (**1**) to obtain the dissociation constant ($K_{D1}$) for the fluorescein-labeled peptide **12** and **IZN17** complex:

$$K_{D1} = \frac{R_T \times (1 - F_{SB}) + L_{ST} \times F_{SB}^{\,2}}{F_{SB}} - L_{ST} \qquad \textbf{(1)}$$

$$K_{D2} = K_{D1} \times F_{SB} \times \left(\frac{L_T}{L_{ST} \times F_{SB}^{\,2} - (K_{D1} + L_{ST} + R_T) \times F_{SB} + R_T} - \frac{1}{1 - F_{SB}}\right) \qquad \textbf{(2)}$$

Eq (2) is obtained by rearranging eq 16 from reference 6:

$$L_T = \frac{[(K_{D1} - K_{D2})F_{SB} + K_{D2}][(L_{ST}F_{SB}^{\,2} - (K_{D1} + L_{ST} + R_T)F_{SB} + R_T]}{(1 - F_{SB})F_{SB}K_{D1}} \qquad (\textbf{16}) \text{ from ref 6.}$$

$K_{D1}$: $K_D$ fluorescein-labeled peptide **12**;

$K_{D2}$: $K_D$ of peptides **1-10**;

$R_T$: Total concentration of **IZN17**;

$L_{ST}$: Total concentration of fluorescein-labeled peptide **12**;

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6. M. H. Roehrl, J. Y. Wang, G. Wagner, *Biochemistry* **2004**, *43*, 16056-16066

$F_{SB}$: Fraction of bound fluorescein-labeled peptide **12**;

$L_T$: Total concentration of peptide **1-10**.

The saturation binding curve was used to calculate the optimum concentration of the probe (15 nM fluorescein-labeled peptide **12**) and **IZN17** (25 μM) needed for the competition binding anisotropy assays. Optimum concentrations are necessary to develop a highly sensitive fluorescence polarization assay. The sensitivity and usability of a polarization assay largely depends on two important considerations: (a) concentration of the complex should be chosen so that the polarized probe affords an observable signal (beyond experimental error) over background, and (b) the concentration of **IZN17** should be lower than that needed for saturation of the probe because excess protein concentrations would lead to inaccurately high $IC_{50}$ values. Based on the $K_{D1}$ of fluorescein-labeled peptide **12** and **IZN17** complex, at 25 μM **IZN17** and 15 nM probe peptide **12**, 51% of the fluorescent probe should be bound to **IZN17** while a measurable change in polarization signal (100-114 mP) is expected upon inhibition of the probe **12/IZN17** complex by the antagonists **1-10**.

*Competition polarization assay:* A solution of 25 μM **IZN17** and 15 nM fluorescein-labeled peptide **12** was incubated at 25 °C. After 1 hour, appropriate concentrations (10 nM to 500 μM) of the antagonists (**1-10**) were added; total volume of the incubation solution was 60 μL. After 1 hour, the amount of the dissociated fluorescent probe **12** was determined by the Beckman DTX 880 fluorescence plate reader. The experimental dose response data for a given antagonist were fit to a sigmoidal dose-response nonlinear regression model on GraphPad Prism 4.0. The $IC_{50}$

value obtained from this analysis was used in equation (**2**) along with other parameters to calculate the $K_{D2}$ value of the antagonist.

**Lowest $K_d$ that may be accurately determined from the 1ZN17 assay employed:**

Competitive binding assay does not allow accurate estimates of Kd values much lower than binding values of the fluorescent probe. Figure S4 shows graph of fraction of bound probe versus ligand concentration as a function of ligand binding affinity.[6] The narrowing of the space between curves below Kd of 5 μM illustrates the limits of this assay.

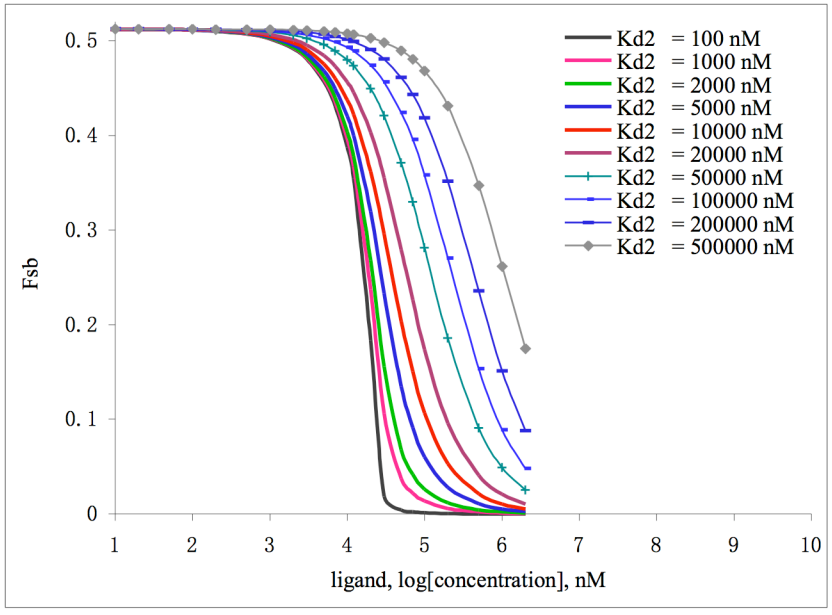


**Figure S4.** Simulation of fraction of bound probe versus ligand concentration as a function of ligand binding affinity.

**Cell-Cell Fusion Inhibition Assay**

Cell-cell fusion (i.e., syncytium formation) was assayed by coculturing CHO[HIVe] (clone 7d2) cells expressing HXB2 envelope and tat with U373-MAGI cells (M. Emerman and A. Geballe, National Institutes of Health AIDS Research and Reference Reagent Program) in the presence of different concentrations of peptides **1-10**. Cell fusion allows the expression of nuclear β-galactosidase from the U373-MAGI indicator cell line and can be quantitated by monitoring β-galactosidase activity. After an overnight incubation at 37°C after coculture, β-galactosidase enzymatic activity was measured with the Mammalian beta-galactosidase Chemiluminescent Assay Kit (Gal-Screen from Applied Biosystems). The peptide inhibitor concentrations at which activities were reduced by 50% ($IC_{50}$) relative to control samples lacking peptide inhibitor were calculated by fitting data to the variable-slope-sigmoid equation using the Prism program.

**Cytotoxity of peptides on U373-MAGI**

The cytotoxic effect of HBS peptides on U373-MAGI cells was measured in the presence of a series of diluted inhibitors for 6 days, and cell viability was quantitated with an MTT assay.[7] No cytotoxicity was observed for peptides up to 200 μM concentrations.

7. T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55-63.