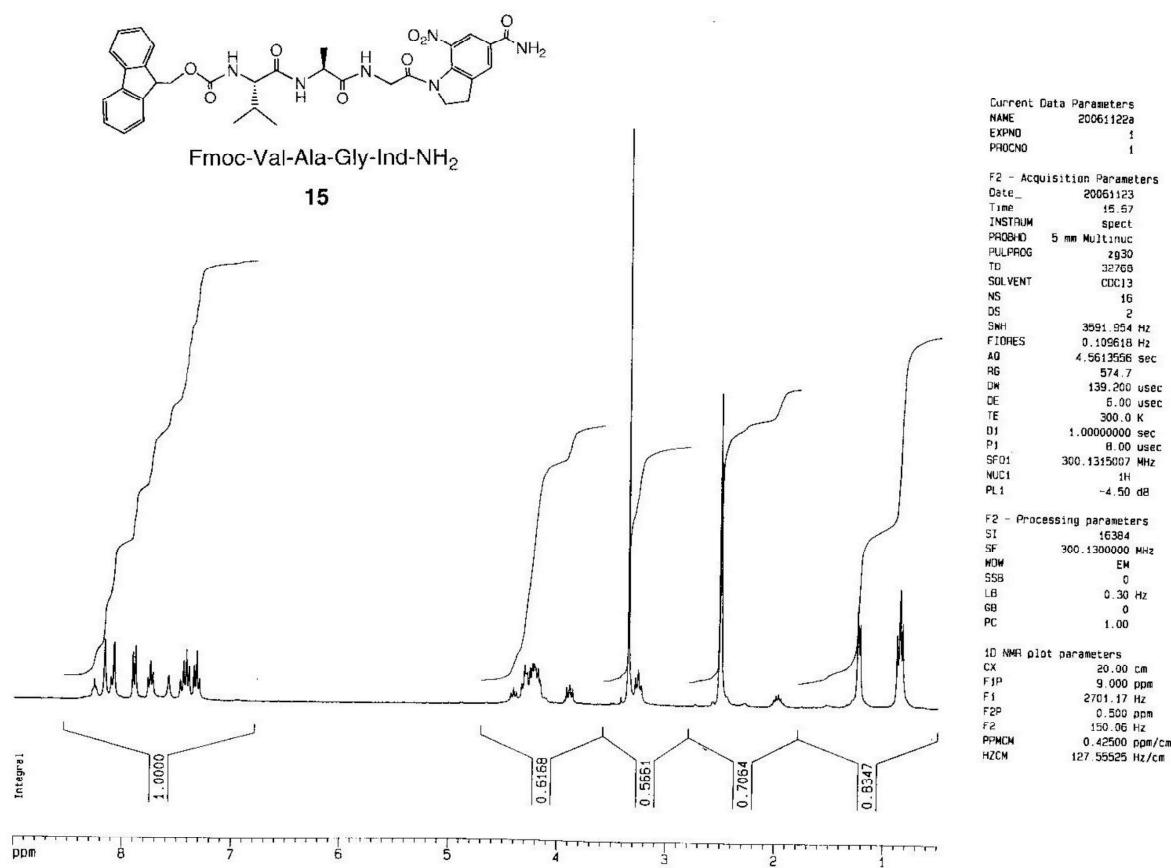


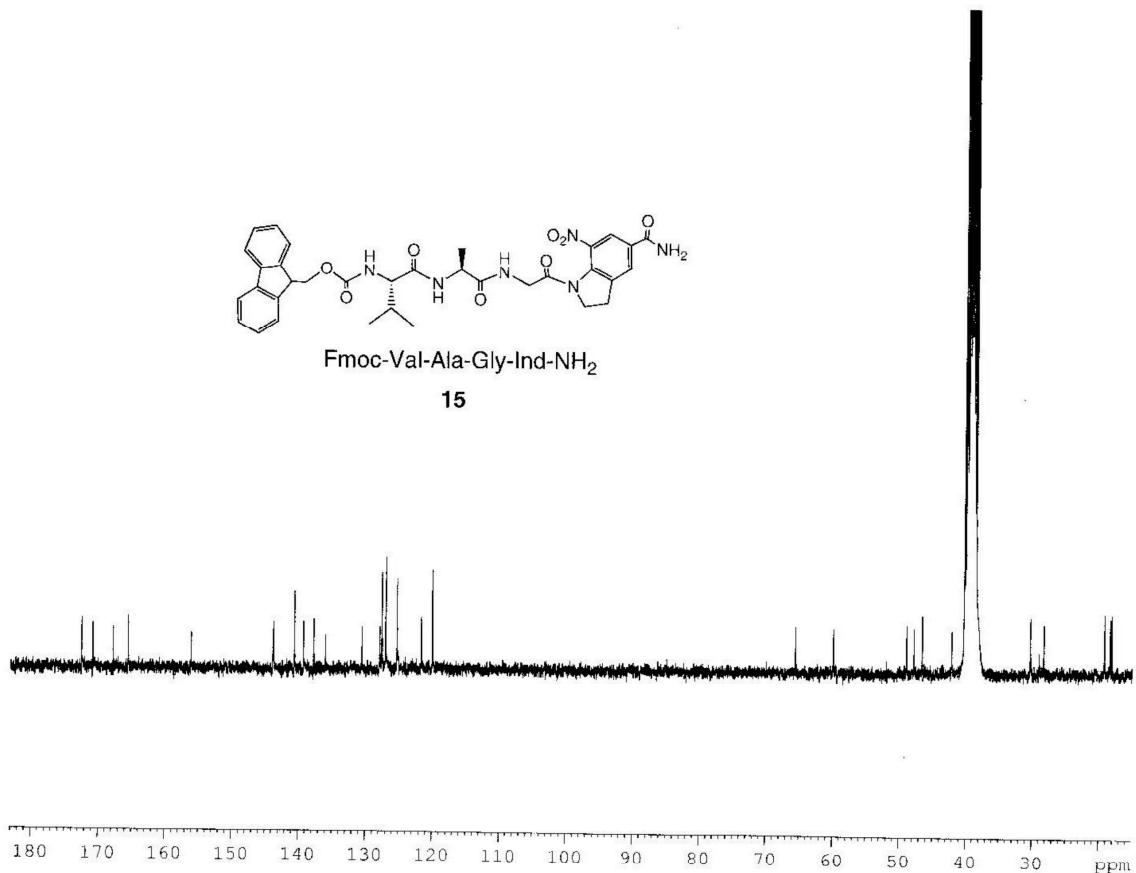
## Supplementary Information

### Virtually Epimerization-Free Synthesis of Peptide-alpha-Thioesters

Tyrone J. Hogenauer, Qianli Wang, Aditya K. Sanki, Amy J. Gammon, Cherie H. Chu, Clyde M. Kaneshiro, Yasuhiro Kajihara, and Katja Michael\*

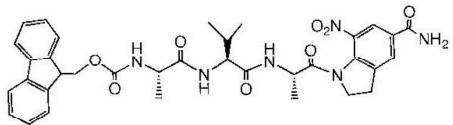


<sup>1</sup>H NMR (dmso-d<sub>6</sub>, 298K, 300 MHz) δ 8.26 (m, 1H, NH-Gly); 8.16 (s, 2H, H-4, H-6); 8.10 (d, 1H NH-Ala, <sup>3</sup>J<sub>NH,α</sub> 8.3 Hz); 8.08 (s, 1H, C-terminal NH); 7.89 (d, 2H, Fmoc, <sup>3</sup>J 7.4 Hz); 7.70 (m, 2H, Fmoc); 7.74 (t, 2H, Fmoc, <sup>3</sup>J 6.7 Hz); 7.58 (s, 1H, C-terminal NH'); 7.48-7.38 (m, 3H, NH-Val, Fmoc); 7.32 (t, 2H, Fmoc); 4.41 (t, 1H, Hα-Ala); 4.35-4.14 [m, 7H, Hα-Gly, Hα'-Gly, CH, CH<sub>2</sub> (Fmoc), H-2, H-2']; 3.89 (dd, 1H, Hα-Val), 3.26 (t, 2H, H-2, H-2'), 1.98 (m, 1H, H-β-Val); 1.23 (d, 3H, 3×Hβ-Ala); 0.65 (t, 6H, 3×Hγ-Val, 3×H-γ'-Val) ppm.



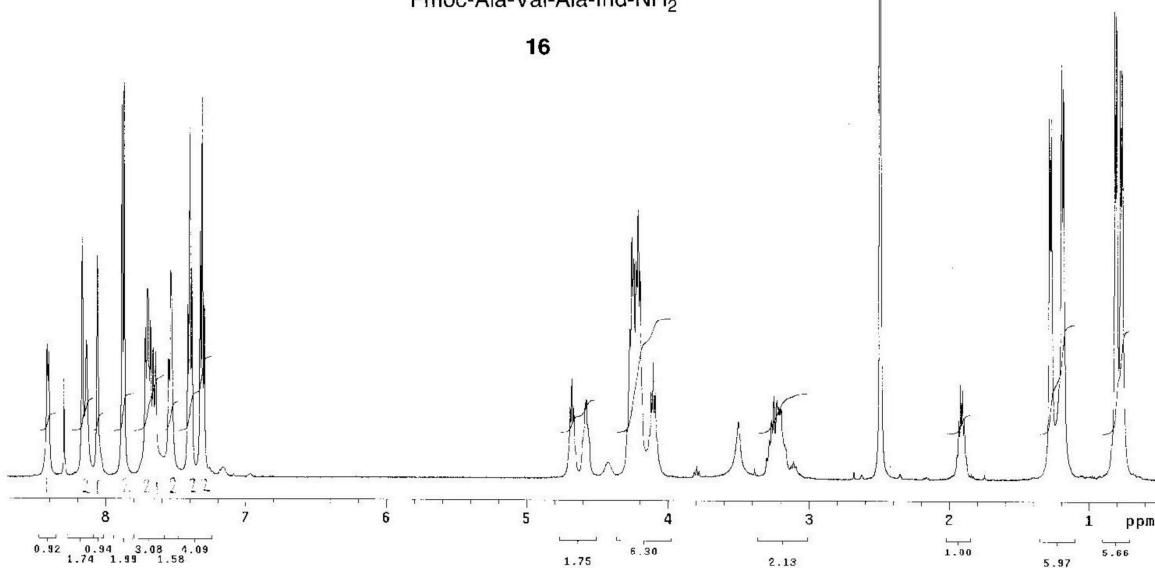
$^{13}\text{C}$  NMR ( $\text{dmso-d}_6$ , 298K, 75 MHz)  $\delta$  172.5; 170.9; 167.8; 165.6; 156.1; 143.9; 143.8; 140.7; 139.4; 137.8; 136.1; 130.6; 128.0; 127.7; 127.1; 125.4; 121.8; 120.1; 65.7; 60.0; 49.0; 47.9; 46.7; 42.2; 30.4; 28.4; 19.3; 18.4; 18.2 ppm.

Pulse Sequence: presat  
 Solvent: dmso  
 Temp. 25.0 C / 298.1 K  
 File: km-8-14-1h1  
 INOVA-500 "xenon"  
 Relax. delay 0.100 sec  
 Pulse 73.0 degrees  
 Acq. time 3.199 sec  
 Width 5500.2 Hz  
 32 repetitions  
 OBSERVE H-1 500.1137913 MHz  
 DATA PROCESSING  
 Line broadening 0.3 Hz  
 FT size 32768  
 Total time 2 min, 36 sec



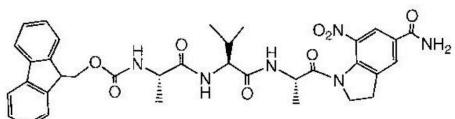
Fmoc-Ala-Val-Ala-Ind-NH<sub>2</sub>

**16**



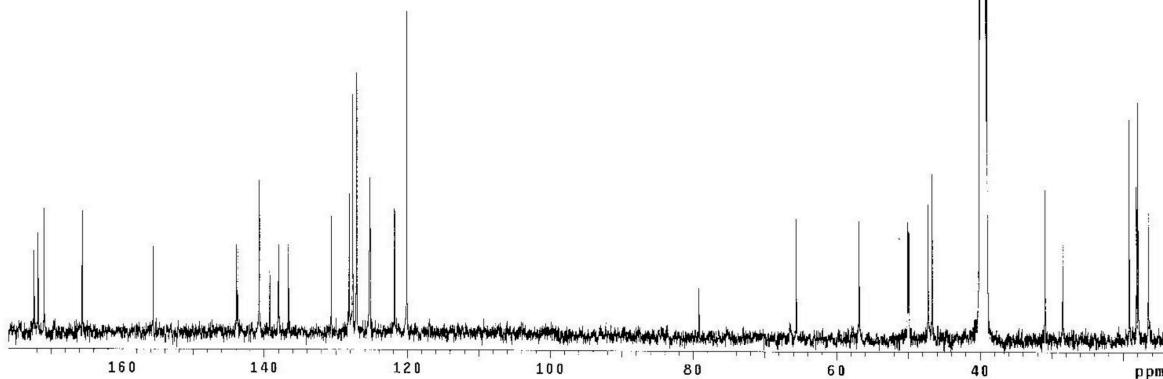
<sup>1</sup>H NMR (dmso-d<sub>6</sub>, 298K, 500 MHz) δ 8.41 (d, 1H, NH-Ala-2, <sup>3</sup>J<sub>NH,α</sub> 6.1 Hz); 8.17, 8.14, 8.06 (3s, 3H, H-4, H-6, C-terminal NH); 7.87 (d, 2H, Fmoc, <sup>3</sup>J 7.4 Hz); 7.70 (m, 2H, Fmoc); 7.65 (d, 1H, NH-Val, <sup>3</sup>J<sub>NH,α</sub> 9.0 Hz); 7.54 (m, 2H, NH-Ala-1, C-terminal NH'); 7.40 (t, 2H, Fmoc, <sup>3</sup>J 7.3 Hz); 7.31 (t, 2H, Fmoc); 4.68 (m, 1H, H-α-Ala-2); 4.58 (m, 1H, H-2); 4.29-4.17 (m, 5H, H-α-Val, CH-Fmoc, CH<sub>2</sub>-Fmoc, H-2'); 4.10 (m, 1H, H-α-Ala-1); 3.29-3.15 (m, 2H, H-3, H-3'); 1.91 (m, 1H, H-β-Val); 1.28, d, 3H, 3×H-β-Ala-2, <sup>3</sup>J<sub>α,β</sub> 6.7 Hz); 1.18 (d, 3H, 3×H-β-Ala-1, <sup>3</sup>J<sub>α,β</sub> 7.0 Hz); 0.81 (d, 3H, 3 × H-γ-Val, <sup>3</sup>J<sub>α,β</sub> 6.7 Hz); 0.77 (d, 3H, 3 × H-γ'-Val, <sup>3</sup>J<sub>α,β</sub> 6.4 Hz) ppm.

Pulse Sequence: s2pul  
 Solvent: dmso  
 Temp: 25.0 °C / 298.1 K  
 File: km-8-14-13c  
 INOVA-500 "xenon"  
 Relax. delay 1.500 sec  
 Pulse 56.8 degrees  
 90°/Pulse 1.5 sec  
 Width 23392.5 Hz  
 15048 repetitions  
 OBSERVE: C13, 125.7537712 MHz  
 CONVERSE: 500.1162863 MHz  
 Power 40 dB  
 continuously on  
 WALTZ-16 modulated  
 DATA PROCESSING:  
 FID processing 3.0 Hz  
 FT size: 131072  
 Total time 12 hr, 50 min, 52 sec



Fmoc-Ala-Val-Ala-Ind-NH<sub>2</sub>

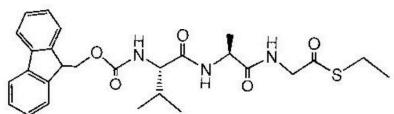
**16**



<sup>13</sup>C NMR (dmso-d<sub>6</sub>, 298K, 126 MHz) δ 172.3; 171.8; 170.9; 165.6; 155.6; 143.9; 143.7; 140.7; 139.2; 138.0; 136.6; 130.6; 128.1; 127.6; 127.1; 125.3; 125.2; 121.8; 120.1; 79.2; 65.6; 56.9; 50.1; 49.9; 47.2; 46.6; 30.9; 28.4; 19.1; 18.1; 17.9; 16.5 ppm.

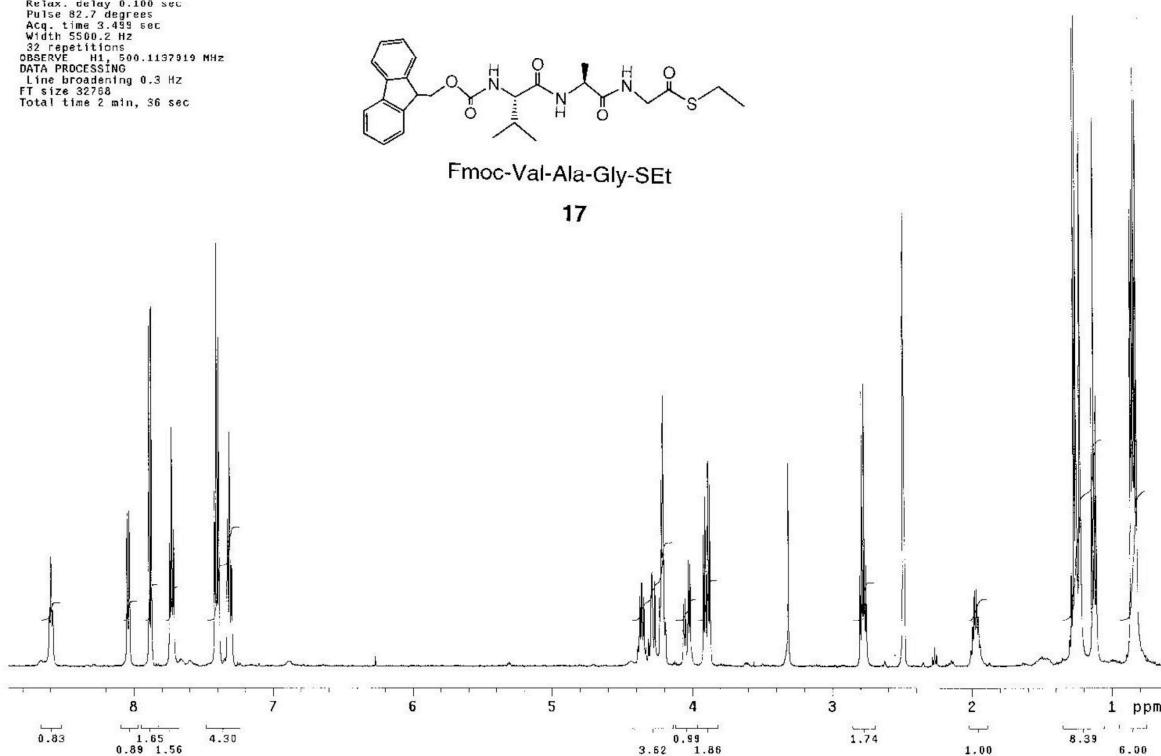
Pulse Sequence: presat  
 Solvent: dmso  
 Temp. 25.0 C / 298.1 K  
 File: km-gly-1h  
 INOVA-500 "xenon"

Relax. delay 0.100 sec  
 Pulse 82.7 degrees  
 Acq. time 3.495 sec  
 Width 5500.2 Hz  
 N repetition 1  
 OBSERVE H1 500.1137919 MHz  
 DATA PROCESSING  
 Line broadening 0.3 Hz  
 FT size 32768  
 Total time 2 min, 36 sec

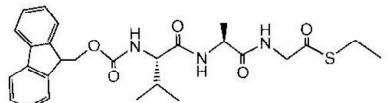


Fmoc-Val-Ala-Gly-SEt

**17**

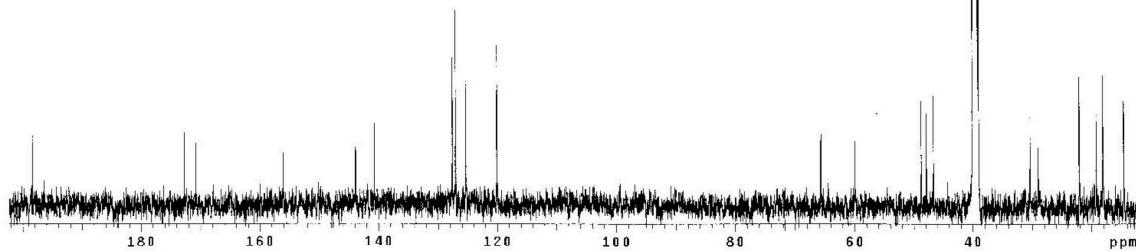


<sup>1</sup>H NMR (dmso-d<sub>6</sub>, 298K, 500 MHz) δ 8.53 (m, 1H, NH-Gly); 8.04 (d, 2H, NH-Ala, <sup>3</sup>J<sub>NH,α</sub> 7.4); 8.10 (d, 1H NH-Ala, <sup>3</sup>J<sub>NH,α</sub> 8.3 Hz); 7.88 (d, 2H, Fmoc, <sup>3</sup>J 7.7 Hz); 7.23 (t, 2H, Fmoc); 7.43-7.38 (m, 3H, NH-Val, Fmoc); 7.31 (t, 2H, Fmoc); 4.36 (m, 1H, Hα-Ala); 4.32-4.18 [m, 3H, CH, CH<sub>2</sub> (Fmoc)]; 4.04 (dd, 1H, Hα-Gly, <sup>2</sup>J<sub>α,α</sub> 17.5Hz, <sup>3</sup>J<sub>α,NH</sub> 6.4Hz), 3.93-3.86 (m, 2H, Hα'-Gly, Hα-Val), 2.78 [q, 2H, CH<sub>2</sub> (Et), <sup>3</sup>J 7.4 Hz]; 1.98 (m, 1H, Hβ-Val); 1.26 (d, 3H, 3×HβAla); 1.12 [t, 3H, CH<sub>3</sub> (Et)]; 0.85 (d, 3H, 3×Hγ-Val, <sup>3</sup>J<sub>β,γ</sub> 6.7Hz); 0.83 (d, 3H, 3×H-γ'-Val, <sup>3</sup>J<sub>β,γ'</sub> 7.1Hz) ppm.



### Fmoc-Val-Ala-Gly-SEt

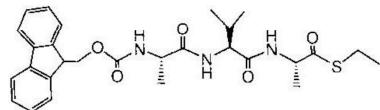
17



<sup>13</sup>C NMR (dmso-d<sub>6</sub>, 298K, 126 MHz) δ 198.4; 172.7; 170.8; 156.1; 143.9; 143.8; 140.7; 127.6; 127.0; 125.3; 120.1; 65.6; 59.9; 48.7; 47.9; 46.7; 30.3; 29.0; 22.1; 19.2; 18.1; 14.6 ppm.

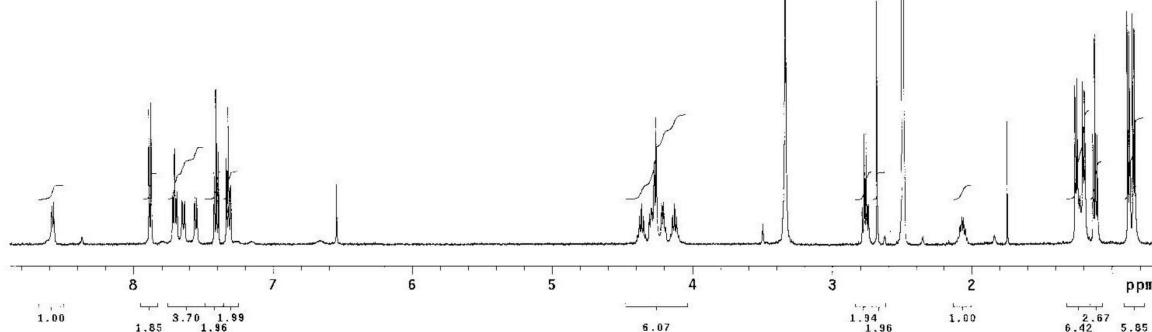
Pulse Sequence: presat  
Solvent: dmso  
Temp. 25.0 C / 298.1 K  
File: km-al-a-1h  
TNOVA-560 "xenon"

Relax. delay 0.100 sec  
Pulse 83.5 degrees  
Acq. time 3.499 sec  
Width 5500.2 Hz  
32 repetitions  
OBSERVE: H1 500.1137913 MHz  
DATA PROCESSING  
Line broadening 0.3 Hz  
FT size 32768  
Total time 2 min, 36 sec



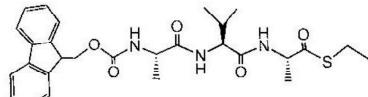
Fmoc-Ala-Val-Ala-SEt

**18**



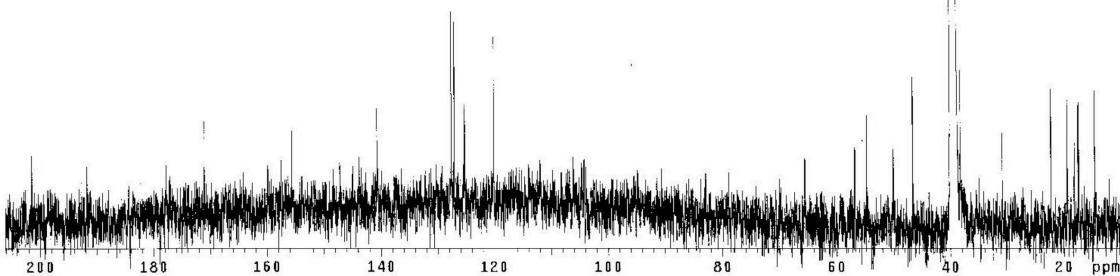
<sup>1</sup>H NMR (dmso-d<sub>6</sub>, 298K, 500 MHz) δ 8.57 (d, 1H, NH-Ala-2, <sup>3</sup>J<sub>NH,α</sub> 7.1 Hz); 7.88 (d, 2H, Fmoc, <sup>3</sup>J 7.4 Hz); 7.70 (m, 2H, Fmoc); 7.64 (d, 1H, NH-Val, <sup>3</sup>J<sub>NH,α</sub> 9.4 Hz); 7.55 (d, 1H, NH-Ala-1, <sup>3</sup>J<sub>NH,α</sub> 8.1 Hz); 7.41 (t, 2H, Fmoc, <sup>3</sup>J 7.4 Hz); 7.32 (t, 2H, Fmoc); 4.36 (m, H-α-Ala-2); 4.31-4.18 (m, 4H, H-α-Val, CH-Fmoc, CH<sub>2</sub>-Fmoc); 4.13 (m, 1H, H-α-Ala-1); 2.76 (q, 2H, CH<sub>2</sub>-SEt, <sup>3</sup>J 7.4 Hz); 2.06 (m, 1H, H-β-Val); 1.25 (d, 3H, 3×H-β-Ala-2, <sup>3</sup>J<sub>α,β</sub> 7.1 Hz); 1.20 (d, 3H, 3×H-β-Ala-1, <sup>3</sup>J<sub>α,β</sub> 7.1 Hz); 1.12 (t, 3H, CH<sub>3</sub>-SEt); 0.87 (d, 3H, 3×H-γ-Val, <sup>3</sup>J<sub>α,β</sub> 6.7 Hz); 0.84 (d, 3H, 3 × H-γ-Val, <sup>3</sup>J<sub>α,β</sub> 6.7 Hz) ppm.

Pulse Sequence: s2pul  
 Solvent: dmso  
 Temp. 25.0 C / 298.1 K  
 File: km-al-a-13.v1  
 INQVA-500 "xenon"  
 Relax. delay 1.500 sec  
 Pulse 55.8 degrees  
 Acq. time 0.004 sec  
 Width 25862.5 Hz  
 27364.0 ref. time  
 OBSERVE: U13, U23, S33//13 MHz  
 DECOUPLE: H1, S00.1162863 MHz  
 Power 40 dB  
 Control: 100% on  
 WALTZ-16 modulated  
 DATA PROCESSING:  
 Line broadening 3.0 Hz  
 FT size 131072  
 Total time 15 hr, 16 min, 17 sec



Fmoc-Ala-Val-Ala-SEt

**18**



<sup>13</sup>C NMR (dmso-d<sub>6</sub>, 298K, 126 MHz) δ 201.5; 191.8; 177.8; 171.0; 159.8; 155.6; 147.3; 144.9; 143.9; 140.7; 131.2; 127.6; 127.1; 125.2; 120.1; 65.6; 56.8; 54.7; 50.0; 46.6; 38.2; 30.8; 22.3; 19.4; 18.2; 17.5; 17.4; 14.6 ppm.