

# Total Synthesis and Biological Evaluation of Modified Ilamycin Derivatives

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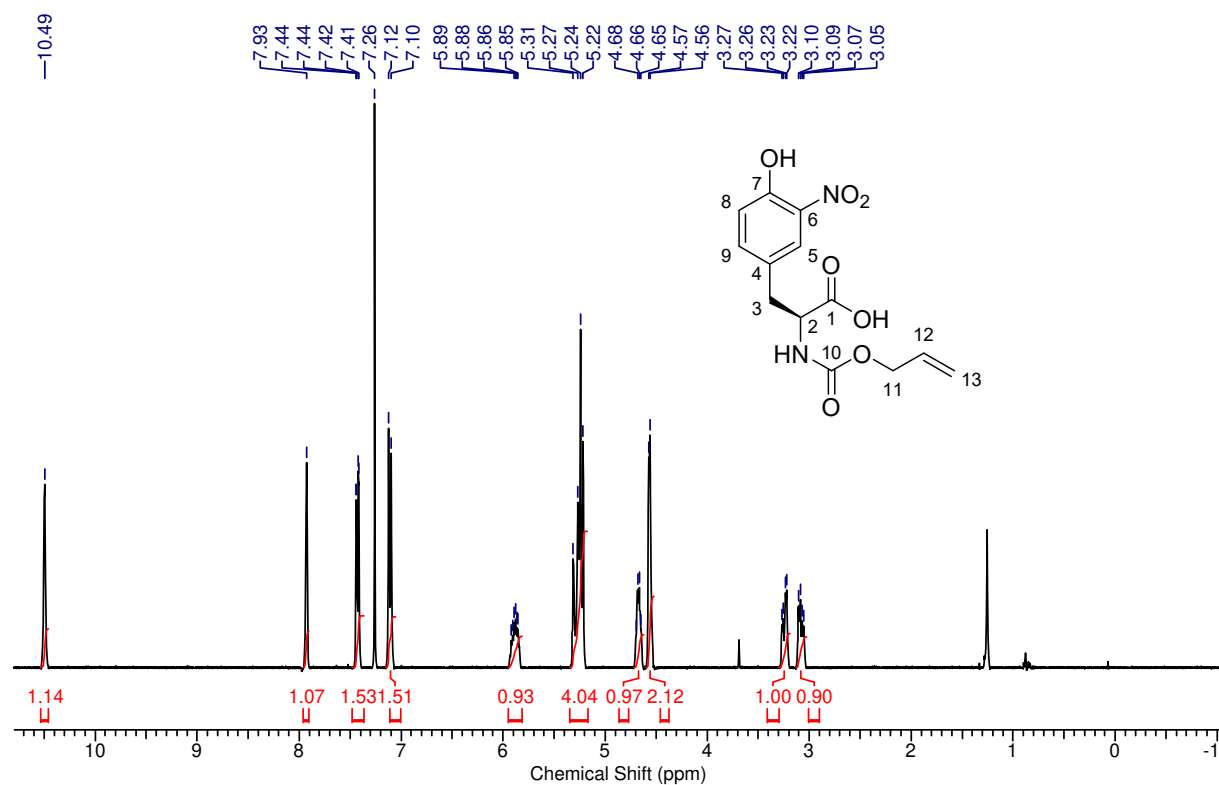
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## Copies of the NMR spectra and GC chromatogram

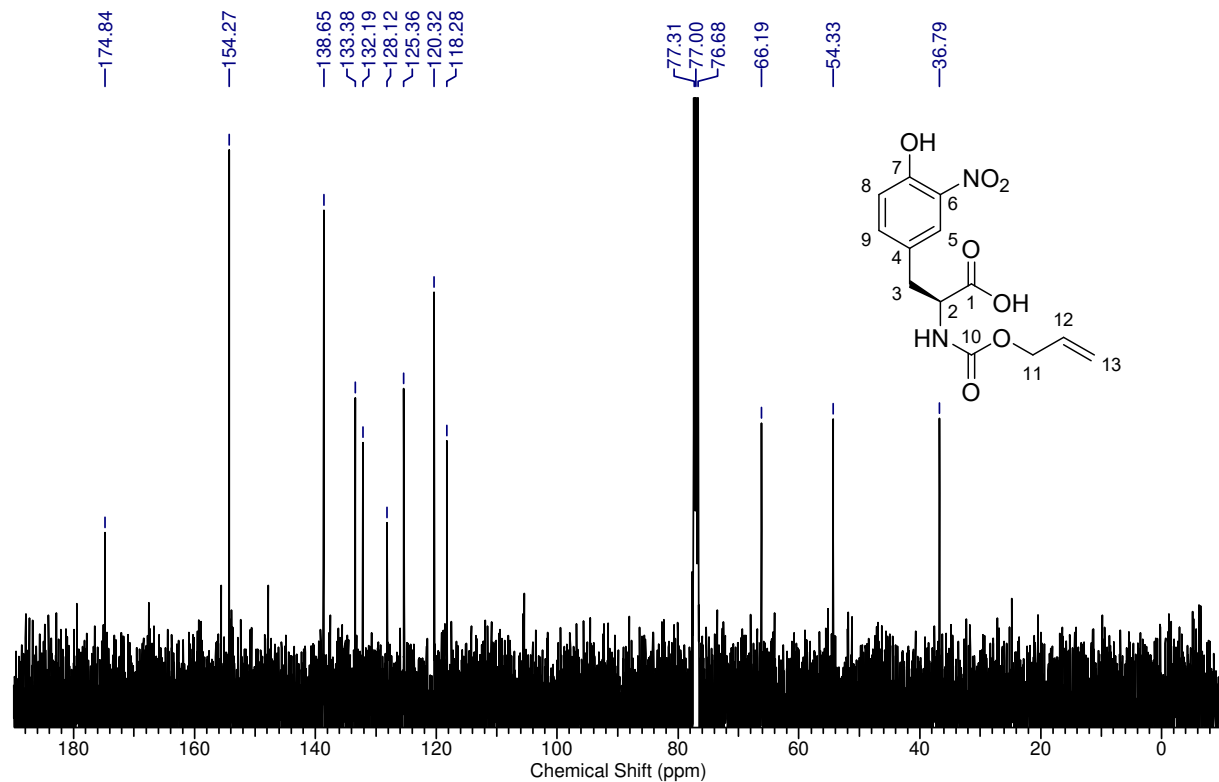
<sup>1</sup> H/ <sup>13</sup> C-NMR Compound 2 .....	S1
<sup>1</sup> H/ <sup>13</sup> C-NMR Compound 3 .....	S2
<sup>1</sup> H/ <sup>13</sup> C-NMR Compound 4 .....	S3
<sup>1</sup> H/ <sup>13</sup> C-NMR Compound 5 .....	S4
<sup>1</sup> H/ <sup>13</sup> C-NMR Compound 7 .....	S5
<sup>1</sup> H/ <sup>13</sup> C-NMR Compound 9 .....	S6
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<sup>1</sup> H/ <sup>13</sup> C-NMR Compound 11 .....	S9
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<sup>1</sup> H/ <sup>13</sup> C-NMR Compound 19 .....	S16
<sup>1</sup> H/ <sup>13</sup> C-NMR Compound 20 .....	S17
<sup>1</sup> H/ <sup>13</sup> C-NMR Compound 21 .....	S18
<sup>1</sup> H/ <sup>13</sup> C-NMR Compound 22 .....	S19
<sup>1</sup> H/ <sup>13</sup> C-NMR Compound 23 .....	S20
<sup>1</sup> H/ <sup>13</sup> C-NMR Compound 24 .....	S21
<sup>1</sup> H/ <sup>13</sup> C-NMR Compound 26 .....	S22/23
<sup>1</sup> H/ <sup>13</sup> C-NMR Compound 27 .....	S24/25
Supplementary Figure S1 .....	S26

**(S)-2-[[Allyloxy]carbonyl]amino-3-(4-hydroxy-3-nitrophenyl)propanoic acid (2)**

$^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ ):

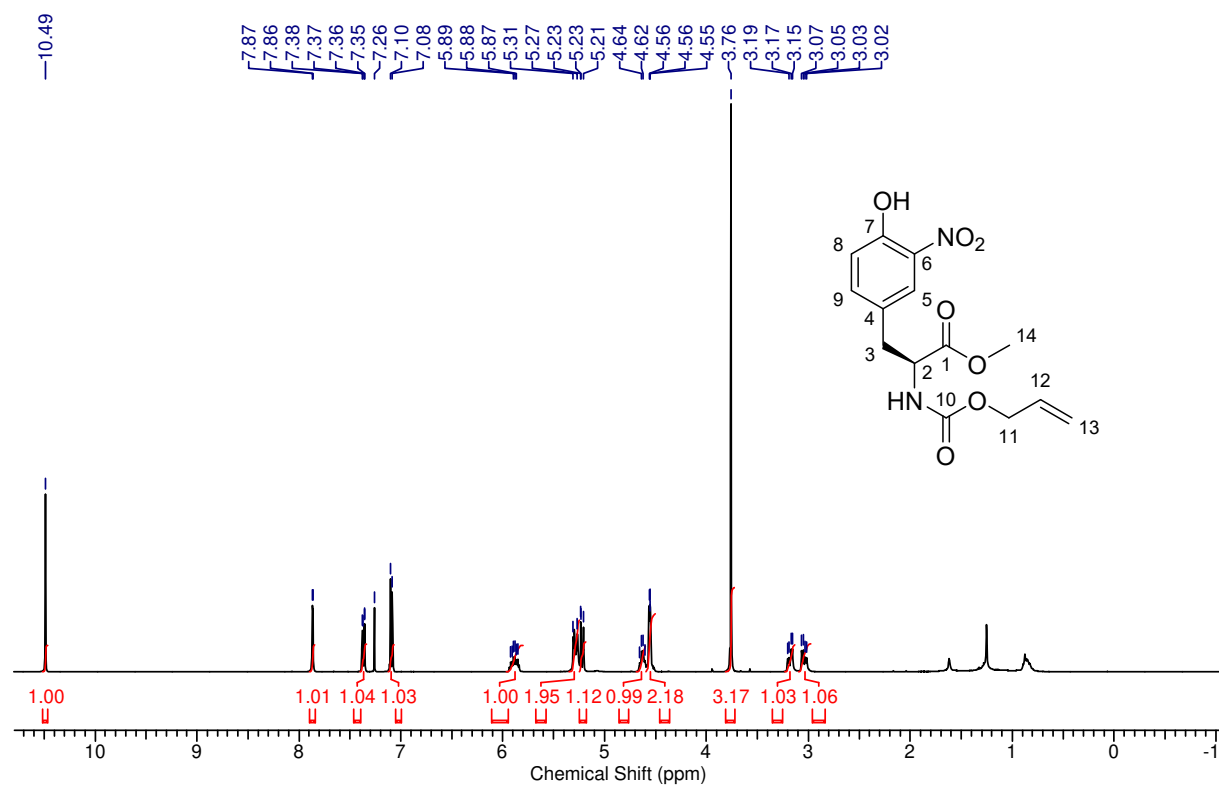


$^{13}\text{C-NMR}$  (100 MHz,  $\text{CDCl}_3$ ):

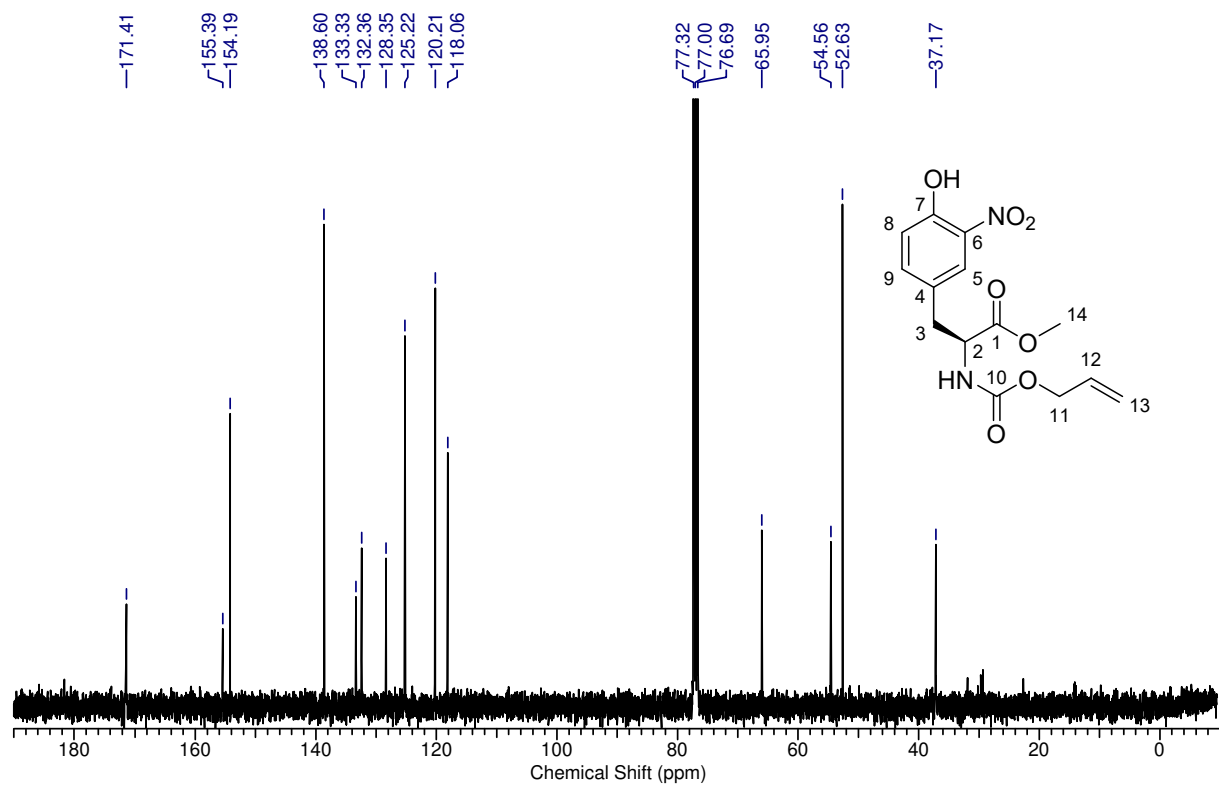


**Methyl (S)-2-[(allyloxy)carbonylamino]-3-(4-hydroxy-3-nitrophenyl)propanoate (3)**

$^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ ):

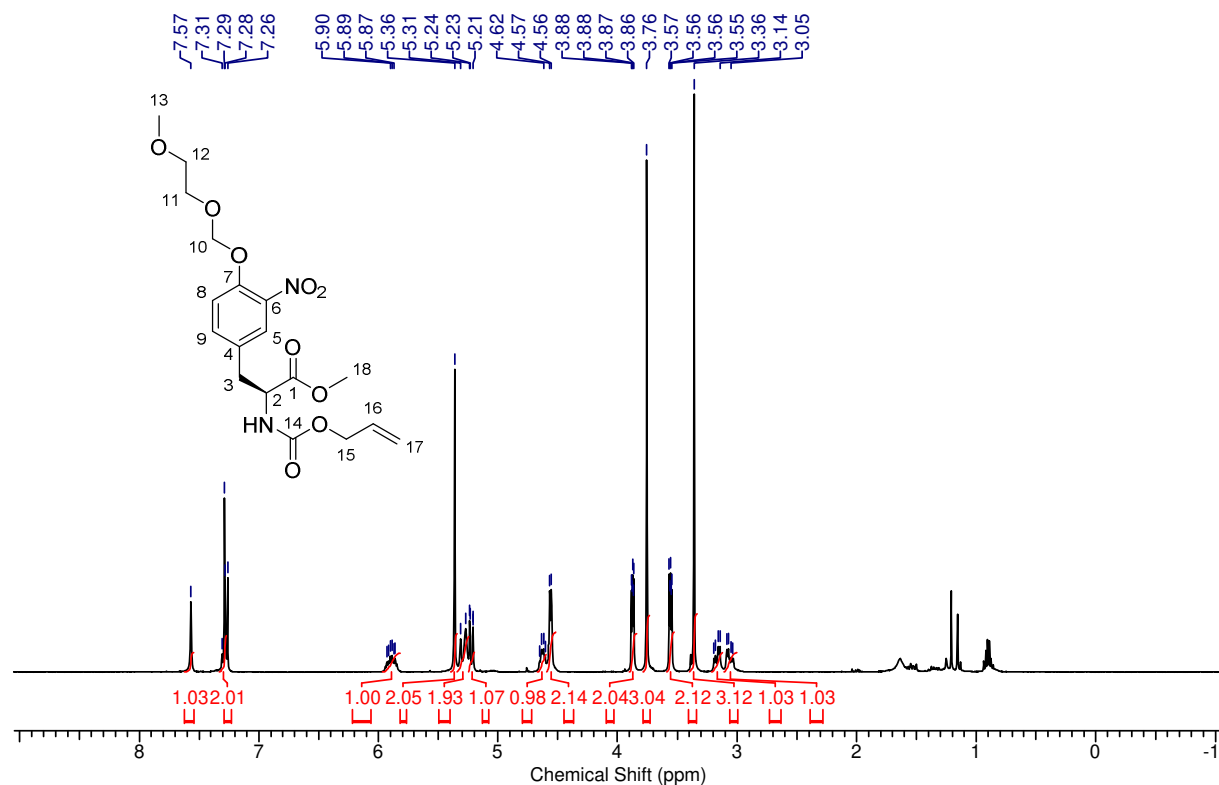


$^{13}\text{C-NMR}$  (100 MHz,  $\text{CDCl}_3$ ):

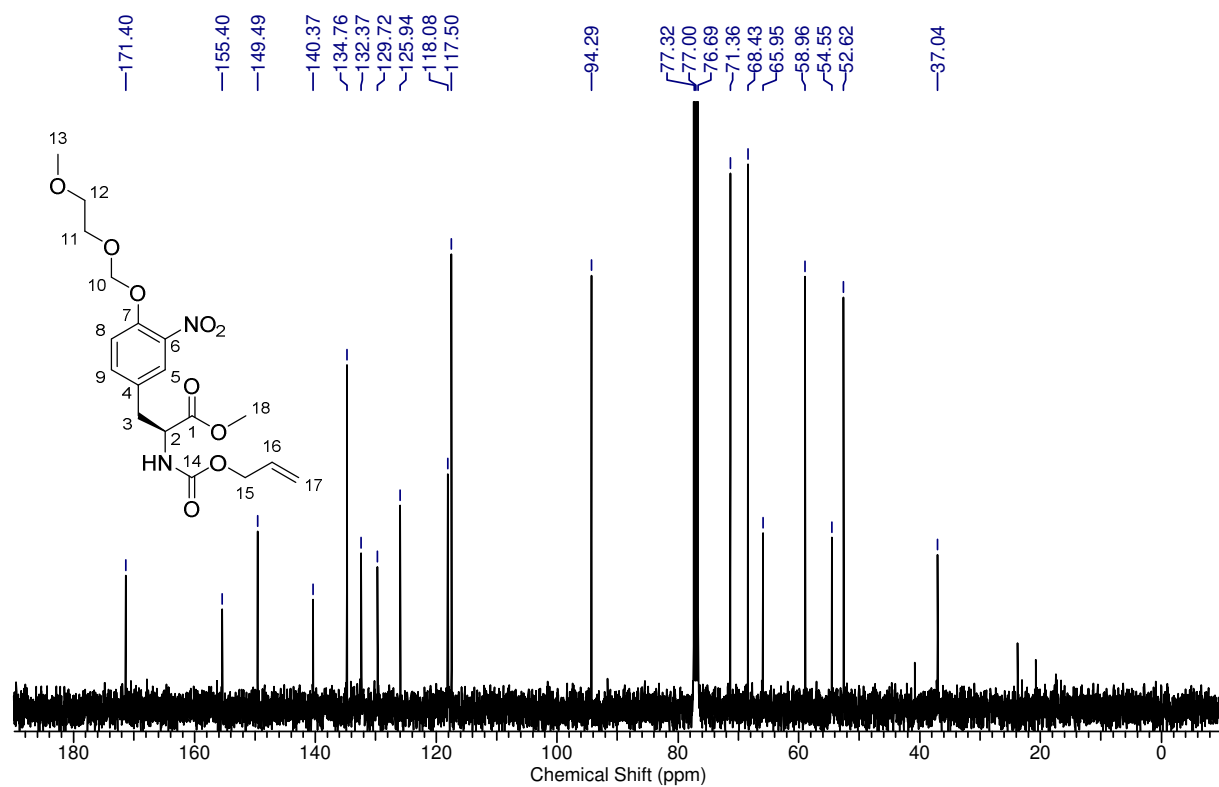


**Methyl (S)-2-[(allyloxy)carbonyl]amino-3-[4-[(2-methoxyethoxy)methoxy]-3-nitrophenyl]-propanoate (4)**

$^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ ):

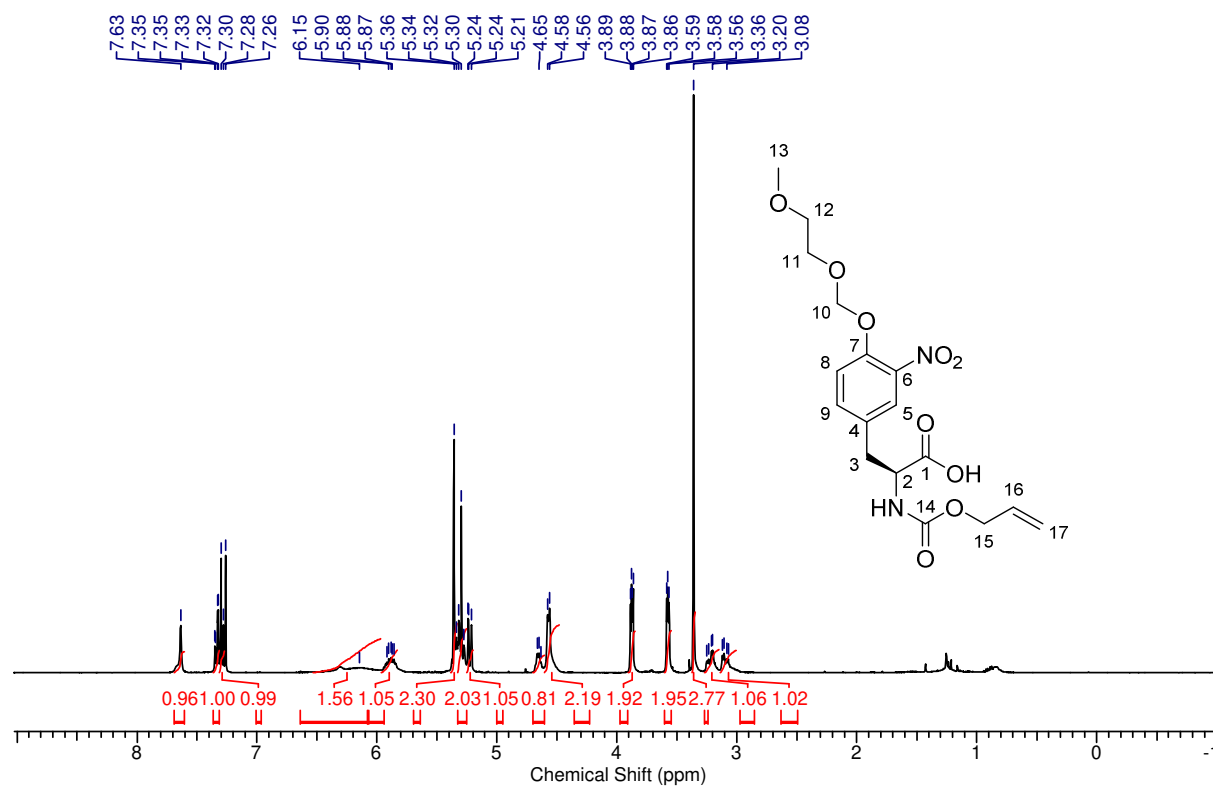


$^{13}\text{C-NMR}$  (100 MHz,  $\text{CDCl}_3$ ):

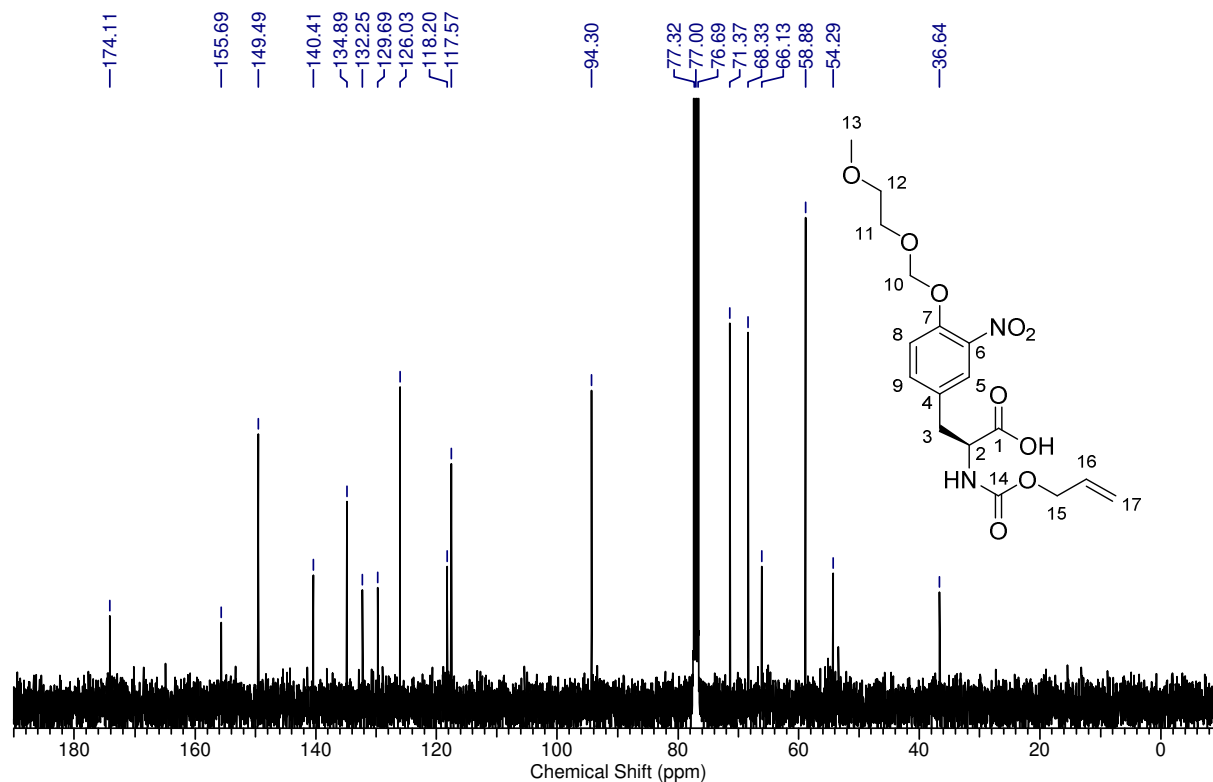


**(S)-2-[[[(Allyloxy)carbonyl]amino]-3-{4-[(2-methoxyethoxy)methoxy]-3-nitro-phenyl}]propanoic acid (5)**

<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>):

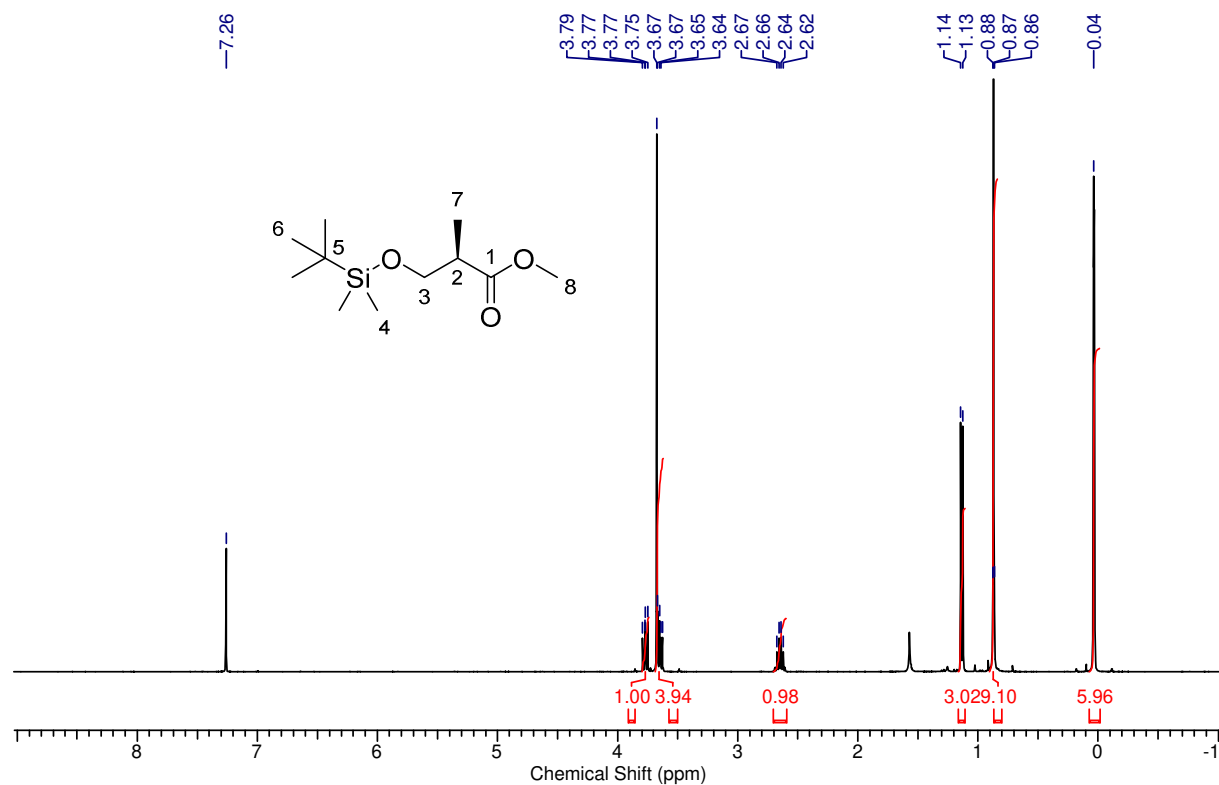


<sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>):

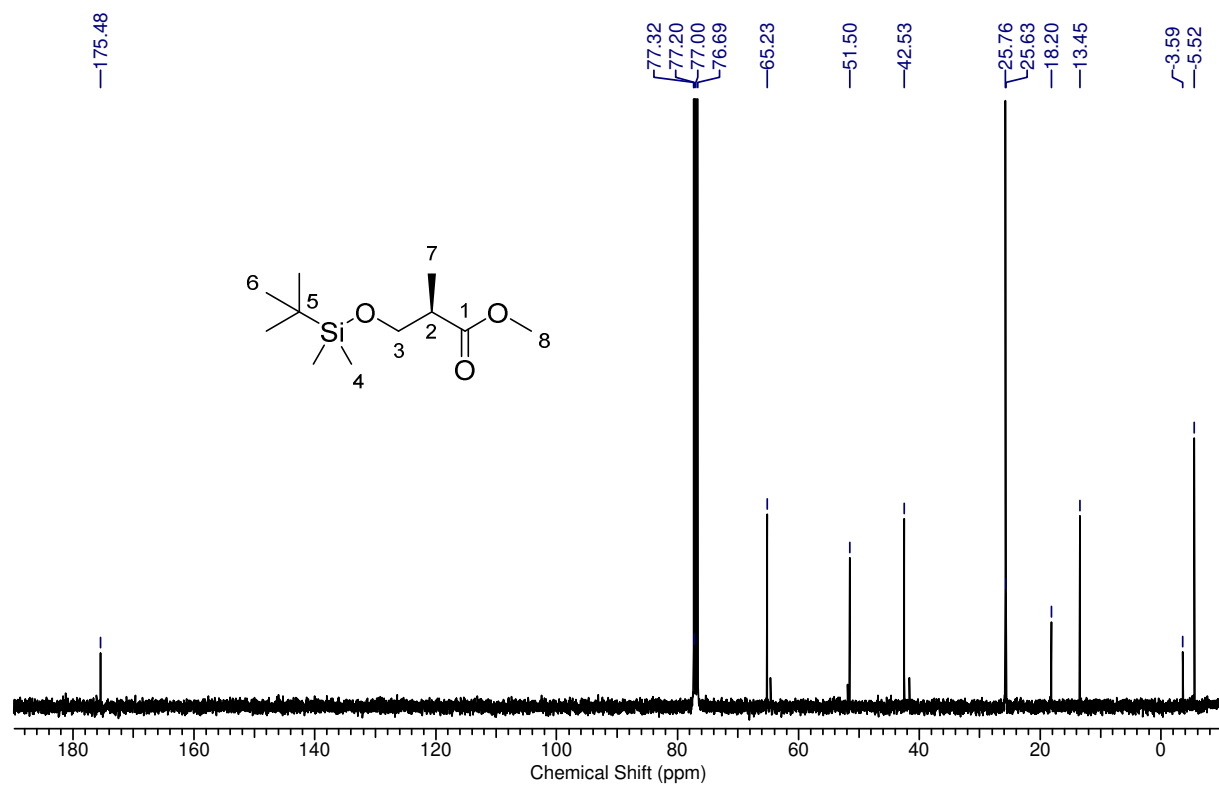


**Methyl (R)-3-[(*tert*-butyldimethylsilyl)oxy]-2-methylpropanoate (7)**

$^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ ):

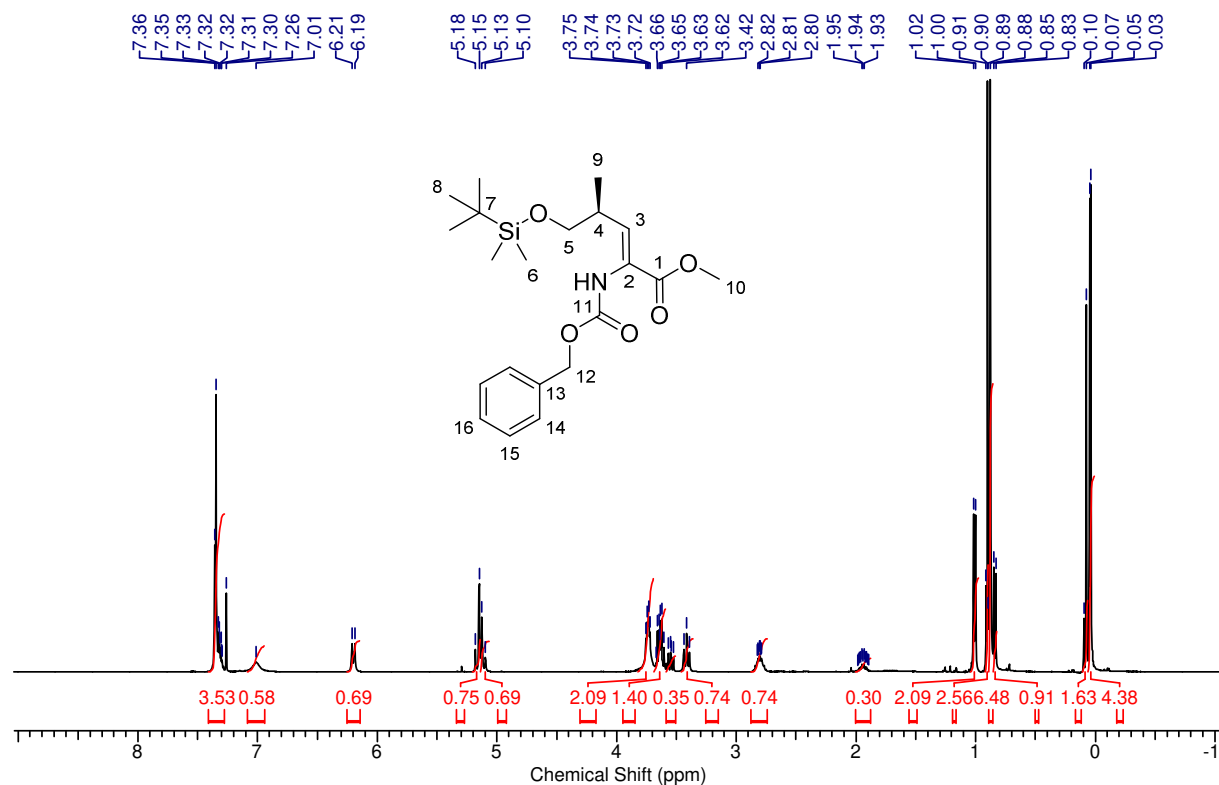


$^{13}\text{C-NMR}$  (100 MHz,  $\text{CDCl}_3$ ):

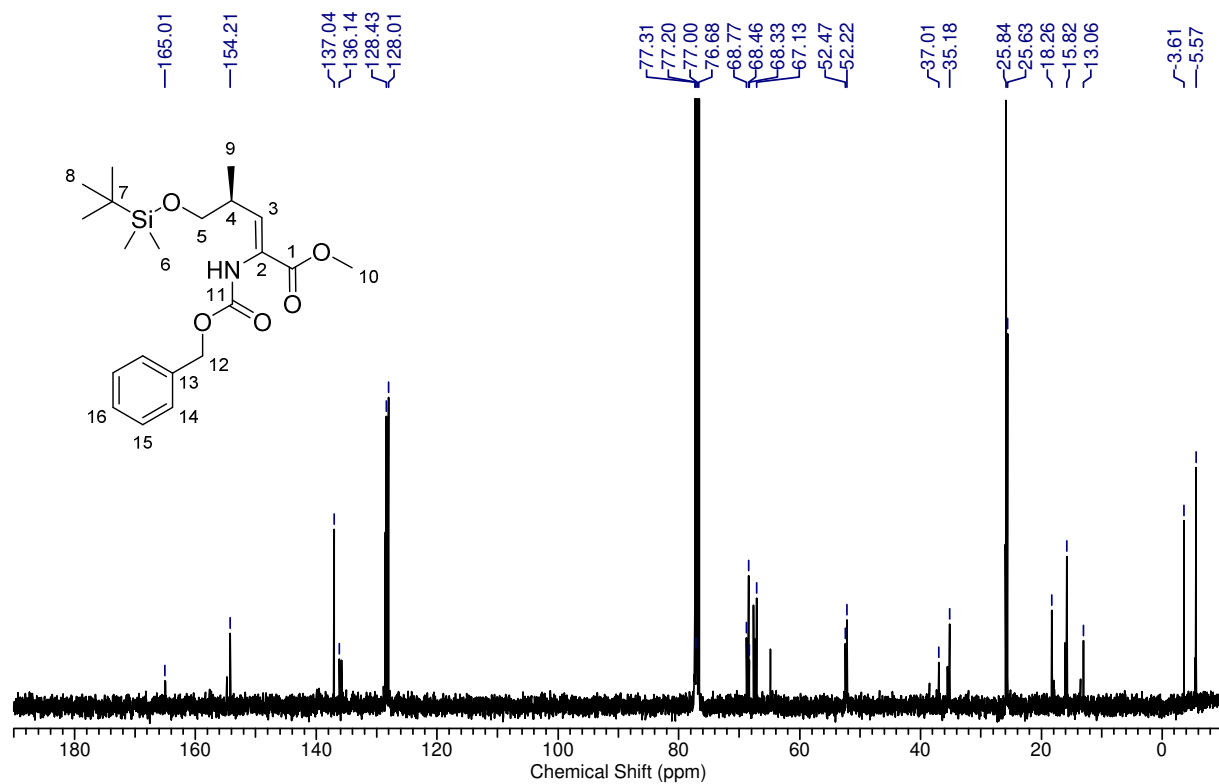


**Methyl (S,Z)-2-[[[(benzyloxy)carbonyl]amino]-5-[(*tert*-butyldimethylsilyl)oxy]-4-methylpent-2-enoate (9; *E/Z* ratio 1:3)**

$^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ ):

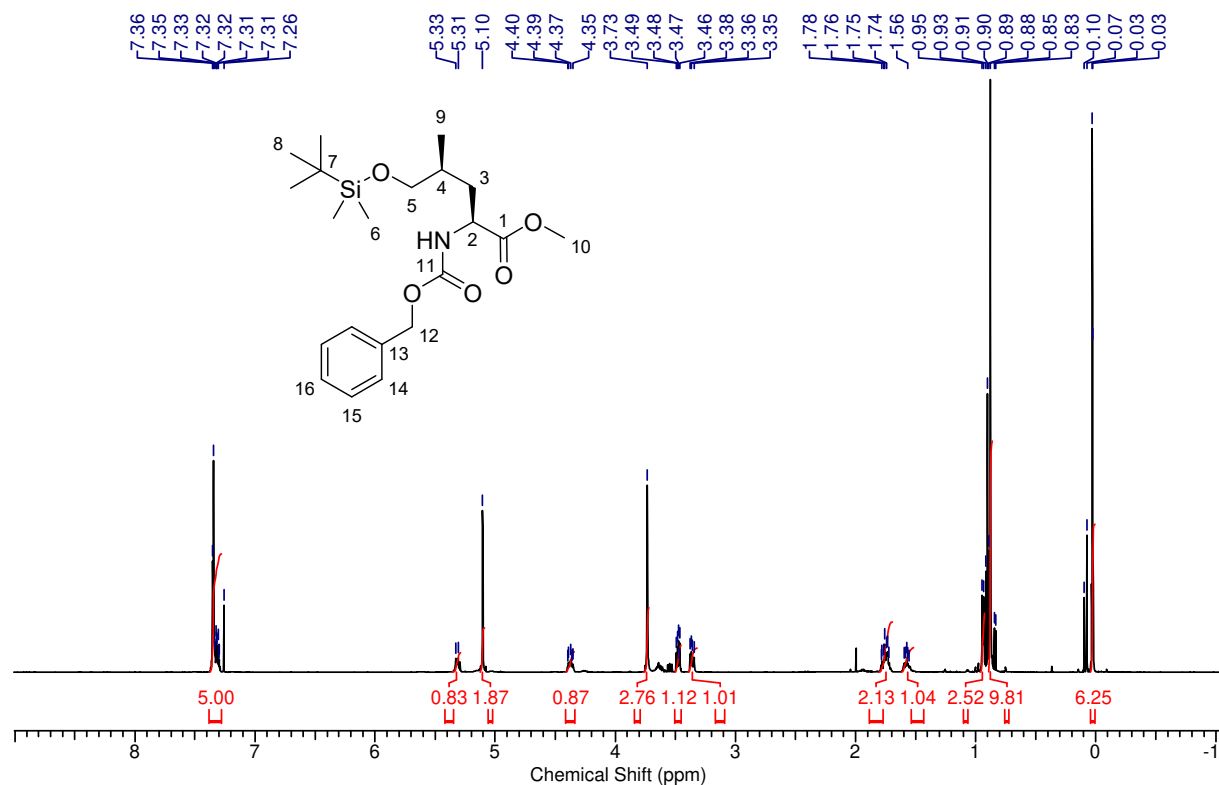


$^{13}\text{C-NMR}$  (100 MHz,  $\text{CDCl}_3$ ):

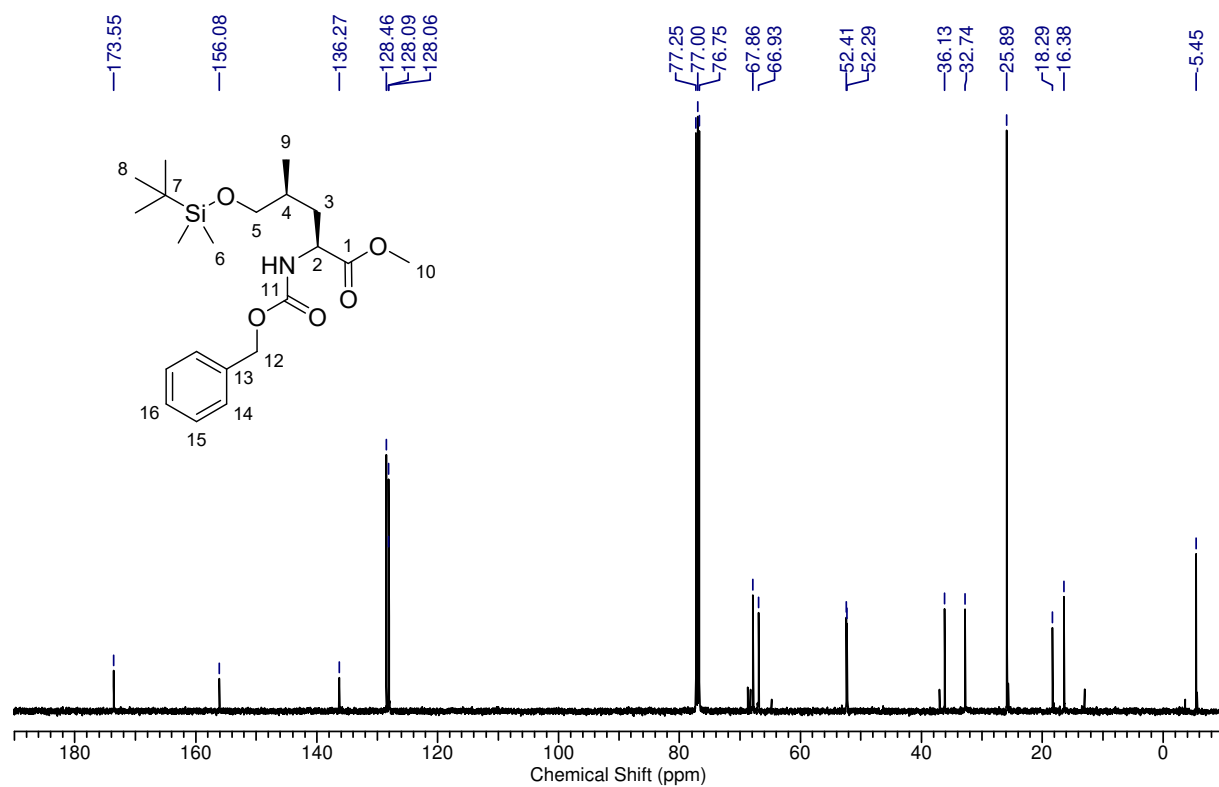


**Methyl (2*S*,4*S*)-2-[(benzyloxy)carbonyl]amino-5-[(*tert*-butyldimethylsilyl)oxy]-4-methyl-pentanoate (10)**

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>):



<sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>):



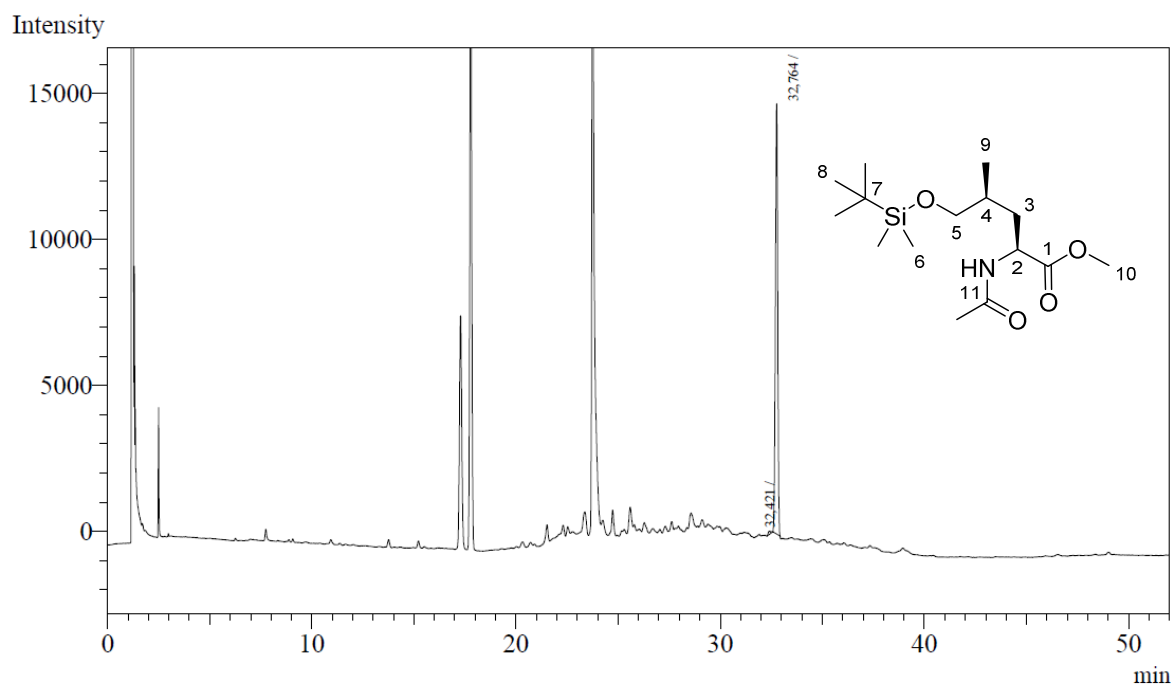


GC-FID:

**Column:** Agilent CP-Chirasil-Dex CB (25 m x 0.25 mm, 0.25 µm ID); **Carrier gas:** N<sub>2</sub>

T<sub>0</sub> [1 min] = 110 °C, 2.0 °C/min to 180 °C, injector: 250 °C, detector: 275 °C

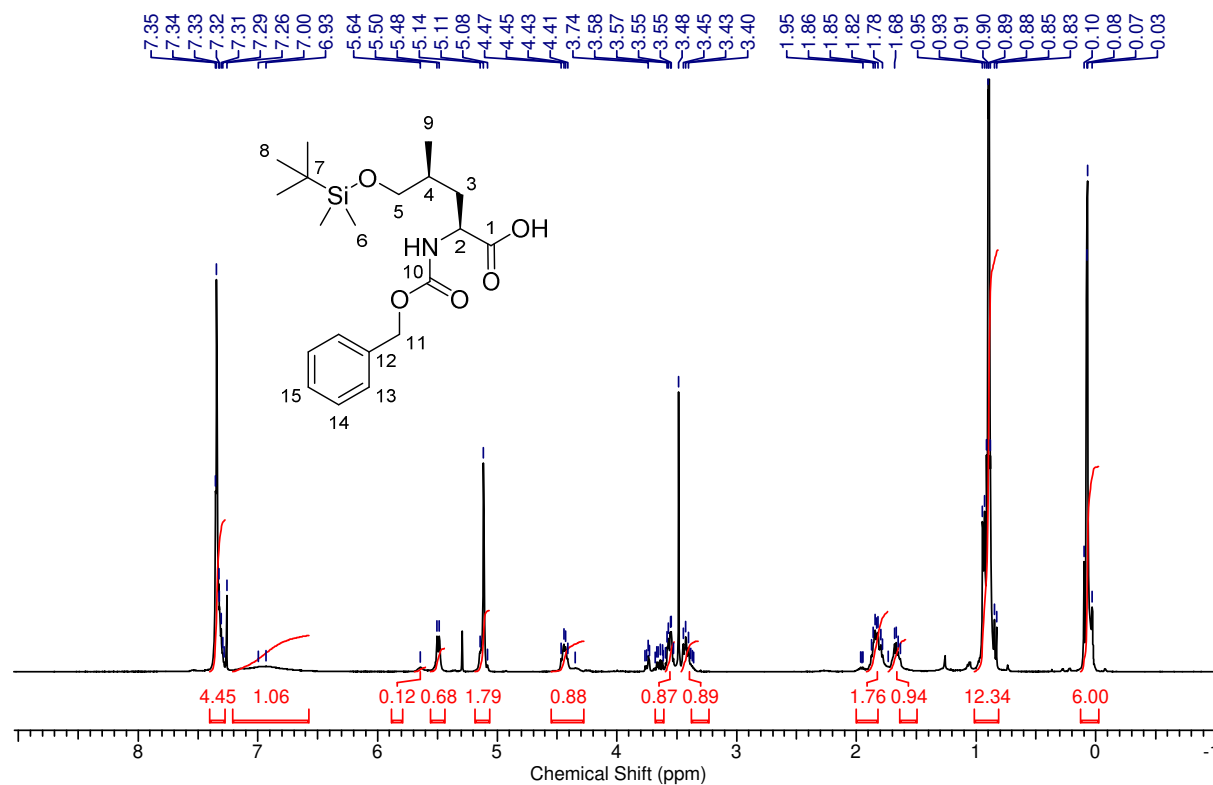
*N*-acetyl-(2*R*,4*S*): t<sub>r</sub> = 32.42 min, *N*-acetyl-(2*S*,4*S*): t<sub>r</sub> = 32.76 min.



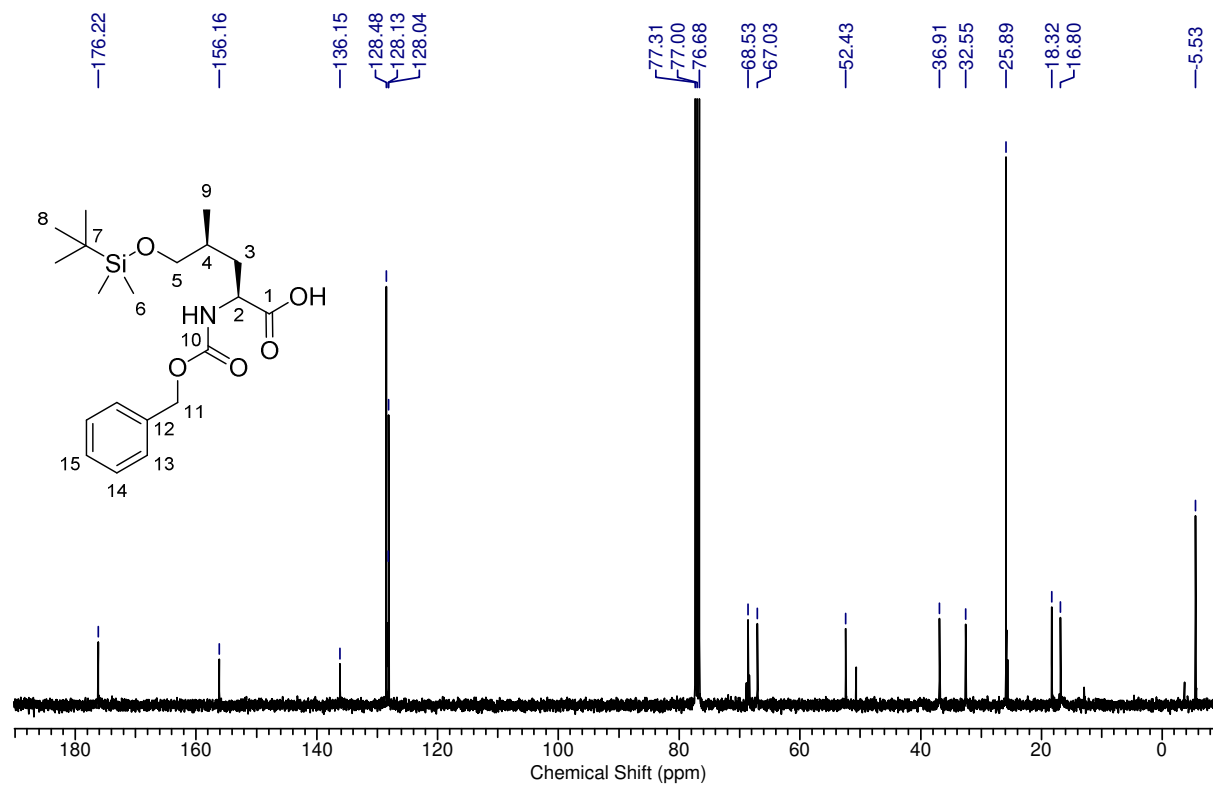
Peak#	Ret.Time	Area	Height	Conc.	Unit Mark	ID#	Cmpd Name
1	32.421	977	105	0.841			
2	32.764	115222	14255	99.159			
Total		116199	14360				

**(2*S*,4*S*)-2-[[*(*Benzyloxy)carbonyl]amino]-5-[(*tert*-butyldimethylsilyl)oxy]-4-methylpentanoic acid (11)**

<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>):

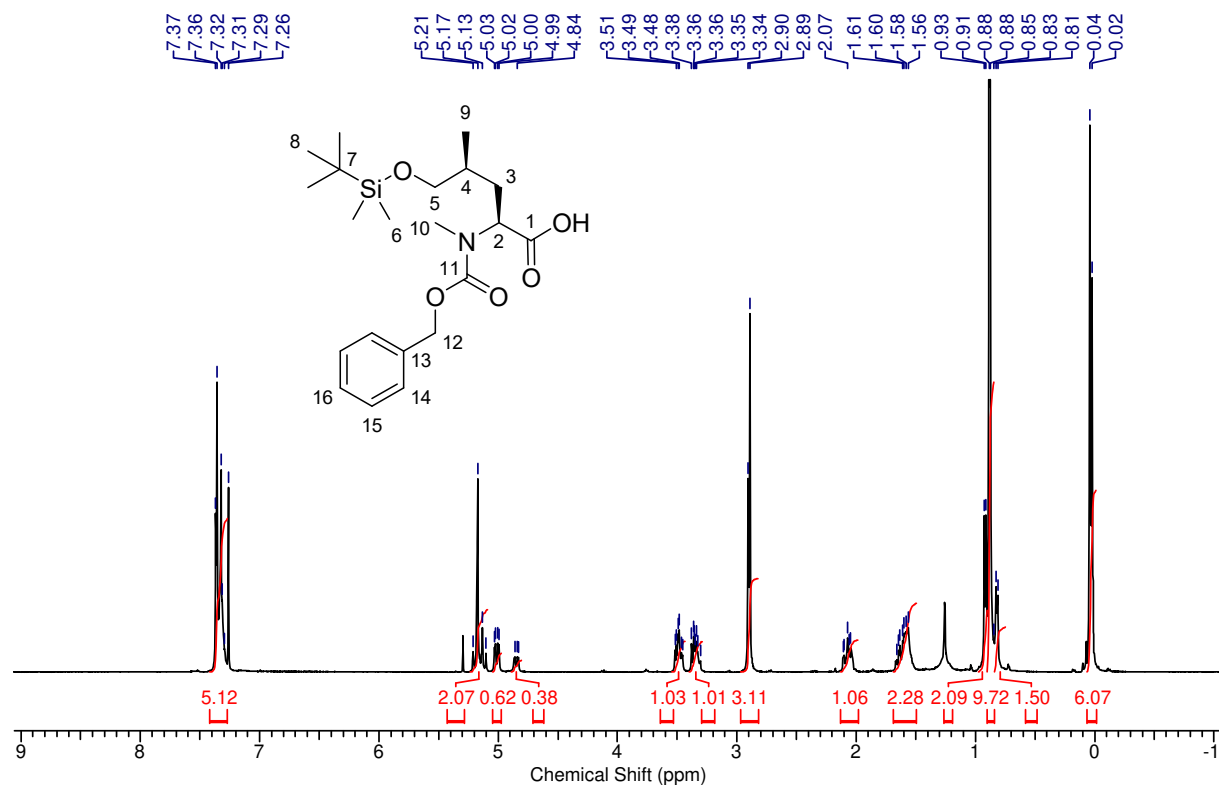


<sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>):

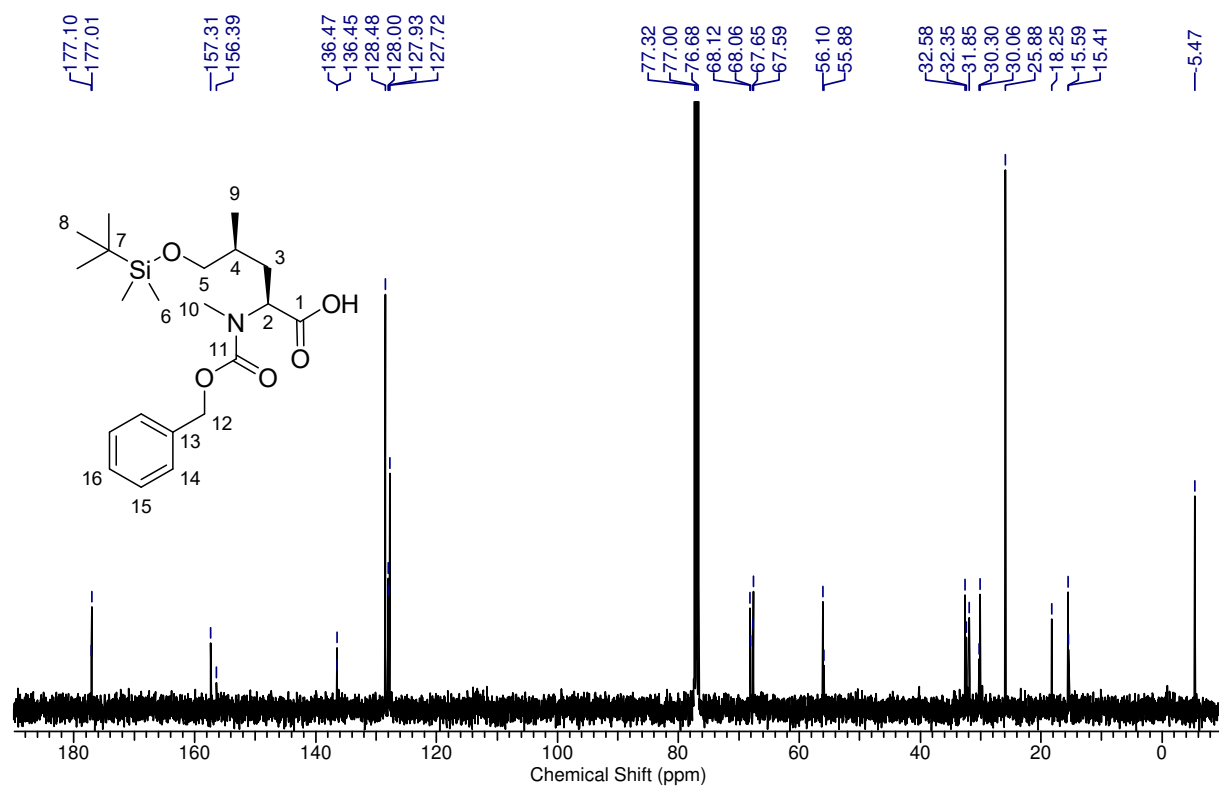


**(2*S*,4*S*)-2-[[*(*Benzyloxy)carbonyl](methyl)amino]-5-[[*tert*-butyldimethylsilyl]oxy]-4-methylpentanoic acid (12)**

<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>):

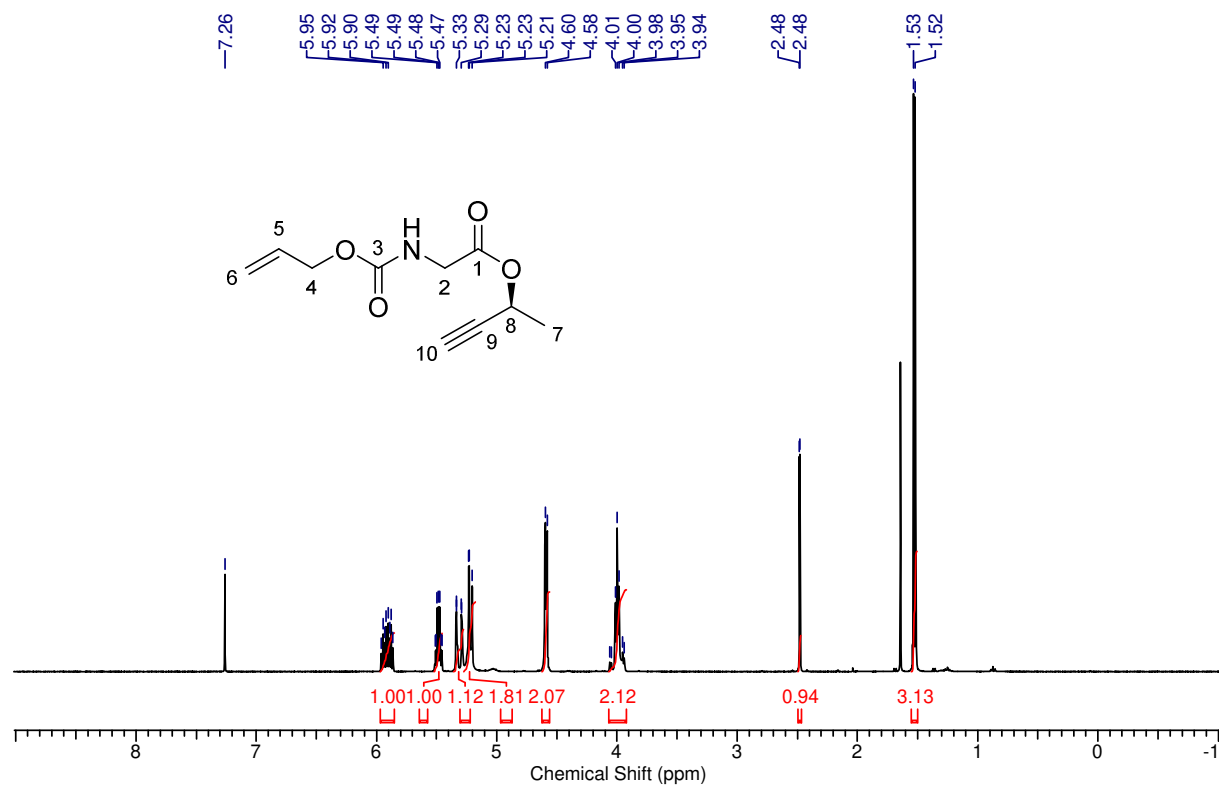


<sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>):

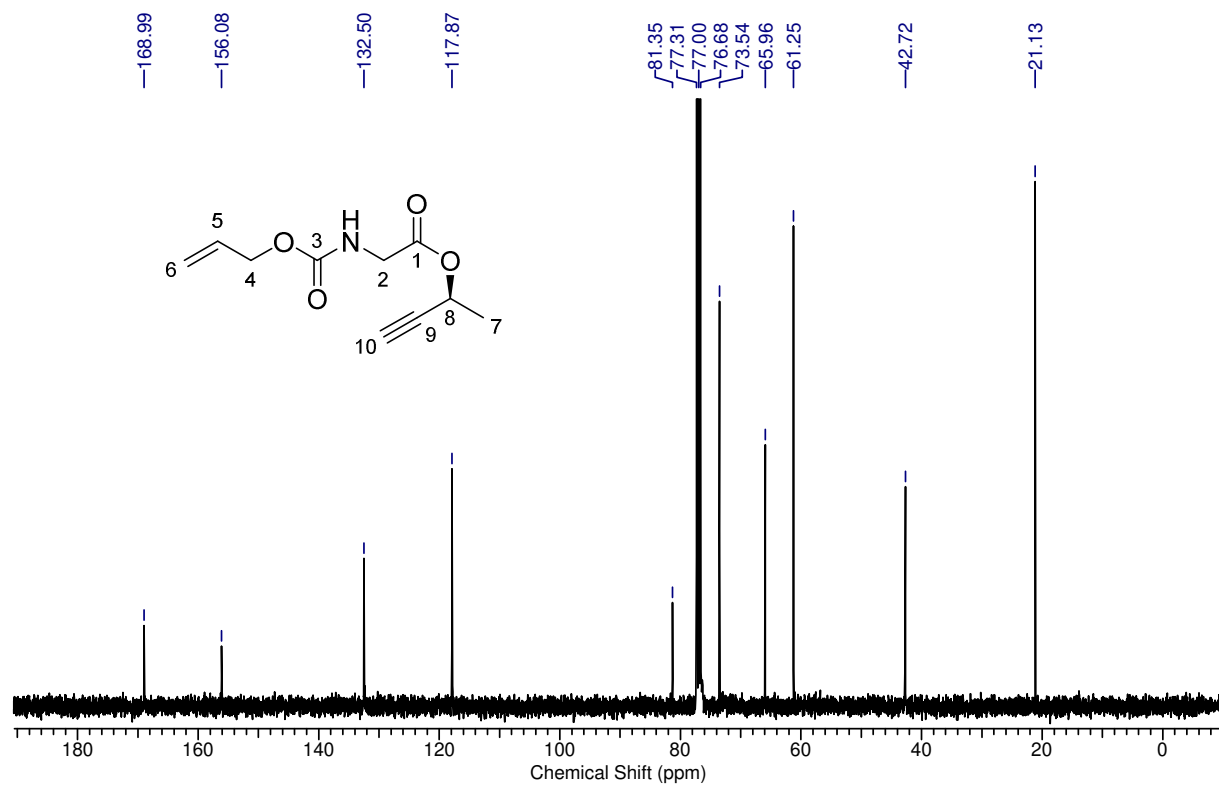


**(S)-But-3-yn-2-yl [(allyloxy)carbonyl]glycinate (14)**

<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>):

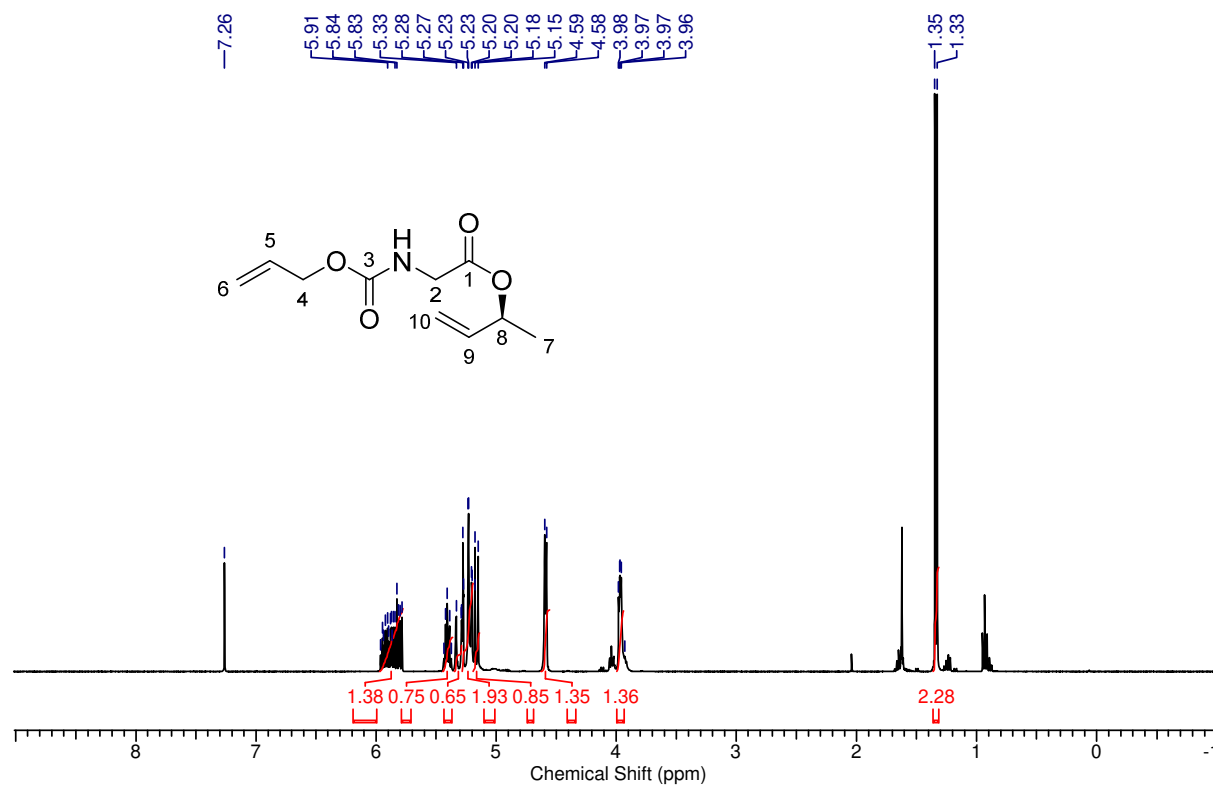


<sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>):

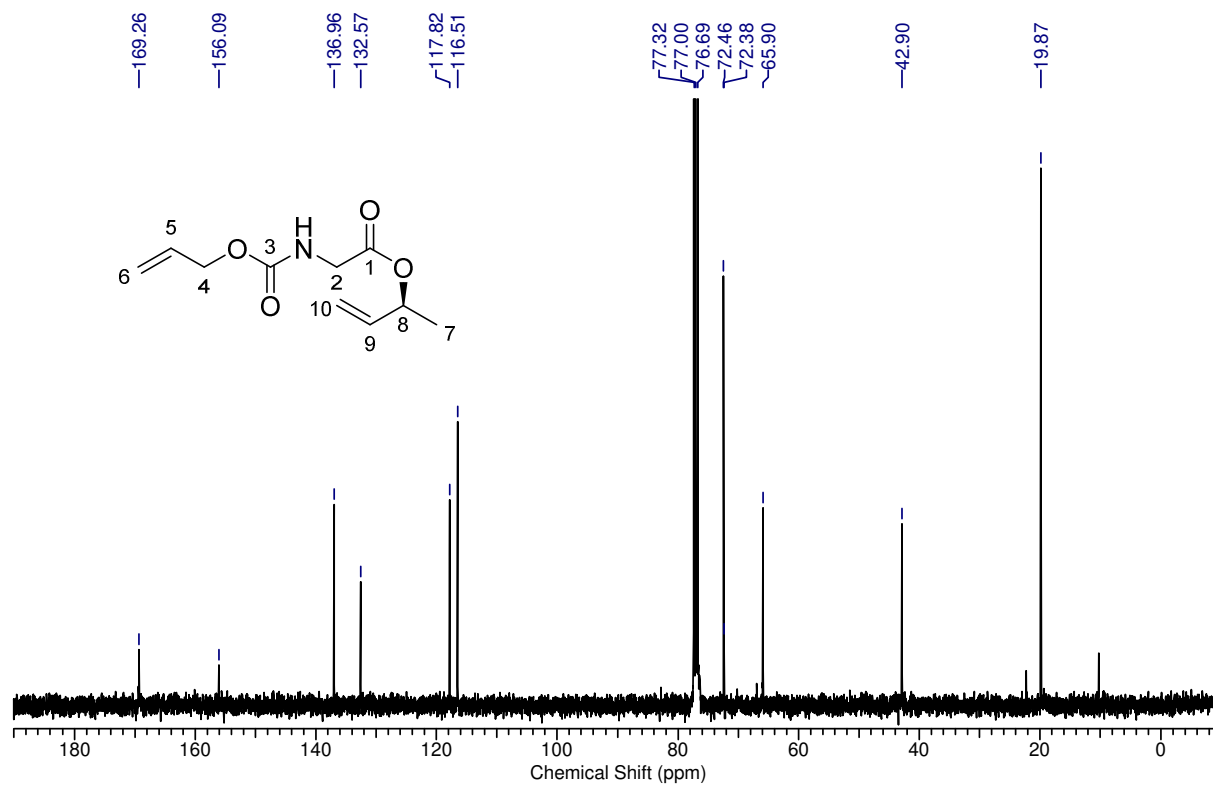


**(S)-But-3-en-2-yl [(allyloxy)carbonyl]glycinate (15)**

<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>):

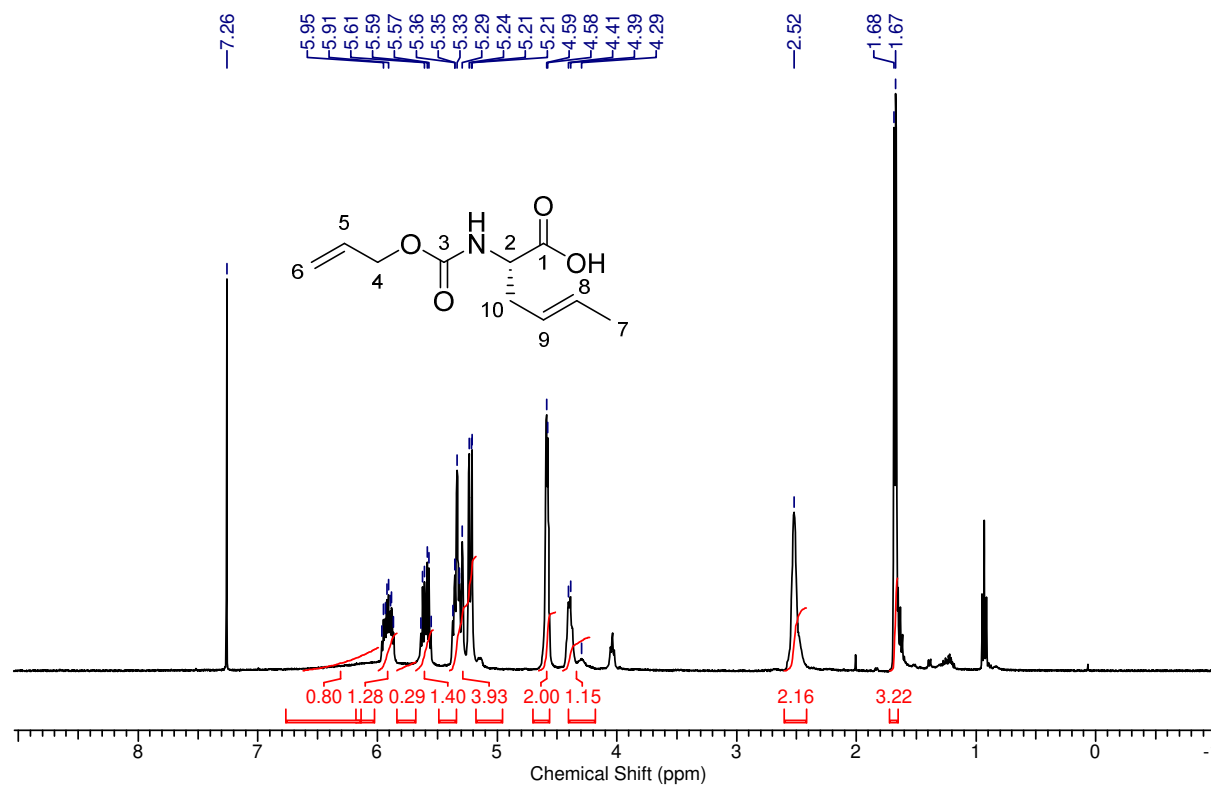


<sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>):

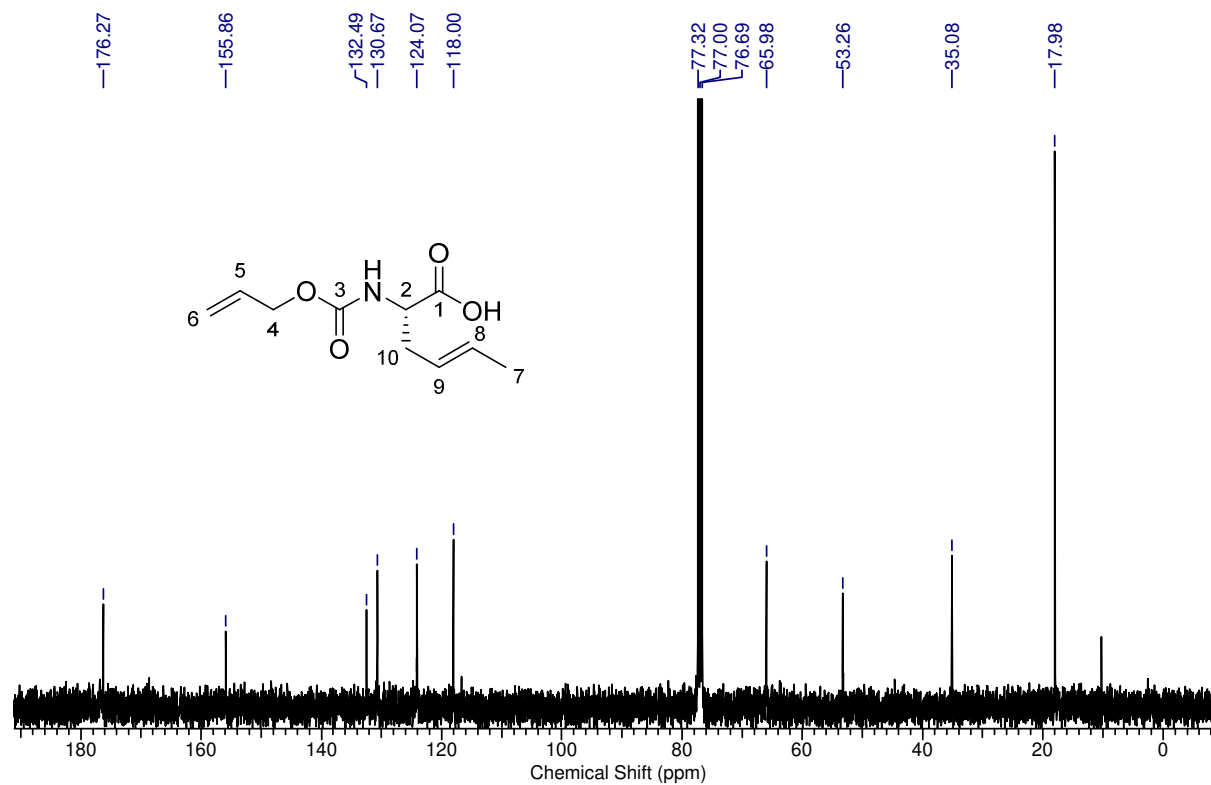


**(S,E)-2-[(Allyloxy)carbonylamino]hex-4-enoic acid (16)**

<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>):

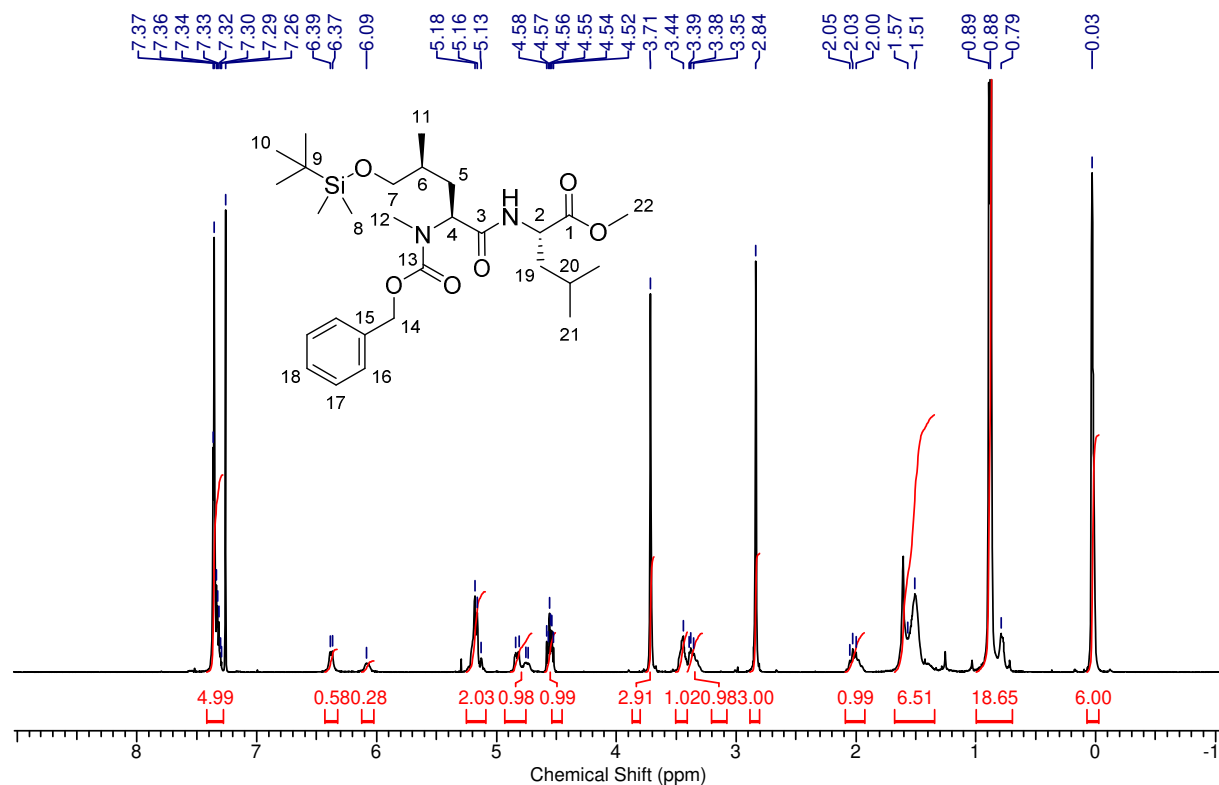


<sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>):

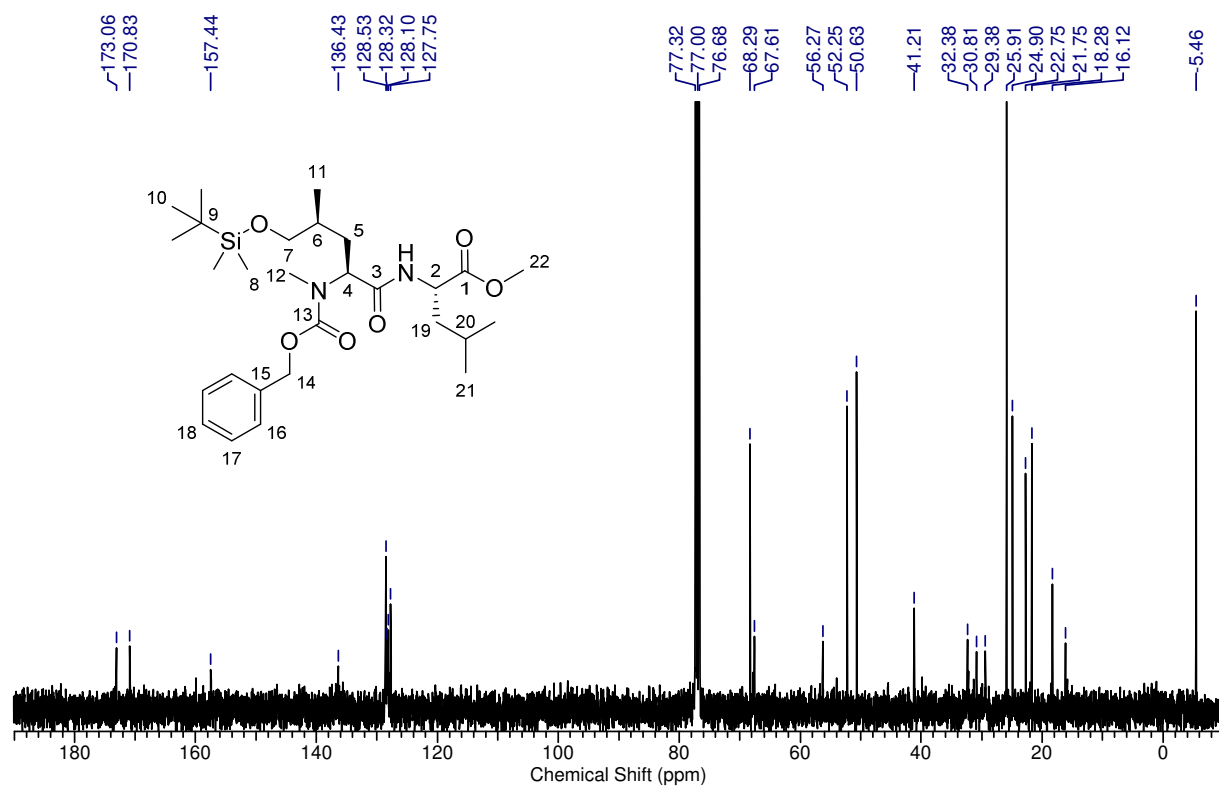


**Methyl {[2*S*,4*S*]-2-([benzyloxy]carbonyl)(methyl)amino)-5-[(*tert*-butyldimethyl-silyl)oxy]-4-methylpentanoyl]-L-leucinate (17)**

<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>):

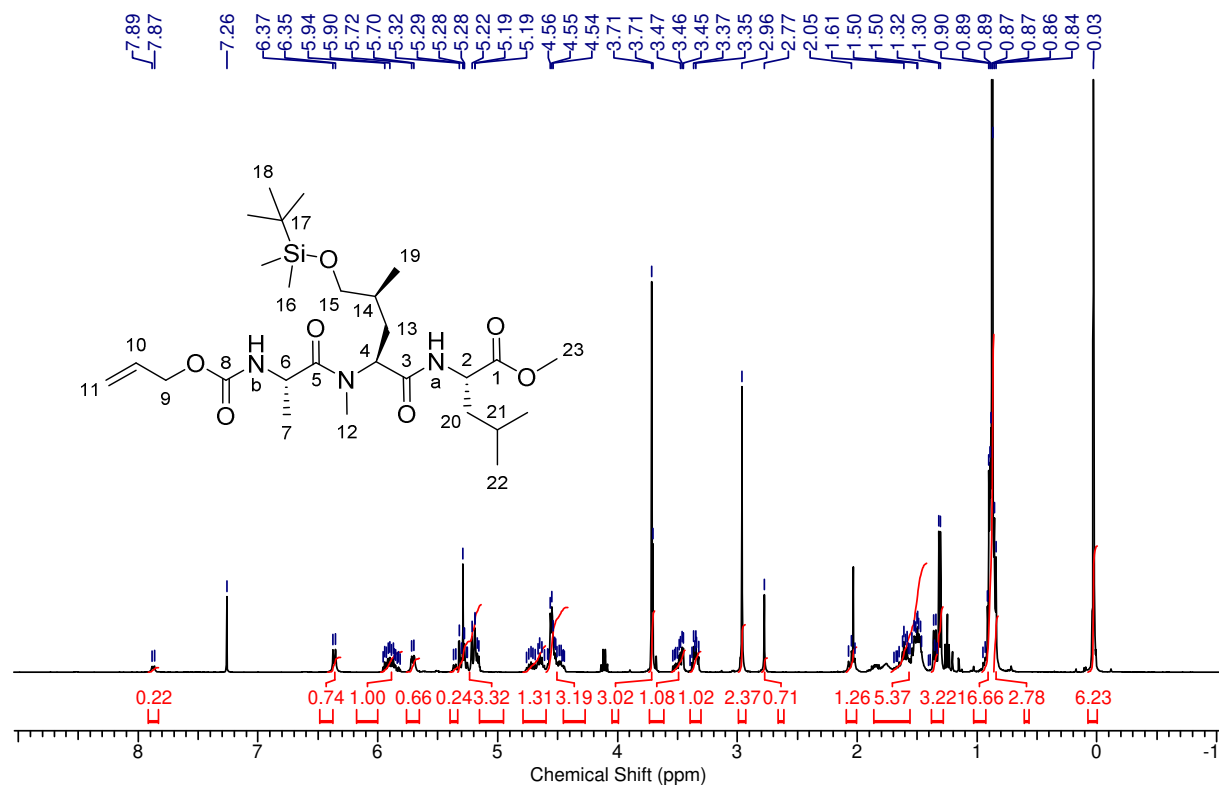


<sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>):

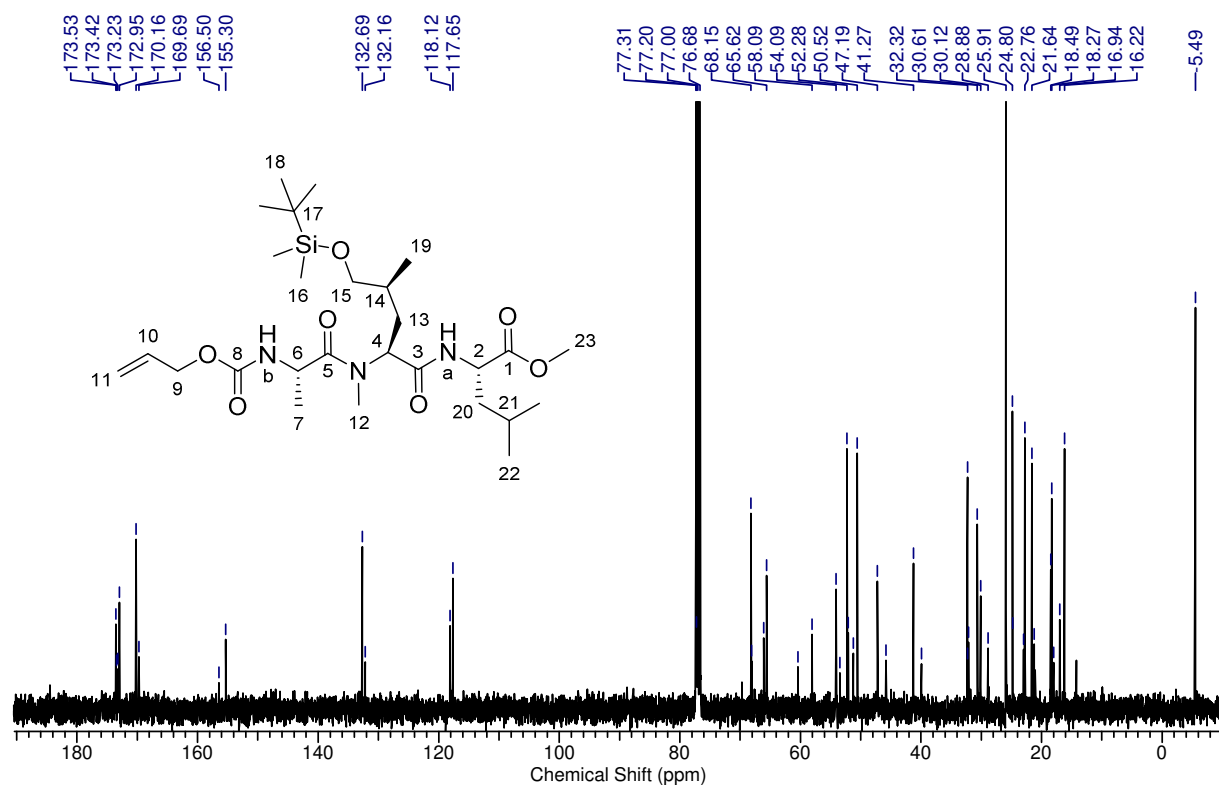


**Methyl {[2*S*,4*S*]-2-[(*S*)-2-[(allyloxy)carbonyl]amino]-*N*-methylpropanamido]-5-[(*tert*-butyldimethylsilyl)oxy]-4-methylpentanoyl]-*L*-leucinate (18)**

<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>):



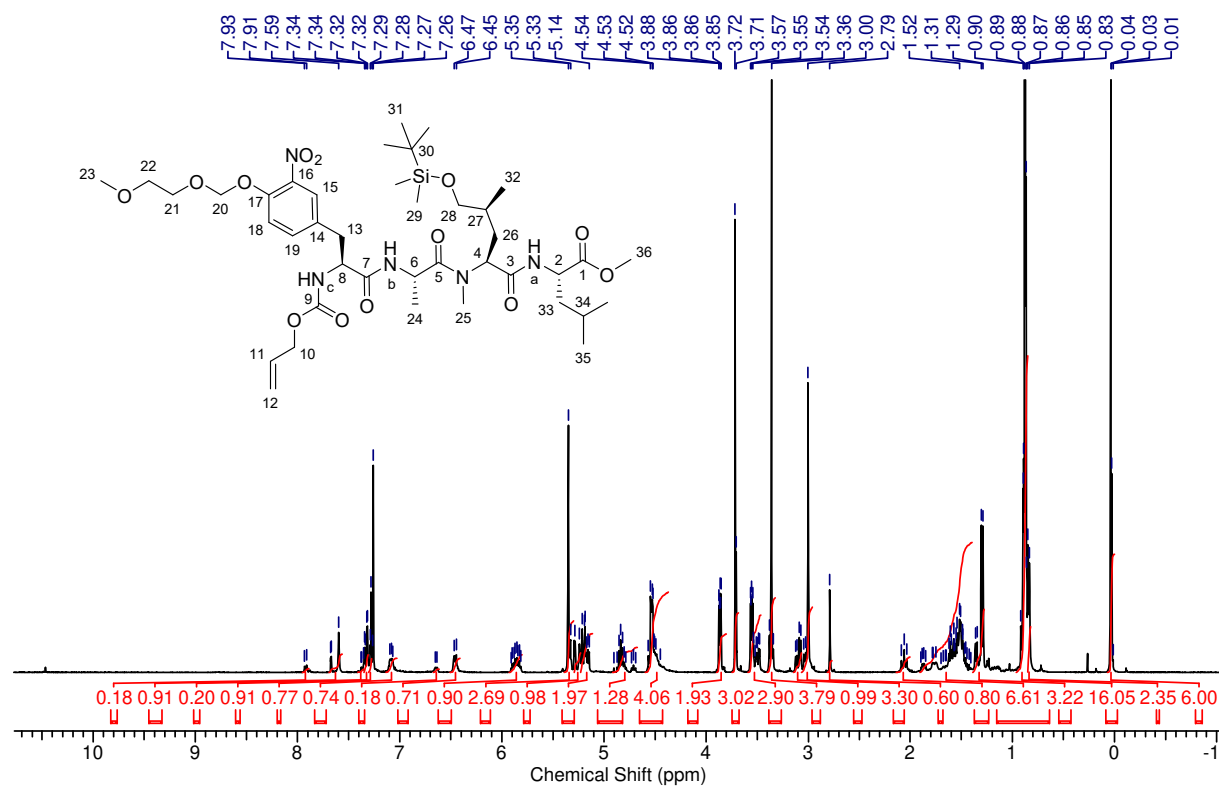
<sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>):



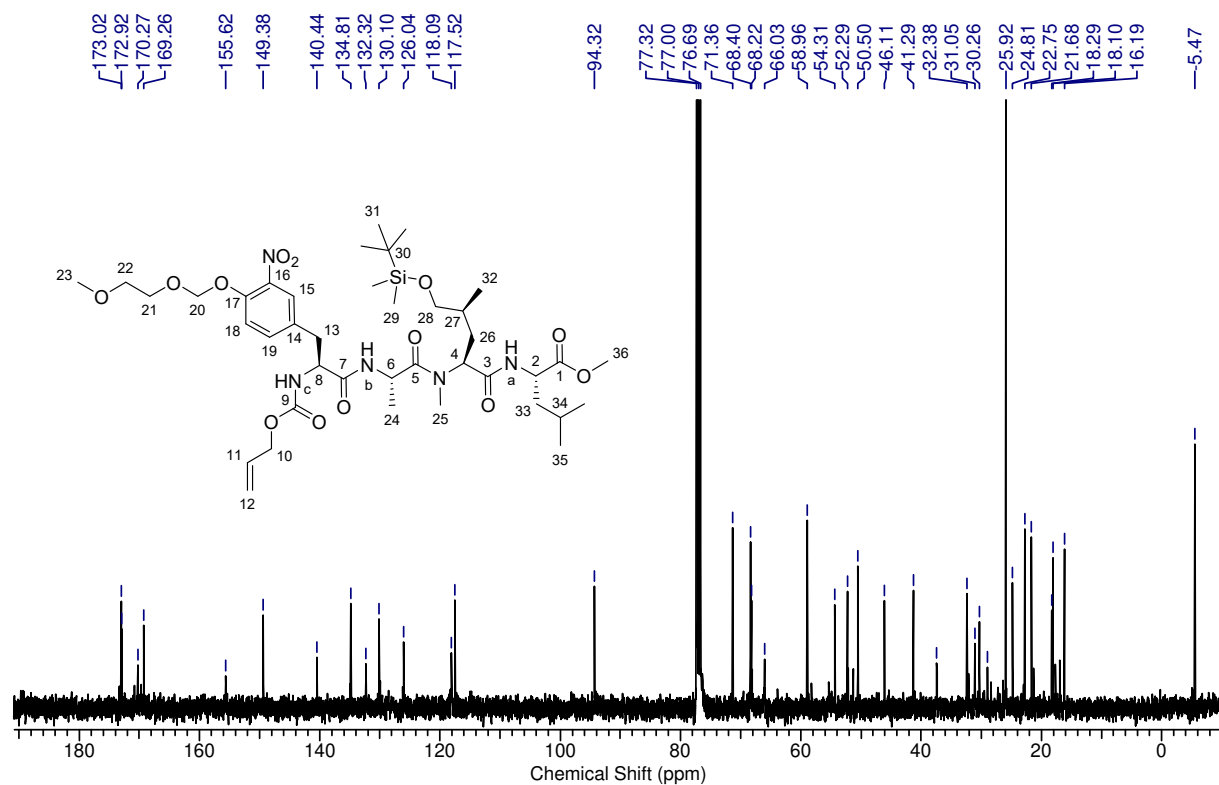


**Methyl {[2*S*,4*S*]-2-[(*S*)-2-[(*S*)-2-[(allyloxy)carbonyl]amino]-3-[4-[(2-methoxy-ethoxy)methoxy]-3-nitrophenyl]propanamido]-*N*-methylpropanamido]-5-[(*tert*-butyldimethylsilyl)oxy]-4-methyl-pentanoyl]-*L*-leucinate (19)**

$^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ ):

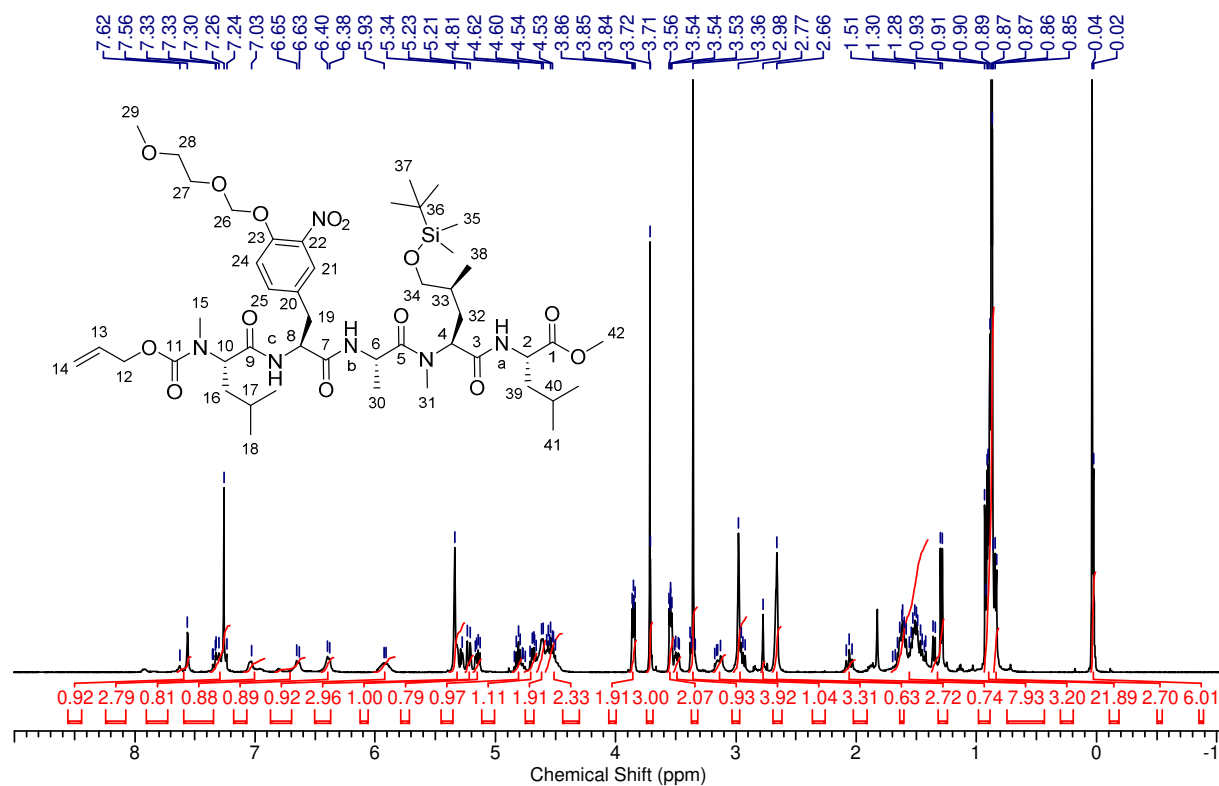


$^{13}\text{C-NMR}$  (100 MHz,  $\text{CDCl}_3$ ):

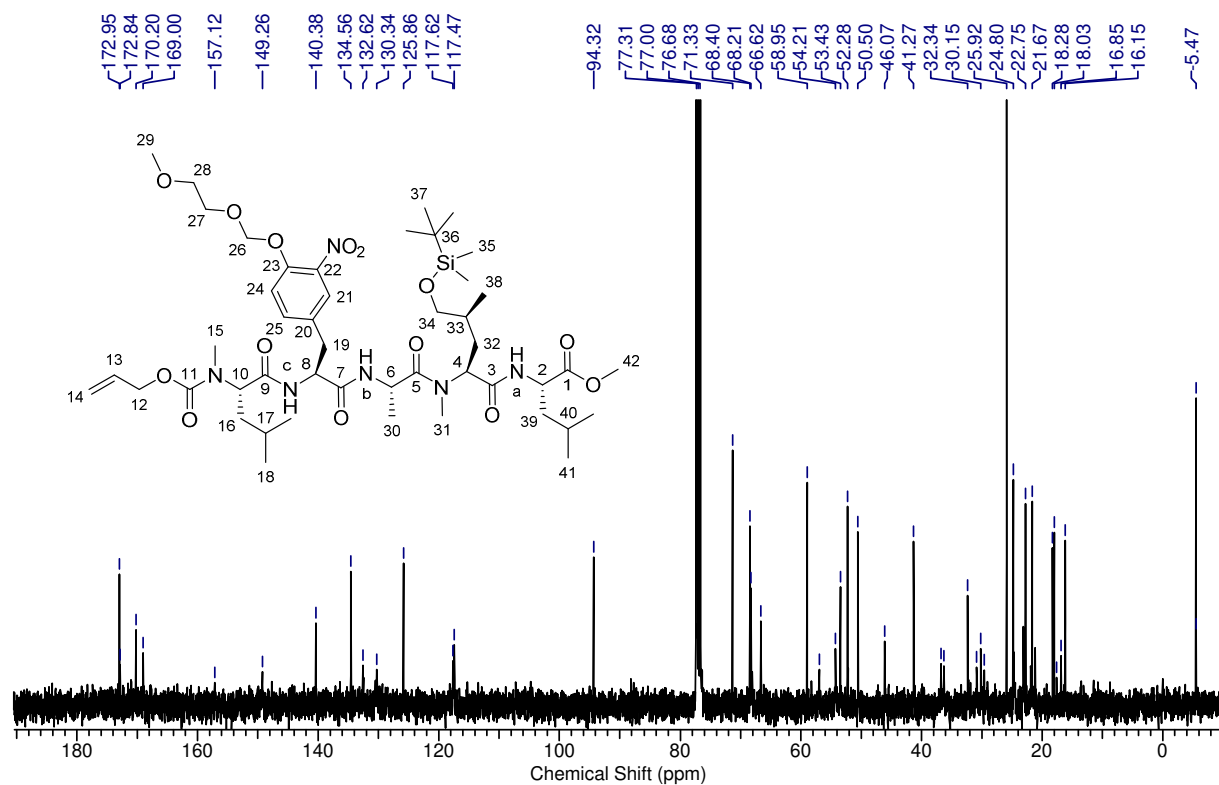


**Methyl {[2*S*,4*S*]-2-[(*S*)-2-[(*S*)-2-[(*S*)-2-[(allyloxy)carbonyl](methyl)amino)-4-methylpentanamido]-3-[4-[(2-methoxyethoxy)methoxy]-3-nitrophenyl]propan-amido]-*N*-methylpropanamido)-5-[(*tert*-butyldimethylsilyl)oxy]-4-methyl-pentanoyl]-L-leucinate (20)**

<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>):

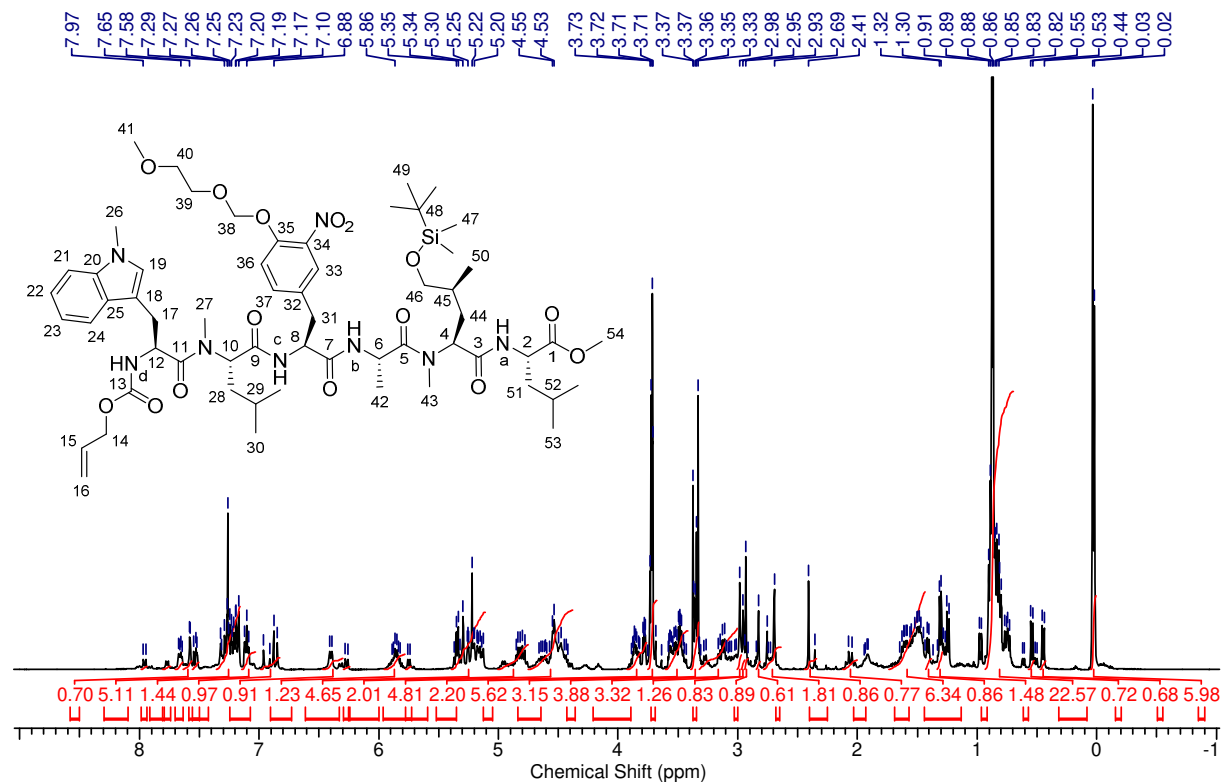


<sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>):

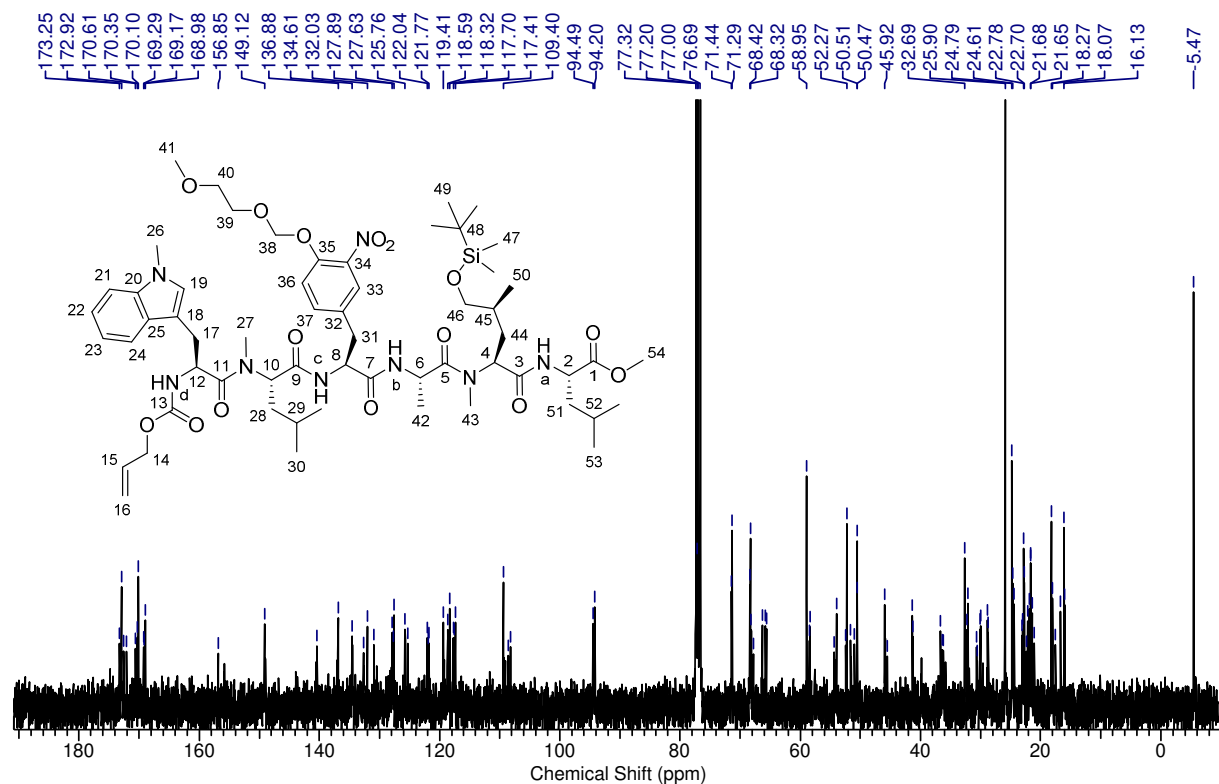


**Methyl [(2S,4S)-2-((S)-2-[(S)-2-[(S)-2-[(allyloxy)carbonyl]amino]-N-methyl-3-[1-methyl-1H-indol-3-yl]propanamido)-4-methylpentanamido]-3-(4-[[2-methoxyethoxy]methoxy]-3-nitrophenyl)-propanamido]-N-methylpropanamido)-5-[(*tert*-butyldimethylsilyl)oxy]-4-methylpentanoyl]-L-leucinate (21)**

<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>):

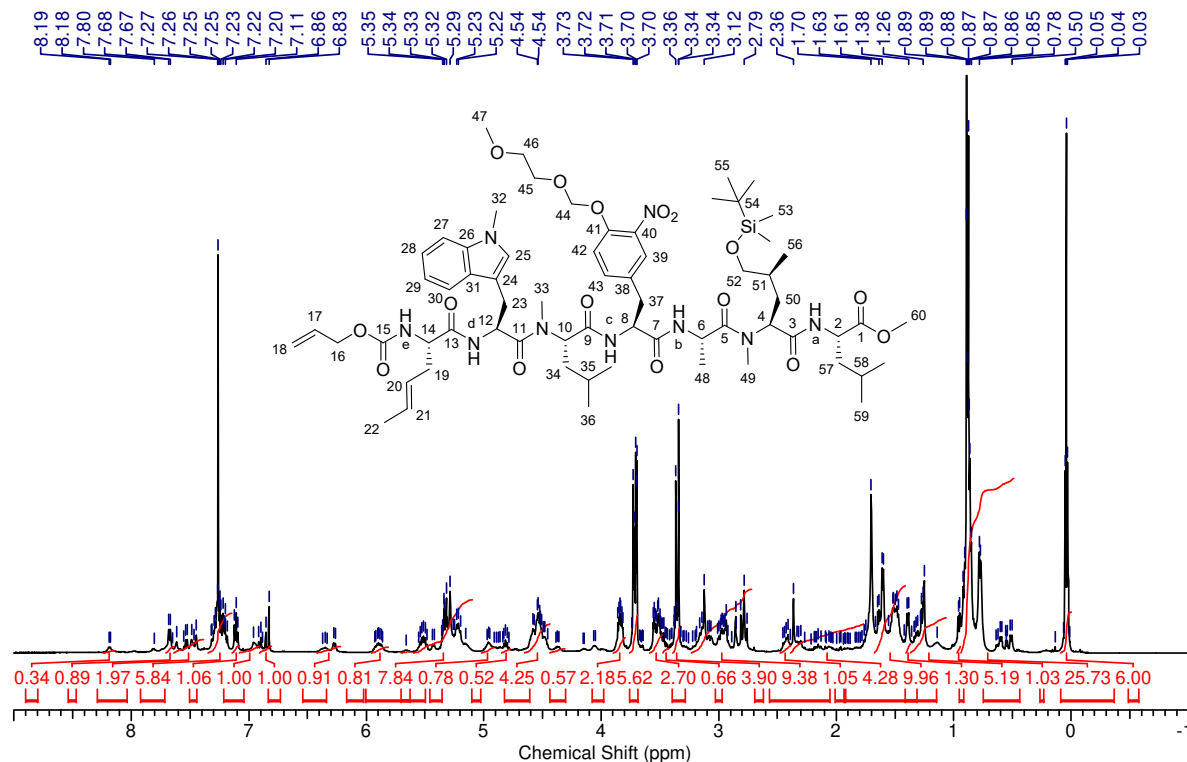


<sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>):

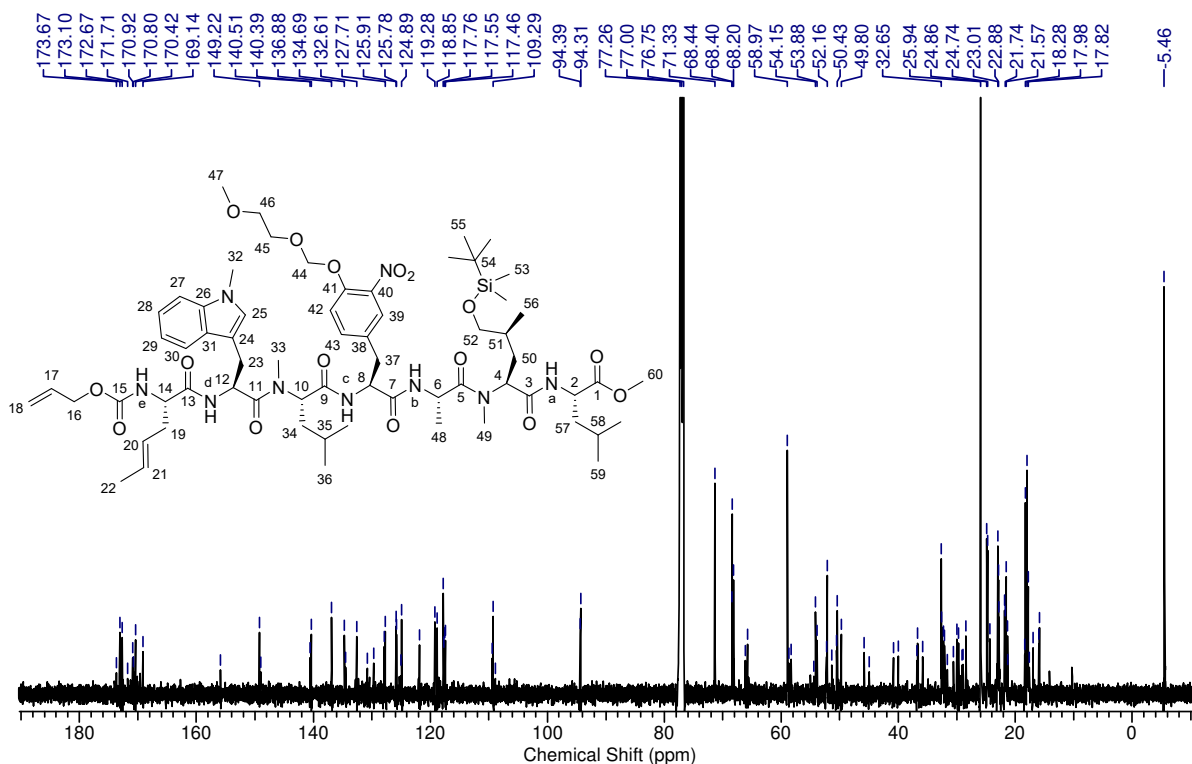


Methyl {[2*S*,4*S*]-2-[(*S*)-2-[(*S*)-2-[(*S*)-2-[(*S*,*E*)-2-[(allyloxy)carbonyl]amino]-hex-4-enamido]-*N*-methyl-3-[1-methyl-1*H*-indol-3-yl]propanamido]-4-methyl-pentanamido]-3-[4-[(2-methoxyethoxy)-methoxy]-3-nitrophenyl]propanamido]-*N*-methylpropanamido]-5-[(*tert*-butyldimethylsilyl)oxy]-4-methylpentanoyl]-*L*-leucinate (22)

$^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ ):

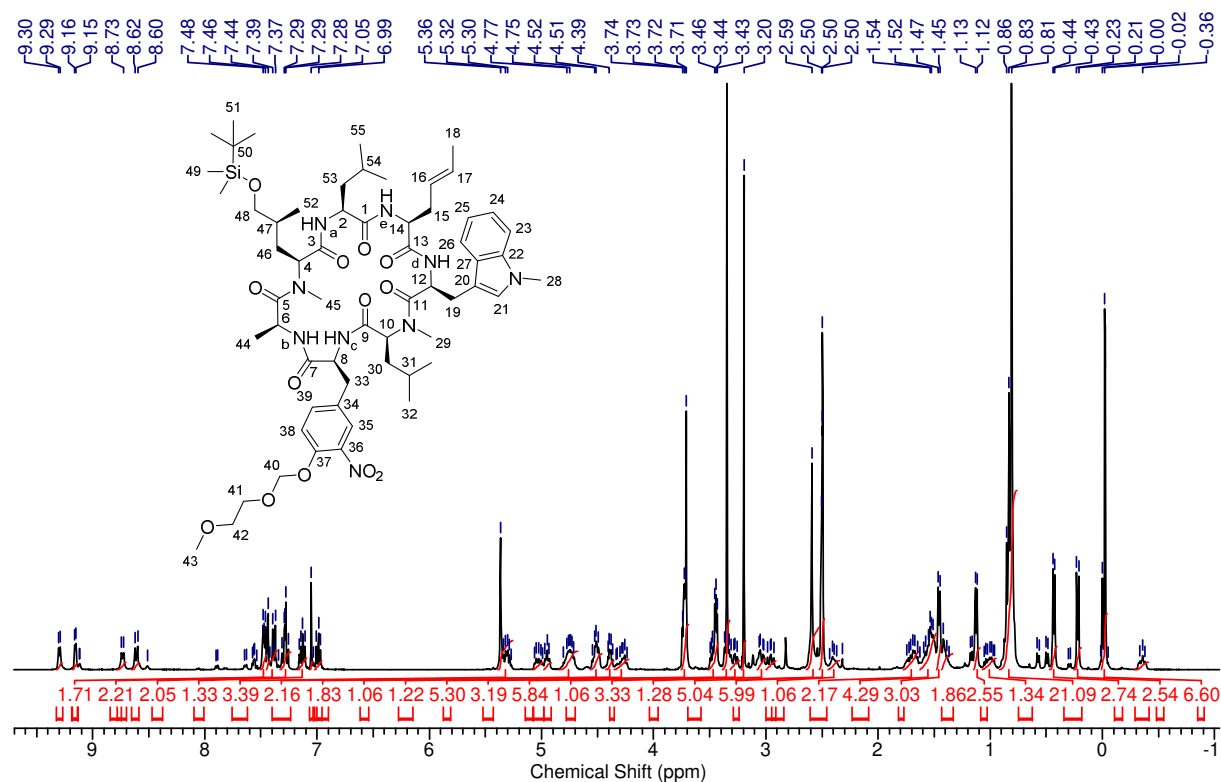


$^{13}\text{C-NMR}$  (125 MHz,  $\text{CDCl}_3$ ):

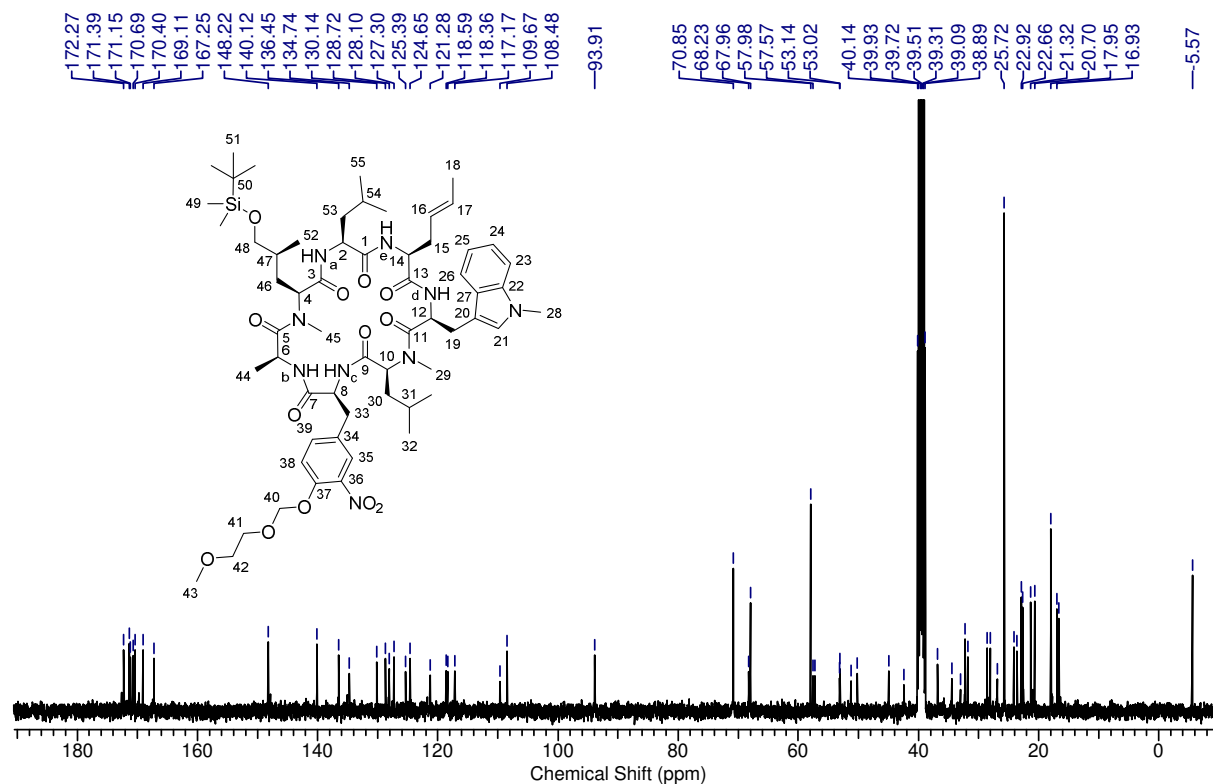


(3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,21*S*)-15-[(*E*)-But-2-en-1-yl]-21-[(*S*)-3-[(*tert*-butyldimethyl-silyl)oxy]-2-methylpropyl]-9,18-diisobutyl-6-[4-[(2-methoxyethoxy)methoxy]-3-nitrobenzyl]-1,3,10-trimethyl-12-[(1-methyl-1*H*-indol-3-yl)methyl]-1,4,7,10,13,16,19-heptaazacyclohenicosane-2,5,8,11,14,17,20-heptaone (23)

$^1\text{H-NMR}$  (400 MHz,  $(\text{CD}_3)_2\text{SO}$ ):

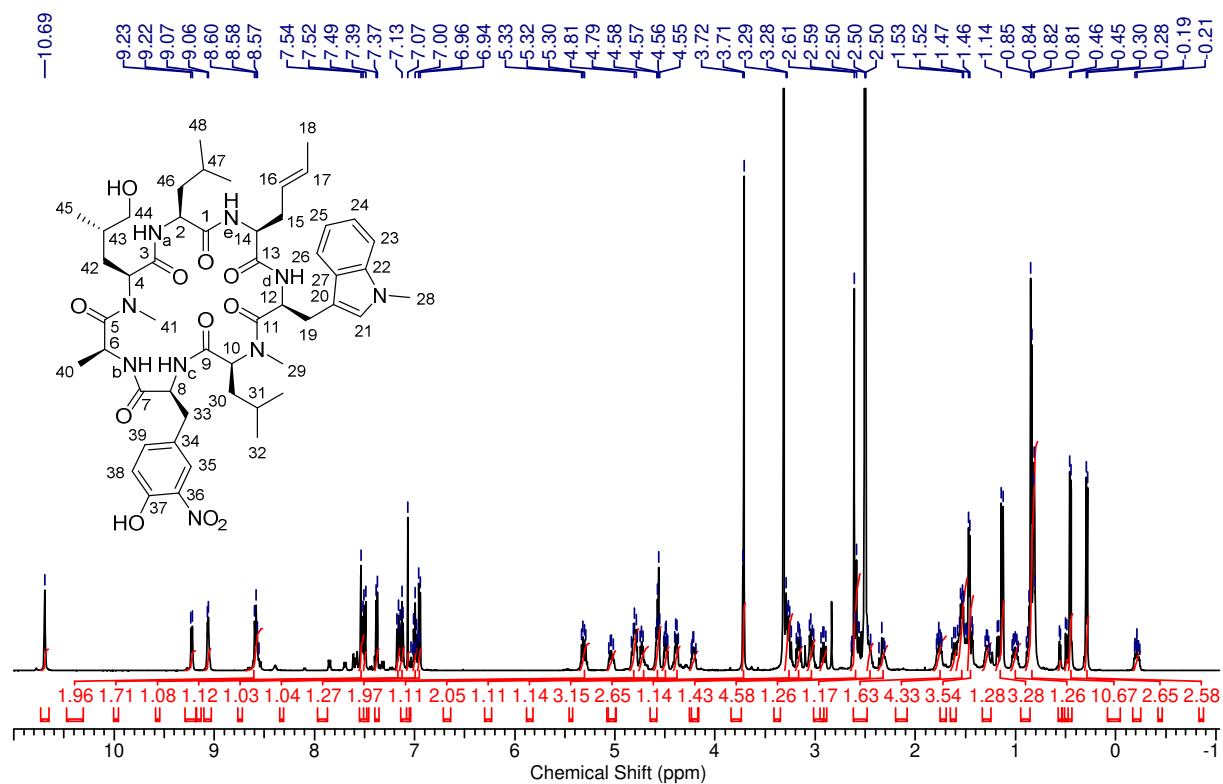


$^{13}\text{C-NMR}$  (100 MHz,  $(\text{CD}_3)_2\text{SO}$ ):

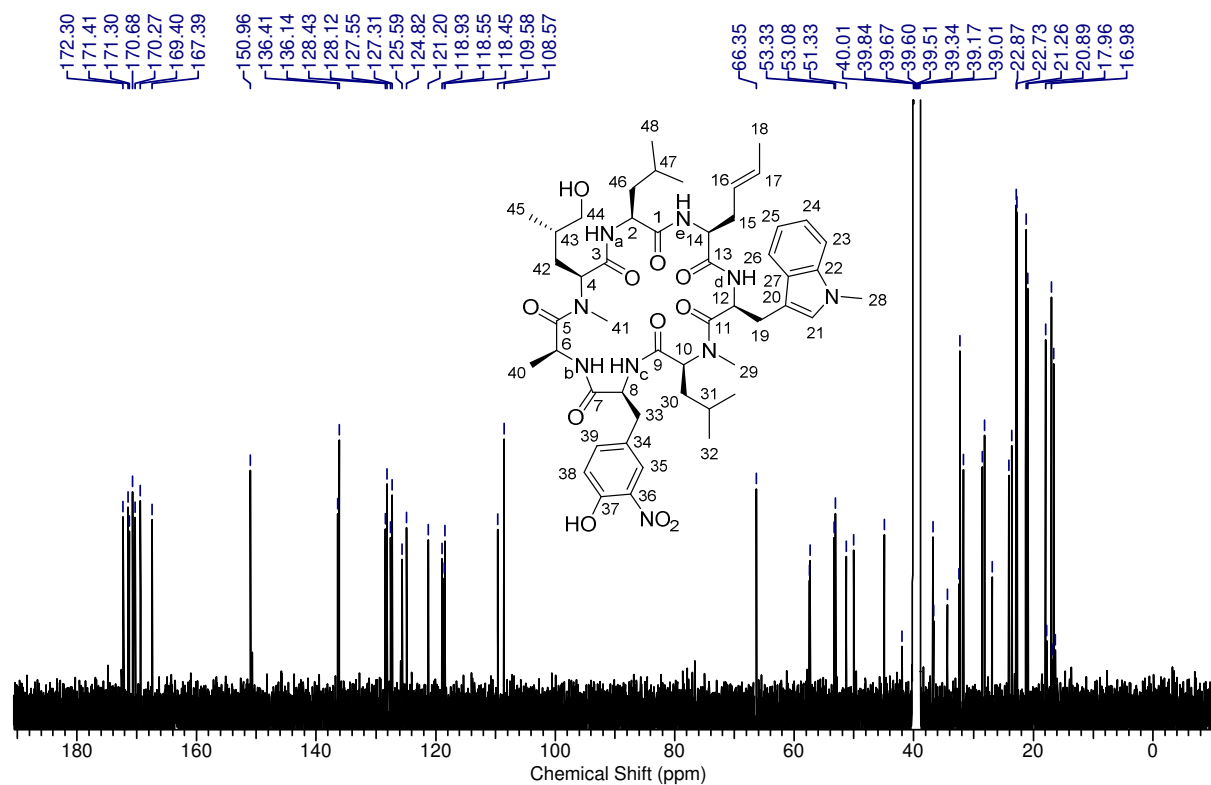


(3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,21*S*)-15-[(*E*)-But-2-en-1-yl]-21-[(*S*)-3-hydroxy-2-methyl-propyl]-6-(4-hydroxy-3-nitrobenzyl)-9,18-diisobutyl-1,3,10-trimethyl-12-[(1-methyl-1*H*-indol-3-yl)methyl]-1,4,7,10,13,16,19-heptaazacyclohenicosane-2,5,8,11,14,17,20-heptaone (24)

$^1\text{H-NMR}$  (500 MHz,  $(\text{CD}_3)_2\text{SO}$ ):

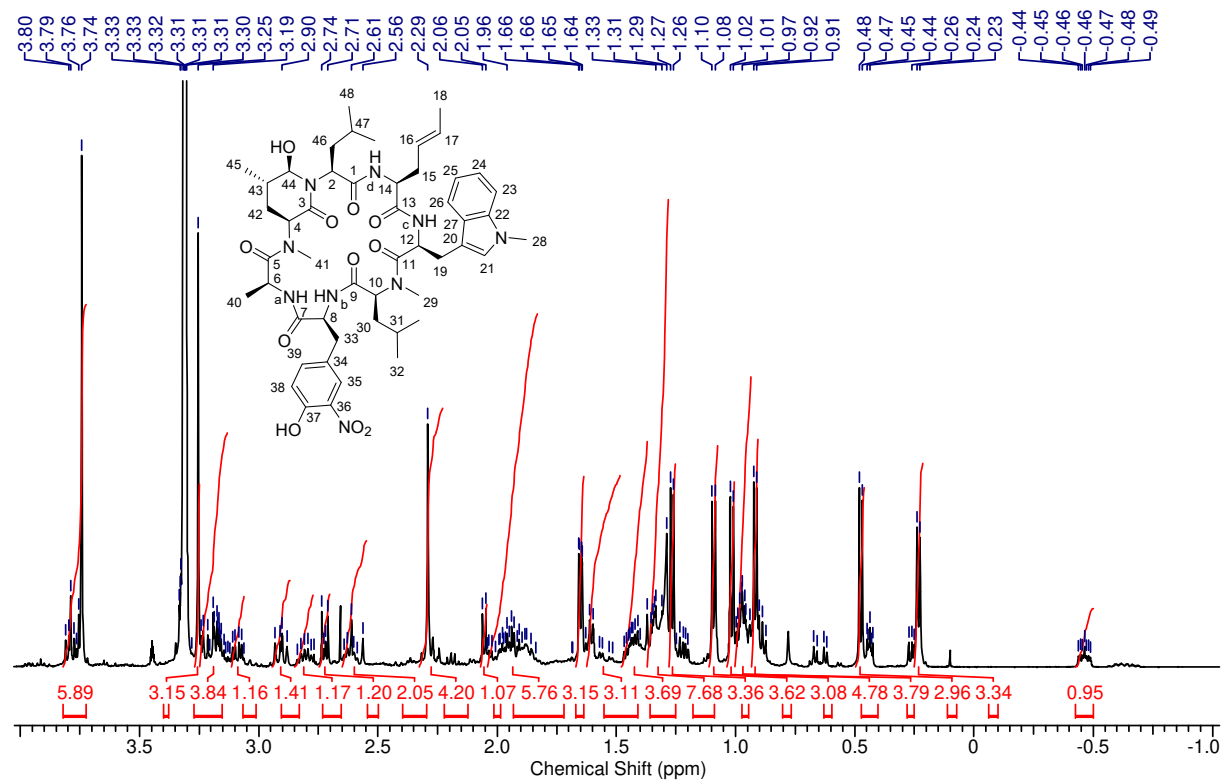
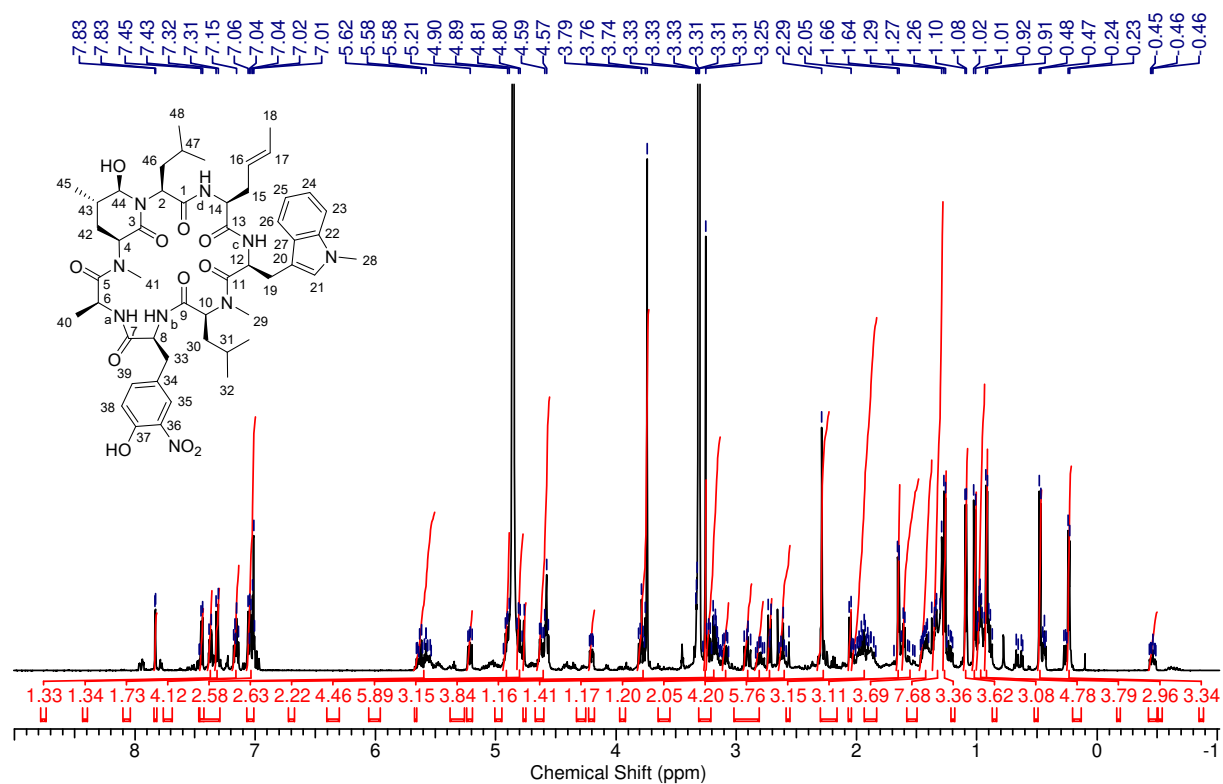


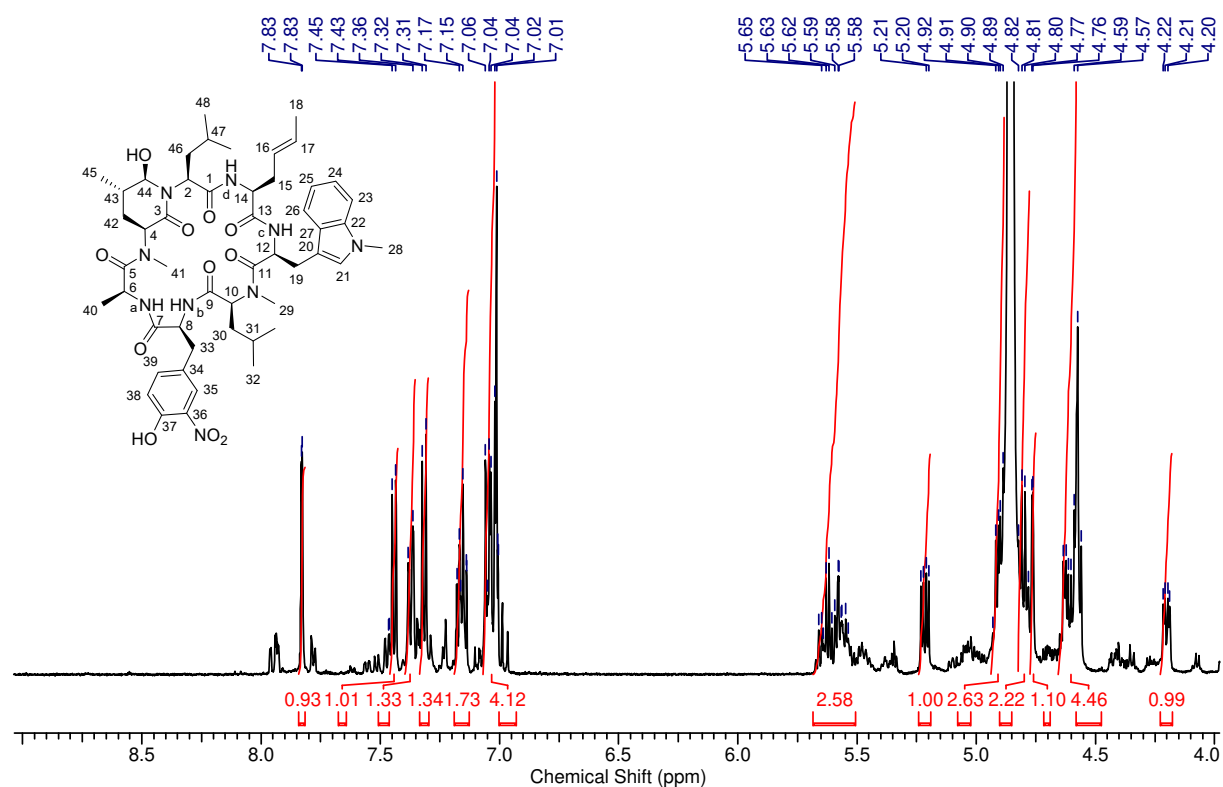
$^{13}\text{C-NMR}$  (125 MHz,  $(\text{CD}_3)_2\text{SO}$ ):



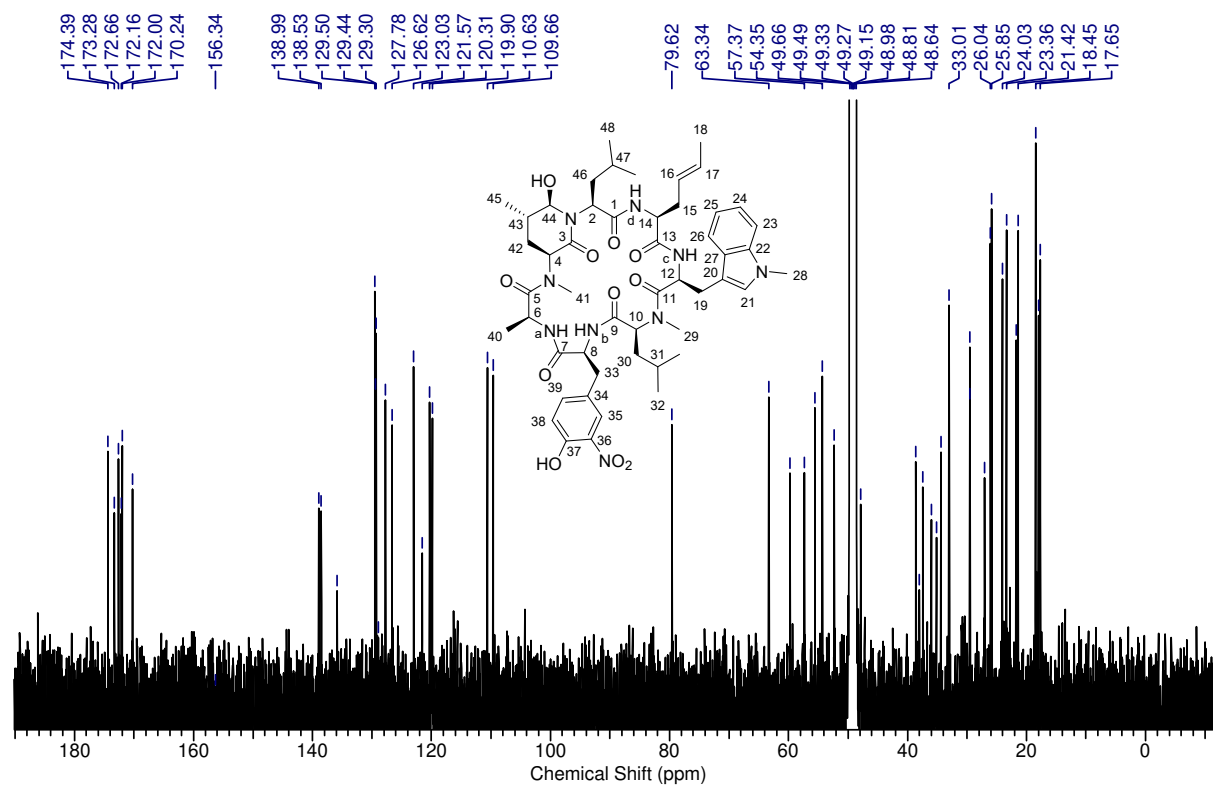
**(2*S*,5*S*,8*S*,11*S*,14*S*,17*S*,20*S*,22*S*,23*R*)-5-[(*E*)-But-2-en-1-yl]-23-hydroxy-14-(4-hydroxy-3-nitrobenzyl)-2,11-diisobutyl-10,17,19,22-tetramethyl-8-[(1-methyl-1*H*-indol-3-yl)methyl]-1,4,7,10,13,16,19-heptaazabicyclo[18.3.1]tetracosane-3,6,9,12,15,18,24-heptaone (26)**

<sup>1</sup>H-NMR (500 MHz, CD<sub>3</sub>OD):





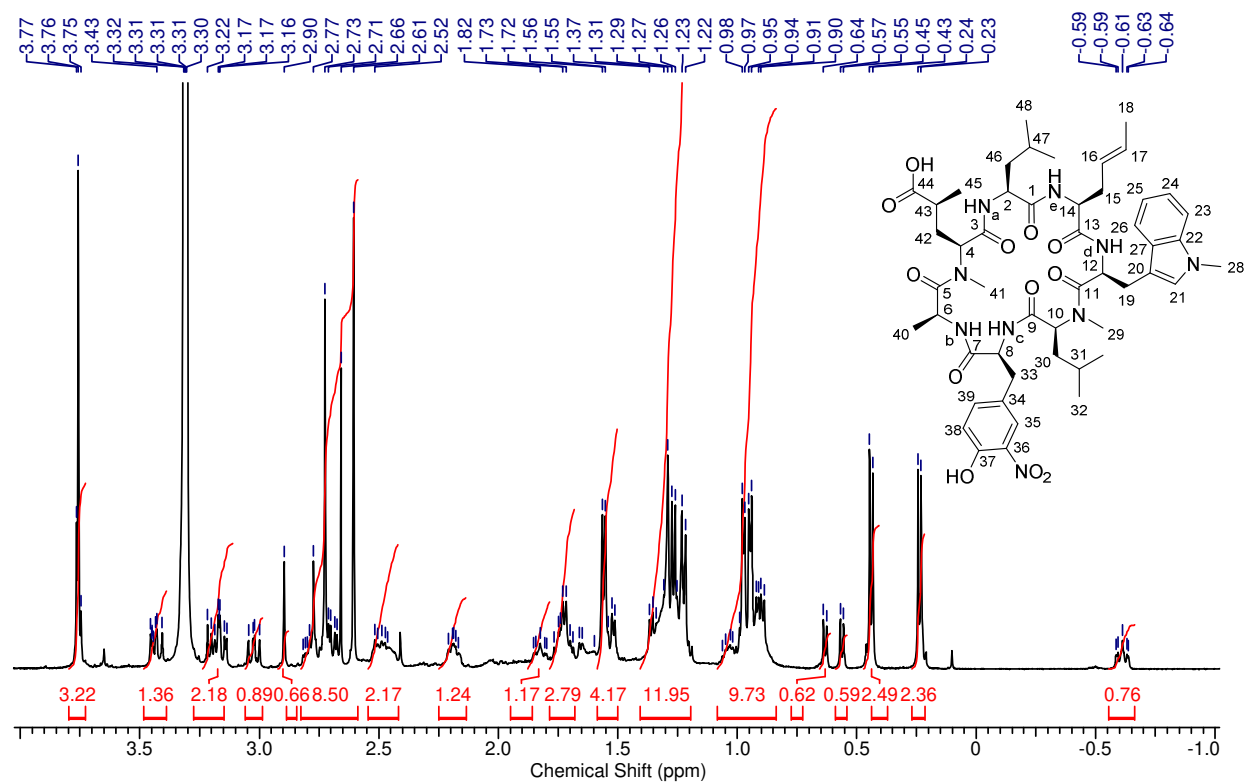
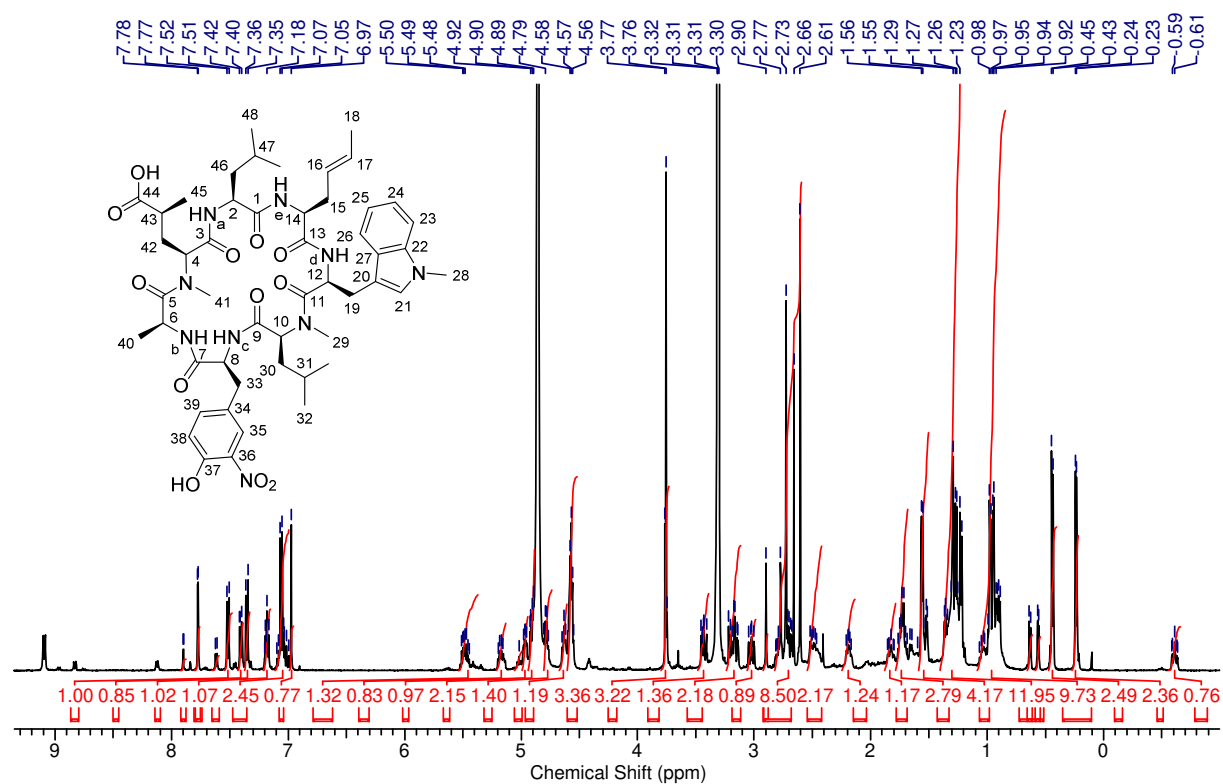
**<sup>13</sup>C-NMR (125 MHz, CD<sub>3</sub>OD):**

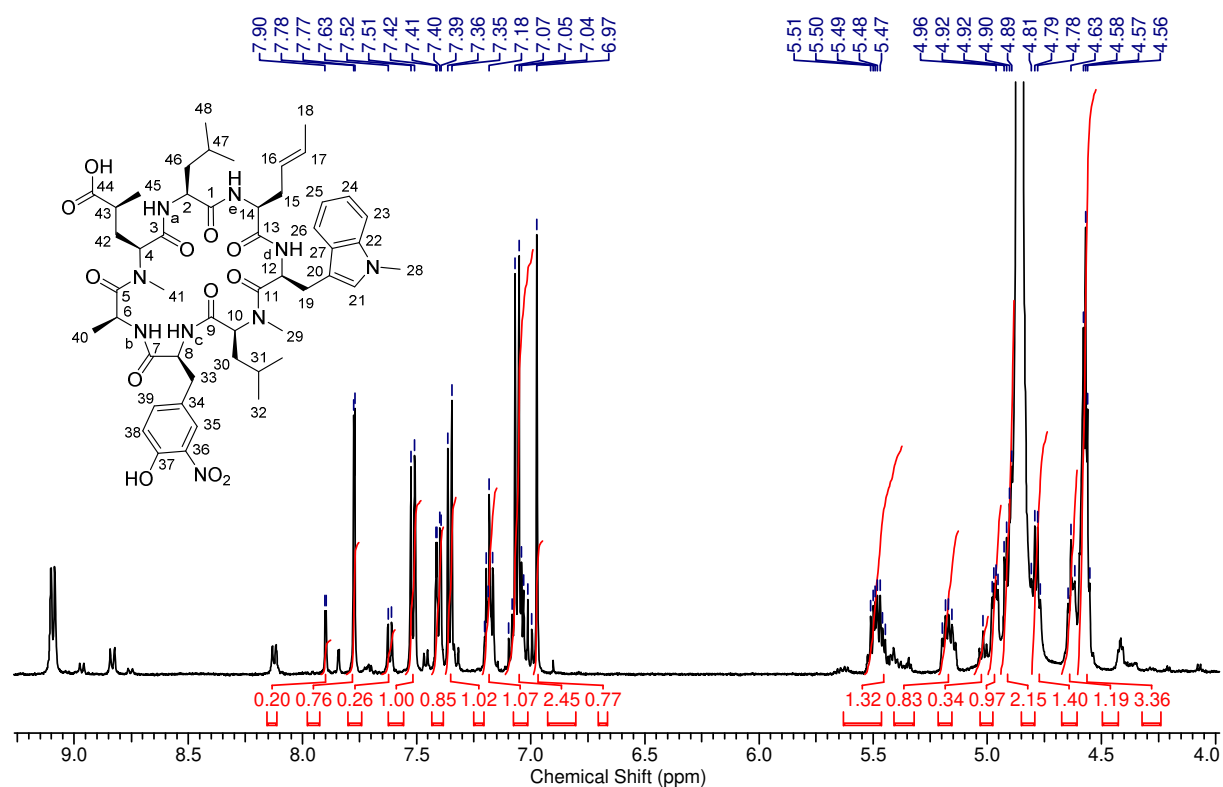




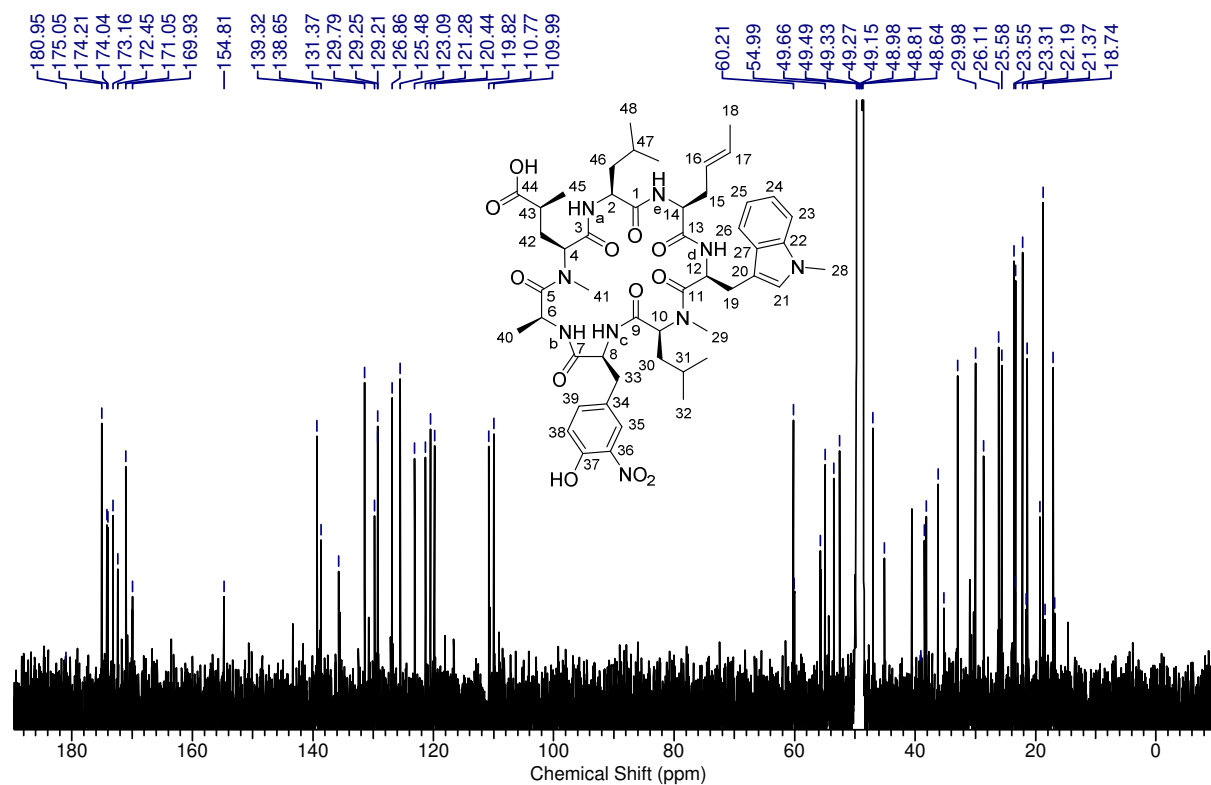
**(S)-3-[(2S,5S,8S,11S,14S,17S,20S)-8-[(E)-But-2-en-1-yl]-17-[4-hydroxy-3-nitro-benzyl]-5,14-diisobutyl-1,13,20-trimethyl-11-[(1-methyl-1H-indol-3-yl)-methyl]-3,6,9,12,15,18,21-heptaazacyclo-henicosan-2-yl]-2-methylpropanoic acid (27)**

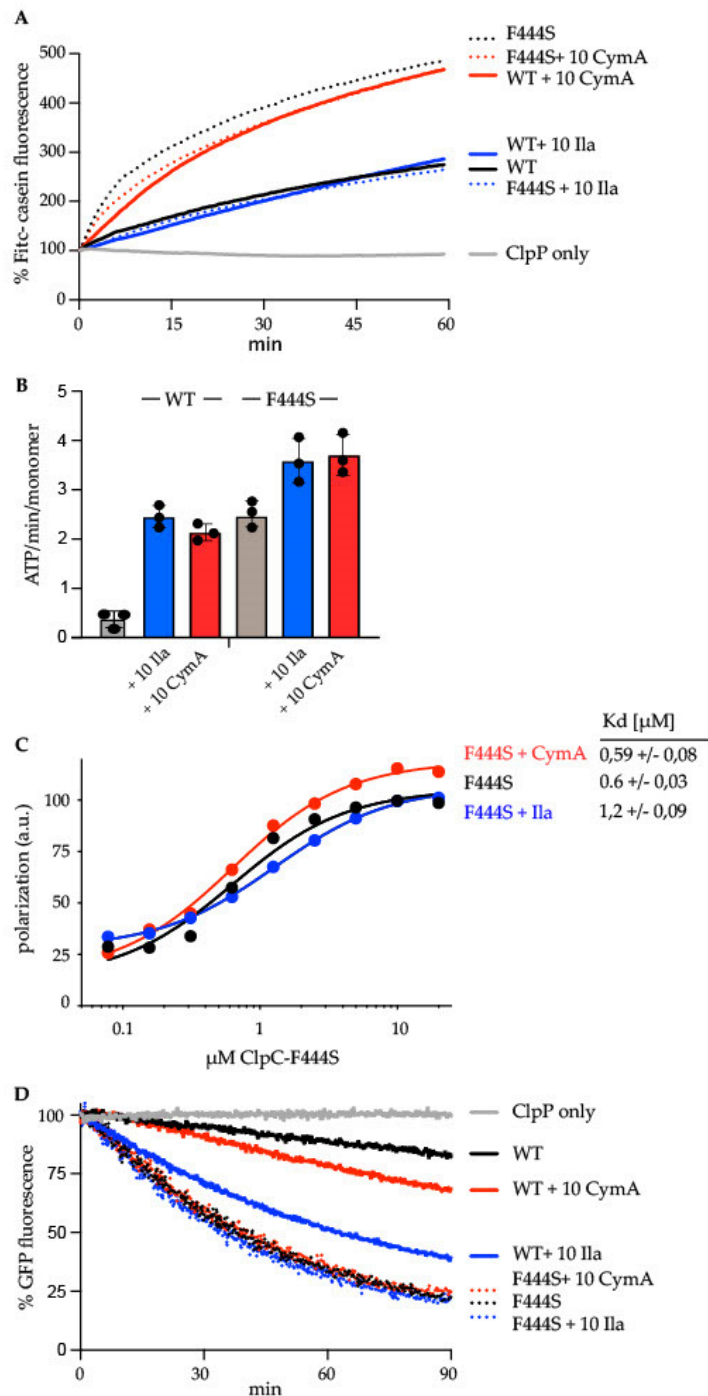
<sup>1</sup>H-NMR (500 MHz, CD<sub>3</sub>OD):





**<sup>13</sup>C-NMR (125 MHz, CD<sub>3</sub>OD):**





### Supplementary Figure S1

Ilamycin (Ila) (**26**) deregulates ClpC1 activities. (A) FITC-casein degradation by ClpC1-WT and ClpC1-F444S was monitored in the presence of ClpP and indicated components (10  $\mu\text{M}$  Ilamycin (Ila) or CyclomarinA (CymA)). Initial FITC-casein fluorescence was set to 100. FITC-casein degradation is linked to increase in fluorescence intensity, which is quenched in full-length casein. (B) ATPase activities of ClpC1-WT and ClpC1-F444S were determined in presence of ClpP and indicated components (as in (A)). (C) Binding of ClpC1-F444S to FITC-casein was monitored in presence of ATP $\gamma$ S and Ilamycin (Ila) or Cyclomarin A (CymA) by determining changes in FITC-casein anisotropy. Calculated binding affinities are provided. (D) GFP-SsrA degradation was monitored by determining the loss of GFP fluorescence in presence of ClpC1-WT or ClpC1-F444S and ClpP in presence of indicated components (as in (A)).