

## Supporting Information

*Revised version, MS# JO 026310C*

### **Design and synthesis of novel fluoropeptidomimetics as potential mimics of the transition state during peptide hydrolysis**

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## Experimental Section.

Procedure to synthesize compounds **15-20** is described below.

***N*-Benzyl-2-bromo-2,2-difluoro-acetamide (15):** A solution of  $\text{BnNH}_2$  (0.05 g, 0.46 mmol) in anhydrous DMF (1.0 mL) was treated with ethyl bromodifluoroacetate (0.059 mL, 0.46 mmol) at 0 °C. The reaction mixture was stirred for 15 min. and was purified as described for **2** to obtain compound **15** as a syrup (0.1 g, 82%):  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  4.52 (d, 1H,  $J = 6.0$  Hz), 6.48 (brs, 1H), 7.26-7.39 (m, 5H); MS-EI  $m/z$  265 (5), 263 (5), 184 (100), 141 (28), 91 (64).

***N*-Benzyl-2-bromo-2,2-difluoro-*N*-methyl acetamide (16):** A solution of *N*-benzylmethyl amine (0.05 g, 0.41 mmol) in anhydrous DMF (1.0 mL) was treated with DMAP (0.05 g, 0.41 mmol) and ethyl bromodifluoroacetate (0.052 mL, 0.41 mmol) at 0 °C and stirred for another 1 h at rt. The reaction mixture was extracted into ethyl acetate and was purified as described for **2** to obtain compound **16** as a syrup (0.021 g, 19%):  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ )  $\delta$  3.10 (s, 3H), 4.65 (s, 2H), 7.20-7.46 (m, 5H).

**Benzoic acid 2-bromo-2-fluoro-ethyl ester (17):** A solution of ethyl bromofluoroacetate (0.1 g, 0.54 mmol) in anhydrous MeOH (1.0 mL) was treated with  $\text{NaBH}_4$  (5 mg, 0.13 mmol) at 0 °C. The reaction mixture was stirred for another 2 h at rt and treated with water. The reaction mixture was extracted into ether, and the organic layer was washed with brine and dried ( $\text{Na}_2\text{SO}_4$ ). Organic layer was concentrated to a volume of 5 mL, and  $\text{Et}_3\text{N}$  (0.075 mL, 0.54 mmol) and  $\text{BzCl}$  (0.062 mL, 0.54 mmol) were added at 0 °C. The reaction mixture was stirred for another 30 min at 0 °C. The reaction mixture was dissolved in ethyl acetate, washed with sat  $\text{NaHCO}_3$  solution, water, brine and dried ( $\text{Na}_2\text{SO}_4$ ). The concentrated organic layer was purified by column chromatography (EtOAc:Hex, 3:97) to obtain compound **17** as an oil (0.12 g, 91%):

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  4.69-4.83 (m, 2H), 6.65 (ddd, 1H,  $J = 3.3, 6.6, 51.1$  Hz), 7.44-7.62 (m, 3H), 8.05-8.09 (m, 2H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  67.08 (d,  $J = 22.5$  Hz), 89.46 (d,  $J = 253.5$  Hz), 128.55, 128.96, 129.90, 133.62, 165.53; MS-EI  $m/z$  248 (6), 246 (6), 122 (75), 105 (100), 77 (51).

**2-(2-Bromo-2-fluoro-ethoxy)-tetrahydro-pyran (18):** A solution of ethyl bromofluoroacetate (0.1 g, 0.54 mmol) in anhydrous MeOH (1.0 mL) was treated with  $\text{NaBH}_4$  (0.005 g, 0.13 mmol) at 0 °C. The reaction mixture was stirred for another 2 h at rt, and worked-up as described for **17**. The resulting organic layer was further treated with catalytic PTSA and DHP (0.04 mL, 0.54 mmol) at 0 °C. The reaction mixture was stirred for 1 h at rt and was purified as described for **17** to obtain compound **18** as an oil (0.044 g, 36%):  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  1.51-1.85 (m, 6H), 3.51-3.58 (m, 1H), 3.81-4.20 (m, 3H), 4.72 (s, 1H), 6.52 (ddd, 1H,  $J = 51.4$  Hz); MS-EI  $m/z$  228 (14), 226 (14), 127 (23), 125 (23), 85 (100), 56 (88).

**Benzoic acid 2-azido-2-fluoro-ethyl ester (19):** A solution of compound **17** (0.02 g, 0.08 mmol) in anhydrous DMSO (1.0 mL) was treated with  $\text{NaN}_3$  (0.026 g, 0.40 mmol) and stirred for 18 h at 50 °C. The reaction mixture was brought to rt, treated with water and extracted into ethyl acetate. The organic layer was washed with brine and dried ( $\text{Na}_2\text{SO}_4$ ). The concentrated organic layer was purified by column chromatography (EtOAc:Hex, 2:98) to obtain compound **19** as a syrup (0.016 g, qua):  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  4.36-4.53 (m, 2H), 5.82 (dt, 1H,  $J = 4.8, 55.8$  Hz), 7.44-7.49 (m, 2H), 7.57-7.62 (m, 1H), 8.05-8.08 (m, 2H); MS-EI  $m/z$  209 (7), 167 (42), 161 (19), 122 (100).

**2-(2-Azido-2-fluoro-ethoxy)-tetrahydro-pyran (20):** A solution of compound **18** (0.4 g, 1.75 mmol) in anhydrous DMSO (2.0 mL) was treated with  $\text{NaN}_3$  (0.45 g, 7.01 mmol) and stirred for 18 h at 50 °C. The reaction mixture was purified as described for **19** to obtain compound **20** as a

syrup (0.198 g, 60%):  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  1.51-1.84 (m, 6H), 3.50-3.69 (m, 2H), 3.75-3.92 (m, 2H), 4.67 (brs, 1H), 5.44-5.50, 5.62-5.69 (2m, 1H).

98-200-7

Pulse Sequence: s2pul

Solvent: CDCl<sub>3</sub>

Temp. 25.0 C / 298.1 K

Mercury-300 "mercury300"

PULSE SEQUENCE

Pulse 45.0 degrees

Acq. time 2.279 sec

Width 2645.5 Hz

16 repetitions

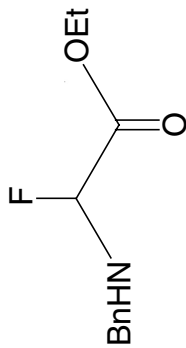
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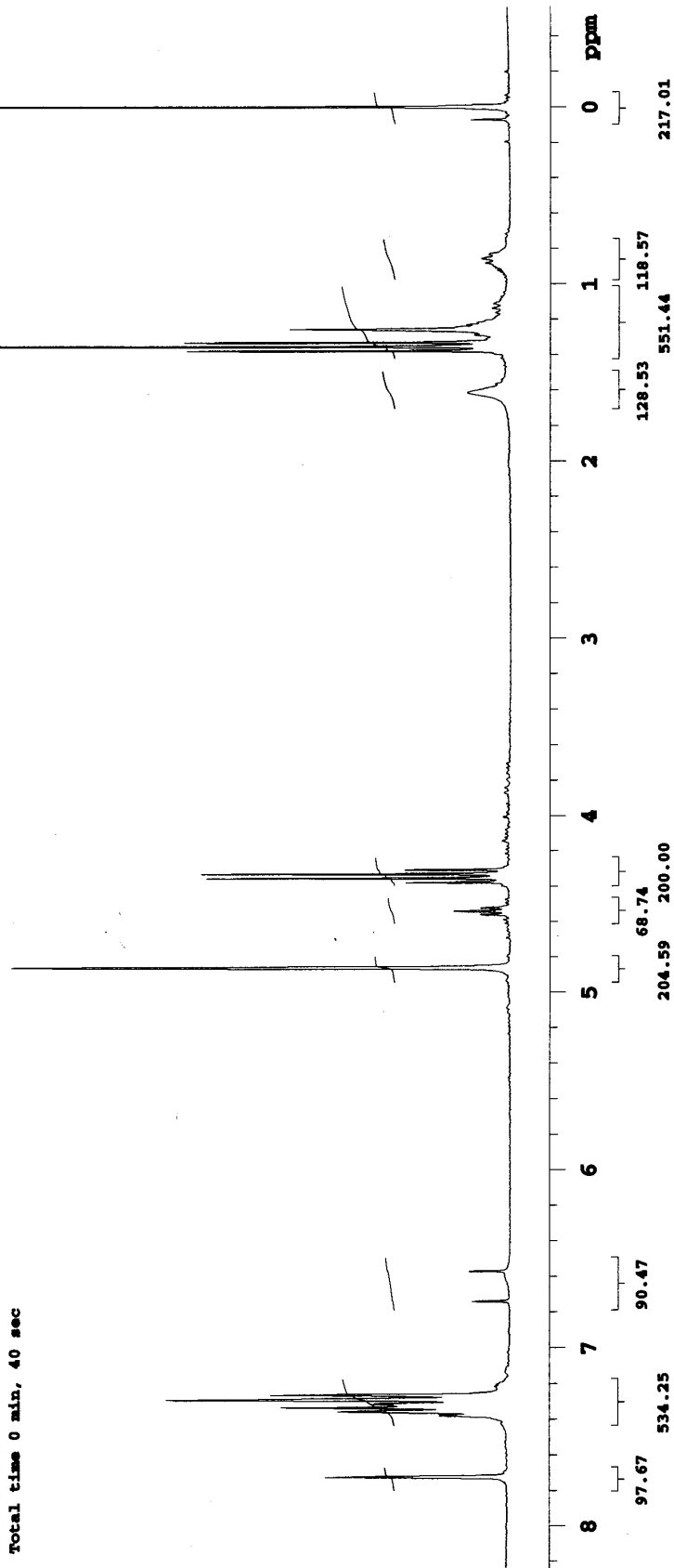
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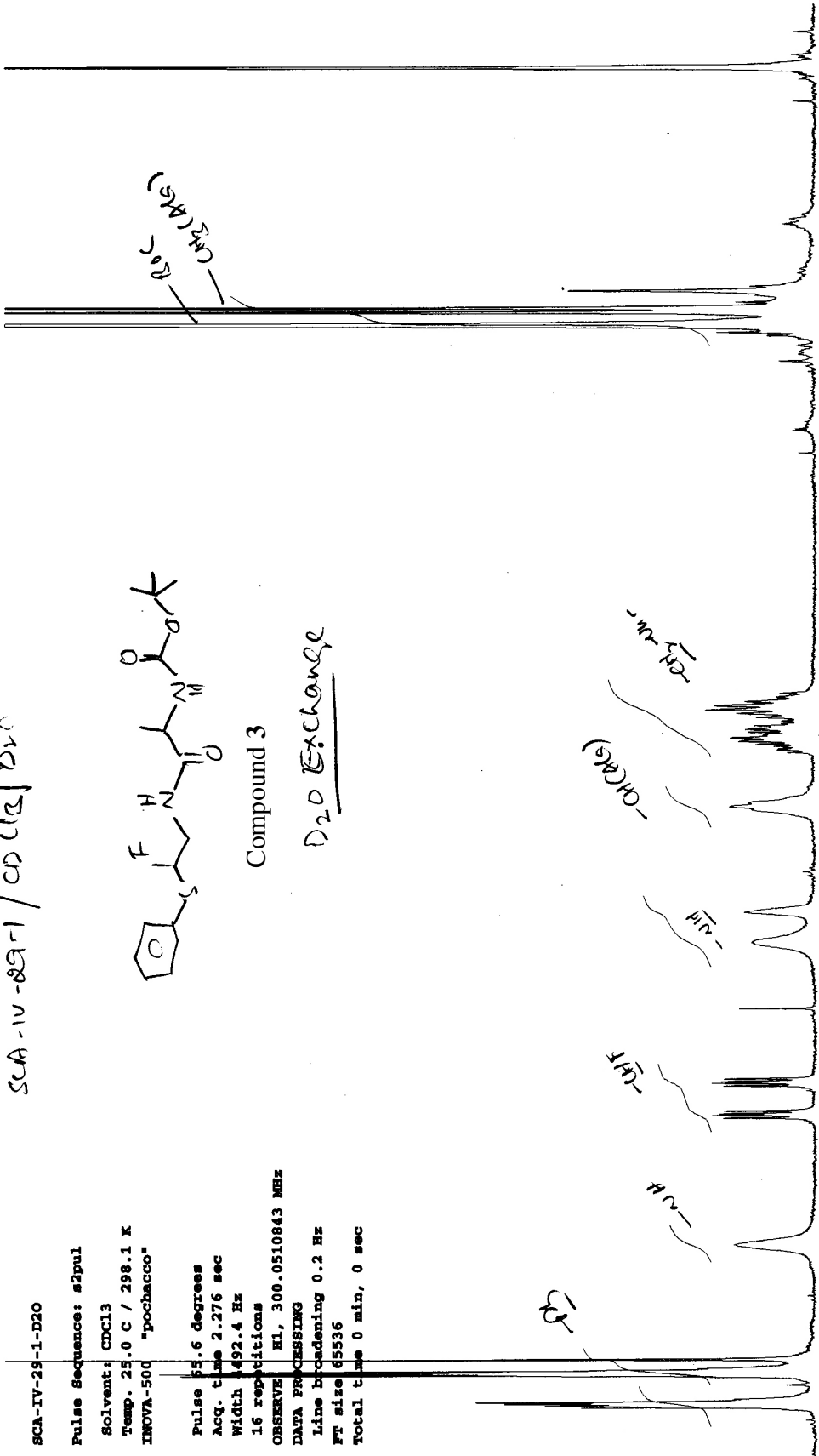


Compound 2



7

SCA-IV-29-1-D2O



SCA-IV-29-1-D2O

Pulse Sequence: s2pul

Solvent: CDCl<sub>3</sub>

Temp. 25.0 C / 298.1 K

INOVA-500 "pochacco"

Pulse 55.6 degrees

Acq. time 2.276 sec

Width 492.4 Hz

16 repetitions

OBSERVE H1, 300.0510843 MHz

DATA PROCESSING

Line broadening 0.2 Hz

FT size 65536

Total time 0 min, 0 sec

Compound 3

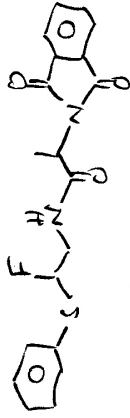
D<sub>2</sub>O Exchange

ppm	1	2	3	4	5	6	7
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24.06							
7.82							
10.00							
12.74							
19.32							
90.17							

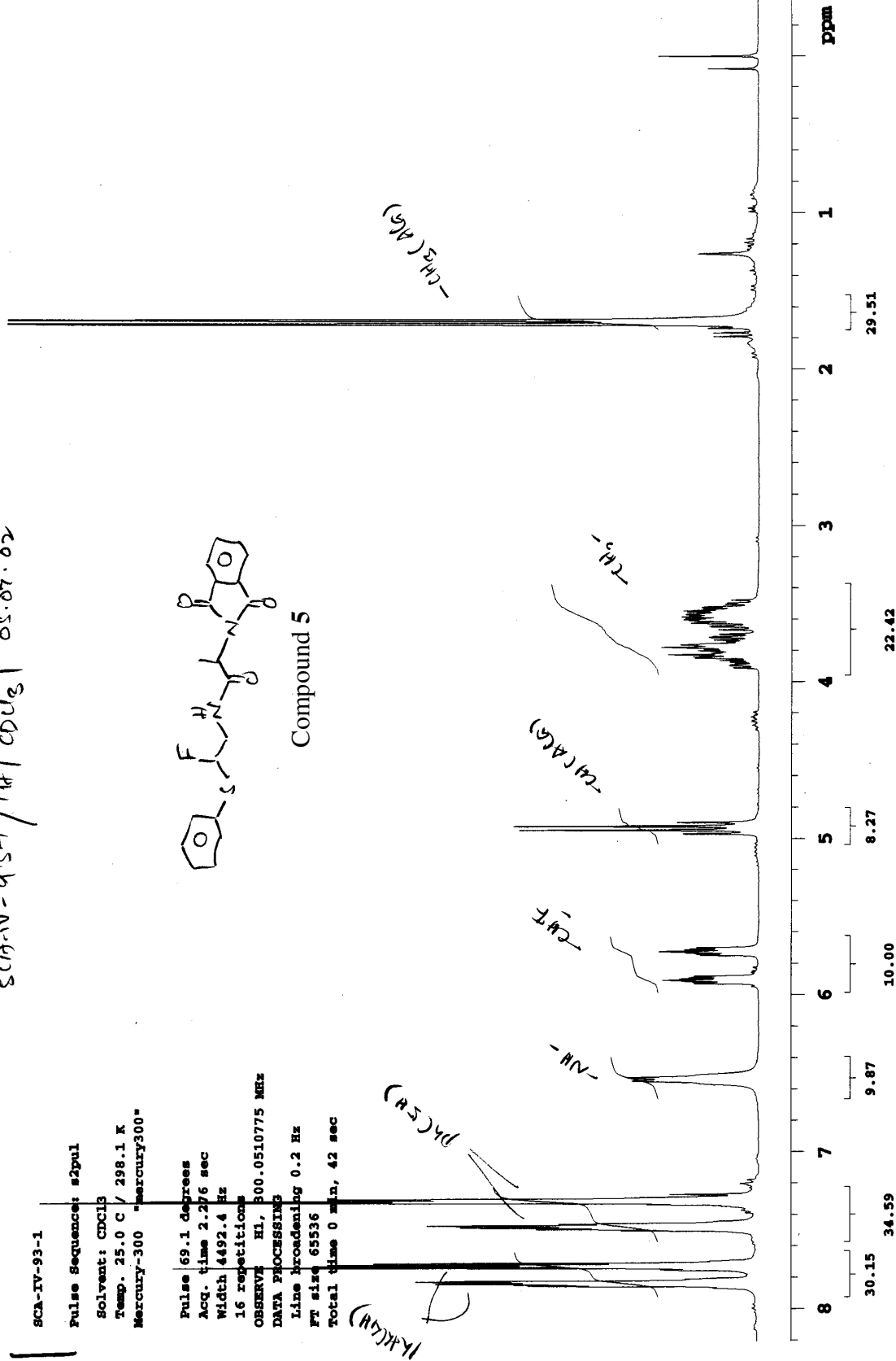


SCA-NV-93-1 / 141 CDUg | 05.07.02

SCA-IV-93-1  
Pulse Sequence: s2pul  
Solvent: CDCl<sub>3</sub>  
Temp. 25.0 C / 298.1 K  
Mercury-300 Mercury300-  
Pulse 69.1 degrees  
Acq. time 2.276 sec  
Width 4492.4 Hz  
16 repetitions  
OBSERVE H1, 300.0510775 MHz  
DATA PROCESSING  
Line broadening 0.2 Hz  
Ft size 65536  
Total time 0 min, 42 sec



Compound 5



SCA-IV-95-1

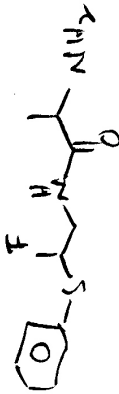
Pulse Sequence: s2pul

Solvent: CDCl<sub>3</sub>

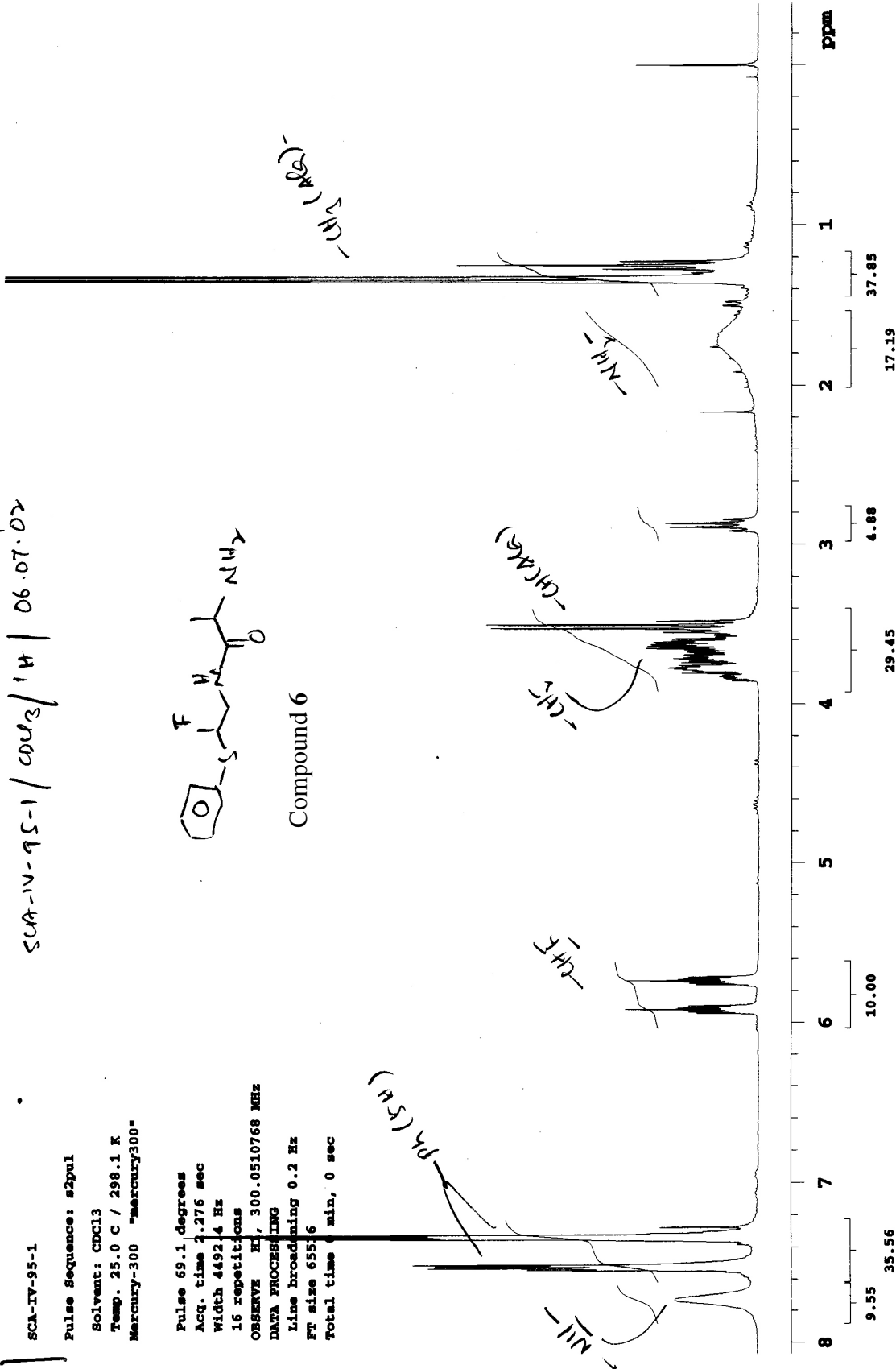
Temp. 25.0 C / 298.1 K

Mercury-300 "mercury300"

Pulse 69.1 degrees  
Acq. time 2.276 sec  
Width 4492.4 Hz  
16 repetitions  
OBSERVE H1, 300.0510768 MHz  
DATA PROCESSING  
Line broadening 0.2 Hz  
FT size 65536  
Total time 6 min, 0 sec



Compound 6



SCA-IV-62-1

Pulse Sequence: s2pul

Solvent: CDCl3

Temp. 25.0 C / 298.1 K

File: SCA-IV-62-1

INOVA-500 "pochacco"

Pulse 69.1 degrees

Acq. time 2.276 sec

Width 4492.4 Hz

16 repetitions

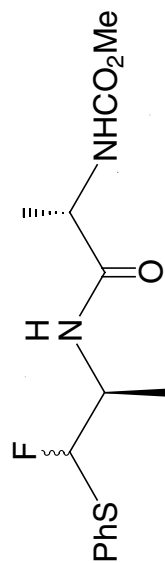
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DATA PROCESSING

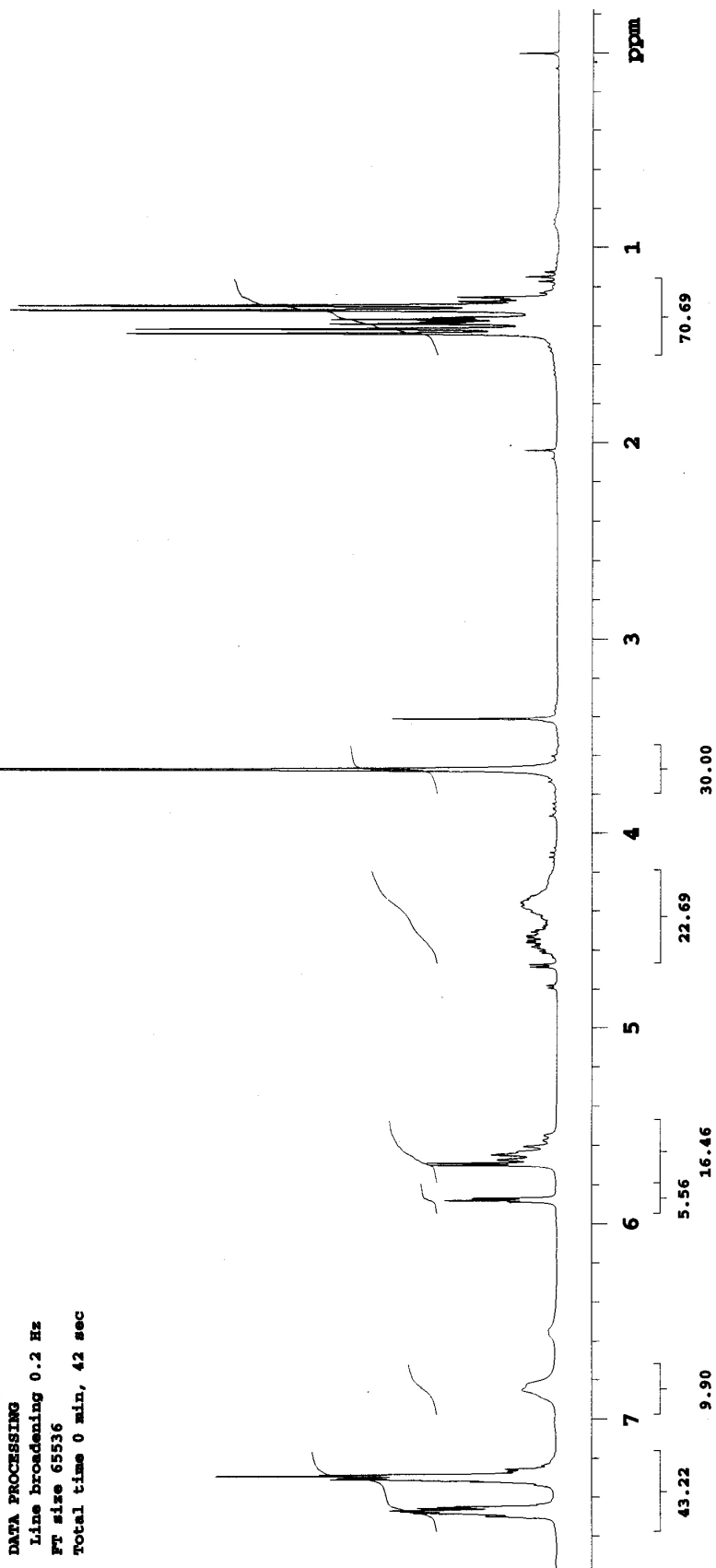
Line broadening 0.2 Hz

FT size 65536

Total time 0 min, 42 sec



Compound 7



SCA-IV-50-1f

Pulse Sequence: s2pul

Solvent: CDCl3

Temp. 25.0 C / 298.1 K

File: SCA-IV-50-1f

INOVA-500 "pochacco"

Pulse 69.1 degrees

Acq. time 2.276 sec

Width 4497.4 Hz

16 repetitions

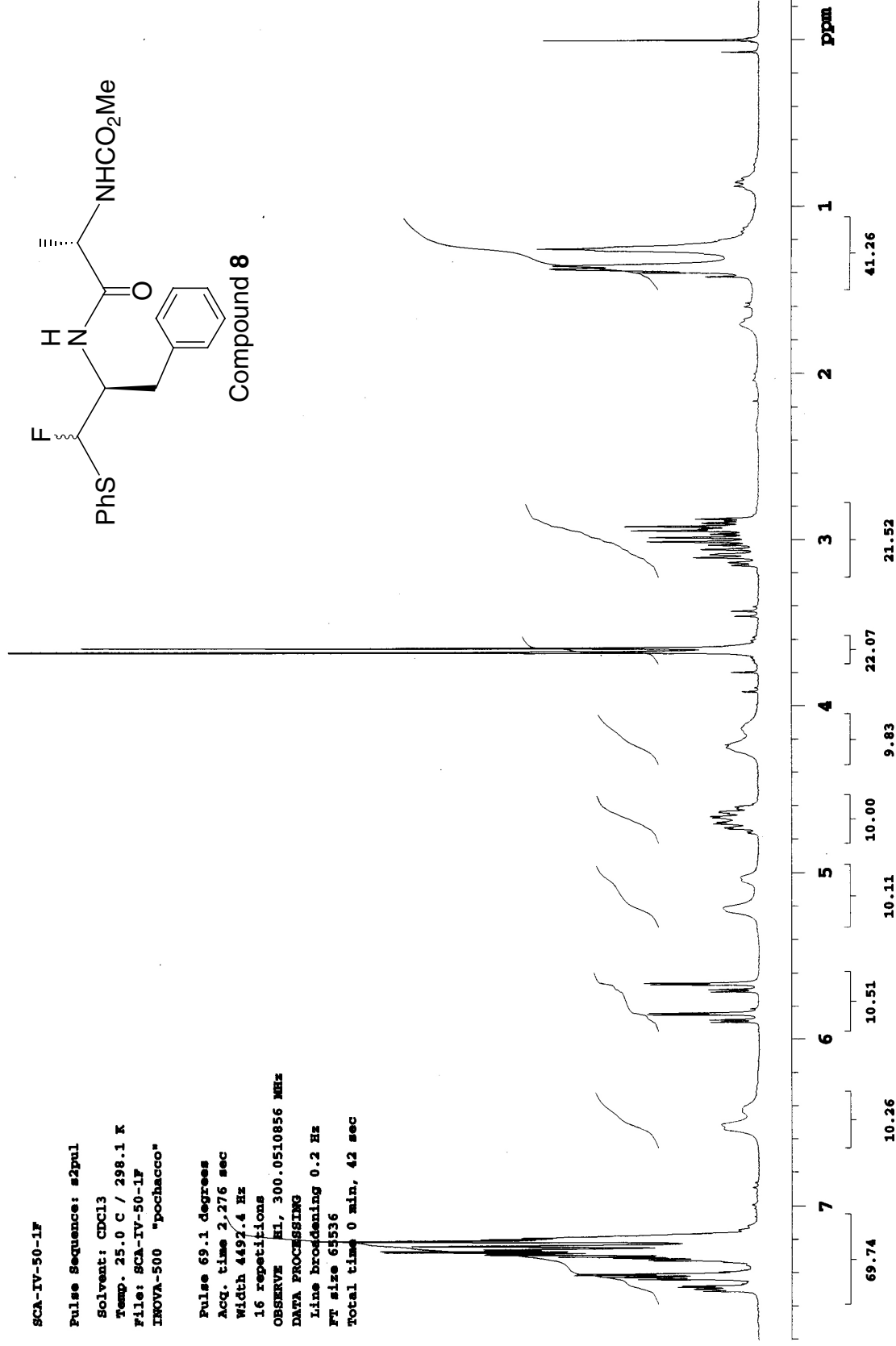
OBSERVE M1, 300.0510856 MHz

DATA PROCESSING

Line broadening 0.2 Hz

FT size 65536

Total time 0 min, 42 sec



SCA-IV-61-1

Pulse Sequence: s2pul

Solvent: CDCl3

Temp. 25.0 C / 298.1 K

File: SCA-IV-61-1

INOVA-500 "pochacco"

Pulse 69.1 degrees

Acq. time 2.276 sec

Width 4492.4 Hz

16 repetitions

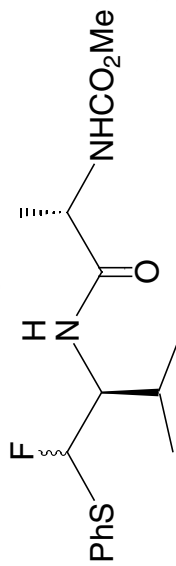
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DATA PROCESSING

Line broadening 0.2 Hz

FT size 65536

Total time 0 min, 42 sec



Compound 9

