

Supporting Information

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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 BnNH₂ (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%): ¹H NMR (300 MHz, CDCl₃) δ 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%): ¹H NMR (200 MHz, CDCl₃) δ 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 NaBH₄ (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 (Na₂SO₄). Organic layer was concentrated to a volume of 5 mL, and Et₃N (0.075 mL, 0.54 mmol) and 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 NaHCO₃ solution, water, brine and dried (Na₂SO₄). The concentrated organic layer was purified by column chromatography (EtOAc:Hex, 3:97) to obtain compound **17** as an oil (0.12 g, 91%):

^1H NMR (300 MHz, CDCl_3) δ 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}C NMR (75 MHz, CDCl_3) δ 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 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%): ^1H NMR (300 MHz, CDCl_3) δ 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 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 (Na_2SO_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): ^1H NMR (300 MHz, CDCl_3) δ 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 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%): ^1H NMR (300 MHz, CDCl_3) δ 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: CDCl3

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

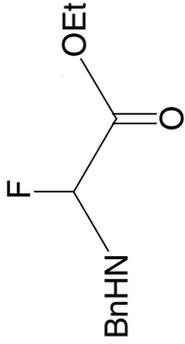
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DATA PROCESSING

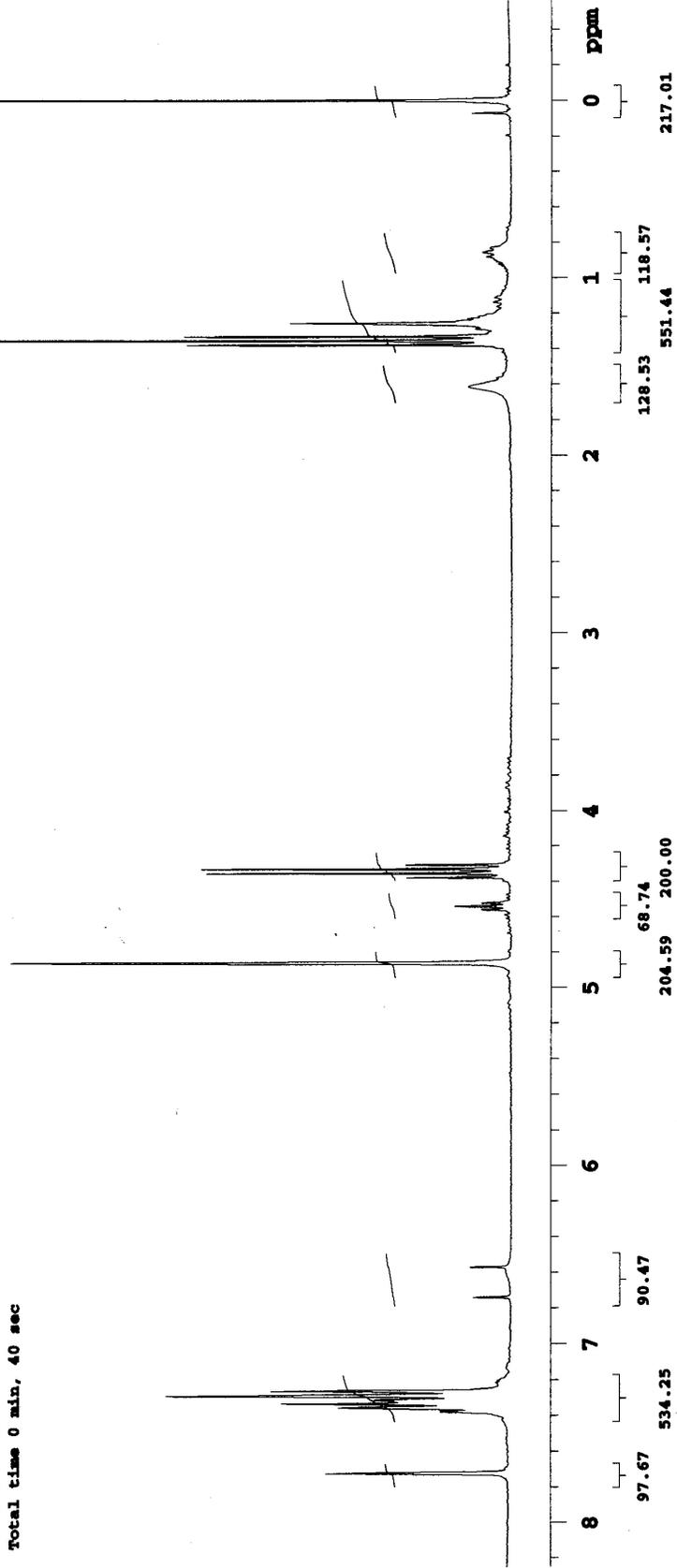
Line broadening 0.2 Hz

FT size 65536

Total time 0 min, 40 sec



Compound 2



SCA-IV-29-1-D2O

SCA-IV-29-1-D2O

Pulse Sequence: s2pul

Solvent: CDCl3

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

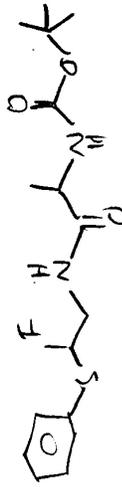
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DATA PROCESSING

Line broadening 0.2 Hz

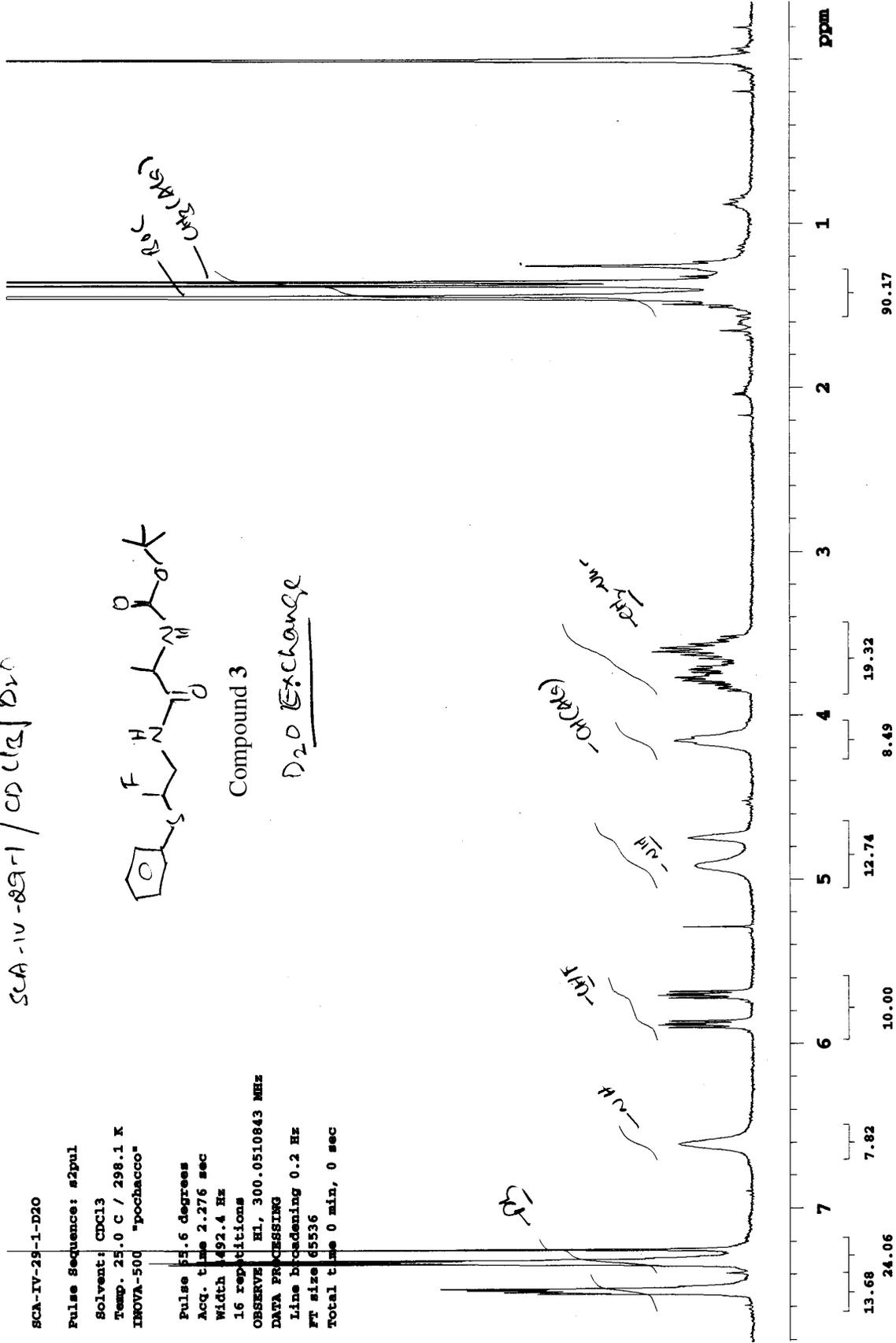
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Total time 0 min, 0 sec



Compound 3

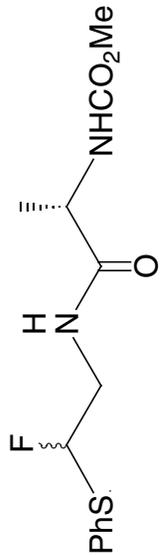
D₂O Exchange



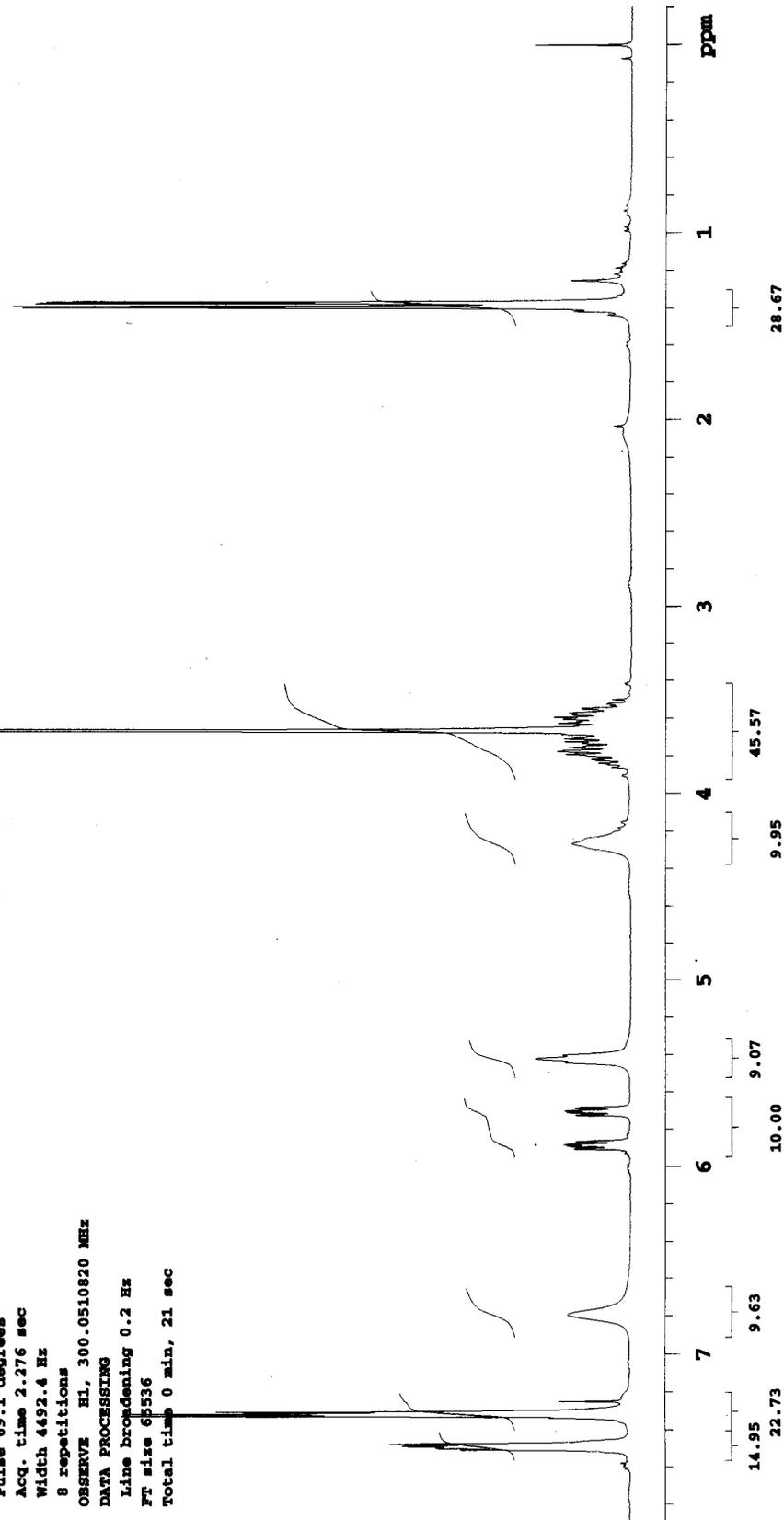
SCA-IV-86-1

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
File: SCA-IV-86-1
INOVA-500 "pochacco"

Pulse 69.1 degrees
Acq. time 2.276 sec
Width 4492.4 Hz
8 repetitions
OBSERVE F1, 300.0510820 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 65536
Total time 0 min, 21 sec



Compound 4



SCAN-93-1 / 1H CDUG | 05.07.02

SCA-IV-93-1

Pulse Sequence: s2pul

Solvent: CDCl3

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

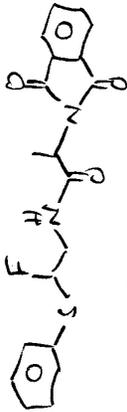
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DATA PROCESSING

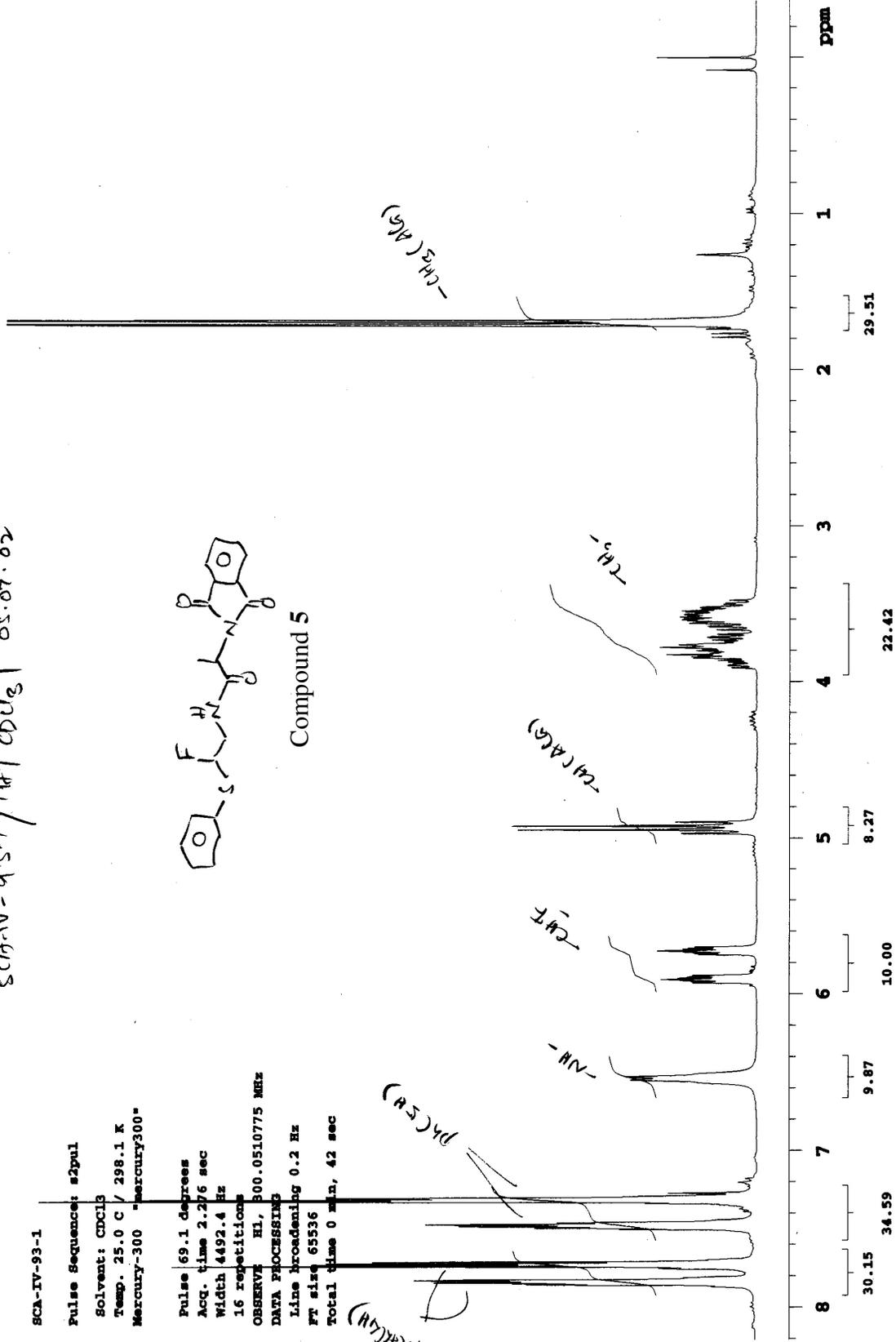
Line broadening 0.2 Hz

FT size 65536

Total time 0 min, 42 sec



Compound 5



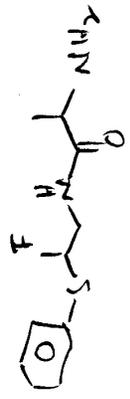
SCA-IV-95-1 / CDCl₃ / 1H / 06.07.02

SCA-IV-95-1

Pulse Sequence: s2pul

Solvent: CDCl₃
Temp. 25.0 C / 298.1 K
Mercury-300 "mercury300"

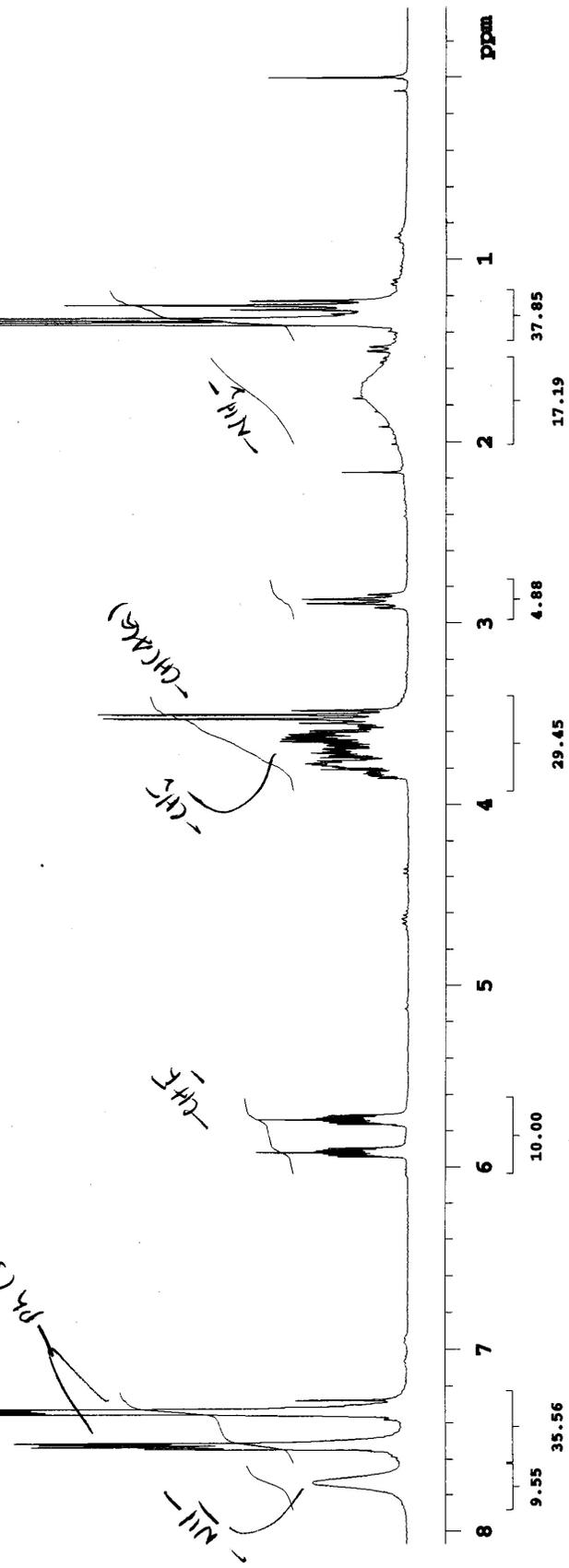
Pulse 69.1 degrees
Acq. time 2.276 sec
Width 4492.4 Hz
16 repetitions
OBSERVE H1, 300.0510768 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 65516
Total time 6 min, 0 sec



Compound 6

(CH₃)₂C-

(H₂)₂C



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

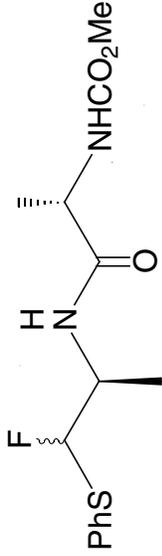
OBSERVE EL, 300.0510809 MHz

DATA PROCESSING

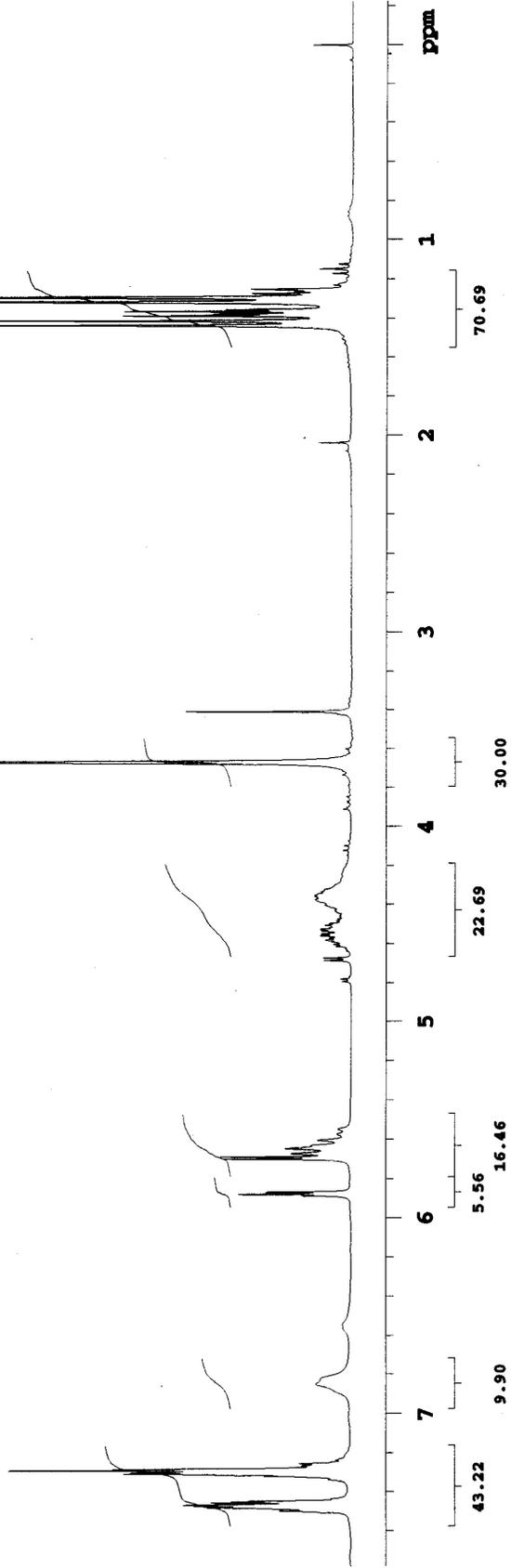
Line broadening 0.2 Hz

Ft size 65536

Total time 0 min, 42 sec



Compound 7



SCA-IV-50-1f

Pulse Sequence: #2pul

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

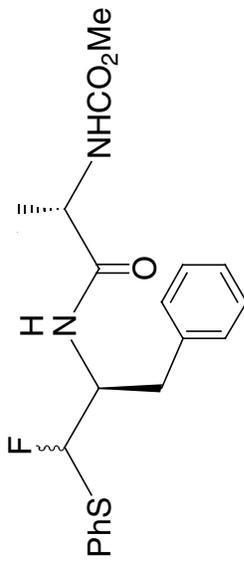
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DATA PROCESSING

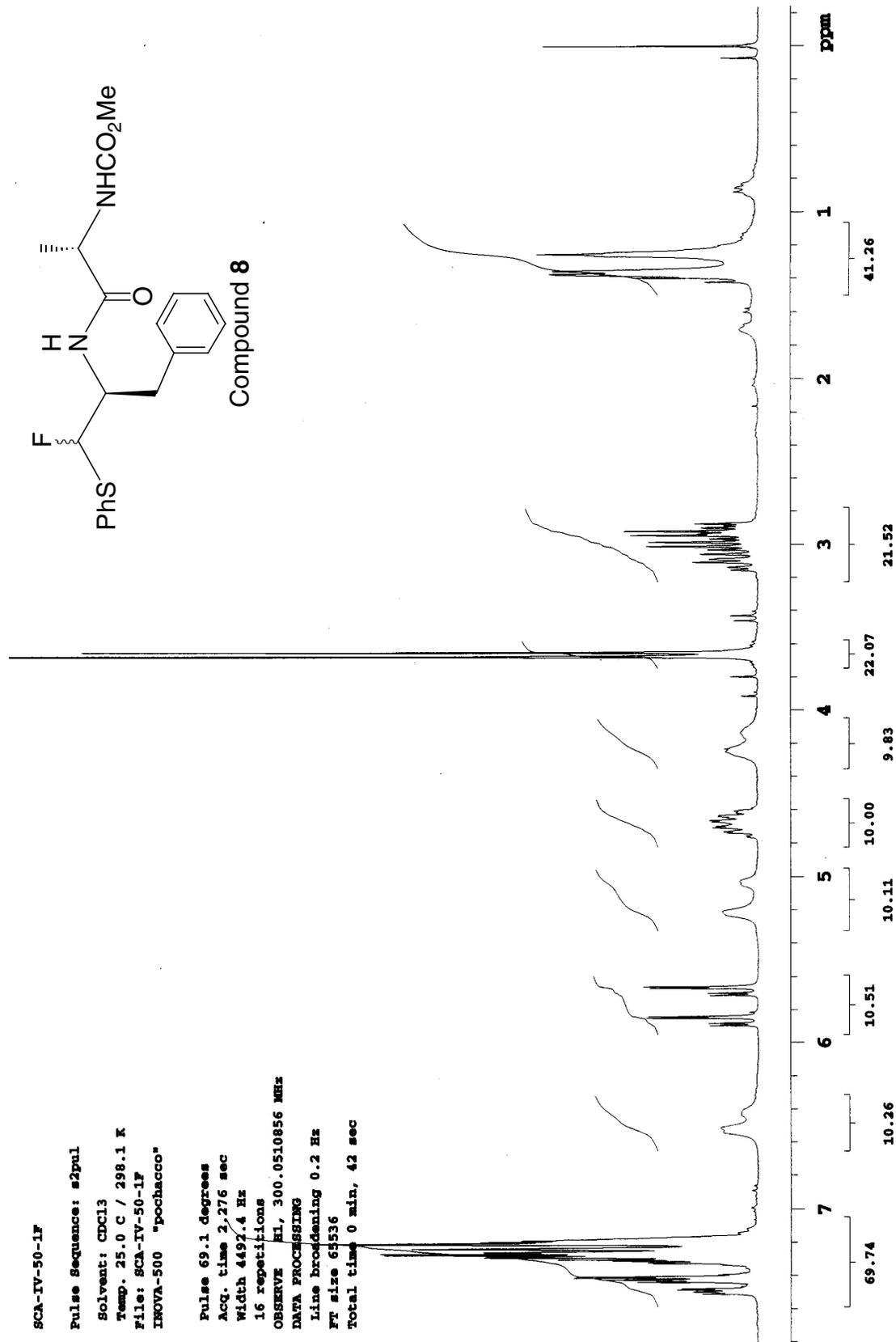
Line broadening 0.2 Hz

FT size 65836

Total time 0 min, 42 sec



Compound 8



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 492.4 Hz

16 repetitions

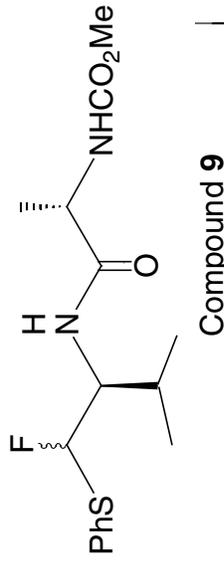
OBSERVE H1, 300.0510813 MHz

DATA PROCESSING

Line broadening 0.2 Hz

FT size 65536

Total time 0 min, 42 sec



Compound 9

