The synthesis of a geminally perfluoro-*tert*-butylated β-amino acid and its protected forms as potential pharmacokinetic modulator and reporter for peptide-based pharmaceuticals

Zhong-Xing Jiang, Y. Bruce Yu

Department of Pharmaceutics and Pharmaceutical Chemistry, College of Pharmacy, University of Utah, Salt Lake City, UT 84112, U. S. A.

bruce.yu@utah.edu

SUPPORTING INFORMATION

General Procedure

RP-HPLC runs for compounds **4**, **5** and their co-injection were carried out on a Kromasil C18 column (150 × 2.1 mm I.D.; 5-μm particle size; 100-Å pore size) where eluent A was 0.2% aqueous TFA and eluent B was 0.2% TFA in acetonitrile at a flow rate of 1 mL/min and a gradient of 2% B/min, starting from 0% B.

Synthetic Procedures:

Compound 7. Pentaerythritol (136.2 g, 1.0 mol) was dissolved in water (1000 mL) with heating. After the hot solution was cooled to room temperature, stirring was started and concentrated hydrochloric acid (5 mL) was added, followed by *p*-anisaldehyde (13.5 mL, 111.2 mmol). When the precipitate of mono-*p*-methoxylbenzilidene-pentaerythritol started forming, dropwise addition of *p*-anisaldehyde (115.0 mL, 947.1 mmol) was begun. After the addition of *p*-anisaldehyde was completed (2 h), the mixture was stirred for additional 2 h. The precipitate was collected and washed with diluted sodium carbonate aqueous solution and ethyl ether. The solid was dried over phosphorus pentoxide to give **7** as a white solid (222.7 g, 87%). ^{1}H NMR (400 MHz, acetone-d_6) δ 3.46 (s, 2H), 3.79 (s, 4H), 3.84 (s, 2H), 3.92 (s, 3H), 5.36 (s, 1H), 6.89-6.91 (m, 2H), 7.37-7.39 (m, 2H).

Compound 9. To a stirred mixture of compound **8** (33.1 g, 48.0 mmol) and anisole (20.7 g, 192.0 mmol) in dichloromethane (200 mL) at 0 ^{o}C was slowly added powdered aluminum chloride anhydrous (19.5 g, 144.0 mmol). After the addition, the reaction mixture was allowed to warm to room temperature and stirred for additional 30 min. The reaction mixture was quenched by slow addition of 1N HCl (100 mL) and the resulted

mixture was extracted with ether (100 mL, 3 times). The combined organic layer was concentrated in vacuo and the residue was purified by flash column chromatography on silica gel (*n*-hexane/ethyl acetate = 8/1) to give compound **9** as a clear oil (27.4 g, 99%). ^{1}H NMR (400 MHz, CD_3OD) δ 3.63 (s, 4H), 4.14 (s, 4H); ^{19}F NMR (376 MHz, CD_3OD) δ -71.14 (s); ^{13}C NMR (100.7 MHz, CD_3OD) δ 47.8, 60.4, 69.1, 80.9 (m), 121.8 (q, J = 292.6 Hz); MS (CI) *m/z* 573 (M^++1, 100); HRMS (CI) Calcd for $C_{13}H_{11}F_{18}O_4$: 573.0374, Found: 573.0374.

Compound 10. To a stirred solution of compound **9** (22.9 g, 40.0 mmol) and triethyl amine (16.2 g, 160.0 mmol) in dichloromethane (100 mL) and ethyl ether (100 mL) at room temperature was added dropwise a solution of thionyl chloride (9.52 g, 80.0 mmol, in 10 mL of dichloromethane). The resulting mixture was stirred for an additional 20 min and quenched with cold water (100 mL). The organic and aqueous phases were separated and the aqueous phase was extracted with ether (100 mL, three times). The combined organic phase was dried over magnesium sulfate and concentrated in vacuo. The residue was purified by column chromatography on silica gel to give the cyclic sulfite intermediate (24.7 g). The cyclic sulfite (24.7 g, 40 mmol) was then dissolved with stirring in acetonitrile (50 mL), carbon tetrachloride (50 mL) and water (75 mL) at 0 ^{o}C. Ruthenium trichloride (500 mg) was added to the mixture, followed by sodium periodate (17.1 g, 80.0 mmol). The reaction mixture was then stirred at room for 1 h. The reaction was quenched with ether (200 mL) and saturated sodium bicarbonate solution (100 mL). The organic and aqueous phases were separated and the aqueous phase was extracted with ether (100 mL, three times). The combined organic phase was dried over

magnesium sulfate and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (*n*-hexane/ethyl acetate = 10/1) to give the cyclic sulfate **10** as a solid (21.2 g, 84%). mp. 90-91 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.24 (s, 4H), 4.63 (s, 4H); ^{19}F NMR (376 MHz, $CDCl_3$) δ -72.91 (s); ^{13}C NMR (100.7 MHz, $CDCl_3$) δ 39.8, 64.9, 73.0, 80.1 (m), 120.2 (q, *J* = 292.6 Hz); MS (CI) *m/z* 635 (M^{+}+1, 100); HRMS (CI) Calcd for $C_{13}H_9F_{18}O_6S$: 634.9832, Found: 634.9843.

Compound 12. To a stirred solution of alcohol **11** (19.0 g, 31.8 mmol) at 0 ^{o}C was added dropwise a solution of Jones reagent (2.7 N, 31 mL). After addition, the reaction mixture was stirred at room temperature for 1 h and 2-propanol (10 mL) was slowly added. The solvent was removed under vacuo and the residue was dissolved in ether. The ether solution was washed with water, the organic and the aqueous phases were separated and the aqueous phase was extracted with ether. The combined organic phase was dried over magnesium sulfate and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (*n*-hexane/ethyl acetate = 1/1) to give the acid **12** as a solid (18.5 g, 95%). mp. 97-98 ^{o}C; ^{1}H NMR (400 MHz, CD_3OD) δ 3.62 (s, 2H), 4.25 (d, *J* = 8.8 Hz, 2H), 4.33 (d, *J* = 8.4 Hz, 2H); ^{19}F NMR (376 MHz, CD_3OD) δ -71.16 (s); ^{13}C NMR (100.7 MHz, CD_3OD) δ 50.9, 53.6, 67.5, 81.2 (m), 121.6 (q, *J* = 292.6 Hz), 171.9; MS (CI) *m/z* 612 (M^{+}+1, 100), 584 (80); HRMS (CI) Calcd for $C_{13}H_8F_{18}N_3O_4$: 612.0227, Found: 612.0204.

Formyl-Gly-βFa-Gly-amide 4. The peptide was synthesized using standard Fmoc chemistry[i] on Rink Amide MBHA resin (which gives *C*-terminal amide). TBTU and

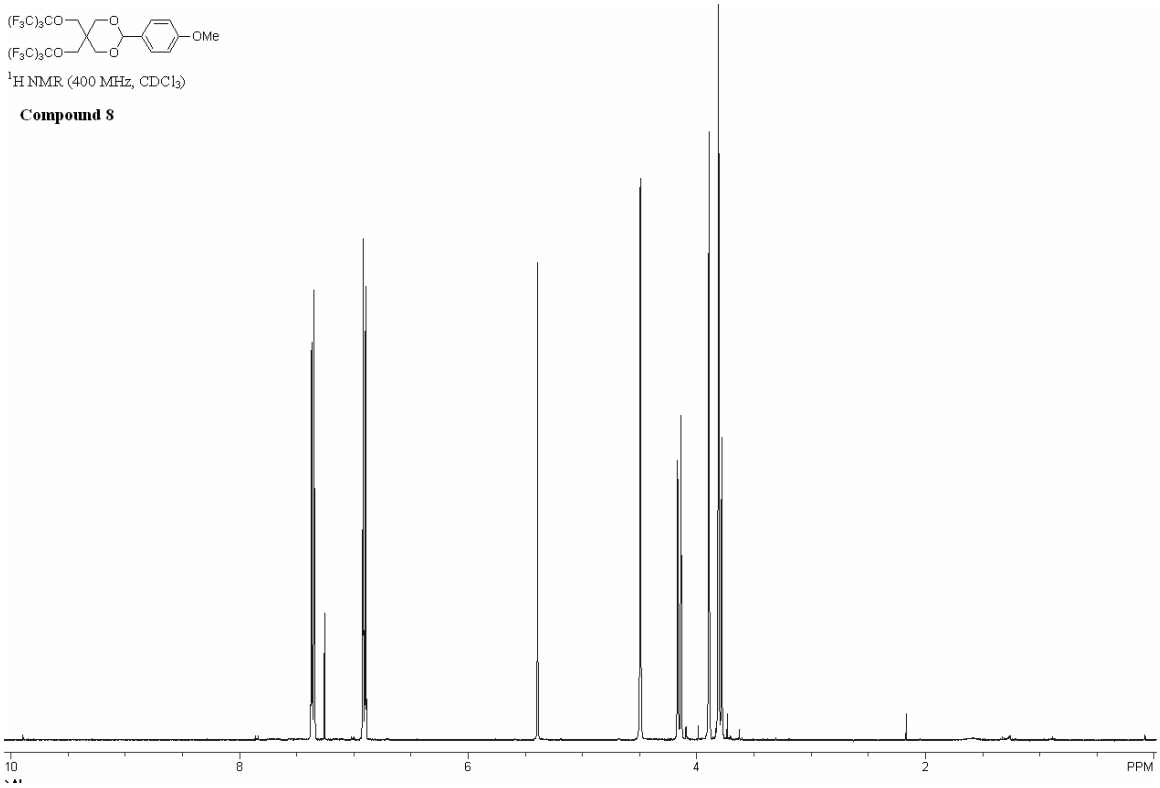
HOBt were employed as coupling reagents. Coupling time for each residue is 1 h with 5 fold of Fmoc-amino acid except for βFa (which is 8 h with 2 fold of βFa). The tripeptide was cleaved off the resin using 90% trifluoroacetic acid with 2.5% each of *tri*-isopropyl silane, water, anisole and dichloromethane. The cleavage product was concentrated under vacuo and purified using normal-phase chromatography using a TSK-GEL Amide-80 column (300 × 21.5 mm I.D.; 10-μm particle size) with methanol and acetonitrile as the eluents (2% MeOH/min, starting from 0% MeOH, at a flow rate of 5 mL/min, at 25 ^{o}C). ^{1}H NMR (400 MHz, CD_3OD) δ 3.50 (s, 2H), 3.80 (s, 2H), 3.81 (s, 2H), 4.19 (d, J = 9.2 Hz, 2H), 4.29 (d, J = 9.2 Hz, 2H), 8.03 (s, 1H); ^{19}F NMR (376 MHz, CD_3OD) δ -70.97 (s); MS (MALDI-TOF) *m/z* 749 (M^++Na).

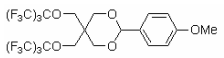
Formyl-Gly-Trp-Gly-amide 5. The peptide was synthesized and cleaved by the same method as for compound **4.** The cleavage product was concentrated under vacuo, precipitated out with cold ether and the precipitate was purified by reversed-phase chromatography using a Zorbax 300SB-C18 column (250 × 21.2 mm I.D.; 7-μm particle size) with water and acetonitrile as the eluents at pH 2 (2% acetonitrile/min, starting from 0% acetonitrile , at a flow rate of 5 mL/min, at 25 ^{o}C). ^{1}H NMR (400 MHz, CD_3OD) δ 3.54-3.84 (m, 4H), 4.46-4.50 (m, 1H), 6.91-7.03 (m, 3H), 7.23 (d, J = 8.4 Hz, 1H), 7.48 (d, J = 7.6 Hz, 1H), 7.99 (s, 1H); MS (MALDI-TOF) *m/z* 368 (M^++Na).

Determination of 1-octanol/water partition coefficients of tripeptides 4 and 5. To 20 mg of tripeptide **4** or **5** were added 2 mL of water and 2mL of 1-octanol. The resulting mixture was shaken with a mixer for 10 min. After the organic and the aqueous phases

were fully separated, 50 μL of solution was taken out from each phase for HPLC analysis. Formyl-Gly-βFa-Gly-amide **4** can only be detected in the 1-octanol phase. Formyl-Gly-Trp-Gly-amide **5** can be detected in both the 1-octanol phase and the aqueous phase and the partition coefficient P_{oct} is 1/9.5 (1-octanol/water), based on the ratio of the areas of HPLC peaks monitored at 280 nm. Solutions of the tripeptide formyl-Gly-βFa-Gly-amide **4** were also taken for ^{19}F NMR analysis and ^{19}F NMR signal was detected only in the 1-octanol phase.

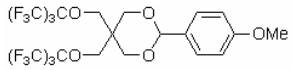
P_{oct} for **4** and **5** were also determined at a concentration 10 times lower than the above, using similar procedure. Partition profiles of **4** and **5** at these two different concentrations are comparable, according to HPLC analysis.





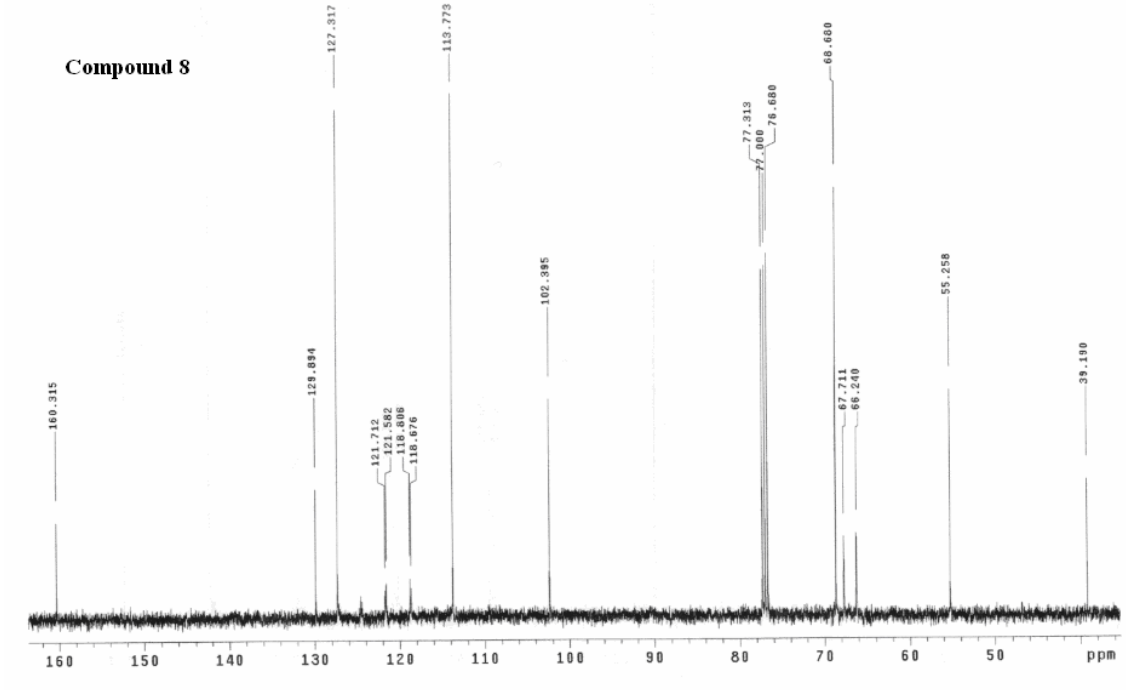
^{19}F NMR (376 MHz, $CDCl_3$)

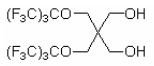
Compound 8



^{13}C NMR (100.7 MHz, $CDCl_3$)

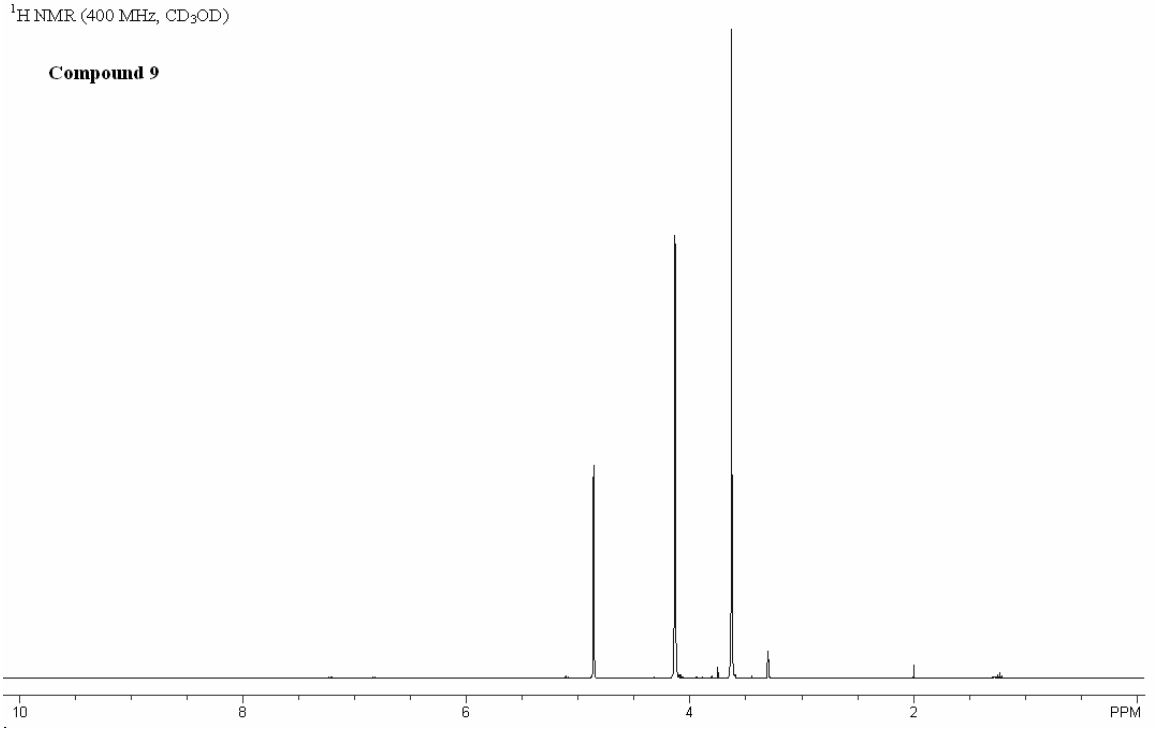
Compound 8

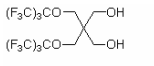




^{1}H NMR (400 MHz, CD_3OD)

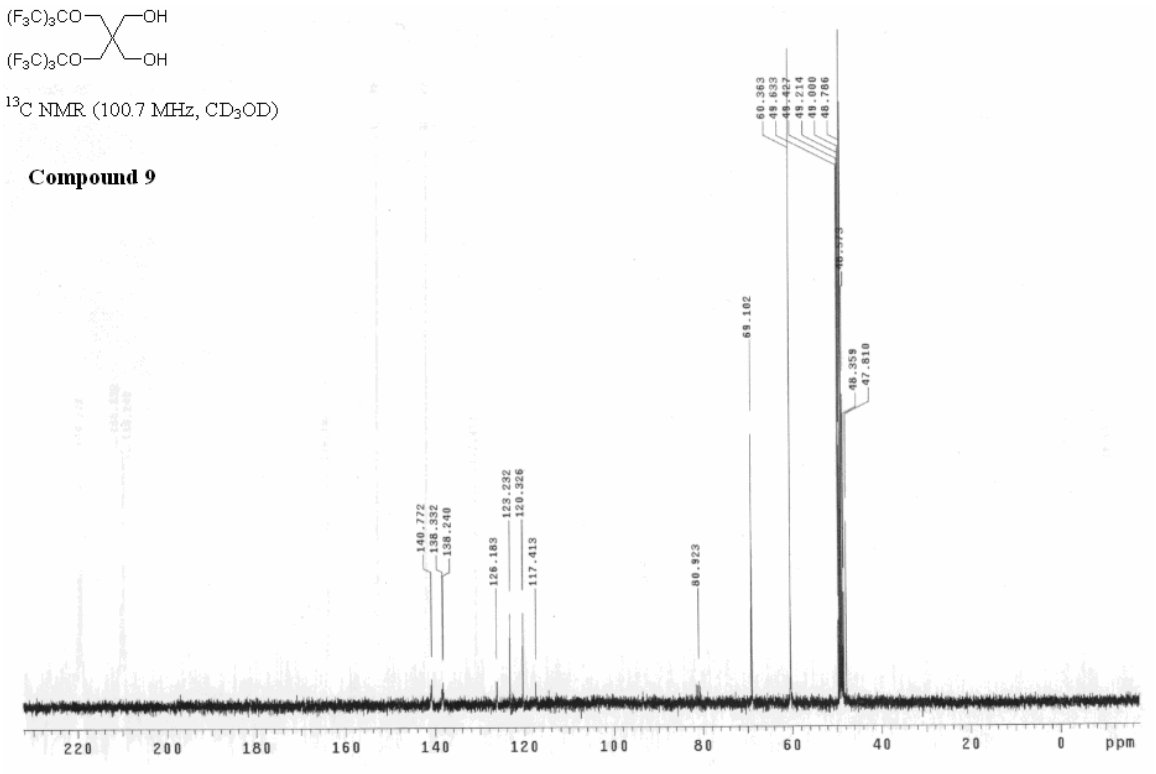
Compound 9





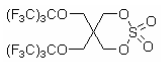
^{19}F NMR (376 MHz, CD_3OD)

Compound 9



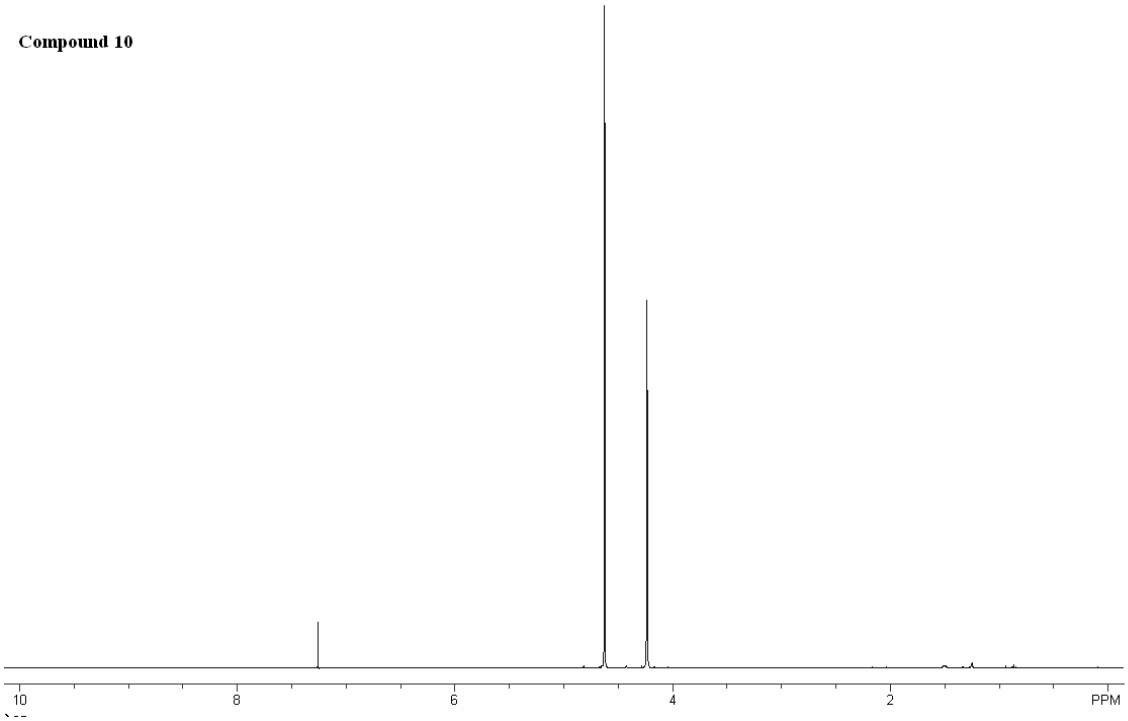
^{13}C NMR (100.7 MHz, CD_3OD)

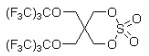
Compound 9



^{1}H NMR (400 MHz, $CDCl_3$)

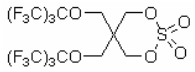
Compound 10





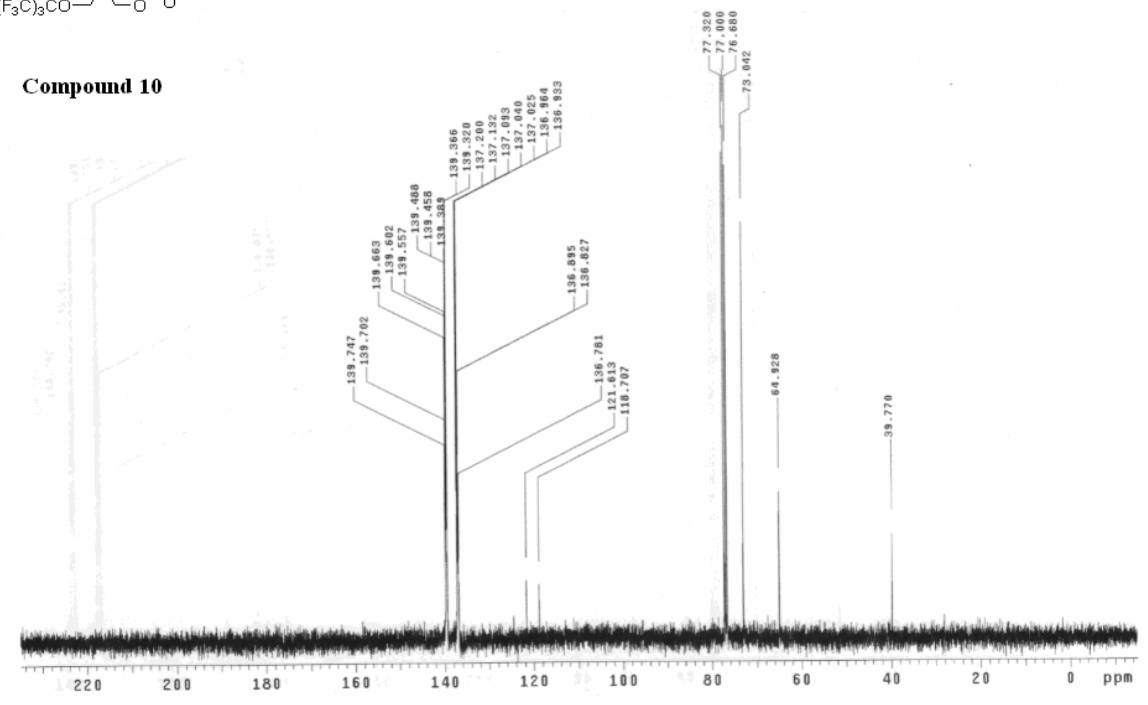
^{19}F NMR (376 MHz, $CDCl_3$)

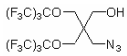
Compound 10



^{13}C NMR (100.7 MHz, $CDCl_3$)

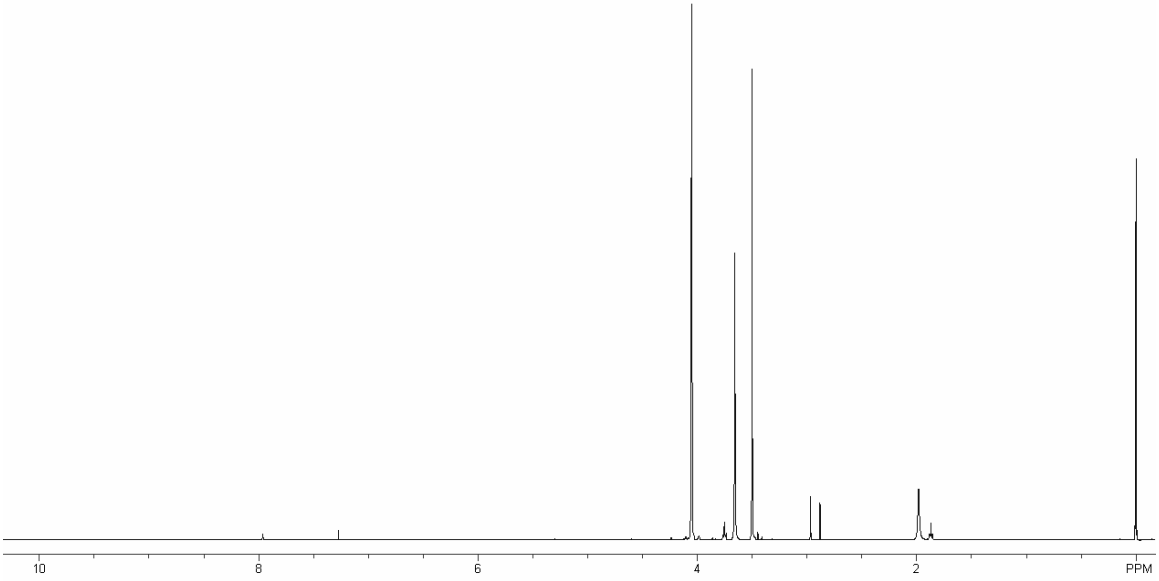
Compound 10

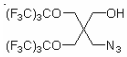




^{1}H NMR (400 MHz, $CDCl_3$)

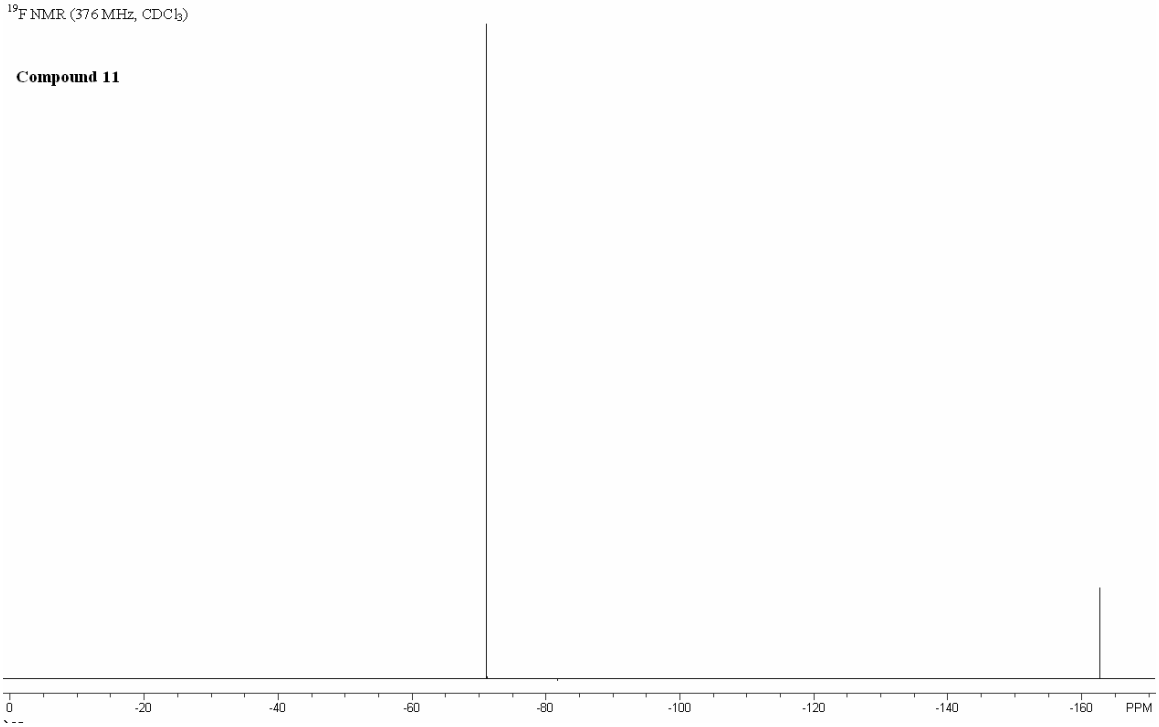
Compound 11

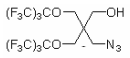




^{19}F NMR (376 MHz, $CDCl_3$)

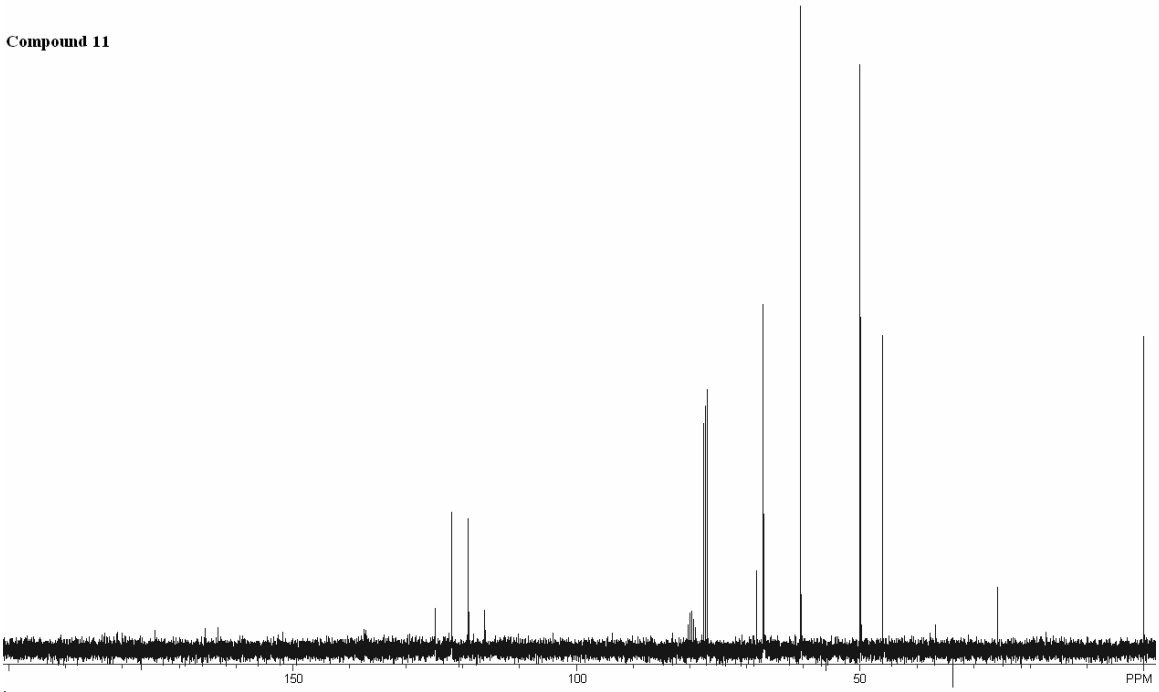
Compound 11

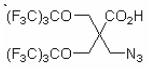




^{13}C NMR (100.7 MHz, $CDCl_3$)

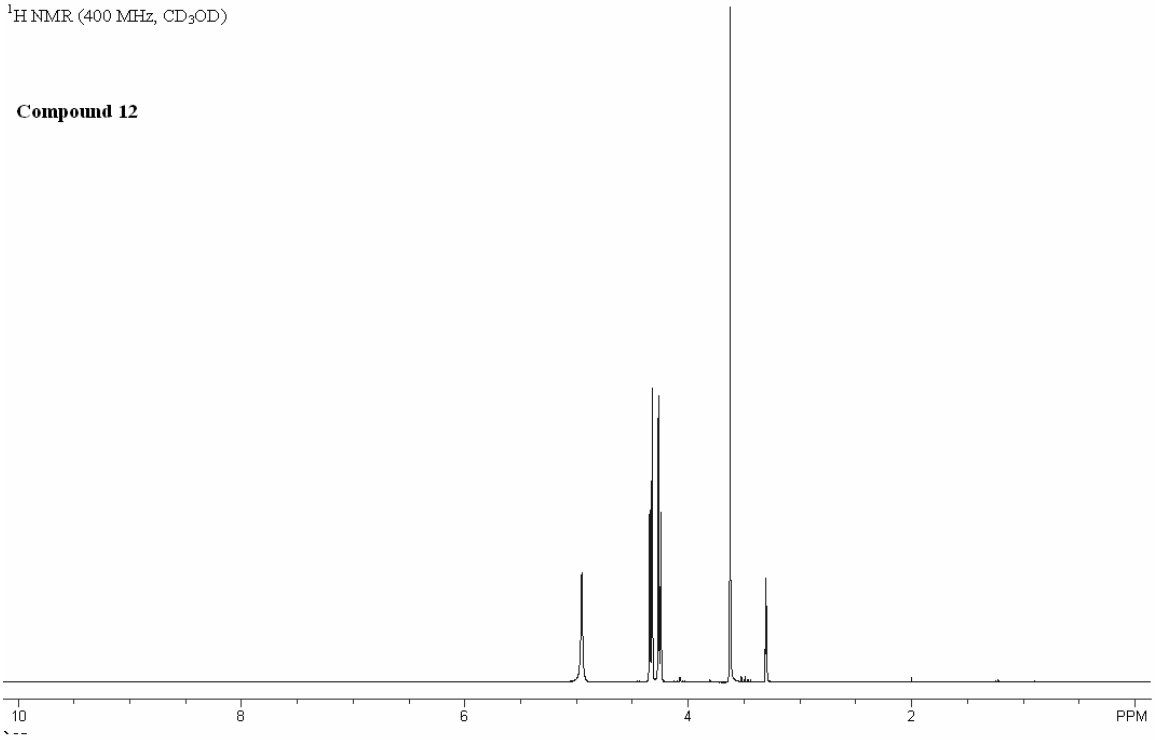
Compound 11





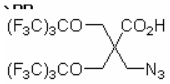
^{1}H NMR (400 MHz, CD_3OD)

Compound 12



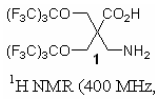
^{19}F NMR (376 MHz, CD_3OD)

Compound 12



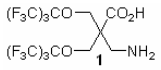
^{13}C NMR (100.7 MHz, CD_3OD)

Compound 12



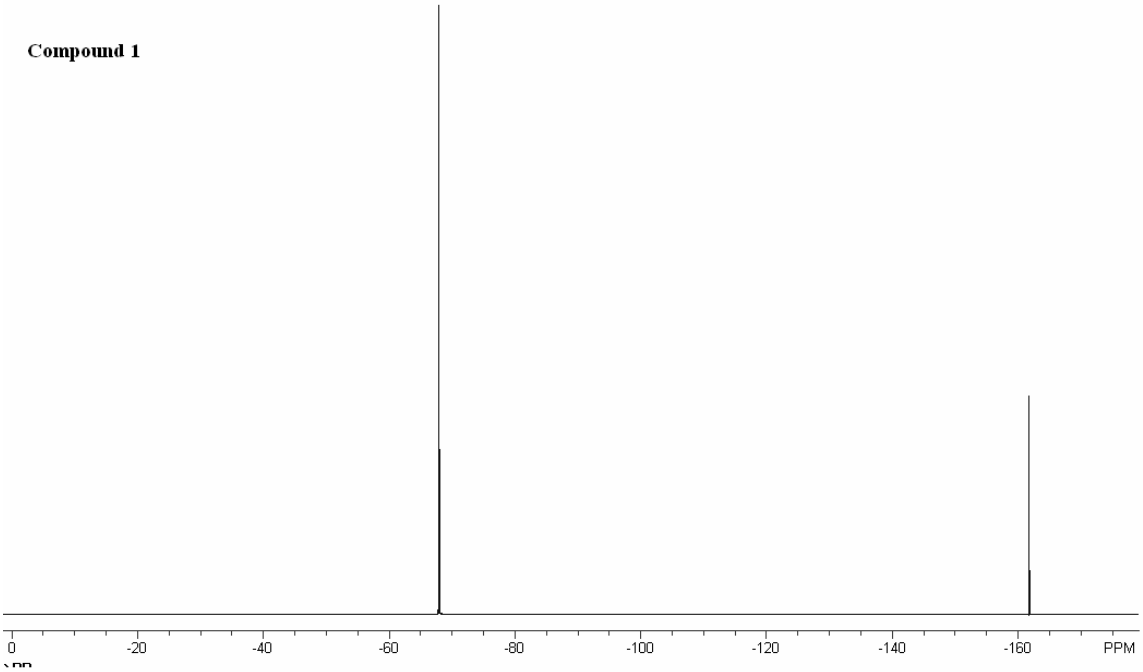
^{1}H NMR (400 MHz, CD_3OD)

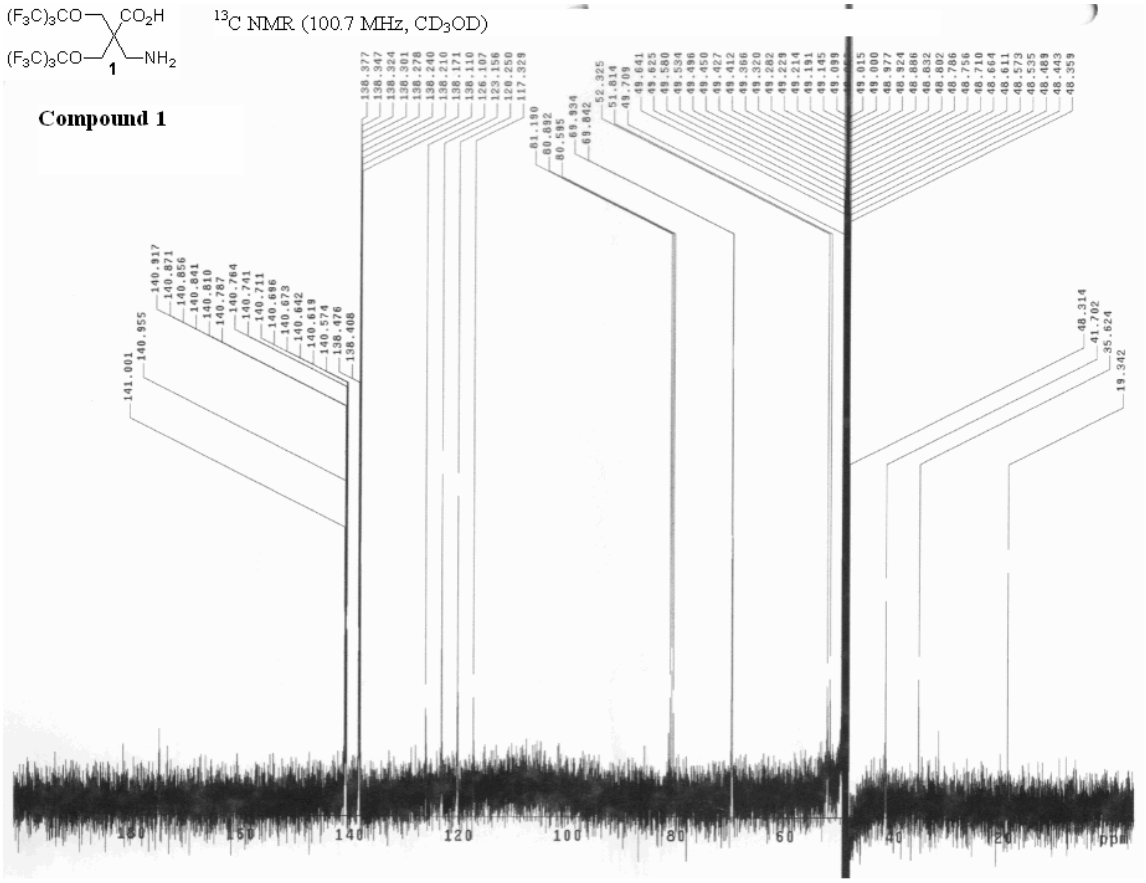
Compound 1

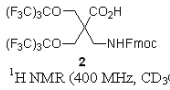


^{19}F NMR (376 MHz, CD_3OD)

Compound 1

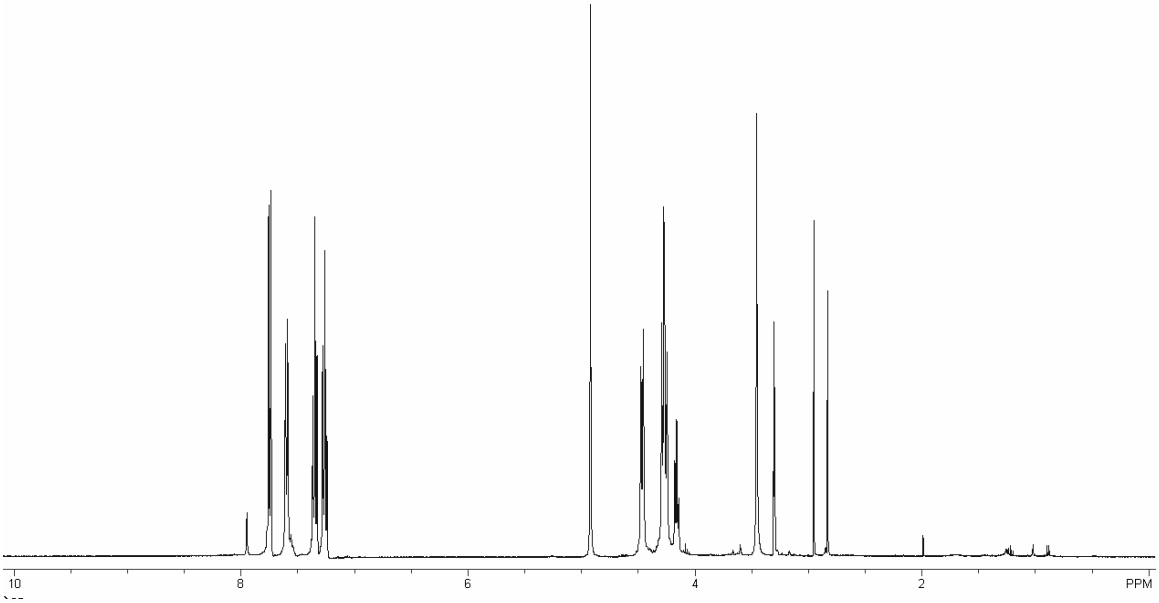


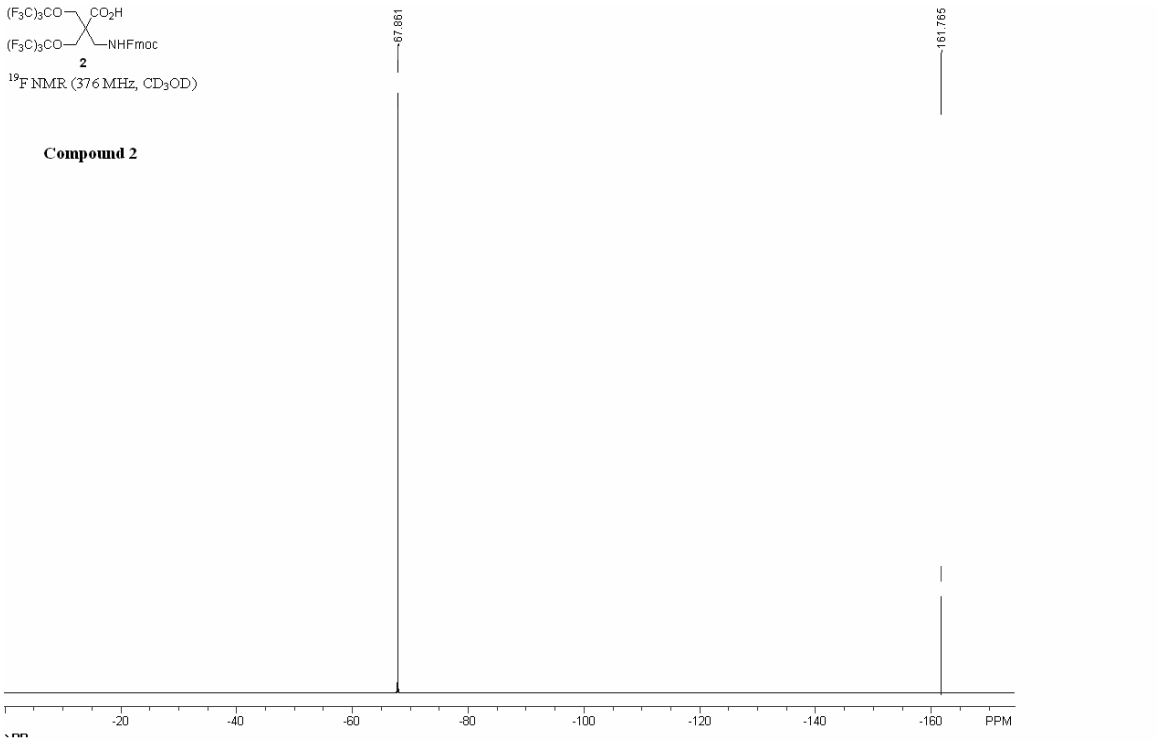




^{1}H NMR (400 MHz, CD_3OD)

Compound 2

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Compound 2
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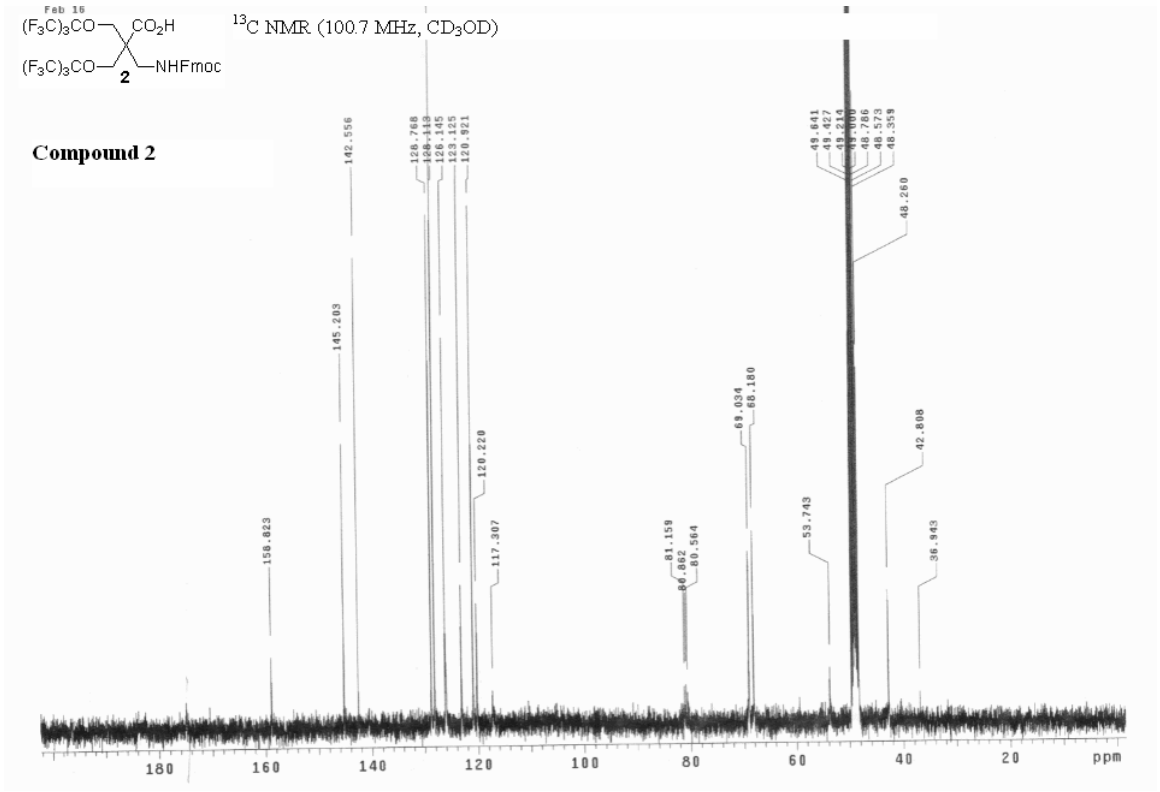
PPM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Compound 2
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145.203
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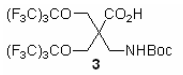
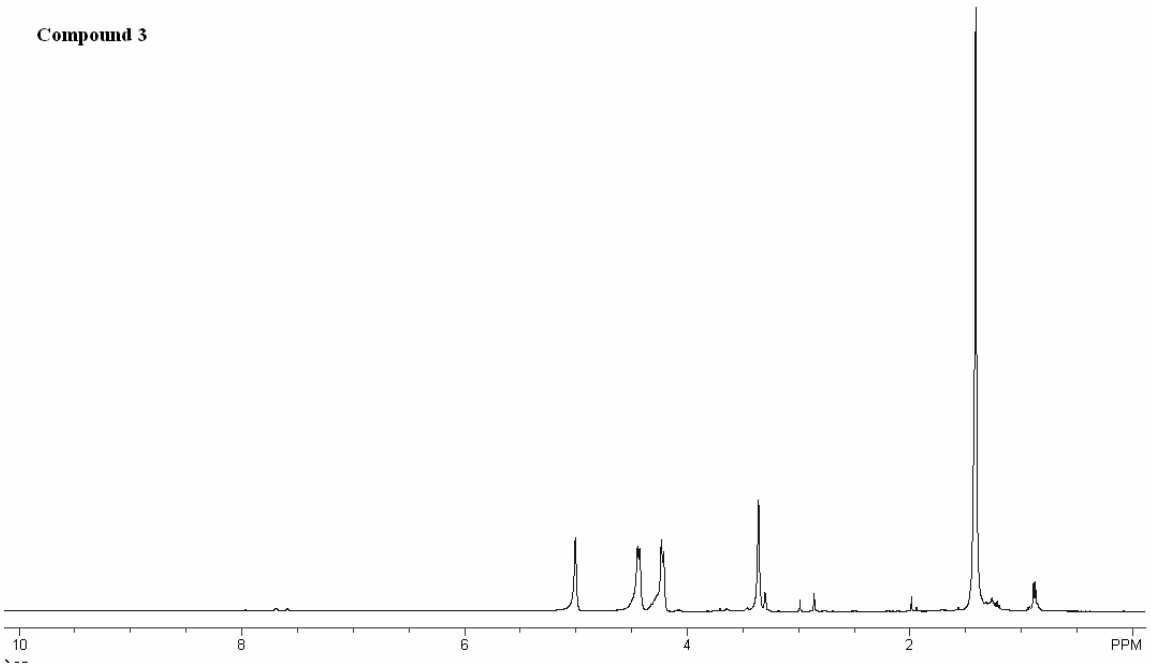
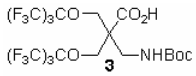


^{1}H NMR (400 MHz, CD_3OD)

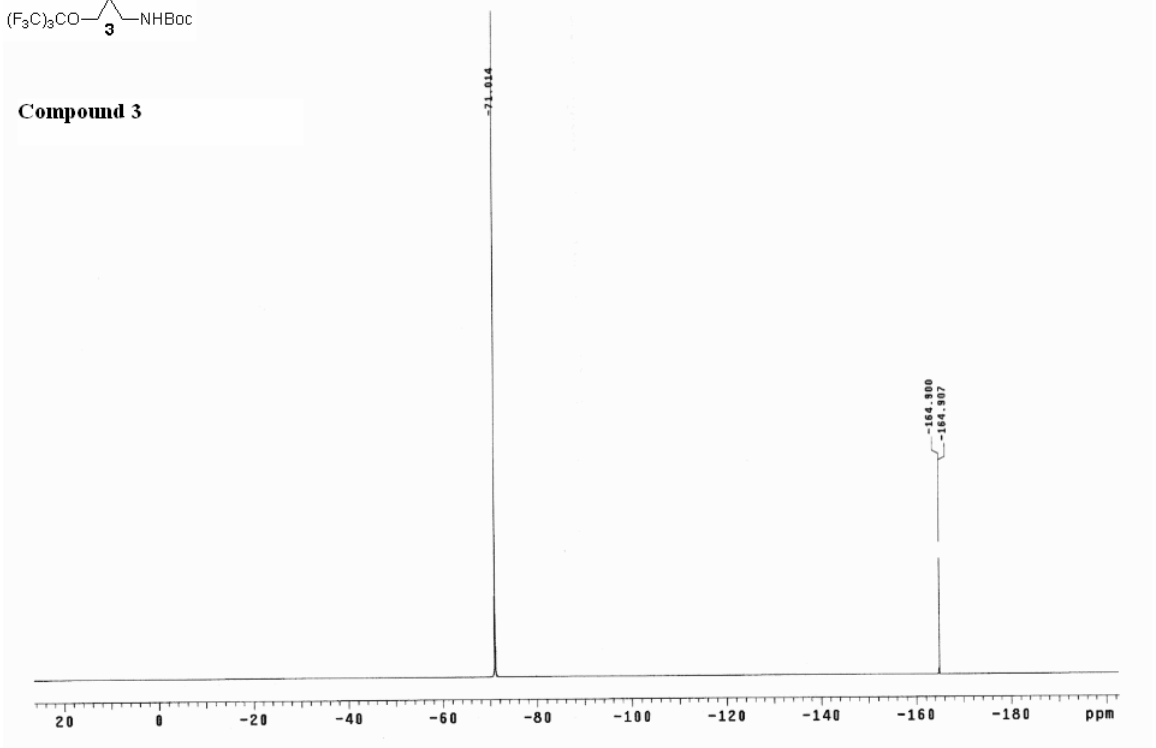
Compound 3

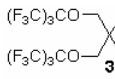




^{19}F NMR (376 MHz, CD_3OD)

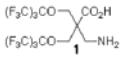
Compound 3





^{13}C NMR (100.7 MHz, CD_3OD)

Compound 3



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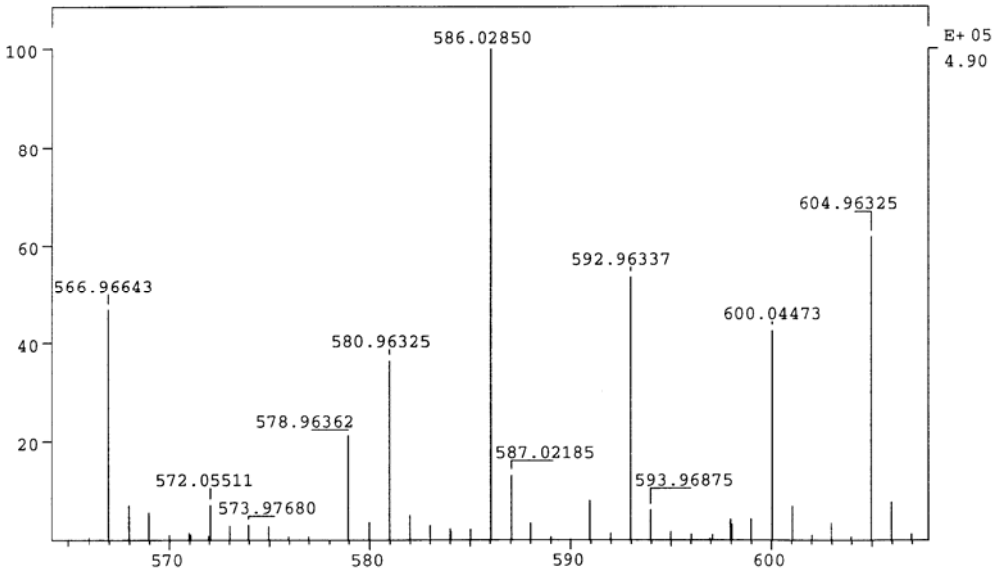
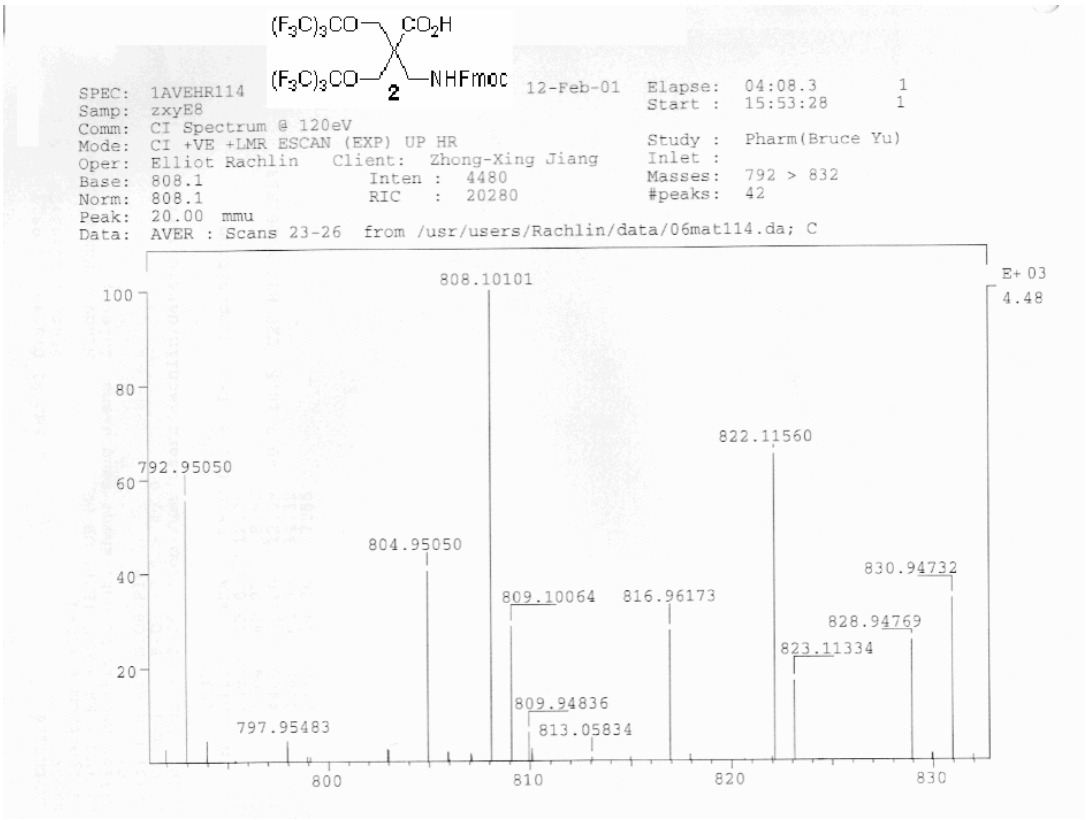
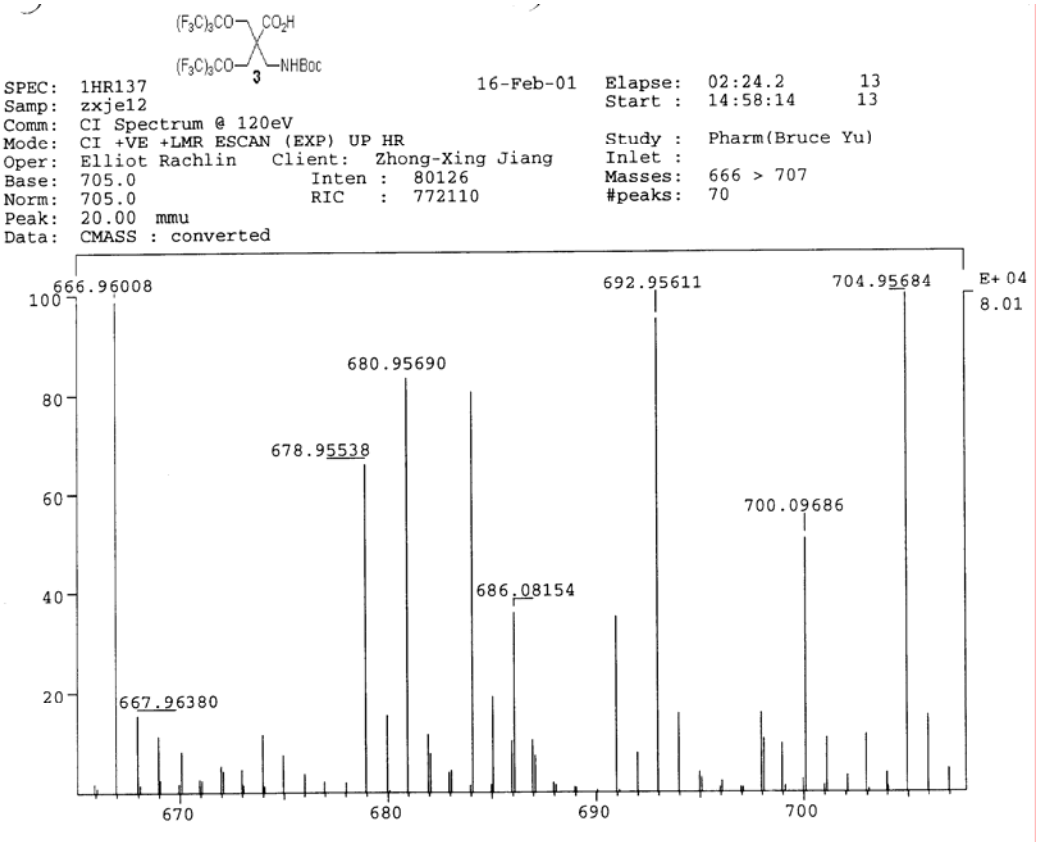
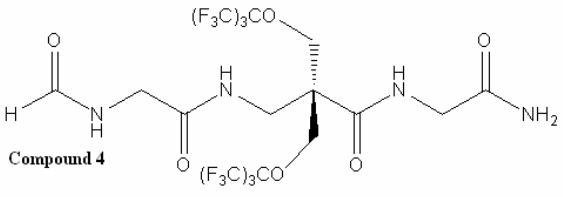
Comm: CI Spectrum @ 120eV
Mode: CI +VE +LMR ESCAN (EXP) UP HR Study : Pharm(Bruce Yu)
Oper: Elliot Rachlin Client: Zhong-Xing Jiang Inlet :
Base: 586.0 Inten : 490297 Masses: 565 > 607
Norm: 586.0 RIC : 2382308 #peaks: 52
Peak: 20.00 mmu
Data: CMASS : converted




(F3C)3CO
CO2H
(F3C)3CO
NHFmoc
2
SPEC: 1AVEHR114
Samp: zxyE8
Comm: CI Spectrum @ 120eV
Mode: CI +VE +LMR ESCAN (EXP) UP HR
Oper: Elliot Rachlin Client: Zhong-Xing Jiang
Base: 808.1 Inten : 4480
Norm: 808.1 RIC : 20280
Peak: 20.00 mmu
Data: AVER : Scans 23-26 from /usr/users/Rachlin/data/06mat114.da; C
12-Feb-01
Elapse: 04:08.3 1
Start : 15:53:28 1
Study : Pharm(Bruce Yu)
Inlet :
Masses: 792 > 832
#peaks: 42
E+ 03
4.48
808.10101
822.11560
792.95050
804.95050
830.94732
809.10064
816.96173
828.94769
823.11334
809.94836
797.95483
813.05834


(F3C)3CO
CO2H
(F3C)3CO
NHBoc
3
SPEC: 1HR137
Samp: zxje12
Comm: CI Spectrum @ 120eV
Mode: CI +VE +LMR ESCAN (EXP) UP HR
Oper: Elliot Rachlin Client: Zhong-Xing Jiang
Base: 705.0 Inten : 80126
Norm: 705.0 RIC : 772110
Peak: 20.00 mmu
Data: CMASS : converted
16-Feb-01
Elapse: 02:24.2 13
Start : 14:58:14 13
Study : Pharm(Bruce Yu)
Inlet :
Masses: 666 > 707
#peaks: 70
E+ 04
8.01
666.96008
692.95611
704.95684
680.95690
678.95538
700.09686
686.08154
667.96380

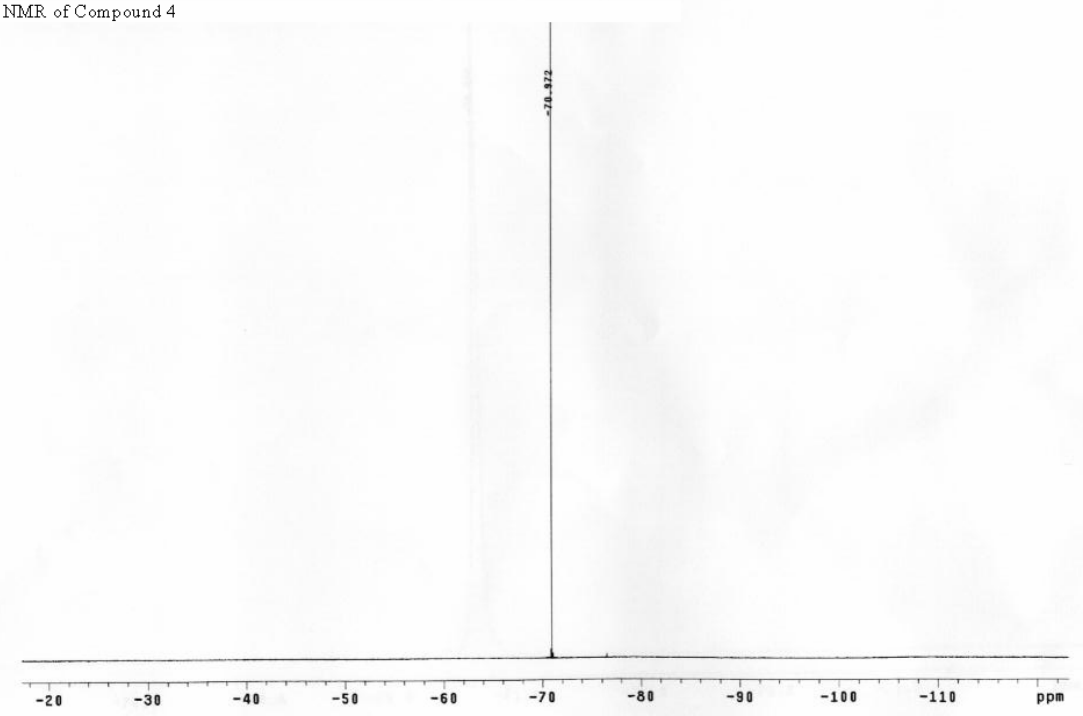
^{1}H NMR (400 MHz, CD_3OD)

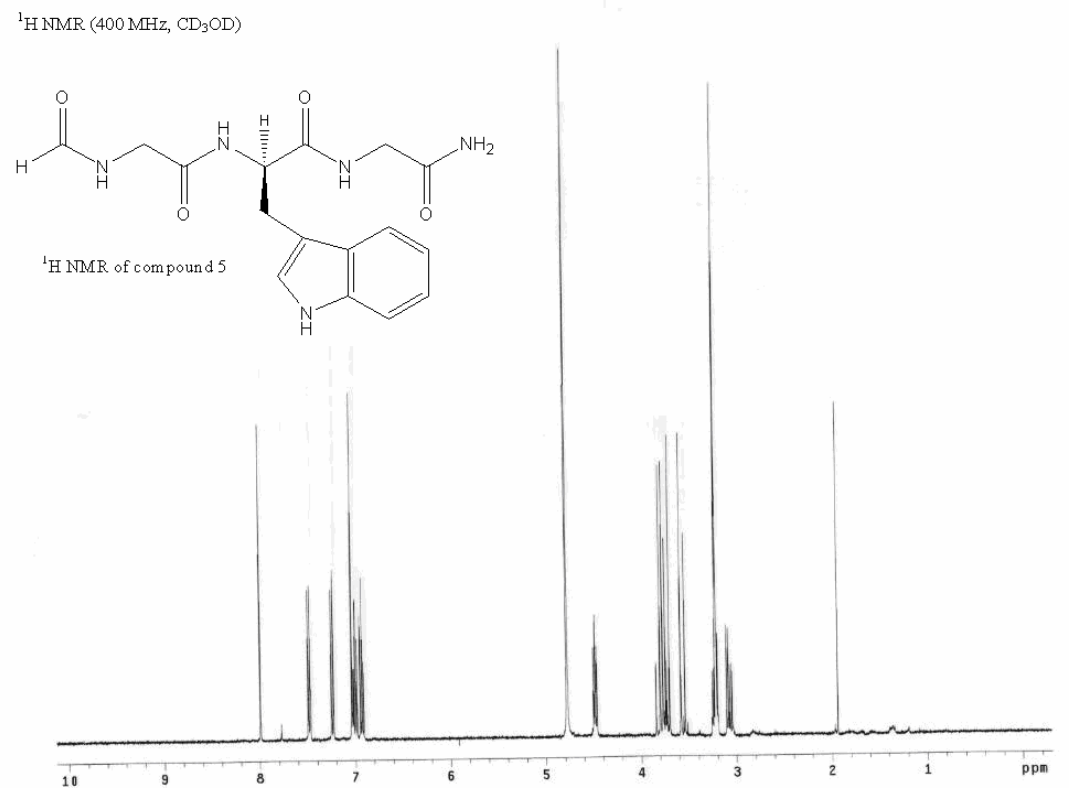


Compound 4

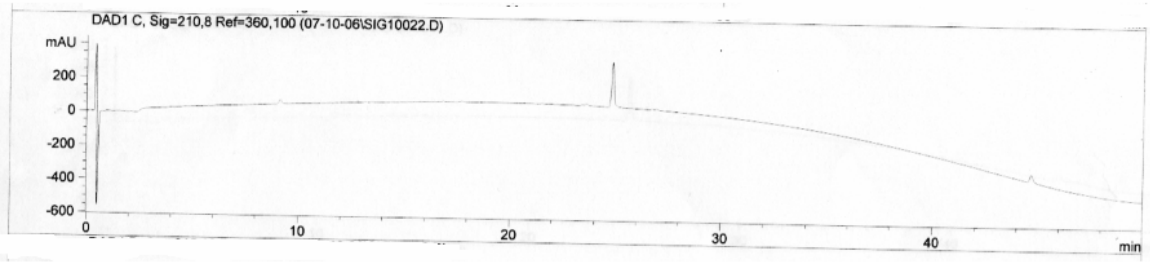
G19FG
19F-MeOH

^{19}F NMR of Compound 4

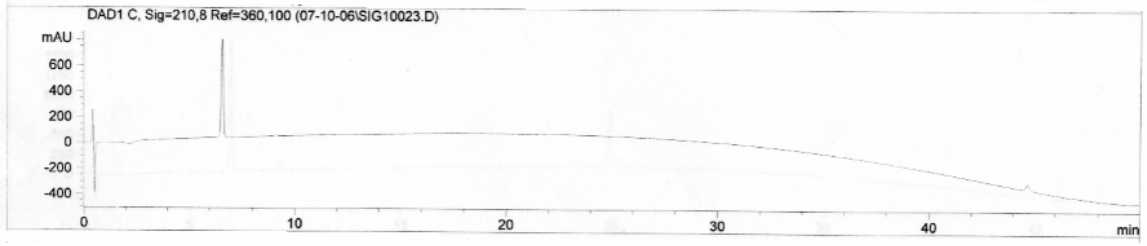




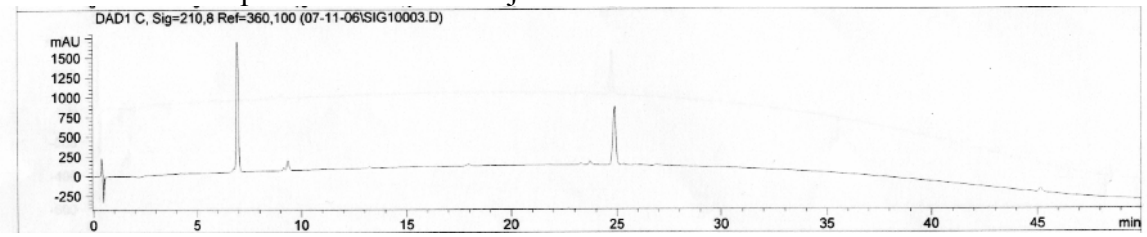
RP-HPLC for compounds **4**



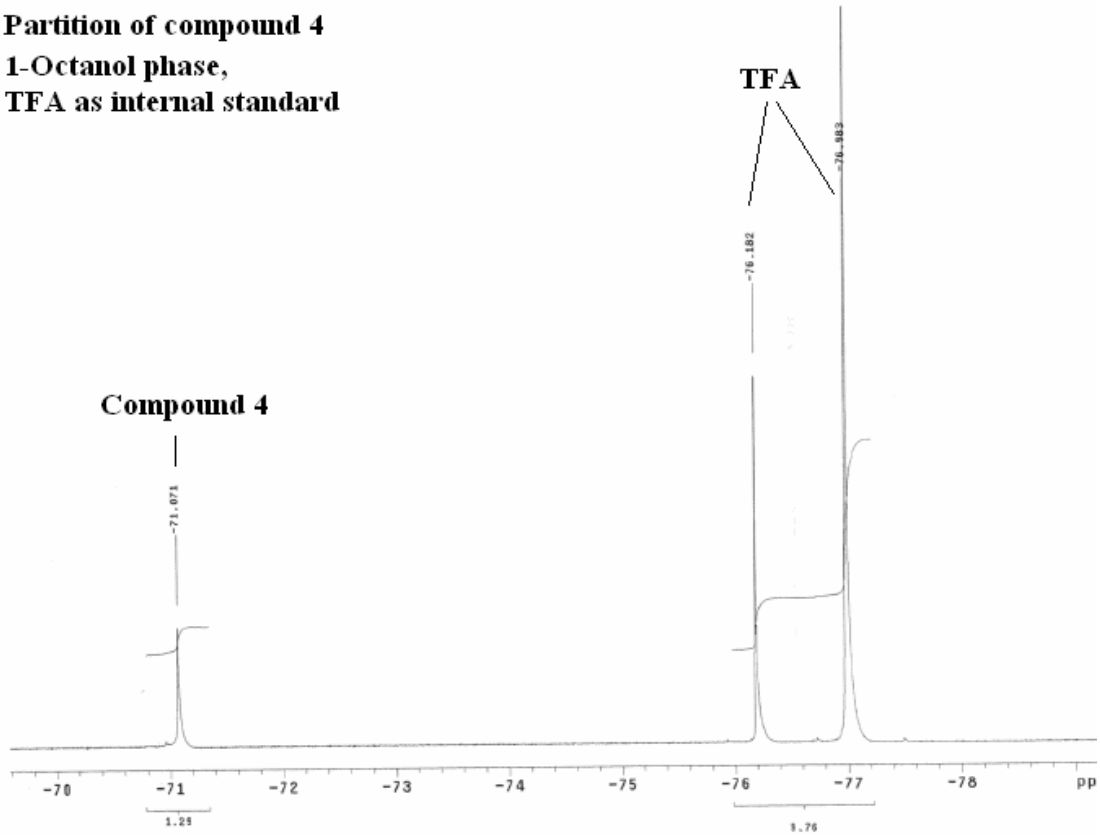
RP-HPLC for compounds **5**



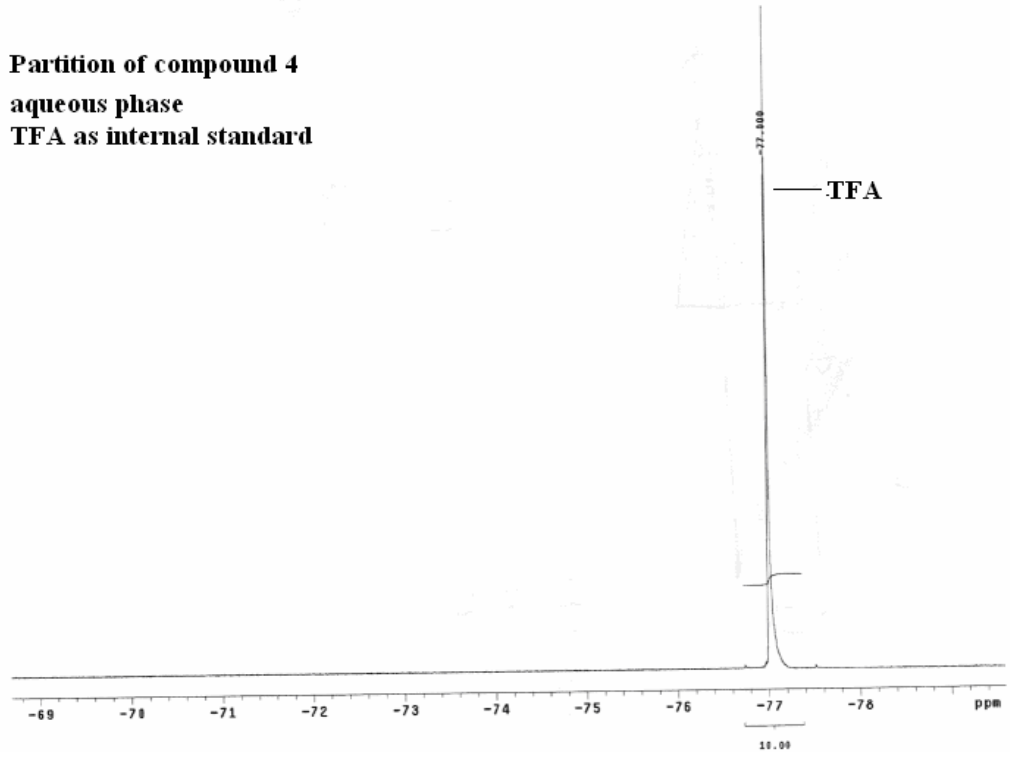
RP-HPLC for compounds **4** and **5** co-injection

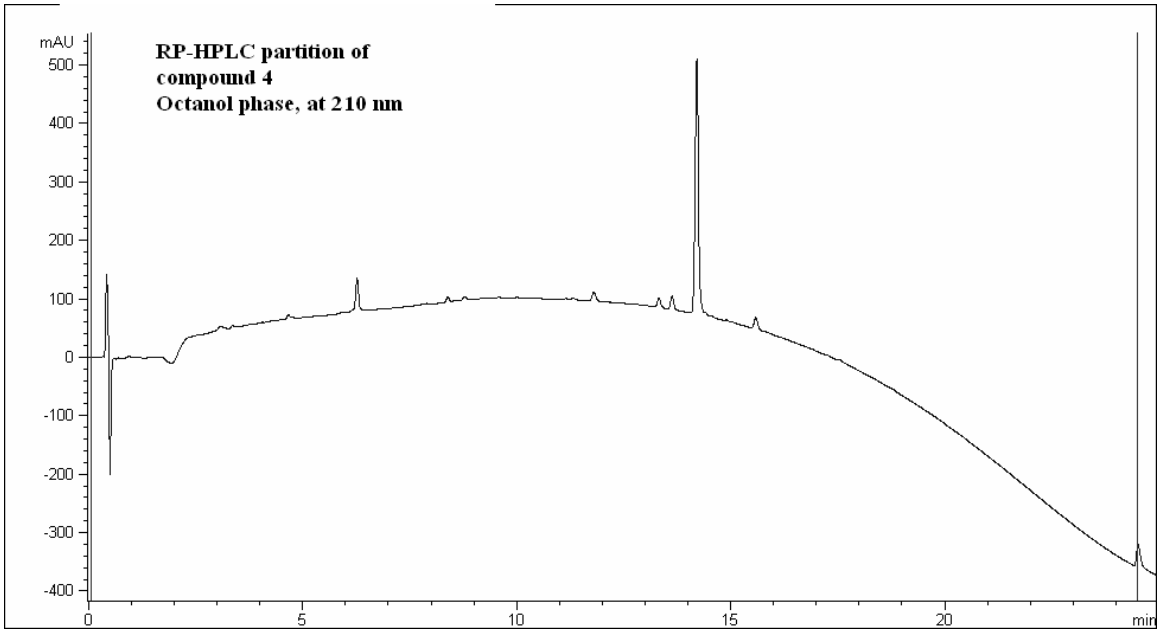


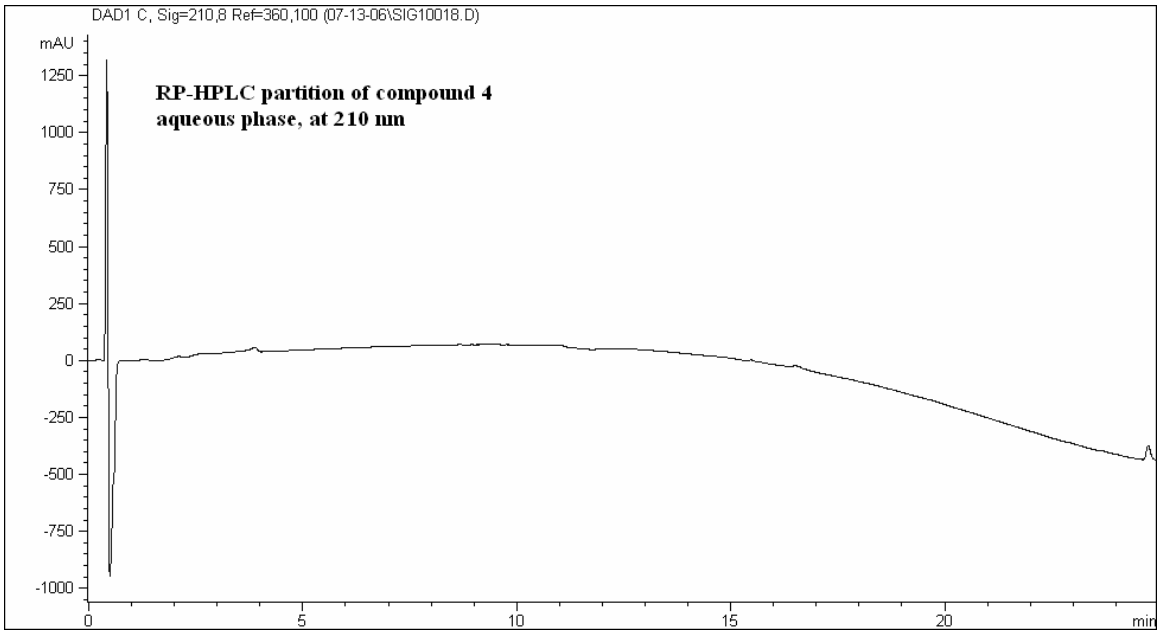
Partition of compound 4
1-Octanol phase,
TFA as internal standard

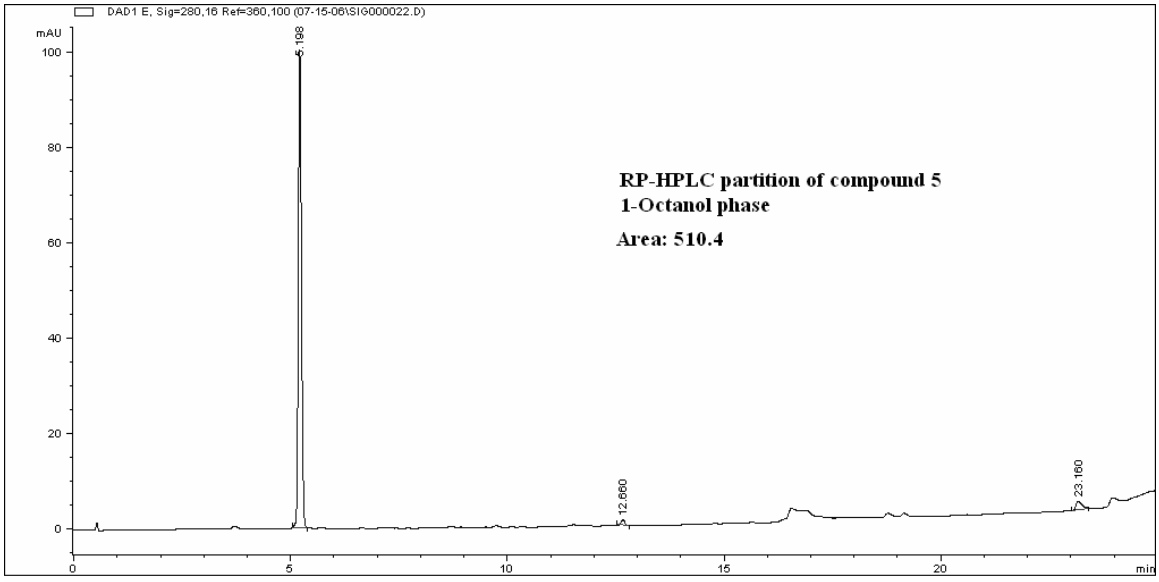


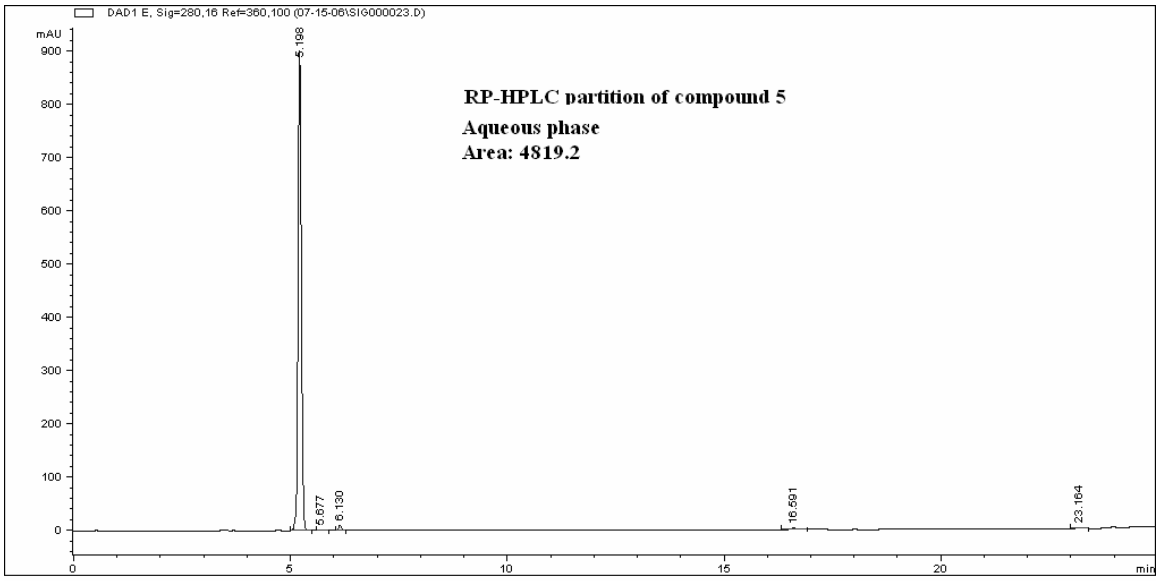
Partition of compound 4
aqueous phase
TFA as internal standard




RP-HPLC partition of
compound 4
Octanol phase, at 210 nm
mAU
500
400
300
200
100
0
-100
-200
-300
-400
0
5
10
15
20
min


DAD1 C, Sig=210,8 Ref=360,100 (07-13-06\SIG10018.D)
RP-HPLC partition of compound 4
aqueous phase, at 210 nm
mAU
1250
1000
750
500
250
0
-250
-500
-750
-1000
0
5
10
15
20
min





P_{oct} (**5**) = 510/4819 = 1/9.5

Reference

[i] Chan, W. C.; White, P. D. *Fmoc Solid Phase Peptide Synthesis: A Practical Approach*.; Oxford University Press: New York: **2000**; pp 1-75.