

(1) Experimental section. General.

All reagents used are commercially available and were employed without further purification. Fmoc-protected amino acids and PS-Trityl chloride resin (200-400 mesh, 1% DVB, 0.80 mmol/g) were purchased from Novabiochem (San Diego, California) and Rapp-Polymere (Tuebingen, Germany) respectively. All glassware used in solid-phase reactions had been silanized by treatment with 20% chlorotrimethylsilane/toluene for 12h and then dried under vacuum. Polypropylene(PP) filter vessels were obtained from Bio-Rad. THF was dried by distillation over sodium/benzophenone ketyl, CH_2Cl_2 over sodium hydride. Anhydrous DMF was obtained commercially from Aldrich. NMR spectra were recorded at Bruker AM-400 and AM-300, Varian Inova 300 or Varian Unity 500 MHz. Low and high resolution ES-MS were done on a Hewlett-Packard 1100 MSD and ZabSpecETOF respectively. Compound purity analysis was carried out by RP-HPLC on a Hewlett-Packard 1100 system using conditions described in the manuscript. Abbreviations: HBTU – 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate, HOBt – N-Hydroxybenzo-triazole, DIPEA – Diisopropylethyl-amine, γ -Abu – 4-Aminobutyric acid, Tyr(t-Bu) – O-t-butyl L-tyrosine, Dde-OH – 2-Acetyldimedone, $(\text{Boc})_2\text{O}$ – Di-t-butyl-dicarbonate, TFA – Trifluoroacetic acid.

(2) Experimental details for the synthesis of 1.

PS-Trityl-NH(CH₂)₃NH₂ resin (4) 1,3-Diaminopropane (4.0 mL, 48.0 mmol) was dissolved in 4 mL of dry CH_2Cl_2 in a PP filter vessel. PS-Trityl chloride resin (1.20g, 0.96 mmol, 0.80 mmol/g) was then added to the solution in 4 portions over one hour with vortexing in between additions. After vortexing for an additional hour, 2 mL of methanol was added followed by another 20 min. of vortexing. The resin was then filtered and rinsed with MeOH, 1:4 Et₃N/DMF, MeOH and CH_2Cl_2 (3 times each), and dried under high-vacuum for over 12 hours to give PS-Trityl-NH(CH₂)₃NH₂ resin **4** (1.22g, 0.73 mmol/g, as determined by Fmoc release U.V. assay after derivatizing with Fmoc-Cl). A ninhydrin assay gave a positive result.

PS-Trityl-NH(CH₂)₃NH₂ resin-bound triamide (5) To the resin **4** (0.382g, 0.279 mmol, 0.73 mmol/g) in a PP filter vessel was added N-Fmoc- β Alanine (0.349g, 1.12 mmol) as a solution in 3 mL of dry DMF. The vessel was shaken for 10 min. before a solution of HBTU (0.43g, 1.12 mmol) and HOBt (0.16g, 1.12 mmol) in 5 mL of dry DMF, and DIPEA (0.39 mL, 2.24 mmol) were added. After vortexing for an hour, the resin was filtered and rinsed with DMF, MeOH and CH_2Cl_2 (3 times each). A ninhydrin assay gave a negative result. The resin was rinsed with dry DMF (3 times) and then treated with 20% piperidine/DMF (3 mL for 3 min. then 3 mL for 25 min.). After

washing with MeOH, CH₂Cl₂ and DMF (3 times each), the coupling procedure was exactly repeated followed by the second repetition as above with 3 mL solution of Fmoc- γ Abu (0.37g, 1.12 mmol), a 5 mL DMF solution of HBTU (0.43g, 1.12 mmol) and HOBt (0.16g, 1.12 mmol), and DIPEA (0.39 mL, 2.24 mmol). Subsequently, the resin was again rinsed with dry DMF, MeOH and CH₂Cl₂ (3 times each), and dried under vacuum overnight to afford the resin-bound triamide **5** (0.430g, 0.59 mmol/g, calculated from the loading of its Fmoc-protected precursor, obtained from Fmoc release U.V. assay). A ninhydrin assay gave a negative result. A portion of the Fmoc-protected precursor of **5** was cleaved from the resin and its ES-MS analysis validated the efficiency of synthesis of the tripeptide **5**.

PS-Trityl resin-bound tetraamine (6) The resin-bound triamide **5** (0.276g, 0.163 mmol, 0.59mmol/g) was weighed into a 25 mL silanized round bottom flask and swelled in dry THF (1.5 mL) under nitrogen. The diborane solution (1M in THF, 6.0 mL, 6.0 mmol) was added dropwise at rt over 2 min. The flask was then equipped with a condenser and the suspension was gently refluxed at 65°C for 48h. Upon cooling to rt, the suspended resin was rapidly transferred into a PP filter vessel via a silanized pipette using dry THF to rinse out the flask and to wash the resin extensively. Then, dry THF (2.0 mL), anhydrous DIPEA (0.3 mL) and glacial AcOH (0.6 mL) were added successively. After shaking the suspension the iodine was added (1.53g, 6.0 mmol, as a concentrated THF solution) and the vessel was vortexed for 4h. The resin was then filtered and rinsed with THF, 1:3 Et₃N/DMF, MeOH and CH₂Cl₂ (3 times each) and dried under high-vacuum overnight to give the resin-bound tetraamine **6** (0.220g). A portion of the tetraamine **6** was acetylated (6 eq. Et₃N, 22 eq. Ac₂O, DMF, rt, 12h) and then cleaved from the resin. The ES-MS analysis of the acetylated derivative confirmed the efficiency of the reduction reaction (see attached MS spectra).

PS-Trityl resin-bound Boc-protected tetraamine (7) To the resin-bound tetraamine **6** (0.184g) in a PP filter vessel was added Dde-OH (40 mg, 0.22 mmol) as a solution in 2 mL of dry DMF. After vortexing for 2h, the resin was filtered and rinsed with DMF, MeOH and CH₂Cl₂ (3 times each). A ninhydrin assay gave a negative result. Then, a solution of DIPEA (0.24 mL, 1.32 mmol) in 1 mL dry CH₂Cl₂ was added, after shaking for an minute, followed by addition of 1 mL dry CH₂Cl₂ solution of (Boc)₂O (0.58g, 2.64 mmol). The suspension was vortexed overnight. Then, the resin was filtered and rinsed with CH₂Cl₂, MeOH and DMF (3 times each) and treated with 2% hydrazine in DMF (3 mL for 10 min. then 3 mL for 30 min.) to remove the Dde-protecting group. The resin was rinsed with DMF, MeOH and CH₂Cl₂ (3 times each) and dried under vacuum for over 12h to afford the PS-Trityl resin-bound selectively Boc-protected tetraamine **7** (0.195g). A ninhydrin assay gave a positive result.

PS-Trityl resin-bound Boc-protected HO-416b (9) The resin-bound selectively Boc-protected tetraamine **7** (182 mg) was swelled in 1 mL CH₂Cl₂ in a PP filter vessel. Et₃N (24 μ L, 0.15 mmol) and DMAP (20mg, 0.15 mmol) as a solution in 1 mL of dry CH₂Cl₂ were added successively. After vortexing for a few minutes, a CH₂Cl₂ solution of mixed anhydride **8** was added, prepared by reacting 3-indoleacetic acid (0.14g, 0.80 mmol) in 2 mL dry CH₂Cl₂ with trimethylacetyl chloride (99 μ L, 0.80 mmol) in the presence of Et₃N (0.12 mL, 0.80 mmol) at rt for 1h. After shaking overnight the resin was filtered and rinsed with DMF, MeOH and CH₂Cl₂ (4 times each) and dried under high-vacuum for over 12h to give the PS-Trityl resin-bound selectively Boc-protected HO-416b **9** (184 mg). A ninhydrin assay gave a negative result.

HO-416b (1) The resin-bound Boc-protected HO-416b **9** (41 mg) was weighed into a 5 mL silanized round bottom flask and stirred in a freshly prepared TFA/H₂O/*i*-Pr₃SiH (95:2.5:2.5) cleavage cocktail (2 mL) for 2h at rt. After removing the solution by a pipette from the flask, the above-mentioned cleavage cocktail (2 mL) was added to the resin left. The suspension was then stirred for an additional 2h. The contents were filtered through a glasswool plug and the resin rinsed extensively with TFA/MeOH/CH₂Cl₂ (5:30:65). The combined filtrates from two rounds of cleavage were evaporated and dried over high-vacuum for >12h to give crude HO-416b **1** as a penta(trifluoroacetate) ammonium salt (13.5 mg, 57% from tripeptide **5**). Its purity was estimated to be 81% according to RP-HPLC analysis. Two rounds of precipitation with methanol/ether finally afforded HO-416b **1** (8.1 mg) of 89% purity with a 37% overall yield from tripeptide **5**.

Analytical data for 1:

¹H NMR (400 MHz, CD₃OD):

1, \cdot 5TFA salt: δ = 1.50-1.85 (4H, m), 2.01-2.22 (6H, m), 2.94-3.27 (16H, m), 3.65 (2H, s), 7.02 (1H, t, J=7.0Hz), 7.11 (1H, t, J=7.0Hz), 7.18 (1H, s), 7.36 (1H, d, J=8.0Hz), 7.53 (1H, d, J=8.0Hz).

ES-MS (C₂₃H₄₀N₆O): m/z 416.3 (M+H)⁺

RP-HPLC: (a) Retention time: 11.70 min;
(b) Conditions: Column Zorbax SB-C18 (4.6X150mm, 5 μ m);
Eluent (Isocratic) 12.5% MeCN (0.1% TFA) and 87.5% H₂O (0.1% TFA);
Flow rate 1.5 mL/min.; Detection 279 nm.

Selected NMR, ES-MS and HPLC spectra are shown on pages 11 to 15.

(3) Experimental details for the synthesis of PhTX-433 (2)

PS-Triyl-NH(CH₂)₃NH₂ resin-bound diamide (10) To the resin **4** (0.338g, 0.247mmol, 0.73 mmol/g) in a PP filter vessel was added N-Fmoc-βAlanine (0.314g, 1.0 mmol) as a solution in 3 mL of dry DMF. The vessel was shaken for 10 min. before a solution of HBTU (0.38g, 1.0 mmol) and HOBt (0.14g, 1.0 mmol) in 5 mL of dry DMF, and DIPEA (0.35 mL, 2.0 mmol) were added. After vortexing for an hour, the resin was filtered and rinsed with DMF, MeOH and CH₂Cl₂ (3 times each). A ninhydrin assay gave a negative result. The resin was rinsed with dry DMF (3 times) and then treated with 20% piperidine/DMF (3 mL for 3 min. then 3 mL for 25 min.). After washing with MeOH, CH₂Cl₂ and DMF (3 times each), the coupling procedure was repeated except for the use of Fmoc-γAbu (0.33g, 1.0 mmol) instead of Fmoc-βAlanine. Subsequently, the resin was again rinsed with dry DMF, MeOH and CH₂Cl₂ (3 times each), and dried under vacuum overnight to afford the resin-bound diamide **10** (0.385g, 0.63 mmol/g, calculated from the loading of its Fmoc-protected precursor, obtained from Fmoc release U.V. assay). A ninhydrin assay gave a negative result. A portion of the Fmoc-protected precursor of **10** was cleaved from the resin and its ES-MS analysis validated the efficiency of synthesis of the dipeptide **10**.

PS-Triyl resin-bound triamine (11) The resin-bound diamide **10** (0.316g, 0.20 mmol, 0.63mmol/g) was weighed into a 25 mL silanized round bottom flask and swelled in dry THF (1.5 mL) under nitrogen. The diborane solution (1M in THF, 5.0 mL, 5.0 mmol) was added dropwise at rt over 2 min. The flask was then equipped with a condenser and the suspension was gently refluxed at 65°C for 48h. Upon cooling to rt, the suspended resin was rapidly transferred into a PP filter vessel via a silanized pipette using dry THF to rinse out the flask and to wash the resin extensively. Then, dry THF (2.0 mL), anhydrous DIPEA (0.3 mL) and glacial AcOH (0.6 mL) were added successively. After shaking the suspension the iodine was added (1.27g, 5.0 mmol, as a concentrated THF solution) and the vessel was vortexed for 4h. The resin was then filtered and rinsed with THF, 1:3 Et₃N/DMF, MeOH and CH₂Cl₂ (3 times each) and dried under high-vacuum overnight to give the resin-bound triamine **11** (0.269g). A portion of the triamine **11** was acetylated (6 eq. Et₃N, 22 eq. Ac₂O, DMF, rt, 12h) and then cleaved from the resin. The ES-MS analysis of the acetylated derivative confirmed the efficiency of the reduction reaction (see attached MS spectra).

PS-Triyl resin-bound Boc-protected triamine (12) To the resin-bound triamine **11** (0.152g) in a PP filter vessel was added Dde-OH (38 mg, 0.21 mmol) as a solution in 2 mL of dry DMF. After vortexing for 2h, the resin was filtered and rinsed with DMF, MeOH and CH₂Cl₂ (3 times each). A ninhydrin assay gave a negative result. Then, a

solution of DIPEA (68 μ L mL, 0.38 mmol) in 1 mL dry CH_2Cl_2 was added, after shaking for an minute, followed by addition of 1 mL dry CH_2Cl_2 solution of $(\text{Boc})_2\text{O}$ (0.17g, 0.76 mmol). The suspension was vortexed overnight. Then, the resin was filtered and rinsed with CH_2Cl_2 , MeOH and DMF (3 times each) and treated with 2% hydrazine in DMF (3 mL for 10 min. then 3 mL for 30 min.) to remove the Dde-protecting group. The resin was rinsed with DMF, MeOH and CH_2Cl_2 (3 times each) and dried under vacuum for over 12h to afford the PS-Trityl resin-bound selectively Boc-protected triamine **12** (0.185g). A ninhydrin assay gave a positive result.

PS-Trityl resin-bound Boc-protected PhTX-433 (13) To the resin-bound selectively Boc-protected triamine **12** (83 mg) in a PP filter vessel was added N-Fmoc-Tyr(t-Bu) (88 mg, 0.19 mmol) as a solution in 2 mL of dry DMF. The vessel was shaken for 10 min. before a solution of HBTU (73 mg, 0.19 mmol) and HOBt (27 mg, 0.19 mmol) in 3 mL of dry DMF, and DIPEA (68 μ L mL, 0.38 mmol) were added. After vortexing for an hour, the resin was filtered and rinsed with DMF, MeOH and CH_2Cl_2 (3 times each). A ninhydrin assay gave a negative result. The resin was rinsed with dry DMF (3 times) and then treated with 20% piperidine/DMF (3 mL for 3 min. then 3 mL for 25 min.). After washing with MeOH, CH_2Cl_2 and DMF (3 times each), the coupling procedure was repeated except for the use of butyric acid (18 μ L, 0.19 mmol) instead of Fmoc-Tyr(t-Bu). Subsequently, the resin was again rinsed with dry DMF, MeOH and CH_2Cl_2 (3 times each), and dried under vacuum overnight to afford the resin-bound selectively Boc-protected PhTX-433 **13** (86 mg). A ninhydrin assay gave a negative result.

PhTX-433 (2) The resin-bound Boc-protected PhTX-433 **13** (38 mg) was weighed into a 5 mL silanized round bottom flask and stirred in a freshly prepared TFA/ H_2O /*i*-Pr₃SiH (95:2.5:2.5) cleavage cocktail (2 mL) for 2h at rt. After removing the solution by a pipette from the flask, the above-mentioned cleavage cocktail (2 mL) was added to the resin left. The suspension was then stirred for an additional 2h. The contents were filtered through a glasswool plug and the resin rinsed extensively with TFA/MeOH/ CH_2Cl_2 (5:30:65). The combined filtrates from two rounds of cleavage are evaporated and dried over high-vacuum for >12h to give crude PhTX-433 **2** as a tris(trifluoroacetate) ammonium salt (14.0 mg, 77% from dipeptide **10**). Its purity was estimated to be 80% according to RP-HPLC analysis. Two rounds of precipitation with methanol/ether finally afforded PhTX-433 **2** (8.5 mg) of 92% purity with a 54% overall yield from dipeptide **10**.

Analytical data for 2:

¹H NMR (500 MHz, CD₃OD):

Synthetic product 2, 3TFA salt: δ = 0.84 (3H, t, J=7.4Hz), 1.54 (2H, q, J=7.4Hz), 1.46-1.64 (4H, m), 2.04-2.18 (4H, m), 2.16 (2H, dt, J₁=1.5Hz, J₂=7.4Hz), 2.80 (2H, dd, J₁=8.5Hz, J₂=14Hz), 2.93-3.20 (12H, m), 4.39 (1H, dd, J₁=7.0Hz, J₂=8.5Hz), 6.70 (2H, d, J=8.5Hz), 7.04 (2H, d, J=8.5Hz).

Natural product 2, 3TFA salt: δ = 0.84 (3H, t, J=7.4Hz), 1.56 (2H, q, J=7.4Hz), 1.46-1.64 (4H, m), 1.98-2.08 (4H, m), 2.16 (2H, dt, J₁=1.5Hz, J₂=7.4Hz), 2.80 (2H, dd, J₁=8.5Hz, J₂=14Hz), 2.94-3.20 (12H, m), 4.38 (1H, dd, J₁=7.0Hz, J₂=8.5Hz), 6.70 (2H, d, J=8.5Hz), 7.04 (2H, d, J=8.5Hz).

¹³C NMR (75 MHz, CD₃OD) for Synthetic product 2, TFA salt: δ = 13.90, 20.24, 24.11, 24.29, 25.36, 26.01, 27.24, 27.57, 37.79, 38.20, 38.71, 39.25, 45.65, 45.99, 56.86, 116.23, 129.08, 131.27, 157.31, 174.13, 176.10.

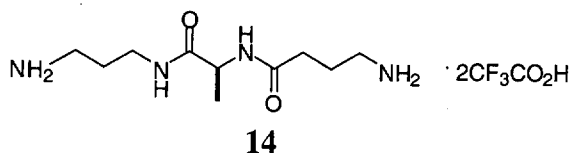
ES-MS (C₂₃H₄₁N₅O₃): Synthetic product 2: m/z 436.3 (M+H)⁺
Natural product 2: m/z 436.3 (M+H)⁺

RP-HPLC: (a) Retention time: synthetic product 2 11.83 min., natural product 2 11.83 min., co-injection of synthetic and natural products eluted as one peak at 11.83 min.
(b) Conditions: Column SB-C18 (4.6X150mm, 5 μ m);
Eluent 10% MeCN (0.1% TFA) and 90% H₂O (0.1% TFA); Flow rate 1.5 mL/min.; Detection 274 nm.

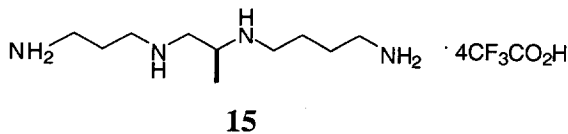
Selected NMR, ES-MS and HPLC spectra are shown on pages 16 to 23.

(4) Characterization of 3, an ethylenediamine structural analogue of PhTX-433.

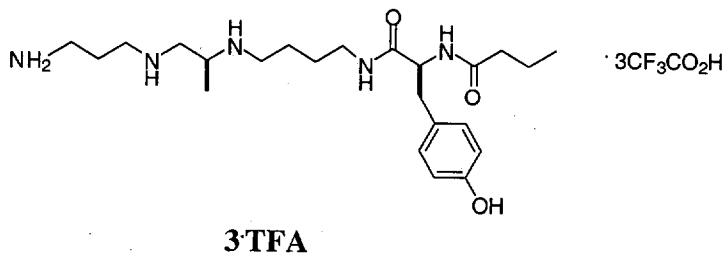
Synthesis of **3** was similar to that outlined for **2** except for the use of L-Ala instead of β -Ala in the first amino acid coupling.



H₂N-(CH₂)₃-NH-LAla- γ Abu (14). ¹H NMR (300 MHz, CD₃OD) δ 4.22 (q, J = 7.2 Hz, 1H), 3.30 (t, J = 7.0 Hz, 2H), 2.97 (t, J = 7.0 Hz, 2H), 2.94 (t, J = 7.0 Hz, 2H), 2.37 (t, J = 7.1 Hz, 2H), 1.92 (quintet, J = 7.2 Hz, 2H), 1.84 (quintet, J = 7.2 Hz, 2H), 1.35 (d, J = 7.2 Hz, 3H). ESMS M+H 231.1.



Tetraamine tetrakis(flouroacetate) salt (15). Cleavage from the resin followed by precipitation from methanol/ether gave the salt as a white solid in 77 % yield from the diamide. ¹H NMR (300 MHz, CD₃OD) δ 3.71 (multiplet, 1H), 3.48 (dd, J = 5.9 Hz, 13.4 Hz, 1H), 3.31 (dd, J = 5.9 Hz, 13.4 Hz, 1H), 3.24 – 2.94 (multiplet, 8H), 2.12 (quintet, J = 7.8 Hz, 2H), 1.90 – 1.65 (multiplet, 4H), 1.46 (d, J = 6.9 Hz, 3H). ¹³C NMR (75.5 MHz, CD₃OD) δ 163.4 (C), 163.0 (C), 52.9 (CH), 50.8 (CH₂), 46.9 (CH₂), 45.9 (CH₂), 39.9 (CH₂), 37.9 (CH₂), 25.5 (CH₂), 24.3 (CH₂), 15.0 (CH₃). ESMS 203.2 (M+H), 102.2 (M+2H)/2. ESMS of acetylated tetraamine 329.2 (M+H).



PhTX-433 analogue (3TFA). Two rounds of precipitation from methanol/ether afforded **3** as a yellow amorphous solid in 88 % purity, as determined by HPLC, with a

75 % overall yield from the diamide. ^1H NMR (300 MHz, CD_3OD) δ 7.04 (d, $J = 8.1$ Hz, 2H), 6.70 (d, $J = 8.4$ Hz, 2H), 4.40 (dd, $J = 6.9$ Hz, 8.5 Hz, 1H), 3.62 – 3.50 (broad s, 1H), 3.28 – 2.96 (multiplet, 10H), 2.97 (dd, $J = 6.6$ Hz, 13.8 Hz, 1H), 2.80 (dd, $J = 8.7$ Hz, 13.8 Hz, 1H), 2.16 (t, $J = 7.2$ Hz, 2H), 2.12 – 1.98 (m, 2H), 1.89 – 1.73 (m 2H), 1.70 – 1.46 (m, 8H), 1.42 (d, $J = 6.6$ Hz, 3H), 0.84 (t, $J = 7.5$ Hz, 3H). ^{13}C NMR (75.5 MHz, CD_3OD) δ 176.1 (C), 174.1 (C), 163.4 (C), 163.0 (C), 157.3 (C), 131.3 (CH), 129.1 (C), 120.0 (CF_3), 116.2 (CH), 116.2 (CF_3), 56.8 (CH), 52.7 (CH), 50.6 (CH_2), 46.9 (CH_2), 39.2 (CH_2), 38.7 (CH_2), 37.8 (CH_2), 27.2 (CH_2), 25.5 (CH_2), 24.5 (CH_2), 20.2 (CH_2), 15.5 (CH_3), 13.9 (CH_3). ES-MS 436.3 (M+H). HRMS-ES-MS M+H for $\text{C}_{23}\text{H}_{42}\text{N}_5\text{O}_3$ calcd. 436.328766, obsd. 436.329035.

Selected NMR, ES-MS and HPLC spectra are shown on pages 24 to 33.

Print of window 80: MS Spectrum
F. Wang

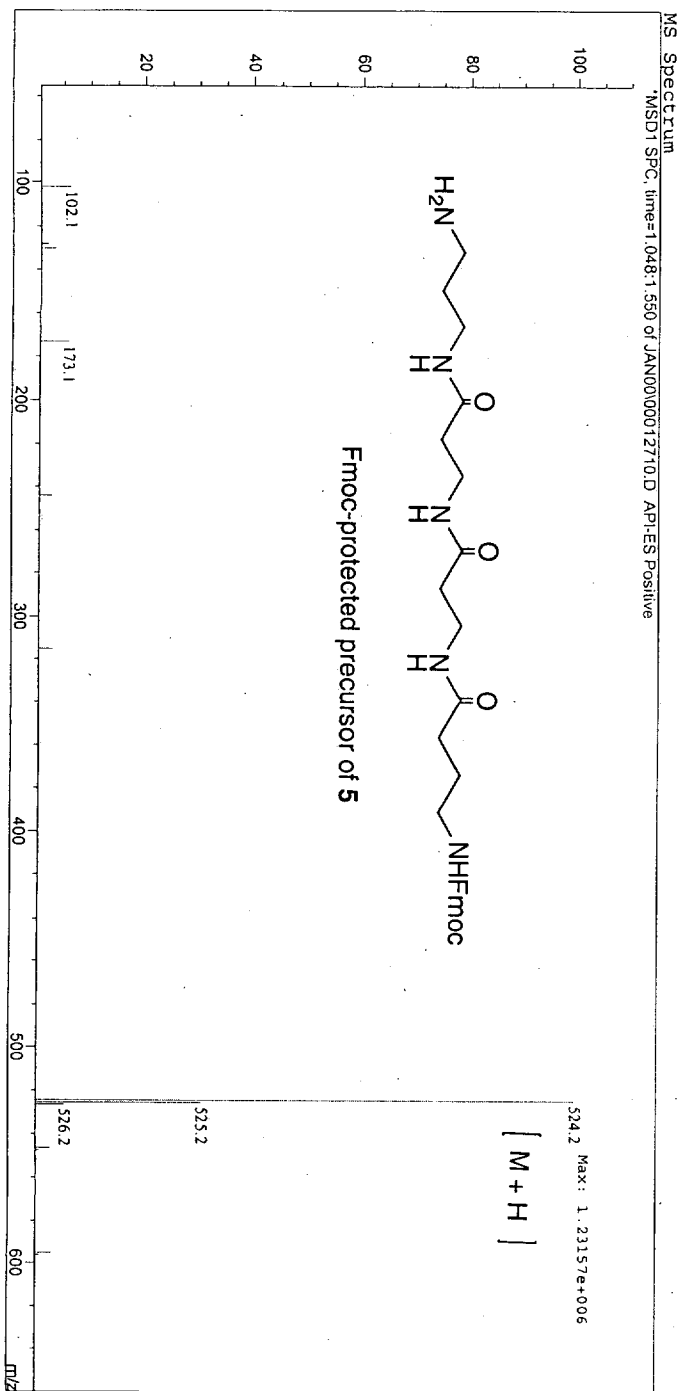
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Flow Injections :

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Print of window 80: 1 Mass Spectra of Peak 2.059 of 00020305.D
Fan Wang

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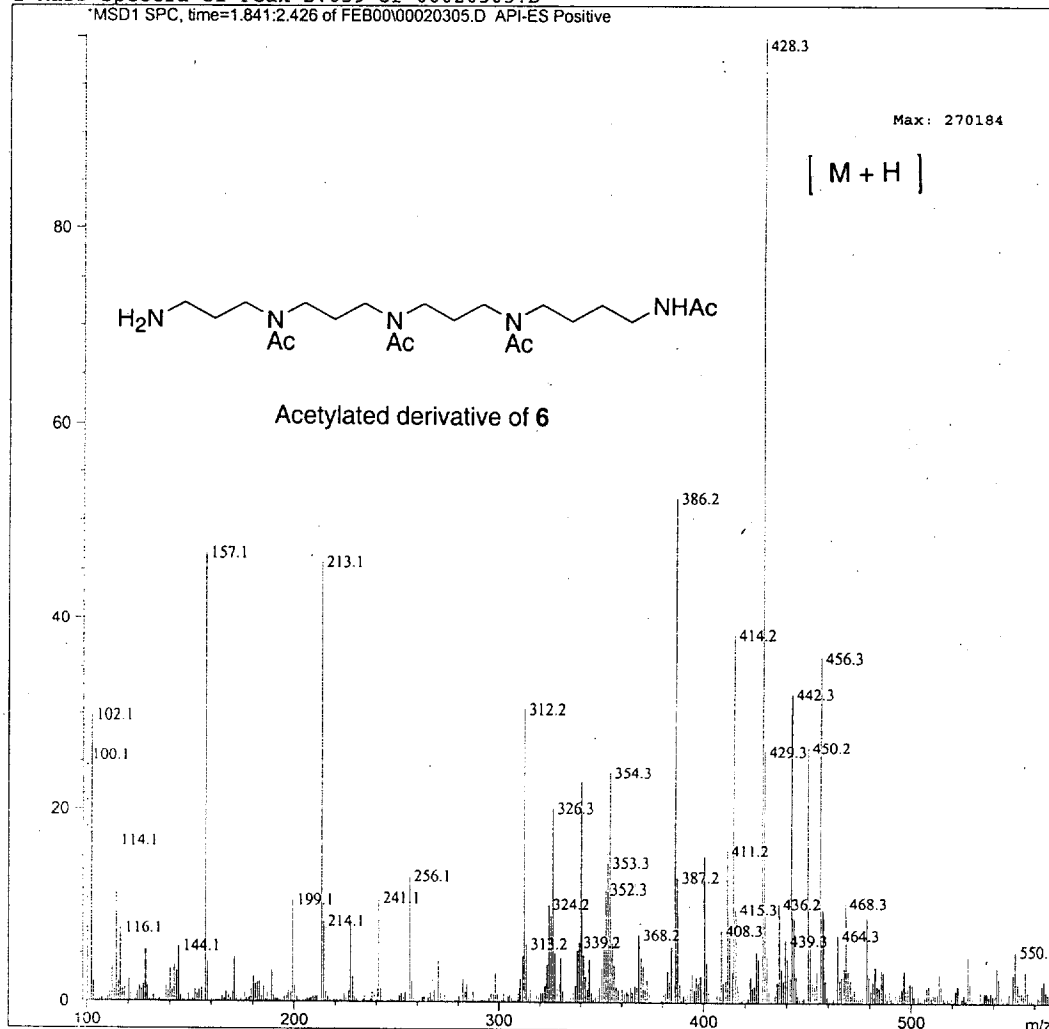
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                (modified after loading)
    
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MeOH 100uL/min

Flow Injections :

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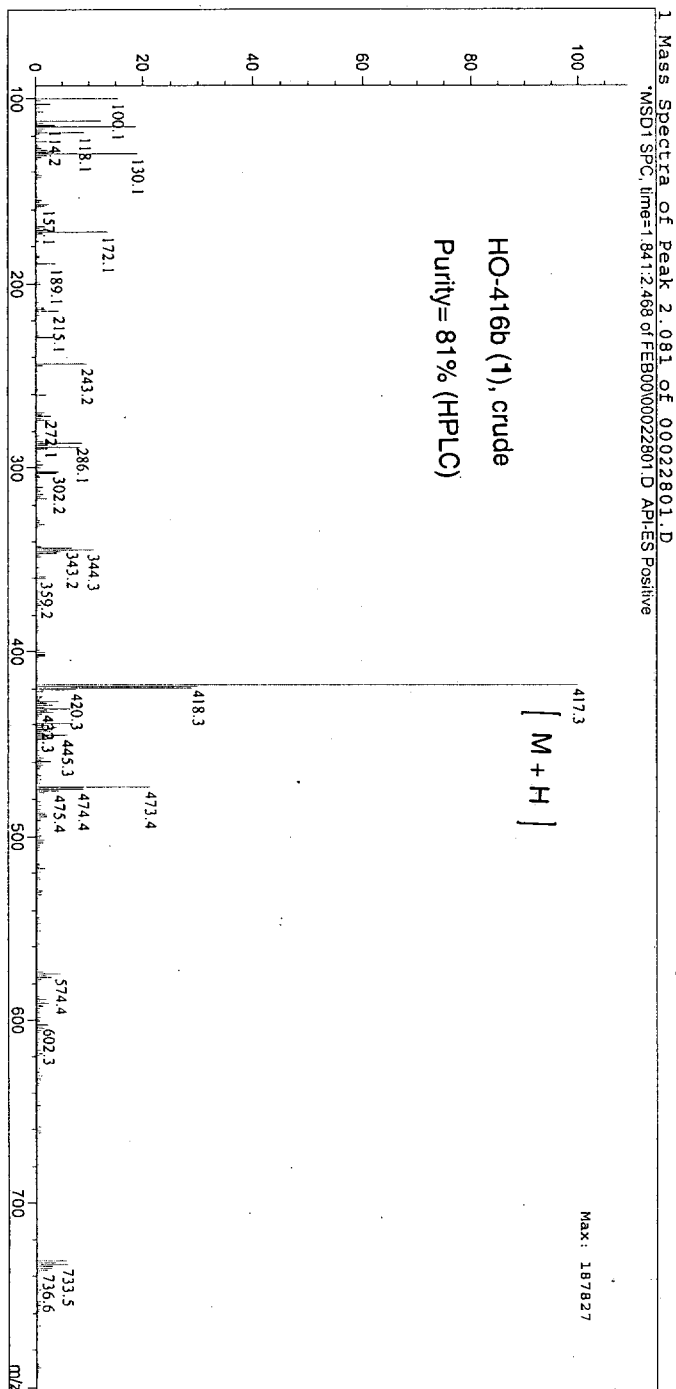


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 Fan Wang

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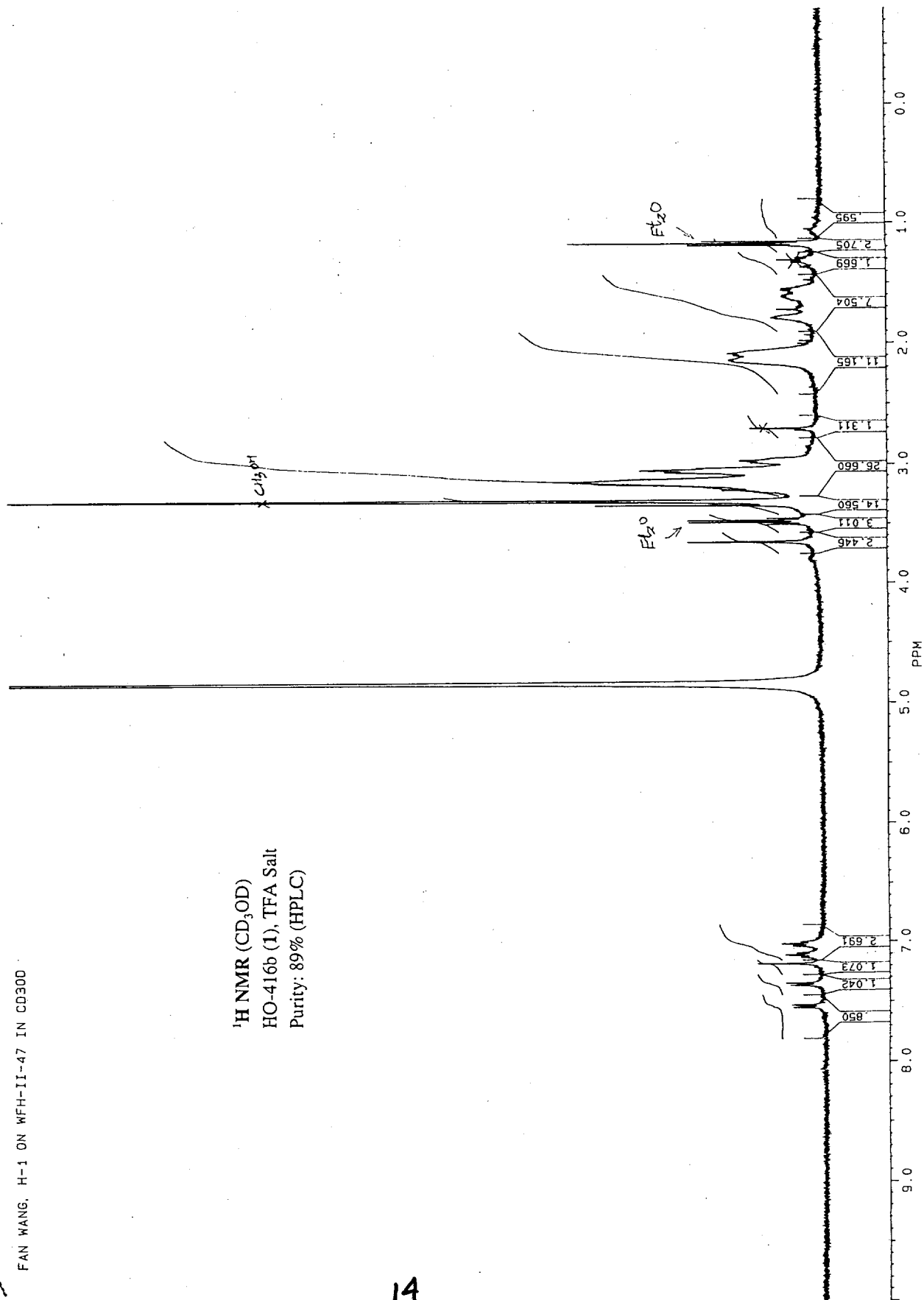
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3	1.720	81		120





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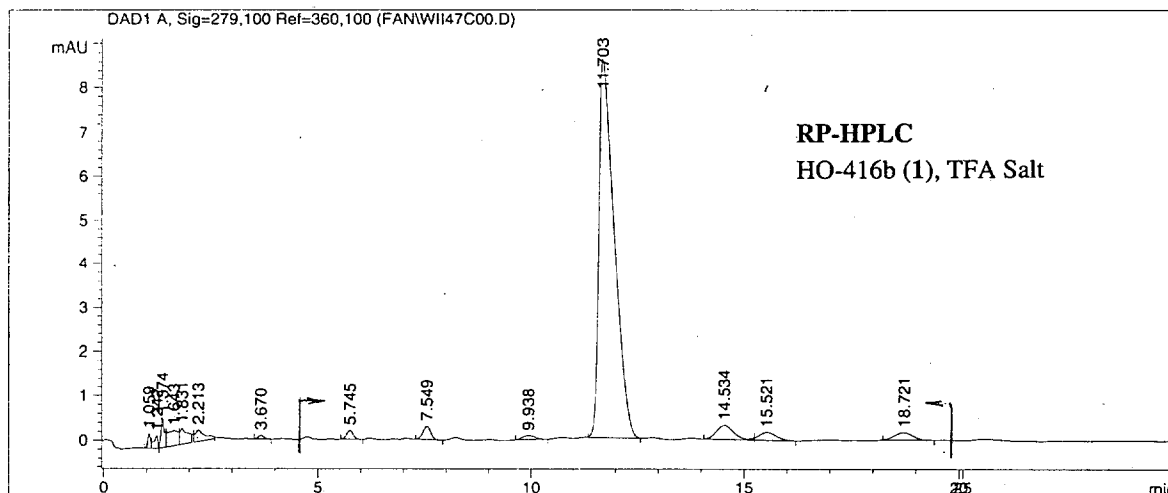


¹H NMR (CD₃OD)
 HO-416b (1), TFA Salt
 Purity: 89% (HPLC)

FAN WANG, H-1 ON WFH-II-47 IN CD300

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SB-C18 (4.6 X 150 mm, 5 µm), 12.5 % MeCN (0.1% TFA) and 87.5% water (0.1 % TFA) for 30
minutes; Flow rate 1.50 mL/min; Det. 279 nm.
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Area Percent Report
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Sorted By      : Signal
Multiplier     : 1.0000
Dilution       : 1.0000
    
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Purity = 214.56078/241.46072X100%= 89%

Signal 1: DAD1 A, Sig=279,100 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.059	PV	0.0627	1.42823	3.41830e-1	0.5413
2	1.242	VP	0.0895	1.79066	2.84265e-1	0.6786
3	1.374	VV	0.0802	3.75576	6.61398e-1	1.4233
4	1.643	VV	0.2125	5.86137	3.44469e-1	2.2213
5	1.831	VB	0.1630	4.68653	3.67038e-1	1.7761
6	2.213	BB	0.1968	4.15304	2.70946e-1	1.5739
7	3.670	PP	0.1187	7.34811e-1	8.68439e-2	0.2785
8	5.745	BP	0.1524	2.13680	2.07074e-1	0.8098
9	7.549	PP	0.1890	3.77129	3.03430e-1	1.4292
10	9.938	BP	0.2397	1.88940	9.43205e-2	0.7160
11	11.703	BP	0.3603	214.56078	8.62219	81.3127
12	14.534	PB	0.3414	9.13115	3.19572e-1	3.4605
13	15.521	BP	0.3279	4.91266	1.80300e-1	1.8618
14	18.721	BB	0.3514	5.05864	1.71873e-1	1.9171

Area = 241.46072

Totals : 263.87112 12.25554

Print of window 80: 1 Mass Spectra of Peak 2.883 of 99112601.D
Fan Wang

```

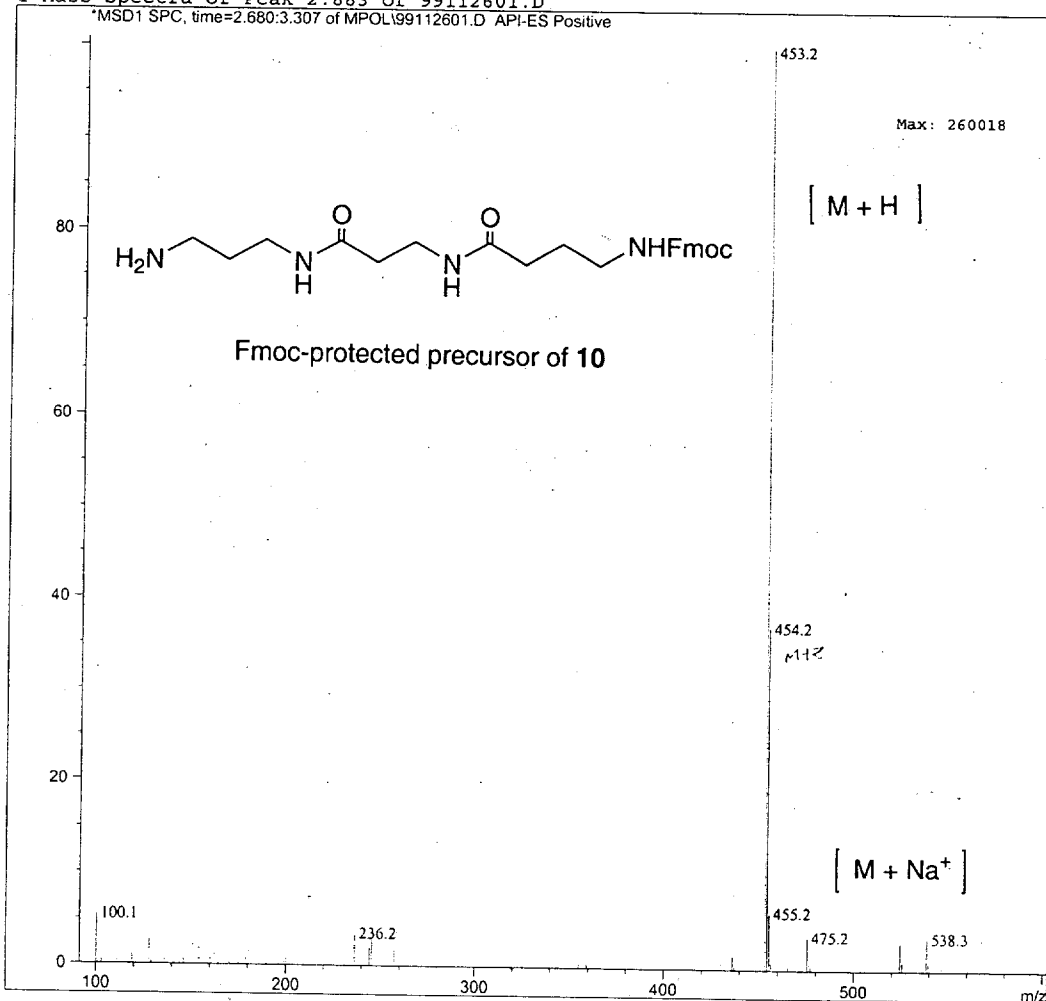
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Injection Date : 11/26/99 8:38:37 AM
Sample Name    : WFH-I-021
Acq. Operator  : Don
Vial           : FIA
Inj Volume    : 2 µl
Method        : D:\HPCHEM\1\METHODS\MEOHP.M
Last changed   : 11/26/99 8:27:25 AM by mike
                  (modified after loading)
    
```

MeOH 100uL/min

Flow Injections :

Inj.	InjTime [min]	Vial	FIA Sample Name	Fragmentor [V]
1	0.000	81		20
2	0.912	81		40
3	1.727	81		60
4	2.549	81		80

1 Mass Spectra of Peak 2.883 of 99112601.D



Print of window 80: 1 Mass Spectra of Peak 3.519 of 99121603.D

fan Wang

```

=====
Injection Date   : 12/16/99 2:14:37 PM
Sample Name     : WFH-I-067
Acq. Operator   : Don
Vial            : FIA
Inj Volume     : 2 µl

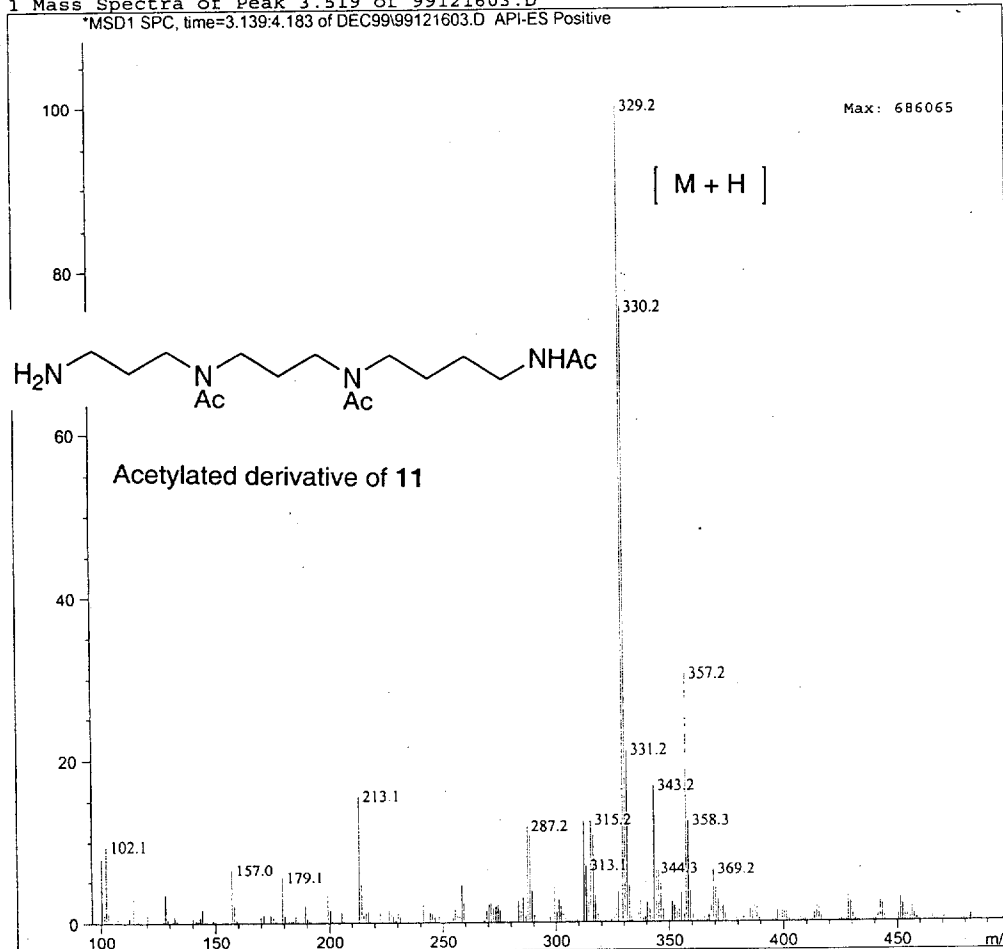
Acq. Method    : D:\HPCHEM\1\METHODS\MEOHP.M
Last changed   : 12/16/99 2:08:53 PM by Don
Analysis Method: D:\HPCHEM\1\METHODS\MEOHP.M
Last changed   : 12/16/99 2:19:29 PM by Don
                (modified after loading)
    
```

MeOH 100uL/min

Flow Injections :

Inj.	InjTime [min]	Vial	FIA Sample Name	Fragmentor [V]
1	0.000	81		20
2	1.113	81		40
3	2.120	81		60
4	3.132	81		80

1 Mass Spectra of Peak 3.519 of 99121603.D



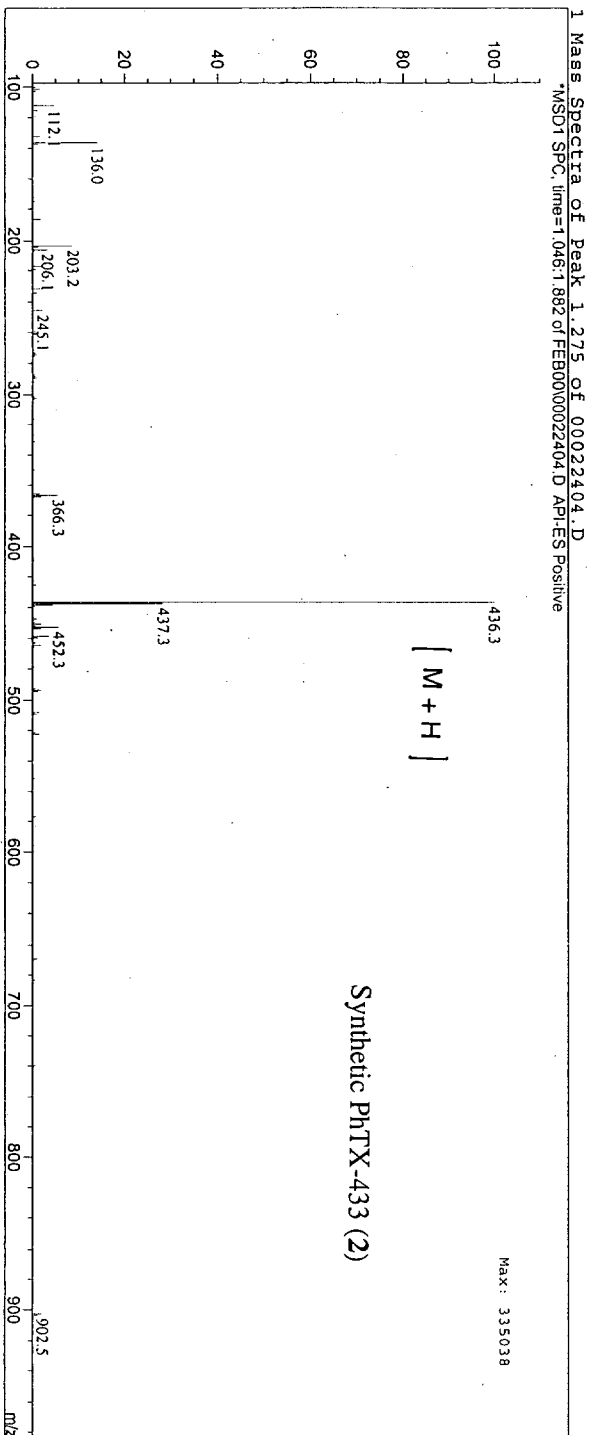
Print of window 80: 1 Mass Spectra of Peak 1.275 of 00022404.D
Fan Wang

=====
Injection Date : 2/24/00 11:04:15 AM
Sample Name : WPH-I-167B
Acq. Operator : Don
Vial : FIA
Inj Volume : 2 µl

Acq. Method : D:\HPCHEM\1\METHODS\MEOH.P.M
Last changed : 2/24/00 11:02:08 AM by Don
(modified after loading)
Analysis Method : D:\HPCHEM\1\METHODS\MEOH.P.M
Last changed : 2/24/00 11:22:42 AM by Don
(modified after loading)

MeOH 100ul/min
sample in MeOH
Flow Injections :

Inj.	InjTime [min]	Vial	FIA Sample Name	Fragmentor [V]
1	0.000	81		40
2	0.912	81		80
3	1.721	81		120



Print of window 80: 1 Mass Spectra of Peak 1.268 of 00022405.D
 Fan Wang

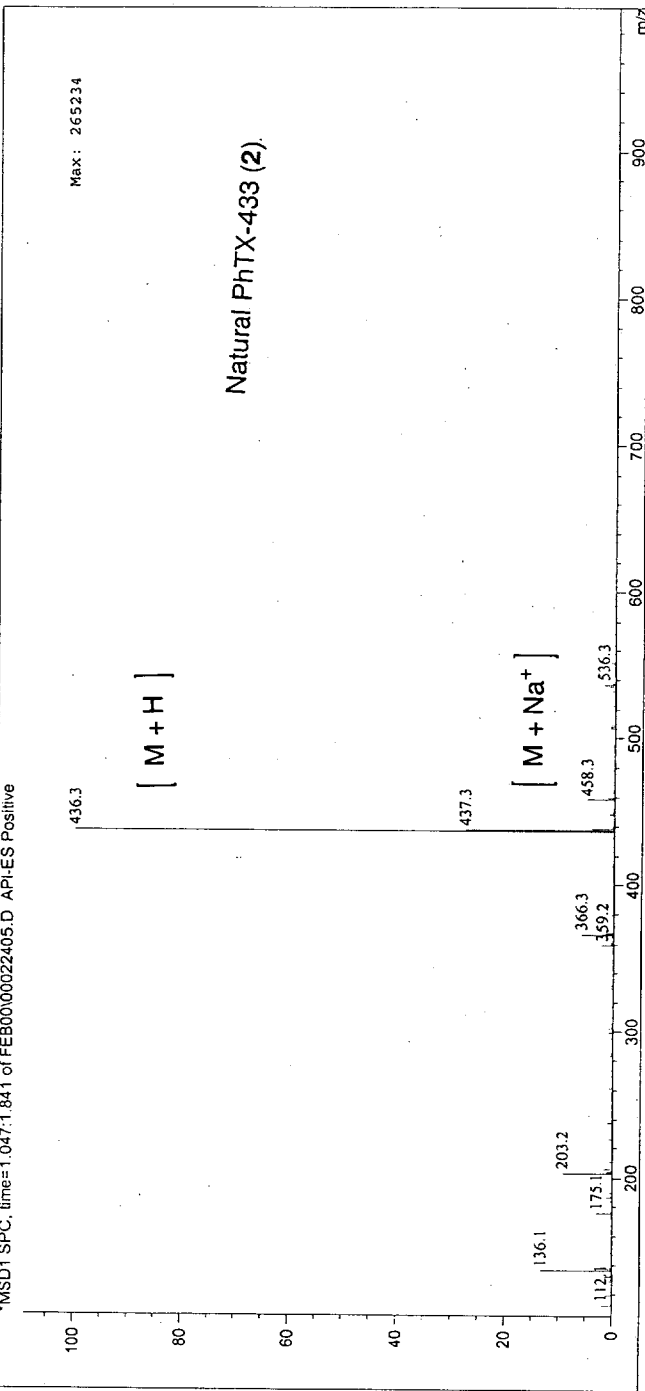
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=====
Injection Date : 2/24/00 11:25:43 AM
Sample Name    : WPH-KN-PhTX 433
Acq. Operator  : Don
Vial : FIA
Inj Volume : 2 µl
Method        : D:\HPCHEM\1\METHODS\MEOH.P.M
Last changed  : 2/24/00 11:22:42 AM by Don
                (modified after loading)
    
```

MeOH 100uL/min
 sample in MeOH
 Flow Injections :

Inj.	InjTime (min)	Vial	FIA	Sample Name	Fragmentor [V]
1	0.000	81			40
2	0.908	81			80
3	1.719	81			120

1 Mass Spectra of Peak 1.268 of 00022405.D
 MSDI SPC. time=1.047:1.841 of FEB00\00022405.D API-ES Positive

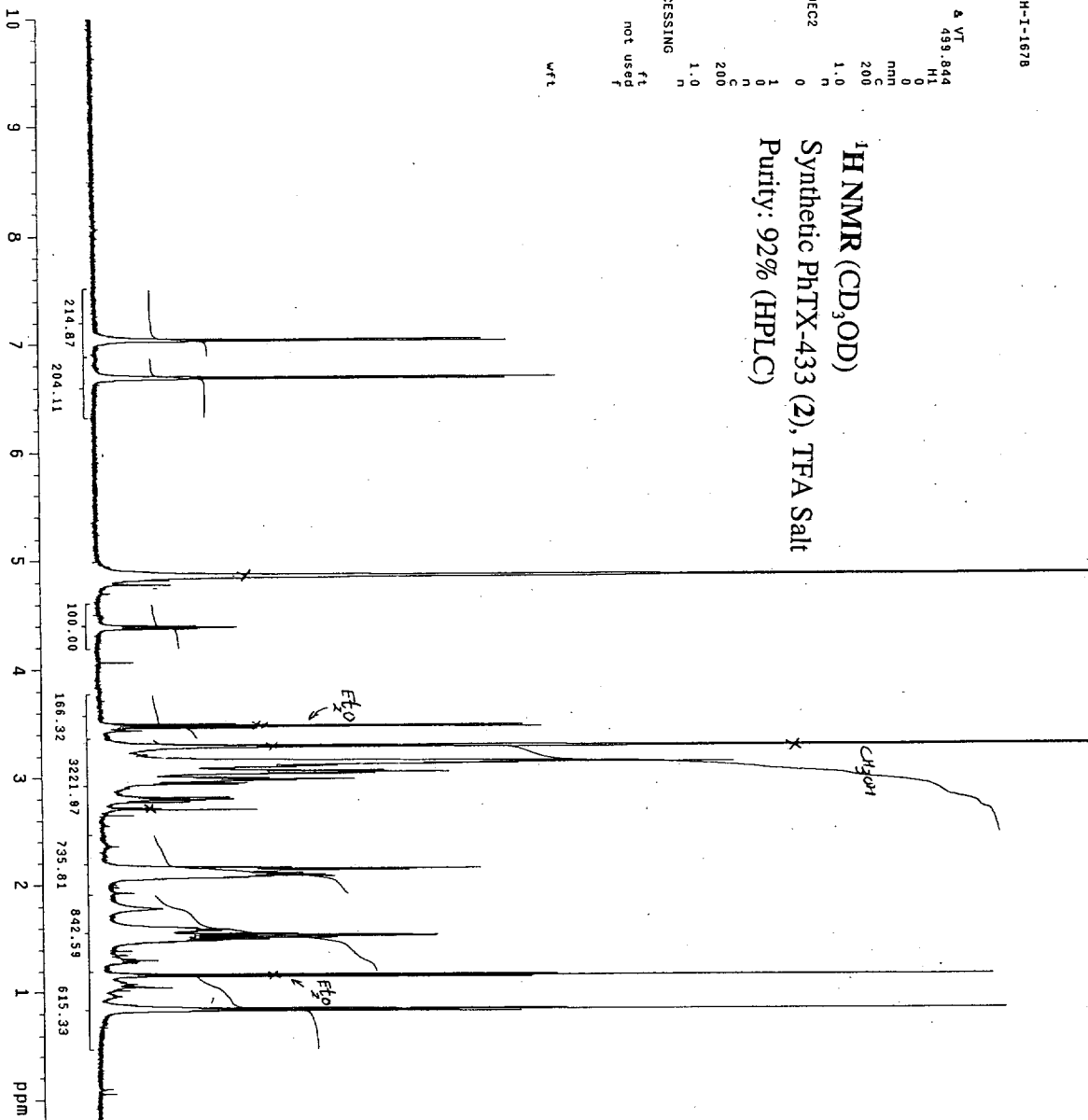


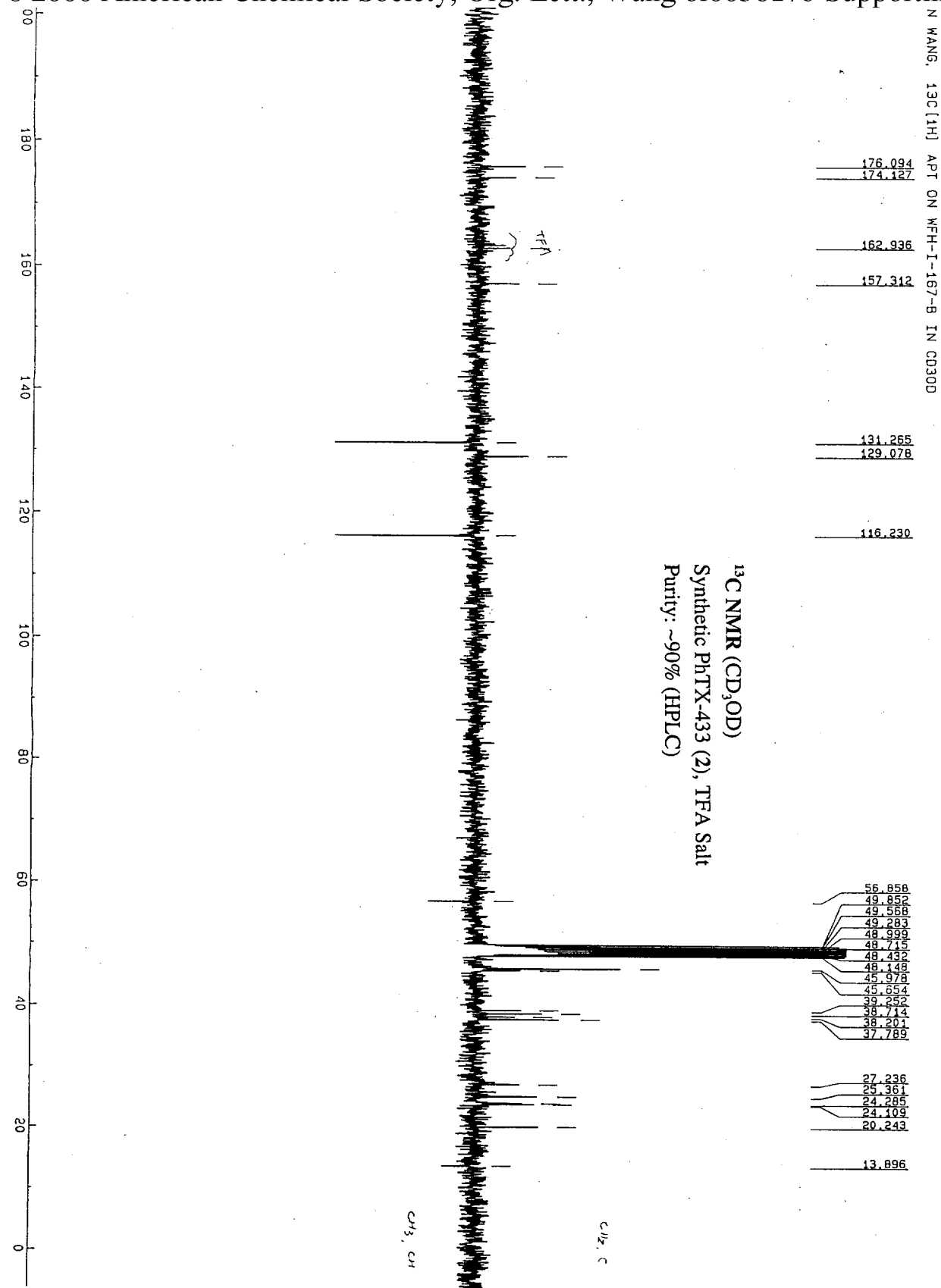
STANDARD PROTON PARAMETERS
 Fan Wang Proton on sample WFH-1-1678

```

expt s2pu1
SAMPLE Feb 28 00 dfrq DEC. & VT 499.844
date Feb 28 00 dfrq 499.844
solvent Feb CD3OD dn H1
116 km/4560 dn H1
116 km/4560 dn H1
labdate/MSCELLNAME CD3
ONS/7Feb25505h dm nnn
ACQUISITION dmm C
sfrq 499.844 dmf 200
ln H1 dseq 1.0
at 3.000 dres 1.0
np 48000 homo
sw 8000.0 dfrq2 DEC2 0
sb 4400 dn2
td 58 dpwr2 1
tpwr 4.0 ddf2 0
dl 0 dmm2 0
d1 0 ddf2 0
tof 32 dmf2 200
nt 32 dseq2
ct 32 dres2
alock not used n HOMO PROCESSING 1.0
galt not used n HOMO PROCESSING 1.0
ll n wfill n
in n proc n
dp y fn not used f
hs nm math
DISPLAY -100.1 wbrf
sp 5088.3 wbrf
vp 4988 wds
vc 0 wti
wc 200
h2mn 25.49
ls 1222.13
rfi 3158.8
rfp 1849.5
tms 100.000
nm cdc ph
  
```

¹H NMR (CD₃OD)
 Synthetic PhTX-433 (2), TFA Salt
 Purity: 92% (HPLC)



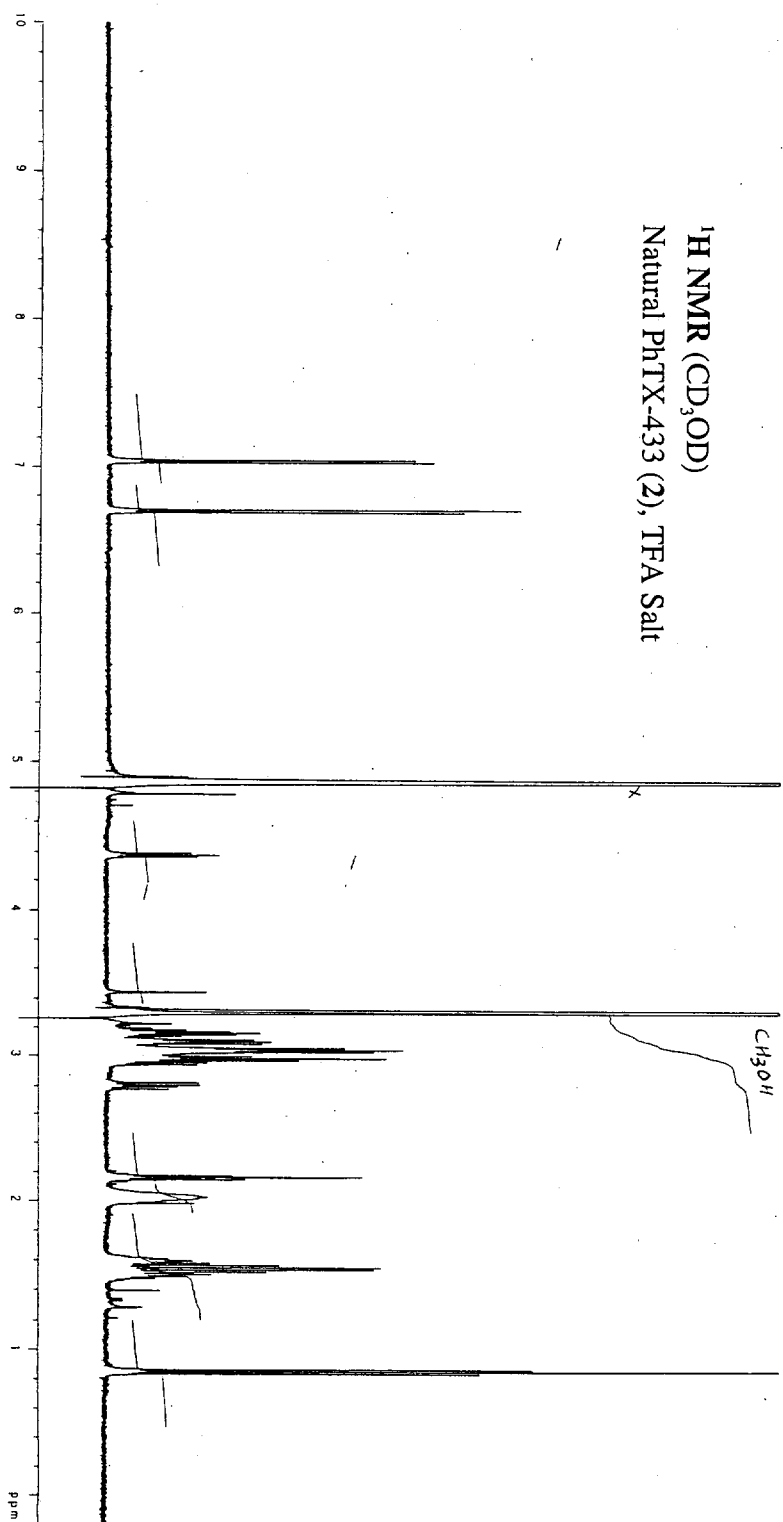


¹³C NMR (CD₃OD)
 Synthetic PhTX-433 (2), TFA Salt
 Purity: ~90% (HPLC)

MR09301C.001	SF	75.469
AU PR06:	SY	112.0
X25.AU	O1	7478.000
DATE 9-3-0	S1	32768
TIME 9:35	T0	32768
	SW	18518.519
	HZ/PT	1.130
	PW	0.0
	RD	0.0
	AG	.885
	RG	800
	NS	6032
	TE	297
	FW	23200
	O2	5595.000
	DP	18H CPD
	LB	2.000
	GB	.010
	CX	36.00
	CY	0.0
	E1	210.001P
	F2	-5.995P
	HZ/CM	452.803
	PPM/CM	6.000
	SR	-1214.88
	D1	1.0000000
	S1	18H
	P9	100.00
	D2	.0010000
	S2	18H
	P0	1.70
	D3	.0065000
	P6	8.40
	D4	.0010000
	RGA	0.0
	RD	0.0
	PW	35.30
	DE	6032
	NS	2
	DS	

STANDARD PROTON PARAMETERS
 FOR NMR PROTON ON SAMPLE W-404-N-1A-33
 exp1 12jul

SAMPLE	Feb 28 00	dlq	DEC 4 VI
date	Feb 28 00	dlq	489 844
solvent	CD3OD	en	H1
file	/mnt/4800/mw-489		0
name	005/1203507b	dlq	0
date	05/12/2007		000
ACQUISITION	dm		200
freq	489.844	dm	200
nu	489.844	dm	200
nu1	489.844	dm	200
nu2	489.844	dm	200
nu3	489.844	dm	200
nu4	489.844	dm	200
nu5	489.844	dm	200
nu6	489.844	dm	200
nu7	489.844	dm	200
nu8	489.844	dm	200
nu9	489.844	dm	200
nu10	489.844	dm	200
nu11	489.844	dm	200
nu12	489.844	dm	200
nu13	489.844	dm	200
nu14	489.844	dm	200
nu15	489.844	dm	200
nu16	489.844	dm	200
nu17	489.844	dm	200
nu18	489.844	dm	200
nu19	489.844	dm	200
nu20	489.844	dm	200
nu21	489.844	dm	200
nu22	489.844	dm	200
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nu25	489.844	dm	200
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nu28	489.844	dm	200
nu29	489.844	dm	200
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nu35	489.844	dm	200
nu36	489.844	dm	200
nu37	489.844	dm	200
nu38	489.844	dm	200
nu39	489.844	dm	200
nu40	489.844	dm	200
nu41	489.844	dm	200
nu42	489.844	dm	200
nu43	489.844	dm	200
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nu46	489.844	dm	200
nu47	489.844	dm	200
nu48	489.844	dm	200
nu49	489.844	dm	200
nu50	489.844	dm	200
nu51	489.844	dm	200
nu52	489.844	dm	200
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nu56	489.844	dm	200
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nu72	489.844	dm	200
nu73	489.844	dm	200
nu74	489.844	dm	200
nu75	489.844	dm	200
nu76	489.844	dm	200
nu77	489.844	dm	200
nu78	489.844	dm	200
nu79	489.844	dm	200
nu80	489.844	dm	200
nu81	489.844	dm	200
nu82	489.844	dm	200
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nu86	489.844	dm	200
nu87	489.844	dm	200
nu88	489.844	dm	200
nu89	489.844	dm	200
nu90	489.844	dm	200
nu91	489.844	dm	200
nu92	489.844	dm	200
nu93	489.844	dm	200
nu94	489.844	dm	200
nu95	489.844	dm	200
nu96	489.844	dm	200
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nu99	489.844	dm	200
nu100	489.844	dm	200



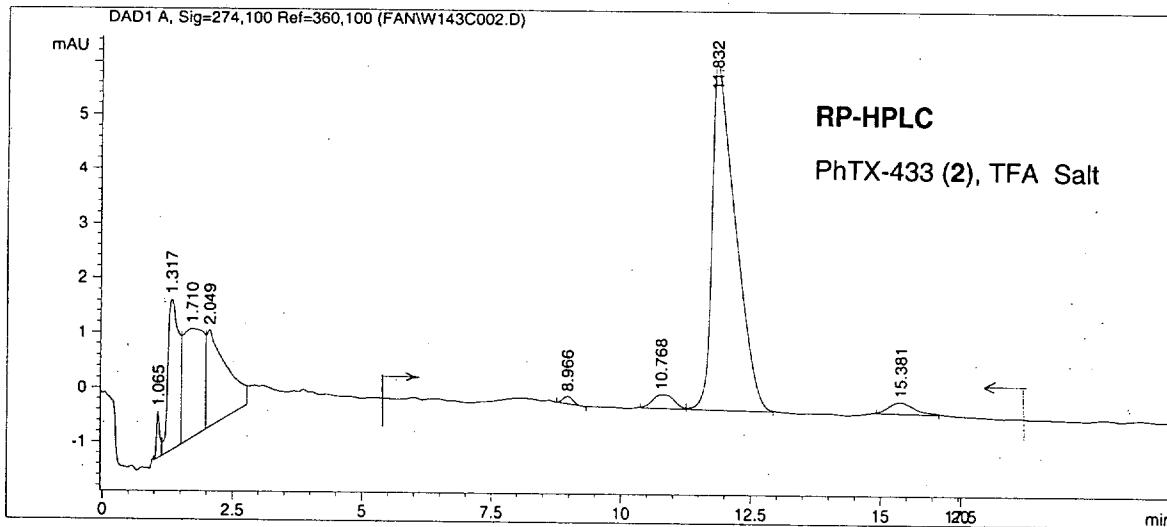
Data File C:\HPCHEM\1\DATA\FAN\W143C002.D

Sample Name: WFH-I-143C

Injection Date : 1/28/00 10:59:26 AM
 Sample Name : WFH-I-143C Vial : 1
 Acq. Operator : Fan Inj Volume : 5 µl

Method : C:\HPCHEM\1\METHODS\OLIGOA-1\SPIDER.M
 Last changed : 1/28/00 10:34:32 AM by Fan
 (modified after loading)

SB-C18 (4.6 X 150 mm, 5 µm), 10.0 % MeCN (0.1% TFA) and 90.0 % water (0.1 % TFA) for 20 minutes; Flow rate 1.50 mL/min; Det. 274 nm. Column temperature: 20 C.



Area Percent Report

Sorted By : Signal
 Multiplier : 1.0000
 Dilution : 1.0000

Purity= 198.6078/215.5185X100%= 92%

Signal 1: DAD1 A, Sig=274,100 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.065	VV	0.0596	3.51574	8.61245e-1	0.9750
2	1.317	VV	0.2086	40.08689	2.77405	11.1166
3	1.710	VV	0.3448	54.66890	2.00064	15.1604
4	2.049	VB	0.3260	46.81400	1.78497	12.9821
5	8.966	PP	0.2000	1.79255	1.37546e-1	0.4971
6	10.768	BV	0.3721	7.59059	2.55888e-1	2.1050
7	11.832	VB	0.4229	198.60780	6.42105	55.0764
8	15.381	BB	0.4317	7.52757	2.14335e-1	2.0875

Area= 215.5185

Totals : 360.60404 14.44973

Results obtained with enhanced integrator!

*** End of Report ***

smh-IV-23 6 march 2000

Pulse Sequence: szpu1

Solvent: cd3od
Temp: 27.5 C / 300.6 K
INOVA-300 "1300"

PULSE SEQUENCE

Relax: delay 3.000 sec

Pulse: 77.1 degrees

Acq: time 1.994 sec

Width 3001.2 Hz

16 repetitions

DSERVE H1, 299.9597708 MH

DECUPLE H1, 299.9612155 MH

Power 3 db

off during acquisition

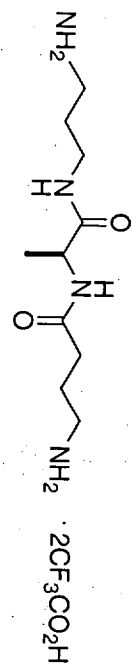
on during delay

single frequency

DATA PROCESSING

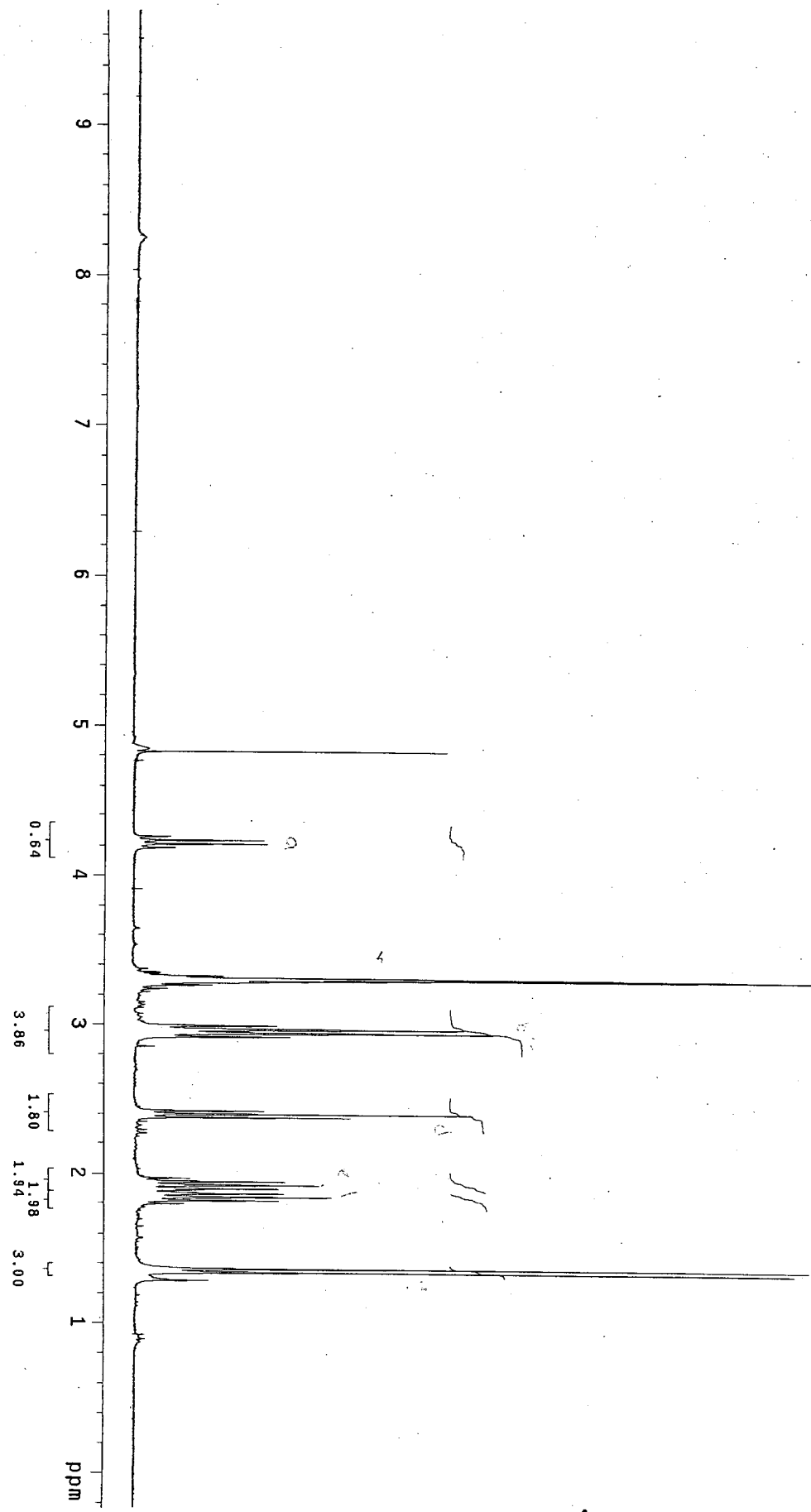
FT size 65536

Total time 1 min, 30 sec



14

¹H NMR (300 MHz, CD₃OD) of 14



```

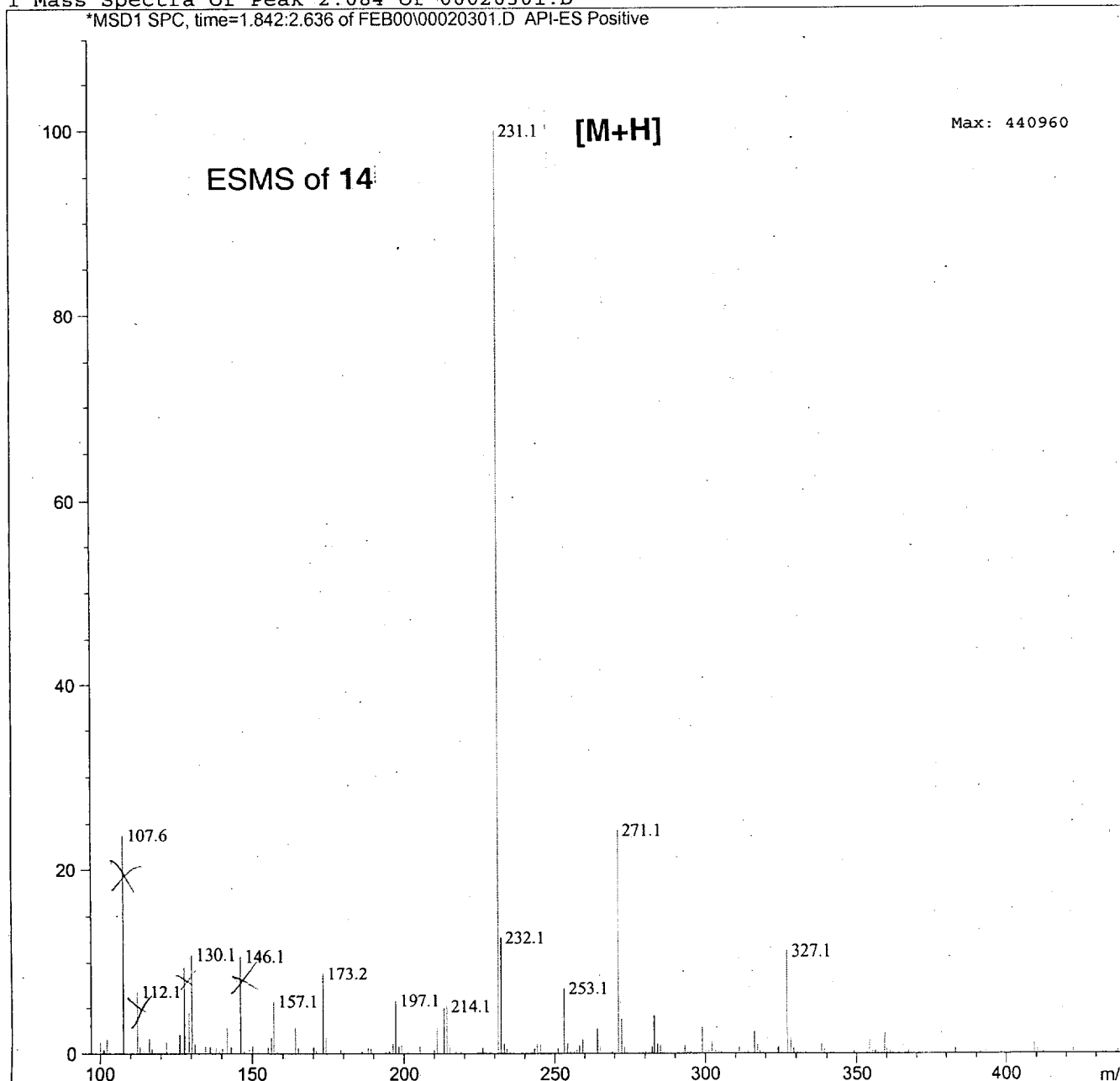
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Injection Date : 2/3/00 9:32:10 AM
Sample Name    : SMH (IV) 23
Acq. Operator  : Don
Method         : D:\HPCHEM\1\METHODS\MEOHP.M
Last changed   : 2/3/00 9:30:25 AM by Don
                (modified after loading)
Vial           : -
Inj Volume     : 2 µl
    
```

MeOH 100uL/min

Flow Injections :

Inj.	InjTime [min]	Vial	FIA Sample Name	Fragmentor [V]
1	0.000	81		20
2	0.913	81		40
3	1.722	81		60
4	2.529	81		80

1 Mass Spectra of Peak 2.084 of 00020301.D
 *MSD1 SPC, time=1.842:2.636 of FEB00\00020301.D API-ES Positive



=====

Printed on: 2/8/00 5:41:53 PM

Vial : FIA

Injection Date : 2/8/00 5:41:53 PM

Sample Name : SMH(IV)31

Operator : Inj Volume : 2 µl

=====

Method : D:\HPCHEM\1\METHODS\MEOH.P.M

Changed : 2/8/00 5:35:48 PM by Don

(modified after loading)

Analysis Method : D:\HPCHEM\1\METHODS\MEOH.P.M

Changed : 2/8/00 5:46:02 PM

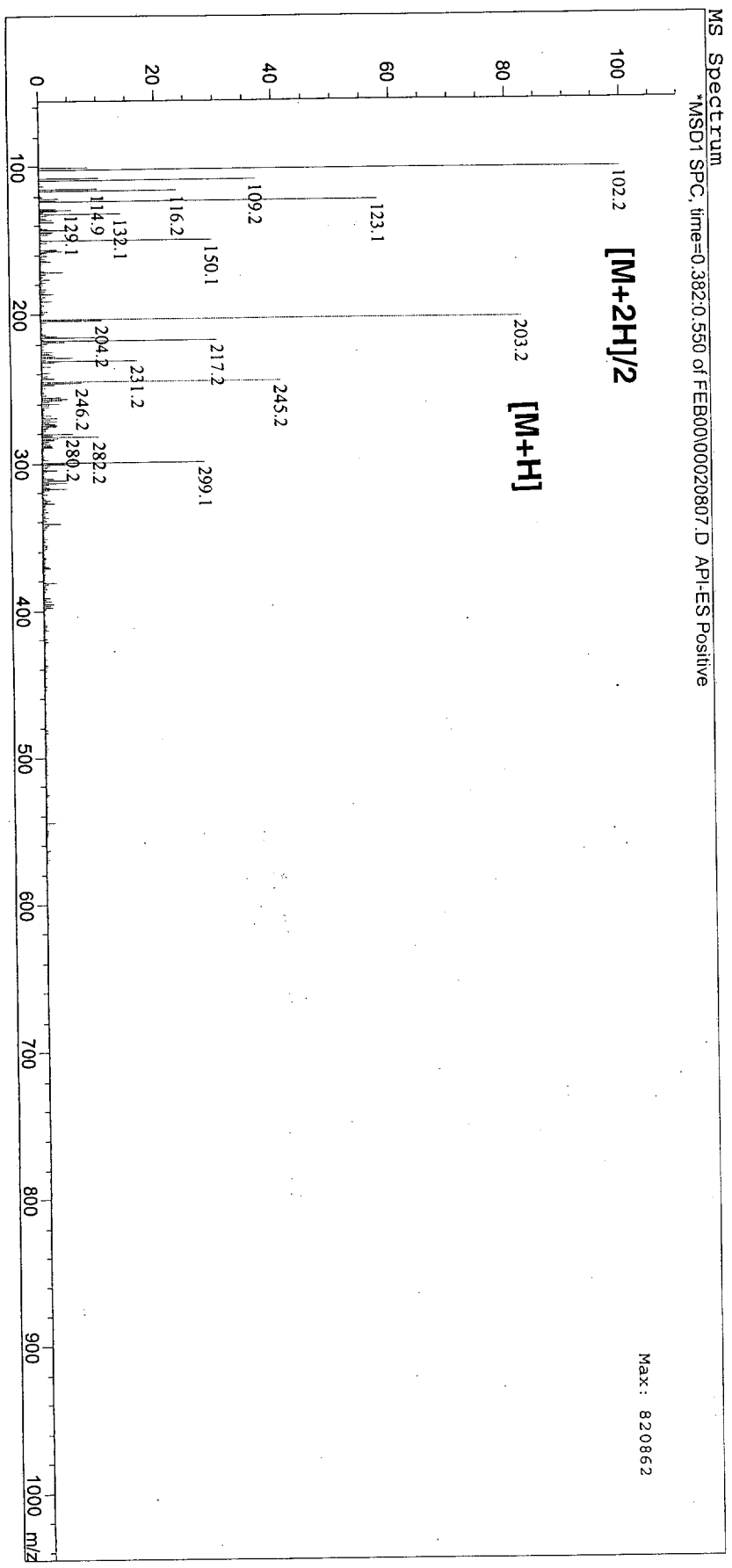
(modified after loading)

Flow Rate : 100 µL/min

Fl Injections :

Inj	Time [min]	Vial	FIA Sample Name	Fragmentor [V]
1	0.000	81		40
2	0.907	81		80
3	1.718	81		120

ESMS of 15



Injection Date : 2/9/00 5:38:05 PM

Vial : FIA

Sample Name : SMH(IV) 36

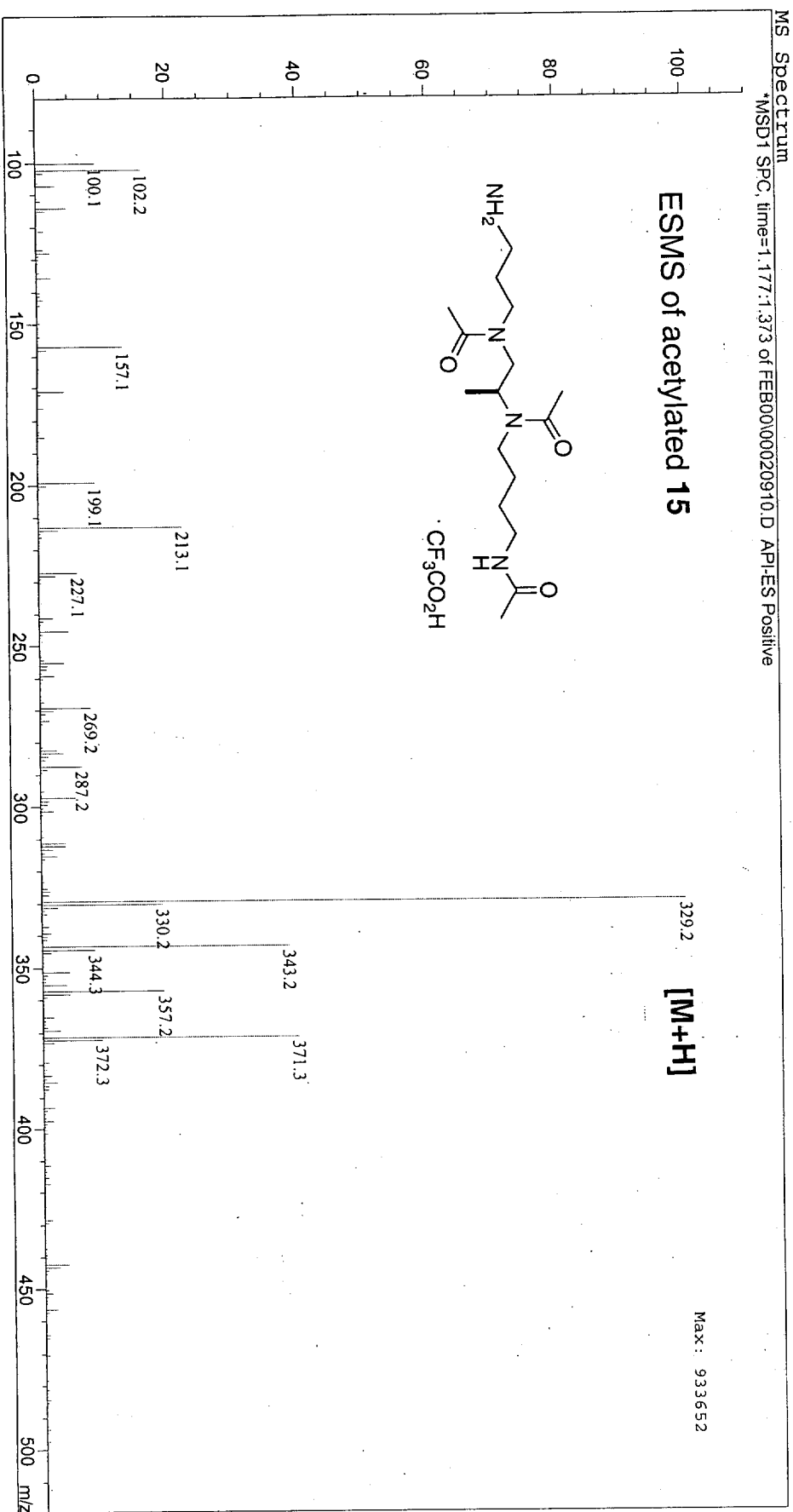
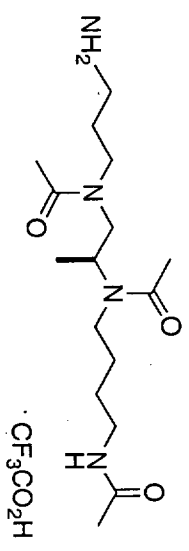
Inj Volume : 2 µl

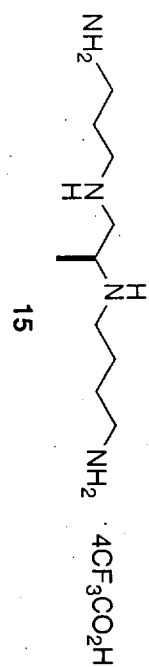
Operator : Don
Method : D:\HPCHEM\1\METHODS\MEOH.P
Label changed : 2/9/00 5:35:58 PM by Don
(modified after loading)

Flow Rate : 100µL/min

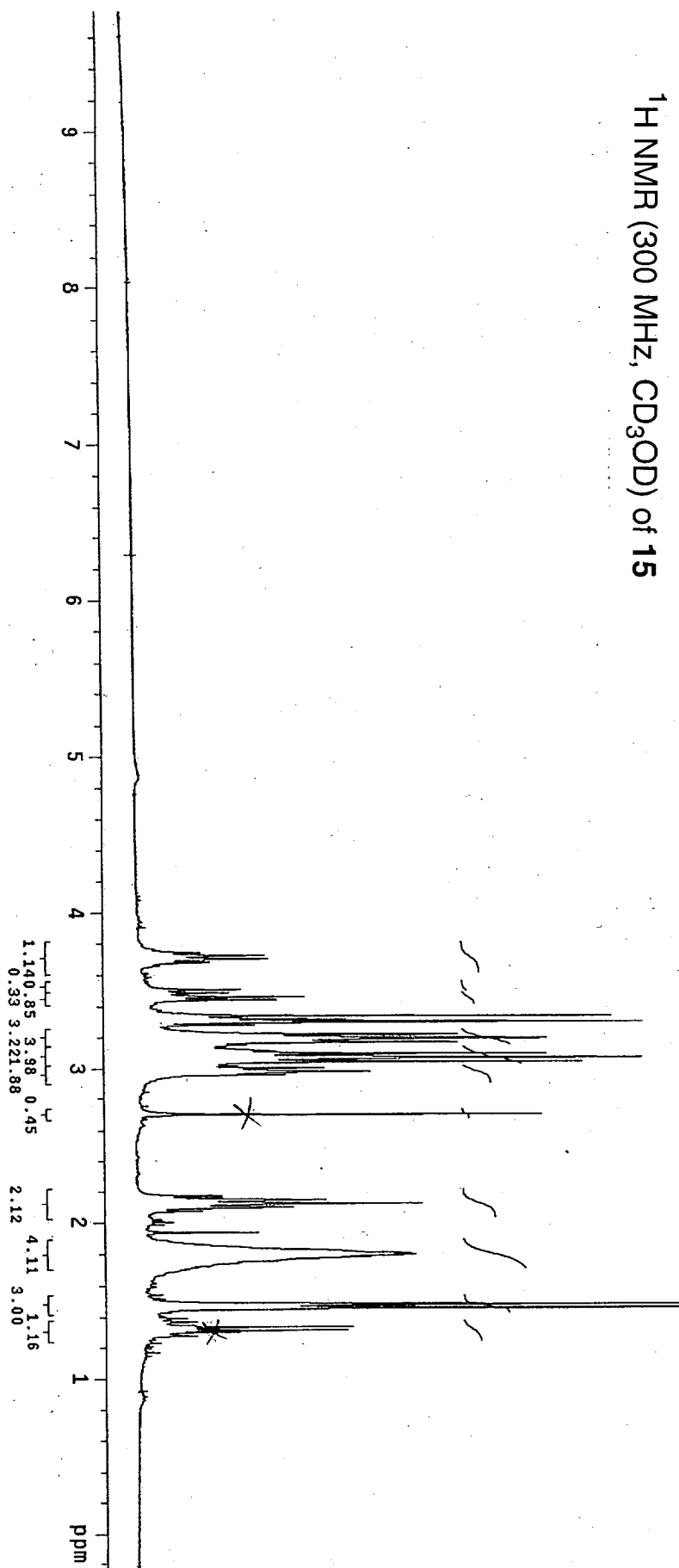
Injections	Inj. InjTime [min]	Vial	FIA Sample Name	Fragmentor [V]
1	0.000	81		40
2	0.907	81		80

ESMS of acetylated 15

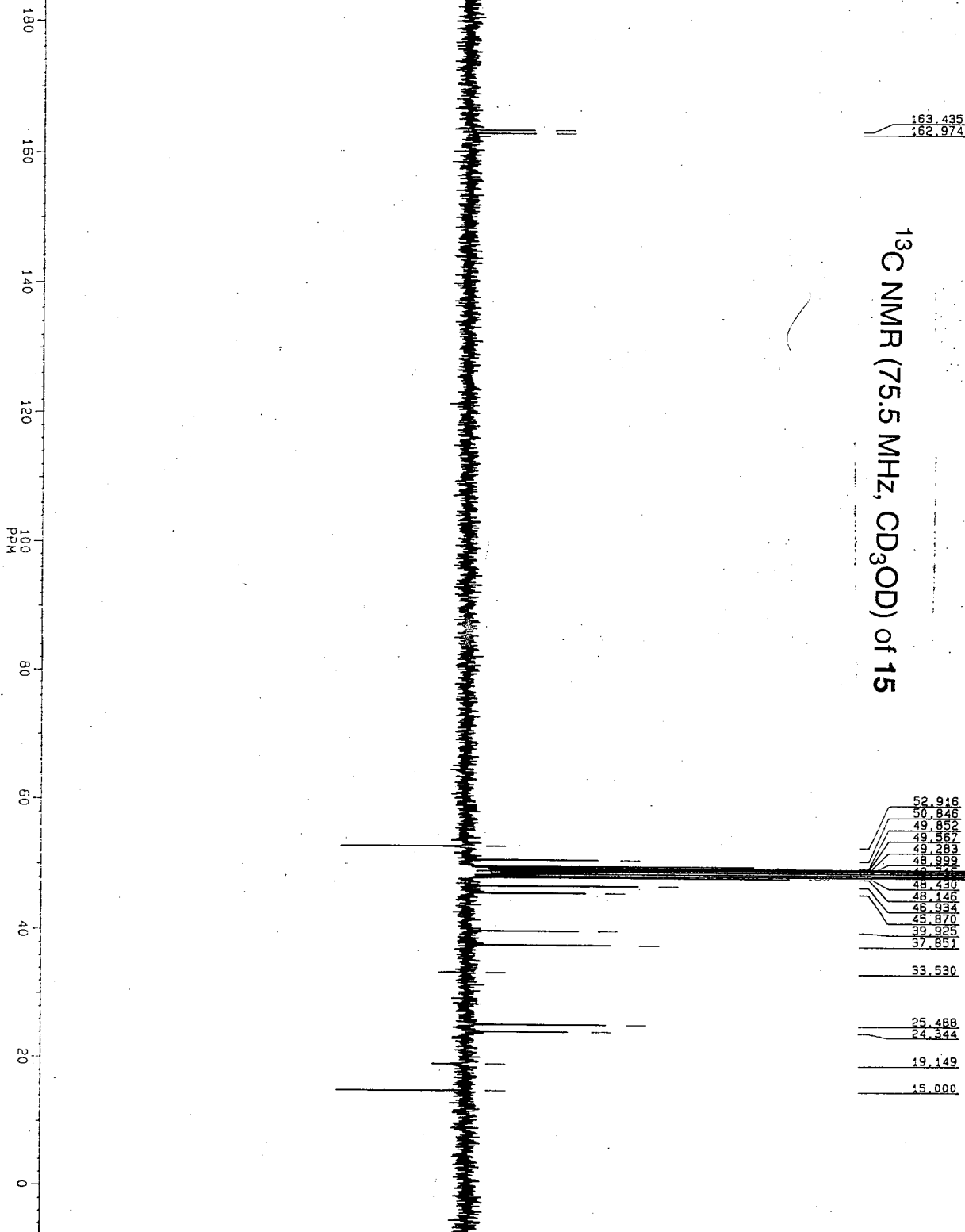




¹H NMR (300 MHz, CD₃OD) of 15



¹³C NMR (75.5 MHz, CD₃OD) of 15



162.974
153.435

52.916
50.846
49.852
49.567
49.283
48.999
48.717
48.430
48.146
46.934
45.870
39.925
37.851

33.530

25.488
24.344

19.149

15.000



MR090F.102
AU PR06:
X251.AU
DATE 9-3-0
TIME 6:13

SF 75.469
SY 112.0
O1 7478.000
SI 32768
TD 32768
SM 18518.519
HZ/PT 1.130

PW 0.0
RD 0.0
AQ .885
RG 800
NS 10000
TE 297

FM 23200
O2 3395.000
DP 18H CPD

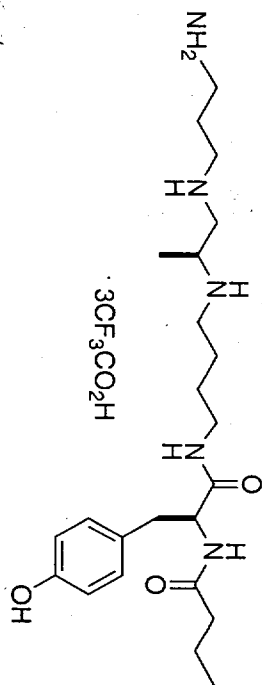
LB 1.200
GB 0.0
CX 36.00
CY 0.0

F1 208.099P
F2 -7.987P
HZ/CM 452.803
PPM/CM 6.000
SR -1214.68

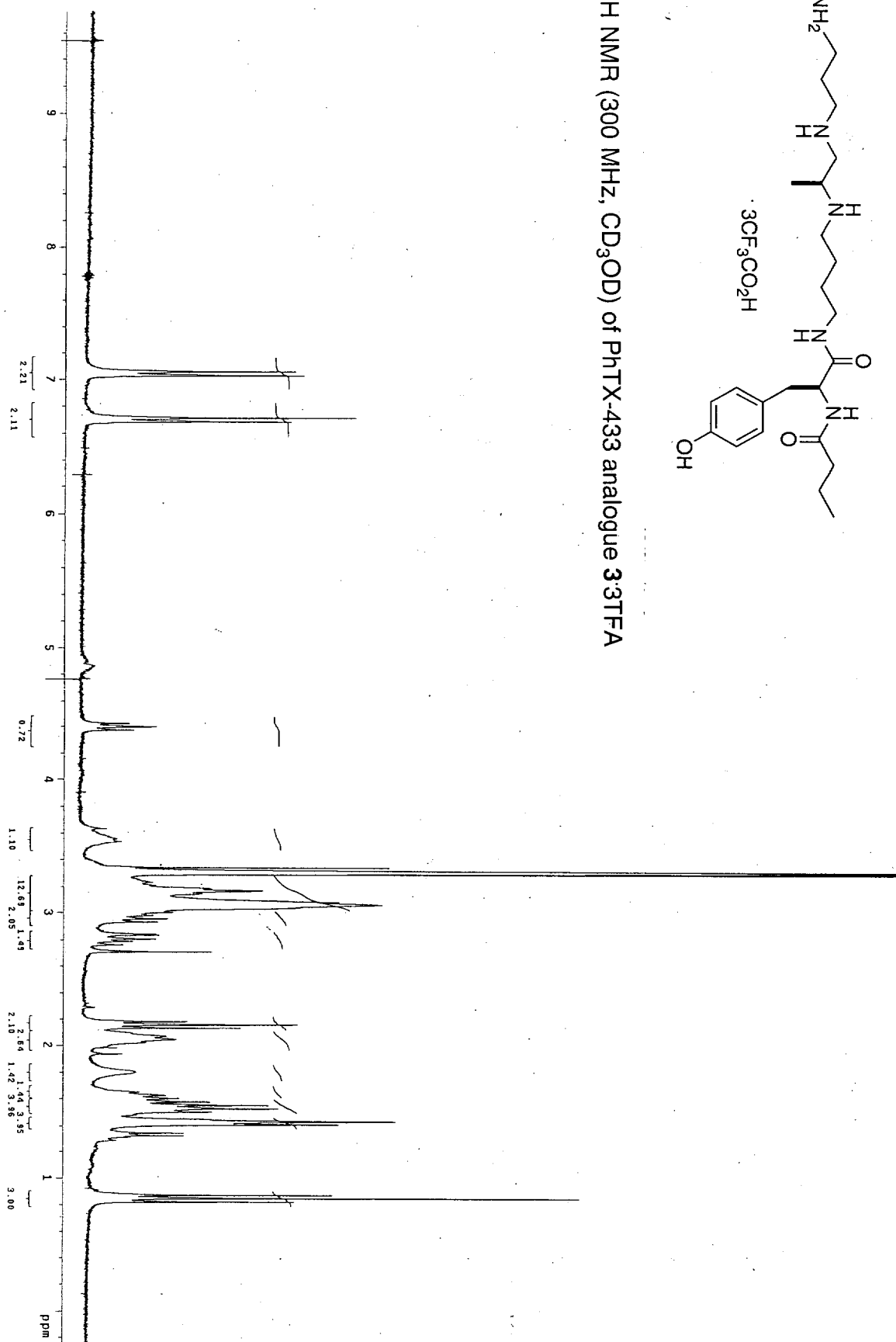
D1 1.0000000
S1 18H
P9 100.00
D2 0010000
S2 18H

P0 1.70
D3 .005000
P8 8.40
D4 .0010000
RGA

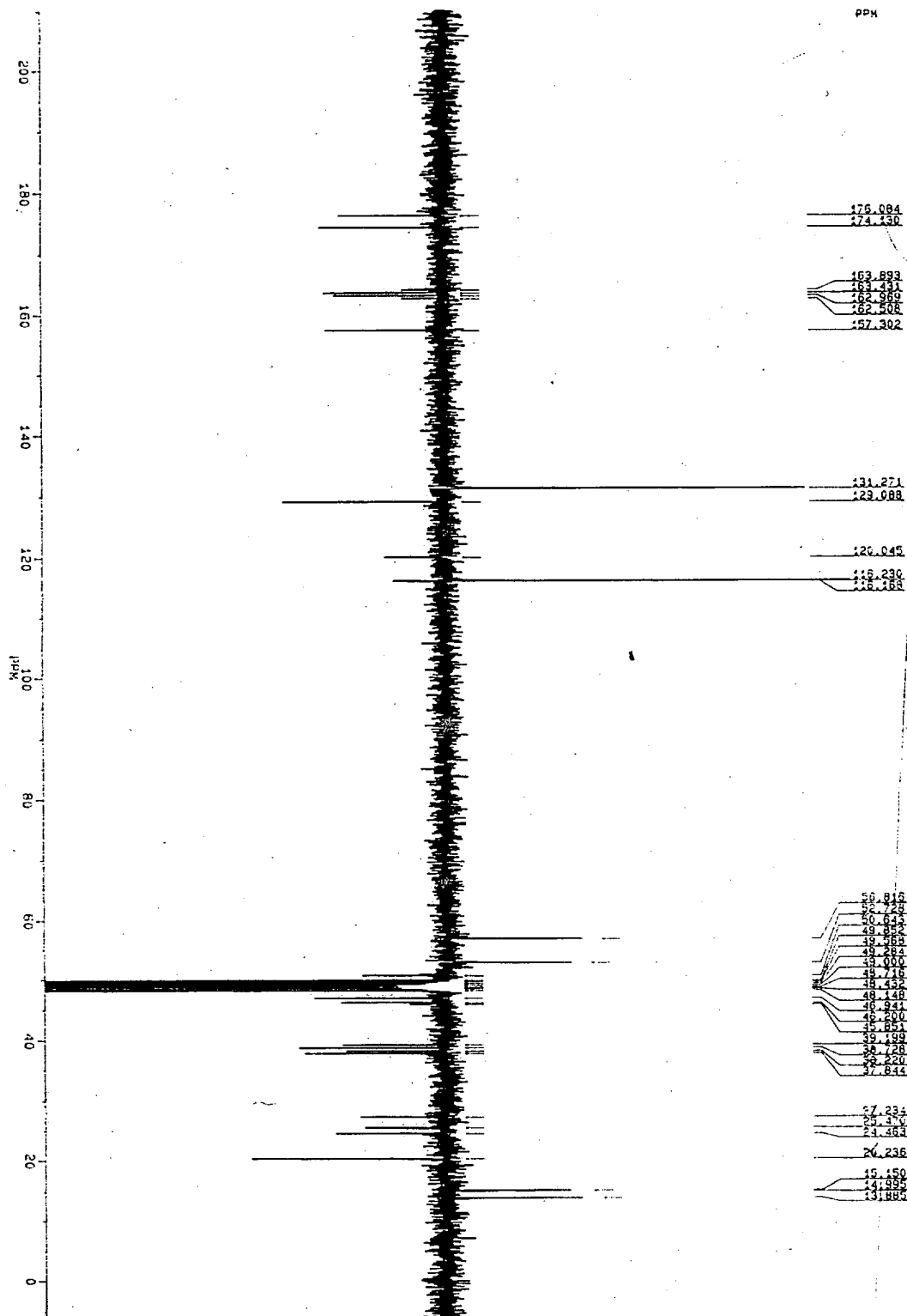
RD 0.0
PW 0.0
DE 36.30
NS 10000
DS 2



¹H NMR (300 MHz, CD₃OD) of PhTX-433 analogue 33TFA



¹³C NMR (75.5 MHz, CD₃OD) of PhTX-433 analogue 3-3TFA



BIOXIA
 FE230F.101
 AU PRGR: X25.AU
 DATE 23-2-0
 TIME 21:49
 SF 112.75.459
 SV 112.75.000
 F1 63926
 F2 55205
 F3 55205.513
 HZ/PI 15218.1805
 RX 0
 RD 0.0
 A2 1.88E
 HG H3
 NS 6000
 TE 297
 F4 23200
 F2 5595.000
 DP 18H CPD
 L8 1.000
 L9 0.0
 L0 0.0
 L1 56.05
 L2 210.00E
 L3 153.885E
 L4 452.000
 L5 452.000
 SM -121.112
 D1 2.0000000
 S1 18H
 P9 100.00
 D2 0.010000
 S2 18H
 P5 1.76
 O3 .0085000
 P5 8.40
 D4 .0010000
 RGA
 RD 0.0
 HD 0.0
 FW 36.39
 GC 6000
 MS 2

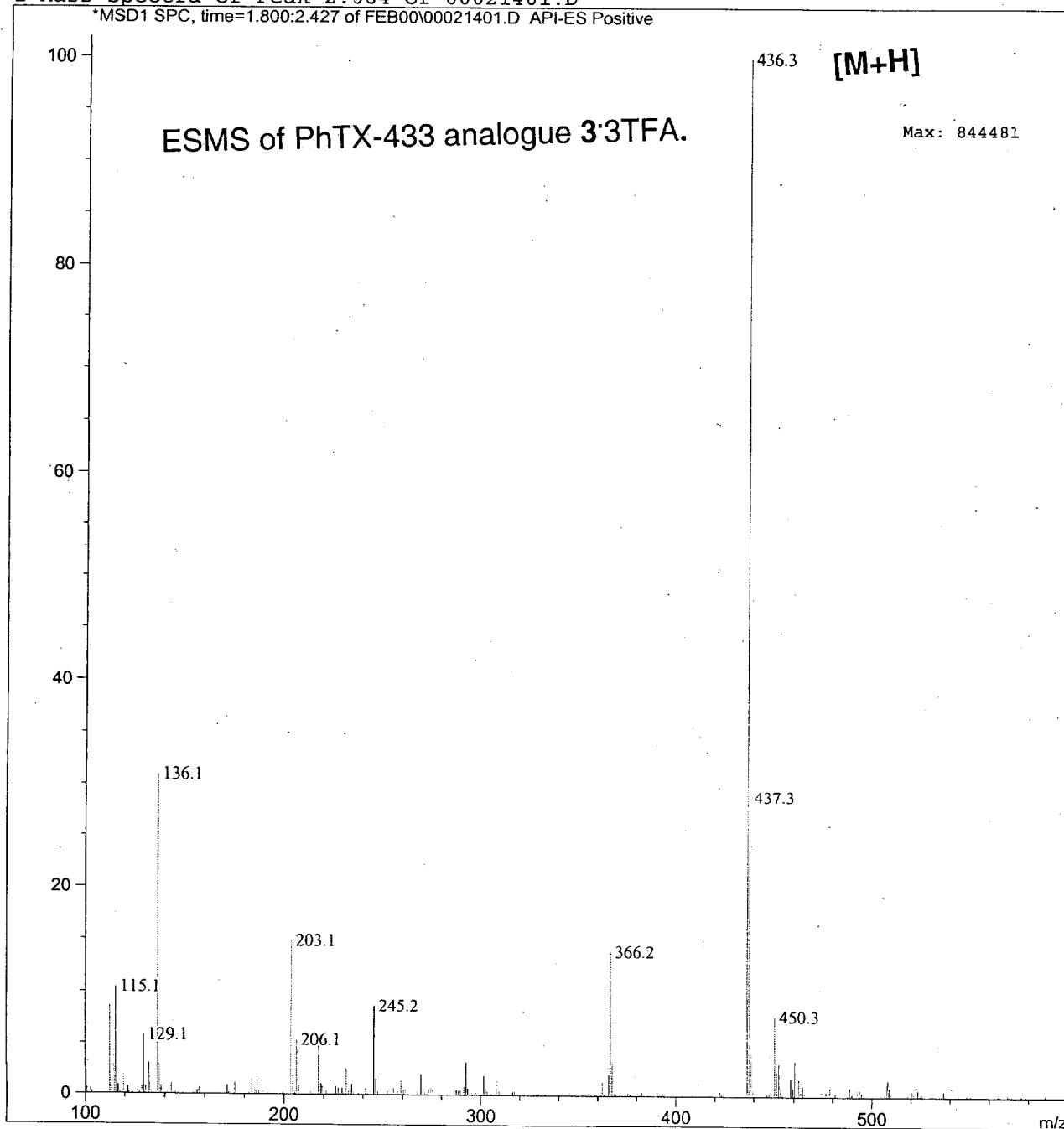
Injection Date : 2/14/00 11:31:19 AM
Sample Name : SMH (IV) 41 Vial : FIA
Acq. Operator : Don Inj Volume : 2 µl
Method : D:\HPCHEM\1\METHODS\MEOHP.M
Last changed : 2/14/00 11:29:01 AM by Don
(modified after loading)

MeOH 100uL/min

Flow Injections :

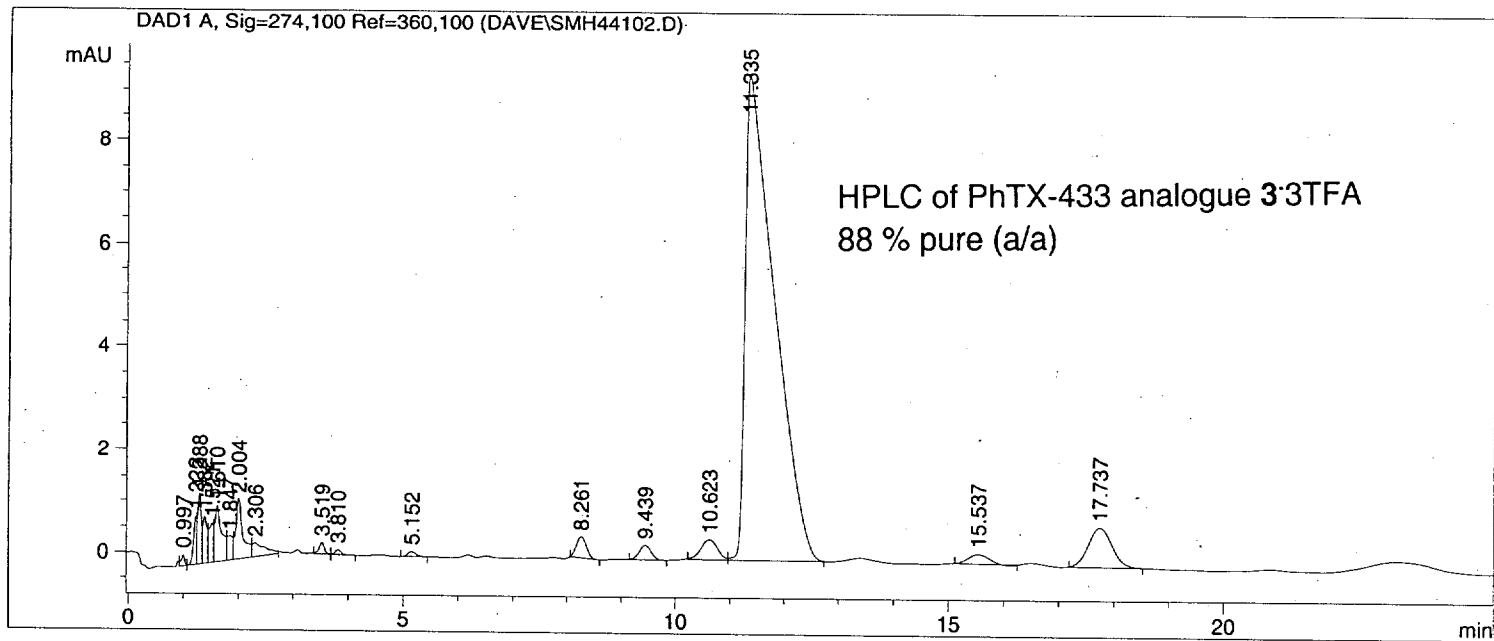
Inj.	InjTime [min]	Vial	FIA Sample Name	Fragmentor [V]
1	0.000	81		40
2	0.909	81		60
3	1.716	81		80

1 Mass Spectra of Peak 2.064 of 00021401.D



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 Injection Date : 3/8/00 3:40:30 PM
 Sample Name : SMH-IV-41 Vial : 1
 Acq. Operator : Dave Inj Volume : 3 µl
 Method : C:\HPCHEM\1\METHODS\OLIGOA~1\SPIDER.M
 Last changed : 3/8/00 3:39:59 PM by Dave
 (modified after loading)

SB-C18 (4.6 X 150 mm, 5 µm), 10.0 % MeCN (0.1% TFA) and 90.0 % water (0.1 % TFA) for 20 minutes; Flow rate 1.50 mL/min; Det. 274 nm.
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 Area Percent Report
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Sorted By : Signal
 Multiplier : 1.0000
 Dilution : 1.0000

Signal 1: DAD1 A, Sig=274,100 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.997	VV	0.0613	9.59547e-1	2.18396e-1	0.2153
2	1.223	VV	0.0572	3.59909	9.29570e-1	0.8075
3	1.288	VV	0.0651	6.24334	1.37078	1.4007
4	1.382	VV	0.0743	4.83231	9.04669e-1	1.0841
5	1.521	VV	0.0809	4.45011	7.53612e-1	0.9984
6	1.610	VV	0.1190	9.51404	1.07776	2.1345
7	1.847	VV	0.1041	3.29648	4.57337e-1	0.7396
8	2.004	VV	0.1249	10.44565	1.16208	2.3435
9	2.306	VB	0.2081	4.23864	2.60144e-1	0.9510
10	3.519	BV	0.1003	1.45094	2.16165e-1	0.3255
11	3.810	VP	0.1133	6.97699e-1	8.91485e-2	0.1565
12	5.152	PB	0.1380	9.60853e-1	9.34538e-2	0.2156
13	8.261	BP	0.1987	5.05943	3.97078e-1	1.1351
14	9.439	BP	0.2133	4.21318	2.71073e-1	0.9452
15	10.623	BV	0.2681	8.10489	3.78021e-1	1.8184
16	11.335	VB	0.4861	349.57321	9.43930	78.4280
17	15.537	BP	0.3096	4.80211	1.86988e-1	1.0774
18	17.737	BB	0.3898	23.28341	7.63509e-1	5.2237