

Short Total Synthesis of (±)-Sceptrin

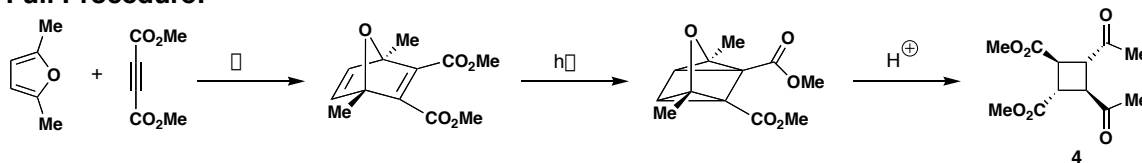
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North Torrey Pines Road, La Jolla, California 92037***Supporting Information**

General Procedures. All reactions were carried out under an nitrogen atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Dry tetrahydrofuran (THF), toluene, acetonitrile (CH₃CN), dimethylformamide, methanol, diethyl ether (Et₂O) and methylene chloride (CH₂Cl₂) were obtained by passing commercially available pre-dried, oxygen-free formulations through activated alumina columns. Yields refer to chromatographically and spectroscopically (¹H NMR) homogeneous materials, unless otherwise stated. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light as visualizing agent and either an ethanolic solution of phosphomolybdic acid and cerium sulfate or vanillin in ethanol/aqueous H₂SO₄, and heat as developing agents. NMR spectra were recorded on either Bruker DRX-600, DRX-500, and AMX-400 or Varian Inova-400 instruments and calibrated using residual undeuterated solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, quin = quintuplet, sext = sextet, sep = septet, b = broad. IR spectra were recorded on a Perkin-Elmer 1600 series or a Perkin-Elmer Spectrum BX FT-IR spectrometer. Electrospray ionization (ESI) mass spectrometry (MS) experiments were performed on an API 100 Perkin Elmer SCIEX single quadrupole mass spectrometer at 4000V emitter voltage. High-resolution mass spectra (HRMS) were recorded on a VG ZAB-ZSE mass spectrometer using MALDI (matrix-assisted laser-desorption ionization). Melting points

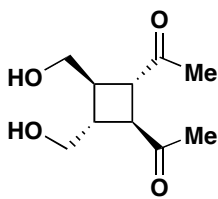
(m.p.) are uncorrected and were recorded on a Fisher-Johns 12-144 melting point apparatus.

Full Procedure:



Cyclobutane ketoester 4: To a solution of 2,5-dimethylfuran (4.5 mL, 42 mmol) in dioxane (5.5 mL) was added dimethyl acetylenedicarboxylate (4.14 mL, 32.3 mmol). The solution was heated in a sealed tube at 100 °C for 24 hours, then solvent and excess dimethylfuran were removed *in vacuo* to afford the Diels-Alder adduct (7.7 g, quantitative) as a slightly yellow oil. To a portion of the crude Diels-Alder adduct (1.0 g, 4.2 mmol) was dissolved in Et₂O (120 mL) in a sealed tube, and then the solution was degassed by bubbling with nitrogen for 30 min. The solution was irradiated for 16 hours at 23 °C; due to the instability of oxaquadricyclane **3** to silica gel, the reaction was monitored by taking ¹H NMR spectra of aliquots (0.1 mL) from the reaction. The reaction was then concentrated *in vacuo* to give oxaquadricyclane **3** (1.0 g, quantitative) as a yellow oil. Care should be taken in handling **3**, as prolonged contact with this substance can cause chemical burns. The sequence was repeated (4x) and the crude oxaquadricyclane **3** (5.0 g, 21 mmol) was dissolved in MeOH (250 mL) and H₂SO₄ (3.0 mL) was added. The resulting solution was stirred for 24 hours at 23 °C and then concentrated to a red oil, which was diluted with EtOAc (250 mL), washed with H₂O (3 x 250 mL), dried (Na₂SO₄), and concentrated. TLC analysis of the reaction mixture shows at least 10 compounds. Recrystallization (Et₂O) of the crude product yielded cyclobutane **4** (2.69 g, 50%). **Cyclobutane ketoester 4:** white needles; m.p. 73-75 °C (Et₂O); R_f = 0.44 (silica gel, 1:1 EtOAc: Hexanes); IR (film) $\bar{\nu}_{\text{max}}$ 2957, 1728, 1706, 1439, 1360, 1216 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 3.76 (s, 6 H), 3.52 (A₂B₂, J = 9.6, 2.4 Hz, 2 H), 3.41

(A₂B₂, $J = 9.6, 2.4$ Hz, 2 H), 2.21 (s, 6 H); ¹³C NMR (100 MHz, CDCl₃) δ 204.8, 171.6, 52.5, 46.3, 38.9, 27.7; HRMS (MALDI) calcd. for C₁₂H₁₆O₆ [M + Na⁺] 279.0839, found 279.0842.



Cyclobutane diol diketone 5: Diketodiester **4** (2.2 g 8.58 mmol) was

dried azeotropically (toluene) and then dissolved in MeOH (90 mL).

To the resulting solution was added CH(OMe)₃ (14 mL, 128 mmol)

and TsOH (180 mg, 1.28 mmol). The solution was heated to 50 °C for

24 hours, then poured into saturated aqueous NaHCO₃ (90 mL) and extracted with CH₂Cl₂

(2 x 90 mL). The combined organic layers were dried (Na₂SO₄) and evaporated to give

the ketal of cyclobutane **4**. To a portion of this crude ketal (0.19 g, 0.55 mmol) in CH₂Cl₂

at -78 °C was added DIBAL (2.18 mL, 1.0 M in CH₂Cl₂, 2.18 mmol) under a nitrogen

atmosphere. The reaction was stirred for 1 hour, and then additional DIBAL (1 mL, 1

mmol) was added. After 30 minutes, the reaction was quenched with MeOH and

extracted from saturated aqueous sodium potassium tartrate (20 mL) with EtOAc (2 x 20

mL), dried (Na₂SO₄), and concentrated *in vacuo* to give a mixture of partially cyclized

ketals. This mixture was treated with aqueous AcOH (80%, 2 mL) for 5 min, and then

the AcOH was evaporated to give cyclobutane diol **5** (110 mg, quantitative).

Cyclobutane diol diketone 5: light yellow oil; R_f = 0.35 (silica gel, 1:1 acetone: CH₂Cl₂);

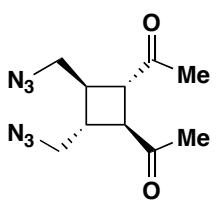
IR (film) ν_{max} 3385, 2924, 1694, 1420, 1360, 1188, 1017 cm⁻¹; ¹H NMR (400 MHz,

CDCl₃) δ 3.80 (dd, $J = 10.4, 4.4$ Hz, 2 H), 3.59 (dd, $J = 10.4, 7.6$ Hz, 2 H), 3.14 (A₂B₂, J

= 9.2, 2.8 Hz, 2 H), 2.26 – 2.33 (m, 2 H), 2.15 (s, 6 H); ¹³C NMR (100 MHz, CDCl₃) δ

207.5, 64.3, 46.7, 41.0, 28.1; HRMS (MALDI) calcd. for C₁₀H₁₆O₄ [M + H⁺] 201.1121,

found 201.1122.

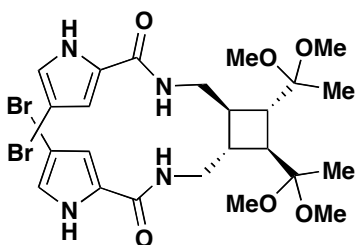


Ketoazide 6: To a solution of diol **5** (1.66 g, 8.29 mmol) in pyridine

(30 mL) at 0 °C was added methanesulfonyl chloride (2.85 mL, 36.5

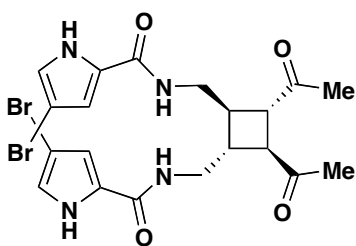
mmol). The reaction was stirred for 30 minutes at 0 °C, then allowed to

warm to room temperature. After a further 30 minutes, the reaction was diluted with 30 mL CH_2Cl_2 and cooled to 0 °C. Water (10 mL) was added slowly to quench the remaining methanesulfonyl chloride, then the layers were separated. The aqueous layer was reextracted with CH_2Cl_2 (30 mL), and then the combined organic layers were dried (Na_2SO_4) and evaporated to give the crude mesylate of alcohol **6** (2.95 g, quantitative). To a portion of the crude mesylate (1.2 g, 3.37 mmol) in dimethylformamide (30 mL) was added sodium azide (1.30 g, 20 mmol). Although we have never experienced complications with this reaction, sodium azide is known to be explosive, so a blast shield was used for large-scale experiments. The solution was heated to 50 °C for 24 hours, and then extracted from H_2O (300 mL) using CH_2Cl_2 (300 mL). The organic layer was washed with H_2O (2 x 300 mL), dried (Na_2SO_4), and evaporated to give the crude azide **6** (843 mg, *ca* 80% pure by ^1H NMR) as a brown oil. The crude azide could be purified by flash column chromatography to obtain an analytically pure sample. **Ketoazide 6**: slightly yellow oil; $R_f = 0.53$ (silica gel, 1:1 EtOAc: Hexanes); IR (film) ν_{max} 2923, 2097, 1703, 1671, 1540, 1507, 1418, 1358, 1272, 1186, 1092 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 3.47 (A_2B_2 , $J = 6.0, 2.4$ Hz, 4 H), 3.15 (A_2B_2 , $J = 6.0, 2.4$ Hz, 2 H), 2.32 – 2.36 (m, 2 H), 2.17 (s, 6 H); ^{13}C NMR (100 MHz, CDCl_3) δ 206.1, 53.8, 47.3, 37.3, 28.2; MS (ESI) calcd. for $\text{C}_{10}\text{H}_{14}\text{N}_6\text{O}_4$ $[\text{M} + \text{Na}^+]$ 273.1, found 273.2, for $[\text{M} - \text{H}]$ 249.1 found 249.0.

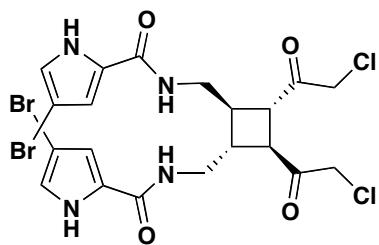


Pyrroleketal 8: Toluenesulfonic acid (0.22 g, 1.16 mmol) was dried azeotropically (toluene) and added to a solution of crude azide **6** (2.7 g, 10.80 mmol) in MeOH (90 mL) and $\text{CH}(\text{OMe})_3$ (16.5 mL, 151 mmol). The solution was heated to 50 °C for 24 hours, and then extracted from saturated aqueous NaHCO_3 (90 mL) with CH_2Cl_2 (2 x 90 mL). The organic layers were combined, dried (Na_2SO_4), and concentrated to give the ketal of **6** (3.7 g) as a yellow oil. To the

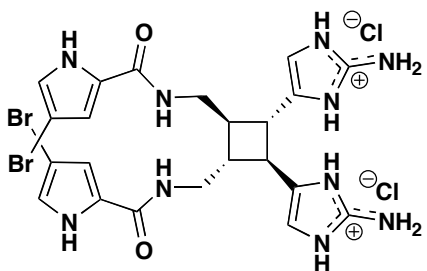
crude ketal in MeOH (35 mL) was added Pd on CaCO₃ (Lindlar catalyst, 1.1 g). The reaction was stirred under an atmosphere of H₂ for 12 hours at 23 °C. The solvent was removed *in vacuo*, and the residue was dissolved in CH₃CN (14 mL) and bromopyrrole **7** (6.92 g, 23.8 mmol) was added under a nitrogen atmosphere. After 4 hours, a white precipitate formed. The precipitate was filtered off to give pyrrole ketal **8** (4.80 g, 70% yield from **5**) as a white powder. **Pyrroleketal 8**: m.p. 177-179 °C (CH₃CN); R_f = 0.43 (silica gel, 3:1 EtOAc: hexanes); IR (film) $\bar{\nu}_{\max}$ 3224, 1630, 1567, 1527, 1109 cm⁻¹; ¹H NMR (600 MHz, CD₃OD) δ 6.89 (d, *J* = 1.8 Hz, 2 H), 6.77 (d, *J* = 1.8 Hz, 2 H), 3.37 (m, 4 H), 3.17 (s, 6 H), 3.18 (s, 6 H), 2.40 (A₂B₂, *J* = 10.2, 3.0 Hz, 1.35 (s, 6 H)); ¹³C NMR (100 MHz, DMSO-d₆) δ 161.1, 127.8, 122.2, 112.3, 103.2, 96.0, 49.0, 48.8, 43.2, 41.4, 36.7, 19.4; HRMS (MALDI) for C₂₂H₂₈Br₂N₄O₅ [M – C₂H₆O + Na] 609.0324 found 609.0294, C₂₀H₂₂Br₂N₄O₄, for [M – C₄H₁₂O₂ + Na] 562.9905 found 562.9895; MS(ESI) calcd. for C₂₄H₃₄Br₂N₄O₆ [M + Na⁺] 655.0737 found 655, for [M – H] 631.0772, found 631, for [M + Cl] 667.0539, found 667.



Pyrroleketone 8': To pyrroleketal **8** (5 mg, .008 mmol) was added aqueous AcOH (80%, 1 mL). The resulting solution was stirred for 5 min., and then evaporated to give **8'** as white crystals (4.3 mg, quantitative). **Pyrroleketone 8'**: m.p. 225 – 227 °C; R_f = 0.45 (silica gel, EtOAc: hexanes 4:1); IR (film) $\bar{\nu}_{\max}$ 3266, 1700, 1630, 1566, 1528, 1388, 1328, 1121, 921 cm⁻¹; ¹H NMR (400 MHz, CD₃OD) δ 6.90 (d, *J* = 1.6 Hz, 2 H), 6.78 (d, *J* = 1.6 Hz, 2 H), 3.45 – 3.42 (m, 4 H), 3.16 (A₂B₂, *J* = 8.8, 2.4 Hz, 2 H), 2.36 – 2.33 (m, 2 H), 2.11 (s, 6 H); ¹³C NMR (100 MHz, CD₃OD) δ 210.6, 163.8, 128.2, 123.8, 114.2, 98.4, 50.0, 43.9, 40.8, 29.0; HRMS (MALDI) calcd. for C₂₀H₂₂Br₂N₄O₄ [M + H⁺] 541.0080 found 541.0082. For X-ray analysis, see Figure 1 and attached CIF file. Structure deposited in Cambridge crystallography database: CCDC 228756.

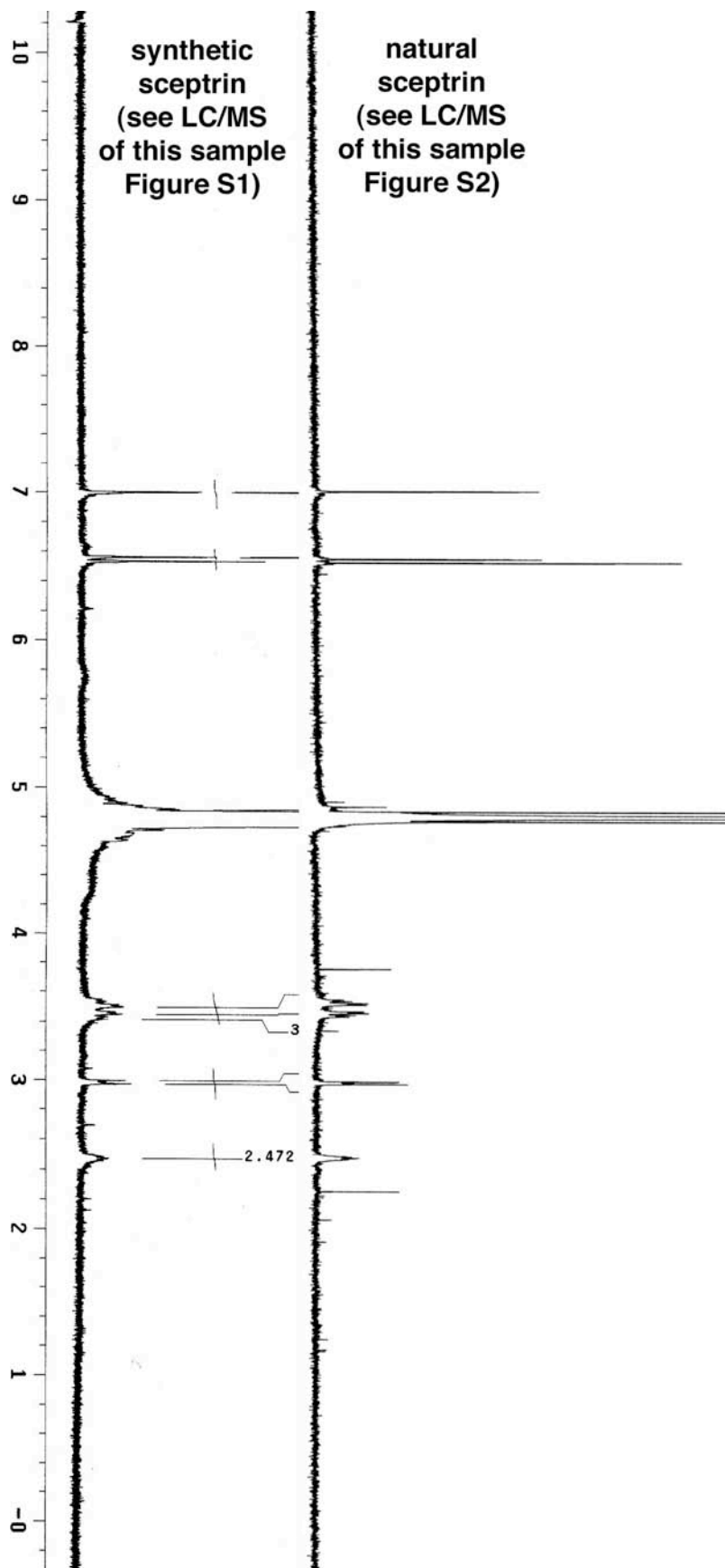


Chloroketone 10: To ketalpyrrole **8** (30 mg, 0.047 mmol) in THF (2.0 mL) was added benzyltrimethylammonium dichloroiodate (54 mg, 0.155 mmol). The resulting orange solution was heated at 60 °C for 1.75 hours, then quenched with 5% aqueous Na₂S₂O₄ (10 mL) and extracted with EtOAc (10 mL). In quenching this reaction is important to use a large excess of Na₂S₂O₄ and completely remove all color from the organic layer, as excess dichloroiodate and byproducts from the reaction are capable of decomposing the product upon concentration. The organic layer was washed with brine (10 mL), then dried with MgSO₄ and evaporated to give pure chloroketone **10** as a white powder (28 mg, 97%). Note: when running this reaction on a large scale, it is useful (but not required) to pass the EtOAc layer of the extraction through a small plug of MgSO₄ and silica before evaporation to remove trace impurities. **Chloroketone 10:** m.p. 174 – 176 °C (dec.); R_f = 0.89 (silica gel, EtOAc: hexanes 4:1); IR (film) $\bar{\nu}_{\max}$ 3303, 2916, 1720, 1630, 1569, 1519, 1429, 1384, 1323, 1104 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 9.41 (br s, 2 H, D₂O exchangeable), 6.94 (br t, 2 H, D₂O exchangeable), 6.86 (dd, *J* = 1.6, 2.8 Hz, 2 H), 6.69 (dd, *J* = 1.6, 2.8 Hz, 2 H), 4.06 (d, *J* = 2.4 Hz, 4 H), 3.58 – 3.42 (m, 4 H), 3.38 (A₂B₂, *J* = 9.2, 2.4 Hz, 2 H), 2.39 – 2.36 (m, 2 H); ¹H NMR (400 MHz, DMSO-d₆) δ 8.28 (br t, 2 H, D₂O exchangeable), 7.04 (dd, *J* = 1.6, 2.8 Hz, 2 H), 6.88 (dd, *J* = 1.6, 2.8 Hz, 2 H), 4.59 (s, 4 H), 3.38 – 3.52 (m, 4 H) peak under water peak, 3.22 (A₂B₂, *J* = 9.2, 2.4 Hz, 2 H), 2.38 – 2.35 (m, 2 H); ¹³C NMR (100 MHz, DMSO-d₆) δ 201.3, 161.1, 127.5, 122.4, 112.5, 96.0, 49.4, 45.1, 39.6, 39.3; HRMS (MALDI) calcd for C₂₀H₂₀Br₂Cl₂N₄O₄ [M + H⁺] 608.9301, found 608.9322.



(±)-Sceptrin 1: To chloroketone **10** (350 mg, 0.573 mmol) in CH₃CN (35 mL) was added sodium diformylamide (300 mg, 3.157 mmol). The resulting solution was stirred at 35 °C for 40 hours. The solvent was removed *in vacuo*, and the residue was washed with H₂O (30 mL) and CH₂Cl₂ (30 mL). The crude formamide **11** (400 mg) was then dissolved in MeOH (30 mL) and concentrated aqueous HCl (3 mL). The resulting solution was stirred at 23 °C for 16 hours. The solvent was removed *in vacuo*, and the residue was dissolved in H₂O (10 mL) and cyanamide (260 mg, 6.184 mmol) was added. The reaction was heated to 95 °C for 4 h, then the solvent was removed *in vacuo*, and the residue was washed three times with CH₃CN (50 mL), DCM (50 mL), CH₃CN (50 mL) and then the residue was redissolved in 5 °C H₂O (30 mL). Reaction time varies from 3-4 hours and was monitored by TLC (silica gel, CHCl₃-MeOH-H₂O 20:6:1, saturated with NH₃) in order not to let the reaction go too long (resulting in lower yield due to decomposition). After evaporation the residue was redissolved in -20 °C n-BuOH to give pure sceptrin **1** (256 mg, 72% yield from **10**) as a pale yellow powder. We have prepared *ca.* 350 mg so far using this route and we recommend storing material at compound **8** and preparing **1** as needed. **Sceptrin 1:** m.p. 223 – 225 °C (dec.), lit. 215 – 225 °C (dec.); R_f = 0.13 (silica gel, CHCl₃-MeOH-H₂O 20:6:1, saturated with NH₃); ¹H NMR (400 MHz, D₂O) □ 7.00 (d, *J* = 1.6 Hz, 2 H), 6.56 (d, *J* = 1.6 Hz, 2 H), 6.53 (s, 2 H), 3.40 – 3.52 (m, 4 H), 2.98 (A₂B₂, *J* = 9.6, 2.4 Hz, 2 H), 2.46 – 2.48 (m, 2 H); ¹³C NMR (125 MHz, D₂O) □ 160.8, 145.4, 125.3, 123.6, 121.5, 111.0, 108.0, 94.8, 41.3, 40.5, 37.2; HRMS (MALDI) calcd. for C₂₂H₂₄Br₂N₁₀O₂ [M + H⁺] 619.0523, found 619.0526.

See following figures for spectral and LCMS comparisons, stability studies, and copies of spectra for all intermediates.



NMR COMPARISON

synthetic scep trin

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Inj Volume : 10 µl

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(modified after loading)
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MA-method
    
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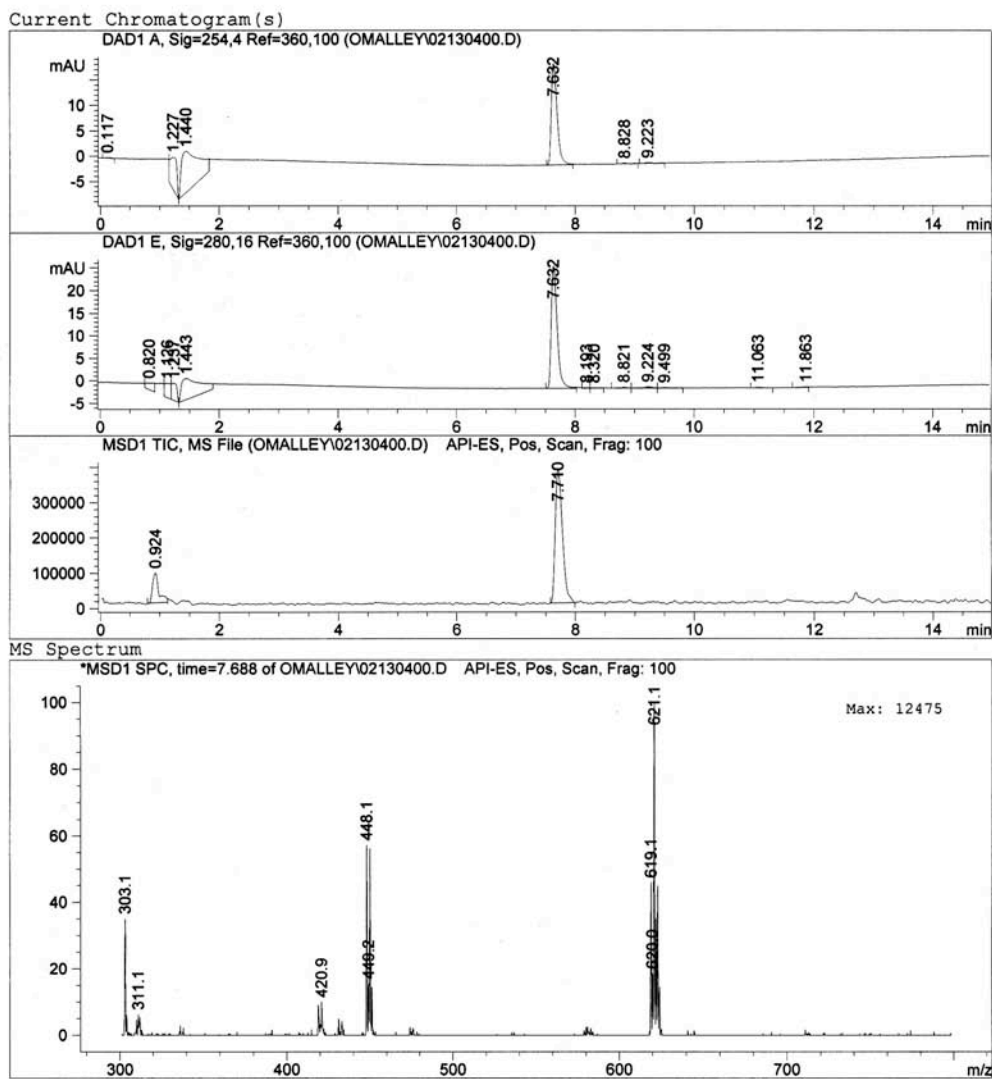


Figure S1. Synthetic scep trin.

Print of all graphic windows

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Acq. Operator   : Dan
Location        : Vial 1
Inj Volume     : 5 µl

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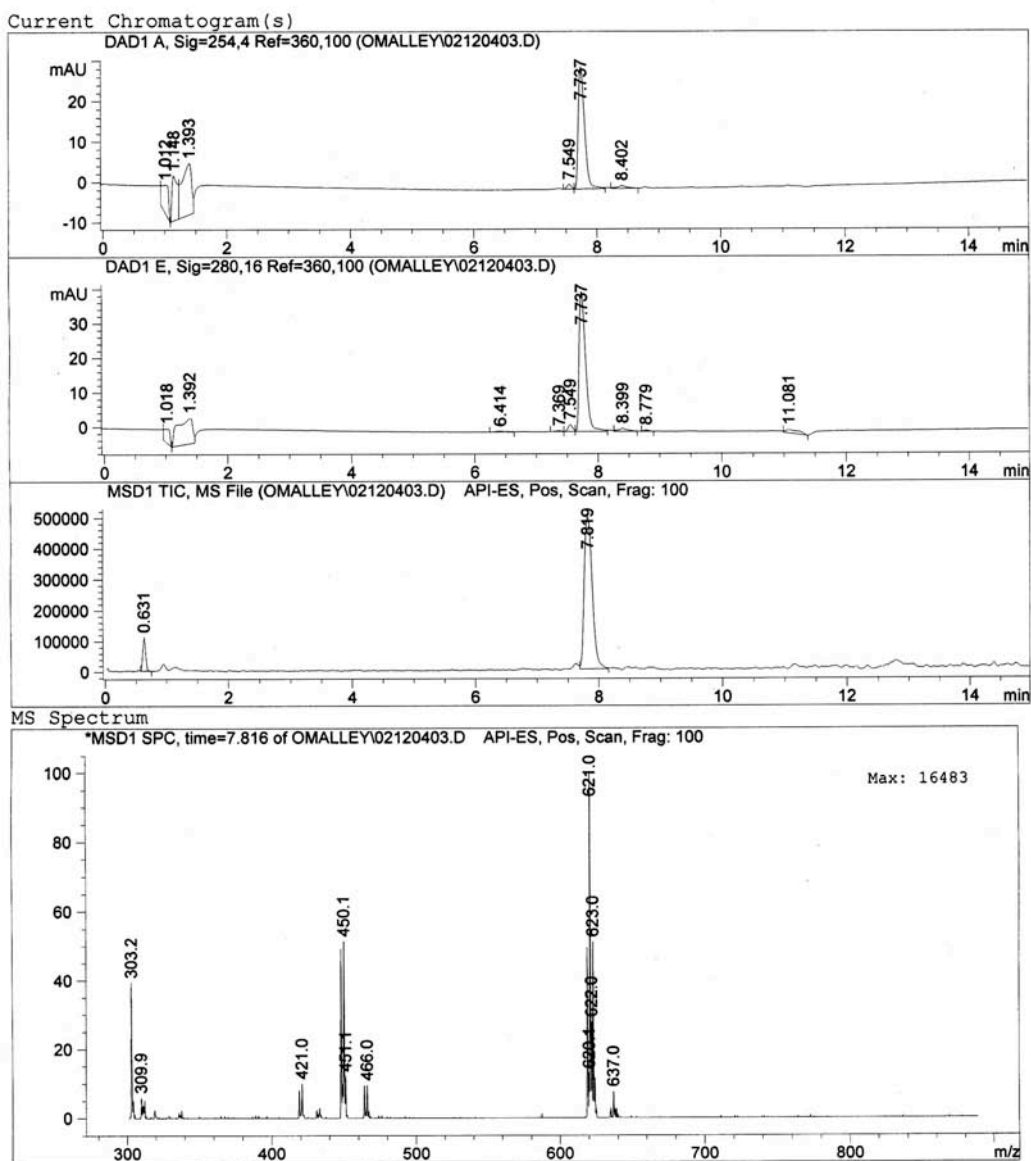


Figure S2. Natural scepterin.

coinjection of natural and synthetic sceptrin

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Inj Volume     : 10 µl

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MA-method
    
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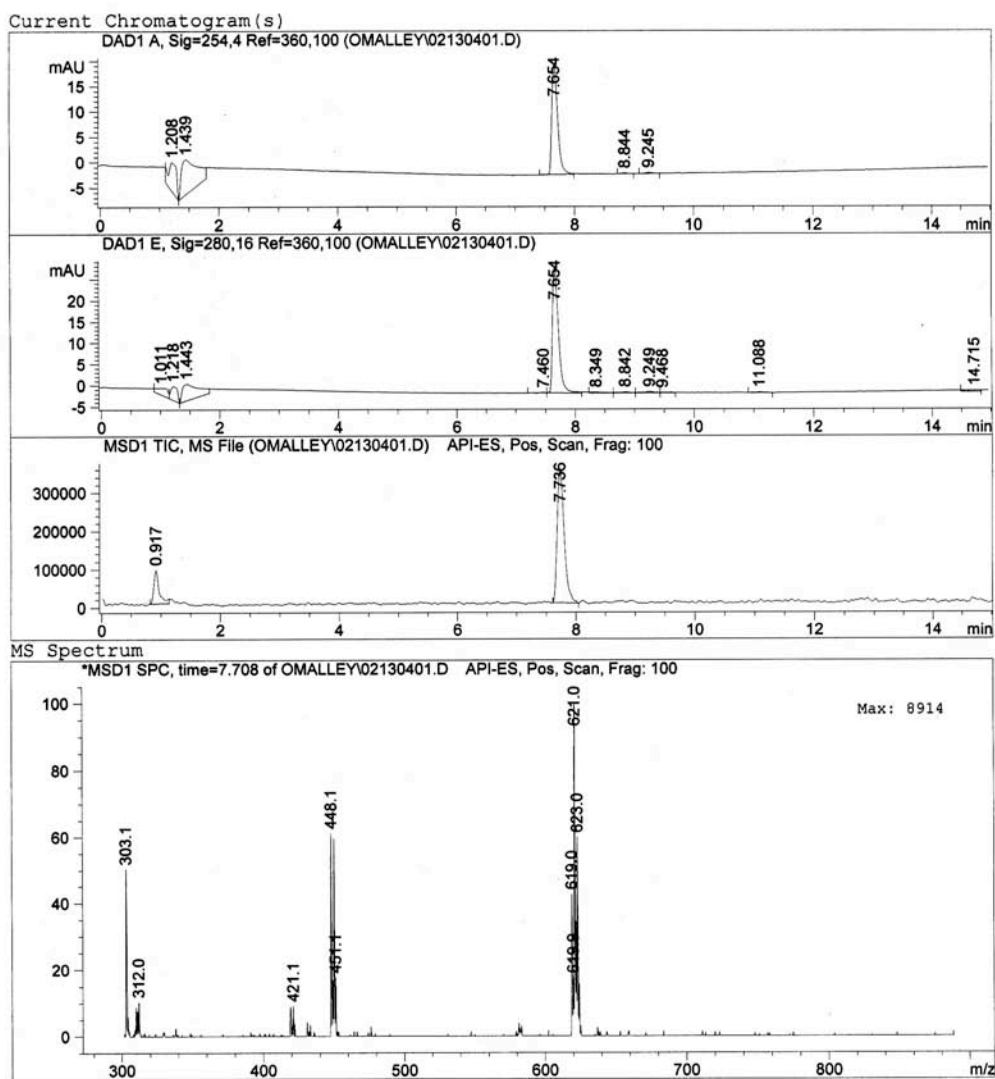


Figure S3. Co-injection of natural and synthetic.

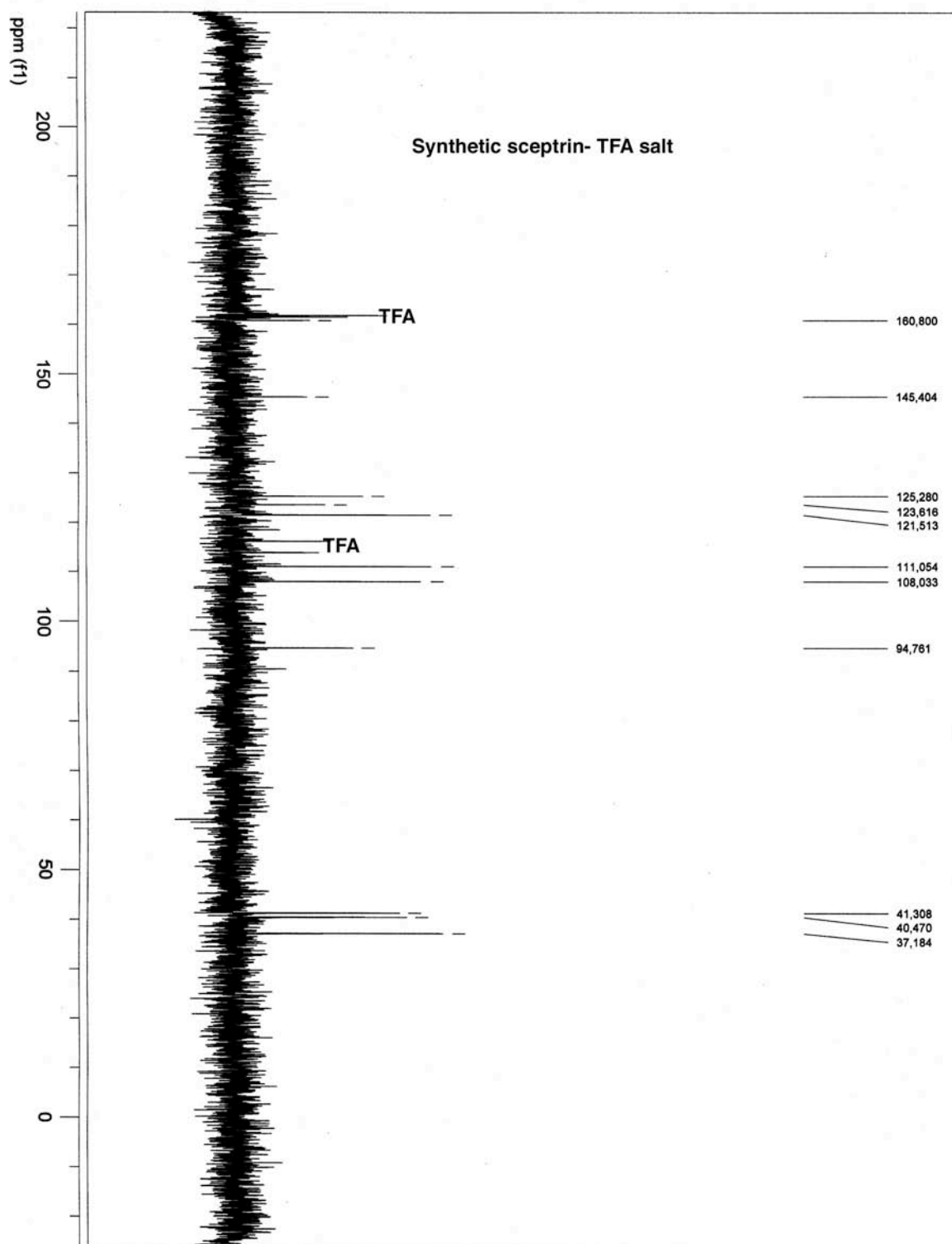


Figure S4. ^{13}C NMR of synthetic scep trin.

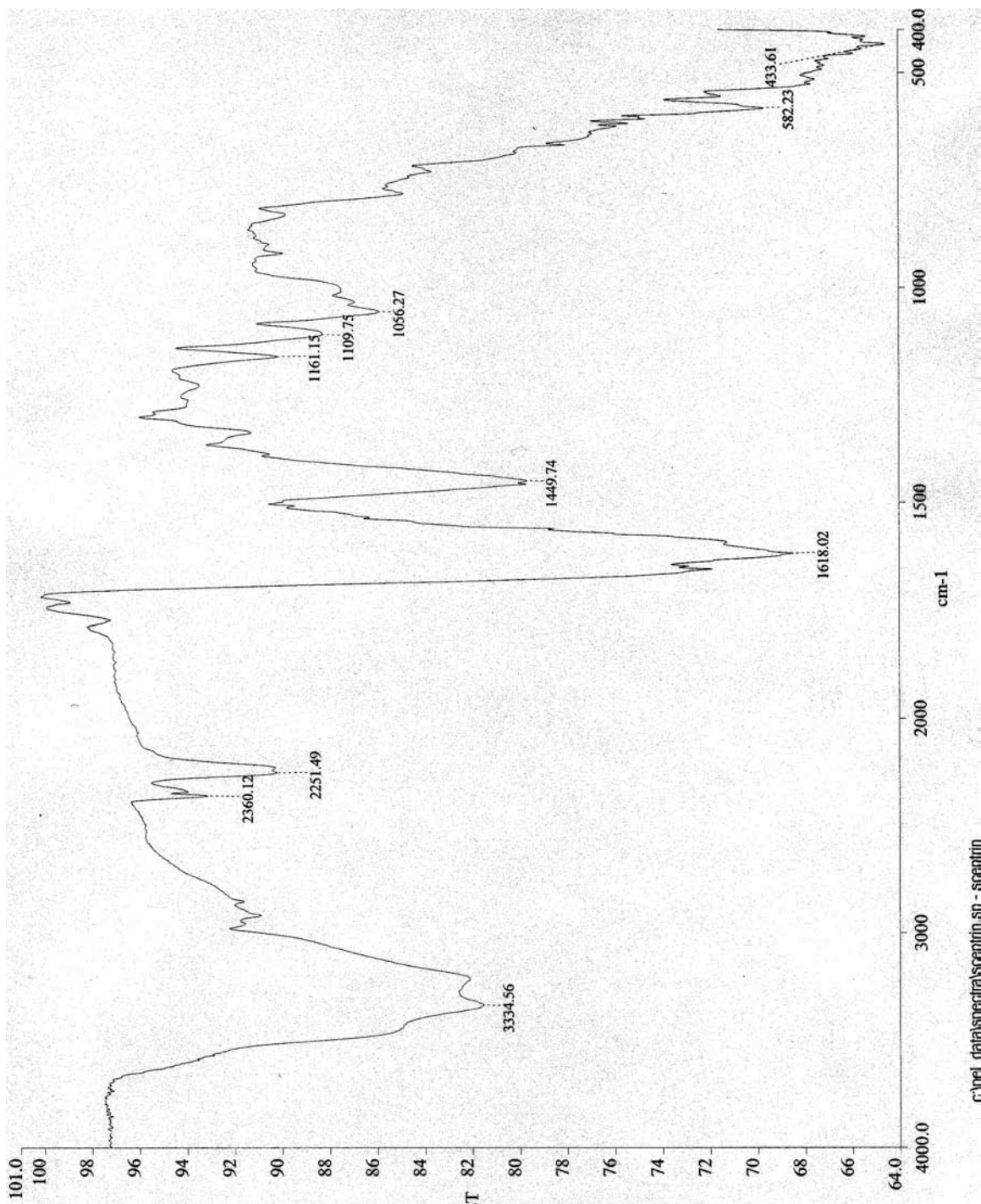


Figure S5. IR of scep trin.

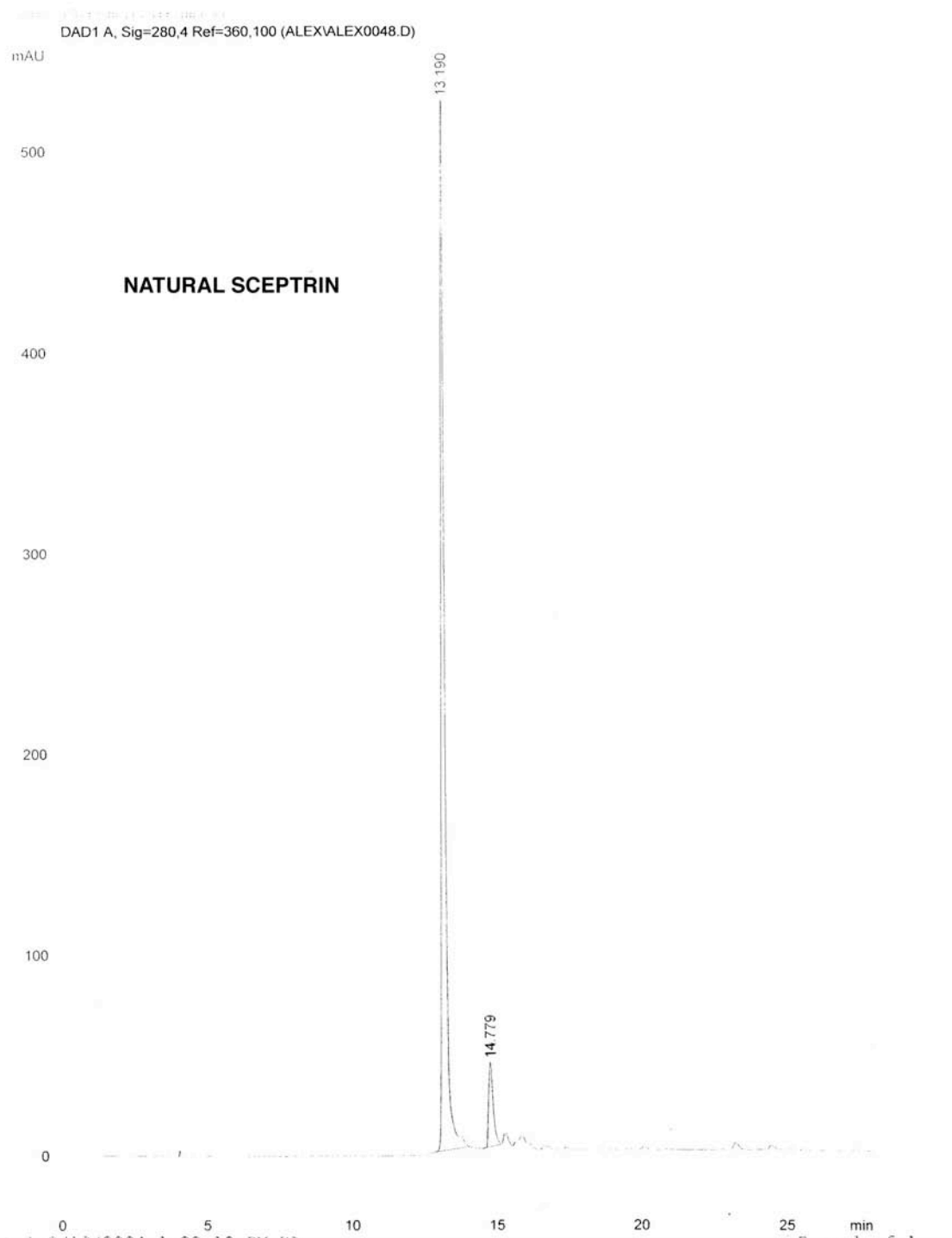


Figure S6.

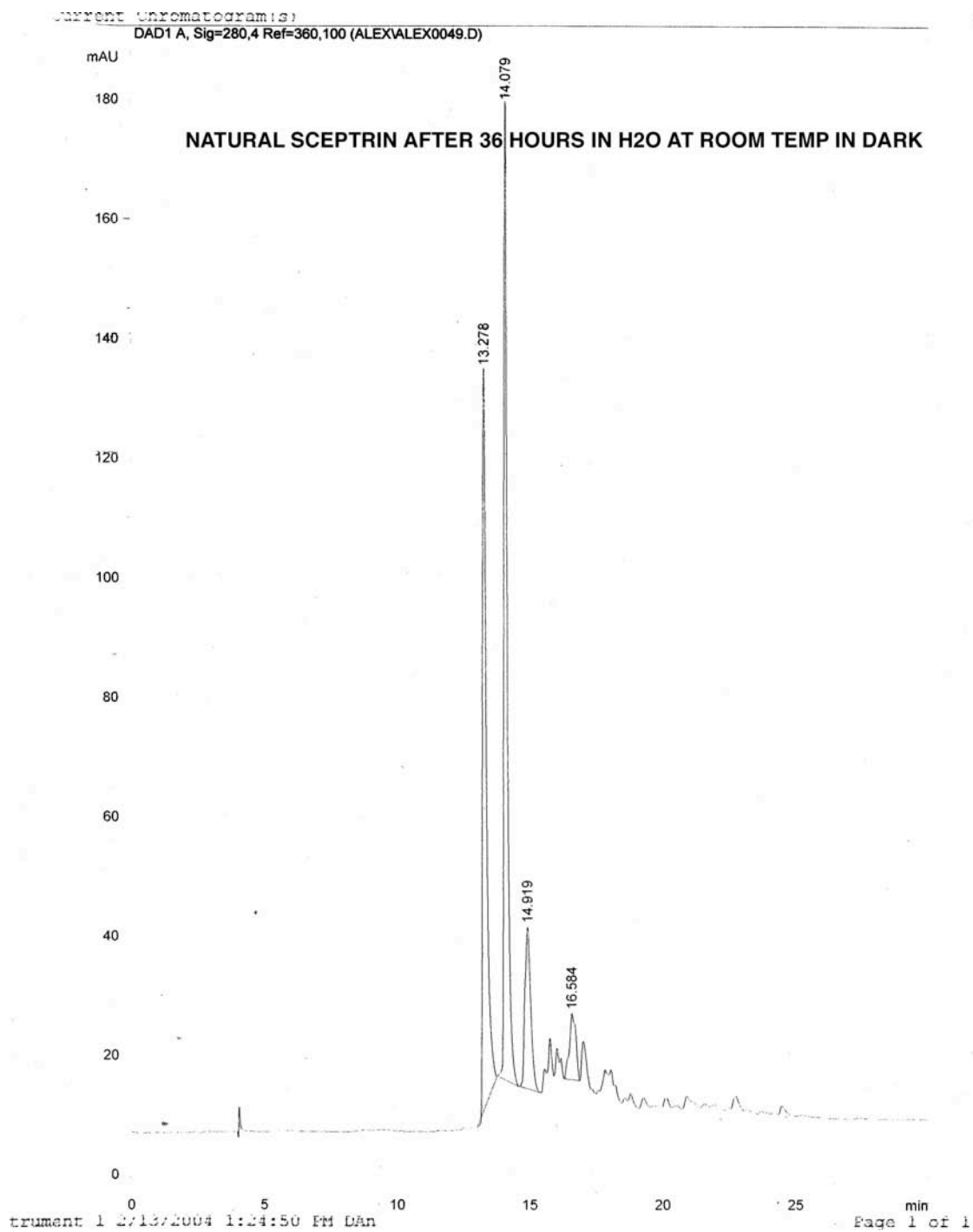


Figure S7.

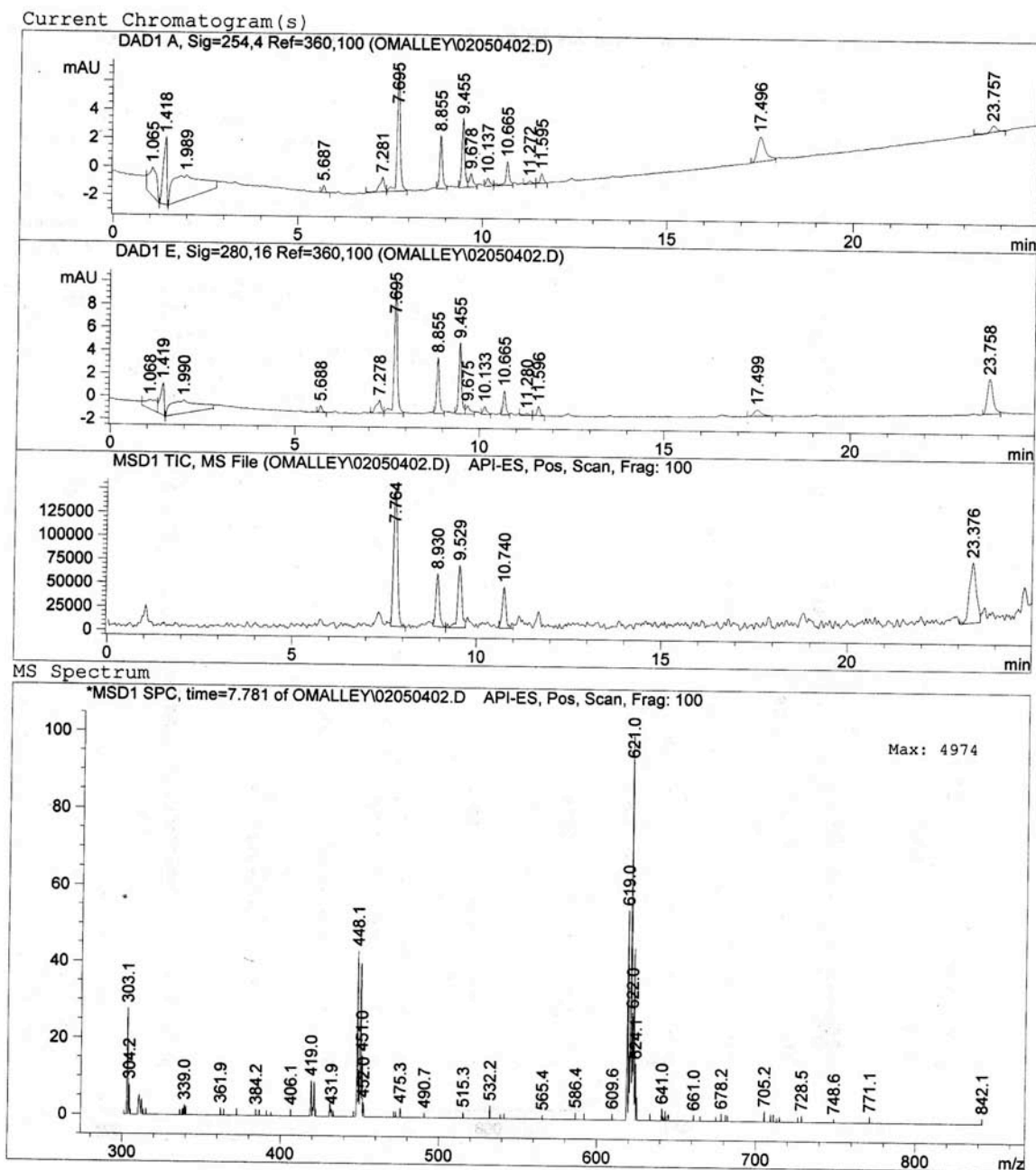
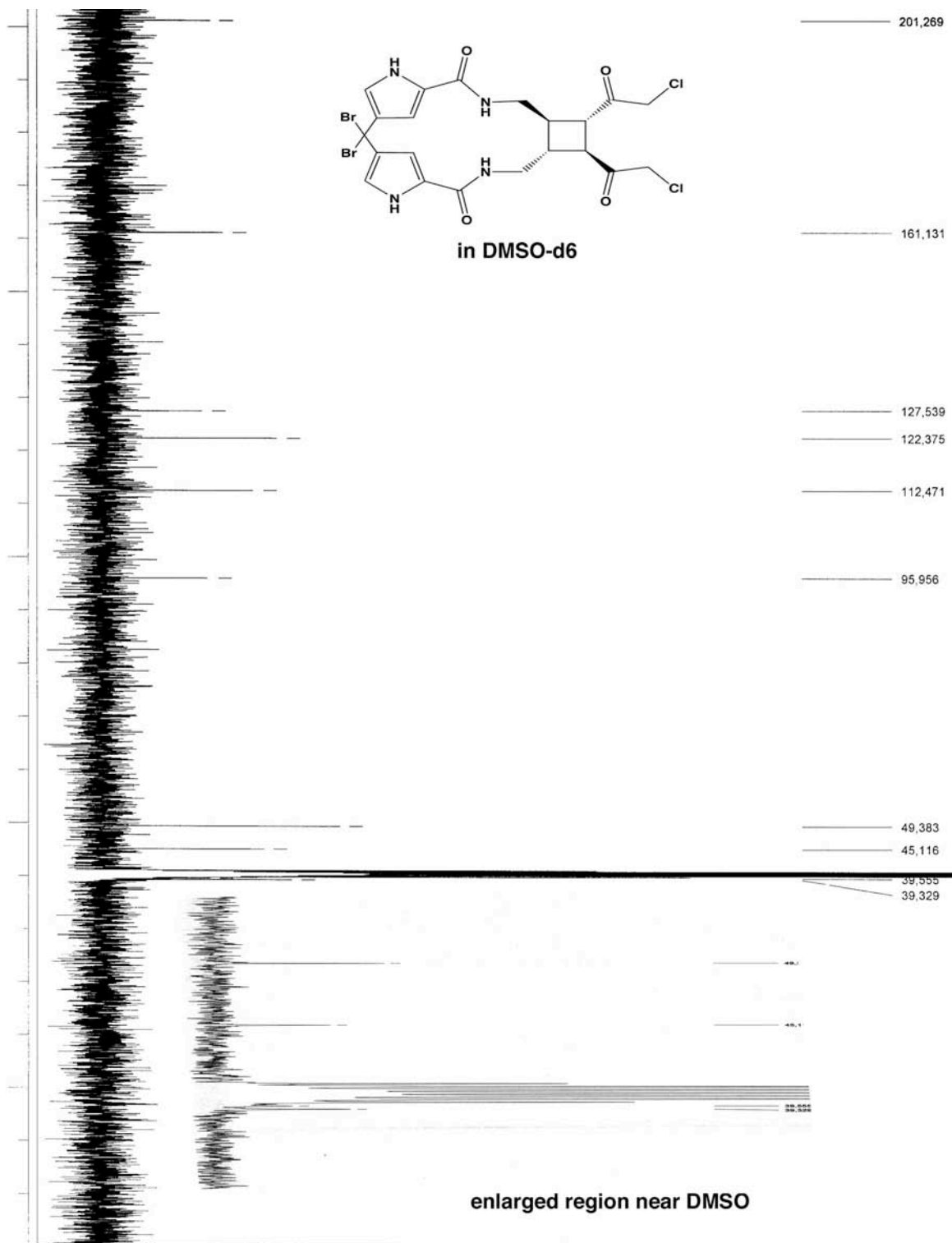
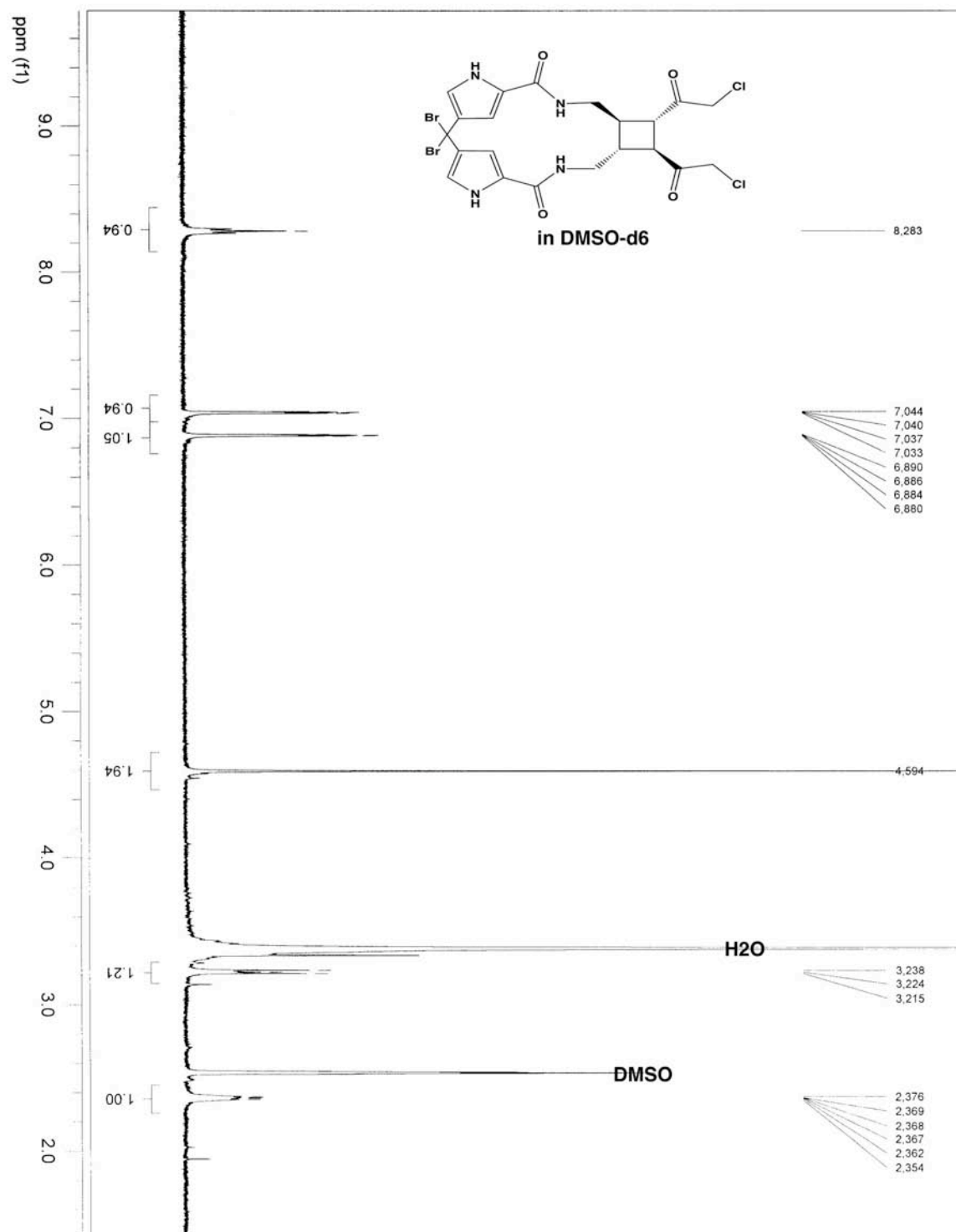
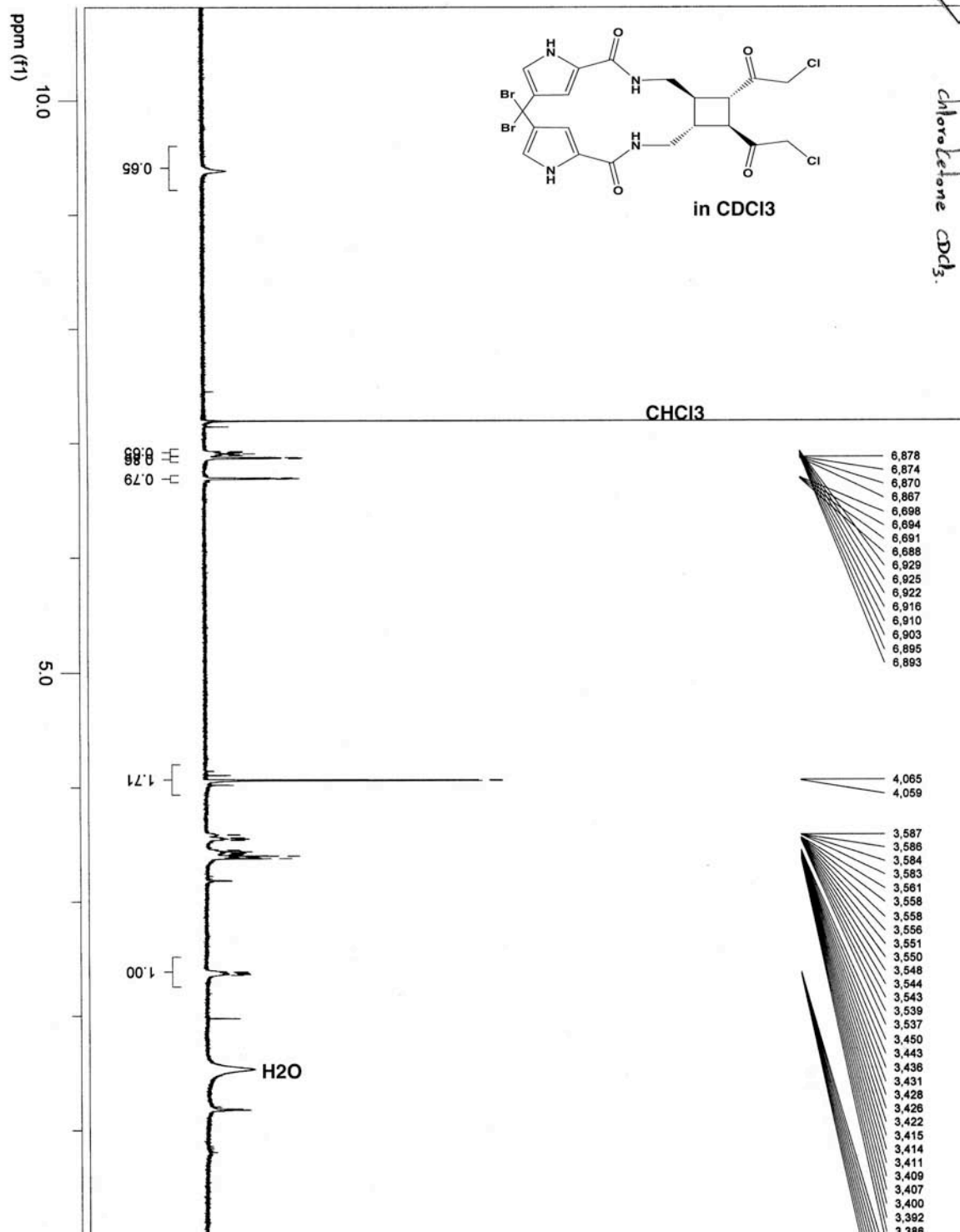
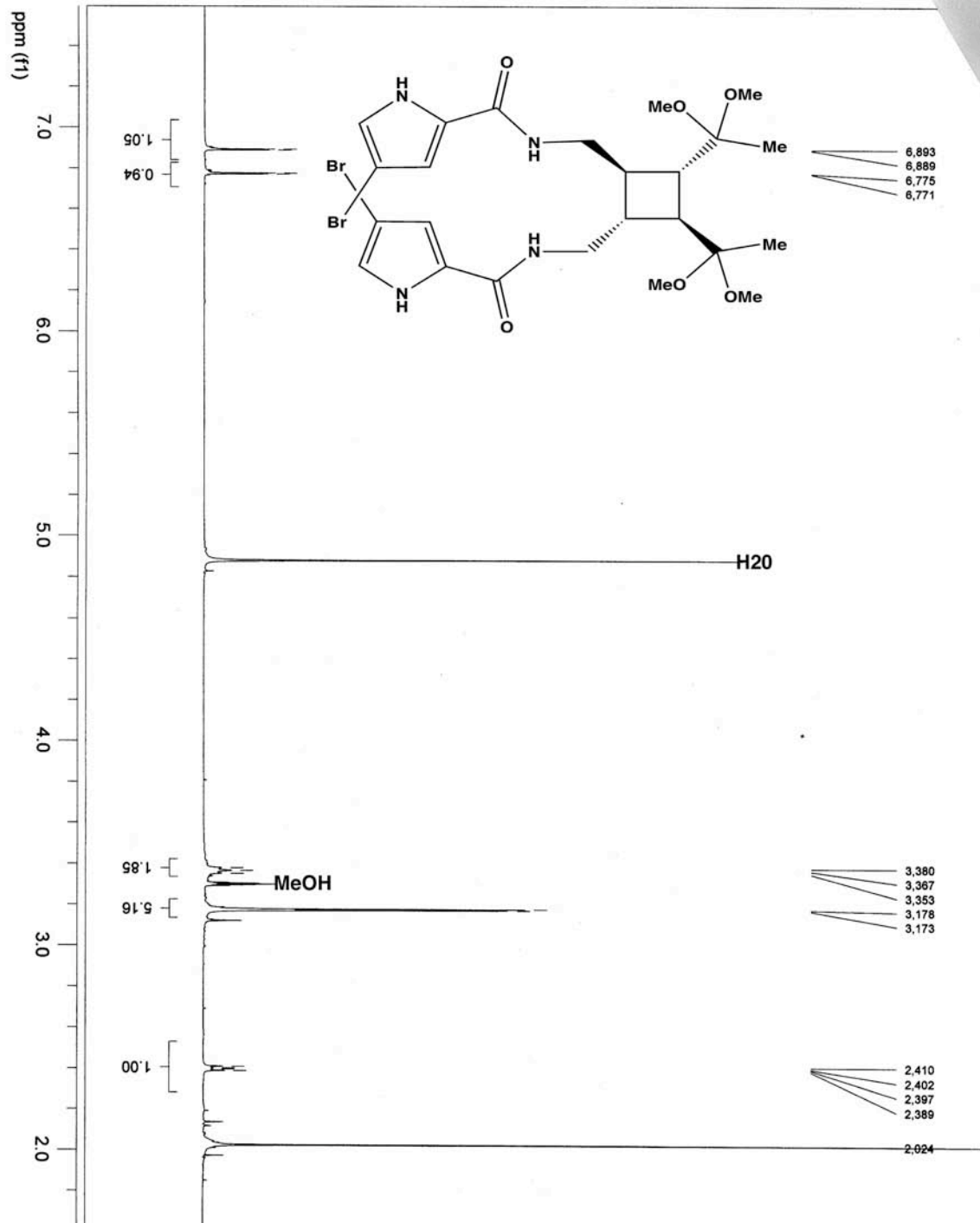


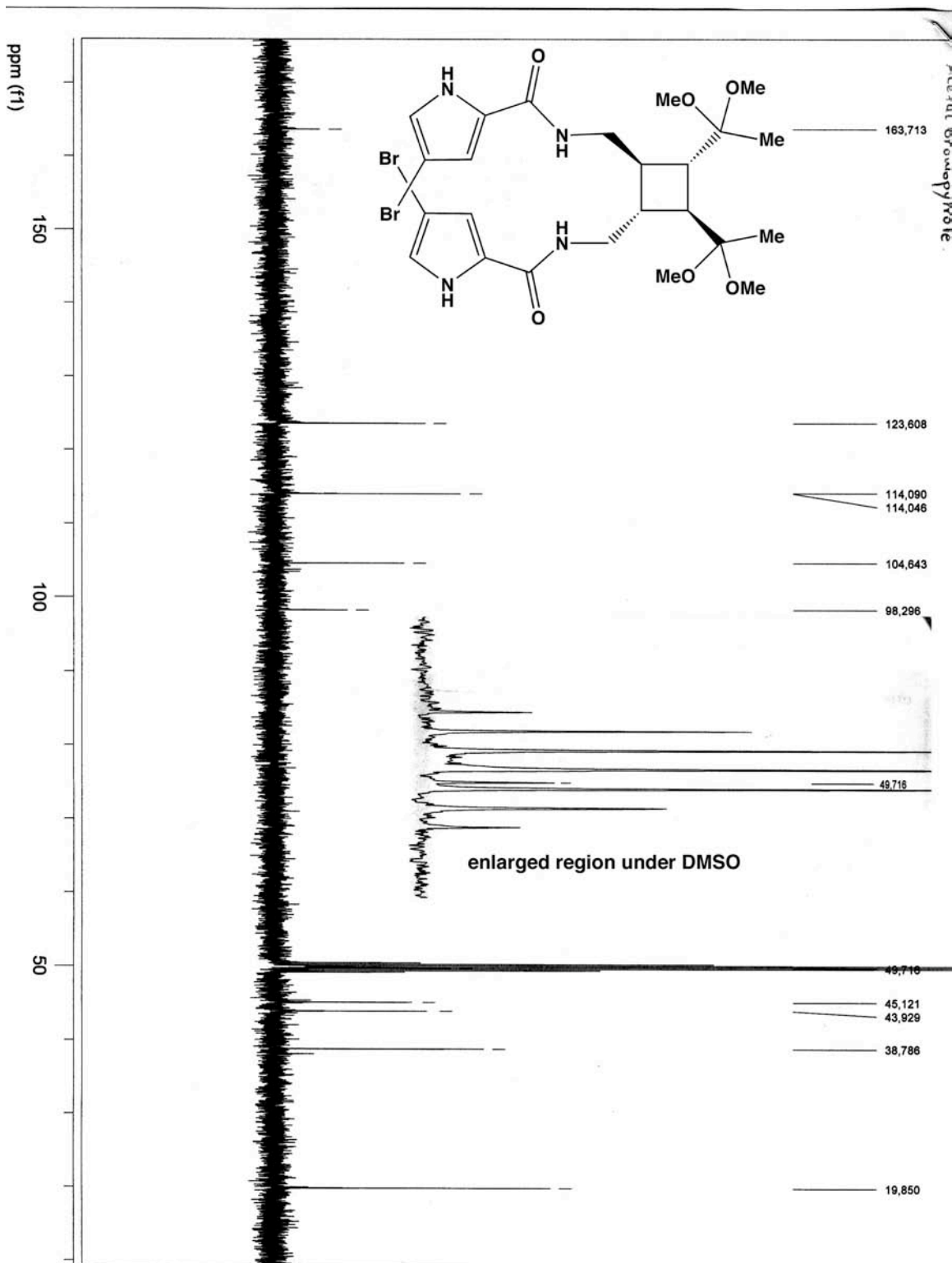
Figure S8. Synthetic sceptrin after 48 hours at room temperature in the dark in H₂O. The peak at 7.7 min is sceptrin, the rest are new peaks which formed during this period. For HPLC of natural sceptrin, see previous pages.

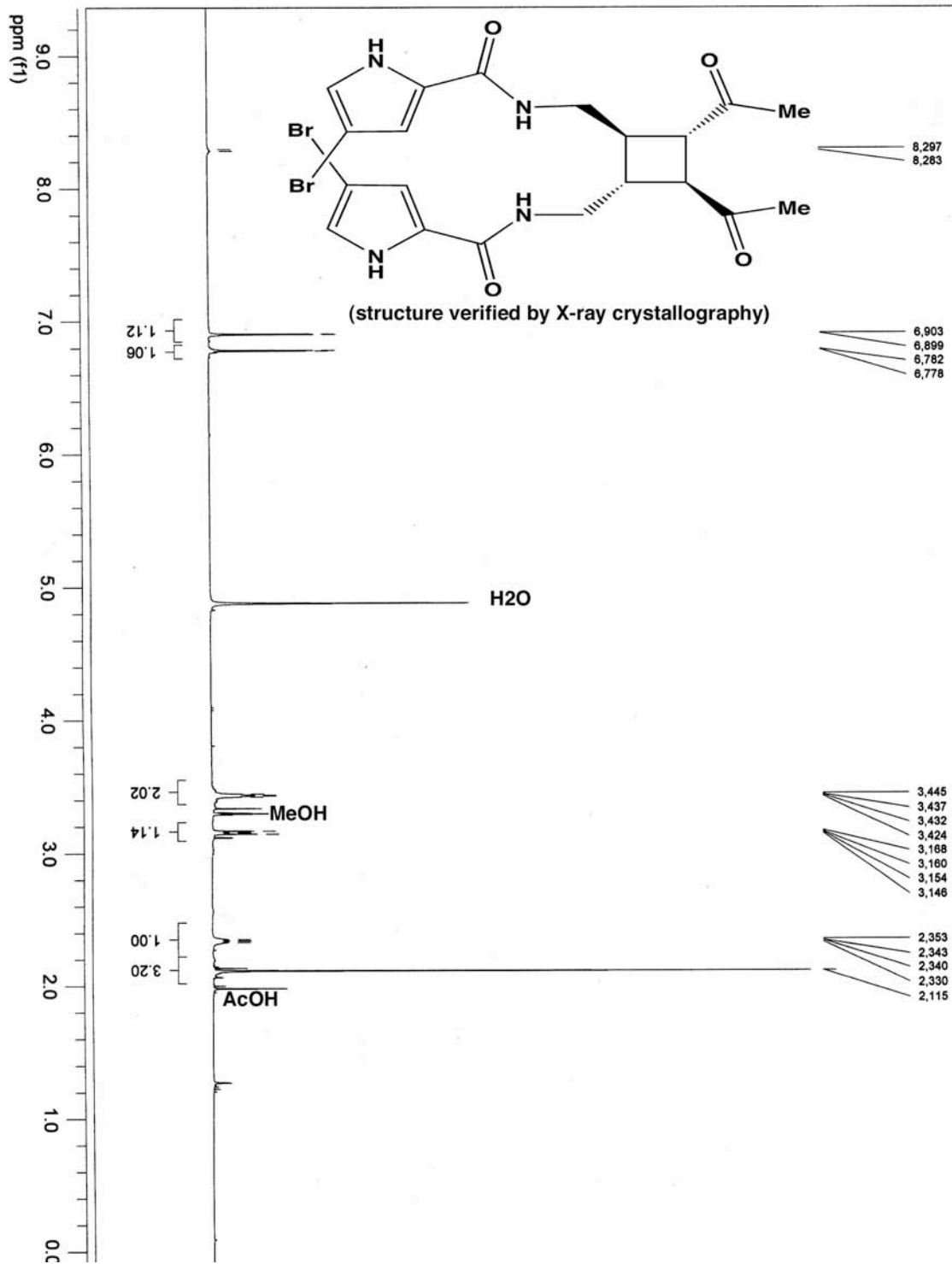


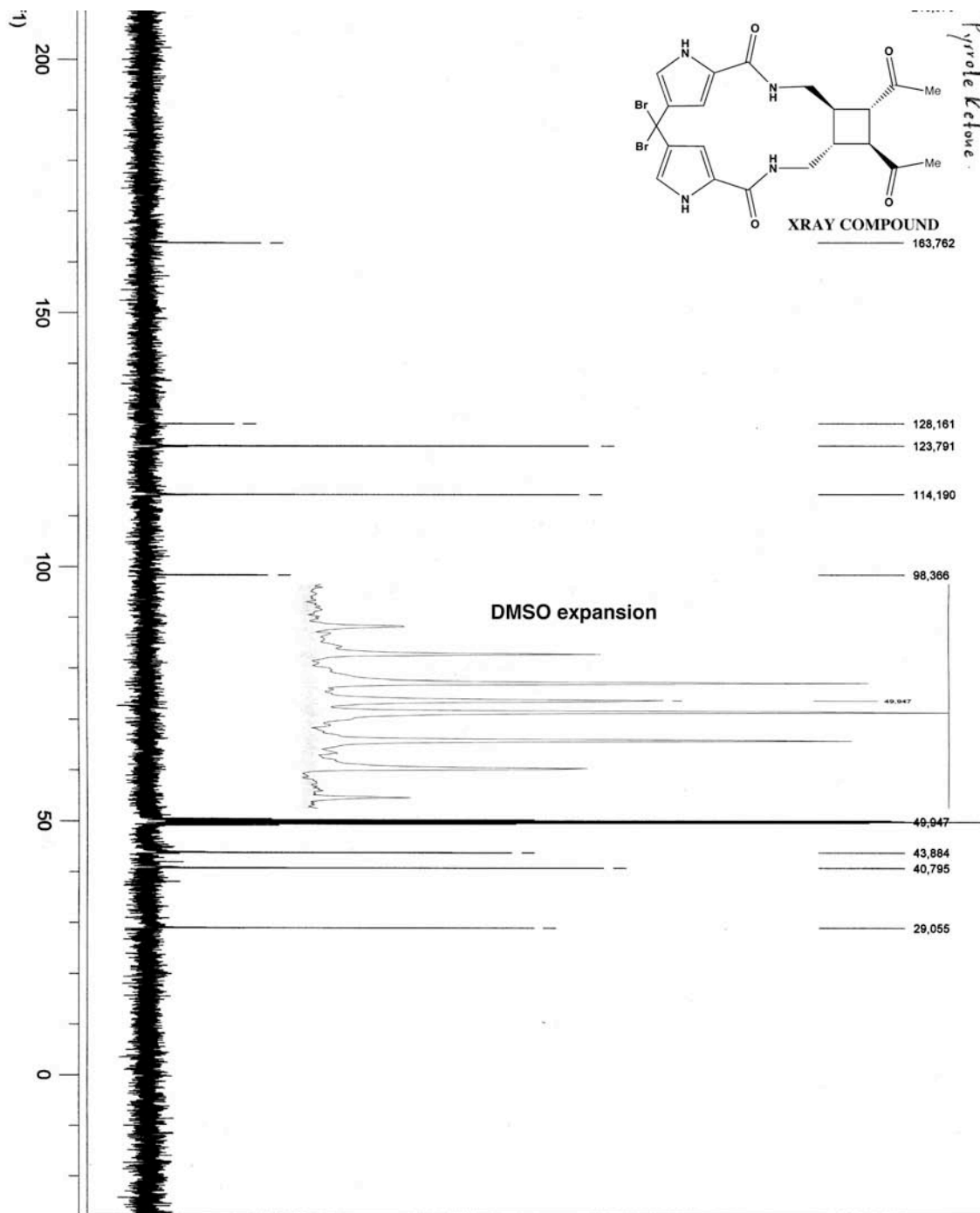


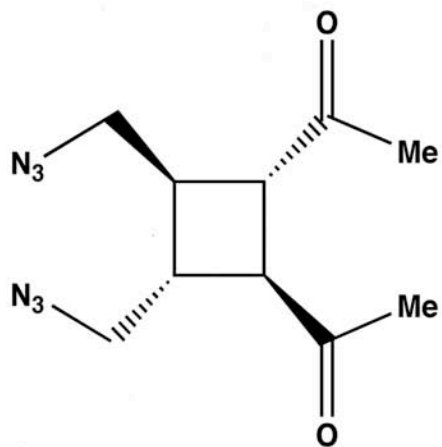
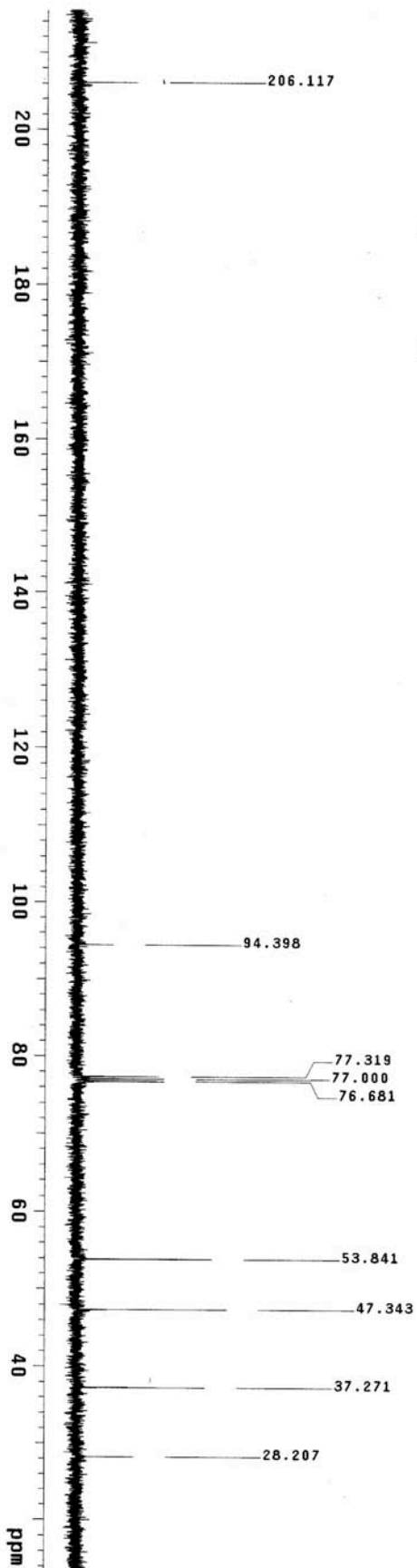


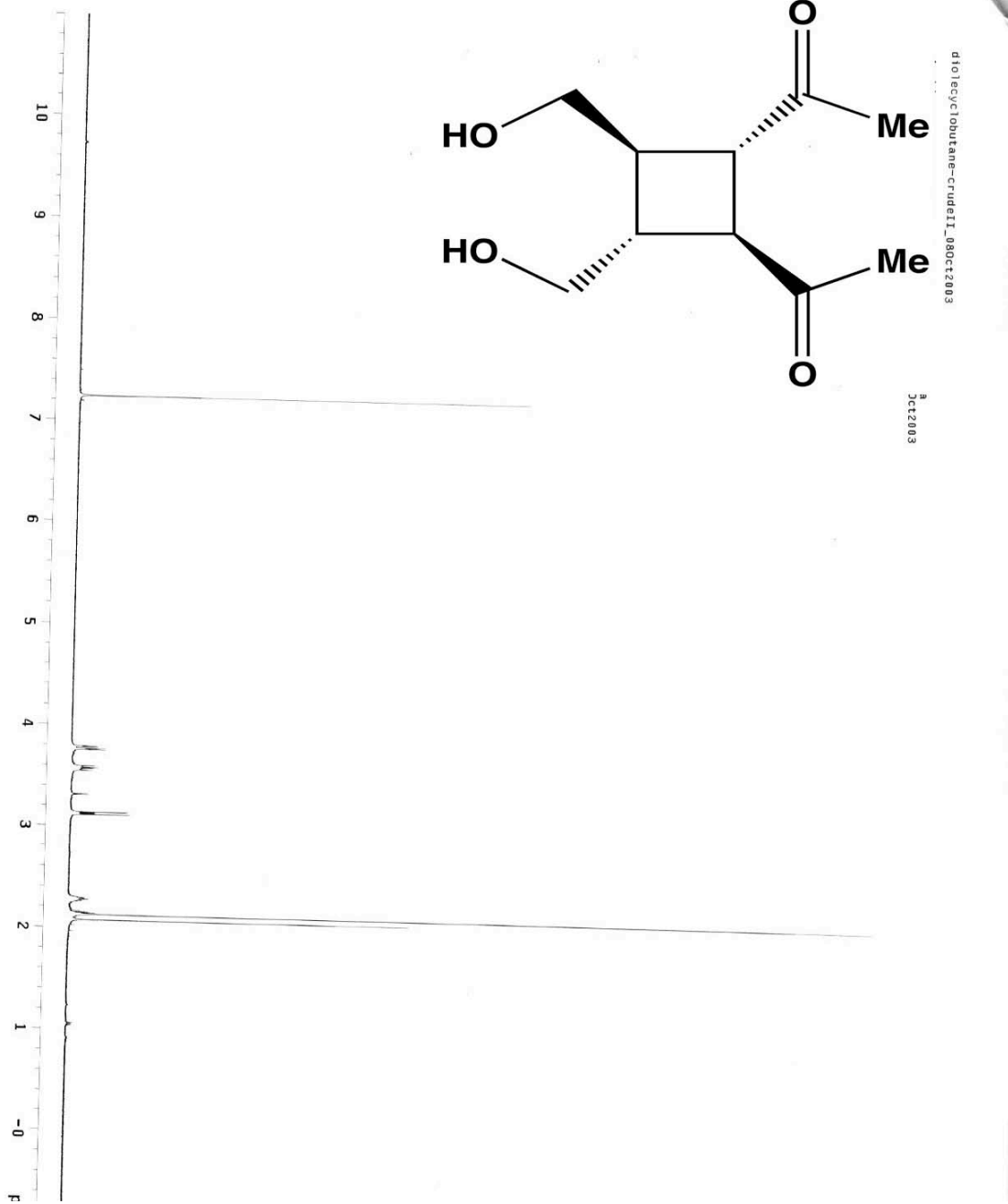


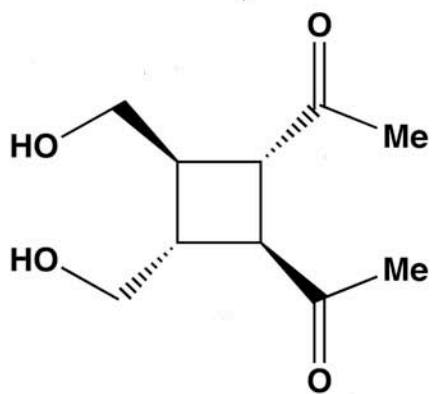
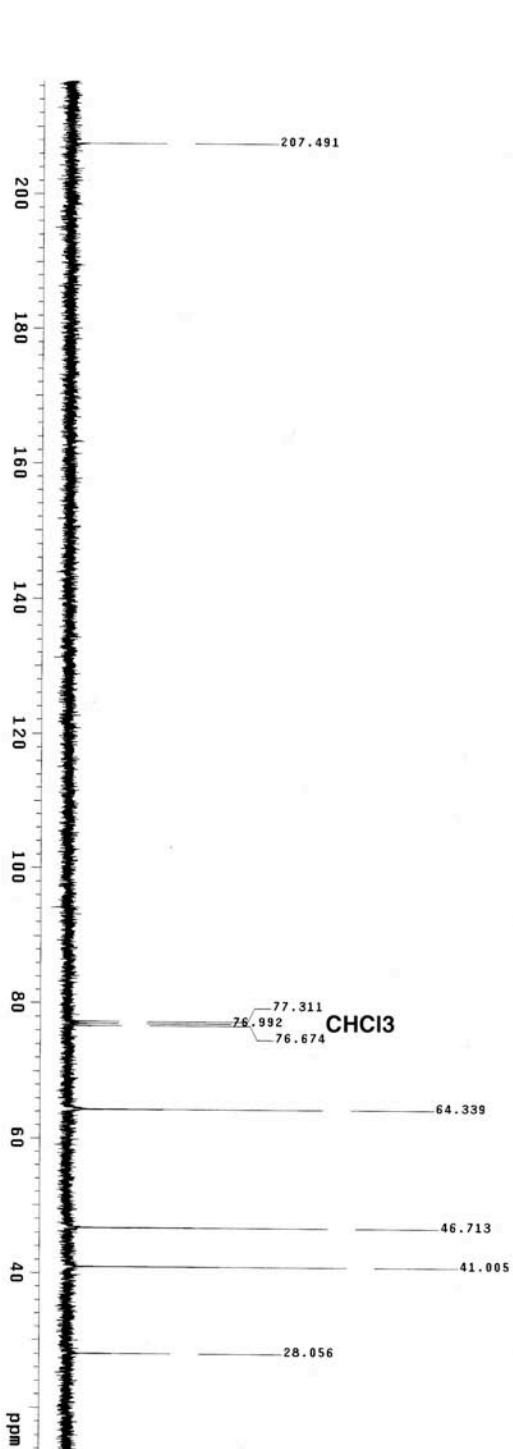












CRUDE (directly carried to next step)

