# INHIBITION OF PROTEIN SYNTHESIS BY DIDEMNIN B: HOW EF-1α MEDIATES INHIBITION OF TRANSLOCATION

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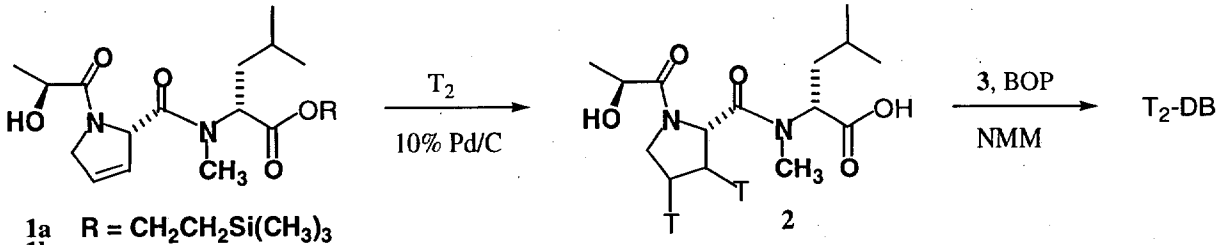
## Supplementary Material

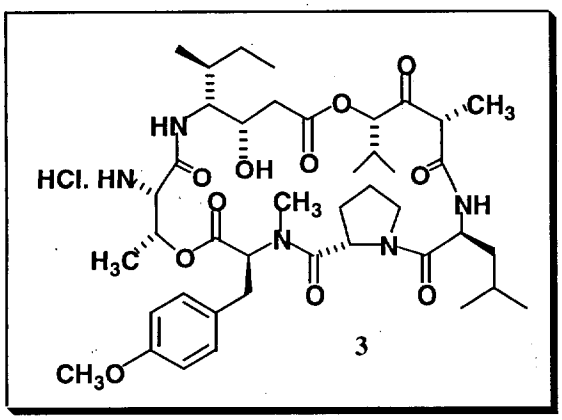
**Synthesis of $T_2$-DB**

Scheme **1**.




**1a** R = $CH_2CH_2Si(CH_3)_3$
**1b** R = H

**2**




**3**

*(S)-Lactyl-(S)-4,5-dehydroprolyl-(R)-N-methyl-leucine trimethylsilylethyl ester (**1a**).* (*S*)-*O*-(*tert*-butyldimethylsilyl)-Lactyl-(*S*)-4-hydroxyprolyl-(*R*)-*N*-Methyl-leucine (trimethylsilyl)ethyl ester was prepared as described previously with minor modifications (*51*; Scheme **1**). This tripeptide (0.330 g, 0.636 mmol) was dissolved in THF (2 mL) and treated with 3:1 AcOH:$H_2O$ (8 mL) at rt overnight then concentrated under reduced pressure. The residue was redissolved in toluene and concentrated repeatedly under reduced pressure until no AcOH remained. Flash chromatography on silica gel (3:7 acetone:hexanes) gave the alcohol (**1a**) as a yellow oil: $R_f$ 0.28 (40:60 acetone:hexanes); $^1$H NMR (500 MHz, $CDCl_3$) δ 0.01-0.37 (m, 9H), 0.85-1.03 (m, 8H), 1.34 and 1.39 (d, J=6.6 Hz, 3H), 1.42-1.44 and 1.67-1.73 (m, 2H), 1.75-1.80 (m, 1H), 2.70 (brs, 1H), 2.83 and 2.98 and 3.11 (s, 3H), 4.14-4.30 (m, 2H), 4.31-4.65 (m, 3H), 5.08 and 5.23 and 5.28 (dd,

J=5.5, 9.9 Hz, 1H), 5.60-5.61 and 5.63-5.65 (m, 1H), 5.75-5.84 (m, 1H), 5.96- 6.09 (m, 1H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ -1.6, -1.5, 17.4, 20.3, 20.4, 21.4, 23.2, 25.1, 31.7, 37.4, 38.4, 53.0, 55.4, 55.6, 57.9, 63.5, 64.9, 65.0, 65.5, 65.6, 66.4, 124.2, 124.8, 127.9, 128.9, 169.4, 170.9, 171.4 (Rotamers present in NMR); MS (FAB) calc'd for $C_{20}H_{36}N_2SiO_5$+Na: m/e 435.2291, found 435.2280; $[\alpha]_D^{20}$ -121° (c=1.202, $CHCl_3$).

*(S)-Lactyl-(S)-4,5-dehydroprolyl-(R)-N-methyl-leucine (**1b**).* A solution of ester **1a** (0.120 g, 0.291 mmol) in distilled THF (7.5 mL) was cooled to 0 °C and 0.1M LiOH (7.45 mL, 0.727 mmol) was added dropwise. The temperature was kept at 0 °C for 1 h and then allowed to rise gradually to rt Stirring was continued overnight. The mixture was concentrated to half its volume under reduced pressure, then extracted with ether (10 mL). The ether layer was back-extracted once with saturated sodium bicarbonate (10 mL). The aqueous layers were combined, acidified to pH 2 by careful addition of 6 M HCl, then extracted with ethyl acetate (4 x 10 mL). The ethyl acetate extracts were washed with acidified saturated sodium chloride (5 mL), dried with anhydrous magnesium sulfate, and concentrated under reduced pressure to yield **1b** as an amorphous solid (0.065 g, 71%) which was used without further purification: $R_f$ 0.13 (15:85 methanol:chloroform); $^1$H NMR (500 MHz, methanol-$d_4$) δ 0.87-1.05 (m, 6H,), 1.32 (d, 6.5H), 1.36 (d, 6.6H), 1.48 (br s, 1H), 1.70-1.85 (m, 3H), 2.83 and 3.12 and 3.17 (s, 3H), 4.40-4.53 (m, 3H), 4.99-5.03 (m, 1H), 5.65-5.67 (m, 1H), 5.88-5.91 (m, 1H), 6.08-6.10 (m, 1H); $^{13}$C NMR (125 MHz, methanol-$d_4$) δ 19.8, 21.7, 23.6, 25.8, 26.1, 32.4, 38.2, 38.8, 39.5, 54.5, 66.3, 66.7, 67.2, 121.0, 124.7, 130.0, 172.0, 174.4, 174.5 (Rotamers present in NMR); MS (FAB) calc'd for $C_{15}H_{24}N_2O_5$Na: m/e 335.1583, found 335.1571; $[\alpha]_D^{20}$ -182° (c=0.675, methanol).

*(S)-Lactyl-(S)-4,5-ditritioprolyl-(R)-N-methyl-leucine (2).* Compound **1b** (11.4 mg, 0.037 mmol) was dissolved in dry methanol (1.5 mL) containing 10% Pd/C catalyst (11.5 mg). This sample was degassed by two cycles of freezing and thawing then placed under an atmosphere of tritium gas for 2.5 h with brief warming to 45 °C. At the end of this time, the tritium was removed and the solvent was evaporated. The residue was redissolved in methanol and pumped dry three times, then filtered and lyophilized to dryness to provide the crude tritiated side chain compound **2** (1.9 Ci) which was characterized by tritium NMR and used without further purification.

*Tritiated didemnin B ($T_2$-DB).* To compound **2** (1 Ci) was added didemnin macrocycle as its HCl salt (**3**, 10 mg, 12 μmol) and BOP (7.96 mg, 18 μmol), followed by dry $CH_3CN$ (200 μL). The resulting solution was degassed then placed under an atmosphere of nitrogen and cooled to 0 °C. NMM (6 μL, 54 μmol) was added and this mixture was stirred at 0 °C for 30 min then at rt for 3.6 h. The $CH_3CN$ was removed *in vacuo* and replaced by EtOAc (1 mL). This solution was rinsed with 5% $HCl_{(aq)}$ (1.5 mL), 5% $NaHCO_{3(aq)}$ (1.5 mL), and brine (1.5 mL). Each aqeuous layer was back-extracted with EtOAc (3 x 1.5 mL). The combined organic layers were dried ($MgSO_4$), then filtered, and the solvent was removed *in vacuo*. The tritiated product was purified by RP-HPLC (C-18, 1.5 x 25 cm; 60% $CH_3CN$: 40% $H_2O$; 205 nm), and lyophilized to dryness to provide $T_2$-DB (specific activity = 53 Ci $mmol^{-1}$): $^3H$ NMR (320 MHz methanol-$d_4$) δ 1.95-2.01 (1H, m), 2.25-2.30 (1H, m); $^1H$ NMR (300 MHz, methanol-$d_4$) δ 0.86 –0.97 (m), 1.03-1.07 (t), 1.30 (d, J = 7 Hz, 3H), 1.35 (d, J = 7 Hz, 3H), 1.37 (d, J = 7 Hz, 3H), 1.58 – 1.63 (m), 1.95 – 2.19 (m), 2.21 – 2.33 (m), 2.37 (d, J = 7 Hz), 2.45 (d, J = 9Hz), 2.62 (s, 3H), 3.19 (s, 3H), 3.77 (3H), 3.77 (s), 4.46 (q, J = 6.6 Hz, 1H), 5.04 (q, J = 6.9 Hz, 1H), 5.25 – 5.40 (m, 1H), 6.87 (d, J = 8.4 Hz, 2H_, 7.14 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 9 Hz, 1H).

Figure **S1**.

Tritium NMR spectrum of $T_2$-DB recorded at 320 MHz.

Figure **S2**.

Proton NMR spectrum of $T_2$-DB recorded at 300 MHz.

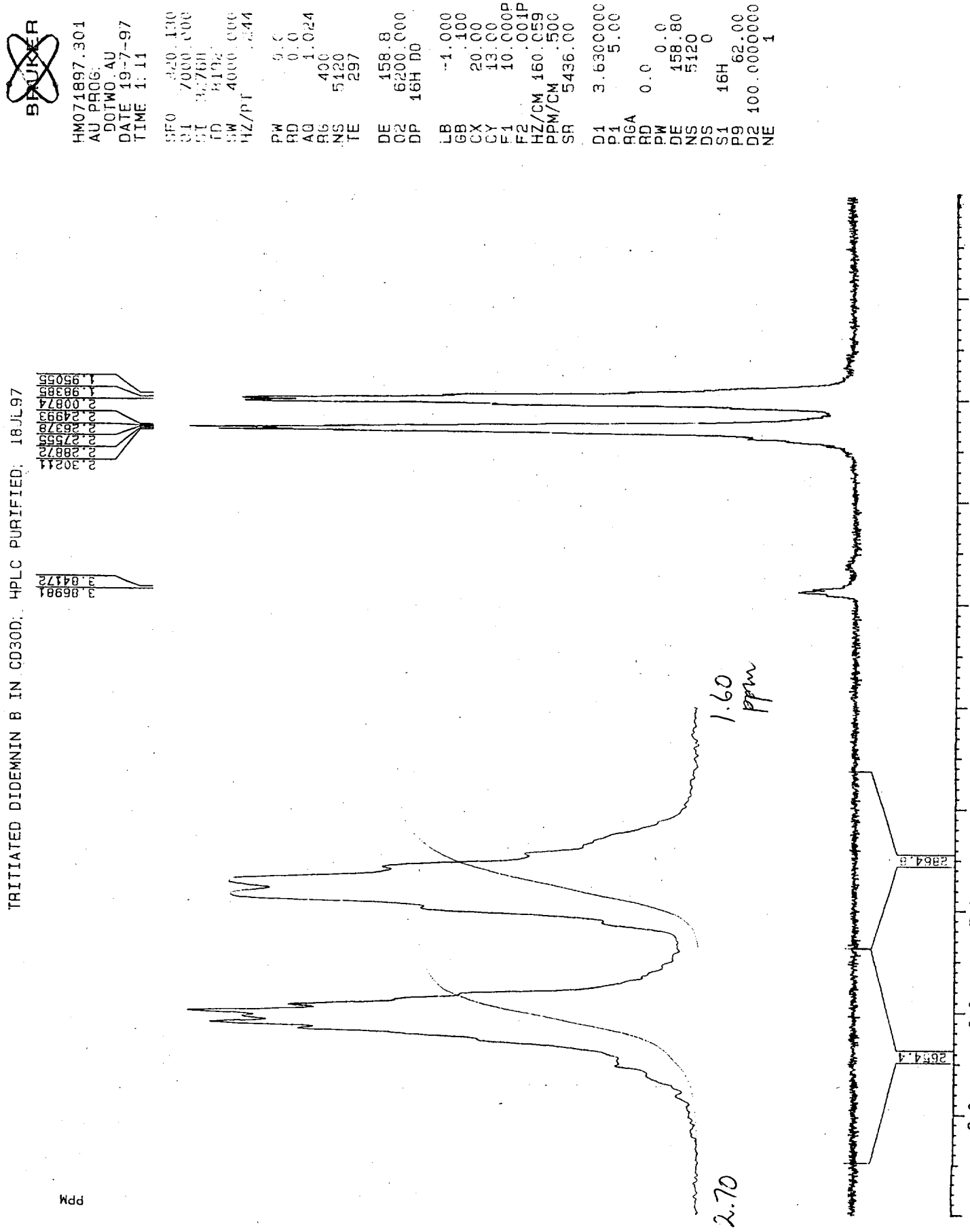



Figure S1.
Tritium NMR spectrum of $T_2$-DB recorded at 320 MHz.

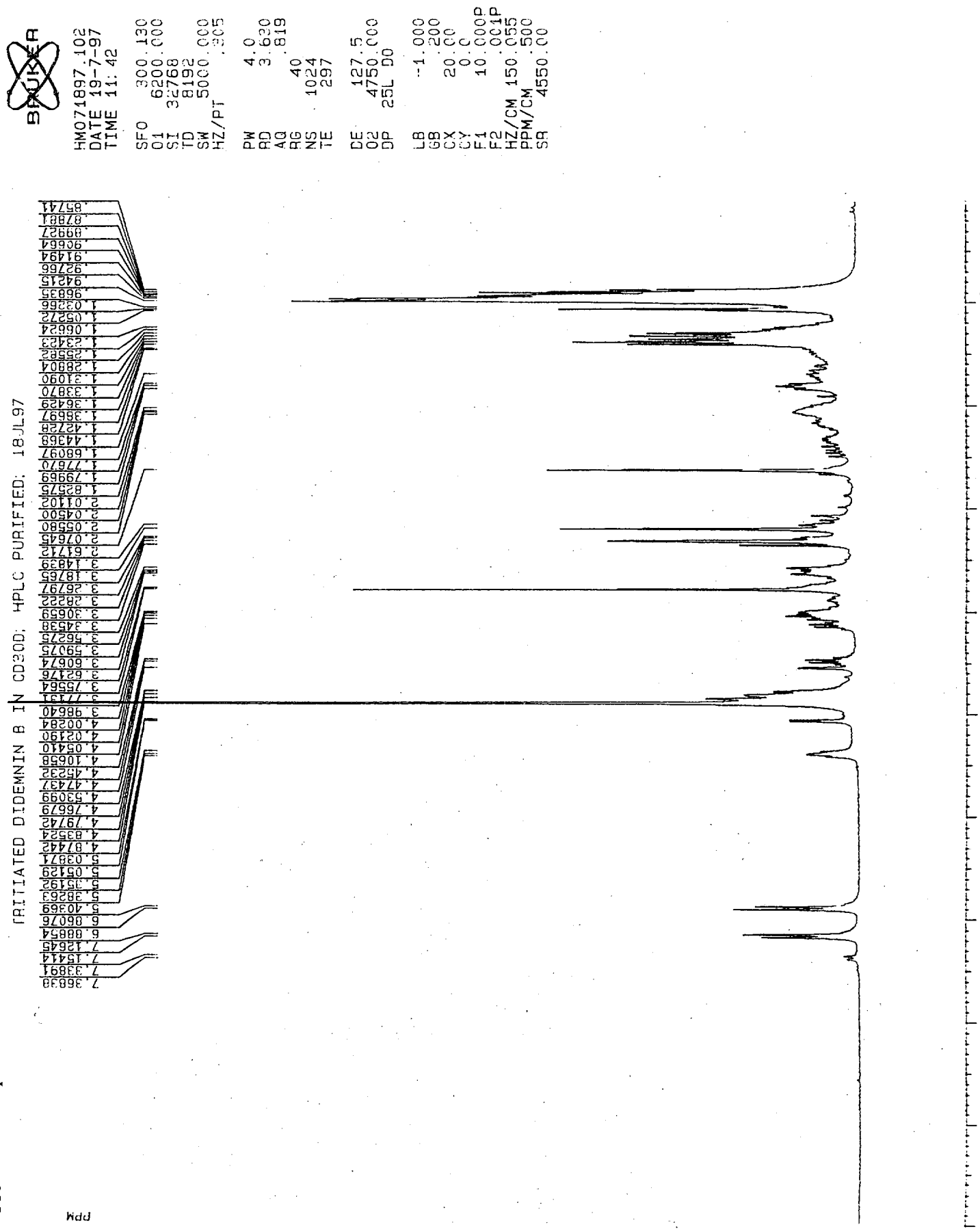



Figure S2.
Proton NMR spectrum of $T_2$-DB recorded at 300 MHz.