Cyclic Homooligomers of Furanoid Sugar Amino Acids

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Supporting Information

Contents

General Experimental Procedures. Reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm silica gel plates with UV light, I_2, 7% ethanolic phosphomolybdic acid-heat and 2.5% ethanolic anisaldehyde (with 1% AcOH and 3.3% conc. H_2SO_4)-heat as developing agents. Silica gel finer than 200 mesh was used for flash column chromatography. Yields refer to chromatographically and spectroscopically homogeneous materials unless otherwise stated. Melting points are uncorrected. IR spectra were recorded as neat liquids or KBr pellets. Mass spectra were obtained under liquid secondary ion mass spectrometric (LSIMS) technique, electron spray ionisation (ESI) and MALDI techniques.

NMR spectroscopy. NMR spectra were recorded on 500 MHz spectrometers at 30 °C with 2-10 mM solutions in appropriate solvents using TMS as internal standard or the solvent signals as secondary standards and the chemical shifts are shown in δ scales. Multiplicities of NMR signals are designated as s (singlet), d (doublet), t (triplet), q (quartet), br (broad), m (multiplet, for unresolved lines), etc. ^{13}C NMR spectra were recorded on 75 MHz spectrometers with complete proton decoupling. The chemical shift assignments were carried out with the help of two-dimensional total correlation spectroscopy (TOCSY)[1] and rotating frame nuclear Overhauser effect spectroscopy (ROESY) experiments,[1] the later also provided the information on the proximity of protons. All the experiments were carried out in the phase sensitive mode.[2] The spectra were acquired with 2 × 256 or 2 × 192 free induction decays (FID) containing 8-16 transients with relaxation delays of 1.0 to 1.5 sec. The ROESY experiments were performed with mixing time of 0.3 sec. For ROESY experiments a spin-locking field of about 2 kHz was used. The TOCSY experiments were performed with the spin locking fields of about 10 kHz and a mixing time of 0.08 sec. The two-dimensional data were processed with Gaussian apodization in both the dimensions. To obtain the temperature coefficients of NH-chemical shifts, the spectra were recorded between 30 and 70 °C in DMSO-d_6 and between 30 and 55 °C in $CDCl_3$.

Molecular dynamics. Molecular mechanics/dynamics calculations were carried out using Sybyl 6.8 program on a Silicon Graphics O2 workstation. The Tripos force field, with default parameters, was used throughout the simulations. Minimizations were done first with steepest decent, followed by conjugate gradient methods for a maximum of 2000 iterations each or RMS deviation of 0.005 kcal/mol, whichever was earlier. The energy-minimized structures were then subjected to MD studies.

A number of inter atomic distances and torsional angle constraints obtained from NMR data were used. Distance constraints with a force constant of 15 kcal/Å were applied in the form of flat bottom potential well with a common lower bound of 2.0 Å and the upper bound of 2.8, 3.5, 4.0 and 4.5 Å, in accordance with the nOe intensities. Force constants of 30 kcal/Å and 5 kcal/Å were employed for H-bond distance and dihedral angle constraints, respectively.[3] The energy-minimized structures were subjected to constrained MD simulations for duration of 300 ps using 50 cycles, each of 6 ps period, of the Simulated Annealing protocol. The atomic velocities were applied following Boltzmann distribution about the center of mass, to obtain a starting temperature of 700 °K. After simulating for 1 ps at high temperature, the system temperature was reduced exponentially over a 5 ps period to reach a final temperature of 300 °K. Structures were sampled after every two cycle, leading to an ensemble of total 25 structures. The sampled structures were energy-minimized using the above-mentioned protocol and the superimposed structures obtained by backbone alignment are shown in Figures 3, 4 and 5. To determine the backbone and the average pair-wise heavy atom RMSD, the structures were analyzed using the MOLMOL program.

Ion flux study. The ion transporting abilities of the cyclic peptides **3b**, **4b**, **8b** and **9b** and the bicyclic lactam **5b** across model membranes comprising of large unilamellar vesicles (LUVs) from palmitoyl-oleoyl phosphatidylcholine (POPC)[4] were assessed by the dissipation of valinomycin-mediated K^+ diffusion potential on adding these substrates as monitored by the fluorescence of cyanine dye diS-C_3-(5).[5] LUVs were made by the rapid extrusion procedure in Hepes buffer (pH 7.4) and KCl (150 mM). The vesicles were diluted (100 fold) using the same buffer (pH 7.4) containing NaCl (150 mM). To an aliquot of this LUV preparation (1 mL, 40 μM), cyanine dye (diS-C_3-(5); 1 μM final concentration) was added, followed by valinomycin (0.01 μM, final concentration) to create a diffusion potential which is reflected by a sharp fall in fluorescence (point Vm) as shown in Figure 6. Addition of **5b** (40 μM) at point C in the profile resulted in the dissipation of the diffusion potential as seen by the increase in the fluorescence again indicating that the molecule is able to cause the influx of Na^+ ions from the suspension medium into the vesicles. The spikes seen on the profile at Vm and C resulted from the opening of the chamber door. The excitation and emission wavelengths were 620 and 670 nm, respectively. As already pointed out, other cyclic homoologomers did not show any significant ion influx.

References

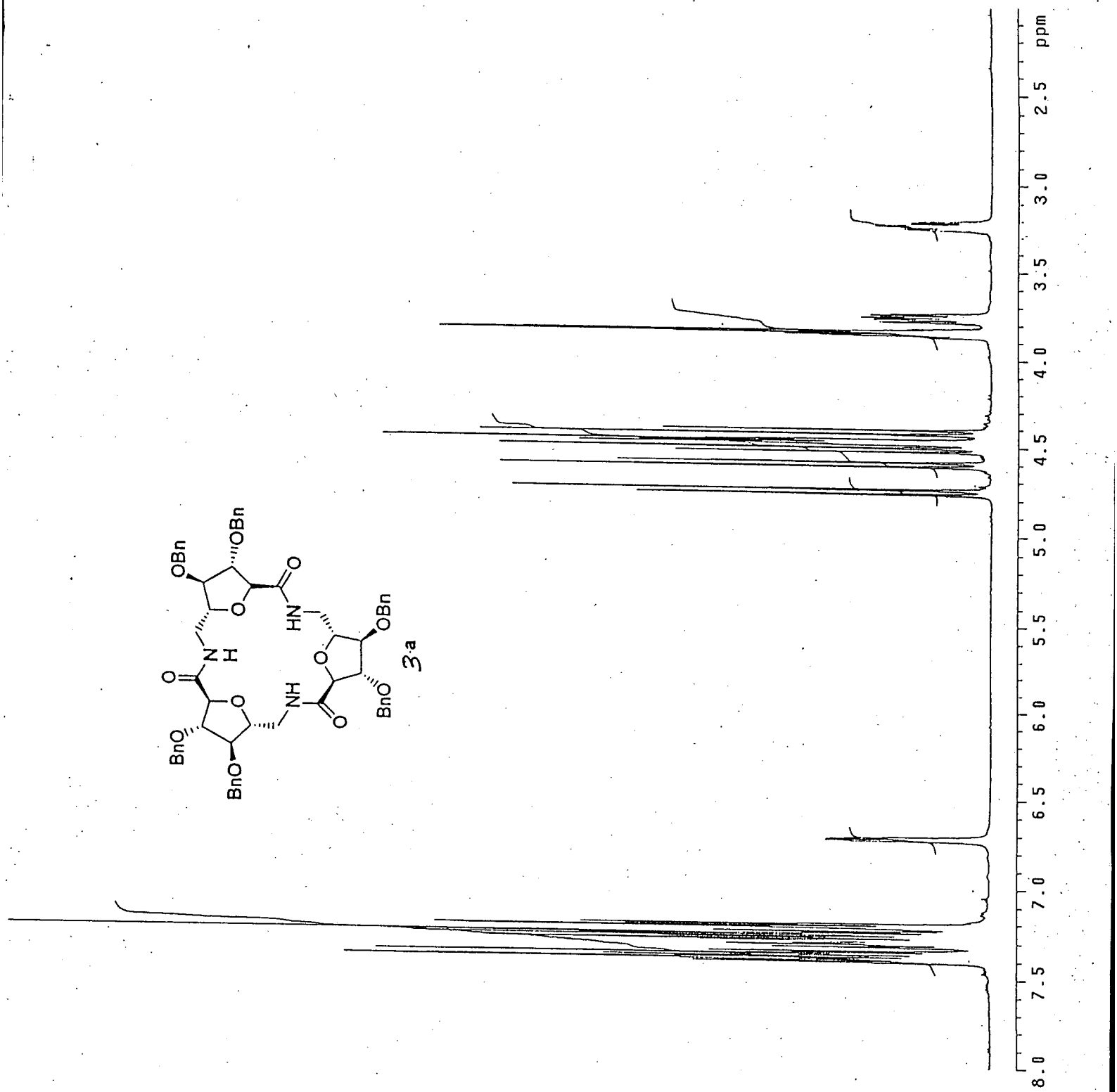
(1) (a) Cavanagh, J.; Fairbrother, W. J.; Palmer III, A. G.; Skelton, N. J. *Protein NMR Spectroscopy*, Academic Press: San Diego, 1996. (b) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986.

(2) States, D. J.; Haberkorn, R. A.; Ruben, D. J. *J. Magn. Reson.* **1982**, *48*, 286.

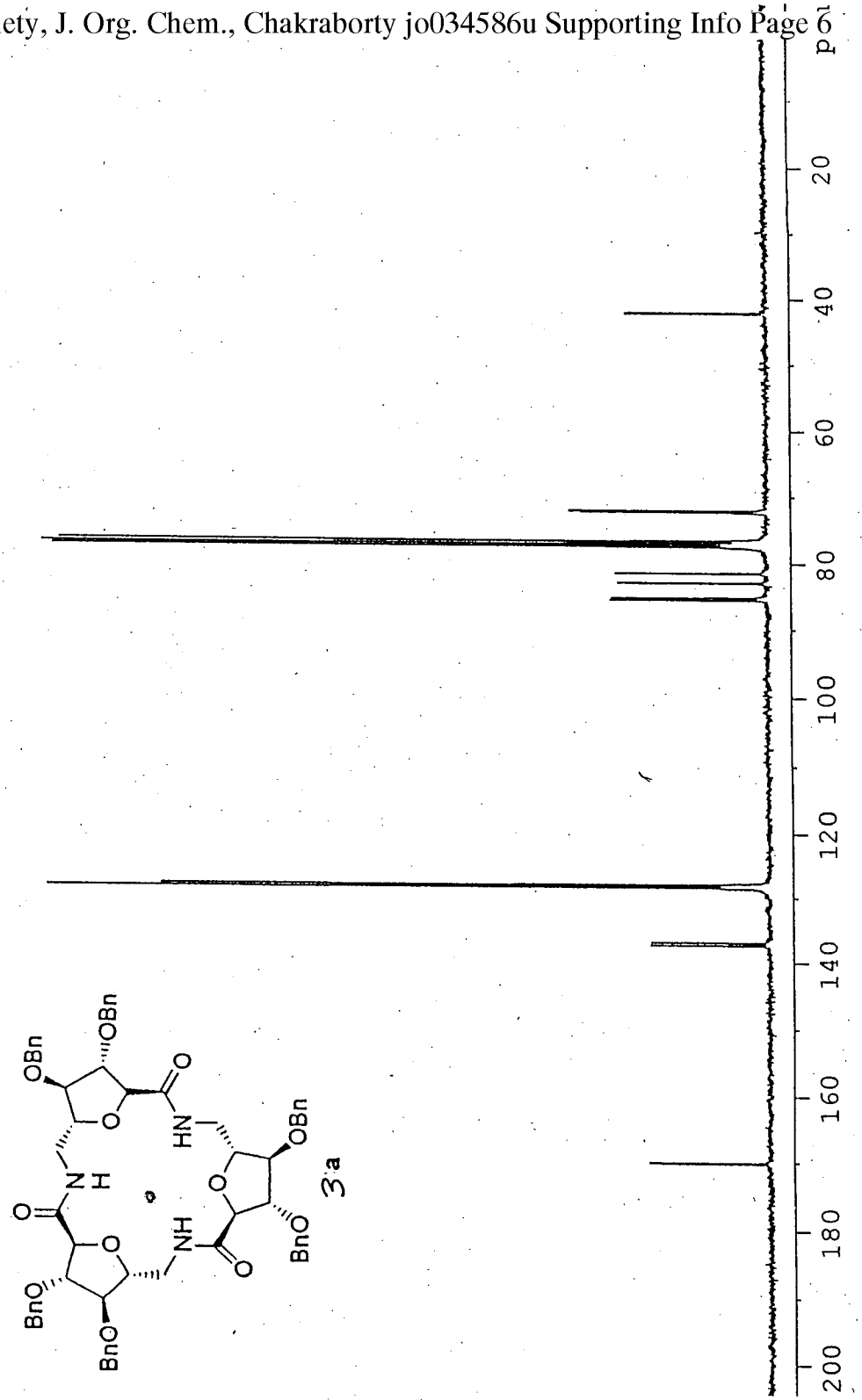
(3) Kessler, H.; Griesinger, C.; Lautz, J.; Müller, A.; F. van Gunsteren, W.; Berendsen, H. J. C. *J. Am. Chem. Soc.* **1988**, *110*, 3393.

(4) MacDonald, R. C.; MacDonald, R. I.; Menco, B. Ph.M.; Takeshita, K.; Subbarao, N. K.; Hu, L.-R *Biochim. Biophys. Acta* **1991**, *1061*, 297.

(5) Sims, P. J.; Waggoner, A. S.; Wang, C. H.; Hoffman, J. F. *Biochemistry* **1974**, *13*, 3315.



^{1}H NMR (500 MHz) spectrum of 3a in $CDCl_3$.



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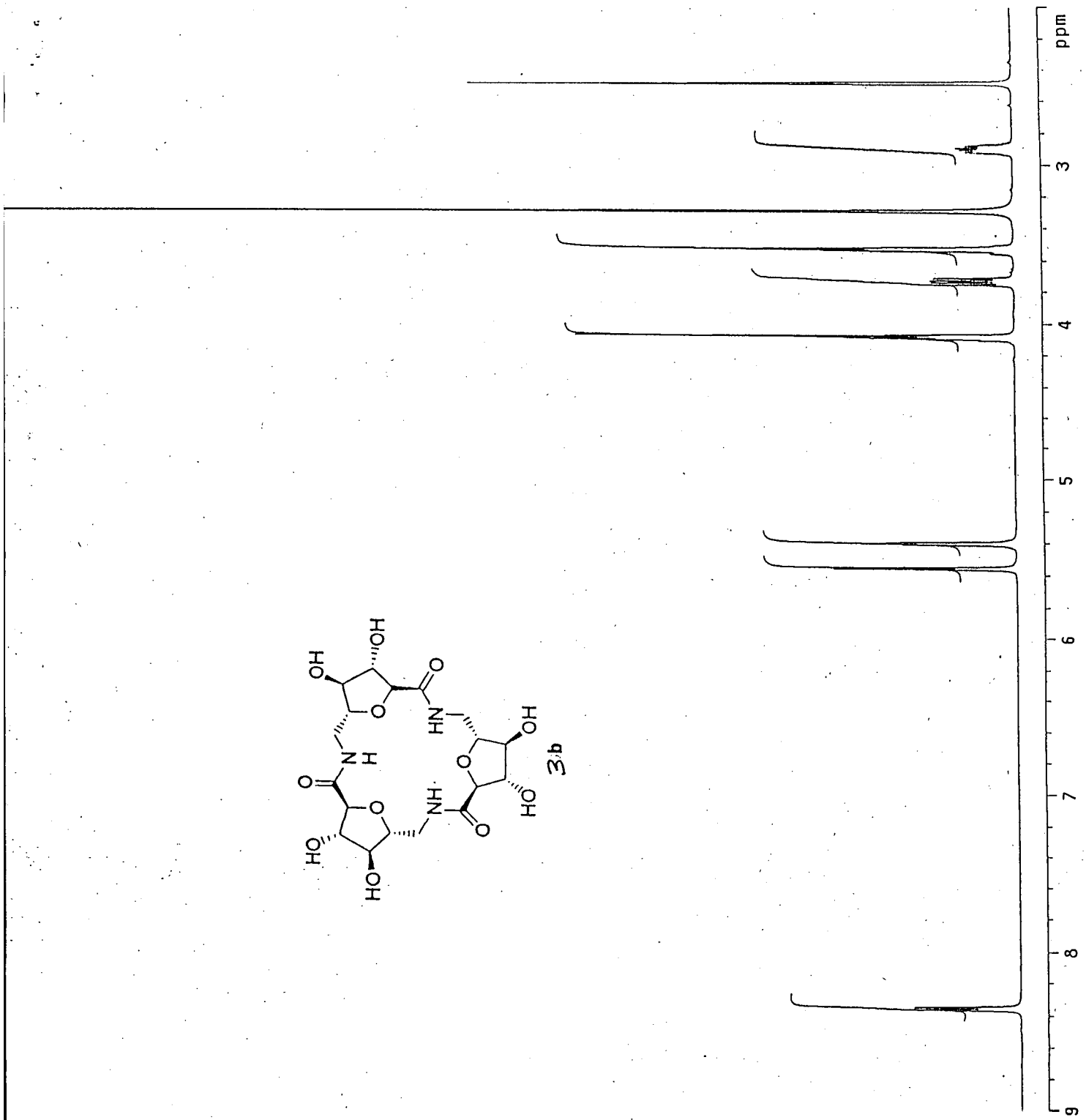
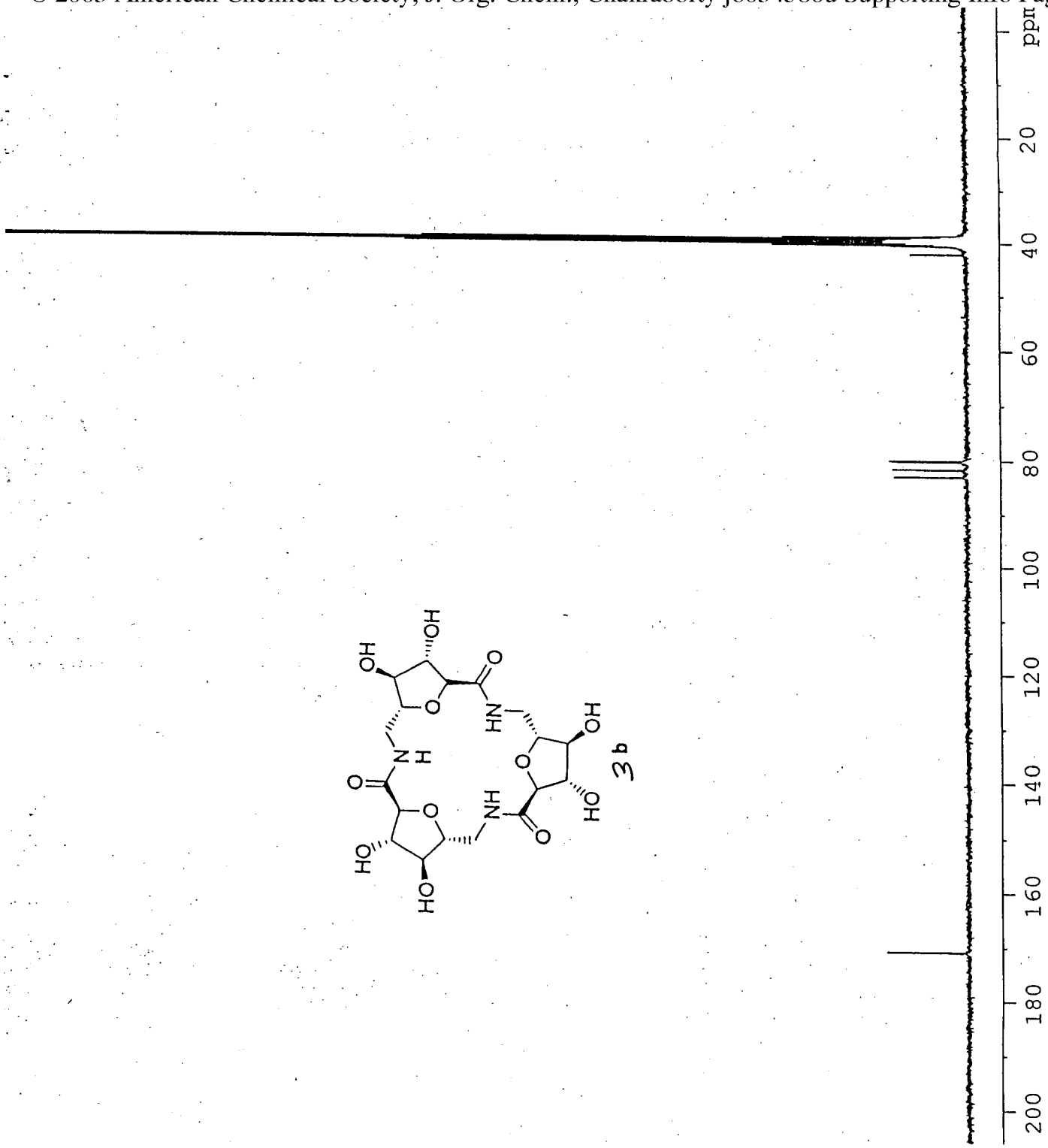
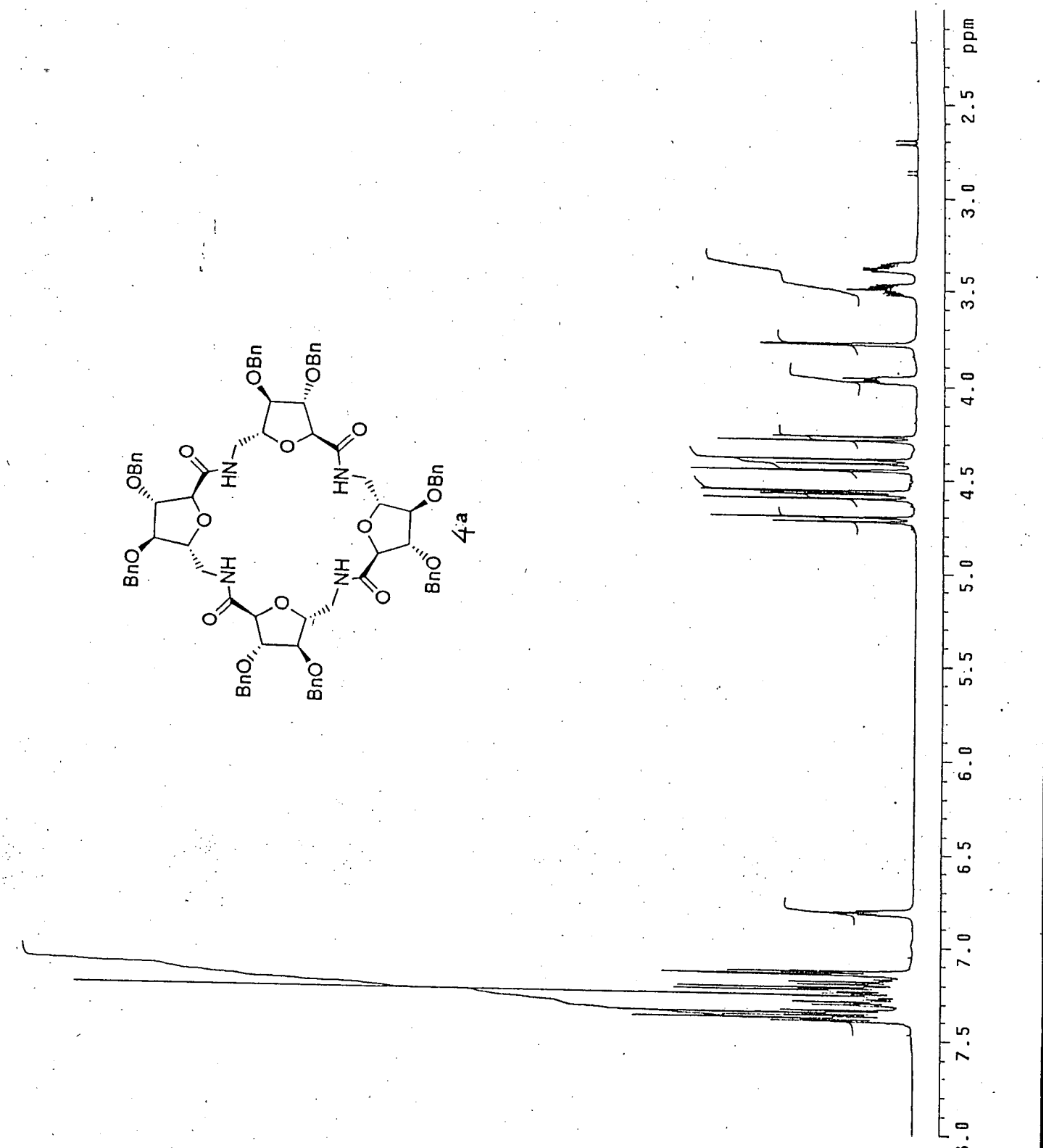


3b

^{1}H NMR (500 MHz) spectrum of **3b** in DMSO-d_6.



^{13}C NMR (75 MHz) spectrum of **3b** in DMSO-d_6.



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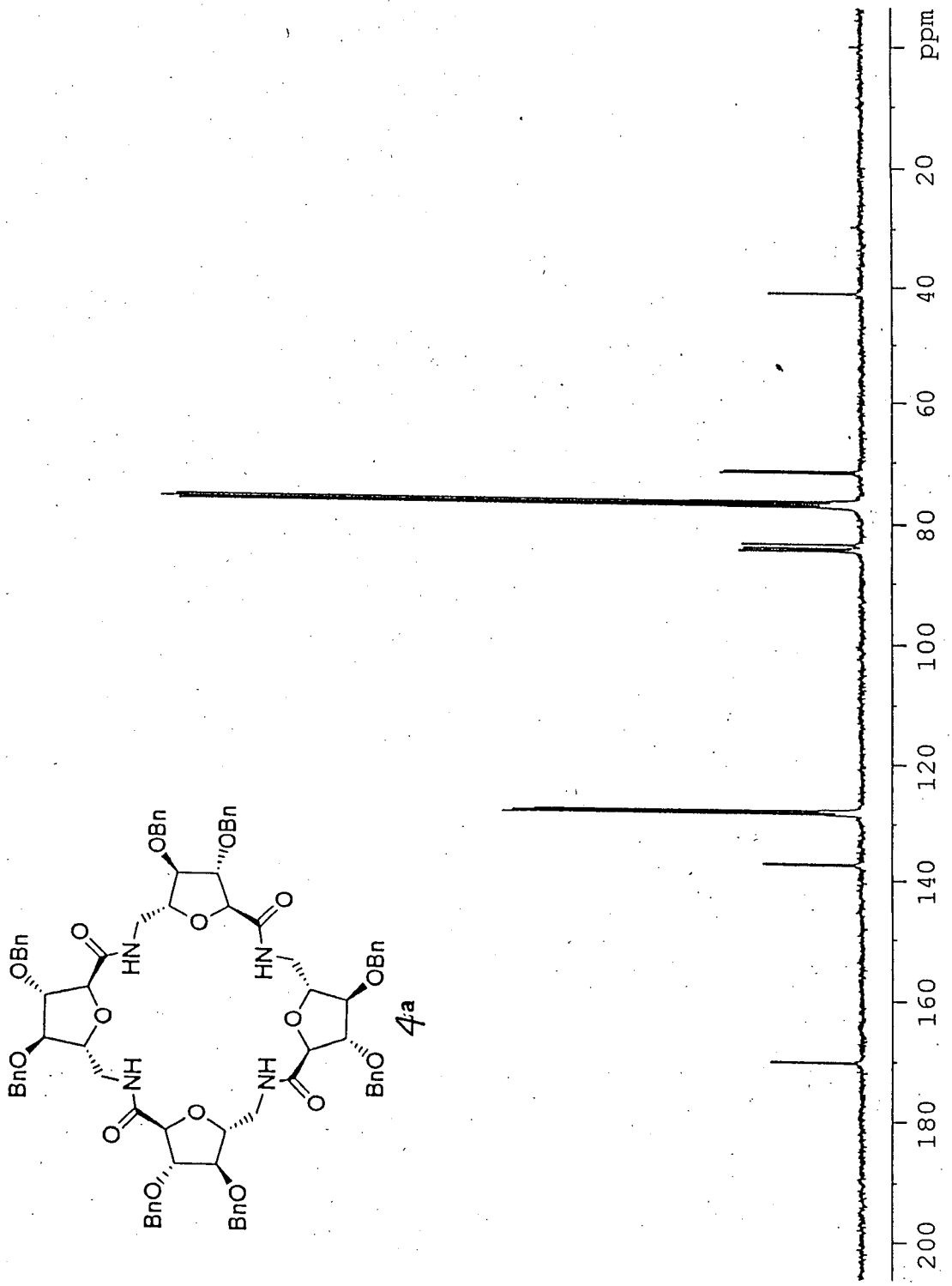
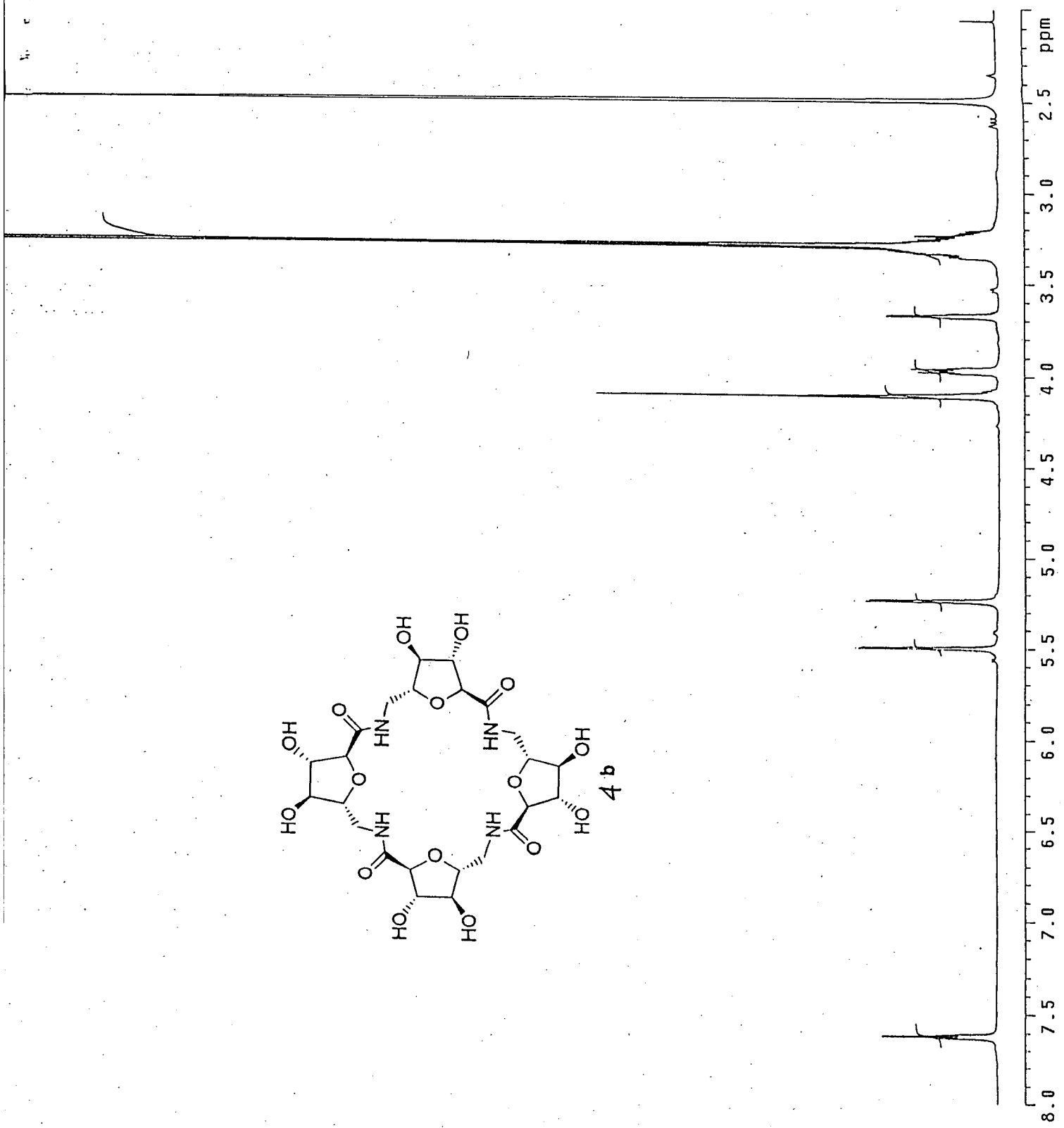
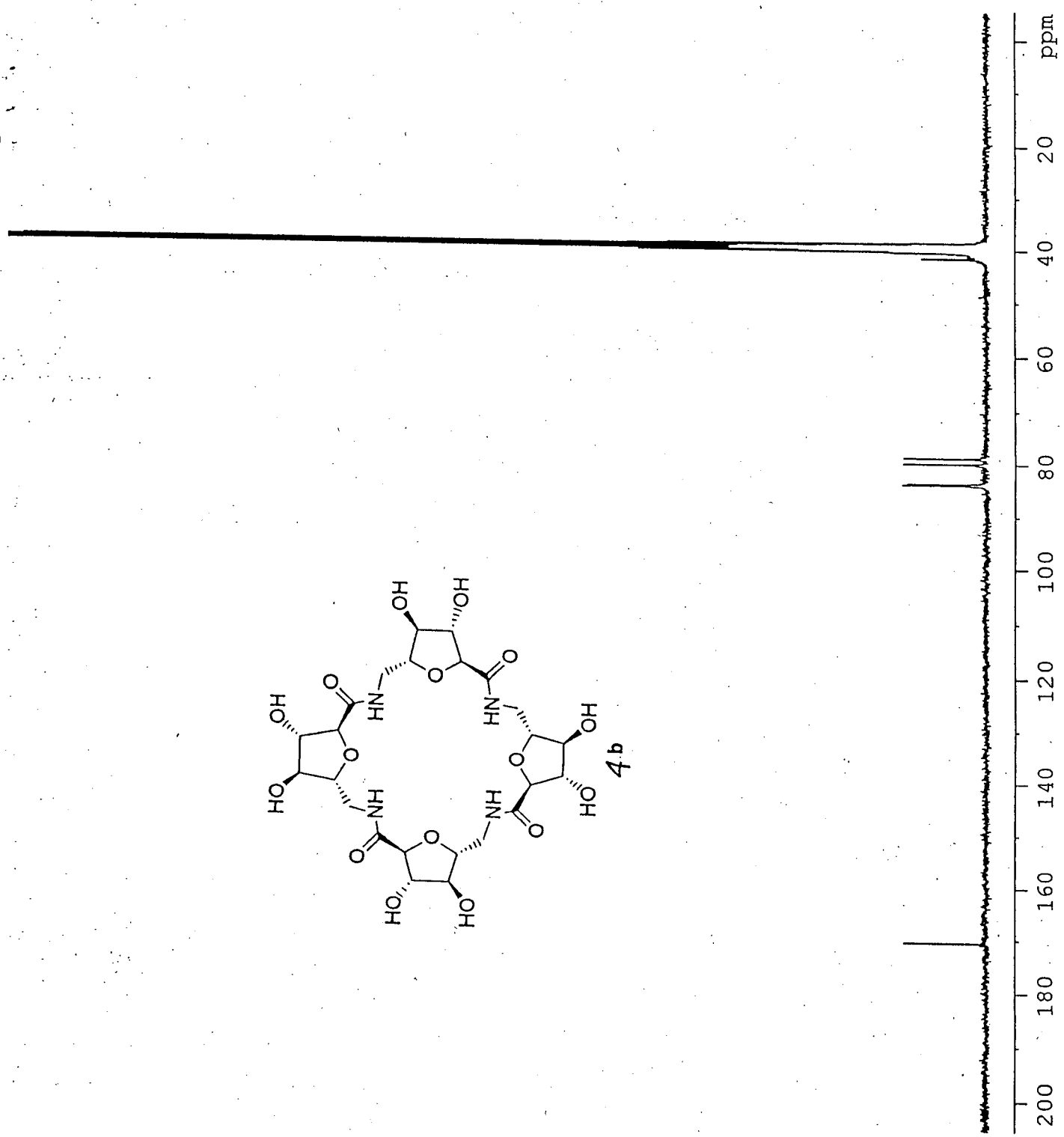


^{13}C NMR (75 MHz) spectrum of **4a** in $CDCl_3$.

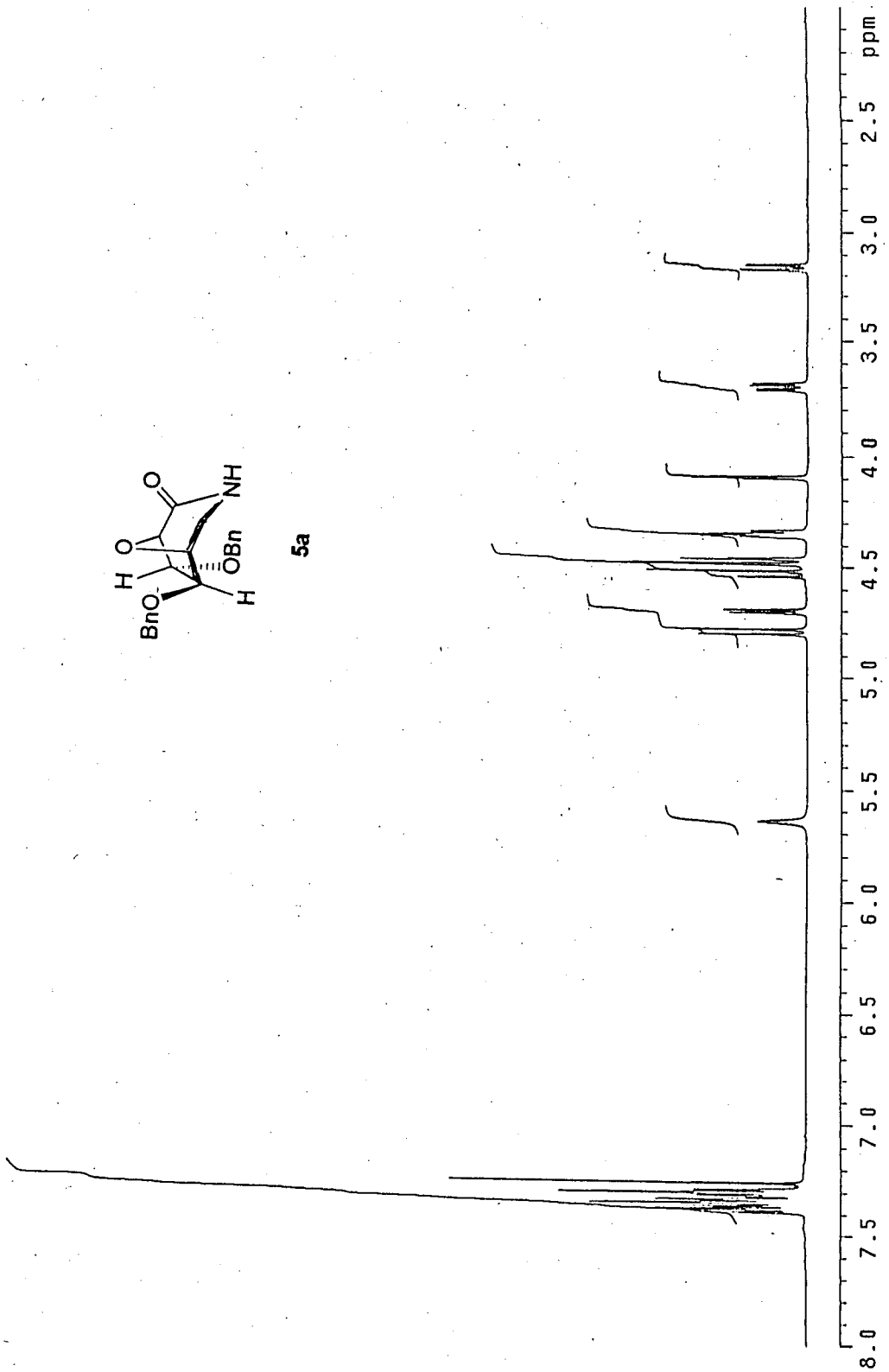


4b

^{1}H NMR (500 MHz) spectrum of **4b** in DMSO-d_6.



^{13}C NMR (75 MHz) spectrum of **4b** in DMSO-d_6.



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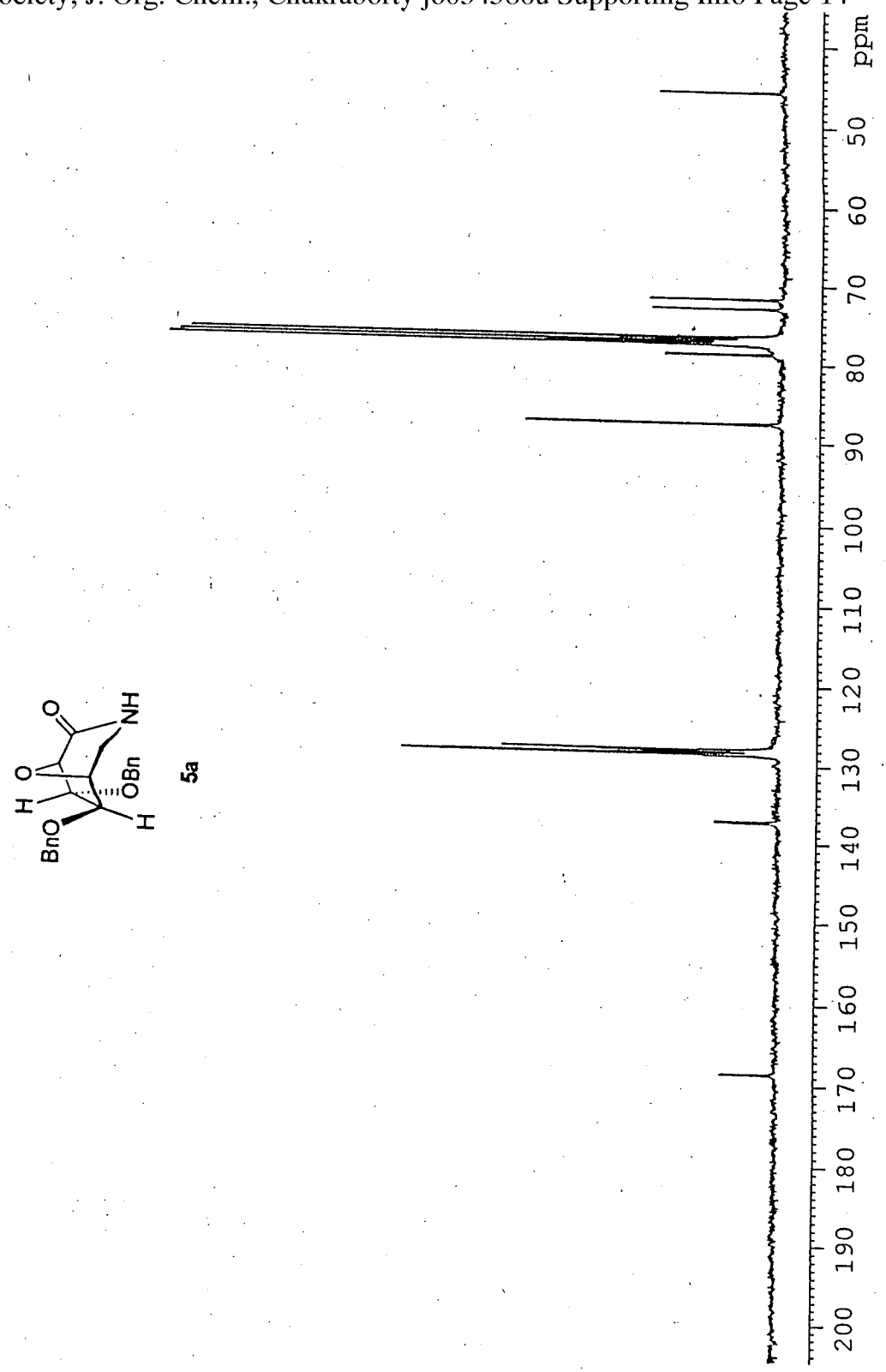

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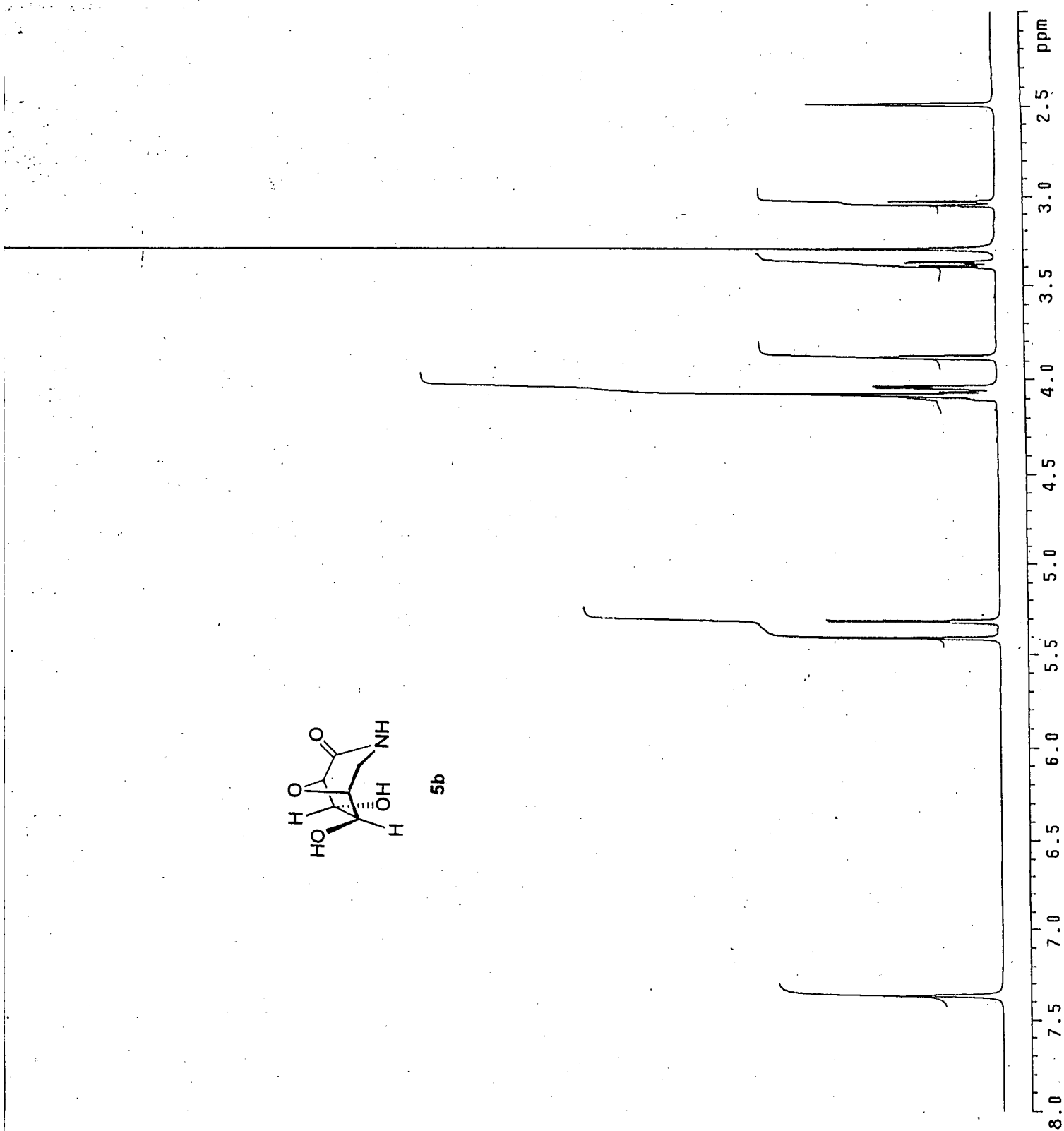
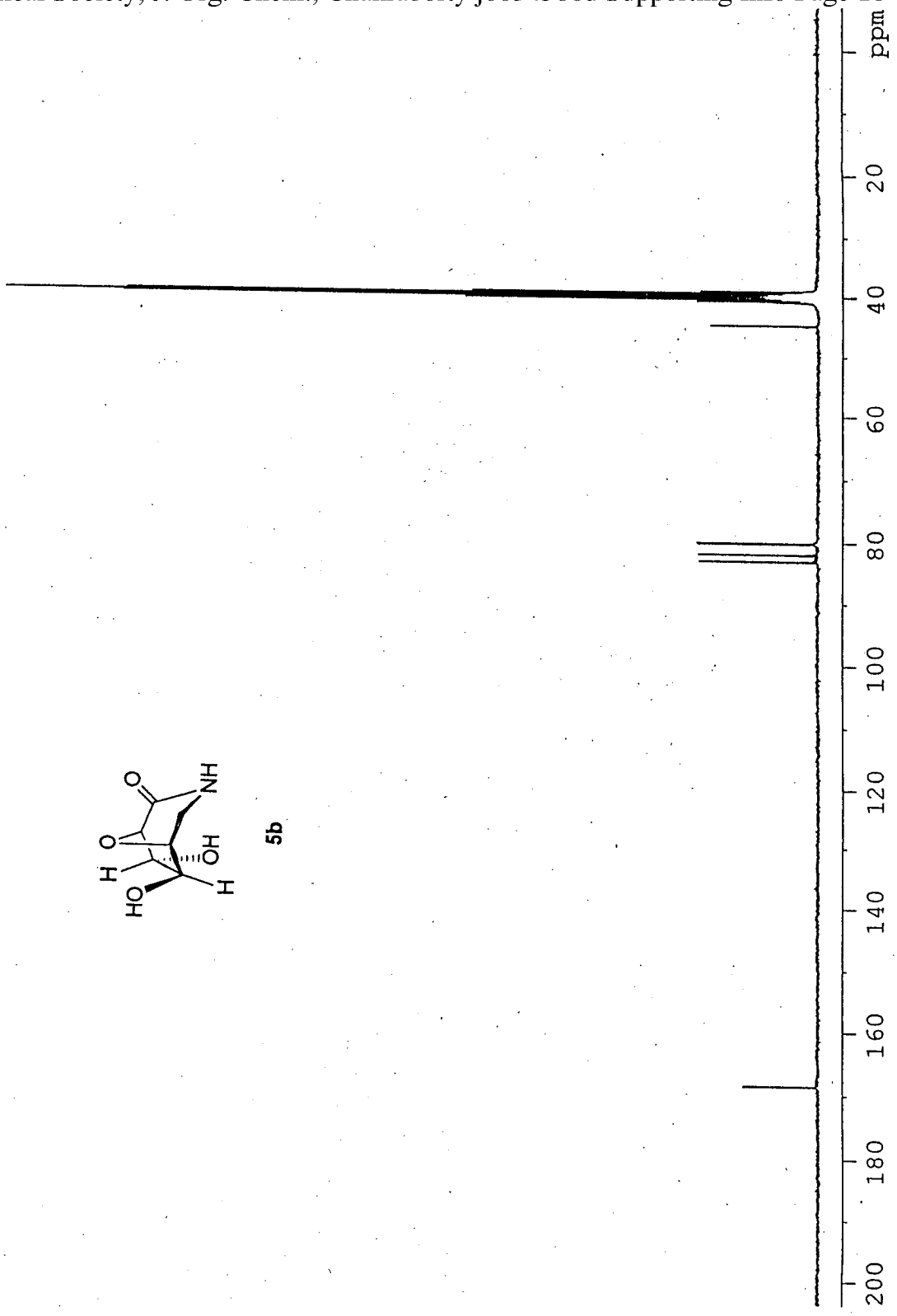


5b

^{1}H NMR (500 MHz) spectrum of **5b** in DMSO-d_6.



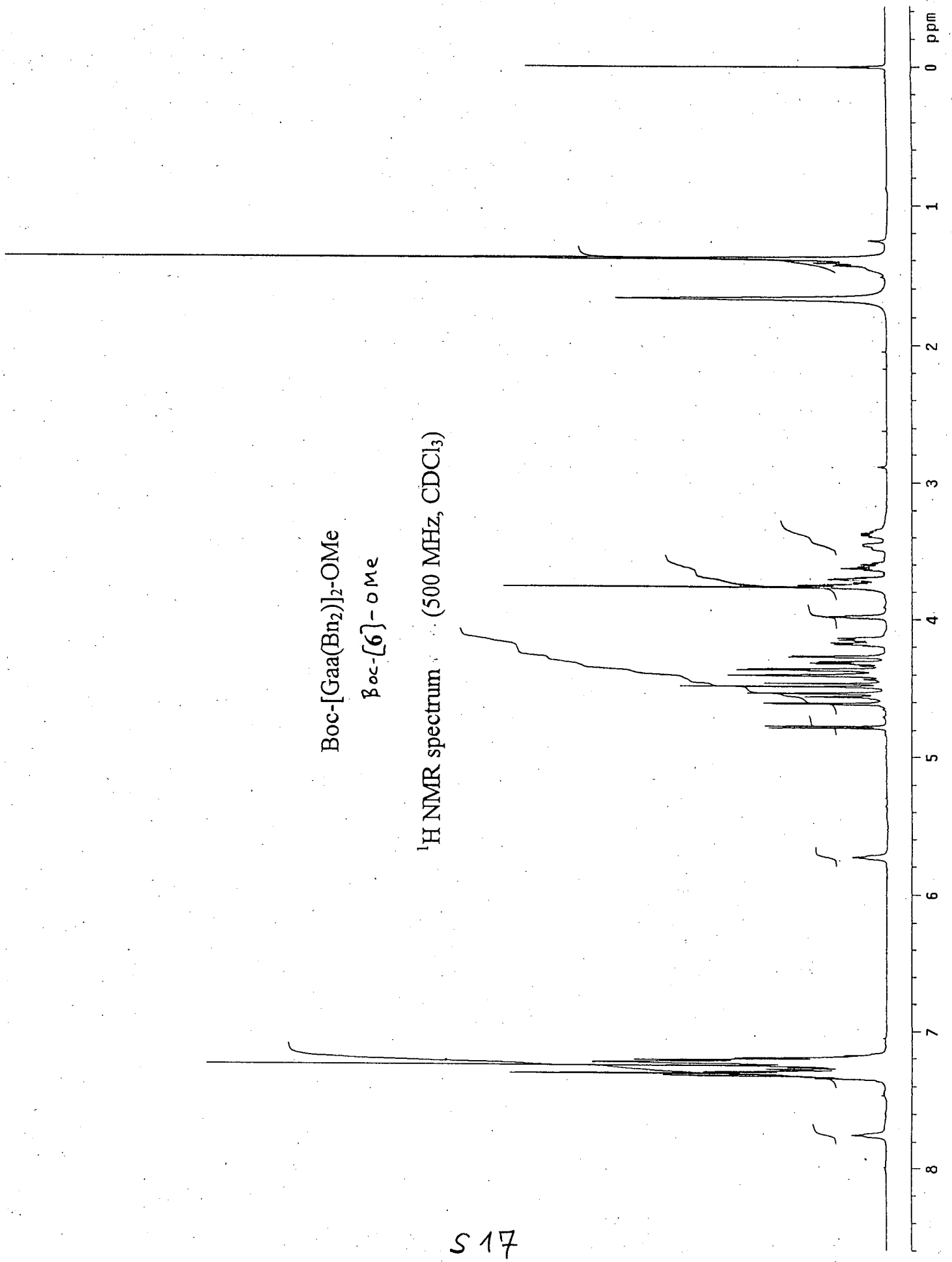
5b

^{13}C NMR (75 MHz) spectrum of **5b** in DMSO-d_6.

Boc-[Gaa(Bn$_2$)]$_2$-OMe

Boc-[6]-OMe

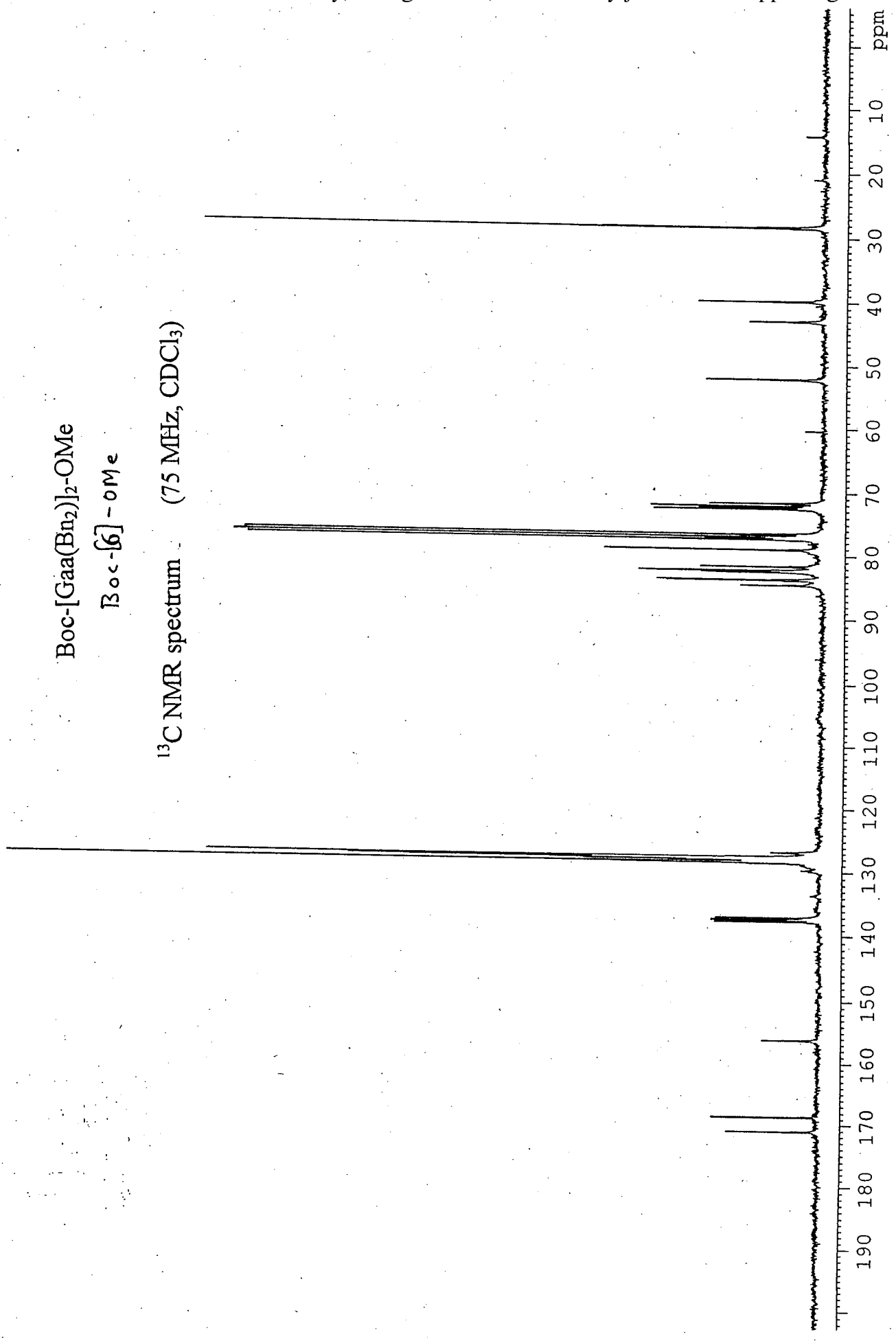
^{1}H NMR spectrum (500 MHz, $CDCl_3$)



Boc-[Gaa(Bn$_2$)]$_2$-OMe

Boc-[6]-OMe

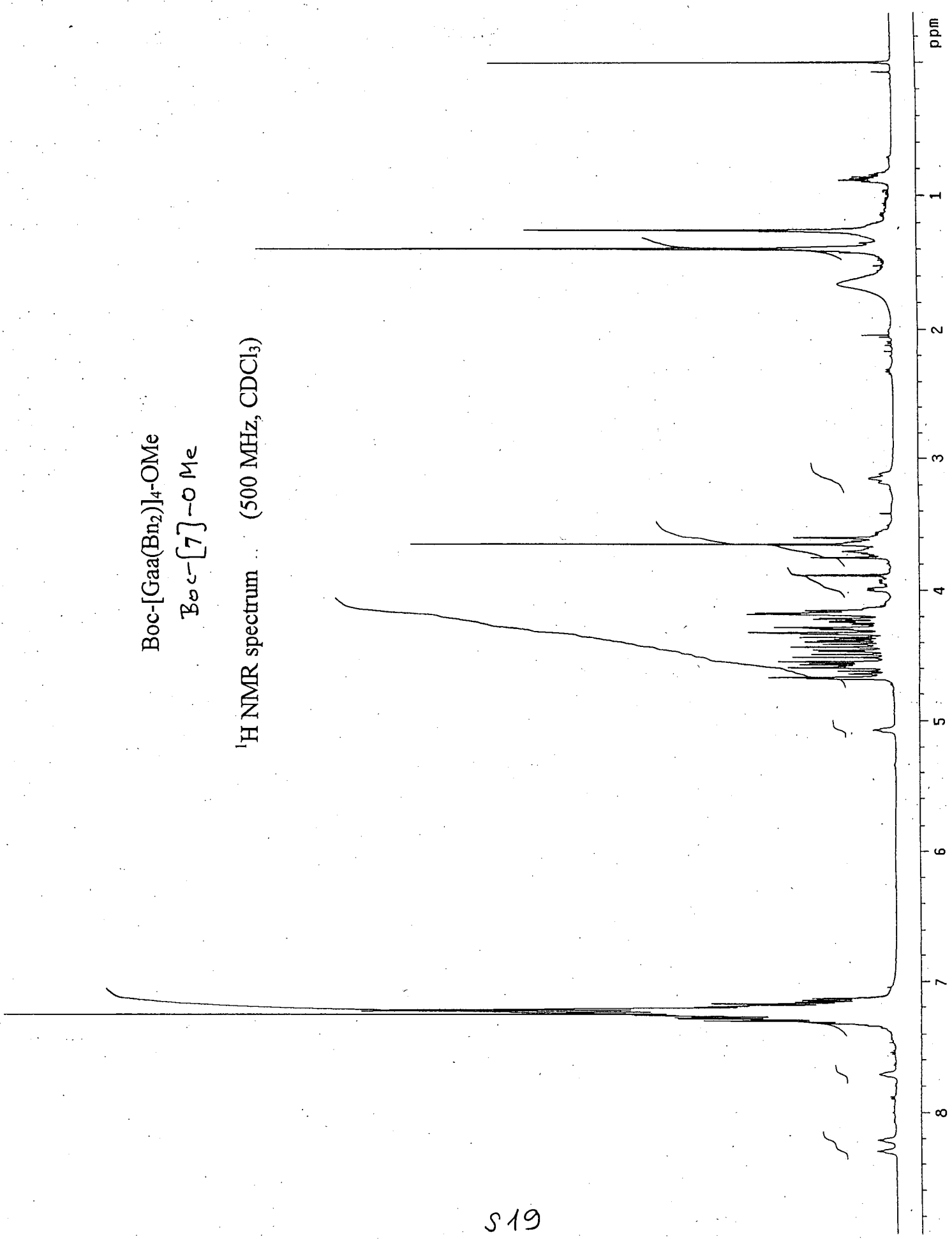
^{13}C NMR spectrum (75 MHz, $CDCl_3$)



Boc-[Gaa(Bn$_2$)]$_4$-OMe

Boc-[7]-O Me

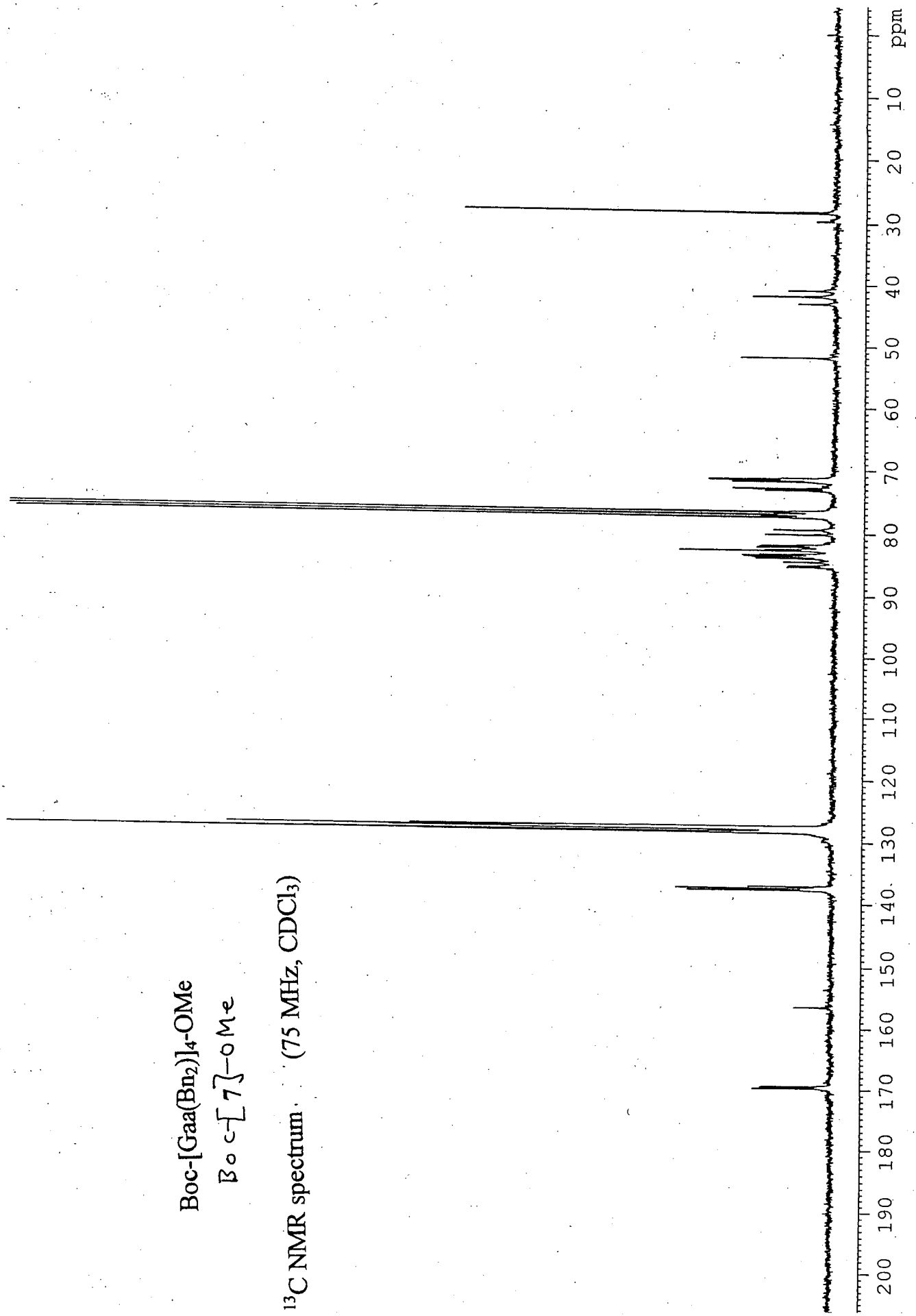
^{1}H NMR spectrum (500 MHz, CDCl$_3$)

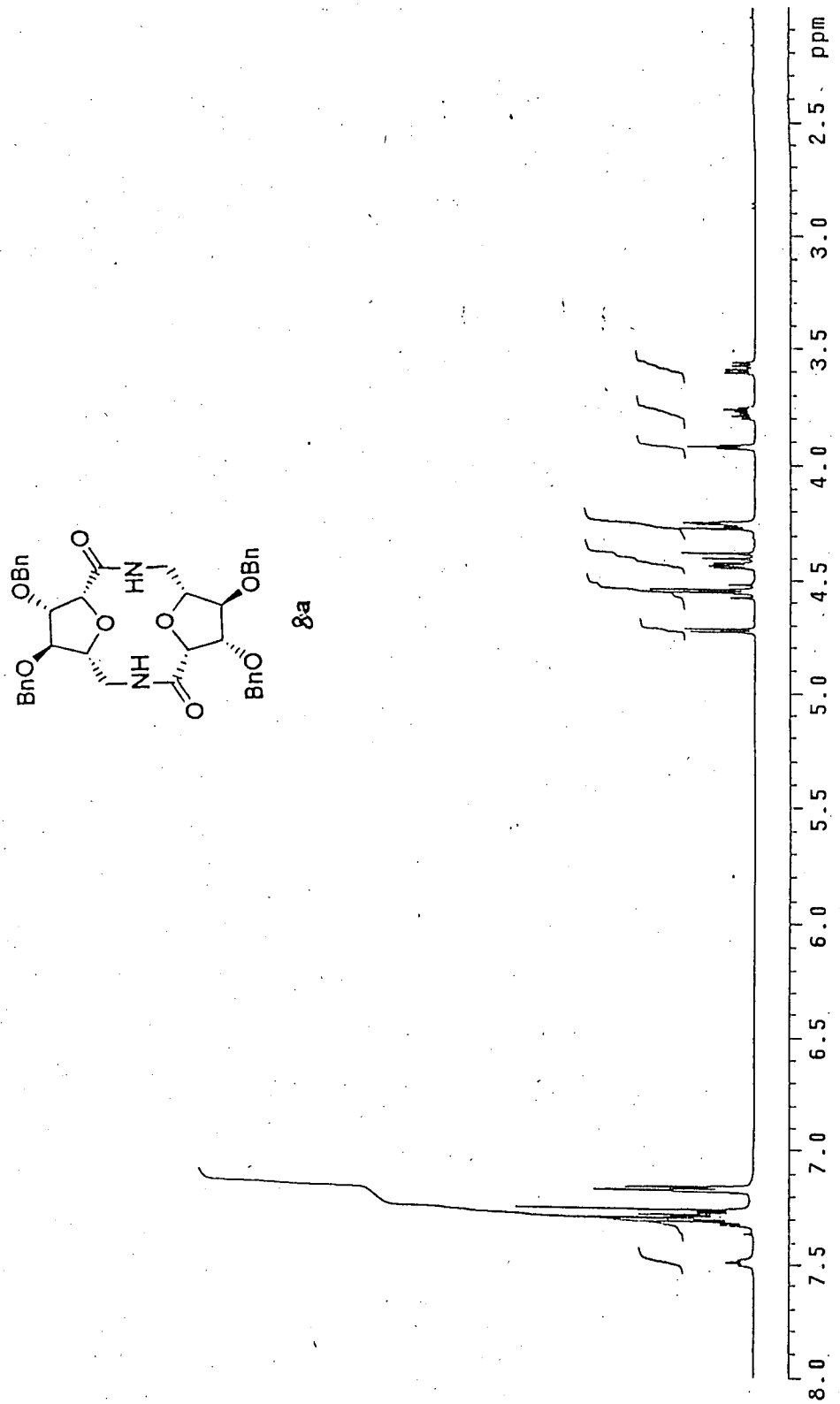


Boc-[Gaa(Bn$_2$)]$_4$-OMe

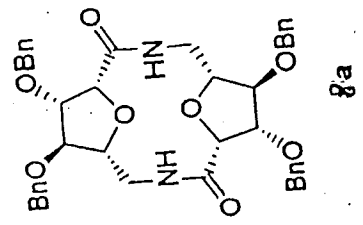
Boc-[ ]$_4$-OMe

^{13}C NMR spectrum (75 MHz, $CDCl_3$)

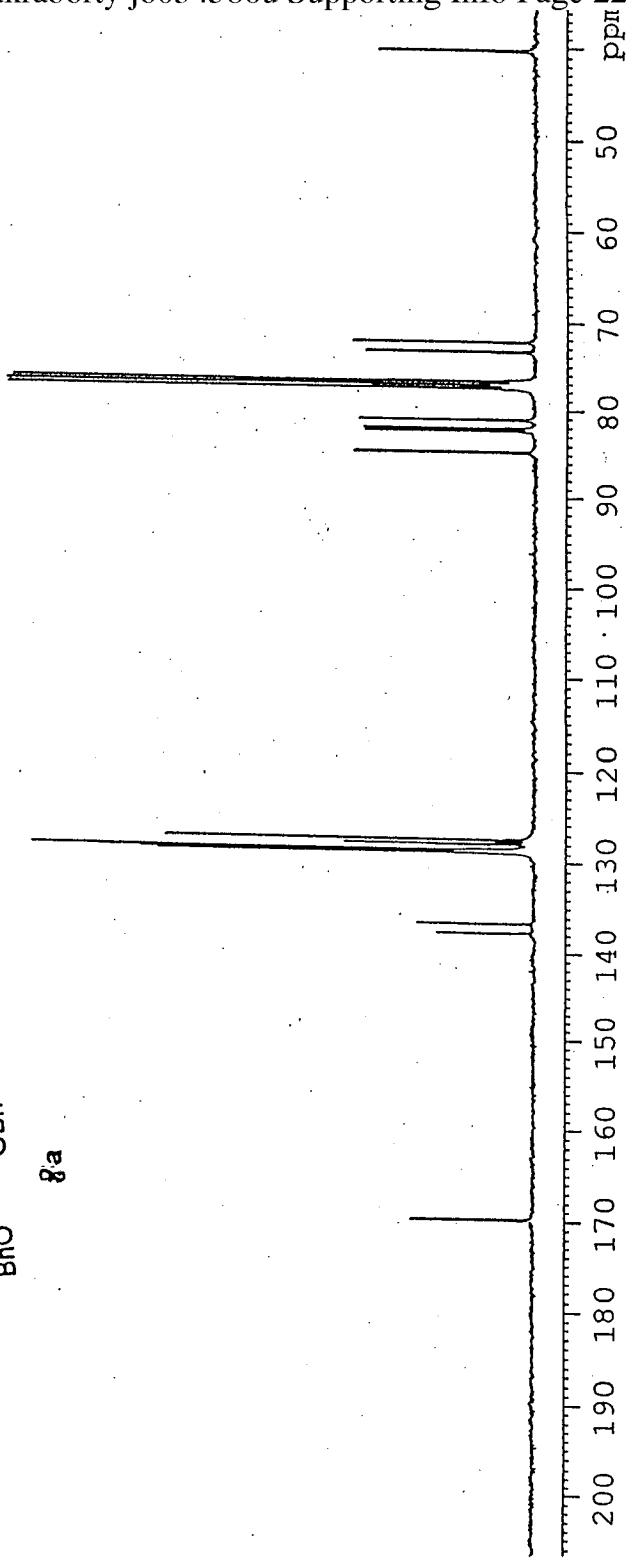




^{1}H NMR (500 MHz) spectrum of **8a** in $CDCl_3$.



8a



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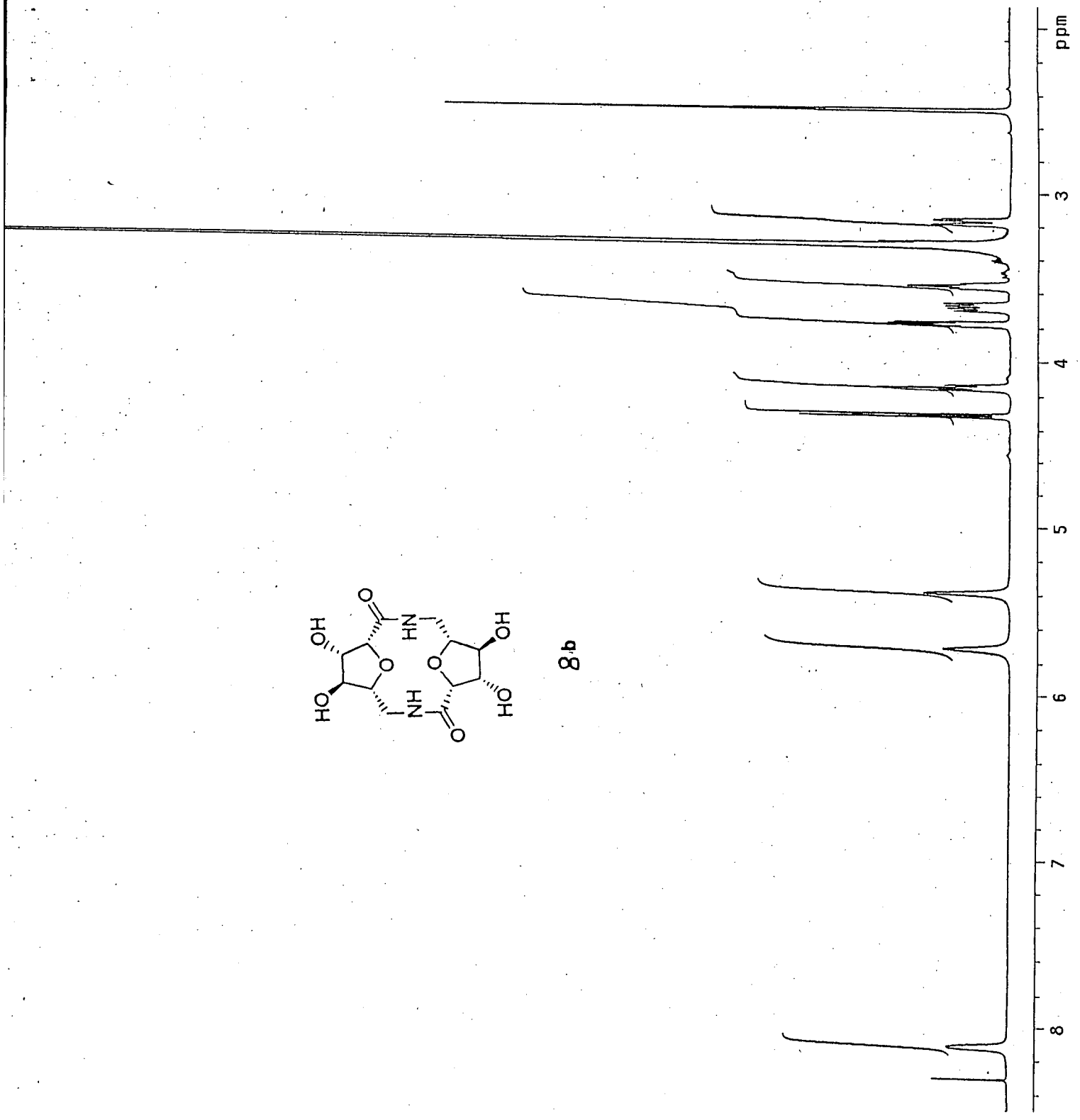

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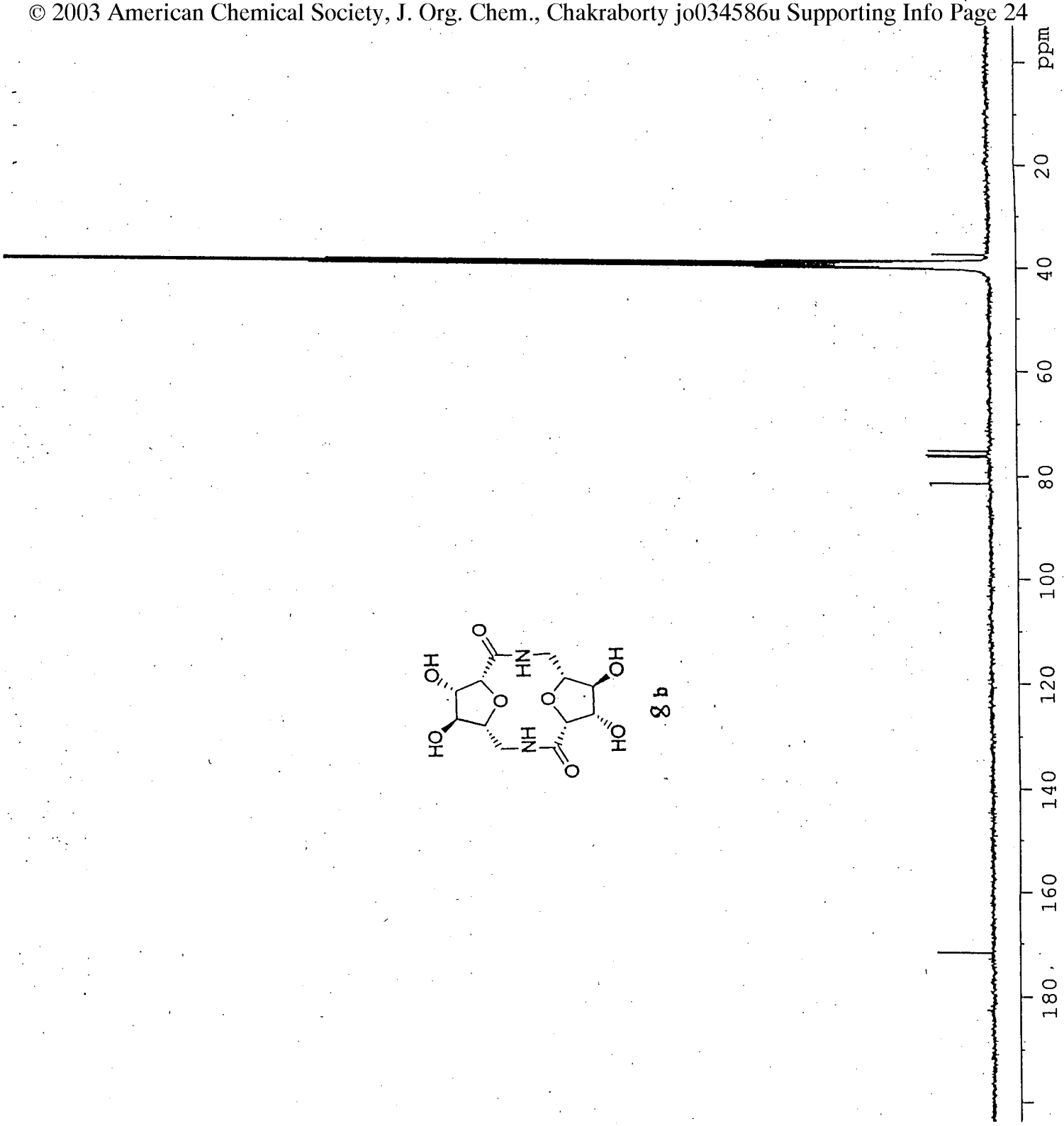
^{1}H NMR (500 MHz) spectrum of **8b** in DMSO-d_6.



8b

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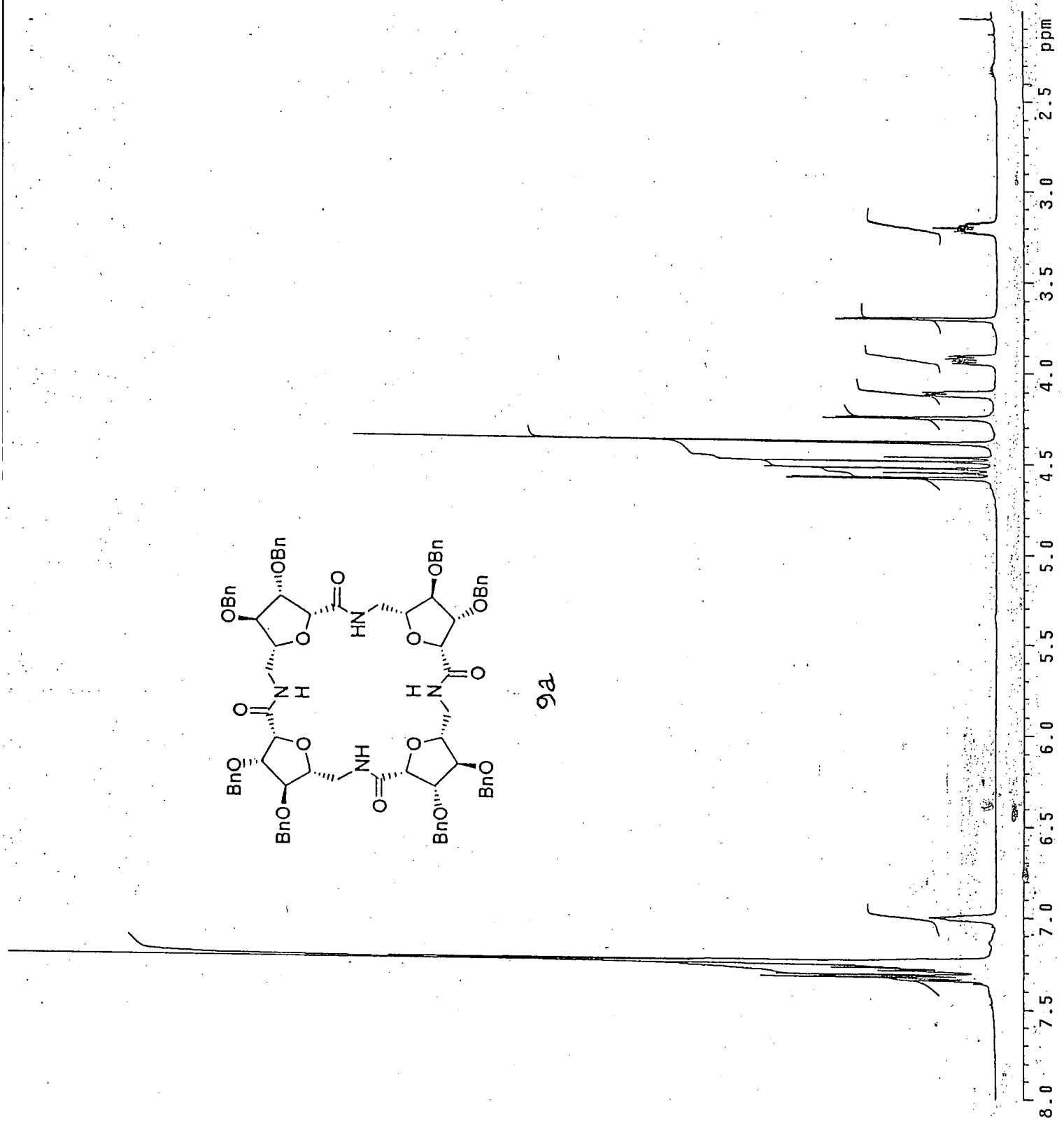
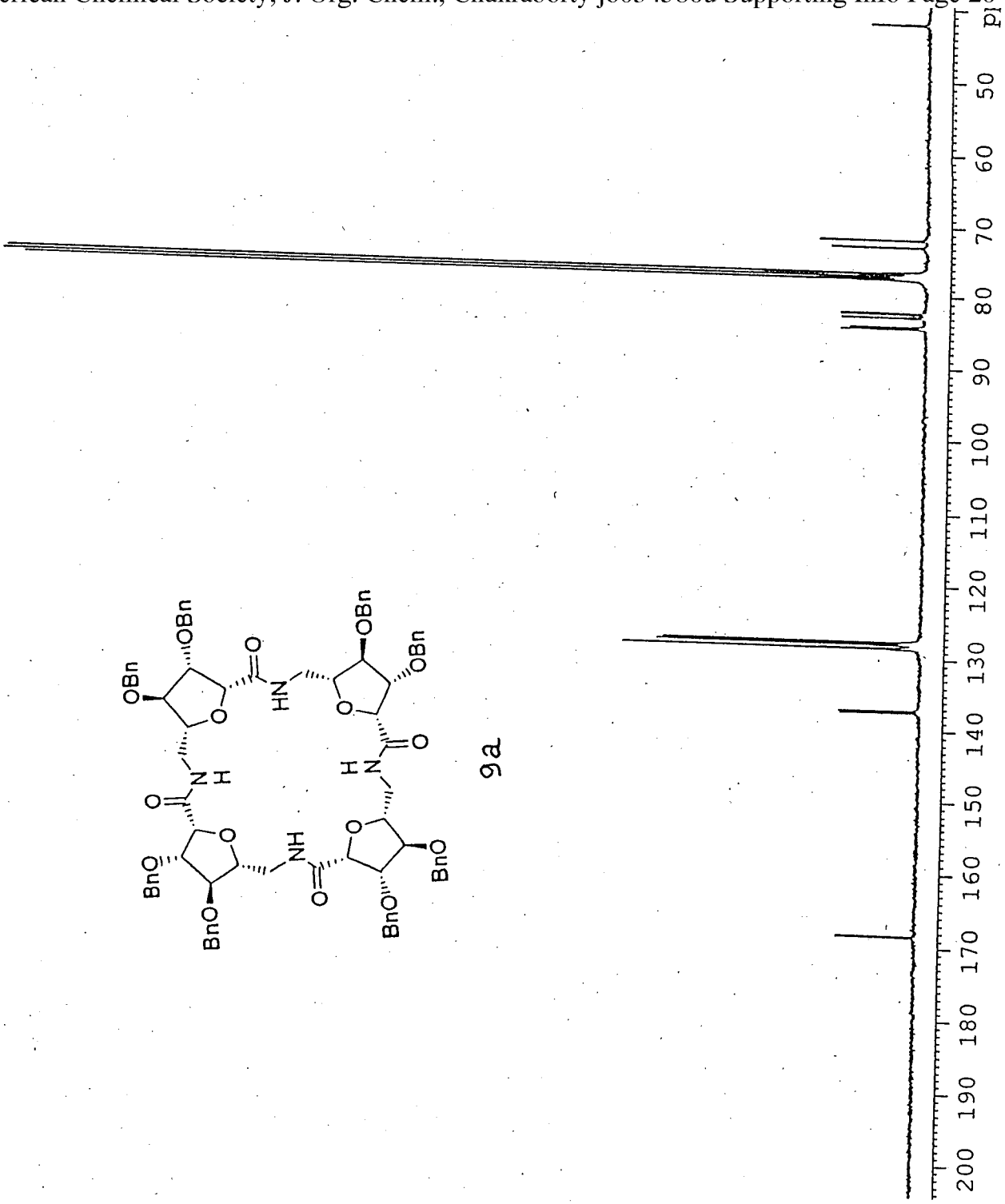


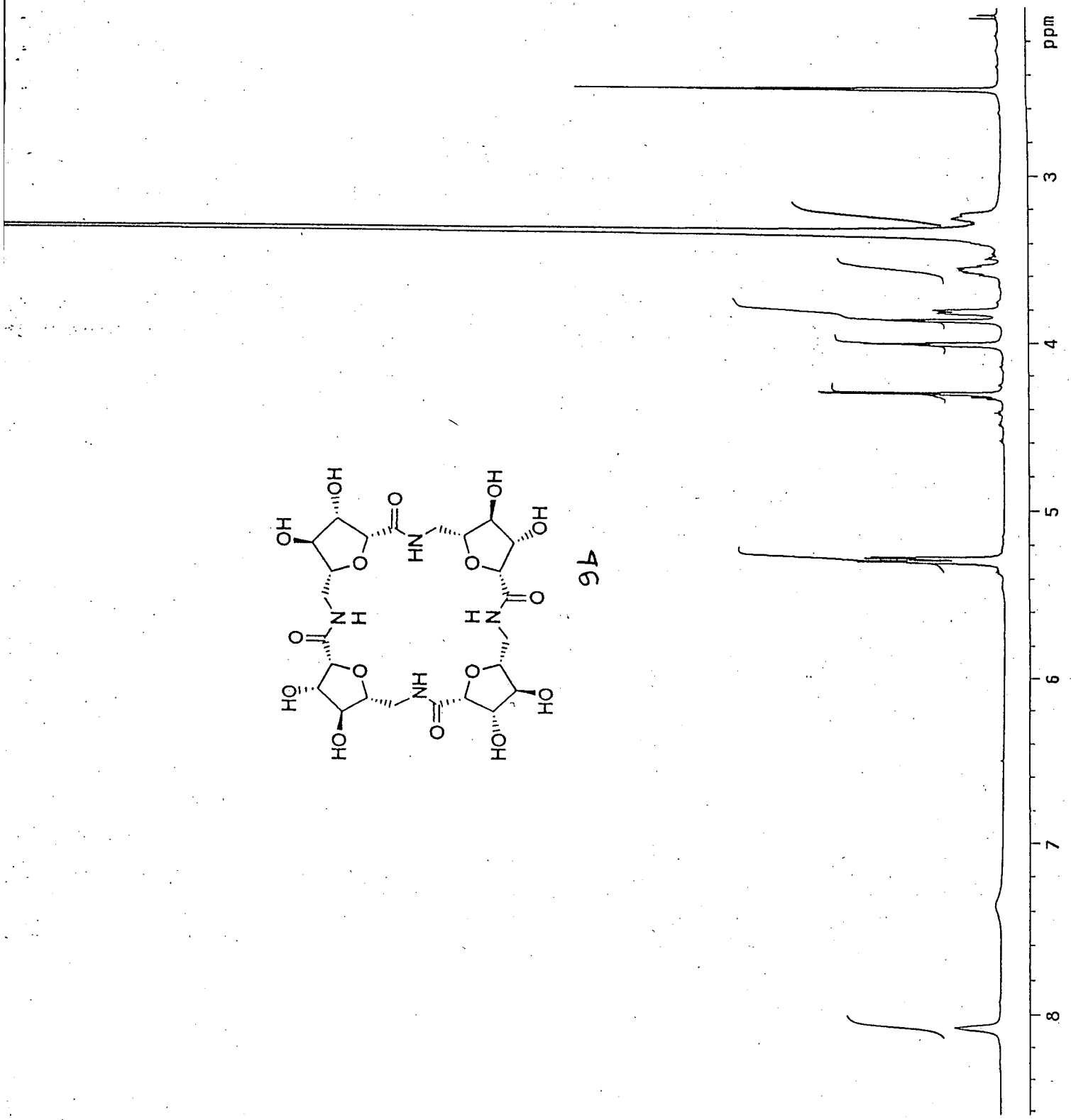
9a

^{1}H NMR (500 MHz) spectrum of 9a in $CDCl_3$.



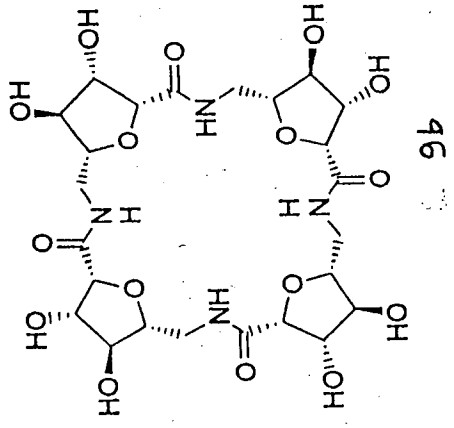
9a

^{13}C NMR (75 MHz) spectrum of 9a in $CDCl_3$.



9b

^{1}H NMR (500 MHz) spectrum of **9b** in DMSO-d_6.



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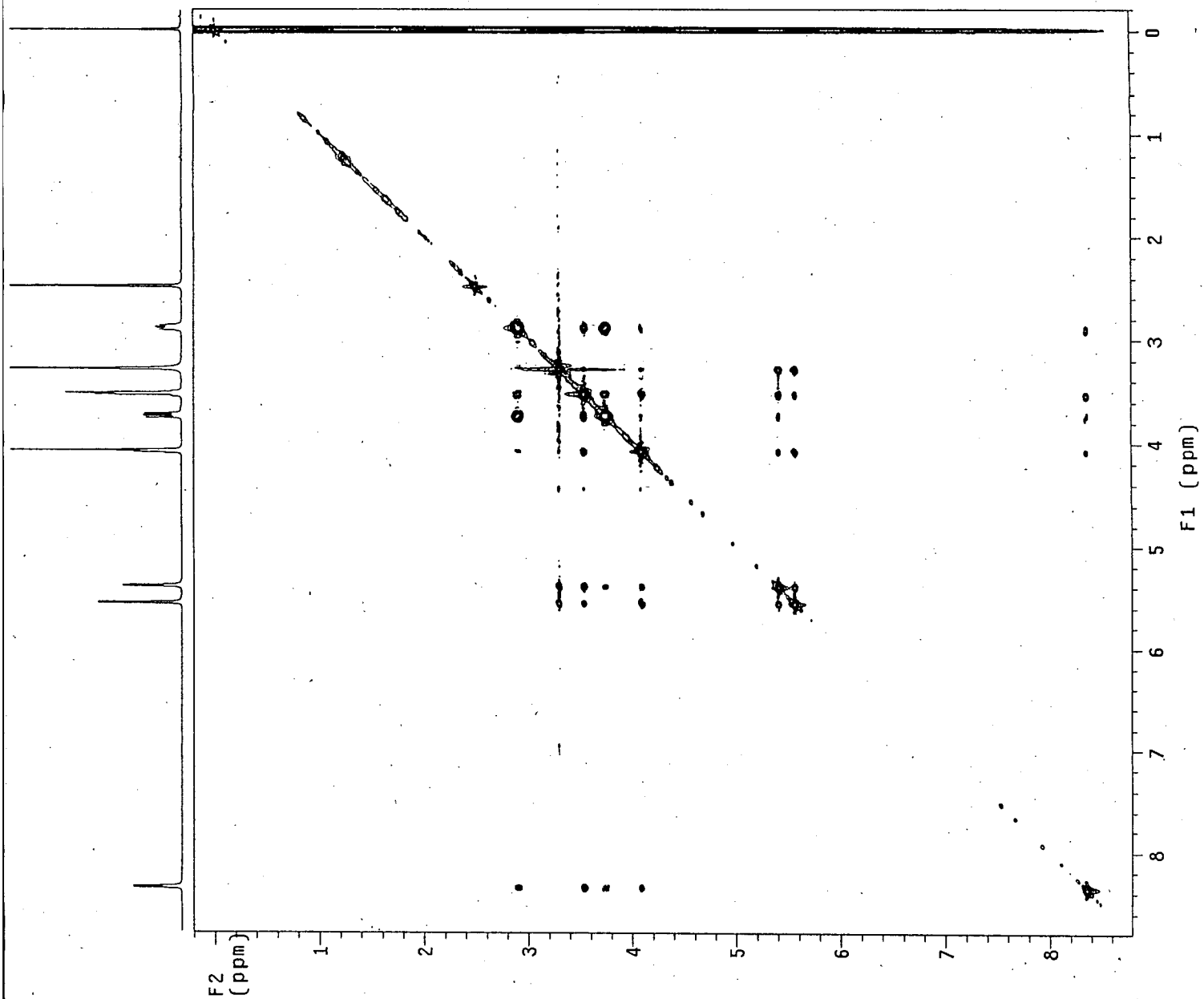


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STANDARD PROTON PARAMETERS

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sample                  PFGflg          y
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sw          4842.6      SPECIAL
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np              2048    gain           34
fb       not used       spin            0
ss                 8        F2 PROCESSING
d1             1.000    gf          0.070
nt                16    gfs      not used
   2D ACQUISITION       fn           4096
swl         4842.6          F1 PROCESSING
ni             192      gfl           0.035
   TRANSMITTER          gfs1     not used
tn              H1      proc1          lp
sfrq       499.904      fn1          4096
tof        -280.9               DISPLAY
tpwr            55      sp          -100.4
pw           7.000      wp          4494.9
        ROESY           sp1         -100.4
mix            0.300    wp1         4494.9
slpw           98.9     rfl          199.8
slpwr         32.000    rfp              0
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satpwr           0                  PLOT
satdly           0      wc          150.0
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                        th              1
                        ai     ph
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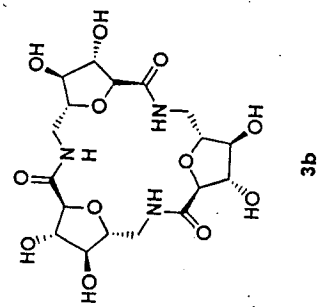
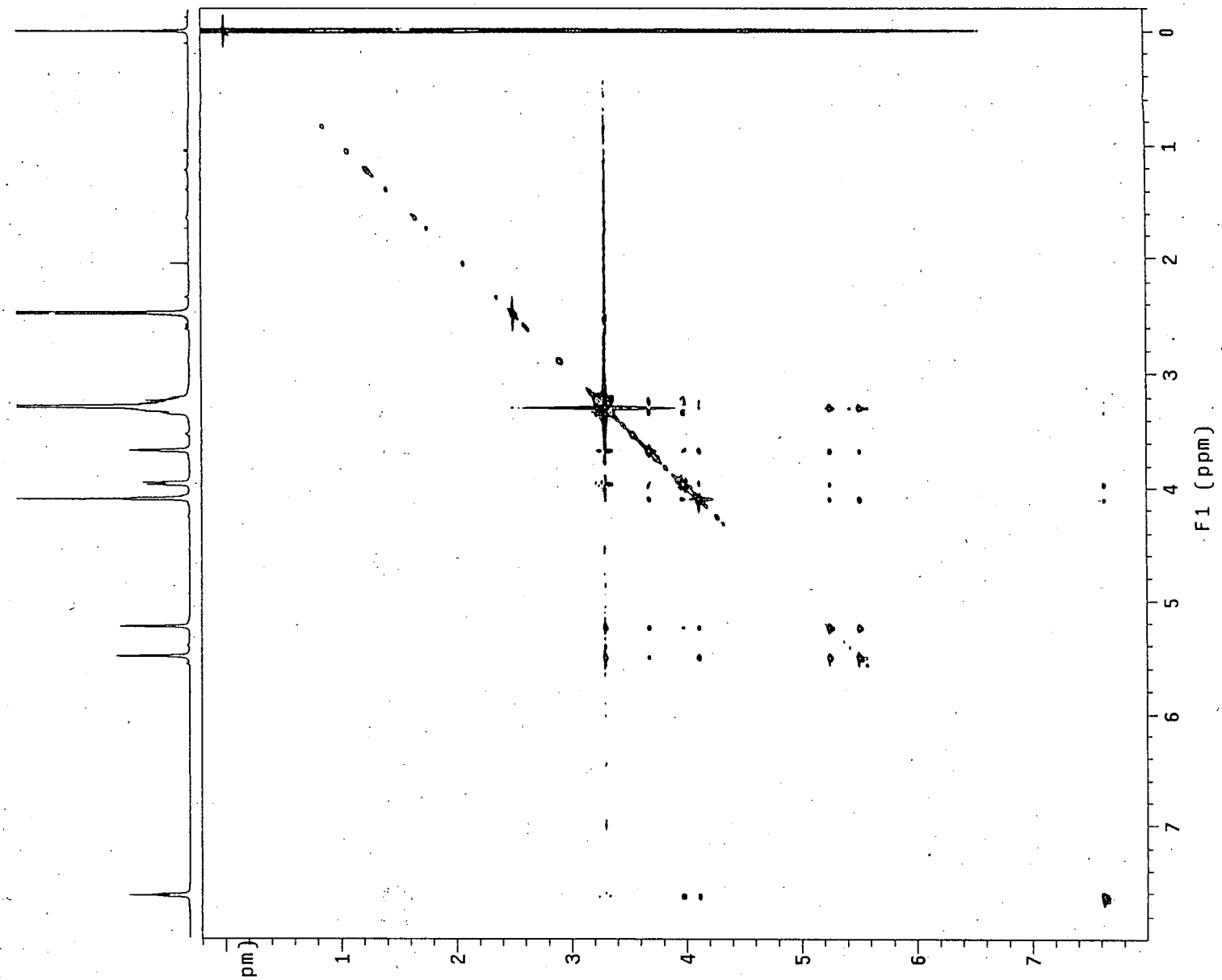


ROESY spectrum of **3b** (500 MHz, DMSO-d_6)

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STANDARD PROTON PARAMETERS

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sample             PFGflg                  y
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fb     not used     spin              0
ss               8      F2 PROCESSING
d1           1.000  gf             0.079
nt              16  gfs        not used
  2D ACQUISITION   fn               4096
sw1         4304.5      F1 PROCESSING
nl             256  gf1            0.040
   TRANSMITTER      gfs1       not used
tn               H1  proc1             lp
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tpwr             55  sp             -101.1
pw            7.000  wp             4098.5
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mix          0.300  wp1            4098.5
slpw          98.9  rf1            195.7
slpwr       32.000  rfp                 0
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satmode          n  rfp1           1244.8
satpwr           0          PLOT
satdly           0  wc           150.0
satfrq           0  sc                0
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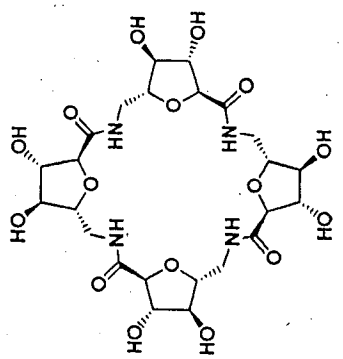
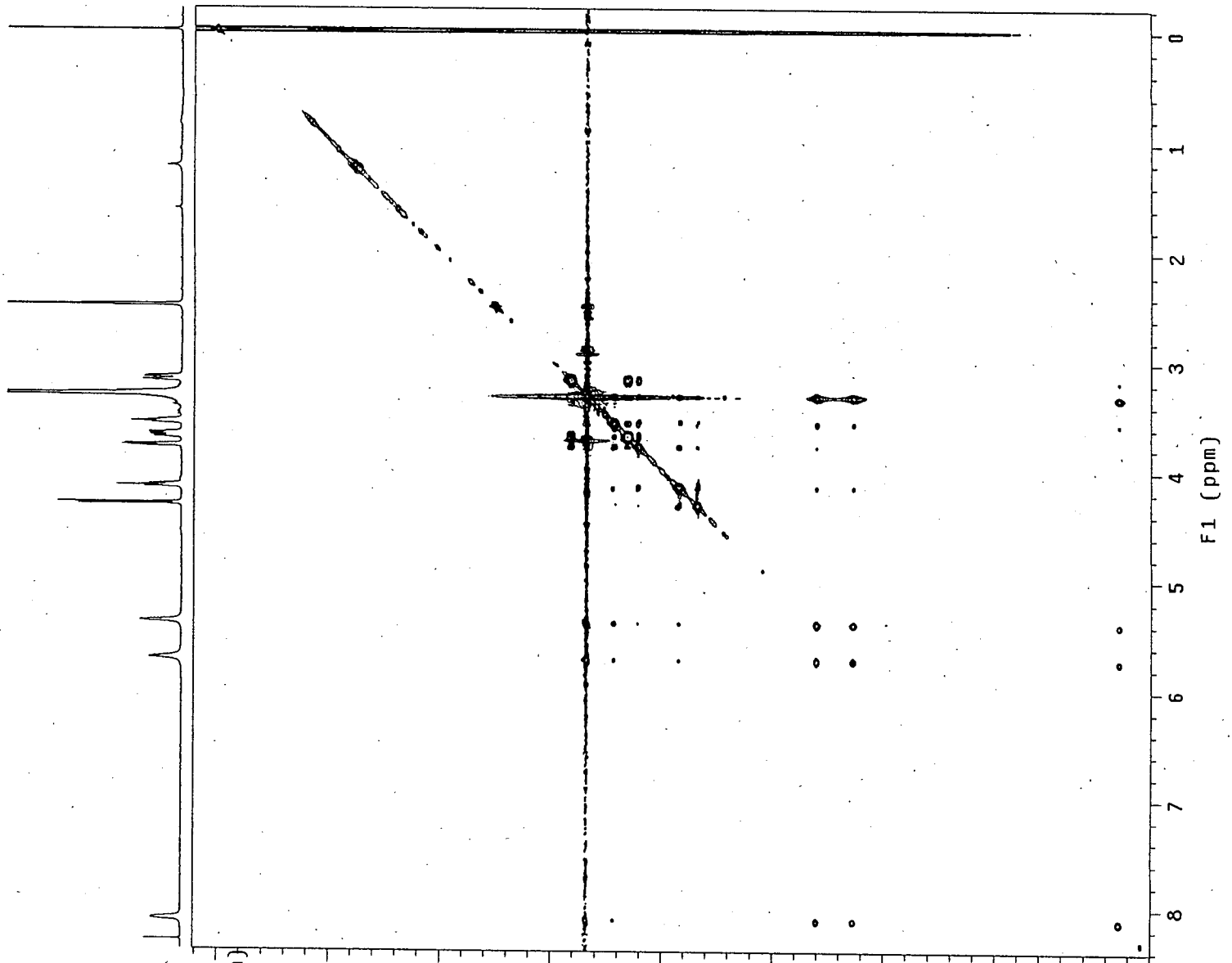


4b

ROESY spectrum of **4b** (500 MHz, DMSO-d_6)

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STANDARD PROTON PARAMETERS

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sample                  PFGflg          y
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np            2048      gain           34
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d1           1.000      gf          0.075
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                        wp1         4304.5
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slpw         103.1      rfp             0
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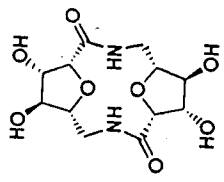

8b

ROESY spectrum of **8b** (500 MHz, DMSO-d_6)