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Synthesis of unsymmetrical pendant-armed and phosphonic acid-pendant-armed cross-bridged tetraazamacrocycles

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SYNTHESIS OF UNSYMMETRICAL PENDANT-ARMED AND PHOSPHONIC ACID-PENDANT-ARMED CROSS-BRIDGED TETRAAZAMACROCYCLES

By

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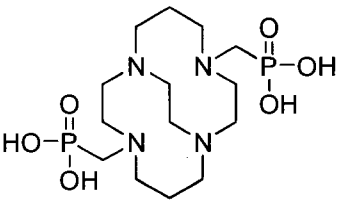
University of New Hampshire, December 2008

The synthesis of a variety of cross-bridged tetraazamacrocycles bearing phosphonate pendant arms and their corresponding phosphonic acid derivatives as well as their Cu(II) complexes are presented. The half-life of decomplexation of Cu(II)·**51** in 5M HCl at 90 °C was found to be 3.8 hours. Ligand **51** showed faster complexation kinetics relative to known cross-bridged tetraazamacrocycles bearing ionizable pendant



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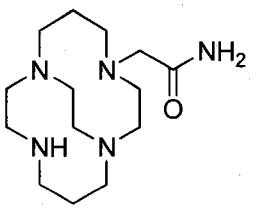
arms. The biodistribution of ^{64}Cu(II)·**51** was studied in male rats and indicated an improved blood and liver clearance and higher bone retention of ^{64}Cu(II)·**51** relative to ^{64}Cu(II)·**10**.

The formation of a single pendant-armed cross-bridged cyclam is presented through an unusual single alkylation of cross-bridged cyclam. Several experiments were

implemented to probe the origin of the selectivity of the reaction including computational studies, 1D ^{1}H NMR and 2D NMR experiments. Monoamide ligand **64** was used as a



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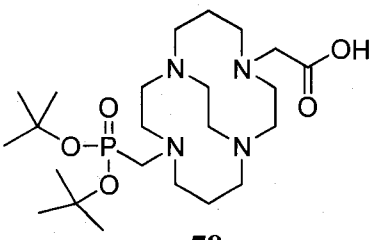
springboard for the formation of several unsymmetrical pendant-armed cross-bridged cyclams.

Attempts were made toward the synthesis of two potential bifunctional chelators for radiopharmaceuticals, each bearing only one phosphonic acid pendant arm and



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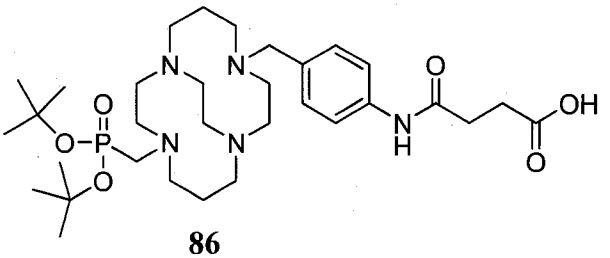

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one free carboxylate for conjugation to somatostatin analogues.

Chapter I

SYNTHESIS OF CROSS-BRIDGED TETRAAZAMACROCYCLES BEARING PHOSPHONIC ACID PENDANT ARMS

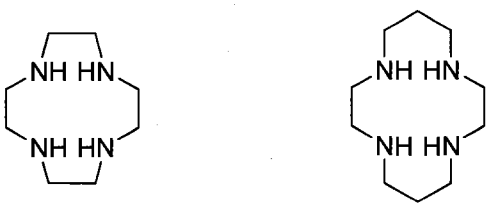
I. Introduction

Cyclic polyamines are an interesting class of compounds that have attracted a great deal of attention in recent years. Specifically, the coordination chemistry community has been interested in the study of such compounds due to the increased kinetic inertness and thermodynamic stability of their small metal cation complexes compared to their acyclic analogues.[1-10] These effects have allowed many variations of such species to be used in a broad range of applications such as synthetic ribonucleases[11], luminescent sensors[11], contrast agents[12], radiopharmaceuticals[2, 13] and models for biomolecules.[14]

The increase in thermodynamic stability has been attributed, in part, to the macrocyclic effect.[2, 15, 16] In order for an acyclic compound to coordinate a metal, its donor atoms must adopt a specific geometry around the ion in order to fill its inner coordination sphere. Macrocycles, on the other hand, have a pre-arranged set of donor atoms, thus lowering the entropic barrier of formation and providing a more stable complex in comparison to their acyclic analogues.

A large portion of recent literature has focused on the design, synthesis and coordination chemistry of tetraazamacrocyclic systems, particularly derivatives of cyclen and cyclam[17] (**Figure 1**). Extensive work on these ligands alone has shown that they

adopt a range of configurations when complexed to a metal cation. In 1964, Bosnich and coworkers developed a nomenclature[18] for the various configurational geometries of cyclam when complexed to a small metal cation (**Figure 2**). Due to the flexible nature of the macrocyclic ring, complexes of cyclam tend to adopt a range of configurational geometries in both the solid state and in solution. Most cyclam complexes exist in the *trans-I*, *trans-III* or *cis-V* coordination configurations in the solid state,[19-21] while in solution, a number of configurational diastereomers can exist in equilibrium.[22, 23] This phenomenon makes prediction of complex geometry difficult in such complexes.



1 2

Figure 1: Cyclen (**1**) and Cyclam (**2**).

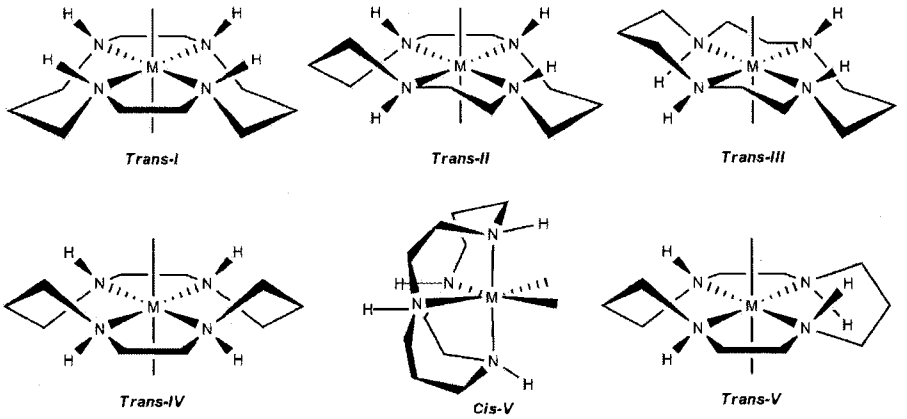


Figure 2: Bosnich-Tobe nomenclature of coordination geometries of six cyclam complexes.

In order to fully envelope an octahedral metal, a cyclic polyamine bearing only four donor atoms must possess further functionality. Typically, this functionality is added to the amine nitrogens, however, ligands that are "*C*-functionalized" have been studied as well.[24, 25] The term "pendant arm" refers to additional donor groups attached to the periphery of a macrocyclic chelator. They serve several purposes: to fill the first coordination sphere of a metal complexed by the ligand, to balance the positive charge of the metal cation and to act as a site for further chemistry.[17] Syntheses and coordination chemistries of pendant-armed polyamines have been extensively studied and reviewed;[14, 17, 24, 26-36] a small sample of these ligands bearing four pendant arms is shown in **Figure 3**. Specifically, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate (DOTA) (**3**) and 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetate (TETA) (**4**) have received a great deal of interest with respect to their use as chelators for radiopharmaceuticals,[37-39] in both imaging and therapy applications.

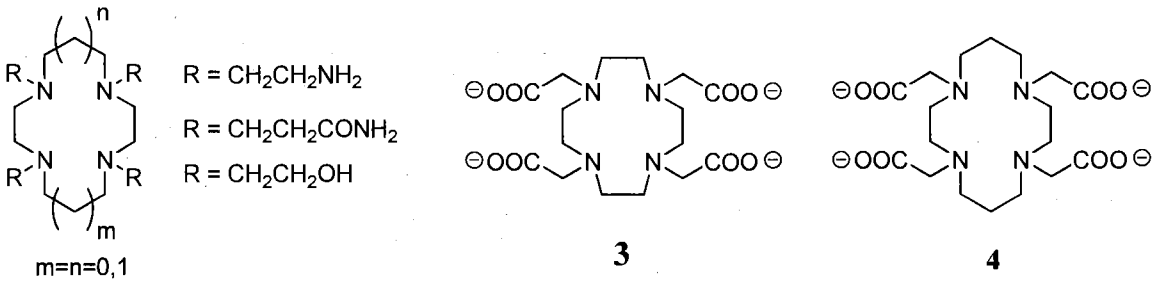
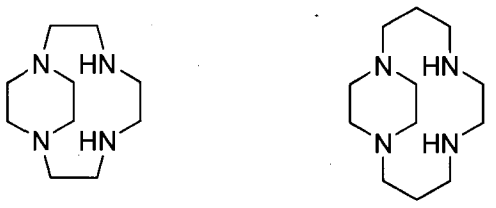


Figure 3: Some known tetra-substituted azamacrocyclic systems; common pendant-armed ligands DOTA (**3**) and TETA (**4**).

The use of radioisotopes of Cu, Ga, In, and Tc in imaging applications has created a need for strong bifunctional chelators for complexing these metals to biological molecules.[40, 41] Both DOTA and TETA act as bifunctional chelators (BFCs) in radiopharmaceuticals; they simultaneously bind to a radioactive metal and can be

covalently linked to a biomolecule. The binding of a BFC to copper must remain intact *in vivo* in order for the radioisotope to reach the desired target, thus the factors that support demetallation must be studied in order to optimize performance. While both the thermodynamic and kinetic stabilities of BFC complexes determined *in vitro* are useful gauges for assessing the viability of a ligand for use in radiopharmaceuticals, the kinetic inertness of copper(II) complexes has been shown to be more predictive of *in vivo* stability.[40, 42] Complexes of ligands such as TETA demonstrate a higher kinetic inertness towards demetallation relative to complexes of acyclic analogues such as EDTA (ethylenediamine tetraacetic acid).[40, 42, 43] However, Anderson and coworkers have shown with model experiments that Cu(II) dissociates from TETA *in vivo* and binds strongly to proteins such as superoxide dismutase, found in rat livers, limiting its use as an effective BFC in imaging agents in humans.[44] Thus in order to utilize pendant-armed azamacrocycles in radiopharmaceuticals, BFCs that form kinetically inert complexes *in vitro* must also resist transchelation to peptides *in vivo*.

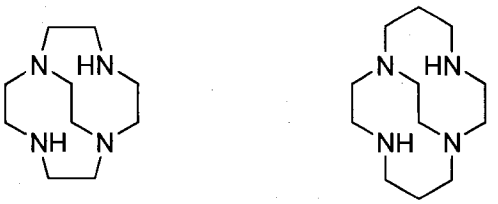
In 1980, Wainwright *et al.* developed a synthetic method to add reinforcement to macrocyclic tetraamines without losing the saturated character of the ring (**Figure 4**) by joining two adjacent nitrogens with an ethylene bridge.[45, 46] By limiting the flexibility of



5 **6**

Figure 4: Reinforced chelators designed by Wainwright and coworkers with two non-adjacent nitrogens bound through an ethylene bridge.

the macrocycle, only the *trans* configurations of the ligand were observed in various Ni(II) complexes.[45, 46] Bridged cyclen **5**, also studied by Hancock and coworkers, has shown a dramatic enhancement in the formation constants of certain transition metal complexes when compared to cyclen.[47, 48] Weisman, Wong and coworkers subsequently synthesized ligands where two non-adjacent nitrogens are bridged by an ethylene "cross-bridge"[49, 50] (**Figure 5**). It was anticipated that this type of bridging would allow the ligand to adopt several low energy conformations where "all four nitrogen lone pairs were convergent upon a cleft"[50], suitable for *cis-V* coordination of small metal cations (**Figure 6**). The Cu(II) complex of **8** showed that the ligand adopted a distorted [2323]/[2323] diamond lattice conformation.[49]



7 **8**

Figure 5: Ligands synthesized by Weisman, Wong and coworkers: cross-bridged cyclen (CB-cyclen) and cross-bridged cyclam (CB-cyclam)

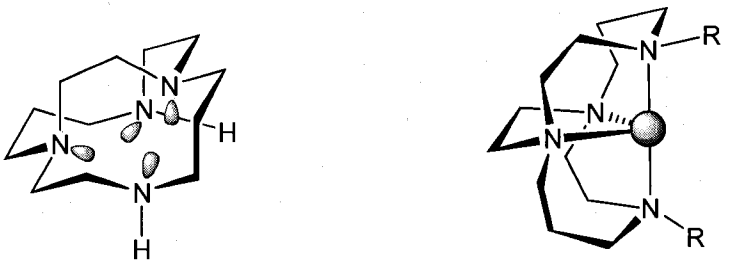
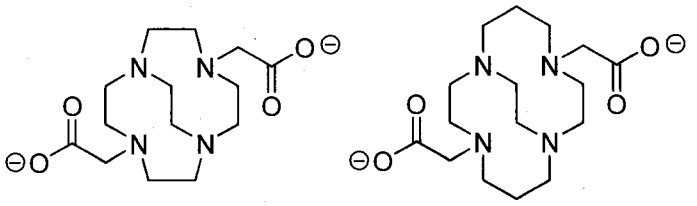


Figure 6: Three-dimensional representation of CB-cyclam (**8**) with all four nitrogen lone pairs convergent upon a molecular cleft. The ligand adopts a *cis*-V folded configuration when complexed to a small metal cation.

The parent cross-bridged ligands, **7** and **8**, have been converted to a number of *N,N'*-difunctionalized pendant-arm derivatives,[51-54] with the most attention directed towards carboxymethyl pendant arms. The free carboxylate in these ligands serves to both fill the first coordination sphere of the metal and to neutralize the charge of the overall complex. The Cu(II) complexes of both CB-DO2A (**9**) and CB-TE2A (**10**) (**Figure 7**) have been prepared and characterized.[51, 55] The solid state structures of both Cu-CB-DO2A and Cu-CB-TE2A show N_4O_2 donor sets with both carboxylates bound to the metal. However, only in Cu-CB-TE2A does the ligand fully encapsulate the metal, as the cleft of CB-DO2A is a bit smaller.[51]



9 **10**

Figure 7: Ligands CB-DO2A (**9**) and CB-TE2A (**10**).

In order to assess the potential of cross-bridged ligands as BFCs in radiopharmaceuticals, the *in vivo* behaviors of the corresponding ^{64}Cu(II) complexes were determined through biodistribution studies in rats and compared with those of non-cross-bridged analogues. ^{64}Cu-CB-TE2A was found to have the most efficient rat liver and blood clearance when compared to that of ^{64}Cu-CB-DO2A, ^{64}Cu-TETA and ^{64}Cu-DO2A.[52, 55] These data were consistent with metabolism studies that confirmed greater *in vivo* stability correlated with decreased transchelation of ^{64}Cu(II) to proteins.[55] Weisman,

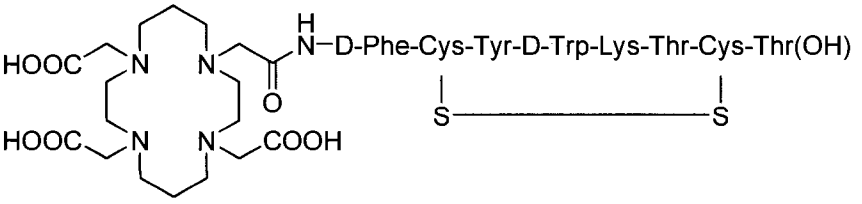
Wong and coworkers developed a convenient, yet qualitative assay to determine *in vitro* stability of these complexes by measuring the half-life of acid decomplexation of various

Complex	Half-life
Cu-CB-TE2A	154(6) h
Cu-TETA	4.5(5) min
Cu-DOTA	< 1 min
Cu-CB-Cyclam	11.8(2) min

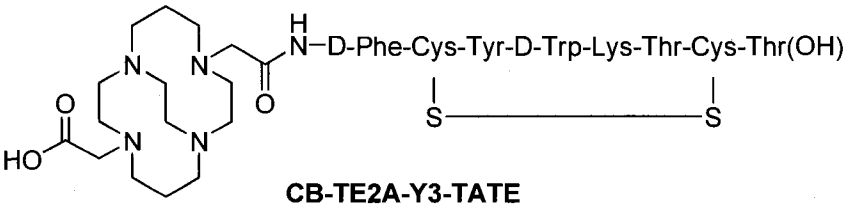
Table 1: Half lives of various Cu(II) complexes at 90 °C in 5 M HCl.

Cu(II) complexes under pseudo-first order conditions in 5M HCl.[56] These kinetic data (**Table 1**) coupled with resistance towards Cu(II)/Cu(I) reduction and subsequent Cu(I) loss were found to correlate well with the results of the biodistribution study confirming that CB-TE2A was a superior BFC for radio-imaging.[56]

Both TETA and CB-TE2A were evaluated as BFCs of ^{64}Cu in bioconjugates of the somatostatin analogue, tyrosine-3-octreotate (Y3-TATE), for use in positron emission tomography (PET) to image neuroendocrine tumors with somatostatin receptors (**Figure**



TETA-Y3-TATE



CB-TE2A-Y3-TATE

Figure 8: Structures of TETA-Y3-TATE and CB-TE2A-Y3-TATE.

8).[57] The Cu(II) complexes of these bioconjugates showed similar affinity for somatostatin receptors *in vitro*, however a 4.4x greater tumor uptake was detected with ^{64}Cu-CB-TE2A-Y3-TATE than with ^{64}Cu-TETA-Y3-TATE at 4 hours post injection.[57] These data coupled with the differences in biodistribution of ^{64}Cu-CB-TE2A-Y3-TATE and ^{64}Cu-CB-TETA-Y3-TATE confirmed that the cross-bridged type ligands are much more suitable BFCs for radiolabeling biomolecules and as such require further study.

II. Background

A. Cyclic Polyamines bearing α-Aminophosphonic Acids

α-Aminophosphonic acids represent an important class of biologically relevant compounds due to their resemblance to naturally occurring amino acids and have been extensively reviewed (**Figure 9**).[58-77] α-Aminophosphonic acids differ from their α-amino

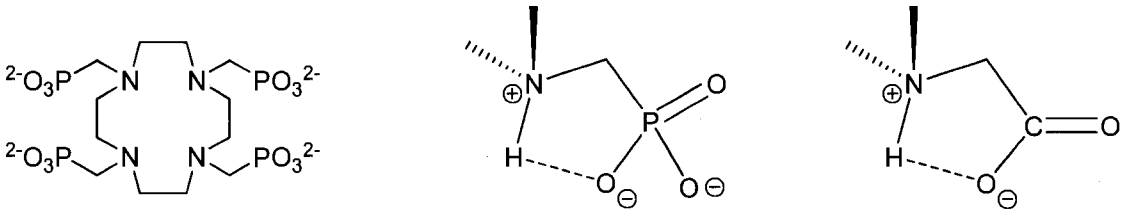
11 **12**

Figure 9: Simple amino acid glycine, **11** and phosphonic acid analogue **12**.

acid analogues in several ways: the phosphorus is tetrahedral while the carbonyl carbon is trigonal planar, the phosphonic acid functionality of aminomethylphosphonic acid is more acidic (pKa_1(**12**) = 0.5, pKa_2(**12**) = 5.38)[78] than the acetic acid functionality of glycine (pKa(**11**) = 2.36)[78], the phosphorus atom is larger than the carbon and an additional hydroxyl group is present in the phosphonic acid.[64, 77] These compounds exhibit very little mammalian toxicity and efficiently mimic amino acids, making them important antimetabolites that can compete with their carboxylic counterparts for active sites on cell receptors.[64] Their unique properties allow them to encompass a broad range of applications. Aminophosphonic acids have been used as agrochemicals since 1954. AMPA (aminomethylphosphonic acid), **12**, acts in similar ways to glycine in suppressing the growth of tobacco rootlets.[77] Derivatives of aminophosphonic acids have been

used as antithrombotic agents, which aid in the breakdown of thrombin, a biological compound necessary for blood clotting.[77, 79] Open chain aminopolyphosphonates act as multidentate ligands for selective cation complexation in several applications such as corrosion inhibitors[80]and removal of metal overload from organisms.[81] Most notably, metal complexes of aminophosphonates have attracted interest due to their biomedical applications in anti-cancer agents,[82, 83] NMR imaging agents[84] and radiopharmaceuticals.[85]

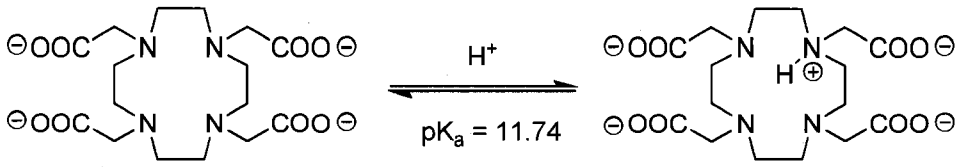
The coordination chemistry of tetraazamacrocyclic aminophosphonates has been studied extensively due to their ability to form both very thermodynamically stable and kinetically inert complexes.[86] The first such compound, DOTP (**13**, **Figure 10**) (H_8-DOTP = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(methylene phosphonic acid) was originally synthesized in 1984[87]; a modified synthesis was published later by Sherry



13

Figure 10: Structure of DOTP (**13**); Representations of hydrogen bonding in aminophosphonate and amino acid derivatives.[77]

and coworkers.[88] A comparison of the pKa's of the first protonation of ligand **3** and ligand **13** has been reported[86] as shown in **Scheme 1**. The first pKa of **3** (11.74) is lower than the first pKa of **13** (13.7) by two orders of magnitude indicating **13** is a stronger base. This increased basicity was directly related to the increased thermodynamic stability of the corresponding Cu(II) complexes. The explanation proposed by Sherry stated that an electrostatic effect of the double negative charge on the neighboring



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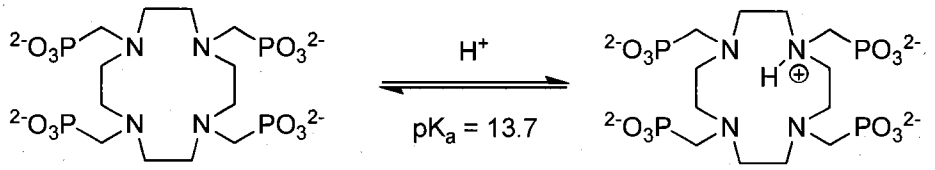
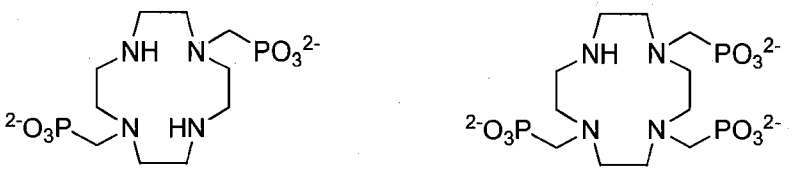


13

Scheme 1: Protonation of ligands **3** and **13**.

phosphonate group increased the electron density of the amine nitrogen, thus increasing its basicity through induction.[86, 89] An alternative explanation of the increased basicity could stem from the increased hydrogen bonding ability of the phosphonate versus the acetate[90] (**Figure 10**). Furthermore, the C-P and P-O bond lengths in the phosphonate functionality are longer than the C-C and C-O bond lengths of the acetate. This could allow for a stronger hydrogen bond between the negatively charged oxygen and the ammonium proton and potentially decrease the strain in the H-bonded ring (**Figure 10**). Azamacrocycles bearing acetate pendant arms have been known to possess this type of intramolecular hydrogen bond.[91, 92] Extensive study of DOTP as a chelator for lanthanides has been conducted by Sherry[93-96] for use as ligands for MRI contrast agents.

Anderson and coworkers, in collaboration with Sherry, have measured the biodistribution of three ^{64}Cu(II) complexes of cyclen ligands bearing methylene phosphonic acid pendant arms in an investigation of their possible use in radiopharmaceuticals.[52] The ^{64}Cu complexes of DOTP (**13**), DO2P (**14**)

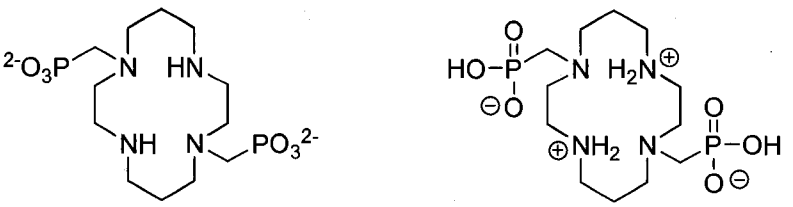


14 **15**

Figure 11: Ligands DO2P (**14**) and DO3P (**15**).

(1,4,7,10-tetraazacyclododecane-1,7-di(methylenephosphonic acid)) and DO3P (**15**) (1,4,7,10-tetraazacyclododecane-1,4,7-tri(methylenephosphonic acid)) were all found to have significant uptake in bone, likely due to the binding of the methylenephosphonate groups to hydroxyapatite (**Figure 11**).[97] Of the three, ^{64}Cu-DO2P possessed the fastest blood and liver clearance; ^{64}Cu-DO3P and ^{64}Cu-DOTP showed higher liver uptake, possibly due to the large negative charge of the complexes under physiological conditions.[97] Metabolism studies of ^{64}Cu-DO2P suggested that DO2P can be used as a BFC for radiopharmaceuticals.[97]

In 2000, Hermann and coworkers synthesized a cyclam ligand bearing two methylenephosphonic acids (**Figure 12**).[90] The solid state structure of 1,8-disubstituted



16 H_4-**16**

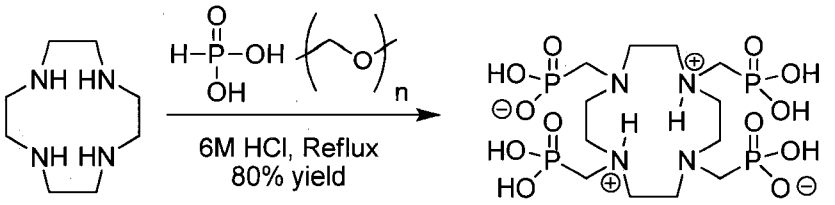
Figure 12: Ligands TE2P (**16**) and H_4-TE2P (H_4-**16**).

H_4-TE2P (H_4-**16**) indicated the presence of two intramolecular hydrogen bonds, between the negatively charged phosphonate oxygen and an ammonium proton on nitrogen 4. The hydrogen bond was also present in solution, confirmed by values of protonation

constants, titration experiments and VT-NMR.[90] The source of the increased basicity reported by Sherry and Hermann of cyclic polyamines bearing phosphonic acid pendant arms relative to acetate pendant arms can be explained by this intramolecular hydrogen bond but not by the dispersion of electron density by the highly charged phosphonate, an explanation reported previously.[86, 89] At 25 °C, the Cu(II) complex of TE2P was found to exist with pentacoordinated Cu(II) with the cyclam ring in the *trans*-I configuration.[98] When heated, the complex isomerizes to a hexacoordinate isomer with the cyclam ring in the *trans*-III configuration.[98] The kinetic acid decomplexation data of the *trans*-III complex indicated a half-life of approximately seven months in 5M $HClO_4$ at 25 °C.[98] This impressive kinetic inertness makes TE2P an attractive candidate as a BFC for radioimaging.

B. Synthesis of Phosphonic Acid Pendant Arms on Cyclic Polyamines

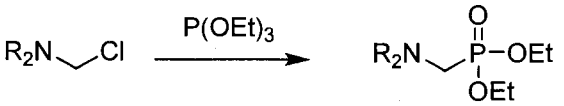
The direct synthesis of α-aminophosphonic acids was first reported by Moedritzer in 1965 through a Mannich-like mechanism.[99] Primary and secondary amines were allowed to reflux in the presence of aqueous formaldehyde, phosphorous acid and 6M HCl and the isolated aminophosphonic acids were recovered in a wide range of yields (35-75%).[99] H_8-DOTP (H_8-**13**), the first polyamine bearing phosphonic acid pendant arms was synthesized using these conditions.[89] Subsequently, Sherry and coworkers were able to optimize the synthesis of H_8-DOTP by substituting dry paraformaldehyde for aqueous formaldehyde. The isolated ligand required no further purification and was obtained in good yield (**Scheme 2**), however the harsh conditions limit the use of this procedure to systems with sensitive functionality.[88]



1 H_8-**13**

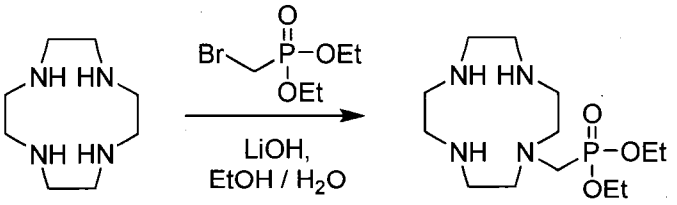
Scheme 2: Synthesis of H_8-DOTP (H_8-**13**).

An alternative approach to the formation of aminophosphonic acids involves the formation of phosphonate esters followed by ester cleavage to yield the acid. Formation of α-aminophosphonates and their transformation to α-aminophosphonic acids has been extensively reviewed.[58, 62, 77, 100-105] S_N2 displacement of substituted chloro- and bromomethanamines has recently been reported (**Scheme 3**).[106, 107] The reaction was



Scheme 3: Formation of α-aminophosphonic acid ethyl ester.

reported to be carried out in high yield and purity, however formation of either chloro- or bromomethanamines on cyclic polyamines would likely cause polymerization or lead to aminals. Parker and coworkers have recently reported a novel synthesis of aminophosphonate esters by the addition of diethyl-2-bromoethylphosphonate to cyclen

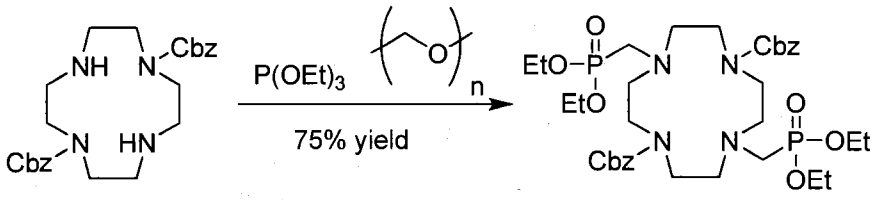


1 **17**

Scheme 4: Formation of ligand **17**.

(**1**) with LiOH (**Scheme 4**). [108] The procedure was selective for the formation of only a single pendant arm, but a low yield (43%) makes this approach not very attractive.

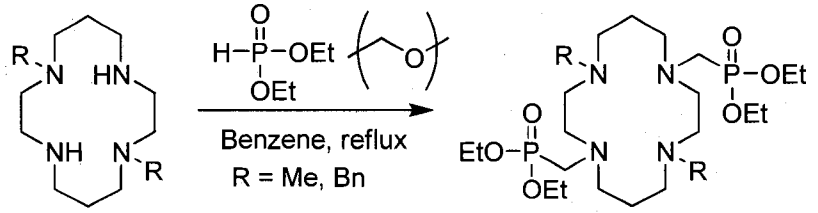
The more common approach towards formation of aminophosphonates involves the use of trialkyl[109-119] or dialkyl[90, 106, 120-127] phosphites and aqueous formaldehyde or paraformaldehyde. Sherry reported the synthesis of a 1,7-di-protected cyclen derivative with triethyl phosphite and paraformaldehyde to form ligand **19** (**Scheme 5**).[115] The crude material was purified on a silica gel column in 75% yield. Sherry has also used these



18 **19**

Scheme 5: Formation of di-pendant armed ligand **19**.

conditions for the formation of a trifluoroethyl ester derivative of **19**, by substituting tris(2,2,2-trifluoroethyl) phosphite for triethyl phosphite, in order to utilize ^{19}F NMR for the determination of the solution structure of various lanthanide complexes.[128] Hermann and coworkers have reported the synthesis of a series of bis(methylphosphonate) esters with the use of diethyl phosphite and paraformaldehyde (**Scheme 6**).[90] The reactions



20 **21**

Scheme 6: Formation of phosphonates ester **21** with diethyl phosphite.

were run in benzene at reflux in order to azeotropically remove the water produced, forcing the equilibrium towards product formation.

The hydrolysis of an alkylphosphonate can be carried out under basic or acidic conditions. In the case of ethyl phosphonates, basic hydrolysis yields exclusively partially-hydrolyzed phosphonates.[115, 122, 128, 129] There are several distinct mechanisms possible for hydrolysis in base. In one mechanism, the hydroxide displaces an alkoxide by S_N2 attack at the phosphorus atom[130, 131] and as another equivalent of hydroxide removes the new acidic proton from the phosphonate, the negative charge built up around the phosphorus makes further attack of hydroxide at the phosphorus atom a higher energetic process (**Figure 13**). In a second possible mechanism, hydroxide can perform

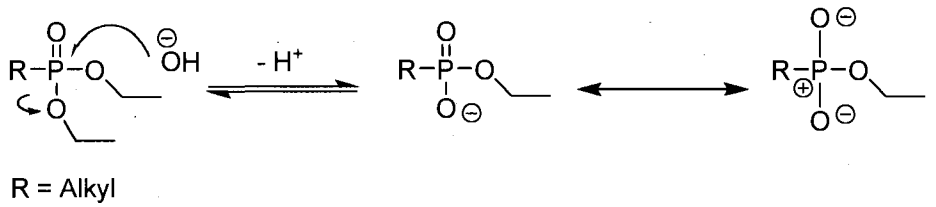


Figure 13: Mechanism of basic hydrolysis of an ethyl phosphonates by S_N2 displacement.

an E2 elimination, abstracting a methyl proton while the phosphonate acts as a leaving group (**Figure 14**). Ethylene gas is formed as a byproduct of this reaction. Presence of ethylene in a reaction flask strongly suggests the phosphonates is formed through an E2 elimination. A third possibility is an addition/elimination mechanism which would require attack of hydroxide at the phosphorus, forming a pentacoordinate intermediate, followed by expulsion of alkoxide (**Figure 14**). Alkaline hydrolysis of ethyl phosphonates has been reported to occur exclusively with P-O bond cleavage.[132-135] Currently, no published reports confirm the presence of an E2 elimination. Reported

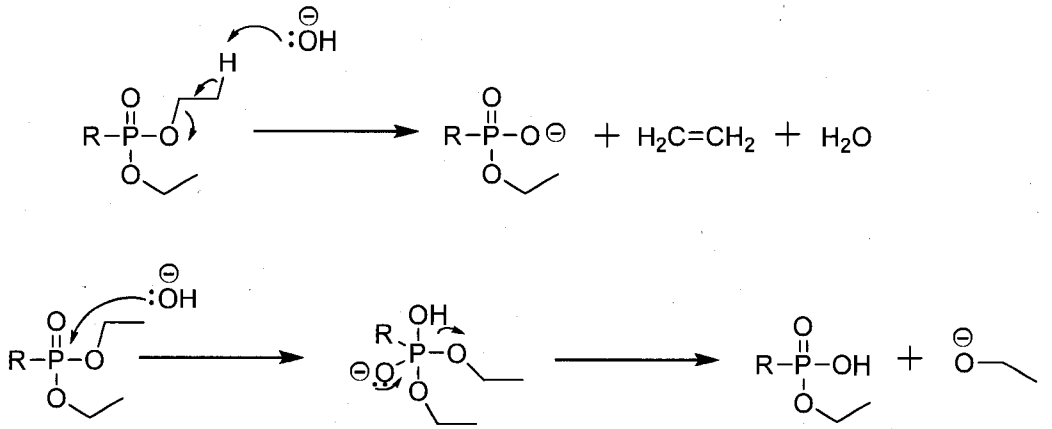
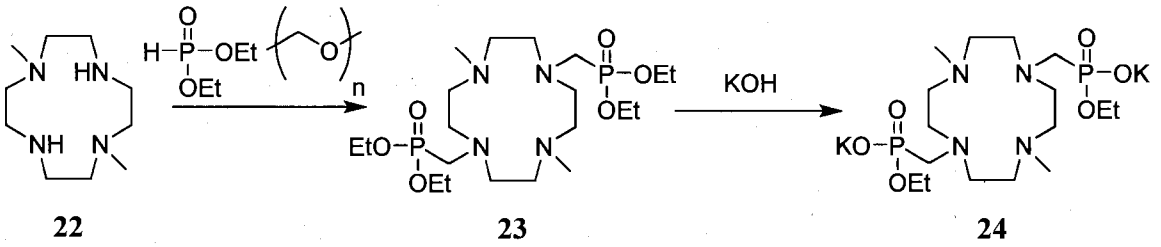


Figure 14: E2 elimination and addition/elimination mechanisms of base hydrolysis.

procedures for the base hydrolysis of cyclic amines bearing phosphonates pendant arms typically involve highly basic solutions of either potassium hydroxide or sodium hydroxide.[115, 122, 129] Vizza and coworkers appended ethyl phosphonate pendant arms on 1,7-dimethylcyclen and were able to isolate the partially-hydrolyzed phosphonate **24** after hydrolysis with potassium hydroxide (**Scheme 7**). The authors noted that methylene



22 **23** **24**

Scheme 7: Formation of ligand **24** by Vizza and coworkers.

protons on each ethyl ester were diastereotopic, as seen in the ^{1}H NMR spectrum. After basic hydrolysis, the time-averaged structure makes the remaining ethyl ester methylene protons enantiotopic. Coincidentally, the coupling between these protons and the methyl protons and the coupling between these protons and the ^{31}P were very similar, simplifying the ^{1}H resonance to a pentet.[122]

Acid hydrolysis of alkyl phosphonates yields the fully hydrolyzed phosphonic acid.[134] Similar to basic hydrolysis, acid hydrolysis can follow through multiple possible pathways (**Figure 15**). In all three mechanisms, the phosphoryl is protonated by the acid and water can either remove a methyl proton in an E2 elimination, producing ethylene gas, attack the methylene carbon of the ethyl ester by an S_n2 displacement or attack the phosphorus to form a pentacoordinated species that expels ethanol after a proton transfer.[134, 136, 137] According to Gerrard[133] and Blumenthal,[138] acid hydrolysis of phosphonates occurs with approximately 70% C-O cleavage and 30% P-O cleavage. Cadogan reported that C-O was predominate as well, but didn't report to what extent.[139] Currently, there is little known as to whether an E2 elimination mechanism or an S_N2 mechanism or both are the cause for the C-O bond cleavage.

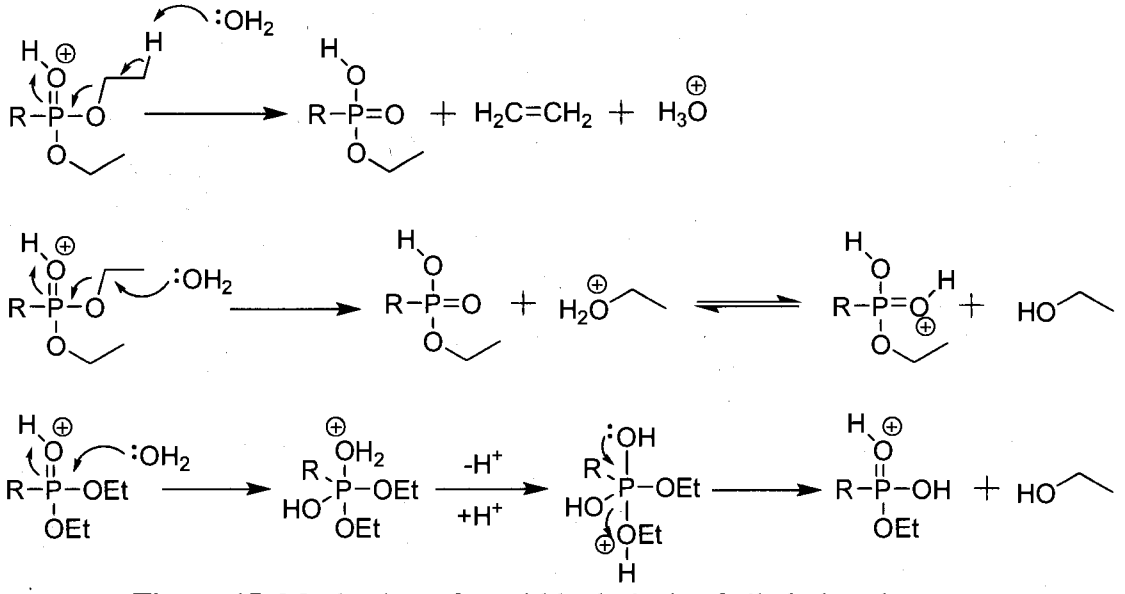
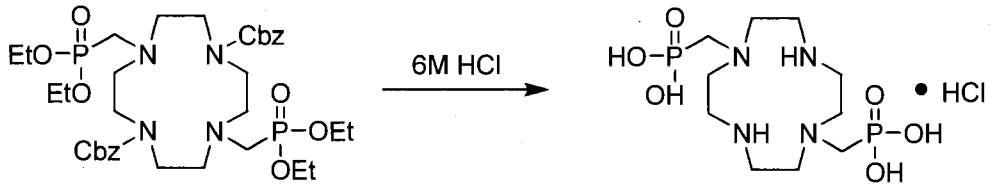


Figure 15: Mechanisms for acid hydrolysis of alkyl phosphonates.

Acid hydrolysis of aminophosphonate esters on cyclic polyamines has been reported.[77, 90, 108, 115, 116, 127, 140, 141] Sherry reported the acid hydrolysis of ligand **19** with 6M HCl (**Scheme 8**). The acid simultaneously hydrolyzed the ethyl ester and removed

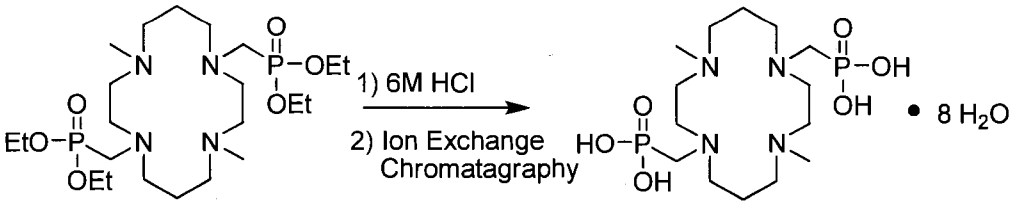
the protecting group; ligand **25** was isolated in 90% yield after recrystallization as an HCl salt.[115] The ligand was complexed with lanthanides for use as MRI contrast agents.



19 **25**

Scheme 8: Acid hydrolysis of ligand **19** performed by Sherry.[115]

In 2000, Hermann and coworkers described the hydrolysis of ethyl phosphonates on a 1,7-dimethyl cyclam.[90] The ester was hydrolyzed with 6M HCl, acids were removed on a Dowex 50 ion exchange column and the fractions containing the product were transferred onto an Amberlite 50 column (carboxylic ion exchanger) and eluted with 10% aqueous ammonia. The product was isolated in a 70% yield from the column (**Scheme 9**). The authors did not report the identity of any byproducts of the reaction and used ion exchange chromatography in both subsequent publications involving acid hydrolysis of alkyl phosphonates to purify their compounds.[116, 126]



26 **27**

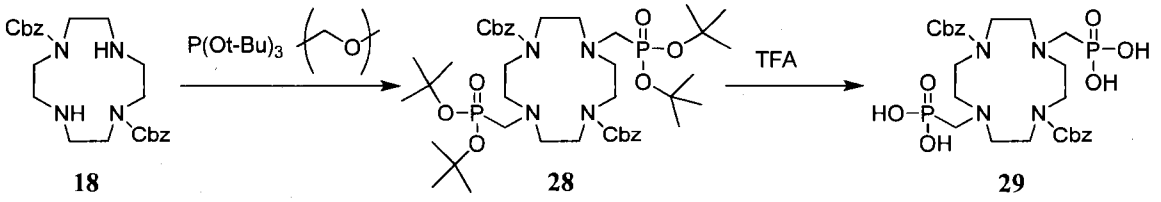
Scheme 9: Hydrolysis of ligand **26** performed by Hermann.[90]

Formation of methyl phosphonate esters has been reported previously using very similar conditions as for the ethyl analogues.[58, 62, 77, 100-102, 104, 105] Alkaline hydrolysis of methyl esters was found to occur with P-O bond cleavage exclusively.[132, 133] The hydrolysis in acid, however was found to be similar to ethyl phosphonates where both

P-O and C-O cleavage were reported.[132, 135] Interestingly, there are no reports in the literature of direct S_N2 displacement of hydroxide at the methyl carbon, a plausible pathway for basic hydrolysis. Work done by Gerrard and coworkers indicated that basic hydrolysis occurred only through P-O bond cleavage.[133] Hydrolysis of phosphonates formed with optically active *sec* alcohols revealed a complete retention of configuration of the alcohol after the hydrolysis of the phosphonates was complete.[133] According to Westheimer, this mechanistic requirement can be transferred to methyl esters.[132] The rates of methyl hydrolysis have been reported to be faster than ethyl hydrolysis in both acid and base solutions.[135]

Other reagents have been shown to dealkylate alkyl phosphonates without the need for the presence of water during the course of the reaction. Reaction with HBr in AcOH has been reported to cleave alkyl phosphonates in high yield and purity.[77, 90, 113] Alkyl phosphonates react with trimethylsilyl bromide or trimethylsilyl iodide to form trimethylsilyl esters, which cleave easily in water.[117-119] Boron tribromide has also been reported to cleave alkyl phosphonates.[142, 143]

The attachment of *t*-butyl phosphonate ester pendant arms to a cyclen has been recently reported. Manning and coworkers reacted tri-t-butyl phosphite, paraformaldehyde and a 1,7-di-protected cyclen to form ligand **28** (**Scheme 10**).[144]



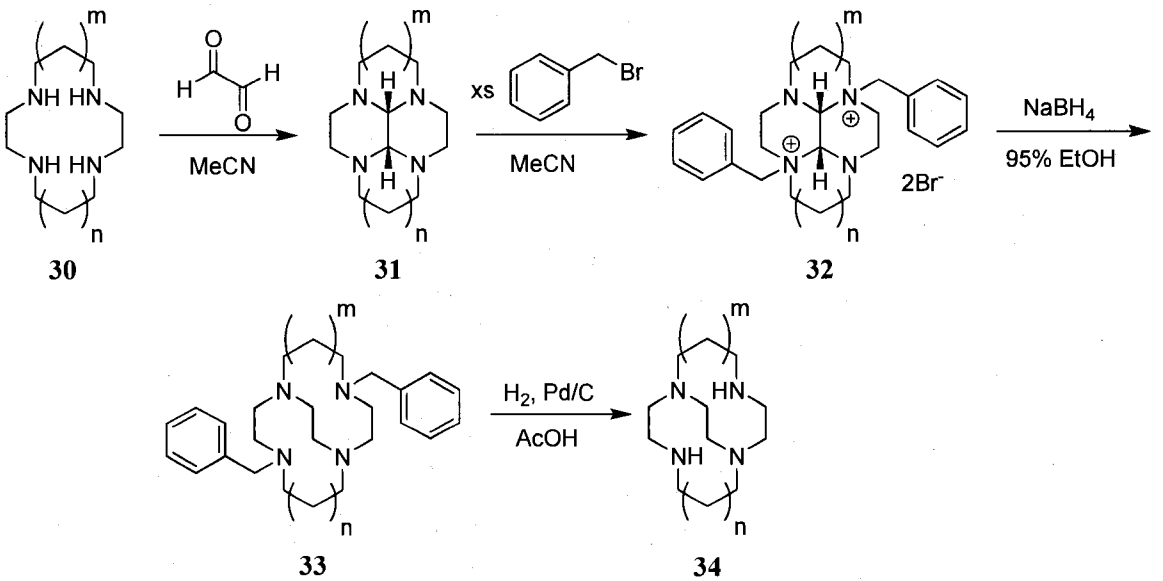
18 **28** **29**

Scheme 10: Formation of t-butyl phosphonates **28** followed by deprotection with TFA.

t-Butyl phosphonate formation has been reported in the literature with the use of either tri-*t*-butyl phosphite or di-*t*-butyl phosphite as the nucleophile for the Mannich-type reaction.[145-151] Deprotection of *t*-butyl esters with mild acid trifluoroacetic acid (TFA) produced phosphonic acid **29**. Manning also reported an improved synthesis of tri-*t*-butyl phosphite, a reagent originally synthesized by Mark and coworkers.[152] The sensitivity of tri-*t*-butyl phosphite towards air, water and heat was reported by Manning.

C. Phosphonic Acid Pendant Arms on Cross-Bridged Tetraamines.

The goal of the work herein is to synthesize methylphosphonic acid pendant armed derivatives of CB-cyclen (**7**) and CB-cyclam (**8**) in order to make model compounds for the determination of the corresponding Cu(II) complexes' *in vivo* and *in vitro* stability. The syntheses of CB-cyclen (**7**) and CB-cyclam (**8**) were reported by Weisman, Wong and coworkers (**Scheme 11**).[49, 51, 153] They utilized a condensation between tetraamines **30** and aqueous glyoxal to afford bisaminals **31** with aminal



Scheme 11: Synthetic sequence to form cross-bridged tetraamines. m=n=0, (CB-cyclen, **7**), m=n=1 (CB-cyclam, **8**).

hydrogens in the *cis* configuration. Regioselective alkylation on two non-adjacent nitrogens with benzyl bromide produced dibenzyl salts **32** in high yield and purity. Double reductive ring opening with excess $NaBH_4$ yielded the dibenzyl cross-bridged ligands **33** which were debenzylated via hydrogenolysis to obtain the parent cross-bridged ligands. A key step in this sequence lies in the highly regioselective alkylation[51] of **31**. As shown in **Figure 16**, the three-dimensional structure of **31** is a *cis* fused, all chair conformation possessing a cleft. Two equivalent nitrogen lone pairs are oriented *exo* to the cleft while the other two lone pairs are oriented *endo*.[51] The molecule undergoes an enantiomerization where the *exo* and *endo* nitrogen lone pairs interconvert through a conformational process involving two ring flips and the inversion of all four nitrogens.[51] The first alkylation takes place at a more sterically accessible *exo* lone pair, conformationally locking that nitrogen from inversion. The regioselective second alkylation then occurs on the other *exo* nitrogen. Double reductive ring opening of

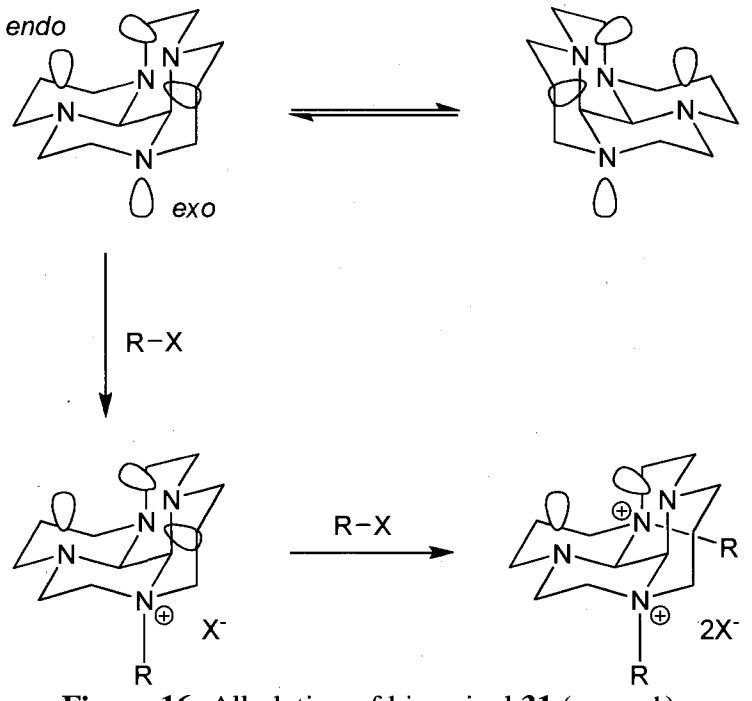


Figure 16: Alkylation of bisaminal **31** (m=n=1).

ligands **32** is described in **Figure 17**. Dialkylated salt is converted into a tricyclic iminium ion via a twist-boat conformation in order to arrange the lp-N-C-N+ torsion angle into a stereoelectronically idealized arrangement for cleavage.[51] **7** and **8** were synthesized by this reported procedure.[49, 51, 153]

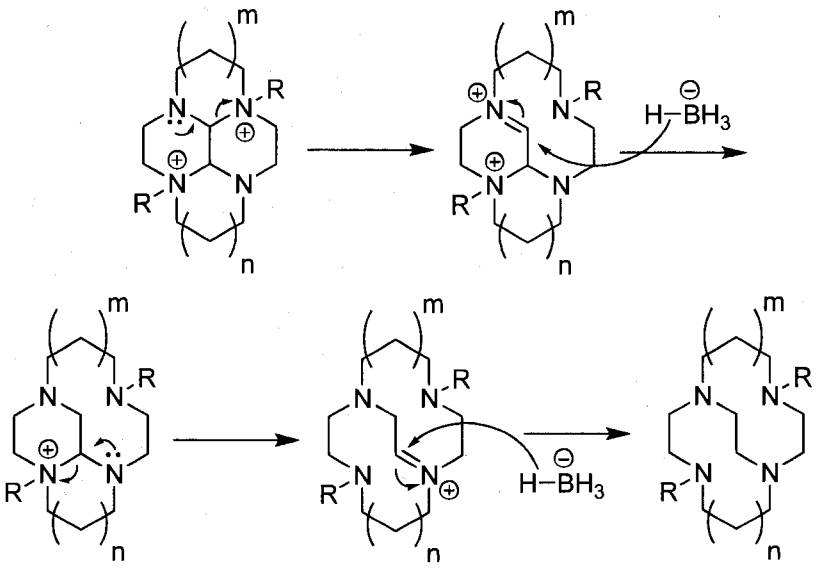
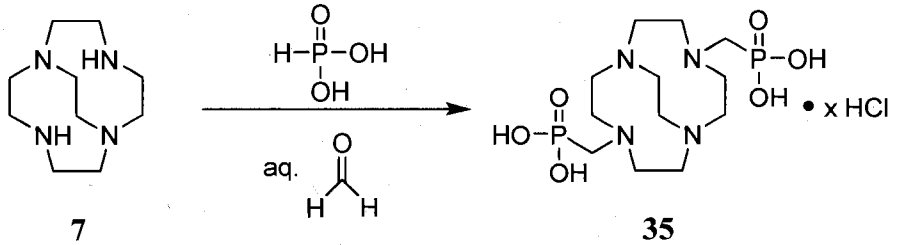


Figure 17: Mechanism of double-reductive ring opening of ligands **32**.

The formation of ligand **35** has been reported in the patent literature (details not reported) by Winchell and coworkers (**Scheme 12**).[154] The procedure listed involved the use of phosphorous acid and aqueous formaldehyde. No further details were listed with regards to purification, yield or speciation.



7 **35**

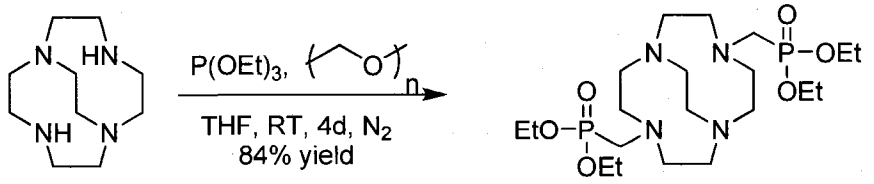
Scheme 12: Formation of ligand **35**.

III. Results and Discussion

A. Synthesis of Phosphonate Ester Pendant Arms on CB-Cyclen (7) and CB-Cyclam (8).

(1) The synthesis and characterization of 1,4,7,10-tetraazabicyclo[5.5.2]tetradecane-4,10-bis(methanephosphonic acid diethyl ester) (**36**)

The most common synthetic technique for appending polyamines with phosphonic acid pendant arms is the use of the Moedritzer conditions that form the aminophosphonic acid through a Mannich-type mechanism.[99] These reaction conditions are highly acidic (conc HCl) and produce a broad range of yields, making them less attractive for use with expensive cross-bridged tetraamines.[88, 89, 99] Weisman proposed the formation of phosphonate esters as synthetic intermediates due to their ease of synthesis (mild formation conditions and typical high yields). This was to be followed by phosphonate ester hydrolysis to obtain the ionizable ligands. Compound **7** was allowed to react with triethyl phosphite and paraformaldehyde in THF at room temperature for four days under N_2 (**Scheme 13**). Ethyl phosphonate **36** was isolated after a basic extraction as a yellow oil in 84% yield and characterized by ^{1}H, ^{13}C, ^{31}P NMR and high resolution



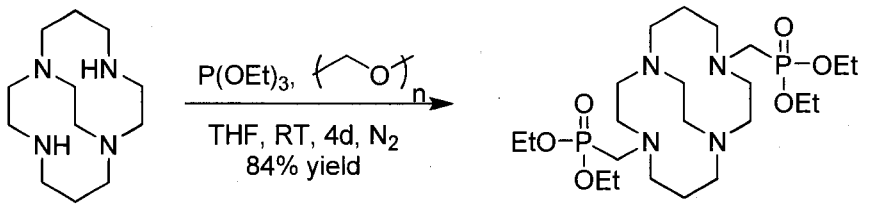
7 **36**

Scheme 13: Synthesis of **36**.

FAB mass spectrometry (HRFABMS). As the spin of ^{31}P is ½, some ^{1}H's and ^{13}C's couple to the phosphorus allowing better identification of NMR resonances. The methylene protons flanked by the nitrogen and phosphorus are enantiotopic by symmetry (structure is time-averaged C_{2V}). However, the resonance in the ^{1}H NMR that corresponds to these protons is a doublet, due to the coupling with the NMR active ^{31}P ($^{2}J_{HP}$ = 7.6 Hz).[155] The methylene protons on the ester substituent are diastereotopic based on symmetry, and couple to each other, as well as to the methyl protons and the ^{31}P. The coupling constants are difficult to extract from the complicated resonance in the ^{1}H NMR spectrum. In the ^{13}C NMR, the carbon flanked by the phosphorus and the nitrogen possesses a one bond coupling constant with the phosphorus, which is typically on the order of 150-160 Hz.[115, 116, 122, 128] This doublet is used as a diagnostic peak for the formation of the pendant arm. ^{31}P coupling with other carbons is seen as well, however, those coupling constants are typically on the order of 4-8 Hz. As shown by ^{1}H NMR, the yellow oil is quite hygroscopic, a characteristic seen with many cross-bridged tetraamines prepared thus far. The isolated material contained a small amount of mono-armed product, 1,4,7,10-tetraazabicyclo[5.5.2]tetradecane-4-(methanephosphonic acid diethyl ester) (0.1 molar equivalents relative to 1.0 of product). An increase in reaction time to 6 days produced a similar result, as did the addition of extra equivalents of reagents. Because the reaction is controlled by thermodynamic conditions, Le Chatelier's principle suggests the byproduct present should fully convert to product with the addition of further equivalents of reagents. No further purification was attempted.

(2) The synthesis and characterization of 1,4,8,11-tetraazabicyclo[6.6.2]hexadecane-4,11-bis(methanephosphonic acid diethyl ester) (**37**)

CB-cyclam (**8**) was subjected to the same set of conditions as in the formation of **36** (**Scheme 14**). Compound **8** was stirred in THF for 4 days under N_2 in the presence of



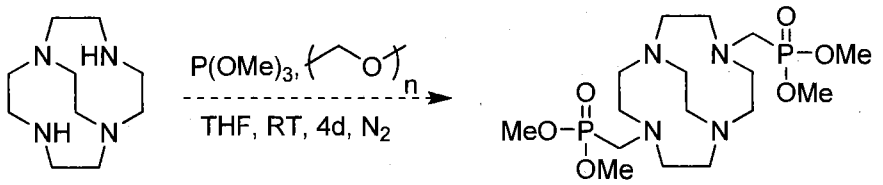
8 **37**

Scheme 14: Synthesis of **37**

triethyl phosphite and paraformaldehyde. The product was isolated from a basic extraction and solidified under vacuum in good yield (84%). The ^{1}H NMR spectrum of ligand **37** indicated no observable impurities. This was in contrast to **36**, which contained a small percentage of mono-armed material. ^{1}H NMR assignments were made with the assistance of 2D homo- and heteronuclear NMR experiments (^{1}H-COSY and gHMQC). The time-averaged C_2 symmetry of the ligand can be seen in the ^{1}H NMR spectrum by the appearance of an AA′XX′ spin system corresponding to the ethylene cross-bridge. As in **36**, the methylene protons on the ethyl ester are diastereotopic and their resonances overlap, thus they have a complicated resonance pattern due to couplings with ^{31}P and the methyl protons.

(3) Attempted synthesis and characterization of 1,4,7,10-tetraazabicyclo[5.5.2]tetradecane-4,10-bis(methanephosphonic acid methyl ester) (**38**)

Methyl phosphonates are known to hydrolyze faster than their ethyl analogues in both acidic and basic solutions, making them attractive precursors to ionizable methyl phosphonic acid pendant arms.[132, 133, 135, 156] As the conditions used for the preparation of **36** produced only a slightly impure product, the synthesis of **38** was attempted under the same conditions. CB-cyclen **7** was stirred in THF for four days under N_2 in the presence of trimethyl phosphite and paraformaldehyde (**Scheme 15**). The initial solution became opaque ten minutes after the addition of the trimethyl phosphite and paraformaldehyde,



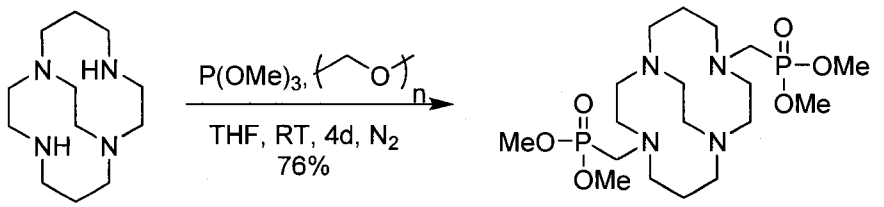
7 **38**

Scheme 15: Attempted synthesis of **38**.

then after 1 hour, the solution became transparent and was allowed to stir for 1 day. This unusual behavior was never seen in any other phosphonate ester forming reaction with cross-bridge ligands. A basic extraction afforded the ligand in poor yield (~10%) and purity. It is quite possible that the methyl phosphonate functionality hydrolyzed, either partially or fully, in the strongly basic (pH=14) aqueous phase during the course of the extraction. The crude ^{1}H NMR spectrum also indicated the presence of more than one ligand containing a methyl phosphonate. Attempts to extract the product from an ice cold aqueous phase (pH = 14) produced similar results. Further synthetic work is required in order isolate pure ligand in respectable yield.

(4) The synthesis and characterization of 1,4,8,11-tetraazabicyclo[6.6.2]hexadecane-4,11-bis(methanephosphonic acid dimethyl ester) (**39**)

CB-cyclam (**8**) was dissolved in THF and stirred with paraformaldehyde and trimethyl phosphite under N_2 for four days (**Scheme 16**). The product was extracted



8 **39**

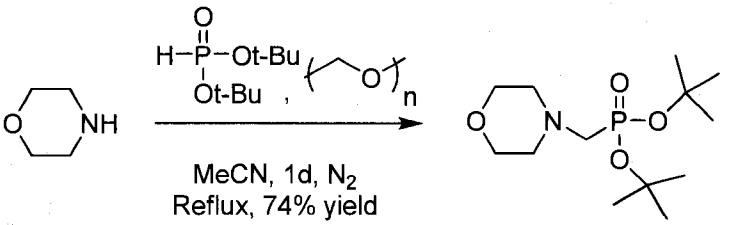
Scheme 16: Synthesis of **39**.

from a strongly basic solution and solidified to a white solid upon the removal of solvent. Ligand **39** was formed in 76% yield and high purity. Resonance assignments were aided by 2D NMR experiments (^{1}H-COSY and gHMQC). The relative ease of formation of both ligands **37** and **39** with respect to ligands **36** and **38** was not expected.

(5) The synthesis of di-tert-butyl morpholinomethylphosphonate (**40**)

Although the synthesis of ligands **36** through **39** utilized trialkyl phosphites, the same product could be formed through the use of dialkyl phosphites due to similar mechanisms. The acidic proton on the dialkyl phosphite (pKa = 1.3)[157] can protonate the aminomethanol intermediate, making formation of the iminium ion easier due to the loss of H_2O instead of hydroxide. Water can also be removed in the presence of molecular sieves, forcing the equilibrium towards product. To test this, morpholine was allowed to reflux overnight in the presence of paraformaldehyde and commercially available di-*t*-butyl phosphite (1.5 molar equivalents relative to 1.0 molar equivalents of morpholine)

over 3Å sieves (**Scheme 17**). Compound **40** was isolated from a basic extraction in 74% yield with 0.17 molar equivalents of di-*t*-butyl phosphite still present. The addition of *t*-butyl phosphonates could be attempted on a cross-bridged tetraamaine.

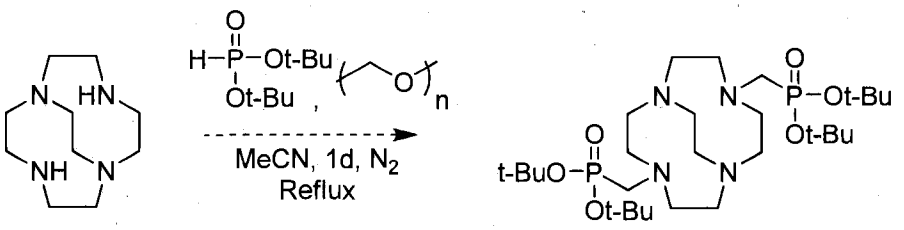


40

Scheme 17: Synthesis of **40**.

(6) Attempted synthesis of 4,11-bis-(4-carbo-t-butoxymethyl)-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane(**41**).

The synthesis of compound **41** was attempted under the conditions used for the synthesis of **40** (**Scheme 18**). The reaction became a very dark red color after only a few



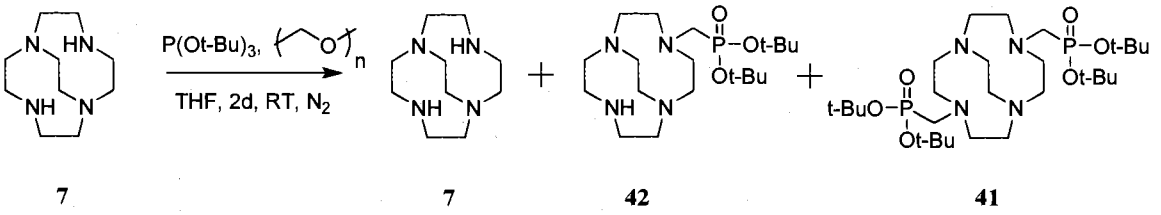
7 **41**

Scheme 18: Attempted synthesis of **41**.

hours. The ^{1}H NMR of the crude mixture as well as the material collected after a basic extraction indicated a complex mixture of products. It is quite possible that the strong base **7** was merely protonated by the acidic di-*t*-butyl phosphite, inhibiting the Mannich-like reaction. However, if this was the case, then after a basic extraction, the starting material should have been recovered. Since the ^{1}H NMR spectrum revealed a mixture of

materials, clearly some other reaction occurred. The reaction to form **41** was attempted in dry THF due to the success of the formation of **36**, **37** and **39** under similar conditions (*vide supra*). Reactions were run at room temperature for both 3 days and 4 days utilizing 2.5 equivalents of both paraformaldehyde and di-*t*-butyl phosphite. Reactions were also attempted in MeCN, giving similar results. A new synthetic direction was thus necessary.

The synthesis of tri-*t*-butyl phosphite has been reported in the literature[152] and its reaction with paraformaldehyde and cyclen have also been reported.[144] The authors described the sensitivity of the reagent to heat, air and water, both once it was formed, as well as during the course of reaction.[144] With this in mind, tri-*t*-butyl phosphite was synthesized according to literature procedures in 63% yield with approx 91% purity based on ^{1}H NMR (the presence of tri-*t*-butyl phosphate accounted for the 9% impurity). The procedure used to add *t*-butyl phosphonate arms to cyclen[144] was followed with CB-cyclen (**Scheme 19**). The reaction was run for 2 days under N_2 and the products were isolated from a basic extraction. The ^{1}H NMR and ^{13}C{^{1}H}NMR spectra indicated the



7 **7** **42** **41**

Scheme 19: Attempted synthesis of **41** with tri-t-butyl phosphite.

presence of **7**, **42**, and **41**, with **42** being the dominant species in solution. The same result was obtained when the reaction was allowed to run for 6 days and as well as with an excess of reagents. Further work in this area is paramount, as is the preparation of tri-*t*-butyl phosphite in higher purity.

(7) Attempted synthesis of 1,4,8,11-tetraazabicyclo[6.6.2]hexadecane-4,11-bis(ethanephosphonic acid dimethyl ester) (**46**).

In 2007, Heroux *et al.* reported their exploration of the role pendant arm length played in the kinetic inertness of various cross-bridged tetraamine Cu(II) complexes. Their assays involved acid dissociation experiments and resistance to reduction of Cu(II).[158] Previous experiments on *N*-carboxyethyl pendant-armed cyclen and cyclam derivatives indicated a change in coordination modes, dissociation kinetics and redox chemistry relative to their *N*-carboxymethyl analogues like DOTA (**3**) and TETA (**4**).[159-163] The cross-bridged ligands studied are shown in **Figure 18**. The lengthening of the pendant arms (from **9** to **43** and from **10** to **44**, respectively) resulted in a small decrease on the kinetic inertness (half-life) of their Cu(II) complexes relative to the short armed Cu(II) complexes.[158] However, [Cu·**44**] was less resistant to reduction by 400 mV compared with [Cu·**10**], suggesting that [Cu·**44**] could be susceptible to physiological reductants.[158] Biodistribution studies of the ^{64}Cu complexes revealed poorer tissue

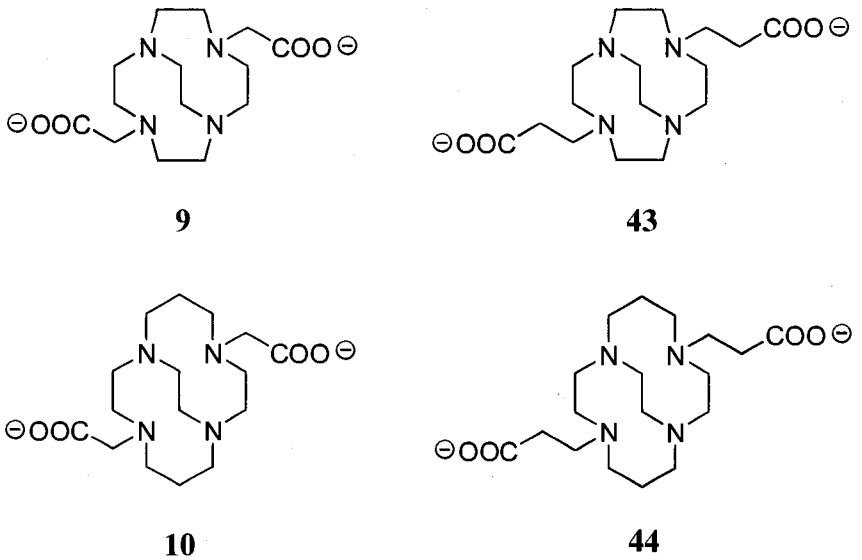
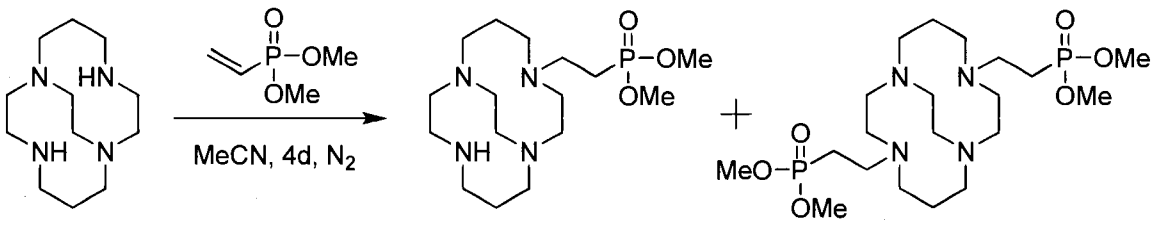


Figure 18: Ligands of the Cu(II) complexes studied by Heroux *et al.* to determine effects of *N*-carboxyethyl pendant arms versus *N*-carboxymethyl pendant arms on kinetic inertness and resistance to Cu(II)/Cu(I)

clearance of the "long-armed" complexes with respect to the "short-armed" complexes, which indicated that resistance to reduction is a useful tool to assess, qualitatively, the *in vivo* stability of these complexes.

The addition of ethanephosphonic acid pendant arms on CB-cyclam (**8**) would serve as an appropriate model to determine if the presence of the phosphonate moiety could influence the kinetic inertness and/or resistance to reduction similar to the results obtained for carboxylate-armed analogues. The conditions reported for the formation of the *t*-butyl ester precursor to **44** were implemented with CB-cyclam (**8**) and ten equivalents of commercially-available dimethyl vinylphosphonate (**Scheme 20**). The ^{1}H



Scheme 20: Attempted synthesis of **46**.

and $^{13}C\{^{1}H\}$ NMR spectra of the crude product indicated the presence of predominantly mono-armed ligand **45**, with less di-armed ligand **46** (approximately 0.3 molar equivalents of **46** to 1.0 molar equivalent of **45**). It was hypothesized that the large amount of reagent present was affecting the polarity of the solvent, which affected the rate of the Michael addition of dimethyl vinylphosphonate. Several different reaction conditions were attempted as summarized in **Table 2**. The reaction was run with less reagent and more polar solvents, but the crude ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra indicated the crude still contained predominantly **45**. The same result was seen with microwave heating. The recovered yields were always poor as well (~ 25-50% after workup). Two

Equiv. Ligand 8	Equiv. Dimethyl vinyl phosphonate	Conditions	Result
1	2.2	MeCN, RT, N_2, 2d	Mostly **45**
1	2.5	DMF, RT, N_2, 2d	Mostly **45**
1	2.2	Microwave, MeCN, 30 min, 80 °C	Mostly **45**
1	8	MeCN, reflux, 4d, N_2	Mostly **45**

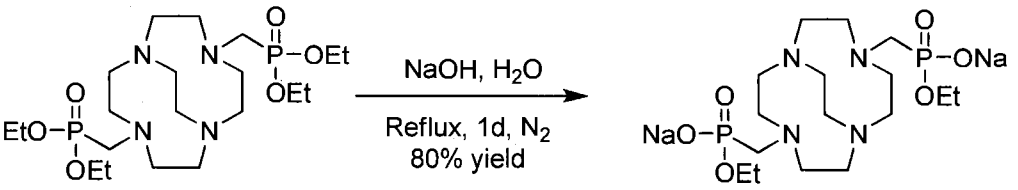
Table 2: Various reaction conditions used for the attempted synthesis of **46**.

possible explanations involve either the hydrolysis of the methyl esters during the course of extraction from a pH 14 aqueous solution, preventing the resulting phosphonic salt from being extracted into the organic phase, or the de-arming of the ligand by hydroxide (reverse Michael addition). In order to prevent these, the extraction was attempted from an ice-cold aqueous phase (pH = 14). However, the recovered yields didn't improve and the isolated material was similar in composition to those reactions described previously. Further work in this area is clearly required.

B. Formation of Ionizable Phosphonate Pendant Arms on CB-cyclen (7) and CB-cyclam (8).

(1) The synthesis and characterization of 1,4,7,10-tetraazabicyclo[5.5.2]tetradecane-4,10-bis(methanephosphonic acid monoethyl ester sodium salt) (47)

Compound **36** was brought to reflux for 1 day in the presence of 4 equivalents of NaOH to form sodium salt **47** (**Scheme 21**). The sensitivity of basic hydrolysis of **36** was not expected as only 4 equivalents of sodium hydroxide were needed for hydrolysis in 1 day. Reported procedures of base hydrolysis of aminophosphonates on macrocycles



36 **47**

Scheme 21: Synthesis of partially-hydrolyzed phosphonate **47**

generally involved highly basic solutions.[115, 122] Ligand **47** was characterized by ^{1}H, $^{13}C\{^{1}H\}$ and $^{31}P\{^{1}H\}$ NMR, HRFABMS and elemental analysis. The once diastereotopic methylene protons on the ethyl ester became enantiotopic, due to the time-averaged

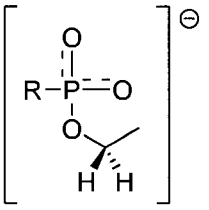
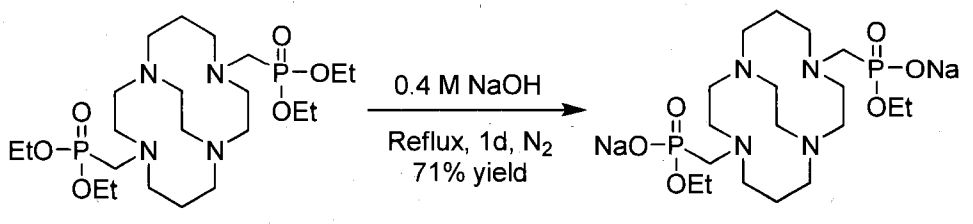


Figure 19: Time-averaged structure of a partially-hydrolyzed phosphonate. The methylene protons shown are interconverted by a sigma plane in ligand **47**.

structure in solution (**Figure 19**). Interestingly, the coupling constant between those methylene protons and the methyl protons was very similar to the coupling constant between the methylene protons and the phosphorus, simplifying the resonance in the ^{1}H NMR to a pentet. This feature has been previously reported on an analogous system.[122] The $^{31}P\{^{1}H\}$ spectrum indicated the presence of 0.05 molar equivalents of the monoarmed ligand relative to 1.0 molar equivalent of **47**. No further attempts to purify this mixture were attempted.

(2) The synthesis and characterization of 1,4,8,11-tetraazabicyclo[6.6.2]hexadecane-4,11-bis(methanephosphonic acid monoethyl ester sodium salt) (**48**)

Attempts to partially hydrolyze ligand **37** with 4 equivalents of NaOH, as was done with **36**, failed. The starting phosphonate **37** was dissolved in a 0.4 M NaOH (12 equivalents of NaOH to 1 equivalent of **37**) solution and refluxed for 24 hours (**Scheme 22**). The solvent was removed and ice cold methanol was added to the crude isolated solids to dissolve the ligand and remove it from the bulk NaOH. However, some NaOH dissolved as well, which made analysis difficult. The crude mixture was dissolved in a minimal amount of -78 °C MeOH in order to dissolve the ligand and not the excess NaOH. The ligand was characterized by ^{1}H, $^{13}C\{^{1}H\}$, $^{31}P\{^{1}H\}$ NMR, and HRFABMS. Elemental analysis revealed 24 molar equivalents of NaOH, 7 molar equivalents of MeOH and 20 molar equivalents of water present in the sample relative to 1 molar equivalent of **48**. Titration of the sample with 0.1 N HCl, performed by Prof. Ed Wong, indicated the presence of 19 ± 5 molar equivalents of NaOH relative to 1 molar equivalent of **48**. Unlike ligand **47**, the diastereotopic methylene protons on the ethyl ester stay diastereotopic upon base hydrolysis of compound **37**, due to the time-averaged C_2 symmetry of the solution structure. However, the resonance for these protons simplified into a pentet as well, which indicated that the coupling constant between the accidentally isochronous methylene ester protons and the methyl protons was very



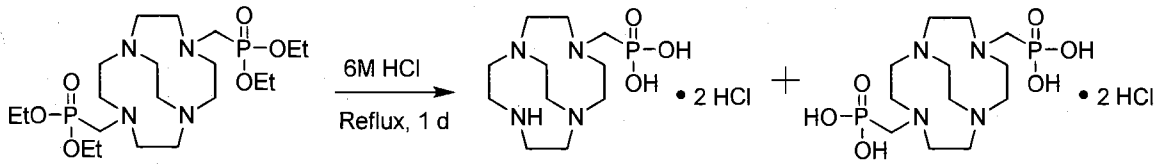
37 **48**

Scheme 22: Synthesis of ligand **48**.

similar to the coupling with the ^{31}P. Ligand **48** was used without further purification for Cu(II) complexation.

(3) The synthesis and characterization of 1,4,7,10-tetraazabicyclo[5.5.2]tetradecane-4,10-bis(methanephosphonic acid) (**37**·2HCl·3H_2O)(H_4-CB-DO2P)

The hydrolysis of ligand **36** was carried out under a series of different conditions in order to attempt to produce **50** and prevent any reverse Mannich from taking place (**Scheme 23**). Those reaction trials are summarized in **Table 3** with a listing of the



36 **49** **50**

Scheme 23: Acid hydrolysis of ligand **36** produced various amounts of monoarmed ligand **49**.

HCl conc.	Temperature	Reaction time	Result
6 M	reflux	1 d	~50% **49**, ~50% **50**
6 M	50 °C	1 d	~50% **49** and incompletely hydrolyzed ligand
6 M	rt	1 d	mostly **36**
6 M	50 °C	5 h	mostly **36**
4 M	reflux	1 d	~20% **49**, ~80% **50**
2 M	reflux	1 d	~20% **49** and incompletely hydrolyzed ligand
1 M	reflux	1 d	mixture of **36**, **49** and incompletely hydrolyzed ligand
12 M	reflux	1 d	~66% **49**, ~33% **50**

Table 3: Summery of reaction trials for the acid-assisted hydrolysis of **36**.

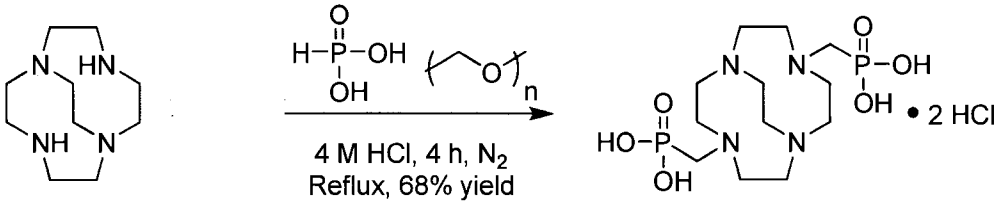
percentage of products of each reaction. In all trials, either a significant amount of reverse Mannich occurred or very little hydrolysis occurred. In the two trials in 6 M HCl run at 50 °C and room temperature, the ^1H NMR of the crude indicated the presence of a small

amount of phosphorous acid which suggested that increases in reaction time should still produce some **49**. The hydrolysis of **36** was attempted at lower acid concentrations in order to form less **49**, however the presence of phosphorous acid in the crude ^{1}H NMR spectra suggested reverse Mannich had taken place, only to a lesser extent. The attempted purification of a select few reaction trials with ion-exchange chromatography (Amberlite 50, carboxylic acid exchanger, eluted with water) failed to separate the mixtures.

Alkyl phosphonates have been converted to their corresponding phosphonic acids by means other than HCl hydrolysis. Reactions with HBr in AcOH and with trimethylsilyl bromide (TMS-Br) have successfully cleaved alkyl phosphonates to form phosphonic acids in high yield and purity.[113, 119] The conversion of **36** to **50** was attempted with an HBr/AcOH solution using a published procedure for the cleavage of another aminophosphonate.[113] The reaction was run for both 1 day and for 6 days, however these reactions were incomplete and the crude ^{1}H NMR spectrum again indicated the presence of **49**. Cleavage of **36** was also attempted with TMS-Br following a published procedure.[119] The room temperature reaction cleanly cleaved the ethyl phosphonate with little formation of **49** (~5%). The $^{31}P\{^{1}H\}$ NMR spectrum, however, indicated the presence of five distinct peaks, four of them integrated for approximately the same amount while the fifth peak was much smaller than the other four (peak at 14.3 ppm was ligand **50**). These impurities were not identified.

The synthesis of ligand **50** has been reported in the patent literature (details not reported) utilizing the Moedritzer conditions.[154] The procedure reported for the synthesis of **13** was followed for the formation of **50·2HCl** (**Scheme 24**). The crude material contained 0.08 molar equivalents of ligand **49·2HCl** and 0.04 molar equivalents of H_3PO_3

relative to 1.0 molar equivalent of **50** as shown in the ^{1}H and ^{31}P NMR spectra. Attempts to separate the impurities with ion-exchange chromatography (Amberlite 50, carboxylic acid exchanger) failed.

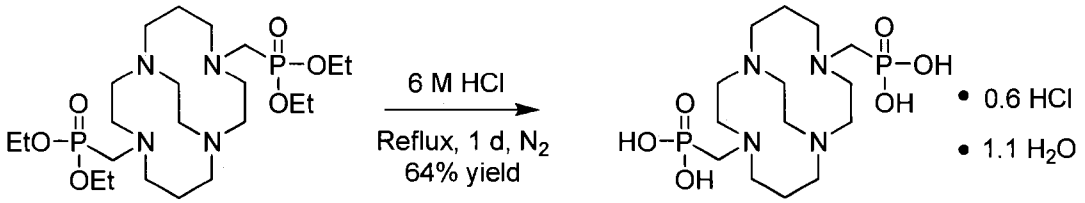


7 **50**

Scheme 24: Synthesis of **50·2HCl**.

(4) The synthesis and characterization of 1,4,8,11-tetraazabicyclo[6.6.2]hexadecane-4,11-bis(methanephosphonic acid) (**51**·0.6HCl·1.1H_2O) (H_4-CB-TE2P)

The hydrolysis of ligand **37** was carried out under reflux in 6 M HCl for 24 hours (**Scheme 25**). The ^{1}H NMR spectrum of crude product indicated the presence of 0.08 molar equivalents of monoarmed material relative to 1.0 equivalent of **51**, however the



37 **51**

Scheme 25: Synthesis of ligand **51**.

impurities were separated on an ion-exchange column (Amberlite 50, carboxylic ion exchanger) by elution with water. Microwave-assisted hydrolysis afforded product with ~12% de-arming according to ^{1}H NMR analysis. The purified ligand was characterized by ^{1}H, $^{13}C\{^{1}H\}$ and $^{31}P\{^{1}H\}$ NMR. ^{1}H-COSY and gHMQC experiments were used to

identify every resonance in the ^{1}H NMR spectrum. This ^{1}H NMR spectrum is consistent with a solution state [2323/2323] diamond lattice conformation or a [2233/2233] conformation, both di-inside-protonated. As shown in **Figure 20**, the two upfield patterns correspond to the pseudo-equatorial and pseudo-axial protons on the central methylene of the three carbon bridge. In either conformation, the pseudo-equatorial proton exhibits one

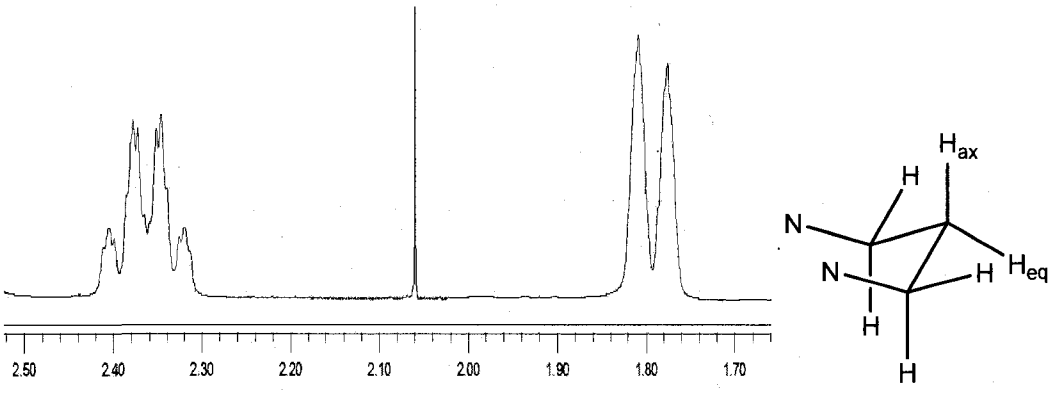


Figure 20: Upfield region of the ^{1}H NMR spectrum of **51**.

large coupling, with the pseudo-axial proton, and four smaller gauche couplings with the rest of the protons in the spin system (**Figure 20**) giving a resonance that is a doublet of multiplets. The pseudo-axial proton, on the other hand, experiences three large couplings and two smaller gauche couplings giving a resonance that appears like a quartet of multiplets (**Figure 20**). An x-ray quality crystal was grown by slow diffusion of acetone into water; the structure is shown in **Figure 21**. The close proximity of the two phosphonic acid pendants suggested a hydrogen bond between a phosphonic acid proton and an oxygen on the other phosphonic acid moiety. The distance between the placed proton and the oxygen was measured at 1.6 Å, the distance between the two oxygen atoms was found to be 2.4 Å, and the angle between the oxygen/hydrogen/oxygen atoms was measured to be 166°. Interestingly, the ligand backbone was found to be in the

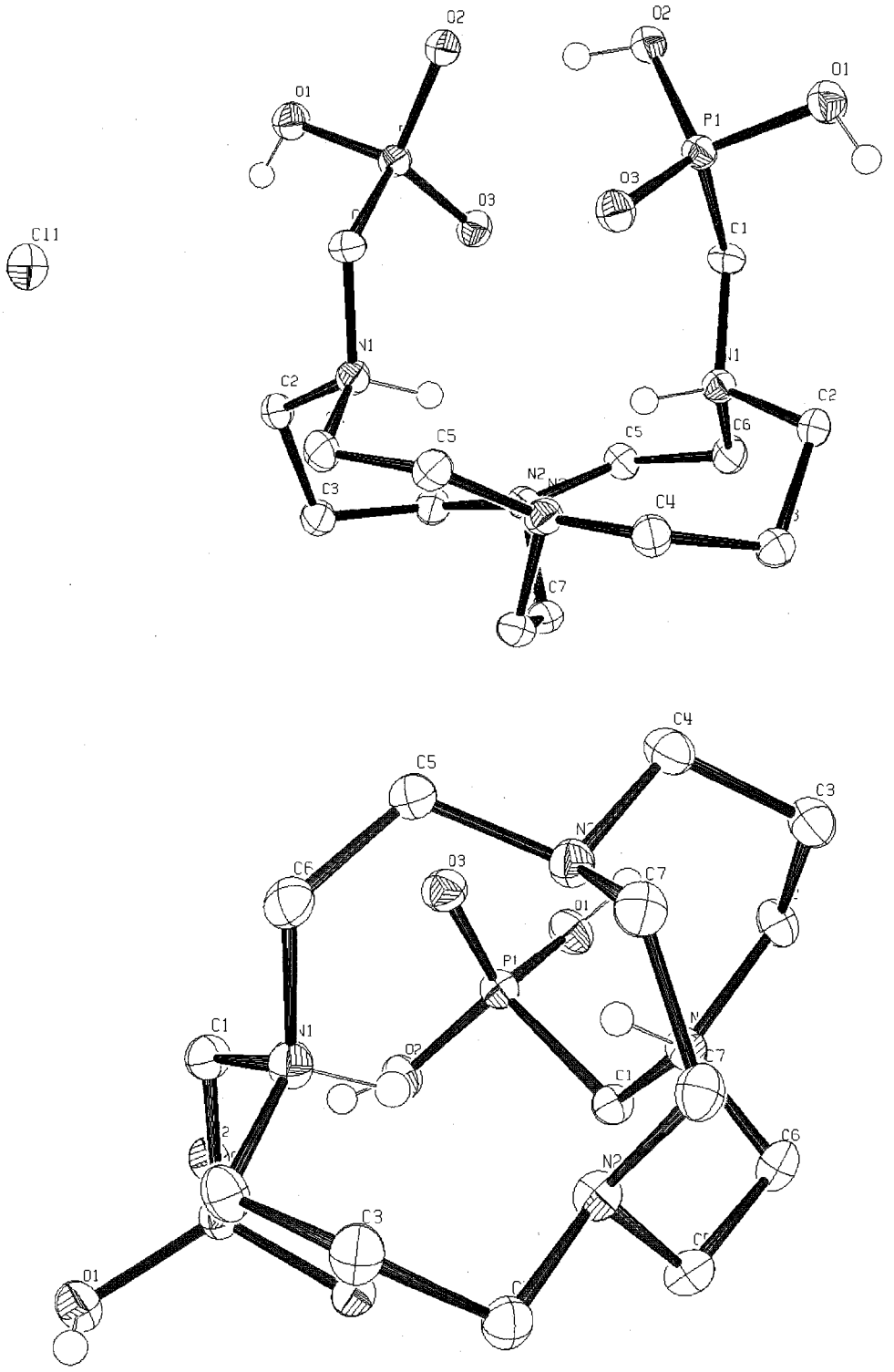


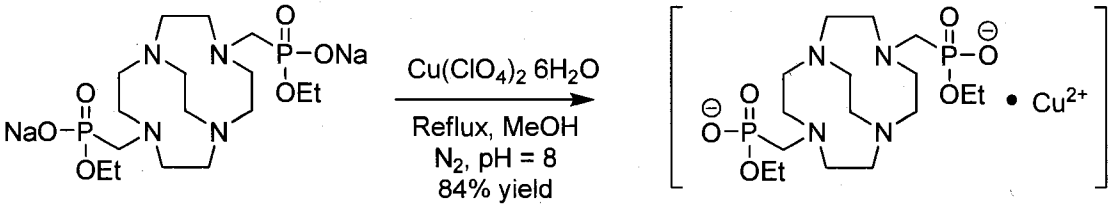
Figure 21: X-ray structure of H_4-CB-TE2P (**51**). Side view revealed a hydrogen bond between a phosphonic acid proton on one pendant arm and an oxygen atom on the other arm. Back view showed a [2233/2233] ligand conformation. Hydrogens were removed for clarity.

[2233/2233] conformation (**Figure 21**). A possible explanation for this conformational preference could be due to the hydrogen bond between the pendant arms in the solid state forcing the ligand into this geometry.

C. Copper (II) Complexation of Cross-Bridged Ligands Containing Ionizable Phosphonate Pendant Arms.

(1) Cu(II) Complexation of CB-DO2P-Et (**47**).

Ligand **47** was refluxed in MeOH in the presence of $Cu(ClO_4)_2 \cdot 6H_2O$ for 1 day (**Scheme 26**). The purified blue solids were isolated from slow diffusion of Et_2O into 95% EtOH and were characterized by UV-vis spectroscopy (λ_{max} = 672 nm, ε = 44 M^{-1}



47 **52**

Scheme 26: Complexation of ligand **34**.

cm^{-1}), HRFABMS and elemental analysis. The pH of the solution was lowered to 8 using 0.1 M NaOH. pH was monitored with a pH electrode calibrated with aqueous solutions of buffer at pH = 7 and 10 using the so called "practical method".[164, 165] The presence of 0.05 molar equivalents of the mono-armed Cu(II) complex was inferred by elemental analysis. The formation of mono-armed complex occurred from the presence of 0.05 molar equivalents relative to 1.0 molar equivalent of **52** of the single pendant armed version of **47** present in the reaction mixture. A low-quality X-ray crystal was obtained from slow diffusion of Et_2O into 95% EtOH. The X-ray structure (**Figure 22**) indicated the coordination of the ligand nitrogens around the Cu(II) with both pendant arms

coordinating to the Cu(II) as well. However, the unit cell also indicated the presence of mono-armed complex, present in a 1:3 ratio with the di-armed complex. Unfortunately, the structure refinement was not well behaved. The R factor was found to be very high so bond lengths and bond angles are not accurate from this highly disordered structure.

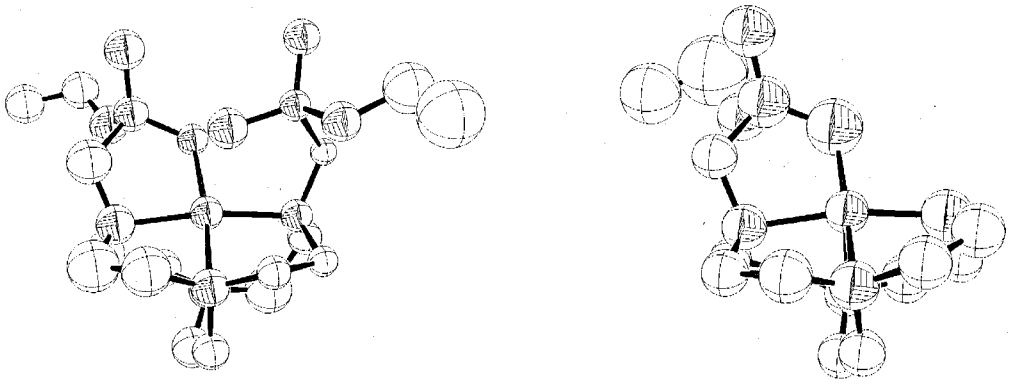
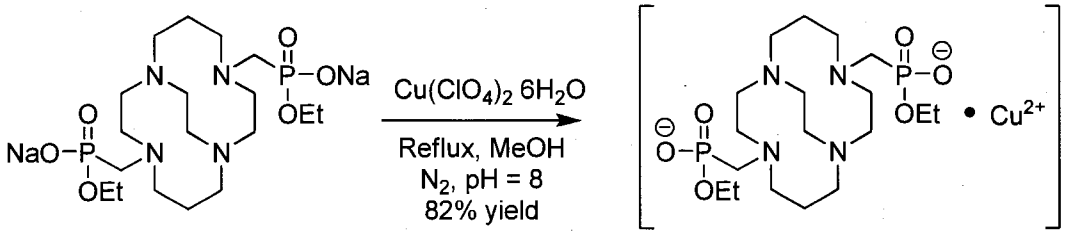


Figure 22: X-ray crystal structure of complex **52**. The left-hand structure is a di-armed complex, The right hand structure is a monoarmed complex.

(2) Cu(II) Complexation of CB-TE2P-Et (**48**)

Ligand **48** was refluxed in MeOH in the presence of $Cu(ClO_4)_2 \cdot 6H_2O$ for 1 day (**Scheme 27**). The complex was characterized by UV-vis spectroscopy (λ_{max} = 646 nm, ε = 18 M^{-1} cm^{-1}), HRFABMS and elemental analysis. 1,1,1,3,3,3-hexafluoroisopropanol



48 **53**

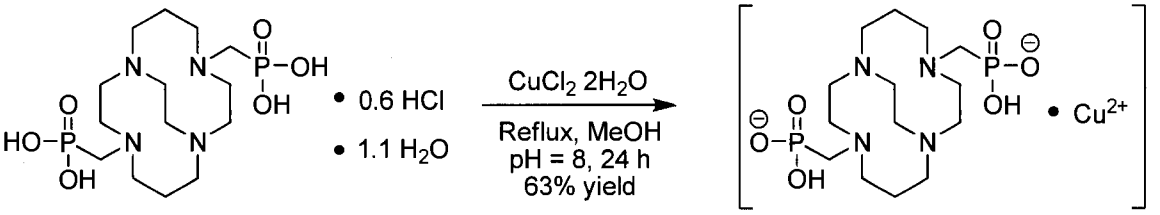
Scheme 27: Complexation of ligand **48**.

was present as indicated by the elemental analysis of the complex. It had been used to separate the complex from the NaCl byproduct. Several attempts were made to isolate an X-ray quality crystal. Et_2O diffusion into MeOH, MeCN, 95% EtOH and abs EtOH

produced blue oils. Et_2O diffusion into 95% EtOH at 0 °C produced a blue oil as well. Further work to obtain an X-ray quality crystal is required.

(3) Cu(II) Complexation of CB-TE2P (**51**)

Ligand **51** was complexed with $CuCl_2 \cdot 2H_2O$ in MeOH following the procedures described for the formation of **52** and **53** at pH = 8 (**Scheme 28**). The resultant blue solid



51 **54**

Scheme 28: Complexation of **51**.

was characterized by UV-vis spectroscopy (λ_{max}(MeOH) = 639 nm; $\varepsilon = 35\ M^{-1}\ cm^{-1}$) and HRFABMS. The blue color did not appear to change over the course of the reaction. Typically, most cross-bridged pendant armed ligands are complexed in basic solution because the complexation kinetics in acidic solution tend to be very slow due to the protonation of the highly basic cross-bridged macrocycle.[51, 54] Even at pH = 8, the cross-bridged ligands are still protonated which requires that the complexation reaction take place at reflux for 1 day. Hermann and coworkers have shown that azamacrocycles bearing phosphonic acid pendant arms tend to have faster complexation kinetics than analogous systems with acetate pendant arms.[166] The deprotonated phosphonates rapidly coordinate the divalent metal in solution, followed by a rate-limiting step involving the removal of the proton on the nitrogen and insertion of the metal into the macrocycle.[166, 167] Since the phosphonate-containing ligand can form this reaction intermediate faster

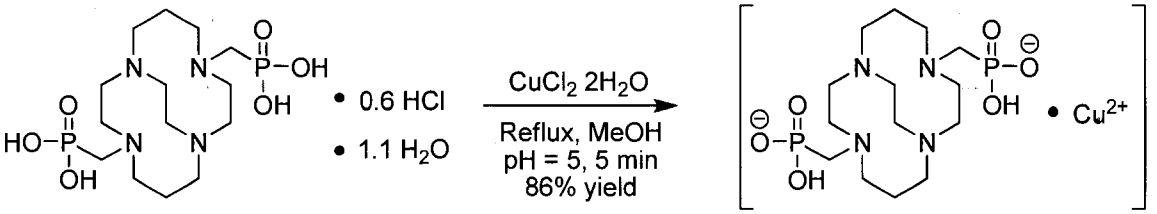
(due in part to the higher negative charge present in the phosphonate moiety with respect to the acetate moiety), and the increased hydrogen bonding ability of the phosphonate relative to the acetate makes removal of the ammonium proton faster, the overall complexation kinetics tend to be faster than for those ligands containing acetate pendant arms.

In order to determine if H_4-CB-TE2P (**51**) can exhibit faster complexation kinetics than CB-TE2A (**10**), a series of complexations were performed under various conditions, summarized in **Table 4**. The reactions were analyzed by UV-vis spectroscopy, specifically the presence of the complex absorption band and the lack of an aqueous Cu(II) absorption band. First, H_4-CB-TE2P (**51**) was allowed to complex with $CuCl_2 \cdot 2H_2O$ in MeOH at pH 8. The pH electrode used to measure the acidity was calibrated with aqueous buffers at pH = 7 and 10. The electrode was placed into the methanolic solution of **51** and the pH was raised with 0.1 M NaOH solution. No correction for methanol was used. The UV-vis spectrum taken 5 minutes after the addition was identical to that of the fully complexed ligand. When the pH was lowered to 5, the same result was observed. This was unexpected, as complexation of CB-TE2A (**10**) in acidic solution typically required very long reaction times and high temperatures. When the solvent was switched to EtOH, full complexation took 60 min at room temperature. The full characterization of the complex was completed for a sample of the complex formed in MeOH at pH = 5 (**Scheme 29**). Cyclic voltammetry conducted in 0.1

Concentration of ligand	Solvent	pH	Time to completion
0.02 M	MeOH	8	< 5 min
0.02 M	MeOH	5	< 5 min
0.02 M	EtOH	5	~ 60 min

Table 4: Time required for full complexation of H_4-CB-TE2P (**51**) under various conditions.

N sodium acetate revealed a quasi-reversible reduction with an E_p = -0.96 V (vs. Ag/AgCl), indicating a good resistance to Cu(II)/Cu(I) reduction. Numerous attempts



51 **54**

Scheme 29: Complexation of ligand **51** in MeOH at pH = 5.

were made to grow an X-ray crystal. Et_2O diffusion into solutions of **54** in MeOH, MeCN, 95% EtOH and abs EtOH at both room temperature and 0 °C produced oils. Evaporation from a 0.1 M $HClO_4$ solution produced oils as well. $Ba(OH)_2$ and $Ca(OH)_2$ solutions were added to methanolic solutions of **54** in order to form barium and calcium salts. Slow evaporation produced non-X-ray quality solids. Solids were produced upon very slow diffusion (performed in NMR and EPR tubes) of Et_2O into a 1,1,1,3,3,3-hexfluoroisopropanol solution of the complex, however, neither case produced crystals that diffracted X-rays. Et_2O diffusion into a 1,1,1,3,3,3-hexfluoroisopropanol solution of complex in an EPR tube at 0 °C produced solids that were still not X-ray quality. Further work to obtain an X-ray quality crystal is required.

D. Kinetic Dissociation of Cu-CB-TE2P (54)

As discussed previously, the acid dissociation kinetics of cross-bridged Cu(II) complexes *in vitro* have been a useful gauge for the prediction of *in vivo* stability.[56] The half-life of decomplexation of **54** was conducted under pseudo-first order conditions (5M

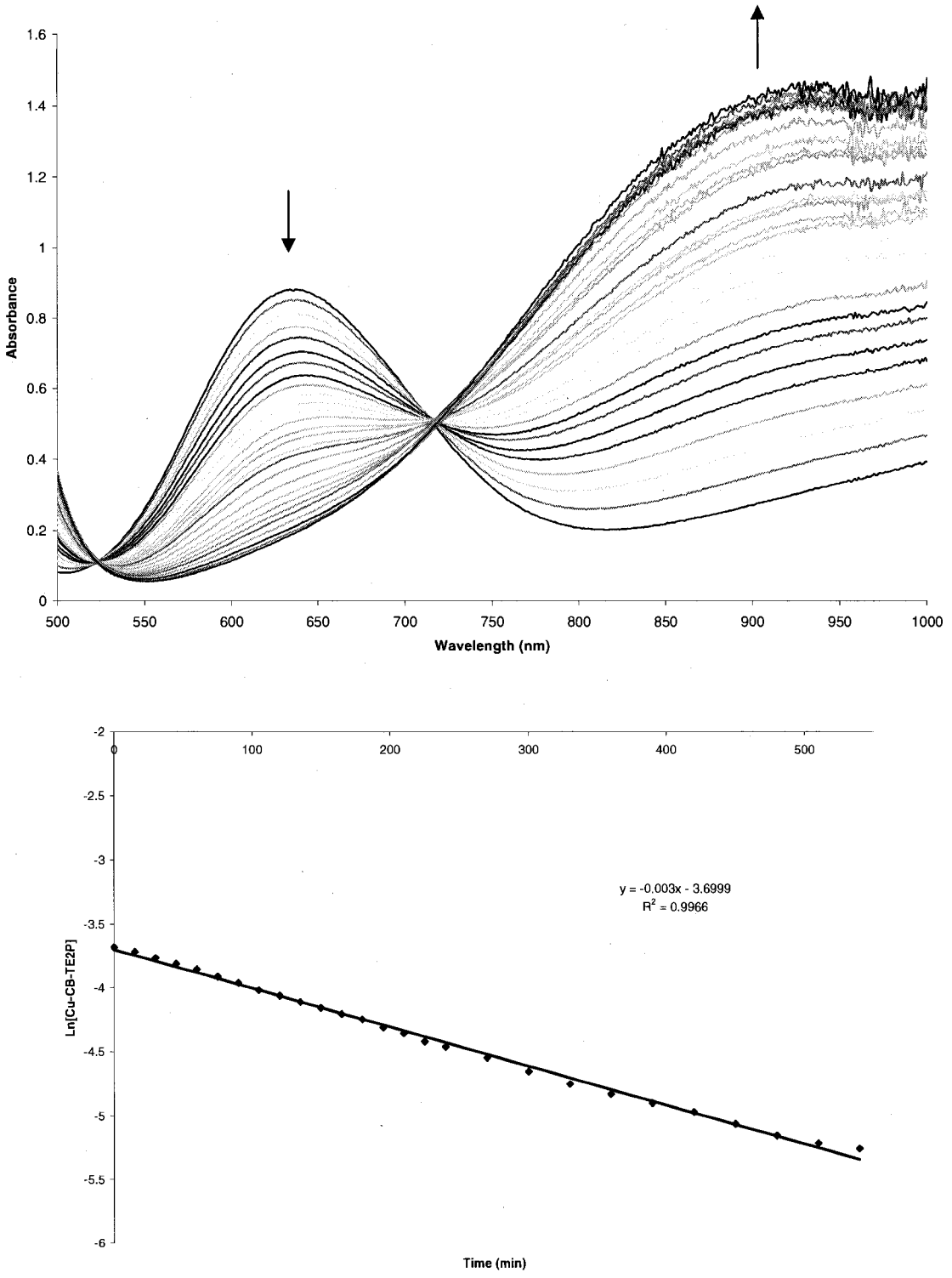
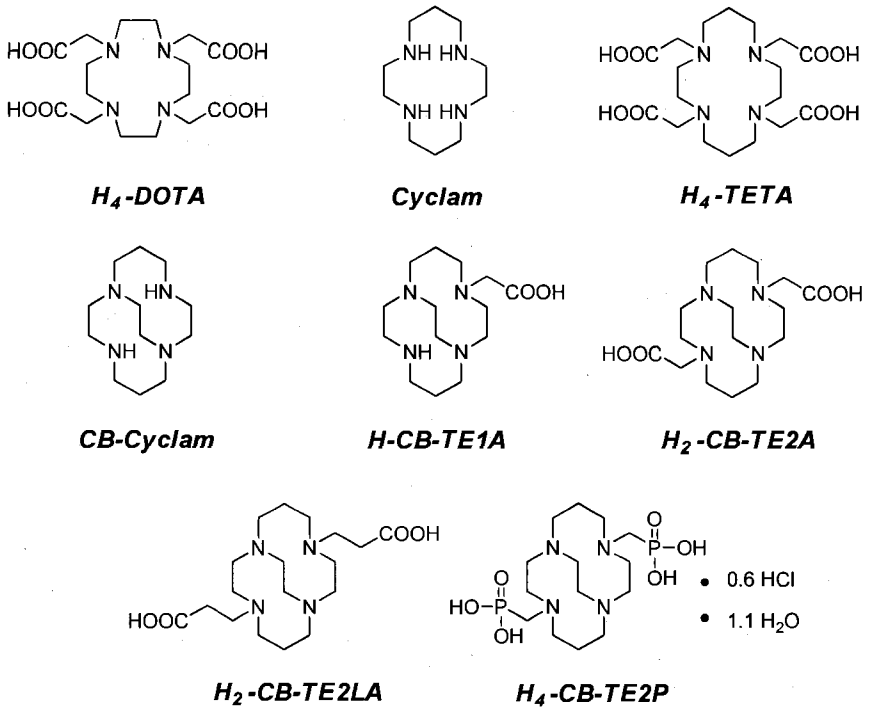


Figure 23: Isosbestic plot and pseudo-first order fit of the kinetic data for the half-life of decomplexation of Cu-CB-TE2P (**54**) in 5M HCl at 90 °C.



Ligand	Half-life of Cu(II) Complex
DOTA	< 1 min
Cyclam	3.8(2) min
TETA	4.5(5) min
CB-Cyclam	11.8(2) min
CB-TE1A	51.3(1) min
CB-TE2A	154(6) h
CB-TE2LA	~ 120 h
H_2-CB-TE2P	3.8 h

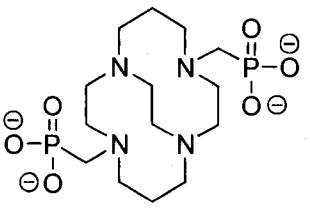
Figure 24: Half-lives of Cu(II) complexes of various tetraamine ligands in 5M HCl at 90 °C.

HCl) at 90 °C. The UV-vis trace was monitored over the course of several hours by Prof. Ed Wong of the University of New Hampshire and the isosbestic plot and the pseudo-first order plot are shown above (**Figure 23**, black arrows indicate the loss complex and the increase of free Cu(II)). The half life was found to be 3.8 hours in 5 M HCl at 90 °C. **Figure 24** shows the half-life of decomplexation of several related cross-bridged Cu(II) complexes. It is interesting to note that clearly Cu-CB-TE2A (**Cu·10**) remains the most kinetically-inert complex of the series ($t_{1/2}$ = 154 h), the half-life of Cu-CB-TE2P (**54**)

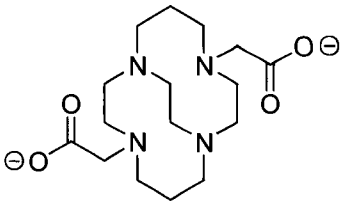
was only 3.8 hours. This result was not expected as the presence of the phosphonate has been shown to result in a more kinetically inert complex as described previously.[98] A possibility for the short half life could stem from the close proximity of the phosphonate arms when bound to the Cu(II). As the phosphonate moiety is larger than the acetate moiety, there may not be enough room to accommodate both phosphonates as they are bound to the Cu(II) which could make the first mechanistic step (removal of one pendant arm from the Cu(II)) faster with coordinating phosphonates relative to coordinating carboxymethyl pendant arms. Complexation of CB-TE2P with Zn(II) would allow NMR to be used as a means to probe the kinetics of decomplexation. However, the 3.8 hour half-life under such acidic conditions suggests that CB-TE2P (**54**) is still a viable ligand for use in radiopharmaceutical applications.

E. Biodistribution of ^{64}Cu-CB-TE2P (54)

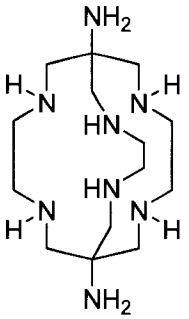
Since CB-TE2P (**51**) serves as a model for a BFC in a radiopharmaceutical, the *in vivo* behavior of the ^{64}Cu complex was investigated. The biodistribution of ^{64}Cu-CB-TE2P was performed by Riccardo Ferdani under the guidance of Prof. Carolyn Anderson at the Washington University School of Medicine, St. Louis, MO. The tissue clearance of ^{64}Cu-CB-TE2P was compared against that of the ^{64}Cu complexes of CB-TE2A (**10**), diamsar and NOTA (**Figure 25**). Male Lewis rats were injected with a solution of the ^{64}Cu complexes made from the ligands above and the amounts of ^{64}Cu remaining in selected tissues were determined at 1 hour, 4 hours and 24 hours post-injection. The data (**Figure 26**) are listed as %ID (percent injected dose) versus time. Significantly, the blood clearance of ^{64}Cu-CB-TE2P indicated a slightly improved clearance with respect to ^{64}Cu-



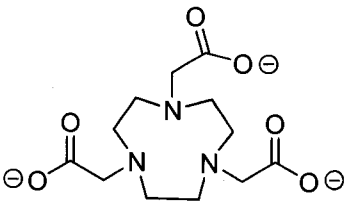
CB-TE2P



CB-TE2A



DIAMSAR



NOTA

Figure 25: Ligands used in the biodistribution study of ^{64}Cu-CB-TE2P.

CB-TE2A, a requirement of a radiopharmaceutical in order to create a background contrast for a PET scan, while the kidney clearances of these two complexes were similar. The liver and marrow clearances of ^{64}Cu-CB-TE2P proved to be the most interesting. The significant enhancement in the liver clearance of ^{64}Cu-CB-TE2P versus those of ^{64}Cu-CB-TE2A indicated that a phosphonate-containing BFC in a bioconjugate should have improved liver clearance behavior.[57] The slight decrease in bone-marrow clearance of ^{64}Cu-CB-TE2P versus that of ^{64}Cu-CB-TE2A indicated the possibility of the phosphonate containing ligand to be a candidate for bone imaging. The uptake of phosphonate containing ligands in bone has been previously documented.[168]

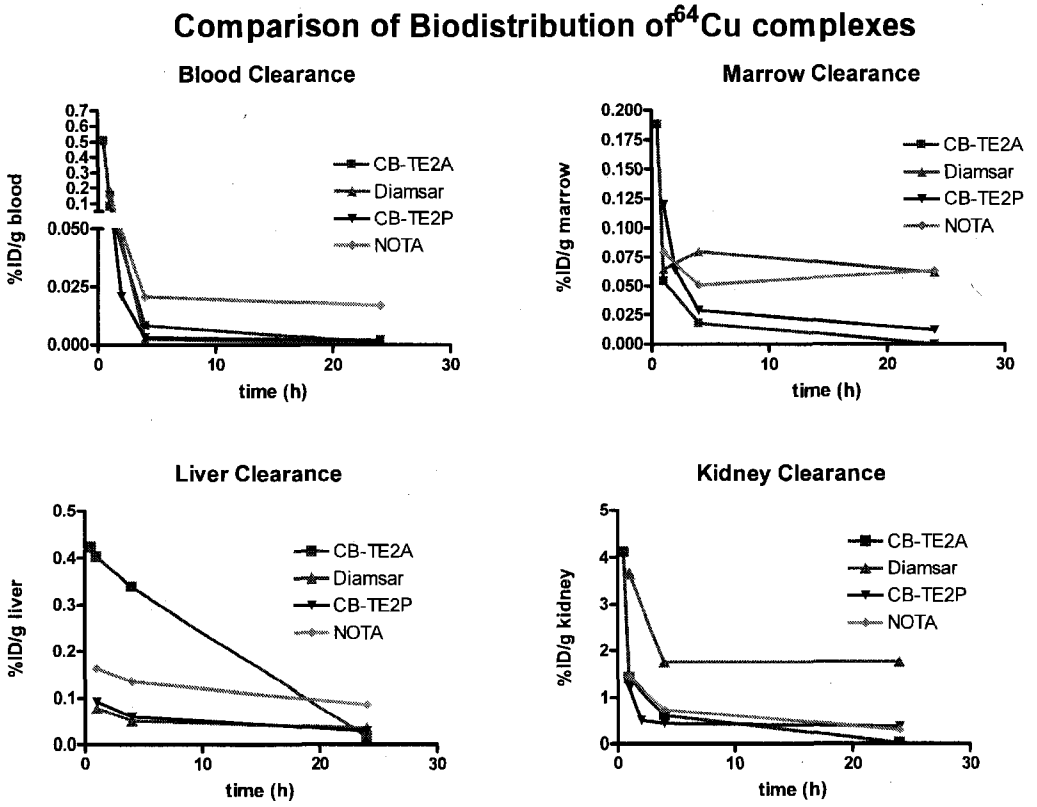


Figure 26: Biodistribution of the ^{64}Cu complexes of CB-TE2A, CB-TE2P, Diamsar and NOTA.

IV. Conclusions

Phosphonate ester pendant arms were appended onto CB-cyclen (**7**) and CB-cyclam (**8**). Attempts were made at synthesizing *t*-butyl phosphonates, and long-arm phosphonates. The base hydrolysis of ligands **36** and **37** afforded the partially-hydrolyzed ligands **47** and **48** which were complexed with Cu(II). Acid hydrolysis of ligand **36** caused significant de-arming of the ligand while the acid hydrolysis of ligand **37** caused a small amount of de-arming, however, the impurity can be separated on an ion-exchange column. Ligand **51** was complexed with Cu(II) and experiments under various conditions revealed that complexation of ligand **51** can occur quickly under conditions in which other cross-bridge ligands complex slowly. The acid-assisted decomplexation kinetics of Cu-CB-TE2P in 5 M HCl at 90 °C indicated a half-life of 3.8 hours, less kinetically inert than Cu-CB-TE2A, however inert enough to indicate promise for a phosphonate-armed cross-bridged cyclam as a BFC for radiopharmaceuticals. The biodistribution of ^{64}Cu-CB-TE2P indicated an improved liver clearance, when compared to ^{64}Cu-CB-TE2A, as well as a potential to be used as a BFC for bone imaging.

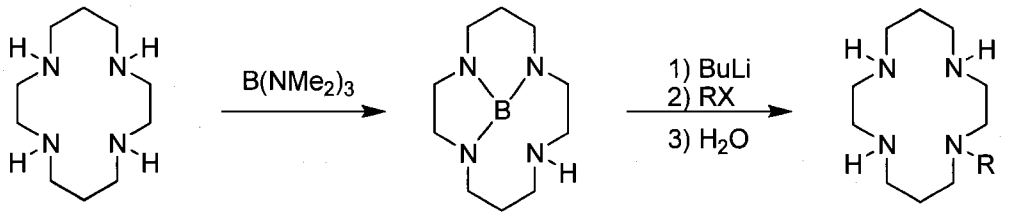
CHAPTER II

SYNTHESIS OF BIFUNCTIONAL CHELATORS BEARING UNSYMMETRICAL PENDANT ARMS ON CROSS-BRIDGE CYCLAM

I. Introduction

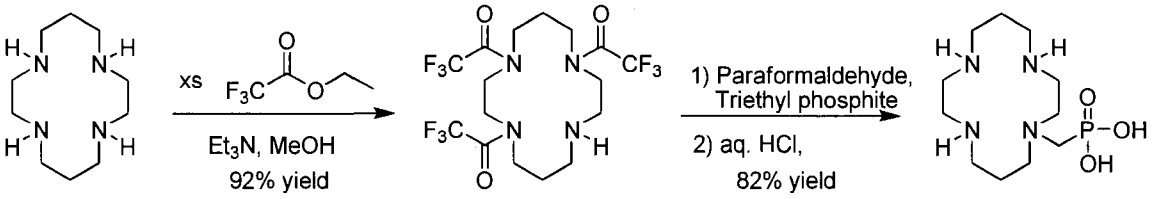
In order to use azamacrocycles as bifunctional chelators (BFCs) for the purpose of radioimaging and targeted therapy, they must be covalently bound to either a peptide or monoclonal antibody.[169-171] Typically, this addition is done by coupling the biomolecule to the BFC via a reaction with the pendant arm resulting in a new functionality present on the BFC. There are examples of *C*-functionalized BFCs that conjugate via an extra pendant arm attached to the macrocyclic backbone; however this arm will typically not be involved in coordination of the radiometal.[24, 25, 172] As shown in **Figure 8** (**Chapter I**), the bioconjugate of both TETA (**4**) and CB-TE2A (**10**) possess an amide as the linking functionality between the BFC and the somatostatin derivative Y3-TATE. The amide is formed during the coupling of the N-terminus of the peptide to a free carboxylate on the BFC. In both cases, the overall charge of the bioconjugate is now different than the overall charge of the ligand-metal complex itself, due to the conversion of an ionizable pendant arm to a neutral amide. A further complication exists from a synthetic standpoint. Both TETA and CB-TE2A have more than one free carboxylate present for coupling with the N-terminus of Y3-TATE, resulting in poor yields of the desired bioconjugate (~ 10% yield).[57] In order to sidestep this problem, macrocyclic chelators need be synthesized with only one free carboxylate in order to prevent the over-coupling with biomolecules.

Formation of mono-pendant-armed azamacrocyclic systems has been known in the literature for some time.[173-177] From a synthetic standpoint, it is clearly easier to fully substitute polyamines with excess alkylating agent; however, preparation of single pendant armed polyamines requires a more selective synthetic approach. Weisman and coworkers developed novel mono *N*-formyl protected cyclic polyamines from the hydrolytic cleavage of the corresponding orthoamides of 1,4,7-triazacyclononane and 1,5,9-triazacyclododecane.[178] Parker synthesized tritosyl cyclam, which was functionalized at the remaining secondary amine, and then detosylated to yield a mono-pendant-armed derivative of cyclam.[179] Kaden and coworkers synthesized a polyamine with one pendant arm by attaching it to a ring nitrogen before cyclization of the macrocycle.[180] Unfortunately, the synthetic sequence was time consuming and required the cyclization procedure to be carried out every time a new derivative was made. Studer was able to prepare several tetraazamacrocycles with one pendant arm by utilizing a five-fold excess of the macrocycle with an alkylating agent.[181] The procedure relied on the ability to separate the mono-armed material from the starting polyamine which limits its use in more complex systems, as well as increasing the cost of expensive reagents. Handel and coworkers were able to form mono N-substituted derivatives of several tetraazamacrocycles through the coordination of a boron atom by three of the nitrogens in the ring, leaving the fourth nitrogen to react with the appropriate electrophile (**Scheme 30**).[182] The use of *n*-butyllithium as well as the sensitivity of the boron complex to H_2O



Scheme 30: General synthetic sequence developed by Handel to mono-alkylate cyclam.

made this procedure only applicable to systems with robust functionality. Handel also reported a procedure similar to the one described previously in which a tetraamine was used to coordinate to $Mo(CO)_3$ or $Cr(CO)_3$ instead of boron to occupy thee nitrogens of cyclam while the fourth could be alkylated.[183] Although the reaction could be performed in "one-pot", over-alkylated material was found to be present in the crude product.[183] Hermann and coworkers were able to produce a cyclam derivative with one methylphosphonate pendant arm (**Scheme 31**).[116] They utilized a procedure developed by Yang and coworkers which involved the tri-protection of cyclam with ethyl

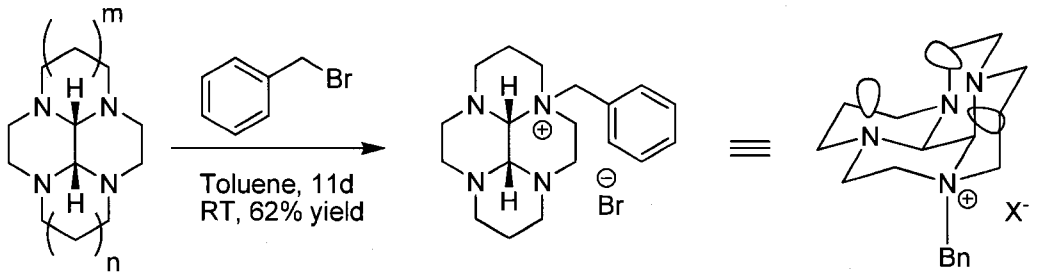


Scheme 31: Synthetic sequence to produce a methylphosphonate on cyclam. The first step was developed by Yang *et al.*,[184] the second step developed by Hermann *et al.*[116]

trifluoroacetate.[184] The reaction was run in neat ethyl trifluoroacetate yet interestingly, showed no presence of the tetraamide. This is in contrast to most reactions with cyclam in the presence of excess electrophile where the tetrasubstituted molecule is produced in significant quantities.[184] The goal of this work will be to attain a synthesis of a single pendant arm derivative of CB-cyclam (**8**) and use that as a springboard for the synthesis of a range of unsymmetrical pendant-armed cross-bridged macrocycles.

II. Background

The synthesis of mono-pendant-armed derivatives of CB-cyclen (**7**) and CB-cyclam (**8**) and their conversion to unsymmetrical pendant-armed ligands has been reported and are based on two different synthetic strategies. The first is based on the benzylation/ reductive ring opening/ debenzylation procedure described previously (**Scheme 11, Chapter 1**).[51] The synthetic sequence, developed by Weisman, Wong and coworkers, relies on the regioselective dibenzylation of **31** (**Figure 16, Chapter 1**) in MeCN to yield dibenzyl salt **32** with benzyl protecting groups on non-adjacent nitrogens. A double reductive ring opening with $NaBH_4$ yields the dibenzyl cross-bridged ligand (**33**) which when subjected to hydrogenolysis forms the parent ligand **34**. As described by Weisman, Wong and coworkers, the monobenzylation of bisaminal **31** (m=n=1) can be performed under kinetic control with benzyl bromide in toluene; dibenzylation was

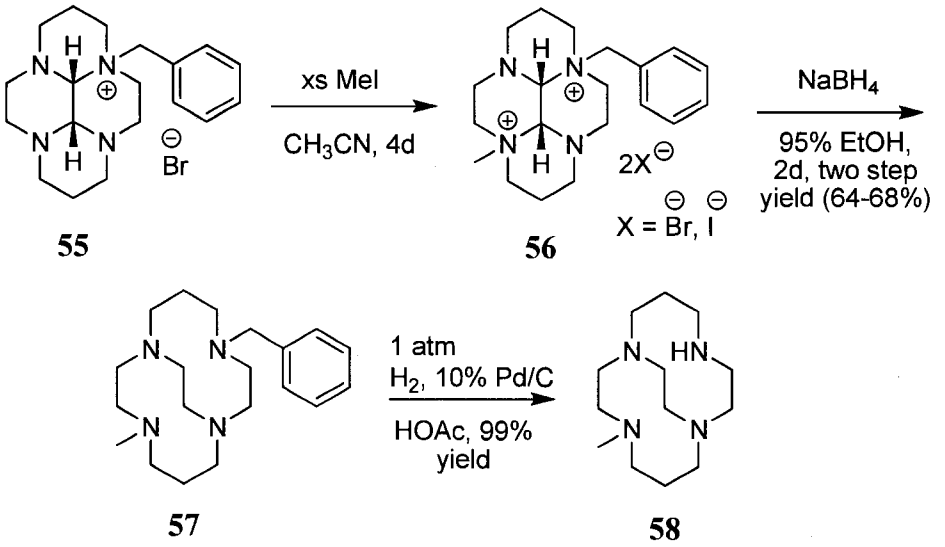


32 (m=n=1) **55**

Scheme 32: Synthetic sequence to form monobenzyl salt **55**; 3-D representation of **55**.

prevented by precipitation of **55**.[51] As seen in **Scheme 32**, the three-dimensional representation of **55** revealed the presence of a single *exo* lone pair which can be used for further chemistry, as confirmed by an X-ray crystal structure.[51] Methylation of **55** was carried out with excess MeI in MeCN to yield mixed benzyl/methyl salt **56** which was

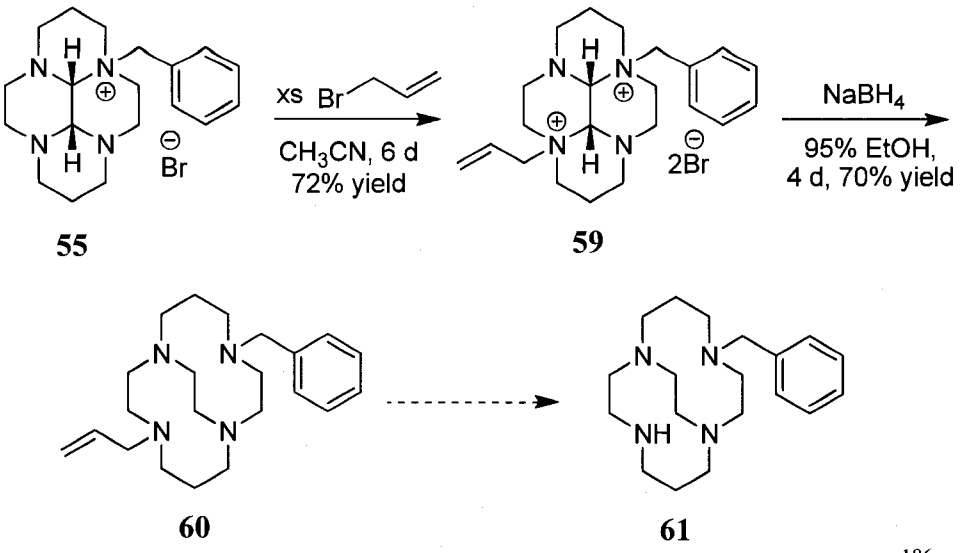
reduced with $NaBH_4$ to yield **57**, as reported by Hill,[185] in a two-step overall yield of 64-68% (**Scheme 33**). Hydrogenolysis of **57** was accomplished in excellent yield to produce



Scheme 33: Synthetic sequence to form single pendant-armed ligand **58**.[185]

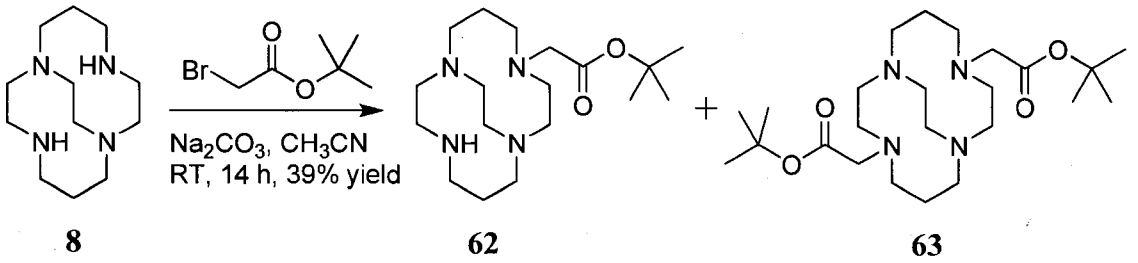
single pendant-armed ligand **58**.[185] This synthetic sequence was the first reported to form a single pendant-armed cross-bridged ligand, however the methyl substituent can not coordinate a metal in the cleft of the ligand and is difficult to remove or functionalize.

A variation of this procedure was developed by Bist that involved the addition of excess allyl bromide instead of methyl iodide to form monoallyl / monobenzyl salt **59** **Scheme 34**).[186] This salt was reduced in the presence of $NaBH_4$ in good yield to afford unsymmetrical monoallyl/monobenzyl ligand **60**. Unfortunately, attempts to remove the allyl protecting group were unsuccessful. Bist was also able to produce a variant of **55** with a 4-nitrobenzyl protecting group instead of benzyl. The nitrobenzyl group could be reduced to an aniline pendant arm, providing a site to be used for further chemistry.



Scheme 34: Attempted preparation of monobenzyl CB-cyclam (**61**).[186]

A second strategy to form single pendant-armed derivatives of CB-cyclam (**8**) involved statistical alkylation of the parent ligand. Sprague *et al.* reported the synthesis (carried out by coauthor Yijie Peng) of *t*-butyl ester **62** from the statistical alkylation of **8**



Scheme 35: Preparation of mono-*t*-butyl ester **62**.

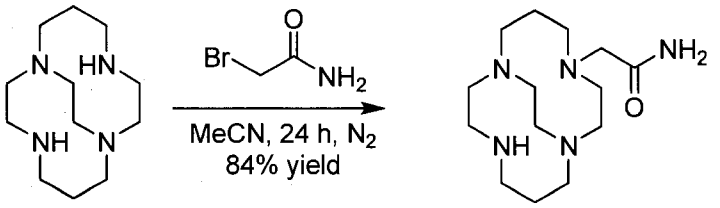
in a 0.1 M solution of MeCN with *t*-butyl bromoacetate (**Scheme 35**).[54] The analysis of the crude mixture indicated the presence of 45-50% **62**, dialkylated byproduct **63**, and starting material **8**.[54] The desired product was isolated in a modest 39% yield after flash chromatography. Compound **62** was used to prepare a range of unsymmetrical pendant-armed species and the *in vivo* stabilities of the corresponding ^{64}Cu(II) complexes were analyzed.[54] While the synthesis of **62** afforded a single pendant-armed ligand which

could be used as a starting point for a host of different unsymmetrical pendant-armed ligands, the yield of the reaction, as well as the method of purification, argues for the development of a new strategy for the formation of new single pendant-armed macrocycles. Herein, an unusually selective monoalkylation of CB-cyclam (**8**) with bromoacetamide will be discussed. The origin of the selectivity of the reaction has been probed and this new ligand has been used as a springboard for the synthesis of a range of new unsymmetrical pendant-armed species.

III. Results and Discussion

A. Synthesis and Cu(II) Complexation of 4-(carbamoylmethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane(64).

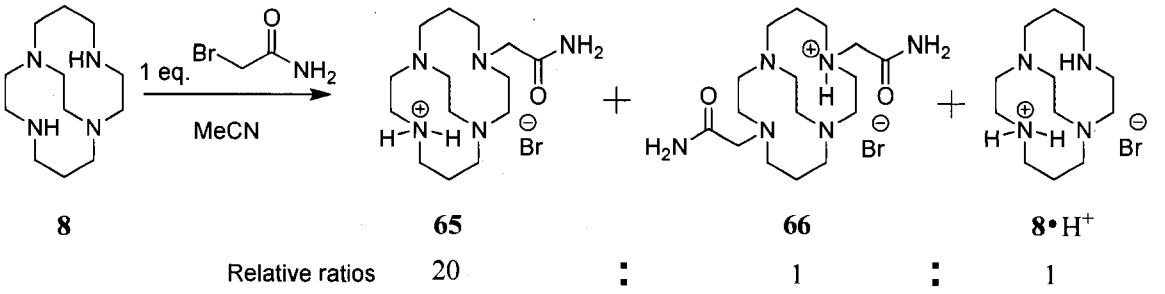
CB-cyclam (**8**) was dissolved in MeCN and a solution of one equivalent of 2-bromoacetamide in MeCN was added dropwise to **8** (final concentration of **8** in MeCN was 55 mM) in order to form ligand **64** (**Scheme 36**). The reaction was stirred at room temperature for 24 h under N_2. The crude product was extracted from a strongly basic (pH = 14) solution with $CHCl_3$. Recrystallization of monoamide **64** from hot MeCN afforded the pure ligand in 84% yield. The ^1H NMR of the crude solid (before extraction)



8 **64**

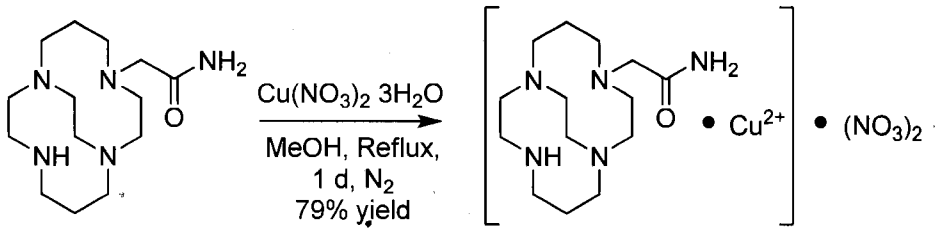
Scheme 36: Synthesis of monoamide **64**.

indicated the presence of dialkylated ligand **66** in a 20:1 ratio of **65** to **66** (**Scheme 37**) as determined by the relative integrations of the AB resonances for the pendant-armed methylenes of **65** and **66**. For every mole of **66** formed, one mole of protonated starting ligand **8**·H^+ should be present. Although the presence of **8** was difficult to see in the ^1H NMR spectrum, the ^{13}C NMR spectrum indicated the presence of another species in addition to **65** or **66**. Although the reaction was expected to give a statistical alkylation, the reaction was clearly selective towards formation of **65**.



Scheme 37: Ratios of products in the alkylation of CB-cyclam with 2-bromoacetamide as indicated by the crude ^{1}H NMR spectrum.

The Cu(II) complex of ligand **64** was readily prepared from $Cu(NO_3)_2$ in methanol (**Scheme 38**). Diethyl ether diffusion into a methanol solution of the



Scheme 38: Complexation of ligand **64**.

crude product yielded the X-ray quality crystals which were used to determine the solid state structure (**Figure 27**), which confirmed an expected *cis-V* configuration of the ligand around the metal. Important bond angles and bond lengths are listed in **Table 5**. The Jahn-Teller elongation of the six-coordinate Cu(II) is shown in the Cu(1) - O(6) (nitrate oxygen) bond at 2.662(2) Å and the Cu(I) – N(1) bond at 2.177(0) Å, which are longer than the Cu(1) - O(1) (amide oxygen) distance of 1.983(4) Å. The slightly distorted octahedral geometry of Cu(II) in the cleft of ligand **64** is confirmed by the near-linear bond angles for N(1) - Cu(1) - O(6) of 174.17(83)°, N(6) - Cu(1) - O(1) of 170.16(49)°, and N(3) - Cu(1) - N(2) of 177.45(31)°. Both nitrate counter ions are present in the structure which neutralize the charge from the Cu(II) and hydrogen bond with the

ligand. The nitrate bound to the Cu(II) is hydrogen-bonded with the amine hydrogen (H(42) – O(5)) with a bond distance of 2.105 Å. The second nitrate counter ion is hydrogen-bonded with the cis amide proton (H(40) – O(3)) with a bond distance of 2.008 Å.

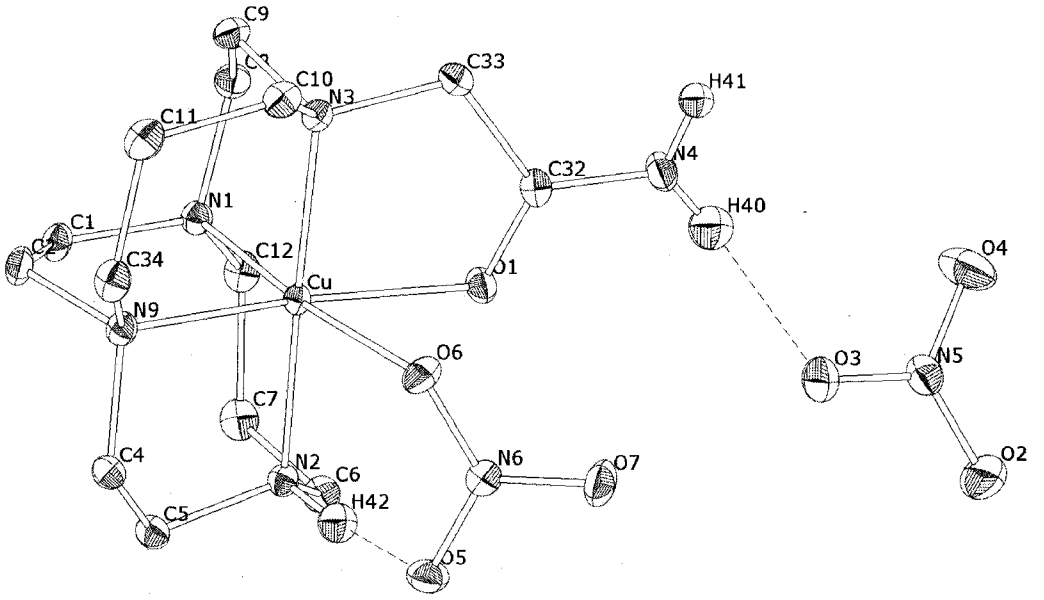


Figure 27: ORTEP diagram of **67**. The hydrogens are omitted except those on the nitrogens.

Bond	Length (Å)	Bond angle	Degrees (°)
Cu(1) - O(1)	1.9834	N(2) - Cu(1) - N(9)	87.04(46)
Cu(1) - N(2)	2.0003	N(2) - Cu(1) - N(1)	92.18(78)
Cu(1) - N(9)	2.0214	N(9) - Cu(1) - N(1)	87.78(21
Cu(1) - N(3)	2.0262	N(9) - Cu(1) - N(3)	95.36(35)
Cu(1) - N(1)	2.1770	N(1) - Cu(1) - N(3)	87.08(48)
Cu(1) - O(6)	2.6622	N(1) - Cu(1) - O(6)	174.17(83)
		N(9) - Cu(1) - O(1)	170.16(49)
		N(3) - Cu(1) - N(2)	177.45(31)

Table 5: Selected bond distances (Å) and bond angles (°) for **67**.

B. Experiments to Probe the Selectivity of the Formation of 64.

(1) Computational analysis of [64·H]$^+$.

The following experiments were carried out in an attempt to determine why the rate of the second alkylation of **8** with 2-bromoacetamide is so much slower than the rate of the first alkylation of **8** with bromoacetamide. As stated above, the alkylation of **8** with one equivalent of 2-bromoacetamide was expected to be a statistical one, but instead was selective for monoalkylation. To gain further insight as to why the second alkylation is significantly slower than the first, a molecular model of the reaction intermediate was studied using density functional theory (DFT). The XYZ coordinates of the X-ray crystal structure were used as a starting geometry. The Cu(II) and the two nitrate counter ions were subsequently removed from the input and a proton was added to the tertiary nitrogen bearing the pendant arm. This cationic species represents the reaction intermediate resultant from S_N2 displacement. A Merck Molecular Force Field (MMFF) minimization produced a structure possessing a hydrogen bond between the lone pair of the secondary nitrogen and an amide proton with the amine proton *exo* to the cleft of the molecule (**Figure 28 A**). The secondary nitrogen was inverted, then reoptimized using MMFF to produce a second conformation; one with the amine proton *endo* to the cleft and a hydrogen bond between an amide proton and the *exo* lone pair of the secondary nitrogen (**Figure 28 B**). Both isomers (**A** and **B**) are in the [2323/2323] ligand conformation, **B** was found to be lower in energy by 3 kcal/mol. The distance between the amide NH and the secondary amine was 2.01 Å in **B** and the angle from the amide nitrogen to the secondary amine nitrogen through the amide proton was 167°. Both of

these values are within the acceptable range for hydrogen bonding.[187] Both geometries were reoptimized at the B3LYP/6-31+G* level. The DFT energies suggest that geometry B is lower in energy than geometry A by 1 kcal/mol in the gas phase.

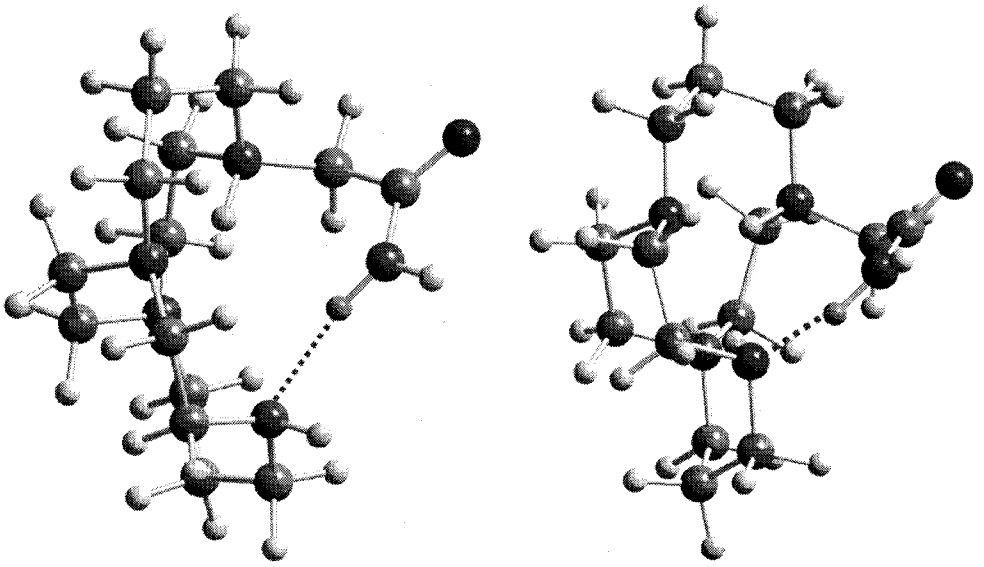


Figure 28: B3LYP/6-31+G* minimized geometries of [**64**·H]$^+$, A and B. Geometry B is lower in energy by 1 kcal/mol, computed using Spartan '02.

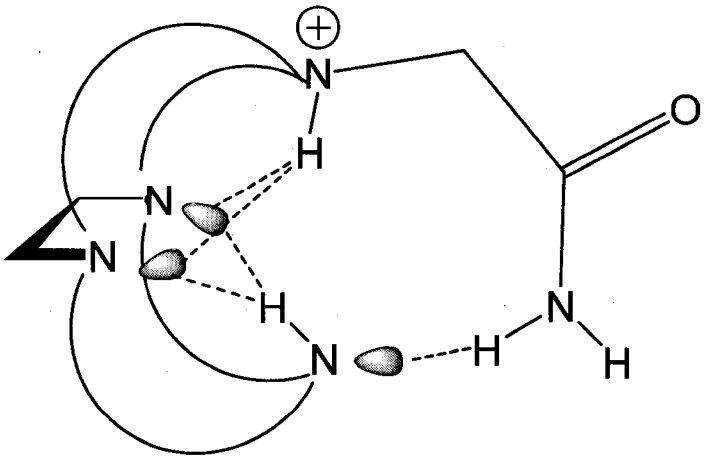
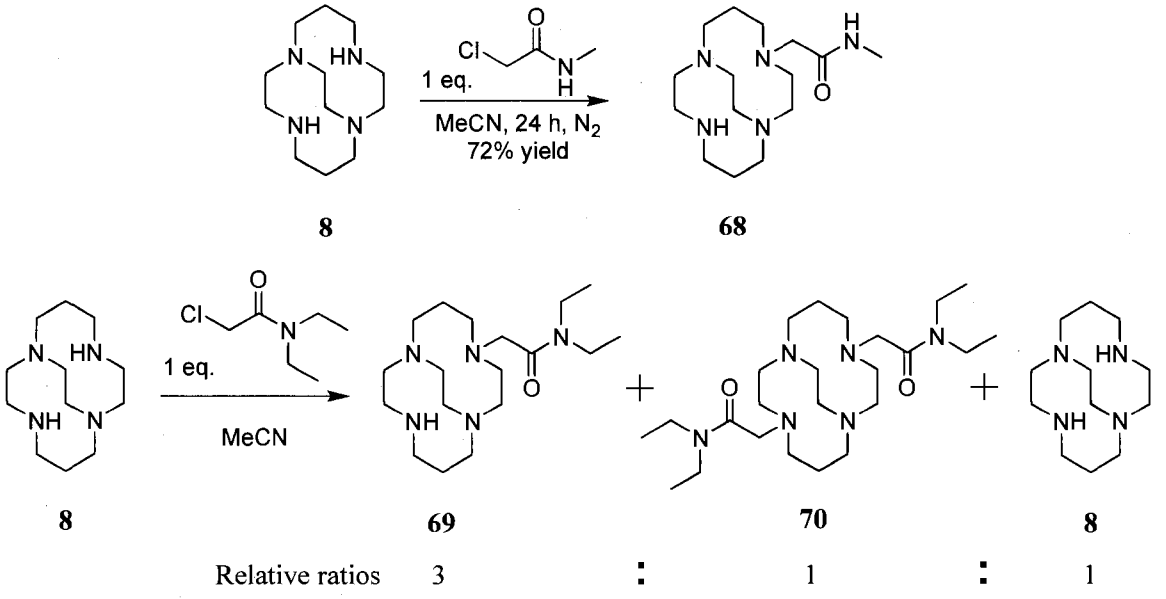


Figure 29: Representation of an intramolecular hydrogen bond between an amide NH and the lone pair of the secondary amine.

If an intramolecular hydrogen bond was present that tied up the lone pair of the secondary amine (**Figure 29**), the difference in rate of the second alkylation could be explained.

(2) Synthesis of 4-(*N*-methylcarbamoylmethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (**68**) and 4-(N,N-diethylcarbamoylmethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (**69**).

In order to obtain additional experimental evidence for or against the involvement of an intramolecular hydrogen bond, the same reaction conditions for the formation of **64** were used with chloro-*N*-methylacetamide and chloro-*N*,*N*-diethylacetamide instead of 2-



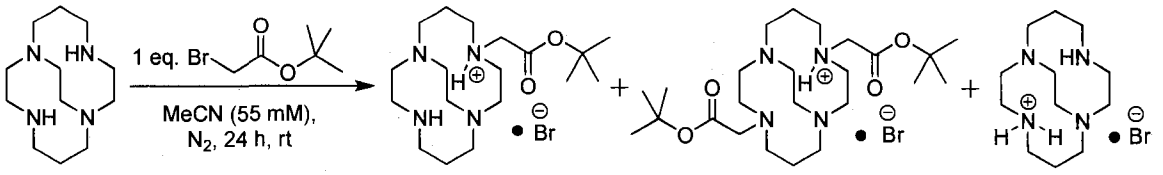
Scheme 39: Synthesis of **68** and attempted synthesis of **69**.

bromoacetamide (**Scheme 39**). If the intramolecular hydrogen bond with an amide proton is the source of the selectivity, then the reaction with a secondary amide should show selectivity towards monoalkylation while the tertiary amide should not. The crude reaction mixture of the synthesis of **68** indicated a ratio of 8:1 of **68** relative to the

dialkylated species as determined by integrations of the AB resonances of the pendant-arm methylene protons. Although the selectivity for monoalkylation was not as high as was the case for **64**, the reaction was still reasonably selective. The product was isolated by recrystallization from MeCN which contained a ratio of 33:1 of **68** relative to the dialkylated species. The reaction of CB-cyclam (**8**) with one equivalent of chloro-*N*,*N*-diethylacetamide revealed a 3:1:1 ratio of **69**:**70**:**8** based on the ^{1}H NMR spectrum of the crude reaction mixture, indicating a much decreased selectivity.

(3) Synthesis (as part of a mixture) of 4-carbo-*t*-butoxymethyl-1,4,8,11-tetraazabicyclo[6.6.2]-hexadecane (**62**).

The alkylation of **8** with *t*-butyl bromoacetate was carried out under the exact conditions (concentrations, temperature, etc.) used for the formation of **64** (55 mM MeCN) in order to determine the selectivity of the reaction. CB-cyclam (**8**) was stirred in



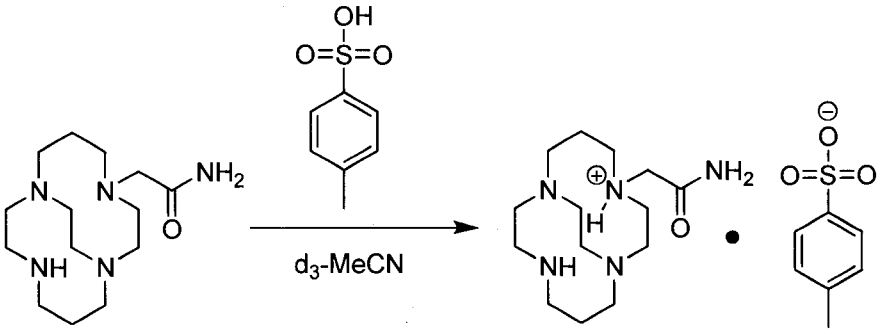
Scheme 40: Attempted formation of mono-*t*-butyl ester **62** under the conditions for the formation of monoamide **64**.

MeCN with the alkylating agent for 1 day under N_2 (**Scheme 40**). An analysis of the crude mixture by ^{1}H NMR indicated the presence of a 2.7:1:1 ratio of **62**:**63**:**8**, clearly indicating an approximately statistical alkylation of **8**. The fact that the reactions of **8** with 2-bromoacetamide and chloro-*N*-methylacetamide show selectivity for monoalkylation while those with chloro-*N*,*N*-diethylacetamide and *t*-butyl bromoacetate

are much less so, indicates that the amide NH appears to play a major role in the decrease in rate of the second alkylation of **64** and **68**.

(4) Analysis of ^{1}H NMR chemical shifts of [**64**·H]$^+$ under various concentrations.

^{1}H NMR has been used for the determination of the existence of intramolecular hydrogen bonds in solution.[188-191] In order to differentiate between intra- and intermolecular hydrogen bonds, the chemical shift of any proton involved in a hydrogen bond can be plotted against various concentrations of the hydrogen-bonded species. A change in chemical shift upon a change in concentration suggests that an intermolecular hydrogen bond exists.[190, 191] Conversely, if the chemical shift of the hydrogen-bonded proton does not change over the course of the same concentration range, then that proton



64 $\left[\mathbf{64} \cdot H^+\right] \cdot TsO^-$

Scheme 41: Synthesis of reaction intermediate model [**64**·H$^+$]·TsO$^-$.

could be involved in an intramolecular hydrogen bond or no hydrogen bond at all. In order to determine if [**64**·H]$^+$ possessed an intramolecular hydrogen bond, a model of the reaction intermediate was studied in a range of concentrations in d_3-MeCN and the amide chemical shifts were recorded. Monoamide **64** was dissolved in d_3-MeCN to make a 55 mM sample with one equivalent of anhydrous tosic acid producing [**64**·H$^+$]·TsO$^-$

(**Scheme 41**). The ^{1}H NMR chemical shifts were recorded for the downfield NH protons in a 55 mM (A), 5.5mM (B) and 0.55 mM (C) solution in d_3-MeCN (**Figure 30**). The chemical shift data are summarized in **Table 6**. The "inside proton" corresponds to the ammonium hydrogen and its chemical shift is in the downfield region due to the hydrogen bonding character of the molecular cleft as well as the positive charge from the nitrogen (**Figure 30**). The change in chemical shift of the $^+$NH proton over the course of two ten-fold dilutions is quite small ($\Delta\delta = 0.14$), suggesting its involvement in an

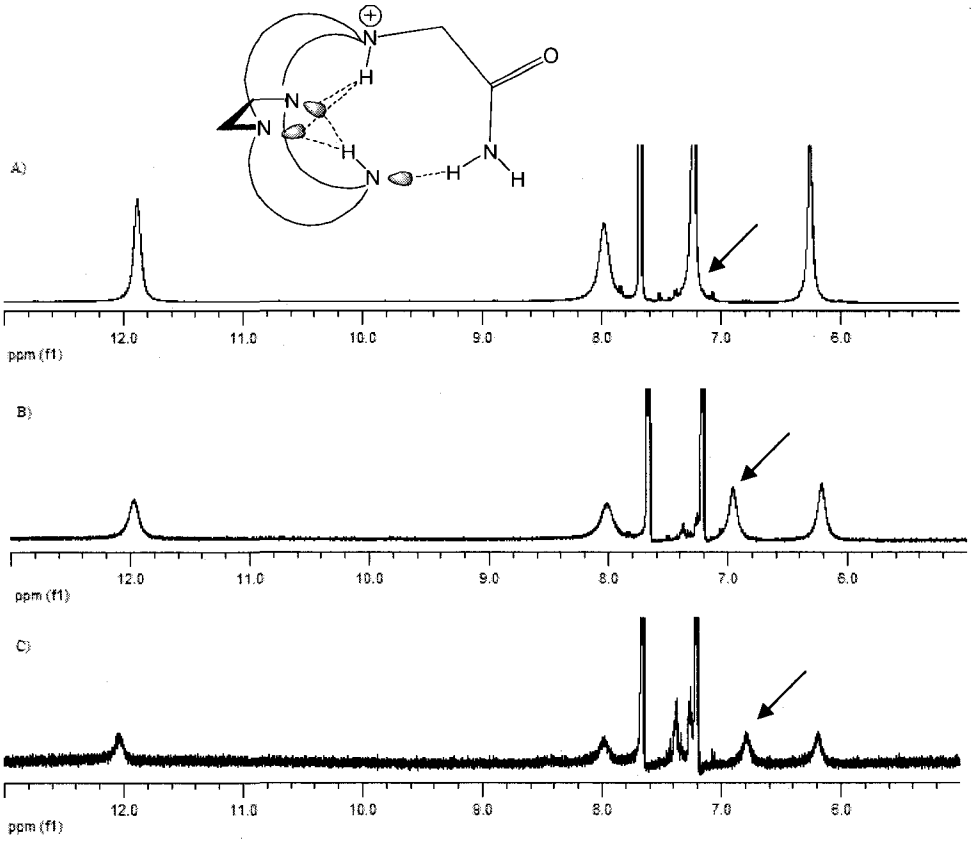


Figure 30: Downfield region of ^{1}H NMR of **[64**·H^+]·TsO^-. Compound concentration in A is 55 mM; concentration in B is 5.5 mM, concentration in C is 0.55 mM (The black arrows indicate the amide peak that shifts upfield upon dilution); a representation of the hypothesized reaction intermediate is shown on top.

	55 mM	5.5 mM	0.55 mM	Δδ
Upfld amide NH	6.19	6.14	6.06	0.13
Dnfld amide NH	7.15	6.87	6.73	0.42
***sec* NH**	7.93	7.95	7.92	0.01
$^{+}$NH Inside proton	11.82	11.90	11.96	0.14

Table 6: Chemical shift data (ppm) from **Figure 17**.

intramolecular hydrogen bond. The secondary amine proton at δ 7.93 ppm changes very little upon dilution (Δδ = 0.01), again suggesting participation in an intramolecular hydrogen bond. This is supported by the computationally-derived structure (**Figure 28 B**). The downfield amide NH resonance (black arrow in **Figure 30**) shifts upfield considerably over the course of the two ten-fold dilutions (Δδ = 0.42 ppm) with respect to the upfield amide NH (Δδ = 0.13). The change in chemical shift for the upfield amide NH is comparable in magnitude to the change in chemical shift of the secondary amine proton, leading to the conclusion that the upfield amide proton could also be involved in an intramolecular hydrogen bond, while the downfield amide peak could be involved in an intermolecular hydrogen bond, most likely in the form of a dimer.[192] However, Perrin has suggested that the H_E proton tends to be downfield of the H_Z proton in acetamide, a model amide for this species (**Figure 31**).[193, 194] This is in direct contrast to the

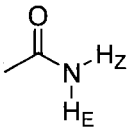


Figure 31: Nomenclature of amide hydrogens in acetamide; H_Z is *cis* to carbonyl, H_E is *trans*.

assignments made by this ^{1}H NMR dilution study. The chemical shifts of the amide protons of acetamide are reported to be H_E = δ 6.7 ppm and H_Z = δ 6.4 ppm as a 3M solution in MeCN.[195] However, there is evidence that as the concentration of amides decreases, the relative position of the chemical shifts of their amide protons switch (i.e.

the chemical shift of H_E will decrease upon dilution eventually becoming upfield from the H_Z proton).[196] Further experimentation is required to solve this discrepancy.

(5) Influence of d_6-DMSO on the chemical shifts of [**64**·H^+]·TsO^-.

The addition of incremental amounts of a strong hydrogen bond accepter has helped in the differentiation between hydrogens that are involved in intra- and intermolecular hydrogen bonds.[191] According to Anslyn and Dougherty, DMSO has one of the largest hydrogen bond accepting abilities (β) of common solvents, thus its influence on chemical shift can be seen with the addition of small quantities of it.[197] All NH protons in [**64**·H^+]·TsO^- should hydrogen bond with DMSO to force a downfield shift in their ^{1}H NMR resonances, however, those involved in an intramolecular hydrogen bond should show less change upon the addition of DMSO, due to the competition between hydrogen bond acceptors. Ligand **64** was dissolved in d_3-MeCN with one equivalent of anhydrous tosic acid to make a 55 mM solution, the concentration at which the alkylation of **8** and bromoacetamide was run. d_6-DMSO was added to create 5% and 10% (vol:vol) solutions in d_3-MeCN and the chemical shifts of the NH protons were measured (**Figure 32, Table 7**).

The overall change in chemical shift ($\Delta\delta$) of the $^+$NH inside proton was negligible upon the addition of d_6-DMSO. This was expected as the inside proton is encapsulated in the molecular cleft of [**64**·H^+]·TsO^-, minimizing the exposure to solvent. The overall downfield shift of the *sec* NH resonance indicated a slight equilibrium exposure to the DMSO. The ^{1}H NMR dilution study described previously revealed that the *sec* NH was

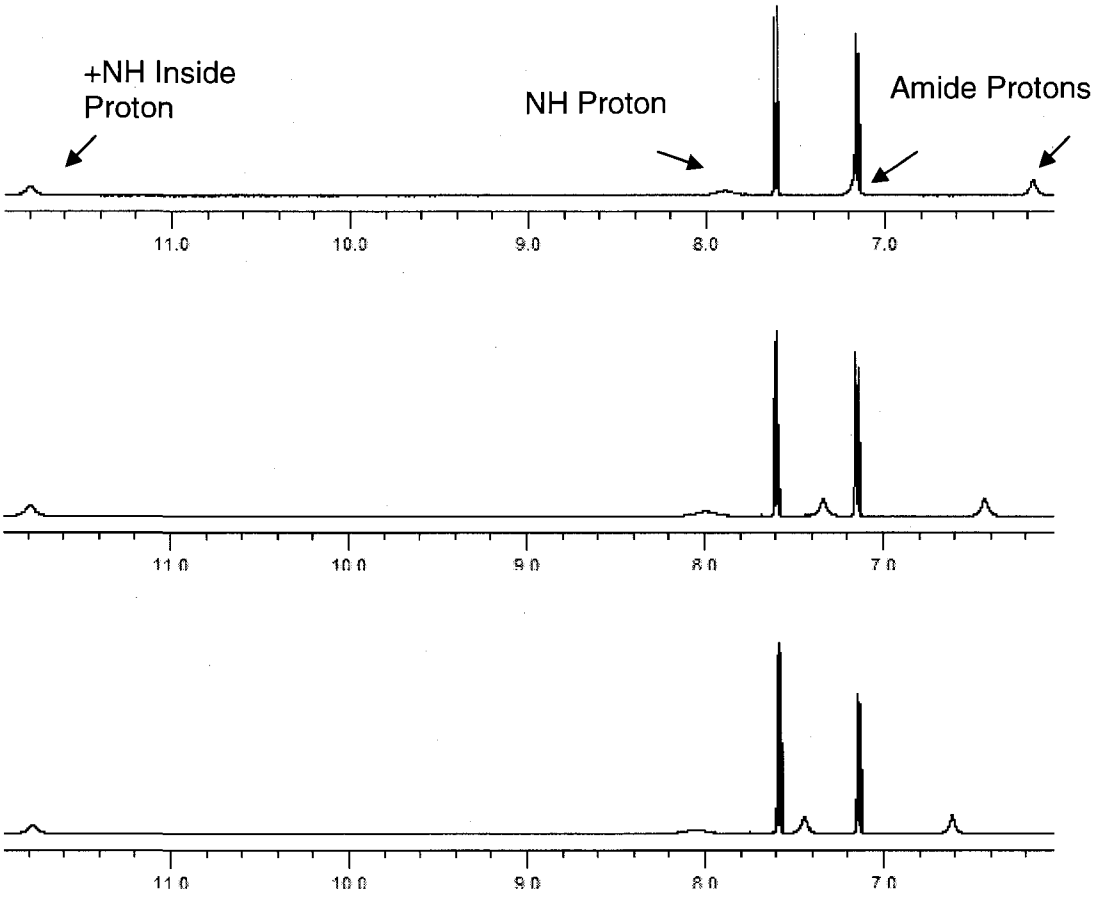


Figure 32: Downfield region of ^{1}H NMR of **[64**·H$^+$]·TsO$^-$, A: 55mM in d_3-MeCN, B: 5% d_6-DMSO, C: 10% d_6-DMSO.

	NMR A: Control	5% DMSO	10% DMSO	Δδ
Amide NH upfield	6.17	6.43	6.62	0.45
Amide NH downfield	7.16	7.33	7.44	0.28
***sec* NH**	7.89	8.01	8.05	0.16
$^+$NH Inside proton	11.79	11.79	11.78	0.01

Table 7: Chemical shifts of NH protons in ^{1}H NMR of **[64**·H$^+$]·TsO$^-$.

intramolecularly hydrogen-bonded, however, this DMSO study suggested that this NH was also partially exposed to solvent. Both the upfield amide NH resonance and downfield amide NH resonance shift downfield upon the addition of d_6-DMSO, however, the upfield resonance shifts downfield 1.6 times further than the downfield resonance.

The difference in the change of chemical shift ($\Delta\delta$) indicated that one of these amide NH's was involved in an intramolecular hydrogen bond, most likely the downfield amide resonance due to the smaller change of chemical shift over the additions of d_6-DMSO. However, the ^1H dilution experiment suggested that the upfield resonance was involved in an intramolecular hydrogen bond. Alternative experiments were required to solve the discrepancy in the data.

(6) Attempted determination of the rate of H/D exchange of NH protons in [**68**·H]$^+$.

Proton exchange rates have been utilized for the determination of protein folding for several years.[198-201] Professor Brian Linton (Bowdoin College) has used H/D exchange rates to quantify hydrogen bonding in small amides.[202] Based on personal correspondence with Prof. Linton, the H/D exchange rate of [**68**·H]$^+$ could provide insight into the presence or absence of an intramolecular hydrogen bond when compared to that of a model amide. Due to the nature of a hydrogen bond, the rate of exchange of a proton involved in a hydrogen bond should be slower than that of one that does not hydrogen bond under the same set of conditions. The measurement of the H/D exchange rate of *N*-methylacetamide, (which is an appropriate model for the secondary amide in **68**) was attempted (**Figure 33**). The experiment was carried out in a 55 mM solution of *N*-methylacetamide in MeCN with 100 molar equivalents of d_4-MeOD relative to 1 molar equivalent of *N*-methylacetamide in order to maintain pseudo-first order kinetics. Under these conditions, the equilibrium was reached in less than 2 min, preventing the kinetic measurement. The reaction was then carried out with only 30 molar equivalents of d_4-

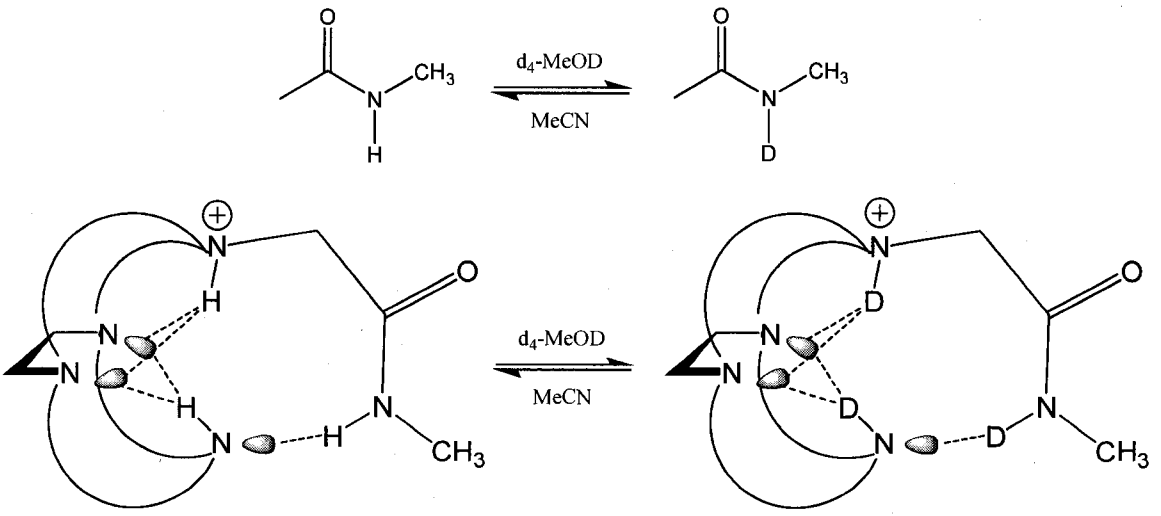


Figure 33: Proposed H/D exchange of *N*-methylacetamide and **68** with d_4-MeOD to determine the presence of an intramolecular hydrogen bond.

MeOD relative to 1 molar equivalent of *N*-methylacetamide. According to Anslyn and Dougherty, in order to maintain pseudo-first order conditions, there must be at least a 10:1 molar ratio of reagents.[197] Comparison of the H/D exchange rates of *N*-methylacetamide and that of $[\mathbf{68}\cdot H]^+$ required that the conditions be identical, thus with three exchangeable protons on the protonated ligand $[\mathbf{68}\cdot H]^+$, 30 equivalents of d_4-MeOD provided enough deuterium to maintain pseudo-first order conditions over the course of this reaction. However, the reaction with *N*-methylacetamide reached equilibrium in less than 2 min, making measurement of the reaction rate by NMR too difficult. An attempt was made to measure the H/D rate of exchange on *N*-methylacetamide in $CDCl_3$, a less polar solvent also preferred by Prof Linton. However, equilibrium was still reached in less than 2 min in a 55 mM solution of *N*-methylacetamide in $CDCl_3$. Due to the inability to measure an H/D exchange rate of a model molecule, further experiments in this respect were abandoned.

(7) Antiperiplanar coupling of pendant arm methylene carbon and amide hydrogen in a gHMBC.

In order to fully understand the results obtained from the ^{1}H NMR dilution experiment described above (*vide supra*), the identification of amide resonances must be made via an independent experiment. ^{1}H-coupled ^{13}C NMR spectroscopy has been useful for the determination of dihedral dependence in coupling though an amide functionality.[203] In the case of acetamide, coupling was seen between the methyl carbon and an amide N*H* (**Figure 34**), however, no coupling was seen between the acetyl methyl carbon and the amide proton in N-methylacetamide (**Figure 34**).[203] Because N-methyl acetamide is known to exist in predominantly the trans conformation, the coupling in acetamide must be seen only between the so-called *cis* amide proton and the acetyl

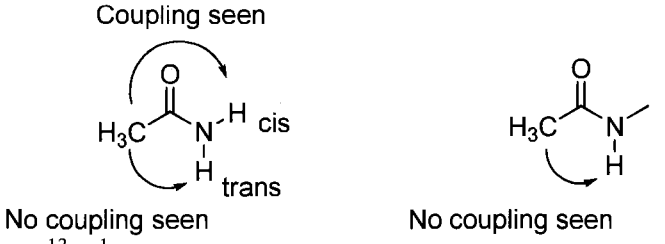


Figure 34: $^{13}C/^{1}H$ coupling in acetamide and N-methyl acetamide.

carbon. The observation of coupling between the acetyl methyl carbon and an antiperiplanar amide N*H* would allow for the identification of both amide resonances in the ^{1}H NMR spectrum of **[64**·H^{+}]·TsO^{-}. A gHMQC spectrum was used to identify the methylene carbon of the pendant arm of **[64**·H^{+}]·TsO^{-}. The gHMBC (**Figure 35**) spectrum revealed no coupling between either amide resonance and the pendant arm methylene (methylene carbon and amide resonances identified by black arrows). The exchange of the two amide resonances could have averaged out the appearance of any coupling in the 2D experiment. Attempts to increase the amplitude of the cross peaks

only made the contour plot noisier. As such, the gHMBC failed to help assign the amide resonances and further experimentation was required.

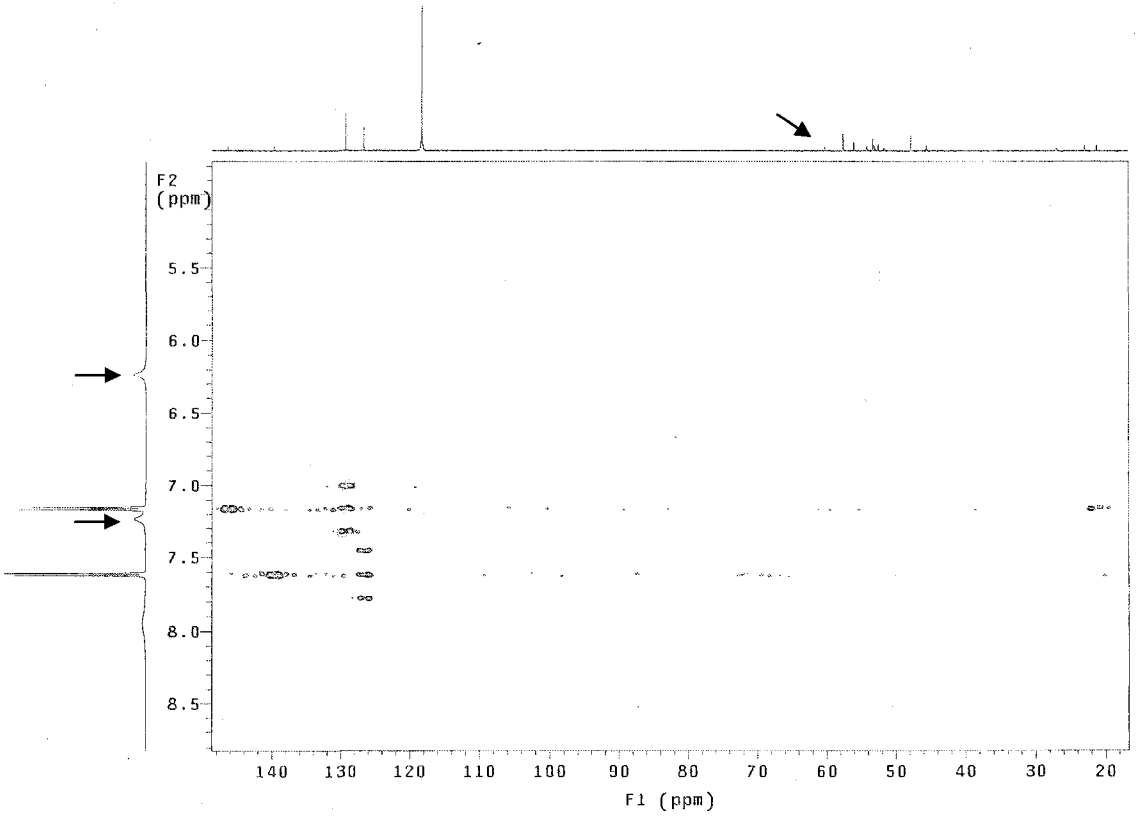


Figure 35: The gHMBC spectrum of [**64**·H^+]·TsO^-.

(8) Through-space coupling of NH protons of [**64**·H^+]·TsO^- in a 2D NOESY.

Nuclear Overhauser Enhancement Spectroscopy (NOESY) is a useful technique to aid in the determination of the three-dimensional solution structure of hydrogen-bonded systems.[191] Presumably, if an amide NH of [**64**·H^+]·TsO^- is hydrogen-bonded to the lone pair of the secondary amine, then there should be a difference in enhancement between one amide N*H* and the amino proton and the other amide N*H* and the amino proton. The 2D NOESY plot is shown in **Figure 36**. The spectrum indicated that both amide resonances contained an NOE with the amino proton. Attempts to quantify the size

of the enhancements were unsuccessful. Independent verification of the amide resonance identity of **[64**·H^+]·TsO^- was not obtained.

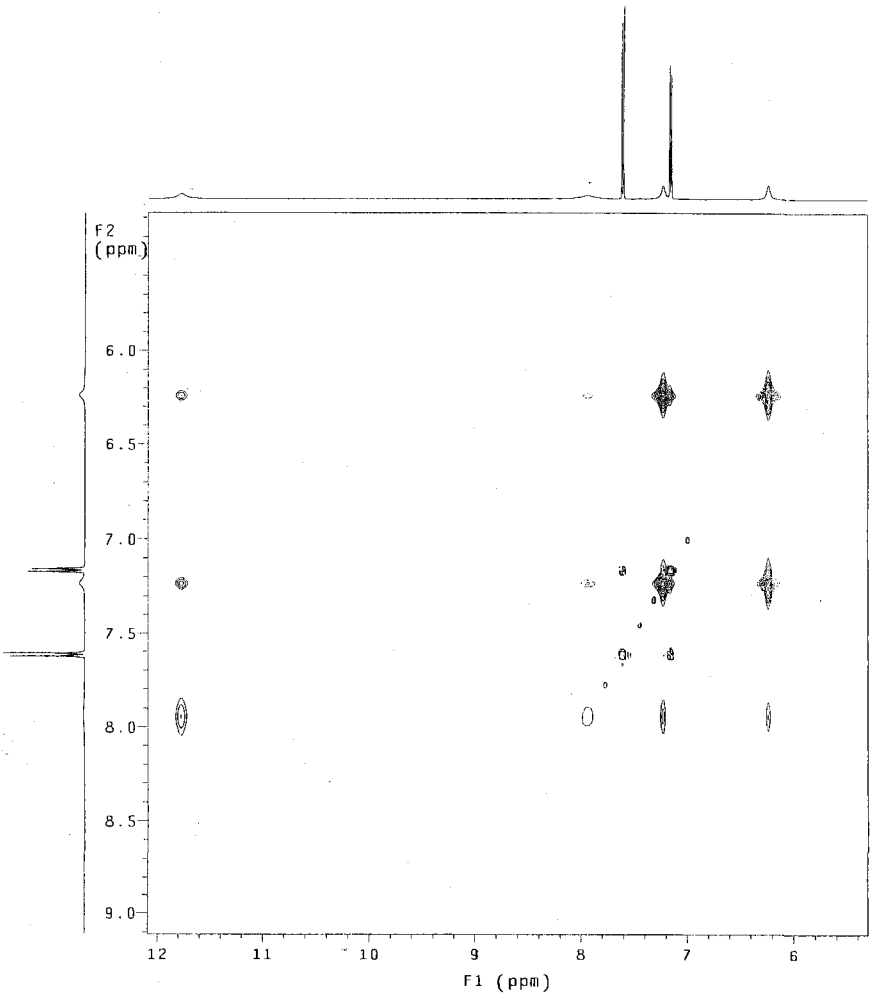


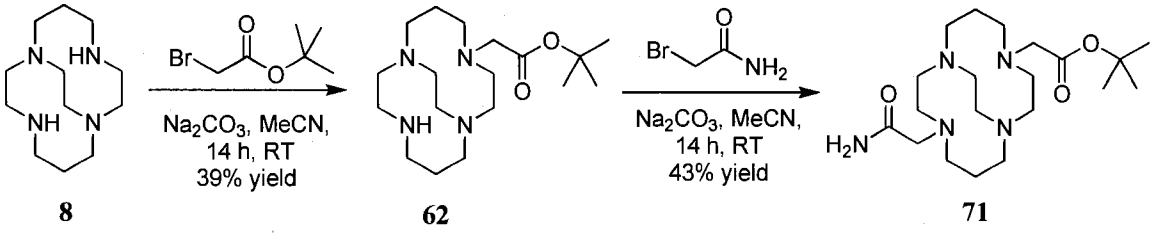
Figure 36: 2D NOESY of **[64**·H^+]·TsO^-.

C. Synthetic Derivatives of Monoamide 64.

(1) The synthesis of 4-acetoamido-11-carbo-*t*-butoxymethyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (**71**).

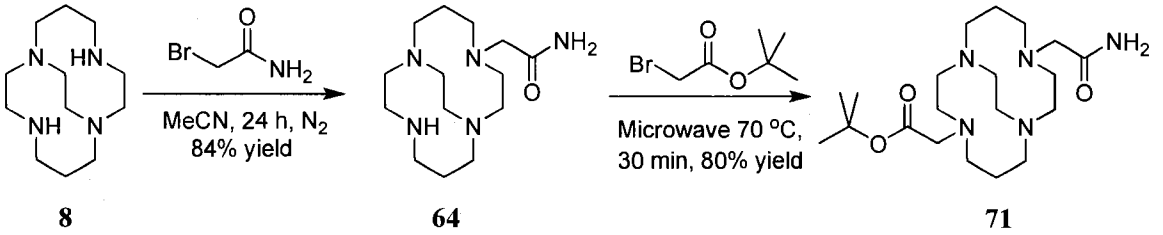
Monoamide **64** can be used as a springboard for the synthesis of a range of unsymmetrical pendant-armed cross-bridged ligands, taking advantage of the high yield single alkylation reaction. The preparation of monoamide / mono-*t*-butyl ester ligand **71**

was previously reported and isolated in a 17% overall yield from CB-cyclam (**8**) (**Scheme 42**).[54] The low yield of the first reaction was due to the statistical alkylation



Scheme 42: Preparation of ligand **71** as previously reported. **71** was isolated in a 17% overall yield from ligand **8**.

of ligand **8** and necessary column chromatography to isolate pure ligand **62**. However, when the synthetic approach towards ligand **71** utilized monoamide **64** as the synthetic intermediate, ligand **71** was isolated in an overall yield of 68%, making this a viable and much improved approach to cross-bridged derivatives (**Scheme 43**).

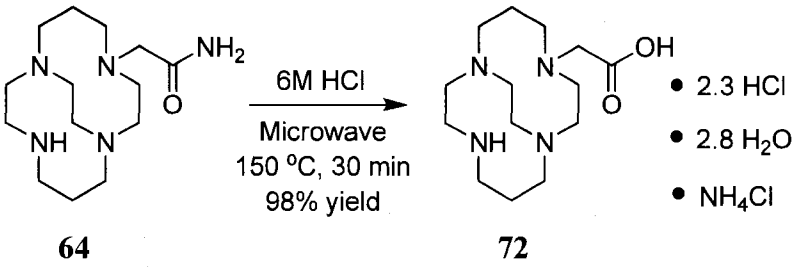


Scheme 43: Alternative synthetic route towards the formation of mixed pendant armed ligand **71**.

(2) The synthesis and characterization of 4-carboxymethyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane dihydrochloride trihydrate (**72**·2.3HCl·2.8H_2O).

The monoarmed ligand, CB-TE1A (**72**), has been prepared from the trifluoroacetic acid mediated deprotection of ligand **62** and was isolated as a

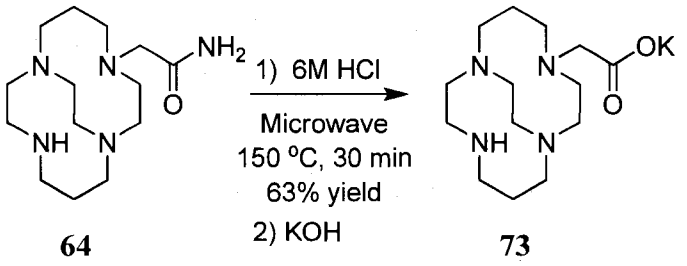
trifluoroacetate salt. Acid hydrolysis of ligand **64** with 6M HCl produced ligand **72** as an HCl salt in a 98% yield (**Scheme 44**). CB-TE1A was characterized by ^{1}H, $^{13}C\{^{1}H\}$ NMR



64 **72**

Scheme 44: Acid mediated hydrolysis of monoamide ligand **64**.

and Laser Desorption Ionization Mass Spectrometry. One molar equivalent of ammonium chloride was present in the sample as well. The free amine potassium carboxylate salt of ligand **72** was isolated by acid hydrolysis of ligand **64**, followed by a $CHCl_3$ extraction from a strongly basic aqueous KOH solution (**Scheme 45**). Ligand **73** was characterized by ^{1}H, $^{13}C\{^{1}H\}$ NMR and HRFABMS.



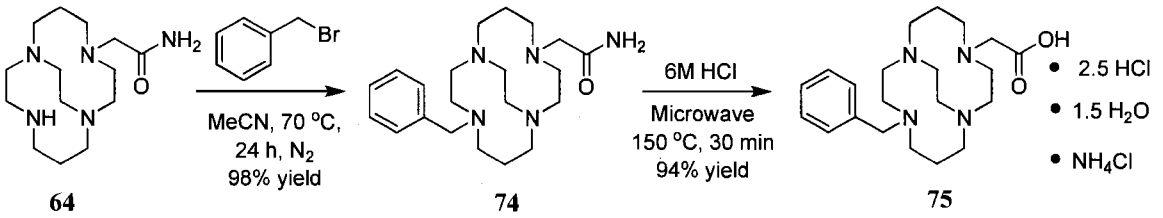
64 **73**

Scheme 45: Synthesis of ligand **73**.

(3) The synthesis and characterization of 4-benzyl-11- carboxymethyl -1,4,8,11-tetraazabicyclo[6.6.2]hexadecane dihydrochloride monohydrate (**75**·2.5HCl·1.5H_2O).

Weisman, Wong and coworkers have shown that Cu-CB-TE1A has comparable acid dissociation kinetics and resistance to Cu(II)/Cu(I) reduction relative to Cu-CB-TE2A indicating that only one carboxylate is required in order to retain the kinetic

inertness of the complex.[204] Recent reports have suggested that increasing the lipophilic character of a BFC can impart improved biodistribution properties of the complex.[205, 206] Ligand **75**, an appropriate model ligand for such work, was synthesized from a two-step sequence starting from monoamide **64** (**Scheme 46**). Unsymmetrical pendant-armed

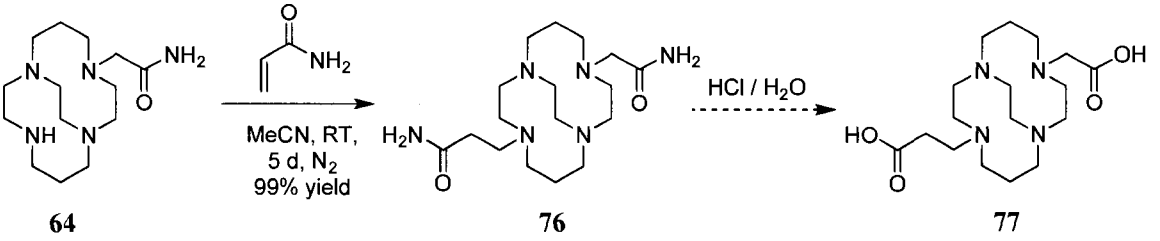


Scheme 46: Two step synthesis for ligand **75** from monoamide **64**.

ligand **74** was prepared by alkylation with a small excess of benzyl bromide in MeCN at reflux for 1 day. The ligand was isolated by extraction from a strongly basic aqueous solution in excellent yield. The white solid was characterized by ^{1}H, $^{13}C\{^{1}H\}$ NMR and HRFABMS and required no further purification. The acid-catalyzed hydrolysis of ligand **74** was completed under microwave conditions (150 °C, 30 min) in a closed vessel microwave reactor. The residue was dissolved in H_2O and the solvent was removed under reduced pressure in order to remove excess HCl from the sample. Ligand **75** was isolated as a white solid in excellent yield and characterized by ^{1}H, $^{13}C\{^{1}H\}$ NMR and electrospray ionization mass spectrometry. The elemental analysis of ligand **75** indicated the presence of 2.5 molar equivalents of HCl, 1.5 molar equivalents of water and 1 molar equivalent of ammonium chloride relative to 1.0 molar equivalent of **75**.

(4) The synthesis and characterization of 4-(2-carbamoylethyl)-11-(carbamoylmethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane(**76**·0.5H_2O)

As described previously (**Chapter 1**), Heroux and coworkers compared the kinetic inertness of Cu(II) complexes of ligands containing *N*-carboxyethyl pendant arms and *N*-carboxymethyl pendant arms. The results of the experiments indicated that the *N*-carboxyethyl pendant arms caused a decrease in both the half-life of decomplexation of the complex in 5M HCl and the resistance to Cu(II)/Cu(I) reduction. The Cu(II) complex of a ligand containing one *N*-carboxymethyl pendant arm and one *N*-carboxyethyl pendant arm could provide more insight into the relationship between pendant arm length and kinetic inertness. Monoamide ligand **64** and acrylamide were allowed to stir in MeCN for 5 days (**Scheme 47**). The crude solid was washed with hot EtOAc to remove



64 **76** **77**

Scheme 47: Synthesis of ligand **76** and proposed synthesis of ligand **77**.

excess acrylamide. White solid, **76**, required no further purification and was characterized by ^{1}H, $^{13}C\{^{1}H\}$ NMR and HRFABMS. Elemental analysis indicated the presence of 0.5 molar equivalents of water relative to 1.0 molar equivalent of **76**. Acid hydrolysis of ligand **76** to ionizable ligand **77** was never attempted and should be followed up in the Weisman-Wong group in the future.

IV. Conclusion

An unusually selective mono-alkylation of parent ligand CB-cyclam (**8**) with 2-bromoacetamide in MeCN allows preparation of monoamide ligand **64** in high yield. An X-ray structure of **67** was obtained and indicated a six-coordinate geometry about the Cu(II) with a Jahn-Teller elongation along the Cu(1) - O(6) (nitrate counterion) bond. The selectivity of the monoalkylation was hypothesized to stem from an intramolecular hydrogen bond that could exist from the amide proton to the secondary amino nitrogen lone pair (**Figure 29**). B3LYP/6-31+G* minimized geometries of [**64**·H^+] indicated the presence of a hydrogen bond in a low energy conformation. Alkylations of CB-cyclam with chloro-*N*-methylacetamide, chloro-*N*,*N*-diethylacetamide and *t*-butyl bromoacetate indicated the requirement of one amide proton in order to observe high selectivity for monoalkylation. The chemical shifts of the N*H* protons of [**64**·H^+]·TsO^- were measured as a function of concentration to determine which protons were involved in intra- and intermolecular hydrogen bonds. Only one amide N*H* was found to significantly change chemical shift upon dilution, which suggested that the other amide N*H* was involved in an intramolecular hydrogen bond. The N*H* resonances in the ^1H NMR spectrum of [**64**·H^+]·TsO^- were measured upon the addition of d_6-DMSO. Both amide resonances shifted downfield upon this addition, however, the chemical shift of the resonance identified as that involved in an intramolecular hydrogen bond shifted further downfield than the other amide N*H* peak. This result is in conflict with the conclusions based upon ^1H NMR dilution study. The measurement of the H/D rate of exchange of N-methylacetamide was attempted for comparison with the H/D rate of exchange with

[**68**·H]$^+$ to indicate the presence of a hydrogen bond. However, conditions could not be found to measure the H/D exchange rate of the model compound. gHMBC and NOESY were utilized to attempt to independently identify both amide resonances and find coupling through the proposed hydrogen bond, respectively. Unfortunately both experiments failed to provide information to either validate or invalidate the hypothesis. The origin of the selectivity of the monoalkylation is still not fully understood. Several other ligands were shown to be obtainable utilizing monoamide **64** as a synthetic starting point. The synthesis of ligand **71** was improved utilizing monoamide **64** as a synthetic intermediate. Acid-catalyzed hydrolysis of **64** afford CB-TE1A (**72**) and, following an extraction from a strongly basic KOH solution, potassium carboxylate salt **73**. Ligand **75** was formed in a two step synthesis from monoamide **64**. The corresponding Cu(II) complex can be used as a model for bioconjugates possessing one carboxylate pendant arm and one lipophilic pendant arm. Michael addition of acrylamide with **64** afforded ligand **76**, however the hydrolysis was not attempted.

Chapter III

ADVANCES IN THE SYNTHESIS OF CROSS-BRIDGED CYCLAMS BEARING PHOSPHONIC ACID PENDANT ARMS FOR USE IN RADIOPHARMACEUTICALS

I. Introduction

Radiopharmaceutical agents have the ability to be used both as imaging agents and in targeted therapy applications.[41, 207] Those that act through receptor binding are classified as receptor-based target-specific radiopharmaceuticals.[205] These types of systems have the general structure shown in **Figure 37**. The radionuclide is coordinated

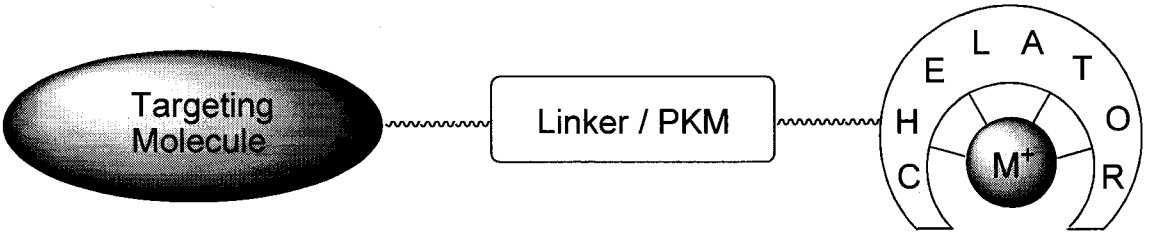
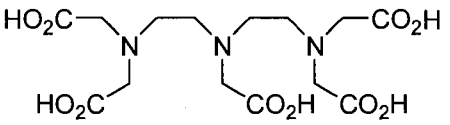


Figure 37: General structure of a receptor-based target-specific radiopharmaceutical.

to a bifunctional chelator (BFC), an organic molecule that both coordinates with the metal as well as provides a covalent linkage to the rest of the system. The targeting molecule can be large biological molecules such as a peptide, protein or antibody, or a smaller organic molecule such as dopamine or folic acid.[205, 208] These two major components are covalently linked by a pharmacokinetic modifier (PKM). The choice of

this linking functionality is dependent on the desired properties of the overall radiopharmaceutical.[205] The linker can be a simple hydrocarbon chain to tune lipophilicity or a peptide to increase the overall hydrophilicity. The biodistribution properties of several ^{111}In and ^{99m}Tc complexes have been reported to change through alteration of the PKM.[209, 210] The 8-amino acid peptide, "Octreotide", has been labeled with ^{111}In using diethylene triamine pentaacetic acid (DTPA) as a BFC and is available for human use as a diagnostic imaging agent for neuroendocrine tumors (**Figure 38**).[205] Unfortunately, DTPA was found to be a poor ligand for ^{64}Cu(II) complexation as a



DTPA

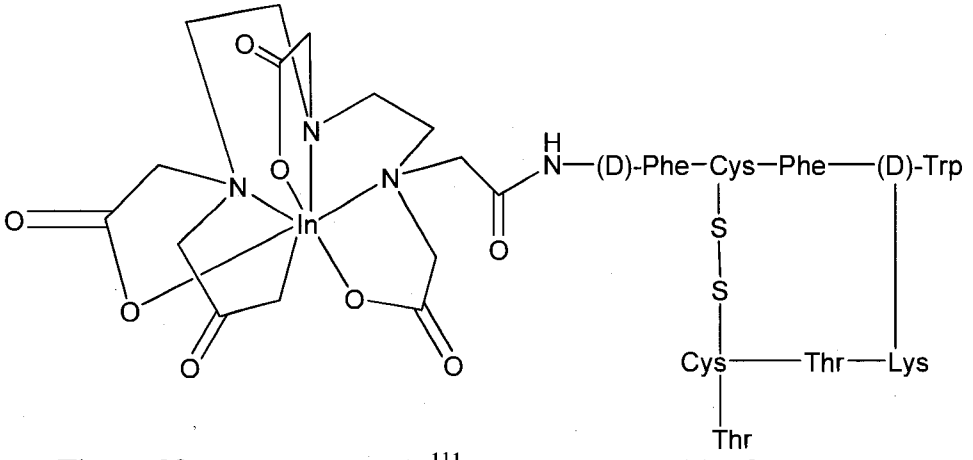


Figure 38: BFC DTPA and [^{111}In]DTPA-octreotide (OctreoScan).

large amount of the ^{64}Cu(II) complex was found to dissociate in human serum.[42, 211] It has been demonstrated that cyclic BFCs form kinetically more inert complexes than their acyclic analogues, a necessary trait for ^{64}Cu(II)-containing radiopharmaceuticals used in PET (Positron Emission Tomography) imaging.[42]

Positron Emission Tomography is a nuclear medicine imaging technique that can image structures and physiological processes in a living organism.[208] Generally, it relies on the ability of a biologically-significant molecule to deliver a positron-emitting radiometal to a target organan. As the metal decays (**Figure 39**), it ejects a positron from the nucleus which will annihilate upon contact with an electron, releasing two 511 keV

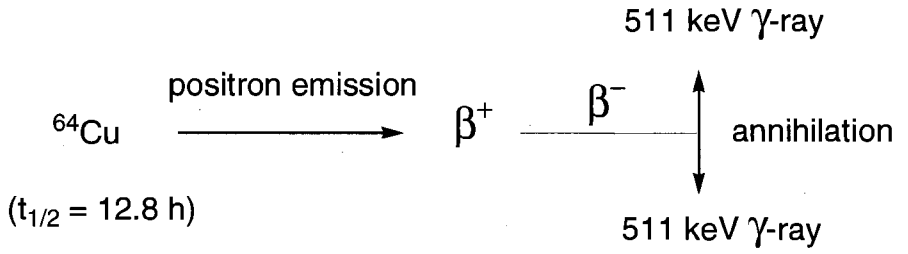


Figure 39: Radioactive decay of ^{64}Cu; emitted positron (β^+) is annihilated with an electron (β^-) releasing two gamma rays in opposite directions.

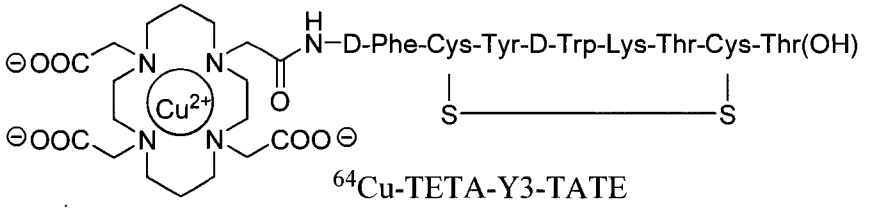
gamma rays 180° apart.[208] The gamma rays are detected by a circular array of photomultiplier tubes and the location of the radionuclide is determined through tomographic image reconstruction, a process that creates 2D cross-section data.[212] The selection of a radiometal is critical in the design of a radiopharmaceutical used as a PET imager because the half-life should be long enough to allow for significant organ uptake and radioactive decay to yield considerable contrast.[208, 213] The benefit of utilizing radioisotopes of copper lies in the well-established coordination chemistry of the metal, the range of half-lives and the range of energies of emitted particles.[208, 211] **Table 8** lists the decay characteristics of various copper isotopes, however, only ^{64}Cu is used in PET imaging. ^{60}Cu, ^{61}Cu and ^{62}Cu are all positron emitters and can emit photons by electron capture, making them useful in imaging applications. ^{67}Cu can emit an electron from its nucleus while ^{64}Cu emits positrons, electrons and photons by electron capture, making

them useful for therapeutic applications. ^{60}Cu, ^{61}Cu ^{62}Cu and ^{64}Cu can all be used for SPECT (Single-Photon Emission Computed Tomography) imaging as they all emit X-ray photons by electron capture.

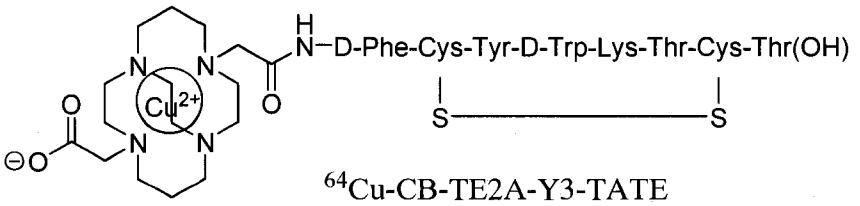
Isotope	$t_{1/2}$	β^- MeV (%)	β^+ MeV (%)	EC (%)
^{60}Cu	23.4 min	---	2.00 (69%) 3.00 (18%) 3.92 (6%)	7.4(%)
^{61}Cu	3.32 h	---	1.22 (60%)	40%
^{62}Cu	9.76 min	---	2.91 (97%)	2%
^{64}Cu	12.7 h	0.573 (39.6%)	0.655 (17.4%)	41%
^{67}Cu	62.0 h	0.395 (45%) 0.484 (35%) 0.577 (20 %)	---	---

Table 8: Decay characteristics of copper radionuclides.

As described previously (**Chapter 1**), ^{64}Cu-TETA-Y3-TATE and ^{64}Cu-CB-TE2A-Y3-TATE were evaluated as radiopharmaceuticals for the detection of neuroendocrine tumors (**Figure 40**).[57] The data reported suggested that cross-bridged



^{64}Cu-TETA-Y3-TATE



^{64}Cu-CB-TE2A-Y3-TATE

Figure 40: Bioconjugates ^{64}Cu-TETA-Y3-TATE and ^{64}Cu-CB-TE2A-Y3-TATE. Overall charges are -1 and +1 respectively.

ligands are superior BFC's for use in radiopharmaceuticals due to their kinetic inertness and favorable biodistribution properties.[57] However, a larger retention of ^{64}Cu was found in the liver after administration of ^{64}Cu-CB-TE2A-Y3-TATE compared to ^{64}Cu-TETA-Y3-TATE. The authors suggest that the difference in the overall charge of the bioconjugate was related to the difference in renal retention.[57] Studies have reported a correlation between charge and renal uptake of ^{64}Cu and ^{111}In compounds.[40, 214, 215] Renal uptake of ^{111}In was largest for positively charged peptide conjugates and lowest for negatively charged peptide conjugates,[215] and a similar result was seen with azamacrocyclic complexes of ^{64}Cu.[40] Tuning the lipophilicity of bioconjugates as well as maintaining charge neutrality are interesting modifications that could be made to the complexed bioconjugates of cross-bridged BFC.

II. Background

Weisman, Wong and coworkers proposed the formation of a second generation of cross-bridged azamacrocyclic systems bearing only one ionizable pendant arm.[204] Their idea was based on the retention of solution inertness and reduction stability *in vivo* of the BFC of ^{64}Cu-CB-TE2A-Y3-TATE, as described previously.[216] This was unexpected as the bioconjugates' overall charge was +1 (**Figure 40**) while the acid-assisted dissociation kinetics and resistance to Cu(II)/Cu(I) reduction were determined on charge neutral Cu-CB-TE2A. The model compound, CB-TE1A (**72**), was complexed with Cu(II) and exhibited a reduction potential ($E_{1/2}$) of only 120 mV higher than Cu-CB-TE2A (-0.95 versus -1.07 V respectively) while maintaining quasi-reversibility. This suggests that single-pendant arm carboxylate ligands should form stable Cu(II) complexes for *in vivo* applications.

Phosphonic acid pendant arms on CB-cyclam (**8**) have been studied for their role in Cu(II) coordination chemistry (*vide supra*). Cu-CB-TE2P (**51**) was evaluated as a model complex for radiopharmaceutical applications by acid-assisted decomplexation ($t_{1/2}$(5 M HCl, 90 °C) = 3.8 h), resistance to Cu(II)/Cu(I) reduction (quasi-reversible, E_p = -0.96 V) and biodistribution in male rats (**Chapter 1**). These data confirm that phosphonic acid pendant arms are a suitable alternative to carboxylate pendant arms on cross-bridged ligands. Replacement of the carboxylate arm on CB-TE2A-Y3-TATE with

a phosphonic acid arm allows for the formation of a charge neutral bioconjugate (**Figure 41**) which has the potential to show improved biodistribution properties compared with charged analogues.[40, 57, 215]

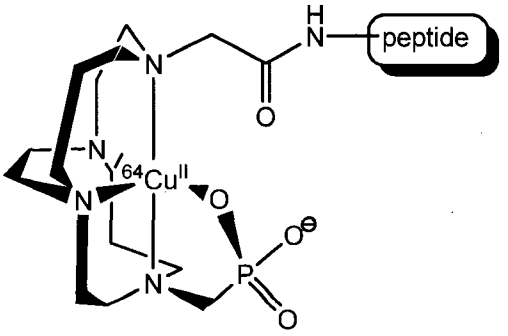
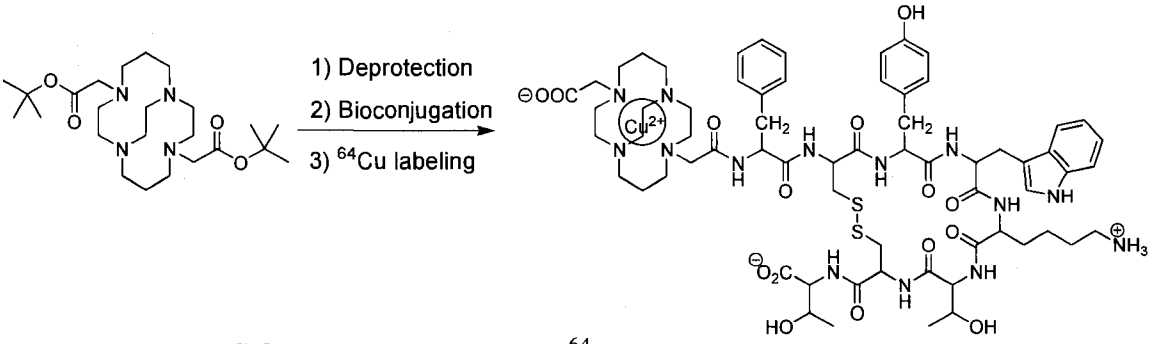


Figure 41: BFC complex of proposed bioconjugate with a single phosphonic acid pendant arm.

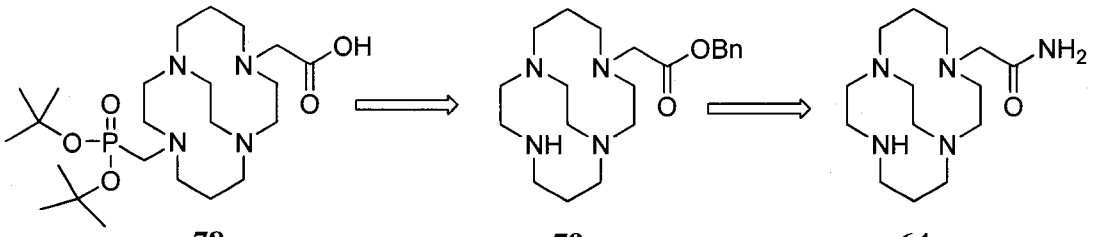
The formation of ^{64}Cu-CB-TE2A-Y3-TATE from a diprotected CB-TE2A has been reported and is shown in **Scheme 48**.[57] The *t*-butyl protected CB-TE2A is



Scheme 48: Formation of ^{64}Cu-CB-TE2A-Y3-TATE.

deprotected in the presence of weak acid trifluoroacetic acid (TFA), isolated as a TFA salt and then conjugated to Y3-TATE using solid-phase peptide synthesis methods.[216] Complexation with ^{64}Cu was done with a large excess of CB-TE2A-Y3-TATE and radiochemical purity was determined by radio-TLC. Unfortunately, the hydrolysis of an ethyl phosphonate on CB-cyclam (*vide supra*), which required strong acid and high temperatures, was isolated as an HCl salt. The presence of HCl could denature a peptide

during the course of bioconjugation. As such, preparation of a *t*-butyl phosphonate was proposed. **Scheme 49** shows the proposed retrosynthetic analysis for the formation of potential BFC **78**, a mixed-pendant arm ligand with a *t*-butyl protected phosphonic acid



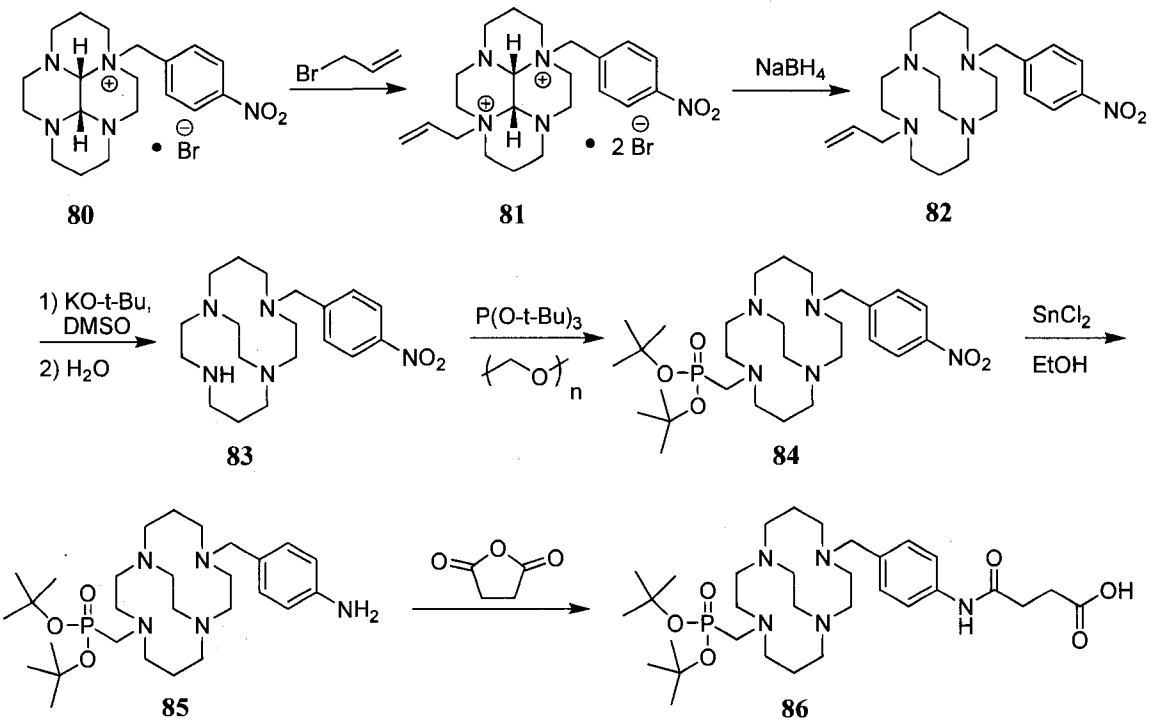
78 **79** **64**

Scheme 49: Retrosynthetic analysis of the formation of potential BFC **78**.

pendant arm for coordinating a metal and a methyl carboxylate pendant arm for coupling with the N-terminus of Y3-TATE. The proposed synthesis is based upon the formation of monoamide **64**, described previously (**Chapter 2**). Hydrolysis of **64** yields ligand CB-TE1A (**74, Chapter 2**). Esterification of carboxylic acid **72** with benzyl alcohol would form ligand **79**. Formation of a *t*-butyl protected phosphonate pendant arm on ligand **79** followed by hydrogenolysis to remove the benzyl protecting group would yield ligand **78**, which can be further coupled with Y3-TATE then deprotected in order to prepare a bioconjugate for study.

Recent literature reports have indicated that an increase in lipophilicity of a BFC can impart improvements, such as faster liver clearance, in the biodistribution of either the BFC or related bioconjugates.[205, 206] In order to determine the effect of linker character on cross-bridged ligands with phosphonic acid pendant arms, the formation of compound **86** was proposed (**Scheme 50**). This synthesis was based on previous work by Shanta Bist,[186] as well as current research being developed in the Weisman research group.[217] The proposed synthetic sequence involves the isolation of mono-*p*-nitrobenzyl

salt **80**, a compound originally synthesized by Bist, but has also been reported by Hermann.[218] The addition of an allyl protecting group on ligand **80** could afford mixed pendant-armed salt **81**. Double reductive ring opening of ligand **81** can yield unsymmetrical cross-bridged ligand **82** which can be deallylated in the presence of potassium *t*-butoxide and DMSO to yield mono *p*-nitrobenzyl CB-cyclam **83**. Deallylation can occur through the formation of a dimsyl anion *in situ* which can isomerize the allyl functionality to an enamine followed by cleavage with H_2O. These conditions have been reported previously,[219-223] but have been carried out by current members of the Weisman research group.[217] Mannich-like conditions can be used for the formation of *t*-butyl protected phosphonates followed by reduction of the *p*-nitro



Scheme 50: Proposed formation of ligand **86**.

substituent to a *p*-amino functionality forming ligand **85**. Addition of succinic anhydride yields ligand **85** which can be coupled to a biological molecule via the N-terminus.

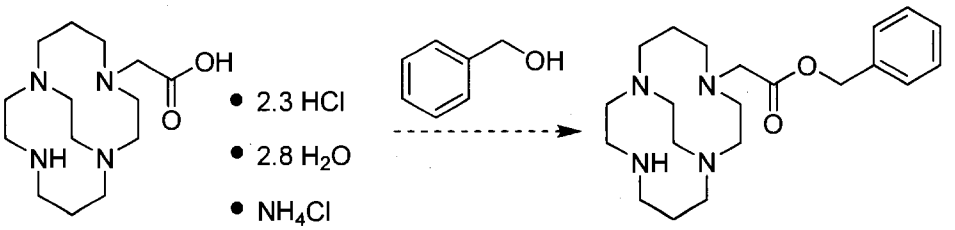
Herein are reported the attempted synthetic formations of two single phosphonate pendant-armed cross-bridged ligands **78** and **86** as potential BFCs for use in radiopharmaceuticals.

III. Results and Discussion

A. Attempted Preparation of Ligand 79.

(1) Fischer esterification of CB-TE1A (**72**).

The synthesis of a benzyl ester of ligand CB-TE1A (**Scheme 51**) is critical because a free carboxylate can attack tri-*t*-butyl phosphite, the reagent used to form *t*-butyl protected phosphonates in the following step, allowing multiple products to form.[77] Unfortunately, ligand **72** is soluble in only MeOH, EtOH and water. The presence of either alcohol will compete with benzyl alcohol (BnOH) for Fischer esterification and the presence of water will force the equilibrium towards ligand **72**. Several different reaction



72 **79**

Scheme 51: Proposed formation of ligand **79** by Fischer esterification.

trials were attempted and are summarized in **Table 9**. Ligand **72** was isolated as a hydrochloride salt (2.3 molar equivalents) and attempts at Fischer esterification were carried out without the presence of another acid. Removal of water produced by the Fischer esterification has been accomplished by azeotropic distillation with toluene or benzene in a Dean-Stark trap.[224] After 6 days at reflux with a Dean-Stark trap, only starting material could be seen in the ^{1}H NMR spectrum of the crude product. The ^{1}H NMR spectrum of the reaction with BnOH in d_6-DMSO at room temperature was

analyzed daily. The reaction afforded no product after 7 days. Microwave heating conditions were implemented, and Et_2O was used to wash away excess BnOH. ^1H NMR spectrum of the crude reaction material indicated the presence of starting material and some BnOH, but no product.

Ligand	Equiv. of BnOH	Conditions	Result
72	1.4	Toluene, reflux, D-S Trap, 6d	Starting material
72	2	d_6-DMSO, RT, 7d	Starting material
72	NEAT	microwave, 60 min, 125 °C	Starting material

Table 9: Reaction trials with ligand **72** and BnOH to form **79**.

Fischer esterification was then attempted in the presence of catalytic amounts of anhydrous tosic acid. Reaction trials are summarized in **Table 10**. Reactions were carried

Ligand	Equiv. of BnOH	Equiv. of TsOH	Conditions	Result
72	NEAT	0.1	70 C, 3 Å sieves	Starting material
72	NEAT	0.1	microwave, 30 min 125 °C	Starting material
72	10	0.1	MeCN, reflux, 1d	Starting material
72	10	3	DMF, 100 °C, 1d	Starting material
72	NEAT	0.1	microwave, 60 min, 125 °C	Mostly starting material

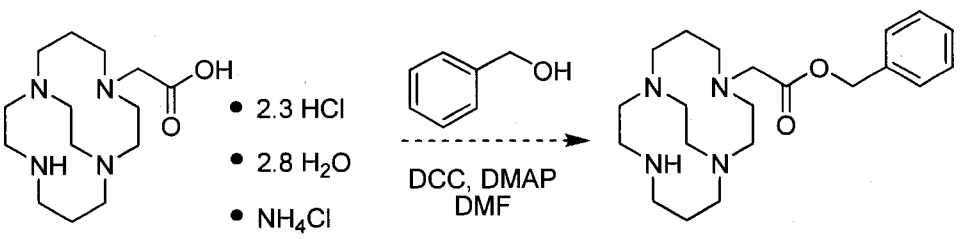
Table 10: Reaction trials with ligand **72**, BnOH and TsOH to form **79**.

out in neat BnOH at 70 °C with conventional heating and 30 min at 125 °C with microwave heating. The ^1H NMR spectrum in both cases, after removal of BnOH with Et_2O, indicated no reaction had taken place. The polarity of the solvent in these cases could not have been high enough to stabilize the transition state of the reaction (the dielectric constant of BnOH is 4.5 at 30 °C)[225] thus increasing the time required to reach equilibrium. As such, reactions were attempted in more polar solvents MeCN (dielectric

constant = 37.50 at 20 °C) and DMF (dielectric constant = 36.70 at 20 °C). Starting material **72** does not appear to be soluble in either solvent, but may have some slight solubility at high temperatures. However, both trials indicated only the presence of starting material and benzyl alcohol. The only reaction trial that indicated the presence of some product was conducted under microwave heating in neat BnOH with 0.1 molar equivalents of TsOH relative to 1.0 molar equivalents of **72**. LDI (Laser Desorption Ionization) mass spectrometry of the crude product indicated the presence of benzyl ester. However, after a basic extraction from an ice cold aqueous phase, only starting material was isolated.

(2) Coupling of CB-TE1A (**72**) with benzyl alcohol in the presence of DCC and DMAP.

Coupling reactions are a common technique for the formation of esters utilizing dicyclohexylcarbodiimide (DCC) and dimethylamino pyridine (DMAP). The carboxylate adds to DCC allowing the newly formed ester to be easily cleaved by any alcohol present. Ligand **72** was partially dissolved in DMF in the presence of DCC and DMAP following a procedure for the formation of benzyl esters of amino acids (**Scheme 52**).[226] The crude reaction mixture indicated no product had formed after 2 days. The same result was seen after 7 days.

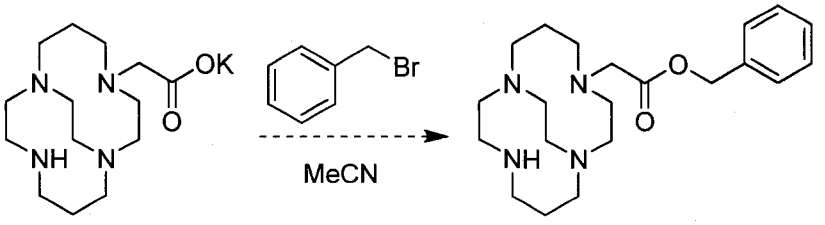


72 **79**

Scheme 52: Proposed formation of ligand **79** by a coupling reaction.

(3) Alkylation of CB-TE1A-K^+ (**73**) with benzyl bromide

Alkylation of amino acids with benzyl bromide is well known and several reported procedures have been recently published.[227-232] Unfortunately, CB-TE1A (**72**) possesses two nucleophilic sites: a free carboxylate and a secondary amine. In order to impart some enhanced nucleophilic character to the carboxylate, alkylation with one equivalent of benzyl bromide was carried out with the potassium salt of CB-TE1A (**73**)



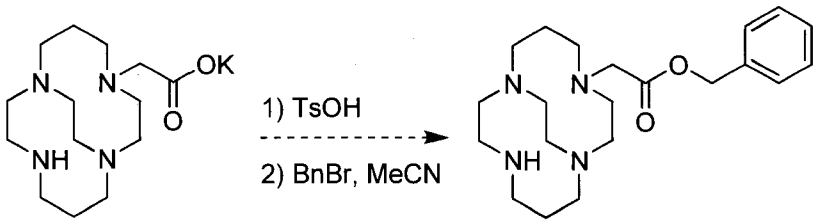
73 **79**

Scheme 53: Alkylation of ligand **73** with benzyl bromide.

(**Scheme 53**). The crude ^{1}H NMR spectrum indicated the presence of three AB resonances and no free benzyl bromide. This indicated that the rates of alkylation of the secondary amine and the free carboxylate are similar under these conditions.

In order to attempt to slow down the rate of alkylation at the secondary amine relative to the rate of alkylation of the free carboxylate, another alkylation was carried out with ligand **73**, but as a tosic acid salt (**Scheme 54**). The highly basic nature of the cross-bridged ligand should ensure that once protonated, the lone pair of the secondary nitrogen would be involved in a hydrogen bond, slowing down its rate of alkylation relative to the free amine. Ligand **73** was protonated with one equivalent of anhydrous tosic acid in MeCN and stirred for 10 minutes. One equivalent of benzyl bromide was added and the reaction was stirred for 24 hours. ^{1}H NMR of the crude product indicated the same result

as previously described. Further attempts at formation of ligand **79** via alkylation were abandoned.

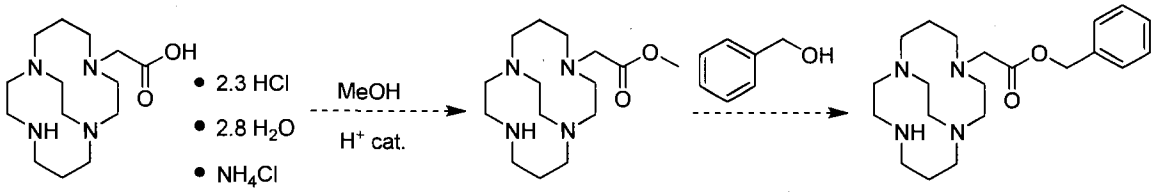


73 **79**

Scheme 54: Attempted synthesis of ligand **79**.

(4) Fischer Esterification of ligand **72** with MeOH

An alternative approach towards the formation of ligand **79** involves the esterification of **72** with MeOH in order to form methyl ester **87** followed by transesterification with benzyl alcohol (**Scheme 55**). Although this synthetic



74 **87** **79**

Scheme 55: Proposed formation of ligand **79** by Fischer esterification with MeOH followed by transesterification.

pathway adds a step to the synthesis of **78**, it avoids the use of large amounts of high boiling benzyl alcohol, a difficult solvent to remove. Also, the solubility of ligand **87** may be greater in a larger range of solvents compared to that of **72** which has limited solubility. Reaction trials for the formation of ligand **87** are summarized in **Table 11**. Since **72** is isolated with 2.8 molar equivalents of HCl relative to 1.0 molar equivalents of the ligand, the HCl already present could act as the necessary catalyst. Compound **72** was

dissolved in MeOH and heated at reflux for 1 day over 3 Å sieves. The crude ^{1}H NMR spectrum indicated no reaction had taken place. Fischer esterification of ligand **72** was attempted with both an ion exchange resin and a drop of 0.1 M HCl as the acid catalyst, however, both reactions failed to esterify as indicated by the ^{1}H NMR spectra of the crude products.

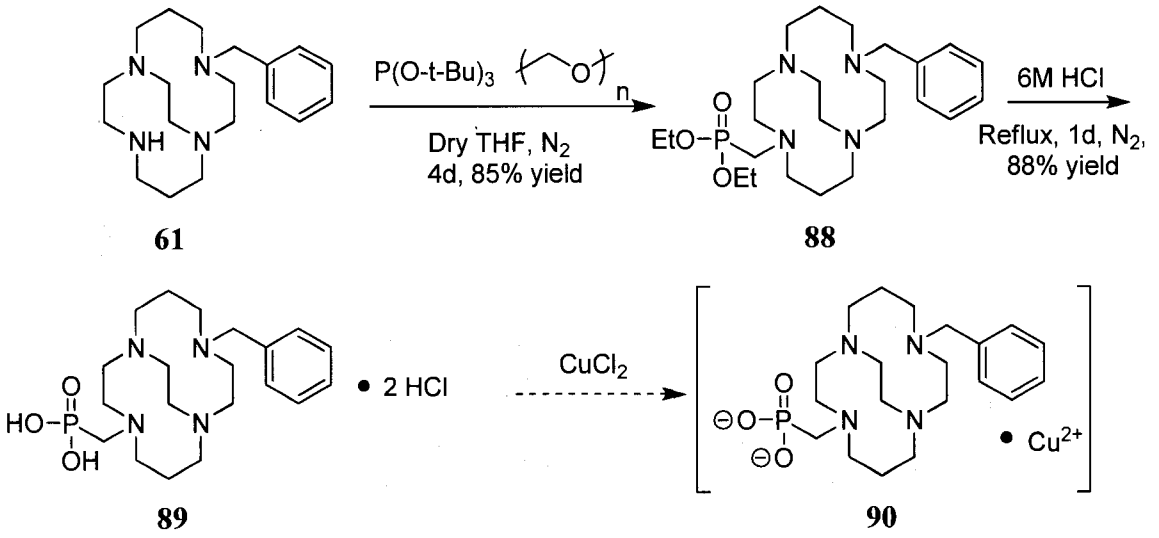
Ligand	Acid catalyst	Solvent	Conditions	Result
72	---	MeOH	Reflux, 1d, N_2, 3 Å sieves	Starting material
72	Dowex 50x2-100 ion exchange resin	MeOH	Reflux, 1d, N_2, 3 Å sieves	Starting material
72	1 drop 0.1 M HCl	MeOH	Reflux, 1d, N_2, 3Å sieves	Starting material

Table 11: Reaction trials for the formation of methyl ester **87**.

B. <u>Attempted Formation of Mono p-nitrobenzyl Ligand 83</u>.

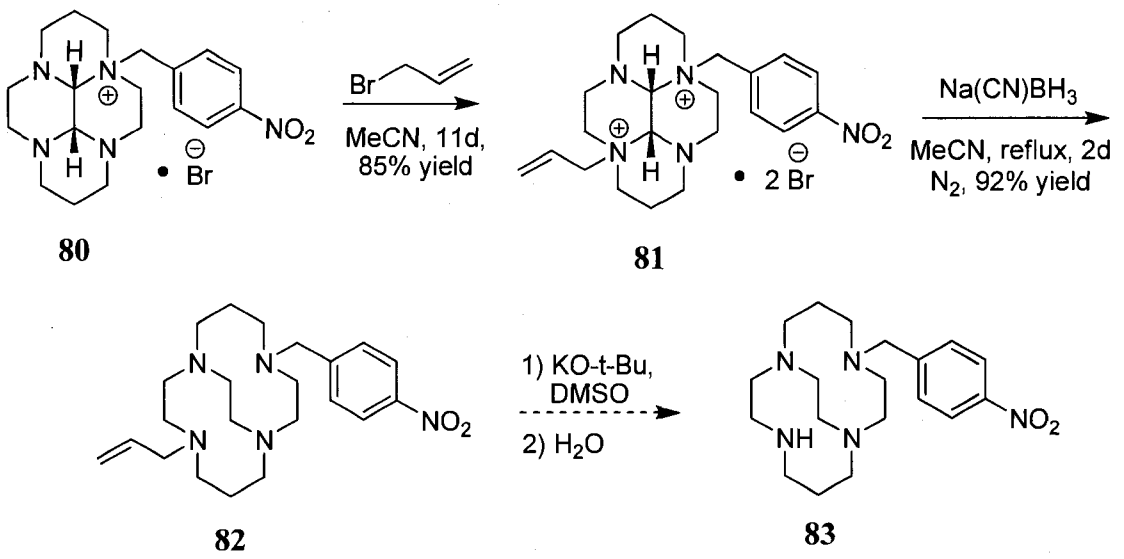
In order to determine the kinetic inertness and biodistribution properties of a single phosphonic acid pendant-armed cross-bridged cyclam, model ligand **90** was prepared from monobenzyl ligand **61**, prepared by Matthew Young.[217] The conditions used for the preparation of ligand **37** (**Chapter 1**) were used to prepare ligand **88** (**Scheme 56**), which was isolated in 85% yield. Ligand **88** was characterized by ^{1}H, ^{13}C and ^{31}P NMR and HRFABMS. The hydrolysis of ligand **88** was carried out under conditions identical to those used for the formation of ligand **51**, refluxing 6 M HCl for one day. Ligand **89** was characterized by ^{1}H, ^{13}C{^{1}H}, and ^{31}P{^{1}H} NMR, HRFABMS and elemental analysis. Elemental analysis indicated the isolated sample had 2 molar equivalents of HCl relative to 1.0 molar equivalent of ligand **89**. Both the ^{1}H and ^{31}P{^{1}H} NMR spectra and elemental analysis indicated the presence of 0.08 molar equivalents of

89·3HCl relative to 1.0 molar equivalent of **89**. Purification of ligand **89** with ion exchange chromatography was not attempted, nor was Cu(II) complexation.



Scheme 56: Formation of ligand **89**, Cu(II) complexation was not attempted.

As described previously, ligand **80** can be converted into ligand **83**. The synthetic sequence carried out is shown in **Scheme 57**. Ligand **80** was synthesized using previously reported conditions.[186] Mono *p*-nitro salt **80** was stirred in MeCN with 60 molar equivalents of allyl bromide relative to 1.0 molar equivalent of **80** for 11 days in a sealed flask which was wrapped in foil. Ligand **81** slowly precipitated from solution over the 11 day reaction period and was isolated by vacuum filtration. Characterization was completed with ^{1}H and $^{13}C\{^{1}H\}$ NMR, HRFABMS and elemental analysis. Interestingly, ligand **81** was found to be unstable in water. The ^{1}H NMR spectrum of **81** in D_2O indicated the presence of approximately 0.05 ± 0.01 molar equivalents of starting ligand **80** and 0.05 ± 0.01 molar equivalents of allyl alcohol relative to 1.0 molar equivalents of **81**. However, the ^{1}H NMR spectrum of the exact same sample in d_4-MeOD revealed no observable impurities. Sensitivity of analogous systems towards water has been reported.[217, 233]



80 **81** **82** **83**

Scheme 57: Attempted synthesis of ligand **83**.

Double reductive ring openings of unsymmetrical di-armed salts (analogues of **81**) have been reported with the use of $NaBH_4$.[186, 234] As described by Martin and Young, the reaction of $NaBH_4$ in 95% EtOH formed small amounts of hydroxide and ethoxide which caused deallylation of allyl analogues of **81**.[217, 233] Reductive ring opening with the less basic reducing agent sodium cyanoborohydride in MeCN was found to be effective. Double reductive ring opening of ligand **81** was carried out in MeCN for 2 days in the presence of excess sodium cyanoborohydride (care must be taken when working with reagents that can produce HCN). Once the reaction solvent was removed, neutralization of the excess cyanoborohydride was done with dropwise addition of a 25% HBr solution. HBr was used because HCl resulted in the mono HCl salt of ligand **82**, which was insoluble in H_2O. Water solubility is a necessity in order to isolate free amine **82**.

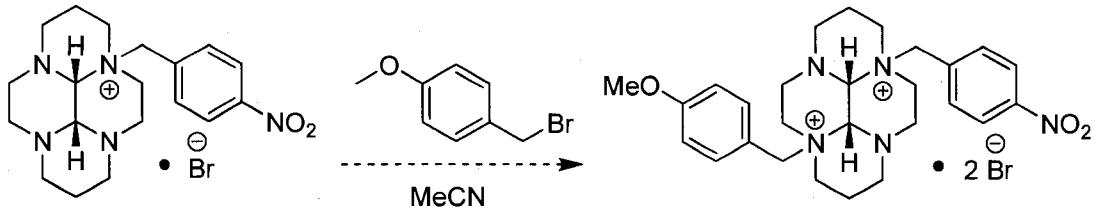
Removal of allyl protecting groups from amines has been recently reviewed.[223] Potassium *t*-butoxide and DMSO have been reported to deprotect allyl amines with the aid of a dimsyl anion in situ. This highly basic species causes isomerization of the allyl

amine to an enamine which is hydrolyzed upon subsequent reaction with water. Conditions for the removal of allyl protecting groups on cross-bridged ligands have been reported.[217] Utilizing these same conditions, ligand **81** and potassium *t*-butoxide were heated in DMSO under microwave conditions (25 min, 100 °C, open vessel) (care must be taken when heating DMSO in a microwave as it can be explosive)[235]. The ^{1}H NMR spectrum of the crude product indicated no aromatic signals suggesting that the ligand had de-*p*-nitrobenzylated instead of deallylated. Since, para-nitro benzyl substituents are good S_N2 substrates it is possible that dimsyl anion could have attacked the benzyl carbon displacing the rest of the ligand. Since this deallylation reaction was crucial to the formation of ligand **82**, a new synthetic strategy was adopted in order to form potential BFC ligand **85**.

C. Alternative Syntheses Towards the Formation of Potential BFC 86.

(1) The use of a *p*-methoxyl benzyl (PMB) protecting group on ligand **80**.

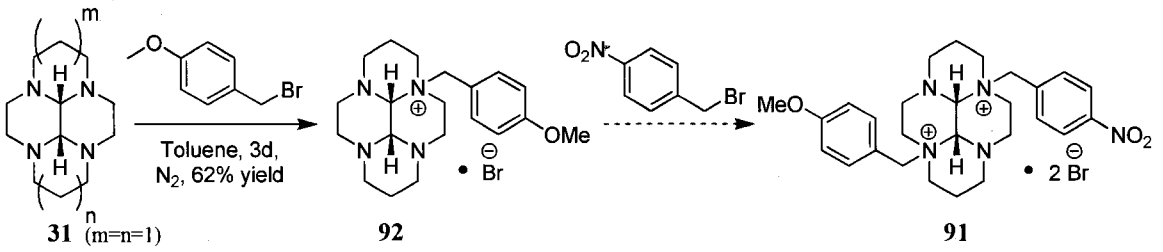
TFA (trifluoroacetic acid) has been shown to remove *p*-methoxy benzyl (PMB) protecting groups from cyclic heterocycles through the formation of a PMB cation.[236-238] Addition of anisole to the reaction mixture to trap the PMB cation in situ has been shown to improve the reaction.[239] Recently, a PMB protecting group of an amine in a cross-bridged tetraamine has been shown to be cleaved by TFA.[217] Since the attempted deallylation of ligand **82** removed the p-nitro benzyl group instead, PMB was chosen as a substitute for the allyl protecting group. The synthesis of compound **91** was attempted with 10 molar equivalents of PMB-Br relative to 1 molar equivalent of compound **80** (**Scheme 58**) under the same conditions used for the formation of ligand **81**. An aliquot



80 **91**

Scheme 58: Attempted synthesis of ligand **91**.

was removed after 11 days and revealed the presence of only starting material. The reaction was attempted with 20 molar equivalents of PMB-Br relative to 1.0 molar equivalent of **80** in MeCN. However, no precipitate was seen after 14 days and the ^{1}H NMR spectrum of a small aliquot showed the presence of *p*-nitrobenzyl bromide, indicating the substituent had been removed in solution. Another synthetic pathway towards the formation of dibromide salt **91** involves the formation of mono-*p*-methoxy



31 (m=n=1) **92** **91**

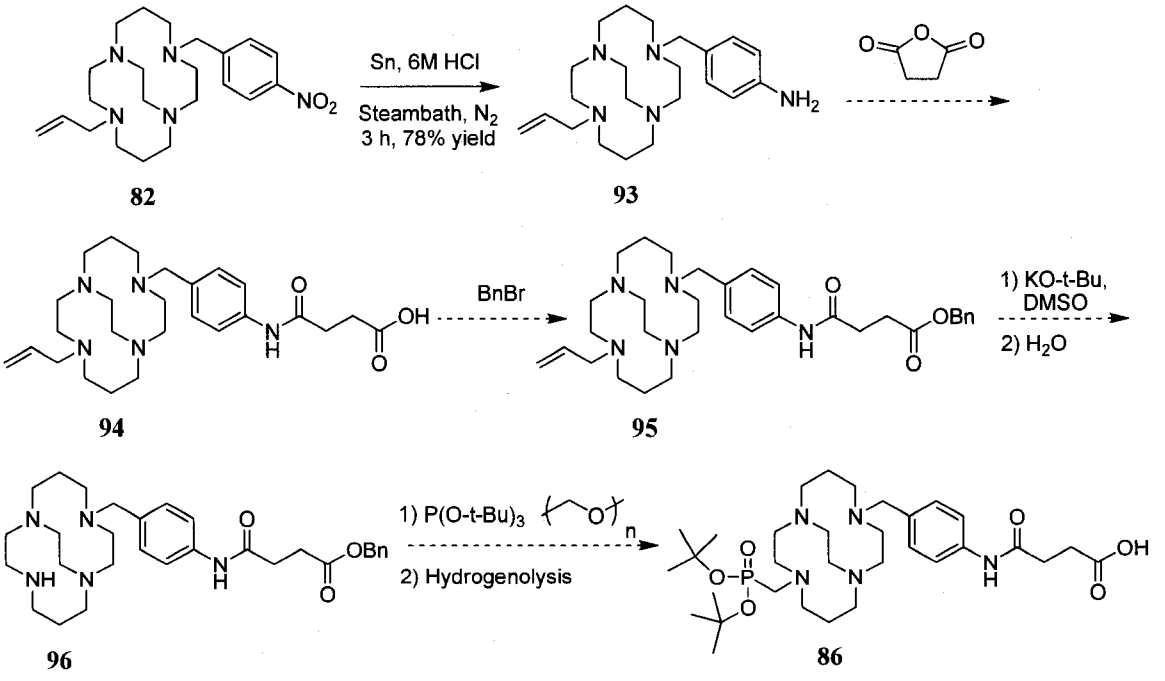
Scheme 59: Attempted synthesis of ligand **91** from ligand **92**.

salt ligand **92** followed by alkylation with *p*-nitrobenzyl bromide (**Scheme 59**). Compound **92** was prepared in 62% yield by the mono-alkylation of ligand **31** (m=n=1) in toluene, precipitated from solution, isolated by vacuum filtration and characterized by ^{1}H and ^{13}C NMR, HRFABMS and elemental analysis. Both the ^{1}H NMR spectrum and the analysis indicated the presence of 0.16 molar equivalents of toluene relative to 1.0 molar equivalent of ligand **92**. Six molar equivalents of *p*-nitrobenzyl bromide were added to a stirred solution of ligand **92** in MeCN at room temperature. An aliquot was

removed after 6 days and revealed a complex mixture of products. Only compound **92** was identified in the mixture. The reaction was stirred for an additional 6 days and the ^{1}H NMR of an aliquot indicated that the mixture had become more complex. Further attempts to prepare compound **91** were abandoned.

(2) Attempted synthesis of ligand **86** via ligand **93**.

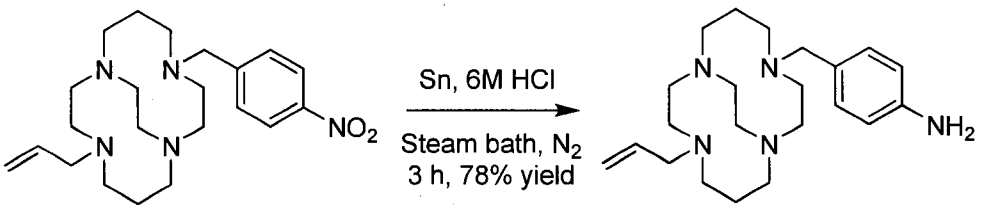
A second alternative synthetic sequence towards the formation of ligand **86** involves the intermediacy of mono-anilino/ mono-allyl cross-bridged tetraamine **93** (**Scheme 60**). Tin-mediated reduction of compound **82** forms compound **93**. Unfortunately, deallylation of ligand **93** with potassium *t*-butoxide and DMSO would produce a single-pendant-arm ligand with both anilino and amine functionalities. Recent reports have indicated that the nucleophilicities of both tend to be quite similar in water



Scheme 60: Alternative synthetic sequence towards the formation of ligand **86**.

making regioselective reactions difficult.[240] Even though these reactions would not be run in water, any competition between these two nucleophiles will cause a mixture of products to form. Similar to the approach in **Scheme 50**, succinic anhydride could be reacted with **93** to form compound **94**. However, unlike the synthesis described in **Scheme 50**, the carboxylate moiety in ligand **94** must be protected in order to prevent a reaction with the free carboxylate and tri-*t*-butyl phosphite two steps later. De-allylation of ligand **95** followed by phosphonate formation and acid-free hydrogenolysis of ligand **96** should yield potential BFC **86**.

Compound **82** was dissolved in 6 M HCl and stirred in the presence of mossy tin over a steam bath for 3 hours (**Scheme 61**). The free amine was isolated by extraction from a strongly basic solution (pH = 14) and characterized by ^{1}H and $^{13}C\{^{1}H\}$ NMR and HRFABMS. The ^{1}H NMR spectrum of the product was found to be almost identical

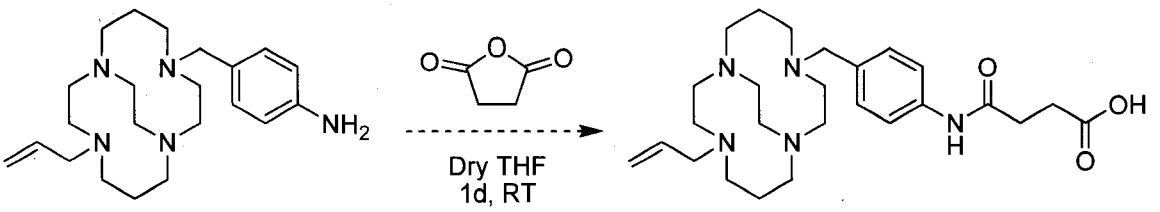


82 **93**

Scheme 61: Tin-mediated reduction of ligand **82**.

to the starting material with the exception of the chemical shifts of the aromatic AA′XX′ of each species. The electron withdrawing nature of the *p*-nitro substituent forces the resonances of AA′XX′ downfield relative to the resonances of the AA′XX′ of the electron donating anilino substituent.

The attempted synthesis of ligand **94** was carried out in freshly distilled THF (**Scheme 62**). After the reaction was complete, an extraction was attempted from a



93 **94**

Scheme 62: Attempted synthesis of ligand **94**.

strongly basic solution (pH = 14) with chloroform and toluene. Neither solvent extracted any material from the aqueous solution. The solvent was removed from the aqueous solution and a sample of the white solids remaining was analyzed by ^{1}H NMR. The product was present with approximately 0.1 molar equivalents of starting material **93** relative to 1.0 molar equivalent of **94**. Further purification was not attempted.

IV. Conclusions

The syntheses of two different mono-phosphonic acid pendant arm ligands were attempted through various synthetic routes in order to produce ligands for use in radiopharmaceuticals. The attempted esterification of CB-TE1A (**72**) with BnOH or MeOH failed under various conditions. The attempted alkylation of CB-TE1A-K (**73**) with BnBr produced multiple products indicating that both the secondary amine and the free carboxylate were alkylated. The attempted synthesis of ligand **86** via ligand **83** failed due to the unexpected de-*p*-nitrobenzylation of ligand **82** under the conditions for deallylation. Two alternative synthetic routes towards the formation of ligand **86** were proposed. First, substitution of an allyl protecting group with a *p*-methoxy benzyl protecting group was attempted and failed to produce ligand **91** via two different sequences. Second, the synthesis of ligand **86** was attempted via the formation of ligand **93**, however, ligand **94** could not be isolated.

CHAPTER 4

EXPERIMENTAL DETAILS

I. General Methods

Melting points (mp) were obtained on a Thomas Hoover capillary melting point apparatus and were uncorrected.

Infrared Spectra (IR) were run on a Nicolet MX-1 FT-IR spectrometer and absorptions are reported in wavenumber (cm^{-1}).

^{1}H NMR Spectra (^{1}H NMR) were acquired on a Varian Mercury-400BB NMR spectrometer or a Varian INOVA-500 NMR spectrometer. Chemical shifts are reported in parts per million (ppm) relative to internal Me_4Si (TMS) unless otherwise noted and coupling constants (J values) are in Hertz (Hz).

$^{13}C\{^{1}H\}$ NMR Spectra ($^{13}C\{^{1}H\}$ NMR) were acquired on a Varian Mercury-400BB NMR spectrometer or a Varian INOVA-500 NMR spectrometer. Chemical shifts are reported in parts per million (ppm) relative to internal Me_4Si (TMS) unless otherwise noted and coupling constants (J values) are in Hertz (Hz).

$^{31}P\{^{1}H\}$ NMR Spectra ($^{31}P\{^{1}H\}$ NMR) were acquired on a Varian Mercury-400BB NMR spectrometer. Chemical shifts are reported in parts per million (ppm) relative to an external 85% phosphoric acid standard

Low-Resolution Mass Spectra (LRMS) were performed by the University of New Hampshire Instrumentation Center on a Hitachi-Perkin-Elmer RMU-60 mass spectrometer.

Electrospray Ionization Mass Spectra (ESIMS) was obtained on a Thermoquest LCQ mass spectrometer at the University of New Hampshire.

High-Resolution Mass Spectra (HRMS) were obtained from the Mass Spectroscopy Facility at the University of Notre Dame using a JEOL AX505HA high resolution mass spectrometer.

Elemental Analysis (CH&N) was performed by Atlantic Labs Inc., Georgia, USA.

X-ray Crystallography (X-ray) was performed by Jonathan M. White at the University of Melbourne, Parkville, Australia, for compound **67**, and Arnold L. Rheingold at the University of California, San Diego, La Jolla, California, USA, for compounds **51** and **52**.

Electrochemical Analysis was collected on a BAS100B electrochemical analyzer. A glassy carbon working electrode was used in combination with a Pt auxiliary electrode.

Ultraviolet-visible spectra (UV-Vis) were acquired on a Varian Cary 50 spectrometer and absorptions are reported in nanometers (nM).

Microwave Reactions were carried out on a CEM® Discover research scale manual microwave synthesizer.

II. Solvents

Absolute Ethanol (EtOH, ACS/USP grade) was obtained from Pharmco Products Inc. and was used without further purification.

Acetonitrile (CH_3CN) was obtained from EMD Chemicals Inc. and stored in an Innovative Technology Inc. Pure-Solv Solvent Purification System. Prior to use, the solvent was passed through the system's alumina column under low pressure to remove trace impurities.

Benzene (C_6H_6, ACS grade) was obtained from EMD Chemicals Inc. and was used without further purification.

Chloroform ($CHCl_3$, HPLC grade) was obtained from EMD Chemicals Inc. and was used without further purification.

Diethyl Ether (Et_2O) was obtained from EMD Chemicals Inc. and stored in an Innovative Technology Inc. Pure-Solv Solvent Purification System. Prior to use, the solvent was passed through the system's alumina column under low pressure to remove trace impurities.

Dimethyl Sulfoxide: (DMSO, ACS grade) was obtained from EMD Chemicals Inc. and was stored over 3Å molecular sieves.

Deuterated NMR Solvents were obtained from Cambridge Isotope Laboratories and stored over 3Å molecular sieves.

Ethanol (95% EtOH, ACS grade) was obtained from EMD Chemicals Inc. and was used without further purification.

Methanol (MeOH, Reagent/ACS/USP/NF grade) was obtained from Pharmco Products Inc. and stored in an Innovative Technology Inc. Pure-Solv Solvent Purification System. Prior to use, the solvent was passed through the system's alumina column under low pressure to remove trace impurities.

Tetrahydrofuran (THF) was obtained from EMD Chemicals Inc. and stored in an Innovative Technology Inc. Pure-Solv Solvent Purification System. Prior to use, the solvent was passed through the system's alumina column under low pressure to remove trace impurities.

Toluene ($PhCH_3$, ACS grade) was obtained from EMD Chemicals Inc. and stored in an Innovative Technology Inc. Pure-Solv Solvent Purification System. Prior to use, the solvent was passed through the system's alumina column under low pressure to remove trace impurities

III. Reagents

Acrylamide was obtained from Aldrich Chemical Company.

Allyl bromide was obtained from Aldrich Chemical Company.

Benzyl Bromide was obtained from Alfa Aesar.

2-Bromoacetamide was obtained from Aldrich Chemical Company.

Bromotrimethylsilane was obtained from Alfa Aesar.

tert-Butyl bromoacetate was obtained from Aldrich Chemical Company.

2-Chloro-*N*-methylacetamide was obtained from Pfaltz and Bauer, Inc.

Cupric Chloride Dihydrate was obtained from Aldrich Chemical Company.

Dimethyl vinylphosphonate was obtained from Alfa Aesar.

Di-t-butyl phosphite was obtained from Alfa Aesar.

1,1,1,3,3,3-hexafluoroisopropanol was obtained from Alfa Aesar.

Hydrobromic Acid was obtained from Alfa Aesar.

Hydrochloric Acid was obtained from Fischer Scientific.

4-Methoxybenzyl bromide was obtained from Aldrich Chemical Company.

N-Methylacetamide was obtained from Aldrich Chemical Company.

Morpholine was obtained from Fisher Scientific.

4-Nitrobenzyl bromide was obtained from Aldrich Chemical Company.

Paraformaldehyde was obtained from Acros Organics.

Phosphorus trichloride was obtained from Alfa Aesar.

Potassium Hydroxide was obtained from EM Science Company.

Sodium Cyanoborohydride ($NaBH_3CN$) was obtained from Aldrich Chemical Company.

Sodium Hydroxide was obtained from EM Science Company.

Succinic Anhydride was obtained from Aldrich Chemical Company.

Trace Metal™ Grade Hydrochloric Acid was obtained from Fischer Scientific.

Triethyl phosphite was obtained from Acros Organics.

Trimethyl phosphite was obtained from Aldrich Chemical Company.

IV. Column Chromatography Solid Supports

Amberlite CG50 was obtained from Sigma.

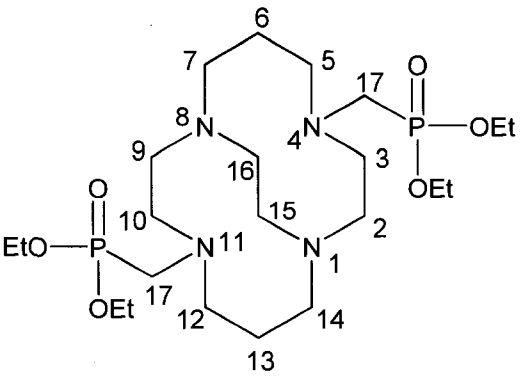
V. Experimental Procedures

1,4,7,10-Tetraazabicyclo[5.5.2]tetradecane-4,10-bis(methanephosphonic acid diethyl ester) (36). Triethyl phosphite (0.25 mL, 1.4 mmol) and paraformaldehyde (43.4 mg, 2.50 molar equivalents of CH_2O) were added to a solution of **7** (0.0955 g, 0.482 mmol) in 1.5 mL of dry THF and the mixture was stirred under N_2 for 4 days. The solvent was removed under aspirator pressure and volatile phosphites were removed under vacuum. The residue was dissolved in H_2O (10 mL), and the solution was made

strongly basic (pH=14, slow addition of KOH pellets). The resulting solution was extracted with toluene (5 x 10 mL), the combined organic phases were dried over anhyd Na_2SO_4, the solvent was removed under aspirator pressure, and the residual solvent was removed under vacuum to yield 0.2015 g (81%) of clear oil **36**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.26 (t_{app}, 12H, J = 7.1 Hz, P(O)OCH$_2$C*H*$_3$), 2.71-2.92 (m, 16H), 2.88 (s, 4H, NC*H*$_2$C*H*$_2$N, cross-bridge), 2.96 (d, 4H, $^2J_{PH}$ = 7.6 Hz, NC*H*$_2$P), 3.99-4.10 (m, 8H, P(O)OC*H*$_2$CH$_3$); ^{13}C{^{1}H} NMR ($CDCl_3$, 125.68 MHz) δ 15.59 (d, $^3J_{PC}$ = 5.8 Hz, P(O)OCH$_2$*C*H$_3$), 50.59 (d, $^1J_{PC}$ = 155.5 Hz, NCH$_2$P), 55.13, 55.73, 58.29 (d, $^3J_{PC}$ = 4.8 Hz, *C*3), 60.43 (d, $^2J_{PC}$ = 6.7 Hz, P(O)OCH$_2$CH$_3$); ^{31}P{^{1}H} NMR ($CDCl_3$, 161.83 MHz) δ 22.13; (The ^{31}P{^{1}H} and ^{13}C{^{1}H} spectra revealed the presence of 0.03 ± 0.01 molar equivalents triethyl phosphate, formed from the oxidation of residual triethyl phosphite and 0.1 ± 0.01 molar equivalents of 1,4,7,10-tetraazabicyclo[5.5.2]tetradecane-4-methanephosphonic acid diethyl ester relative to 1.0 molar equivalents of **36**); IR (thin film) 3448, 2981, 2916, 2820, 1653, 1445, 1374, 1223, 1027, 963; HRFABMS, *m/z* $(M+H)^+$ exact mass calcd for $C_{20}H_{45}N_4O_6P_2$: 499.2814; Found: 499.2803 (error -1.2 mmu/-2.3 ppm).

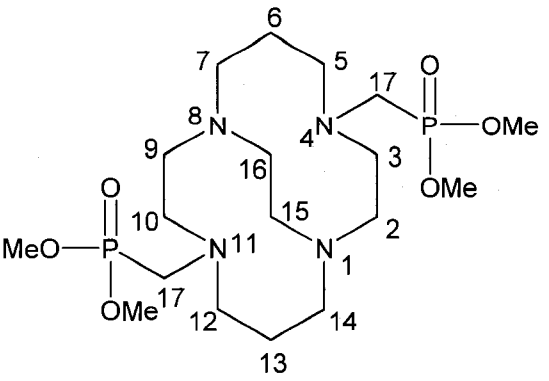
1,4,8,11-Tetraazabicyclo[6.6.2]hexadecane-4,11-bis(methanephosphonic acid diethyl ester) (37). Triethyl phosphite (0.18 mL, 1.0 mmol) and paraformaldehyde (32.5 mg, 2.5 molar equivalents of CH_2O) were added to a solution of **8** (0.0982 g, 0.4338 mmol) in 1.5 mL of dry THF and stirred under N_2 for 4 d. The solvent was removed, the residue was dissolved in H_2O (20 mL), and the solution was made strongly basic (pH=14, slow addition of KOH pellets). The resulting solution was extracted with toluene (5 x 11

mL), the combined organic phases were dried over anhyd Na_2SO_4, the solvent was removed under aspirator pressure, and the residual solvent was removed under vacuum to yield 0.2156 g (94%) of waxy white solid **37**: mp 42-44 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.22-1.32 (m, 14H), 1.32-1.41 (m, 2H), 2.20-2.28 (m, 4H), 2.27-2.35 (XX′ of AA′XX′, 2H, NCH*H*CH*H*N, cross-bridge), 2.38 (dt, 2H, J = 12.0, 3.2 Hz), 2.50-2.75 (m, 6H), 2.60 (dd, 2H, $^2J_{HH}$ = -15.6 Hz, $^2J_{HP}$ = -2.2 Hz, NC*H*HP) 2.77-2.87 (m, 2H), 2.82 (t_{app}, 2H, $^2J_{HH}$ = -16.0 Hz, $^2J_{HP}$ = -16.0 Hz, NCH*H*P), 3.04-3.13 (AA′ of AA′XX′, 2H, NCH*H*CH*H*N, cross-bridge), 3.70 (tt, 2H, J = 11.7, 4.2 Hz), 3.99-4.10 (m, 8H, P(O)OCH_2CH$_3$); ^{13}C{^{1}H} NMR ($CDCl_3$, 125.68 MHz) δ 15.54 ($^3J_{PC}$ = 4.8 Hz, OCH$_2$*C*H$_3$), 15.59 ($^3J_{PC}$ = 5.8 Hz, OCH$_2$*C*H$_3$), 27.10 ($C_{6,13}$), 49.24 ($^1J_{PC}$ = 160.3 Hz, N*C*H$_2$P), 49.88 (coincident with downfield line of doublet at 49.24), 54.94 ($^3J_{PC}$ = 15.4 Hz, $C_{5,12}$), 55.55, 55.79 ($C_{15,16}$), 59.41, 60.24 ($^2J_{PC}$ = 6.7 Hz, O*C*H$_2$CH$_3$), 60.47 ($^2J_{PC}$ = 7.7 Hz, O*C*H$_2$CH$_3$); [Note: ^{1}H-COSY and gHMQC experiments aided resonance assignments (see figure below for compound numbering)]; ^{31}P {^{1}H} NMR ($CDCl_3$, 161.83 MHz) δ 26.98; IR (CH_2Cl_2) 3476, 2980, 2905, 2799, 1669, 1446, 1390, 1366, 1343, 1323, 1292, 1245, 1161, 1126, 1098, 1028; HRFABMS, *m/z* $(M+H)^+$ exact mass for $C_{22}H_{49}N_4O_6P_2$: 527.3127; Found: 527.3127 (error -0.8 mmu/-1.6 ppm).



Numbering for **37**.

1,4,8,11-Tetraazabicyclo[6.6.2]hexadecane-4,11-bis(methanephosphonic acid dimethyl ester) (39). Trimethyl phosphite (0.1053 g, 0.8487 mmol) and paraformaldehyde (0.0255 g, 2.5 molar equivalents of CH_2O) were added to a solution of **8** (0.0768 g, 0.3393 mmol) in 1.5 mL of dry THF and stirred under N_2 for 4 d. The solvent was then removed under reduced pressure, the residue was dissolved in H_2O (7 mL), and the solution was made strongly basic (pH=14, slow addition of KOH pellets). The resulting solution was extracted with toluene (5 x 5 mL), the combined organic phases were dried over anhyd Na_2SO_4, the solvent was removed under aspirator pressure, and the residual solvent was removed under vacuum to yield 0.1218 g (76%) of waxy white solid **39**: mp = 105-108 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.31-1.48 (m, 4H), 2.28-2.37 (m, 4H), 2.35-2.43 (XX′ of AA′XX′, 2H, NCH*H*CH*H*N, cross-bridge), 2.47 (dt, 2H, J = 12.1, 3.5 Hz), 2.59-2.66 (m, 2H), 2.68-2.78 (m, 4H), 2.71 (dd, 2H, $^2J_{HH}$ = -15.8 Hz, $^2J_{HP}$ = -2.3 Hz, NC*H*HP), 2.86 (ddd, 2H, J = 14.6, 11.0, 3.9 Hz), 2.91 (t_{app}, 2H, $^2J_{HH}$ = -15.8 Hz, $^2J_{HP}$ = -15.8 Hz, NCH*H*P), 3.06-3.13 (AA′ of AA′XX′, 2H, NCH*H*CH*H*N, cross-bridge), 3.74-3.83 (m, 1H), 3.74 (d, 6H, $^3J_{HP}$ = 14.9 Hz, P(O)OC*H*$_3$), 3.76 (d, 6H, $^3J_{HP}$ = 14.9 Hz, P(O)OC*H*$_3$); ^{13}C{^{1}H} NMR ($CDCl_3$, 125.68 MHz) δ 28.00 ($C_{6,13}$) , 49.38 (P*C*H$_2$N, $^1J_{PC}$ = 160.3 Hz), 50.90, 52.02 (O*C*H$_3$, $^2J_{PC}$ = 6.7 Hz), 52.36 (O*C*H$_3$, $^2J_{PC}$ = 6.7 Hz), 55.70 ($C_{5,12}$, $^3J_{PC}$ = 15.4 Hz), 56.58, 56.66 (N*C*H$_2$*C*H$_2$N, cross-bridge), 60.25; [Note: ^{1}H-COSY and gHMQC experiments aided resonance assignments (see figure below for compound numbering)]; ^{31}P{^{1}H} NMR ($CDCl_3$, 161.83 MHz) δ 27.30; IR (KBr) 3421, 2954, 2899, 2841, 2815, 2772, 1466, 1447, 1367, 1296, 1230, 1126, 1032, 832, 693; HRFABMS, *m/z* $(M+H)^+$ exact mass for $C_{18}H_{41}N_4O_6P_2$: 471.2501; Found: 471.2503 (error -1.2 mmu/-2.6 ppm).



Numbering for **39**.

Di-*tert*-butyl morpholinomethylphosphonate (40). Di-tert-butyl phosphite (0.47 mL, 2.2 mmol) and paraformaldehyde (0.0656 g, 2.50 molar equivalents of CH_2O) were added to a stirred solution of morpholine (0.1270 g, 1.458 mmol) in 4.8 mL of dry MeCN and heated to reflux for 1 day under N_2 over 3Å molecular sieves. The solution was filtered, the solvent was removed from the filtrate under aspirator pressure, the residue was dissolved in H_2O (20 mL), and the solution was made strongly basic (pH=14, slow addition of KOH pellets). The resulting solution was extracted with $CHCl_3$ (5 x 20 mL), the combined organic phases were dried over anhyd Na_2SO_4, the solvent was removed under aspirator pressure, and the residual solvent was removed under vacuum to yield 0.3542 g (74%) of yellow oil **40**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.52 (s, 18H, $OC(CH_3)_3$), 2.64 (d, 2H, $^2J_{PH}$ = 11.9 Hz, NCH_2P), 2.61-2.66 (m, 4H, CH_2N), 3.69 (AA′ of AA′XX′, 4H, CH_2OCH_2); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125.68 MHz) δ 29.52 (d, $^3J_{PC}$ = 3.8 Hz, $POC(CH_3)_3$); 54.02 (d, $^3J_{PC}$ = 10.6 Hz, CH_2NCH_2P); 56.53 (d, $^1J_{PC}$ = 167.9 Hz, NCH_2P); 66.04 (CH_2O); 81.14 (d, $^2J_{PC}$ = 8.6 Hz, POC); (The ^{1}H and $^{13}C\{^1H\}$ spectra indicated the presence of 0.17 ± 0.01 molar equivalents of residual di-*tert*-butyl phosphite relative to **40**); IR (CCl_4) 3448, 2979, 2934, 2854, 2803, 1664, 1453, 1417, 1370, 1259, 1172, 1119,

984; HRFABMS, *m/z* $(M+H)^+$ exact mass calcd for $C_{13}H_{29}O_4NP$: 294.1834; Found: 294.1815 (error -1.9 mmu/-6.6 ppm).

1,4,7,10-Tetraazabicyclo[5.5.2]tetradecane-4,10-bis(methanephosphonic acid monoethyl ester sodium salt) (47·1.5H_2O·NaOH). 1,4,7,10-Tetraazabicyclo[5.5.2]tetradecane-4,10-bis(methanephosphonic acid diethyl ester) (0.0908 g, 0.1821 mmol; contained 0.1 ± 0.01 molar equivalents of 1,4,7,10-tetraazabicyclo[5.5.2]tetradecane-4-methanephosphonic acid diethyl ester and 0.03 ± 0.01 molar equivalents of triethyl phosphate relative to **47**) and sodium hydroxide (0.0291 g, 0.728 mmol) were dissolved in H_2O (2 mL) and heated to reflux for 24 h. The solvent was removed under reduced pressure, the crude was dissolved in cold methanol (1 mL) and removed from insoluble solids (sodium hydroxide). The methanol was removed under reduced pressure and residual solvent was removed under vacuum to yield white solid **47·1.5H_2O·NaOH** (0.0835 g, 80% yield): ^{1}H NMR (D_2O, 500 MHz, internal ref CH_3CN set to δ 2.06) δ 1.41 (t, 6H, J = 7.1 Hz, P(O)OCH_2CH_3), 3.08-3.20 (m, 7H), 3.17 (d, 4H, $^2J_{PH}$ = 7.1 Hz, NCH_2P), 3.22-3.41 (m, 7H), 3.23 (s, 4H, NCH_2CH_2N, cross-bridge), 4.08 (p, 4H, J = 7.1 Hz, P(O)OCH_2CH_3); $^{13}C\{^1H\}$ NMR (D_2O, 125.68 MHz, internal ref CH_3CN set to δ 1.47) δ 16.80 (d, $^3J_{PC}$ = 5.8 Hz, P(O)OCH_2CH_3), 48.02, 48.72 (d, $^1J_{PC}$ = 140.1 Hz, NCH_2P), 51.28, 54.85 61.05 (d, $^2J_{PC}$ = 5.8 Hz, P(O)OCH_2CH_3); $^{31}P\{^1H\}$ NMR (D_2O, 161.83 MHz) 23.60; (The $^{31}P\{^1H\}$ spectrum indicated the presence of 0.07 ± 0.02 molar equivalents of 1,4,7,10-tetraazabicyclo[5.5.2]tetradecane-4-methanephosphonic acid monoethyl ester sodium salt relative to desired product of 1.0 molar equivalents); IR (KBr) 3423, 2974, 2927, 2894,

2863, 1482, 1389, 1171, 1065, 1050, 943; HRFABMS, *m/z* $(M+H)^+$ exact mass for $C_{16}H_{35}N_4O_6P_2Na_2$: 487.1827; Found: 487.1826 (error -0.2 mmu/-0.3 ppm); Anal. Calcd for $C_{16}H_{35}N_4O_6P_2Na_2 \cdot (C_{13}H_{28}N_4O_3PNa)_{0.05} \cdot (H_2O)_{1.5} \cdot (NaOH)$: C, 35.05; H, 6.96; N 10.31. Found: C, 34.98; H, 7.24; N, 10.16.

1,4,8,11-Tetraazabicyclo[6.6.2]hexadecane-4,11-bis(methanephosphonic acid monoethyl ester sodium salt) (48). 1,4,8,11-Tetraazabicyclo[6.6.2]hexadecane-4,11-bis(methanephosphonic acid diethyl ester) (0.0511 g, 0.0970 mmol, **37**) was dissolved in a 0.4 M NaOH solution (3 mL) and the mixture was brought to reflux for 1 day. The solvent was removed under reduced pressure and the residue was washed with 0 °C methanol (1 mL), the solvent was removed under reduced pressure and residual solvent was removed under vacuum. The crude product was dissolved in 3 mL MeOH and the pH was lowered to 7 with 1 mL of 1M HCl solution. White solids were separated by centrifugation, and the pH of the supernatant was raised to 14 with crushed NaOH. The solvent was removed under reduced pressure and residual solvent was removed under vacuum to yield white solid **2** (0.1433 g, 71%): ^{1}H NMR (D_2O, 500 MHz, internal ref CH_3CN set to δ 2.06) δ 1.23 (t-like, 6H, J = 7.0 Hz, P(O)OCH$_2$C*H*$_3$), 1.60-1.76 (m, 4H), 2.79-3.17 (m, 22H), 3.38-3.49 (m, 2H), 3.90 (p, 4H, J = 7.1, P(O)OC*H*$_2$CH$_3$); ^{13}C{^{1}H} NMR (D_2O, 100.53 MHz, internal ref CH_3CN set to δ 1.47) δ 17.09 (d, $^3J_{PC}$ = 5.4 Hz, P(O)OCH$_2$*C*H$_3$), 24.69, 49.69 (d, $^1J_{PC}$ = 140.0 Hz, N*C*H$_2$P), 52.17, 52.84 (d, $^3J_{PC}$ = 5.4 Hz), 53.12, 56.56, 56.97, 61.20 (d, $^2J_{PC}$ = 6.1 Hz, P(O)O*C*H$_2$CH$_3$); ^{31}P{^{1}H} NMR (D_2O, 161.83) δ 21.98; IR (KBr) 3448, 1645, 1450, 1392, 1318, 1175, 1086; HRFABMS, *m/z* $(M+Na)^+$ exact mass for $C_{18}H_{38}N_4O_6P_2Na_3$: 537.1960; Found: 537.1970 (error +1.0

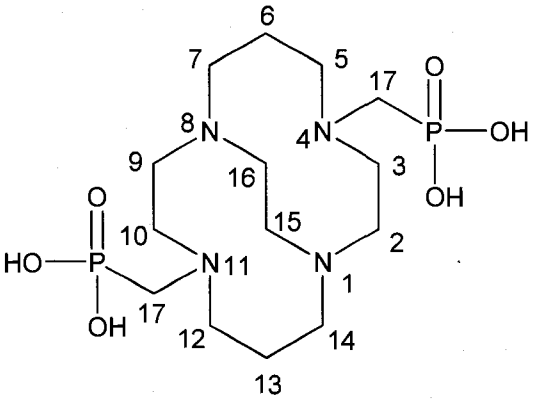
mmu/+1.8 ppm); Anal Calcd for $C_{18}H_{38}N_4O_6P_2Na_2 \cdot (NaOH)_{24} \cdot (H_2O)_{20} \cdot (NaCl)_{0.3} \cdot (CH_3OH)_7$: C, 14.46; H, 6.31; N, 2.70; Cl, 0.51. Found: C, 14.60; H, 6.02; N, 2.30; Cl, 0.64. (Titration of **48** with 0.101 N HCl revealed 19 ± 5 molar equivalents of NaOH relative to 1 molar equivalents of **48**)

1,4,7,10-Tetraazabicyclo[5.5.2]tetradecane-4,10-bis(methanephosphonic acid) (50·2HCl·3H$_2$O). This compound was prepared previously (U.S. patent 5874573, 1999) and listed with an incomplete procedure. The procedure reported here is similar to a procedure reported by Sherry *et al.* (Inorg. Chem. **1992**, 31(21) 4422-4). 1,4,7,10-tetraazacyclo[5.5.2]tetradecane (0.0828 g, 0.418 mmol) and H_3PO_3 (0.1370 g, 1.671 mmol) were dissolved in 4 M HCl (0.8 mL) and brought to reflux. Paraformaldehyde (0.0376 g, 2.50 molar equivalents of CH_2O) was added in small portions over the course of 1 hour. The reaction was allowed to stay at reflux for an additional 30 min, the heat was removed, the reaction mixture was cooled to room temperature, acetone (7 mL) was added in one portion and the cloudy white mixture was allowed to stand for 24 h. The white crystalline solids were isolated by suction filtration, washed with hot acetone (2 x 6 mL), and dried under vacuum to yield 0.1493 g (68%) of **50·2HCl·3H$_2$O**: ^{1}H NMR (D_2O, 500 MHz, internal ref CH_3CN set to δ 2.06) δ 3.17-3.28 (m, 4H), 3.20 (s, 4H, NCH_2CH_2N, cross-bridge), 3.37-3.52 (m, 8H), 3.40 (d, 4H, $^2J_{PH}$ = 10.8 Hz, NCH_2P), 3.57-3.63 (m, 4H); $^{13}C\{^1H\}$ NMR (D_2O, 125.68 MHz, internal ref CH_3CN set to δ 1.47) δ 46.63 54.66 (d, $^1J_{PC}$ = 146.8 Hz, NCH_2P), 54.78, 56.06 (d, $^3J_{PC}$ = 4.8 Hz, C_3); $^{31}P\{^1H\}$ NMR (D_2O, 161.83 MHz) δ 14.30; (The ^{1}H and $^{31}P\{^1H\}$ NMR spectra indicated the presence of 0.04 ± 0.02 molar equivalents of H_3PO_3 and 0.08 ± 0.01 molar equivalents of

1,4,7,10-tetraazabicyclo[5.5.2]tetradecane-4-(methanephosphonic acid) dihydrochloride relative to **50**. Attempted purification by ion-exchange chromatography failed to separate these impurities.); IR (KBr) 3410, 3008, 2968, 2926, 2754, 2627, 1499, 1488, 1415, 1325, 1144, 1096, 945; HRFABMS, *m/z* $(M+H)^+$ exact mass calcd for $C_{12}H_{29}N_4O_6P_2$: 387.1562; Found: 387.1538 (error -2.5 mmu/-6.4 ppm); Anal. Calcd for $C_{12}H_{28}N_4O_6P_2 \cdot (HCl)_2 \cdot (H_2O)_3 \cdot [C_{11}H_{25}N_4O_6P \cdot (HCl)_2]_{0.04} \cdot (H_3PO_3)_{0.02}$: C, 28.22; H, 7.07; N 11.00; Cl, 13.93. Found: C, 28.36; H, 6.84; N, 10.88; Cl, 14.02.

1,4,8,11-Tetraazabicyclo[6.6.2]hexadecane-4,11-bis(methanephosphonic acid) ($H_4$51·0.6HCl·1.1H_2O). 0.3440 g (0.6533 mmol) of **37** was dissolved in 32 mL of a 6M HCl solution prepared from TraceMetal™ grade hydrochloric acid (Fisher Scientific), and refluxed under N_2 for 1 d. The solvent was removed under reduced pressure and the crude product was dissolved in 4 mL of H_2O and chromatographed using a weakly acidic cation exchange column (Amberlite CG50, H^+ form, 7.7 g, column size = 27 cm x 2 cm). The product was eluted with H_2O (first 30 mL of elutant was discarded), fractions containing the product were combined (35 fractions, 3 mL each), the solvent was removed under reduced pressure, and residual solvent was removed under vacuum to yield 0.1919 g (64%) of white solid **$H_4$51·0.6HCl·1.1H_2O**: mp 182-185 °C; ^{1}H NMR (D_2O, 500 MHz, internal ref CH_3CN set to δ 2.06) δ 1.74-1.83 (dm, 2H, J = 16.6 Hz, H_{6eq}), 2.29-2.42 (qm-like, 2H, H_{6ax}), 2.49-2.58 (dm, 2H, J = 13.9 Hz, H_{2eq}), 2.80-2.93 (m, 4H, $H_{15\ BB'\ of\ AA'BB'}$, H_7), 2.95-3.18 (m, 8H, $H_{15\ AA'\ of\ AA'BB'}$, H_7, H_{3eq}, NC*H*HP), 3.29-3.37 (dm, 2H, J = 13.2 Hz, H_{5eq}), 3.45 (td, 2H, J = 13.7, 3.7 Hz, H_{2ax}), 3.76 (tt_{app}, 2H, J = 14.6, 3.7 Hz, H_{3ax}), 3.96 (td, 2H, J = 13.0, 3.4 Hz, H_{5ax}), 4.17 (t_{app}, 2H, $^2J_{HH}$ = -14.6 Hz, $^2J_{PC}$ =

-14.6 Hz, NCH*H*P); ^{13}C {^{1}H} NMR (D_2O, 125.68 MHz, internal ref CH_3CN set to δ 1.47) δ 20.23 (C_6), 48.75 (C_{15}), 52.01 ($^{1}J_{PC}$ = 137.2 Hz, NCH_2P), 52.34 ($^{3}J_{PC}$ = 9.6 Hz, C_3), 52.64 (C_2), 58.17 (br, C_5), 58.47 (C_7) [Note: ^{1}H-COSY and gHMQC experiments aided resonance assignments (see figure below for compound numbering)]; ^{31}P {^{1}H} NMR (D_2O, 161.83 MHz) δ 9.48; IR (KBr) 3397 (br), 2983, 2851, 1655, 1491, 1470, 1241, 1183, 1097, 1073, 1047, 903; HRFABMS, *m/z* $(M+H)^+$ exact mass for $C_{14}H_{33}N_4O_6P_2$: 415.1875; Found: 415.1877 (error -1.2 mmu/-2.9 ppm); Anal. Calcd for $C_{14}H_{32}N_4O_6P_2 \cdot (HCl)_{0.6} \cdot (H_2O)_{1.1}$: C, 36.87; H, 7.69; N 12.28; Cl, 4.66. Found: C, 36.56; H, 7.84; N, 12.06; Cl, 4.81. Diffusion of acetone into an aqueous solution of **$H_4$51·0.6HCl·1.1H_2O** produced crystals suitable for X-ray diffraction.



Numbering for H_4-CB-TE2P

52. A solution of $Cu(ClO_4)_2 \cdot 6H_2O$ in 3.5 mL MeOH (0.2341 g, 0.6318 mmol) was added to a 7.7 mL MeOH solution of **47** (0.3235 g, 0.5670 mmol; contained 0.07 ± 0.02 molar equivalents of 1,4,7,10-tetraazabicyclo[5.5.2]tetradecane-4-methanephosphonic acid monoethyl ester sodium salt relative to 1.0 molar equivalents of **47**). Aq NaOH (0.3 mL of a 0.4 M NaOH solution) was added to raise the pH of the solution to 8 and the solution was brought to reflux for 24 h. The solution was allowed to cool to room

temperature and insoluble solids were removed by centrifugation. Diethyl ether (30 mL) was added to the supernatant and blue solids formed after the solution was stirred for 20 min. X-ray quality crystals were obtained from Et_2O diffusion into a 95% EtOH solution of the product. **52** was collected and dried under vacuum (0.3165 g, 84% yield). X-ray quality crystals were obtained from Et_2O diffusion into a 95% EtOH solution of the product: Anal Calcd for $C_{16}H_{34}N_4O_6P_2Cu\cdot(NaClO_4)_{1.1}\cdot(H_2O)_{1.3}\cdot[C_{13}H_{28}N_4O_3PCu\cdot(ClO_4)]_{0.05}$: C, 29.14; H, 5.58; N, 8.57; Cl, 5.94. Found: C, 29.18; H, 5.54; N, 8.42; Cl, 6.17 (Elemental analysis indicated the presence of 0.05 molar equivalents of 1,4,7,10-tetraazabicyclo[5.5.2]tetradecane-4-methanephosphonic acid monoethyl ester copper (II) complex); HRFABMS, m/z $(M+H)^+$ exact mass $C_{16}H_{35}N_4O_6P_2\cdot Cu$: 504.1339; Found: 504.1338 (error +1.0 mmu/+1.9 ppm); IR (KBr) 3397, 2976, 2899, 1653, 1483, 1199, 1086, 1035, 948, 857, 787, 624, 577; Electronic data: (MeOH) λ_{max}, 672 nm ($\varepsilon = 44\ M^{-1}\ cm^{-1}$).

53. A solution of $Cu(ClO_4)_2\cdot 6H_2O$ in 2.0 mL MeOH (0.0685 g, 0.185 mmol) was added to a 6 mL MeOH solution of **48** (0.1001 g, 0.0482 mmol, contained 24 molar equivalents of NaOH, 20 molar equivalents of water, 0.3 molar equivalents of NaCl and 7 molar equivalents of methanol all relative to 1 molar equivalent of **48**). Aq HCl (0.3 mL of a 1.0 M HCl solution) was added to lower the pH of the solution to 8 and the solution was brought to reflux for 24 h. The solution was allowed to cool to room temperature and insoluble solids were removed by centrifugation. The resultant blue solution was evaporated to dryness, 1,1,1,3,3,3-hexafluoro-2-propanol was added, and solid NaCl was removed by centrifugation. The solvent was removed under reduced pressure and residual

solvent was removed under vacuum to yield 0.0300 g (82% yield) of **53**: Anal Calcd for $C_{18}H_{38}N_4O_6P_2Cu\cdot(C_3H_2F_6O)_1\cdot(H_2O)_2$: C, 34.27; H, 6.02; N, 7.61. Found: C, 34.26; H, 5.75; N, 7.29. HRFABMS, m/z $(M+H)^+$ exact mass $C_{18}H_{39}N_4O_6P_2\cdot Cu$: 532.1652; Found: 532.1664 (error +2.4 mmu/+4.4 ppm); IR (KBr) 3569, 3411, 2981, 2904, 1483, 1210, 1097, 1071, 1041, 949, 787, 625; Electronic data: (MeOH) λ_{max}, 646 nm (ε = 18 M^{-1} cm^{-1}).

54. A solution of $CuCl_2\cdot 2H_2O$ in 2 mL MeOH (13.2 mg, 0.077 mmol) was added to a 2 mL MeOH solution of **51** (35.0 mg, 0.077 mmol). Aq NaOH (0.3 mL of a 0.4 M NaOH solution) was added to raise the pH of the solution to 5. The resultant blue solution was evaporated to dryness, 1 mL of 1,1,1,3,3,3-hexafluoro-2-propanol was added, and solid NaCl was removed by centrifugation. The supernatant was placed in an Et_2O diffusion chamber, resulting in formation of blue crystals which were collected and dried (47.0 mg, 86% yield). Anal Calcd for $C_{14}H_{30}N_4O_6P_2Cu\cdot(NaCl)_{0.3}\cdot(H_2O)\cdot(C_3H_2F_6O)_{1.2}$: C, 29.64; H, 4.86; N, 7.86; Cl, 1.49; P, 8.69. Found: C, 29.48; H, 4.96; N, 7.91; Cl, 1.78; P, 8.66. HRFABMS, m/z $(M+H)^+$ exact mass $C_{14}H_{31}N_4O_6P_2\cdot Cu$: 476.1015; Found: 476.1009 (error -0.6 mmu/-1.2 ppm); IR (KBr) 3421 (br), 2867, 1654, 1482, 1150, 1095, 1055, 972. Electronic data: (MeOH) λ_{max}, 639 nm (ε = 35 M^{-1} cm^{-1}); Cyclic voltammetry conducted in 0.1 N sodium acetate, reduction was quasi-reversible with E_p = -0.96 V (Ag/AgCl).

4-(Carbamoylmethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (64). A solution of 2-bromoacetamide (0.6365 g, 4.613 mmol) in dry MeCN (20 mL) was added

dropwise to a solution of **8** (1.0432 g, 4.6085 mmol) in dry MeCN (60 mL) under N_2, over the course of 20 min. The resulting solution was stirred at room temperature for 1 day. The solvent was removed, the residue was dissolved in H_2O (60 mL), and the solution was made strongly basic (pH=14, slow addition of KOH pellets with cooling). The resulting solution was extracted with $CHCl_3$ (5 x 25 mL), the combined organic phases were dried over anhyd Na_2SO_4, the solvent was removed via aspirator pressure, and residual solvent was removed from the crude viscous oil under vacuum for 2 days. The resulting white solid was recrystallized from MeCN affording 1.0980 g (84%) of white solid **64**: mp 137-139 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.33-1.43 (m, 1H), 1.49-1.59 (m, 1H), 1.63-1.82 (m, 2H), 2.45-2.80 (m, 19H), 2.86-2.94 (m, 1H), 2.92 and 3.18 (AB, 2H, J = 16.0 Hz, NC*H*$_2$CONH$_2$), 3.03-3.13 (m, 1H), 4.93 (br s, 1H, amide N*H*), 5.60 (br s, 1H, amide N*H*), 8.51 (br s, 1H, *sec* N*H*); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ 24.39, 25.58, 45.88, 49.91, 50.33, 52.28, 53.84, 53.90, 54.14, 54.42, 56.05, 58.59, 59.93, 174.17; IR (KBr) 3446 (br), 2948, 2919, 2855, 2824, 1677 (C=O, amide I), 1545 (NH_2 bend, amide II) cm^{-1}; MS (ESI) *m/z* 284.4 (M+H$^+$); Anal. Calcd for $C_{14}H_{29}N_5O\cdot(H_2O)_{0.1}$: C, 58.96; H, 10.32; N, 24.55. Found: C, 58.62; H, 10.28; N, 24.62.

67. A solution of **64** (0.0531 g, 0.1874 mmol) and $Cu(NO_3)_2\cdot 3H_2O$ (0.0453 g, 0.1875 mmol) was refluxed in 6 mL of MeOH for 1 d. The resulting blue solution was cooled to room temperature, centrifuged, and the supernatant was put in an Et_2O diffusion chamber. The resulting blue solids were suitable for X-ray analysis (0.0700 g, 79% yield): Anal Calcd for $C_{14}H_{29}N_7O_7\cdot Cu$: C, 35.70; H, 6.21; N, 20.82. Found: C, 35.76; H, 6.02; N, 20.86; HRFABMS, m/z [$Cu(NO_3)\cdot$**64**]$^+$ exact mass $C_{14}H_{29}N_6O_4\cdot Cu$:

408.1546; Found: 408.1526 (error -2.1 mmu/-5.1 ppm); IR (KBr) 3244, 3063, 2969, 2879, 2771, 1664, 1602, 1463, 1383, 1175, 1103, 1014, 825, 703. Electronic data: (MeOH) λ_{max}, 600 nm (ε = 88.5 M^{-1} cm^{-1}), 974 nm (ε = 55.8 M^{-1} cm^{-1}); Cyclic voltammetry conducted in 0.1 N sodium acetate, reduction was quasi-reversible with E_p = -0.76 V (Ag/AgCl).

4-(*N*-methylcarbamoylmethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (68). A solution of Chloro-*N*-methylacetamide (0.0770 g, 0.7160 mmol) in dry MeCN (2 mL) was added dropwise to a stirred solution of **8** (0.1621 g, 0.7161 mmol) in dry MeCN (10 mL) under N_2 over the course of 20 min. The resulting solution was stirred at room temperature for 1 day. The solvent was removed, the residue was dissolved in H_2O (20 mL), and the solution was made strongly basic (pH=14, slow addition of KOH pellets with cooling). The resulting solution was extracted with $CHCl_3$ (5 x 20 mL), the combined organic phases were dried over anhyd Na_2SO_4, the solvent was removed under aspirator pressure, and residual solvent was removed from the crude viscous oil under vacuum for 2 days (the crude ^1H spectrum indicated the presence of 0.09 ± 0.01 molar equivalents of 4,11-bis(*N*-methylcarbamoylmethyl)-1,4,8,11-tetraazabycyclo[6.6.2]hexadecane. The resulting yellow solid was recrystallized from MeCN affording 0.1620 g (72% yield) of white solid **68**: ^1H NMR ($CDCl_3$, 500 MHz) δ 1.36-1.45 (m, 1H), 1.49-1.64 (m, 2H), 1.67-1.76 (m, 1H), 2.47-2.96 (m, 19H), 2.83 (s, 3H, C(O)NHCH_3), 2.96 and 3.15 (AX, 2H, *J* = 15.9 Hz, NCH_2C(O)NHCH$_3$), 3.07-3.15 (m, 1H), 5.11 (br s, 1H, amide N*H*), 8.36 (br s, 1H, N*H*); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125.68 MHz) δ 24.82, 25.78, 46.36, 50.02, 50.69, 51.58, 53.66, 54.02 (2*C*), 54.47, 54.74, 55.98,

58.34, 60.44, 171.56 (The ^{1}H spectrum indicated the presence of 0.03 ± 0.01 of 4,11-bis(*N*-methylcarbamoylmethyl)-1,4,8,11-tetraazabycyclo[6.6.2]hexadecane); IR (KBr) 3274, 3075, 2916, 2807, 1654, 1560, 1460, 1411, 1114; HRFABMS, *m/z* ($M+H^+$) exact mass for $C_{15}H_{32}N_5O$: 298.2607; Found : 298.2594 (error -1.3 mmu/ -4.2 ppm).

4-Acetoamido-11-carbo-*t*-butoxymethyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (71). *t*-Butyl bromoacetate (0.0685 g, 0.3512 mmol) was added to a solution of 4-(carbamoylmethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (0.0663 g, 0.2339 mmol) in 2 mL of dry MeCN and heated to 70 °C in a closed vessel (10 mL tube) microwave reactor for 30 min. After this reaction period, the mixture was cooled to room temperature and the solvent was removed under reduced pressure. The residue was dissolved in H_2O (3 mL), washed with Et_2O (3 x 3 mL) and the solution was made strongly basic (pH=14, slow addition of KOH pellets with cooling). The resulting solution was extracted with cold $CHCl_3$ (5 x 5 mL), the combined organic phases were dried over anhyd Na_2SO_4, the solvent was removed under aspirator pressure, and the residual solvent was removed under vacuum to yield **71** (0.0746 g, 80% yield); (^{1}H spectrum indicated the presence of 5% (+/- 1%) of 4-(carbamoylmethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane); Spectra previously reported: Sprague, J.E.; Peng, Y.; Fiamengo,A.L.; Woodin, K.S.; Southwick, E.A.; Weisman, G.R.; Wong, E.H.; Golen, J.A.; Rheingold, A.L.; Anderson, C.J.; *J. Med. Chem.* **2007**, *50*, 2527-2535.

4-Carboxymethyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane Dihydrochloride Trihydrate (72·2.3HCl·2.8H_2O·NH_4Cl). Compound **64** (0.0495 g, 0.1747 mmol) was dissolved in 6 N HCl (9.5 mL) and raised to 150 °C in a closed vessel microwave reactor for 30 min. The solvent was removed under reduced pressure. The crude was dissolved in 15 mL of H_2O and the solvent was removed via reduced pressure. This was carried out three times. Residual solvent was removed from the white solid under vacuum (0.0810 g, 98% yield); ^{1}H NMR (D_2O, 500 MHz, internal ref CH_3CN set to δ 2.06) δ 1.69-1.78 (m, 2H, $NCH_2CH_{eq}HCH_2N$), 2.29-2.48 (m, 2H, $NCH_2CH_{ax}HCH_2N$), 2.57-2.70 (ddm, 1H, J = 14.2, 3.2 Hz), 2.66 (ddd, 1H, J = 13.0, 9.0, 3.7 Hz), 2.87-3.04 (m, 5H), 3.08-3.37 (m, 8H), 3.40-3.51 (m, 2H), 3.53-3.62 (m, 3H), 3.67 and 4.22 (AX, 2H, J = 17.6 Hz, NCH_2COOH); $^{13}C\{^1H\}$ NMR (D_2O, 100 MHz, internal ref CH_3CN set to δ 1.47) δ 18.63, 19.31, 41.50, 47.99, 48.64, 48.79, 49.04, 54.77, 54.99, 55.50, 57.73, 58.35, 59.03, 172.25; IR (KBr) 3406, 3186, 3093, 2988, 2956, 2840, 2753, 2636, 2363, 1728, 1491, 1480, 1403, 1354, 1241, 1044 cm^{-1}; MS (LDI) *m/z* 285.1 ($M+H^+$); Anal. Calcd for $C_{14}H_{28}N_4O_2 \cdot (HCl)_{2.3} \cdot NH_4Cl \cdot (H_2O)_{2.8}$: C, 35.61; H, 8.52; N, 14.83; Cl, 24.78. Found: C, 35.90; H, 8.20; N, 14.80; Cl, 24.92.

4-Acetoxymethyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane potassium salt (73). 4-(Carbamoylmethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (0.0920 g, 0.3246 mmol) was dissolved in 6 N HCl (6.8 mL) and raised to 150 °C in a closed vessel microwave reactor for 30 min. The solvent was removed under reduced pressure. The crude product was dissolved in 10 mL H_2O and made strongly basic (pH=14, slow addition of a large excess of KOH pellets). The resulting solution was extracted with

chloroform (5 x 10 mL), the combined organic fractions were dried over anhyd Na_2SO_4, the solvent was removed under aspirator pressure and the residue was repeatedly triturated with Et_2O followed by removal of Et_2O under reduced pressure (6 x 10 mL). Residual solvent was removed under vacuum to yield 0.0656 g (63% yield) of white solid **73**: mp = 156-160 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.47-1.57 (m, 1H), 1.62-1.70 (m, 1H), 1.71-1.89 (m, 2H), 2.36 (ddd, 1H, *J* = 11.1, 6.3, 4.8 Hz), 2.45 (ddd, 1H, *J* = 13.2, 6.2, 2.1 Hz), 2.57-2.65 (m, 2H), 2.65-2.87 (m, 12H), 2.92 (ddd, 1H, *J* = 13.8, 8.8, 6.5 Hz), 2.97-3.05 (m, 1H), 3.00 and 3.20 (AX, 2H, *J* = 13.7 Hz, benzylic C*H*$_2$), 3.13-3.23 (m, 1H), 3.27-3.38 (m, 1H), 12.02 (br s, 1H, N*H*); ^{13}C{^{1}H} NMR ($CDCl_3$, 125.68 MHz) δ 23.70, 26.73, 44.79, 46.90, 49.60, 52.39, 52.61, 53.18, 54.62, 55.60, 55.75, 58.53, 64.48, 175.92; IR (KBr) 3415, 2958, 2850, 1646, 1570, 1484, 1400, 1319, 1063; HRFABMS, *m/z* $(M+H)^+$ exact mass for $C_{14}H_{28}N_4O_2K$: 323.1849; Found: 323.1833 (error -1.6 mmu/-5.0 ppm).

4-(Carbamoylmethyl)-11-benzyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (74). A solution of benzyl bromide (0.0279 g, 0.1631 mmol) in dry MeCN (0.5 mL) was added to a stirred solution of **64** (0.0309 g, 0.1090 mmol) in dry MeCN (1.0 mL) at 70 °C. The solution was brought to reflux and stirred under N_2 for 24 h. The solvent was removed under aspirator pressure and the crude residue was dissolved in MeOH (4 mL) and made strongly basic (pH=14, slow addition of KOH pellets). The solvent was removed, the crude white solid was dissolved in H_2O (4 mL) and extracted with $CHCl_3$ (6 x 5 mL), and the combined organic phases were dried over anhyd Na_2SO_4. The solvent was removed under aspirator pressure and residual solvent was removed under vacuum to

yield white solid **74** (0.0400 g, 98%): mp 94-97 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.36-1.66 (m, 4H), 2.30-2.50 (m, 10H), 2.54-2.68 (m, 4H), 2.70-2.77 (m, 1H), 2.82-2.95 (m, 2H), 2.90 and 3.15 (AX, 2H, *J* = 16.9 Hz), 3.21-3.28 (m, 1H), 3.74 and 3.25 (AX, 2H, *J* = 13.3 Hz), 3.69-3.78 (m, 1H), 4.17 (ddd, 1H, *J* = 13.8, 9.7, 4.4 Hz), 6.06 (br s, 1H, amide N*H*), 7.18-7.25 (m, 2H), 7.27-7.34 (m, 4H); ^{13}C{^{1}H} NMR ($CDCl_3$, 125.68 MHz) δ 27.74 (2*C*), 52.79, 53.21, 53.71, 53.84, 56.15, 56.44, 56.63, 57.46, 57.87, 58.22, 60.09, 60.62, 126.61, 128.04 (2*C*), 128.99 (2*C*), 140.62, 175.56; IR (KBr) 3442, 3326, 3252, 3176, 3059, 3025, 2917, 2802. 2784, 1702, 1677, 1452, 1360, 1260, 1117; HRFABMS, *m/z* ($M+H^+$) exact mass for $C_{21}H_{36}N_5O$: 374.2920; Found : 374.2919 (error -1.4 mmu/ -3.7 ppm).

4-Benzyl-11- carboxymethyl -1,4,8,11-tetraazabicyclo[6.6.2]hexadecane Dihydrochloride Monohydrate (75·2.5HCl·1.5H_2O·NH_4Cl). Compound **74** (0.0241 g, 0.0645 mmol) was dissolved in 6 N HCl (2 mL) and heated to 150 °C in a closed vessel (10 mL tube) microwave reactor for 30 min. After this reaction period, the mixture was cooled to room temperature and the solvent was removed under reduced pressure. The residue was dissolved in 15 mL of H_2O and the solvent was removed under reduced pressure. This process was carried out two additional times. Residual solvent was removed under vacuum to yield white solid product (0.0333 g, 94% yield): ^{1}H NMR (D_2O, 500 MHz, internal ref CH_3CN set to δ 2.06) δ 1.76-1.85 (dm, 2H, *J* = 16.6 Hz, $NCH_2CH_{eq}HCH_2N$), 2.27-2.39 (qm-like, 1H, $NCH_2CH_{ax}HCH_2N$), 2.39-2.51 (qm-like, 1H, $NCH_2CH_{ax}HCH_2N$) 2.61-2.70 (dm, 1H, *J* = 14.4 Hz), 2.75 (ddd, 1H, *J* = 14.7, 9.2, 4.6 Hz), 2.88-3.50 (m, 15H), 3.59-3.82 (m, 4H), 3.78 and 4.27 (AX, 2H, *J* = 17.6 Hz),

4.56 and 4.58 (AB, 2H, J = 14.2 Hz), 7.40-7.46 (m, 2H), 7.51-7.58 (m, 3H); $^{13}C\{^{1}H\}$ NMR (D_2O, 125 MHz, internal ref CH_3CN set to δ 1.47) δ 19.81, 19.91, 46.06, 48.71, 49.05, 49.49, 54.01, 54.94, 55.97, 56.33, 56.81, 58.10, 58.30, 58.85, 127.81, 129.91 (2*C*), 130.85, 132.06 (2*C*), 172.10; IR (KBr) 3386, 3155, 3043, 2918, 2850, 2618, 1725, 1494, 1457, 1406, 1051, 707; MS (ESI) *m/z* 375.5 ($M+H^+$); Anal. Calcd. for $C_{21}H_{34}N_4O_2 \cdot (HCl)_{2.5} \cdot (NH_4Cl) \cdot (H_2O)_{1.5}$: C, 46.18; H, 8.03; N, 12.82; Cl, 22.72. Found: C, 46.00; H, 8.12; N, 12.57; Cl, 23.03.

4-(2-carbamoylethyl)-11-(carbamoylmethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (76) Compound **64** (0.0470 g, 0.1658 mmol) was added to a stirred solution of acrylamide (0.0140 g, 0.1969 mmol) in MeCN (3.5 mL) under N_2. After 5 days, white precipitate had formed. Solvent was removed under aspirator pressure and the crude product was washed with hot EtOAc to remove excess acrylamide. Product was isolated as a white solid (0.0584 g, 99%): mp 154-156 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.40-1.70 (m, 4H), 2.26-2.74 (m, 18H), 2.75-3.01 (m, 3H), 2.94 and 3.13 (AB, 2H, J = 16.8 Hz), 3.99 (dt, 1H J = 13.1, 6.6 Hz), 4.07 (ddd, 1H, J = 12.7, 8.4, 5.1 Hz), 5.91 (br s, 1H, amide N*H*), 6.23 (br s, 1H, amide N*H*), 7.17 (br s, 1H, amide N*H*), 7.42 (br s, 1H, amide N*H*); $^{13}C\{^{1}H\}$ NMR ($CHCl_3$, 100 MHz) δ 25.53, 26.19, 32.64, 51.06, 51.28, 52.31 (2*C*), 52.59, 53.99, 54.54, 54.64, 55.34, 57.37, 57.60, 59.60, 174.47, 174.52; IR (KBr) 3452, 3395, 3322, 3261, 3178, 2962, 2902, 2794, 1689, 1667, 1460, 1405, 1359, 1253, 1119; HRFABMS, *m/z* ($M+H^+$) exact mass for $C_{17}H_{35}N_6O_2$: 355.2821; Found: 355.2836 (error +1.5 mmu/ +4.1 ppm); Anal. Calcd for $C_{17}H_{34}N_6O_2 \cdot (H_2O)_{0.5}$: C, 56.17; H, 9.70; N, 23.12. Found: C, 56.07; H, 9.46; N, 22.88.

(10bα,10cα)-Decahydro-3a-allyl-8a-[(4-nitrophenyl)methyl]-1H,6H-3a,5a,8a,10a-tetraazapyrenium dibromide (81). Allyl bromide (12.4 mL, 0.1433 mol) was added via syringe to a stirred solution of **80** (1.0470 g, 2.388 mmol) in 35 mL of MeCN. The flask was stoppered, wrapped in aluminum foil and the reaction mixture was stirred for 11 d. The resultant white precipitate was collected by vacuum filtration, washed with 40 mL of MeCN, and allowed to air dry on the funnel to yield 1.14 g (85% yield) of **81**: mp = 188-194 °C (dec); ^{1}H NMR (CD_3OD, 500 MHz) δ 1.81-1.89 (dm, 1H, J = 15.1 Hz, $NCH_2CH\mathit{H}_{eq}CH_2N$), 1.91-1.98 (dm, 1H, J = 15.0 Hz, $NCH_2CH\mathit{H}_{eq}CH_2N$), 2.20-2.32 (m, 1H, $NCH_2CH\mathit{H}_{ax}CH_2N$), 2.36-2.49 (1H, m, $NCH_2CH\mathit{H}_{ax}CH_2N$), 2.99 (td, 1H, J = 12.3, 3.4 Hz), 3.05 (td, 1H, J = 12.3, 3.2 Hz), 3.07-3.26 (m, 4H), 3.41 (dd, 1H, J = 14.2, 3.7 Hz), 3.61 (td, 1H, J = 13.6, 3.5 Hz), 3.73-3.83 (m, 2H), 3.94 (td, 1H, J = 13.1, 3.8 Hz), 4.40 (dd, 1H, J = 13.4, 7.6 Hz, C*H*HCH=CHH), 4.51-4.62 (m, 2H), 4.65 (dd, 1H, J = 13.4, 7.1 Hz, CH*H*CH=CHH), 4.93 and 5.55 (AX, 2H, J = 12.9 Hz, benzylic CH_2), 5.24 and 5.37 (AX, 2H, J = 1.6 Hz, $N_2C\mathit{H}C\mathit{H}N_2$), 5.85 (dd, 1H, J = 10.1, 0.8 Hz, $CH_2CH{=}CH\mathit{H}_E$), 5.99 (dd, 1H, J = 16.9, 1.0 Hz, $CH_2CH{=}CH\mathit{H}_Z$), 6.13-6.22 (m, 1H, $CH_2C\mathit{H}$=CHH), 6.17 (ddt, 1H, J = 17.2, 10.0, 7.3 Hz, $CH_2C\mathit{H}$=CHH), 7.91-7.95 (AA′ of AA′XX′, 2H), 8.35-8.39 (XX′ of AA′XX′, 2H); ^{13}C{^{1}H} NMR (CD_3OD, 125.68 MHz) δ 19.78 (2*C*), 47.27, 47.64, 47.90, 48.09, 52.22, 52.36, 61.32, 61.97, 62.02, 62.53, 77.05 (*C*H), 78.84 (*C*H), 124.12, 125.31 (2*C*), 131.15, 133.77, 136.06 (2*C*), 150.99; IR (KBr) 3404, 3106, 3056, 2981, 2946, 2914, 2852, 1606, 1518, 1346, 1123, 854, 708; HRFABMS, *m/z* (M^+) exact mass for $C_{22}H_{33}N_5O_2Br$: 478.1818; Found: 478.1810 (error -1.5 mmu/-0.7 ppm); Anal. Calcd for $C_{22}H_{33}N_5O_2Br_2$: C, 47.24; H, 5.95; N, 12.52; Br, 28.57. Found: C, 47.33; H, 5.95; N, 12.64; Br, 28.43.

4-(Allyl)-11-[(4-nitrophenyl)methyl]-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (82). (10bα,10cα)-Decahydro-3a-allyl-8a-[(4-nitrophenyl)methyl]-1H,6H-3a,5a,8a,10a-tetraazapyrenium dibromide (0.0504 g, 0.0901 mmol) (**81**) was added to a stirred solution of sodium cyanoborohydride (0.2260 g, 3.596 mmol) in 4.5 mL of dry MeCN. The solution was brought to reflux and stirred under N_2 for 48 h. The solvent was removed under aspirator pressure and 7 mL of a 25% HBr solution was added dropwise to the residue (the mono-HCl salt was found to be insoluble in H_2O). 2 mL of MeOH were added and the solution was made strongly basic (pH=14, slow addition of KOH pellets). The resulting solution was extracted with toluene (5 x 10 mL), the combined organic phases were dried over anhyd Na_2SO_4, the solvent was removed under aspirator pressure, and the residual solvent was removed under vacuum to yield **82** (0.0332 g, 92% yield), which solidified upon standing: mp = 58-60 °C; ^{1}H NMR (C_6D_6, 500 MHz) δ 1.25-1.51 (m, 4H), 2.12 (ddd, 1H, *J* = 13.4, 3.7, 2.1 Hz), 2.15-2.28 (m, 4H), 2.28-2.58 (m, 10H), 2.71-2.86 (m, 4H), 2.86 and 3.45 (AX, 2H, *J* = 14.3 Hz, benzylic C*H*$_2$), 3.11-3.20 (m, 2H), 3.27 (td, 1H, *J* = 12.3, 3.9 Hz), 3.64 (td, 1H, *J* = 11.7, 4.2 Hz), 4.09 (td, 1H, *J* = 11.6, 4.2 Hz), 5.05-5.09 (dm, 1H, *J* = ~10 Hz, CH_2CH=CH*H*$_E$), 5.12-5.18 (dm, 1H, *J* = ~17, CH_2CH=CH*H*$_Z$), 5.89 (dddd, 1H, *J* = 17.1, 10.3, 7.8, 4.9 Hz, CH_2C*H*=CHH), 7.00-7.04 (m, 2H, aromatic *H*), 7.87-7.90 (m, 2H, aromatic *H*); ^{13}C{^{1}H} NMR (C_6D_6, 125.68 MHz) δ 28.42, 28.71, 51.89, 51.95, 54.26, 54.70, 57.14, 57.30, 57.74, 57.82, 57.99, 58.01, 58.31, 59.60, 116.29, 123.46 (2*C*), 129.34 (2*C*), 137.50, 147.36, 148.49 (Although the ^{1}H NMR did not reveal significant impurities other than silicon grease, ^{13}C{^{1}H} NMR showed the presence of impurities at the ≤ 5% level); IR (C_6D_6) 3417, 2959, 2917, 2849, 2800, 1606, 1520, 1456, 1344, 1260, 1115, 1020;

HRFABMS, *m/z* ($M+H^+$) exact mass for $C_{22}H_{36}O_2N_5$: 402.2869; Found: 402.2885 (error +1.6 mmu/+ 4.1 ppm).

4-benzyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecyl-11-(methanephosphonic acid diethyl ester) (88). Triethyl phosphite (0.0354 g, 0.213 mmol) and paraformaldehyde (6.4 mg, 2.5 molar equivalents of CH_2O) were added to a solution of 4-benzyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (0.0450 g, 0.142 mmol) (**61**) in 1.0 mL of THF (freshly distilled from benzophenone ketyl) and the mixture was stirred under N_2 for 4 days. The solvent was removed under aspirator pressure and excess triethyl phosphite was removed under vacuum. The residue was dissolved in H_2O (10 mL), and the solution was made strongly basic (pH=14, slow addition of KOH pellets). The resulting solution was extracted with toluene (5 x 10 mL), the combined organic phases were dried over anhyd Na_2SO_4, the solvent was removed under aspirator pressure, and the residual solvent was removed under vacuum to yield 0.0564 g (85%) of product as clear oil **88**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.32 (t, 3H, J = 7.0 Hz, P(O)OCH_2CH_3), 1.33 (t, 3H, J = 7.0 Hz, P(O)OCH_2CH_3), 1.29-1.50 (m, 3H), 1.51-1.63 (m, 1H), 2.17-2.31 (m, 2H), 2.32-2.50 (m, 9H), 2.60-2.81 (m, 4H), 2.82-2.95 (m, 3H), 3.09-3.27 (m, 2H), 3.15 and 3.79 (AX, 2H, J = 13.4 Hz, benzylic CH_2), 3.76-3.86 (m, 1H), 3.92 (td-like, 1H, J = ~ 11.5, 4.2 Hz), 4.05-4.71 (m, 4H, P(O)CH_2CH_3), 7.18-7.24 (m, 1H, aromatic *H*), 7.26-7.35 (m, 4H, aromatic *H*); ^{13}C{^{1}H} NMR ($CDCl_3$, 125.68 MHz) δ 16.53 (d, $^3J_{PC}$ = 5.8 Hz, P(O)OCH_2CH_3), 16.58 (d, $^3J_{PC}$ = 5.8 Hz, P(O)OCH_2CH_3, 16.53 and 16.58 overlap to give the appearance of a triplet), 28.02, 28.15, 50.33 (d, $^1J_{PC}$ = 162.2 Hz, NCH_2P), 51.10, 51.79, 54.35, 55.86 (d, $^3J_{PC}$ = 15.4 Hz), 56.60, 56.76, 56.95, 57.23, 57.46, 59.92, 60.16,

61.28 (d, $^2J_{PC}$ = 6.7 Hz, P(O)O*C*H$_2$CH$_3$), 61.51 (d, $^2J_{PC}$ = 6.7 Hz, P(O)O*C*H$_2$CH$_3$), 126.49, 127.98 (2*C*), 128.93 (2*C*), 140.95; ^{31}P{^{1}H} NMR ($CDCl_3$, 161.83 MHz) δ 27.24; IR (thin film) 3449, 2978, 2906, 2796, 1452, 1364, 1244, 1124, 1056, 1028, 962; HRFABMS, *m/z* $(M+H)^+$ exact mass calcd for $C_{24}H_{44}N_4O_3P$: 467.3151; Found: 467.3135 (error -1.6 mmu/ -3.4 ppm).

4-benzyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecyl-11-(methanephosphonic acid) (89). 4-benzyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecyl-11-(methanephosphonic acid diethyl ester) (**88**) (0.0511 g, 0.101 mmol) was dissolved in 4 mL of a 6M HCl solution prepared from TraceMetal™ grade hydrochloric acid (Fisher Scientific), and refluxed under N_2 for 1 d. The solvent was removed and the crude solid was dissolved in 10 mL H_2O. This was repeated three times. Residual solvent was removed from the yellow solid under vacuum (0.0576 g, 88% yield); ^{1}H NMR (D_2O, 500 MHz, internal ref *C*H$_3$CN set to δ 2.06) δ 1.75-1.86 (tm-like, 2H, *J* = ~17.0 Hz), 2.21-2.33 (qm-like, 1H, *J* = ~13 Hz), 2.38-2.50 (qm-like, 1H, *J* = ~13 Hz), 2.56-2.70 (m, 3H), 2.72-2.78 (dm, 1H, *J* = 14.2 Hz), 2.88-2.95 (m, 1H), 2.96-3.10 (m, 3H), 3.11-3.40 (m, 8H), 3.41-3.54 (m, 3H), 3.55-3.66 (m, 2H), 3.67-3.79 (m, 2H), 4.53 and 4.80 (AX, 2H, J = 13.9 Hz, benzylic C*H*$_2$), 7.48-7.61 (m, 5H, aromatic H); ^{13}C{^{1}H} NMR (D_2O, 100.52 MHz, internal ref *C*H$_3$CN set to δ 1.47) δ 20.12 (2*C*), 45.02, 48.38 49.04, 50.60 (2*C*), 51.70 (d, $^2J_{PC}$ = 143.0 Hz, N*C*H$_2$P), 54.30, 55.35, 55.48, 57.67, 58.55, 58.89, 127.28, 129.79 (2*C*), 131.02, 133.05 (2*C*); ^{31}P{^{1}H} NMR (D_2O, 161.83) δ 12.76 (major), 23.26 (minor) (The ^{1}H, ^{31}P{^{1}H} spectra and analysis indicated the presence of 0.08 ± 0.01 molar equivalents of 4-benzyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecyl-11-(methanephosphonic acid)

trihydrochloride relative to 1.0 molar equivalents of **89**); IR (KBr) 3376, 3963, 2858, 1634, 1494, 1456, 1208, 1096, 1044, 1002, 931, 733, 707; HRFABMS, *m/z* $(M+H)^+$ exact mass calcd for $C_{20}H_{36}N_4O_3P$: 411.2525; Found: 411.2534 (error +0.9 mmu/ +2.1 ppm); Anal. Calcd for $C_{20}H_{37}N_4O_3PCl_2 \cdot (C_{20}H_{38}N_4O_3Cl_3P)_{0.08}(HCl)_{0.64}(H_2O)_{2.64}$: C, 43.54; H, 7.77; N 10.15; Cl, 17.13. Found: C, 43.18; H, 7.70; N, 10.38; Cl, 17.13.

(10bα,10cα)-Decahydro-3a-[(4-methoxyphenyl)methyl]-1H,6H-3a,5a,8a,10a-tetraazapyrenium bromide (92·0.5H$_2$O·0.2CH$_3$Ph). 4-Methoxybenzyl bromide (0.1387 g, 0.6898 mmol) was added to a solution of **31** (0.1180 g, 0.5307 mmol) in 4 mL of toluene and stirred for 3 d under N_2. The resultant mixture was vacuum filtered and the crude white solids were washed with 5 mL toluene, 10 mL of Et_2O and 1.5 mL of ice cold MeCN and put under vacuum for 1 d to yield 0.1495 g (62% yield) of **92·0.5H$_2$O·0.2CH$_3$Ph**: mp = 155-160 °C (dec); ^{1}H NMR (D_2O, 500 MHz, internal ref CH_3CN set to δ 2.06) δ 1.43-1.49 (dm, 1H, J = 14.2 Hz, $NCH_2CHH_{eq}CH_2N$), 1.74-1.81 (dm, 1H, J = 13.9 Hz, $NCH_2CHH_{eq}CH_2N$), 2.11-2.30 (m, 3H), 2.43-2.50 (m, 2H), 2.62 (td, 1H, J = 12.3, 3.4 Hz), 2.97-3.13 (m, 7H), 3.22 (td, 1H, J = 13.6, 3.0 Hz), 3.28-3.34 (dm, 1H, J = 12.9 Hz), 3.46-3.55 (m, 2H), 3.62-3.65 (s-like, 1H, C*H*), 3.88, (s, 3H, OCH_3), 4.16 (td, 1H, J = 13.2, 3.9 Hz), 4.34 (d, 1H, J = 2.2 Hz, C*H*), 4.73 and 4.99 (AX, 2H, J = 13.6 Hz), 7.09-7.13 (XX′ of AA′XX′, 2H), 7.46-7.50 (AA′ of AA′XX′, 2H); $^{13}C\{^1H\}$ NMR (D_2O, 125.68 MHz, internal ref CH_3CN set to δ 1.47) δ 18.57, 18.95, 42.55, 47.19, 48.92, 51.90, 52.58, 56.13, 60.12, 62.87, 70.13, 82.22, 115.28 (2*C*), 118.40, 135.43 (2*C*), 161.45; (The ^{1}H spectrum indicated the presence of 16% (+/- 4%) residual toluene.) IR (KBr) 3410, 3007, 2989, 2940, 2867, 2836, 2778, 1612, 1518, 1437, 1275,

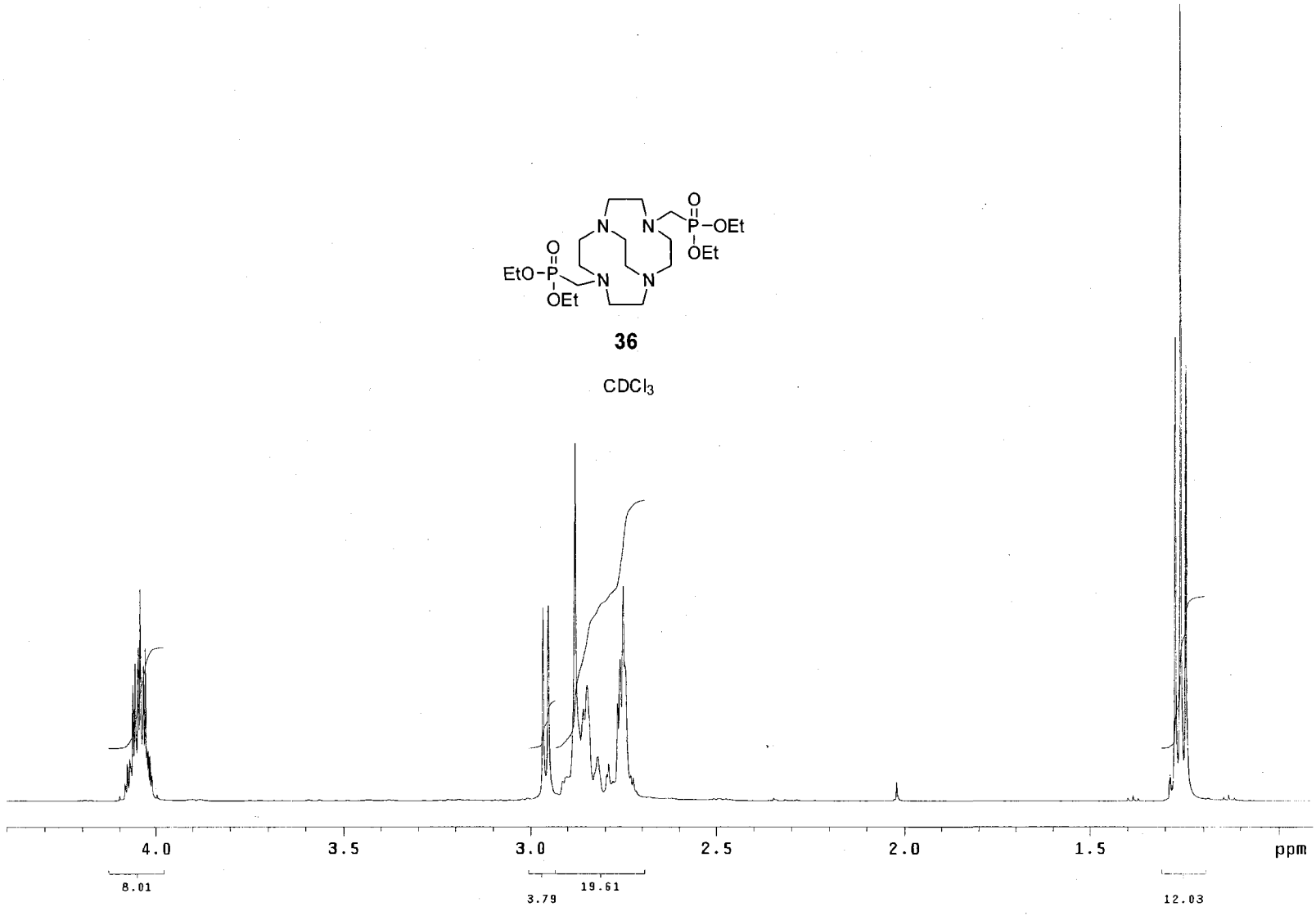
1264, 1251, 1187; HRFABMS, *m/z* (M^+) exact mass for $C_{20}H_{31}N_4O$: 343.2498; Found: 343.2505 (error +0.7 mmu/+2.0 ppm); Anal. Calcd for $C_{20}H_{31}N_4OBr{\cdot}(H_2O)_{0.5}{\cdot}(CH_3Ph)_{0.2}$: C, 57.01; H, 7.51; N 12.43; Br, 17.72. Found: C, 57.42; H, 7.45; N, 12.28; Br, 17.31.

4-(Allyl)-11-[(4-aminophenyl)methyl]-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (93). 4-(Allyl)-11-[(4-nitrophenyl)methyl]-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (0.0707 g, 0.1761 mmol) (**82**) and mossy tin (0.1045 g, 0.8803 mmol) were heated in 6M HCl (6 mL) with a steam bath for 3 h under N_2. The solvent was removed under reduced pressure and the crude solid was dissolved in H_2O and made strongly basic (pH=14, slow addition of KOH pellets). The resulting solution was extracted with toluene (5 x 10 mL), the combined organic phases were dried over anhyd Na_2SO_4, the solvent was removed under aspirator pressure, and the residual solvent was removed under vacuum to yield product **93** as a clear oil. (0.0510 g, 78%): ^{1}H NMR (C_6D_6, 500 MHz) δ 1.27-1.48 (m, 3H), 1.54-1.62 (m, 1H), 2.20-2.28 (m, 2H), 2.35 (ddd, 1H, *J* = 13.7, 6.1, 2.0 Hz), 2.38-2.46 (m, 5H), 2.47-2.61 (m, 5H), 2.68-2.84 (m, 3H), 2.80 (br s, 2H, N*H*$_2$), 3.00 (ddd, 1H, *J* = 13.0, 12.1, 4.0 Hz), 3.06 (d, 2H, *J* = 12.9 Hz, benzylic CH*H*), 3.16 (ddt, 1H, *J* = 13.92, 4.7, 1.5 Hz), 3.27 (td, 1H, *J* = 12.4, 3.8 Hz), 3.38 (td, 1H, *J* = 12.3, 3.9 Hz), 3.76 (d, 1H, *J* = 12.9 Hz, benzylic C*H*H), 3.77 (td, 1H, *J* = 11.7, 4.4 Hz), 4.01 (td, 1H, *J* = 11.7, 4.0 Hz), 5.01-5.05 (dm, 1H, *J* = ~ 10.1 Hz, $CH_2CH{=}CH\mathit{H}_E$), 5.10-5.16 (dm, 1H, *J* = ~ 17.1 Hz, $CH_2CH{=}CH\mathit{H}_Z$), 5.88 (dddd, 1H, *J* = 17.3, 10.0, 7.6, 4.9 Hz, $CH_2C\mathit{H}{=}CHH$), 6.38-6.41 (XX′ of AA′XX′, 2H), 7.18-7.22 (AA′ of AA′XX′, 2H); ^{13}C{^{1}H} NMR (C_6D_6, 125.68 MHz) δ 28.48, 28.79, 51.82, 52.07, 54.26, 54.83, 57.22, 57.34, 57.82 (2*C*), 57.86, 58.06, 58.28, 59.85, 114.85 (2*C*), 116.04,

130.28 (2*C*), 130.50, 137.71, 145.90; IR (thin film) 3451, 3363, 3217, 3073, 2911, 2793, 1622, 1516, 1458, 1363, 1279, 1126, 1060, 999, 916; HRFABMS, *m/z* ($M+H^+$) exact mass for $C_{22}H_{38}N_5$: 372.3127; Found: 372.3150 (error +2.3 mmu/+ 6.1 ppm).

Appendix A:

Spectral Index


36
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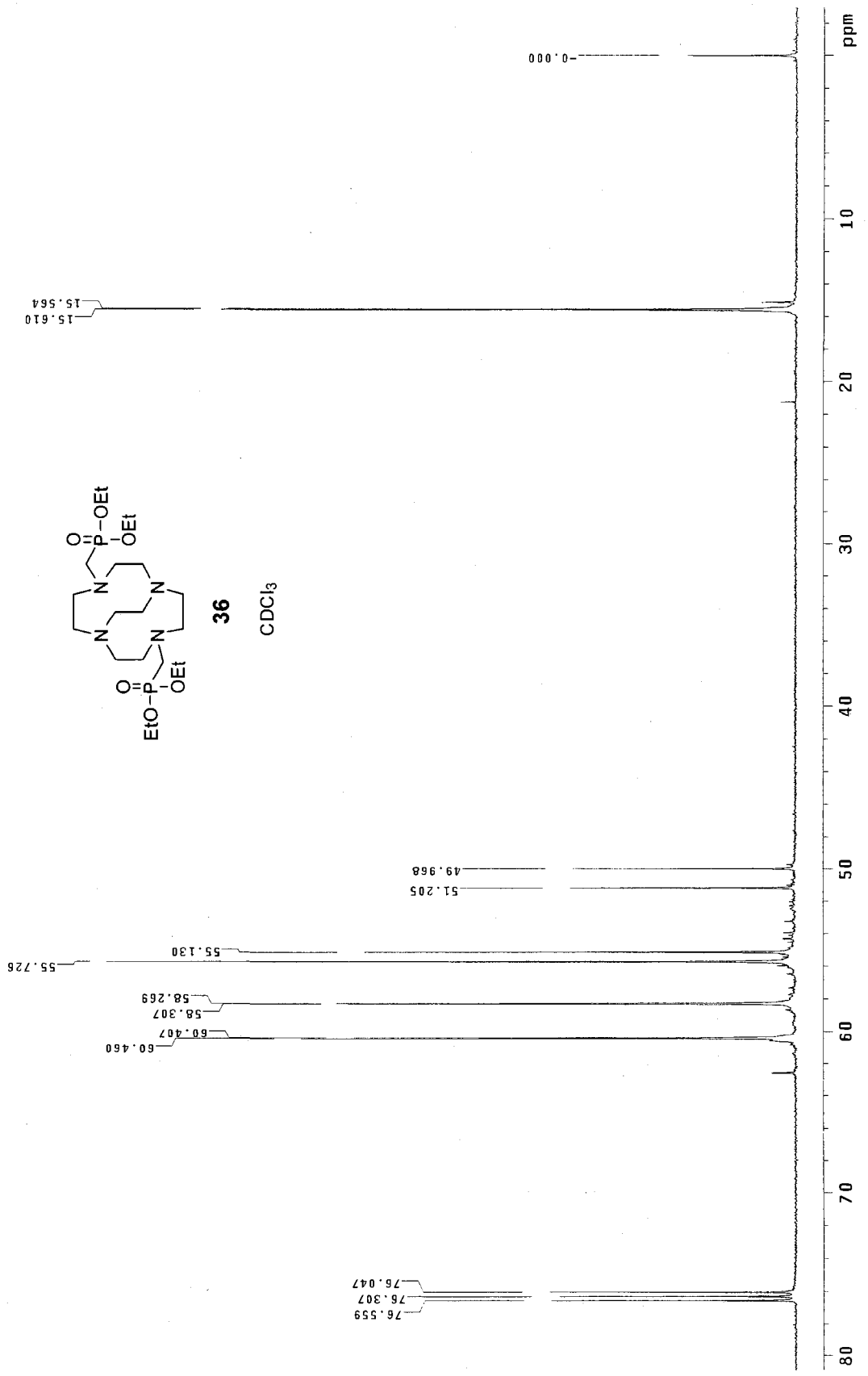
3.5
3.0
2.5
2.0
1.5
ppm
8.01
3.79
19.61
12.03


ppm
10
20
30
40
50
60
70
80
-0.000
15.610
15.564
49.968
51.205
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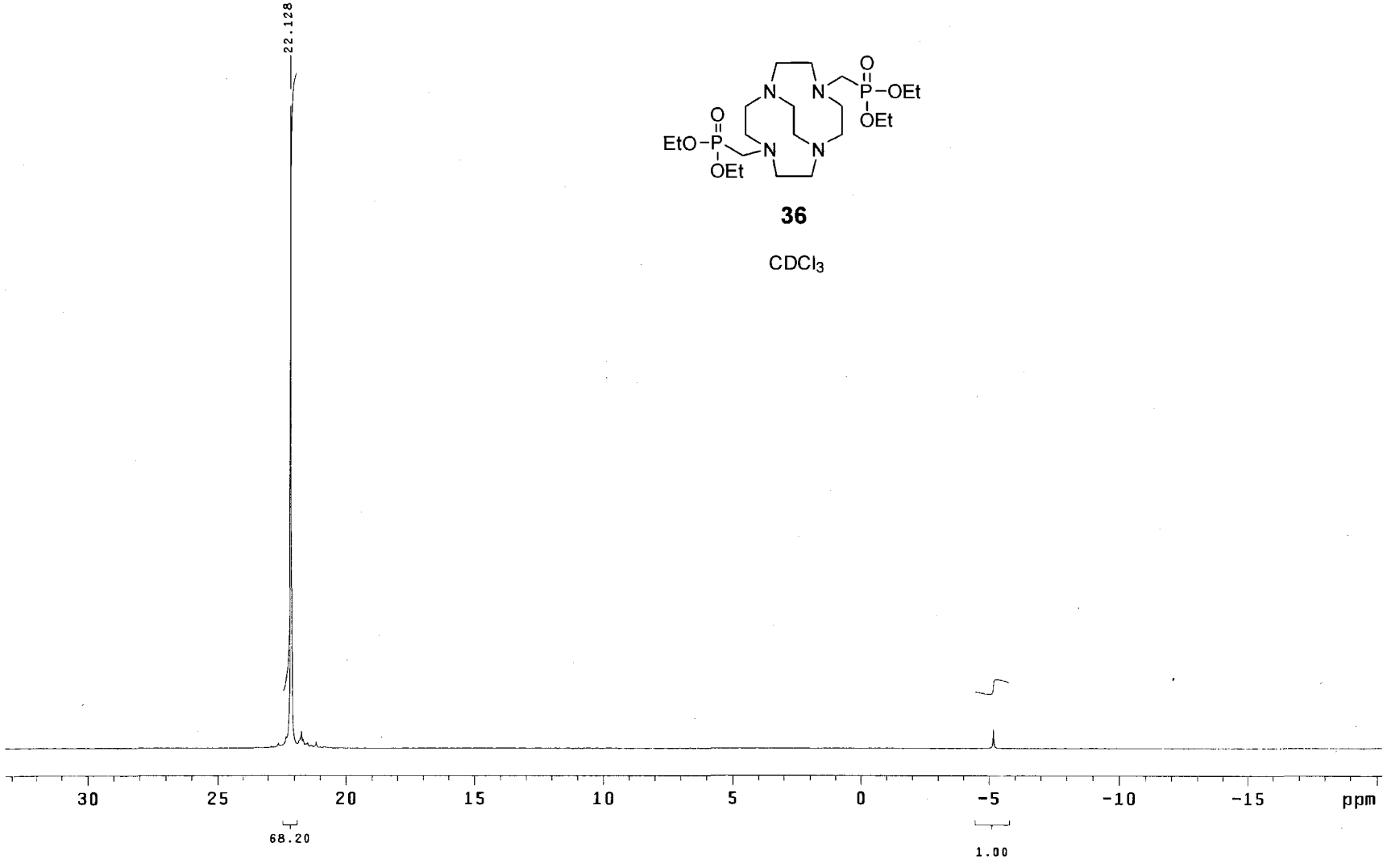
55.726
58.269
58.307
60.407
60.460
76.559
76.307
76.047
36
CDCl3


22.128
EtO–P(=O)–OEt
N
N
N
N
P(=O)–OEt
OEt
36
CDCl3
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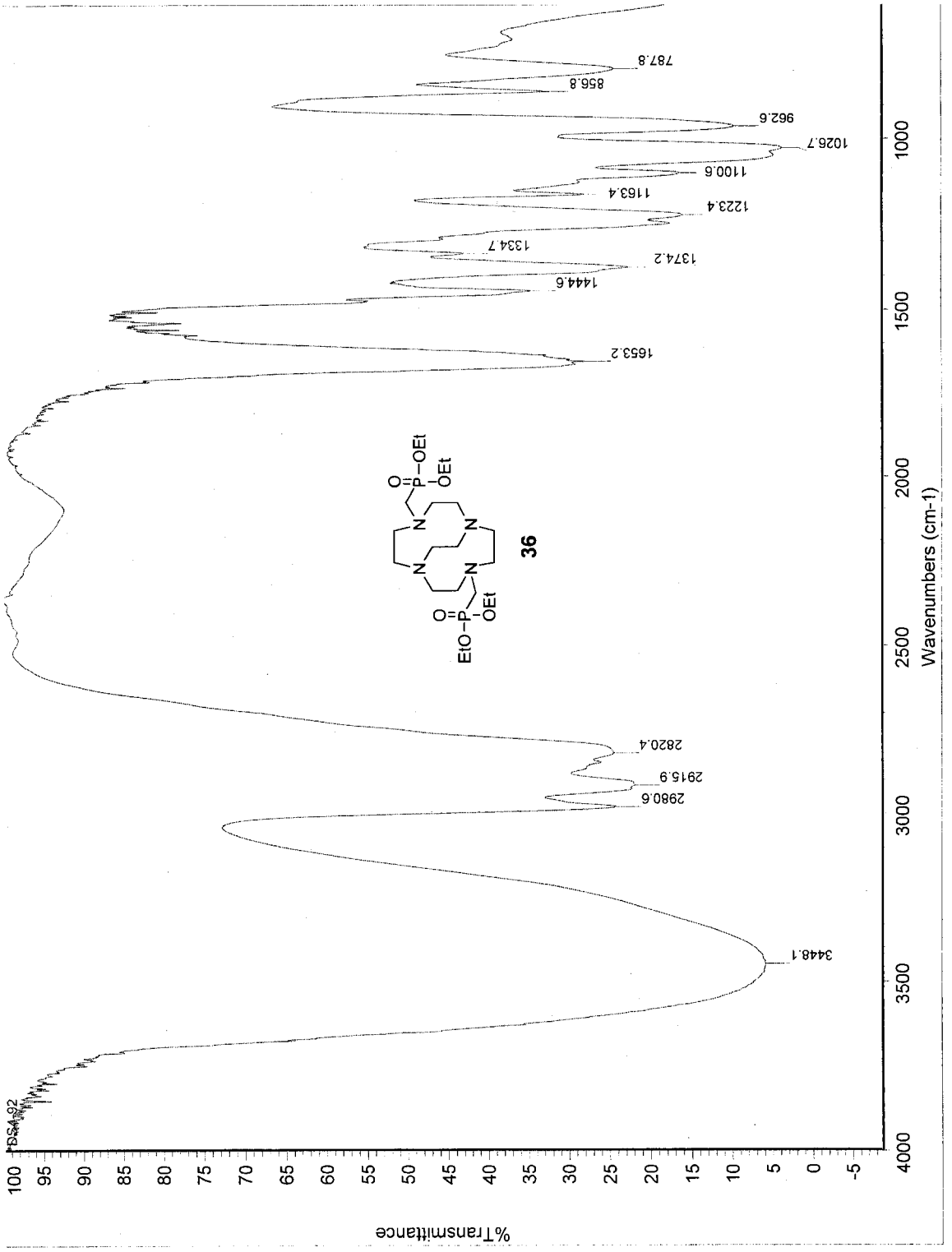
25
20
15
10
5
0
-5
-10
-15
ppm
68.20
1.00


PBSA-92
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Wavenumbers (cm-1)
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2820.4
1653.2
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1334.7
1223.4
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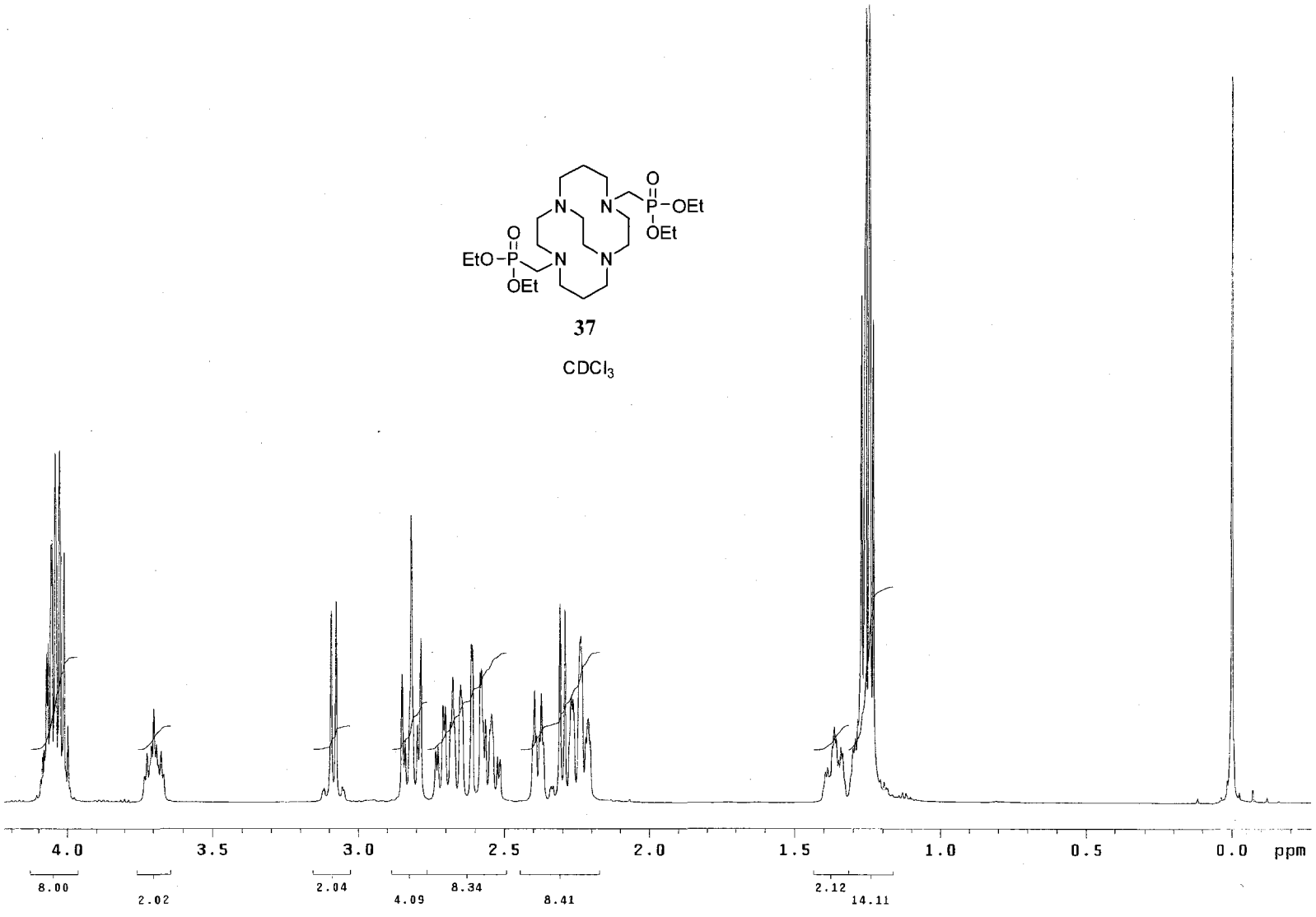
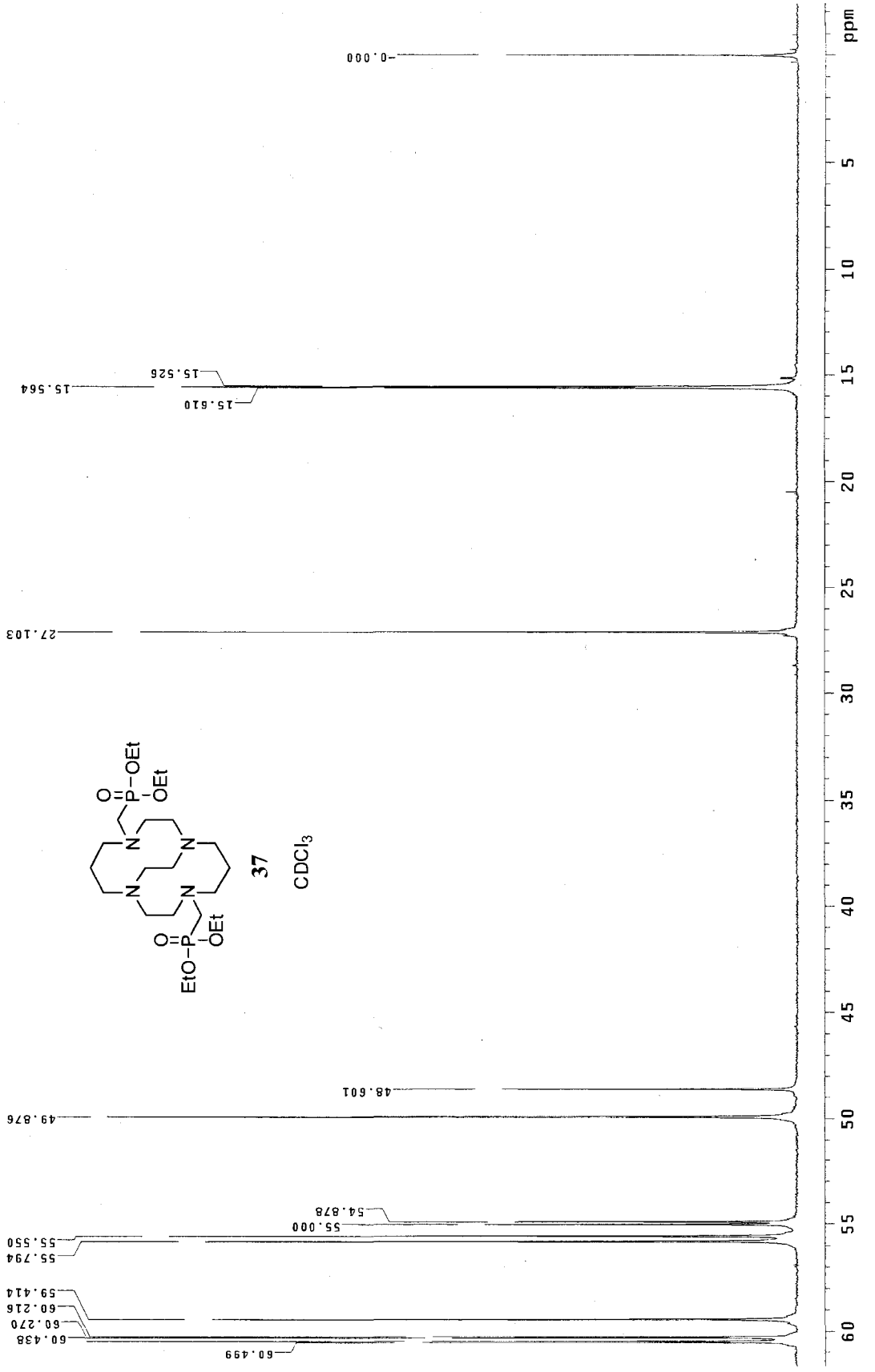
1100.6
1026.7
962.6
856.8
787.8
36


37
CDCl3
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
8.00
2.02
2.04
4.09
8.34
8.41
2.12
14.11


ppm
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15.526
15.510
27.103
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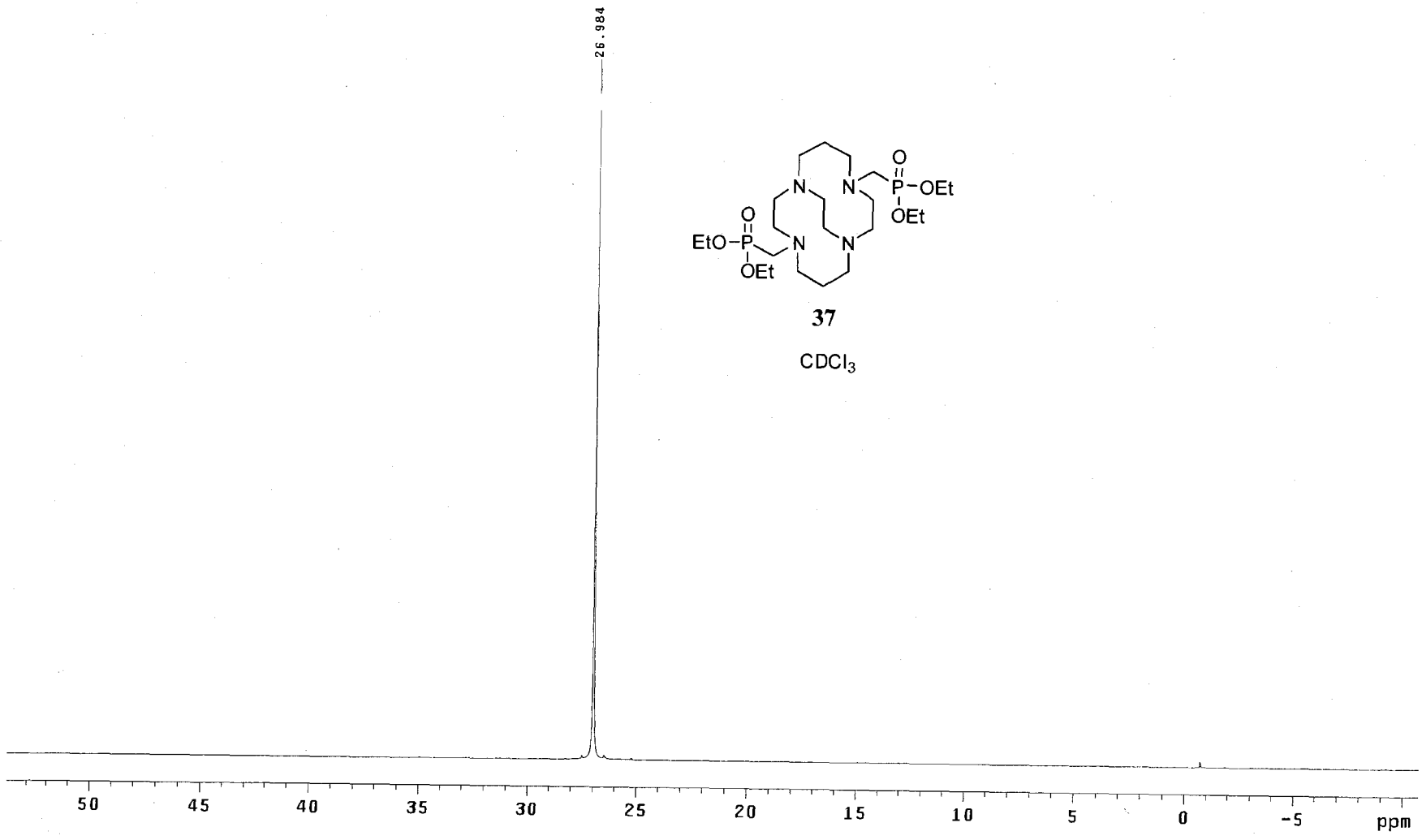
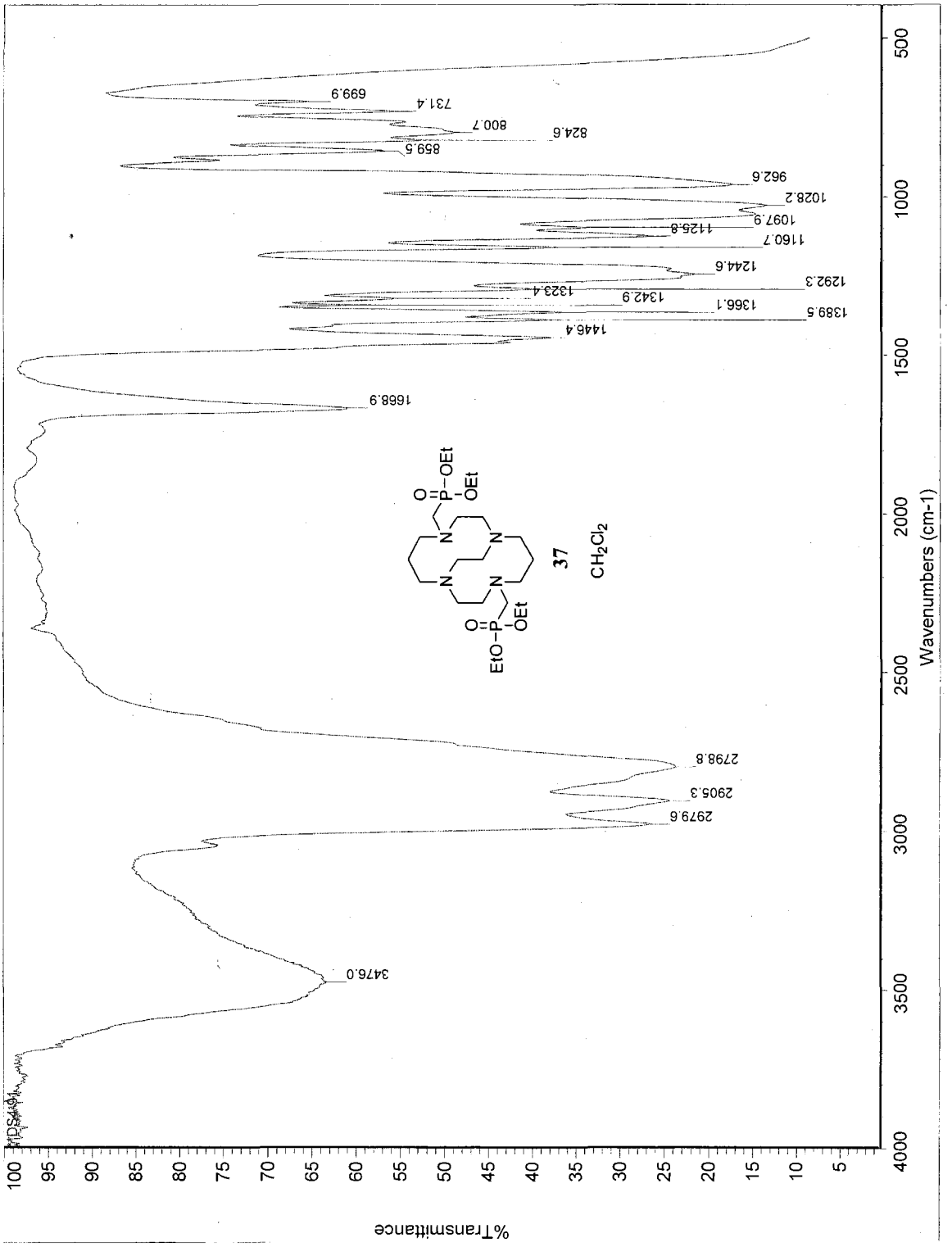
55.000
55.550
55.794
59.414
60.216
60.270
60.438
60.499
37
CDCl3



37

$CDCl_3$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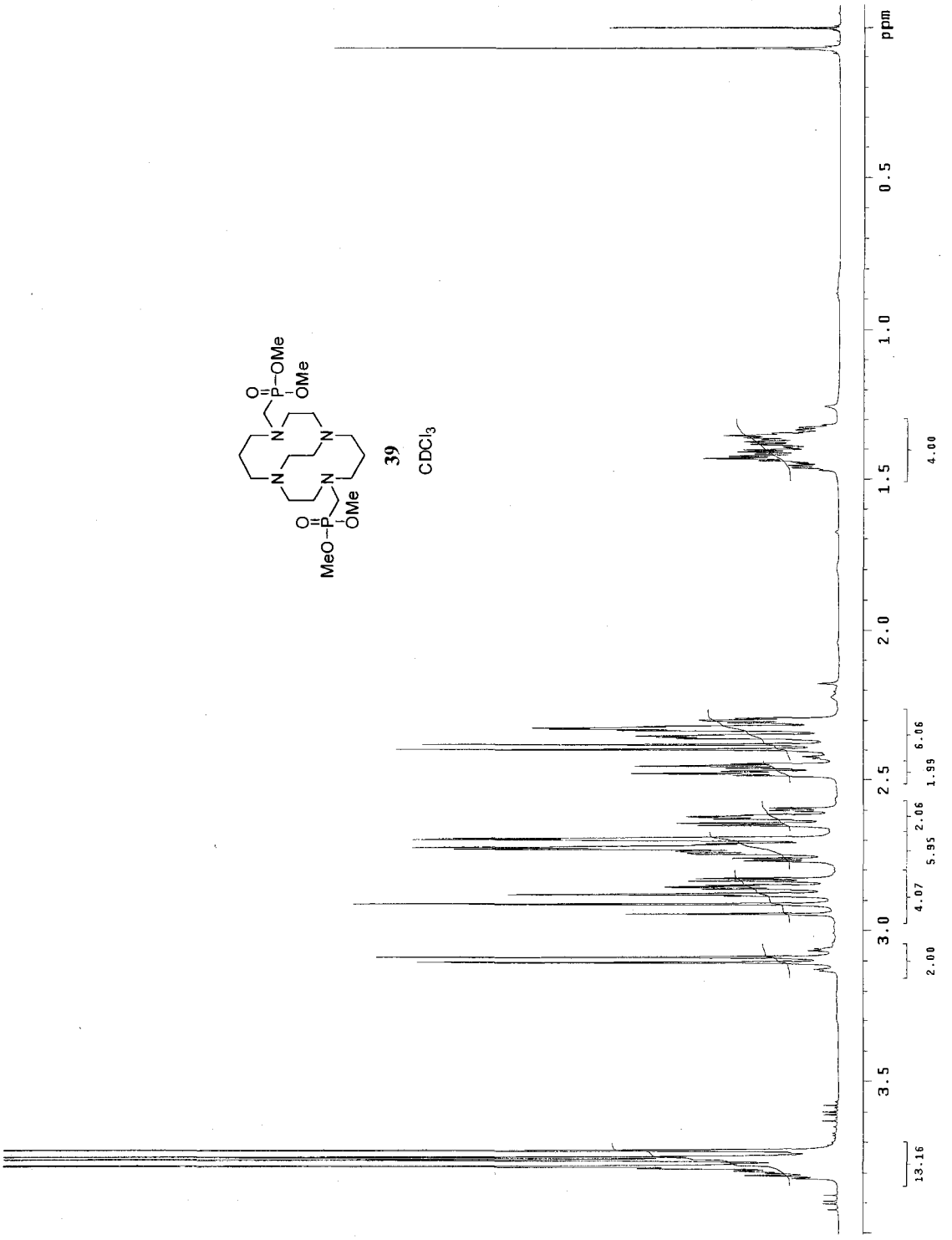
1366.1
1342.9
1323.4
1292.3
1244.6
1160.7
1125.8
1097.9
1028.2
962.6
859.5
824.6
800.7
731.4
699.9
37
CH_2Cl_2


39
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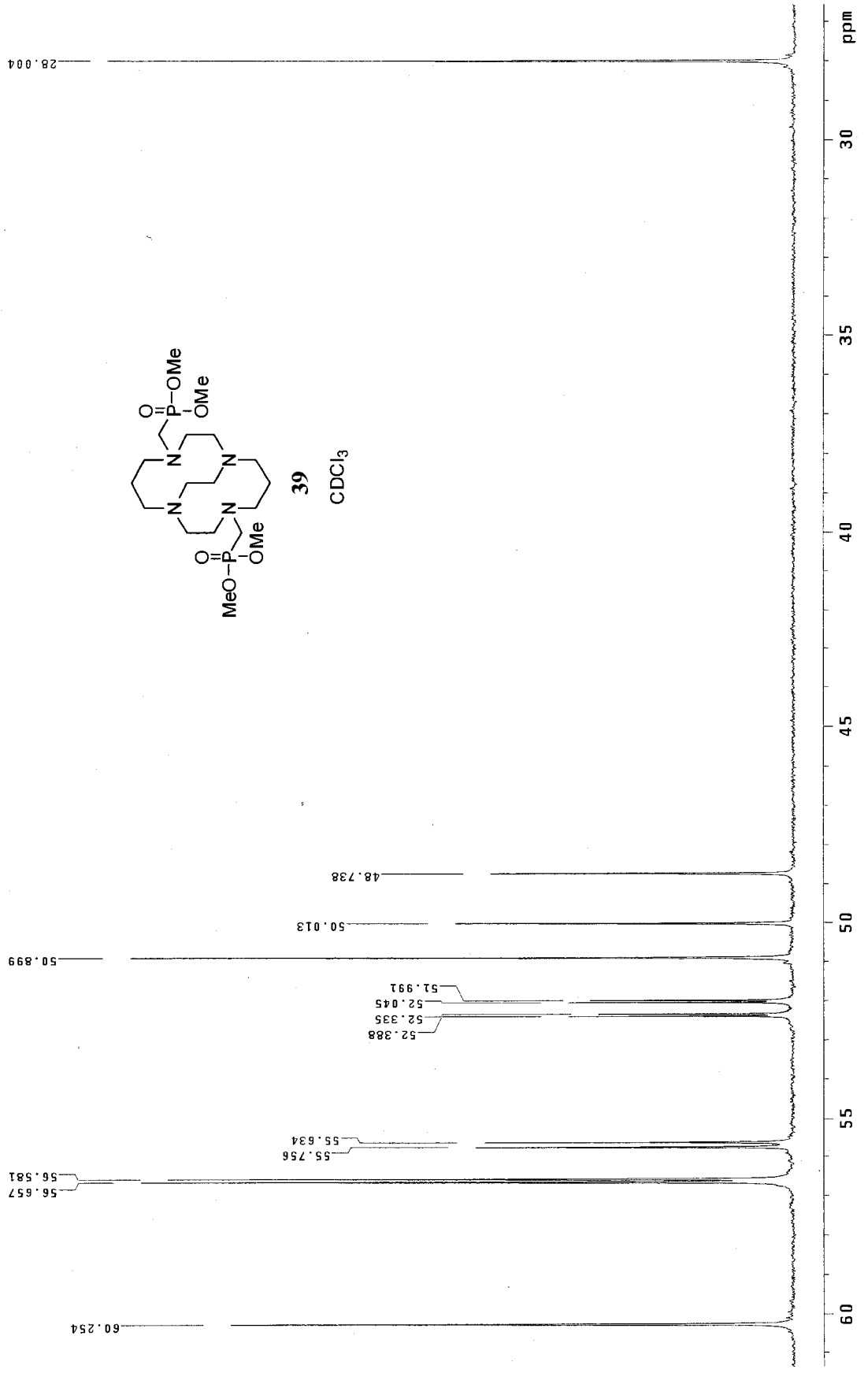
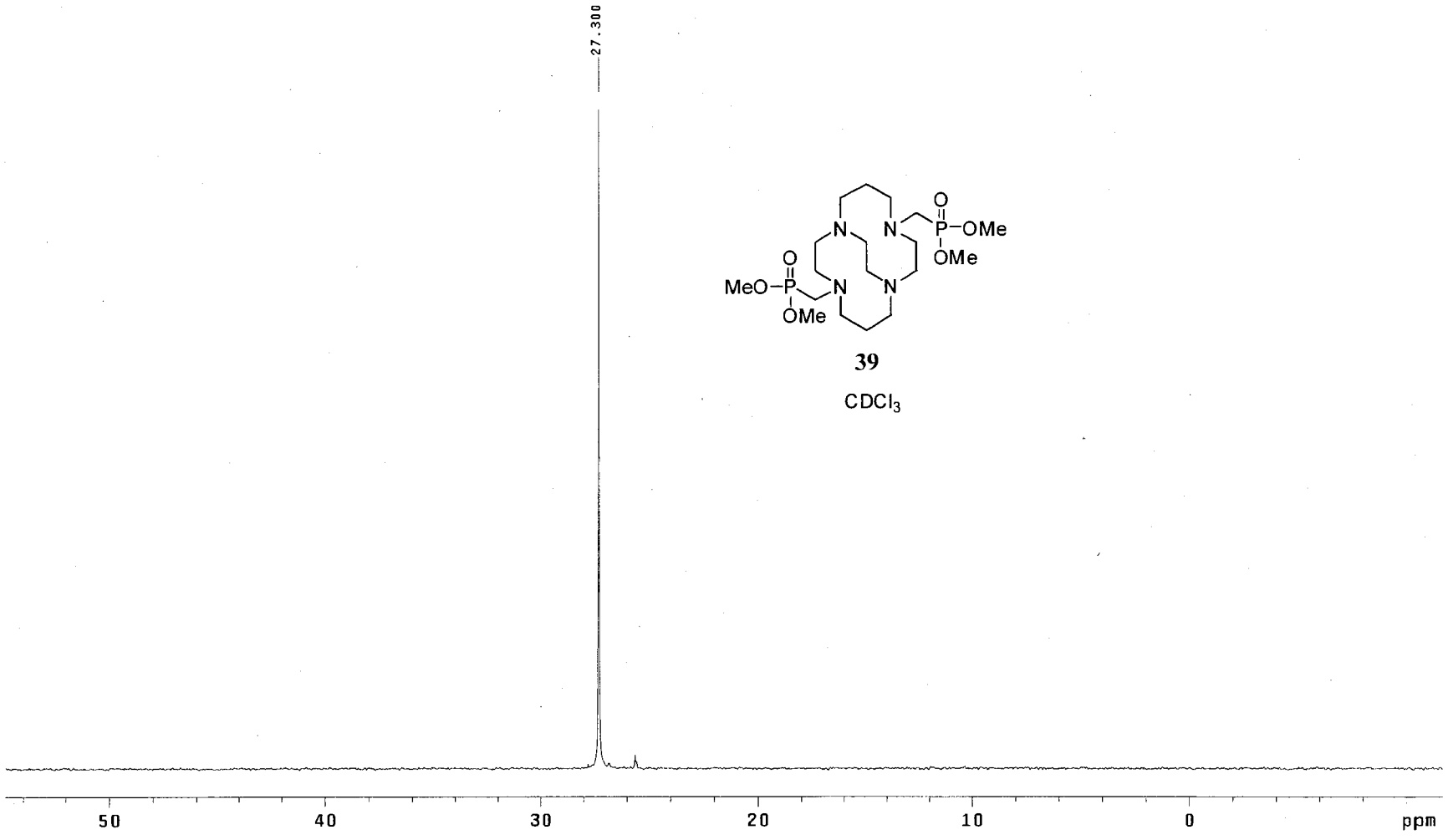
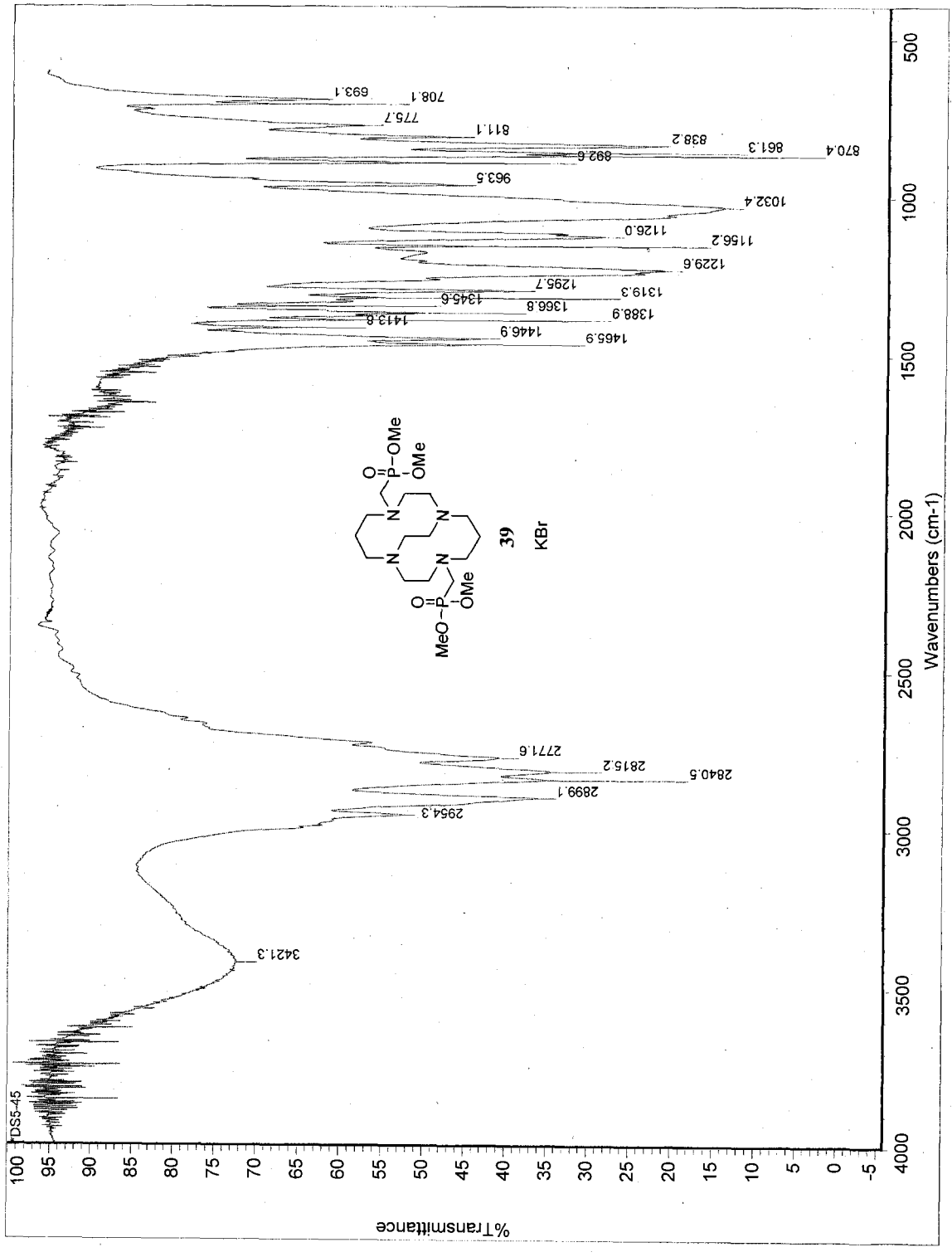
4.07
5.95
2.06
1.99
6.06
4.00


28.004
60.254
56.657
56.581
55.756
55.634
52.388
52.335
52.045
51.991
50.899
50.013
48.738
ppm
60
55
50
45
40
35
30
39
CDCl3



39

$CDCl_3$


DS5-45
%Transmittance
Wavenumbers (cm-1)
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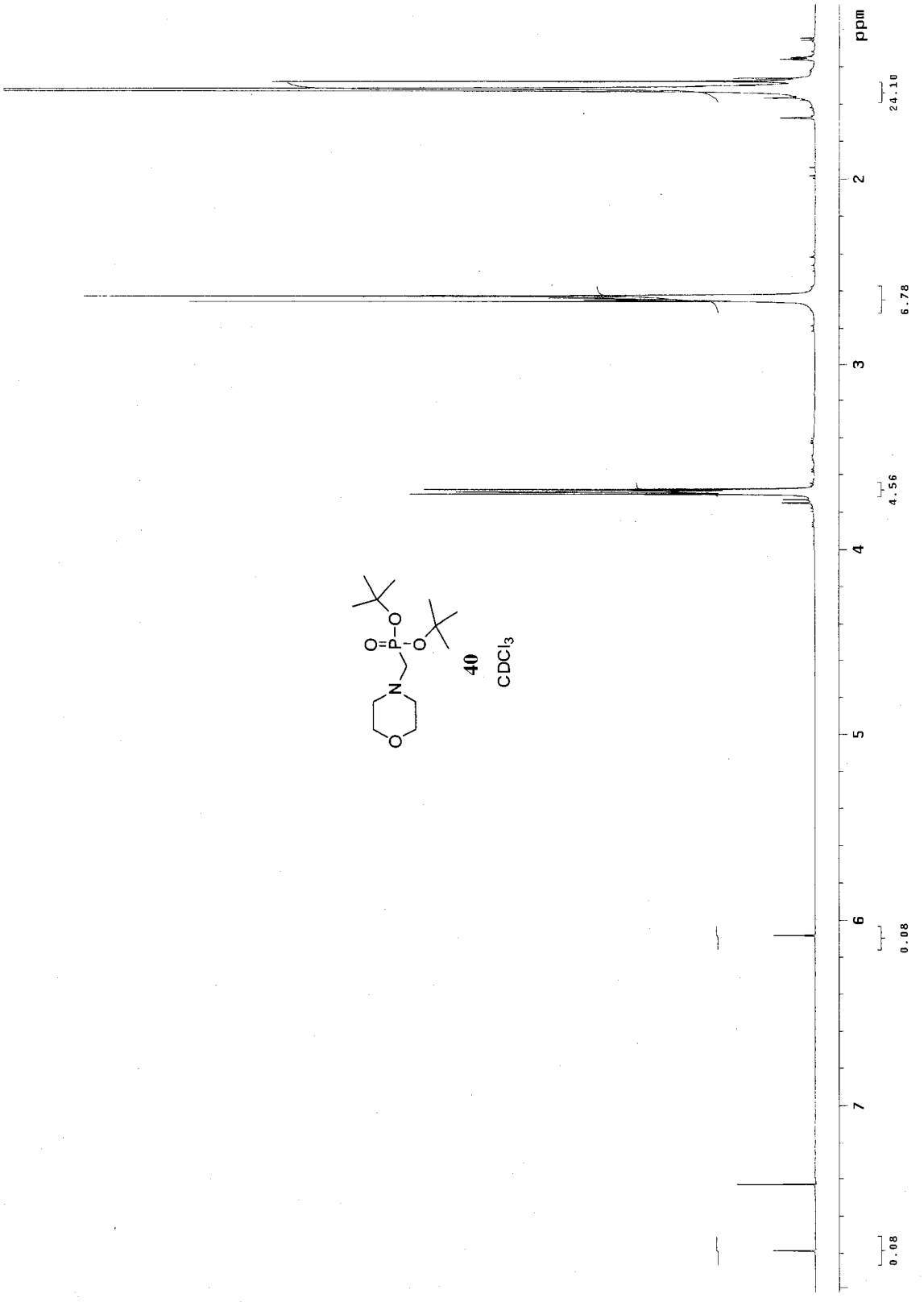
2954.3
2899.1
2840.5
2815.2
2771.6
1465.9
1446.9
1413.8
1388.9
1366.8
1345.6
1319.3
1295.7
1229.6
1156.2
1126.0
1032.4
963.5
892.6
870.4
861.3
838.2
811.1
775.7
708.1
693.1
39
KBr


40
CDCl3
ppm
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6.78
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0.08
0.08

81.179
81.110
76.437
76.177
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66.043
57.200
55.863
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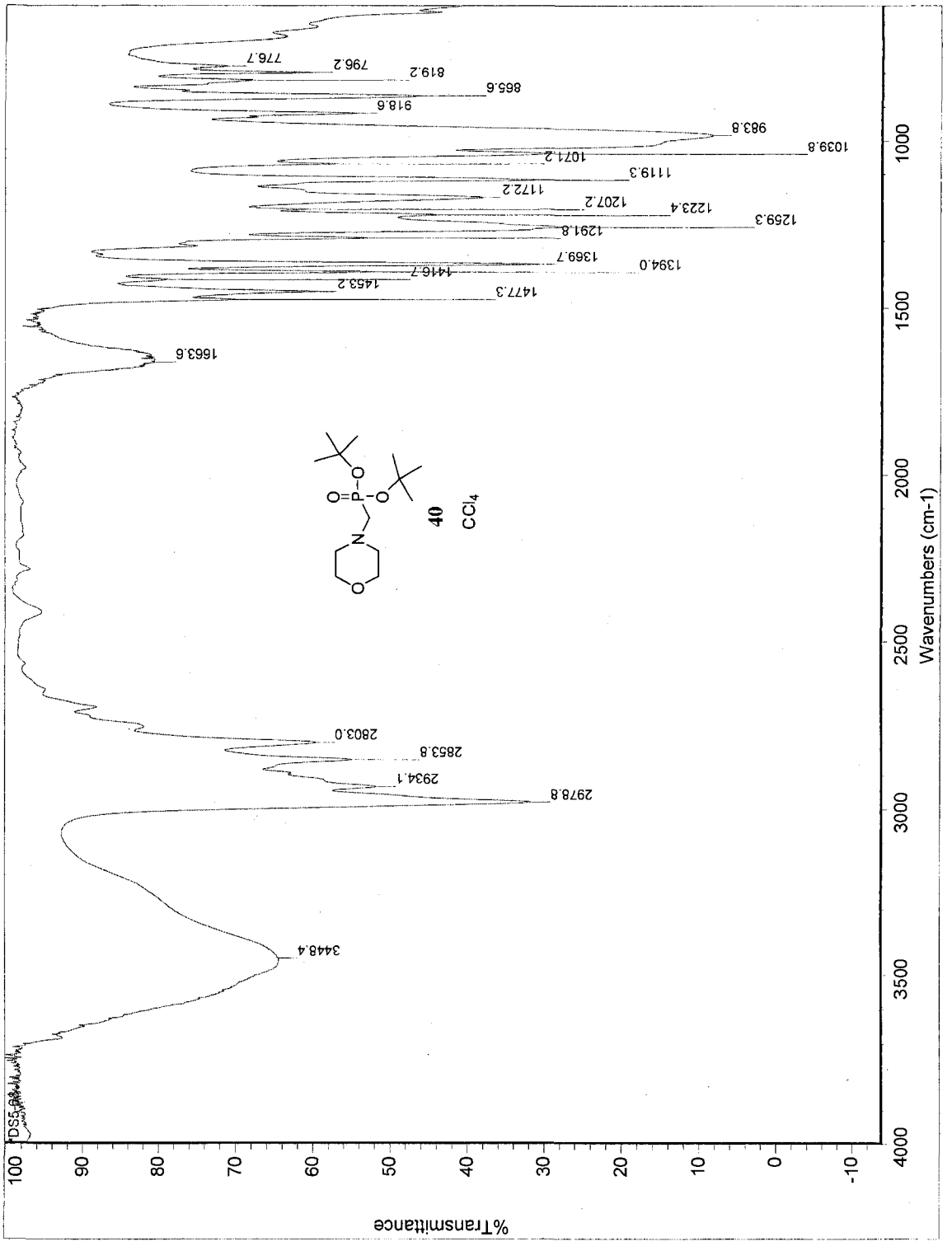
29.531
29.501
29.447
29.356
29.318
28.905
28.875
ppm
40
CDCl3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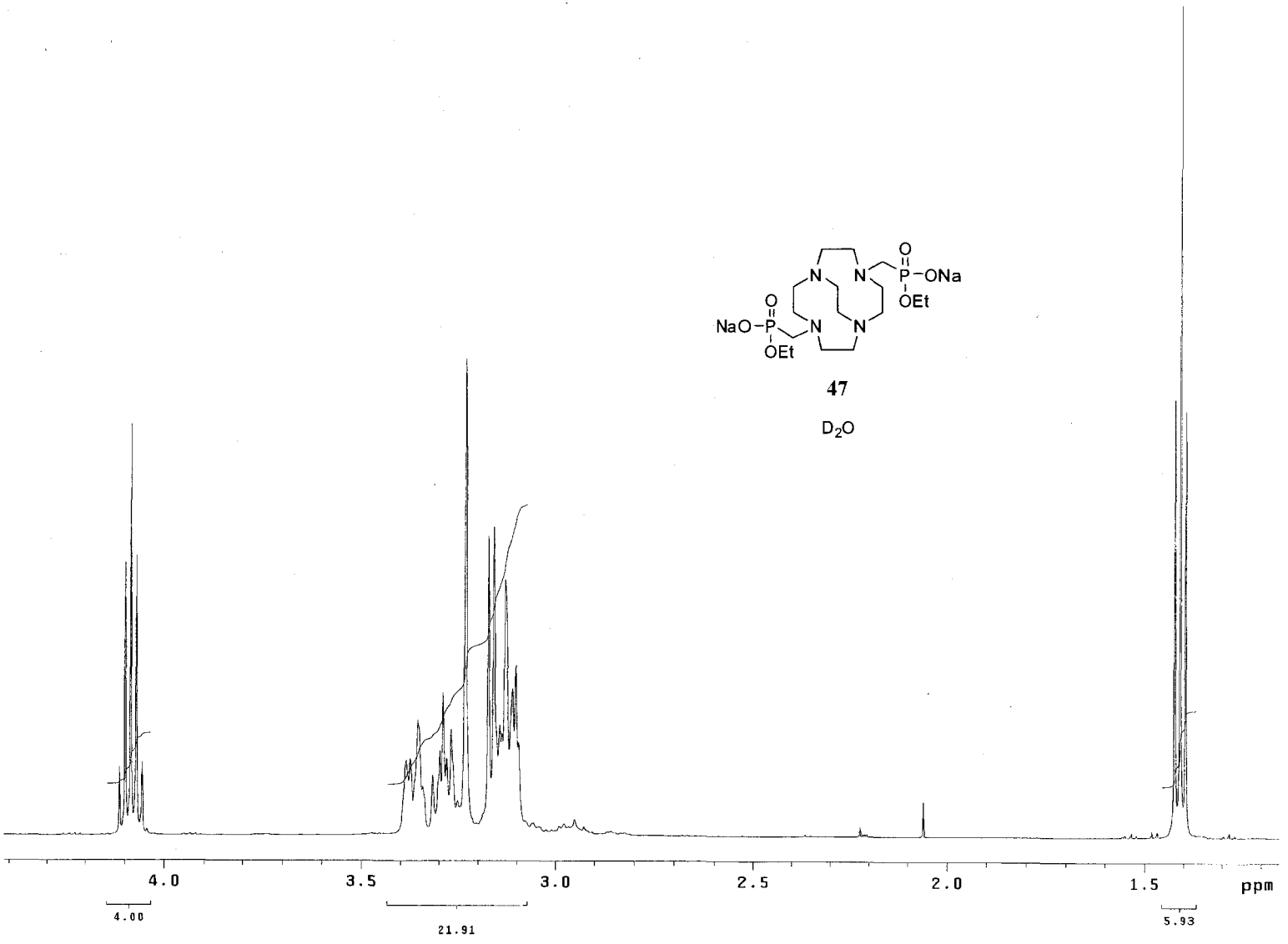
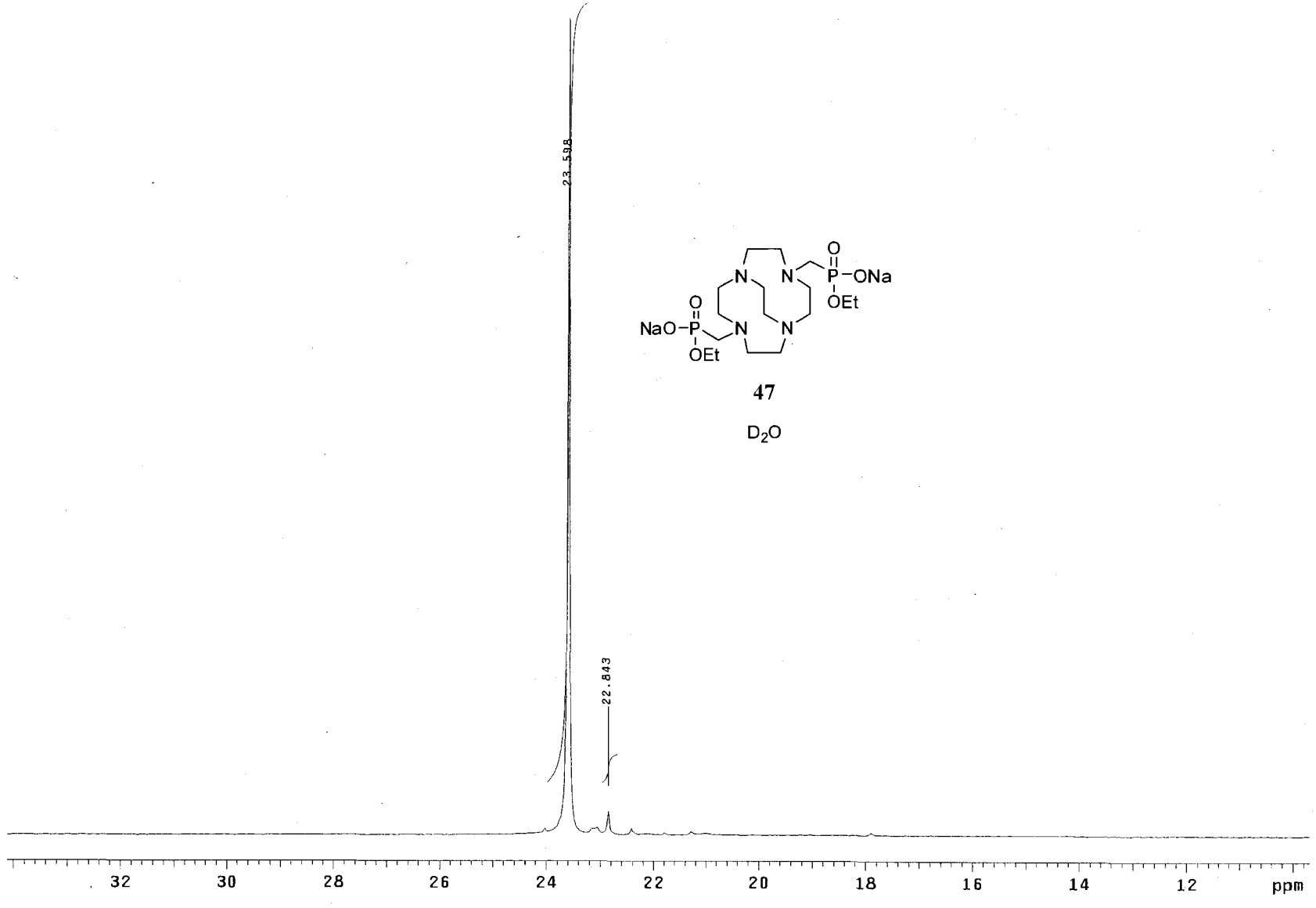
Wavenumbers (cm-1)
4000
3500
3000
2500
2000
1500
1000
100
90
80
70
60
50
40
30
20
10
0
-10
3448.4
2978.8
2934.1
2853.8
2803.0
1663.6
1477.3
1453.2
1416.7
1394.0
1369.7
1291.8
1259.3
1223.4
1207.2
1172.2
1119.3
1071.2
1039.8
983.8
918.6
865.6
819.2
796.2
776.7
40
CCl_4


NaO–P
OEt
O
N
N
N
N
P–ONa
OEt
O
47
D2O
4.0
3.5
3.0
2.5
2.0
1.5
ppm
4.00
21.91
5.93

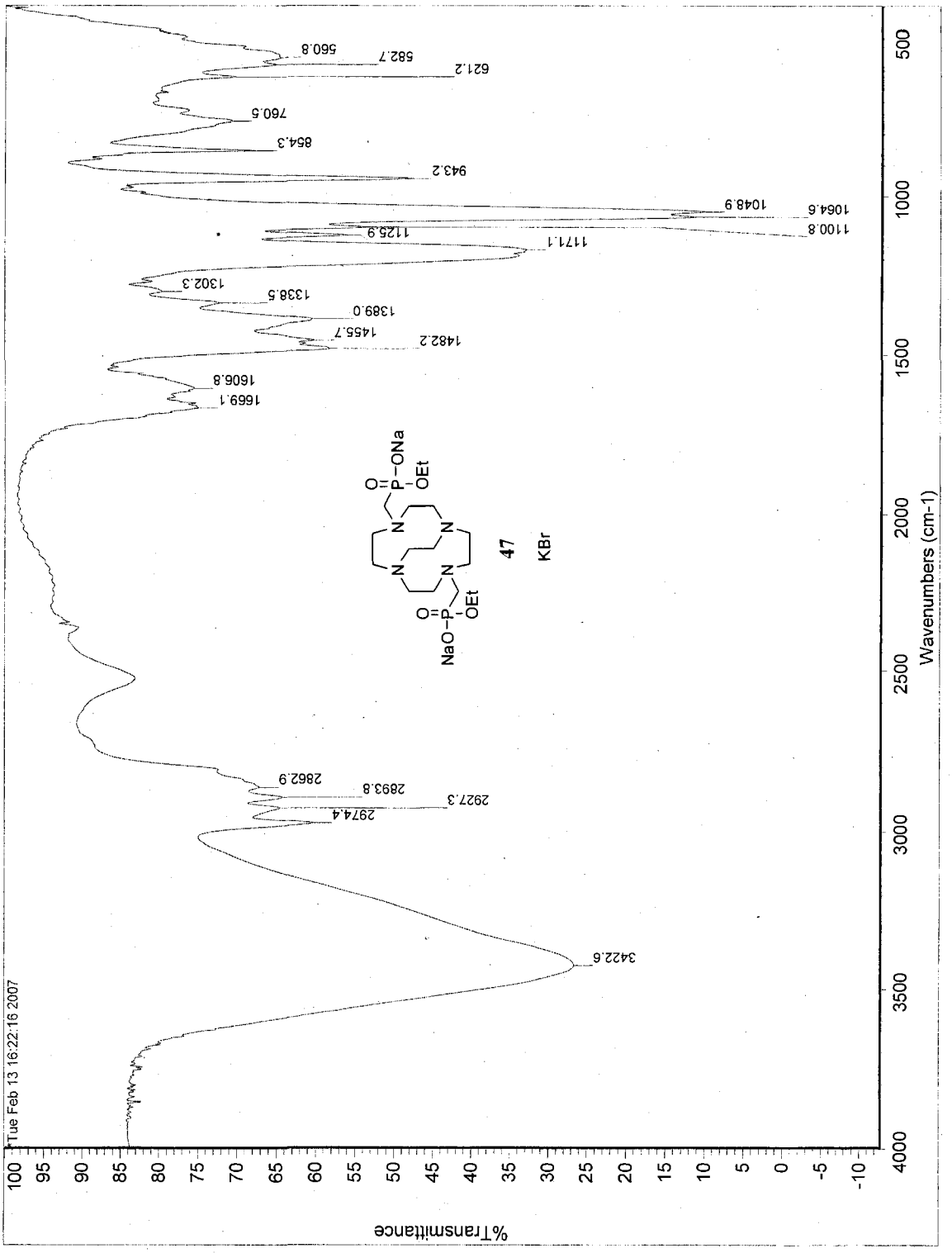
61.075
61.029
54.851
51.285
48.024
48.161
49.276
16.820
16.774
1.470
ppm
10
20
30
40
50
60
O=P–ONa
OEt
N
N
N
N
O=P–OEt
NaO–P
47
D2O


23.598
22.843
32
30
28
26
24
22
20
18
16
14
12
ppm

NaO–P
O
OEt
N
N
N
N
P–ONa
O
OEt

47

D_2O


47
KBr
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Wavenumbers (cm-1)
Tue Feb 13 16:22:16 2007
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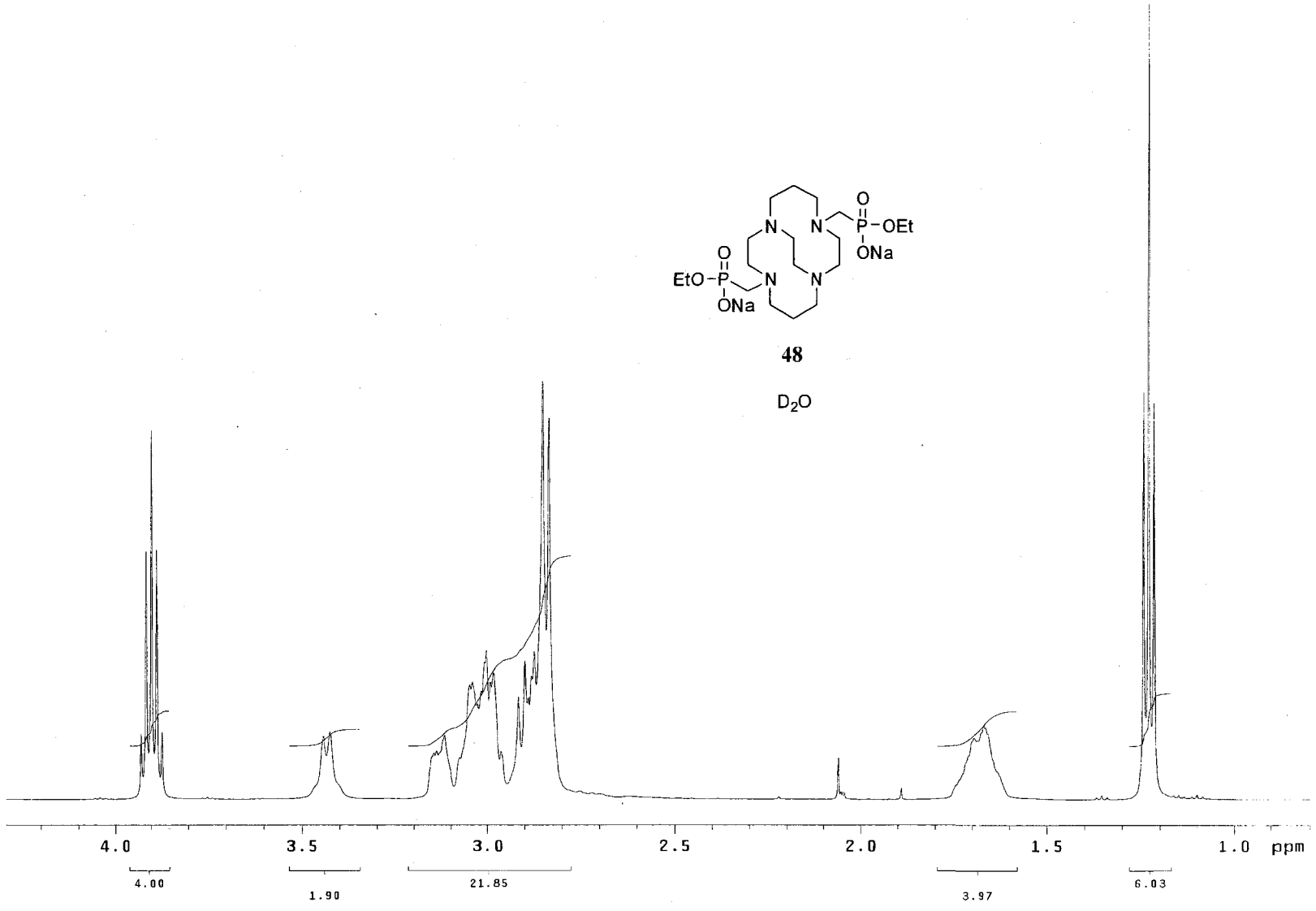
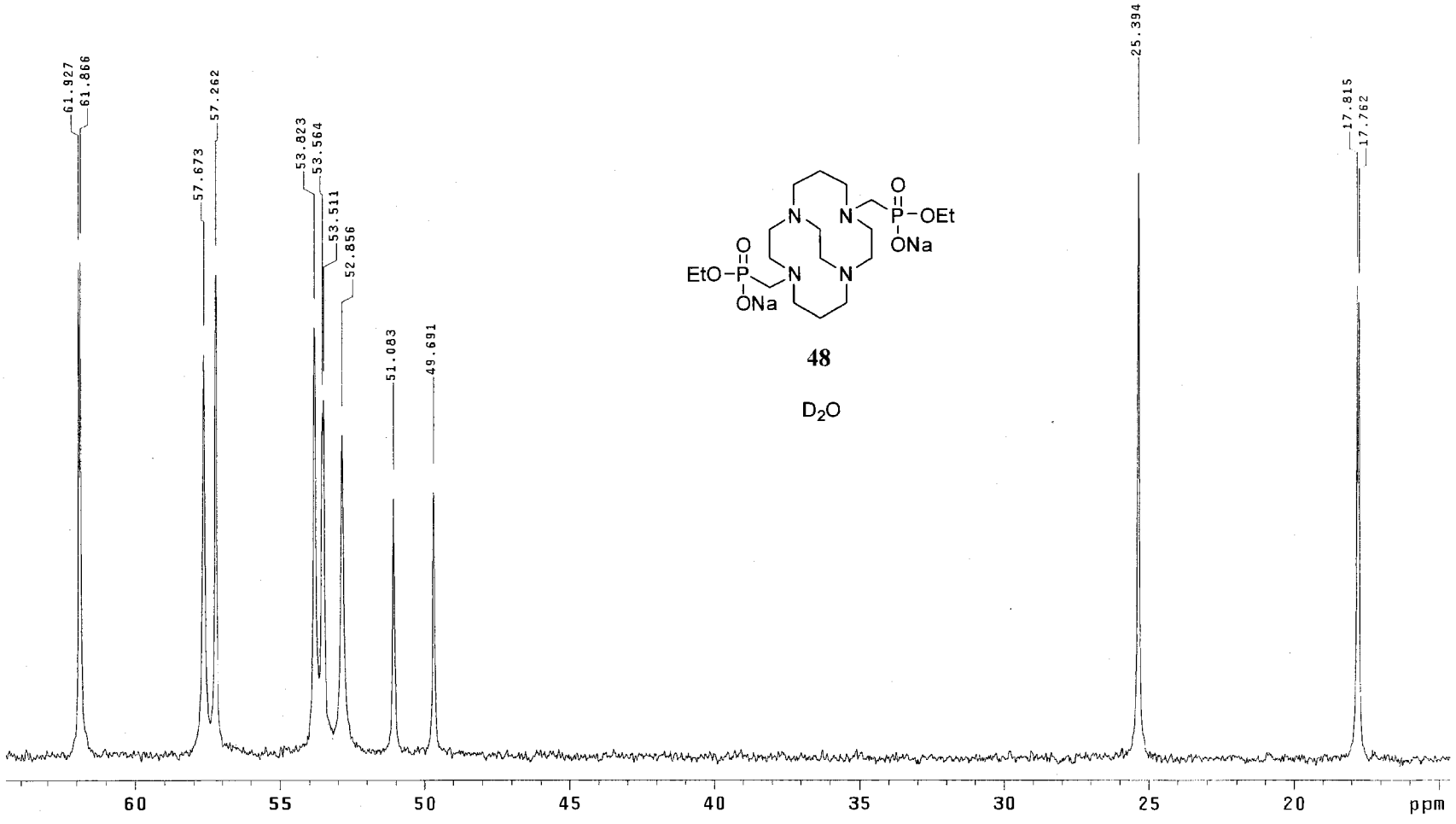
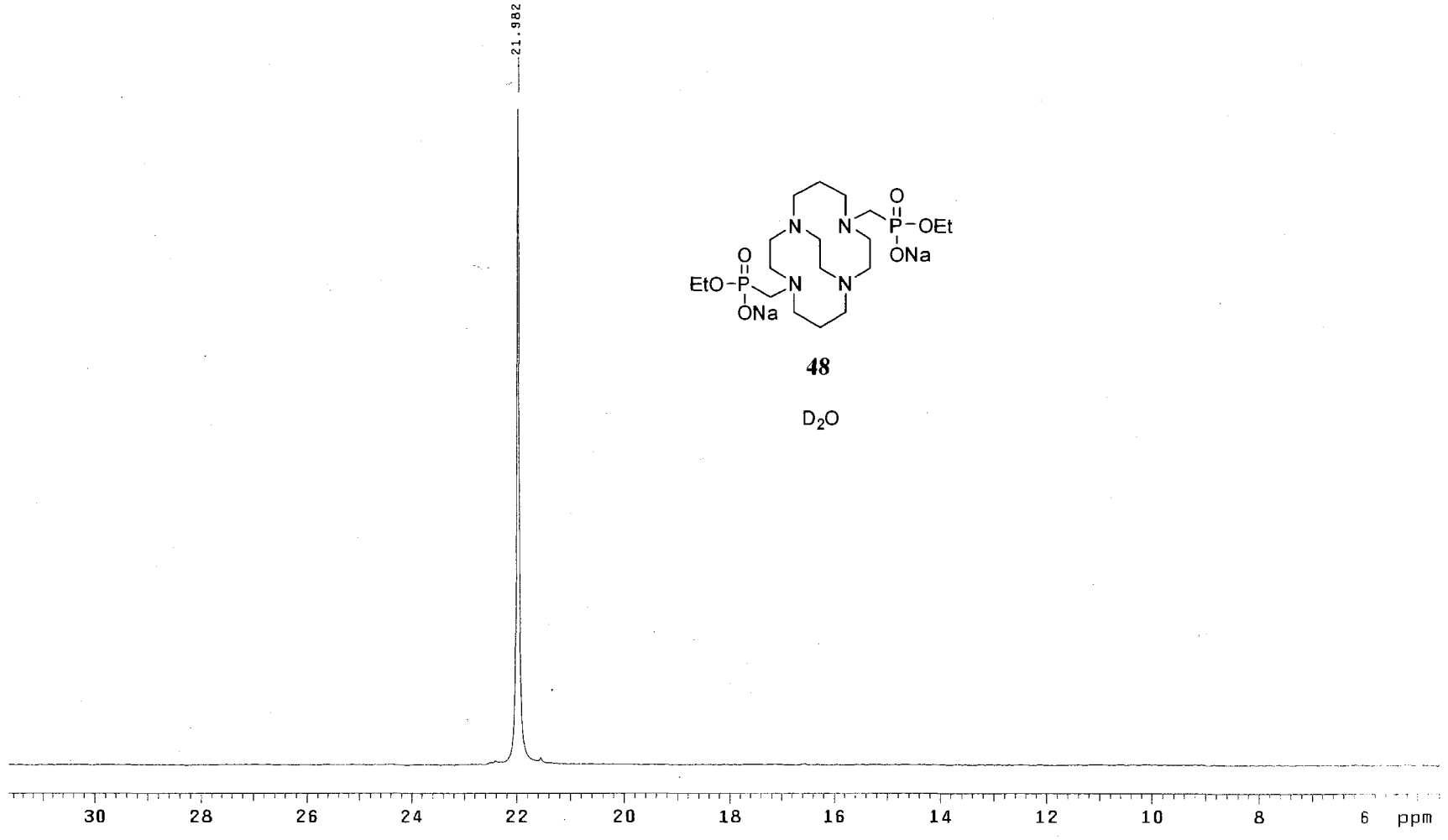
2927.3
2893.8
2862.9
1669.1
1606.8
1482.2
1455.7
1389.0
1338.5
1302.3
1171.1
1125.9
1100.8
1064.6
1048.9
943.2
854.3
760.5
621.2
582.7
560.8

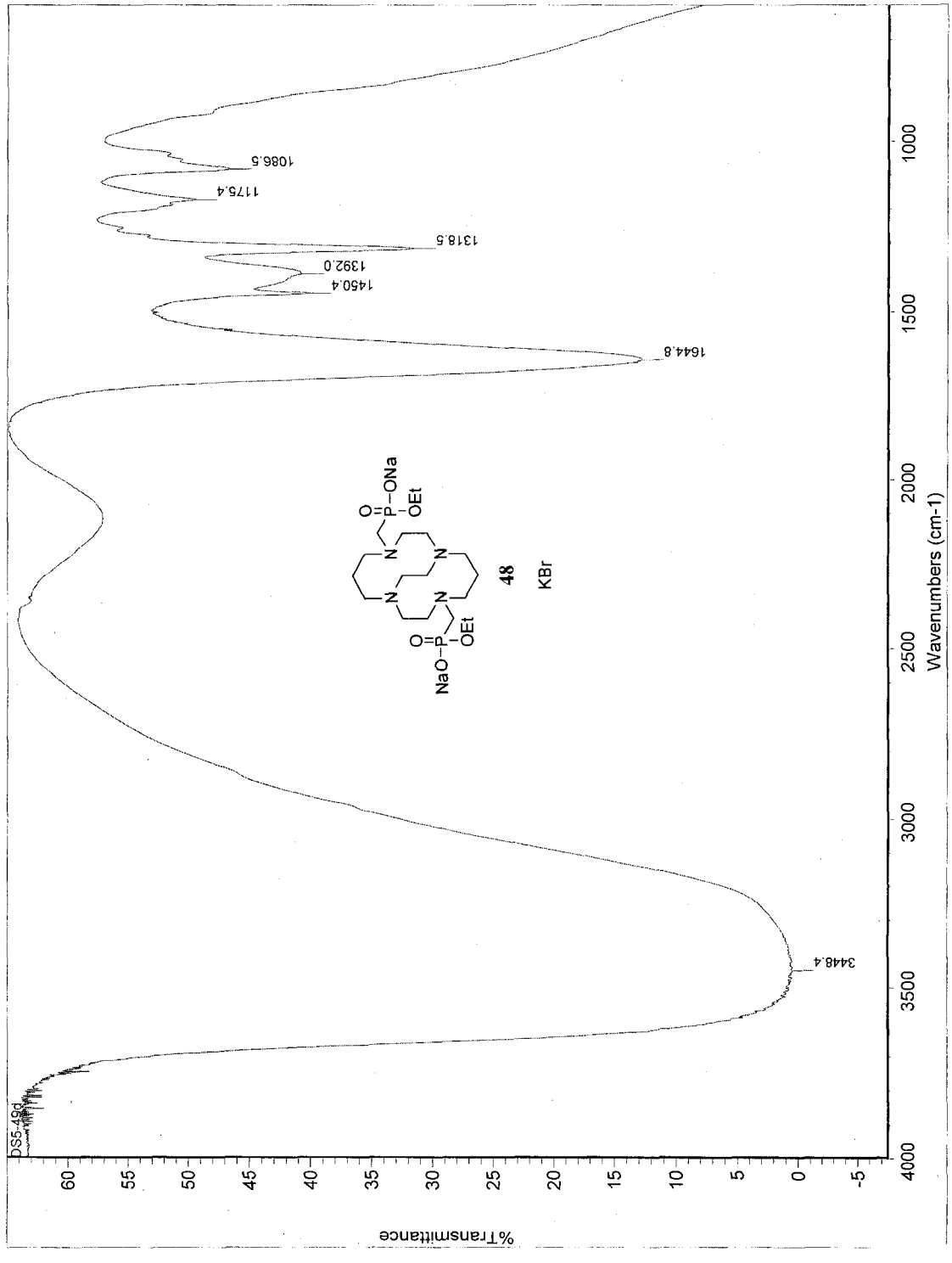

EtO-P
ONa
O
N
N
N
N
P-OEt
ONa
O
48
D2O
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
4.00
1.90
21.85
3.97
6.03


61.927
61.866
57.673
57.262
53.823
53.564
53.511
52.856
51.083
49.691
25.394
17.815
17.762
EtO–P(=O)(ONa)
N
N
N
N
P(=O)(OEt)ONa
48
D2O
60
55
50
45
40
35
30
25
20
ppm


21.982
EtO–P
O
ONa
N
N
N
N
P–OEt
O
ONa
30
28
26
24
22
20
18
16
14
12
10
8
6
ppm

48

D_2O


DS5-48d
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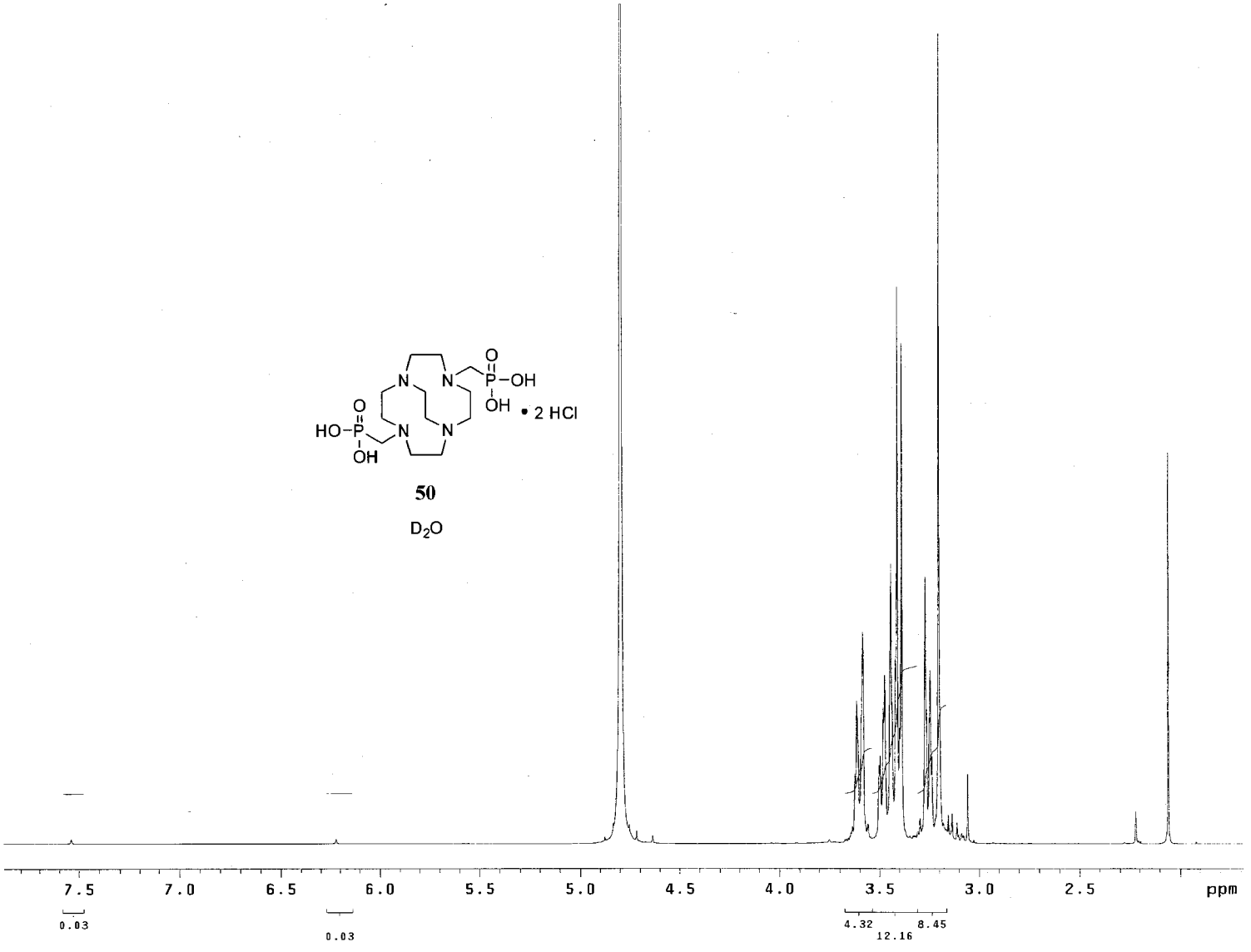
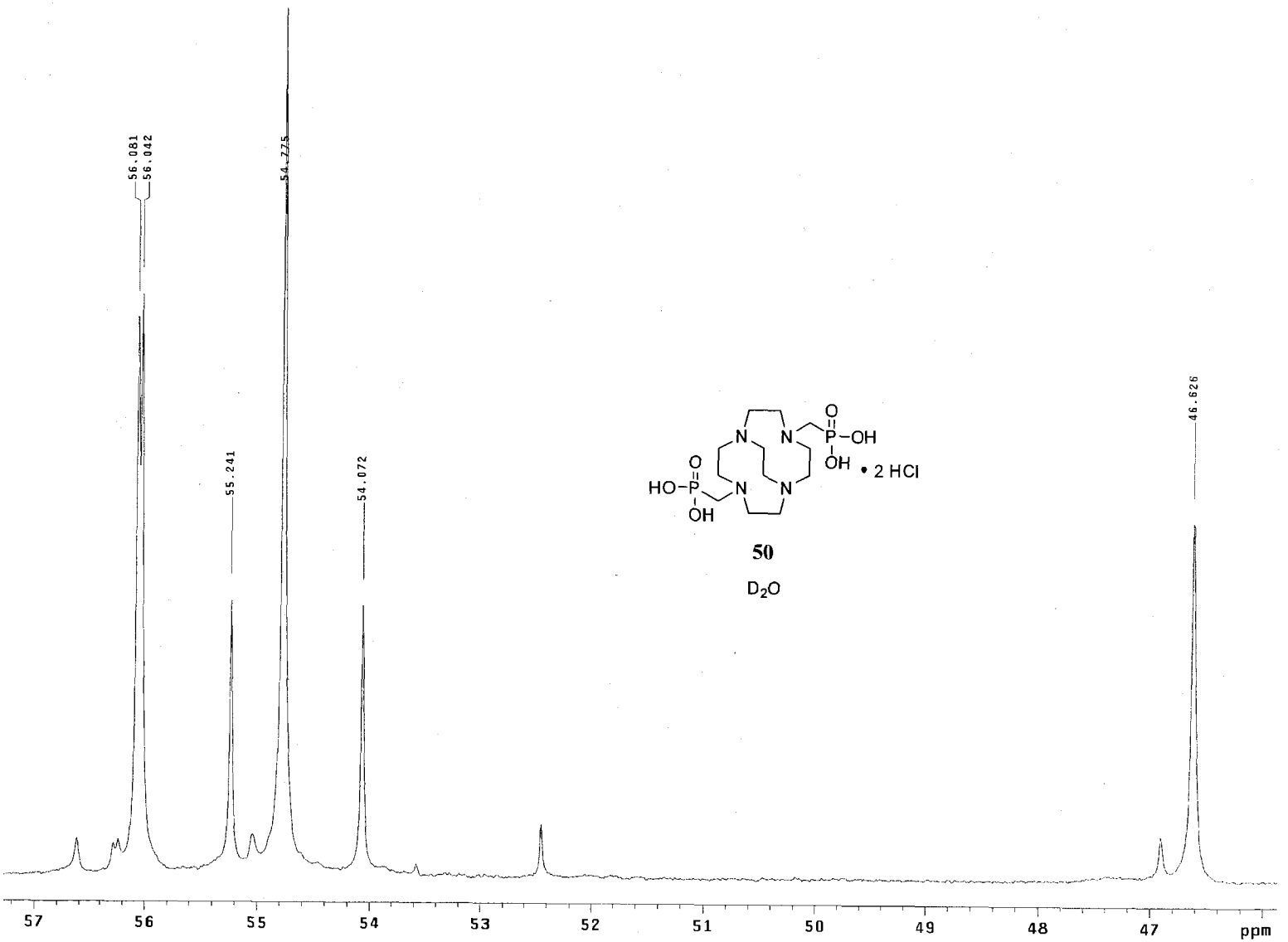
1318.5
1175.4
1086.5
48
KBr


HO–P
O
OH
N
N
N
N
P–OH
O
OH
• 2 HCl
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
ppm
0.03
0.03
4.32
8.45
12.16

50

D_2O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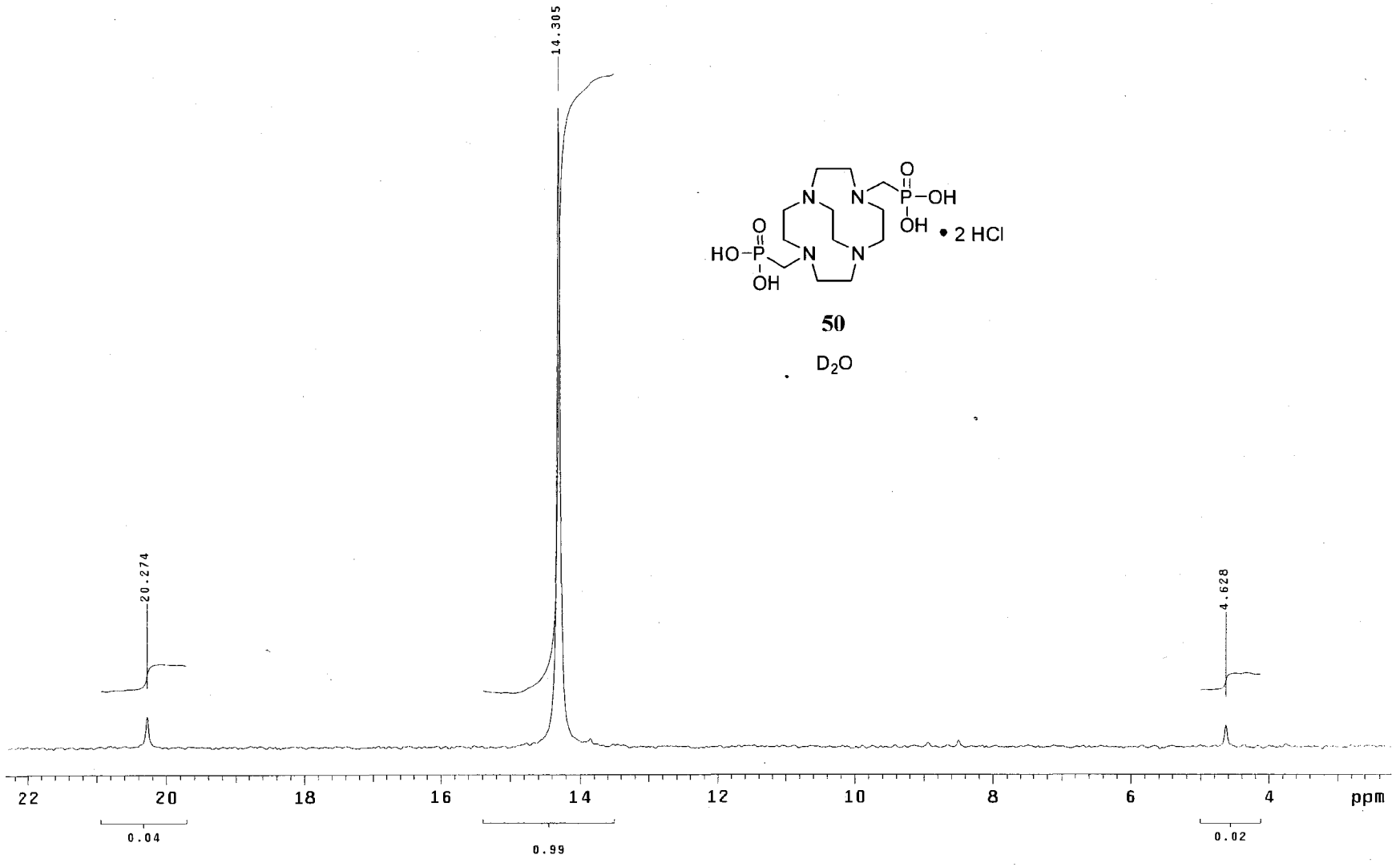
56.042
55.241
54.775
54.072
46.626
57
56
55
54
53
52
51
50
49
48
47
ppm
HO-P
O
OH
N
N
N
N
P-OH
O
OH
• 2 HCl

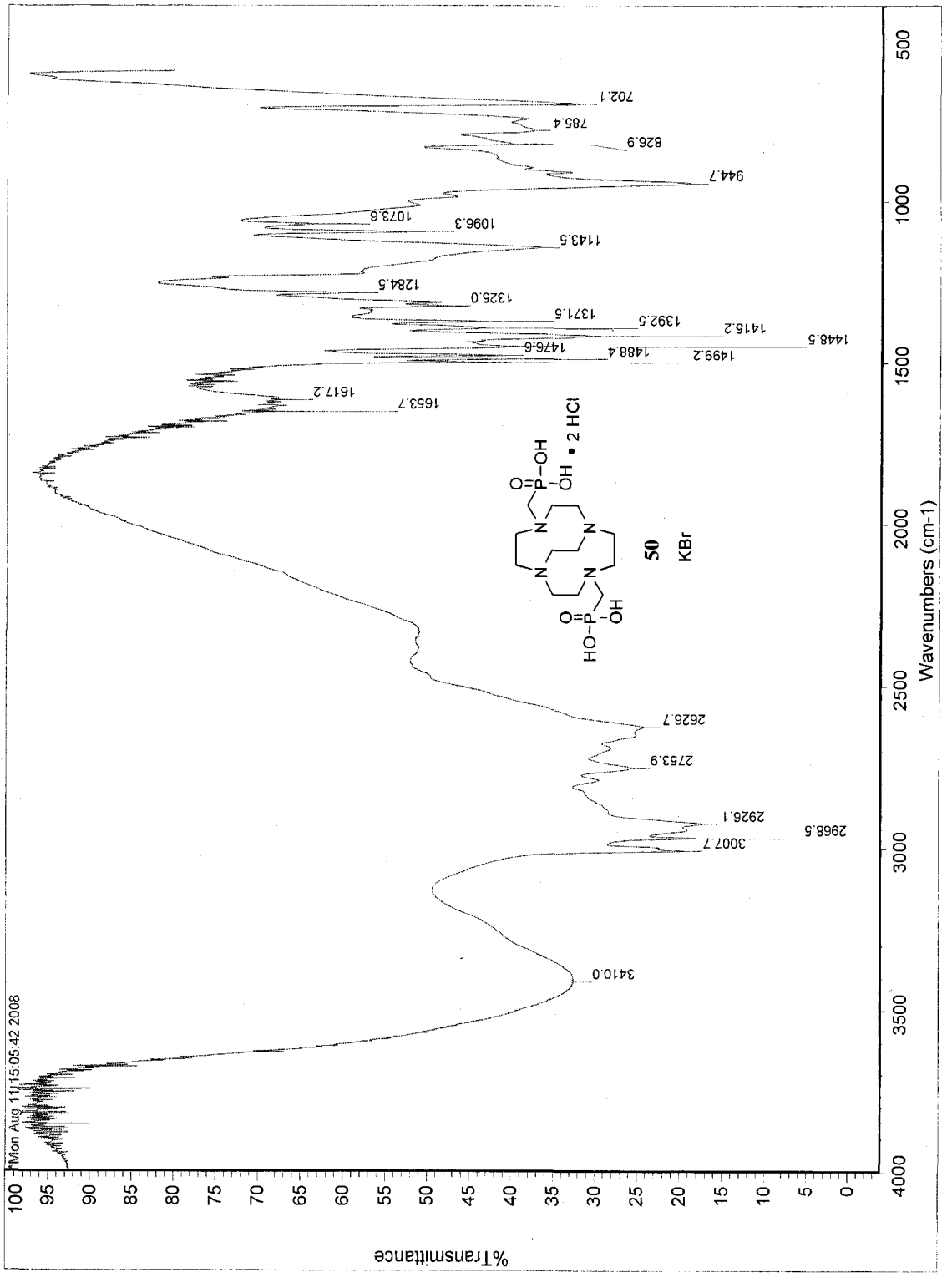
50

D_2O


14.305
20.274
4.628
HO–P(=O)(OH)
N
N
N
N
P(=O)(OH)–OH
• 2 HCl
22
20
18
16
14
12
10
8
6
4
ppm
0.04
0.99
0.02

50

D_2O


Mon Aug 11 15:05:42 2008
%Transmittance
Wavenumbers (cm-1)
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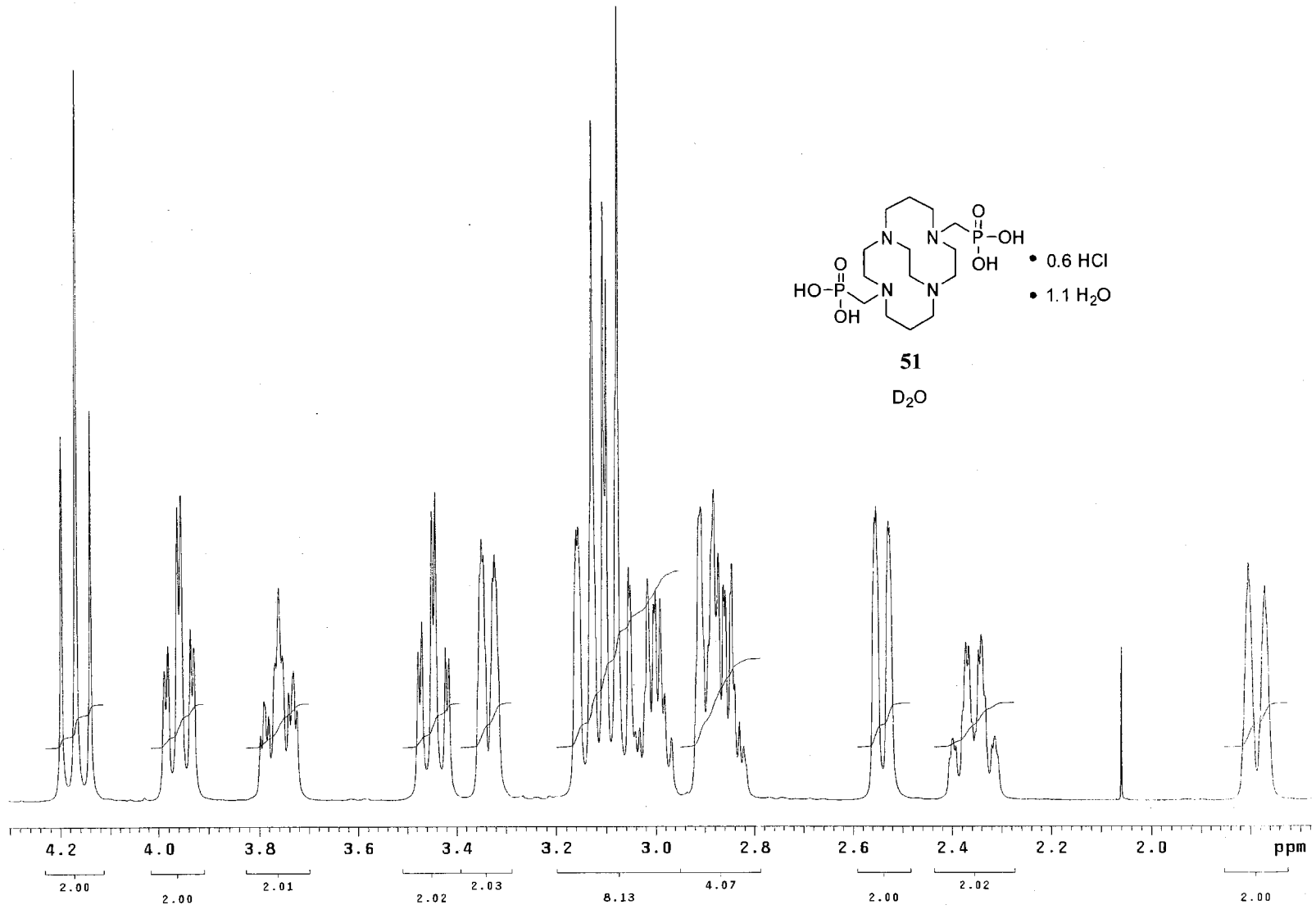
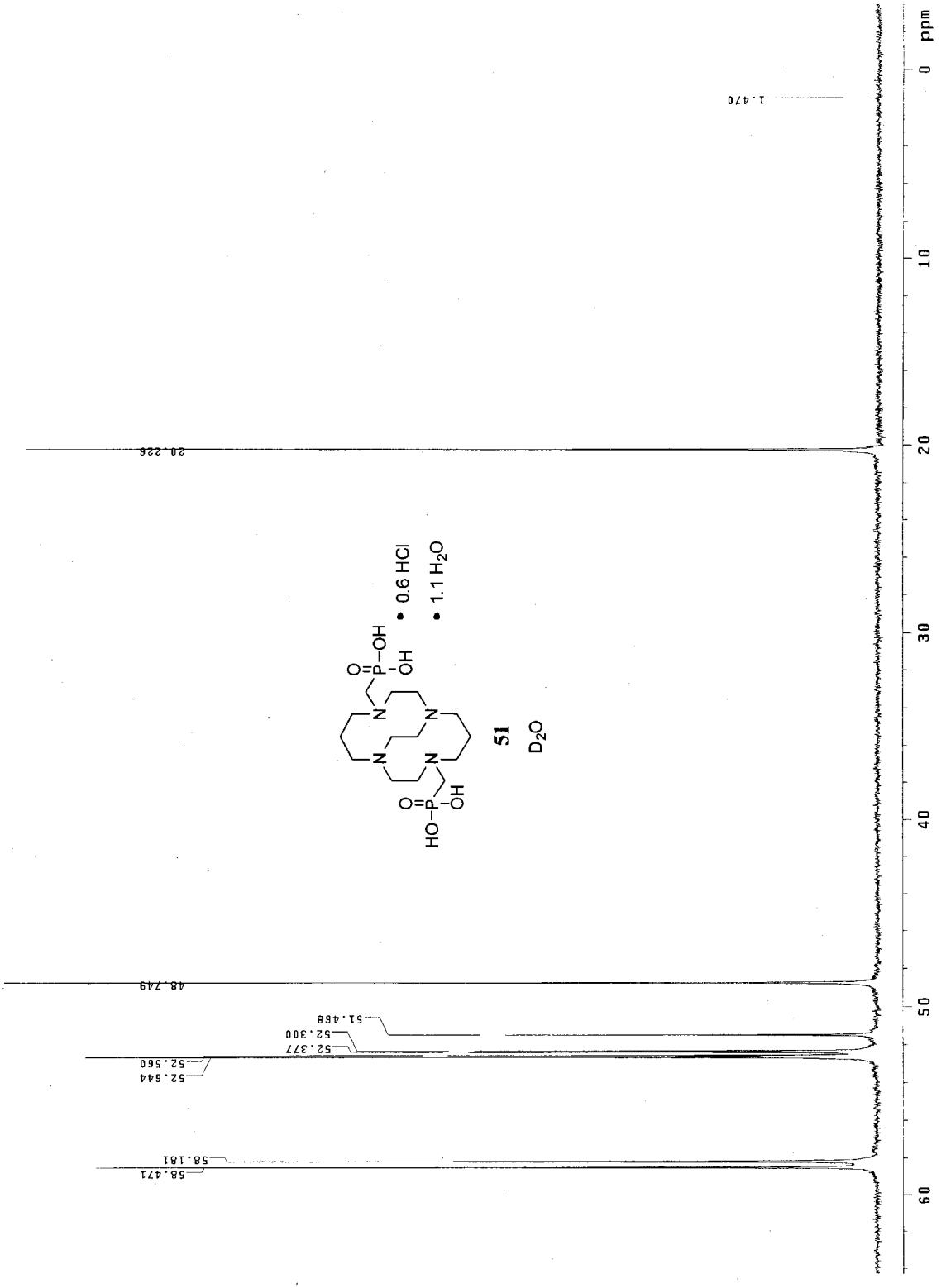
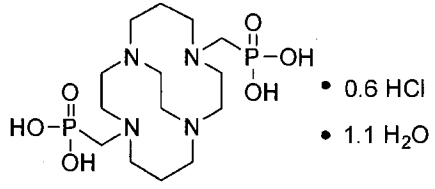
3000
2500
2000
1500
1000
500
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
3410.0
3007.7
2968.5
2926.1
2753.9
2626.7
1653.7
1617.2
1499.2
1488.4
1476.6
1448.5
1415.2
1392.5
1371.5
1325.0
1284.5
1143.5
1096.3
1073.6
944.7
826.9
785.4
702.1
50
KBr
• 2 HCl

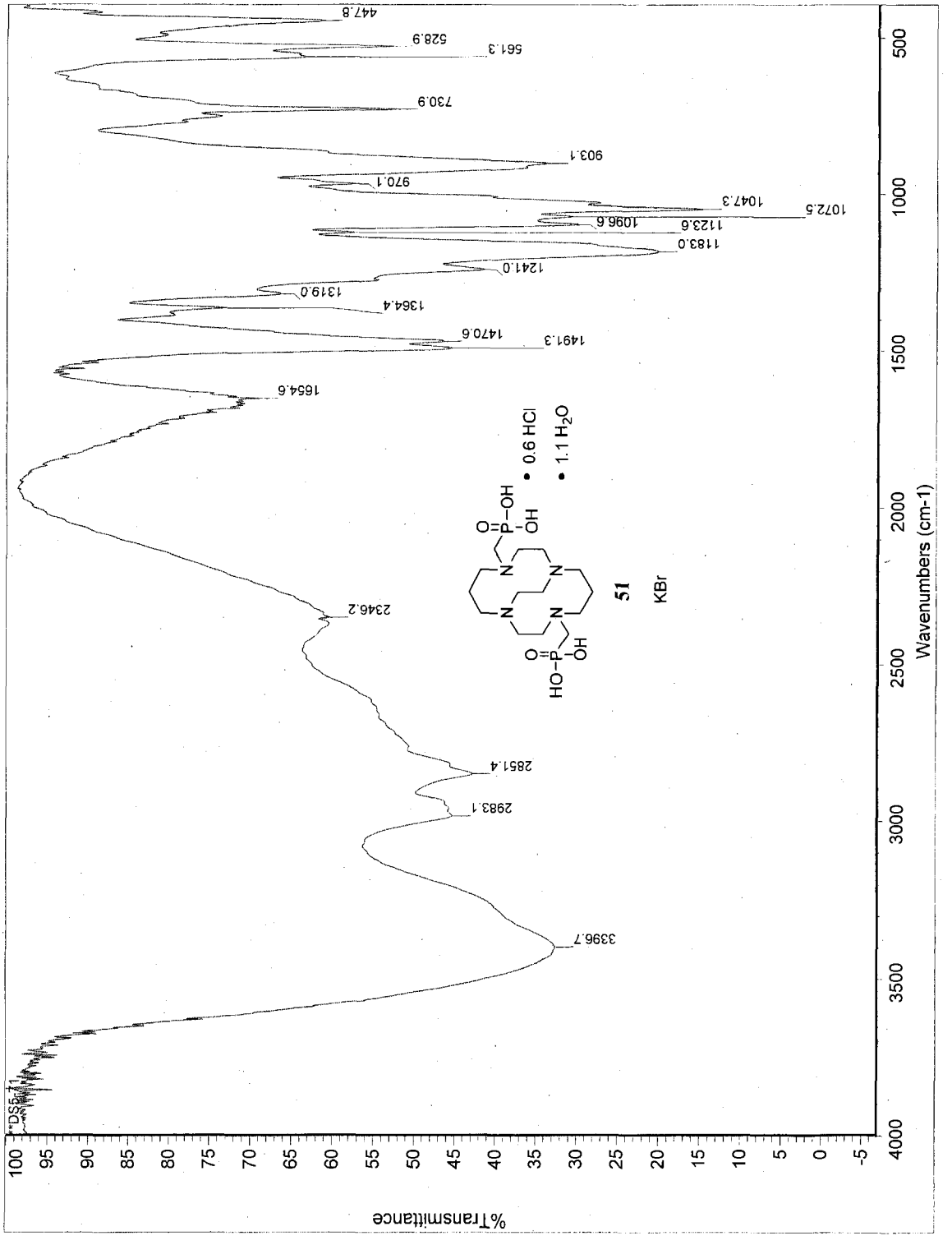

HO–P
OH
O
N
N
N
N
P–OH
OH
O
• 0.6 HCl
• 1.1 H2O
51
D2O
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
ppm
2.00
2.00
2.01
2.02
2.03
8.13
4.07
2.00
2.02
2.00

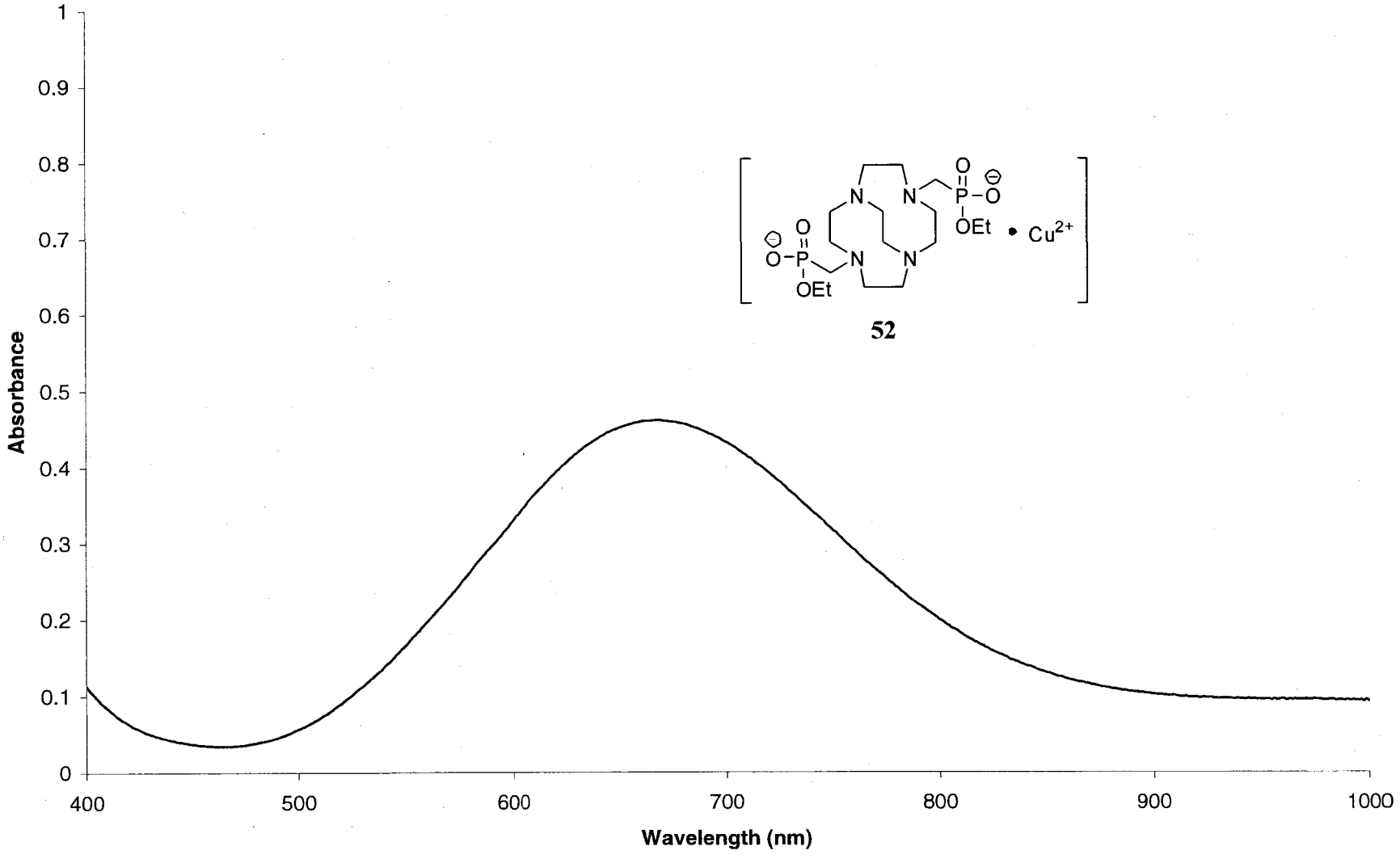

1.470
20.226
48.749
51.468
52.377
52.300
52.560
52.644
58.181
58.471
0 ppm
10
20
30
40
50
60
• 0.6 HCl
• 1.1 H2O
51
D2O



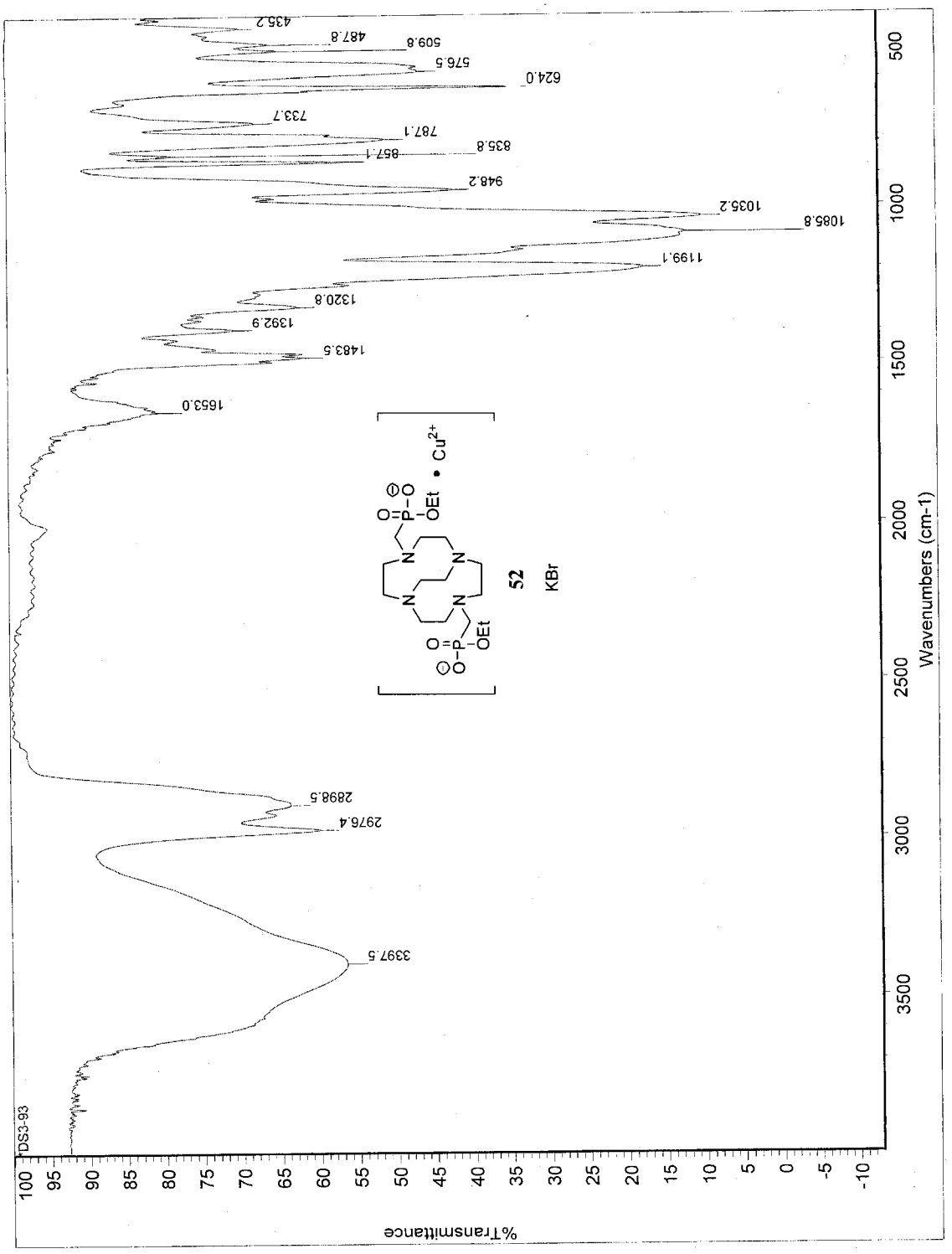
51

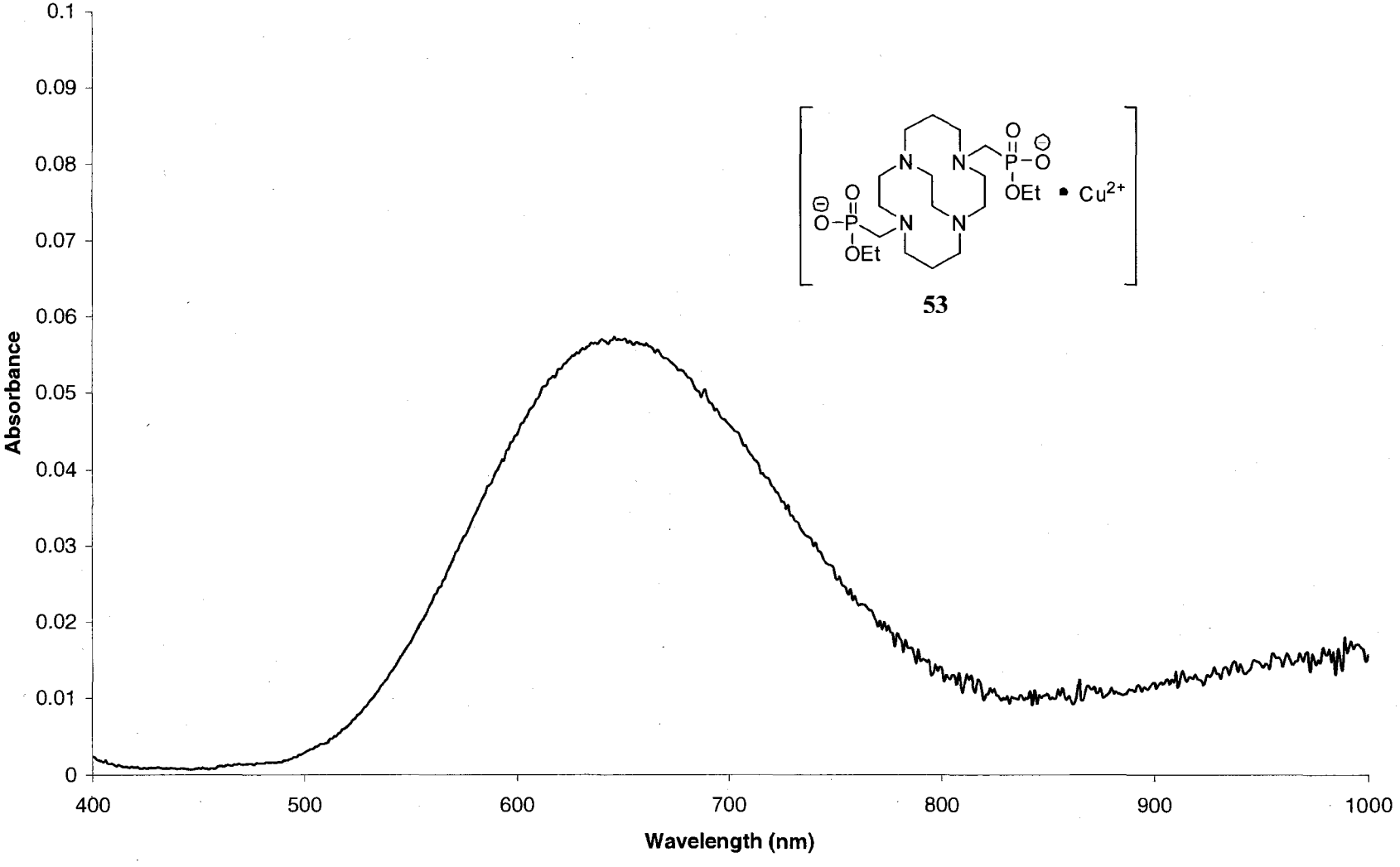
D_2O


%Transmittance
Wavenumbers (cm-1)
DS5-1
3396.7
2983.1
2851.4
2346.2
1654.6
1491.3
1470.6
1364.4
1319.0
1241.0
1183.0
1123.6
1096.6
1072.5
1047.3
970.1
903.1
730.9
561.3
528.9
447.8
• 0.6 HCl
• 1.1 H2O
51
KBr

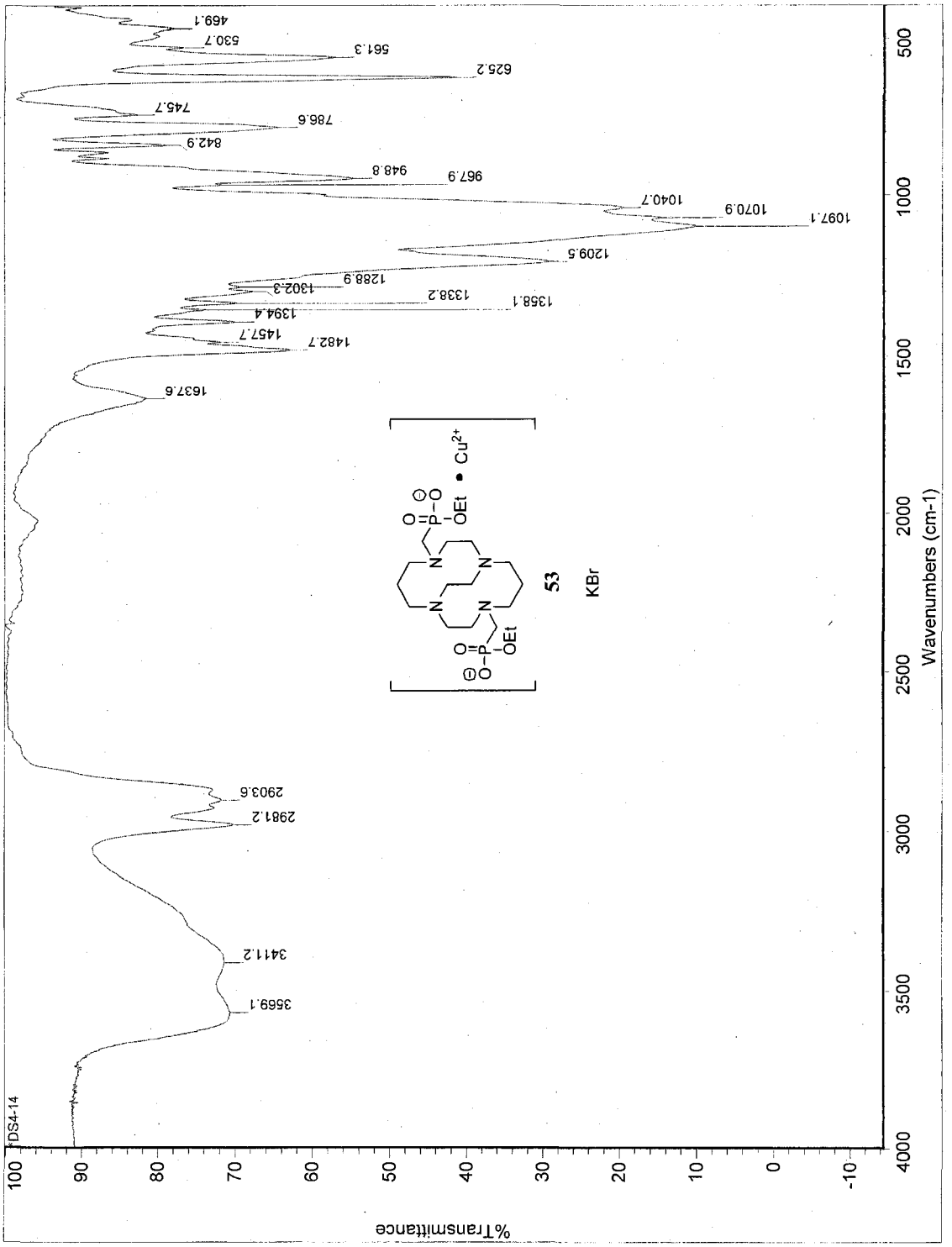

Absorbance
Wavelength (nm)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
400
500
600
700
800
900
1000
N
N
N
N
O
P
O
OEt
• Cu2+

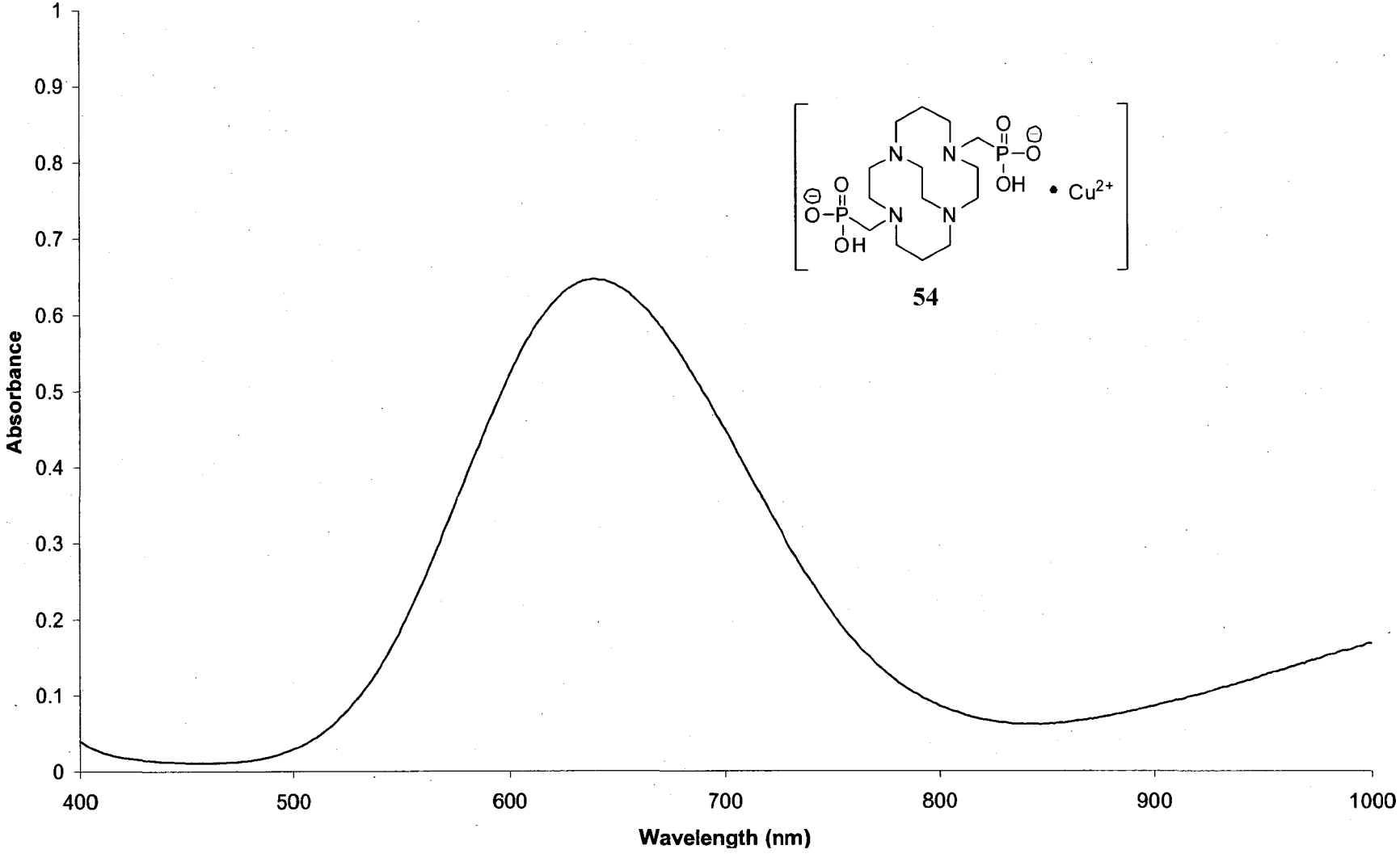
52


DS3-93
%Transmittance
Wavenumbers (cm-1)
3397.5
2976.4
2898.5
1653.0
1483.5
1392.9
1320.8
1199.1
1085.8
1035.2
948.2
857.1
835.8
787.1
733.7
624.0
576.5
509.8
487.8
435.2
52
KBr

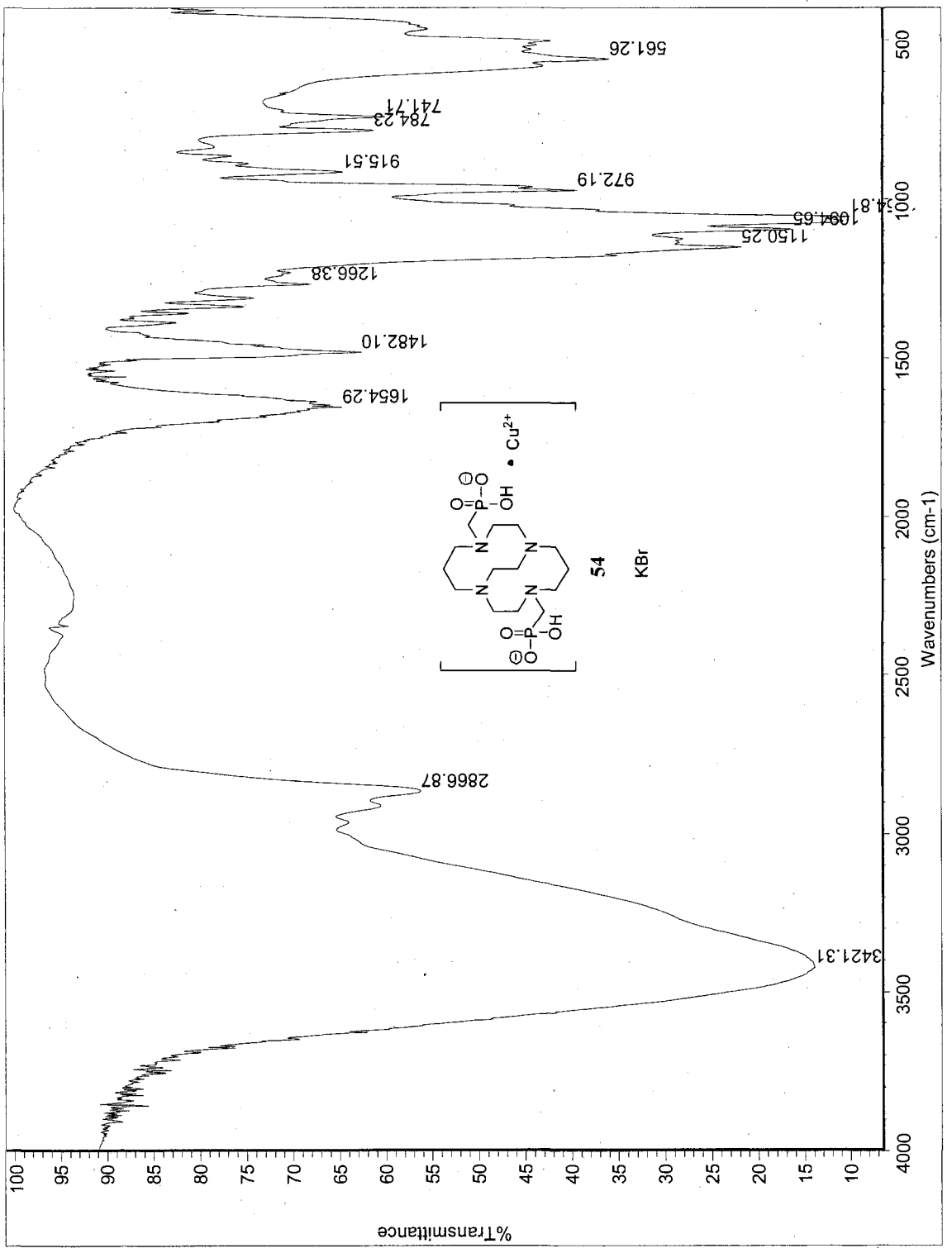

0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Absorbance
400
500
600
700
800
900
1000
Wavelength (nm)
O
O
P
O
OEt
N
N
N
N
O
O
P
O
OEt
• Cu2+

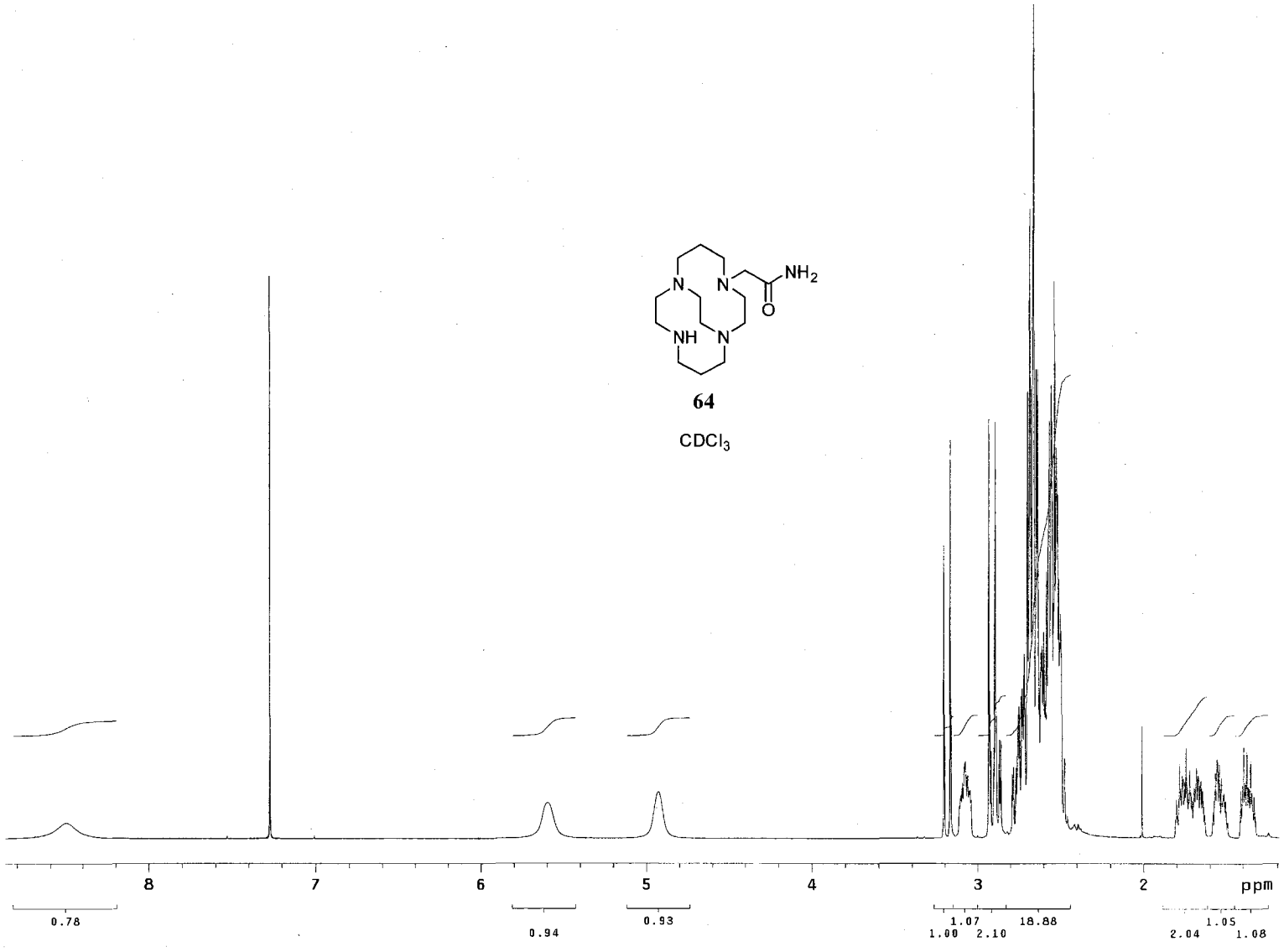
53

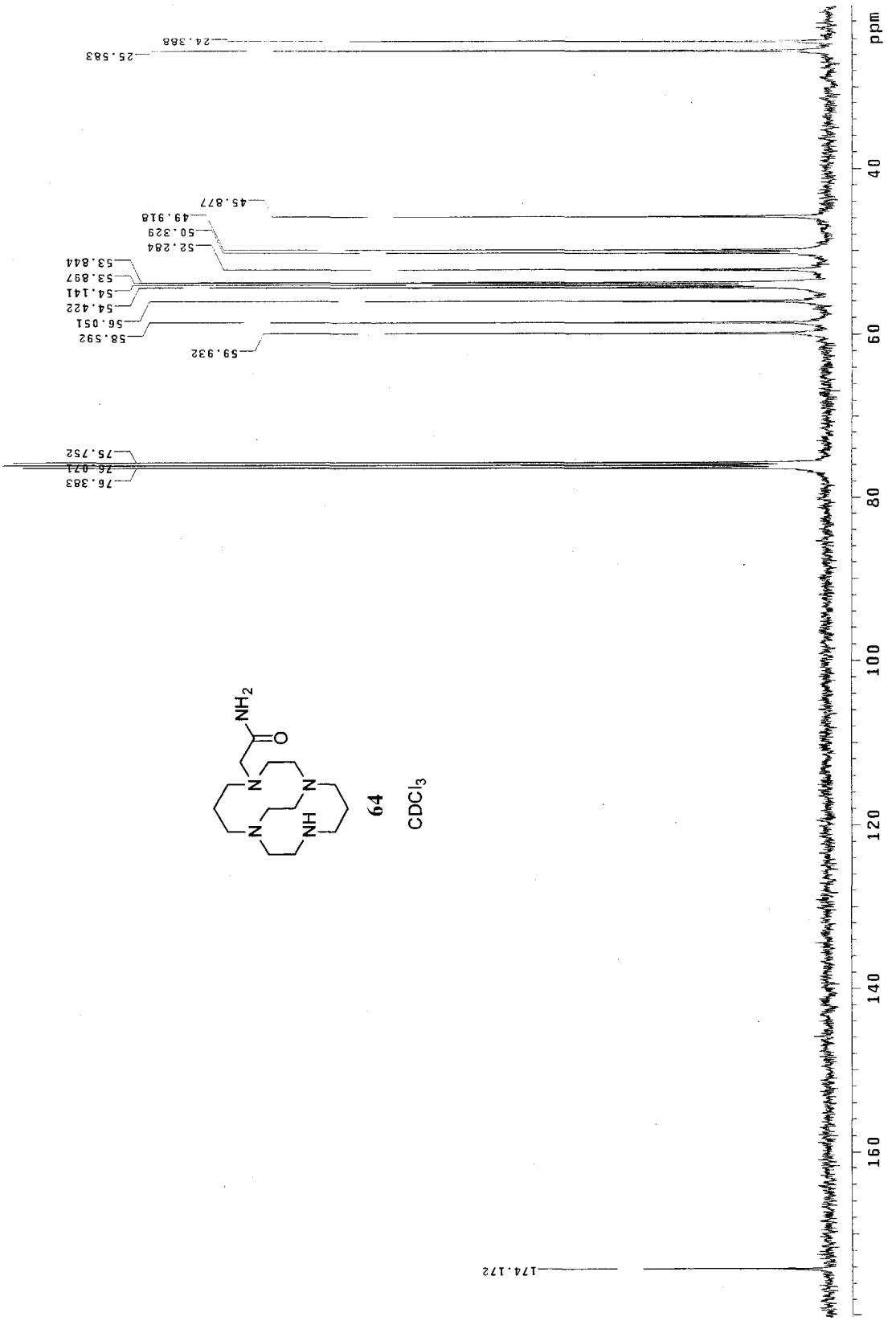

%Transmittance
Wavenumbers (cm-1)
DS4-14
3569.1
3411.2
2981.2
2903.6
1637.6
1482.7
1457.7
1394.4
1358.1
1338.2
1302.3
1288.9
1209.5
1097.1
1070.9
1040.7
967.9
948.8
842.9
786.6
745.7
625.2
561.3
530.7
469.1
KBr

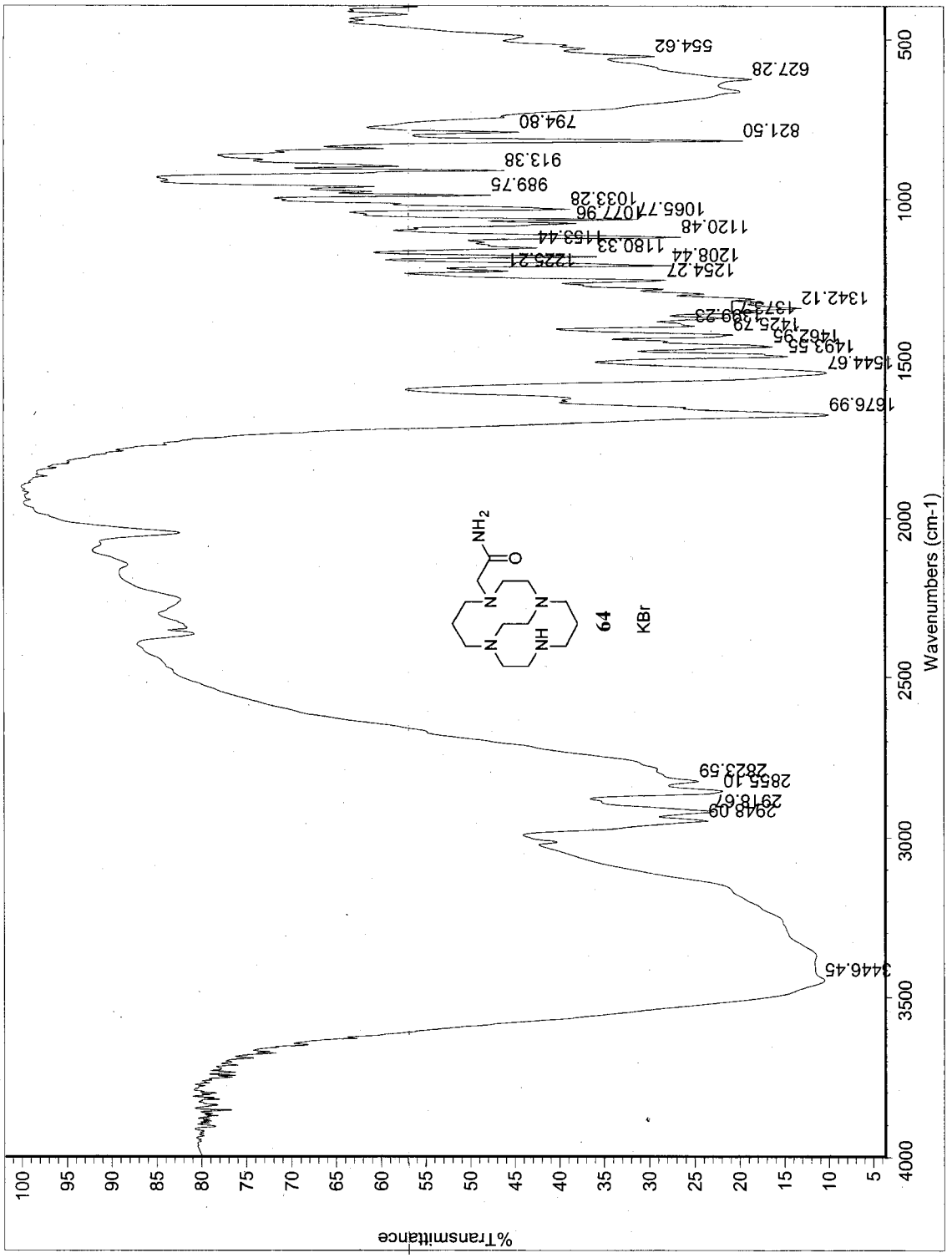

Absorbance
Wavelength (nm)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
400
500
600
700
800
900
1000
• Cu2+

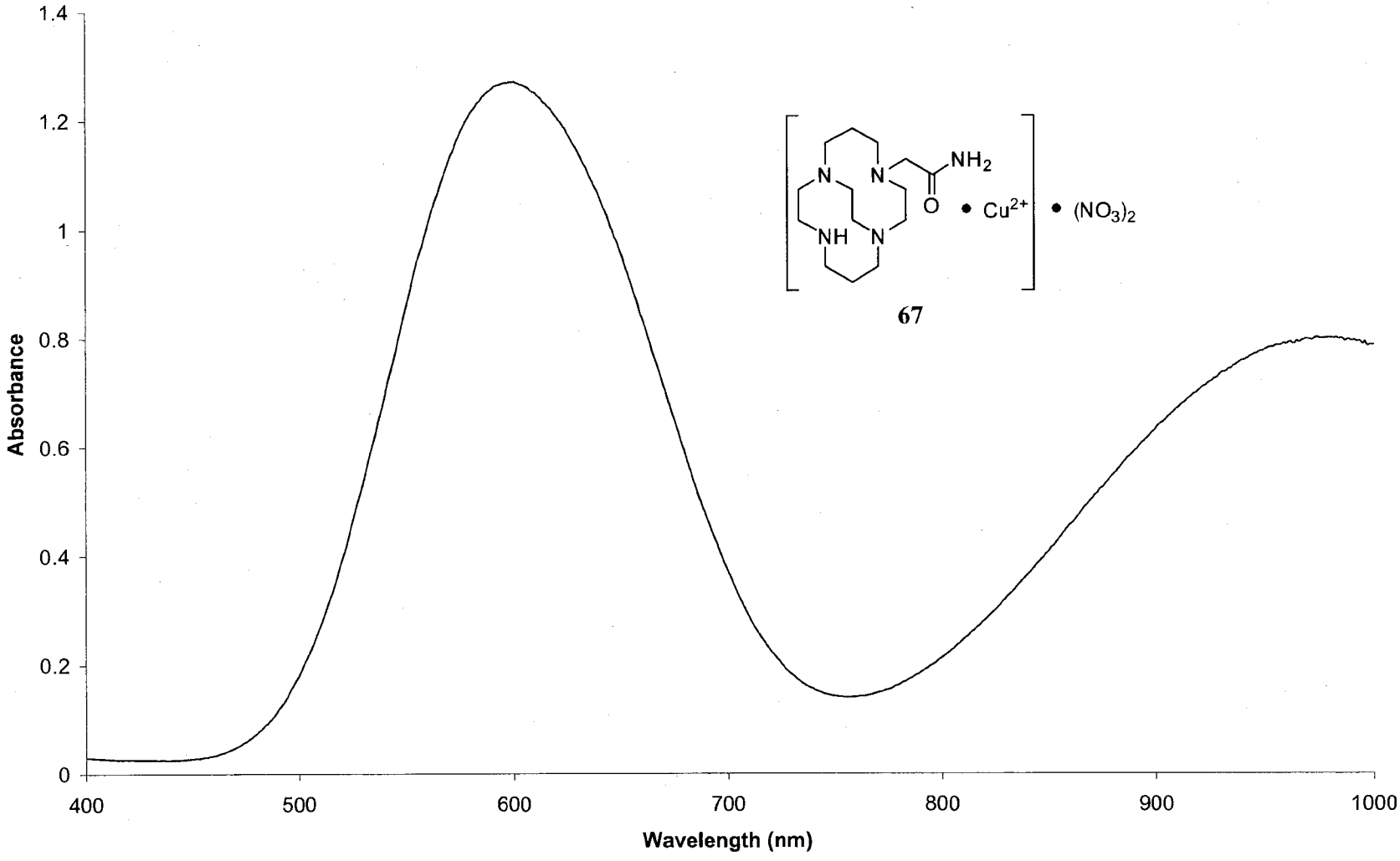
54


%Transmittance
Wavenumbers (cm-1)
3421.31
2866.87
1654.29
1482.10
1266.38
1150.25
1094.65
1044.81
972.19
915.51
784.23
741.71
561.26
54
KBr

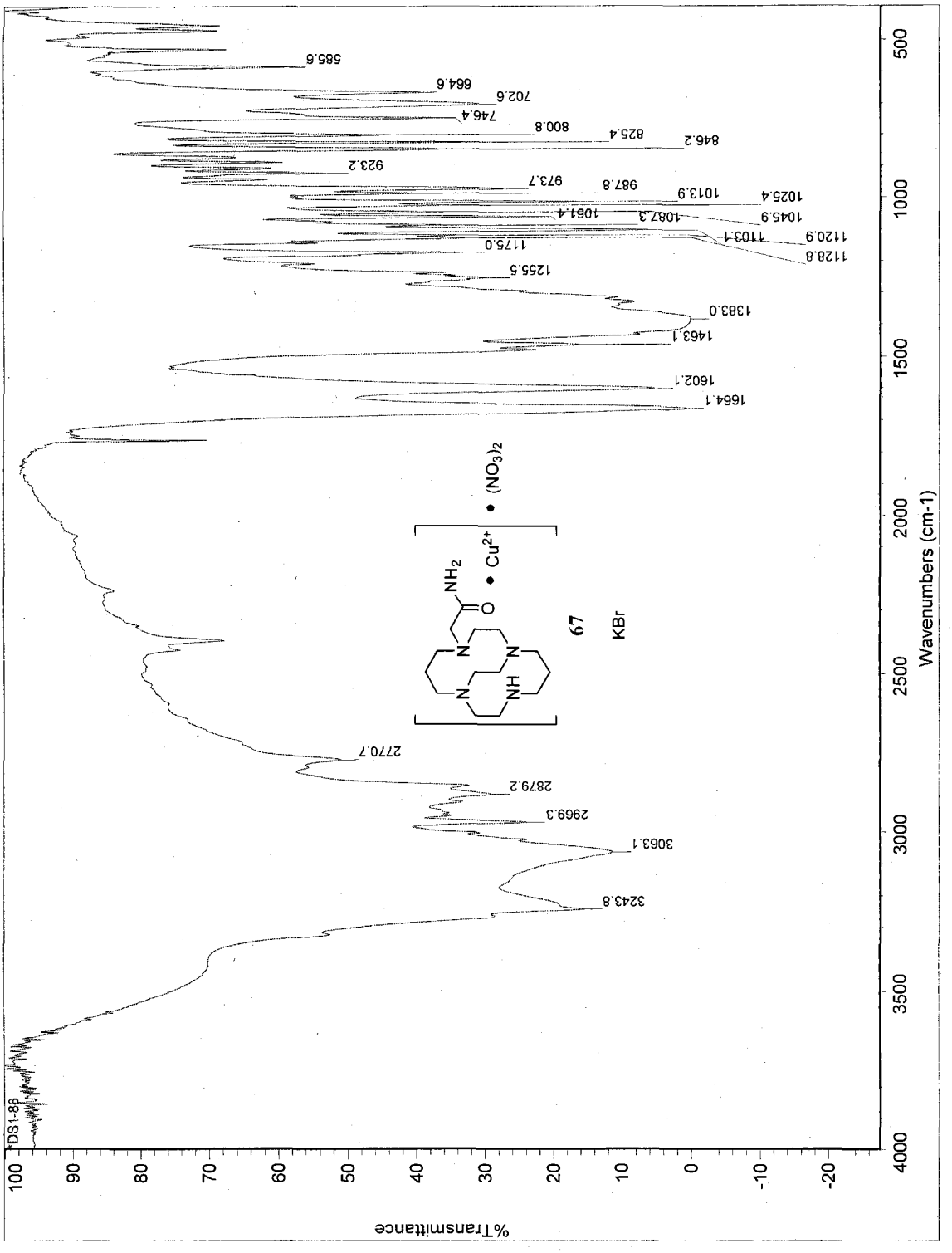

NH2
O
N
N
NH
N
64
CDCl3
8
7
6
5
4
3
2
ppm
0.78
0.94
0.93
1.07
18.88
1.00
2.10
1.05
2.04
1.08

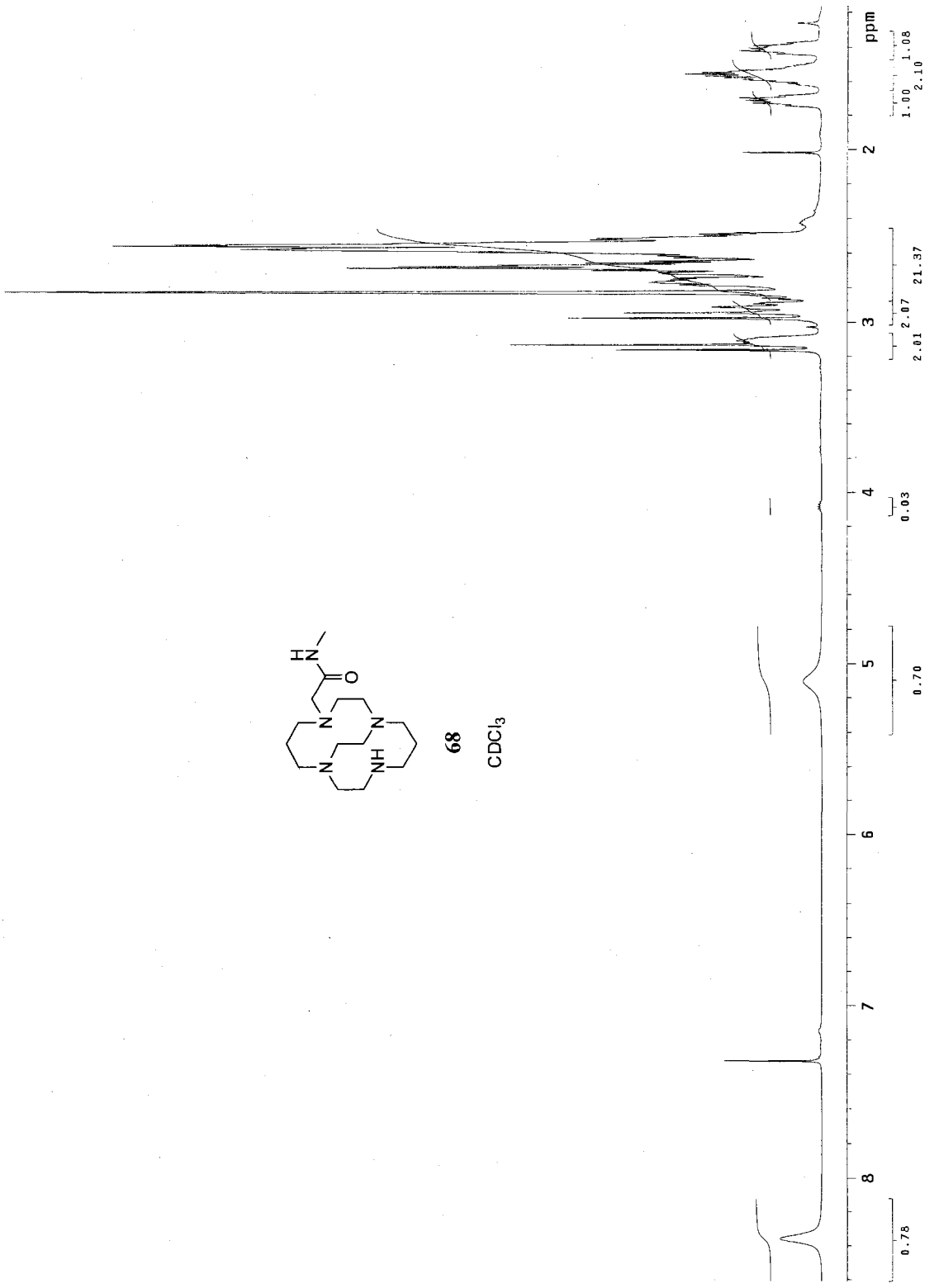

24.388
25.583
45.877
49.918
50.329
52.284
53.844
53.897
54.141
54.422
56.051
58.592
59.932
75.752
76.071
76.383
174.172
ppm
40
60
80
100
120
140
160
NH2
O
N
N
N
NH
64
CDCl3

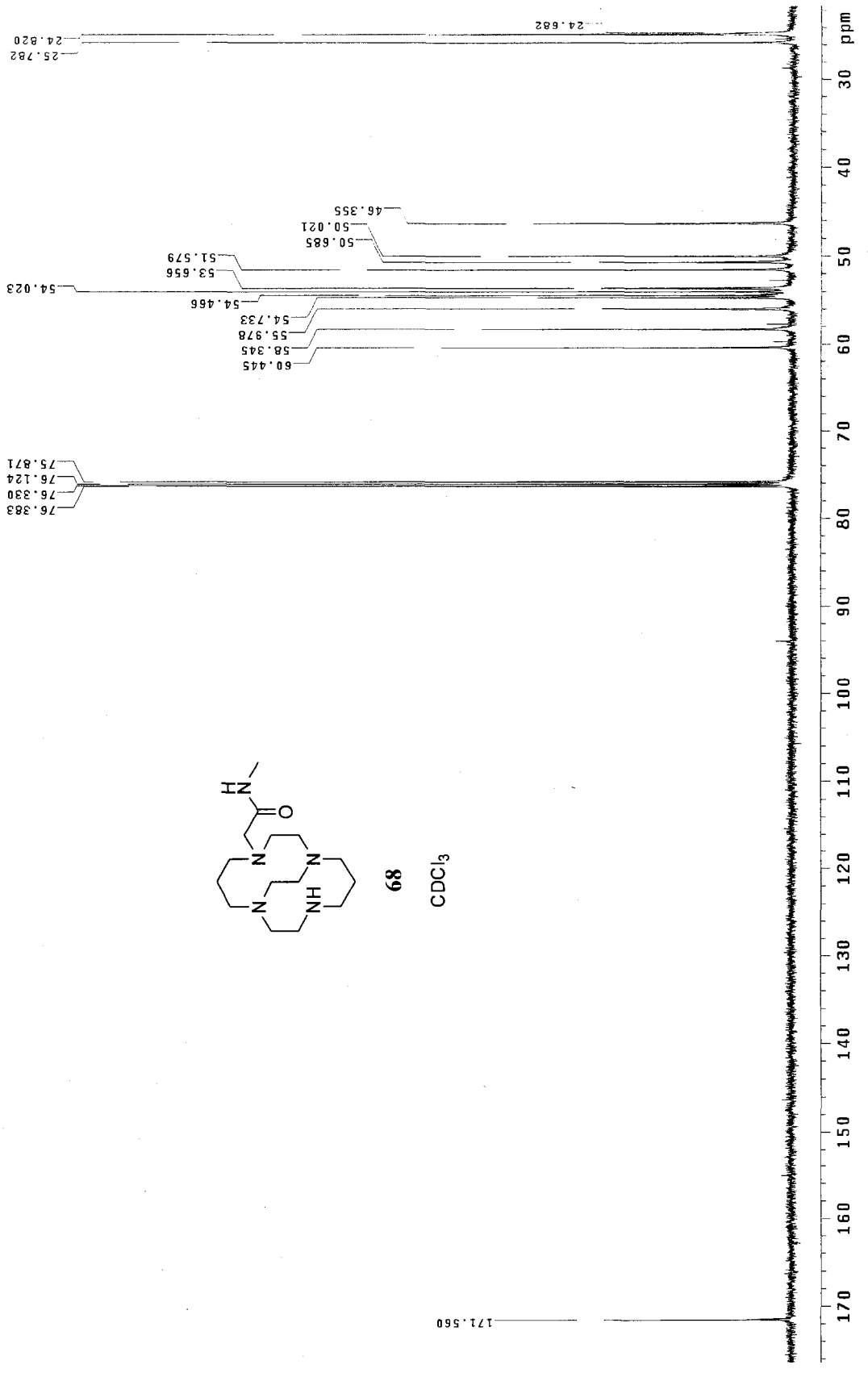

%Transmittance
Wavenumbers (cm-1)
64
KBr
NH2
O
N
NH
3446.45
2948.09
2918.67
2855.10
2823.59
1676.99
1544.67
1493.55
1462.95
1425.79
1389.23
1373.27
1342.12
1254.27
1225.21
1208.44
1180.33
1153.44
1120.48
1077.96
1065.77
1033.28
989.75
913.38
821.50
794.80
627.28
554.62

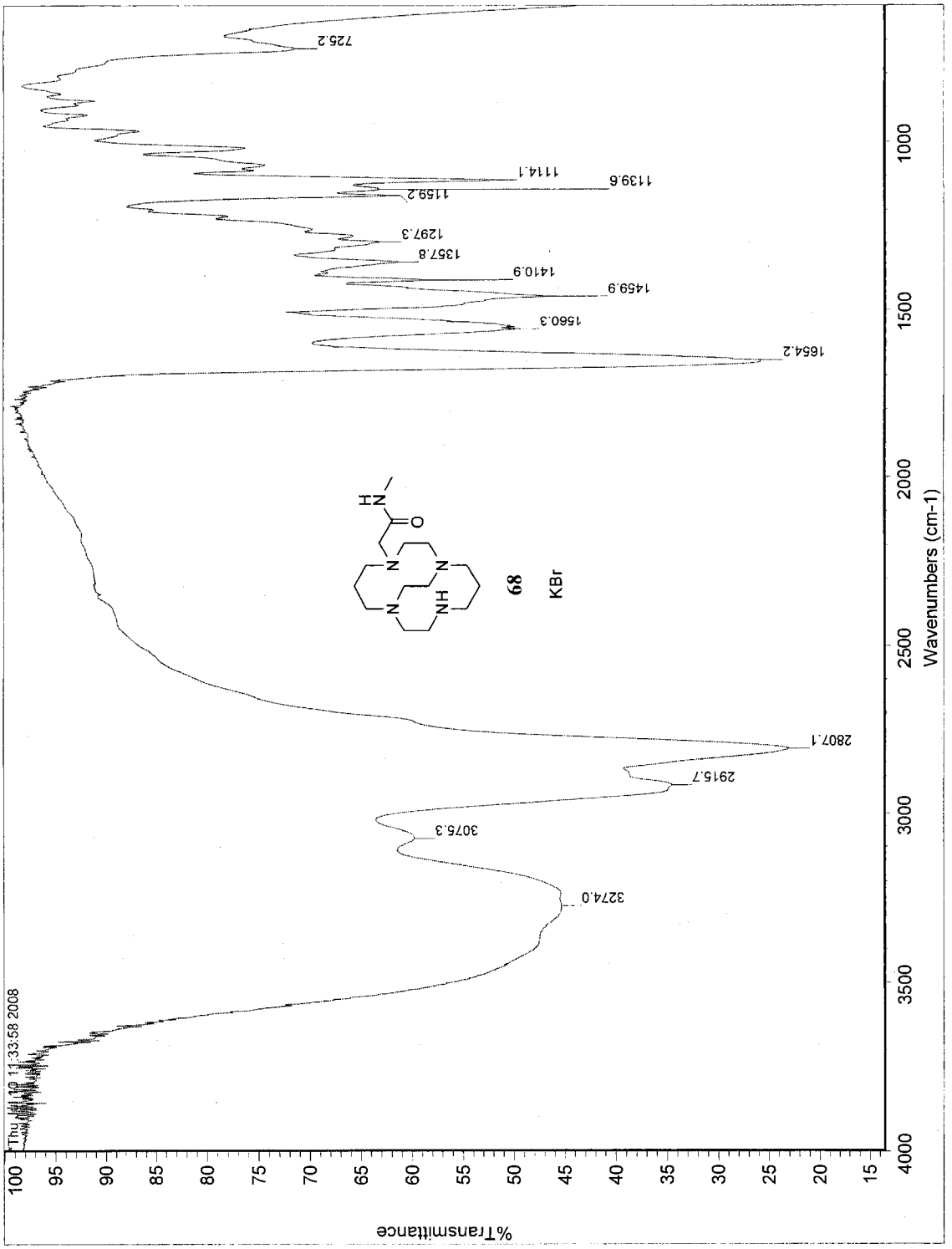

1.4
1.2
1
0.8
0.6
0.4
0.2
0
Absorbance
400
500
600
700
800
900
1000
Wavelength (nm)
N
N
NH
N
NH2
O
• Cu2+
• (NO3)2

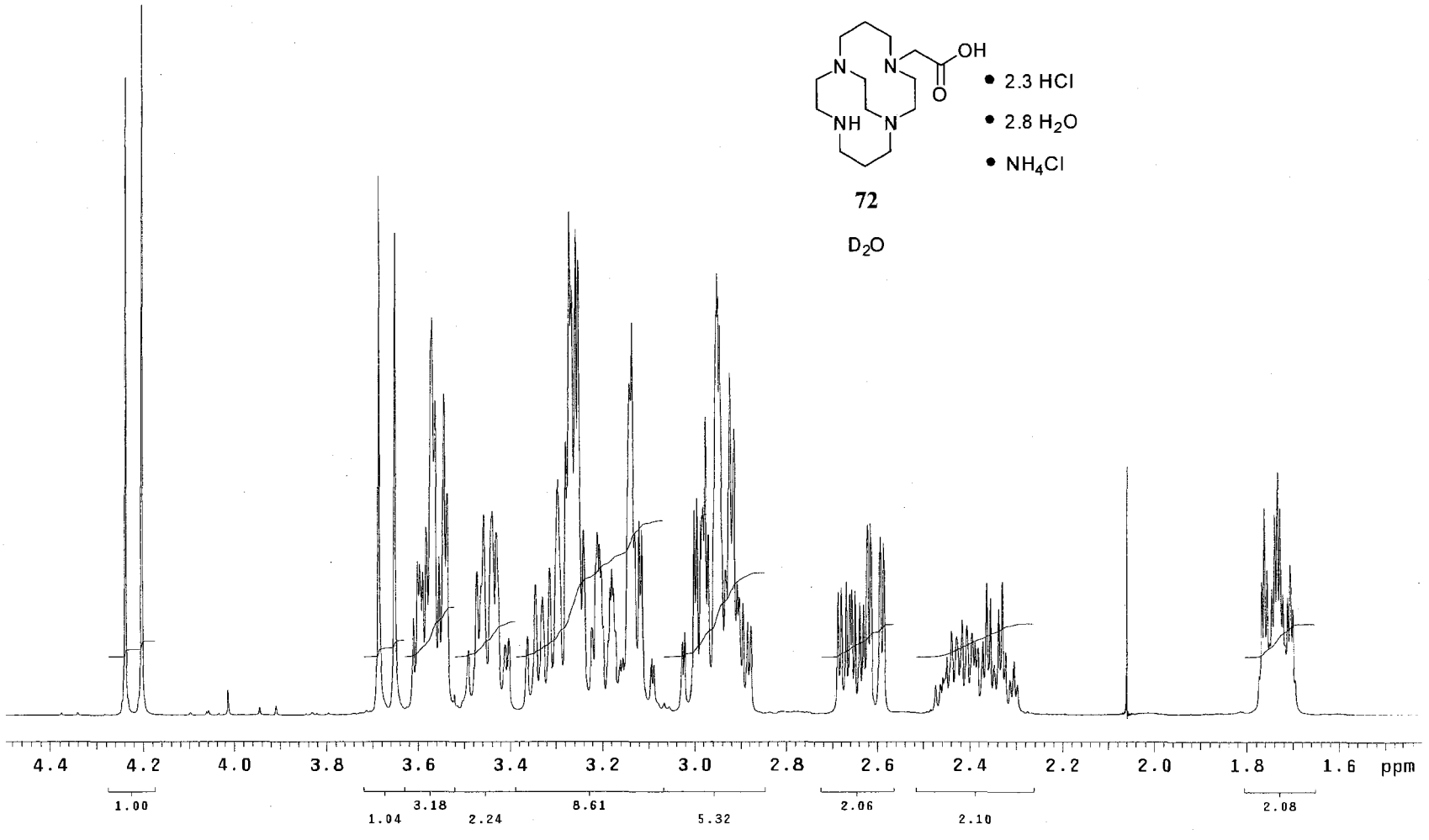
67

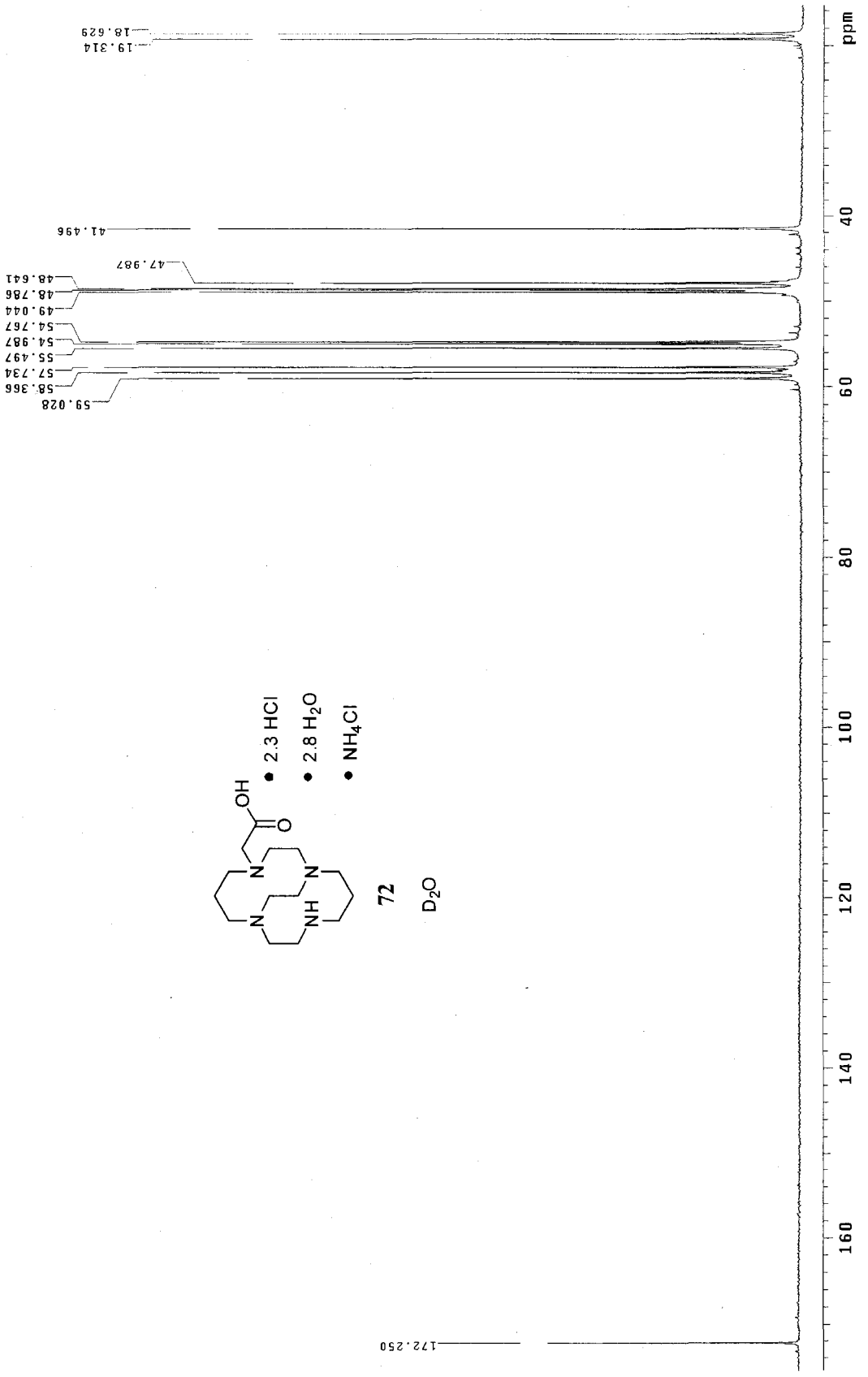

%Transmittance
Wavenumbers (cm-1)
DS1-88
KBr
67
$(NO_3)_2$
Cu^{2+}
NH_2
O
N
NH
3243.8
3063.1
2969.3
2879.2
2770.7
1664.1
1602.1
1463.1
1383.0
1255.5
1175.0
1128.8
1120.9
1103.1
1045.9
1087.3
1061.4
1025.4
1013.9
987.8
973.7
923.2
846.2
825.4
800.8
746.4
702.6
664.6
585.6

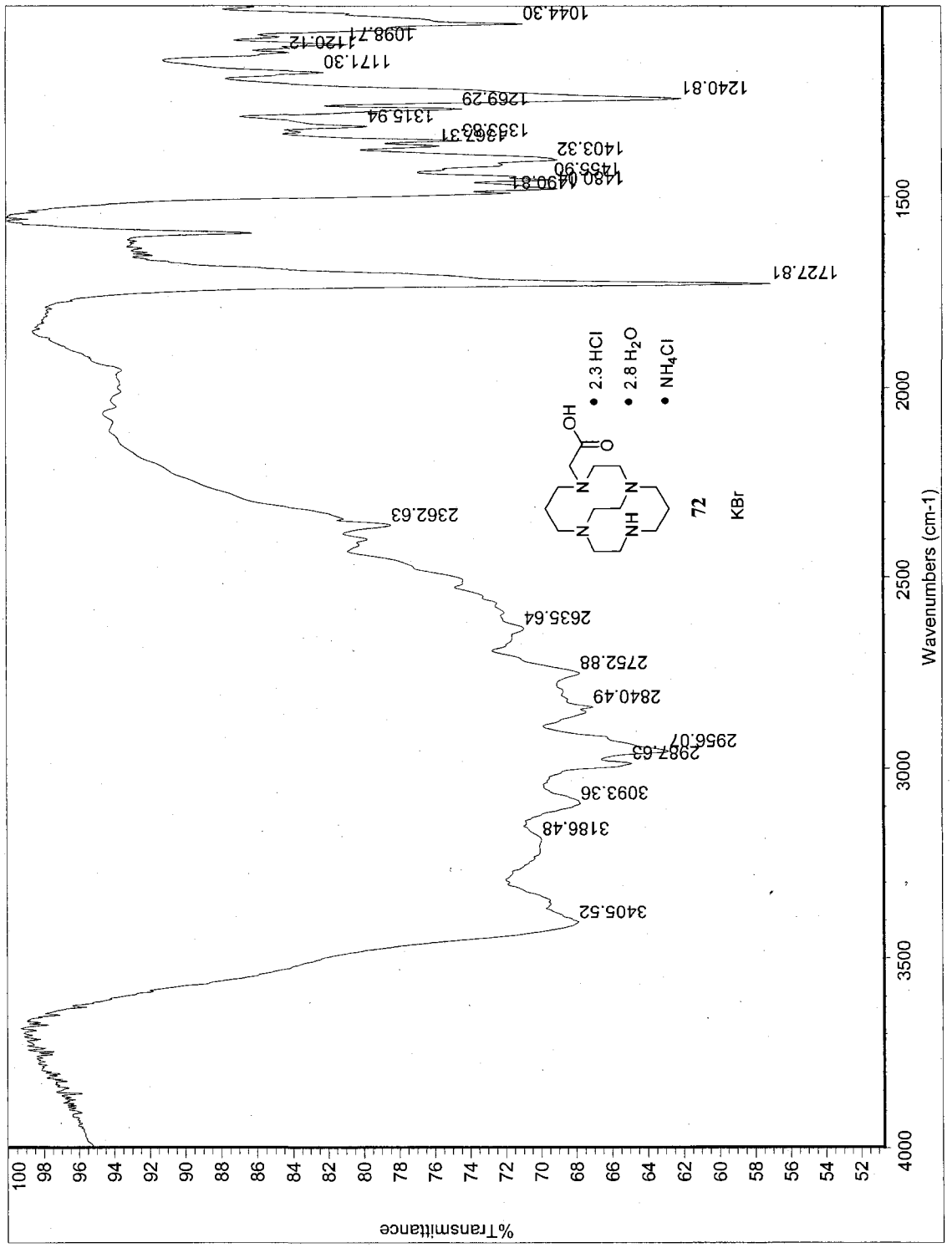

68
CDCl3
ppm
1.00
2.10
1.08
2.01
2.07
21.37
0.03
0.70
0.78

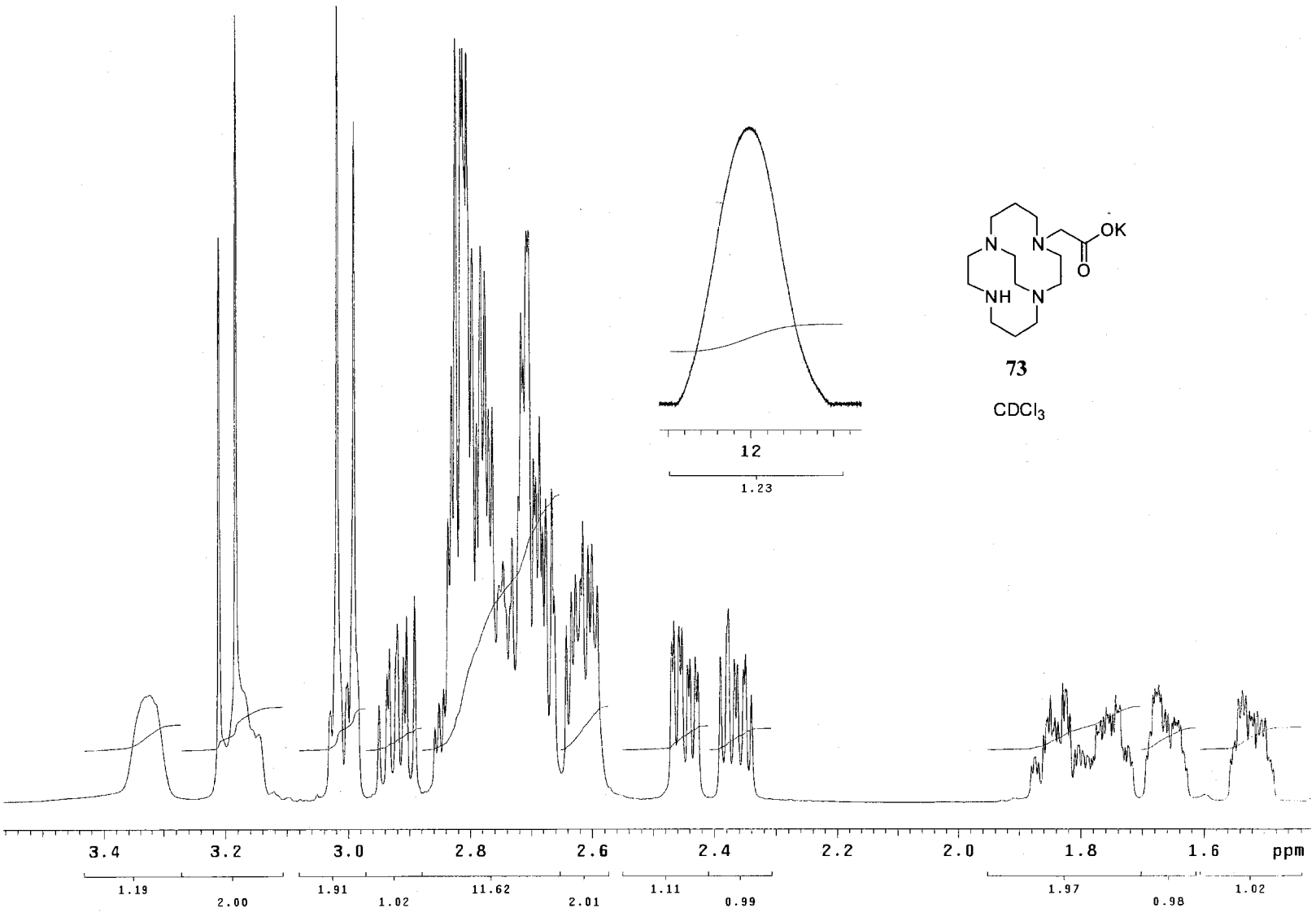

24.682
24.820
25.782
46.355
50.021
50.685
51.579
53.656
54.023
54.466
54.733
55.978
58.345
60.445
75.871
76.124
76.330
76.383
171.560
ppm
68
CDCl3

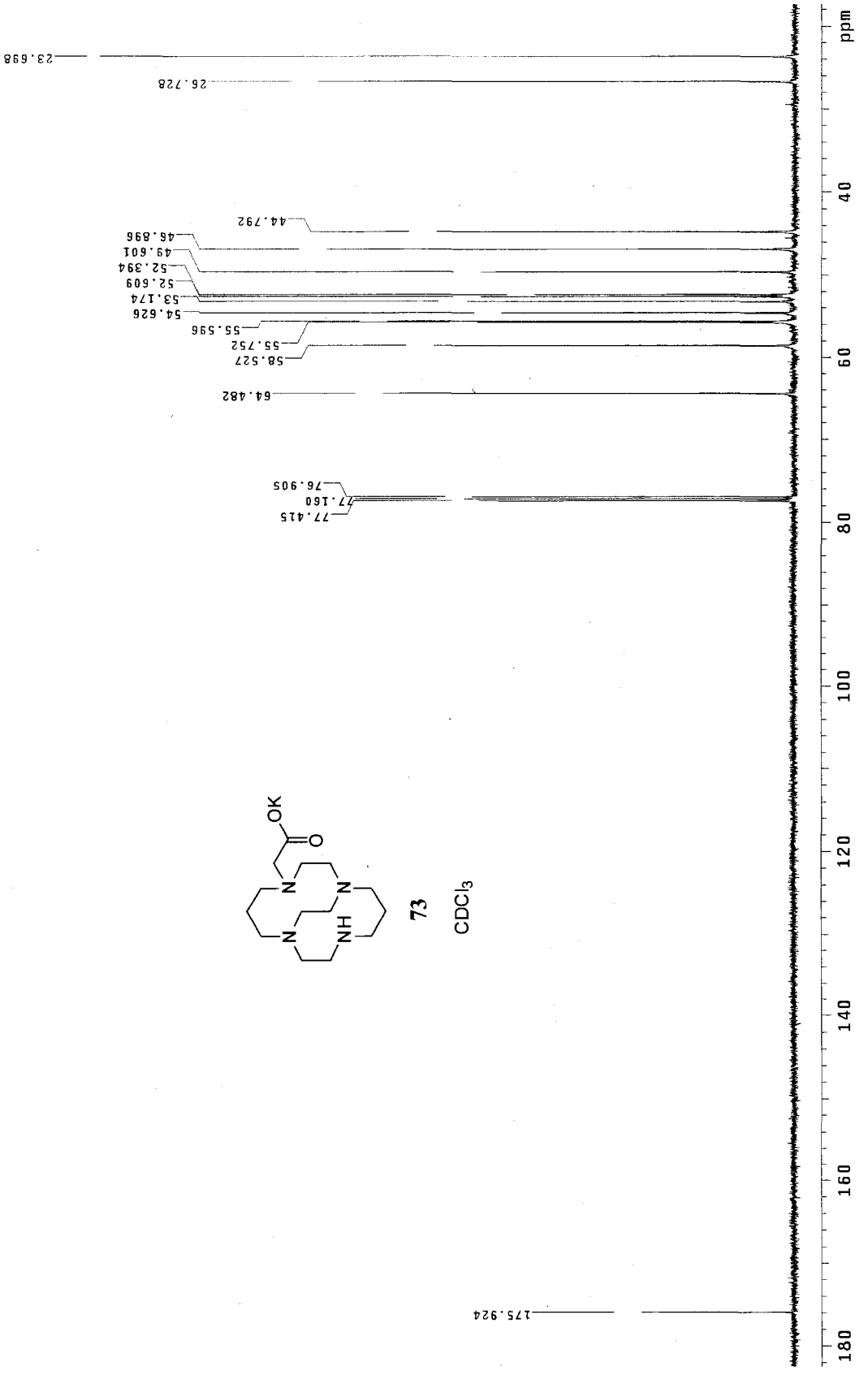

%Transmittance
Wavenumbers (cm-1)
Thu 10 11:33:58 2008
3274.0
3075.3
2915.7
2807.1
1654.2
1560.3
1459.9
1410.9
1357.8
1297.3
1159.2
1139.6
1114.1
725.2
68
KBr

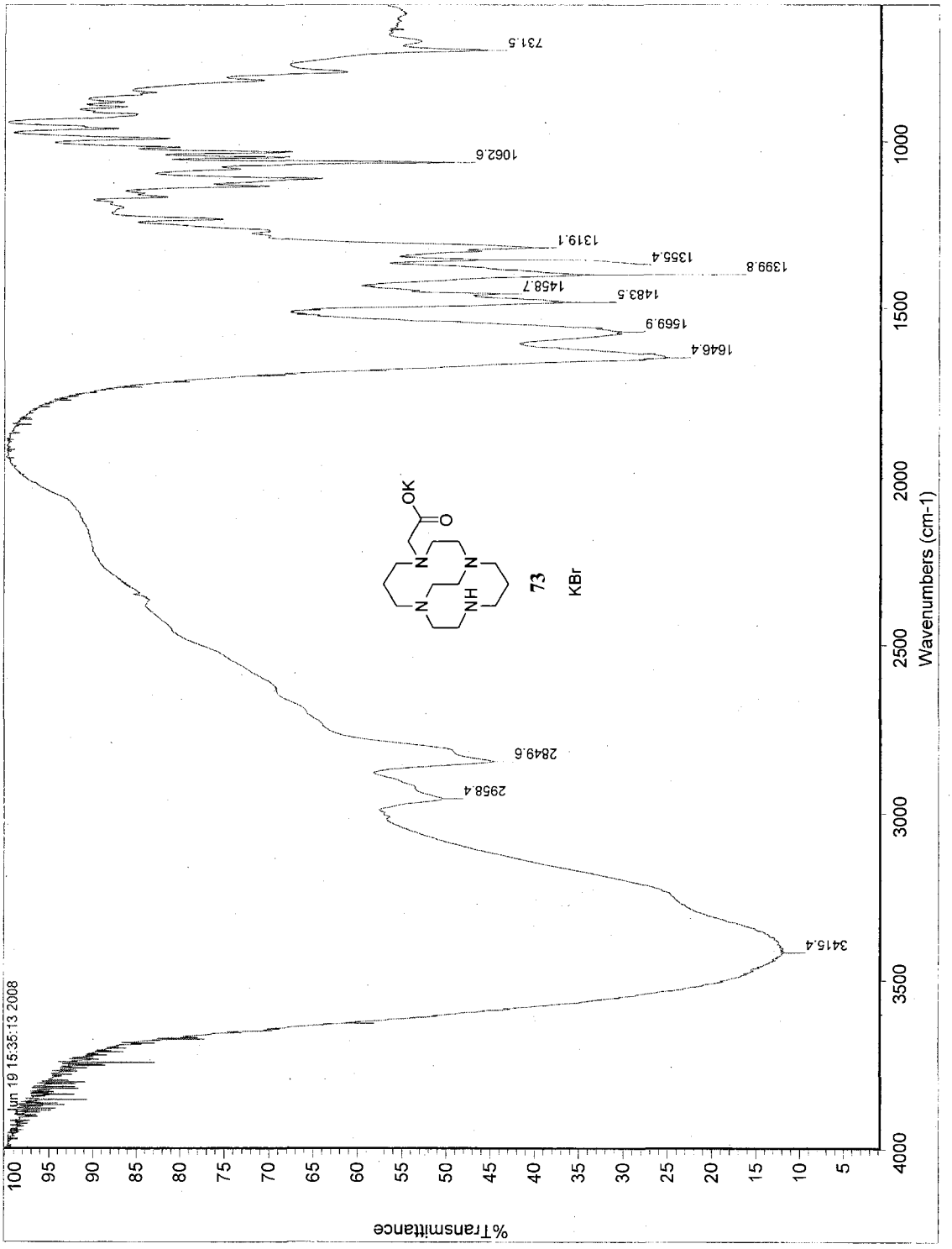

OH
N
N
O
NH
N
• 2.3 HCl
• 2.8 H_2O
• NH_4Cl
72
D_2O
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
ppm
1.00
1.04
3.18
2.24
8.61
5.32
2.06
2.10
2.08

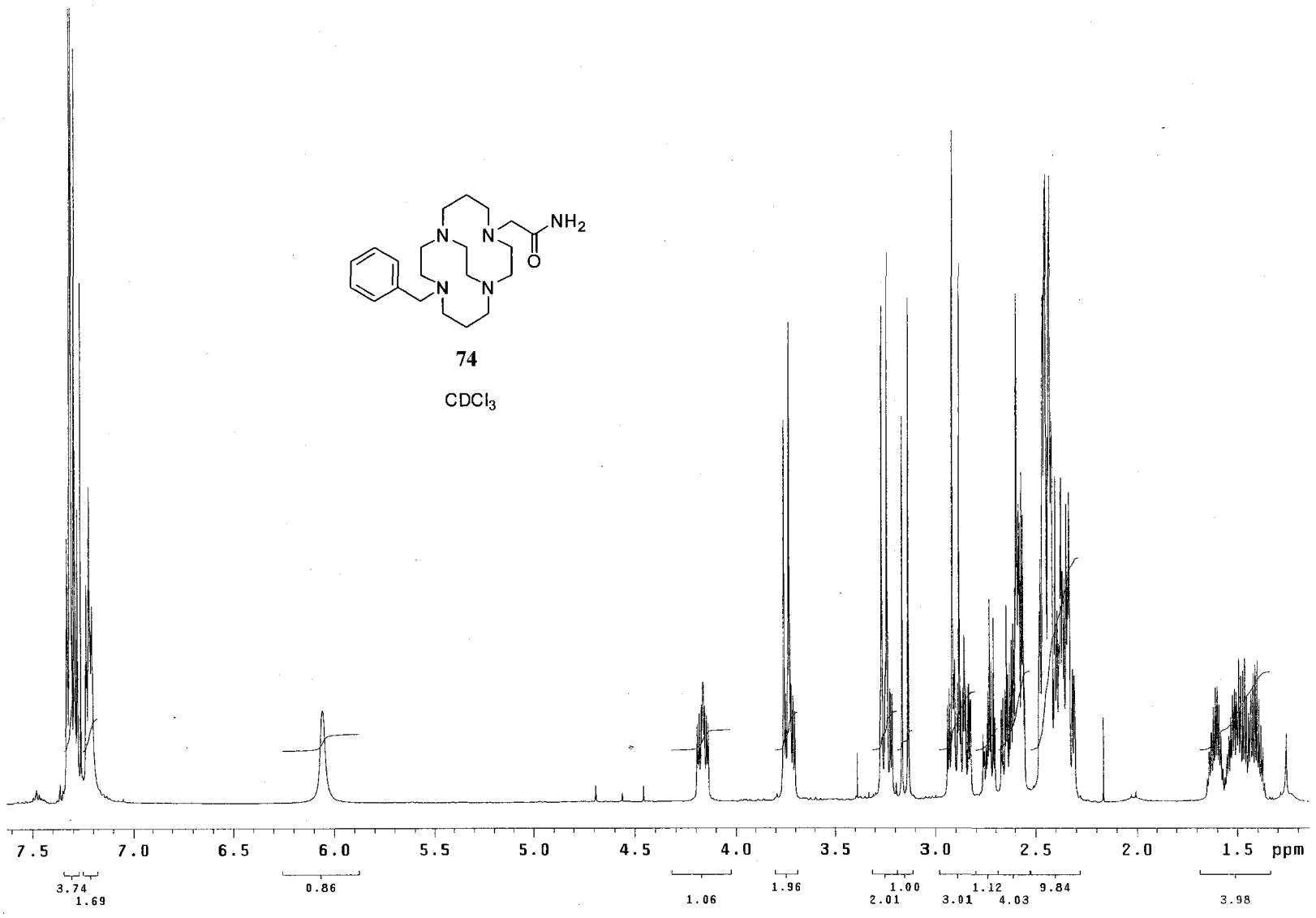

18.629
19.314
41.496
47.987
48.641
48.786
49.044
54.767
54.987
55.497
57.734
58.366
59.028
172.250
ppm
40
60
80
100
120
140
160
• 2.3 HCl
• 2.8 H2O
• NH4Cl
72
D2O

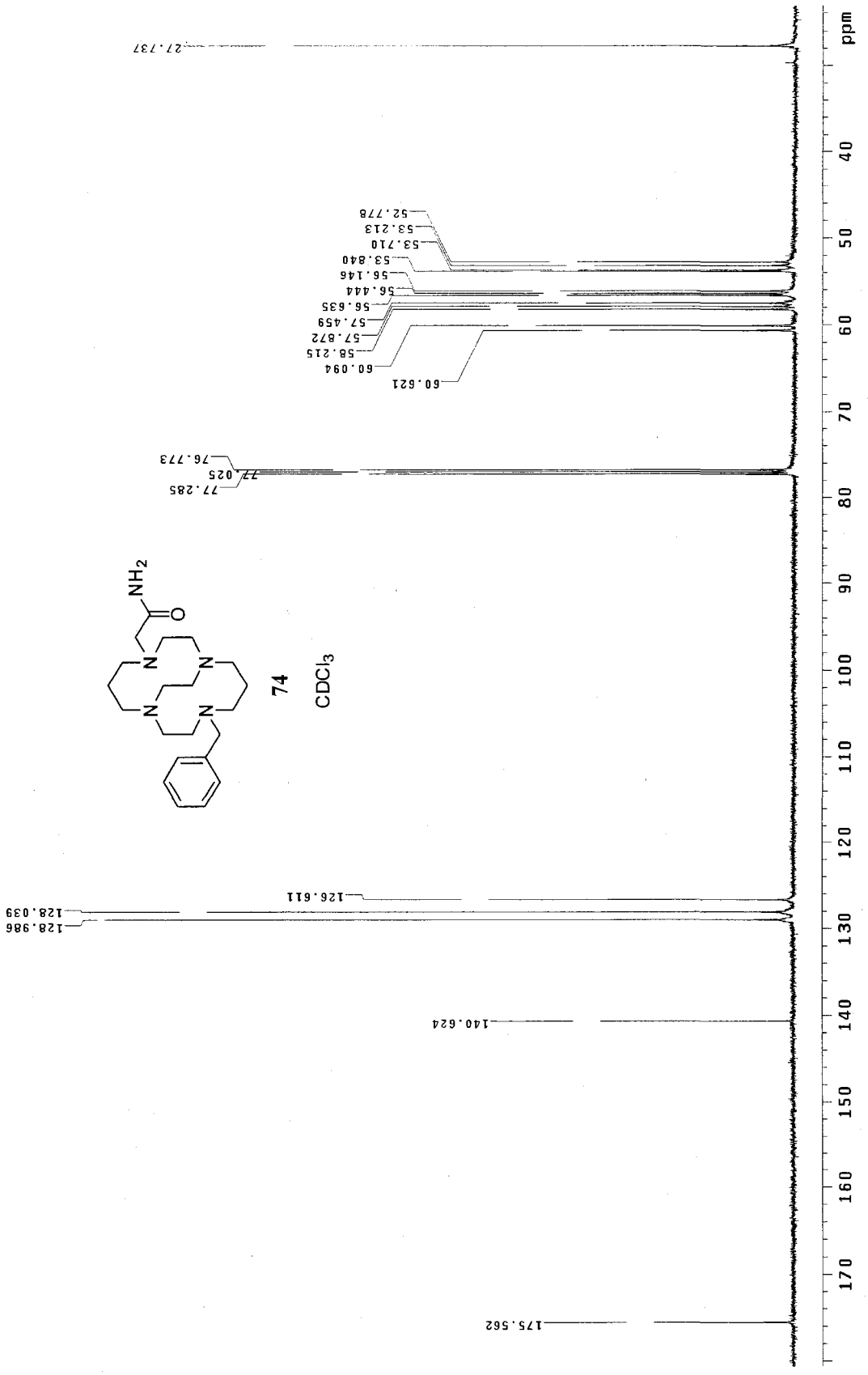

%Transmittance
Wavenumbers (cm-1)
4000
3500
3000
2500
2000
1500
100
98
96
94
92
90
88
86
84
82
80
78
76
74
72
70
68
66
64
62
60
58
56
54
52
3405.52
3186.48
3093.36
2987.63
2956.07
2840.49
2752.88
2635.64
2362.63
1727.81
1480.04
1455.90
1403.32
1240.81
1269.29
1315.94
1171.30
1098.71
1044.30
• 2.3 HCl
• 2.8 H_2O
• NH_4Cl
72
KBr

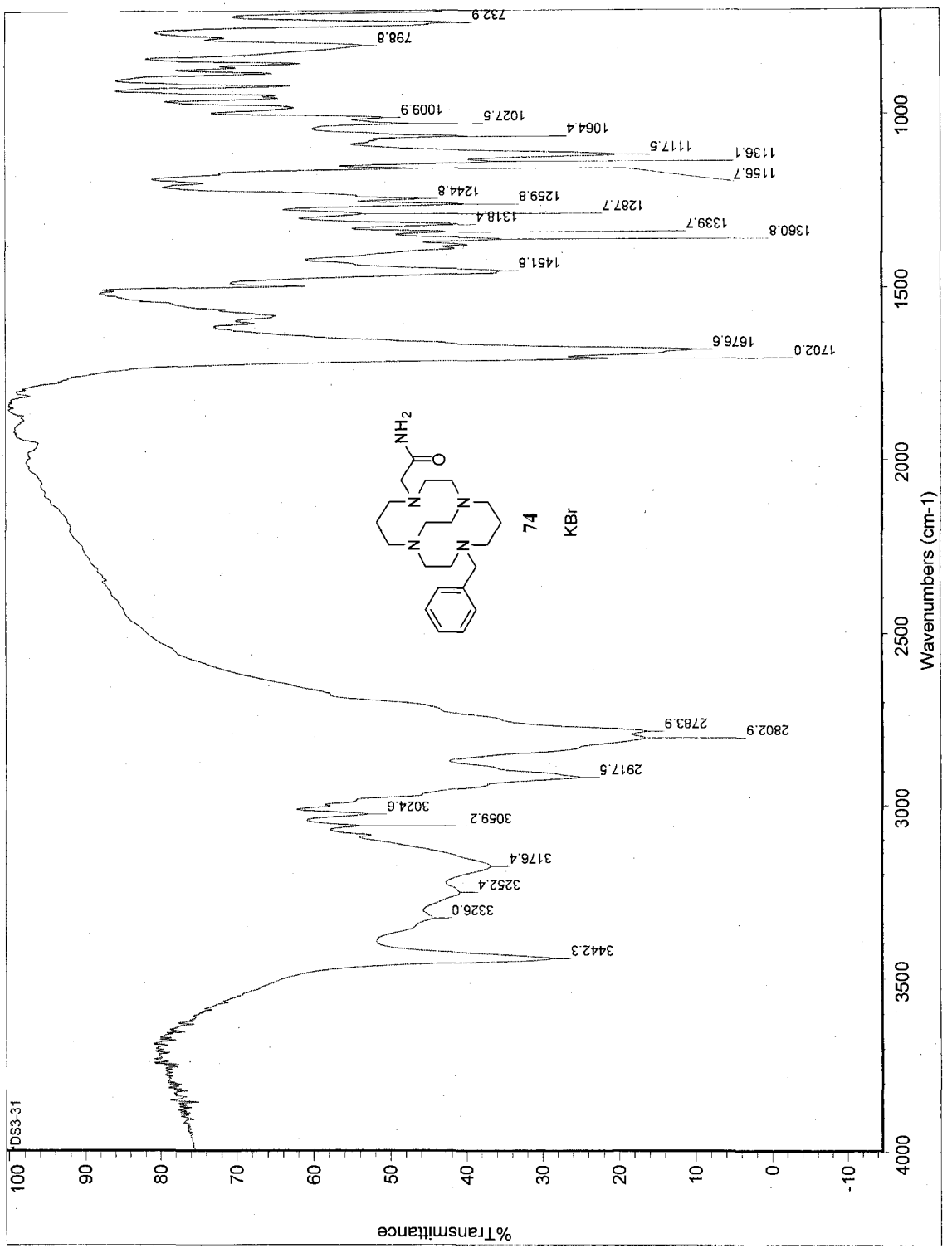

12
1.23
OK
O
N
N
NH
N
73
CDCl3
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
ppm
1.19
2.00
1.91
1.02
11.62
2.01
1.11
0.99
1.97
0.98
1.02

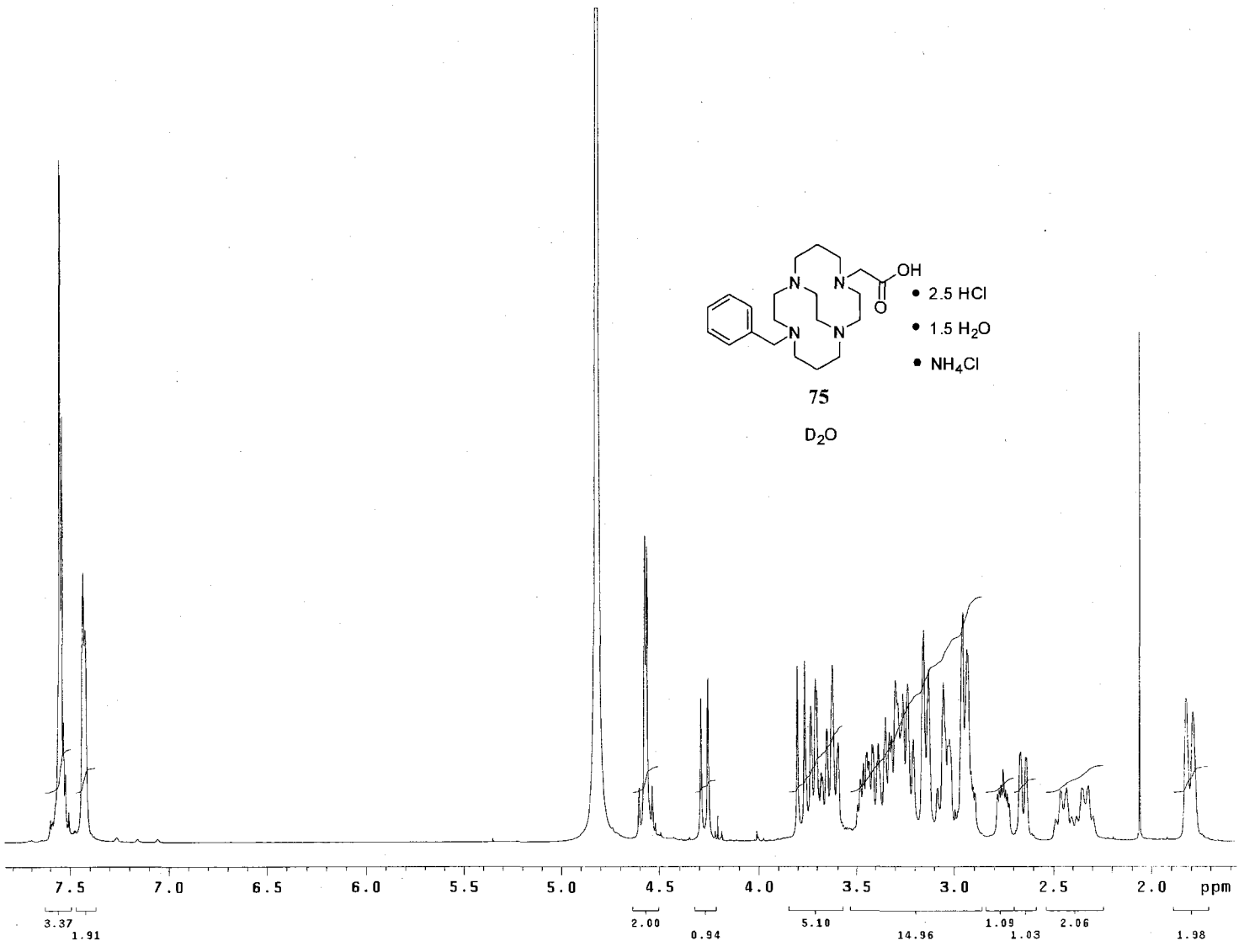

23.698
26.728
44.792
45.896
49.601
52.394
52.609
53.174
54.626
55.596
55.752
58.527
64.482
76.905
77.160
77.415
175.924
ppm
40
60
80
100
120
140
160
180
OK
O
N
N
N
NH
73
CDCl3

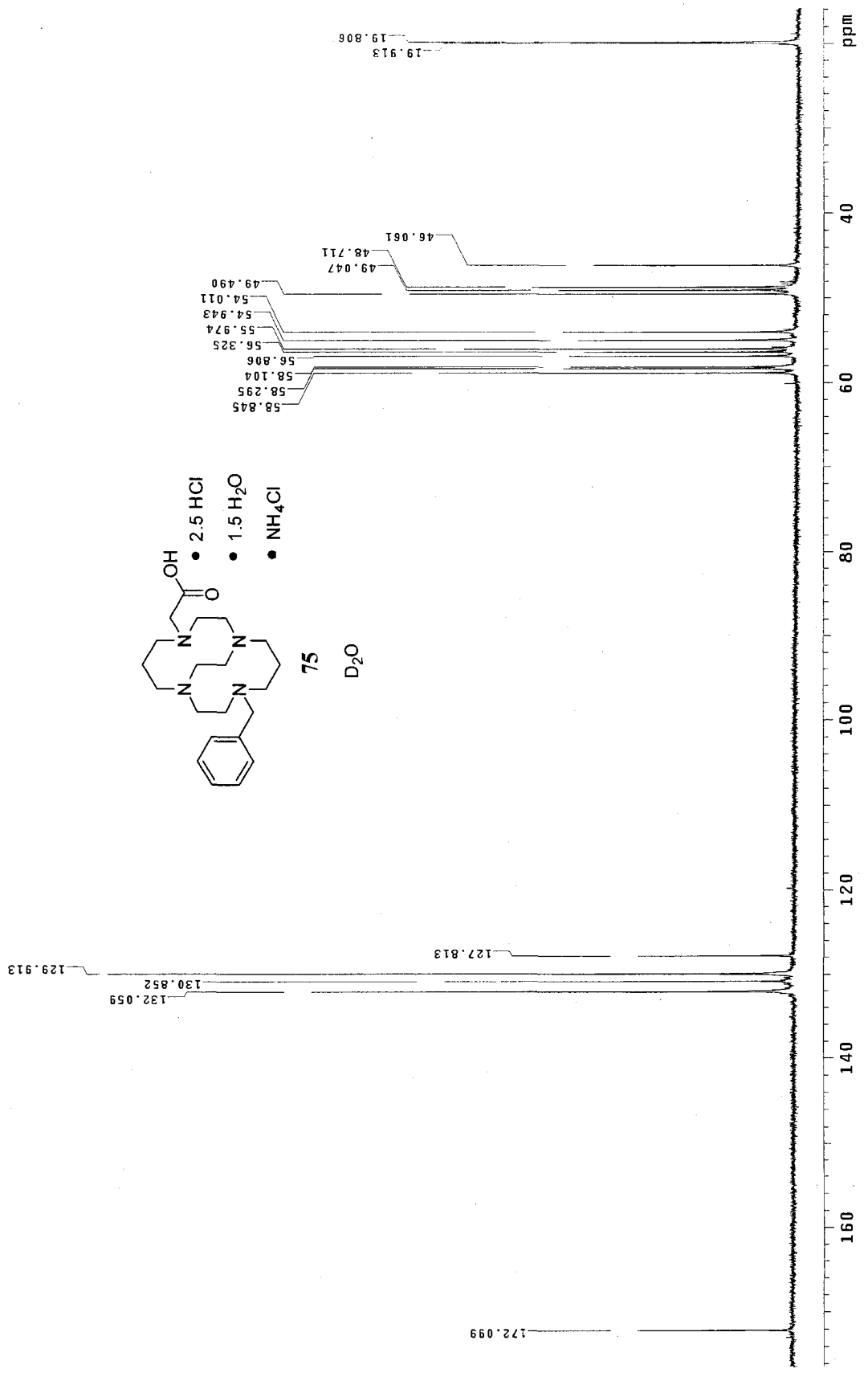

%Transmittance
Wavenumbers (cm-1)
Tue Jun 19 15:35:13 2008
3415.4
2958.4
2849.6
1646.4
1569.9
1483.5
1458.7
1399.8
1355.4
1319.1
1062.6
731.5
OK
O
N
N
NH
N
73
KBr

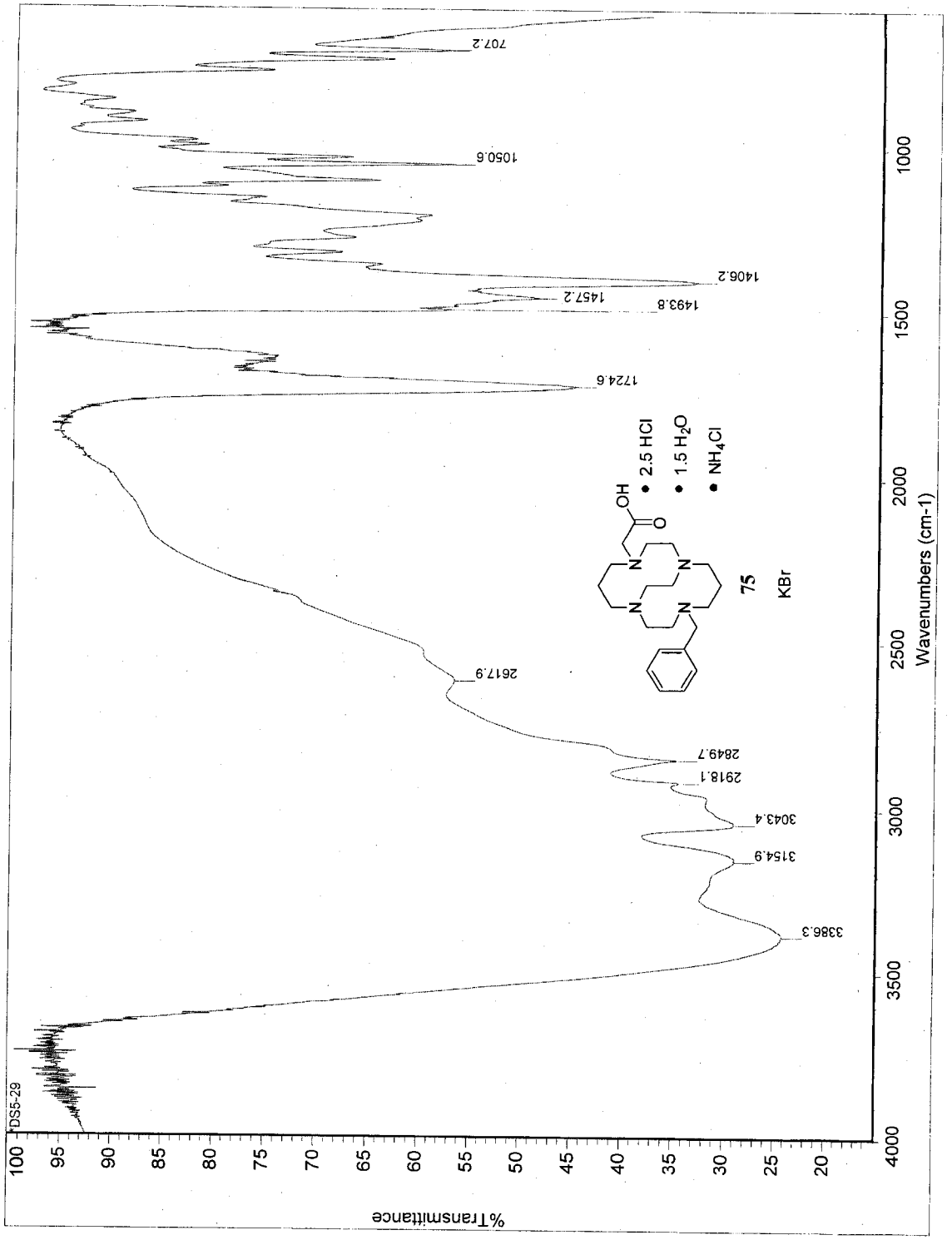

NH2
O
N
N
N
N
74
CDCl3
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
3.74
1.69
0.86
1.06
1.96
2.01
1.00
3.01
1.12
4.03
9.84
3.98

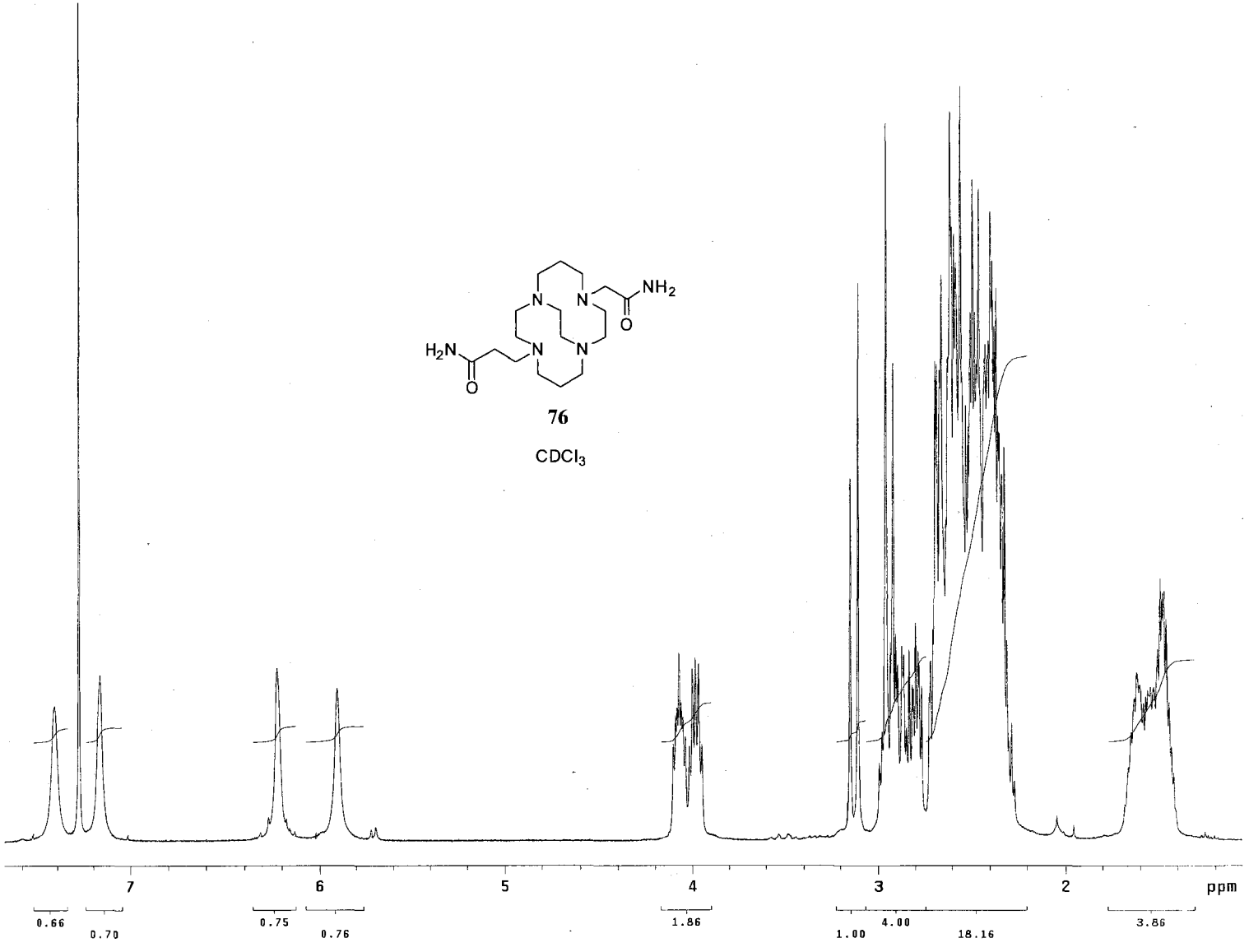

27.737
52.778
53.213
53.710
53.840
56.146
56.444
56.635
57.459
57.872
58.215
60.094
60.621
76.773
77.025
77.285
128.039
128.986
126.611
140.624
175.562
ppm
NH2
O
N
74
CDCl3

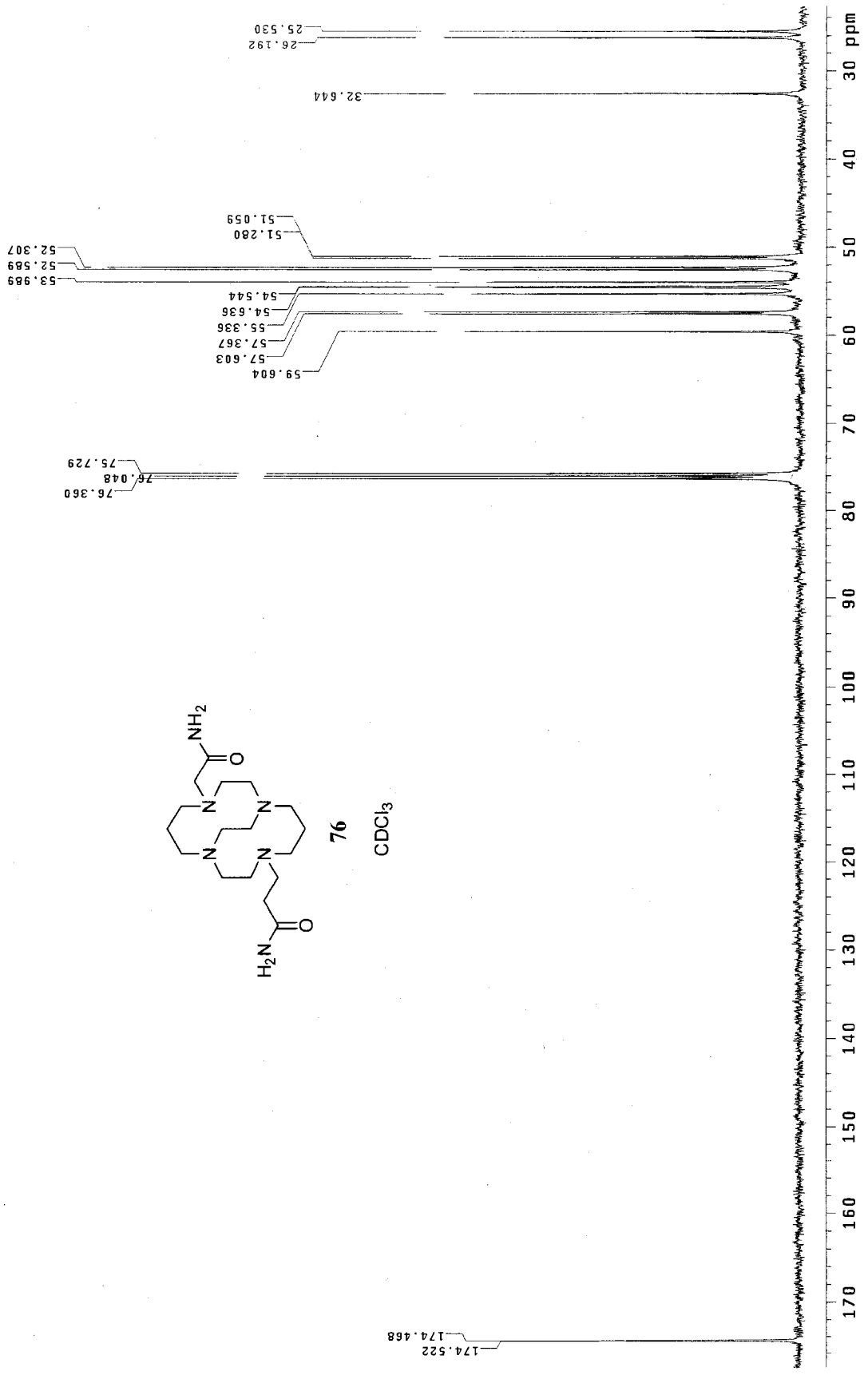

DS3-31
%Transmittance
Wavenumbers (cm-1)
4000
3500
3000
2500
2000
1500
1000
100
90
80
70
60
50
40
30
20
10
0
-10
3442.3
3326.0
3252.4
3176.4
3059.2
3024.6
2917.5
2802.9
2783.9
1702.0
1676.6
1451.8
1360.8
1339.7
1318.4
1287.7
1259.8
1244.8
1156.7
1136.1
1117.5
1064.4
1027.5
1009.9
798.8
732.9
74
KBr

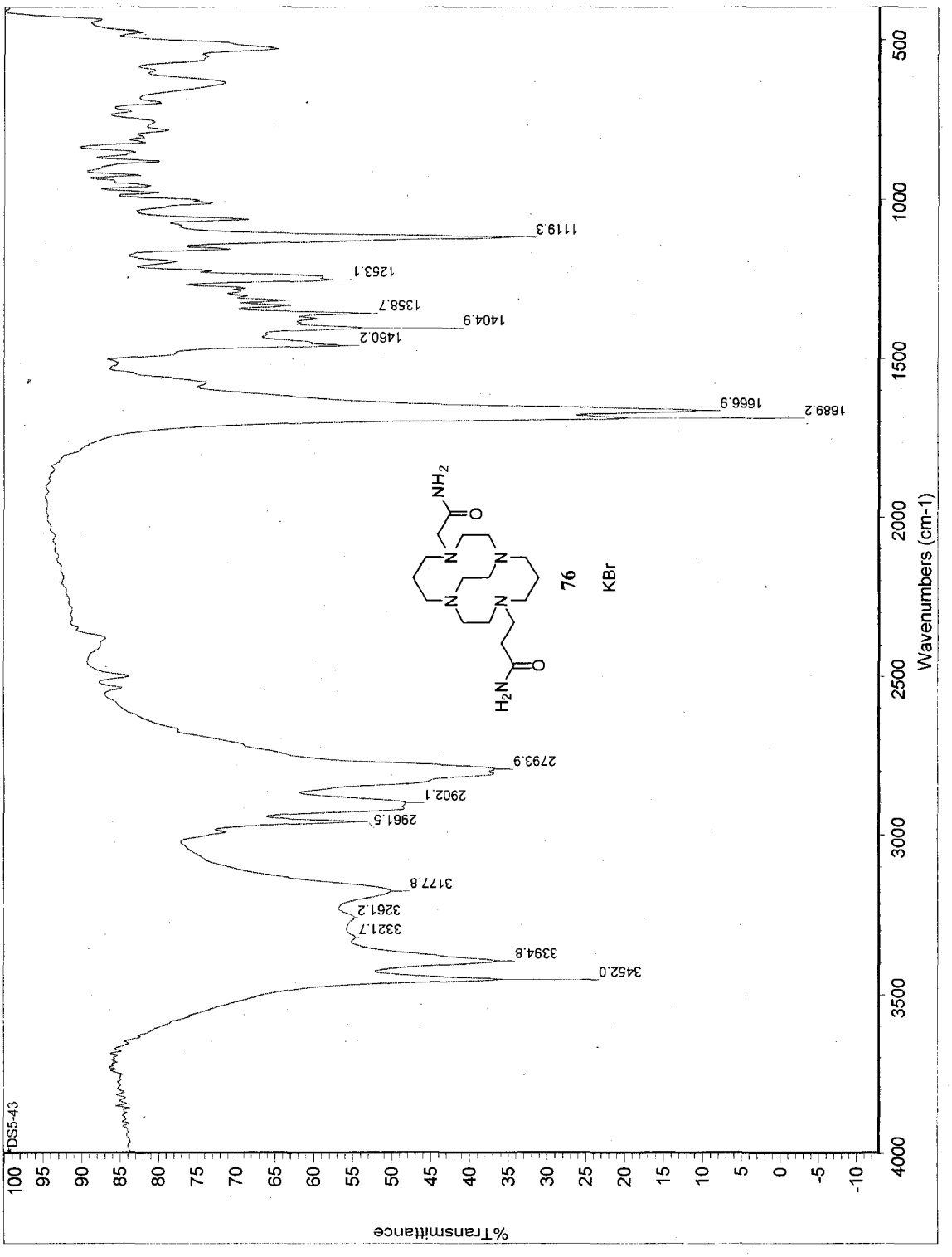

OH
O
• 2.5 HCl
• 1.5 H_2O
• NH_4Cl
75
D_2O
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
3.37
1.91
2.00
0.94
5.10
14.96
1.09
1.03
2.06
1.98

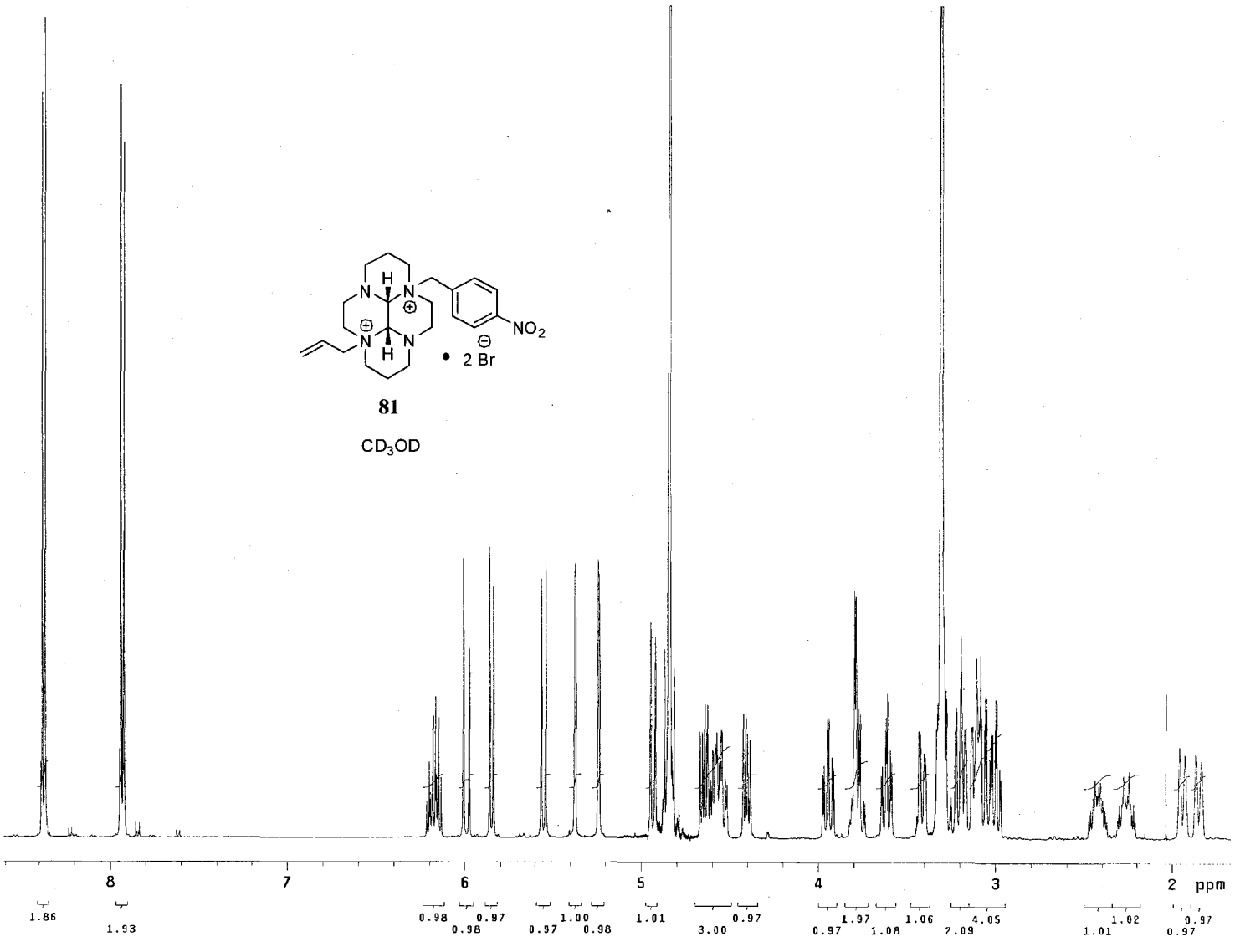

19.806
19.913
46.061
48.711
49.047
49.490
54.011
54.943
55.974
56.325
56.806
58.104
58.295
58.845
127.813
129.913
130.852
132.059
172.099
ppm
40
60
80
100
120
140
160
• 2.5 HCl
• 1.5 H_2O
• NH_4Cl
75
D_2O
OH
O
N

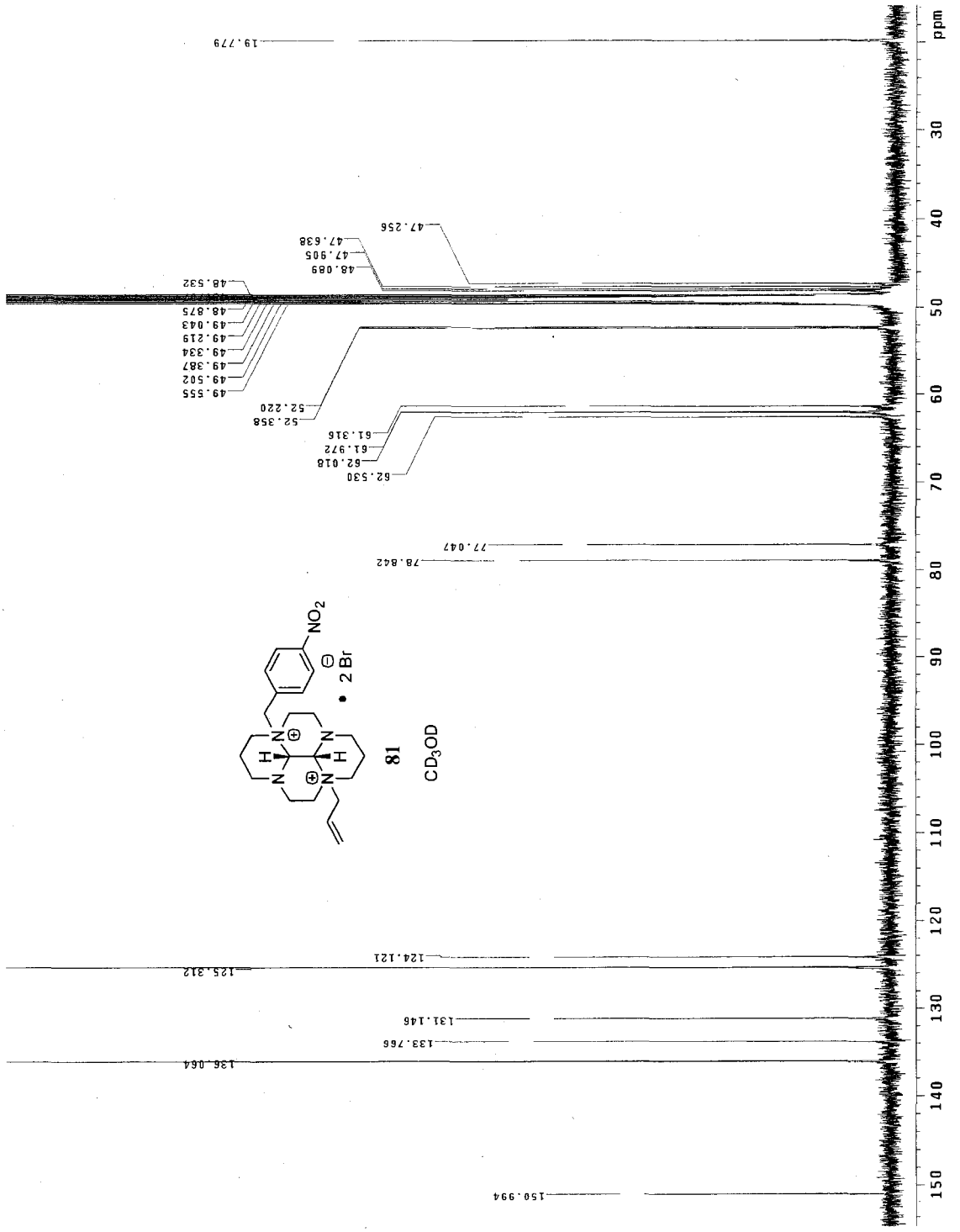

DS5-29
%Transmittance
Wavenumbers (cm-1)
4000
3500
3000
2500
2000
1500
1000
3386.3
3154.9
3043.4
2918.1
2849.7
2617.9
1724.6
1493.8
1457.2
1406.2
1050.6
707.2
• 2.5 HCl
• 1.5 H2O
• NH4Cl
OH
O
N
75
KBr

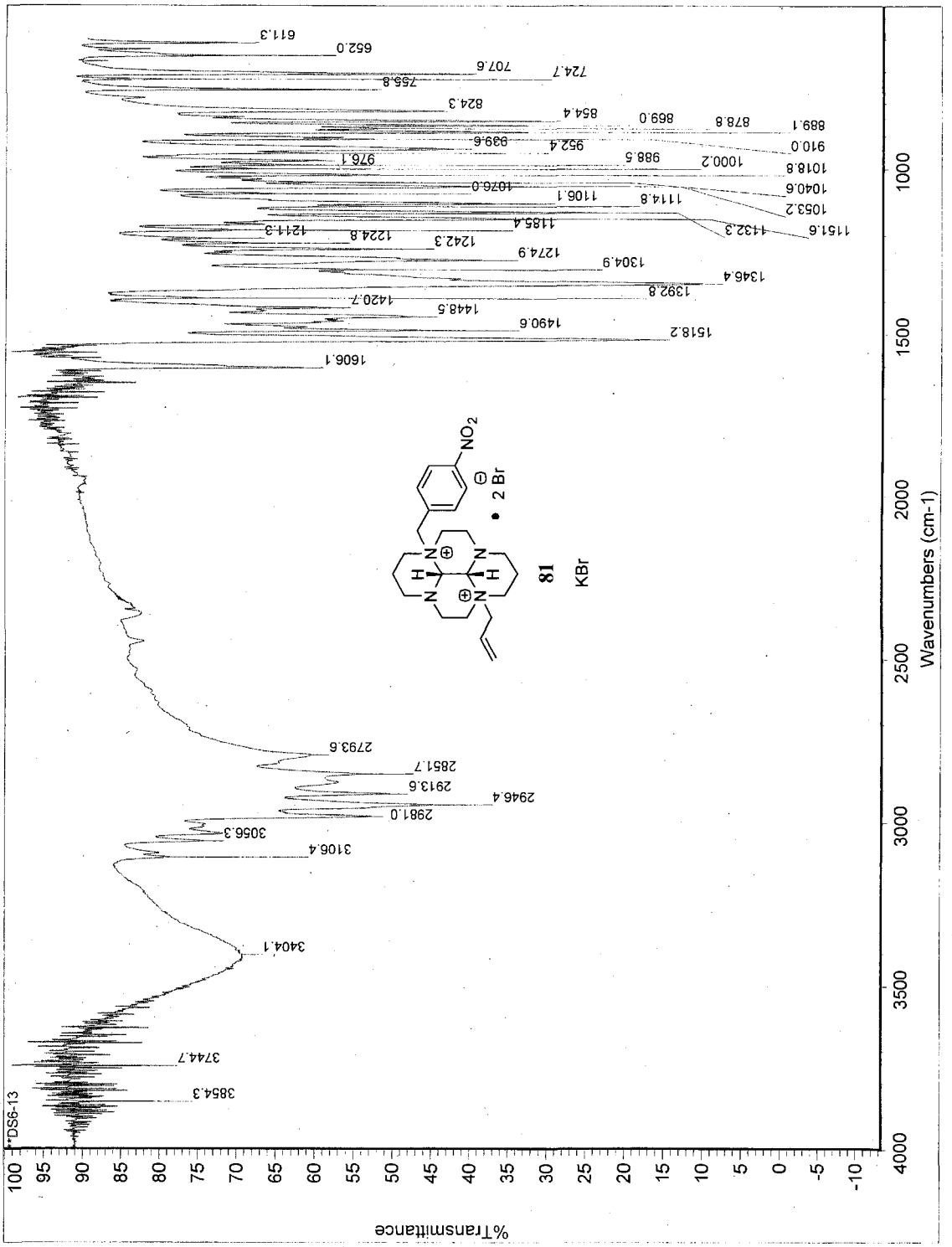

NH2
O
N
N
N
N
N
H2N
O
76
CDCl3
7
6
5
4
3
2
ppm
0.66
0.70
0.75
0.76
1.86
1.00
4.00
18.16
3.86

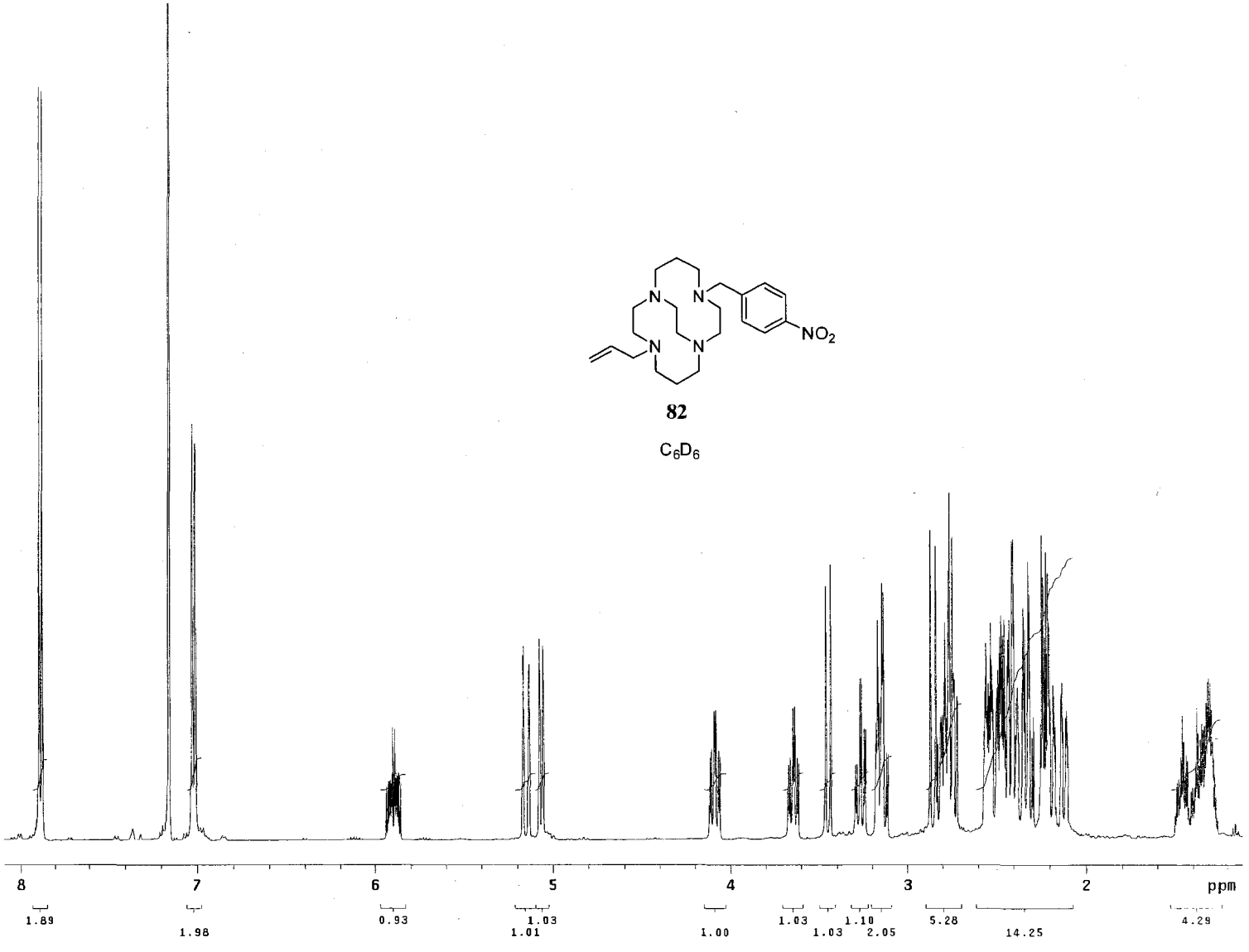

174.522
174.468
76.360
76.048
75.729
59.604
57.603
57.367
55.336
54.636
54.544
53.989
52.589
52.307
51.280
51.059
32.644
26.192
25.530
ppm
NH2
O
N
N
N
N
O
H2N
76
CDCl3

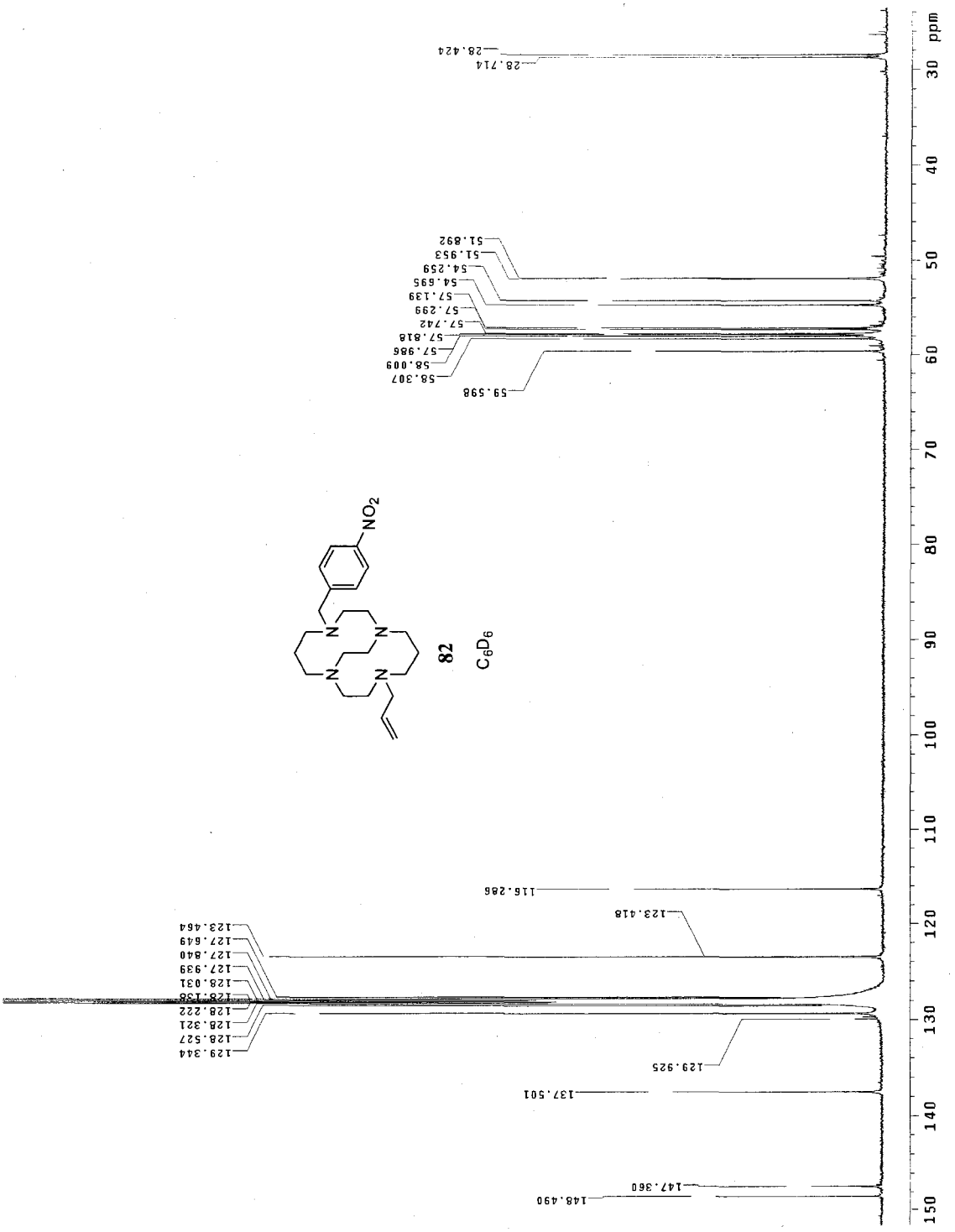

FDS5-43
%Transmittance
Wavenumbers (cm-1)
3452.0
3394.8
3321.7
3261.2
3177.8
2961.5
2902.1
2793.9
1689.2
1666.9
1460.2
1404.9
1358.7
1253.1
1119.3
76
KBr

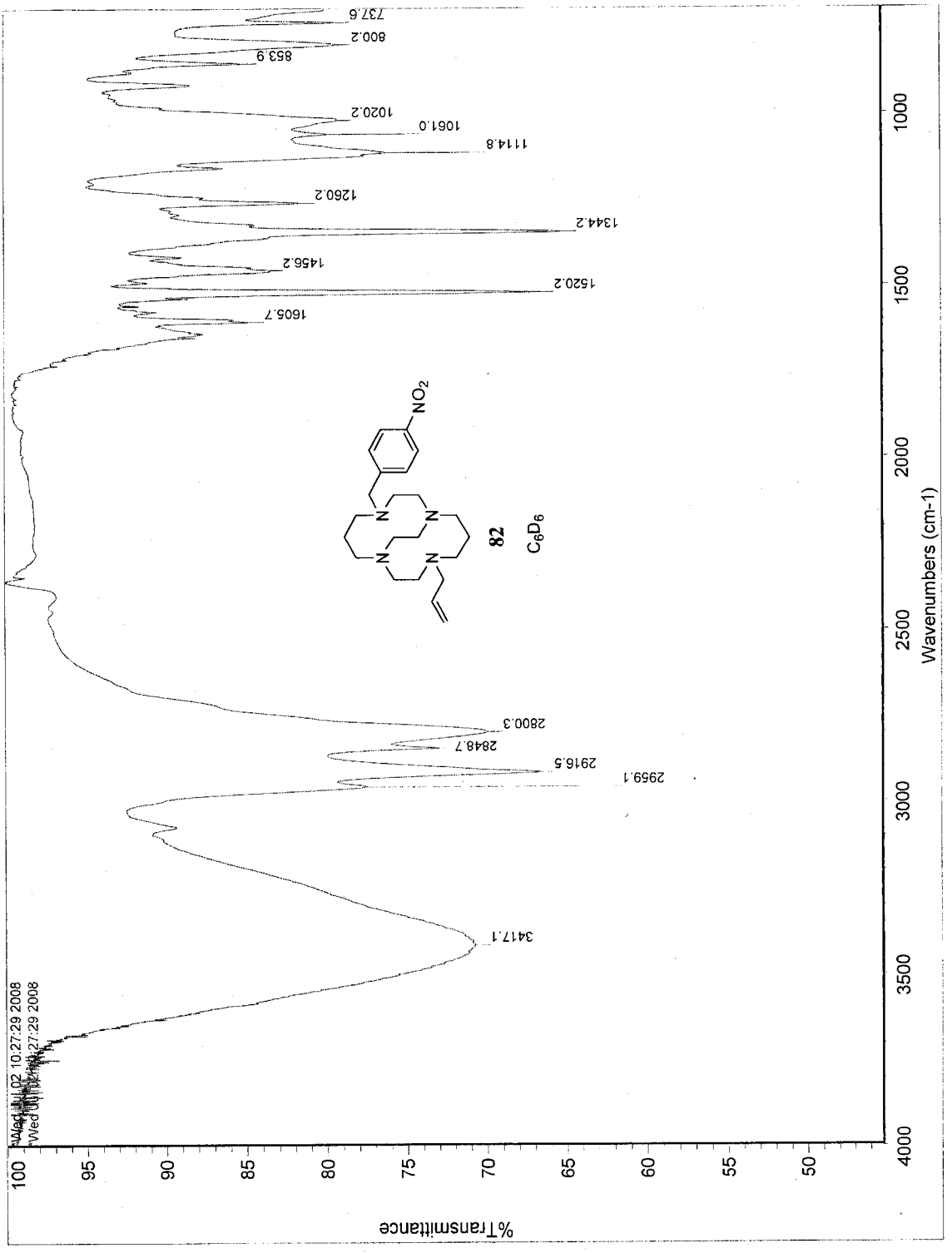

81
CD3OD
8
7
6
5
4
3
2 ppm
1.86
1.93
0.98
0.98
0.97
0.97
1.00
0.98
1.01
3.00
0.97
0.97
1.97
1.08
1.06
2.09
4.05
1.01
1.02
0.97

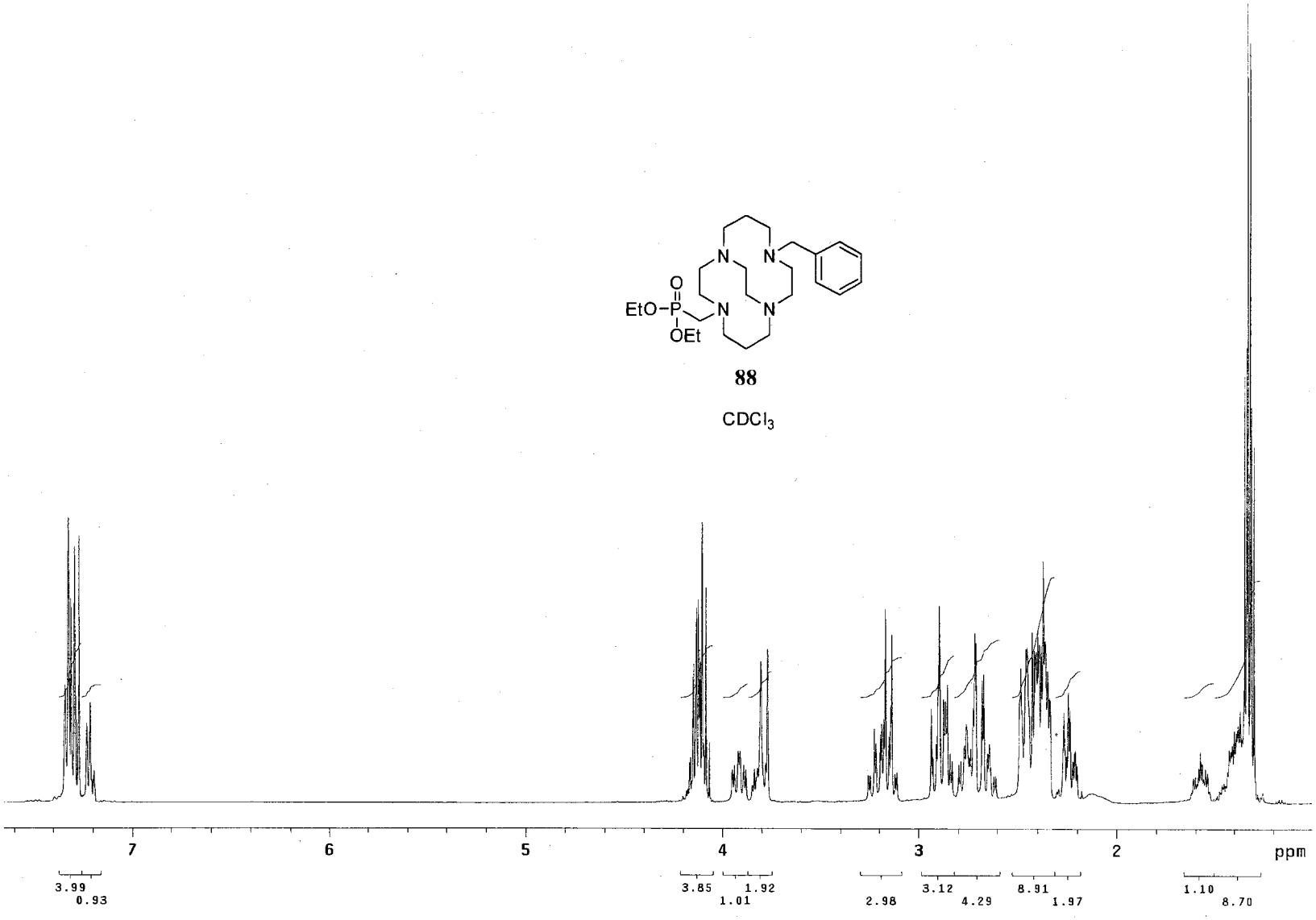

19.779
47.256
47.638
47.905
48.089
48.532
48.875
49.043
49.219
49.334
49.387
49.502
49.555
52.220
52.358
61.316
61.972
62.018
62.530
77.047
78.842
124.121
125.312
131.146
133.766
136.064
150.994
ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
NO_2
2 Br
81
CD_3OD

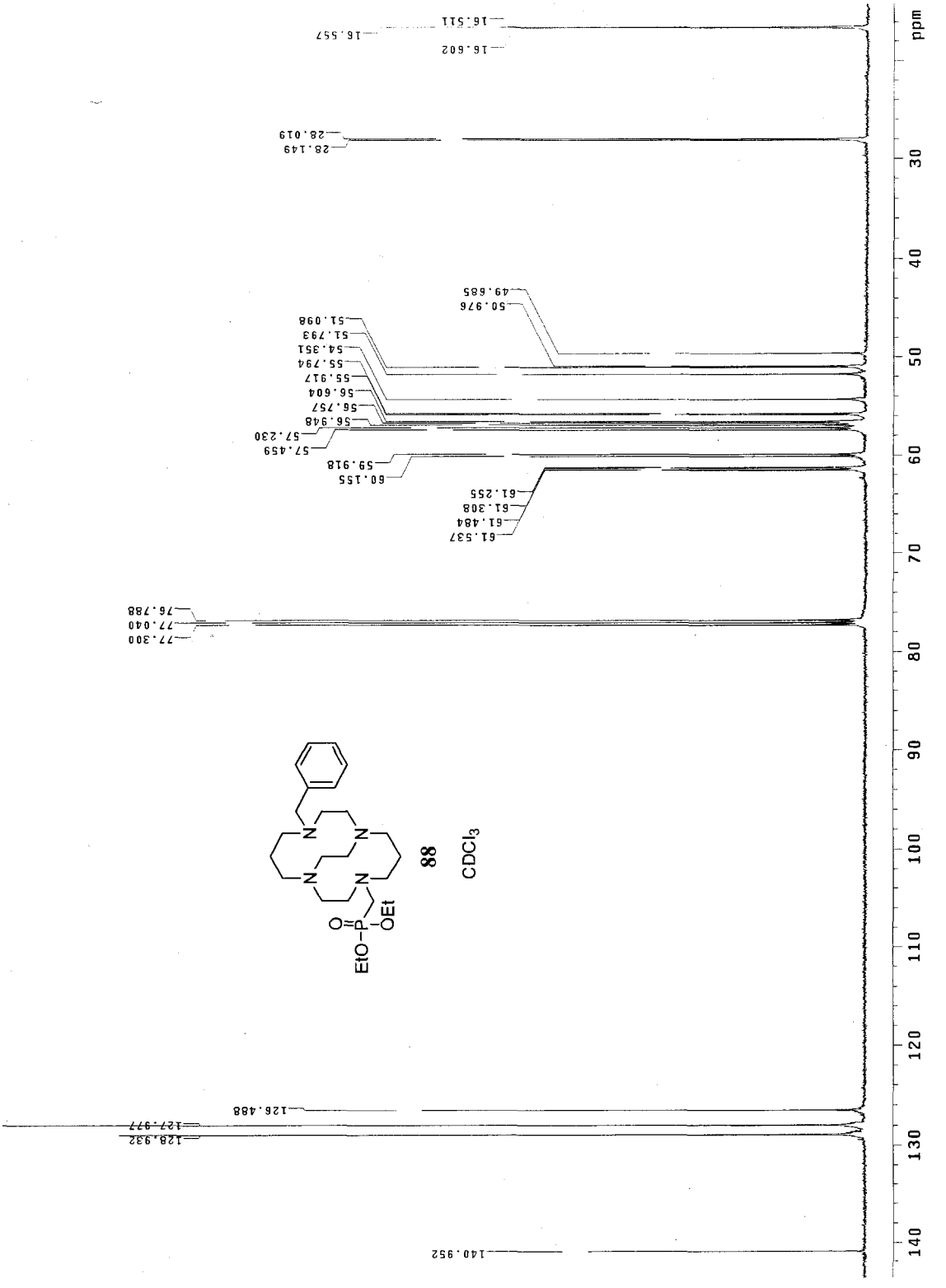

DS6-13
%Transmittance
Wavenumbers (cm-1)
4000
3500
3000
2500
2000
1500
1000
3854.3
3744.7
3404.1
3106.4
3056.3
2981.0
2946.4
2913.6
2851.7
2793.6
1606.1
1518.2
1490.6
1448.5
1420.7
1392.8
1346.4
1304.9
1274.9
1242.3
1224.8
1211.3
1185.4
1151.6
1132.3
1053.2
1040.6
1018.8
1000.2
988.5
976.1
952.4
939.6
910.0
889.1
878.8
869.0
854.4
824.3
755.8
724.7
707.6
652.0
611.3
NO_2
2 Br
81
KBr


N
N
N
N
NO2
82
C6D6
8
7
6
5
4
3
2
ppm
1.89
1.98
0.93
1.03
1.01
1.00
1.03
1.03
1.10
2.05
5.28
14.25
4.29

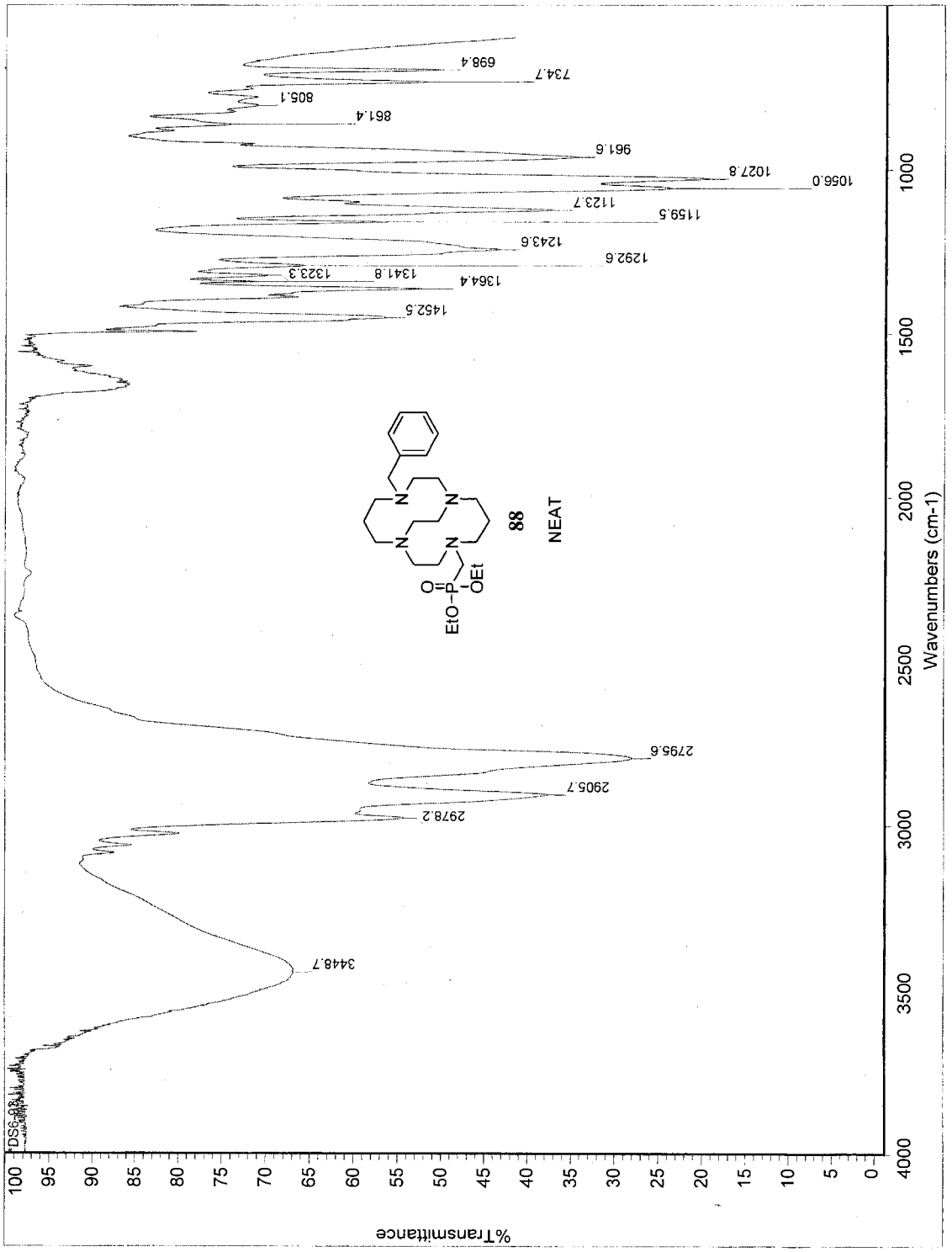

28.424
28.714
51.892
51.953
54.259
54.695
57.139
57.299
57.742
57.818
57.986
58.009
58.307
59.598
116.286
123.418
123.464
127.649
127.840
127.939
128.031
128.138
128.222
128.321
128.527
129.344
129.925
137.501
147.360
148.490
82
C_6D_6
NO_2
N
ppm
30
40
50
60
70
80
90
100
110
120
130
140
150

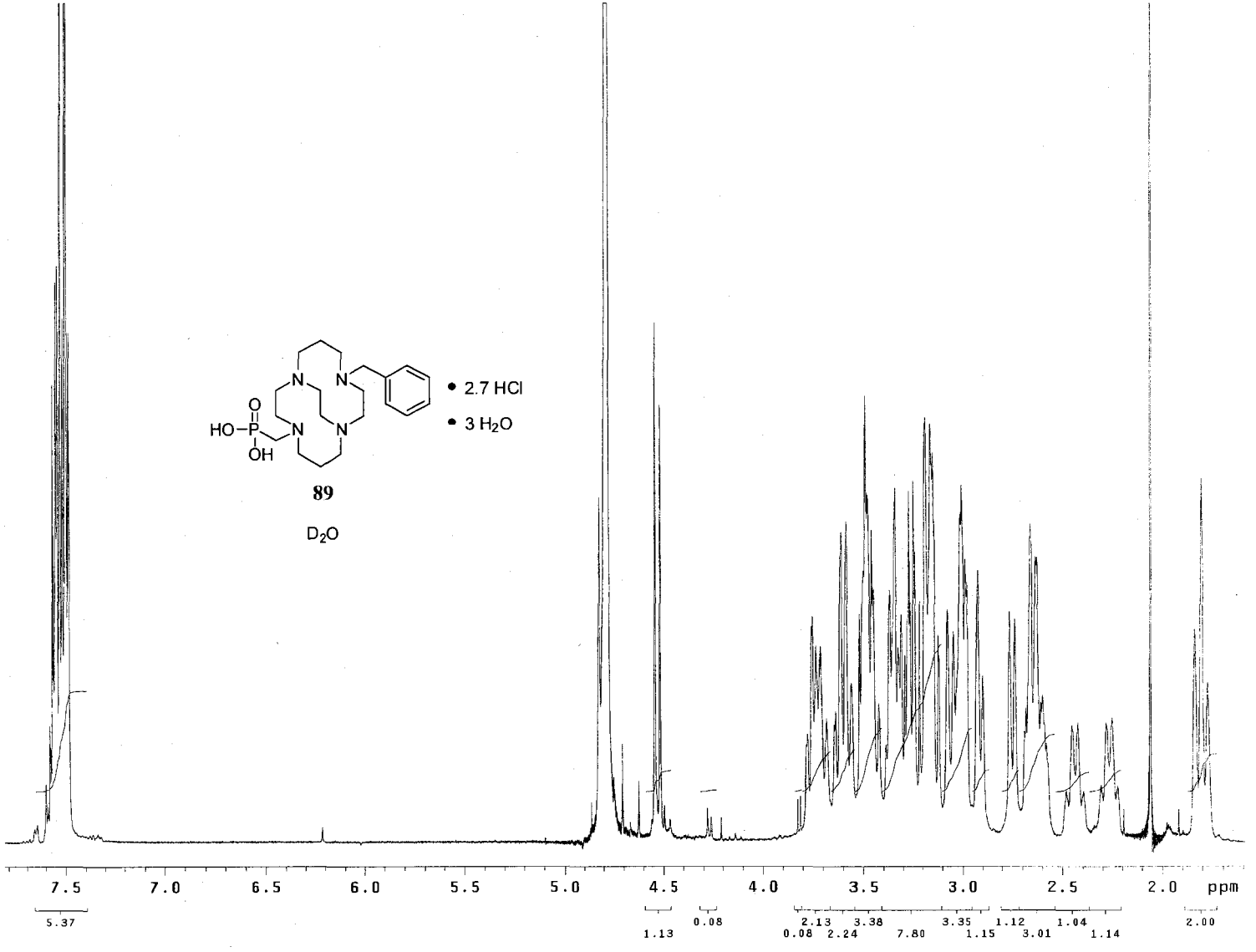

%Transmittance
Wavenumbers (cm-1)
3417.1
2959.1
2916.5
2848.7
2800.3
1605.7
1520.2
1456.2
1344.2
1260.2
1114.8
1061.0
1020.2
853.9
800.2
737.6
82
C_6D_6
NO_2


EtO–P
O
OEt
N
N
N
N
88
CDCl3
7
6
5
4
3
2
ppm
3.99
0.93
3.85
1.92
1.01
2.98
3.12
4.29
8.91
1.97
1.10
8.70

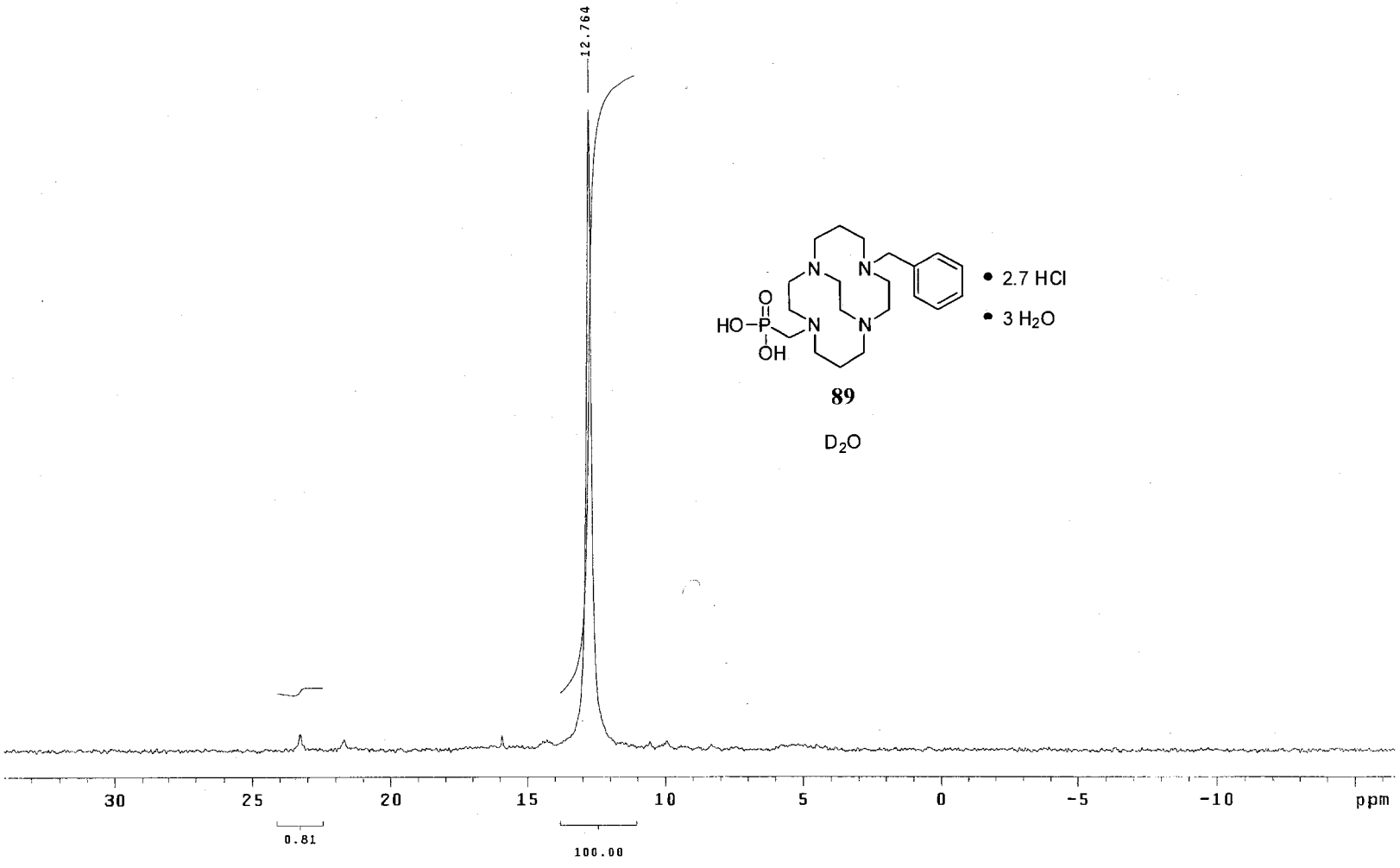

16.511
16.557
16.602
28.019
28.149
49.685
50.976
51.098
51.793
54.351
55.794
55.917
56.604
56.757
56.948
57.230
57.459
59.918
60.155
61.255
61.308
61.484
61.537
76.788
77.040
77.300
126.488
127.977
128.932
140.952
ppm
88
CDCl3

27.244
88
CDCl3
EtO–P–OEt
O
N
N
N
N
ppm
38
36
34
32
30
28
26
24
22
20
18
16
14
12


%Transmittance
Wavenumbers (cm-1)
NEAT
88
EtO–P–OEt
O
3448.7
2978.2
2905.7
2795.6
1452.5
1364.4
1341.8
1323.3
1292.6
1243.6
1159.5
1123.7
1056.0
1027.8
961.6
861.4
805.1
734.7
698.4

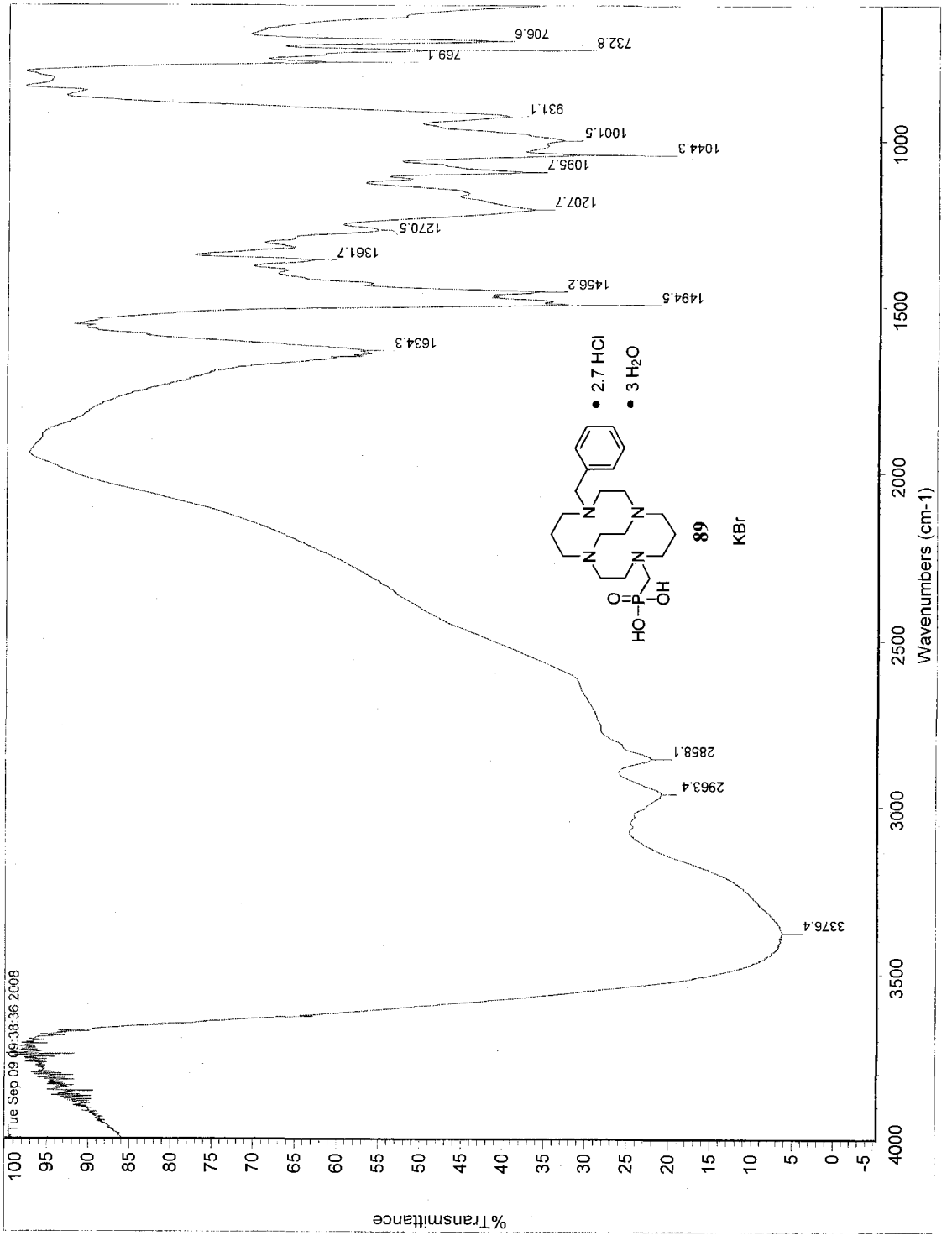

• 2.7 HCl
• 3 H2O
89
D2O
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
5.37
1.13
0.08
2.13
3.38
3.35
1.12
1.04
0.08
2.24
7.80
1.15
3.01
1.14
2.00

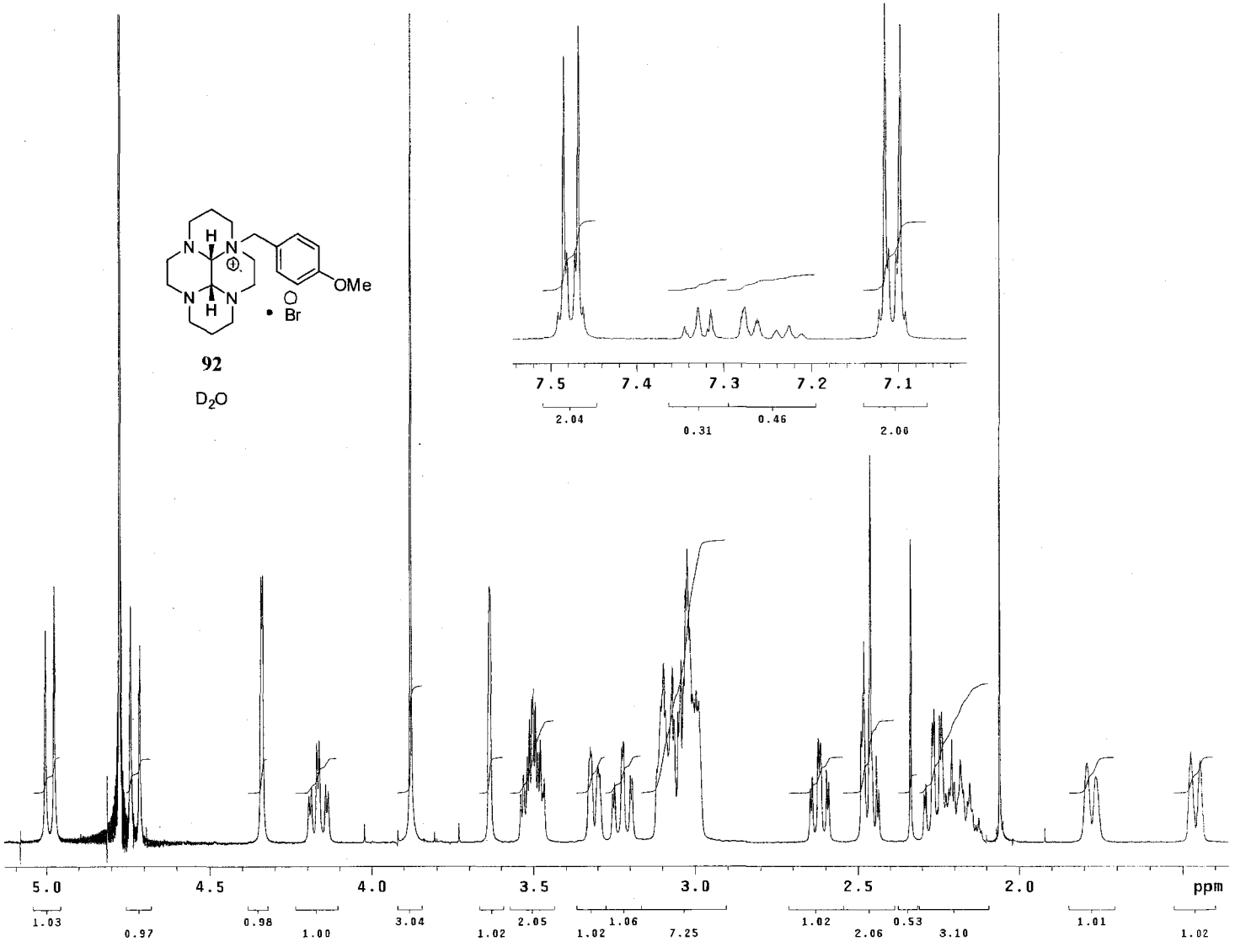
20.121
45.019
48.383
49.045
50.597
50.993
52.416
54.295
55.353
55.482
57.674
58.549
58.892
127.278
129.789
131.015
133.046
ppm
• 2.7 HCl
• 3 H2O
89
D2O


12.764
HO–P
O
OH
N
N
N
N
• 2.7 HCl
• 3 H2O
30
25
20
15
10
5
0
-5
-10
ppm
0.81
100.00

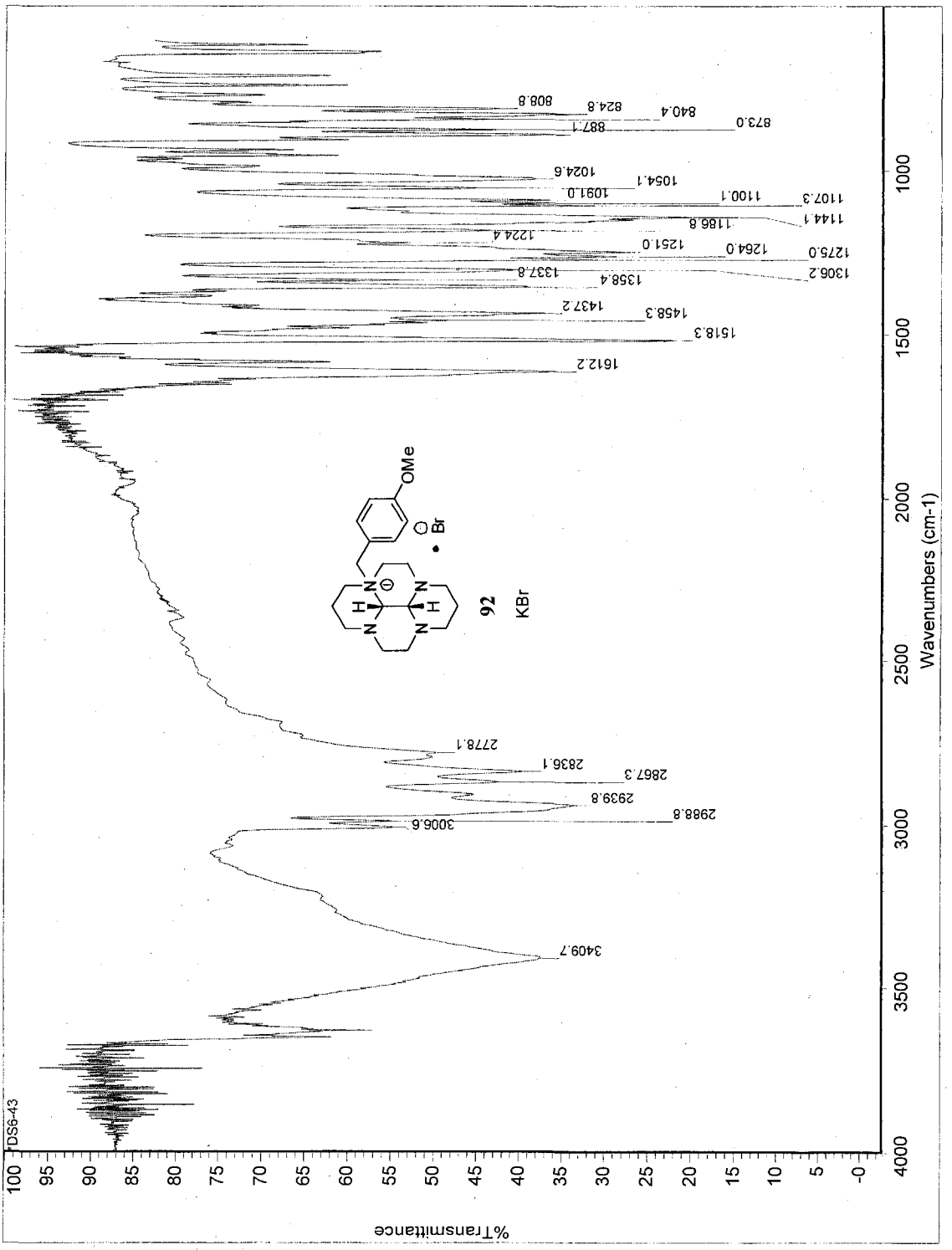
89

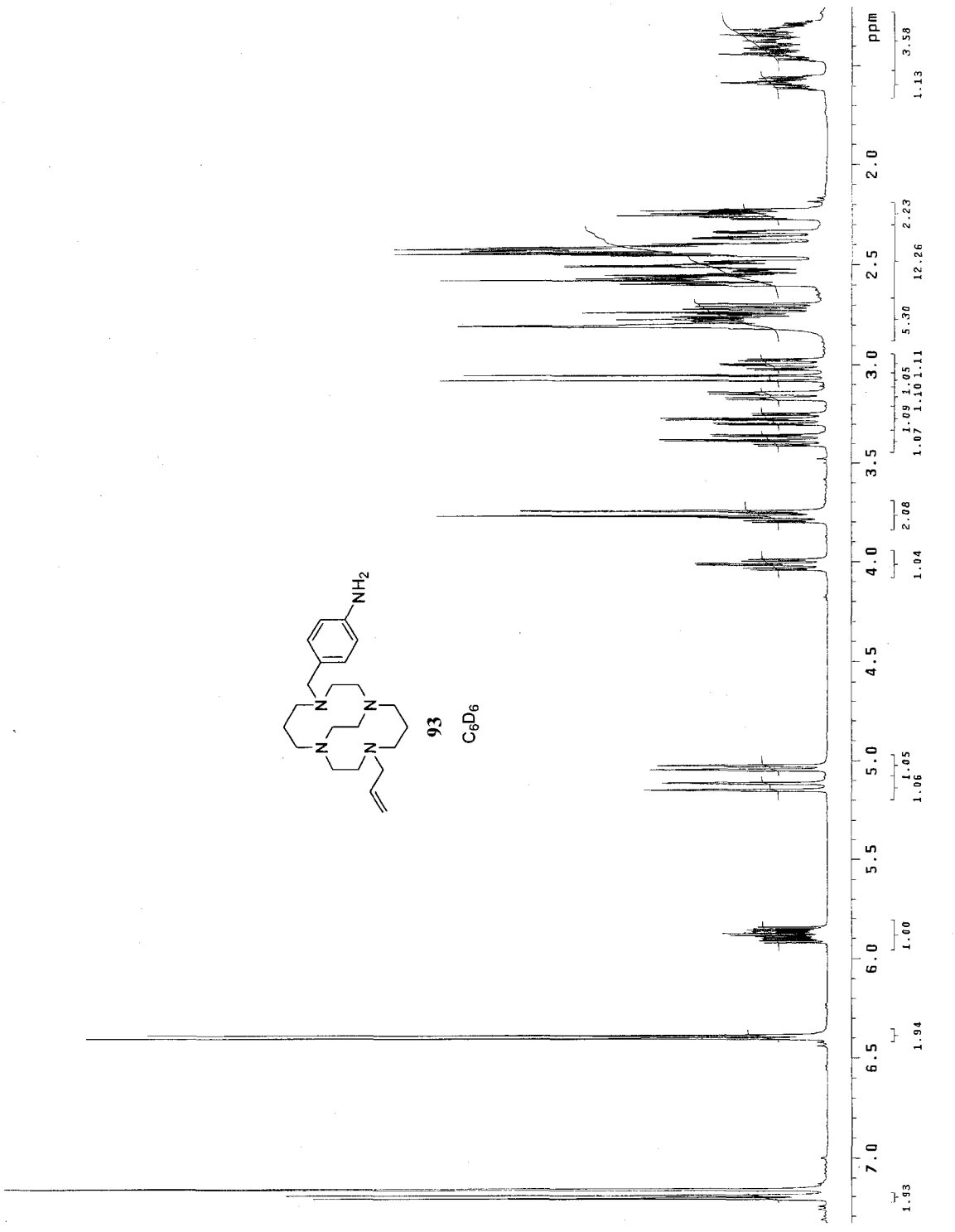
D_2O

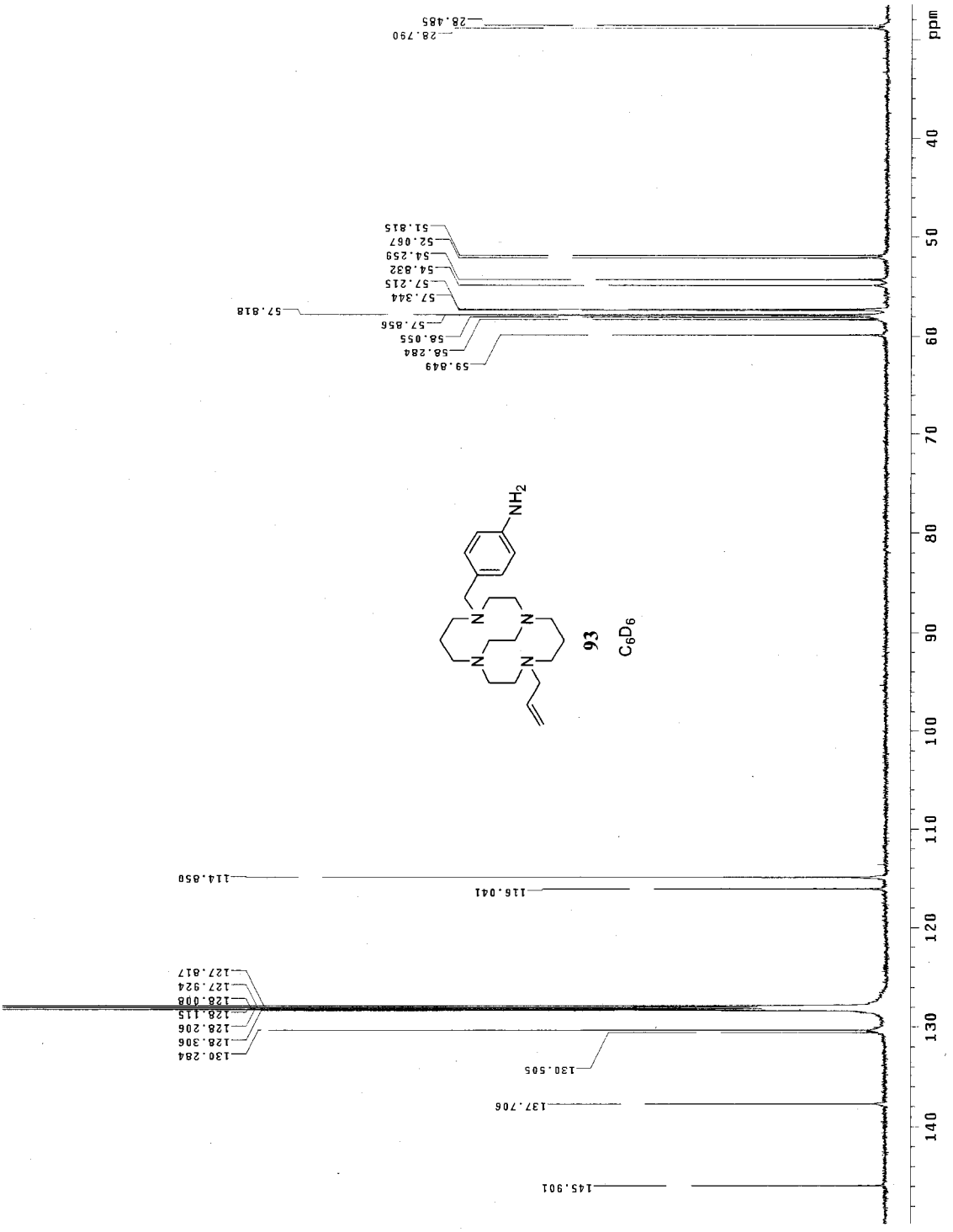

Tue Sep 09 09:38:36 2008
%Transmittance
Wavenumbers (cm-1)
4000
3500
3000
2500
2000
1500
1000
100
95
90
85
80
75
70
65
60
55
50
45
35
30
25
20
15
10
5
0
-5
3376.4
2963.4
2858.1
1634.3
1494.5
1456.2
1361.7
1270.5
1207.7
1096.7
1044.3
1001.5
931.1
769.1
732.8
706.6
• 2.7 HCl
• 3 H2O
89
KBr

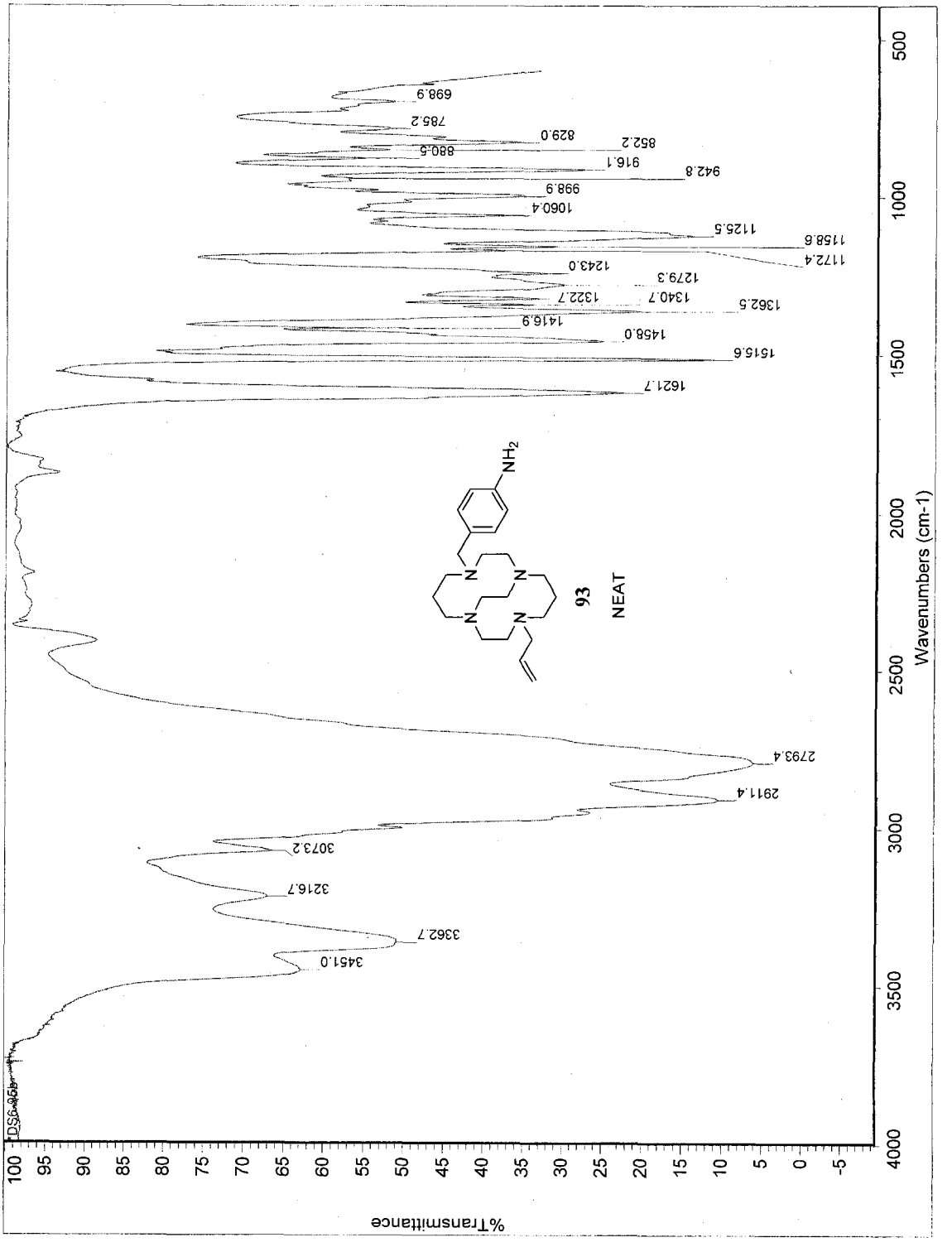

OMe
O
Br
92
D2O
7.5
7.4
7.3
7.2
7.1
2.04
0.31
0.46
2.00
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
1.03
0.97
0.98
1.00
3.04
1.02
2.05
1.06
1.02
7.25
1.02
2.06
0.53
3.10
1.01
1.02

OMe
Br
92
D2O
18.569
18.951
42.548
47.192
48.918
51.896
52.576
53.859
54.538
56.127
60.121
62.870
70.133
82.222
115.261
118.397
135.427
161.446
ppm


DS6-43
%Transmittance
Wavenumbers (cm-1)
4000
3500
3000
2500
2000
1500
1000
3409.7
2988.8
3006.6
2939.8
2867.3
2836.1
2778.1
1612.2
1518.3
1458.3
1437.2
1358.4
1337.8
1306.2
1275.0
1264.0
1251.0
1224.4
1186.8
1144.1
1107.3
1100.1
1091.0
1054.1
1024.6
887.1
873.0
840.4
824.8
808.8
OMe
Br
92
KBr


NH2
93
C6D6
ppm

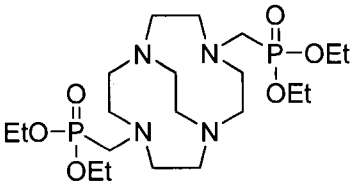
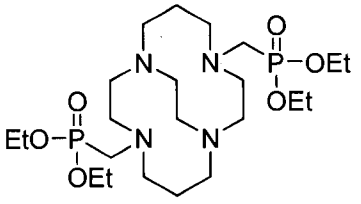
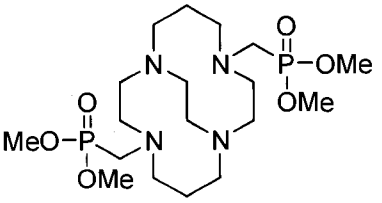
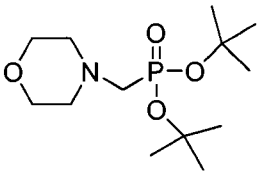
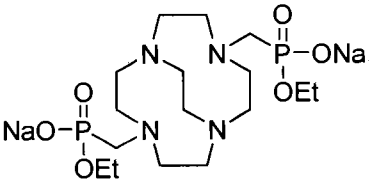
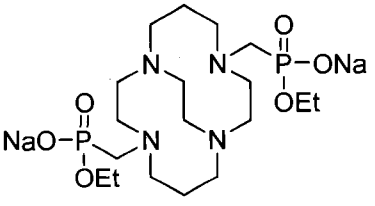

28.485
28.790
51.815
52.067
54.259
54.832
57.215
57.344
57.818
57.856
58.055
58.284
59.849
114.850
116.041
127.817
127.924
128.008
128.115
128.206
128.306
130.284
130.505
137.706
145.901
ppm
40
50
60
70
80
90
100
110
120
130
140
NH2
N
N
N
N
93
C6D6

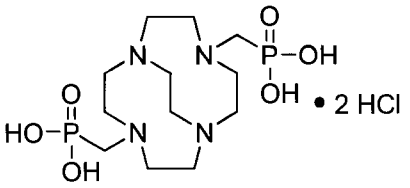
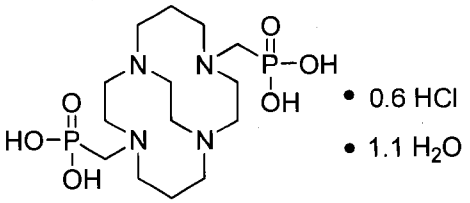
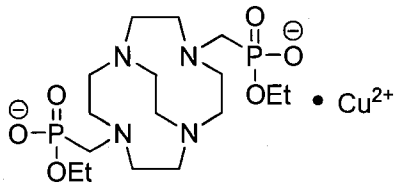
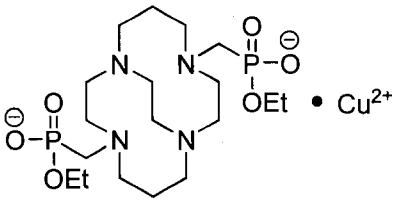
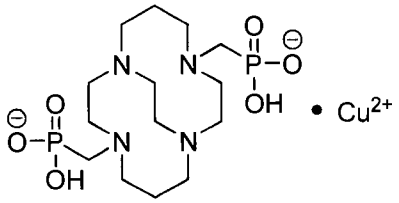
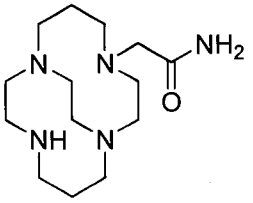
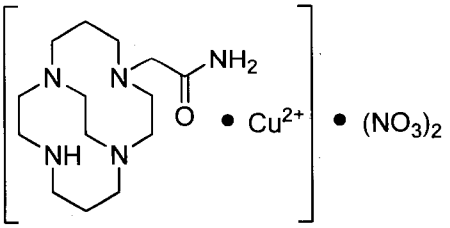

%Transmittance
Wavenumbers (cm-1)
NEAT
93
NH2
3451.0
3362.7
3216.7
3073.2
2911.4
2793.4
1621.7
1515.6
1458.0
1416.9
1362.5
1340.7
1322.7
1279.3
1243.0
1172.4
1158.8
1125.5
1060.4
998.9
942.8
916.1
880.5
852.2
829.0
785.2
698.9

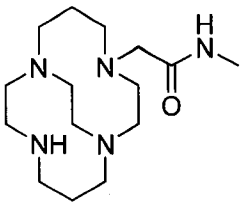
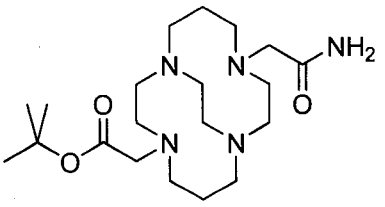
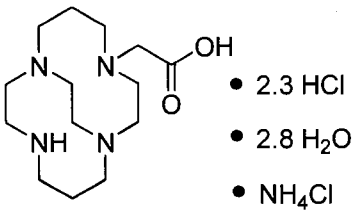
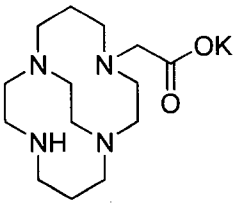
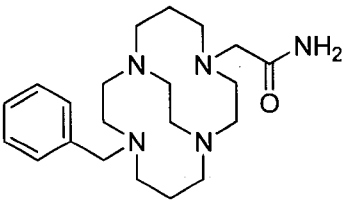
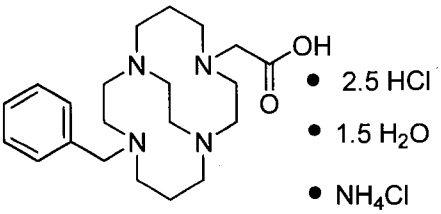
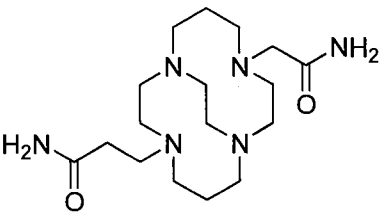
Appendix B:

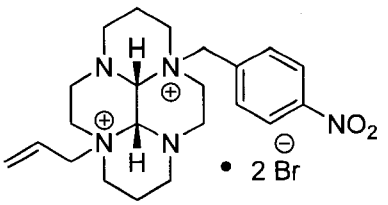
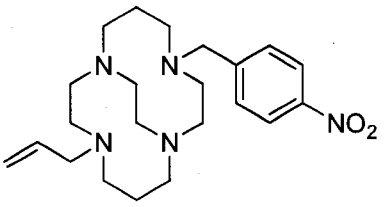
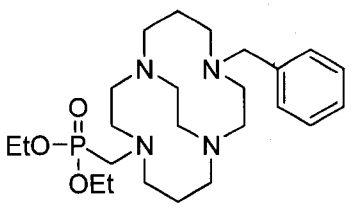
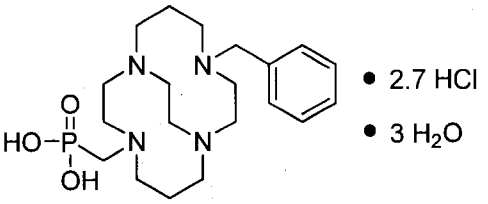
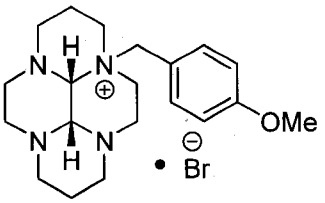
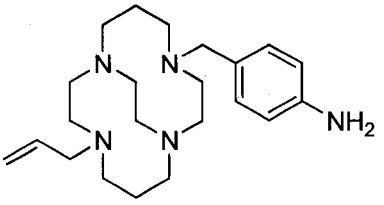
Compound Index

COMPOUND INDEX

Compound	Structure	Experimental	Spectra
36		110	137
37		111	141
39		113	145
40		114	149
47		115	152
48		116	156

50		117	160
51		118	164
52		119	168
53		120	170
54		121	172
64		121	174
67		122	177

68		123	179
71		124	---
72	 • 2.3 HCl • 2.8 H_2O • NH_4Cl	125	182
73		125	185
74		126	188
75	 • 2.5 HCl • 1.5 H_2O • NH_4Cl	127	191
76		128	194

81		129	197
82		130	200
88		131	203
89		132	207
92		133	211
93		134	214

REFRENCES

1. Busch, D. H., Distinctive coordination chemistry and biological significance of complexes with macrocyclic ligands. *Acc. Chem. Res.* **1978,** 11, (10), 392-400.

2. Liang, X.; Sadler, P. J., Cyclam complexes and their applications in medicine. *Chem. Soc. Rev.* **2004,** 33, (4), 246-266.

3. Alder, R. W., Medium-ring bicyclic compounds and intrabridgehead chemistry. *Acc. Chem. Res.* **1983,** 16, (9), 321-7.

4. Bencini, A.; Bianchi, A.; Paoletti, P.; Paoli, P., Encapsulation of cations and anions by azacrowns: thermodynamic and structural aspects. *Pure Appl. Chem.* **1993,** 65, (3), 381-6.

5. Black, D. S. C.; Hartshorn, A. J., Ligand design and synthesis. *Coord. Chem. Rev.* **1973,** 9, (3-4), 219-74.

6. Dietrich, B.; Viout, P.; Lehn, J.-M., *Macrocyclic Chemistry.* VCH: Weinheim, 1993.

7. Hancock, R. D., Macrocycles and their selectivity for metal ions on the basis of size. *Pure Appl. Chem.* **1986,** 58, (11), 1445-52.

8. Izatt, R. M.; Bradshaw, J. S.; Nielsen, S. A.; Lamb, J. D.; Christensen, J. J.; Sen, D., Thermodynamic and kinetic data for cation-macrocycle interaction. *Chem. Rev.* **1985,** 85, (4), 271-339.

9. Lindoy, L. F., *The Chemistry of Macrocyclic Ligand Complexes.* Cambridge University Press: New York, 1989.

10. Melson, G. A., *Coordination Chemistry of Macrocyclic Compounds.* Plenum Press: New York, 1979.

11. Wainwright, K. P., Applications for polyaza macrocycles with nitrogen-attached pendant arms. *Adv. Inorg. Chem.* **2001,** 52, 293-334.

12. Reichert, D. E.; Lewis, J.; Anderson, C. J., Metal complexes as diagnostic tools. *Coord. Chem. Rev.* **1999,** 184, 3-66.

13. Hancock, R. D.; Martell, A. E., The chelate, cryptate and macrocyclic effects. *Comments on Inorg. Chem.* **1988,** 6, (5-6), 237-84.

14. Costamagna, J.; Ferraudi, G.; Matsuhiro, B.; Campos-Vallette, M.; Canales, J.; Villagran, M.; Vargas, J.; Aguirre, M. J., Complexes of macrocycles with pendant arms as models for biological molecules. *Coord. Chem. Rev.* **2000,** 196, 125-164.

15. Cabbiness, D. K.; Margerum, D. W., Macrocyclic effect on the stability of copper(II) tetramine complexes. *J. Am. Chem. Soc.* **1969,** 91, (23), 6540-1.

16. Hancock, R. D.; Martell, A. E., The chelate, cryptate and macrocyclic effects. *Comments Inorg. Chem.* **1988,** 6, (5-6), 237-84.

17. Wainwright, K. P., Synthetic and structural aspects of the chemistry of saturated polyaza macrocyclic ligands bearing pendant coordinating groups attached to nitrogen. *Coord. Chem. Rev.* **1997,** 166, 35-90.

18. Bosnich, B.; Tobe, M. L.; Webb, G. A., Complexes of nickel(II) with a cyclic tetradentate secondary amine. *Inorg. Chem.* **1965,** 4, (8), 1109-12.

19. Bakaj, M.; Zimmer, M., Conformational analysis of copper(II) 1,4,8,11-tetraazacyclotetradecane macrocyclic systems. *J. Mol. Struct.* **1999,** 508, (1-3), 59-72.

20. Cooper, C. G., Jr.; Zimmer, M., Cluster and molecular mechanical analysis of cobalt(III) 14-membered macrocyclic complexes. *Struct. Chem.* **1999,** 10, (1), 17-27.

21. Donnelly, M. A.; Zimmer, M., Structural analysis of all the nickel 14-membered tetraaza acrocycles in the Cambridge Structural Database. *Inorg. Chem.* **1999,** 38, (8), 1650-1658.

22. Liang, X.; Parkinson, J. A.; Parsons, S.; Weishaeupl, M.; Sadler, P. J., Cadmium cyclam complexes: Interconversion of cis and trans configurations and fixation of CO_2. *Inorg. Chem.* **2002,** 41, (17), 4539-4547.

23. Liang, X.; Parkinson, J. A.; Weishaeupl, M.; Gould, R. O.; Paisey, S. J.; Park, H.-S.; Hunter, T. M.; Blindauer, C. A.; Parsons, S.; Sadler, P. J., Structure and dynamics of metallomacrocycles: Recognition of zinc xylyl-bicyclam by an HIV coreceptor. *J. Am. Chem. Soc.* **2002,** 124, (31), 9105-9112.

24. Bernhardt, P. V.; Lawrance, G. A., Complexes of polyaza macrocycles bearing pendent coordinating groups. *Coord. Chem. Rev.* **1990,** 104, (2), 297-343.

25. Lewis, E. A.; Allan, C. C.; Boyle, R. W.; Archibald, S. J., Efficient N- and C-functionalization of cyclam macrocycles utilizing bisaminal methodology. *Tetrahedron Lett.* **2004,** 45, (15), 3059-3062.

26. Ferraudi, G.; Canales, J. C.; Kharisov, B.; Costamagna, J.; Zagal, J. G.; Cardenas-Jiron, G.; Paez, M., Synthetic N-substituted metal aza-macrocyclic complexes: Properties and applications. *J. Coord. Chem.* **2005,** 58, (1), 89-109.

27. Marques, F.; Gano, L.; Campello, M. P.; Lacerda, S.; Santos, I., Biological evaluation of ^{153}Sm and ^{166}Ho complexes with tetraazamacrocycles containing methylcarboxylate and/or methylphosphonate pendant arms. *Radiochim. Acta* **2007,** 95, (6), 335-341.

28. Schroder, M.; Lippolis, V., *Binding and structural aspects of nitrile- and amino-functionalized pendant arm derivatives of 1,4,7-triazacyclononane ([9]aneN$_3$).* 2005; p 67-86.

29. Haines, R. I., Pendant-arm tri- and tetraazamacrocycles: synthesis, structure and properties of their first-row transition metal complexes. *Rev. Inorg. Chem.* **2001,** 21, (3-4), 165-205.

30. Kaden, T. A., Synthesis and metal complexes of aza-macrocycles with pendant arms having additional ligating groups. *Top. Curr. Chem.* **1984,** 121, (Host Guest Complex Chem. 3), 157-79.

31. Bianchi, A.; Micheloni, M.; Paoletti, P., Thermodynamic aspects of the polyazacycloalkane complexes with cations and anions. *Coord. Chem. Rev.* **1991,** 110, (1), 17-113.

32. Chaudhuri, P.; Wieghardt, K., The chemistry of 1,4,7-triazacyclononane and related tridentate macrocyclic compounds. *Prog. Inorg. Chem.* **1987,** 35, 329-436.

33. Kaden, T. A., Functionalized tetraazamacrocycles: ligands with many aspects. *Adv. Supramol. Chem.* **1993,** 3, 65-96.

34. Kaden, T. A., Structural aspects of metal complexes with functionalized azamacrocyclic ligands. *Pure Appl. Chem.* **1993,** 65, (7), 1477-83.

35. Kimura, E., Developments in functionalization of macrocyclic polyamines. *Pure Appl. Chem.* **1989,** 61, (5), 823-8.

36. Weighardt, K., 1,4,7-Triazacyclononane and N,N',N"-trimethyl-1,4,7-triazacyclononane - two versatile macrocycles for the synthesis of monomeric and oligomeric metal complexes. *Pure Appl. Chem.* **1988,** 60, (4), 509-16.

37. Tanaka, K.; Fukase, K., PET (positron emission tomography) imaging of biomolecules using metal-DOTA complexes: a new collaborative challenge by chemists, biologists, and physicians for future diagnostics and exploration of in vivo dynamics. *Org. Bio. Chem.* **2008,** 6, (5), 815-828.

38. Virgolini, I.; Traub-Weidinger, T.; Gabriel, M.; Heute, D.; Rodrigues, M., Somatostatin receptor-mediated radionuclide therapy for cancer: therapy with ^{90}Y-DOTA-lanreotide. *Radionuclide Peptide Cancer Therapy* **2006**, 209-219.

39. Anderson, C. J., Metabolism of radiometal-labeled proteins and peptides: What are the real radiopharmaceuticals in vivo? *Cancer Biotherapy & Radiopharmaceuticals* **2001,** 16, (6), 451-455.

40. Jones-Wilson, T. M.; Deal, K. A.; Anderson, C. J.; McCarthy, D. W.; Kovacs, Z.; Motekaitis, R. J.; Sherry, A. D.; Martell, A. E.; Welch, M. J., The in vivo behavior of copper-64-labeled azamacrocyclic complexes. *Nucl. Med. Biol.* **1998,** 25, (6), 523-30.

41. Kaden, T. A., Labelling monoclonal antibodies with macrocyclic radiometal complexes. A challenge for coordination chemists. *Dalton Trans.* **2006**, (30), 3617-3623.

42. Cole, W. C.; DeNardo, S. J.; Meares, C. F.; McCall, M. J.; DeNardo, G. L.; Epstein, A. L.; O'Brien, H. A.; Moi, M. K., Comparative serum stability of radiochelates for antibody radiopharmaceuticals. *J. Nucl. Med.* **1987,** 28, (1), 83-90.

43. Moi, M. K.; Meares, C. F.; McCall, M. J.; Cole, W. C.; DeNardo, S. J., Copper chelates as probes of biological systems: stable copper complexes with a macrocyclic bifunctional chelating agent. *Anal. Biochem.* **1985,** 148, (1), 249-53.

44. Bass, L. A.; Wang, M.; Welch, M. J.; Anderson, C. J., In vivo transchelation of copper-64 from TETA-octreotide to superoxide dismutase in rat liver. *Bioconjugate Chem.* **2000,** 11, (4), 527-32.

45. Wainwright, K. P., Bridging alkylation of saturated polyaza macrocycles: a means for imparting structural rigidity. *Inorg. Chem.* **1980,** 19, (5), 1396-8.

46. Ramasubbu, A.; Wainwright, K. P., Structurally reinforced cyclen: a rigidly trans-coordinating twelve-membered macrocycle. *J. Chem. Soc., Chem. Commun.* **1982**, (5), 277-8.

47. Hancock, R. D.; Dobson, S. M.; Evers, A.; Wade, P. W.; Ngwenya, M. P.; Boeyens, J. C. A.; Wainwright, K. P., More rigid macrocyclic ligands that show metal ion size-based selectivity. Crystallographic, molecular mechanics, and formation constant study of the complexes of bridged cyclen. *J. Am. Chem. Soc.* **1988,** 110, (9), 2788-94.

48. Hancock, R. D.; Pattrick, G.; Wade, P. W.; Hosken, G. D., Structurally reinforced macrocyclic ligands. *Pure Appl. Chem.* **1993,** 65, (3), 473-6.

49. Weisman, G. R.; Wong, E. H.; Hill, D. C.; Rogers, M. E.; Reed, D. P.; Calabrese, J. C., Synthesis and transition-metal complexes of new cross-bridged tetraamine ligands. *Chem. Comm.* **1996**, (8), 947-948.

50. Weisman, G. R.; Rogers, M. E.; Wong, E. H.; Jasinski, J. P.; Paight, E. S., Cross-bridged cyclam. Protonation and lithium cation (Li^+) complexation in a diamond-lattice cleft. *J. Am. Chem. Soc.* **1990,** 112, (23), 8604-5.

51. Wong, E. H.; Weisman, G. R.; Hill, D. C.; Reed, D. P.; Rogers, M. E.; Condon, J. S.; Fagan, M. A.; Calabrese, J. C.; Lam, K.-C.; Guzei, I. A.; Rheingold, A. L., Synthesis and characterization of cross-bridged cyclams and pendant-armed derivatives and structural studies of their copper(II) complexes. *J. Am. Chem. Soc.* **2000,** 122, (43), 10561-10572.

52. Sun, X.; Wuest, M.; Weisman, G. R.; Wong, E. H.; Reed, D. P.; Boswell, C. A.; Motekaitis, R.; Martell, A. E.; Welch, M. J.; Anderson, C. J., Radiolabeling and in vivo behavior of copper-64-labeled cross-bridged cyclam ligands. *J. Med. Chem.* **2002,** 45, (2), 469-477.

53. Niu, W.; Wong, E. H.; Weisman, G. R.; Zakharov, L. N.; Incarvito, C. D.; Rheingold, A. L., Copper(II) and zinc(II) complexes of amide pendant-armed cross-bridged tetraamine ligands. *Polyhedron* **2004,** 23, (6), 1019-1025.

54. Sprague Jennifer, E.; Peng, Y.; Fiamengo Ashley, L.; Woodin Katrina, S.; Southwick Evan, A.; Weisman Gary, R.; Wong Edward, H.; Golen James, A.; Rheingold Arnold, L.; Anderson Carolyn, J., Synthesis, characterization and in vivo studies of Cu(II)-64-labeled cross-bridged tetraazamacrocycle-amide complexes as models of peptide conjugate imaging agents. *J. Med. Chem.* **2007,** 50, (10), 2527-35.

55. Boswell, C. A.; Sun, X.; Niu, W.; Weisman, G. R.; Wong, E. H.; Rheingold, A. L.; Anderson, C. J., Comparative in vivo stability of copper-64-labeled cross-bridged and conventional tetraazamacrocyclic complexes. *J. Med. Chem.* **2004,** 47, (6), 1465-74.

56. Woodin, K. S.; Heroux, K. J.; Boswell, C. A.; Wong, E. H.; Weisman, G. R.; Niu, W.; Tomellini, S. A.; Anderson, C. J.; Zakharov, L. N.; Rheingold, A. L., Kinetic inertness and electrochemical behavior of copper(II) tetraazamacrocyclic complexes: Possible implications for in vivo stability. *Eur. J. Inorg. Chem.* **2005**, (23), 4829-4833.

57. Sprague Jennifer, E.; Peng, Y.; Sun, X.; Weisman Gary, R.; Wong Edward, H.; Achilefu, S.; Anderson Carolyn, J., Preparation and biological evaluation of copper-64-labeled tyr3-octreotate using a cross-bridged macrocyclic chelator. *Clin. Cancer Res.* **2004,** 10, (24), 8674-82.

58. Lewkowski, J., Synthesis, chemistry and applications of alpha -aminophosphonic and alpha -aminophosphonous acids derivatives bearing furan moiety. *Focus Organomet. Chem. Res.* **2005**, 167-195.

59. Mikolajczyk, M., Acyclic and cyclic aminophosphonic acids: asymmetric syntheses mediated by chiral sulfinyl auxiliary. *J. Organomet. Chem.* **2005,** 690, (10), 2488-2496.

60. Song, B.-A.; Jiang, M.-G., Recent advances in synthesis and biological activity of alpha -aminophosphonic acids and their esters. *Youji Huaxue* **2004,** 24, (8), 843-856.

61. Kamecka, A.; Kurzak, B., Coordination properties of phosphonate ligands with some metal ions. *Wiadomosci Chemiczne* **2003,** 57, (9-10), 797-825.

62. Song, B.-A.; Wu, Y.-L.; He, X.-Q.; Hu, D.-Y.; Liu, G.; Yang, S.; Jin, L.-H.; Jiang, M.-G., Recent progress in the synthesis of novel α-aminophosphonic acids and α-aminophosphonates with optical activity. *Youji Huaxue* **2003,** 23, (9), 933-943.

63. Yuan, C.; Li, S.; Xiao, J.; Cui, S.; Wang, G., Asymmetric synthesis of aminophosphonic acids and trifluoromethylated derivatives thereof. *Phosphorus, Sulfur Silicon Relat. Elem.* **2002,** 177, (6-7), 1731-1734.

64. Kafarski, P.; Lejczak, B., Aminophosphonic acids of potential medical importance. *Curr. Med. Chem. Anti-Cancer Agents* **2001,** 1, (3), 301-312.

65. Huang, J.; Chen, R., An overview of recent advances on the synthesis and biological activity of alpha -aminophosphonic acid derivatives. *Heteroat. Chem.* **2000,** 11, (7), 480-492.

66. Hoffmann, M., Esters of phosphonic analogs of α-amino acids and α-hydroxy acids as substrates in the synthesis of phosphonopeptides and phosphonodepsipeptides. *Zeszyty Naukowe Politechniki Gdanskiej, Chemia* **1999,** 41, 3-59.

67. Mikolajczyk, M.; Lyzwa, P.; Drabowicz, J., Asymmetric synthesis of aminophosphonic acids. *Phosphorus, Sulfur Silicon Relat. Elem.* **1999,** 144-146, 157-160.

68. Xu, J.-X.; Yu, L., Synthesis of aminophosphonic acids and their esters. *Hecheng Huaxue* **1999,** 7, (2), 153-158.

69. Uziel, J.; Genet, J. P., Synthesis of racemic and optically active alpha -aminophosphonic acids. *Russ. J. Org. Chem.* **1997,** 33, (11), 1521-1542.

70. Gobec, S.; Urleb, U., Aminophosphonic acids and phosphapeptides. *Farmacevtski Vestnik (Ljubljana)* **1996,** 47, (4), 391-404.

71. Kiss, T., Metal complexes of aminophosphonic acids. *Bioinorg. Chem.* **1995,** 1, 717-24.

72. Kafarski, P.; Lejczak, B., Biological activity of aminophosphonic acids. *Phosphorus, Sulfur Silicon Relat. Elem.* **1991,** 63, (1-2), 193-215.

73. Sinyavskaya, E. I., Coordination compounds with aminophosphonic acids. *Koord. Khim.* **1991,** 17, (12), 1599-626.

74. Yamauchi, K., Aminophosphonic acids. Synthesis and biological activities of phosphonopeptides. *Yuki Gosei Kagaku Kyokaishi* **1988,** 46, (7), 654-6.

75. Prajer, K.; Rachon, J., α-Aminophosphonic acids. *Zeitschrift fuer Chemie* **1975,** 15, (6), 209-15.

76. Collinsova, M.; Jiracek, J., Phosphinic acid compounds in biochemistry, biology and medicine. *Curr. Med. Chem.* **2000,** 7, (6), 629-647.

77. Kukar, V. P.; Hudson, H. R., *Aminophosphonic and Aminophosphinic Acids* John Wiley and Sons: West Sussex, 2000.

78. Smith, R. M.; Martell, A. E., *Critical Stability Constants, vols. 1-6*. Plenum Press: New York, 1974-1989.

79. Kimball, S. D., Challenges in the development of orally bioavailable thrombin active site inhibitors. *Blood Coagulation & Fibrinolysis* **1995,** 6, (6), 511-19.

80. Kalman, E., Routes to the development of low toxicity corrosion inhibitors for use in neutral solutions. *European Federation of Corrosion Publications* **1994,** 11, 12-38.

81. Motekaitis, R. J.; Murase, I.; Martell, A. E., Equilibriums of ethylenediamine-N,N,N',N'-tetrakis(methylenephosphonic) acid with copper(II), nickel(II), cobalt(II), zinc(II), magnesium(II), calcium(II), and iron(III) ions in aqueous solution. *Inorg. Chem.* **1976,** 15, (9), 2303-6.

82. Rizkalla, E. N., Metal chelates of phosphonate-containing ligands. *Rev. Inorg. Chem.* **1983,** 5, (3), 223-304.

83. Pasini, A.; D'Alfonso, G.; Manzotti, C.; Moret, M.; Spinelli, S.; Valsecchi, M., Cytotoxic diamine-platinum(II) complexes with methylsulfinyl carboxylates as the leaving groups. Synthesis, characterization and reactivity toward chloride ions, 5'-GMP, and 9-methylguanine. *Inorg. Chem.* **1994,** 33, (18), 4140-8.

84. Lauffer, R. B., Paramagnetic metal complexes as water proton relaxation agents for NMR imaging: theory and design. *Chem. Rev.* **1987,** 87, (5), 901-27.

85. Hayes, R. L.; Huebner, K. F., Basis for the clinical use of gallium and indium radionuclide. *Met. Ions Biol. Syst.* **1983,** 16, 279-315.

86. Lukes, I.; Kotek, J.; Vojtisek, P.; Hermann, P., Complexes of tetraazacycles bearing methylphosphinic/phosphonic acid pendant arms with copper(II), zinc(II) and lanthanides(III). A comparison with their acetic acid analogues. *Coord. Chem. Rev.* **2001,** 216-217, 287-312.

87. Kabachnik, M. I.; Medved, T. Y.; Pisareva, S. A.; Bel'skii, F. I. 1,4,7,10-Tetra(dihydroxyphosphorylmethyl)-1,4,7,10-tetraazacyclododecane as a complexing agent for binding copper(II), cobalt(II), cadmium(II), lead(II), and lanthanum(III) cations. SU-1098937, June 23, 1984.

88. Lazar, I.; Hrncir, D. C.; Kim, W. D.; Kiefer, G. E.; Sherry, A. D., Optimized synthesis, structure, and solution dynamics of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(methylenephosphonic acid) (H_8DOTP). *Inorg. Chem.* **1992,** 31, (21), 4422-4.

89. Geraldes, C. F. G. C.; Sherry, A. D.; Cacheris, W. P., Synthesis, protonation sequence, and NMR studies of polyazamacrocyclic methylenephosphonates. *Inorg. Chem.* **1989,** 28, (17), 3336-41.

90. Kotek, J.; Vojtisek, P.; Cisarova, I.; Hermann, P.; Jurecka, P.; Rohovec, J.; Lukes, I., Bis(methylphosphonic acid) derivatives of 1,4,8,11-tetraazacyclotetradecane (cyclam). Synthesis, crystal and molecular structures, and solution properties. *Collect. Czech. Chem. Commun.* **2000,** 65, (8), 1289-1316.

91. Letkeman, P.; Martell, A. E., Nuclear magnetic resonance and potentiometric protonation study of polyaminopolyacetic acids containing from two to six nitrogen atoms. *Inorg. Chem.* **1979,** 18, (5), 1284-9.

92. Desreux, J. F.; Merciny, E.; Loncin, M. F., Nuclear magnetic resonance and potentiometric studies of the protonation scheme of two tetraaza tetraacetic macrocycles. *Inorg. Chem.* **1981,** 20, (4), 987-91.

93. Sherry, A. D.; Ren, J.; Huskens, J.; Bruecher, E.; Toth, E.; Geraldes, C. F. C. G.; Castro, M. M. C. A.; Cacheris, W. P., Characterization of lanthanide(III) DOTP complexes: thermodynamics, protonation, and coordination to alkali metal ions. *Inorg. Chem.* **1996,** 35, (16), 4604-4612.

94. Geraldes, C. F. G. C.; Sherry, A. D.; Kiefer, G. E., The solution structure of Ln(DOTP)5- complexes. A comparison of lanthanide-induced paramagnetic shifts

with the MMX energy-minimized structure. *J. Magn. Reson.* **1992,** 97, (2), 290-304.

95. Buster, D. C.; Castro, M. M. C. A.; Geraldes, C. F. G. C.; Malloy, C. R.; Sherry, A. D.; Siemers, T. C., Tm(DOTP)5-: a sodium-23 ion shift agent for perfused rat hearts. *Magn. Reson. Med.* **1990,** 15, (1), 25-32.

96. Sherry, A. D., MR imaging and spectroscopy applications of lanthanide complexes with macrocyclic phosphonate and phosphonate ester ligands. *J. Alloys Compd.* **1997,** 249, (1-2), 153-157.

97. Sun, X.; Wuest, M.; Kovacs, Z.; Sherry, A. D.; Motekaitis, R.; Wang, Z.; Martell, A. E.; Welch, M. J.; Anderson, C. J., In vivo behavior of copper-64-labeled methanephosphonate tetraaza macrocyclic ligands. *J. Biol. Inorg. Chem.* **2003,** 8, (1-2), 217-225.

98. Kotek, J.; Lubal, P.; Hermann, P.; Cisarova, I.; Lukes, I.; Godula, T.; Svobodova, I.; Taborsky, P.; Havel, J., High thermodynamic stability and extraordinary kinetic inertness of copper(II) complexes with 1,4,8,11-tetraazacyclotetradecane-1,8-bis(methylphosphonic acid): Example of a rare isomerism between kinetically inert penta- and hexacoordinated copper(II) complexes. *Chem. Eur. J.* **2003,** 9, (1), 233-248.

99. Moedritzer, K.; Irani, R. R., The direct synthesis of α-aminomethylphosphonic acids. Mannich-type reactions with orthophosphorous acid. *J. Org. Chem.* **1966,** 31, (5), 1603-7.

100. Zhang, G. p.; Zi, Y. q.; Wang, Y. q.; Wang, L.; Song, B. a., Progress in synthesis of α-aminophosphonates. *Hecheng Huaxue* **2008,** 16, (3), 247-251, 263.

101. Syamala, M., A decade of advances in three-component reactions. A review. *Org. Prep. Proced. Int.* **2005,** 37, (2), 103-171.

102. Shibuya, S., Synthesis of phosphonic acids and phosphinic acids derivatives toward to biologically active compounds. *Yakugaku Zasshi* **2004,** 124, (11), 725-750.

103. Gajda, T.; Sikora, D.; Nonas, T., Application of 1-azidoalkylphosphonates in the synthesis of phosphonodipeptides and other N-substituted aminophosphonates. *Phosphorus, Sulfur Silicon Relat. Elem.* **1999,** 144-146, 581-584.

104. Boduszek, B., Synthesis and biological activity of heterocyclic aminophosphonates. *Phosphorus, Sulfur Silicon Relat. Elem.* **1999,** 144-146, 433-436.

105. Gancarz, R., Kabachnik-Fields reaction. Synthesis of biologically active aminophosphonates. *Prace Naukowe Instytutu Chemii Organicznej, Biochemii i Biotechnologii Politechniki Wroclawskiej* **1997,** 39, 1-78.

106. Prishchenko, A. A.; Livantsov, M. V.; Novikova, O. P.; Livantsova, L. I., Reaction of 1,3,5-tris[N-(alkoxycarbonylalkyl)]hexahydrotriazines with PH-acids. *Russ. J. Gen. Chem.* **2006,** 76, (12), 1960-1962.

107. Kalman, F. K.; Woods, M.; Caravan, P.; Jurek, P.; Spiller, M.; Tircso, G.; Kiraly, R.; Bruecher, E.; Sherry, A. D., Potentiometric and relaxometric properties of a gadolinium-based MRI contrast agent for sensing tissue pH. *Inorg. Chem.* **2007,** 46, (13), 5260-5270.

108. Mamedov, I.; Mishra, A.; Angelovski, G.; Mayer, H. A.; Palsson, L.-O.; Parker, D.; Logothetis, N. K., Synthesis and characterization of lanthanide complexes of DO3A-alkylphosphonates. *Dalton Trans.* **2007**, (45), 5260-5267.

109. Lebduskova, P.; Hermann, P.; Helm, L.; Toth, E.; Kotek, J.; Binnemans, K.; Rudovsky, J.; Lukes, I.; Merbach, A. E., Gadolinium(III) complexes of mono- and diethyl esters of mono-phosphonic acid analogue of DOTA as potential MRI contrast agents: solution structures and relaxometric studies. *Dalton Trans.* **2007**, (4), 493-501.

110. Chaudhary, P.; Kumar, R.; Verma, A. K.; Singh, D.; Yadav, V.; Chhillar, A. K.; Sharma, G. L.; Chandra, R., Synthesis and antimicrobial activity of N-alkyl and N-aryl piperazine derivatives. *Biorg. Med. Chem.* **2006,** 14, (6), 1819-1826.

111. Skwierawska, A.; Paluszkiewicz, E.; Przyborowska, M.; Ossowski, T., The synthesis of 1,4,7,10-tetraazacyclododecanes with acetylsalicylic side arm as potential cobalt(II) fluorophores. *J. Inclusion Phenom. Macrocyclic Chem.* **2004,** 49, (1-2), 21-26.

112. Skwierawska, A., Synthesis of new trifunctional ligands based on 1,4,7,10-tetraazacyclododecane. *Pol. J. Chem.* **2004,** 78, (1), 75-82.

113. Li, X.; Zhang, S.; Zhao, P.; Kovacs, Z.; Sherry, A. D., Synthesis and NMR studies of new DOTP-like lanthanide(III) complexes containing a hydrophobic substituent on one phosphonate side arm. *Inorg. Chem.* **2001,** 40, (26), 6572-6579.

114. Kovacs, Z.; Sherry, A. D., A general synthesis of 1,7-disubstituted 1,4,7,10-tetraazacyclododecanes. *J. Chem. Soc., Chem. Commun.* **1995**, (2), 185-6.

115. Burai, L.; Ren, J.; Kovacs, Z.; Bruecher, E.; Sherry, A. D., Synthesis, potentiometry, and NMR studies of two new 1,7-disubstituted

tetraazacyclododecanes and their complexes formed with lanthanide, alkaline earth metal, Mn^{2+}, and Zn^{2+} Ions. *Inorg. Chem.* **1998,** 37, (1), 69-75.

116. Fuezerova, S.; Kotek, J.; Cisarova, I.; Hermann, P.; Binnemans, K.; Lukes, I., Cyclam (1,4,8,11-tetraazacyclotetradecane) with one methylphosphonate pendant arm: a new ligand for selective copper(II) binding. *Dalton Trans.* **2005**, (17), 2908-2915.

117. Kim, Y.-C.; Lee, J.-S.; Sak, K.; Marteau, F.; Mamedova, L.; Boeynaems, J.-M.; Jacobson, K. A., Synthesis of pyridoxal phosphate derivatives with antagonist activity at the P2Y13 receptor. *Biochem. Pharmacol.* **2005,** 70, (2), 266-274.

118. Tesson, N.; Dorigneux, B.; Fadel, A., Synthesis of (1S,2S)- and (1R,2R)-1-amino-2-methylcyclopropane-phosphonic acids from racemic methylcyclopropanone acetal. *Tetrahedron: Asymmetry* **2002,** 13, (20), 2267-2276.

119. Rajwanshi, V. K.; Prhavc, M.; Fagan, P.; Brooks, J. L.; Hurd, T.; Cook, P. D.; Wang, G., Synthesis of 5'-triphosphate mimics (P3Ms) of 3'-azido-3',5'-dideoxy-thymidine and 3',5'-dideoxy-5'-difluoromethylene-thymidine as HIV-1 reverse transcriptase inhibitors. *Nucleosides, Nucleotides & Nucleic Acids* **2005,** 24, (3), 179-189.

120. Prauda, I.; Greiner, I.; Ludanyi, K.; Keglevich, G., Efficient synthesis of phosphono- and phosphinoxidomethylated N-heterocycles under solvent-free microwave conditions. *Synth. Commun.* **2007,** 37, (2), 317-322.

121. Ouadi, A.; Bultel, K.; de France-Robert, A.; Loussouarn, A.; Morandeau, L.; Gestin, J.-F., Synthesis of a new bifunctional chelating agent for samarium complexation. *Tetrahedron Lett.* **2004,** 45, (7), 1395-1397.

122. Bianchini, C.; Giambastiani, G.; Laschi, F.; Mariani, P.; Vacca, A.; Vizza, F.; Zanello, P., Synthesis, characterization and coordination chemistry of the new tetraazamacrocycle 4,10-dimethyl-1,4,7,10-tetraazacyclododecane-1,7-bis (methanephosphonic acid monoethyl ester) dipotassium salt. *Org. Bio. Chem.* **2003,** 1, (5), 879-886.

123. Pudovik, M. A.; Terent'eva, S. A.; Pudovik, A. N., Reaction of N-aminoalkylated 1,3,2-oxazaphosphacyclanes with proton-donating reagents. *Zh. Obshch. Khim.* **1991,** 61, (5), 1056-8.

124. Osapay, G.; Szilagyi, I.; Seres, J., Conversion of amino acids and dipeptides into their phosphonic analogs. Aminoalkylphosphonic acids and peptides. II. *Tetrahedron* **1987,** 43, (13), 2977-83.

125. Phillion, D. P.; Andrew, S. S., Synthesis and reactivity of diethyl phosphonomethyl triflate. *Tetrahedron Lett.* **1986,** 27, (13), 1477-80.

126. Rudovsky, J.; Cigler, P.; Kotek, J.; Hermann, P.; Vojtisek, P.; Lukes, I.; Peters, J. A.; Vander Elst, L.; Muller, R. N., Lanthanide(III) complexes of a mono(methylphosphonate) analogue of H_4dota: The influence of protonation of the phosphonate moiety on the TSAP/SAP isomer ratio and the water exchange rate. *Chem. Eur. J.* **2005,** 11, (8), 2373-2384.

127. Loussouarn, A.; Ouadi, A.; Morandeau, L.; Remaud, P.; Giles, R.; Gestin, J.-F.; Webb, J., Synthesis of new semi-rigid chelating agents for samarium-153. *Tetrahedron Lett.* **2003,** 44, (17), 3539-3541.

128. Kim, W. D.; Kiefer, G. E.; Huskens, J.; Sherry, A. D., NMR Studies of the Lanthanide(III) Complexes of 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetrakis(methanephosphonic acid mono(2',2',2-trifluoroethyl) ester). *Inorg. Chem.* **1997,** 36, (18), 4128-4134.

129. Lazar, I.; Sherry, A. D., Synthesis of ester derivatives of 1,4,7-triazacyclononane-1,4,7-tris(methylenephosphonic acid) and 1,4,7-triazacyclononane-1,4,7-tris(methyleneethylphosphinic acid). *Synthesis* **1995**, (4), 453-7.

130. Tsubouchi, A.; Bruice, T. C., Phosphonate ester hydrolysis catalyzed by two lanthanum ions. Intramolecular nucleophilic attack of coordinated hydroxide and lewis acid activation. *J. Am. Chem. Soc.* **1995,** 117, (28), 7399-411.

131. Kovach, I. M., Solution dynamics of phosphonate ester hydrolysis. *Phosphorus, Sulfur Silicon Relat. Elem.* **1993,** 75, (1-4), 131-4.

132. Westheimer, F. H.; Huang, S.; Covitz, F., Rates and mechanisms of hydrolysis of esters of phosphorous acid. *J. Am. Chem. Soc.* **1988,** 110, (1), 181-5.

133. Gerrard, W.; Green, W. J.; Nutkins, R. A., Dealkylation and hydrolysis of alkyl phosphites, phosphates, and phosphonates. *J. Chem. Soc.* **1952**, 4076-8.

134. Cox, J. R., Jr.; Ramsay, O. B., Mechanisms of nucleophilic substitution in phosphate esters. *Chem. Rev.*
1964, 64, (4), 317-52.

135. Hudson, R. F.; Keay, L., Hydrolysis of phosphonate esters. *J. Chem. Soc.* **1956**, 2463-9.

136. Kovach, I. M.; Bennet, A. J.; Bibbs, J. A.; Zhao, Q., Nucleophilic and protolytic catalysis of phosphonate hydrolysis: isotope effects and activation parameters. *J. Am. Chem. Soc.* **1993,** 115, (12), 5138-44.

137. Epstein, J.; Cannon, P. L., Jr.; Sowa, J. R., Catalysis of the hydrolysis of isopropyl methylphosphonofluoridate in aqueous solutions by primary amines. *J. Am. Chem. Soc.* **1970,** 92, (25), 7390-3.

138. Blumenthal, E.; Herbert, J. B. M., Mechanism of the hydrolysis of trimethyl orthophosphate. *Trans. Faraday Soc.* **1945,** 41, 611-17.

139. Cadogan, J. I. G.; Eastlick, D.; Hampson, F.; Mackie, R. K., Reactivity of organophosphorus compounds. XXIV. Acidic hydrolysis of dialkyl methylphosphonates. *J. Chem. Soc. B* **1969,** 2, 144-6.

140. Baudy, R. B.; Fletcher, H., III; Yardley, J. P.; Zaleska, M. M.; Bramlett, D. R.; Tasse, R. P.; Kowal, D. M.; Katz, A. H.; Moyer, J. A.; Abou-Gharbia, M., Design, synthesis, SAR, and biological evaluation of highly potent benzimidazole-spaced phosphono-α-amino acid competitive NMDA antagonists of the AP-6 type. *J. Med. Chem.* **2001,** 44, (10), 1516-1529.

141. Giambastiani, G.; Oberhauser, W.; Bianchini, C.; Laschi, F.; Sorace, L.; Brueggeller, P.; Gutmann, R.; Orlandini, A.; Vizza, F., Synthesis and characterization of a novel copper(II) azamacrocycle-phosphonate 3D polymeric network. *Eur. J. Inorg. Chem.* **2005**, (11), 2027-2031.

142. Gauvry, N.; Mortier, J., Dealkylation of dialkyl phosphonates with boron tribromide. *Synthesis* **2001**, (4), 553-554.

143. Mortier, J., Recent developments on the mechanism of the reaction of dialkyl phosphonates with haloboranes and their implications for synthesis. *Recent Research Developments in Organic Chemistry* **2002,** 6, (Pt. 1), 169-176.

144. Manning, H. C.; Bai, M.; Anderson, B. M.; Lisiak, R.; Samuelson, L. E.; Bornhop, D. J., Expeditious synthesis of 'P'-protected macrocycles en route to lanthanide chelate metal complexes. *Tetrahedron Lett.* **2005,** 46, (28), 4707-4710.

145. Krise, J. P.; Zygmunt, J.; Georg, G. I.; Stella, V. J., Novel prodrug approach for tertiary amines: Synthesis and preliminary evaluation of N-phosphonooxymethyl prodrugs. *J. Med. Chem.* **1999,** 42, (16), 3094-3100.

146. Regueiro-Ren, A.; Naidu, B. N.; Zheng, X.; Hudyma, T. W.; Connolly, T. P.; Matiskella, J. D.; Zhang, Y.; Kim, O. K.; Sorenson, M. E.; Pucci, M.; Clark, J.; Bronson, J. J.; Ueda, Y., Novel semi-synthetic nocathiacin antibiotics: synthesis and antibacterial activity of bis- and mono-O-alkylated derivatives. *Bioorg. Med. Chem. Lett.* **2004,** 14, (1), 171-175.

147. Broeren, M. A. C.; de Waal, B. F. M.; van Genderen, M. H. P.; Sanders, H. M. H. F.; Fytas, G.; Meijer, E. W., Multicomponent host-guest chemistry of carboxylic

acid and phosphonic acid based guests with dendritic hosts: An NMR study. *J. Am. Chem. Soc.* **2005,** 127, (29), 10334-10343.

148. Hammerschmidt, F.; Wuggenig, F., Enzymes in organic chemistry. Part 9: Chemo-enzymatic synthesis of phosphonic acid analogues of L-valine, L-leucine, L-isoleucine, L-methionine and L-α-aminobutyric acid of high enantiomeric excess. *Tetrahedron: Asymmetry* **1999,** 10, (9), 1709-1721.

149. Huber, R.; Knierzinger, A.; Obrecht, J. P.; Vasella, A., Nucleophilic additions to N-glycosylnitrones. Asymmetric synthesis of α-aminophosphonic acids. *Helv. Chim. Acta* **1985,** 68, (6), 1730-47.

150. Tweedle, M.; Fan, H.; Lattuada, L.; Ramalingham, K.; Swenson, R. E. Preparation of polyazamacrocyclic compounds useful as radiopharmaceutical imaging agents. Eur. Pat. WO2005062828, July 14, 2005.

151. Franzini, M.; Beltrami, A.; Calabi, L.; Maiocchi, A.; Virtuani, M.; Anelli, P. L.; Ramalingam, K.; Ranganathan, R. S. Preparation of (phosphonomethyl)diethylenetriamine derivative chelating compounds and their complexes with paramagnetic metals for use as MRI contrast agents. Eur. Pat. WO0146207, June 28, 2001.

152. Mark, V.; Van Wazer, J. R., Tri-tert-butyl phosphite and some of its reactions. *J. Org. Chem.* **1964,** 29, (5), 1006-8.

153. Niu, W.; Wong Edward, H.; Weisman Gary, R.; Hill Daniel, C.; Tranchemontagne David, J.; Lam, K.-C.; Sommer Roger, D.; Zakharov Lev, N.; Rheingold Arnold, L., Inside or outside a ligand cleft? Synthetic, structural, and kinetic inertness studies of zinc, cadmium, and mercury complexes of cross-bridged cyclam and cyclen. *Dalton Trans.* **2004**, (21), 3536-47.

154. Winchell, H. S.; Klein, J. Y.; Simhon, E. D.; Cyjon, R. L.; Klein, O.; Zaklad, H. Compounds with chelation affinity and selectivity for first transition series elements for medical use. U.S. Patent 5,874,573, February 23, 1999.

155. Verkade, J. G.; Quin, L. D., *Phosphorus-31 NMR Spectroscopy in Stereochemical Analysis.* VCH Publishers, Inc.: Deerfield Beach, 1987.

156. Behrman, E. J.; Biallas, M. J.; Brass, H. J.; Edwards, J. O.; Isaks, M., Reactions of phosphonic acid esters with nucleophiles. I. Hydrolysis. *J. Org. Chem.* **1970,** 35, (9), 3063-9.

157. Galkin, V. I.; Khabibullina, A. B.; Gaifutdinova, E. R.; Bakhtiyarova, I. V.; Cherkasov, R. A.; Pudovik, A. N., Kinetics of the addition of dialkyl phosphite to butyl cinnamate. *Dokl Akad Nauk SSSR* **1987,** 296, (1), 107-10 [Chem].

158. Heroux, K. J.; Woodin, K. S.; Tranchemontagne, D. J.; Widger, P. C. B.; Southwick, E.; Wong, E. H.; Weisman, G. R.; Tomellini, S. A.; Wadas, T. J.; Anderson, C. J.; Kassel, S.; Golen, J. A.; Rheingold, A. L., The long and short of it: the influence of N-carboxyethyl versus N-carboxymethyl pendant arms on in vitro and in vivo behavior of copper complexes of cross-bridged tetraamine macrocycles. *Dalton Trans.* **2007**, (21), 2150-2162.

159. Kang, S.-G.; Ryu, K.-S.; Nam, K.; Kim, J., Synthesis and coordination behaviors of 14-membered tetraaza macrocyclic copper(II) complexes bearing two N-propionic acid or N-propionic methyl ester groups. *Inorg. Chim. Acta* **2005,** 358, (7), 2224-2230.

160. Keire, D. A.; Jang, Y. H.; Li, L.; Dasgupta, S.; Goddard, W. A., III; Shively, J. E., Chelators for radioimmunotherapy: I. NMR and ab initio calculation studies on 1,4,7,10-tetra(carboxyethyl)-1,4,7,10-tetraazacyclododecane (DO4Pr) and 1,4,7-tris(carboxymethyl)-10-(carboxyethyl)-1,4,7,10-tetraazacyclododecane (DO3A1Pr). *Inorg. Chem.* **2001,** 40, (17), 4310-4318.

161. Tschudin, D.; Basak, A.; Kaden, T. A., Metal complexes with macrocyclic ligands. Part XXVI. Synthesis and spectral properties of Cu^{2+} complexes with mono-N-functionalized 1,4,8-trimethyl-1,48,11-tetraazacyclotetradecanes. *Helv. Chim. Acta* **1988,** 71, (1), 100-6.

162. Lecomte, C.; Dahaoui-Gindrey, V.; Chollet, H.; Gros, C.; Mishra, A. K.; Barbette, F.; Pullumbi, P.; Guilard, R., Prediction of the coordination scheme of lanthanide N-tetrasubstituted tetraazamacrocycles: An X-ray crystallography and molecular modeling study. *Inorg. Chem.* **1997,** 36, (18), 3827-3838.

163. Schatz, M.; Becker, M.; Thaler, F.; Hampel, F.; Schindler, S.; Jacobson, R. R.; Tyeklar, Z.; Murthy, N. N.; Ghosh, P.; Chen, Q.; Zubieta, J.; Karlin, K. D., Copper(I) complexes, copper(I)/O_2 reactivity, and copper(II) complex adducts, with a series of tetradentate tripyridylalkylamine tripodal ligands. *Inorg. Chem.* **2001,** 40, (10), 2312-2322.

164. Gibson, G.; Neverov, A. A.; Brown, R. S., Potentiometric titration of metal ions in methanol. *Can. J. Chem.* **2003,** 81, (6), 495-504.

165. Sigel, H.; Zuberbuehler, A. D.; Yamauchi, O., Comments on potentiometric pH titrations and the relationship between pH-meter reading and hydrogen ion concentration. *Anal. Chim. Acta* **1991,** 255, (1), 63-72.

166. Svobodova, I.; Lubal, P.; Plutnar, J.; Havlickova, J.; Kotek, J.; Hermann, P.; Lukes, I., Thermodynamic, kinetic and solid-state study of divalent metal complexes of 1,4,8,11-tetraazacyclotetradecane (cyclam) bearing two trans 1,8-methylphosphonic acid pendant arms. *Dalton Trans.* **2006**, (43), 5184-5197.

167. Choi, K.-Y., Synthesis, crystal structure and formation kinetics of zinc (II) and copper (II) complexes with 5,12-dimethyl-1,4,8,11-tetraazacyclotetradecane. *Polyhedron* **1997,** 16, (12), 2073-2079.

168. Sun, X.; Wuest, M.; Kovacs, Z.; Sherry, A. D.; Motekaitis, R.; Wang, Z.; Martell Arthur, E.; Welch Michael, J.; Anderson Carolyn, J., In vivo behavior of copper-64-labeled methanephosphonate tetraaza macrocyclic ligands. *J. Biol. Inorg. Chem.* **2003,** 8, (1-2), 217-25.

169. Li, W. P.; Lewis, J. S.; Kim, J.; Bugaj, J. E.; Johnson, M. A.; Erion, J. L.; Anderson, C. J., DOTA-D-Tyr1-Octreotate: A somatostatin analogue for labeling with metal and halogen radionuclides for cancer imaging and therapy. *Bioconjugate Chem.* **2002,** 13, (4), 721-728.

170. De Jong, M.; Breeman, W. A. P.; Bakker, W. H.; Kooij, P. P. M.; Bernard, B. F.; Hofland, L. J.; Visser, T. J.; Srinivasan, A.; Schmidt, M. A.; Erion, J. L.; Bugaj, J. E.; Macke, H. R.; Krenning, E. P., Comparison of ^{111}In-labeled somatostatin analogs for tumor scintigraphy and radionuclide therapy. *Cancer Res.* **1998,** 58, (3), 437-441.

171. De Jong, M.; Bakker, W. H.; Krenning, E. P.; Breeman, W. A. P.; Van Der Pluijm, M. E.; Bernard, B. F.; Visser, T. J.; Jermann, E.; Behe, M.; et al., Yttrium-90 and indium-111 labeling, receptor binding and biodistribution of [DOTA,D-Phe1,Tyr3]octreotide, a promising somatostatin analog for radionuclide therapy. *Eur. J. Nucl. Med.* **1997,** 24, (4), 368-371.

172. Silversides, J. D.; Allan, C. C.; Archibald, S. J., Copper(II) cyclam-based complexes for radiopharmaceutical applications: synthesis and structural analysis. *Dalton Trans.* **2007**, (9), 971-978.

173. Denat, F.; Brandes, S.; Guilard, R., Strategies for the regioselective N-functionalization of tetraazacycloalkanes. From cyclam and cyclen towards more sophisticated molecules. *Synlett* **2000**, (5), 561-574.

174. Suchy, M.; Hudson, R. H. E., Synthetic strategies toward N-functionalized cyclens. *Eur. J. Org. Chem.* **2008,** In Press.

175. Bender, J. A.; Meanwell, N. A.; Wang, T., The mono-functionalization of symmetrical polyamines. *Tetrahedron* **2002,** 58, (16), 3111-3128.

176. Lee, S. H.; Cheong, C. S., Selective reactions of reactive amino groups in polyamino compounds by metal-chelated or -mediated methods. *Tetrahedron* **2001,** 57, (23), 4801-4815.

177. Wang, T.; Zhang, Z.; Meanwell, N. A., Benzoylation of Dianions: Preparation of Monobenzoylated Derivatives of Symmetrical Secondary Diamines. *J. Org. Chem.* **1999,** 64, (20), 7661-7662.

178. Weisman, G. R.; Vachon, D. J.; Johnson, V. B.; Gronbeck, D. A., Selective N-protection of medium-ring triamines. *J. Chem. Soc., Chem. Commun.* **1987**, (12), 886-7.

179. Helps, I. M.; Parker, D.; Morphy, J. R.; Chapman, J., General routes for the synthesis of mono, di and tri-N-substituted derivatives of cyclam. *Tetrahedron* **1989,** 45, (1), 219-26.

180. Lotz, T. J.; Kaden, T. A., pH-induced coordination geometry change in a macrocyclic nickel(II) complex. *J. Chem. Soc., Chem. Commun.* **1977**, (1), 15-16.

181. Studer, M.; Kaden, T. A., Metal complexes with macrocyclic ligands. Part XXV. One-step synthesis of mono-N-substituted azamacrocycles with a carboxylic group in the side-chain and their complexes with Cu^{2+} and Ni^{2+}. *Helv. Chim. Acta* **1986,** 69, (8), 2081-6.

182. Bernard, H.; Yaouanc, J. J.; Clement, J. C.; Des Abbayes, H.; Handel, H., General route for the synthesis of mono N-alkylated derivatives of tetraazamacrocycles. *Tetrahedron Lett.* **1991,** 32, (5), 639-42.

183. Patinec, V.; Yaouanc, J. J.; Clement, J. C.; Handel, H.; de Abbayes, H., Mono-N-alkylation and N-acylation of cyclen and cyclam via their metal tricarbonyl complexes (metal = Cr, Mo). *Tetrahedron Lett.* **1995,** 36, (1), 79-82.

184. Yang, W.; Giandomenico, C. M.; Sartori, M.; Moore, D. A., Facile N-1 protection of cyclam, cyclen and 1,4,7-triazacyclononane. *Tetrahedron Lett.* **2003,** 44, (12), 2481-2483.

185. Hill, D. C. PhD Dissertation. University of New Hampshire, Durham NH, 1995.

186. Bist, S. Masters Thesis. University of New Hampshire, Durham, NH, 2002.

187. Jeffrey, G. A., *An Introduction to Hydrogen Bonding*. Oxford University Press: New York, 1997.

188. Pople, J. A., Schneider, W.G., Bernstein, H.J., *High Resolution Nuclear Magnetic Resonance*. McGraw-Hill Book Company, Inc.: New York, 1959.

189. Radzicka, A.; Pedersen, L.; Wolfenden, R., Influences of solvent water on protein folding: free energies of solvation of cis and trans peptides are nearly identical. *Biochemistry* **1988,** 27, (12), 4538-41.

190. Adams, B.; Lerner, L., Observation of hydroxyl protons of sucrose in aqueous solution: no evidence for persistent intramolecular hydrogen bonds. *J. Am. Chem. Soc.* **1992,** 114, (12), 4827-9.

191. Salauen, A.; Favre, A.; Le Grel, B.; Potel, M.; Le Grel, P., Conformation of Nα-substituted hydrazino acetamides in $CDCl_3$, the precious help of the analysis of Δδ between amidic hydrogens, and correlation to the conformation of Aza-β-3-peptides. *J. Org. Chem.* **2006,** 71, (1), 150-158.

192. Del Bene, J. E., Molecular orbital theory of the hydrogen bond. 18. Methyl substituent effects on amide hydrogen bonding. *J. Am. Chem. Soc.* **1978,** 100, (5), 1387-94.

193. Stewart, W. E.; Siddall, T. H., III, Nuclear magnetic resonance studies of amides. *Chem. Rev.* **1970,** 70, (5), 517-51.

194. Perrin, C. L., Proton exchange in amides: Surprises from simple systems. *Acc. Chem. Res.* **1989,** 22, (8), 268-75.

195. Ferris, T. D.; Lee, P. T.; Farrar, T. C., Synthesis of propiolamide and ^{1}H, ^{13}C and ^{15}N NMR spectra of formamide, acetamide and propiolamide. *Magn. Reson. Chem.* **1997,** 35, (9), 571-576.

196. Saito, H.; Tanaka, Y.; Nukada, K., Hydrogen bond studies by nitrogen-14 nuclear magnetic resonance. III. Electron redistribution associated with hydrogen bonding and metal-complex formation of formamide and N-methylacetamide. *J. Am. Chem. Soc.* **1971,** 93, (5), 1077-81.

197. Anslyn, E. V.; Dougherty, D. A., *Modern Physical Organic Chemistry*. Edwards Brothers, Inc.: 1960.

198. Krishna, M. M. G.; Hoang, L.; Lin, Y.; Englander, S. W., Hydrogen exchange methods to study protein folding. *Methods* **2004,** 34, (1), 51-64.

199. Englander, S. W.; Sosnick, T. R.; Englander, J. J.; Leland, M., Mechanisms and uses of hydrogen exchange. *Curr. Opin. Struct. Biol.* **1996,** 6, (1), 18-23.

200. Englander, S. W.; Downer, N. W.; Teitelbaum, H., Hydrogen exchange. *Annu. Rev. Biochem.* **1972,** 41, 903-24.

201. Englander, S. W., Protein folding intermediates and pathways studied by hydrogen exchange. *Annu. Rev. Biophys. Biomol. Struct.* **2000,** 29, 213-238, 3 Plates.

202. Linton, B., H/D Exchange rates to quantify hydrogen bonding *Unpublished work.* In 2007, personal communication.

203. Dorman, D. E.; Bovey, F. A., Proton coupled carbon-13 magnetic resonance spectra. Simple amides. *J. Org. Chem.* **1973,** 38, (9), 1719-22.

204. Weisman, G. R.; Wong E., H.; Anderson C., J., Personal Communication. In 2006.

205. Liu, S.; Edwards, D. S., Bifunctional chelators for therapeutic lanthanide radiopharmaceuticals. *Bioconjugate Chem.* **2001,** 12, (1), 7-34.

206. Yoo, J.; Reichert, D. E.; Welch, M. J., Comparative in vivo behavior studies of cyclen-based copper-64 complexes: Regioselective synthesis, X-ray structure, radiochemistry, log P, and biodistribution. *J. Med. Chem.* **2004,** 47, (26), 6625-6637.

207. Blower, P., Towards molecular imaging and treatment of disease with radionuclides: the role of inorganic chemistry. *Dalton Trans.* **2006**, (14), 1705-1711.

208. Wadas, T. J.; Wong, E. H.; Weisman, G. R.; Anderson, C. J., Copper chelation chemistry and its role in copper radiopharmaceuticals. *Current Pharmaceutical Design* **2007,** 13, (1), 3-16.

209. Quadri, S. M.; Vriesendorp, H. M., Effects of linker chemistry on the pharmacokinetics of radioimmunoconjugates. *Q. J. Nucl. Med.* **1998,** 42, (4), 250-61.

210. Williams, L. E.; Lewis, M. R.; Bebb, G. G.; Clarke, K. G.; Odom-Maryon, T. L.; Shively, J. E.; Raubitschek, A. A., Biodistribution of ^{111}In- and ^{90}Y-Labeled DOTA and maleimidocysteineamido-DOTA conjugated to chimeric anticarcinoembryonic antigen antibody in xenograft-bearing nude mice: Comparison of stable and chemically labile linker systems. *Bioconjugate Chem.* **1998,** 9, (1), 87-93.

211. Anderson, C. J.; Welch, M. J., Radiometal-Labeled Agents (Non-Technetium) for Diagnostic Imaging. *Chem. Rev.* **1999,** 99, (9), 2219-2234.

212. Levin, C. S., Primer on molecular imaging technology. *Eur. J. Nucl. Med. Mol. Imaging* **2005,** 32 325-345.

213. McQuade, P.; Rowland, D. J.; Lewis, J. S.; Welch, M. J., Positron-emitting isotopes produced on biomedical cyclotrons. *Curr. Med. Chem.* **2005,** 12, (7), 807-818.

214. Rogers, B. E.; Anderson, C. J.; Connett, J. M.; Guo, L. W.; Edwards, W. B.; Sherman, E. L. C.; Zinn, K. R.; Welch, M. J., Comparison of four bifunctional

chelates for radiolabeling monoclonal antibodies with copper radioisotopes: Biodistribution and metabolism. *Bioconjugate Chem.* **1996,** 7, (4), 511-522.

215. Akizawa, H.; Arano, Y.; Mifune, M.; Iwado, A.; Saito, Y.; Mukai, T.; Uehara, T.; Ono, M.; Fujioka, Y.; Ogawa, K.; Kiso, Y.; Saji, H., Effect of molecular charges on renal uptake of ^{111}In-DTPA-conjugated peptides. *Nucl. Med. Biol.* **2001,** 28, (7), 761-768.

216. Sprague, J. E.; Peng, Y.; Sun, X.; Weisman, G. R.; Wong, E. H.; Achilefu, S.; Anderson, C. J., Preparation and biological evaluation of copper-64-labeled tyr3-octreotate using a cross-bridged macrocyclic chelator. *Clin. Cancer Res.* **2004,** 10, (24), 8674-82.

217. Young, M.; Weisman, G. R.; Wong, E. H., Unpublished results. In 2008.

218. Plutnar, J.; Havlickova, J.; Kotek, J.; Hermann, P.; Lukes, I., Unsymmetrically substituted side-bridged cyclam derivatives and their Cu(II) and Zn(II) complexes. *New J. Chem.* **2008,** 32, (3), 496-504.

219. Gigg, R.; Conant, R., Allyl group for protection in carbohydrate chemistry. Part 12. N-Allyl and N-benzyl derivatives of 2-amino-2-deoxy-D-glucose. *Carbohydr. Res.* **1982,** 100, C5-C9.

220. Gigg, R.; Conant, R., The allyl group for protection in carbohydrate chemistry. Part 13. The N-allylbenzylamino group for protection in the amino-sugar series. *J. Carbohydr. Chem.* **1983,** 1, (3), 331-6.

221. Hine, J.; Linden, S.-M.; Wang, A.; Thiagarajan, V., Structural effects on rates and equilibriums. 21. Double-bond-stabilizing abilities of dimethylamino, alkylsulfonyl, and acetyl substituents. *J. Org. Chem.* **1980,** 45, (14), 2821-5.

222. Sauer, J.; Prahl, H., Base catalyzed double bond isomerizations. II. Synthesis of simple cis-enamines. *Chem. Ber.* **1969,** 102, (6), 1917-27.

223. Escoubet, S.; Gastaldi, S.; Bertrand, M., Methods for the cleavage of allylic and propargylic C-N bonds in amines and amides - selected alternative applications of the 1,3-hydrogen shift. *Eur. J. Org. Chem.* **2005**, (18), 3855-3873.

224. Otera, J., *Esterification: Methods, Reactions and Applications*. Wiley-VCH: Weinheim, 2003.

225. Buckley, A.; Chapman, N. B.; Dack, M. R. J.; Shorter, J.; Wall, H. M., The separation of polar and steric effects. VIII. The influence of the solvent on the kinetics of the reactions of benzoic acid and meta- or para-substituted benzoic acids with diazodiphenylmethane. *J. Chem. Soc. B* **1968**, (6), 631-8.

226. Mamai, A.; Zhang, R.; Natarajan, A.; Madalengoitia, J. S., Poly-L-proline Type II Peptide Mimics Based on the 3-Azabicyclo[3.1.0]hexane System. *J. Org. Chem.* **2001,** 66, (2), 455-460.

227. Zhang, D.; Yuan, C., An enantioselective nucleophilic addition of α,β-unsaturated trifluoromethyl ketones catalyzed by L-proline derivatives. *Tetrahedron* **2008,** 64, (10), 2480-2488.

228. Huang, J.; Zhang, X.; Armstrong, D. W., Highly efficient asymmetric direct stoichiometric aldol reactions on/in water. *Angew. Chem. Int. Ed.* **2007,** 46, (47), 9073-9077.

229. Owens, N. W.; Braun, C.; Schweizer, F., Tuning of the Prolyl trans/cis-Amide Rotamer Population by Use of C-Glucosylproline Hybrids. *J. Org. Chem.* **2007,** 72, (13), 4635-4643.

230. Norris, T.; Dowdeswell, C.; Johnson, N.; Daia, D., A new efficient stereoselective debromination reaction with trialkylgermanium hydrides useful in the design of short synthetic routes to β-lactamase inhibitor prodrugs. *Org. Process Res. Dev.* **2005,** 9, (6), 792-799.

231. Fillon, Y. A.; Anderson, J. P.; Chmielewski, J., Cell penetrating agents based on a polyproline helix scaffold. *J. Am. Chem. Soc.* **2005,** 127, (33), 11798-11803.

232. Stapon, A.; Li, R.; Townsend, C. A., Carbapenem biosynthesis: Confirmation of stereochemical assignments and the role of CarC in the ring stereoinversion process from L-proline. *J. Am. Chem. Soc.* **2003,** 125, (28), 8486-8493.

233. Martin, D. Masters Thesis. University of New Hampshire, Durham, 2008.

234. Hill, D. Doctoral Thesis. University of New Hampshire, Durham, 1995.

235. Criado, M. R.; Pereiro, I. R.; Torrijos, R. C., Determination of polychlorinated biphenyls in ash using dimethylsulfoxide microwave assisted extraction followed by solid-phase microextraction. *Talanta* **2004,** 63, 533-540.

236. Subramanyam, C., 4-Methoxybenzyl, a versatile protecting group for the regiospecific lithiation and functionalization of pyrazoles. *Synth. Commun.* **1995,** 25, (5), 761-74.

237. Buckle, D. R.; Rockell, C. J. M., Studies on v-triazoles. Part 4. The 4-methoxybenzyl group, a versatile N-protecting group for the synthesis of N-unsubstituted v-triazoles. *J. Chem. Soc. Perkin Trans.* **1982,** (2), 627-30.

238. Kamijo, T.; Yamamoto, R.; Harada, H.; Iizuka, K., An improved and convenient procedure for the synthesis of 1-substituted imidazoles. *Chem. Pharm. Bull.* **1983,** 31, (4), 1213-21.

239. Boger, D. L.; Fink, B. E.; Hedrick, M. P., Total synthesis of distamycin A and 2640 analogs: A solution-phase combinatorial approach to the discovery of new, bioactive DNA binding agents and development of a rapid, high-throughput screen for determining relative DNA binding affinity or DNA binding sequence selectivity. *J. Am. Chem. Soc.* **2000,** 122, (27), 6382-6394.

240. Brotzel, F.; Chu, Y. C.; Mayr, H., Nucleophilicities of primary and secondary amines in water. *J. Org. Chem.* **2007,** 72, (10), 3679-3688.