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Synthetic approaches to cis,cis-1,3,5-triaminocyclohexane (tach) and tach-based ligands

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SYNTHETIC APPROACHES TO *CIS, CIS*-1,3,5-TRIAMINOCYCLOHEXANE (TACH) AND TACH-BASED LIGANDS

BY

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THESIS

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DEDICATION

To my parents, Stephen and Beverly, and to my sisters, Betsy and Paige, who, while they may not have understood exactly what I was researching, never ceased to be interested in it, never ceased to ask questions about it. Without their unremitting curiosity and support throughout, this would not have been.

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CIS, CIS-1,3,5-TRIAMINOCYCLOHEXANE (TACH)
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Cis, cis-1,3,5-triaminocyclohexane (tach) has been synthesized according to various routes, and the methods were evaluated on the basis of product yield, number of steps, and expense of reagents. The conversion of *cis, cis*-1,3,5-cyclohexanetricarboxylic acid to the desired product by the Curtius rearrangement, as previously described in the literature, was found to be the most suitable procedure based on yield and number of steps. From the standpoint of cost, the reagents of the Curtius rearrangement were derived from inexpensive, commercially available starting materials. The yields of the synthesis are variable, and preliminary studies of yield optimization are described.

Three ligands based on the tach framework, tachenPh (*N,N',N"*-tris(2-amino-2-phenylethyl)-*cis, cis*-1,3,5-triaminocyclohexane), tacheniPr (*N,N',N"*-tris(2-amino-2-isopropylethyl)-*cis, cis*-1,3,5-triaminocyclohexane), and tachenNMe (*N,N',N"*-tris[2-(methylamino)ethyl]-*cis, cis*-1,3,5-triaminocyclohexane) were complexed with divalent nickel and copper in solution. The relative ligand field strength of the chelators was assigned for each metal.

For most of the cases, an octahedral geometry of the metal-ligand complexes can be inferred based on UV-Vis data, though there is some suggestion of a five-coordinate species for the copper complex of tachenPh.

The synthesis of another tach-based ligand, tachENSH (*N,N',N"*-tris(2-mercaptoethyl)-*cis, cis*-1,3,5-triaminocylcohexane), containing an N3S3 donor set was evaluated. The thiol arms were derived from a prepared active ester and were combined by peptide linkage with three amine frameworks. The frameworks utilized are triaminocyclohexane (tach), ethylenediamine (en) and *trans*-1,2-diaminocyclohexane (dach). Borane in THF appeared to reduce the prepared amides, however isolation of a borane-free product has not yet proved feasible. Initial *in silico* evaluation of the proposed novel dachENSH ligand shows it to be a promising divalent copper or zinc chelator.

CHAPTER 1

SYNTHETIC APPROACHES TO THE PREPARATION OF *CIS, CIS*-1,3,5-TRIAMINOCYCLOHEXANE

INTRODUCTION

Effective chemotherapy utilizes substances that target the weaknesses of cancerous cells. One category of chemotherapeutics takes advantage of the nature of most tumors to grow more rapidly than normal tissue: an increased metabolic rate means that tumor cells require a constant influx of nutrients in amounts that surpass those of the wild-type cells, and are consequently more sensitive to deprivation.[1] Reducing the amount of nutrients or other essential materials may inhibit tumor growth or promote cell death and tumor regression. Some recent chemotherapeutic agents have focused on inhibition of nucleotide synthesis as a means of halting cell growth,[1] while others currently under examination sequester essential divalent metal ions to similar effect.

Iron is an essential cofactor in many vital cellular processes including respiration and DNA synthesis. Rapidly proliferating cancer cells require large quantities of deoxyribonucleotides for DNA replication. This process is mediated by the enzyme ribonucleotide reductase. Divalent iron is an essential cofactor for the catalytic activity of the enzyme and therefore must be taken up at greater rate by cancer cells than by normal tissue. The intake of iron is mediated by the

cellular surface receptor transferrin, and, in order to meet the high demands of a high rate of cell growth and division, tumor cells have an increased number of transferrin receptors on their cell surface. This renders cancer cells more sensitive to small fluctuations in bio-available iron than normal cells.[2]

Several iron chelators have been considered as potential chemotherapeutic agents. Among the candidates are desferrioxamine (DFO) and ligands of the pyroxidal isonicotinyl hydrazone family.[3] In addition, the novel ligand *N,N',N"*-tris(2-pyridylmethyl)-*cis, cis*-1,3,5- triaminocyclohexane (tachpyr) and analogues based on the *cis, cis*-1,3,5- triaminocyclohexane (tach) framework have been extensively studied in conjunction with our collaborators as iron(II) chelators capable of selective tumor toxicity.[4,5,6,7,8,9,10]

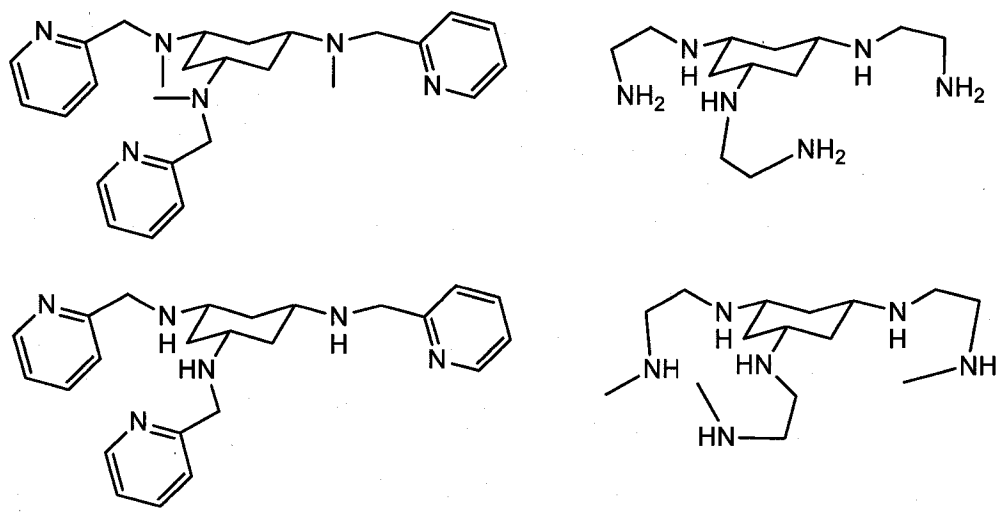


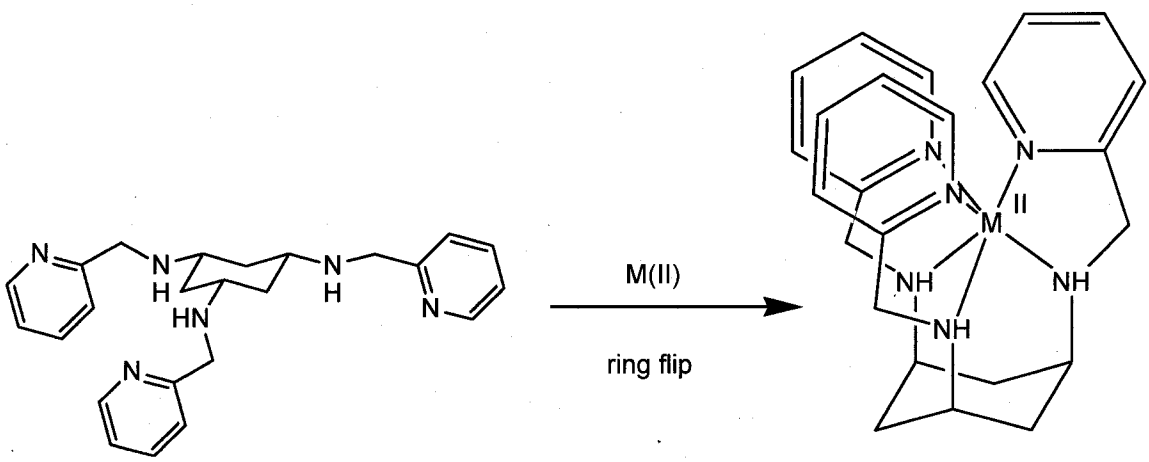
Figure 1.1. *N,N',N"*-tris(2-pyridylmethyl)-*cis, cis*-1,3,5- triaminocyclohexane (tachpyr) and analogues.

In vitro addition of tachpyr to MBT2 and T24 bladder cancer cells induced apoptosis at very low effective concentrations with IC_{50} 2.2 μM and 4.3 μM

respectively. As expected, the chelator required a much greater concentration to cause similar cytotoxicity in normal cell lines (IC_{50} 30.5 μM), and was overall much more potent than DFO (IC_{50} 70 μM with MBT2).[4] Tachpyr has been reported to have similar cytotoxic effects in human cervical and breast cancer cells (HeLa, and SUM149).[9] Data indicate that the ability of tachpyr to sequester iron is the basis of its toxicity, as with DFO; the structure of tachpyr, based on its donor atoms, sterics, and lipophilicity, is an inherent part of its efficacy as an anti-tumor agent.

Introduction of a divalent metal ion induces a conformation change in free tachpyr, providing a preorganized octahedral coordination environment favored by iron(II). The flexibility of the pyridyl arms allows coordination to various metal ions with a radius of 0.76 Å to 0.94 Å; however, iron(II)-, copper(II)-, and zinc(II)-bound tachpyr have been shown to be non-toxic in vitro, indicating that the stability of the complex precludes release of the free ligand.[4] Other divalent metals afford more labile tachpyr complexes and are therefore cytotoxic, as the activity of tachpyr is not masked by the metal. The pyridine rings increase the lipophilicity of the molecule, allowing greater cellular penetration at lower concentrations than more polar ligands.[11] The aminopyridyl donor set of tachpyr is borderline basic and so strongly favors binding to borderline acids including iron(II), zinc(II), nickel(II), and copper(II) over iron(III) or copper(I), providing a degree of metal selectivity. The *N*-alkylated analogues of tachpyr further support iron chelation as the basis of cytotoxicity. The steric hindrance inherent in *N*-

methyl or *N*-ethyl tachpyr weakens the metal bonding, and is therefore labile in solution and non-toxic as it is unable to sequester divalent iron.[7]



Scheme 1.1. Coordination of a divalent metal to free tachpyr.

The promising cytotoxicity and selectivity of tachpyr makes it an excellent candidate for further research. The synthesis of the tach framework, however, is a complex and lengthy process, and in order to continue work on tachpyr and its analogues it was necessary to explore new synthetic pathways. While there are many methodologies present in the literature for preparing tach, none of them were acceptable due to the number of steps, low yields, expense of reagents or some combination of factors. This chapter describes the experimental evaluation of both known and potential novel routes in a search for an efficient and inexpensive high-yield tach synthesis.

EXPERIMENTAL

I. Materials and Methods

All chemicals listed below were of research grade or of spectro-quality grade and were generally obtained from commercial sources and used without further purification except for those described as follows. Acetone was freshly distilled from potassium carbonate under nitrogen. Benzyl alcohol was vacuum distilled prior to use (70.5-72.0°C at 5 mmHg) and then stored under nitrogen in a desiccator. Diethyl ether was refluxed over sodium metal and distilled immediately before use. 1,4-Dioxane was freshly distilled from sodium metal under nitrogen and stored over molecular sieves. Diphenylphosphoryl chloride was vacuum distilled prior to use (168°-172°C at 5 mmHg). ^{31}P NMR (400MHz, $CDCl_3$): δ -4.75. Triethylamine was distilled prior to use and then stored under nitrogen in a desiccator.

The following catalysts were acquired from commercial sources and used without modification except for the Raney nickel. 10% Palladium on carbon and platinum(IV) oxide (Adam's catalyst) were purchased from Aldrich. Raney type nickel aluminum (50% w/w Al:Ni, #87676) was obtained from Alfa Aesar. 5% Rhodium on activated carbon (catalyst formulation G1, Degussa) was purchased from Strem Chemicals.

Sodium metal was stored under mineral oil and washed with hexanes before use. Platinum metal was cut from a disk and then mechanically pressed to yield a thin foil, which was then finely shredded before use.

Chloroform-d_1 ($CDCl_3$), deuterated water (D_2O), and dimethylsulfoxide-d_6 (DMSO-d_6) were obtained from Cambridge Isotope Laboratories and stored in a desiccator.

Water was deionized by reverse-osmosis and by anion and cation exchange (E-pure model D4641, Barnstead).

Melting points (mp) were recorded on a Thomas Hoover capillary melting point apparatus and are uncorrected. ^1H, $^{13}C\{^1H\}$, and $^{31}P\{^1H\}$ NMR spectra were obtained on a Varian Mercury 400MHz FT spectrometer. Proton and C-13 chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS) and ^{31}P chemical shifts are relative to an external standard of 85% H_3PO_4. Proton chemical shifts are described as follows: ppm (multiplicity, integral, and assignment). Electrospray ionization (ESI) mass spectra in the positive ion detection mode were obtained with a Finnegan LCQ Classic instrument with dual optical Paul traps and Picoview nanospray source. Hydrogenations were run in three different systems: a 200 mL Fischer-Porter bottle rated at 100 psi with pressure adaptor threaded head, a Parr shaker-type hydrogenator with a 250 mL borosilicate glass bottle rated at 50 psi, and a PPI 300 mL LC reactor rated to 2000 psi at 340°C equipped with a Dyna-Mag mixer.

II. *Cis, cis*-1,3,5-Triaminocyclohexane Synthesis

A. *Cis, cis*-1,3,5-triaminocyclohexane trihydrochloride from *cis, cis*-1,3,5-cyclohexanetriol.

T-4 Raney nickel (1). Compound **1** was prepared by the method of Nishimura and Urushibara.[12] In an Erlenmeyer flask with stir bar, Raney nickel alloy (50:50

w/w Al:Ni, 5.3 g) was suspended in 25 mL of water under nitrogen. 3 mL of a 40% w/v solution of NaOH was added carefully and let stir until the vigorous gas evolution subsided over several minutes. After 45 min, an additional 15 mL of the alkaline solution was added to the suspension and the alloy was allowed to digest over 2 hr. The solution was decanted off, and the precipitate was washed with 20x50 mL water until pH neutral, and finally washed 2x50 mL 95% EtOH, and 2x50 mL anhydrous EtOH to yield a fine grey suspension, which was stored under anhydrous EtOH. The expected catalyst yield is approximately half of the original mass, and was always used immediately after preparation.

***Cis, cis*-1,3,5-cyclohexanetriol (2).** Compound **2** was prepared by the method of Steinacker and Stetter,[13] and also described by West.[14] Phloroglucinol dihydrate (29.1 g, 111 mmol) was dissolved in 150 mL anhydrous ethanol, to which 2.5 g freshly prepared Raney Nickel catalyst was added with 50 mL ethanol. The mixture was shaken in a Parr flask under 40 psi of hydrogen gas for 3 d. The catalyst was filtered and the solvent was removed by rotary evaporation to yield a pale straw-colored gum, which was dissolved in hot anhydrous ethanol and stored at 4°C overnight. *Cis, cis*-1,3,5-cyclohexanetriol precipitated out of solution as colorless crystals. The crude product was recrystallized from ethanol as 3.96 g of colorless prisms (30.0 mmol, 27%): mp 181-183°C (lit. 184°C).[13] ^{1}H NMR (400MHz, d_6-DMSO): δ 0.95 (dd, 1H, cyclohexyl axial methylene), 1.95 (dt, 1H, cyclohexyl equatorial methylene), 3.33 (m,1H, cyclohexyl axial methine), 4.52 (d, 1H, O<u>H</u>); ^{13}C NMR (400 MHz, d_6-DMSO): δ 45.51, 65.14.

***Cis, cis*-1,3,5-tris(benzylsulfonate)cyclohexane (3).** Compound **3** was prepared by the method of Bollinger[15] and compared to the data reported by Fleischer.[16] *Cis, cis*-1,3,5-cyclohexanetriol (0.601 g, 4.5 mmol) was stirred in 9 mL pyridine at 10°C until dissolved. Excess benzenesulfonyl chloride (5.6 g, 31.8 mmol) was added dropwise over the course of 2.5 hr while maintaining a constant temperature. A wash of 30/50/20 water/ethanol/conc. HCl was added to the thick slurry and stirred for 30 min. The white precipitate was collected on a glass frit, washed with cold anhydrous ethanol, and then dried under vacuum to yield 1.73 g (3.13 mmol, 68.8%) of the crude product. The off-white powder was recrystallized from dichloromethane via vapor-phase ether diffusion as fine white needles in 52% yield. ^{1}H NMR (400MHz, $CDCl_3$): δ 1.59 (dd, 1H, axial cyclohexyl methylene), 2.22 (dt, 1H, equatorial cyclohexyl methylene), 4.30 (m, 1H, axial cyclohexyl methine), 7.57 (m, 2H, aromatic C<u>H</u>), 7.69 (m, 1H, aromatic C<u>H</u>), 7.82 (m, 2H, aromatic C<u>H</u>). ^{13}C NMR (400 MHz, $CDCl_3$): δ 37.73, 73.00, 127.79, 129.68, 134.42, 136.71.

***Cis, cis*-1,3,5-triaminocyclohexane trihydrochloride (4).** Compound **4** was prepared by the method of Golobov and of Martin.[17, 18] Sodium azide (0.78 g, 12 mmol) was added to a solution of *cis, cis*-1,3,5-tris(benzylsulfonate)cyclohexane (0.54 g, 0.98 mmol) in 6 mL of DMF and the mixture was stirred under nitrogen at 100°C for 16 hr. The solution was allowed to cool to room temperature before adding 50 mL H_2O. The aqueous layer was extracted with 3x10 mL diethyl ether and the collected organic fractions were dried over $MgSO_4$ and concentrated to 5 mL under a stream of nitrogen. 10 mL dioxane was added and the solution was

reduced to 5 mL using a portable vacuum pump. To the solution of the triazide an additional 8 mL of dioxane was added, followed by triphenylphosphine (1.31 g, 5 mmol) and 5mL of aqueous ammonia. The solution was stirred at room temperature under nitrogen for 20 hr and loss of the azide was monitored by IR. The solvent was removed by rotary evaporation and the crude amine was taken up in 10 mL chloroform. The solution was extracted with 4x5 mL 2N HCl and the aqueous layers were concentrated under vacuum after which 1 mL of conc. HCl was added and the solution kept at 4°C overnight. The resulting fine white powder was washed with cold conc. HCl, anhydrous EtOH, and ether before drying under vacuum to yield 52.4 mg of the product (0.22 mmol, 22%). ^{1}H NMR (400MHz-D_2O) δ 1.43 (dd, 3H, cyclohexyl axial methylene), 2.25 (dt, 3H, cyclohexyl equatorial methylene), 3.38 (m, 1H, cyclohexyl axial methine), 4.62 (br. s, 2H, $N\underline{H_2}$); ^{13}C NMR (d^6-DMSO) δ 33.9, 45.9.

B. *Cis, cis*-1,3,5-triaminocyclohexane trihydrochloride from 1,3,5-cyclohexanetrione trioxime via a dissolving metal reduction.

1,3,5-Cyclohexanetrione trioxime (5). Hydroxylamine hydrochloride (40.34 g, 0.58 mol) was dissolved in 25 mL water with slight warming and then neutralized with Na_2CO_3 (35.99 g, 0.29 mol) added in portions with stirring. After cooling to 4°C for 1 hr, the suspension was filtered and the salt discarded. Water was added to the hydroxylamine solution for a final volume of 75 mL, which was warmed to 50°C. Phloroglucinol dihydrate (13.38 g, 82.5 mmol) was added with stirring. The mixture was removed from heat and stirring once the product began precipitating out and was covered with foil and allowed to stand at room

temperature for 3 hr. The light tan precipitate was collected on a glass frit, washed with water, and dried under vacuum to yield 13.795 g of the product as a white powder (80.6 mmol, 98%). ^{1}H NMR (400MHz, d_6-DMSO): δ 3.07 (s, 1H, C$\underline{H}_2$), 3.27(s, 1H, C$\underline{H}_2$), 3.49(s, 1H, C$\underline{H}_2$), 10.74 (s, 1H, NO$\underline{H}$), 10.77 (s, 1H, NO$\underline{H}$), 10.84 (s, 1H, NO$\underline{H}$) ; ^{13}C NMR (400 MHz, d_6-DMSO): δ 25.42, 31.17, 36.13, 150.73, 150.83, 151.38.

Mixture of *cis, cis*- and *cis, trans*-1,3,5-triaminocyclohexane (6). The mixture of **6** was prepared by the method of Lions and Martin,[19] as described by Wentworth.[20] 1,3,5-Cyclohexanetrione trioxime (13.70 g, 80 mmol) was dissolved in 650 mL of liquid ammonia in a 2 L three-neck flask fitted with a mechanical stirrer and a dry ice/isopropyl alcohol condenser. Cold absolute ethanol (90 mL) was added in portions over ten minutes with stirring followed by thinly sliced sodium (20.7 g, 900 mmol), which was added carefully over 30 min. The reaction mixture was initially stirred over 1 hr, and then was allowed to stand overnight during which time the ammonia evaporated. Water (50 mL) was carefully added to the brown solids in portions, carefully swirling the flask to fully dissolve all particulates. Sodium hydroxide pellets were added to the brown aqueous solution, and the strongly alkaline mixture was filtered to remove any remaining precipitate. The solution was continuously extracted with diethyl ether for 4 d, and the organic extract was evaporated under vacuum to yield at least 350 mg of brown oil (2.7 mmol, 3.3%) which was a 1:2.1 diastereomeric mixture of *cis, cis*- and *cis, trans*-1,3,5-triaminocyclohexane. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.867 (dd, 3H, *cis, cis* cyclohexyl axial methylene), 0.962 (dd, 1H, *cis, trans*

cyclohexyl axial methylene between equatorial NH_2's), 1.28 (ddd, 2H, *cis, trans* cyclohexyl axial methylene vicinal to axial NH_2), 1.72 (dd, 2H, *cis, trans* cyclohexyl equatorial methylene vicinal to axial NH_2), 1.98 (overlapping m, 4H, *cis, cis* cyclohexyl equatorial methylene and *cis, trans* cyclohexyl axial methylene between equatorial NH_2's), 2.76 (m, 3H, *cis, cis* cyclohexyl axial methine), 3.15 (m, 2H, *cis, trans* cyclohexyl axial methine geminal to equatorial NH_2), 3.44 (m, 1H, *cis, trans* cyclohexyl axial methine geminal to equatorial NH_2).

[Ni(*cis, cis*-1,3,5-triaminocyclohexane)$_2$]X_2 (7). Compound **7** was prepared by altering the method of Wentworth[21] and Urbach.[22] 251 mg of the crude diastereomeric mixture of *cis, cis*- and *cis, trans*-1,3,5-triaminocyclohexane (1:2.1, estimated 0.6 mmol *cis, cis*-tach) was taken up in 5 mL anhydrous methanol to which was added a solution containing $Ni(X)_2 \bullet 6H_2O$ (where X=Cl, ClO_4 or NO_3, 0.3 mmol). The resultant precipitates were washed with methanol and then with diethyl ether and were stored under diethyl ether until used. The chloride anion gave 182 mg of carnation pink microcrystals (0.46 mmol). The perchlorate anion gave 239 mg of raspberry pink needles (0.46 mmol). The nitrate anion gave 126 mg of a salmon pink powder (0.29 mmol).

***Cis, cis*-1,3,5-triaminocyclohexane trihydrochloride (8).** [Ni(*cis, cis*-tach)$_2$]$(ClO_4)_2$ (317 mg, 0.61 mmol) was dissolved in 10 mL 12 M HCl with stirring to yield a white precipitate. The precipitate was washed 3x1 mL cold 12 M HCl and 3x5 mL diethyl ether and then dried under vacuum to give 286 mg of the product as a white powder (1.2 mmol, 98%). ^1H and ^{13}C match the previous spectra of *cis, cis*-1,3,5-triaminocyclohexane trihydrochloride.

C. *Cis, cis*-1,3,5-triaminocyclohexane trihydrobromide from *cis, cis*-1,3,5-cyclohexanetricarboxylic acid.

***Cis, cis*-1,3,5-cyclohexanetricarboxylic acid (9).** Compound **9** was prepared by the method of Steitz.[23] 10% Palladium on carbon (2.5 g, 5 mol% palladium based on substrate) was suspended in 50mL of water and trimesic acid (11.41 g, 47.8 mmol) was added. The mixture was purged with nitrogen and then hydrogenated at 1500 psi and 150°C for 4 hr in a 300 mL PPI LC reactor. The catalyst was collected by filtration and the homogeneous filtrates were dried by vacuum rotary evaporation to yield 9.91 g of an off-white powder as the crude diastereomeric mixture of *cis*- and *trans*-1,3,5-cyclohexanetricarboxylic acid in quantitative yield (45.8 mmol). The predominantly *cis*- product was isolated by recrystallization from ethanol/toluene (64%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.23 (dd, 1H, cyclohexyl axial methylene), 2.09 (d, 1H, cyclohexyl equatorial methylene), 2.35 (t, 1H, cyclohexyl axial methine), 12.22 (br. s, 1H, COO<u>H</u>); ^{13}C NMR (400 MHz, d_6-DMSO): δ 31.33, 41.46, 176.44.

Diphenylphosphoryl azide (10). Compound **10** was prepared by the method of Wolff and Waldvogel[24] and Shioiri and Yamada[25] with notes from Clifford.[26] A suspension of sodium azide (6.24 g, 96 mmol) in 250 mL acetone was stirred in an ice bath under nitrogen while diphenylphosphoryl chloride (16.58 mL, 80 mmol) was added dropwise over 15 min. The ice bath was maintained during the addition, and then the mixture was allowed to stir at room temperature for at least 4 hr, during which time sodium chloride formed. The salt was filtered off and washed with acetone. The filtrates were reduced under rotary evaporation with

no heat applied to yield a translucent oil which was further concentrated under vacuum to yield 19.45 g of product (70.7mmol, 88%). The product was shown pure by phosphorus NMR. ^{31}P NMR (400MHz, $CDCl_3$, 85%H_3PO_4 as an external standard): δ -9.78.

CBZ-protected *cis, cis*-1,3,5-cyclohexanetriamine (11). Compound **11** was prepared according to the method of Brechbiel.[27] To a fine suspension of *cis, cis*-1,3,5-cyclohexanetricarboxylic acid (3.82 g, 17.7 mmol) in 100mL of anhydrous *p*-dioxane was added triethylamine (7.45 mL, 53.5 mmol) while stirring vigorously under nitrogen. After the formation of a pale tan oil, diphenylphosphoryl azide (14.85 g, 54.0 mmol) was added and the mixture was stirred for ½ hr at room temperature, and then an additional hour at 85°C during which time the mixture became homogeneous with slow gas evolution. Benzyl alcohol (6.10 mL, 59.0 mmol) was then added and the solution stirred overnight at temperature. The solution was allowed to cool to room temperature and 250mL water was added to induce precipitation of the product, which fell out as a pale yellow solid. The precipitate was collected by filtration and dried under vacuum to leave 2.746 g of the product (5.16 mmol, 29.3%). ^1H NMR (400 MHz, d_6-DMSO): δ 1.12 (dd, 1H, cyclohexyl axial methylene), 1.90 (dd, 1H, cyclohexyl equatorial methylene), 3.40 (m, 1H, cyclohexyl axial methine), 5.01 (s, 2H, $OC\underline{H}_2$), 7.21-7.41 (m, 5H, $C_6\underline{H}_5$); ^{13}C NMR (400 MHz, d_6-DMSO): δ 38.89, 47.34, 65.85, 128.48, 128.85, 129.03, 137.86, 155.95.

***Cis, cis*-1,3,5-cyclohexanetriamine trihydrobromide (12).** Compound **12** was prepared according to the method of Brechbiel.[27] Under nitrogen, crude CBZ-

protected *cis, cis*-1,3,5-cyclohexanetriamine (2.75 g, 5.16 mmol) was treated with 33% HBr in acetic acid. Gas evolution was noted while stirring for 1 hr at room temperature. The fine precipitate was isolated with multiple washes of diethyl ether by decantation and dried under vacuum as a fine off-white hygroscopic powder of quantitative yield (1.92 g). ^{1}H NMR (400 MHz, D_2O): δ 1.55 (dd, 1H, axial cyclohexyl methylene), 2.36 (d, 1H, equatorial cyclohexyl methylene), 3.37 (m, 1H, axial cyclohexyl methine). ^{13}C NMR is identical to c*is, cis*-1,3,5-cyclohexanetriamine trihydrochloride.

III. Catalytic Heterogeneous Hydrogenation Reactions

1,3,5-Trisacetyltriaminobenzene (13). Compound **13** was prepared according to the method of Arai.[28] Under nitrogen, acetic anhydride (5.6 mL, 60 mmol) was added to 50 mL glacial acetic acid followed by 1,3,5-cyclohexanetrione trioxime (1.71 g, 10 mmol). Zinc dust (5g) was added to the suspension and the mixture was stirred at room temperature. Over 30 min, the trioxime dissolved completely; at which time a second portion of zinc dust (5 g) was added and stirred for an additional hour at 80°C. A final portion of zinc dust (5 g) was added, and the suspension was stirred for overnight at temperature, or until the reaction was complete by TLC (5:1, ethyl acetate:methanol). The solution was filtered hot and the solvent removed by vacuum rotary evaporation to yield a light tan powder. The powder was washed with anhydrous ethanol, and dried under vacuum to yield 2.46 g of the product nearly quantitatively (9.8 mmol, 98%). R_F values (5:1, ethyl acetate:methanol, on silica): 0.65 (starting material), 0.52 (intermediate), 0.47 (intermediate), 0.40 (product). Only the product spot is seen at the

completion of the reaction. ^{1}H NMR (400 MHz, d_6-DMSO): δ 2.02 (s, 3H, C<u>H</u>$_3$), 7.62 (s, 1H, aromatic C<u>H</u>), 9.92 (s, 1H, N<u>H</u>); ^{13}C NMR (400 MHz, d_6-DMSO): δ 29.41, 110.34, 144.97, 173.80.

Ammonium chloroplatinate ($(NH_4)_2[PtCl_6]$) (14). Compound **14** was prepared according to the method of Grube[29] as described by Adams.[30] Thin platinum foil (980 mg, 4.8 mmol) was dissolved in 200 mL aqua regia over 2 hr at 70°C, after which the solution was boiled down to a viscous orange syrup. 12 M HCl (25 mL) was added carefully, releasing brown NO_x gasses, and the solution was again reduced to an orange syrup; this was repeated five times until no further NO_x gas evolution was noted. A final addition of 12 M HCl gave a volume of 60mL and the orange solution was removed from heat. Three mass equivalents of ammonium chloride (3.0 g, 56 mmol) were added with stirring by a glass rod to yield a yellow precipitate and colorless solution. The solution was gently evaporated off over a steam bath to yield a yellow powder, which was further dried under vacuum. The powder was washed on filter paper with 5x10 mL cold sat. NH_4Cl, followed by 3x10 mL anhyd. EtOH, and then dried under vacuum to yield 2.2 g of the product as a bright yellow powder (quantitative).

Nishimura catalyst (7:3 w/w rhodium-platinum oxide) (15). Compound **15** was prepared according to the method of Nishimura.[31, 32] Ammonium chloroplatinate (1.0 g, 440 mg Pt) was ground into a fine homogeneous powder with rhodium trichloride (2.5 g, 1.0 g Rh) and an excess of sodium nitrate (35 g) in a crucible. The powder was heated over a Bunsen burner while stirring with a glass rod until the sodium nitrate began melting (306°) at which point brown NO_x

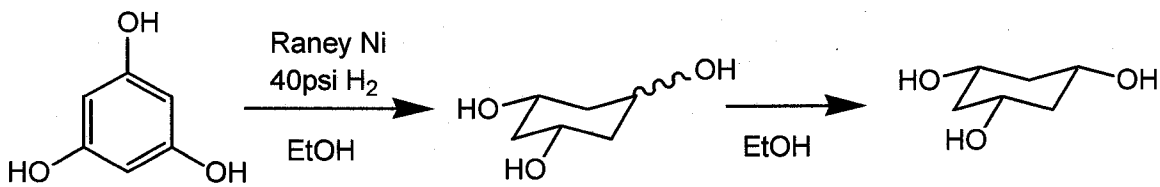
gasses began to evolve. The mixture was stirred continuously and after the gas evolution was complete the temperature was raised to 460°C and held for 20 min. After cooling to room temperature, 50 mL of water was added and the grey solid was broken up with stirring. The black precipitate was collected on filter paper and washed with 0.5% w/w solution of sodium nitrate until excess sodium nitrate had been removed. The black powder was transferred to a desiccator and stored until used.

1,3,5-Triaminobenzene (16). Compound **16** was prepared according to a combination of the methods of Sirpa and Klaus[33] and Arai.[28] To a suspension of T-4 Raney nickel in 75 mL of ethyl acetate in a 200 mL Parr flask was added 1,3,5-cyclohexanetrione trioxime (1.71 g, 10 mmol). Nitrogen gas was bubbled through the mixture and the flask was subsequently pressurized with hydrogen gas. The suspension was stirred under 80 psi of hydrogen gas with warming at 60°C overnight. The catalyst was removed by filtration through Celite® under inert atmosphere, and the Celite® washed with ethyl acetate. The combined filtrates were concentrated under vacuum and the product was collected in quantitative yield as a pale pink powder. ^{1}H NMR (400 MHz, d_6-DMSO): δ 4.31 (br. s, 2H, $N\underline{H}_2$), 5.14 (s, 1H, aromatic C$\underline{H}$); ^{13}C NMR (400 MHz, d_6-DMSO): δ 91.46, 150.02.

RESULTS AND DISCUSSION

I. Utilizing the Starting Material *cis, cis*-1,3,5-Cyclohexanetriol, Low Yields Would Require Scale-Up of a Hazardous Reaction

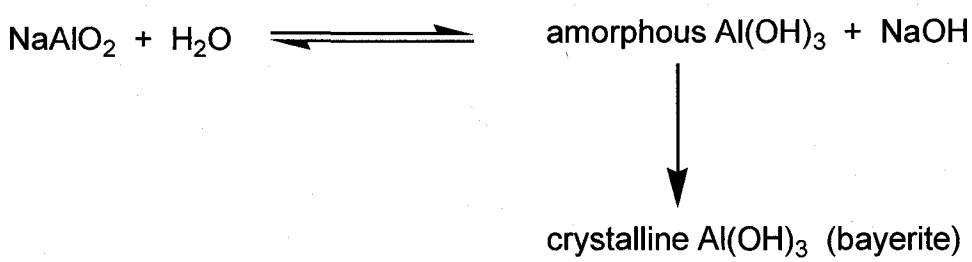
Cis, cis-1,3,5-cyclohexanetriol (**2**) was synthesized according to the literature methods except for an alteration in the preparation of the catalyst.[13] Not only was the starting material readily available but the alcohol was considered a reasonable functional group to convert to the amine. T-4 Raney



Scheme 1.2. Preparation of *cis, cis*-1,3,5-cyclohexanetriol (**2**).

nickel (**1**) was prepared instead of W-7 Raney nickel due to its greater activity and reduced washing complications.[34] Whereas the W-7 Raney nickel requires the alloy to be washed under hydrogen,[35, 36] the T-4 variation may be washed while open to the air. The increase in activity of **1** is related to the initial digestion conditions. It is proposed that a crystalline form of aluminum hydroxide (bayerite), insoluble in alkaline solutions, is produced during the initial addition of a small amount of sodium hydroxide. The Raney alloy initially reacts vigorously with the weakly alkaline solution, and as the bayerite precipitates out of solution, sodium hydroxide is regenerated. The bayerite is noticeable as a white precipitate that forms as a fine suspension while stirring. After the reaction has subsided, the

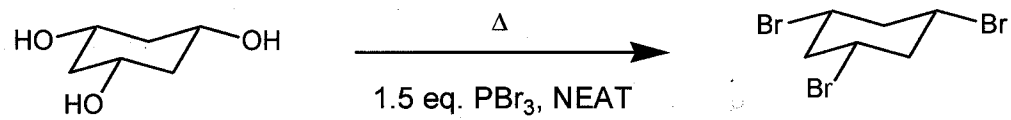
further addition of concentrated alkaline solution reacts only mildly with the alloy and serves to strip the bayerite from the catalyst to complete the development.[32] The characteristically quick-settling mossy precipitate that forms after the alkaline digestion may qualitatively identify the formation of the active catalyst; the fine grey suspension derived after washing will begin to produce hydrogen gas if allowed to stand under ethanol, another indication of the presence of the desired product. However, washing the catalyst to pH neutral appeared to be necessary for successful reductive activity, and as the catalyst could not be prepared in quantity ahead of time and stored, this was a time-consuming and labor-intensive process.



Scheme 1.3. The formation of bayerite in T-4 Raney nickel preparation.[32]

Low-pressure hydrogenation of phloroglucinol with **1** gave a mixture of isomers of 1,3,5-cyclohexane; the *cis, cis*-isomer was isolated as colorless prisms from ethanol recrystallization. The initial batch of crystals was clear of the *cis, trans*- isomer, however second and third batches of crystals were contaminated by the more soluble *cis, trans*- isomer and required a second recrystallization. Three batches of crystals were usually collected in order to maintain a reasonable yield for the reaction, as the ratio of the *cis, cis*- to the *cis, trans*- isomer is reportedly only 1.5:1.[13] The recovery of **2** was less than the

literature yield due to the inability, at that time, to warm the Parr flask while in the shaking hydrogenator; according the Stetter, the suspension should be warmed to 50°C during the 3 d catalytic reduction.

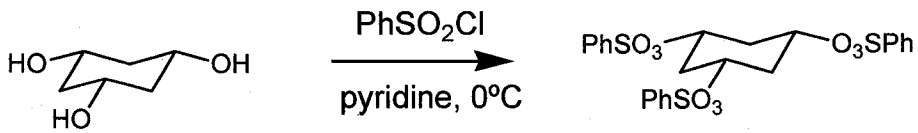


2 **17**

Scheme 1.4. Attempted synthesis of **17**.

The synthesis of the tribromide **17** with PBr_3 was unsuccessfully attempted by adaptation of the methodology of Golobov and Martin.[17, 18] It was hoped that the bromide of **17**, which is not known in the literature, would prove an excellent leaving group for easier conversion to the amine than the alcohol. An excess of the bromide or use of HBr in HOAc might have induced more success in the reaction as noted in other literature reactions.[18]

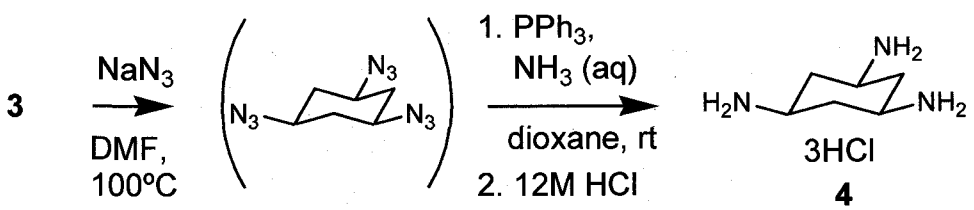
However, conversion to the tris(benzenesulfonate), an alternate leaving group, with benzenesulfonyl chloride gave **3** in good yield as white needles that could be stored long-term without decomposition. Unfortunately there are few literature reactions with **3** or analogous secondary benzenesulfonates, and the typical conversion of RSO_3Ph to $R\text{-}NH_2$ requires an azide intermediate.[17, 37, 38, 39]



2 **3**

Scheme 1.5. Synthesis of **3** using benzenesulfonyl chloride.

Concerns about the azide moiety are founded in the violent reactivity of many aromatic and non-aliphatic azides, but when handled with care they can be useful intermediates in a wide variety of reactions. One such set of reactions is stereospecific conversion. The reaction of a leaving group with an azide takes place by S_N2 displacement and yields a product with inverted absolute configuration: the retention of the *cis, cis* isomer is key in the reaction of **3**. Championed by Sharpless, who has isolated several substituted cyclohexyl polyazides on gram scale, the preparation of an azide is not necessarily an explosive proposition.[40] Nevertheless, all safety precautions were taken in preparing the triazide from **3**. The reaction was run behind a blast shield in a long-necked Schlenk flask to prevent any splashing of the mixture into the ground glass joints, and the triazide was never isolated. The reaction mixture was treated carefully with water and extracted with diethyl ether within the same flask. The diethyl ether was dried and reduced in volume before adding 1,4-dioxane, but the ether solution was never concentrated to dryness. A sample of the dioxane solution indicated the formation of the triazide by the characteristic sharp peak at 2090 cm^{-1} (on KBr), which was the only manipulation of the compound performed before the conversion to the amine. The final solution of the azide was no more than 0.08M in dioxane.



Scheme 1.6. Synthesis of **4** via Staudinger reaction.

In the final step of this preparation the triazide was reduced with triphenylphosphine, a Staudinger reaction in which a phosphazide is formed and subsequently loses N_2 to give the iminophosphorane and then the amine in the presence of water.[17] With an acidic workup, the *cis, cis*-1,3,5-triaminocyclohexane trihydrochloride salt (**4**) was isolated. The ^{1}H NMR spectrum of **4** shows three distinct proton peaks for the cyclohexyl ring, with similar splitting characteristics and shifts as for **2** and **3**. The axial methine is furthest downfield, due to deshielding from the geminal amine. The equatorial methylene is shifted downfield of the axial methylene due to the cone effect from

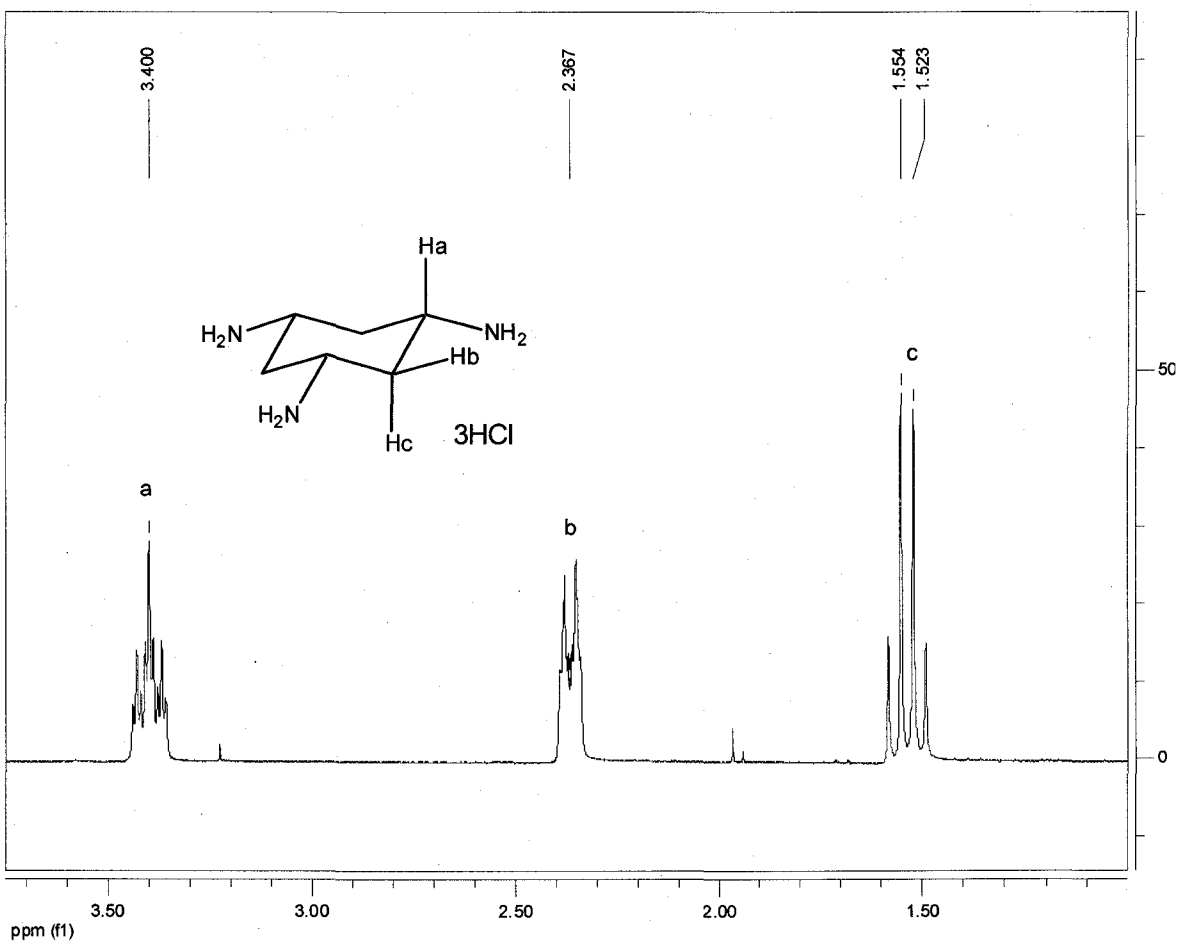


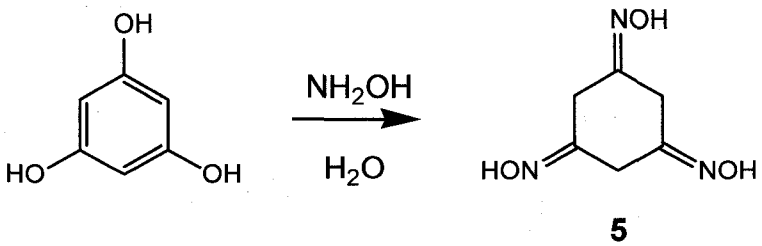
Figure 1.2. ^{1}H NMR of *cis, cis*-1,3,5-triaminocyclohexane trihydrochloride in D_2O.

the cyclohexyl ring.[41] The splitting of the equatorial methylene would be expected to be similar to the axial methylene with both presenting as a doublet of doublets. However, the methine proton is gauche to the equatorial methylene and therefore has a weaker coupling constant than the axial methylene, which is 180° to the methine.[41] This weakened coupling broadens the peaks so that it appears only as a doublet with some fine splitting.

The generation of **4** from **2** proceeds with low yields, and the nature of the triazide intermediate means that the reaction cannot be safely scaled up in these facilities. Since the conversion of **3** to **4** can only be performed on a 1g scale with milligram yields, as well as the preparation of **1** and **2** being both time consuming and labor-intensive, this particular reaction methodology proved to be unsuitable for our needs.

II. *Cis, cis*-1,3,5-Triaminocyclohexane Trihydrochloride *via* a Dissolving Metal Reduction is Time Consuming with Poor Yields

An alternative starting material to **3** was 1,3,5-cyclohexanetrione trioxime (**5**). The trioxime was prepared using parts of the methodology of Baeyer,[42]



5

Scheme 1.7. Preparation of **5** from phloroglucinol dihydrate.

Tokura,[43] and Bottaro.[44] Baeyer's initial synthesis required keeping the reaction mixture at 0°C for several days to a week in order to crystallize out the trioxime. The more recent preparations by Tokura and Bottaro require 24h or less to complete with excellent yields of better than 80%. Bottaro's preparation utilized the free hydroxylamine, as opposed to neutralizing the hydroxylamine hydrochloride in the presence of phloroglucinol per Baeyer and Tokura.

The trioxime was prepared via an optimization of Bottaro's methods. As hydroxylamine solution was not available for use in our laboratory, hydroxylamine hydrochloride was neutralized with sodium carbonate in water to yield a concentrated solution of the free hydroxylamine after filtration. This solution was diluted with a small amount of water and phloroglucinol dihydrate was added with slight warming. The mixture was stirred to give a golden yellow solution concomitant with precipitation of the product as an off-white powder. The flask was immediately removed from heat and covered with foil, whereupon the off-white powder that was the product (**5**) continued to precipitate out of solution nearly quantitatively over an hour. At this point the product was promptly filtered, washed with scant amounts of cold water, and dried under vacuum. It was noted that **5** may be stored for extended periods of time without decomposition at 4°C in a foil wrapped bottle, but if left on the benchtop, the off-white powder turns brown over night. This decomposition is even more pronounced if heat is applied.

Bottaro added phloroglucinol to a 50% w/w aq. solution of hydroxylamine and let **5** precipitate out of solution as a brown solid.[44] Both Tokura[43] and

Baeyer[42] report their product as an off-white or grey solid with light and heat sensitivity, and it appears that Bottaro may not have taken precautions against light, meaning that recrystallization was necessary, converse to the optimized methods in the above methodology where pure **5** was generated. Compared to all three procedures in the literature, less solvent was used overall and therefore the product fell out of solution in greater quantity. This concurs with observations in preparations by the optimized methodology, that in cases where the hydroxylamine solution was diluted to more than 75mL of total volume, the product yield decreased significantly.

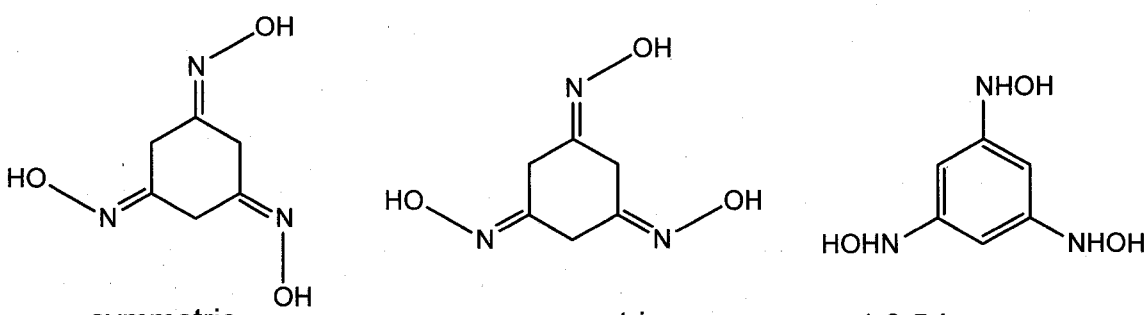


Figure 1.3. Rotational isomers of 1,3,5-cyclohexanetrione trioxime, including a literature proposed tautomer, 1,3,5-benzenetrihydroxylaminol.

The ^{1}H NMR data of the trioxime product is an unexpectedly complex and interesting spectrum with multiple rotomers due to the locked position of the oxime at room temperature. While it has been proposed that tautomerisation may convert the oxime to 1,3,5-benzenetrihydroxylaminol, spectroscopic data does not support the existence of the aromatic form.[45] Therefore there are two rotational isomers possible: the symmetric and the asymmetric form, whose relative population within the product may be ascertained by NMR. The

asymmetric isomer is expected to show three hydroxyl and three aliphatic peaks, while the symmetric isomer would only show one of each species. The ^{1}H NMR in d_6-DMSO shows two sets of peaks. The three peaks furthest downfield have been assigned to the hydroxyl protons, as confirmed by their disappearance upon the addition of a small amount of D_2O. The peaks are not uniform in integration, with the central peak being slightly taller than the other two whose integrations are identical. In the aliphatic region there is another set of three

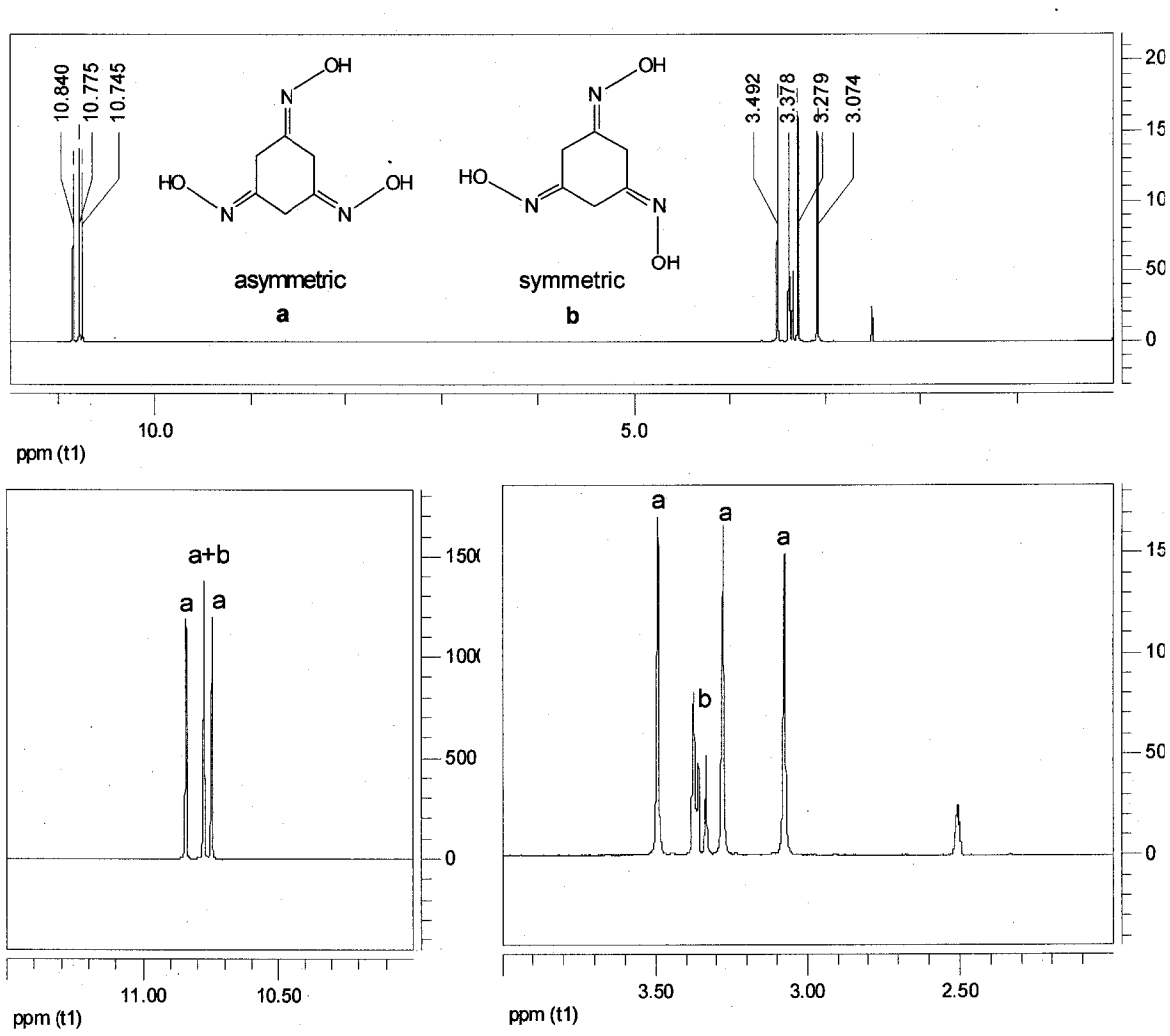
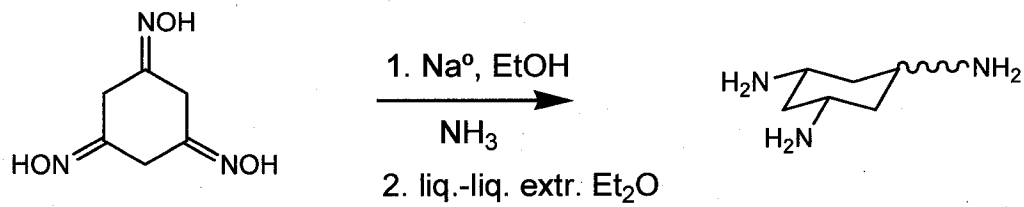


Figure 1.4. ^{1}H NMR spectrum of **5** in d_6-DMSO.

strong peaks that are associated with the methylene protons of the ring and have the same integrations. A considerably smaller peak is visible partially overlapped by the signal for water. The height of the small peak adjacent to water and the additional height of the central downfield hydroxyl peak appear to have similar integrations, while each of the other peaks integrate to one proton. From this information, the asymmetric rotomer may be identified as the major product, while only 20% of the product formed is the symmetric isomer according to the hydroxyl proton integrations. The ^{13}C spectrum further supports the assignment of the major product, having two sets of three major peaks and two sets of singular minor peaks.

According to the method of Lions and Martin[19] with further notes from Fuhrer and Günthard,[46] 1,3,5-cyclohexanetrione trioxime was reduced to *cis, trans* and *cis, cis*-1,3,5-triaminocyclohexane by a Birch reduction.



5 **6**

Scheme 1.8. Dissolving metal reduction (Birch) of **5**.

After the dissolving metal reduction, the brown liquid was carefully treated with water and then continuously extracted with diethyl ether over 4 d to give a tan oil after removal of the solvent. This oil contained both isomers of 1,3,5-triaminocyclohexane, with the desired *cis, cis* isomer as the minor product in a ratio of 1:1.2 (average 1:2.1) in favor of the *trans*. The ^{1}H NMR spectrum shows

that the oil contains few impurities, and the peaks of the *cis, cis* isomer are easily picked out in reference to spectrum of the trihydrochloride salt (Figure 1.1). The *cis, trans* isomer shows five distinct proton peaks, and the shift and splitting patterns follow a similar rationale to the *cis, cis* trihydrochloride salt.[47] Due to a combination of the ring-effect and the geminal amine, the equatorial methine of *cis, trans* tach is shifted furthest downfield. The axial methine of *cis, trans* tach is shifted further downfield than the axial methine in the *cis, cis* isomer due to the steric effect of the axial amine, which is significantly more pronounced than the effect of axial protons in the *cis, cis* isomer. The rest of the proton assignments are noted in Figure 1.5.

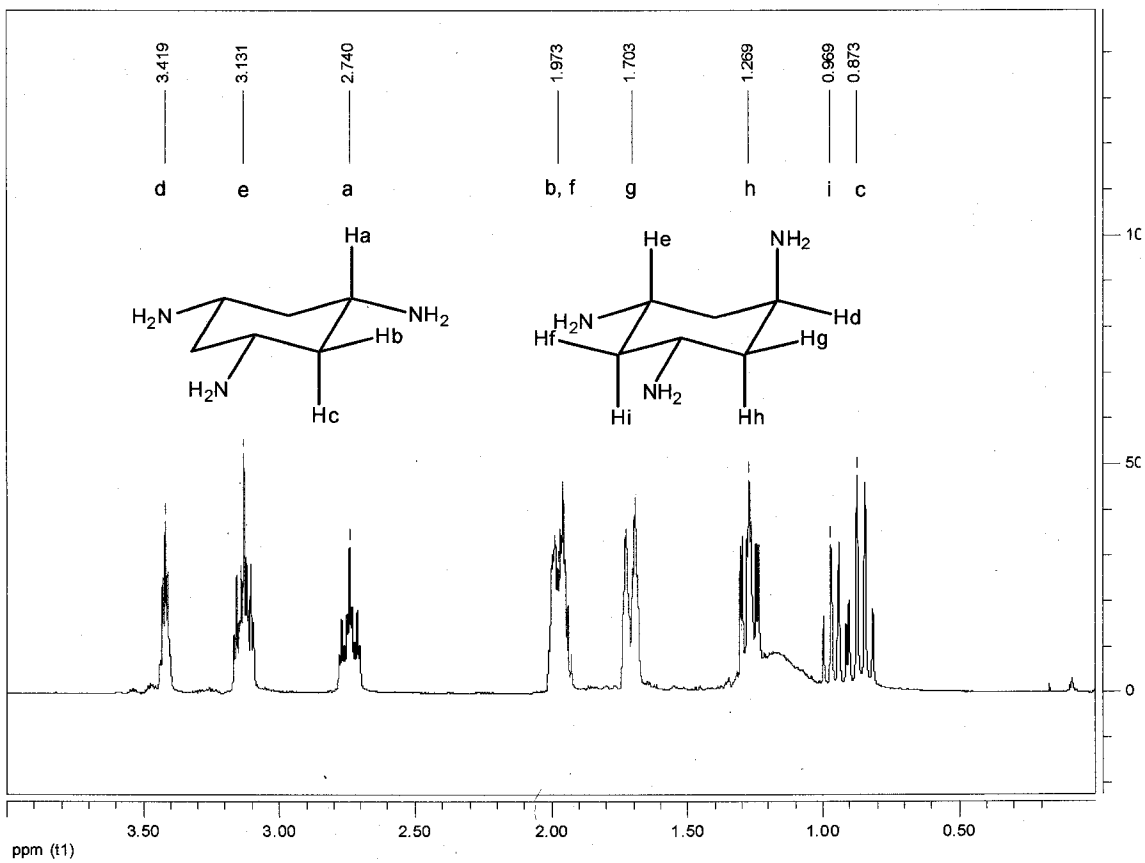
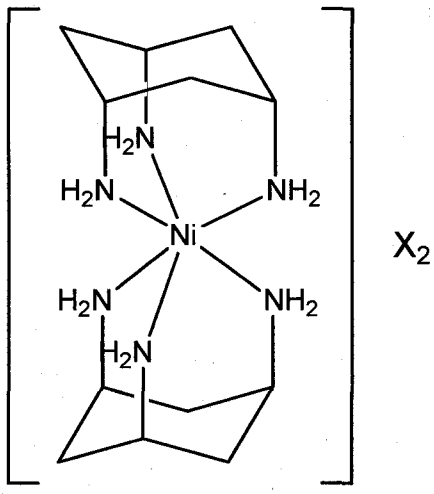


Figure 1.5. ^{1}H NMR of **6** with peak assignments in D_2O.

While Lions and Martin report distilling the oil, the amount of crude oil generated was so small (3-8% yield), it was important to avoid the distillation step. By adding a metal salt directly to a methanolic solution[22] of the crude oil the *cis, cis* isomer could be precipitated out as the bis-adduct (**18**). The amount of divalent metal necessary to complex the *cis, cis* isomer was calculated by evaluating the ^{1}H NMR spectra and estimating the ratio of the isomers. Noting that the bis-*cis, cis* adduct is more stable than the *cis, trans* and therefore more likely to easily crystallize out of solution, Wentworth utilized this method with the distilled oil to isolate **18** with $Ni(NO_3)_2 \bullet 6H_2O$ from water.[21]



18

Figure 1.6. Nickel(II) bis-*cis, cis*-1,3,5-triaminocyclohexane with anion.

To isolate **18** from **6**, three different nickel salts were tried: $Ni(NO_3)_2 \bullet 6H_2O$, $NiCl_2 \bullet 6H_2O$, and $Ni(ClO_4)_2 \bullet 6H_2O$. All three gave crystalline material out of the crude brown methanolic solution of **6**, however $NiCl_2 \bullet 6H_2O$ and $Ni(ClO_4)_2 \bullet 6H_2O$ gave better crystals than $Ni(NO_3)_2 \bullet 6H_2O$, requiring no recrystallization in most cases. Analysis of all three complexes by UV-Vis was attempted, but the nitrate

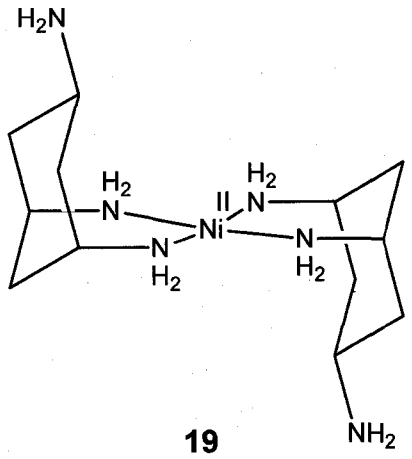
salt proved insoluble in water, methanol and acetonitrile and therefore no data was obtained for that species. It did appear as a salmon pink crystalline powder, and the pink color is typical of Ni-N6 octahedral complexes. The other two complexes gave results typical of Ni-N6 octahedral complexes in solutions, with three peaks whose assignments follow.

Solution complexation of Ni(II) with *cis, cis*-tach	υ/ $10^{-3}cm^{-1}$	Assignment	Color
$[Ni(tach)_2](ClO_4)_2$	11.7 18.9 29.8	$^3A_{2g} \rightarrow {}^3T_{2g}$ (F), $^3A_{2g} \rightarrow {}^1E_g$ (D) $^3A_{2g} \rightarrow {}^3T_{1g}$ (F) $^3A_{2g} \rightarrow {}^3T_{1g}$ (P)	Raspberry pink
$[Ni(tach)_2]Cl_2$	11.7 18.9 30.0	$^3A_{2g} \rightarrow {}^3T_{2g}$ (F), $^3A_{2g} \rightarrow {}^1E_g$ (D) $^3A_{2g} \rightarrow {}^3T_{1g}$ (F) $^3A_{2g} \rightarrow {}^3T_{1g}$ (P)	Carnation pink

Table 1.1. UV-Vis data and peak assignments for Ni(II) bis-*cis, cis*-tach complexes, 15mM in H_2O.

As the needles formed by perchlorate salt were easier to handle than the chloride microcrystals, this was the salt selected for later isolations of *cis, cis*, tach. Since perchlorate salts are known to be somewhat shock sensitive when dry, they were stored under diethyl ether until used.

The *cis, trans* isomer was also subsequently isolated by further additions of the nickel salt to the methanolic solution decanted away from **17**. The yellow powder that formed was extremely hygroscopic, and tentative UV-Vis data shows a single broad peak indicative of a square-planar complex.[48] However, due to the ability of *cis, trans*, tach to form polynuclear complexes,[49, 50] investigation of this byproduct is on hold.



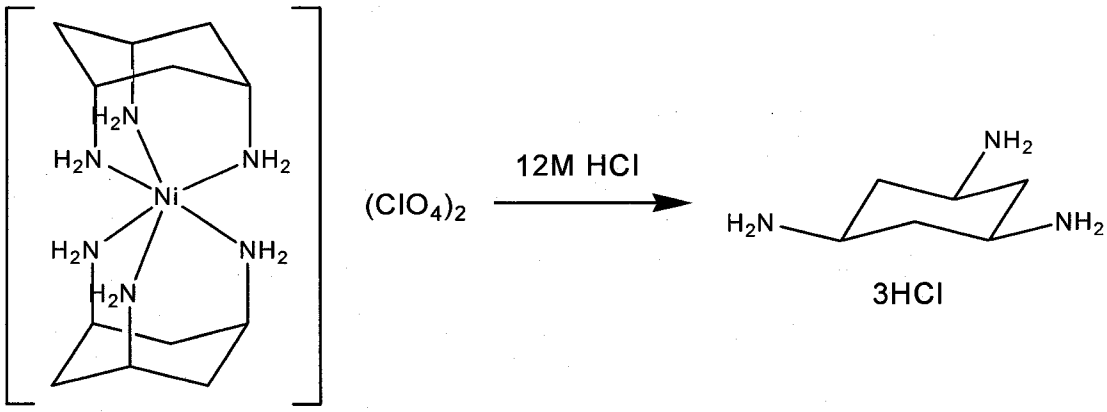
19

Figure 1.7. Proposed structure of nickel(II) bis-*cis, trans*-1,3,5-triaminocyclohexane. The non-coordinated amines may bind other nickel cations, creating polynuclear complexes.

It was hoped that the free amine might be directly acquired by treating the metal salts in aqueous solution with NaCN as was successfully reported with other amines.[51] The cyanide anion is a better ligand than *cis, cis,* tach for nickel and treatment of a pink aqueous solution of one of the complexes gave a yellow solution indicative of the square-planar $Ni(CN)_4^{2-}$ and the free amine. However, the excellent water solubility of the free tach prevented extraction in reasonable yield.

The trihydrochloride salt was acquired instead of the free amine by treating the Ni(*cis, cis*-tach)$_2$]$(ClO_4)_2$ crystals under diethyl ether directly with 12M HCl. The trihydrochloride salt could be recrystallized from hot 12M HCl, but this was usually unnecessary.

The poor yields of this route rendered it unsuitable for generating gram quantities of *cis, cis*-tach. One of the pitfalls of the dissolving metal reduction is that the stereoselectivity of the reaction, seen in the literature[14] as well in these



1 equivalent
18

2 equivalents
8

Scheme 1.9. Acidification of the nickel-complexed *cis, cis*-tach.

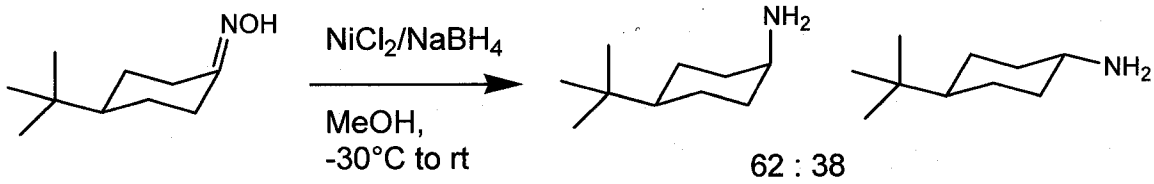
trials, is usually preferential for the *cis, trans* isomer, and therefore the overall yield is reduced by at least half. According to Lions and Martins, they only received the *cis, cis* isomer, however, according to the literature preparations done following their methodology,[20] this report was in error, and the presence of the *cis, trans* isomer had been overlooked. The multiple-day liquid-liquid extraction was not time effective for the amount of product obtained, but it was the only effective method for extracting the crude product mixture. Other methods attempted included Soxhlet extraction, direct chloroform and dichloromethane extraction, and sublimation of dried samples of crude **6**. The diminished yield of the crude isomeric mixture compared to West, 3.3.% versus 16%, may be due to the amount of water added following the reduction. The isomers are extremely water soluble, and are resistant to extraction. Addition of excess water may severely inhibit recovery of the desired product. However, without sufficient addition of water, any residual sodium metal remains active and unquenched – a hazard in further workup.

The critical step to improve this route in the future may be to ensure a homogeneous mixture during the dissolving metal reduction, a difficult adaptation considering the grey clumping solids that form during the solvation of sodium in the liquid ammonia.

III. Attempted Reductions of *cis, cis*-1,3,5-Cyclohexanetrione Trioxime Using $NaBH_4$ and Metal Cofactors

Alternate methods to reduce 1,3,5-cyclohexanetrione trioxime to the triamine using sodium borohydride and metal cofactors proved unsuccessful. The aromatic nature of this particular oxime appears to render it resistant to these reductive methods. This oxime is in a conjugated aromatic system where it is isoelectronic to 1,3,5-benzenetrihydroxylaminol, as was shown in Figure 1.3. It was hoped that the trioxime would react similarly to other single oximes that were successfully reduced in the literature. While oximes cannot be reduced by sodium borohydride alone, the presence of certain transition metal salts result in amine products. The reduction of oximes may follow a similar mechanism to that of nitriles in this reaction: it has been shown that, for nitriles, metal borides are formed and that these catalyze the reduction by hydride transfer.[52,53] While these reductive pathways do not provide any more stereoselectivity than the dissolving metal reduction, the easy availability of the starting material combined with the short reaction durations made them attractive preparative methodologies. It was anticipated that the resulting mixture of isomers could be

isolated in the same manner as those in the dissolving metal reduction, but starting material was recovered in all cases.

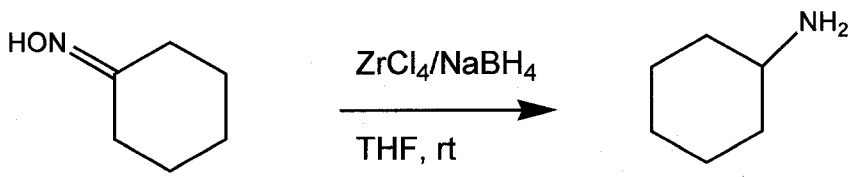


Scheme 1.10. Example of oxime reduction to an amine with $NiCl_2/NaBH_4$.[54]

Hydroxyiminosteroids containing an oxime functionality were reduced by Szendi using nickel chloride and sodium borohydride in methanol.[55] A similar method was used by Ipaktschi with various singular oximes.[54] In these cases the oxime was dissolved in methanol and then treated with nickel chloride followed by the sodium borohydride added over time. After addition was complete, the reaction mixture was allowed to stir overnight. The inorganic precipitate was filtered away, and the reaction mixture worked up appropriately.

Although this method was tried with the trioxime, it proved ineffective, and only returned starting material. Varying the amount of metal and borohydride to attempt to find a more reactive mixture exhibited no change in results.

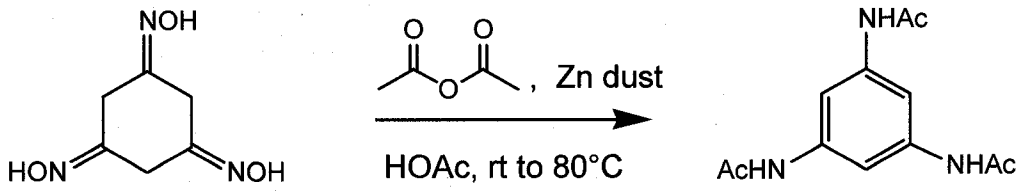
Zirconium tetrachloride was used as a metal cofactor in lieu of nickel chloride to reduce some oxime moieties in work by Itsuno.[56] This method was attempted for use with the trioxime. Sodium borohydride was added directly to a solution of zirconium chloride, which immediately began evolving gas, and after the formation of a cream colored suspension, the oxime was added to the solution. Though freshly sublimed zirconium chloride was used, only starting material was returned, as for the nickel reduction trials.



Scheme 1.11. Example of oxime reduction to an amine with $ZrCl_4/NaBH_4$.[56]

IV. Attempted Catalytic Heterogeneous Hydrogenations of *cis, cis*-1,3,5-Cyclohexanetrione Trioxime and 1,3,5-Trisacetyltriaminobenzene

According to Stetter, 1,3,5-trisacetyltriaminobenzene could be reduced to 1,3,5-triaminocyclohexane under mild reductive conditions using the Nishimura catalyst.[57] Since the Nishimura catalyst was reported to be effective in this case at atmospheric hydrogen pressures, 1,3,5-trisacetyltriaminobenzene was prepared according to the more recent synthesis by Arai in order to evaluate this method.[28]



5 **13**

Scheme 1.12. Reduction of trioxime to 1,3,5-trisacetyltriaminobenzene (**13**).

1,3,5-Cyclohexanetrione trioxime was dissolved in glacial acetic acid with acetic anhydride, to which was added several portions of zinc dust while stirring. Initially, when running the reaction according to Arai, yield was poor and the product was contaminated with a large number of impurities. It was thought that the zinc dust, used without prereduction to remove zinc oxides that form over

time, was taking longer to reduce the oxime than was anticipated by the literature report. However, monitoring the reaction by TLC on silica plates (5:1, ethyl acetate: methanol) proved to be an effective method to evaluate the progress of the reaction. It was noted by TLC that the starting material disappeared immediately with formation of two intermediates. After heating with the zinc dust the product also developed in solution, but the two intermediates were still present. After a suitable incubation time of 2-5 hr, the product predominated the reaction mixture and a final addition of zinc dust pushed the remaining intermediates to product. The mixture was filtered hot and the fine zinc metal was reusable for subsequent reductions. After workup, the tan powder was washed with anhydrous ethanol, and shown to be **13** by ^{1}H and ^{13}C NMR with trace impurities of acetic acid.

The acetic acid impurities were not expected to be problematic in later catalytic reductions, as trace amounts of acetic acid have been shown to increase the rate of reaction of aromatic amines with some precious metal catalysts.[32, 58, 59, 60] Often, nitrogen bases show inhibitory effects on the catalysts, however the presence of acetic acid gives the amine salt which is more stable than the aromatic amine, based on the increased basicity of the cyclohexyl amine.[32] As shown in Table 1.2, many hydrogenations were run with the 1,3,5-trisacetyltriaminobenzene substrate using various commercially available metal catalysts under a variety of conditions, but starting material was returned every time.

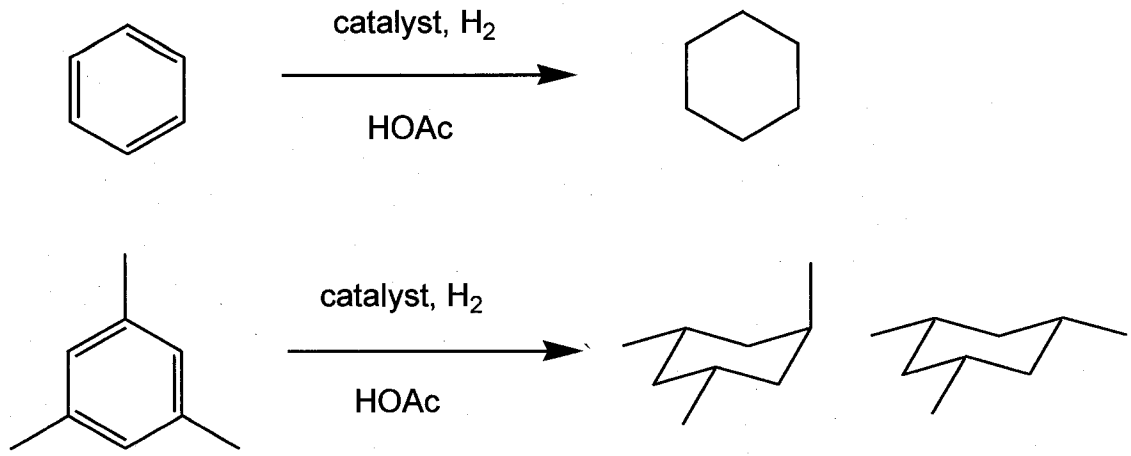
Catalytic reduction conditions applied to 1,3,5-trisacetyltriaminobenzene.					
H_2 pressure	5% Rh on activated C (a)	10%Pd on activated C (b)	(a) and (b)	Pt(IV) oxide Adam's catalyst	Nishimura catalyst
1atm	HOAc, rt, 3d		HOAc, 100°C, 3d		
	HOAc, 100°C, 3d				
	MeOH, rt, 3d				
42psi	HOAc, rt, 3d	HOAc, rt, 3d			
	MeOH, rt, 3d	HOAc, 100°C, 3d			
100psi					HOAc, 50°C, 1d
200psi					HOAc, 50°C, 1d
					HOAc, 50°C, 3d
1000psi	HOAc, rt, 3d	MeOH, rt, 2d	HOAc, 100°C, 3d	HOAc, 50°C, 3d	
		HOAc, 100°C, 3d			
Catalytic reduction conditions applied to 1,3,5-cyclohexanetrione trioxime.					
1000psi	MeOH, rt, 2d	MeOH, rt, 2d			EtOH/HCl, rt, 1d

Table 1.2. Catalytic reduction conditions applied to 1,3,5-trisacetyltriamino-benzene and 1,3,5-cyclohexanetrione trioxime. Only starting material was observed in all cases with 1,3,5-trisacetyltriaminobenzene; 1,3,5-cyclohexanetrione trioxime appeared to decompose. At least 10mol% catalyst based on the substrate was used in each case

The 5% Rh on activated carbon had been the first choice in commercial catalysts due to rhodium's efficacy in reducing aromatic systems with the *cis, cis* isomer predominant at lower temperatures. It is expected that the other catalysts chosen would have similar product distribution due to the nature of heterogeneous catalysis, where the substrate is adsorbed onto one face of the hydrogen rich catalyst and reduced at that point. An increase in temperature

increases the number of both absorptive and desorptive interactions.[61] Thus, the partially reduced substrate may be released from the catalyst before completion, and then adsorb onto another catalytic site from the opposite face, giving mixed products.[32, 57] However, no partial reduction, mixtures of products, or any activity at all was observed with 1,3,5-trisacetyltriaminobenzene; and the substrate could be recovered nearly quantitatively each time.

The commercial catalysts were found to be marginally active for the hydrogenation of mesitylene and benzene. While neither benzene nor mesitylene are directly analogous to 1,3,5-trisacetyltriaminobenzene as far as substituent effects, it was important to ascertain with known systems the efficacy of the catalysts, and therefore note whether they were active at all. According to the hydrogenation methodology of Smith,[62] either benzene or mesitylene was dissolved in glacial acetic acid to which 10 mol% catalyst based on the substrate was added. The reaction vessels were purged with nitrogen before the beginning of each reaction, and carefully vented under nitrogen upon completion. The catalyst was collected on filter paper and the acidic solution was treated with a saturated solution of sodium bicarbonate, which induced the formation of a biphasic system. The organic layer was separated and dried over $MgSO_4$ and then analyzed by ^1H NMR in $CDCl_3$ for conversion to the products, either cyclohexane or 1,3,5-methylcyclohexane depending on the starting material.

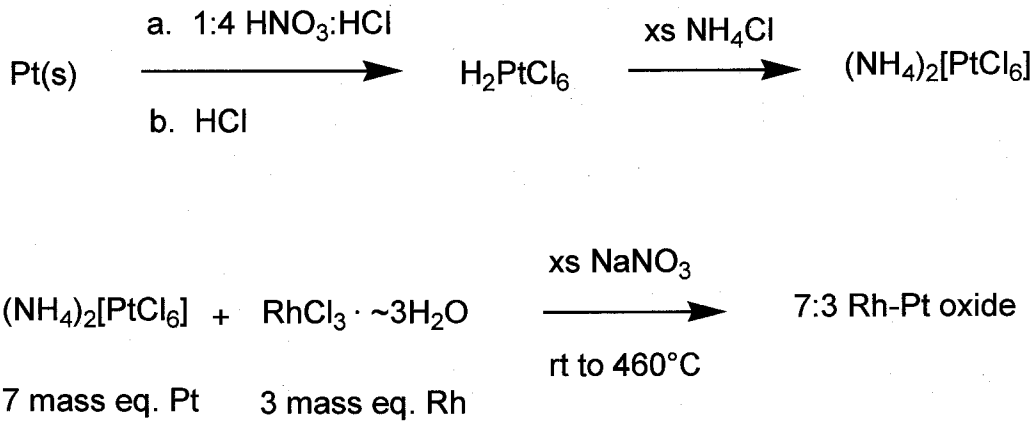


Scheme 1.13. The reduction of benzene and mesitylene.

The 5% Rh on activated carbon catalyst was only partially effective in the reduction of benzene at 1000psi for 24 hr at room temperature, giving cyclohexane in 22% yield. The same catalyst was only minimally effective with mesitylene under the same conditions, with only 3% conversion to the cyclohexyl product. Similar results were also seen with the 10% Pd on activated carbon catalyst. Adam's catalyst proved to be more effective with the reduction of mesitylene when run over 3 d at 1000 psi with heating at 50°, giving 1,3,5-methylcyclohexane in 20% yield. However, none of these catalysts were as effective as would have been expected under the rigorous temperature and pressures.

In light of the poor activity of the commercially available catalysts for this particular system, the Nishimura catalyst that Stetter originally reported to be successful in this hydrogenation reaction was prepared. Synergistic effects of a combination of catalysts are often noted when the precious metals are prepared together or mixed, with an increase in activity and rate of reaction,[32, 60] however,

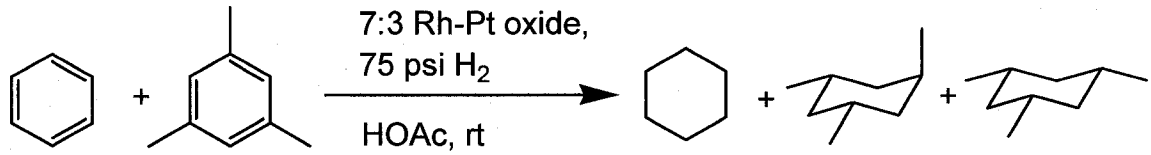
a mixture of the commercially available 5% Rh on carbon and 10% Pd on carbon proved to be an unreactive catalyst when tested in this case.



Scheme 1.14. Preparation of Nishimura catalyst.

The Nishimura catalyst is a combination of rhodium and platinum oxides in a ratio of 7:3 by weights of the metals. It was originally shown to completely reduce toluene and acetophenone in acetic acid at 30°C under atmospheric hydrogen pressure in 16 and 20 min respectively. The catalyst was prepared according the method of Nishimura[31, 32] with minor modification by the method of preparing Adam's catalyst.[30] As with the Nishimura catalyst, Adam's catalyst is a metal oxide prepared by melting sodium nitrate with a metal chloride, and so similar techniques are employed. Chloroplatinic acid was prepared according to Grube,[29] but due to the hygroscopic nature of the acid this was carried on to ammonium chloroplatinate without isolation. The ammonium chloroplatinate is preferred over the chloroplatinic acid due to its stability and non-hygroscopic nature, which allows it to be accurately weighed and stored over long periods of time. The yellow powder was ground into a fine homogeneous powder with excess sodium nitrate and the appropriate ratio of rhodium trichloride by weights

of the metals. The mixture was then fused to promote the evolution of NO_x gases and the formation of the metal oxides. The ammonium chloride salts were washed away with water and the active catalyst was stored under a desiccator.



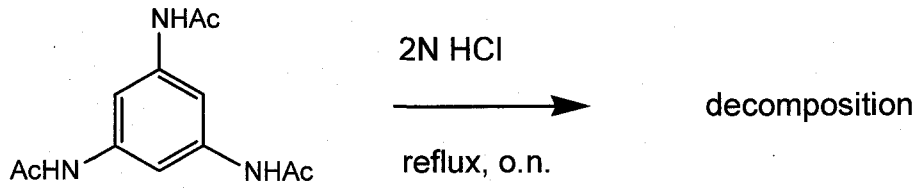
Scheme 1.15. Complete catalytic reduction of benzene and mesitylene by Nishimura's catalyst.

The catalyst was tested with benzene and mesitylene at medium pressures (200 psi and 75 psi) of hydrogen at room temperature. Unlike for the carbon-supported catalysts, Nishimura recommends prereduction of the oxide to the metal by stirring the catalyst in acetic acid under hydrogen for 40 min before addition of the substrate.[31] After prereduction of the catalyst, a mixture of benzene and mesitylene in acetic acid was added to the metal blacks, and the suspension was stirred under hydrogen until uptake ceased. In both cases, this occurred within ten minutes and resulted in complete conversion to the products. However, the repetition of this methodology with the desired substrate, 1,3,5-trisacetyltriaminobenzene, was unsuccessful, returning only starting material. Stetter indicates that he had great difficulty with the hydrogenation and believed that it was linked to the poor solubility of the compound.[57] Examination of the reaction via the glass Parr flask with medium-pressure hydrogenation (100 psi) showed that contrary to Stetter's theory the trisacetyltriaminobenzene dissolved within minutes of addition to the stirred catalyst in acetic acid mixture. Other unknown factors appear to be contributing to the inertness of this compound.

Catalytic reduction of 1,3,5-cyclohexanetrione trioxime was also attempted according to the conditions in Table 1.2. The solvent system was changed to an alcoholic system as the trioxime proved to be unstable in acetic acid, decomposing rapidly. However, none of the commercially available catalysts were effective in reducing the trioxime, which appeared to decompose in all cases. The sensitivity of the trioxime may make it a difficult substrate to work with in this type of reduction.

V. 1,3,5-Triaminobenzene as Another Plausible Starting Reactant in Heterogeneous Hydrogenation

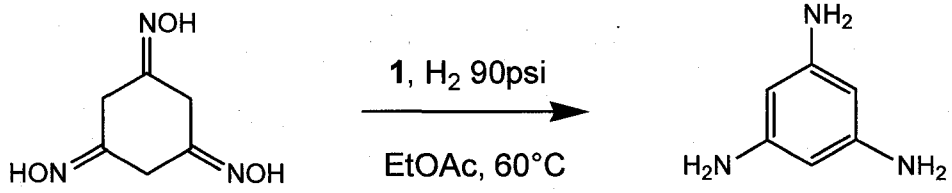
It was theorized that the acetyl protecting groups on the 1,3,5-trisacetyltriaminobenzene were inhibiting the hydrogenation of the compound. Rylander and Nishimura both report that as the substituents on an aromatic ring become larger, the difficulties of hydrogenating the substrate increase exponentially due the sterics of adsorbing onto the catalyst. Deprotecting the trisacetyltriaminobenzene was considered in order to have a less hindered substrate available for hydrogenation. There are several reported deprotecting methodologies for acetyl groups on amines,[63, 64, 65, 66] one of which by Dilbeck involves treating the compound with 2N HCl at reflux overnight.[63] However, this method did not produce any of the expected 1,3,5-triaminobenzene (**16**), only unidentifiable reacted material.



13

Scheme 1.16. Attempted deprotection of 1,3,5-trisacetyltriaminobenzene.

Literature reports that **16** is very unstable in the presence of air, heat, and even upon standing under nitrogen and may be stored for a short period of time under vacuum. It may be most easily prepared by reduction of 1,3,5-trinitrobenzene,[33, 67, 68, 69] 3,5-dinitroaniline,[70, 71, 72] or 1,3,5-cyclohexanetrione trioxime[28] with the appropriate metal catalyst. The explosive nature of 1,3,5-trinitrobenzene and the expense of 3,5-dinitroaniline precluded their use as starting materials, however 1,3,5-cyclohexanetrione trioxime was readily available as an easily accessible reagent.

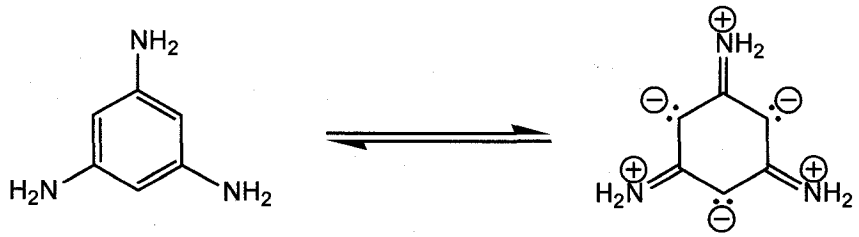


5 **16**

Scheme 1.17. Preparation of 1,3,5-triaminobenzene.

1,3,5-Triaminobenzene was generated from 1,3,5-cyclohexanetrione trioxime by catalytic reduction in ethyl acetate by 10% Pd on C or prepared T-4 Raney nickel (**1**). Raney nickel appeared to give better yields than 10% Pd on C. It was found that ethyl acetate was an easier solvent to work with than the *n*-butyl acetate used by Arai,[28] due to its greater volatility allowing easy concentration

and precipitation of the product as a pale pink powder. Water formed during the reductive process is noted in the ^{1}H NMR spectrum, however it is formed in an inconsequential amount and the amine's poor solubility in ethyl acetate allows it to precipitate out easily.[68]



16

Figure 1.8. Resonance structure of **16**.

Characterization of the product was done by ^{1}H and ^{13}C NMR and matched to reported literature spectra. There are two shifts in the proton spectrum in d_6-DMSO corresponding to the aromatic and the amine protons. The amine protons show up further upfield and their identity was confirmed by the addition of D_2O, which significantly reduced the signal. The aromatic protons show up unexpectedly upfield of their normal range at 5.14ppm, which is related to the presence of the tautomeric form of 1,3,5-triaminobenzene shown in Figure 1.6. The presence of the electron donating amine substituent donates electron density to the ortho and para positions on the aromatic ring,[116] which shifts the aromatic protons upfield of their normal position.[41] As there are three amine substituents affecting the same positions on the ring, this is an especially distinctive effect in this case.

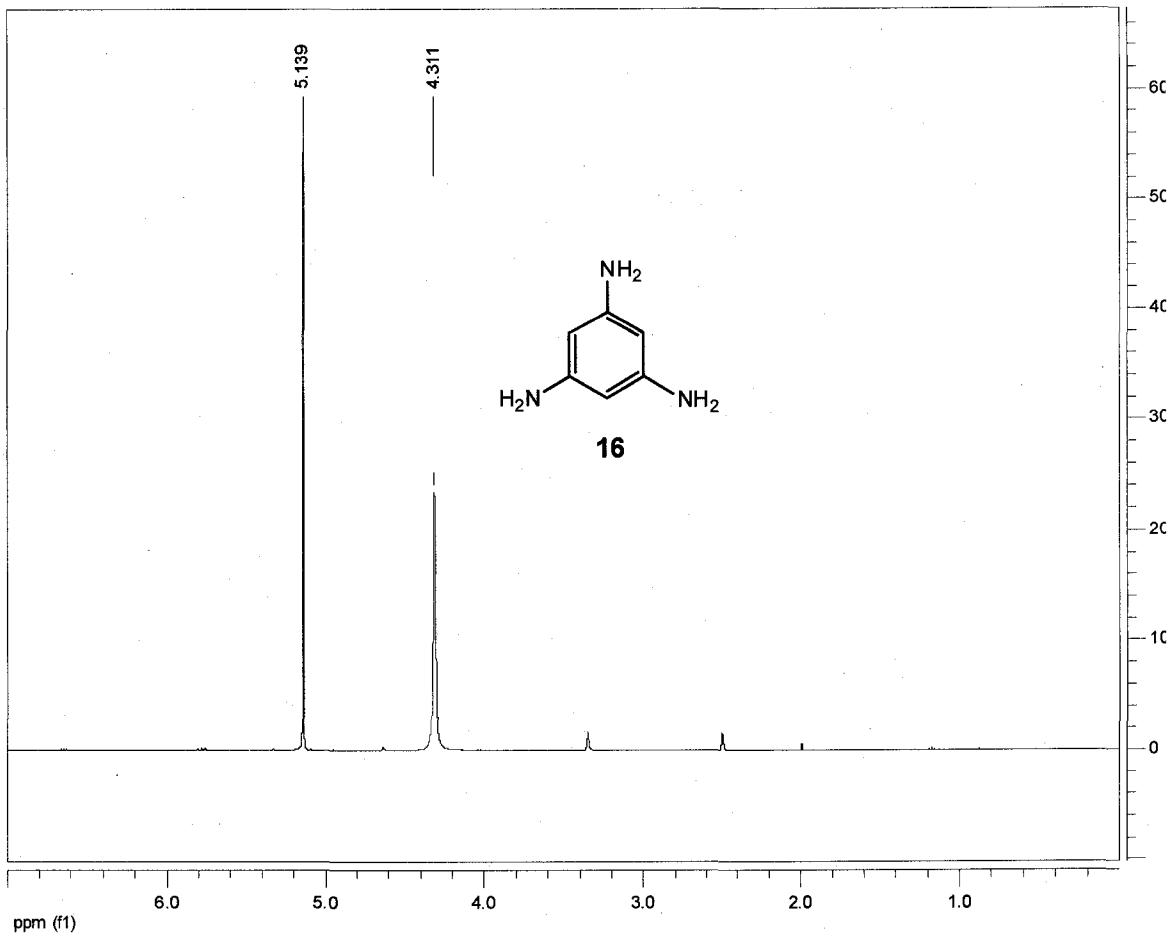
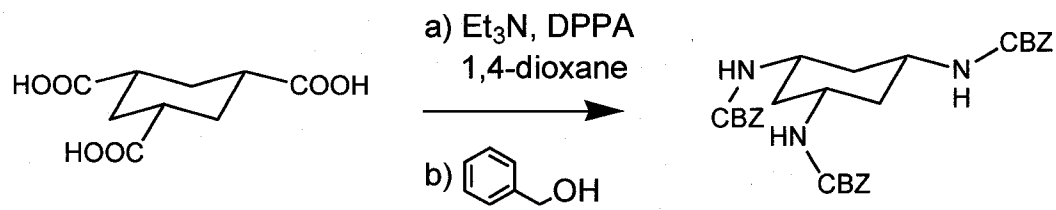


Figure 1.9. ^{1}H NMR of 1,3,5-triaminobenzene in d_6-DMSO.

Unfortunately, there are no literature reports of reducing the triaminobenzene to the triamine. The Nishimura catalyst is not a reasonable candidate as it requires acidic media to be most effective,[32] and the 1,3,5-triaminobenzene is reactive towards acid.[69] Ruthenium is also reported to be an excellent catalyst in the reduction of arylamines in addition to rhodium, and may be a viable avenue to try in the reduction of this substrate. Currently a ruthenium hydroxide catalyst prepared with lithium hydroxide and a rhodium oxide catalyst prepared with lithium nitrate[32] are under consideration.

VI. A Curtius Rearrangement Utilizing *cis, cis*-1,3,5-Cyclohexanetricarboxylic Acid is Currently the Best Synthetic Route to *cis, cis*-Tach

Brechbiel, a collaborator at the NIH, has most recently prepared *cis, cis*-1,3,5-triaminocyclohexane via a Curtius rearrangement from *cis, cis*-1,3,5-cyclohexanetricarboxylic acid to form a carbamate, which was then deprotected to give the amine.[27] This synthesis has many drawbacks including the cost of the reagents and the unpredictability of the reaction yields which are reported, at best, as 57%, but often give no return of product despite care in the reaction.[26] However, among all of the synthetic routes to *cis, cis*-tach reviewed thus far, this reaction shows the most promise with regards to the possibility for improving the methodology, good yield when the reaction is successful, and general safety.

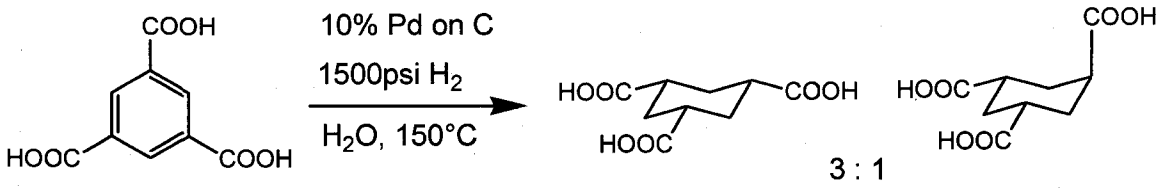


9 **11**

Scheme 1.18. The triple Curtius rearrangement for the synthesis of CBZ-protected *cis, cis*-1,3,5-triaminocyclohexane from 1,3,5-*cis, cis*-cyclohexanetricarboxylic acid. **11** is subsequently deprotected with 33%HBr/HOAc to give the trihydrobromide salt of *cis, cis*-1,3,5-triaminocyclohexane.

The triacid **9** and the azide-transfer reagent diphenylphosphoryl azide (DPPA) may be purchased from Aldrich, but their costs are prohibitively high for a reaction that must be run multiple times and is subject to unreliable returns. However, it was found that both reagents could be synthesized for a fraction of

the cost of the commercially available materials, and, as the synthetic reactions to prepare the triacid and DPPA were not particularly labor intensive and could be run on at least a 10g scale, it was possible to stockpile enough of the materials to have on hand for several trials of the Curtius rearrangement. The ability to synthesize those starting materials quickly and inexpensively made the Curtius rearrangement approach to synthesizing *cis, cis*-1,3,5-cyclohexanetricarboxylic acid the best available route.



Scheme 1.19. Reduction of trimesic acid with 10%Pd on C.

The preparation of 1,3,5-*cis, cis*-cyclohexanetricarboxylic acid (**9**) had been originally reported by Steitz[23] and had been most recently approached by Ye.[73] This straightforward reaction was done in a high-pressure reactor with an internal magnetic stir drive and ceramic heating jacket. Trimesic acid was added to a suspension of the catalyst in water and the mixture was hydrogenated at 150°C for 3 hr at 1500 psi of hydrogen pressure. The commercially available acid was listed as containing trace amounts of acetic acid, which was considered beneficial, as it is often used as a promoter in heterogeneous hydrogenation with platinum metal catalysts.[32] It has been proposed that the acid reacts with residual sodium components in the catalyst, which often act as inhibitors.[59] The catalyst was recovered quantitatively by filtration, washed with water and ethyl acetate and stored in a desiccator; the reaction was run multiple times with the

recovered catalyst, and no loss of activity or selectivity was noted. The water was removed to give an off-white powder which was a mixture of the *cis, cis*- and *cis, trans*-1,3,5- cyclohexanetricarboxylic acid, with the *cis, cis* isomer as the major product in a ratio of 3:1. The reduction proceeded nearly quantitatively, and any residual trimesic acid was removed by filtration with the catalyst.

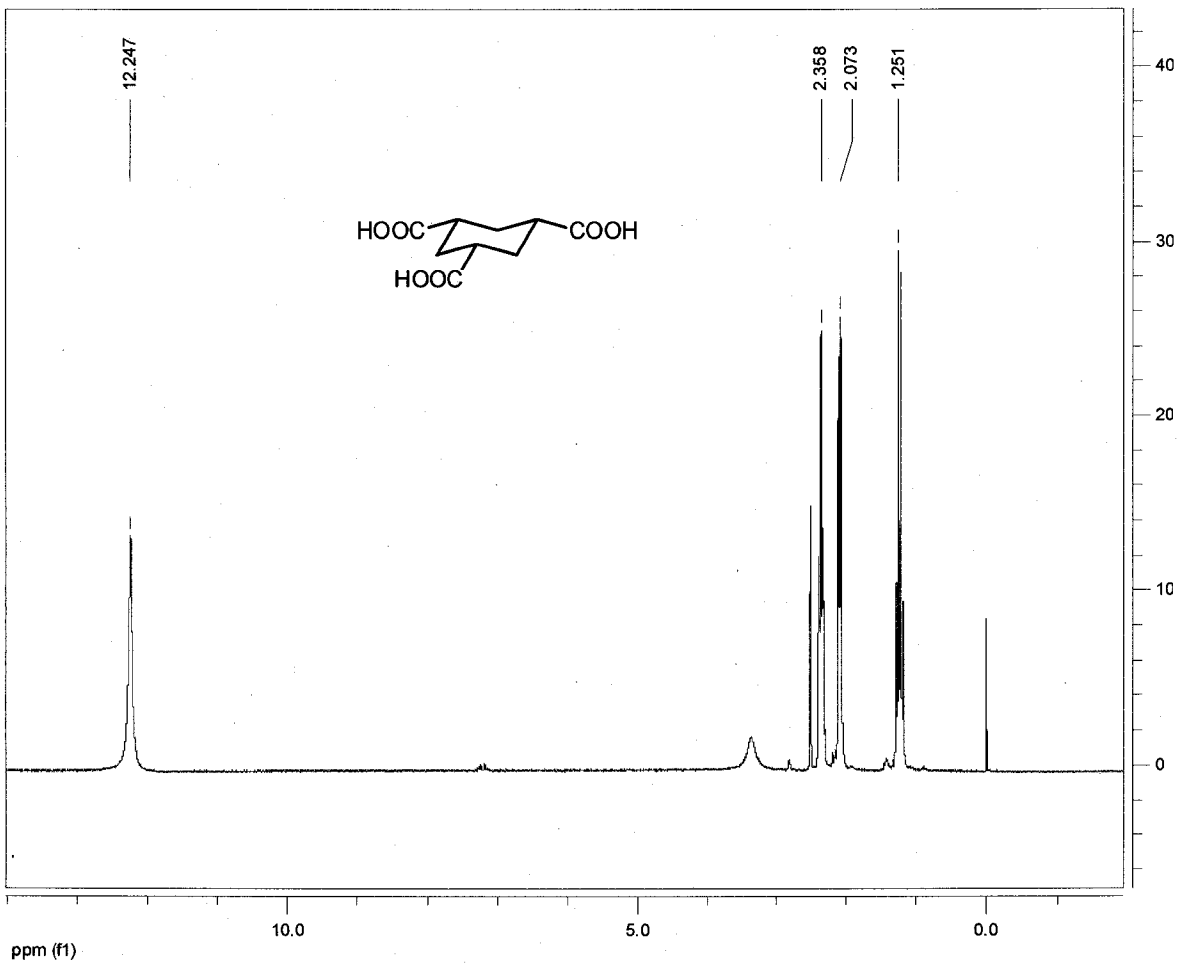


Figure 1.10. ^{1}H NMR spectrum of *cis, cis*-1,3,5- cyclohexanetricarboxylic acid (**9**) in d_6-DMSO, recrystallized.

Recrystallization from ethanol and toluene gave 90-95% *cis, cis*-isomer in 64% yield overall, a distinct improvement over the purchase of the same isomer from Aldrich. For example, 10 g of trimesic acid at a cost of 43¢ per gram was

converted to 6.6 g of at least 90% *cis, cis*-1,3,5-tricarboxylic acid, normally at a cost of $9.52 per gram;[74] thus an initial material input of $4.30 gave $62.83 of product in return (Aldrich Chemicals, 2006 prices).

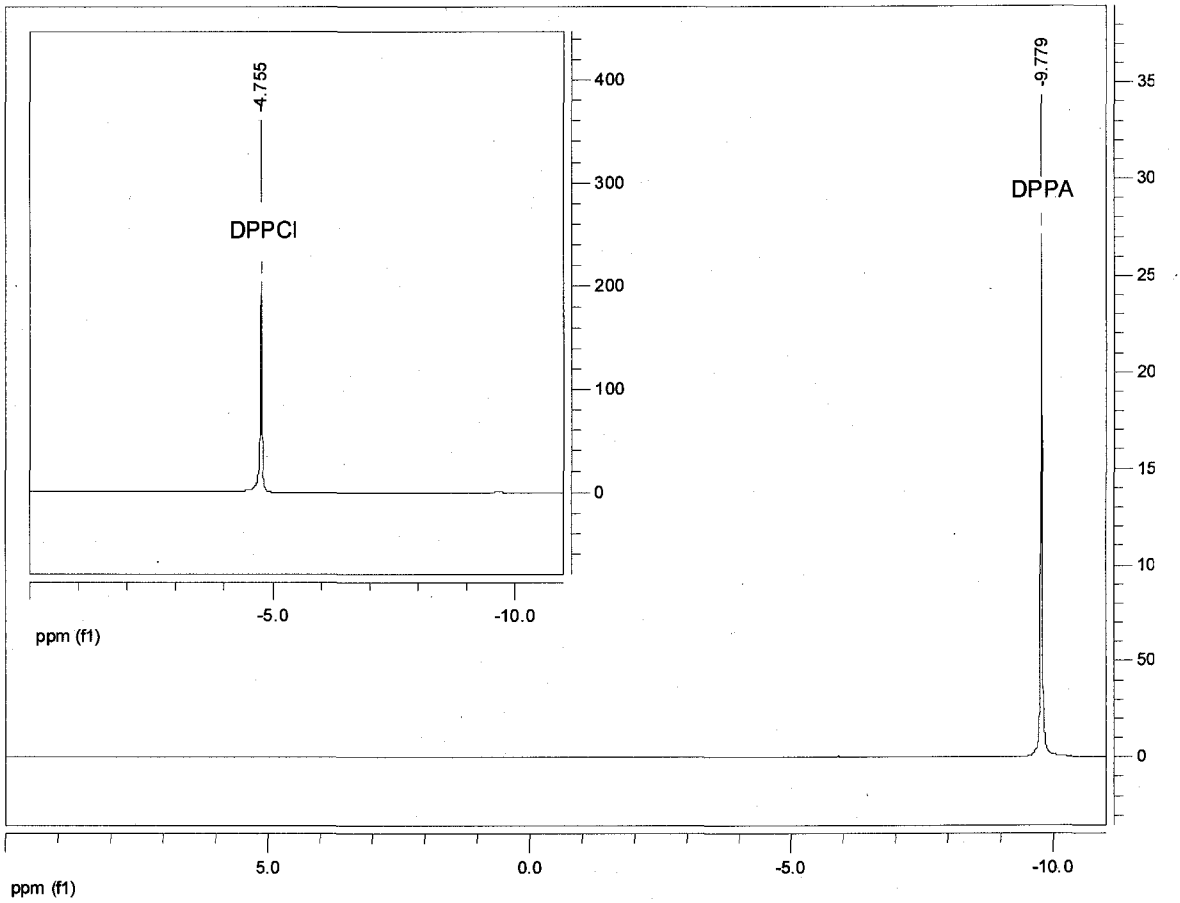


Figure 1.11. ^{31}P NMR spectrum of DPPA in $CDCl_3$ against an external standard of 85% H_3PO_4. Inset is ^{31}P NMR spectrum of distilled DPPCl under the same conditions.

Diphenylphosphorylazide was prepared according to a recent literature preparation.[24] Distilled diphenylphosphoryl chloride was added to a cold suspension of sodium azide in dry acetone over 15 minutes. The formation of the sodium chloride byproduct was noted during the addition, and the reaction

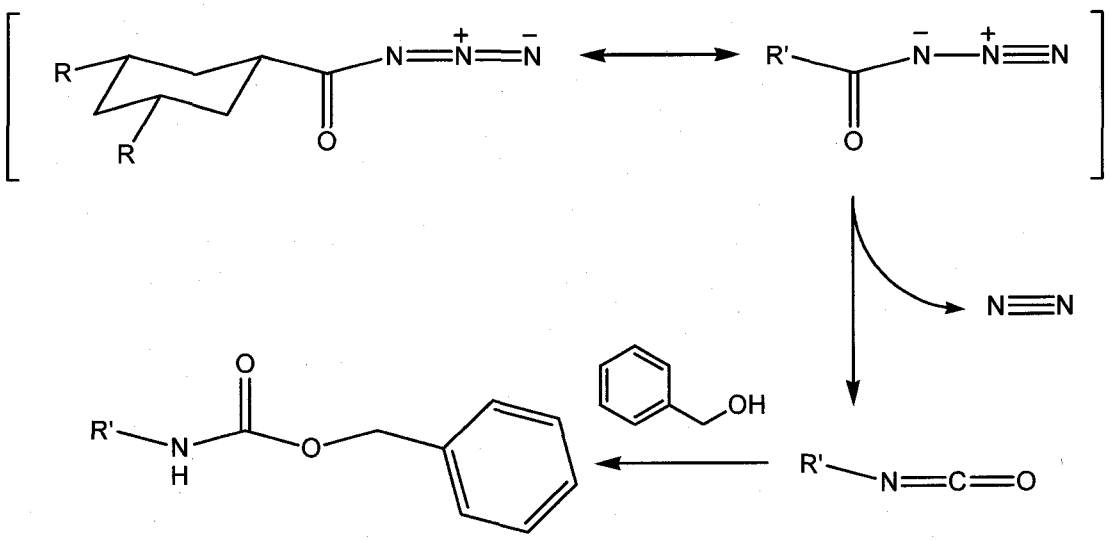
was complete after four hours. The simple workup involved filtration of the salt and removal of the solvent under vacuum to give the desired product.

Evaluation of the ^{31}P NMR spectrum showed the product to be clean of impurities or residual starting material and therefore no further workup was necessary. According to the literature, the conversion from the chlorophosphoryl to the azidophosphoryl moiety occurs nearly quantitatively under the stated conditions.[24] Most of the reduction in the yield likely occurred in the filtration process, as the large amount of salt byproduct was not rigorously washed. It was anticipated that the minor increase in yield from further washing would not offset the risk of product degradation by exposure to atmospheric moisture.

While not quite as cost efficient as the production of **9**, an input of $9.45 in cost of the diphenylphosphoryl chloride gave $45.71 of the diphenylphosphoryl azide in excellent purity, based on 21.5 g of starting material at 43¢ a gram giving 19.4 g of the desired product that commercially costs $2.35 per gram (Aldrich Chemical, 2006 prices).[74]

Utilizing the synthesized starting materials, the *cis, cis*-1,3,5-tricarboxylic acid was suspended in dry dioxane and treated with triethylamine and DPPA according to the literature.[27] The deprotonated acid is reactive enough to facilitate substitution of the hydroxy moiety with the azide on the oxophilic phosphorus center, a reactivity that is enhanced by the electron-withdrawing phenyl substituents.[24, 75] Initially a pentacovalent phosphorus center is formed, which can then undergo S_N1 type rearrangement to the acyl azide by intramolecular transfer of the azide from the phosphorus to the carbonyl.[76, 77]

The reaction is driven by the stability of the formed P-O bond over the initial P-N bond. The tris(acylazide) then undergoes Curtius rearrangement to give the isocyanate upon heating. Addition of benzyl alcohol then gives the benzyl carbamate (CBZ) derivative, which may be isolated and deprotected. However, there were some difficulties observed in the reaction and noted in the literature when attempting this process on the cyclohexyl framework.



Scheme 1.20. Curtius rearrangement of the azide of **9** to isonitrile and subsequent reaction with benzyl alcohol to give the desired CBZ carbamate (**11**).

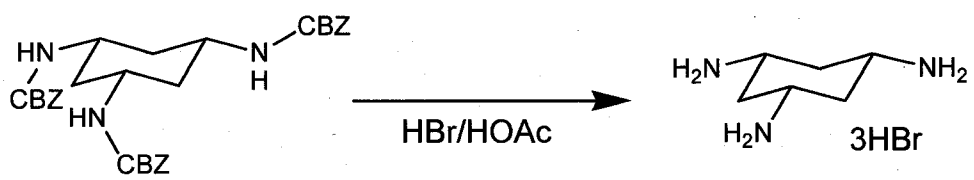
While it was noted that the azide and subsequent isocyanate derivative are soluble,[78] the viscosity and insolubility of the deprotonated acid gum hinders initial reactivity with DPPA. It is reported that diisopropylethylamine used in lieu of triethylamine may afford a more a soluble salt depending on the acid;[79] the effects of diisopropylethylamine with the triacid are currently under examination.

Some work has been done toward isolating the isocyanate as has been successfully done in the literature with analogous systems, including Kemp's triacid.[80, 81] If the triisocyanate is isolated, the amine could be directly generated

by simple hydrolysis. Isocyanates react with water to form unstable carbamic acid which spontaneously decarboxylates to give the amine.[82, 116]

However, isolating the triisocyanate has thus far been unsuccessful due to the multiplicity of possible reaction intermediates. There are three substituents on the triacid that must undergo the azide transfer reaction and subsequent conversion to the isocyanate. While the conversion to the isocyanate proceeds fairly rapidly under heating, the initial azide-transfer reaction does not occur quantitatively, even with single acid systems.[75] Thus, mono-, di-, or the desired triisocyanates may all be present, as seems to be indicated by the complex ^1H NMR spectra of the reaction mixture.[78]

Another improvement under consideration is the use of less bulky alcohols to form the carbamate. The cyclohexyl group attached to the isocyanate inhibits the approach of bulky alcohols via steric interactions, slowing down the reaction rate. It is reported that with t-butanol, the presence of the cyclohexyl monoisocyanate was still observed by IR spectroscopy even after prolonged heating.[75] This is less of a problem with benzyl alcohol due to its primary functional group, but substituting an even less-hindered alcohol may increase the yield of the subsequent carbamate over that of CBZ-tach .



Scheme 1.21. Deprotection of the carbamate (**11**) to give the trihydrobromide salt (**12**).

If tach-CBZ can be obtained reliably, we note that the deprotection of CBZ-tach to the tribromide salt proceeds nearly quantitatively with HBr/HOAc. Even with unreliable yields, this is currently the most promising route to manufacture large quantities of *cis, cis*-1,3,5-triaminocyclohexane for ligand synthesis.

CHAPTER 2

SOLUTION PHASE COMPLEXATION OF TACHENIPR, TACHENME, AND TACHENPH

INTRODUCTION

The triaminocyclohexyl (tach) framework offers an excellent base on which to build octahedral first-row divalent transition metal chelators. The addition of various ligating arms to the three amine groups allows flexibility in design while giving the benefit of a more rigid organized base: the structure favors an octahedral coordination sphere where preorganization increases the stability of the complex.[83] The amine donors on the ring preferentially bind to borderline acids such as divalent iron, copper, and zinc, over harder (iron (III)) or softer (copper(I)) acids, and the electronics of this favorable situation may be adjusted according to the nature of the added arms.[84]

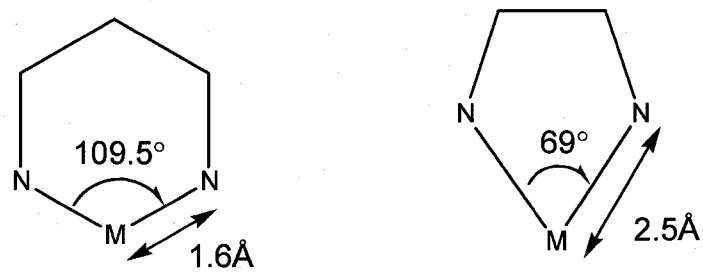
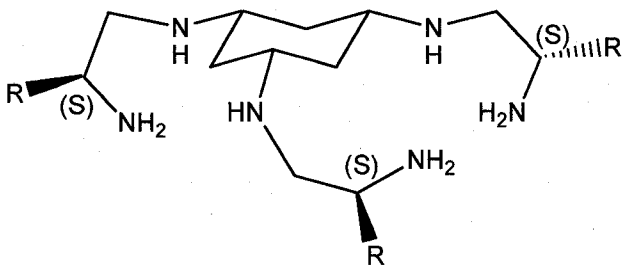
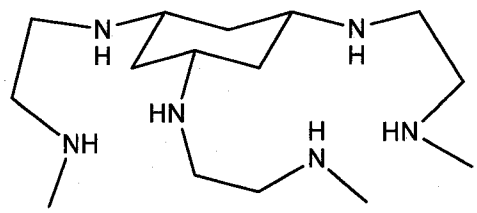


Figure 2.1. Ideal geometries of 5- and 6-member chelate rings containing M-N bonds based on the low strain conformer of cyclohexane and the direction of the nitrogen lone pairs.[83]

The coordination of a metal to the tach framework gives three six-membered chelate rings. Six-membered chelate rings have shown to have decreased complex stability compared to an analogous five-membered ring for divalent zinc, copper, iron and nickel.[85] A six-membered ring is analogous to a cyclohexane ring: it requires a short bond distance as well as a large bite angle, and this is unfavorable to most metals except for those similar in size to carbon.[83] However, this effect is most marked in larger metal ions than the first row transition metals, and the preorganization of the tach framework and the axial ring flip arrangement of the ligand arms gives a stable coordination environment that counteracts some of the strain of the six-membered chelate rings.[86]





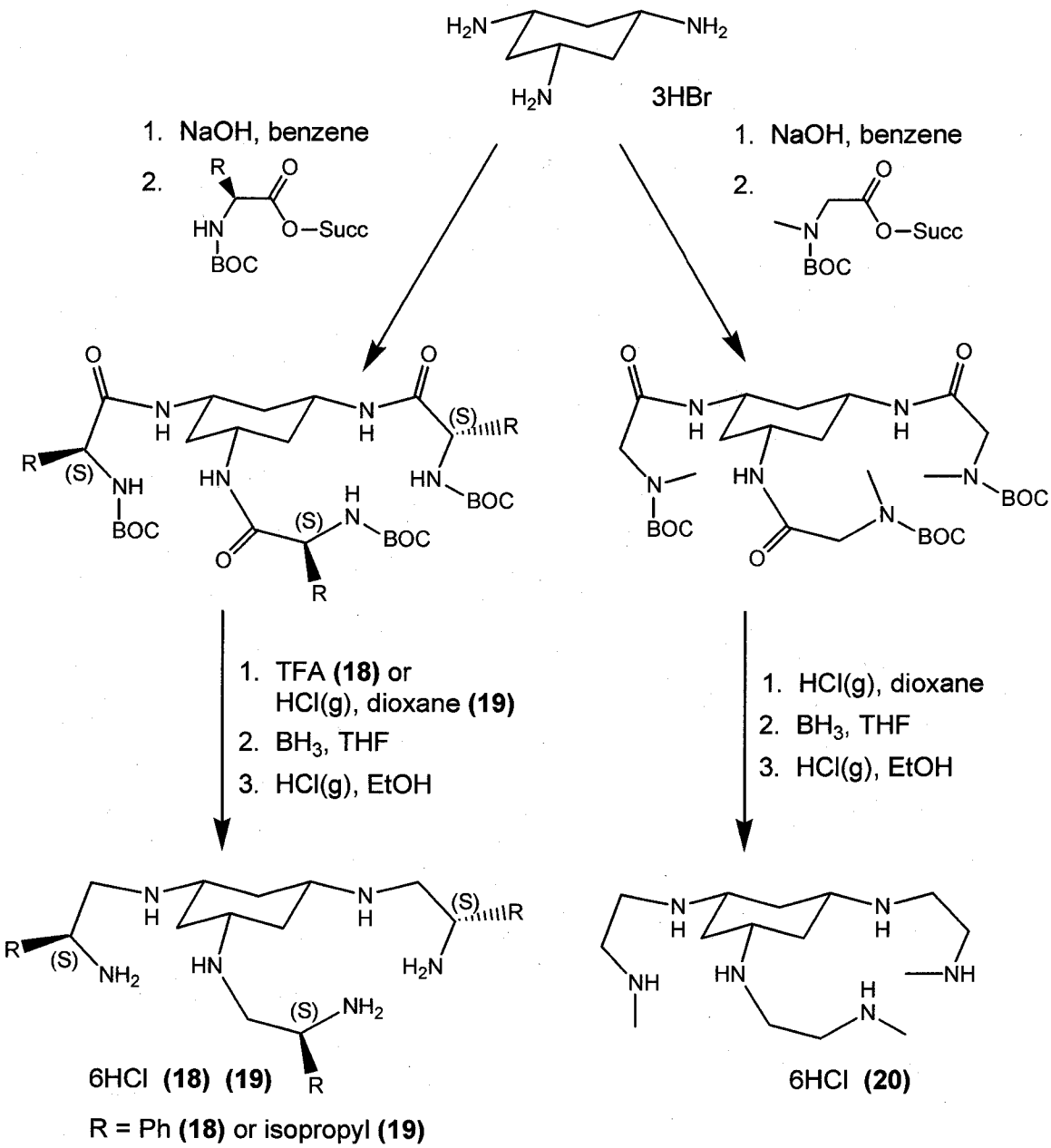
R = Ph tachenPh·6HCl **(18)**
R = isopropyl tacheniPr·6HCl **(19)**
R = H tachen·6HCl
R = methyl tachpn·6HCl
R = benzyl tachbn·6HCl

tachenNMe·6HCl **(20)**

Figure 2.2. Tach-based ligands with substituted aminoethyl arms. The solution phase complexation of **18**, **19** and **20** have been evaluated and will be discussed. The other ligands have been reviewed previously in the literature.[8]

It has been shown that the N6 chelator tachpyr effectively complexes nickel, copper, zinc, and iron, and is likely cytotoxic due its metal binding properties.[9] In response to this finding, other N6 ligand systems based on tach have been synthesized in order to improve upon the cytotoxicity or the selectivity

of tachpyr. One series of these novel chelators were synthesized with 2-aminoethyl pendant arms where various *S*-chiral substituents were present on the ethylene section of the arm.[8, 87]



Scheme 2.1. Synthesis of tachenPh (**18**), tacheniPr(**19**), and tachenNMe(**20**) as reported in the literature.

TachenPh and tachenNMe have been previously evaluated in the literature for their cytotoxicity as anti-angiogenic agents, while tachen, tachpn, and tachbn were also evaluated for their efficacy as iron-complexing agents. The cytotoxicity data is shown in Table 2.1. and is evaluated according to amount of chelator required to induce death in 50% of the cells in the sample (IC_{50}): the less chelator necessary, the more cytotoxic the molecule. While it is apparent that none of these have thus far matched the cytotoxicity of tachpyr, as expected, alteration of the chelate arms to adjust bite angle, donor atoms, and sterics all change the ligating properties of the ligand as well as its cellular distribution and its potential cytotoxic effects.[8]

	Anti-angiogenic assays			Iron deprivation assays	
	HUVEC (human umbilical vein endothelial)	NIH3T3 (normal fibroblast)	U251 (human glioma tumor)	Hela (human cervical tumor)	MBT2 (mouse bladder tumor)
Tachpyr	1	2.1	0.35	5	6
Tachen	1000	1000	1000	200	
Tachpn	8	20	9	28	55
Tachbn	3	4	1	8	10
TachenPh	20	20	20		
TachenNMe	1000	1000	1000		

Table 2.1. Comparison of the cytotoxicity of tach-based chelators based on μM concentration IC_{50} measurement in a variety of cell lines as reported in the literature.[4, 8, 87]

One method for evaluating the potential effect of these chelators is by the assessment of the UV-Vis spectral data from the complex in solution.[7, 8, 10] The

absorbance spectrum of a metal complex is related to the strength of the ligand-metal bonds, and a comparison of similar coordination environments can give a qualitative assessment of the relative strengths of the complexes. This method depends on the ability to monitor the Δ_o transition, which may be obscured by other absorption bands. However, in most cases, the higher the ligand field strength, the more blue-shifted the absorption peaks.[88]

While this technique may give some initial feedback regarding the efficacy of the chelator, there are other important factors in ligand design that influence cell toxicity besides the binding affinity of a molecule. The cellular distribution of the ligand is based on the lipophilicity of the substituents on tach, which alter the cellular distribution of the molecule in vivo. The small size of the chelators allow passage into the cytosol via the cell membrane, but increased lipophilicity increases the facility of crossing cell membranes and thereby increases cellular penetration.[11]

Nonetheless, understanding the metal-complexation of a ligand is the first step in evaluating its potential cytotoxicity. At this time, a preliminary evaluation of tachenPh, tachenNMe, and tacheniPr has been completed involving solution phase complexation with divalent nickel and copper as well as an assessment of the ligand field strengths of the chelators. The data and analysis leading to these assessments are covered in this chapter.

EXPERIMENTAL

I. Materials and Methods

All chemicals listed below were of research grade or of spectro-quality grade and were generally obtained from commercial sources and used without further purification except for those described as follows. Diethyl ether was refluxed over sodium metal and distilled immediately before use. Water was deionized by reverse-osmosis and by anion and cation exchange (E-pure model D4641, Barnstead). TachenMe, tacheniPr, and tachenPh were prepared by Dr. Martin Brechbiel et al. at the NIH and kindly donated.

Solution UV/Vis/NIR spectra were measured using a Varian Cary-50 Bio UV/Vis spectrometer with 1 mL quartz cuvettes (1 cm path length). Spectral data are given in the results and discussion section. Electrospray ionization (ESI) mass spectra in the positive ion detection mode were obtained with a Finnegan LCQ classic instrument with dual optical Paul traps and Picoview nanospray source.

II. Solution phase complexation

Ni(II) species. L•6HCl in 0.1 M MOPS (pH=7.3) (L= tachenPh, tacheniPr, or tachenMe, 100 μL, 0.1 M) was neutralized with Na_2CO_3 (100 μL, 0.3 M), and $NiCl_2•6H_2O$ (100 μL, 0.1 M) was added producing a clear pink solution in the case of tachenMe and tacheniPr and pink-white precipitate with tachenPh. The first two were diluted with 0.1 M MOPS (700 μL) to a final reactant concentration of $1x10^{-2}$ M. Acetonitrile (400 μL) was added to the tachenPh solution followed

by warming (60°C, 5 min) to afford a clear pink solution. A further 300 μL of 0.1 M MOPS was added to the solution for a final concentration of $1x10^{-2}$ M.

Cu(II) species. L•6HCl in 0.1 M MOPS (pH=7.3) (L= tachenPh, tacheniPr, or tachenMe, 20 μL, 0.1 M) was neutralized with Na_2CO_3 (20 μL, 0.3 M), and $Cu(ClO_4)_2•6H_2O$ (20 μL, 0.1 M) was added producing a clear deep purple solution in the case of tachenMe and tacheniPr and blue-purple precipitate with tachenPh. The first two were diluted with 0.1 M MOPS (940 μL) to a final reactant concentration of $2x10^{-3}$ M. Acetonitrile (400 μL) was added to the tachenPh solution to afford a clear lavender solution. A further 540 μL of 0.1 M MOPS was added to the solution for a final concentration of $2x10^{-3}$ M.

III. Isolated Ni(II) complex of tachenPh

[Ni(tachenPh)](ClO$_4$)$_2$ (21). TachenPh•6HCl (0.03528 g, 0.0500 mmol) was dissolved in 0.5 mL of 0.3 M aqueous sodium carbonate. The solution was warmed at 60°C for 5 min and gas evolution was noted. The solvent was removed under vacuum to leave a white residue. Under nitrogen, the neutralized ligand was extracted into 3 mL of anhydrous ethanol and 0.5 mL of 0.1 M $Ni(ClO_4)_2$ in ethanol was added giving a voluminous white precipitate. The solution was stirred at 60°C for 5 min to afford a pale pink solution with some white precipitate and 1 mL of acetonitrile was added to further dissolve the precipitate. The pink supernatant produced pale pink microcrystals by vapor phase ether diffusion. These were recrystallized from acetonitrile and slow ether diffusion as pale pink needles in 11% yield. MS(ESI):m/1, 643 amu ($M - ClO_4^-$); m/2, 272 amu ($M - 2ClO_4^-$).

RESULTS AND DISCUSSION

I. Tach Chelators Complex Ni(II) and Cu(II) in Aqueous Buffered Solution

The metal-complexation properties of tachenPh, tacheniPr, and tachenNMe in solution were evaluated by UV-Vis spectroscopy. These complexes had been previously evaluated in the literature for their cytotoxic effects in vitro,[87] but their metal complexation chemistry had not been probed. The hexahydrochloride salts were neutralized using sodium carbonate to give the free ligand in a buffered aqueous solution (1.0 M MOPS buffer, pH=7.3). The appropriate metal salt was added, predissolved in the same aqueous media. The complexes of tacheniPr and tachenNMe resulted in colored solutions indicative of the soluble nickel or copper complexes, while tachenPh gave a precipitate that was dissolved with the addition of acetonitrile and slight warming to give a clear colored solution. The final analytical concentrations ($1x10^{-2}$ M for the nickel complexes, $2x10^{-3}$ M for the copper complexes) were achieved by addition of further 1.0 M MOPS, and each sample was evaluated immediately. The complexes were evaluated in primarily aqueous buffered solution in order to more closely compare to biological conditions. While the addition of acetonitrile was necessary to induce the solvation of the tachenPh complexes, the solution was primarily aqueous in composition, and previous complexes have been evaluated in a similar manner.[8]

The spectral data was consistent with six-coordinate complexes for all except $[Cu(tachenPh)]^{2+}$, as is discussed below, and the ligands would be expected to react similarly with divalent iron and zinc.

The species were stable at room temperature. There was no change in peak wavelengths and only slight changes were exhibited in absorbance after twenty days. The changes in absorbance were due to small amounts of precipitation of the metal-complexes according to their relative lipophilicity. The most lipophilic molecule, tachenPh, had the greatest overall drop of 13% in the reduction of absorption values since increasing lipophilicity leads to lower solubility in aqueous solutions at pH 7.3.

II. Electronic Spectra of Solution Ni(II) Complexes

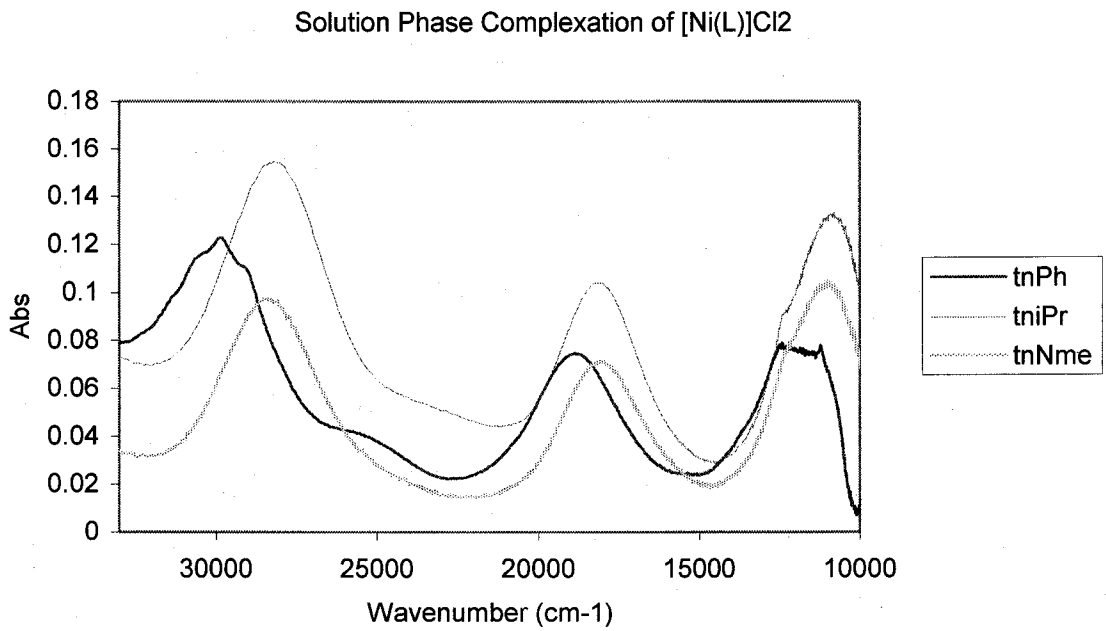


Figure 2.3. UV-Vis spectra of the solution phase nickel(II) complexation of tachenPh (**18**, tnPh), tacheniPr (**19**, tniPr), and tachenNMe (**20**, tnNMe) in aqueous solution.

The solution-phase nickel complexes of tachenPh, tacheniPr, and tachenNMe all gave UV-Vis spectra characteristic of octahedral coordination environments.[89] According to the relative positions of the low energy peaks, as well as the other peaks, tachenPh is a stronger field ligand than either tacheniPr or tachenNMe for nickel(II). There are three distinct peaks resulting from transitions in the *d*-orbitals; these are normally forbidden due to the Laporte selection rule, but the vibrations in the metal-ligand bonds temporarily distort the symmetry of the complex allowing some transitions.[84]

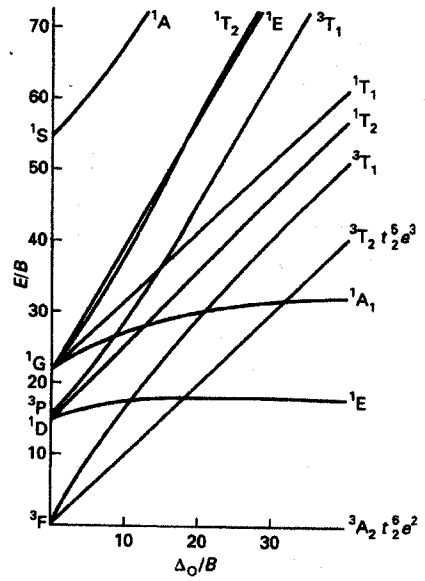


Figure 2.4. Tanabe-Sugano diagram of a d^8-metal, such as nickel(II), showing the available transitions.[90]

Usually, the lowest energy absorption peak may be used to calculate the strength of the metal-ligand bond as it represents the energy gap or ligand field strength (Δ_o) between the lowest energy t_{2g} orbitals and the high energy e_g orbitals in octahedral complexes. However, in these complexes, the band has a shoulder. The shoulder appears most clearly with tacheniPr and tacheNMe and

results from a normally forbidden transition between different spin multiplicities, the $^3A_{2g}$ to the ^1E_g, which is observed due to spin-orbit coupling.[91] The $^3A_{2g}$ to the ^1E_g transition can be seen in the Tanabe-Sugano diagram (Figure 2.4). Because it appears on the high energy side of the lowest energy peak of tacheniPr and tachenNMe, these complexes fall somewhere on the lower energy side of Δ_o/B axis, before the spin crossover of the ^1E_g and the $^3T_{2g}$ transitions. However, it is not possible to distinguish between the two complexes as far as which is the stronger field ligand, as their low energy peaks occur at the same wavelength, and therefore have equivalent values for Δ_o.

TachenPh appears not to exhibit a similar shoulder, showing a broadened low energy peak, however it is likely that the intensity of the $^3A_{2g}$ to the ^1E_g peak has grown as the ligand field strength (Δ_o) of the chelator has increased. As noted in the Tanabe-Sugano diagram (Figure 2.4), as Δ_o increases, the difference in intensity between $^3A_{2g}$ to the ^1E_g and the $^3A_{2g} \rightarrow {}^3T_{2g}$ diminishes as the spin crossover of the ^1E_g and the $^3T_{2g}$ transition occurs; this would account for the broadening of the low-energy peak.[91] The $^3A_{2g} \rightarrow {}^3T_{1g}$ (F) and the $^3A_{2g} \rightarrow {}^3T_{1g}$ (P) peaks are shifted to higher energy consistent with the stronger field strength of tachenPh.[90]

The peaks were all assigned according to previous literature work on other similar N6 octahedral ligands and compared to analogous chelate systems.[7, 92] Their shifts are comparable to the other chelators in the tachen series, tachen, tachpn, and tachbn, whereas the field strengths of the ligands are significantly

stronger than the trisethylenediamine complex due to the preorganization of the cyclohexyl framework.

	υ/ $10^{-3}cm^{-1}$	Assignment	Color
$[Ni(tachenPh)]^{2+}$ [a]	11.2 18.9 29.9	$^3A_{2g} \rightarrow {}^3T_{2g}$ (F), $^3A_{2g} \rightarrow {}^1E_g$ (D) $^3A_{2g} \rightarrow {}^3T_{1g}$ (F) $^3A_{2g} \rightarrow {}^3T_{1g}$ (P)	Pink
$[Ni(tacheniPr)]^{2+}$	10.8 18.1 28.2	$^3A_{2g} \rightarrow {}^3T_{2g}$ (F), $^3A_{2g} \rightarrow {}^1E_g$ (D) $^3A_{2g} \rightarrow {}^3T_{1g}$ (F) $^3A_{2g} \rightarrow {}^3T_{1g}$ (P)	Pink
$[Ni(tachenNMe)]^{2+}$	11.0 18.0 28.5	$^3A_{2g} \rightarrow {}^3T_{2g}$ (F), $^3A_{2g} \rightarrow {}^1E_g$ (D) $^3A_{2g} \rightarrow {}^3T_{1g}$ (F) $^3A_{2g} \rightarrow {}^3T_{1g}$ (P)	Pink
$[Ni(en)_3]^{2+}$	10.6 17.5 28.1	$^3A_{2g} \rightarrow {}^3T_{2g}$ (F), $^3A_{2g} \rightarrow {}^1E_g$ (D) $^3A_{2g} \rightarrow {}^3T_{1g}$ (F) $^3A_{2g} \rightarrow {}^3T_{1g}$ (P)	Pink
$[Ni(tachen)]^{2+}$	11.6 19.2 29.5	$^3A_{2g} \rightarrow {}^3T_{2g}$ (F), $^3A_{2g} \rightarrow {}^1E_g$ (D) $^3A_{2g} \rightarrow {}^3T_{1g}$ (F) $^3A_{2g} \rightarrow {}^3T_{1g}$ (P)	Pink
$[Ni(tachpn)]^{2+}$	11.3 19.6 29.7	$^3A_{2g} \rightarrow {}^3T_{2g}$ (F), $^3A_{2g} \rightarrow {}^1E_g$ (D) $^3A_{2g} \rightarrow {}^3T_{1g}$ (F) $^3A_{2g} \rightarrow {}^3T_{1g}$ (P)	Pink
$[Ni(tachbn)]^{2+}$	11.4 19.1 29.9	$^3A_{2g} \rightarrow {}^3T_{2g}$ (F), $^3A_{2g} \rightarrow {}^1E_g$ (D) $^3A_{2g} \rightarrow {}^3T_{1g}$ (F) $^3A_{2g} \rightarrow {}^3T_{1g}$ (P)	Pink
$[Ni(tachpyr)]^{2+}$ [b]	11.4 19.6	$^3A_{2g} \rightarrow {}^3T_{2g}$ (F), $^3A_{2g} \rightarrow {}^1E_g$ (D) $^3A_{2g} \rightarrow {}^3T_{1g}$ (F)	Violet

[a]Mixture of MOPS and acetonitrile. [b]Methanol.

Table 2.2. Peak assignments for the UV-Vis spectra of nickel(II) complexes of **18**, **19**, **20** and selected reference compounds.[8, 87] Solvent system is 0.1M MOPS (pH=7.3) unless otherwise indicated.

The nickel(II) perchlorate complex of tachenPh was isolated and identified using ESI-mass spectroscopy. Dissolving crystals of $[Ni(tachenPh)](ClO_4)_2$ in DMSO gave a pink solution with a concentration of $1x10^{-2}$ M, equivalent to the concentration expected to have formed in the solution complexation reaction.

The UV-Vis spectrum of the complex in DMSO matched that of the solution phase species.

III. Electronic Spectra of Solution Cu(II) Complexes

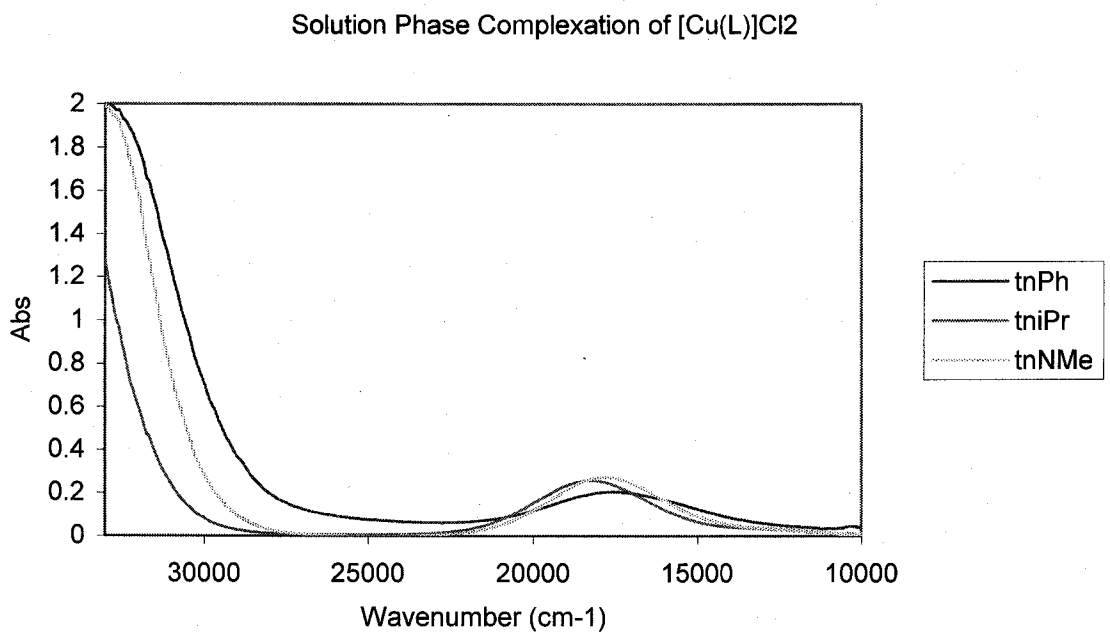


Figure 2.5. UV-Vis spectra of the solution phase copper(II) complexation of tachenPh (**18**, tnPh), tacheniPr (**19**, tniPr), and tachenNMe (**20**, tnNMe) in aqueous solution.

The spectra of the copper(II) solution-phase complexes of tachenPh, tacheniPr, and tachenNMe show a high energy charge transfer band as well as a less strongly absorbing band around 17000-18000 cm^{-1} associated with *d-d* orbital transitions. For all three ligands the *d-d* orbital transition band is broadened, indicating that the peak is made up of more than one absorption band. This is most obvious in tachenPh, where the peak appears to have a shoulder on the low-energy side.

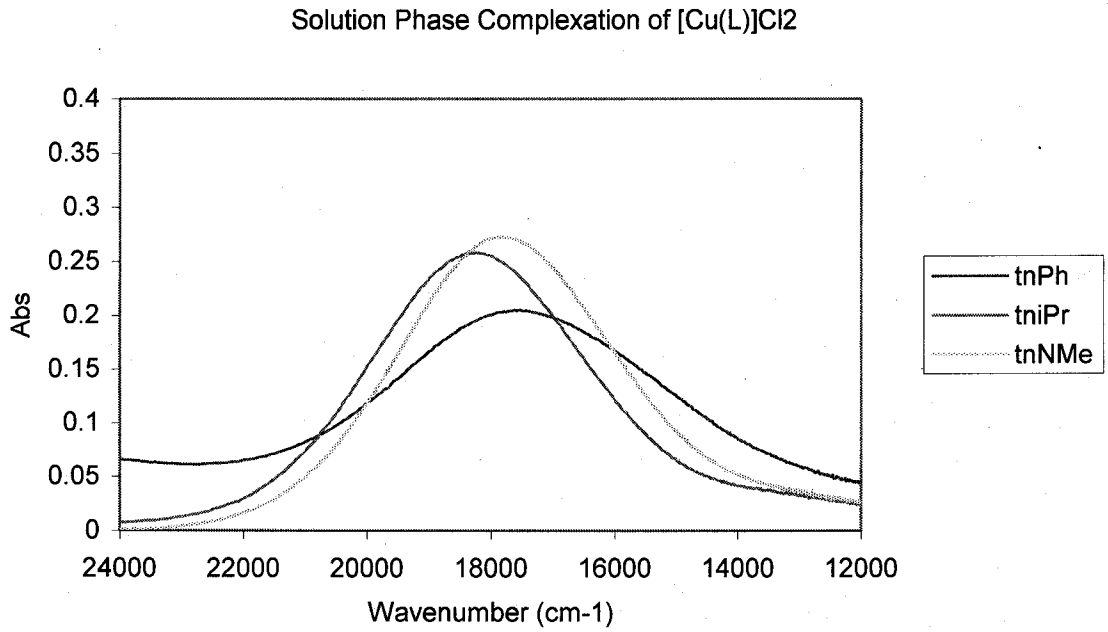


Figure 2.6. UV-Vis spectra of the solution phase copper(II) complexation of **18**, **19**, and **20** in aqueous solution, focusing on the *d-d* transitions band.

Copper complexes typically show one large broad band for six-coordinate complexes.[89] While it might be expected that a perfect octahedral d^9 complex would exhibit only one transition, the metal tends to undergo Jahn-Teller or other distortion, in which the M-L bond along one axis is elongated, resulting in the stabilization of two e_g electrons, while another is destabilized by the same amount.[48] This allows for four transitions and a much more complex spectrum than anticipated, from which the Δ_o can be estimated.

While energy of the broadened bands indicates that the tacheniPr and tachenNMe complexes are tetragonally distorted octahedrons, the geometry of tachenPh is less definite. Identification of the geometry of copper complexes by UV-Vis spectral data is difficult due to the flexibility of copper coordination spheres which allows for multiple species, including five-coordinate.[93] Generally,

square pyramidal (C_{4v}) and elongated tetragonal octahedrons give peaks in the range of 15500 to 19800 cm^{-1},[93, 94] whereas trigonal bipyramidal (D_{3h}) has a lower energy maximum absorbance with a high energy shoulder as is seen with Cu(tachbn)]$^{2+}$.[8, 89] The absorbance peak for tachenPh in the high energy region indicates that it has either square pyramidal or typical elongated octahedral geometry; the asymmetric shape and shoulder on the low energy side suggest that it may be 5-coordinate square pyramidal.[95]

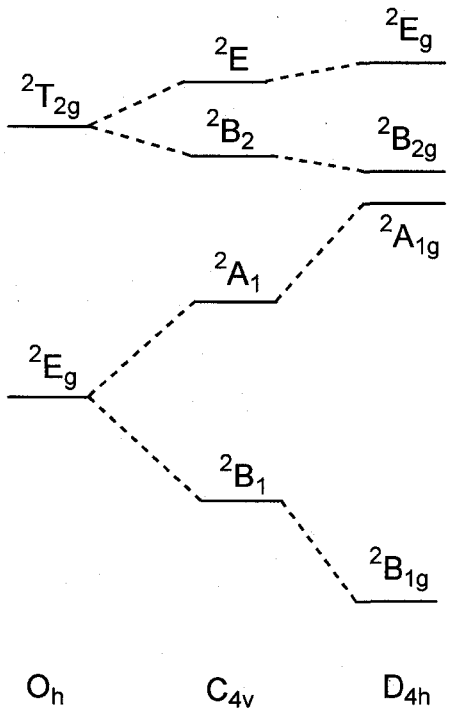


Figure 2.7. Energy-level diagrams for copper(II) in octahedral (O_h), square pyramidal (C_{4v}), and tetragonally distorted octahedral (D_{4h}) coordination spheres.[84, 96]

Since the copper complex of tachenPh gives an absorption peak at lower energy than either tachenNMe or tacheniPr it is a comparatively weaker field ligand. This decrease in energy supports the presence of a five-coordinate copper complex. A decrease in band energy indicates smaller *d*-orbital splitting,

which is typical of a shift from tetragonal (D_{4h}) to square planar geometry (C_{4v}, Figure 2.5).[93]

None of the copper complexes were isolated, but the solution UV-Vis data suggests that their structures would be similar to those reported for the tachen and tachpn copper(II) complexes reported in the literature (Table 2.3).

	υ/ $10^{-3}cm^{-1}$	Assignment	Color
$[Cu(tachenPh)]^{2+}$ [a]	17.5	$^2E_g \rightarrow {}^2T_{2g}$	Lavender
$[Cu(tacheniPr)]^{2+}$	18.2	$^2E_g \rightarrow {}^2T_{2g}$	Purple
$[Cu(tachenNMe)]^{2+}$	17.8	$^2E_g \rightarrow {}^2T_{2g}$	Purple
$[Cu(tachen)]^{2+}$	18.0	$^2E_g \rightarrow {}^2T_{2g}$	Blue
$[Cu(tachpn)]^{2+}$	18.1	$^2E_g \rightarrow {}^2T_{2g}$	Blue
$[Cu(tachbn)]^{2+}$	15.9	$^2E_g \rightarrow {}^2T_{2g}$ with high energy sh.	Blue
$[Cu(tachpyr)]^{2+}$	15.1	$^2E_g \rightarrow {}^2T_{2g}$	Blue

[a]Mixture of MOPS and acetonitrile.

Table 2.3. Peak assignments for the UV-Vis spectra of copper(II) complexes of **18**, **19**, **20** and selected reference compounds.[8, 87] Solvent system is 0.1M MOPS (pH=7.3) unless otherwise indicated.

CONCLUSIONS

TachenPh was shown in the nickel(II) solution phase studies to be a slightly stronger field ligand than either tacheniPr or tachenMe, and performed better in the toxicity assays. However, the greater part of its toxicity is likely linked to its lipophilicity and better cellular distribution rather than differences in substituent effects, as was reported for tachbn.[8] The bulk of the isopropyl group compared with the planar nature of the phenyl ring may indicate a steric reason for the lower field-strength of tacheniPr. The methyl-groups on the primary amines of tachenNMe may also be subject to steric effects; the methyl groups may be close enough in the complex to interact, destabilizing the complex and lowering the absorption band energies. This may be related to the effect of *N*-

methylation on the secondary amine, but in this case the *N*-methylation is on the primary amine and the effects are not as pronounced.[7] Thus for Ni(II), the ligand field strength is tachenPh > tacheniPr ~ tachenNMe.

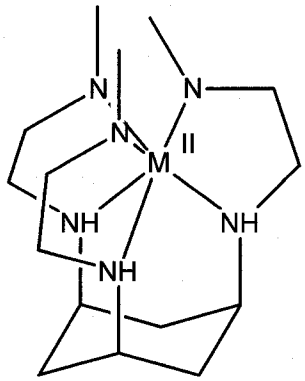


Figure 2.8. A divalent metal complex of tachenNMe illustrating the possible steric interactions of the methyl groups.

For divalent copper, the order changes such that: tacheniPr > tachenNMe > tachenPh. The changes in field strength are likely due to more pronounced steric effects from the distortion in the octahedral coordination sphere typical of Cu(II) complexes. Tetragonal distortion may be inhibited most severely by the phenyl substituents, such that a five-coordinate species predominates, as is indicated by the UV-Vis data. The steric effect of the *N*-methyl groups is apparently worse than the isopropyl groups for copper, as compared to the regular nickel octahedral environment where they give similar ligand field strengths.

The tachenPh, tacheniPr, and tachenNMe ligands are not as cytotoxic as tachpyr either in iron-sequestration related cell deprivation or as anti-angiogenic agents, nor do they show any improved selectivity over tachpyr to counterbalance their decreased toxicity. However, isolation of the complexes

and evaluation of the iron(II) and zinc(II) complexes would give a more complete picture of the metal chemistry associated with these tachen derivatives. Measurement of the lipophilicities of the chelators and complexes is also of interest due to greater cytotoxicities of tachenPh and tachbn as compared to tachen, tachpn, tachiPr, and tachNMe.

CHAPTER 3

SYNTHETIC APPROACHES TOWARDS TACHENSH, DACHENSH, AND ENSH

INTRODUCTION

As noted before, tachpyr and its related analogues, including those with 2-aminopyridyl arms, were evaluated for cytotoxicity based on iron chelation. However, the chelating arms on the cyclohexyl framework have the flexibility to accommodate several first-row, divalent transition metals, and tachpyr has been shown to form stable complexes with nickel, copper and zinc as well as iron. It is not surprising that copper(II) is strongly bound by tachpyr given its place in the Irving-Williams series, which is a qualitative ranking of the magnitude of stability constants based on a high-spin divalent metal in an octahedral coordination environment.[84]

$$Ba^{2+} < Sr^{2+} < Ca^{2+} < Mg^{2+} < Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$$

Figure 3.1. Irving-Williams series.[97]

Divalent copper usually forms the most stable complexes among first row transition metals, better than iron, zinc or nickel. This stability is primarily due to electrostatic effects, where the effective nuclear charge increases across the period such that divalent copper's is the greatest besides zinc. Copper(II) complexes are more stable than zinc because the d^9 metal has one unpaired

electron. Distortion of the normal octahedral coordination sphere alters the energy of the orbitals: it increases the stability of the complex by reducing the energy of the more populated orbitals and raises the energy of the less-populated orbitals. Since zinc has completely filled d-orbitals, there is no further stabilization gained by altering the energy of the orbitals. The splitting of degenerate orbitals and resulting stabilization of the complex is described by ligand-field theory (ligand field stabilization energy, LFSE).[90] The elongation of one of the axes in octahedral copper(II) complexes illustrates the Jahn-Teller distortion typical of the metal as described in Chapter 2.

Altering the donor atoms in a ligand can further support the complexation of copper(II) over other metal cations. Tetrathiomolybdate is a copper chelator that has been used to treat patients suffering from Wilson's disease, a genetic disorder that results in an accumulation of toxic levels of copper in the body.[98, 99] It has been shown to be highly selective towards copper(II) without disruption of other essential bioavailable divalent metal cations, such as iron and zinc.[98] The literature reports that the "soft" base thiol arms are a good electronic match for copper(II),[101] and many different thiol donors have been investigated for copper chelators along these lines.[100] According to this principle, a tach-based chelator with ethane thiol arms, *N,N',N"*-tri(2-mercaptoethyl)-*cis, cis*-1,3,5-triaminocyclohexane, tachENSH, was synthesized on a small scale by Brechbiel et al.[101]

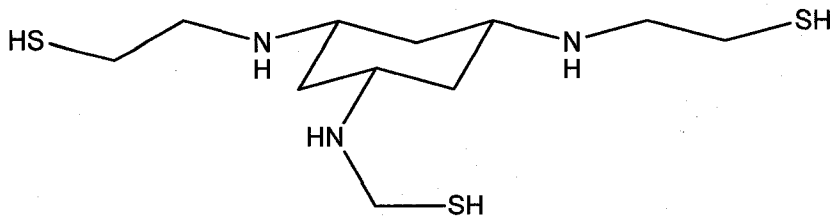


Figure 3.2. *N,N',N"*-tri(2-mercaptoethyl)-*cis, cis*-1,3,5-triaminocyclohexane (tachENSH).

The biological significance of copper chelation is that it opens up another approach to selective metal-deprivation and toxicity in cancer cells. The growth of new blood vessels (angiogenesis) is a tightly regulated process, and only occurs in normal adults during the healing of wounds, menstruation, and pregnancy.[102] Neoplastic cells, however, require the continual development of new blood vessels to sustain their proliferation and rapid metabolic rate. Copper acts as a cofactor in several stimulators of angiogenesis, including fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF),[103, 104] and restricting cellular access to copper(II) by the copper chelator penicillamine or a diet low in copper has been shown to inhibit angiogenesis.[105, 106] As such, several tach-based chelators have been evaluated in the literature for anti-angiogenic effects *in vitro*.[101]

TachENSH showed the greatest promise among all of the chelators tested, as it exhibited both toxicity and specificity compared to tachpyr, which, while toxic, did not show marked selectivity in most cell lines.[87, 101] TachENSH inhibited the proliferation of endothelial cells, the cell type associated specifically with angiogenesis. This inhibition was 10-fold more than the normal wild-type cell line and 18-fold greater than the neoplastic glioma line. Thus, it is selective

for the endothelial cells while exhibiting toxicity towards other cell lines at concentrations ten-fold that of where it is effective against the desired target.[101]

Although tachENSH has been evaluated for its effect *in vitro*, no metal-complexation studies have yet been done on this molecule. As it is anticipated that the ligand is effective due to copper chelation, this is a vital step in probing the use of the molecule as an anti-angiogenic agent; the molecule could become the framework for a new series of N3S3 ligands, analogous to the tachen series but with greater applicability. The limited quantity of tachENSH previously synthesized by our collaborators had already been used in toxicity studies, so it became critical to explore the synthetic pathway to the chelator.

The synthesis of the 1,3,5-*cis,cis*-triaminocyclohexane framework is described in Chapter 1, and the subsequent attempts to form the desired tachENSH product according to the literature[101] is evaluated stepwise in the following sections.

EXPERIMENTAL

I. Materials and Methods

All chemicals listed below were of research grade or of spectro-quality grade and were generally obtained from commercial sources and used without further purification except for those described as follows. (+/-)*Trans*-1,4-diaminocyclohexane was distilled before use. Diethyl ether was refluxed over sodium metal and distilled immediately before use. 1,4-Dioxane was freshly distilled from sodium metal under nitrogen and stored over molecular sieves.

Chloroform-d_1 ($CDCl_3$), deuterated water (D_2O), and dimethylsulfoxide-d_6 (DMSO-d_6) were obtained from Cambridge Isotope Laboratories and stored in a desiccator.

Water was deionized by reverse-osmosis and by anion and cation exchange (E-pure model D4641, Barnstead).

^1H and $^{13}C\{^1H\}$ NMR spectra were obtained on a Varian Mercury 400MHz FT spectrometer. Proton and C-13 chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS). Proton chemical shifts are described as follows: ppm (multiplicity, integral, and assignment). Elemental analyses were run by the Atlantic Microlabs (Atlanta, Georgia).

Molecular modeling was performed using Spartan '04 Version 1.0.3 on a PC workstation.

II. Synthesis of Poly-Mercaptoacetamides

A. Preparation of the thiol arm.

***S*-(1-Ethoxyethyl)mercaptoacetic acid (22).** Compound **22** was prepared according to the method of Kasina.[107] Under nitrogen, a catalytic amount of *p*-toluenesulfonic acid (0.133 g, 0.70 mmol) was added to a solution of thioglycolic acid (7.53 mL, 110 mmol) in 50 mL of dichloromethane while cooling to -24°C in a dry ice/ethylene glycol bath. To this stirred suspension, ethyl vinyl ether (9.70 mL, 100 mmol) in 50 mL of dichloromethane was added dropwise over 90 min while maintaining the bath temperature between -18 and -25°C. Upon completion of the addition, stirring was continued for 30 min at temperature, after which 80 mL of 0.1 M phosphate buffer (pH=7.0) was added. The reaction mixture was allowed to warm to room temperature over 10 min with stirring and then poured into a separatory funnel containing 300 mL ethyl acetate and 80 mL water. The layers were separated and the organic layer was washed 1x200 mL 5% w/w NaCl and then dried over $MgSO_4$. Removal of the solvent left 13.43 g of the product (81.8 mmol, 82% yield) as a clear colorless oil. The oil was used without further purification in the subsequent step. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.21 (t, 3H, $OCH_2C\underline{H}_3$), 1.56 (d, 3H, $CH(C\underline{H}_3)O$), 3.38 (m, 2H, $C\underline{H}_2COOH$), 3.52-3.75 (m, 2H, $OC\underline{H}_2CH_3$), 4.82 (q, 1H, $C\underline{H}(CH_3)O$), 11.79 (s, 1H, $COO\underline{H}$); ^{13}C NMR (400 MHz, $CDCl_3$): δ 14.90. 21.92, 30.03, 62.98, 81.17, 176.1.

***N*-Hydroxysuccinimidyl *S*-(1-ethoxyethyl)mercaptoacetate (23).** Compound **23** was prepared according to the procedure of Kasina.[107] *N*-Hydroxysuccinimide (4.85 g, 42.4 mmol) was added to a solution of *S*-(1-ethoxyethyl)mercaptoacetic acid (6.34 g, 38.6 mmol) in 100 mL of anhydrous THF under nitrogen. A solution of 1,3-dicyclohexylcarbodiimide (8.74 g, 42.4 mmol) in 65 mL THF was added to

the solution and the mixture was stirred for at least 2 hr at room temperature, during which time a voluminous amount of white precipitate formed. The precipitate was filtered away from the solution using an additional wash of 50 mL anhydrous THF and the filtrates were concentrated by rotary evaporation to yield a viscous oil containing trace amounts of precipitate. The oil was taken up in 75 mL ethyl acetate and chilled at 4°C for 1 hr, after which the residual precipitate was removed by filtration. The filtrates were washed 1x50 mL with 5% w/w NaCl and then dried over $MgSO_4$, where removal of the solvent left 8.323 g of a colorless viscous oil (31.9 mmol, 82%) as the product. The gum may be purified by flash chromatography using 2:1 ethyl acetate:hexanes on silica gel, however the crude product is acceptably pure for the subsequent reactions as the NMR spectra show little to no impurities. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.21 (t, 3H, $OCH_2C\underline{H}_3$), 1.57 (d, 3H, $CH(C\underline{H}_3)O$), 2.85 (s, 4H, succinimidyl $C\underline{H}_2$), 3.57 (m, 2H, $OOCC\underline{H}_2$), 3.73 (m, 2H, $OC\underline{H}_2CH_3$), 4.86 (q, 1H, $C\underline{H}(CH_3)O$); ^{13}C NMR (400 MHz, $CDCl_3$): δ 15.13, 22.40, 25.80, 26.74, 63.16, 81.13, 166.66, 169.15.

B. Reaction of active ester with amines.

***Cis, cis*-1,3,5-tris(1-ethoxyethylmercaptoacetamido)cyclohexane (24).**

Compound **23** was prepared according to the method of Camphausen.[101] Triaminocyclohexane tribromide (645 mg, 1.7 mmol) was treated with 5.2 mL 1.0 N NaOH and stirred to yield a clear solution, after which 100 mL benzene was added. Water was distilled off by Dean-Stark trap overnight after which the benzene was removed by vacuum rotary evaporation. Free triaminocyclohexane was extracted from the remaining salt by extracting with 15 mL anhydrous DMF.

N-hydroxysuccinimidyl *S*-(1-ethoxyethyl)mercaptoacetate (5.74 g, 21.9 mmol) in 10 mL of DMF was added dropwise to the solution of triaminobenzene, and was stirred overnight at ca. 45°C. The solvent was removed by vacuum rotary evaporation and the resulting gum was triturated with ethyl acetate to yield an off-white powder. The powder was collected by filtration and dried under vacuum to yield 1.60 g of the product (2.82 mmol, 38.5%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.12 (t, 4H, $OCH_2C\underline{H_3}$ and partial signals of the axial cyclohexyl methylene), 1.45 (d, 3H, $CH(C\underline{H_3})O$), 1.85 (br. d, 1H, equatorial cyclohexyl methylene), 3.13 (s, 2H, $OOCC\underline{H_2}$), 3.41, (m, 1H, axial cyclohexyl methine), 3.64 (m, 2H, $OC\underline{H_2}CH_3$), 4.79 (q, 1H, $C\underline{H}(CH_3)O$), 7.99 (d, 1H, $N\underline{H}$); ^{13}C NMR (400 MHz, $CDCl_3$): δ 15.39, 23.17, 32.06, 37.95, 45.48, 62.71, 81.31, 168.71.

(+/-)*Trans*-1,2-bis(1-ethoxyethylmercaptoacetamido)cyclohexane (25). *N*-hydroxysuccinimidyl *S*-(1-ethoxyethyl)mercaptoacetate (5.13 g, 19.6 mmol) dissolved in 25 mL DMF was added dropwise to a solution of (+/-)*trans*-1,2-diaminocyclohexane (1.13 g, 9.87 mmol) in 25 mL DMF. The pale yellow solution was stirred overnight with slight warming and then concentrated to a viscous gum by vacuum rotary evaporation. The gum was taken up in 50 mL ethyl acetate, washed 2x30 mL with 5% w/w NaCl, and the aqueous layers were backwashed with 1x30 mL ethyl acetate. The combined organic layers were dried over Na_2SO_4, concentrated to a yellow gum and triturated with diethyl ether to yield 946 mg of a light yellow powder as product (2.33 mmol, 23.6%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.10 (t, 4H, $OCH_2C\underline{H_3}$ and partial signals of the axial cyclohexyl methylene $NH_2CHC\underline{H_2}$), 1.19 (br. m, 1H, axial cyclohexyl methylene

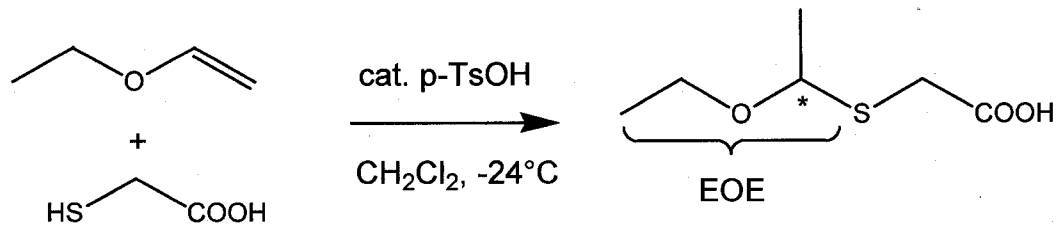
NH2CHCH2CH2),1.41 (d, 3H, CH(CH_3)O), 1.63 (br. m, 1H equatorial cyclohexyl methylene $NH_2CHCH_2CH_2$), 1.78 (br. d, 1H, equatorial cyclohexyl methylene NH_2CHCH_2), 3.06 (s, 2H, $COCH_2$), 3.40-3.63 (m, 2H, OCH_2CH_3), 3.52 (m, 1H, axial cyclohexyl methine), 4.75 (q, 1H, $CH(CH_3)O$), 7.80 (br. s, 1H, NH).

Bis(1-ethoxyethylmercaptoacetamido)ethylenediamine (26). *N*-hydroxysuccinimidyl *S*-(1-ethoxyethyl)mercaptoacetate (2.01 g, 7.69 mmol) suspended in 20 mL of DMF was added dropwise to a solution of ethylenediamine (231 mg, 3.85 mmol) in 20 mL DMF. The pale yellow solution was stirred overnight at room temperature and then concentrated to a crystalline gum. The gum was triturated with ethyl acetate to a pale yellow powder, which was collected by filtration and washed with ethyl acetate and then diethyl ether. The powder was dried under vacuum to yield 485 mg of product (1.2 mmol, 31%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.10 (t, 3H, OCH_2CH_3), 1.43 (d, 3H, $CH(CH_3)O$), 2.58 (s, 2H, $NHCH_2CH_2NH$), 3.14 (s, 2H, $OOCCH_2$), 3.32-3.66 (m, 2H, OCH_2CH_3), 4.77 (q, 1H, $CH(CH_3)O$), 8.03 (d, 1H, NH); ^{13}C NMR (400 MHz, $CDCl_3$): δ 15.58, 23.20, 26.01, 48.18, 62.78, 81.44, 173.54.

RESULTS AND DISCUSSION

I. Synthesis of the Thiol Arm of TachENSH

The synthesis of the thiol arm was completed following the procedures of Kasina in a two step process.[107] Since sulfides are nucleophilic and sensitive to oxidation, forming dimers or *S*-oxides, a suitable protecting group was necessary to carry the sulfur group through the synthetic process.[108] The ethoxyethyl (EOE) protecting group was used in the literature due to its easy removal under acidic conditions;[107] Camphausen also utilized it in the preparation of tachENSH for similar reasons, including its inertness when exposed to boranes.[109] The thioether is the most common thiol protecting group, e.g. benzyl chloride and substituted benzyl derivatives, which give the appropriate substituted *S*-benzyl derivatives.[110] These are attractive protecting groups because the resultant thioethers tend to be solids and are easier to collect and purify than ethoxyethyl-protected thioethers, which are usually oils; they may also be stored long-term without degradation.[111, 112, 113, 114] Unfortunately, the deprotection of benzyl thioethers typically requires sodium in liquid ammonia or reflux in acidic media with mercury(II) salts.[108, 110] Basic workup would make it more difficult to isolate tachENSH, and the use of mercury was to be avoided as much as possible. To avoid this difficulty, mercaptoacetic acid was protected with ethoxyethyl according to the literature procedure.[107]



Scheme 3.1. Synthesis of *S*-(1-ethoxyethyl)mercaptoacetic acid (**21**), as reported in the literature.[107]

Ethyl vinyl ether was added dropwise to a solution of mercaptoacetic acid in anhydrous dichloromethane with a catalytic amount of *p*-toluenesulfonic acid. After an aqueous workup, the desired product, *S*-(1-ethoxyethyl)mercaptoacetic acid, was isolated in good yield (82% yield) as a colorless free-flowing oil. The ^{1}H and ^{13}C NMR spectra matched the literature with only trace impurities that appeared to be from mercaptoacetic acid. Purification of the starting material or product was not attempted because the mercaptoacetic acid impurities were trace and due to the strong odor and corrosive nature of the starting material.

The ^{1}H NMR spectrum of the product clearly shows the two methyl peaks: the terminal methyl as a triplet, and the internal methyl as a doublet. The next set of peaks upfield is associated with the methylene adjacent to the carboxylic acid. Due to the chiral nature of the molecule, the two protons are diastereotopic to one another and would produce two doublets of different chemical shifts, but the peak pattern appears to be a quartet. The intensity of a quartet from an *N*+1 system would be in a ratio of 1:3:3:1, but this system shows a ratio of approximately 1:2:2:1. This is a splitting pattern characteristic of an AB system where the four signals do not have equal intensities, but show a "roof" or "dach" effect related to the coupling of the two protons which increases as the shifts of

the protons become closer together.[41] In this system the $\Delta\nu/|J_{AB}|$ ratio and the $|J_{AB}|$ coupling constant were calculated from the peak frequencies to be 2.6 and 15 Hz respectively.[115] The methylene protons on the ethoxyethyl protecting group show similar diastereotopic effects and present two multiplets between 3.52 and 3.75 ppm. The methine on the chiral carbon shows up further downfield as an easily identifiable quartet, and proved to be an excellent marker of the presence of the protected thiol throughout further manipulations of the product

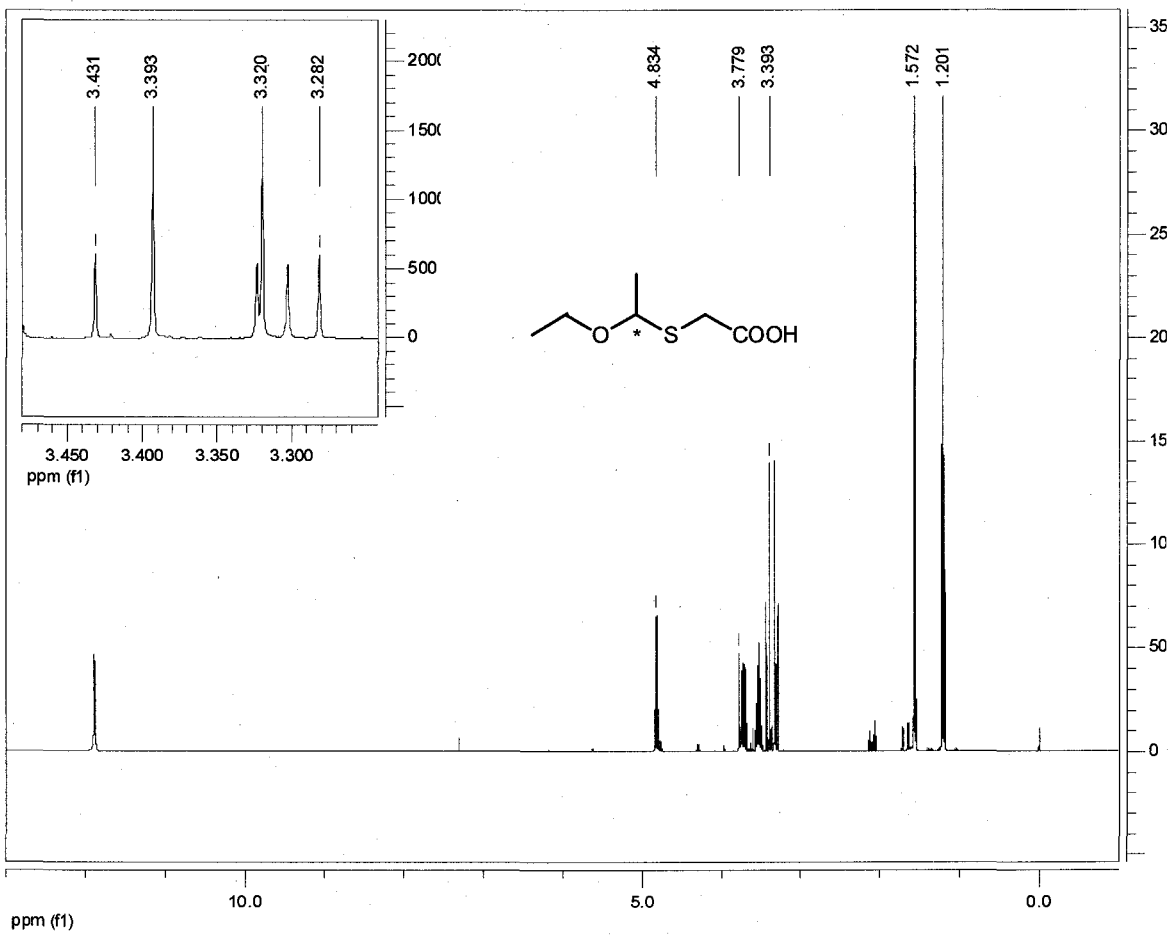
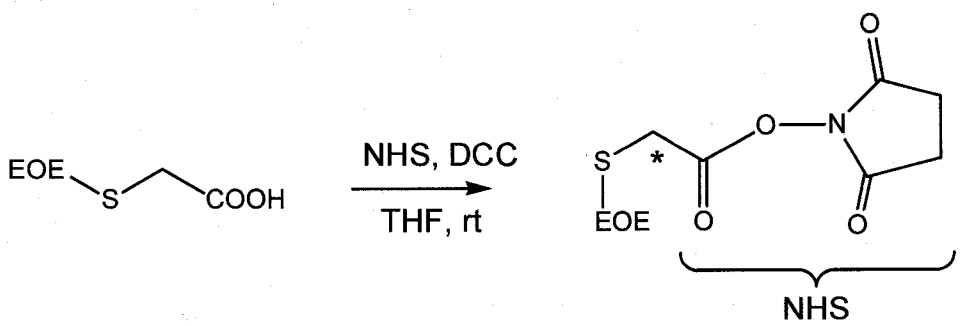


Figure 3.3. ^{1}H NMR spectrum of *S*-(1-ethoxyethyl)mercaptoacetic acid (**21**), with an inset of the AB spin system.

due to its distinct splitting pattern, isolation from other peaks, and its position on the carbon adjacent to the sulfur. The carboxylic proton shows up as the furthest downfield singlet.

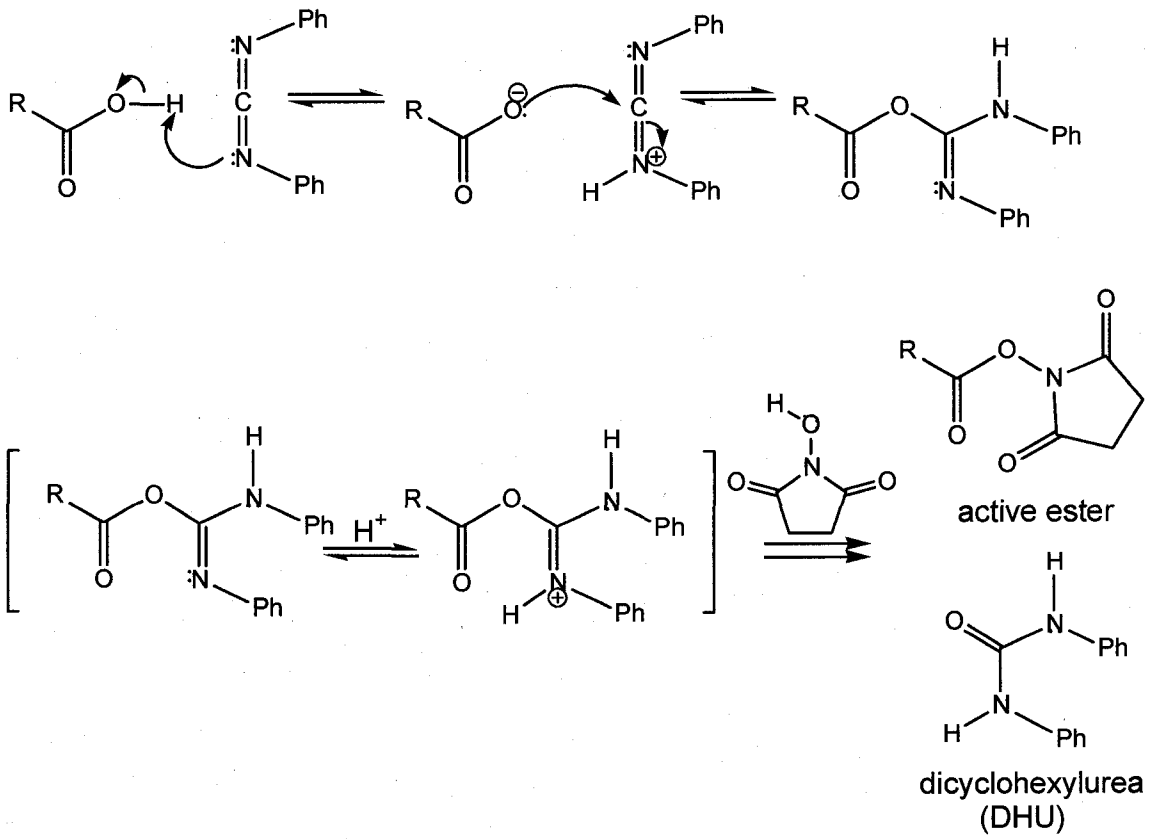


Scheme 3.2. Synthesis of *N*-hydroxysuccinimidyl *S*-(1-ethoxyethyl) mercaptoacetate (**22**), as reported in the literature.[107]

N-Hydroxysuccinimidyl *S*-(1-ethoxyethyl) mercaptoacetate (**22**) was prepared as reported in the literature, where *N*-hydroxysuccinimide (NHS) was added to a solution of the crude protected thiol in THF. A solution of 1,3-dicyclohexylcarbodiimide (DCC) in THF was added whereupon formation of a white precipitate was noted within minutes. The crude active ester was isolated after an aqueous workup as a pale, yellow, viscous oil, often with some residual white precipitate. The yields varied from 26% to 82%, but averaged 64%. Fortunately, the scale of the reaction gave the product in a reasonable amount no matter the yield.

The esterification of an acid with the dehydrating agent DCC and an alcohol is reported to give variable yields due to the formation of *N*-acylureas as side-products.[116] Deprotonation of the acid allows the carbonyl to attack of the electrophilic carbon of DCC to give an *O*-acylurea. The alcohol adds to this activated carboxylic acid, ultimately giving the desired ester and the stable

byproduct dicyclohexylurea (DHU). DHU is mostly insoluble in most solvents and precipitates out of solution as a white, pearlescent solid, driving the reaction to the desired ester product. However, the addition of the alcohol is slow enough that side reactions may occur. The predominant side reaction is acyl-migration in the *O*-acylurea, giving *N*-acylureas as side-products. This reaction may also be performed with amines instead of alcohols to give an amide as the product. In these cases, the reaction is more reliable, as an amine is a stronger nucleophile and the reaction proceeds more quickly, avoiding the slow acyl migration.[116]



Scheme 3.3. Proposed mechanism for the esterification of an acid with DCC and an alcohol.[116]

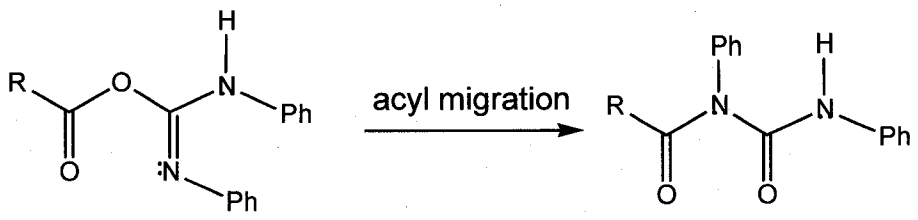
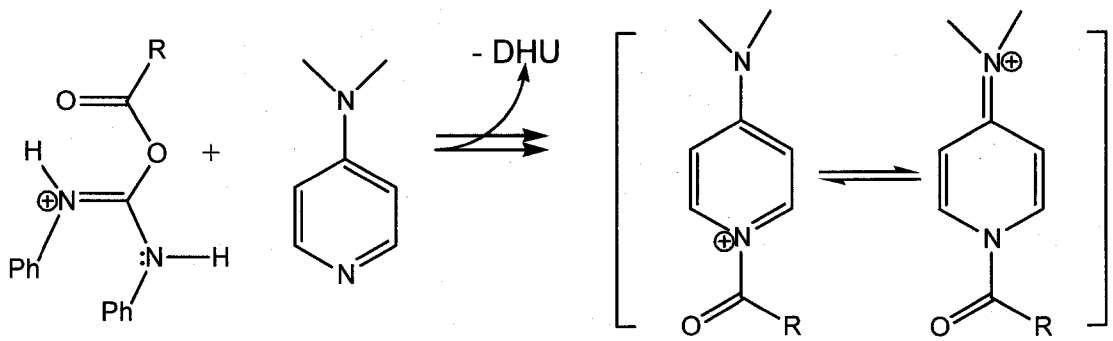


Figure 3.4. *N*-acyl ureas as an undesired side-product from the slow esterification of an acid using an alcohol.

Two different approaches were taken in order to improve the yields of the active ester, though neither of them proved to be more effective than the originally reported procedure. In the first attempt, the carboxylic acid was treated with one equivalent of sodium hydroxide to give the acid salt as a white powder. It was thought that the deprotonation of the acid might speed up the reaction process. The powder was treated with DCC and NHS as per the literature to give the crude active ester in only 49.5% yield. In the second case, 4-dimethylaminopyridine (DMAP) was added catalytically in a 10 mol% ratio as compared to the acid, in addition to the DCC.



Scheme 3.4. Effect of DMAP addition in DCC-coupled esterification reactions.

The literature reported that in some cases the addition of DMAP gave better returns of the desired ester.[117, 118] It has been proposed that DMAP reacts quickly with the *O*-acylurea, as it is a stronger nucleophile than the alcohol, to

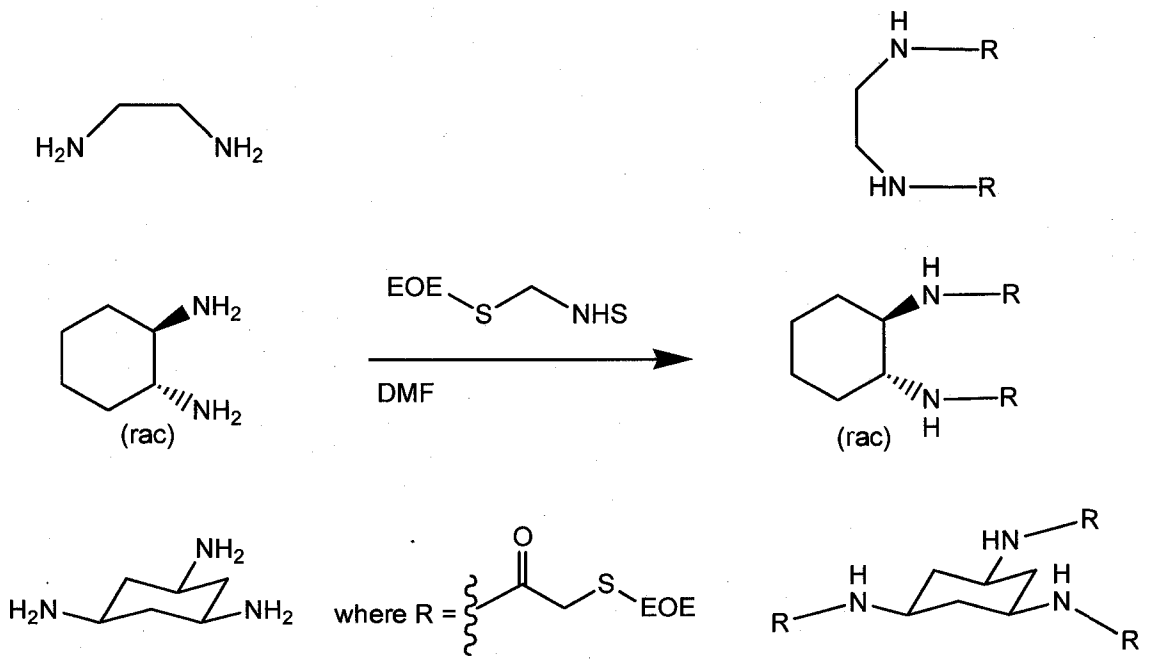
give DHU and an amide which cannot undergo acyl migration. The alcohol is then free to react with the amide, which releases DMAP upon formation of the ester.[117] However, the reaction gave a yield of 65.6% of the crude active ester, which did not show a significant enough improvement to warrant regular use in the reaction.

While the literature reported purifying the crude active ester by column chromatography to remove residual impurities,[107] the greatest impurity observed by ^{1}H NMR spectroscopy was DHU. DHU is a persistent impurity that is mostly insoluble in most solvents and is therefore slightly soluble in most solvents, including THF, dichloromethane, ethyl acetate, and water. Nonetheless, work with similar crude active esters indicates that DHU as a trace impurity does not impair subsequent peptide coupling reactions.[119] For this reason, purification of the active ester was not considered necessary.

II. Reactions of the Active Ester with Polyamines

Cis, cis-1,3,5-triaminocyclohexane was not immediately available for use upon completion of the active ester thiol arm, but two other readily available polyamines were treated with the active ester to test the ease of the peptide-coupling reaction and subsequent reduction to the amine. The N2S2 amide based on ethylenediamine and a *C*-methylated amide based on *trans*-1,2-diaminocyclohexane had been previously reported in the literature and were therefore useful models.[120]

The reactions were set up in a similar fashion to those reported for the synthesis of tachENSH, whereby the free amine was dissolved in DMF and a solution of the active ester was added. The crude amide was subsequently isolated by an aqueous workup in the case of *trans*-1,2-diaminocyclohexane (dach), and by concentration of the reaction solution and trituration with ethyl acetate for ethylenediamine. Similar products had been previously documented in the literature by other synthetic routes, so the reactions were not optimized beyond 40%. As no purification of the tach analogue was done before reduction in the literature procedure,[101] (+/-)*trans*-1,4-bis(1-ethoxyethylmercapto-acetamido)cyclohexane was used without purification to mimic the conditions used in the tach reaction and better evaluate the reaction.



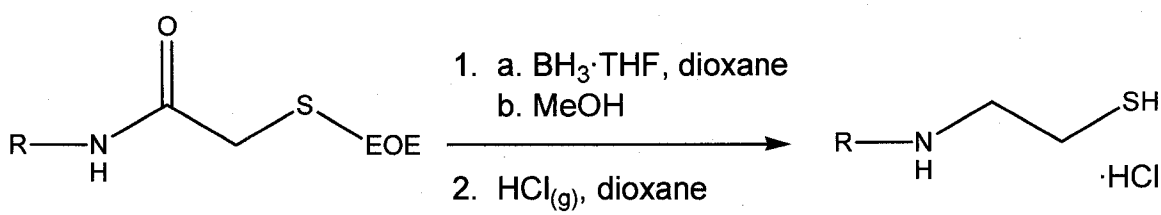
Scheme 3.5. Coupling of the active ester with various polyamines.

Cis, cis-1,3,5-tris(1-ethoxyethylmercaptoacetamido)cyclohexane was prepared according to the literature procedure.[101] The free amine was obtained by neutralization with 3 equivalents of aqueous sodium hydroxide and subsequent drying with benzene in a Dean-Stark trap. The benzene was removed and the amine was taken up in dry DMF, to which the active ester was added. After completion of the reaction, the resultant amide was triturated out of the concentrated solution with ethyl acetate to give a light tan powder that was shown to be the desired product by NMR.

III. Attempts to Reduce the Amide and Deprotect the Thiol

While the literature reports reduction of *cis, cis*-1,3,5-tris(1-ethoxyethylmercapto-acetamido)cyclohexane to the amine by use of borane in THF in good yields,[101] this was not reproducible in our laboratory because the product could not be isolated cleanly from the borane-adducts and some unreduced amide signals were noted in the ^{1}H NMR spectrum. Similar difficulties were also seen when the amide was originally prepared in the literature.[109] The amide was suspended in dry THF under nitrogen and treated with an excess of 1M BH_3•THF by syringe while cooling in an ice bath. After the solution had warmed to room temperature with stirring over one hour, the solution was refluxed for five days, after which methanol was added and the solution was stirred for one hour. The solution was concentrated and the clear, colorless gum was taken up in dioxane and treated with $HCl_{(g)}$ while stirring under nitrogen. The acidic solution was refluxed overnight and then cooled at 4°C to afford a pale pink powder, which was

proposed to be the product. This was never identified clearly by NMR due to the presence of borane adducts, and what appeared to be some unreacted amide. This problem was apparently seen in the initial preparation of the product,[109] but the issue has not yet been successfully resolved.

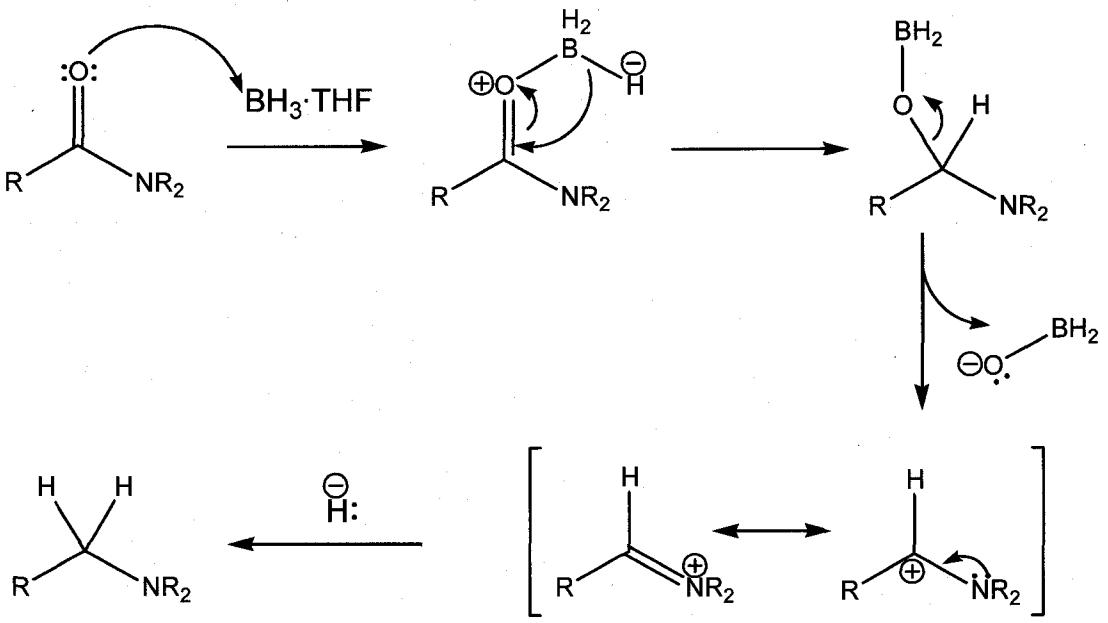


Scheme 3.6. Attempted reduction of the tach amide with borane.

Several steps were taken in order to improve the reduction process, but none proved to be effective in affording a clean product. Most of these trials were run using the prepared (+/-)*trans*-1,2-bis(1-ethoxyethylmercapto-acetamido)cyclohexane (**25**, dach amide), as it was readily accessible via the commercially available *trans*-1,2-dach framework, and was expected to react similarly to tach under the reduction and subsequent acidic workup conditions. Since the molecule has multiple secondary amino groups, it was expected that the dach framework would undergo similar chemistry as the tach framework due to the comparable sterics associated with the cyclohexyl framework.

Borane reduction of the amide with 1.0 M BH_3•THF is based on the oxophilic interaction of borane with the amide oxygen group. Electron-poor borane is initially present in solution as an adduct with tetrahydrofuran (THF) via its vacant p-orbital. However, the amide carbonyl is more reactive than the ether of THF, which promotes the formation of a borane-oxonium adduct over the borane-THF adduct. The electron density in the borane moiety of the new

adduct is located on the hydrogen atoms, being more electronegative than the boron center; this allows for a hydride-shift to the electrophilic oxonium carbon. The subsequent loss of the OBH_2 moiety affords the iminium cation. As the iminium cation is susceptible to reduction, hydride transfer from other borane species in solution gives the desired amine. However, the amine is still a strong Lewis base, and typically forms stable adducts with excess borane in solution that may be difficult to separate, as was found in the reduction of the dach and tach amides. The addition of excess methanol and heating is intended to break up these adducts by introducing a more favorable interaction of oxophilic borane with methanol, as opposed to the amine.



Scheme 3.7. Mechanism of the borane reduction of an amide.

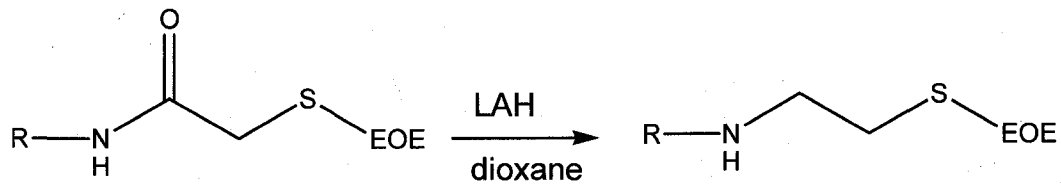
Since both the bisacetamido- and trisacetamidocyclohexanes have multiple reduction sites, it was proposed that several additions of borane over time would be more effective in reducing all of the amides, whereas a single

addition of an excess of borane would result in the formation of borane adducts with the amine product, thereby limiting the amount of available reducing agent remaining in solution. Thus, according to previous literature,[8, 121] the borane-THF solution was added in three separate additions with twelve hours between each addition. ^{1}H NMR spectra of the reaction mixtures showed better reduction of the amide by loss of the amide proton signal as compared to the reactions run with only one addition of the borane. The presence of borane-adducts, however, was persistent.

The dach reaction mixture was subjected to a lengthy methanolysis under reflux, in accordance with other borane reductions in the literature,[122] in order to promote the cleavage of the borane adducts by longer, more vigorous exposure to the alcohol. Initial ^{1}H NMR data indicated some reduction in the amount of borane-adducts, but the lengthy reduction time precluded extensive examination of this improvement, and the hydrochloride salt isolated following acidic workup gave a complex proton spectrum that did not unequivocally support the formation of the desired product.

Preparation of the BOC-protected amine was identified as a method to isolate the amine, but the results have thus far been inconclusive. Following methanolysis, a solution of di-*tert*-butyl dicarbonate was added and the solution was stirred overnight. By taking the resultant crude reaction mixture up in dichloromethane, the remaining borane adducts and other impurities were to be removed by an aqueous wash. The resultant oil was then treated with HCl gas in 1,4-dioxane to give a white salt, which gave a complex proton spectrum similar to

those previously seen. The salt generated may be able to be recrystallized under the appropriate conditions, but the generated thiols are reactive and extremely air sensitive, and thus the benefits of a recrystallization must be weighed against the degradation of the product under further manipulation. Preliminary evaluation of the results of recrystallization would be helpful at this stage in order to resolve the formation of the desired product.



Scheme 3.8. LAH reduction attempt with *cis, cis*-1,3,5-tris(1-ethoxyethyl-mercaptoacetamido)cyclohexane.

Schrock reported the successful reduction of tren-based amides to the corresponding amines using LAH.[123] This reductive method was unsuccessfully attempted with the tach derivative; the reduction was incomplete after 12 hours as noted by the persistence of the amide peak by ^{1}H NMR, and extending the duration of the exposure to LAH resulted in degradation of the starting material and a partially reduced intermediate.[119]

IV. Molecular Modeling of DachENSH

Molecular modeling studies were conducted in order to evaluate dachENSH as a selective zinc chelator. While the *C*-methylated dachenSH molecule is known in the literature, the parent compound has not been isolated. The synthesis of this novel species was initially pursued simply as an analogue to tach, but the steric arrangement of the ligand framework and the nature of the donor set indicated

that it had the potential to act as a better chelator for zinc than for copper, an interesting prospect as it is typically a difficult feat to achieve.

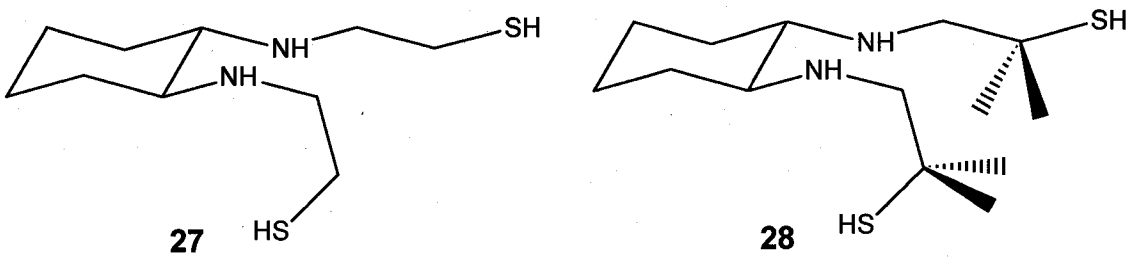


Figure 3.5. DachENSH (**27**) and the *C*-methylated dachENSH (dachEN(Me)$_2$SH **28**) reported in the literature. [130, 131, 132]

In order for a chelator to be selective for zinc, zinc must effectively compete with copper for the ligand, which typically forms more stable complexes than zinc in accordance with the Irving-Williams series.[97] It is preferable to build a ligand that is poorly suited for copper rather than designing one that is suited for zinc since divalent zinc, as a d^{10} metal, does not gain any ligand field stabilization energy (LFSE) in any coordination geometry and therefore shows no preference for a particular coordination environment.[124] Tetrahedral geometry significantly reduces the LFSE of a copper complex as compared to a distorted octahedral environment;[84] this destabilizes the complex and allows zinc to more effectively compete with copper for the ligand.

One key in designing a tetrahedral system is limiting the flexibility of the chelator to preclude the formation of a square-planar complex.[125] Every four-coordinate complex exists somewhere in the spectrum of geometry from tetrahedral to square planar. This can be evaluated by calculating the dihedral angle formed between the two ML_2 planes; a perfect tetrahedron will have a

dihedral angle of 90° compared to 0° for a square planar complex.[126] Typically, a complex will fall somewhere between the two. The more effective a complex is in maintaining a dihedral angle close to that of a tetrahedron, the less preference copper(II) shows for that ligand.[127] Since the two amines in *trans*-1,2-diaminocyclohexane (dach) are gauche to one another in relation to the cyclohexyl ring, this introduces a conformational restriction in ligands based on that framework: even with the addition of flexible pendant arms a square-planar system is not feasible, and thus is likely to show less preference for copper(II).

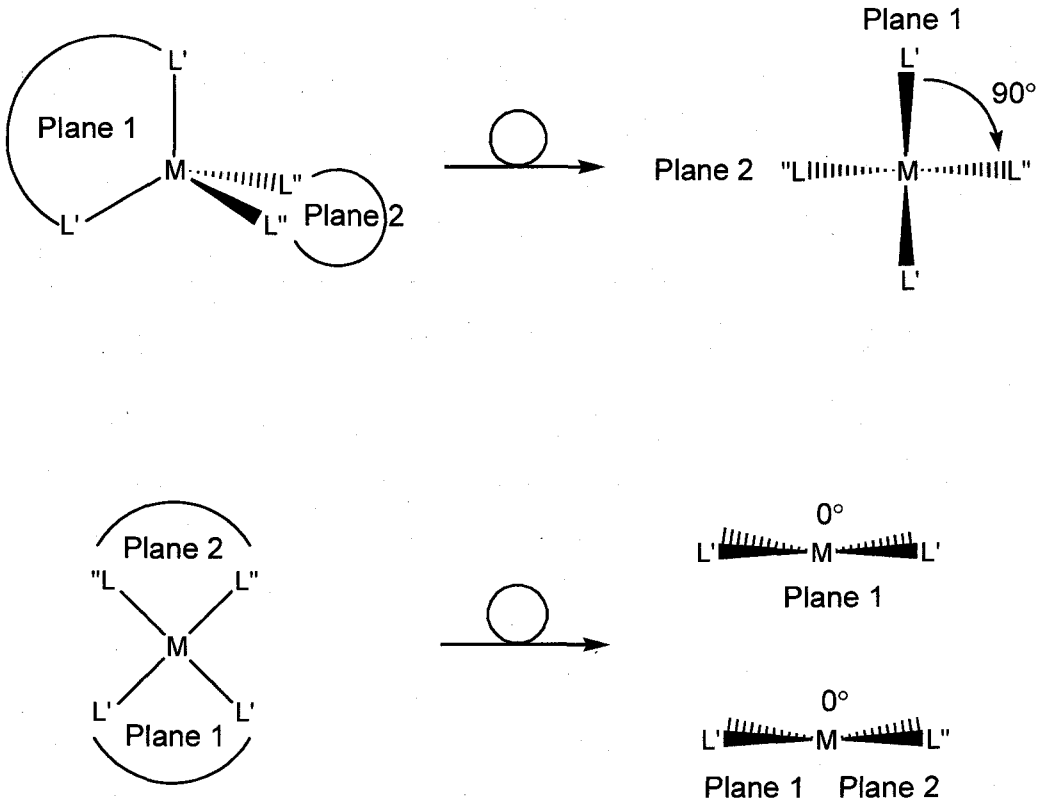


Figure 3.6. Dihedral angles of tetrahedral (top) and square planar (bottom) complexes.

While the geometry of the chelator is of primary importance in reducing the stability of copper complexation, choosing the appropriate donor atoms is important in imparting zinc selectivity.[128] Several zinc chelators are based on a tetrahedral geometry derived from biological systems, such as zinc finger proteins and carbonic anhydrases.[124, 126, 127, 129] The latter systems primarily contain histidine residues for the donor sets, and amino donors are common in synthetic zinc chelators. As divalent zinc is a borderline acid, it shows preference for donor atoms of similar polarizability, such as amines, and thus dach, with two amine donors, is a suitable match. The fourth donor site in zinc finger proteins is made up of a soft-base cysteine donor, thus the ethanethiol arms of dachENSH were considered to be advantageous for zinc chelation.

The *C*-methylated dachENSH analogue has been complexed with both divalent copper and zinc.[130, 131, 132] The addition of bulky substituents to a chelator hinders the introduction of more ligating groups that would otherwise alter the coordination number and geometry, and increase the LFSE of the complex.[133] In this case it may also serve to reduce the formation of thiol bridges or polynuclear complexes by steric hindrance.

In order to test whether dachENSH would be more selective for zinc than copper the ligand was evaluated with divalent copper and zinc according to *ab initio* density functional theory (DFT) geometry optimizations at the B3LYP/6-31G* level. The equilibrium geometries were compared to that of the *C*-methylated complexes in the literature, and were found to give similar M-N and M-S bond lengths and bond angles, as well as similar dihedral angles.

	$[Zn(\mathbf{27})]^0$	$[Zn(\mathbf{28})]^0$	$[Cu(\mathbf{27})]^0$	$[Cu(\mathbf{28})]^0$
	DFT	(KOPYOD)	DFT	(UGUWAU)
Bond lengths (Å)				
N1-M	2.151	2.102 (2)	2.069	2.047 (2)
N2-M	2.147	2.102 (2)	2.071	2.040 (2)
S1-M	2.263	2.2533 (6)	2.245	2.2317 (7)
S2-M	2.264	2.2533 (6)	2.244	2.2296 (6)
S1···S2	4.233	4.240 (1)	3.571	3.5780 (9)
Bite angle (°)				
Dach	82.81	85.6 (1)	84.51	84.54 (7)
N1-M-S1	92.13	92.33	90.35	89.75
N2-M-S2	92.02	92.33	90.32	88.85
Non-coordinating angle (°)				
S1-M-S2	138.44	140.36 (3)	105.42	106.64 (2)
Chelate ring conformations	δ, δ, δ	δ, δ, δ	δ, λ, λ	δ, λ, δ
Chirality of coord. amines	RR	SS	RR	RR
Dihedral Angle (°)				
N1-M-S1/N2-M-S2	68.18	70.99 (5)	33.64	32.77 (6)

Table 3.1. A comparison of structural parameters of tetrahedral zinc(II) and copper(II) complexes. The values for $[M(\mathbf{27})]^0$ are given as calculated by Spartan '04[134] using DFT and those of $[M(\mathbf{28})]^0$ are from the literature[130] as searched in the Cambridge Structural Database (CSDB).

As the calculations for the dachENSH complexes concurred with the literature data for the dachEN$(Me)_2$SH complexes, the equilibrium geometry parameters were considered to be reasonable for use in a preliminary examination of the zinc and copper(II) complexes of dachENSH. The bite angles of both the zinc and copper complexes of dachENSH are significantly distorted from tetrahedral geometry (109.5°), ranging from 82° to 92°, which is due to the constraints of the 5-membered chelate ring system. Measurement of tetrahedrality by way of the dihedral angle does not reflect as great a distortion in the zinc complex, since it only deviates by approximately 20° from 90°. The

copper complex is distorted from the preferred square planar geometry by 30°. While the S1···S2 distances for the zinc complex are beyond that of the Van der Waals diameter of 3.6Å,[135] the distortion of the copper complex towards the square planar geometry brings the thiol atoms close enough to induce a steric interaction. This is illustrated in the significant reduction of S1-M-S2 angles from the zinc complex (138.44°) to the copper complex (105.42°). Thus, the proximity of the thiol atoms hinders further movement of the ligand arms to a more square planar environment.

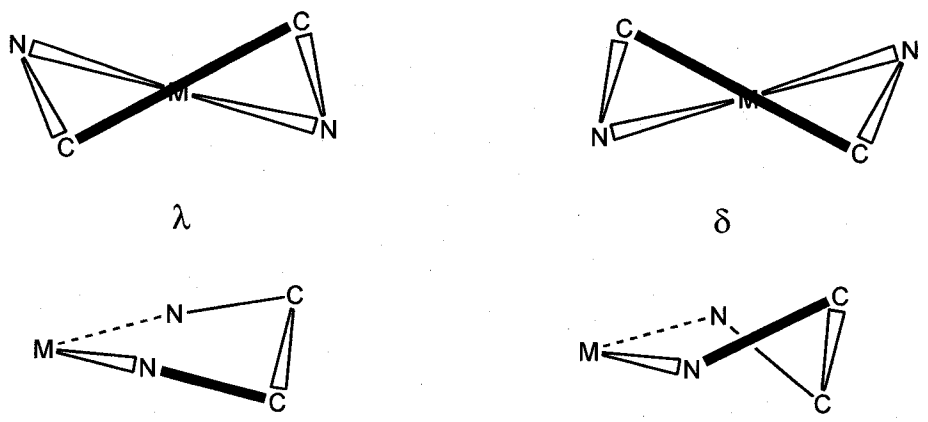


Figure 3.7. Chelate ring conformations, counterclockwise rotation (λ) and clockwise rotation (δ).

In the literature complexes of dachEN(Me)$_2$SH, no additional interactions were reported within the metal coordination sphere according to the crystal structures.[130] Thus, in the solid state, both metals are four-coordinate based only on the ligand. However, the chelator is not sterically bulky enough to preclude the addition of solvent molecules to the coordination sphere in solution.

The inability of the ligand to give a square-planar complex may allow preferential binding with zinc over copper, as indicated experimentally for the *C*-methylated version. A simple competition experiment between divalent copper and zinc with dachEN(Me)$_2$SH, wherein the metal salts were mixed with the chelator in solution, gave primarily colorless crystals of the zinc complex, a dehydrogenated analogue of the same complex, and none of the expected copper species.[130] Since dachENSH is expected to react similarly according to the DFT parameters, it is an interesting chelator to pursue further as a selective zinc chelator.

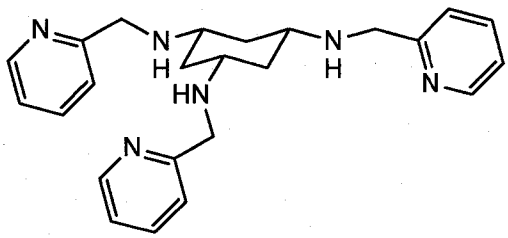
APPENDICES

APPENDIX A

Structures and Abbreviations of Ligands and Other Select Molecules

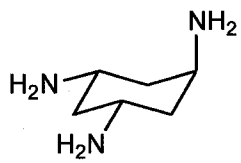
LIGANDS

N,N',N"-tris(2-pyridylmethyl)-*cis, cis*-1,3,5-triaminocyclohexane (tachpyr)

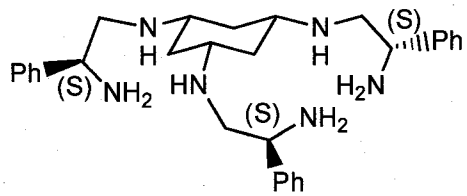


Cis, cis-1,3,5-triaminocyclohexane (tach)

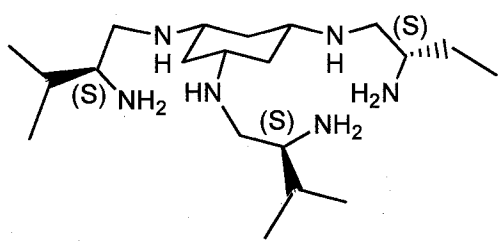
Cis, trans-1,3,5-triaminocyclohexane (*trans*-tach)



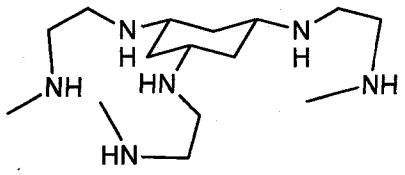
N,N',N"-tris(2-amino-2-phenylethyl)-*cis, cis*-1,3,5-triaminocyclohexane (tachenPh, **18**)



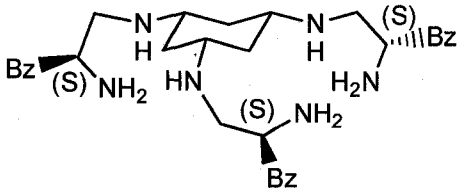
N,N',N"-tris(2-amino-2-isopropylethyl)-*cis, cis*-1,3,5-triaminocyclohexane (tacheniPr, **19**)



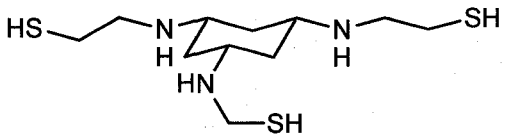
N,N',N"-tris[2-(methylamino)ethyl]-*cis, cis*-1,3,5-triaminocyclohexane (tachenNMe, **20**)



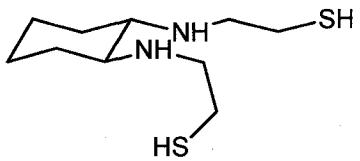
N,N',N"-tris(2-amino-2-benzylethyl)-*cis, cis*-1,3,5-triaminocyclohexane (tachenbn)



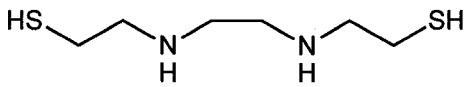
N,N',N"-tris(2-mercaptoethyl)-*cis, cis*-1,3,5-triaminocyclohexane (tachENSH)



(+/-)*Trans*-1,2-diaminocyclohexanediethanethiol (dachENSH)

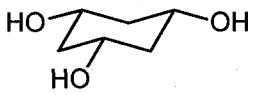


2,2'-(Ethylenediimino)diethanethiol (ENSH)

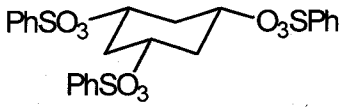


SELECT MOLECULES

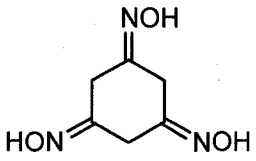
Cis, cis-1,3,5-cyclohexanetriol (**2**)



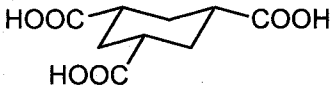
Cis, cis-1,3,5-tris(benzylsulfonate)cyclohexane (**3**)



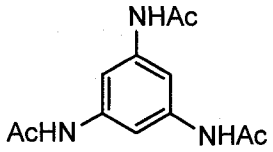
Cis, cis-1,3,5-cyclohexanetrione trioxime (**5**)



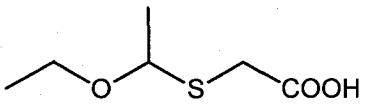
Cis, cis-1,3,5-cyclohexanetricarboxylic acid (**9**)



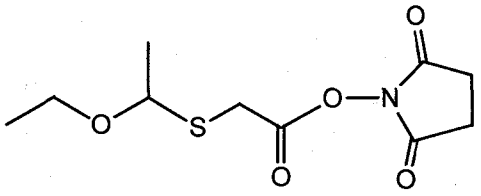
1,3,5-Trisacetyltriaminobenzene (**13**)



S-(1-Ethoxyethyl)mercaptoacetic acid (**22**)

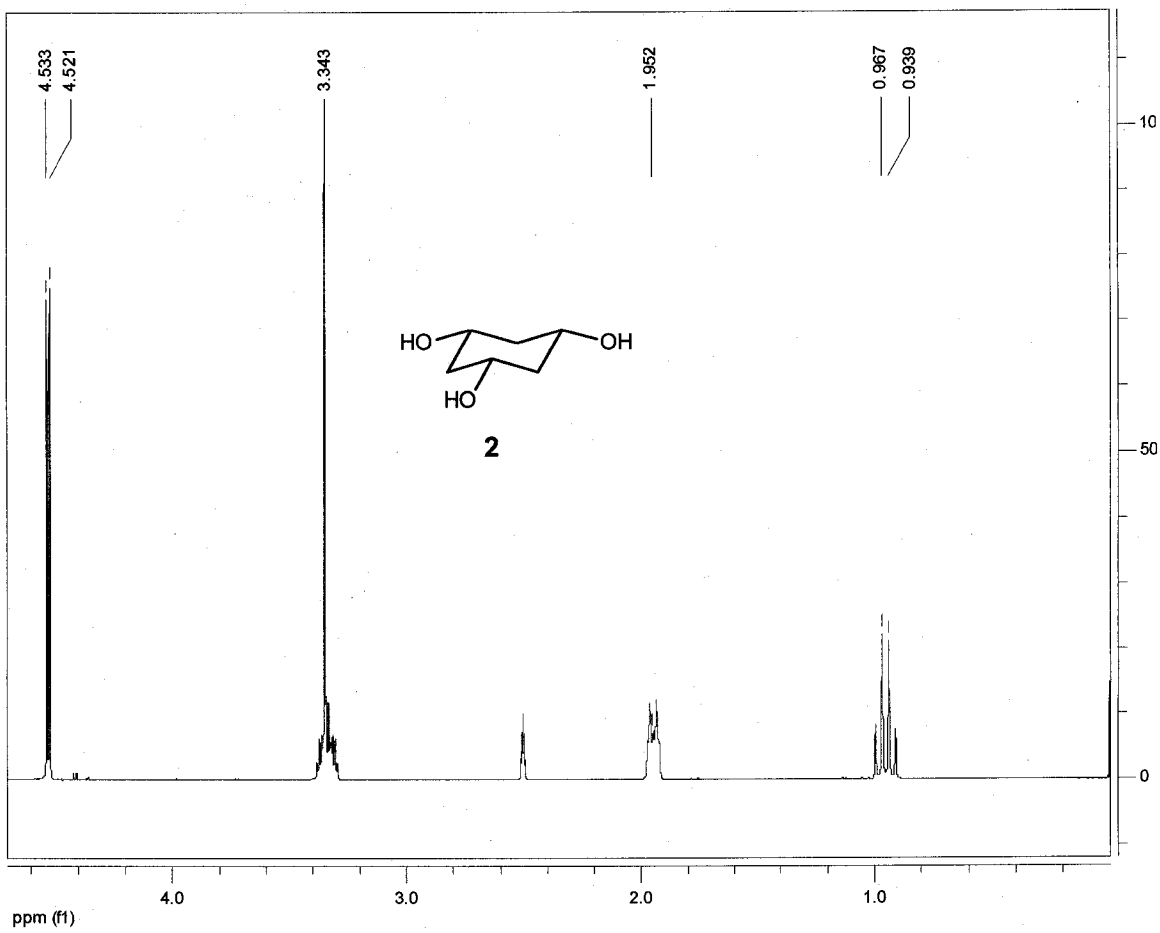


N-Hydroxysuccinimidyl *S*-(1-ethoxylethyl)mercaptoacetate (**23**)

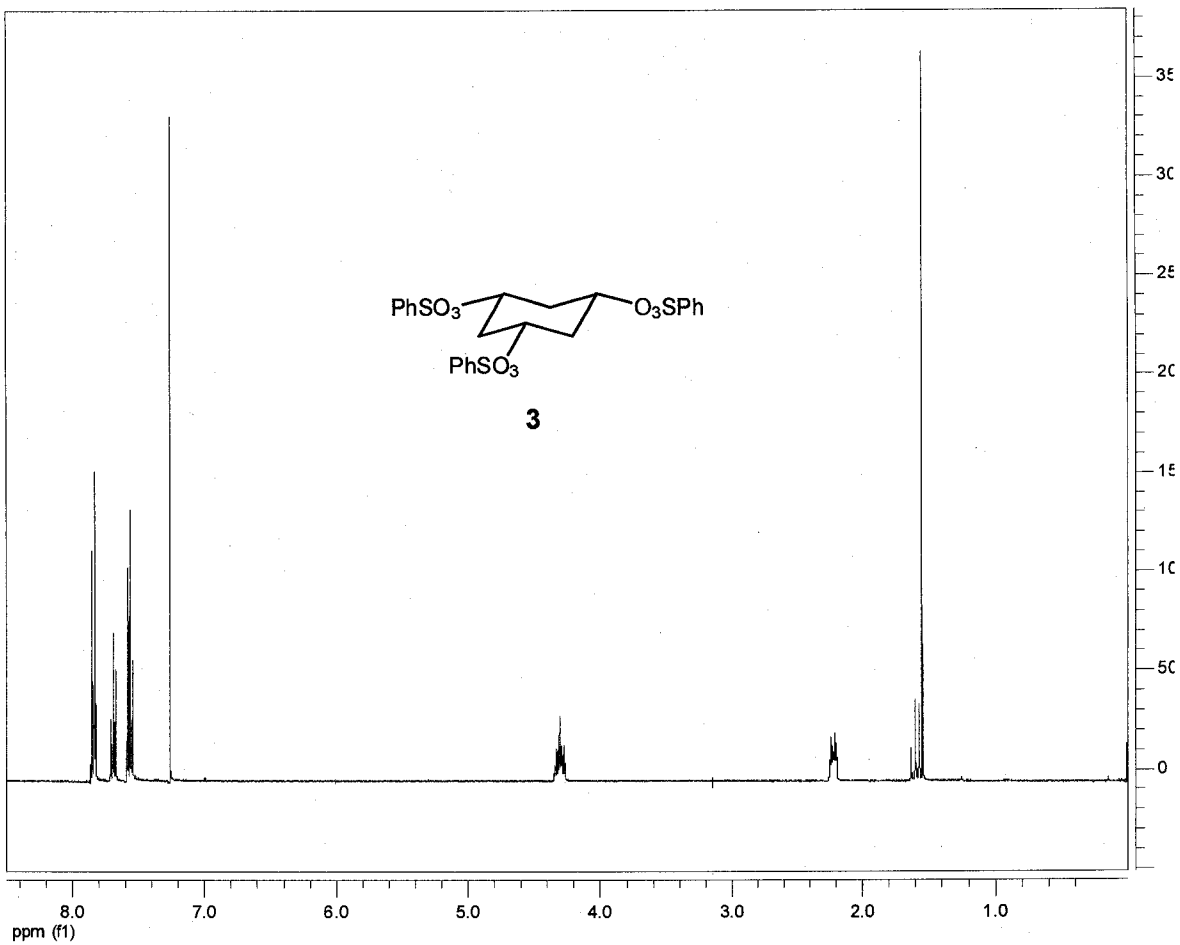


APPENDIX B

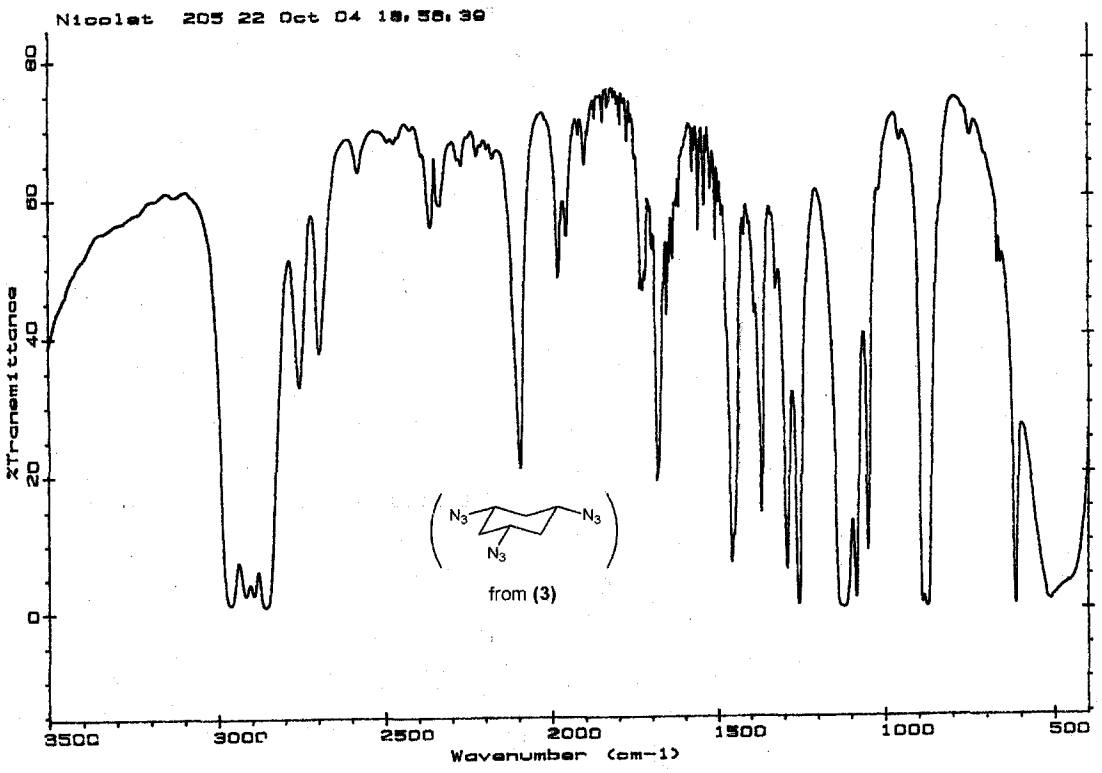
Supplemental Data



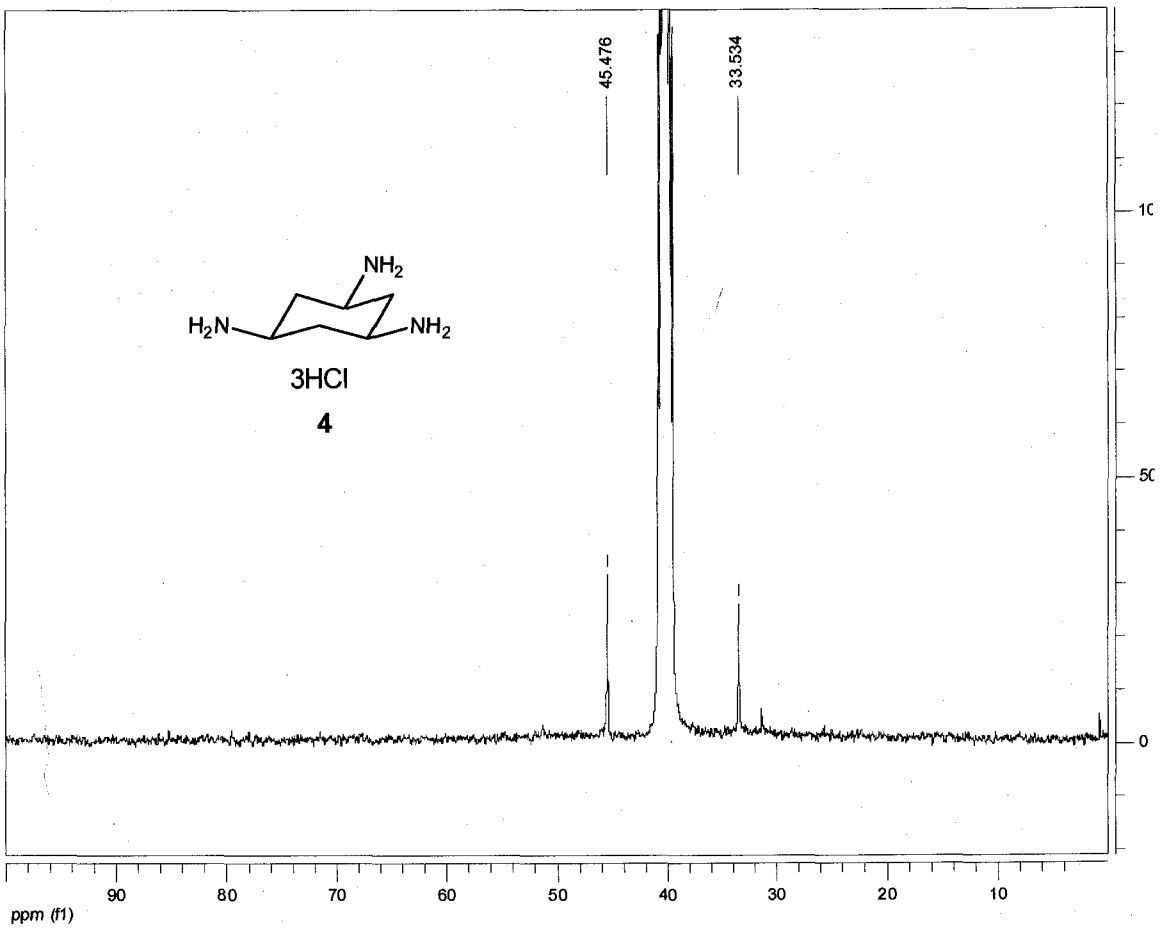
^{1}H NMR spectrum of *cis, cis*-1,3,5-cyclohexanetriol (**2**) in d_6-DMSO.



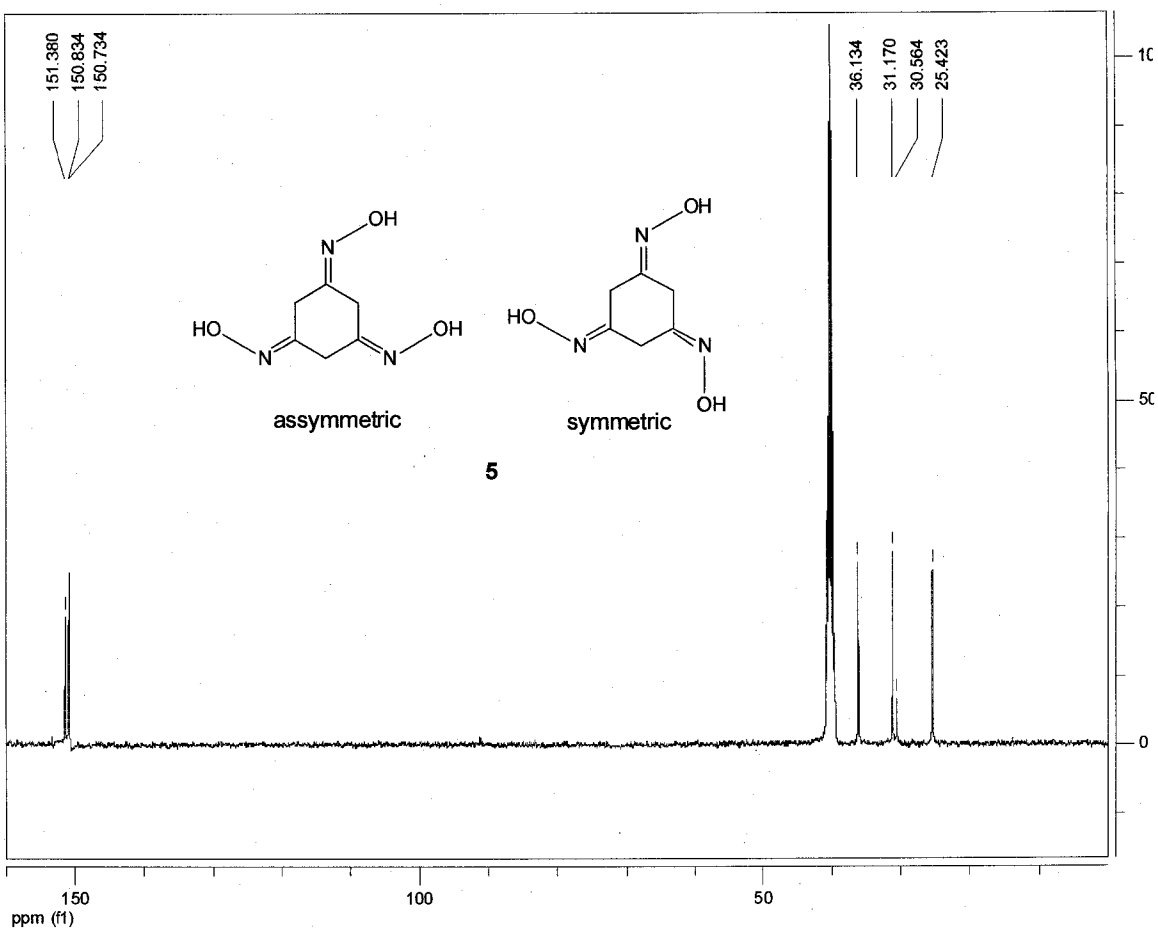
^{1}H NMR spectrum of *cis, cis*-1,3,5-tris(benzylsulfonate)cyclohexane (**3**) in $CDCl_3$.



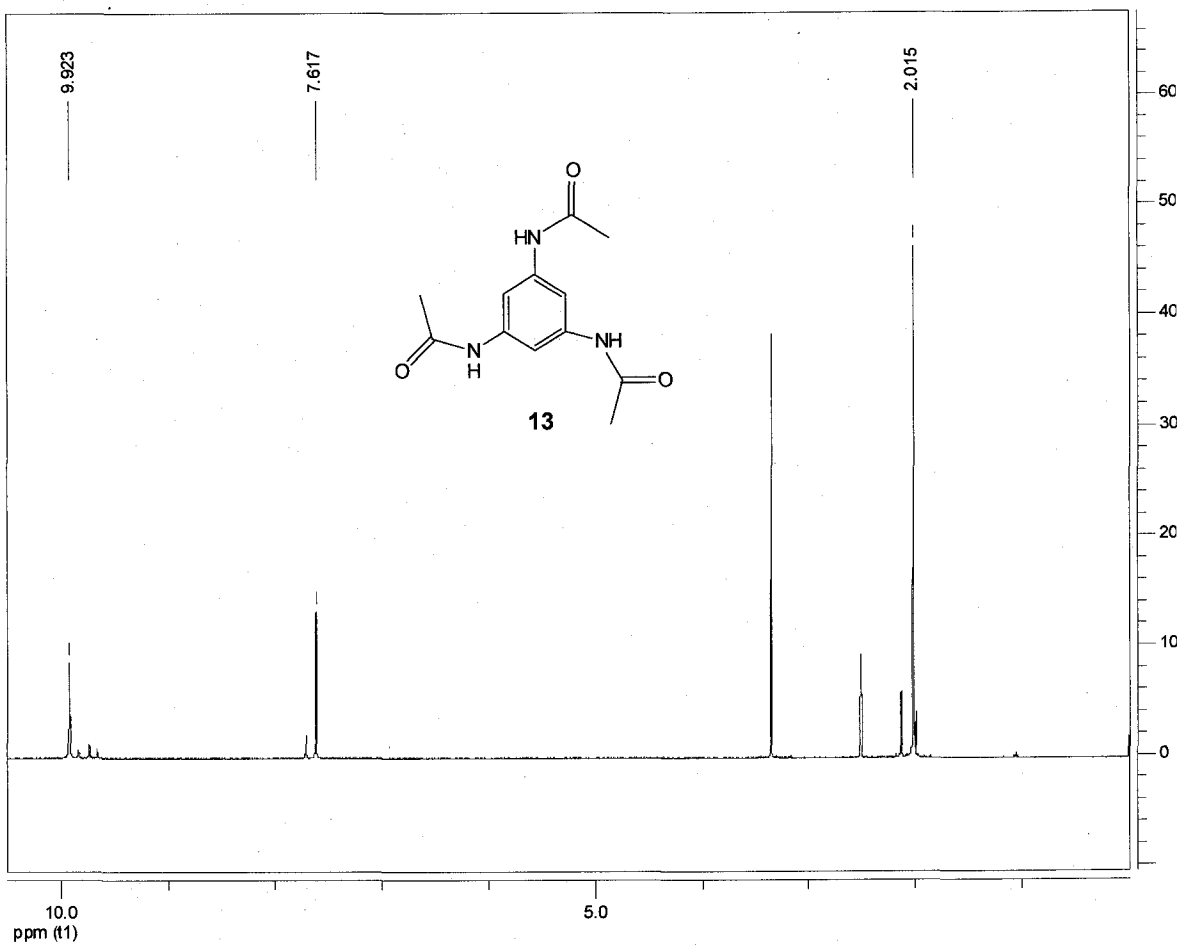
IR monitoring of formation of triazide from **3** by thin film on KBr plate.



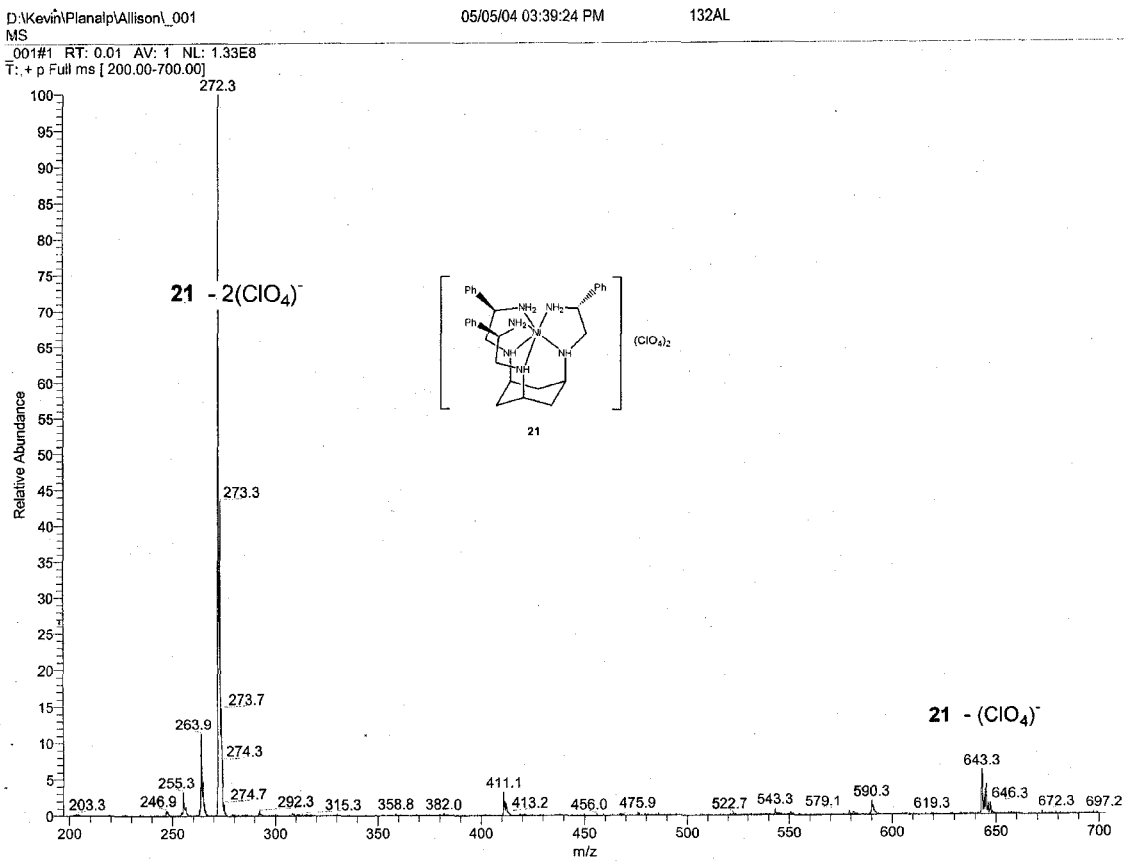
^{13}C NMR spectrum of *cis,cis*-1,3,5-triaminocyclohexane trihydrochloride (**4**) in d_6-DMSO.



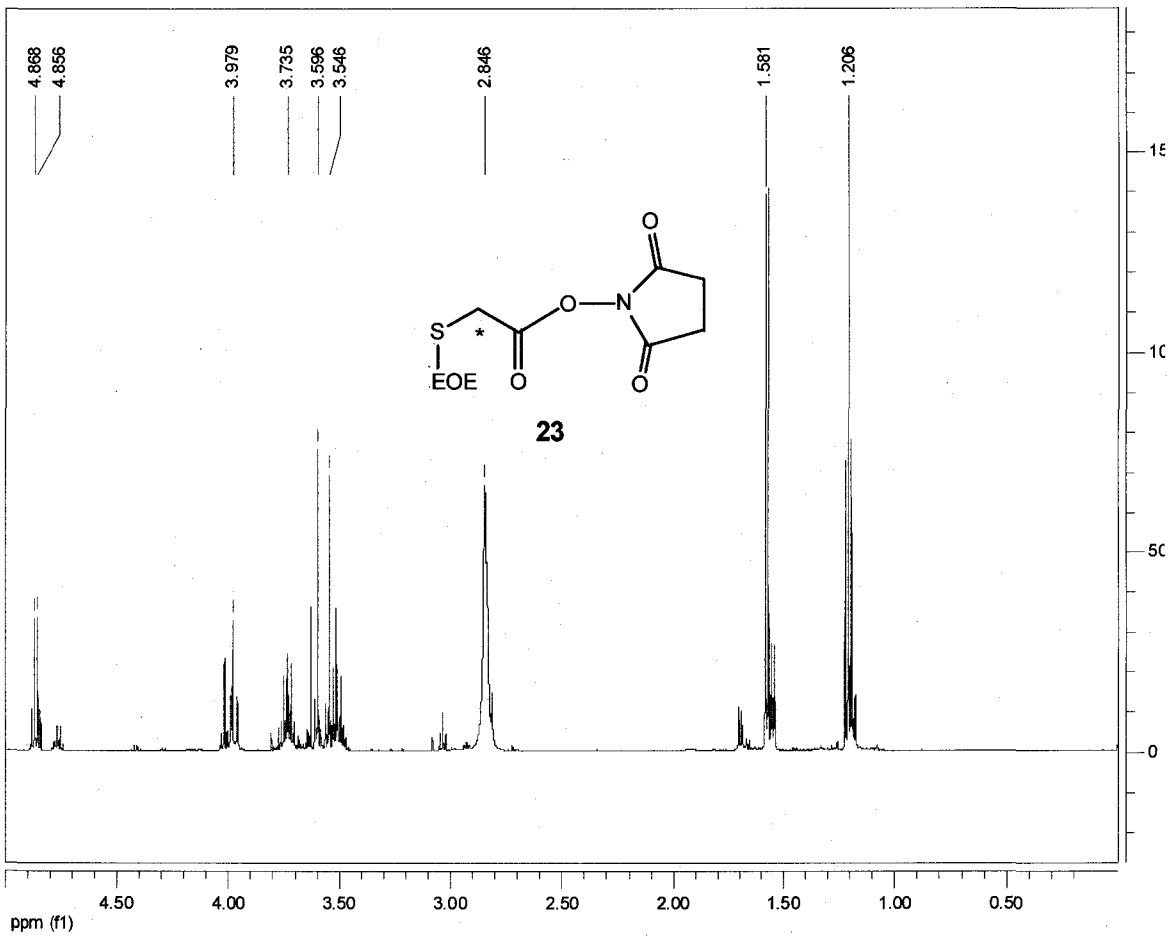
^{13}C NMR spectrum of 1,3,5-cyclohexanetrione trioxime (**5**) in d_6-DMSO.



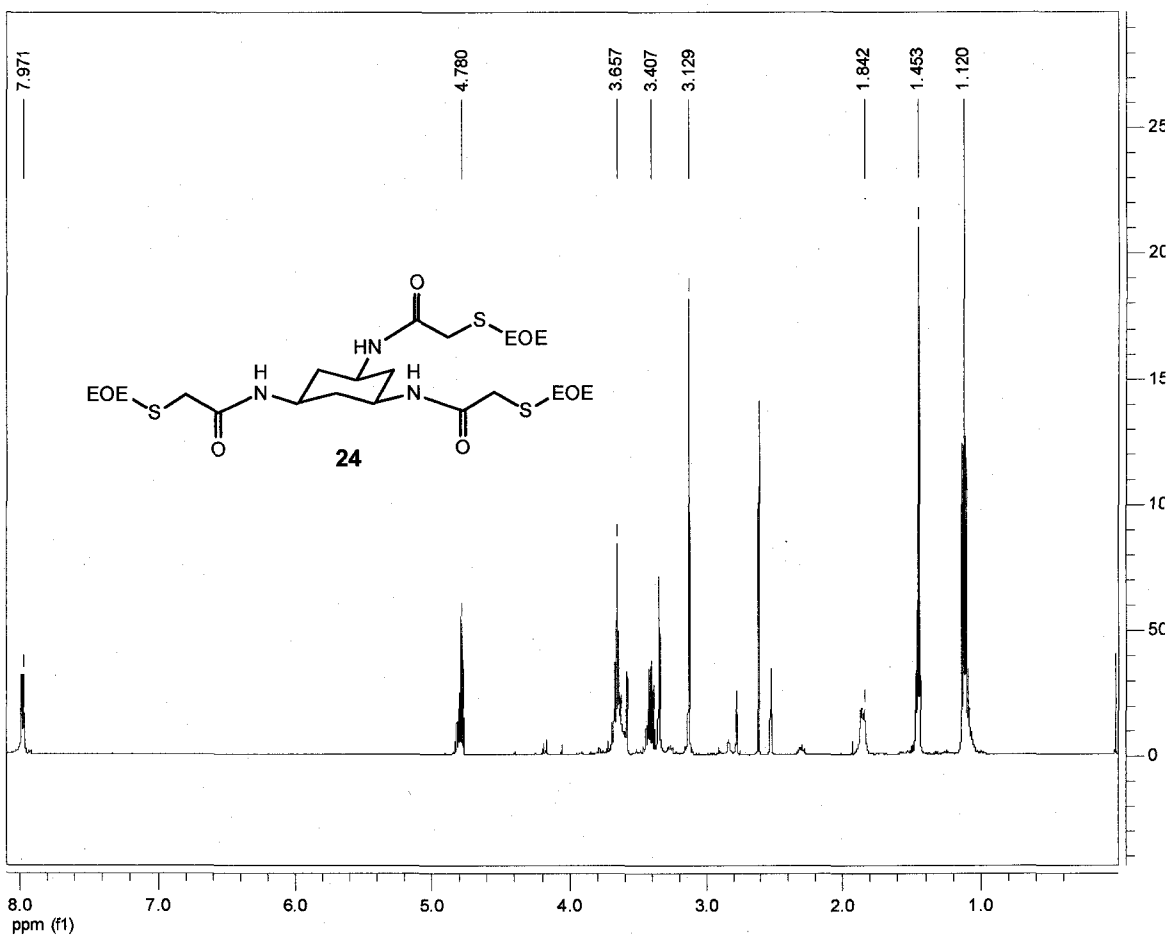
^{1}H NMR spectrum of 1,3,5-trisacetyltriaminobenzene (**13**) in d_6-DMSO.



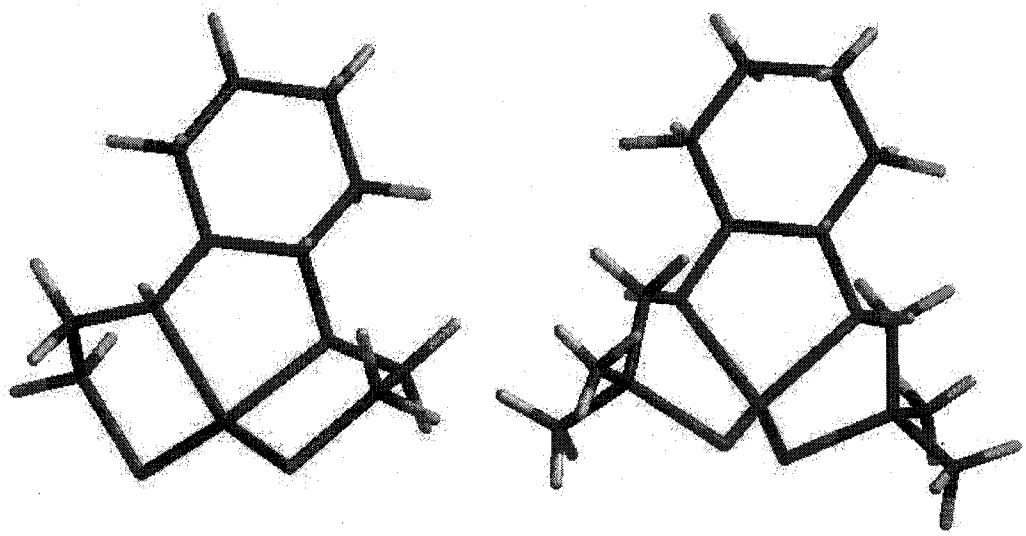
ESI-lo res MS of [Ni(tachenPh)](ClO$_4$)$_2$ (**21**).



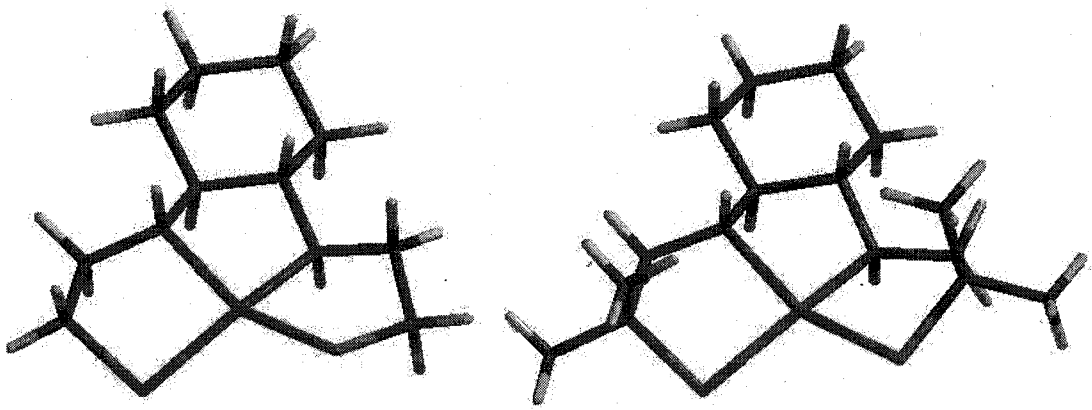
^{1}H NMR spectrum of *N*-hydroxysuccinimidyl *S*-(1-ethoxyethyl)mercaptoacetate (**22**) in $CDCl_3$.



^{1}H NMR spectrum of *cis, cis*-1,3,5-tris(1-ethoxyethylmercaptoacetamido) cyclohexane (**23**) in d_6-DMSO.



The DFT calculated equilibrium geometry for $[Zn(\mathbf{27})]^0$ (left) and the crystal structure of $[Zn(\mathbf{28})]^0$ according to the literature data[130] given in the Cambridge Structural Database (CSDB).



The DFT calculated equilibrium geometry for $[Cu(\mathbf{27})]^0$ (left) and the crystal structure of $[Cu(\mathbf{28})]^0$ according to the literature data[130] given in the Cambridge Structural Database (CSDB).

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