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AN IMPROVED SYNTHESIS OF *cis,cis*-1,3,5-TRIAMINOCYCLOHEXANE. SYNTHESIS OF NOVEL HEXADENTATE LIGAND DERIVATIVES FOR THE PREPARATION OF GALLIUM RADIOPHARMACEUTICALS

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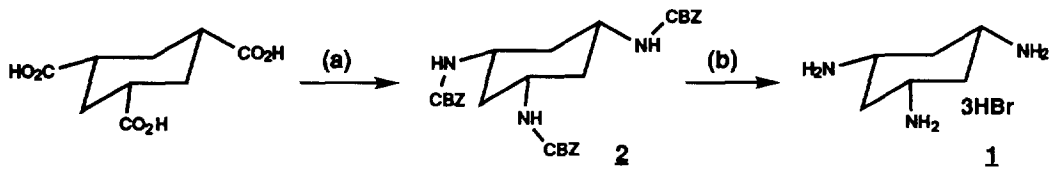
Abstract: An improved synthesis of *cis,cis*-1,3,5-triaminocyclohexane in 82% yield involves reaction of the commercially available *cis,cis*-1,3,5-cyclohexanetricarboxylic acid with DPPA to afford the tri(benzylcarbamate) as the Curtius rearrangement product. Deprotection yields the triamine which serves as a platform from which a variety of chelating structures may be assembled. Novel tris(2-methylenepyridyl)triamine and tris(2-methylenethienyl)triamine ligands are prepared to investigate their suitability for use as gallium radiopharmaceuticals. Elsevier Science Ltd

Considerable effort continues to be devoted to the design and synthesis of novel chelating agents for metal ions such as Ga(III) and In(III), or Gd(III), due to their uses in scintigraphic imaging[1] or as MRI contrast agents.[2] Numerous chelating agents have been reported for the sequestration of the gallium isotopes (Ga-66,67,68) for in vivo imaging by SPECT or PET technology. Recently, reported ligands demonstrate a variety of coordination spheres, including acyclic and macrocyclic derivatives such as N_4O_2 polyaminopyridyl- phenolates,[3] N_3O_3 polyaminophenolates,[3,4] and N_3S_3 or N_2S_2 polyaminothiols,[5,6] all designed to possess varying charge and degrees of lipophilicity to target selected organs such as the heart, kidneys, or brain. In general, high stability of the complex is a fundamental requirement for successful application of such radiopharmaceuticals. To this end we have initiated an investigation into the usefulness of chelating agents based upon the framework of *cis,cis*-1,3,5-triaminocyclohexane. This polyamine appears to be ideal for derivatization, allowing introduction of three or more additional donor groups that would then efficiently encapsulate small trivalent metals.[7-9] Indeed, considerable efforts have been reported in the past, wherein the Zn, Co, Ni, Fe and Mn complexes of the tris(2-iminopyridyl)triamine were prepared and their structures investigated.[10-12]

Several syntheses of *cis,cis*-1,3,5-triaminocyclohexane have been reported, all of which, until recently, depended upon a reductive transformation to introduce the amines into an all *cis* system. The drawback involved in these syntheses, whether from Birch reduction (Na in NH_3) of a tris(oxime) or hydrogenation of the 1,3,5-benzenetriamide (Nishimura catalyst), has been the generation of a mixture of products in which fortunately the *cis* product predominated.[8,9,13] Clearly, this complication coupled with the need to isolate the desired triamine by first forming a metal complex for resolution and subsequent demetallation to obtain the triamine in modest yields has detracted from a systematic study of this system. Recently, an improvement was reported wherein the triamine was prepared from the all *cis* triol by displacement of a sulfonyl ester derivative with azide, followed by $LiAlH_4$ reduction to provide the desired product.[14] While this route completely obviates production of the undesired stereochemical product or of partial hydrogenation products, isolation and use of the tri(azide) intermediate makes large scale production potentially hazardous.

To obviate this difficulty, we have developed a routine synthesis of the requisite triamine by starting with the readily available tricarboxylic acid.[15] In recognizing that the amine would be directly available through the application of a Curtius rearrangement, we subjected a small portion of triacid to a modification of the conditions reported by Yamada[16] (Scheme I) for test purposes and found to our gratification that a significant amount of the desired N,N',N''-tris(benzyl)carbamate had indeed been formed. Repetition of the procedure on a larger scale (8 gr of triacid) has since routinely provided **2** as a flocculent white precipitate, which after collection and drying in vacuo, proved to be analytically pure (86%).[17] The CBZ groups were most efficiently cleaved by treatment of the solid directly with 33% HBr/HOAc.[18] Shortly after a clear solution was achieved a thick suspension of **1** formed, which was diluted with an equal volume of anhydrous ether to ease filtration. The product was collected and dried, providing the desired triamine in a solid, convenient, stable form in just two steps in 82% yield overall.

SCHEME I



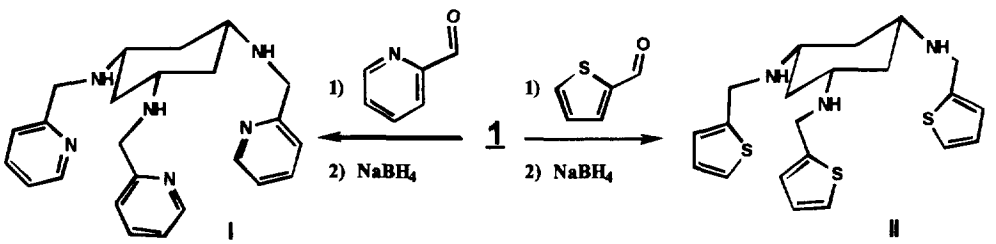
a) Et_3N, DPPA, Bz-OH; b) HBr/HOAc

Application of this process to Kemp's acid resulted in a mixture of products, none of which were identifiable as the expected tris-carbamate. Attempts to expand the options for the cleavage conditions of the carbamate formed in the Curtius rearrangement met with mixed results. Synthesis of the tris-Teoc[19a] analog (F^- cleavage) of **2** was successful when employing 2-trimethylsilylethanol under the same conditions as above. However, this positive result was tempered with a significant reduction in yield (48%) coupled with a requirement of chromatography for isolation from a complex product mixture. Surprisingly, attempts to form the tris-Fmoc[19b] analog of **2** resulted in isolation of 9-fluorenylmethanol as the only major product that could be characterized.

The choice of additional metal binding sites, to add to the triamine framework and to bring the coordination sphere to six, was based upon the desire to form cationic complexes while maintaining 5-membered metal chelate rings in the complex.[20] These criteria were met by the addition of the donor groups pyridine[21] and thiophene[21b] to the nitrogens via a methylene bridge as depicted in Scheme II.

The free amine **1** was reacted with the appropriate aldehyde to form the respective tris(imines).[22] Routine borohydride reduction generated the secondary amines to provide novel hexadentate ligands **I** and **II** (94 and 90%, respectively).[23] Preliminary studies in our laboratories have demonstrated that ligand **I** forms complexes with either Ga(III) or In(III). Preliminary complex stability experiments by NMR have indicated that the Ga(III) complex is stable over a pH range of 2 - 8. To our disappointment, we have been unable to isolate a Ga(III) complex of **II**, although there seems to be some promise of both a Cu(II) complex, which may be of use with Cu-64,62[24] and a Ni(II) complex.

SCHEME II



In conclusion, an efficient and nonhazardous synthesis of *cis,cis*-1,3,5-triaminocyclohexane from commercially available materials is presented. The *cis,cis*-triamine is a useful framework for assembly of hexadentate chelating agents through facile imine formation and reduction. This general route is being explored for a variety of metal binding groups. Evaluation of the metal complexes through both in vitro and in vivo stability and targeting studies with Ga-67, as well as structural studies and solution chemistry of the metal complexes of these ligands, are in progress and will be reported in an appropriate venue.

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17. General Synthesis: *cis,cis*-1,3,5-cyclohexanetricarboxylic acid (8.0 g, 37 mmol) was washed into a round-bottomed flask with benzene (300 mL) and Et_3N (15.6 mL, 112 mmol) was added followed by DPPA (30.85 g, 112 mmol). The mixture was stirred for 0.5 h at room temperature and then refluxed for 0.5 h. Benzyl alcohol (13.34 g, 123.5 mmol) was added and the solution was refluxed for 18 h during which a precipitate formed. After cooling to ambient temperature, the product was collected by vacuum filtration, washed with minimal cold benzene, and dried under vacuum to leave 11.22 g (86%). ^1H NMR (d_6- DMSO) δ 7.40-7.20 (m, 5H), 4.998 (s, 2H), 3.379 (m, 1H), 1.883 (d, 1H, J = 11.1), 1.059 (q, 1H, J=12.0); ^{13}C NMR (d_6- DMSO) δ 155.20, 137.12, 128.37, 128.29, 127.73, 65.13, 46.63, 38.17; CI-MS (*m/z*) 532 M^++1. Anal. Calcd. for $C_{30}H_{33}N_3O_6$: C, 67.77; H, 6.27; N, 7.91. Found: C, 67.63; H, 6.22; N, 8.09. All reagents were used as received. Use of high purity DPPA (Fluka) provides consistant yields and the convenient precipitation of product.
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22. Tris(imine) Synthesis: Triamine **I** (1.00 g, 2.688 mmol) was treated with NaOH (0.323 g, 8.06 mmol) in H_2O (5 mL). Benzene (200 mL) was added to the clear solution along with the appropriate carboxaldehyde (8.1 mmol) and the H_2O was removed via azeotropic distillation with a Dean-Stark trap. The solution was decanted and the solvent removed by rotary evaporaton leaving the product which was further dried under high vacuum. Characterization data for the pyridyl and thienyl tris(imines) follow, respectively: (a) ^{13}C NMR ($CDCl_3$) δ 160.67, 154.71, 149.46, 136.57, 124.73, 121.55, 66.11, 40.51; CI-MS (*m/z*) 397 M^++1. Anal. Calcd. for $C_{24}H_{24}N_6$: C, 72.69; H, 6.11; N, 21.19. Found: C, 72.63; H, 6.07; N, 21.46. (b) ^{13}C NMR ($CDCl_3$) δ 152.52, 142.70, 130.28, 128.81, 127.24, 65.60, 40.56; CI-MS (*m/z*) 412 M^++1. Anal. Calcd. for $C_{21}H_{21}N_3S_3$: C, 61.23; H, 5.15; N, 10.21. Found: C, 61.08; H, 5.22; N, 10.08.
23. (**I**) ^{13}C NMR ($CDCl_3$) δ 159.66, 149.24, 136.46, 121.92, 121.84, 53.80, 52.48, 40.19; Anal. Calc. for $C_{24}H_{30}N_6$: C, 71.59; H, 7.53; N, 20.88. Found: C, 71.46; H, 7.43; N, 20.68. (**II**) ^{13}C NMR ($CDCl_3$) δ 144.39, 126.68, 124.70, 124.31, 52.76, 45.42, 40.16; Anal. Calc. for $C_{21}H_{27}N_6S_3$: C, 60.36; H, 6.53; N, 10.06. Found: C, 60.64; H, 6.63; N, 10.05.
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