# SYNTHESIS OF TRITIATED DIBORANE AND CRYPTAND [2.2.2]

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SUMMARY

In order to provide a method for in vivo and in vitro detection of cryptand [2.2.2], tritiated cryptand was synthesized by reduction of appropriate bicyclic diamide with tritiated diborane. As part of this synthesis, tritiated diborane was synthesized by the reaction of tritiated sodium borohydride with boron trifluoride etherate and trapped in dry THF. This is a potentially useful agent for selective tritiation of molecules with functional groups which are prone to electrophilic attack.

Keywords: Tritiated Cryptand [2.2.2], Trititated Diborane

## INTRODUCTION

Simple macrocyclic organic ligands such as cyclam and the crown ethers have been the subject of investigation in the field of nuclear medicine with the goal of preparing lipophilic complexes of technetium (1,2). Our efforts are directed toward the use of cryptands which are three-dimensional crown ethers with nitrogen bridge heads (3-6). This added dimension results in an almost spherical intramolecular cavity that increases their stability for complexation with spherical cations by several orders of magnitude over planar crown ethers. Cryptands are useful in chemical synthesis as ligands to introduce cations into aprotic solvents and for anion activation in nucleophilic reactions (7,8).

We are investigating the macrobicyclic polyether cryptand [2.2.2] (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo(8.8.8)hexacosane) **5** as a

0362–4803/90/101171–08$05.00

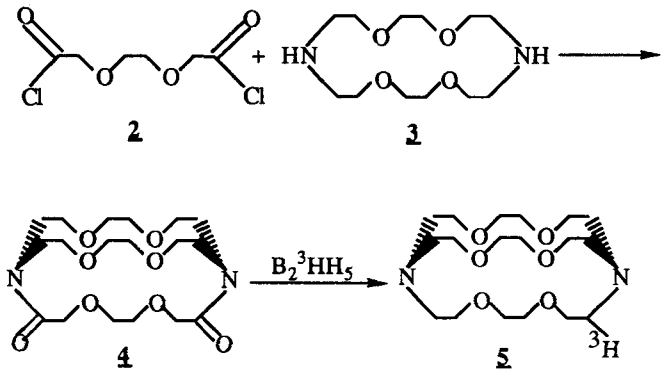

Received March 20, 1990
Revised May 31, 1990

ligand for use with generator produced radionuclides to make a convenient freely-diffusible indicator for imaging blood flow. Cryptates are inclusion complexes formed between cations and cryptands. Our research is based on the hypothesis that the in vivo behavior of the cryptates will be be dominated by the lipophilic characteristics of the cryptands which surround the metal ions. The absence of chromophores or sensitive methods for detection of cryptand [2.2.2] necessitated labeling the ligand with tritium so that we could follow both the complexed radioactive metal ions and the tritiated ligand in vitro and in vivo.

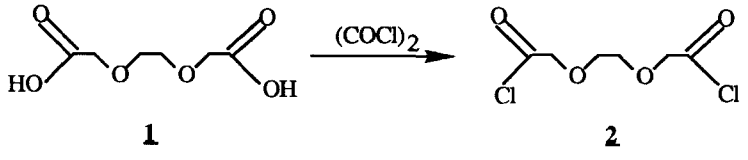
## EXPERIMENTAL

NMR spectra were recorded with a Varian VXR 300 MHz spectrometer. The purity of the precursors was verified with proton nmr. Melting points were measured using an electrothermal melting point apparatus. Unless specified otherwise, the solvents were reagent grade and used as received. Boron trifluoride etherate was purchased from Aldrich Chemical Co. (Milwaukee, WI) and purified by vacuum distillation from $CaH_2$ at 60 $^oC$ and 20 torr. Tetrahydrofuran and benzene were purified by distillation from sodium metal under argon atmosphere. Tritiated sodium borohydride was purchased from DuPont NEN (Boston, MA) as a solid with a specific activity of 12.3 Ci/mmole. Neutral alumina was purchased from Waters Associates Inc.(Framingham, MA). Anhydrous diglyme (2-methoxyethyl ether) was obtained from Aldrich Chemical Co. (Milwaukee,WI). Cryptand [2.2] was purchased from VWR Scientific (Seattle, WA). Triglycolic acid was prepared by oxidation of ethylene glycol with $HNO_3$ (9).

Tritiation was accomplished through reduction of a bicyclic diamide precursor using tritiated diborane. The general reaction scheme was:

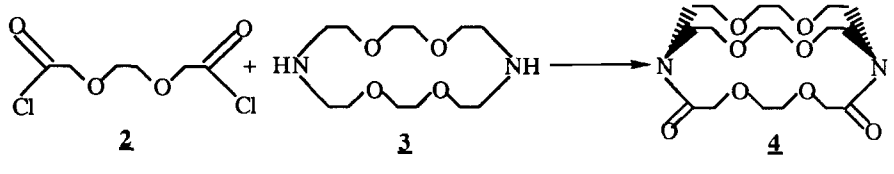



**Preparation of triglycolic diacid dichloride 2**




Triglycolic acid 1 [1,2-bis-(carboxymethoxy) ethane] (5g) was treated with a mixture of oxalyl chloride (10g) and pyridine (one drop) in 33 mL dry benzene (9). The mixture was stirred for 20 hours at room temperature and then was rapidly filtered over a small amount of glass wool. The solvent was rotary evaporated and excess oxalyl chloride was removed by evaporation with two 33 mL portions of dry benzene. The isolated product was recrystallized twice at -70 °C from a mixture of ether and petroleum ether (50:50 v/v) and was dried at 0.1 torr for 30 minutes at room temperature to afford 5g (83% pure yield) triglycolic diacid dichloride 2 [1,2-bis-(chlorocarboxymethoxy) ethane]. NMR (deuterochloroform): δ 3.85 (s), δ 4.52 (s).

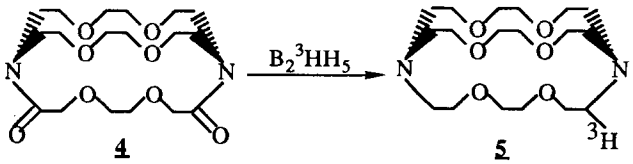
**Preparation of bicyclic diamide 4**



The bicyclic diamide **4** (4,7,13,16,21,24 -hexaoxa-1,10-diaza-2,9-dioxobicyclo[8.8.8]hexacosane) was prepared by the reaction of cryptand [2.2] **3** (1,4,10,13-tetraoxa-7,16-diazacyclooctadecane ) with **2**, employing a high dilution technique (9). The yield of the reaction depends on effective mixing of the reactants and a Morton type flask was used for this purpose. A constant addition funnel was employed to maintain the proper stoichiometric ratio of the reactants to minimize polymerization. In a typical reaction, 400 mL freshly distilled dry benzene was placed in a one-liter three-neck Morton type flask fitted with a mechanical stirrer . Triglycolic diacid dichloride **2** (1.68g) dissolved in 200 mL dry benzene was in one addition funnel; 2g of **3** and 2.3 mL triethylamine in 200 mL of dry benzene were in the second funnel. The system was purged with dry argon for 30 minutes as the speed of a mechanical stirrer was gradually increased to 300 rpm.

The reaction was initiated by the simultaneous addition, at equal flow rates, of both solutions. A constant rate of addition was maintained to deliver the reactants over four hours. Stirring was continued for an additional four hours. The resulting solid was filtered and washed three times with 80 mL of benzene . The filtrate and washings contained the desired products and were evaporated under vacuum. The residue was dissolved in 50 mL benzene, passed through a 60g neutral alumina column, and eluted with 400 mL ethyl acetate-methanol (40:1 v/v). After evaporation of the eluant under reduced pressure, the product was recrystallized from a mixture of benzene and petroleum ether (50:50 v/v) to afford 1.36g (44% yield) bicyclic diamide **4** whose purity was confirmed by proton nmr (9) and melting point (117-120°C ).

**Reduction of bicyclic diamide 4 with tritiated diborane**




**4** **5**

A 250 mL two-neck flask containing tritiated diborane (approximately 12 mmole, produced from 100 mCi tritiated sodium borohydride and 0.83 g of $NaBH_4$ ) in 12 ml THF was fitted with a 25 mL pressure-equalizing addition funnel while being flushed with dry argon. One gram bicyclic diamide **4** dissolved in 10 mL dry THF was added to the addition funnel. An ice bath was placed under the flask and after 20 minutes of flushing with argon, the bicyclic diamide **4** was added dropwise to the diborane over a period of 10 minutes. Formation of a white flaky precipitate was observed. When the addition was complete, the ice bath was removed and the reaction mixture allowed to come to room temperature. After 30 minutes the reaction mixture was boiled for two hours, then allowed to cool to room temperature.

Excess diborane was destroyed by the addition of 3-4 mL of water which resulted in the formation of a white precipitate and the liberation of a small amount of tritiated hydrogen gas which was vented into a radiochemical hood. The THF was removed using a rotary evaporator at 70 °C. The solid residue, which included the desired product, was washed three times with 5 mL of warm chloroform (40 °C). After filtration the $CHCl_3$ was removed under vacuum and a pale yellow oily product was obtained. To this bis(aminoborane) product was added 20 mL 6M HCl and the solution was heated for three hours at 125 °C. The excess HCl was then rotary evaporated (70-75 °C). The bis-dihydrochloride diamine product was dissolved in 10 mL $H_2O$ and passed through 15 g of anion exchange resin ( Dowex 1x8, 50-100 mesh, $OH^-$ form). The column was eluted with 150 mL water. This eluant was rotary evaporated for two hours at 80 °C. The isolated product was oily but crystallized after a few minutes at room temperature. The product was purified by recrystallization in hexane to yield 0.5g tritiated cryptand [2·2·2]. The yield of this reaction is approximately 50% with a specific activity of 0.23 Ci/mole measured by weighing the product and assaying an aliquat of the product for tritium content. The product from an equivalent procedure without tritium had a melting point of 69 - 70 °C and nmr spectra

(CDCl3) δ 2.64 (s), δ 3.59 (t), δ 3.68 (s) consistent with the chemical structure of cryptand [2.2.2] (9). For the tritiated cryptand the only quality control was Silica Gel thin layer chromatography (TLC) using ethylacetate/methanol 40:1 (v/v) as the mobile phase, followed by visualization in an iodine chamber. Authentic standard and the tritiated product migrated in a single band.

**Synthesis of tritiated diborane**

Diborane is a useful agent for reduction of aldehydes and ketones under mild conditions. However trititated diborane is not commercially available except by custom synthesis. We have developed the following system for making diborane from commercially available tritiated sodium borohydride.

$$3\ NaB^3HH_3 + 4\ (C_2H_5)_2O{:}BF_3 \xrightarrow{\text{diglyme}} 2\ B_2{}^3HH_5 + 4\ (C_2H_5)_2O + 3\ NaBF_4$$

$$B_2{}^3HH_5 + 2\ C_4H_8O \longrightarrow 2\ C_4H_8O{:}B_2{}^3HH_5$$

The apparatus for this reaction is shown in figure 1. A 15 mL three neck flask was fitted with a 10 mL pressure-equalizing addition funnel, magnetic stirring bar, Claisen adapter and dry ice condenser. The dry ice condenser was connected via a trap to a 250 mL two-neck flask fitted with a coarse sintered glass dispersion tube. A septum was placed on the side arm. The system was flame dried under vacuum and purged with argon dried by passage through $CaSO_4$ and $P_2O_5$. The flame drying cycle was repeated twice. Dry THF (12 mL) was added to the cooled flask using a 20 mL glass syringe that had been dried in an oven. A mercury bubbler containing acetone was connected to the side arm of the flask by a tygon tube.

Tritiated sodium borohydride (100 mCi, 8.1 μmole) dissolved in 3 mL anhydrous diglyme was placed in the reaction flask through the septum on the Claisen adapter. Carrier $NaBH_4$ (0.83g) was added in a

glove box to a 10 mL airless storage tube and the septum on the Claisen adapter was replaced by this tube. The addition funnel was filled with 3.5 mL freshly distilled $Et_2O{:}BF_3$ and the traps were cooled with dry ice-acetone. The reaction was initiated by very slow (1 drop / 2 second) addition of $Et_2O{:}BF_3$. The flow of argon was cut off after approximately half of the boron trifluoride was added. The reaction flask was heated at 60° C for one hour after the addition was complete. The system was then flushed with argon for 30 minutes. At this time the sintered glass and the tygon tube to the mercury bubbler were removed under a high flow of argon and replaced by a glass stopper. The yield of the reaction was approximately 85%.

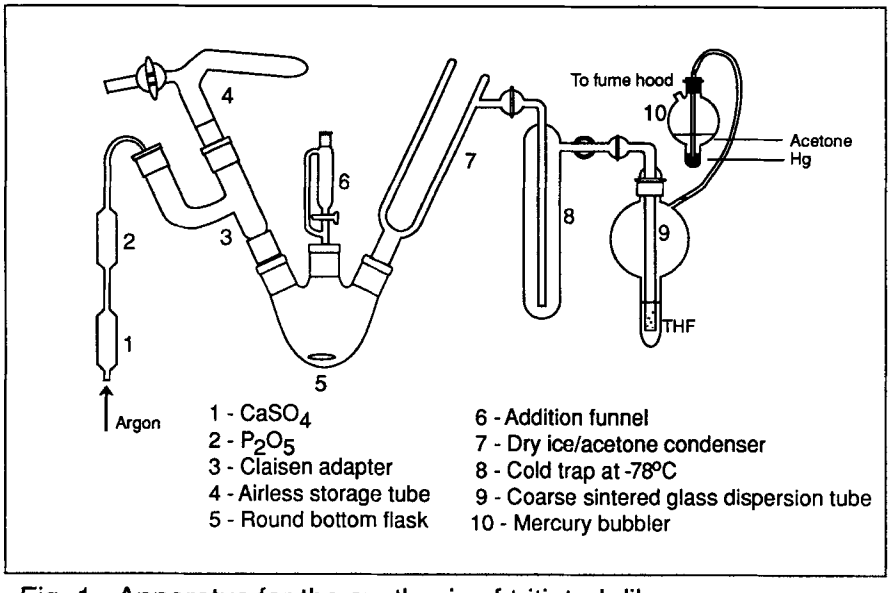



Fig. 1 - Apparatus for the synthesis of tritiated diborane

Tritiated diborane is a useful labeling agent. In contrast to the nucleophilic character of sodium borohydride, diborane functions as an electrophilic hydrogenating agent. This different reducing character could be useful in selective tritiation. The selectivity of diborane permits labeling of one group in the presence of a second. Complete hydrogenation of ketones and t-amides can be achieved by diborane.

Therefore tritiated diborane could be useful for labeling other molecules containing specific functional groups. When high specific activity is desired, tritiated diborane should be prepared in situ without addition of carrier $NaBH_4$.

## ACKNOWLEDGMENTS

This research was supported by NIH grant HL 35343.

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