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[^{11}C]Diazepam Synthesis: N-[^{11}C]Methylation of Desmethyldiazepam is Facilitated by Utilization of a Preformed Sodium Salt/ Benzo-15-Crown-5 Complex

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Summary. The use of a preformed desmethyldiazepam sodium salt/ benzo-15-crown-5 complex in aprotic media allows for rapid and clean [^{11}C]methylation without contamination and loss of activity due to alkylation of solvent residues and other dissolved basic-nucleophilic material.

Keywords. Diazepam, desmethyldiazepam, benzodiazepine, benzo-15-crown-5, crown ether.

We recently began re-investigating the potential of 7-chloro-1,3-dihydro-1-[^{11}C]methyl-5-phenyl-2H-1,4-benzodiazepin-2-one ([^{11}C]Diazepam, [^{11}C]Valium; see Scheme, structure **3**) as a PET tracer to observe changes in benzodiazepine receptor occupancy in GABAergic neurons under conditions of physiological stress. [^{11}C]Diazepam has previously been synthesized (1) by a procedure which calls for deprotonation of desmethyldiazepam (see Scheme, structure **1**) in acetone by 10 N sodium hydroxide, followed by treatment with [^{11}C]methyl iodide. Despite reports of high radiochemical yields in the literature, we were only able to obtain an average of 47% for 4 runs using this procedure (see Table, below). We also noted the concurrent production of varying amounts of [^{11}C]methanol and one or more unidentified labeled products.

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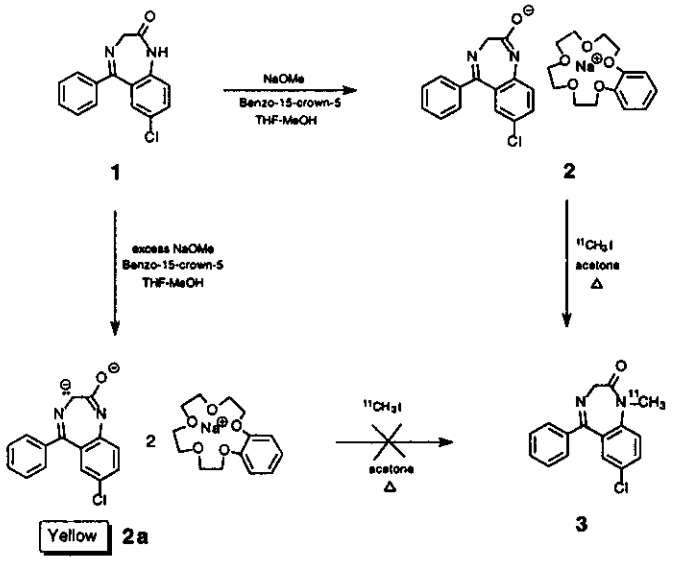
In an attempt to elucidate the course of the reaction and determine the identity of the undesired products, we performed a "macroscale" experiment by dissolving 0.10 mmol **1** in 1 mL acetone-d_6 in an NMR tube and treating it with 1 eq of 40% NaOD in D_2O. In principle, the formation of the anion and alkylation by the addition of methyl iodide could be observed by ^1H NMR. It was not surprising, however, when the introduction of 40% NaOD to the acetone-d_6 solution instantaneously produced an insoluble gelatinous material along the walls of the tube, the product of acetone condensation. It was evident that any condensation product that remained soluble, as well as the acetone enolate and water or residual hydroxide could easily be alkylated by methyl iodide.

We, therefore, elected to eliminate these problems by using a preformed desmethyldiazepam anion (see Scheme, structure **2**). The use of a crown ether to encapsulate the cation would allow the salt-complex a degree of solubility in aprotic solvents (necessary to avoid alkylation of the solvent) and provide appreciable separation of charges to facilitate attack of the anion by the electrophilic methyl iodide. Our decision to use the sodium salt was based on the commercial availability of a 0.5 M solution of sodium methoxide in MeOH, the ease of removing any protic solvent from the complex being an important criterion. Benzo-15-crown-5 was chosen as being the right cavity size to accommodate sodium (2) and because the fused benzo moiety would provide a weak chromophore to allow its identification by a UV detector during the HPLC purification step of radiosynthesis.

In preliminary experiments, we made several hundred milligrams of **2** (which after drying under high-vacuum, is a pale yellow powder). We were able to use this as a source for methylation experiments over the course of several weeks, however, the yields began to decline with time. By the end of the fourth week, no product could be obtained by using the powder. We had also observed that the addition of excess NaOMe results in a bright yellow color due to dianion formation (see Scheme, structure **2a**) from which no N-alkylation product can be obtained. It is likely, in both cases, that oxidation alpha to the carboxamide, which is also a major metabolic pathway for benzodiazepines (3,4), is the contributor to decline and loss of the complex's integrity. When the complex is prepared within several hours of its use, we observe consistently high yields.

The Scheme below, depicts formation and [^{11}C]methylation of the desmethyldiazepam sodium salt/ benzo-15-crown-5 complex **2** and formation of the nonproductive dianion **2a** in the presence of excess base.

SCHEME. Formation and [^{11}C]methylation of desmethyldiazepam sodium salt/ benzo-15-crown-5 complex.



The results of parallel experiments comparing deprotonation of **1** using 10 N NaOH with complex **2** at constant reaction time and temperature are shown in the Table, below. A reaction time of 15 minutes was considered optimal (i.e., yields were not

TABLE. Comparison of deprotonation and preformed complex methods for [^{11}C]methylations of desmethyldiazepam. Structure numbers correspond to those in the Scheme. Values for decay-corrected radiochemical yields (based on [^{11}C]CH_3I) are averages with average deviations and number of determinations, respectively, shown in brackets.

entry	substrate (base)	solvent	rxn temp (°C)	rxn time (min)	yield (%)
1	**1** (10 N NaOH)	acetone	95	15	47 [7, n = 4]
2	**2** (none)	acetone	95	15	89 [5, n = 4]
3	**2** (none)	THF	95	15	78 [3, n = 2]

increased by longer heating) after evaluating times from 10 to 20 minutes. In all cases, complex **2** was freshly prepared (see Experimental section). These data show THF to be an acceptable alternative to acetone; yields are marginally lower but comparable at the same time and temperature.

In conclusion, the use of desmethyldiazepam sodium salt/ benzo-15-crown-5 complex for the production of [^{11}C]diazepam is superior to the method using aqueous sodium hydroxide in acetone in that losses of activity associated with reactions with solvent components and residual basic material are prevented.

EXPERIMENTAL

Desmethyldiazepam was purchased from RBI, Natick, MA. Benzo-15-crown-5 and 0.5 M sodium methoxide in methanol were purchased from Aldrich. THF was distilled from sodium/benzophenone ketyl. All other materials were used without purification.

Complex formation. To a solution of desmethyldiazepam (3.0 mg, 11 μmol) and benzo-15-crown-5 (3.0 mg, 11 μmol) in 2 mL THF is added 0.5 M sodium methoxide in methanol (20 μL, 10 μmol) and the resulting solution stirred 15 min at room temperature. It is important that the amount of substrate be greater than the amount of added base to avoid dianion formation and subsequent decomposition (see text, above). Volatile material is then removed by rotary evaporation, with the bath temperature not exceeding 40 °C. The resulting oil, complex **2**, should be used within 2 hours.

[^{11}C]Methylation of complex **2**. Approximately 15 min prior to delivery of [^{11}C]methyl iodide, complex **2** is dissolved in 300 μL acetone (or THF) and transferred to a 10 mL reacti-vial. [^{11}C]Methyl iodide is trapped in the sealed vial at -15 °C and the temperature subsequently raised to 95 °C for 15 min. After cooling to room temperature, 650 μL HPLC eluant is added (50% CH_3CN/50% 0.1 N ammonium formate buffer) and the product obtained by isocratic elution [Beckman Ultrasphere C-18, 10 x 250 mm, particle size: 5 μm; flow rate: 4 mL/min (t_R = 12 min, total synthesis time: 60 min)]. Yields are given in the Table, above.

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