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Synthesis of [^{11}C]Tetrabenazine, a Vesicular Monoamine Uptake Inhibitor, for PET Imaging Studies

JEAN N. DaSILVA, MICHAEL R. KILBOURN* and THOMAS J. MANGNER

Division of Nuclear Medicine, Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, MI 48109, U.S.A.

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Tetrabenazine (TBZ), a high affinity and specific inhibitor of the vesicular monoamine transporter, has been labeled with carbon-11 as a potential probe for *in vivo* positron emission tomographic imaging of monoaminergic neuronal losses in neurodegenerative diseases. [^{11}C]TBZ was synthesized by *O*-[^{11}C]methylation of the 9-*O*-desmethylTBZ using [^{11}C]methyl iodide in the presence of tetrabutylammonium hydroxide. The radiochemical yields were 35–55% (decay corrected) and the synthesis time 32–37 min from EOB. [^{11}C]TBZ was obtained with specific activities of 2000–2500 Ci/mmol (EOS) and radiochemical and chemical purities were >95%. [^{11}C]Tetrabenazine is a promising new radioligand for the *in vivo* study of monoaminergic neurons using PET.

Introduction

Numerous potential neuronal imaging agents based on the neuronal monoamine reuptake system have been developed, and several of these [[^{11}C]cocaine, Fowler *et al.* (1989); [^{11}C]nomifensine, Salmon *et al.* (1990); [^{18}F]GBR 12909, Koeppe *et al.* (1990)] are currently being evaluated in human subjects using positron emission tomography (PET). The imaging of vesicular neurotransmitter uptake sites would form an alternative method for quantification of neuronal densities. Tetrabenazine (TBZ) is a high affinity [$IC_{50} = 3$ nM, Scherman *et al.* (1988a)] specific inhibitor of the monoamine transporter of the presynaptic vesicular membrane, and shows more CNS specificity, a shorter duration of monoamine depletion and a greater selectivity for depletion of brain catecholamines (dopamine and norepinephrine > serotonin) as compared to reserpine (Pettibone *et al.*, 1984; Scherman, 1986; Henry and Scherman, 1989). [^{3}H]Dihydrotetrabenazine ([^{3}H]TBZOH) has been recently used as a radioligand for *in vitro* homogenate and autoradiographic studies of the monoamine vesicular transporter, and shows high specific binding in the striatum of rodents (Scherman *et al.*, 1988b). In 6-hydroxydopamine-lesioned rats, loss of [^{3}H]TBZOH sites correlated well with loss of tyrosine hydroxylase activity (Masuo *et al.*, 1990), and post-mortem studies of brains of patients with Parkinson's disease revealed lower binding of [^{3}H]TBZOH in the caudate nucleus and the putamen (−73 and −87%) as compared to controls (Scherman *et al.*, 1989).

Tetrabenazine is thus a potential candidate for development as a new PET radiotracer to measure losses of dopaminergic neurons in neurodegenerative disorders, such as Parkinson's disease. The high binding affinity, moderate lipophilicity [log $P = 2.68$, Scherman *et al.* (1988a)] and low toxicity (Pletscher *et al.*, 1962) of this clinically used drug is particularly encouraging. Development of related labeled benzoquinolizine derivatives for single photon emission computed tomography (SPECT) imaging is being pursued by others (Canney *et al.*, 1993). We report here the synthesis of tetrabenazine in carbon-11 form as a potential radioligand for studying monoaminergic terminal losses in neurological diseases using PET.

Experimental

Materials

Tetrabenazine was purchased from Fluka and boron triiodide was obtained from Johnson Matthey Chemicals Ltd; all other chemicals and solvents were obtained from Aldrich Chem. Co., and were used without further purification. Sep-Pak C_{18} cartridges were purchased from Waters Associates. Thin-layer chromatography was performed on 0.25 mm Whatman glass-backed silica gel plates (60A K6F), with $CHCl_3$/methanol (24/1) as eluting solvent mixture. Semi-preparative HPLC was done using a Partisil 10 silica gel column (10 mm, 25 × 0.94 cm) eluted with CH_2Cl_2/hexane/isopropanol 85/14/1 at a flow rate of 6 mL/min. The HPLC system was fitted with in-line

*Author for correspondence.

detectors for u.v. (286 nm) and radioactivity (γ-detector). ^{1}H-NMR chemical shifts are reported relative to tetramethylsilane as an internal reference. Low-resolution mass spectrum (MS) and high-resolution mass spectrum (HRMS) were determined in the chemical ionization mode (ammonia); data are expressed in m/z (intensity relative to base peak = 100). Elemental analysis was performed by Spang Microanalytical Laboratory, Eagle Arbor, MI.

2-Oxo-3-isobutyl-9-hydroxy-10-methoxy-1,3,4,6,7-hexahydro-11bH-benzo[a]quinolizine (desmethyl-TBZ, 2)

Tetrabenazine (**1**, 3.77 g, 11.9 mmol) was dissolved in dichloromethane (10 mL), a N_2 atmosphere introduced and the mixture cooled to $-76°C$ in a dry ice/isopropanol bath. A solution of BI_3 (95%, 4.9 g, 1 equiv.) in CH_2Cl_2 (23.8 mL) was added dropwise over 5–10 min. After stirring for 5 min at room temperature, the reaction was quenched with a saturated aqueous solution (100 mL) of $NaHCO_3$ containing sodium bisulfite. The aqueous layer was extracted with CH_2Cl_2 (3 × 150 mL) and the extracts dried over Na_2SO_4, filtered and evaporated to dryness. The residue was purified (3×) by column chromatography (280 g silica gel, gradient $CHCl_3$/methanol 99/1–$CHCl_3$/methanol 99/5) to give **2** as an oil. DesmethylTBZ (**2**) was further purified by semi-preparative HPLC (CH_2Cl_2/hexane/isopropanol 50/49/1, 4 mL/min, $t_R = 11.6$ min) and dried over KOH under vacuum to provide an oil (72 mg, 2 %): TLC, $R_f = 0.43$; ^{1}H-NMR ($CDCl_3$ 360 MHz) δ 0.95 (d, 3H, $J = 6.60$, CH_3), 0.98 (d, 3H, $J = 6.60$, CH_3), 3.89 (s, 3H, OCH_3), 6.64 (s, 1H, 11-H), 7.20 (s, 1H, 8-H); MS (C.I., NH_3) m/z 304 ($[M+1]^+$, 100%); anal. (HRMS) calcd for $[C_{18}H_{25}NO_3—H]^+$: 304.1913. Found: 304.1900; anal. calcd for $C_{18}H_{25}NO_3$: C, 71.26; H, 8.31; N, 4.62. Found: C, 71.31; H, 8.34; N, 4.55.

Production of [^{11}C]methyl iodide

[^{11}C]Carbon dioxide was produced via the $^{14}N(p,a)^{11}C$ reaction using nitrogen in an all-aluminum target. [^{11}C]CO_2 was then converted to [^{11}C]methyl iodide by $LiAlH_4$ reduction followed by treatment with HI (Crouzel *et al.*, 1987; Jewett, 1987).

*Synthesis of [^{11}C]TBZ ([^{11}C]**1**)*

[^{11}C]Tetrabenazine was prepared by *O*-[^{11}C]methylation of desmethylTBZ with [^{11}C]methyl iodide (Fig. 1), utilizing the apparatus used for the synthesis of [^{11}C](+)2α-tropanylbenzilate (Mulholland *et al.*, 1992). [^{11}C]Methyl iodide was trapped in a reaction vessel containing desmethylTBZ (free base, 1.0 mg) and tetrabutylammonium hydroxide (TBAOH, MeOH solution, 1.8 mL, 0.55 equiv.) in DMF (200 mL) at -30 to $-40°C$. After heating at 20°C for 5 min, water (0.5 mL) was added to the mixture and the solution was transferred onto a short reversed phase column filled with C_{18} Sep-Pak packing that was pre-washed with MeOH (50 mL) and water (100 mL). The extraction column was rinsed with water (1.0 mL) and blown dry with N_2 (80 psi) for 2 min. The HPLC solvent was then passed through this short column onto a silica gel semi-preparative HPLC column. The radioactive peak corresponding to [^{11}C]TBZ ($t_R = 9.0$ min; phenol **2**, $t_R = >20$ min) was collected into a sterile vial placed in a warm water bath (30–40°C) and the solvent removed by N_2 flow. The residue was formulated in a sterile solution of isotonic phosphate buffer (pH 6.0) and then filtered through a 0.2 μm alumina filter (Anotop) into a sterile 10 mL multidose vial (yield 35–55%, decay corrected).

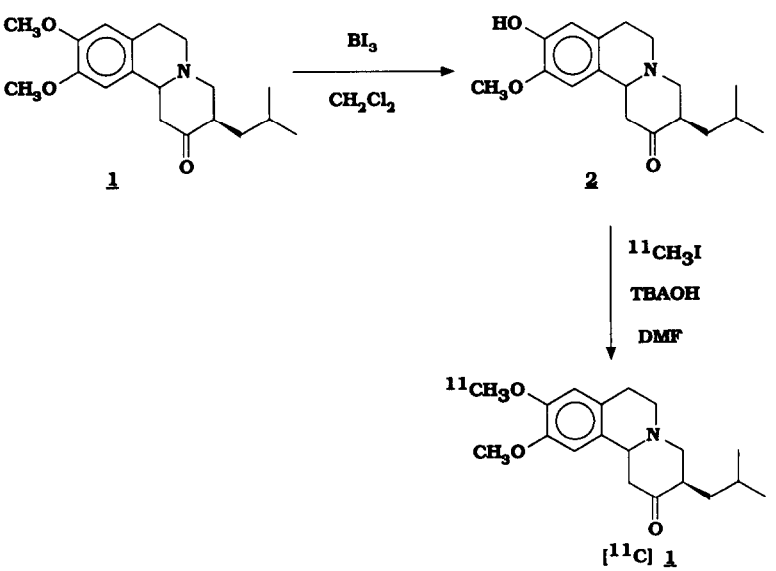


Fig. 1. Synthesis of [^{11}C]TBZ ([^{11}C]**1**).

Analyses of [^{11}C]1

HPLC. An aliquot was removed from the final formulation of [^{11}C]TBZ and assayed for chemical and radiochemical purity, and specific activity determination by analytical HPLC, using an Ultremex column (5 mm, 25 × 0.46 cm) eluted with CH_3CN/KH_2PO_4 (10 mM) 65/35, 1.5 mL/min (t_R = 6.1 min) in series with u.v. (286 nm) and γ-radioactivity detectors. Typical chemical and radiochemical purities were >95%, and specific activities at end of synthesis varied between 2000–2500 Ci/mmol. Identity of the radioactive product as [^{11}C]TBZ was determined by co-injection with authentic TBZ.

TLC. A sample of the radioactive fraction from semi-preparative HPLC after evaporation of the solvent was co-spotted on TLC with authentic TBZ. The radioactive product ([^{11}C]TBZ) showed the same R_f (0.62) as compared to authentic TBZ, and chemical (254 nm u.v.) and radiochemical purities were >99%.

Results and Discussion

Preparation of desmethylTBZ

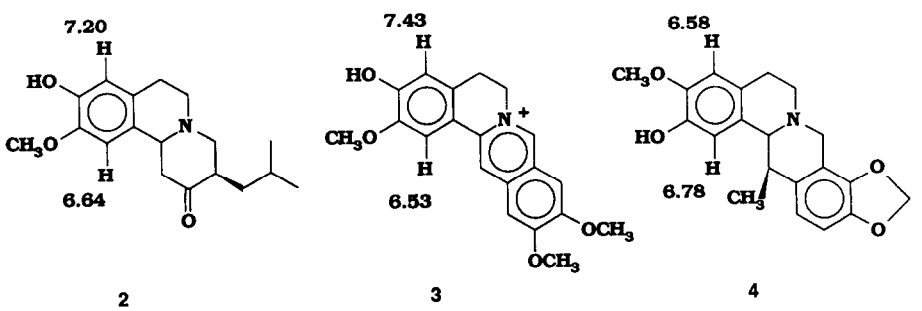
The demethylation of TBZ was carried out in low yields (2–10%) using BI_3 in dichloromethane (Lansinger and Ronald, 1979). The product was repeatedly purified by column chromatography and HPLC until completely free of contamination with unreacted TBZ or catechol; this extensive purification accounted for part of the overall low yield of this synthesis. Demethylation of TBZ with BBr_3 gave a mixture of both 9- and 10-desmethylTBZ in poor yields, while almost no demethylation occurred with iodotrimethylsilane (McOmie *et al.*, 1968; Vickery *et al.*, 1979). Although the overall yield was low, demethylation of TBZ was faster and more convenient than the optional total synthesis of desmethylTBZ.

Assignment of the structure of desmethylTBZ (2)

The structure of desmethylTBZ (**2**) was determined using a combination of ^{1}H-NMR, MS, HRMS and elemental analysis. The ^{1}H-NMR spectrum of TBZ showed two methoxy groups at 3.83 and 3.86 ppm, whereas that of desmethylTBZ exhibited only one methoxy group at 3.89 ppm. The presence of only one methoxy signal for **2** indicates that the demethylation with BI_3 was selective, however, it does not tell which methoxy group had been cleaved. The assignment of the hydroxyl and the methoxyl group was determined by comparing the chemical shifts of the aromatic protons of TBZ and desmethylTBZ with the published NMR spectra of structurally similar demethylated derivatives of protoberberines. The two aromatic protons of desmethylTBZ isolated in this work show singlets at δ720 and δ6.64, consistent with the chemical shifts at δ7.43 and δ6.53 observed for dehydrodiscretine [**3**; Chen *et al.* (1980)] but different from those of apocavidine [**4**: δ6.78 and δ6.58; Yu *et al.* (1970)], which represents an analogue with the other methoxy group cleaved (Fig. 2). Tetrabenazine, with both methoxy groups intact, shows singlets at δ6.55 and δ6.62. Based on these comparisons, we have assigned the hydroxyl group to C-9 and the methoxyl group to C-10 in the desmethylTBZ isolated in this work.

Synthesis of [^{11}C]tetrabenazine

[^{11}C]TBZ was successfully synthesized in high radiochemical yields (35–55%, decay corrected based on [^{11}C]CO_2) and high specific activities (2000–2500 Ci/mmol, end of synthesis) within 32–37 min from end of beam in high chemical and radiochemical purities (>95%). Higher radiochemical yields could be obtained in the production of [^{11}C]TBZ by adding more TBAOH or by increasing the reaction temperature, but more side products were also formed. However, using 0.55 equiv. of base (TBAOH) in DMF at room temperature gave only a few percent of side products and acceptable yields of [^{11}C]TBZ. The identity of [^{11}C]TBZ was confirmed by comparison of HPLC retention times and TLC R_f values, which were identical to those of authentic TBZ. Biodistribution studies in mice with [^{11}C]TBZ showed good brain penetration, with high specific uptake in the striatum (rich in vesicular transporters for dopamine) and lesser uptake in other brain regions, and specific binding could be blocked by coinjection of unlabeled TBZ or pretreatment with reserpine



2 3 4

Fig. 2. Structures and ^{1}H-NMR assignments for aromatic protons of desmethylTBZ (**2**), dehydrodiscretine (**3**) and apocavidene (**4**).

(DaSilva and Kilbourn, 1992). [^{11}C]TBZ is currently being evaluated in human subjects using PET.

In summary, [^{11}C]TBZ has been synthesized by *O*-[^{11}C]methylation of desmethylTBZ with [^{11}C]-methyl iodide as a potential probe for *in vivo* imaging of monoaminergic terminal losses in neurological diseases using PET. Future evaluation of this radiotracer will include *in vivo* displacement studies in mice, determination of possible metabolites in blood and tissues, and kinetic studies of the regional brain distribution in primates and humans using PET.

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