Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 5 November 2010, Revised 18 January 2011, Accepted 21 January 2011 Published online 7 April 2011 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.1881

Synthesis of ^{3}H-Labeled Tetrabenazine (TBZ)

Sung-Whi Rhee, Kenneth J. Ryan, and Mary J. Tanga*

Tetrabenazine (TBZ) (1,3,4,6,7,11b-hexahydro-9,10-dimethoxy-3-(2-methylpropyl)-2H-benzo[a]quinolin-2-one), a vesicular monamine transporter 2 inhibitor, was prepared as a tritium-labeled compound with high specific activity and radiochemical purity. Catalytic hydrogenation of a precursor with the terminal double bond was used to introduce the tritium. This method provides tritium-labeled TBZ with high specific activity and radiochemical purity, which allow the further investigation of a TBZ in the neurological field.

Keywords: tetrabenazine; TBZ; VMAT2-inhibitor; catalytic tritiation of terminal double bond

Introduction

Tetrabenazine (TBZ), which was introduced in 1956 as an antipsychotic drug,[1] is currently used as treatment for hyperkinetic movement disorders.[2,3] TBZ is considered a classical inhibitor of vesicular monamine transporter 2 (VMAT2).[4] VMAT2 is the only transport that moves cytoplasmic dopamine into synaptic vesicles for storage and subsequent release in the central nervous system.[5] It has been shown to deplete cerebral monoamines in rat brain by reversibly inhibiting VMAT2.[1,5] TBZ (Xenazine®) is approved by the U.S. Food and Drug Administration (FDA) to treat chorea, the jerky, involuntary movement, that occurs in people with Huntington's disorder.[6] It is also used to alleviate tics in Tourette's syndrome[7] and movement disorders in tardive dyskinesia.[8]

Experimental

General methods

Ultraviolet spectra were recorded on a Varian DMS-90 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Gemini 300-MHz spectrophotometer using tetramethysilane as the internal standard. In reporting the NMR multiplicities, we use the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; apt, apparent; and br, broad. Reverse-phase HPLC data were obtained using a Waters 2690 Separations Module with photodiode array detector and a Perkin Elmer radioactivity monitor. The mass spectra were obtained with a Ribermag R 10-10 GC/MS. The National Tritium Labeling Facility at Lawrence Berkeley National Laboratory (Berkeley, CA) carried out the tritiation. Atlantic Microlab, Inc. (Norcross, GA) performed microanalysis. TLC analyses were carried out on commercial precoated silica gel 60F254 plates (E. Merck; 5×10 cm and 5×20 cm). The plates were scanned using a Bioscan System 200 Imaging Scanner. Chromatographies were carried out using 230–400 mesh silica gel 60 (E. Merck).

3-(Dimethylaminomethyl)-5-methyl-2-hexanone (2)

A mixture of 5-methyl-2-hexanone (**1**, 130 mL, 115.4 g, 1.01 mol), diethylamine hydrochloride (81.5 g, 1.00 mol), and paraformaldehyde (45 g, 1.5 mol) in absolute ethanol (EtOH, 300 mL) was refluxed for 18 h, protected from moisture. The solution was evaporated to a white solid. The solid was dissolved in dichloromethane (CH_2Cl_2, 500 mL), and the solution was washed with a saturated sodium bicarbonate solution to neutralize the amine hydrochloride. The CH_2Cl_2 solution was dried ($MgSO_4$), filtered, and evaporated to dryness. The NMR showed a mixture of 3-(dimethylaminomethyl)-5-methyl-2-hexanone (**2**) and 1-(dimethylamino)-6-methyl-3-heptanone. The oil was placed on a silica gel column and eluted with CH_2Cl_2 to give 30 g of oil. The oil was dissolved in ethyl ether (Et_2O, 100 mL), and 1 M HCl in Et_2O was added until no further oily precipitate formed. The mixture was evaporated to dryness. The residue was taken up in Et_2O (100 mL) and stirred on an ice bath protected from moisture until a white solid formed; then pentane (100 mL) was added with stirring and chilling. The mixture was chilled at 0°C for 18 h. The very hydroscopic solid was filtered, dissolved in CH_2Cl_2, and then evaporated to oil, which solidified under high vacuum to give 20 g (10%) of the amine hydrochloride (**2**).

^{1}H NMR: (300 MHz, $CDCl_3$) (HCl salt) δ 3.60 (m, 1H), 3.40 (ddt, 1H), 2.87 (dq, 1H), 2.79 (d, 3H, $J = 4.7$ Hz), 2.65 (d, 3H), 2.35 (s, 3H), 1.67 (m, 1H), 1.43 (ddd, 1H), 1.22 (ddd, 1H), 1.03 (d, 3H), 0.95 (d, 3H) ppm.

^{1}H NMR: (300 MHz, $CDCl_3$) (free base) δ 2.75 (m, 1H), 2.56 (dd, 1H), 2.21 (s, 3H), 2.18 (s, 3H), 2.13 (s, 3H), 2.18 (m, 1H), 1.46 (m, 2H), 1.19 (m, 1H), 0.88 (dd, 6H) ppm.

Tetrabenazine (4a)

To a solution of 3,4-dihydro-6,7-dimethoxyisoquinoline hydrochloride (**3**, 3.5 g, 15.4 mmol) in cold H_2O (20 mL) in an ice water bath, was added 3-(dimethylaminomethyl)-5-methyl-2-hexanone (**2**, 3.15 g, 18.3 mmol) as the free base with stirring. Precipitate formed within 3 h, and stirring was continued until the solid-gummy precipitate prevented stirring. The mixture was allowed

Biosciences Division, SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025, USA

**Correspondence to: Mary J. Tanga, Biosciences Division, SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025, USA.*
E-mail: mary.tanga@sri.com

to stand at RT (room temperature) for 3 days. The solid–gum mixture was filtered, and the yellow solid–gum mixture was dissolved in hot MeOH. The solution was chilled at −10°C for 18 h. The pale yellow solid was filtered to give 2.1 g (43%) of TBZ **(4a)**.

TLC: R_f = 0.62; silica gel; 4% MeOH/96% CH_2Cl_2.

MS: (DCl-NH_3) *m/z* 318 (M+H).

UV: (EtOH) λ_{max} 282.0 nm (ε 4431).

^{1}H NMR: (300 MHz, $CDCl_3$) δ 6.61 (s, 1H), 6.55 (s, 1H), 3.85 (s, 3H), 3.82 (s, 3H), 3.51 (br dd, 1H), 3.29 (dd, 1H), 3.13 (m, 2H), 2.90 (dd, 1H), 2.75 (m, 2H), 2.57 (m, 2H), 2.35 (t, 1H), 1.81 (ddd, 1H), 1.65 (m, 1H), 1.04 (ddd, 1H), 0.92 (d, 3H), 0.89 (d, 3H) ppm.

^{13}C NMR: (75 MHz, $CDCl_3$) δ 210.00, 147.86, 147.54, 128.60, 126.11, 111.53, 107.94, 62.48, 61.52, 56.01, 55.92, 50.58, 47.62, 47.57, 36.09, 29.38, 25.44, 23.21, 22.11 ppm.

EA: Anal. Calc for $C_{19}H_{17}NO_3$: C, 71.89; H, 8.57; N, 4.41. Found C, 72.15; H, 8.69; N, 4.47.

HPLC: Brownlee 25 cm × 4.6 mm silica gel column; 30% isopropanol/70% hexane; 1 mL/min; ret. time 5.94 min; purity >99.5%.

1,3,4,6,7,11b-Hexahydro-3-isobutyl-9,10-dimethoxy-2-oxo-2H-benzo[a]quinolizine (5)

To TBZ **(4**, 317 mg, 1.00 mmol) and chloranil (260 mg, 1.05 mmol) was added benzene (30 mL), and mixture was refluxed for 2.5 h. To the dark solution plus insoluble precipitate was added benzene (50 mL) and the mixture was washed with 30 mL of 2 N sodium hydroxide and H_2O. The benzene solution was dried ($MgSO_4$), filtered, and evaporated to dryness. The residue was crystallized from ethyl acetate (EtOAc)/hexane to give 120 mg (38%) of a gray solid **(5)**.

TLC: R_f = 0.25; silica gel; 4% MeOH/96% CH_2Cl_2.

MS: (DCl-NH_3) *m/z* 316 (M+H).

UV: (EtOH) λ_{max} 361.2 nm (ε 17,900), 284.4 (9,932), 238.0 (21,300).

^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.14 (s, 1H), 6.65 (s, 1H), 5.61 (s, 1H), 3.89 (s, 3H), 3.85 (s, 3H), 3.64 (dd, 1H), 3.37 (ddd, 2H), 3.29 (dd, 1H), 2.93 (m, 1H), 2.43 (ddd, 1H), 1.70 (m, 2H), 1.26 (m, 1H), 0.96 (d, 3H); 0.90 (d, 3H) ppm.

^{13}C NMR: (75 MHz, $CDCl_3$) δ 195.43, 156.48, 151.51, 148.11, 128.91, 120.89, 110.44, 108.38, 94.32, 56.06, 55.86, 49.08, 42.10, 37.52, 28.50, 25.57, 23.50, 21.88 ppm.

EA: Anal. Calc for $C_{19}H_{25}NO_3$: C, 72.35; H, 7.99; N, 4.44. Found: C, 72.32 H; 8.04; N, 4.45.

HPLC: Brownlee 25 cm × 4.6 mm silica gel column; 30% isopropanol/70% hexane; 1 mL/min; ret. time 12.87 min; purity >99.5%.

3-(Dimethylaminomethyl)-5-methyl-5-hexen-2-one (8)

A mixture of 5-methyl-5-hexen-2-one **(7**, 25 g, 0.22 mol), diethylamine hydrochloride (18.3 g, 0.223 mol) and paraformaldehyde (10.3 g, 0.35 mol) in EtOH (300 mL) was refluxed for 18 h, protected from moisture. The solution was evaporated to a semisolid. Half the semisolid was dissolved in CH_2Cl_2 (100 mL) and the solution was washed with saturated sodium bicarbonate solution to neutralize the amine hydrochloride. The CH_2Cl_2 solution was dried ($MgSO_4$), filtered, and evaporated to dryness. The NMR showed a mixture of 3-(dimethylaminomethyl)-5-methyl 5-hexen-2-one **(8)** and 1-(dimethylamino)-6-methyl-6-hepten-3-one. The oil was chromatographed on a silica gel column and eluted with CH_2Cl_2 to give 500 mg of oil (3%) of the desired product **(8)**.

^{1}H NMR: (300 MHz, $CDCl_3$) δ 4.75 (d, 2H), 3.10 (m, 1H), 2.88 (dd, 1H), 2.35 (m, 2H), 2.35 (s, 6H), 2.20 (s, 3H), 2.07 (dd, 1H), 1.74 (s, 3H) ppm.

1,3,4,6,7,11b-Hexahydro-3-(2-methylprop-2-enyl)-9,10-dimethoxy-2-oxo-2H-benzo[a]quinolizine (9)

To a solution of 3,4 dihydro-6,7-dimethoxyisoquinoline hydrochloride **(3**, 0.540 g, 15.4 mmol) in cold H_2O (20 mL) on an ice water bath was added 3-(dimethylaminomethyl)-5-methyl 5-hexan-2-one **(8**, 3.15 g, 18.3 mmol) as the free base with stirring. Precipitate formed within 3 h, and stirring was continued for 5 days. The yellow solid was filtered and then dissolved in hot MeOH. The solution was chilled at −10°C for 18 h, resulting in a pale yellow solid, which was filtered to give 140 mg (29%) of 1,3,4,6,7,11b-hexahydro-3-(2-methyl-2-propenyl)-9,10-dimethoxy-2-oxo-2H-benzo[a]quinolizine **(9)**.

TLC: R_f = 0.62; silica gel; 4% MeOH/96% CH_2Cl_2.

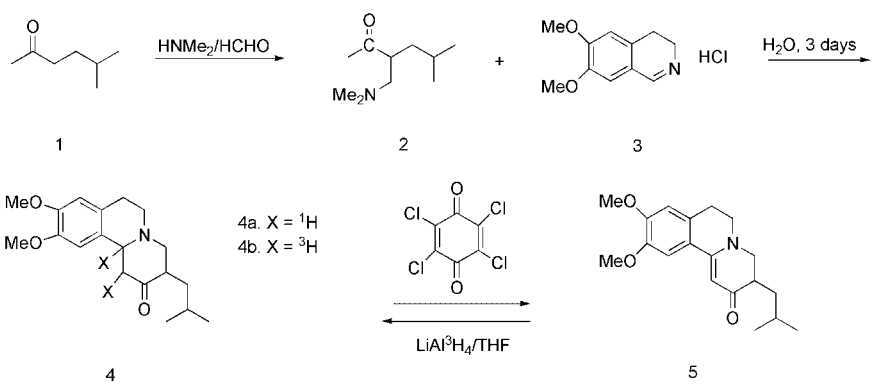
UV: (EtOH) λ_{max} 284.4 nm (ε 38,900).

^{1}H NMR: (300 MHz, $CDCl_3$) δ 6.62 (s, 1H), 6.56 (s, 1H), 4.77 (d, 2H), 3.86 (s, 3H), 3.84 (s, 3H), 3.51 (br dd, 1H), 3.29 (dd, 1H), 3.13 (m, 2H), 2.95 (dd, 1H), 2.88 (m, 1H), 2.60 (m, 2H), 2.32 (t, 1H), 1.95 (dd, 1H), 1.74 (t, 3H) ppm.

^{13}C NMR: (75 MHz, $CDCl_3$) δ 209.3, 147.9, 147.57, 142.75, 128.49, 126.14, 112.21, 111.55, 107.94, 62.47, 60.70, 56.03, 55.94, 50.62, 47.55, 47.09, 34.47, 29.36, 22.26 ppm.

EA: Anal. Calc for $C_{19}H_{17}NO_3 \cdot 0.5\ H_2O$: C, 71.33; H, 8.03; N, 4.38. Found: C, 72.35; H; 7.99; N, 4.44.

HPLC: Brownlee 25 cm × 4.6 mm silica gel column; 30% isopropanol/70% hexane; 1 mL/min; ret. time 5.78 min; purity ⩾ 99.9%.



Scheme 1.

^{3}H-Tetrabenazine (1,3,4,6,7,11b-hexahydro-9,10-dimethoxy-3-(2-methylpropyl-1′,2′-$^{3}H_2$)-2H-benzo[a]quinolizin-2-one) (4b)

The olefinic TBZ, 1,3,4,6,711b-hexahydro-9,10-dimethoxy-3-(2-methylpropenyl)-2H-benzo[a]quinolizin-2-one (**9**, 16 mg, 0.025 mmol), and 10% palladium on carbon (Pd/C; 10 mg), and EtOAc (2 mL) were placed in a tritiation flask. After a freezing, evacuation, and thawing degassing cycle, the mixture was stirred under tritium gas (total activity ~120 Ci) for 3 h at RT. The mixture was cooled on liquid nitrogen, degassed again, and the labile tritium was removed by washing with MeOH (2 × 3 mL). Filtration and concentration of the filtrate resulted in a crude TBZ with a total activity of 6.3 Ci (233.1 GBq). To remove the yellow impurity, ^{3}H-labeled TBZ was purified twice by HPLC (column C_{18}, CN, 4.6 × 250 mm) using a gradient of 15% acetonitrile/85% of 0.1% trifluoroacetic acid (TFA) in H_2O going to 100% of 0.1% TFA in H_2O over 10 min. The ^{3}H-TBZ (**4b**) obtained had a total activity of 2.7 Ci (99.9 GBq), a specific activity of 50 Ci (1,850 GBq/mmol), and radiochemical purity of 97% by HPLC.

As a part of cold chemistry, the olefinic TBZ (**9**) (18 mg) was deuterated under the same conditions (deuterium gas, 10% Pd/C, at RT) to obtain $^{2}H_2$-TBZ, (1,3,4,6,7,11b-hexahydro-9,10-dimethoxy-3-(2-methylpropyl1′,2′-$^{2}H_2$)-2H-benzo[quinolizin-2-one. The identity of the deuterium-labeled TBZ (**4c**) was confirmed by comparison with unlabeled TBZ (**4a**) using NMR, TLC, and HPLC.

Results and discussion

Labeled TBZ (**4a** and **4b**), a hexahydrobenzo[a]quinolizinone,[9–12] was prepared in support of pharmacological studies. In preparing a substrate for tritium labeling we initially used Osbond's approach,[11] as shown in Scheme 1. TBZ (**4**) had been observed to be converted to its corresponding α,β-unsaturated ketone (**5**) in good yield by treatment with chloranil[13] in benzene. Furthermore, the unsaturated compound (**5**) could be reduced back to the saturated ketone (**4**), TBZ, in fair yield with lithium aluminum hydride ($LiAlH_4$) in refluxing tetrahydrofuran (THF). The retention of the ketone group was unexpected: selective reduction of the double bond of α,β-unsaturated ketones or esters with this reagent is rare, although complete reduction to the saturated alcohol is well known. Intrigued by these observations, we decided to use this scheme for incorporating tritium into TBZ by converting TBZ to the corresponding α,β-unsaturated ketone (**5**) and subsequent reduction with lithium aluminum tritide ($LiAl^{3}H_4$) to obtain tritium-labeled TBZ (1,11b-$^{3}H_2$-3,4,6,7-hexahydro-3-isobutyl-9,10-dimethoxy-2-oxo-2H-benzo[a]quinolizine) (**4b**). However, we were unable to duplicate the reduction of the α,β-unsaturated ketone (**5**) to the corresponding saturated ketone (**4a**), TBZ, under three reaction conditions:

- Reduction with refluxing $LiAlH_4$ in THF;
- Reduction with super-hydride (lithium triethylborohydride, $LiBEt_3H$)[13]; and
- Reduction by catalytic hydrogenation with hydrogen or deuterium gas.

The TBZ (**4**) for these reactions was prepared by reacting 3,4-dihydro-6,7-dimethoxyisoquinoline (**3**) and the Mannich base (**2**) as shown in Scheme 1.[14] The α,β-unsaturated TBZ (**5**), which was the original substrate, was obtained by further treatment with chloranil in refluxing benzene.

In each case, after reduction, only a trace amount of the desired saturated ketone (**4**) was detected by thin layer chromatography (TLC) or high-pressure liquid chromatography (HPLC). We suspect that the resonance between (**5**) and (**5′**) may have been responsible for its lack of reactivity for hydrogenation of α,β-unsaturated ketone (**4**) by hydride or hydrogen (Figure 1).

To circumvent this unexpected problem, we prepared a new substrate (**9**)[14] for tritium labeling as shown in Scheme 2. In this approach, we replaced 3-(2-methylpropyl), the alkyl group at 3-position, with 3-(2-methylpropene) (**9**). The methylene group was used successfully in incorporating tritium by catalytic hydrogenation.

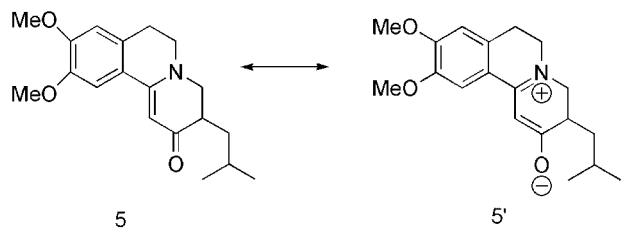
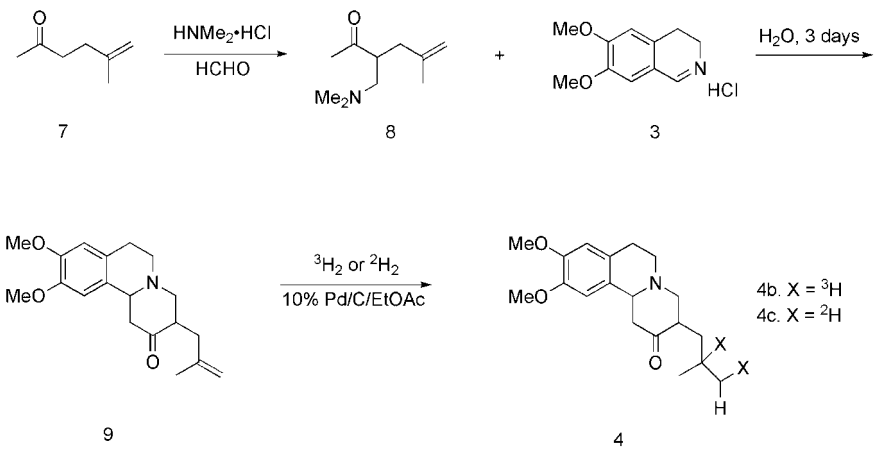


Figure 1. Resonance structures of α,β-unsaturated TBZ.



Scheme 2.

Catalytic hydrogenation was used to tritiate or deuterate the olefinic TBZ substrate (**9**). The resulting tritium-labeled TBZ (**4b**) had a total activity of 2.7 Ci (99.9 GBq), a specific activity of 50 Ci (1850 GBq)/mmol, and 97% radiochemical purity as assessed by radio-HPLC after purification by a preparative HPLC.

Conclusions

An efficient synthesis of tritium-labeled TBZ with high specific activity and radiochemical purity has been developed, which allows for the further investigation of a TBZ in the neurological field.

Acknowledgements

The National Institute of Mental Health supported this work under Contract N01-MH-80015. We would also like to thank Mr. Hiromi Morimoto and Dr. Philip Williams of the National Tritium Labeling Facility (NTLF) at Lawrence Berkeley Laboratory for their assistance.

References

[1] D. J. Pettibone, A. B. Pflueger, J. A. Totaro, *Eur. J. Pharmacol.* **1984**, *102*, 431–436.
[2] C. Kenny, J. Jankovic, *Expert. Rev. Neurother.* **2006**, *6*, 7–17.
[3] J. Jankovic, J. Beach, *Neurology* **1997**, *48*, 358–362.
[4] A. Pletscher, *Br. J. Pharmacol.* **1977**, *59*, 419–424.
[5] G. Zheng, L. P. Dwoskin, P. A. Crooks, *AAPS J.* **2008**, *8*, E682–E692.
[6] A. Fasano, F. Cadeddu, A. Guidubaldi, C. Piano, F. Soleti, P. Zinzi, A. R. Betivoglio, *Clin. Neuropharmacol.* **2008**, *31*, 313–318.
[7] M. Porta, M. Sassi, M. Cavallazzi, M. Fornari, A. Brambilla, D. Servello, *Clin. Drug Investig.* **2008**, *28*, 443–459.
[8] W. G. Ondo, P. A. Hanna, J. Jankovic, *Am. J. Psychiatry* **1999**, *156*, 1279–1281.
[9] A. R. Battersby, H. T. Openshaw, H. C. S. Wood, *J. Chem. Soc.* **1953**, 2463–2470.
[10] A. Brossi, H. Lindlar, M. Water, O. Schneider, *Helv. Chim. Acta* **1958**, *41*, 119–139.
[11] J. M. Osbond, *J. Chem. Soc.* **1961**, 4711–4718.
[12] N. Whittaker, *J. Chem. Soc. C* **1969**, 85–89.
[13] B. E. Blough, F. I. Carrol, *Tetrahedron Lett.* **1993**, *34*, 7239–7242.
[14] E. D. Popp, R. F. Watts, *J. Pharm. Sci.* **1978**, *67*, 871–873.