THE SYNTHESIS OF GLYCINE RECEPTOR RADIOLIGANDS

[21, 22-^{3}H] DIHYDROSTRYCHNINE AND [2-^{3}H]

STRYCHNINE AT HIGH SPECIFIC ACTIVITY[1]

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SUMMARY

Strychnine (1a) was reduced with tritium gas via heterogeneous catalysis to yield [21, 22-^{3}H] dihydrostrychnine (2b) at 31 Ci/mmol and via homogeneous catalysis to yield 2b at 50 Ci/mmol. [2-^{3}H] Strychnine (1b) at 25 Ci/mmol was prepared by the catalytic reductive tritiation of 2-iodostrychnine (4). Other polyhalo strychnine analogues were synthesized in an attempt to prepare [^{3}H] strychnine at even higher specific activity.

Key Words: [21, 22 - ^{3}H] Dihydrostrychnine, [2 - ^{3}H] Strychnine, Tritium, ^{3}H NMR.

INTRODUCTION

Compelling evidence supports the role of glycine as a candidate for an endogenous inhibitory neurotransmitter in spinal cord[2, 3]. The Strychnos alkaloid family has provided the most useful antagonists for the glycine receptor[4] and generally radiolabeled [^{3}H] strychnine was developed as a probe of this receptor at the molecular level[5-9]. Because of our interest in providing radiolabeled tools for the glycine receptor, we prepared [21, 22-^{3}H] dihydrostrychnine 2b at 50 Ci/mmol and specifically radiolabeled [2-^{3}H] strychnine (1b) at 25 Ci/mmol.
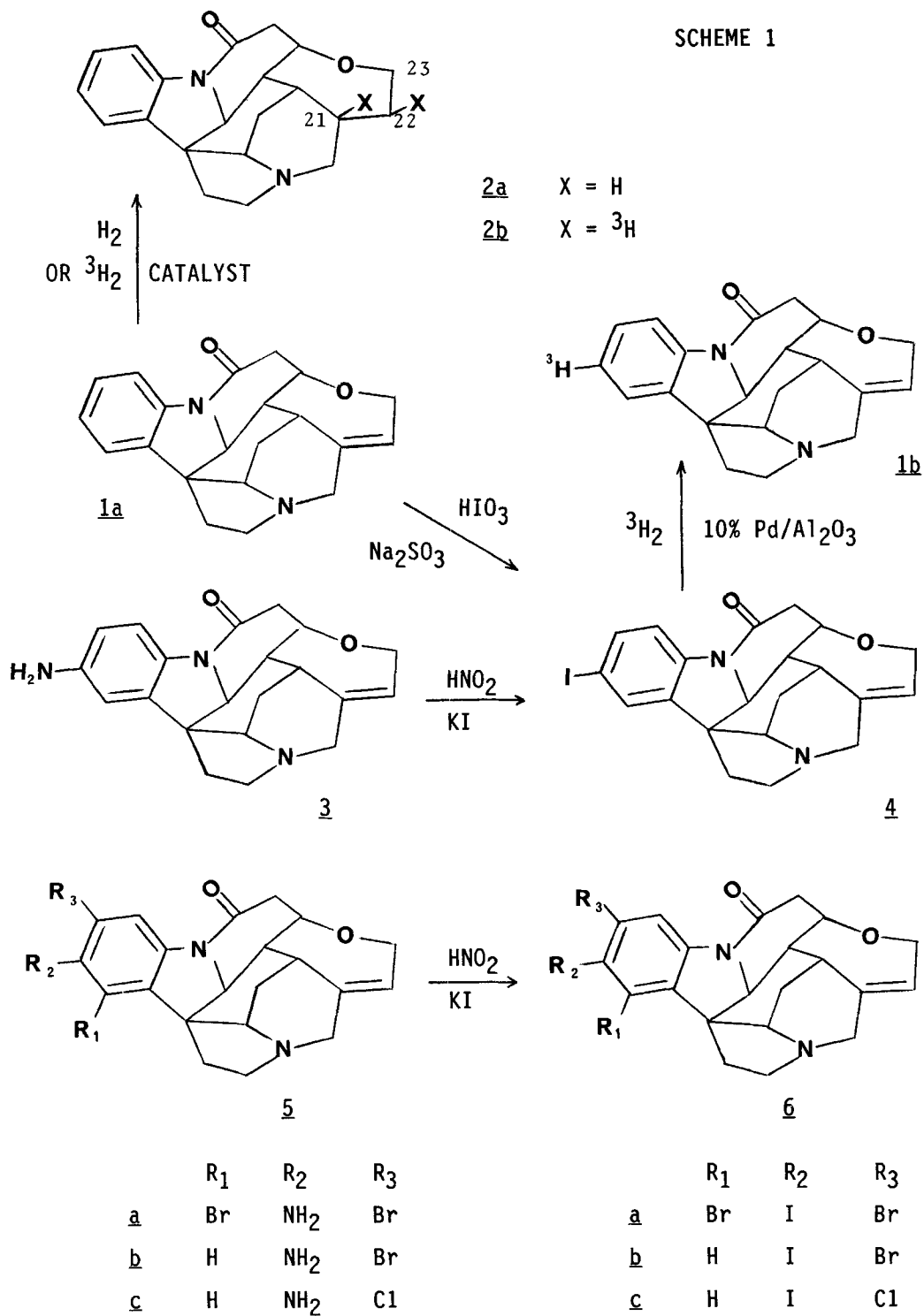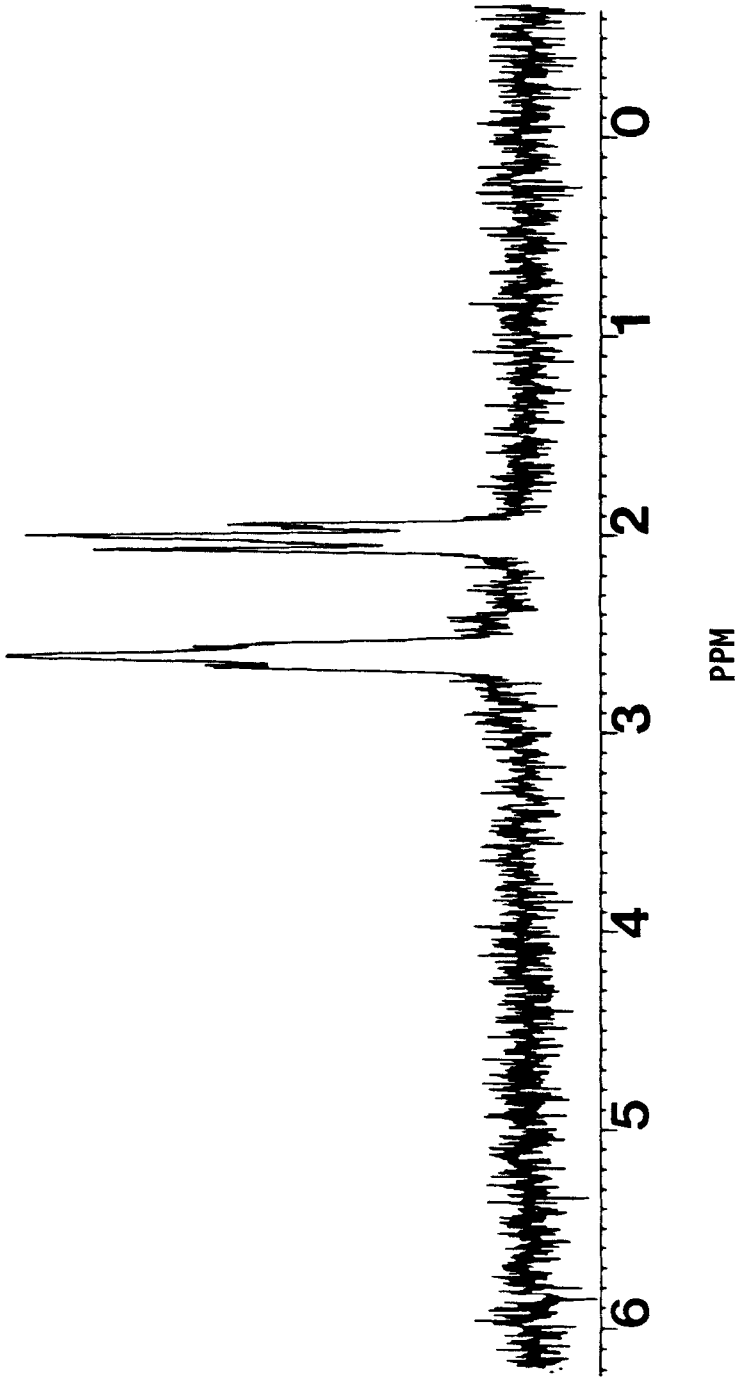
DISCUSSION

The hydrogenation of strychnine (1a) to dihydrostrychnine (2a) via heterogeneous catalysis (Pd) was reported by Skita and Franck as early as 1911[10] and later more thoroughly studied by Robinson[11]. Only recently, however, has ^{1}H and ^{13}C NMR spectroscopy[12] and x-ray crystallography[13] confirmed the stereochemistry of hydrogenation as occurring from the least hindered side of 1a yielding 2a. Our initial attempt at the reduction of 1a with tritium gas utilized 10% Pd/C in THF for 16 h. as shown in Scheme 1. The resulting crude 2b was purified by TLC in a solvent system that clearly separated it from any unreduced 1a. In this way, 2b in 29% radiochemical yield was obtained at 31 Ci/mmol.

A ^{1}H decoupled ^{3}H NMR of 2b at 31 Ci/mmol establishing the existence of a ditritiated radioligand (two coupled doublets) and two monotritiated radioligands (a singlet superimposed on each doublet) has already been published[14a,b]. Double resonance studies performed on 2b at this specific activity confirmed the relative chemical shift assignments made for the 21 and 22 position protons of 2a by Wenkert[12]. A ^{1}H coupled ^{3}H NMR of 2b was taken and as expected it showed more complex multiplets than observed for the ^{1}H decoupled ^{3}H NMR of 2b. However, irradiation of this ^{1}H coupled sample at the resonance frequency (δ 4.00 ppm) of the 23 alpha position proton of 2b afforded a ^{3}H NMR (Figure 1) with an upfield multiplet decidedly less complex than the downfield multiplet. This observation supports Wenkert's assignment of the 21 and 22 position protons of 2a as being the downfield and upfield resonances respectively. Recently another example of ^{3}H NMR assisting in ^{1}H NMR interpretation has been reported[15].

To prepare 2b at even higher specific activity, 1a was tritiated with homogeneous catalyst. The reduction of 1a with tritium gas and $(Ph_3P)_3RhCl$ in benzene for 16 h. yielded a somewhat cleaner crude reaction mixture than obtained by heterogeneous catalysis. TLC purification as before afforded 2b in 40% radiochemical yield at 50 Ci/mmol. The ^{1}H decoupled ^{3}H NMR of 2b at 50 Ci/mmol showed as expected a much higher contribution of ditritiated radioligand.[14b]

SCHEME 1

2a	X = H
2b	X = ³H

H₂ OR ³H₂ | CATALYST

1a

HIO₃ / Na₂SO₃

³H₂ | 10% Pd/Al₂O₃

1b

3

HNO₂ / KI

4

5

HNO₂ / KI

6

	R₁	R₂	R₃
a	Br	NH₂	Br
b	H	NH₂	Br
c	H	NH₂	Cl

	R₁	R₂	R₃
a	Br	I	Br
b	H	I	Br
c	H	I	Cl

FIGURE 1: ^{1}H COUPLED ^{3}H NMR (CDCL$_3$) OF [21,22-^{3}H] DIHYDROSTRYCHNINE 2B PREPARED BY HETEROGENEOUS CATALYSIS, IRRADIATED AT 4.00 PPM.

Besides the preparation of radioligand 2b, we endeavored to facilitate the preparation of [³H] strychnine. Although [³H] strychnine had previously been prepared by general exchange with tritiated water[16], we hoped to synthesize it by means of a specific tritiation method. To insure a consistently high specific activity, we selected as a procedure the catalytic dehalogenation of an iodostrychnine with tritium. As a suitable precursor 2-iodostrychnine (4) was prepared from 2-aminostrychnine (3) by essentially the same method as previously described[17]. Compound 4 was reduced with tritium gas and 10% Pd/Al$_2$O$_3$ in benzene with Et$_3$N for 1.5 h. as shown in Scheme 1. The resulting 1b was first purified by TLC in a solvent system that clearly separated it from any overreduced [2,21,22-³H] dihydrostrychnine and finally HPLC. In this way 1b in 14.5% radiochemical yield was obtained at 25 Ci/mmol. A ¹H decoupled ³H NMR (CDCl$_3$) of 1b indicated exclusive aromatic radiolabeling. Most likely influenced by steric factors, this is a rare example of a reasonably preferential tritium dehalogenation in the presence of a double bond. Radioligand 1b prepared in this way was recently used to assist in mapping the glycine receptor in rat brain by autoradiography[18].

To further elevate the specific activity of [³H] strychnine we explored the use of polyhalogenated strychnines. An examination of the literature disclosed that there were few previous examples of polyhalogenated strychnines reported. Our strategy to prepare them involved mono or dihalogenation of 3 followed by diazotization and iodide displacement. Table 1 summarizes our efforts. Intermediates 5a and 5b were prepared in low yield from bromination of 3. The reaction of 3 and ICl surprisingly afforded 5c but the literature does contain precedent for ICl acting as a chlorinating reagent[19]. Diazotization and iodide displacement of these intermediates afforded polyhalogenated strychnines 6a, 6b and 6c respectively. Unlike the case of 4, we could not work out catalytic tritiation conditions for these precursors that would cleanly yield any [benzene ring-³H] strychnine without concomitant olefin reduction.

In summary, radioligand 2b has been prepared at 50 Ci/mmol, and radioligand 1b has been synthesized at 25 Ci/mmol by a specific tritiation method. ³H NMR has been used to confirm radiolabeling specificity.

TABLE 1

COMPOUNDS[1]	REACTION CONDITIONS	M.P.	YIELD
2-Amino-1,3-dibromostrychnine (5a)	2 Br$_2$, TFA/HOAc, 24°C	279-282°C	11.2%
2-Amino-3-bromostrychnine (5b)	Br$_2$, aq. HBr/HOAc, 24°C	> 300°C	16.8%
2-Amino-3-chlorostrychnine (5c)	2 ICl, TFA/HOAc, 24°C	274-280°C	24.5%
1,3-Dibromo-2-iodostrychnine (6a)	HNO$_2$, KI, 0-100°C	235-240°C	29.7%
3-Bromo-2-iodostrychnine (6b)	" " "	280-284°C	21.5%
3-Chloro-2-iodostrychnine (6c)	"	286-290°C	28.9%

1. All compounds were purified by TLC, demonstrated to be homogeneous by TLC and
   HPLC, and provided ^{1}H NMR, IR, UV and mass spectral data in accord with the
   proposed structures.

EXPERIMENTAL SECTION

General Methods Evaporations were carried out an a Büchi rotary evaporator in vacuo at bath temperatures below 40°C. TLC was performed an Analtech 5 x 15 cm (250 μm, analytical) and 20 x 20 cm (1000 μm preparative) silica gel GF coated glass plates. Common solvent combinations were S_1 (Cyclohexane: EtOAc:Et$_2$NH, 3:7:1), S_2 (PhH:EtOAc:Et$_2$NH, 7:2:1), S_3 (CHCl$_3$:CH$_3$OH:NH$_4$OH, 9:1:0.1). Autoradiography was performed at 0°C after spraying with PPO (DuPont, NEN Products) and exposing the TLC plates to Du Pont Cronex x-ray film. TLC plates were also scanned for radioactivity by using a Packard 7201 scanner. Preparative and analytical HPLC was performed on a Waters instrument using a Zorbax silica column eluted with S_4 (CH$_2$Cl$_2$:CH$_3$OH:Et$_2$NH, 99.1:0.5:0.4) at 1 mL/min. Peak detection was performed simultaneously by UV (280 nm - Waters 440 UV detector) and a liquid scintillation flow monitor. UV spectra were measured on a Beckman Model 25 spectrophotometer. The proton and triton magnetic resonance spectra were obtained on a Bruker WP 200 mHz NMR spectrometer and chemical shifts are expressed in parts per million (ppm) downfield from internal (CH$_3$)$_4$Si. High resolution mass spectra were performed by Shrader Analytical Laboratories (Detroit, Michigan).

[21, 22-³H] Dihydrostrychnine (2b) by Heterogeneous Catalysis A solution of 20 mg (0.06 mmol) of 1a (Aldrich 13492-9) in 4 mL of freshly distilled THF with 10 mg of 10% Pd/C was stirred under an atmosphere of 100 Ci of tritium gas for 16 h at ambient temperature. Catalyst filtration and labile tritium removal with EtOH was performed. The resulting residue was dissolved in 20 mL of EtOH (total radioactivity = 2600 mCi). The entire crude product was purified by preparative TLC on two 1000 μm silica gel GF plates developed with S_1. This system clearly separated 2b from the higher Rf 1a. Standard 2a[20] was allowed to migrate on either side of the hot material to assist in band identification by shortwave UV. After plate development and scraping, the silica gel was eluted with EtOH (total radioactivity = 544 mCi, a 29% radiochemical yield based on 1a). Purified in this way, 2b was found to be 98% radiochemically pure by silica gel TLC (S_1 (Rf 0.19), S_2 (Rf 0.32)) and HPLC (S_4). In both TLC and HPLC 2b cochromatographed with 2a. The UV(EtOH) of 2b was superimposable on that of

2a, and the specific activity of 2b was determined to be 31 Ci/mmol (where ϵ 254 = 12,870 for 2a). A ^{1}H decoupled ^{3}H NMR ($CDCl_3$) of 2b was obtained.[14a, b]

[21, 22-^{3}H] Dihydrostrychnine (2b) by Homogeneous Catalysis A solution of 34 mg (0.1 mmol) of 1a (Aldrich 13492-9) in 4 mL of freshly distilled benzene with 7 mg of $(Ph_3P)_3$ RhCl was stirred under an atmosphere of 70 Ci of tritium gas for 16 h at ambient temperature. Labile tritium removal with EtOH was then performed. The resulting residue was dissolved in 20 mL of EtOH (total radioactivity = 9900 mCi). The entire crude product was purified by preparative TLC on four 1000 μm silica gel GF plates developed with S_1 as before. Standard 2a[20] was allowed to migrate an either side of the hot material to assist in band identification by short wave UV. After plate development and scraping, the silica gel was eluted with EtOH (total radioactivity = 2000 mCi, a 40% radiochemical yield based on 1a). Purified in this way, 2b was found to be 98% radiochemically pure by silica gel TLC (S_1 (Rf 0.19), S_2 (Rf 0.32)) and HPLC (S_4). In both TLC and HPLC, 2b cochromatographed with 2a. The UV (EtOH) of 2b was superimposable on that of 2a and the specific activity of 2b was determined to be 50 Ci/mmol (where ϵ254 = 12,870 for 2a). A ^{1}H decoupled ^{3}H NMR ($CDCl_3$) of 2b was obtained.[14b]

[2-^{3}H] Strychnine (1b) A solution of 46 mg (0.1 mmol) of 4[17] in 2.3 mL of benzene and 40 μL of Et_3N with 36 mg of 10% Pd/Al_2O_3 was reduced with 55 Ci of tritium gas for 1.5 h at 24°C. Following catalyst filtration and labile tritium removal with CH_3OH, the crude product was taken up in 10 mL of EtOH (total radioactivity = 1189 mCi). The entire crude product was purified by preparative TLC on two 1000 μm silica gel GF plates developed with S_1. Again, this system clearly separated 1b from the lower Rf [2,21,22-^{3}H] dihydrostrychnine, typically 25% of the mixture. Standard 1a was allowed to migrate on either side of the hot material to assist in band identification by shortwave UV. After plate development and scraping, the silica gel was eluted with EtOH (total radioactivity = 870 mCi). Final purification of 1b was accomplished by HPLC on a Zorbax silica column eluted with S_4. Typically, HPLC purification of 870 mCi of crude 1b afforded 363 mCi of 1b (a 14.5% radiochemical yield based on 4). Purified in this way, 1b was found to be 98% radiochemically pure by silica gel

TLC (S_2 (Rf 0.53), S_3 (Rf 0.48)) and HPLC (S_4). In both TLC and HPLC, 1b

cochromatographed with 1a. The UV (EtOH) of 1b was superimposable on that of 1a

and the specific activity of 1b was determined to be 25 Ci/mmol (where ϵ 254 =

12,589 for 1a). A ^{1}H decoupled ^{3}H NMR ($CDCl_3$) of 1b was obtained and showed a

single peak at δ 7.09 ppm.

ACKNOWLEDGMENT

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      Na_2SO_3 and 5.63 g (32 mmol) of HIO_3 at 80°C under nitrogen for 16 h.
      Basification, $CHCl_3$ extraction and two TLC purifications (silica gel -
      first S_2 then S_3) afforded 133 mg of <u>4</u> (14.1% yield) whose cochromatography
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20.   Standard <u>2a</u> was prepared by hydrogenation of <u>1a</u> with 10% Pd/C in THF and
      gave an IR, ^{1}H NMR, UV and elemental analysis in accord with its proposed
      structure.