Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **43**, 97-101 (2000)

SYNTHESIS OF 8- [^{10}B] -DIHYDROXYBORYL-HARMINE, A POTENTIAL AGENT FOR BORON NEUTRON CAPTURE THERAPY.

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SUMMARY.- The preparation and spectral properties (MS, IR and NMR) of 8-[^{10}B]-dihydroxyborylharmine (III) are described. A general synthetic method was developed for an efficient production of boronated harmine; a potential agent for boron neutron capture therapy (BNCT) in brain.

Keywords: 8- [^{10}B]- dihydroxyborylharmine; preparation and characterization; boron neutron capture therapy.

INTRODUCTION

The resurgence of interest in boron neutron capture therapy as a treatment for malignant lesions has resulted in the synthesis of numerous boron compounds as candidates for clinical use. BNCT is a selective radiotherapy using boron-10 which absorbs thermal neutrons and releases high Linear Energy Transfer (LET) alpha particles by the ^{10}B (n, α) ^{7}Li reaction. The alpha radiation kills cells in the range of 5-9 μm from the site of the α generation. Therefore, if ^{10}B-compounds could be selectively delivered, it is theoretically possible to kill tumor cells without affecting adjacent healthy tissues. Boron analogs of amino acids constitute a topic of major importance, and also peptides, antibodies, nucleosides and nucleotides[1], etc. Despite of the promising results with *p*-boronophenylalanine (BPA) and $B_{12}H_{11}SH^{2-}(Na^{+})$ (BSH), which presently attracts considerable clinical interest, they display far from optimal selectivity for cancer cells.

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CCC 0362–4803/2000/010097–05$17.50

Received 22 June 1999
Revised 8 July 1999
Accepted 12 July 1999

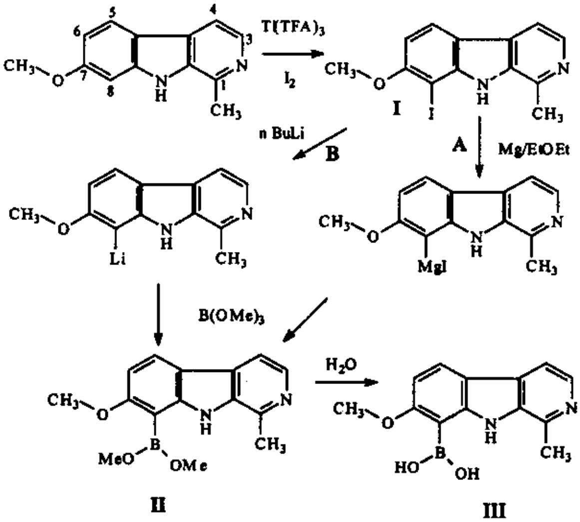
The anatomical distribution of [^{3}H]harmalas binding sites was determined by quantitative autoradiography in rat brain slices[2]. They have a well known brain distribution, so these ^{10}B labelled compounds are potential agents for BNCT. A general synthetic procedure has been developed for a rapid and efficient production of boronated harmine.

RESULTS AND DISCUSSION

Iodination: The methods for iodination have been used previously by us for iodination and radioidination of indolealkylamine[3], phenethylamines[4] and β-carbolines[5].

Boronation: We used the condensation of the Grignard reagent from 8-I-harmine with trimethylborate. This method was previously used for the boronic analogue of choline[6]. An alternative synthesis consists in treating the halogenated substrate with *n*-buthyllithium in THF followed by addition of trimethylborate at – 86 °C. Trimethylborate was prepared by standard procedures[7] either from 95 % ^{10}B-enriched or natural isotope abundance boric acid and methanol, and recovered from the azeotrope.

Scheme I. Synthesis of boronated harmine.



EXPERIMENTAL

General methods. Solvents were of the maximum purity available, checked by gas chromatography and dried by standard methods. Starting materials were from Aldrich. EI-MS was achieved on a Trio2 VG spectrometer operating at 70 eV and HRMS on ZAB-EQ. Melting points were recorded in a Fisher-Jones apparatus and are uncorrected. All ^{1}H and ^{13}C NMR spectra were recorded using a Bruker AC 200 with DMSO-d_6 as solvent HPLC analysis was developed using a column Pharmacia SuperPac Spherisorh ODS 2.5 μm; 4 x 250 mm No. 2134-256, methanol\water: (40:60) as solvent and a flux of 0.5 ml/min. UV-detection was achieved at 254 nm on a Waters Lambda-Max 481.

Synthesis and Characterization. **8-I-Harmine (I):** Thallium trifluoroacetate (2.60 g, 4.80 mmol), in acetonitrile (20 ml) was added slowly at room temperature, to a solution of the harmine in acetonitrile (1.00 g, 4.71 mmol). A solution of iodine in methanol (609 mg, 2.40 mmol) was added afterwards. The reaction mixture was stirred at room temperature for 6 h, and vacuum filtered to remove the remaining solid. The solvent was dried (anh.$MgSO_4$), and removed. The crude solid was crystallized from methanol yielding 1.43 g (90 %) of a mixture of 6- and 8-iodo-harmine as a light yellow solid. The mixture was purified by fractional crystallization from methanol to obtain 550 mg of (I). Mp = 189-190 °C. EI MS m/z (rel. int.) at m/z 338 (M, 100), 323 (M- CH_3, 27.9), 295 (M- C_3H_3O, 31.6), 196 (M- CH_3 - I, 10.7). Rt=3.11 min. (> 98 % of purity).

8-Dimethoxyborylharmine (II) and 8-Dihydroxyborylharmine (III). Method A. 8-I-harmine (400 mg, 1.18 mmol) in dry ether (25 ml) was added slowly to a stirred suspension of Mg (31 mg, 1.30 mmol) and catalytic amounts of iodine in dry ether (15 ml). The mixture was heated under nitrogen for 3 h. The solution of the reaction mixture was injected through a septum slowly to a flask with trimethyl borate (0.150 ml, 1.30 mmol). The reaction mixture was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure, and the crude product recrystallized from ether to obtain a 40 % yield (166 mg, 0.585 mmol) of 8-dimethoxyborylharmine (II), mp. 168 – 170 °C. The ^{1}H-NMR spectrum shows a singlet at δ 10.82 (s, H) due the two methoxy groups at the boron atom. After hydrolysis with D_2O this singlet disappeared and the new spectrum exhibited a singlet at 4.03 ppm corresponding to the resulting CH_3OD. We can conclude that in this case the aryl-boron system behaves as the vinyl group of vinylboranes[8], as a mesomeric donor towards boron in tricoordinate boron compounds,

Table 1. ^{1}H-NMR Data (δ ppm, J Hz), DMSO-d_6 as solvent

Hydrogen Position	**(I)**	**(II)**	**(III)**
3	8.27 (d,1H, J=5.8)	8.22 (d,1H, J=5.6)	8.21 (d,1H, J=5.6)
4	8.03 (d, 1H, J=5.8)	7.89 (d, 1H, J=5.6)	7.93 (d, 1H, J=5.6)
5	8.07 (d, 1H, J=8.5)	8.18 (d, 1H, J=8.4)	8.17 (d, 1H, J=8.4)
6	6.84 (d, 1H, J=8.5)	7.02 (d, 1H, J=8.4)	7.02 (d, 1H, J=8.4)
7-OCH_3	4.02 (s, 3H)	3.96 (s, 3H)	3.98 (s, 3H)
1-CH_3	2.98 (s, 3H)	2.85 (s, 3H)	2.36 (s, 3H)
NH	12.78 (s, 1H)	10.82 (s, 1H)	10.89 (s, 1H)
OH	-	-	6.48 (s, 1H)
$BOCH_3$	-	1.24 (s, 6H)	-

due the delocalization of π-electron density of the aryl system by the vacant p_π-orbital of boron. It can explain the high shielding found for the protons at the methoxy group. The product was hydrolyzed to obtain the corresponding boronic acid derivative **(III)**, mp. 340 ^{0}C (dec.), Rt = 2.05-2.10 min. (> 95 % of purity).

Method B: *n*-Buthyllitium (1.6 M, in hexane; 2.2 ml, 3.52 mmol), was added dropwise with a syringe to a magnetically stirred solution of 8-I-harmine (574 mg, 1.70 mmol), in dry THF (15 ml) under nitrogen at –86 ^{0}C over 3 min. The mixture was stirred for 15 min. Trimethylborate (372 mg, 0.46 ml, 3.58 mmol) and dry THF (10 ml) were added to another flask. This solution was cooled to –86 ^{0}C and the lithioharmine was added dropwise with vigorous stirring over 10-15 min. The mixture was stirred at –86 ^{0}C for 2 h and allowed to warm up at room temperature and stirred for another 6 h. THF was partially removed *in vacuo* with minimal heating. Ether (20 ml) and cold water (5 ml) were added and the contents were further stirred for 20 min. The organic layer was separated, dried (anh. Na_2SO_4) and the solvent was removed. The crude product was recrystallized from ethylacetate to afford a 55 % yield of **(III)**. IR 1335 cm^{-1} and 1235 cm^{-1} (B-O and B-C stretching), 790 cm^{-1} (B-O deformation), 1435 cm^{-1} (boron-aryl sharp). EI MS m/z (rel. int.) 255 (M -H, 20.3), 241 (M- CH_3, 20.3), 213 (M- C_3H_3O, 25.1), 197 (M-HBO_2 - I, 75.2). HRMS [M - H] 254,98717 (Calc. 255,08654). **^{13}C-NMR.**: Unless the boron quadrupole moment is removed, the spectra are further complicated, resulting in a poorly resolved multiplet. Synthesized **(III)** also exhibited this unusual phenomenon, thus, the presence of a dihydroxyboryl function at the assigned position (C-8) was confirmed unequivocally.

Table 2. ^{13}C NMR and DEPT 135 ^{0} data (δ ppm), DMSO-d_6 as solvent.

Carbon Position	**(I)**	**(III)**	**(III)-DEPT 135 ^{0}**
1	142.4	141.9	
1'	134.6	134.2	
3	138.4	137.8	137.8
4	112.1	112.5	112.5
4'	116.3	116.0	
5	122.3	122.7	122.7
5'	144.1	144.2	
6	104.5	105.3	105.3
7	158.3	158.4	
8	67.2	99.3	
8'	134.5	128.8	
OCH_3	56.9	56.9	56.9
CH_3	21.0	19.9	19.9

ACKNOWLEDGMENTS.- Thanks are due to CONICET and Universidad de Buenos Aires (UBA; Argentina) for financial support. One of us, (J.A.S), also thanks CONICET for a research fellowship.

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