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SYNTHESIS OF TRITIUM LABELLED CHOLINE ANALOGS

Srinivasan Rajagopal and Mirko Diksic*
McConnell Brain Imaging Center, Montreal Neurological Institute,
Department of Neurology and Neurosurgery,
McGill University, Montreal, Canada H3A 2B4.

SUMMARY

The synthesis of choline analogs S(+) and R(-) 2-hydroxy, 1-N,N,-dimethyl, N-[^{3}H]-methyl, propylammonium iodide, 3b, 3a; R(+) and S(-) 1-hydroxy, 2-N,N-dimethyl, N-[^{3}H]- methyl, propylammonium iodide, 6b, 6a; and N,N-dimethyl, N-[^{3}H]- methyl, propynylammonium iodide, 8 labelled with [^{3}H] at the N-CH_3 position was achieved with a radiochemical yield of 40% and specific activity of 9.3 Ci/mmol. The preparation of desmethyl compounds, enantiomeric and racemic 1,-N,N-dimethylamino 2-propanol 2a, 2b, 2ab; enantiomeric and racemic 2,-N,N-dimethylamino 1-propanol 5a, 5b, 5ab is also described.

Keywords: [^{3}H]- Choline, labelled cholinergic transmitter.

INTRODUCTION

Choline transport into cholinergic neurons has drawn wide attention due to the viable role of the central cholinergic system in several neurological diseases. A deficiency of cholinergic activity is related to the development of senile dementia of the Alzeimer type psychopathology [1]. Earlier studies suggest a high and a low affinity mechanism by which choline is transported into cholinergic neurons [2,3]. The mechanism of choline uptake manifests itself as a powerful tool in the unravelling of various aspects of acetylcholine synthesis, storage, and release. This tool has been effectively used *in vitro* with cholines and other choline analogs by various groups [4]. Besides various hydroxyl containing analogs, other non hydroxyl compounds (unsaturated amino compounds) have been investigated and found to be preferentially taken up by a high affinity pathway. It is also of interest to note that the unsaturated non hydroxy compounds show pronounced parasympathomimetic activity [5]. Attempts using

*To whom correspondence should be addressed at the Medical Cyclotron Unit, Montreal Neurological Institute, 3801 University St., Montreal, Que. H3A 2B4, Canada.

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^{11}C- methyl choline by positron emission tomography (PET) to image cholinergic neurons have not been successful since choline, in addition to being acetylated to acetylcholine by cholineacetyltransferase (ChAT), is utilized in all cells in the synthesis of membrane phospholipids. Alternatively choline analogs such as ^{11}C-pyrollidino choline was used in the study of the choline analog uptake by PET which enabled to obtain choline influx into brain [6,7]. However, it was not possible to obtain information on the high affinity neuronal system because this analog was also incorporated into phospholipids to a large extent [8,9]. In order to obtain further information in the choline uptake mechanistic pathway, we explored the possibility of examining the stereo requirement in the transport pathway of choline analogs such as the following: R(-)and S(+) 2-hydroxy, 1-N,N,N-trimethylpropylammonium iodides, *β-methylcholine analogs* (**3a**, **3b**) respectively; R(+) and S(-) 1-hydroxy, 2-N,N,N-trimethylpropylammonium iodides, *α-methylcholine analogs* (**6a**, **6b**) respectively. Also the non hydroxyl unsaturated compound N,N,N-trimethylpropynyl ammonium iodide, **8** was included in our study since it cannot be incorporated into phospholipids. The reason for selecting these compounds is to examine a possibility that the membrane transporter might be stereospecific at least to some extent, that different stereoisomers might be incorporated into phopholipids at diffferent rates [10].

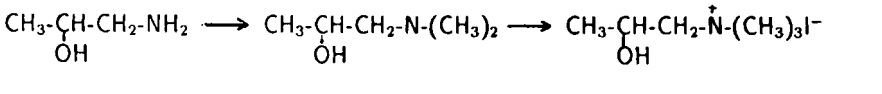
We have labelled these compounds with [^{3}H] in N-CH_3 position. The synthesis of N-methyl, [^{3}H]- labelled compounds is described here. Based on the outcome of invitro measurements for the specificity we plan to label them with ^{11}C- and study their uptake with PET.

MATERIALS AND METHODS

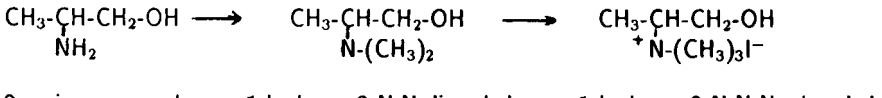
Enantiomerically pure S(+), R(-) and racemic 2-aminopropanol, S(+), R(-), and racemic 1-amino 2-propanol, dimethylamino 2-propyne were obtained from Aldrich Chemical Co. Other chemicals used were of research grade quality. Silica gel plates (AnalTech), Si-C_{18}F (Baker) and alumina plates, Al_2O_3 IB-F (Baker-Flex) were used for thin layer chromatography. Reversed phase Sep-Pak (C_{18}) cartridges from Waters Associates were used for final filtration of the iodide salts. Radio-thin layer chromatography (RTLC) was done with a Packard Model 7221 TLC plate scanner. Liquid scintillation measurements were performed on a LKB-1219 instrument using Fisher Scientific Scintanalyzed Universal cocktail. [^{3}H]-Methyl iodide (with a specific activity of 9.3 Ci/mmole) was obtained from Amersham International. ^{1}H NMR spectra were obtained with a Varian 200 MHz spectrometer using tetramethyl silane (TMS) as an internal standard. Mass spectral analysis were performed on a ZAB-HS20 mass spectrometer using a fast atom bombardment and a glycerol matrix. Optical rotation was analysed in CH_3CN on a JASCO-DTP-140 digital polarimeter. Melting points were done on a Mel-Temp apparatus and are reported uncorrected.

RESULTS AND DISCUSSION

The synthetic scheme for the preparation of the choline analogs is shown in Scheme 1. The preparation of unlabelled precursors **2a**, **2b**, **2ab**, **5a**, **5b**, **5ab** was accomplished by the *Eschweiler-Clarke reaction* (reductive amination) [11]. Methylation of the dimethylamino precursors (desmethyl compounds) using methyl iodide yielded the desired products. The synthesis of the seven quarternary ammonium iodide salts is presented below.



1-amino 2-propanol	2-hydroxy, 1-N,N-dimethyl propylamine	2-hydroxy, 1-N,N,N-trimethyl propylammonium iodide
1a: R(-)	2a: R(-)	3a: R(-)
1b: S(+)	2b: S(+)	3b: S(+)
1ab: D,L	2ab:D,L	3ab: D,L



2-amino propanol	1-hydroxy, 2-N,N-dimethyl propylamine	1-hydroxy, 2-N,N,N-trimethyl propyl ammonium iodide
4a: R(-)	5a: R(-)	6a: R(+)
4b: S(+)	5b: S(+)	6b: S(-)
4ab: D,L	5ab:D,L	6ab: D,L

7 N,N-dimethylamino 2-propyne 8 N,N,N-trimethylamino 2- propyne

SCHEME 1

I. Reductive Amination of hydroxy aminoalcohols

1. *Preparation of R(-), S(+), enantiomeric and racemic 1,-N,N- dimethyl amino 2-propanol* 2a, 2b, 2ab

S(+) N,N-dimethylamino 2-propanol **2b** was prepared by adding S(+) 1-amino 2-propanol (5.3ml, 67 mmol) to a solution containing formaldehyde (2.5 ml, 67 mmol) and formic acid (2.7 ml, 67 mmol) kept in an ice bath. The reaction mixture was allowed to stir overnight and refluxed at 80°C for 10 hours. The progress of the reaction was followed by TLC using silica gel plates developed with MeOH: NH_4OH; 4:1. Subsequently the mixture was treated with KOH (3g) in small portions. The organic portion was separated and subjected to fractional distillation. The fraction distilling at 31-35°C /29 mm, 40% yield, was identified by ^1H NMR as 2b: δ ppm, multiplicity in DMSO-d_6, coupling constants; CH_3, 1.05, d, 7.0 Hz; CH, 3.73, m, 24 Hz; CH_2, 2.10, m, 20 Hz; N-CH_3, 2.14, s. A similar procedure was followed for R(-) N,N-dimethylamino 2-propanol **2a**. The fraction collected between 28-30°C /31 mm, 50% yield was characterised by ^1H NMR. The racemic mixture, D,L-N,N-dimethylamino 2-propanol **2ab** was also obtained (40-44°C/45 mm, 40% yield) using the above procedure.

2. *Preparation of R(-), S(+), enantiomeric and racemic 2-N,N- dimethyl amino 1-propanol* 5a, 5b, 5ab

To a solution containing formaldehyde (1.85 ml, 50 mmol) and formic acid (2.0 ml, 50 mmol) kept in an ice bath, R(-) 2-N,N-dimethylamino propanol (3.9 ml, 50 mmol) was added. The reaction mixture was then refluxed for 15 hours with periodic inspection of product formation by TLC (MeOH: NH_4OH; 4:1). Small

portions of KOH (3g) were added and after separation the aqueous layer was removed. The organic solution was then fractionally distilled to yield **5a**, 60°C /100 mm, 50% yield. The ^{1}H NMR in DMSO- d_6 showed the following signals, δ, multiplicity, coupling constants: CH_3, 0.90, d, 7.0 Hz; CH, 2.50, m, 12 Hz; CH_2, 3.50, m, 15 Hz; N-CH_3, 2.16, s; OH, 4.11, s. S(+) 2-N,N-dimethylamino propanol, **5b** and racemic D,L-2-N,N-dimethylaminopropanol **5ab** were obtained in a similar manner. The conditions were: **5b**, 28-30°C/31 mm, 50%, and **5ab** 37-40°C/30 mm, 40%.

II. Methylation of dimethylamino alcohols (Non radioactive)

1. *Preparation of 2-hydroxy, 1-N,N,N-trimethylpropyl ammonium iodide* **3a, 3b, 3ab**

Compound **3b** was prepared by adding iodomethane (150 μl, 2.4 mmol) to a solution of S(+) N,N-dimethylamino 2-propanol **2b** (400 μl) in 5ml acetonitrile. After stirring for 2 min a white precipitate was obtained which was filtered and recrystallised from acetonitrile, 80% yield, m.p. 172- 173°C. The ^{1}H NMR spectrum of **3b** showed the folowing signals in D_2O: CH_3, 0.60, d, 7.0 Hz; CH, 3.82, m, 45 Hz; CH_2, 2.72, m, 40 Hz; N-CH_3, 2.54, s; OH, 4.11, s. Mass spectrum displayed m/e signal, 118 corresponding to trimethylpropylammonium cation. **3a**, **3ab** were synthesised as described above by methylating the corresponding dimethylamino alcohols, **3a** and **3ab** respectively. The yields and the physical characteristics are as follows: **3a**, 45%, 171-173°C; **3ab**, 78%, 153-155°C.

2. *Preparation of 1-hydroxy, 2-N,N,N-trimethylpropyl ammonium iodide* **6a, 6b, 6ab**

Iodomethane (150 μl, 2.4mmol) was added to a solution of R(-) 2-N,N- dimethylamino 1-propanol **5b** (400 μl) in 5 ml acetonitrile. A white precipitate was obtained after stirring for 5 minutes. The precipitate was filtered and recrystallised from acetonitrile (78% yield), m.p. 284-285° C. The ^{1}H NMR signals in D_2O are as follows: CH_3, 0.79, d, 7.0 Hz; CH, 3.30, m, 48 Hz; CH_2, 2.90, m, 20 Hz; N-CH_3, 2.49, s; OH, 4.11, s. Mass spectrum showed a signal, m/e 118, corresponding to trimethylpropylammonium cation. The product R(+) 1-hydroxy, 2-N,N,N-trimethylpropyl ammonium iodide displayed a reversal in optical rotation. The other S(-) enantiomer **6a** and the racemic mixture **6ab** were prepared in a similar fashion from **5a** (80% yield, 286- 288°C) and from **5ab** (75%, 153-154°C) respectively.

III. Methylation of alkynyl analog (Non radioactive)

1. *Preparation of N,N,N-trimethylamino 2-propyne* **8**

Compound **8** was prepared by adding iodomethane (400 μl, 6.4 mmol) to 5 ml

acetonitrile containing freshly distilled dimethylamino 2-propyne **7**. The reaction mixture immediately produced a white precipitate which was filtered and recrystrallised from ethanol, m.p. 178- 180°C, lit. 180°C [5]. The ^{1}H NMR of **8** showed the following chemical shifts in D_2O, δ (ppm), multiplicity: CH, 4.22, t, 2 Hz; CH_2,4.5, d, 2 Hz; N-CH_3, 3.3, s.

IV. [^{3}H]- Methylation of dimethylamino alcohols

1. *Preparation of S(+) and R(-) 2-hydroxy, 1-N,N-dimethyl N-[^{3}H]- methyl propyl ammonium iodide*, **3b**, **3a**

S(+) 2-N,N-dimethylamino 2-propanol **2b** (20 μl) was added to a solution of [^{3}H]- CH_3I (9.3 Ci/mmol) in 5 ml ether and the mixture was stirred at room temperature for 4 hours. The reaction mixture was extracted with water (4 x 3 ml) and the aqueous layer separated. The aqueous solution was concentrated at reduced pressure to dryness. The residue was dissolved in water and passed through a reversed phase Sep-Pak to yield the desired product, **3b**, 6.5 mCi, 30% yield. Analysis by thin layer radiochromatography showed 99% radiochemical purity, Rf= 0.24. The R(-) isomer **3a** was obtained in a similar manner, 8.5 mCi, 40% yield.

2. *Preparation of R(+) and S(-) 1-hydroxy 2-N,N-dimethyl, N-[^{3}H]- methyl* propyl ammonium iodide, **6a**, **6b**

[^{3}H]- CH_3I (9.3 Ci/mmol) was added to R(-) 2-N,N-dimethylamino 1- propanol **5a** in 5 ml ether. The reaction mixture was stirred at room temperature for 0.5 hours and extracted with 4 x 3 ml water. The aqueous layer was concentrated to dryness in vacuo and an aqueous solution was passed through a reversed phase Sep-Pak to yield **6a**, 4.2 mCi, 46% yield. In a similar fashion, compound **6b** was synthesised, 8.2 mCi, 49% yield.

V. Methylation of [^{3}H]- labelled alkynyl analog

1.*Preparation of N,N-dimethyl, N-[^{3}H]- methyl, propynl ammonium iodide*, **8**

Freshly distilled dimethylamino 2-propyne **7** (20 μl, 3.1 μmol) was added to a solution of [^{3}H]- CH_3I (9.3 Ci/mmol) in 7 ml ether and the mixture was stirred for 0.5 hours. The mixture was then extracted with water (5 x 4 ml) and the aqueous layer was separated and concentrated. The final aqueous solution (2 ml) was passed through a reversed phase Sep-Pak to yield **8**, 7 mCi, 65% yield. Analysis by thin layer radiochromatography showed 98% radiochemical purity, Rf= 0.78.

CONCLUSION

The synthesis of the tritium labelled choline analogs has been performed successfully. A reversal in optical rotation was noticed in the conversion of R(-) 2-N,N-dimethylamino 1- propanol to R(+) 1-hydroxy, 2-N,N,N-trimethylpropyl ammonium iodide (α methylcholine). Also the S(+) 2-N,N-dimethylamino 1-propanol yielded S(-) 1-hydroxy, 2-N,N,N-trimethylpropyl ammonium iodide. This trend was not observed in β methylcholine series.The results of the in vitro measurements using these enantiomeric compounds will be published separately.

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