Synthesis of R-(+)- and S-(-)-8-hydroxy-2-(N,N-dipropylamino)-[2-^{3}H] tetralin. HCl (8-OH-DPAT) a $5HT_{1A}$ receptor agonist.

M. J. Ackland, L. G. Dring, J. R. Jones* and N. Gilon**.

Upjohn Laboratories-U.K., Upjohn Ltd, Fleming Way, Crawley, Sussex, RH10 2NJ and *Department of Chemistry, University of Surrey, Guildford, U.K. and **E.H.I.C.S, Strasbourg, France.

SUMMARY

The title compounds were synthesised in 7 steps from 1,7-dihydroxynaphthalene as follows: 1,7-dihydroxynaphthalene was methylated and subjected to a Birch reduction to yield 8-methoxy-2-tetralone. Reductive amination with sodium cyanoboro[^{3}H]hydride and n-propylamine gave 8-methoxy-2-(n-propylamino)-[2-^{3}H]tetralin which was acylated and reduced to give (±) 8-methoxy-2-(N,N-dipropylamino)-[2-^{3}H]tetralin. Treatment with conc. HCl gave (±)-8-hydroxy-2-(N,N-dipropylamino)-[2-^{3}H]tetralin. The racemate was then resolved by chiral mobile phase chromatography.

Keywords: S-(-)-8-hydroxy-2-(N,N-dipropylamino)-[2-^{3}H]tetralin; R-(+)-8-hydroxy-2-(N,N-dipropylamino)-[2-^{3}H]tetralin; 8-OH-DPAT; tritium labelling; chiral mobile phase chromatography; Z-glycyl-L-proline.

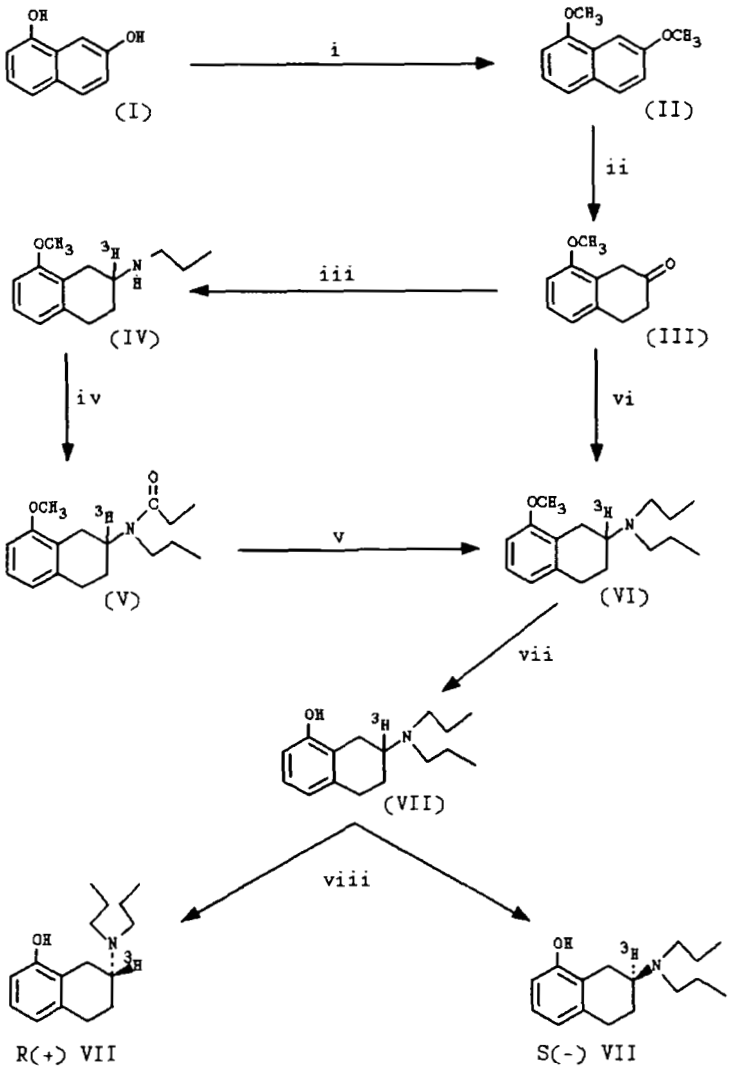
INTRODUCTION

8-Hydroxy-2-(N,N-dipropylamino)tetralin (8-OH-DPAT) is a potent 5-hydroxytryptamine (5HT) receptor agonist with pronounced selectivity for $5HT_{1A}$ receptor sites (1, 2). Considerable knowledge of central 5HT receptors has come from binding studies using labelled ligands of high specific activity, indeed (±)-8-hydroxy-2-(N,N-di[2,3(n)-^{3}H]-propylamino)tetralin is available commercially for such studies. No studies have been reported on the metabolism of 8-OH-DPAT to date. In order to facilitate chiral metabolic studies, the tritiated (R) and (S) enantiomers of 8-OH-DPAT were required. Commercial 8-OH-DPAT is labelled in the chain and is not suitable for our studies as the label is likely to be metabolically unstable. A method was therefore sought to prepare material labelled in the ring at the 2-position (VII; scheme-1). The only risk in this strategy was that oxidative deamination would result in loss of label; however it has been shown that this does not occur (to be described elsewhere). We therefore undertook the synthesis of R-(+)- and S-(-)-8-hydroxy-2-(N,N-dipropylamino)-[2-^{3}H]tetralin.

0362–4803/91/080909–10$05.00

Received 15 March 1991
Revised 16 May 1991

Scheme I



i). Dimethyl sulphate/sodium hydroxide. ii). Sodium/ethanol. iii). Sodium cyanoboro[^{3}H]hydride/n-propylamine/acetic acid/methanol/pH6.7. iv). Propionyl chloride/triethylamine/ dichloromethane. v). Lithium aluminium hydride/dichloromethane. vi). N,N-dipropylamine/ 10% Pd/C/H_2. vii). Conc hydrochloric acid/150°C. viii). Chiral additive HPLC.

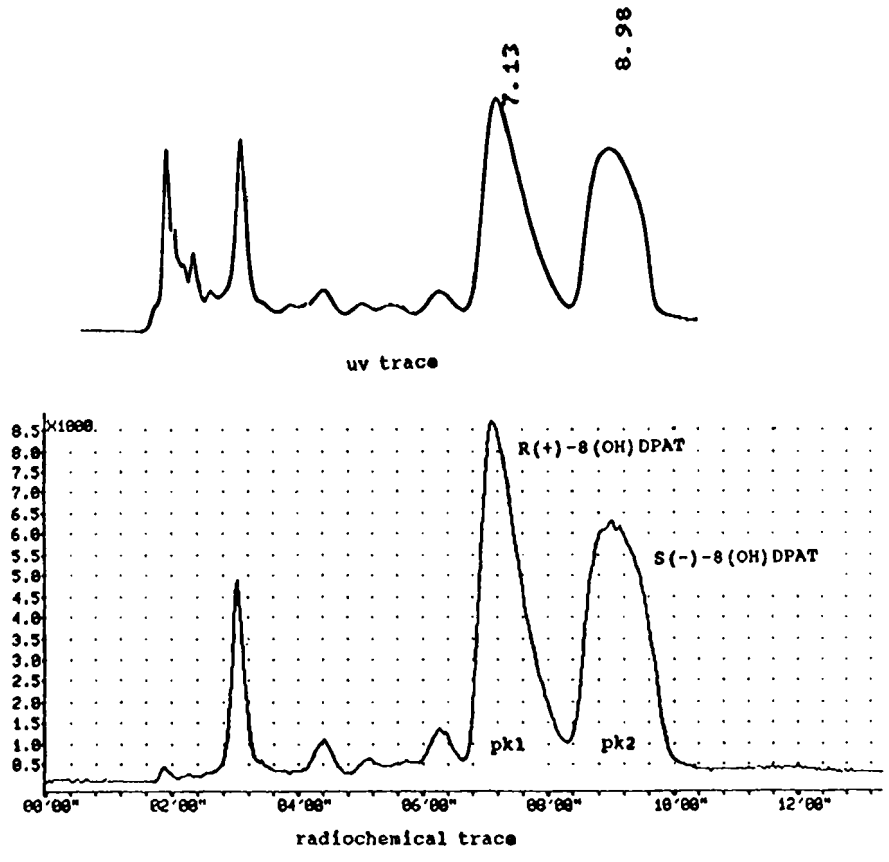
Two syntheses of (±)-8-Hydroxy-2-(N,N-di[2,3(n)-[^{3}H]-propylamino)tetralin have been reported (3-5). In the first method 8-methoxy-2-tetralone was reductively aminated with N,N-diallylamine/Pd/C/H_2, followed by tritium reduction of the terminal alkenes. The second method had a similar strategy but used sodium cyanoborohydride as a reducing agent (unlabelled). Cossery *et al* (6) have reported tritium reduction of allyl side-chains in the chromans (oxygen isosteres of aminotetralins). Koble and Fischer (7) have synthesised 5-hydroxy-6-[^{14}C]methyl-2-(N,N-dipropylamino)tetralin.

DISCUSSION

Scheme I illustrates our approach to the synthesis of 8-OH-DPAT. The tetralone was prepared by the method of Ames *et al* (8; see also Cornforth *et al*: 9, 10). 1,7-Dihydroxynapthalene (I) was methylated with dimethyl sulphate and sodium hydroxide to give 1,7-dimethoxynaphthalene (II), followed by Birch reduction (sodium and ethanol) and purification via the bisulphite complex to give 8-methoxy-2-tetralone (III) as an oil. Investigation of route (vi), i.e. catalytic reductive amination of (III) in the presence of N,N-dipropylamine gave an inefficient synthesis for our purposes. A better method was route (iii) in which the tetralone was reductively aminated with sodium cyanoboro[^{3}H]hydride (11; 1.85GBq, 1.1-5.5 GBq/mg; 0.86mg) and n-propylamine to give (±)-8-methoxy-2-(n-propylamino)-[2-^{3}H]tetralin (IV; 324.12 MBq) in 17.5% radiochemical yield. Treatment of (IV) with propionyl chloride and triethylamine gave amide (V) which was reduced with lithium aluminium hydride to give (±)-8-methoxy-2-(N,N-dipropylamino)-[2-^{3}H]tetralin (VI; 318.2 MBq). O-Dealkylation with concentrated aqueous hydrochloric acid gave (±)-8-hydroxy-2-(N,N-dipropylamino)-[2-^{3}H]tetralin (VII; 293.79 MBq), in 15.9% radiochemical yield from (III). Separation of racemic 8-OH-DPAT into its (R) and (S) enantiomers was achieved using chiral additive chromatography. A modification of the Carlsson *et al* (12) method was used in which chloroform was substituted for dichloromethane in the mobile phase; this did not appear to invert the order of elution. Z-Glycyl-L-proline (ZGP) was employed as the chiral additive. It was possible to achieve almost base line resolution of the enantiomers using this procedure (Figure 1). The enantiomers were further purified by normal phase chromatography (solvent: hexane/ ethyl acetate/ triethylamine; 6:3:1 by volume). The enantiomeric purity was assessed and configuration assigned by reproducing the Carlsson HPLC method (12) exactly. Superimposition of each radioactive peak with one of the UV detected peaks confirmed

Figure 1

Typical HPLC trace. UV trace (upper) and radiochemical trace (lower). Mobile phase: chloroform (500ml), triethylamine (165.9μl), ZGP (1.53g).



assignments. No evidence of cross contamination was seen. Specific activity was assessed by peak collection using a reversed phase chromatography method (see experimental).

The first peak to elute (pk1) by preparative chiral chromatography was found to be R(+)-8-hydroxy-2-(N,N-dipropylamino)-[2-^{3}H]tetralin (specific activity 7.60 GBq/mmol, total activity 26.86 MBq, purity 98.7% by HPLC and 95.93% by tlc). The second peak to elute (pk2) was S-(-)-8-hydroxy-2-(N,N-dipropylamino)-[2-^{3}H]tetralin (specific activity 38.63 GBq/mmol, total activity 6.36 MBq, purity 97.5% by HPLC and 98.3% by tlc). The specific activities of the two enantiomers were significantly different, having an (S)/(R) ratio of 5.1 : 1.0 presumably due to stereoelectronic effects during the reductive amination (13).

EXPERIMENTAL

Reagents

Sodium cyanoboro[^{3}H]hydride (1.85GBq, 1.1-5.5 GBq/mg) was obtained from Amersham International (Aylesbury, Buckinghamshire, UK). Solvents were obtained from F.S.A Laboratory Supplies (Loughborough, Leicestershire, UK) and unlabelled racemic 8-OH-DPAT was obtained from the Sigma Chemical Co (Gillingham, Dorset). Unless otherwise mentioned, all other reagents were obtained from the Aldrich Chemical Co (Gillingham, Dorset, UK).

Thin layer chromatography

T.l.c was performed on silica gel (Kieselgel 60 F_{254}; 20 x 20cm plates; 0.25mm coating; mounted on glass) and visualised by UV lamp. The radioactive areas were located using a RITA linear analyzer (Raytest, Sheffield, UK).

Chiral analytical and preparative HPLC

Z-glycyl-L-proline (ZGP) was obtained from the Sigma Chemical Co (Gillingham, Dorset, UK). Analytical and preparative HPLC was performed with a Waters Associates chromatography pump (Millipore (UK) Ltd, Waters Chromatography Division, Harrow, Middlesex, UK), a Lichrosorb DIOL column (Hichrom Ltd, Reading, Berkshire; 25cm x 4.9mm), LDC Milton Roy Spectromonitor-D variable wavelength detector, Spectraphysics Integrator (Spectraphysics, St Albans, Herts, UK) and a Berthold Radioactivity Monitor (Berthold Labs, Wildbad, German F.R.; LB503).

Reversed phase analytical chromatography (specific activity).

The system used for determination of specific activity was: A Zorbax RX column (C8; deactivated), Spectroflow 400 pump (Spectrochrom, Litchborough, Northants, UK; 1.5ml/min), Waters Wisp 710B autosampler (Millipore, Watford), Spectroflow 773 variable wavelength detector (operating at 278nm), Shimadzu CT02A column oven (40°C; Kyoto, Japan) and a Spectra Physics SP 4290 integrator (Hemel Hempstead, Herts).

Scintillation counting

This was performed using Optiphase HiSafe II scintillant (3ml; Pharmacia Ltd, Milton Keynes, Bucks) and an LKB 1218 Rackbeta scintillation counter (Pharmacia Ltd). The external standard ratio method was used to correct for quenching.

8-Methoxy-2-tetralone (III).

This was prepared by the method of Ames *et al* (8; see also Cornforth *et al*: 9, 10). 1,7-Dihydroxynaphthalene was methylated with dimethyl sulphate and sodium hydroxide, followed by Birch reduction (sodium and ethanol) and purification via the bisulphite complex to give 8-methoxy-2-tetralone as an oil. Bp 119-123 °C (lit ref (3): 120-123 °C). δ(d-$CHCl_3$) 2.5, 3.0 (4H; t, t; $-CH_2-$), 3.5 (1H; s; isolated $-CH_2-$), 3.75 (3H, s, -OMe), 6.75, 7.2 (3H; m; Ar-H). m/e 176 (100%; M^+), 134 (75%), 104 (25%).

(±)-8-Methoxy-2-(n-propylamino)-[2-^{3}H]tetralin, HCl (IV).

A mixture of methanol (2ml) and n-propylamine (2ml) was adjusted to between pH 6-7 with glacial acetic acid. 8-Methoxy-2-tetralone (7mg) was dissolved in the solution (3 ml) and transferred to a vial containing sodium cyanoboro[^{3}H]hydride (1.85GBq, 1.1-5.5 GBq/mg; 0.86mg) and transferred to a 10 ml round bottom flask. Methanol (1.5ml) was used to transfer any remaining material. Freshly activated molecular sieves were added and the reaction placed under nitrogen atmosphere at room temperature. After 18 hr, a solution of saturated sodium bicarbonate was added slowly until effervescence ceased and the mixture extracted with chloroform (3 x 5ml). The extraction created an emulsion which was broken up by filtration through a sinter followed by further extraction with chloroform (3 x 5ml). The chloroform was reduced in volume *in vacuo* (2ml) and extracted with 1M aqueous hydrochloric acid (3 x 2ml). Solid sodium carbonate was added with cooling (acetone/dry ice) until the aqueous phase was basic to litmus and this was re-extracted with chloroform (3 x 5ml). The chloroform phase was dried over sodium sulphate and solvent removed *in vacuo*. The solid was taken up in methanol (2ml), acidified to litmus (3M methanolic hydrochloric acid) and blown down (stream of nitrogen). The sample was reconstituted in methanol and blown down again, finally the sample was reconstituted in methanol (2ml) to give (±)-8-methoxy-2-(n-propylamino)-[2-^{3}H]tetralin, HCl. This had retention characteristics on t.l.c (R_f 0.38; hexane/ethyl acetate/triethylamine, 6:3:1, by vol) identical to an unlabelled reference sample and was 80% radiochemically pure. Total activity: 324.12 MBq.

(±)-8-Methoxy-2-(N-propionyl, N-propylamino)-[2-^{3}H]tetralin (V).

(±)-8-Methoxy-2-(n-propylamino)-[2-^{3}H]tetralin hydrochloride in methanol was treated with triethylamine until basic to litmus and blown gently to dryness. A mixture of

dichloromethane/ toluene (1:1, v/v; 2ml) was added and the sample cooled to 0°C under a nitrogen atmosphere. Triethylamine (22μl) followed by propionyl chloride (10μl) was added and the mixture stirred for 1 hr. Water (2 drops) was added and the organic phase washed with 1M aqueous hydrochloric acid (2 x 2ml), a saturated solution of sodium bicarbonate and a saturated sodium chloride solution. The organic phase was dried over sodium sulphate and solvent removed *in vacuo*. The sample was reconstituted in methanol (2ml) to give (±)-8-methoxy-2-(N-propionyl, N-propylamino)-[2-^{3}H]tetralin. The sample had identical t.l.c retention characteristics as that of an authentic sample (R_f 0.48; hexane/ethyl acetate/triethylamine, 6:3:1, by volume) and was 98% radiochemically pure. Total activity: 321.9 MBq.

(±)-8-Methoxy-2-(N,N-dipropylamino)-[2-^{3}H]tetralin. HCl (VI).

(±)-8-Methoxy-2-(N-propionyl, N-propylamino)-[2-^{3}H]tetralin was taken to dryness *in vacuo* and dissolved in a minimum of dichloromethane (0.2ml). Ether (1ml) was added and the mixture treated with lithium aluminium hydride (2mg) under a nitrogen atmosphere at room temperature for 1 hr. Water (3 drops) followed by 15% aqueous sodium hydroxide (3 drops) was added and the aqueous layer extracted with chloroform (3 x 3ml). The organic layer was washed with a solution of saturated sodium chloride (2 x 3ml), dried (sodium sulphate) and the solvents removed *in vacuo*. Methanol (2ml) was added and the solution acidified (methanolic hydrochloric acid), blown down (nitrogen) and reconstituted in methanol (2ml) to give (±)-8-methoxy-2-(N,N-dipropylamino)-[2-^{3}H]tetralin.HCl. Total activity: 318.2 MBq. The sample had identical retention characteristics to that of a reference sample on t.l.c (R_f 0.70; hexane/ethyl acetate/ triethylamine, 6:3:1, by volume) and was 90% radiochemically pure.

(±)-8-Hydroxy-2-(N,N-dipropylamino)-[2-^{3}H]tetralin. HCl (VII).

(±)-8-Methoxy-2-(N,N-dipropylamino)-[2-^{3}H]tetralin. HCl was blown to dryness (nitrogen) in a Sovirel tube and conc.hydrochloric acid (1ml) added and the vessel sealed. The mixture was heated in an oil bath at 150°C for 3 hr. After cooling, water (1ml) was added followed by dry sodium carbonate (with cooling) until the solution was basic. The mixture was extracted with ether (3 x 3ml), the volume reduced (1ml) and the ether layer extracted with aqueous hydrochloric acid (1M; 2 x 1ml). Solid sodium carbonate was then added to the acid

solution with cooling until basic to litmus and the mixture again extracted with ether (3 x 3ml). The ether layer was dried (sodium sulphate), acidified (3M methanolic hydrochloric acid; a few drops), blown to dryness and reconstituted in methanol to give (±)-8-hydroxy-2-(N,N-dipropylamino)-[2-^{3}H]tetralin. HCl. Total activity: 293.79 MBq. The sample had identical t.l.c characteristics to that of a reference sample (R_f 0.4; hexane/ethyl acetate/triethylamine, 6:3:1, by vol) and was 50% radiochemically pure.

R-(+)-and S-(-)-8-hydroxy-2-(N,N-dipropylamino)-[2-^{3}H]tetralin. HCl R(+) VII and S-(-) VII.

The mobile phase for separation of enantiomers was: carbobenzoxy-glycyl-L-proline (1.53g) and triethylamine (165.9μl) in chloroform (500ml). For each preparative run a methanolic sample (285μl; activity: 42 MBq) of (±)-8-hydroxy-2-(N,N-dipropylamino)-[2-^{3}H]tetralin HCl was basified (excess triethylamine), and gently blown to dryness. Mobile phase (200 µl) was added and the sample injected. Two major peaks (Figure 1) corresponding to the separated enantiomers were collected between 7.0-8.0 min and 9.0-10.0 mins (pk1 and pk2 respectively). Samples from the runs were pooled into their two respective groups and extracted with aqueous hydrochloric acid (1M; 3 x 1ml). Sodium carbonate was added (with cooling) to the acidified mixture until basic to litmus and the sample extracted into fresh chloroform (3 x 1ml), washed with saturated solution of sodium chloride (1 x 1ml), dried (sodium sulphate) and the solvent removed. Samples were reconstituted in methanol (2ml) and acidified (3M methanolic hydrochloric acid). Total activity: pk1 (65.638 MBq), pk2 (48.47 MBq).

Both pk1 and pk2 were subjected to normal phase chromatography respectively. Samples were loaded in mobile phase onto a column (6.5 x 1.6cm) of silica gel (Kieselgel 60; 70-230 mesh), mobile phase: hexane/ethyl acetate/triethylamine, (6:3:1 by vol) and fractions (1ml) collected. Typically, the product eluted pure in fractions 18-36. Each fraction was spotted onto a t.l.c. plate and run in the same mobile phase against a marker. A t.l.c. scanner was used to identify regions of activity. Regions corresponding to product were pooled, the solvent was removed *in vacuo* and the two samples reconstituted in methanol (2ml), acidified to litmus (3M methanolic hydrochloric acid) and stored at -80°C.

Analysis of enantiomers.

a). Identification, total activity and purity of the enantiomers.

Samples of pk1 and pk2 were mixed with unlabelled 8-OH-DPAT and subjected to radio-HPLC in the following mobile phase (12): Z-glycyl-L-proline (ZGP; 1.53g), triethylamine (165.9μl), dichloromethane (500ml). Peaks were found to retain their original elution order (as per ref 9), identifying pk1 and pk2 as the R-(+) and S-(-) isomers respectively. No evidence of contamination by the other isomer was seen in either case; purity was therefore estimated at > 98% ee.

Radiochemical purity: pk1: R-(+)-8-OH-DPAT = 96.0% by t.l.c (system hexane/ethyl acetate/triethylamine; 6:3:1 by vol); 98.7% by radio-HPLC (system chloroform (500ml)/ triethylamine (165.9μl)/ ZGP (1.53g); total activity: 26.862 MBq. pk2: S-(-)-8-OH-DPAT = 98.4% by t.l.c (system hexane/ ethyl acetate/ triethylamine; 6:3:1 by vol); 98.0% by radio-HPLC; total activity = 6.364 MBq.

b). Specific activity.

The mobile phase was 35% acetonitrile/water with 0.1% trifluoroacetic acid. Ten microlitre injections were made. Reference standards of 8-OH-DPAT were prepared at concentrations of 0.13, 0.065, 0.0325 and 0.01625 mg/ml and used to calibrate the HPLC system; these gave a linear correlation (correlation coeff = 1.000). Samples of pk1 and pk2 were injected on column and collected preparatively for scintillation counting. Concentrations were estimated from peak height. Specific activity: pk1: R-(+)-8-OH-DPAT = 7.60 GBq/mmole. pk2: S-(-)-8-OH-DPAT = 38.63 GBq/mmole.

REFERENCES

1). Hamon M., Bourgoin S., Gozlan H., Hall M.D., Goetz C., Atraud F. and Horn A. S.-Eur. J. Pharmacol. 100: 263 (1984)

2). Arvidsson L. E., Hacksell U., Nilsson J. L., Hjorth S., Carlsson A., Lindberg P, Sanchez D., Wikstrom H.-J.Med.Chem. 24: 921-923 (1981)

3). Gozlan H, El Mestikaway S., Pichat L., Glowinski J., Hamon M.-Nature. 305: 140 (1983)

4). Hamon M., Gozlan M., Hall M. D., El Mestkaway S., Emerit M. B., Pichat L.-Regulation of Transmitter Function. Proc. 5th Meeting Eur. Soc. Neurochem. Vizi E. S. and Magyar K. (eds), (1984).

5). Hall M. D., El Mestikaway S., Emerit M. B., Pichat L., Hamon M., Gozlan H.-J. Neurochemistry. 1685-1696 (1985)

6). Cossery J. M., Perdicakis C., Coudert G., Guillaumet G. and Pichat L.-J. Label. Compounds and Radiopharmaceuticals. 25: 833-854 (1988)

7). Koble D. L. and Fischer L. J.-J. Labelled. Compounds and Radiopharmaceuticals. 21: 519 (1984)

8). Ames D. E., Evans D., Grey T. F., Islip P. J. and Richards K. E.-J. Chem. Soc. 2636 (1965)

9). Cornforth J. W., Cornforth R. N. and Robinson R.-J. Chem. Soc. 689 (1942)

10). Cornforth J. W. and Robinson R.-J. Chem. Soc. 1855 (1949)

11). Borch R. F., Bernstein M. D. and Dupont Durst H.-J. Amer. Chem. Soc. 93: 2897-2904 (1971)

12). Karlsson A., Pettersson C., Sundell S., Arvidsson L. E. and Hacksell U.-Acta. Chemica. Scandinavica. B 42: 231-236 (1988)

13). Deslongchamps P.-Stereoelectronic Effects in Organic Chemistry, Organic Chemistry Series, Pergamon Press, Oxford 1983.