LABELING OF (*S*)-DES-4-AMINO-3-[^{125}I]IODOZACOPRIDE (DAIZAC), A HIGH-AFFINITY RADIOLIGAND FOR THE 5-HT-3 RECEPTOR

N. Scott Mason, William A. Hewlett, Michael H. Ebert, Dennis E. Schmidt, Tomas de Paulis*

Departments of Psychiatry and Radiology, Vanderbilt University School of Medicine, Nashville, TN 37232

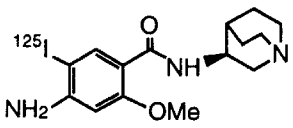
SUMMARY

We have prepared (*S*)-5-chloro-3-[^{125}I]iodo-2-methoxy-*N*-(1-azabicyclo[2.2.2]oct-3-yl)-benzamide ([^{125}I]DAIZAC, [^{125}I]-**3**) as a radioligand for characterization of the 5-HT-3 receptor. Preparation of the 3-tri-*n*-butylstannyl precursor was accomplished from the corresponding unlabeled DAIZAC by reaction with bis(tributyltin). Treatment of the precursor with 5 mCi of Na^{125}I and chloramine-T in dilute HCl gave 2.58 ± 0.22 mCi (52%) of [^{125}I]DAIZAC with >98% radiochemical purity and 1500 Ci/mmol specific activity. Saturation analysis of the binding of [^{125}I]DAIZAC to rat brain homogenates showed a single binding site with a receptor density B_{max} of 0.66 ± 0.03 pmol/g and a receptor affinity K_D of 0.15 ± 0.01 nM. Compared to [^{125}I]iodozacopride, we find the 20-fold higher affinity and easy preparation of [^{125}I]DAIZAC to be advantageous for *in vitro* identification of brain 5-HT-3 receptors.

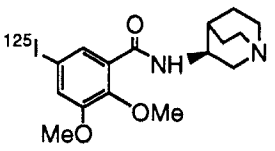
Key Words: [^{125}I]Iodobenzamides, Radioligands, 5-HT-3 receptors, Iododestannylation.

INTRODUCTION

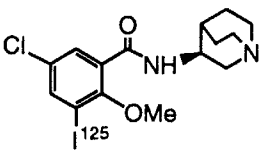
5-HT-3 receptors have been implicated in psychiatric disorders and 5-HT-3 receptor antagonists are able to modify behavior in animal models of anxiety and drug abuse.[1] Several radioligands have previously been developed for identifying 5-HT-3 receptors in the central nervous system.[2] [^{3}H]Zacopride, a potent and selective antagonist of this receptor, has been used to characterize 5-HT-3 receptors in the amygdala and hippocampus of the rat[3] and human brain.[4] Laporte et al. have demonstrated that racemic 5-[^{125}I]iodo analogue (**1**) of zacopride is a useful radioligand for *in vitro* identification of 5-HT-3 receptors in rat brain.[3]



[^{125}I]-**1**



[^{125}I]-**2**



[^{125}I]-**3**

Recently, we have shown that the 3-methoxy-des-4-amino analogue of (*S*)-iodozacopride, **2** (MIZAC), is also a selective 5-HT-3 receptor antagonist.[5] However, neither **1** nor **2** fulfill the requirement of a single photon tomography (SPECT) imaging agent because of their moderate affinities for the 5-HT-3 receptor, K_D

CCC 0362–4803/96/110955–07

Received 11 March 1996
Revised 6 May 1996

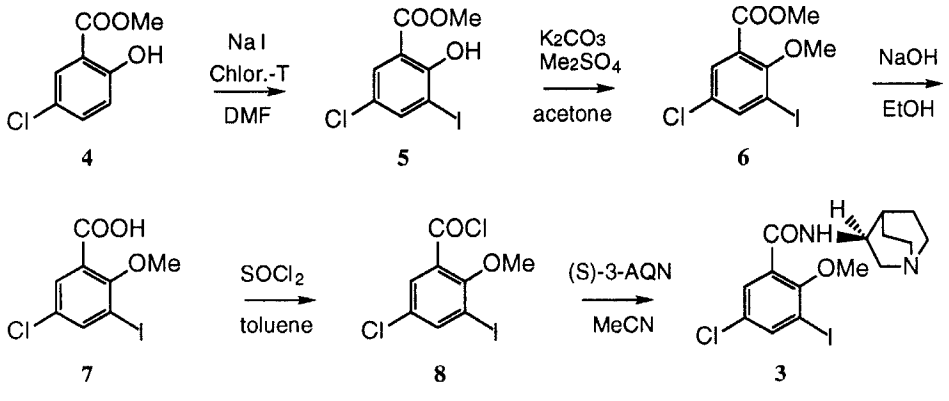
1.5 nM and 1.3 nM, respectively.[5] These ligands are at least one order of magnitude less potent than that required for *in vivo* imaging[7] of the low density of 5-HT-3 receptor populations in amygdala or hippocampus of the human brain (1-3 pmol/g tissue).[4,6]

In the development of MIZAC we have found that a large substiuent in the aromatic 3-position is a prerequisite for high affinity for the 5-HT-3 receptor.[5] We have also confirmed the finding by others[8] that a chloro atom is the optimal substituent in the aromatic 5-position.[9] These structure-activity relationships predict that a combination of optimal aromatic substituents, i.e. (*S*)-5-chloro-3-[^{123}I]iodo-2-methoxy-*N*-(1-azabicyclo[2.2.2]oct-3-yl)benzamide ([^{123}I]DAIZAC, [^{123}I]-**3**), would be a high-affinity radioligand with potential as a SPECT imaging agent for the 5-HT-3 receptor. Further, we postulate that the apparent lipophilicity of DAIZAC (**3**) at pH 7.4 (log P_{app} 2.35)[10] would be sufficient for effective penetration of the blood-brain barrier, but below that causing nonspecific binding to lipids (log P_{app} >2.8) that would obscure the contrast between receptor-rich regions and receptor-poor areas.[11] Because of these considerations, we selected DAIZAC as a candidate for development of a new, high-affinity radioligand for the 5-HT-3 receptor. A preliminary study of the binding properties of [^{125}I]DAIZAC has recently been reported.[12]

RESULTS AND DISCUSSION

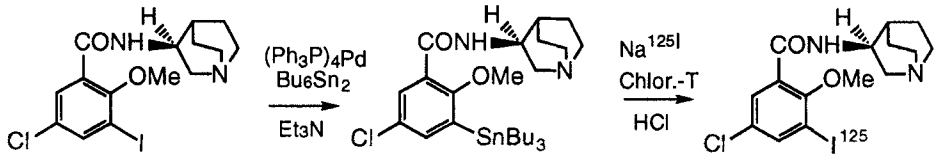
Unlabeled DAIZAC (**3**) was conveniently prepared in five steps from 5-chlorosalicylic acid as shown in Scheme 1 using standard rection conditions. Fisher esterification 5-chlorosalicylic acid gave the methyl ester **4**.[13] Iodination of **4** by a modification of the method used for the preparation of 5-iodo-2,3-dimethoxybenzoic acid[14] gave methyl 5-chloro-3-iodosalicylate (**5**).[15] The generated *p*-toluylsulfonamide was removed by recrystallization of **5** from methanol.

SCHEME 1



Subsequent alkylation of **5** with dimethyl sulfate and hydrolysis of the resulting **6** gave the substituted benzoic acid **7**. Transformation of **7** to the corresponding acid chloride **8** and coupling with (*S*)-3-aminoquinuclidine gave the desired (*S*)-5-chloro-3-iodo-2-methoxy-*N*-(1-azabicyclo[2.2.2]oct-3-yl)benzamide (**3**) in high yields. The identity of **3** was based on its ^{1}H NMR spectrum where the signals were consistant with the structure of **3**.

SCHEME 2



3 **9** [^{125}I]-**3**

Treatment of **3** with tri-*n*-butyltin dimer in the presence of tetrakis(triphenylphosphine) palladium(0) as catalyst[16] gave the required tributyltin precursor **9** (Schème 2). Attempts to use the corresponding 3-bromo derivative as starting material, as described in the preparation of [^{125}I]epidepride,[17] gave unexpected low yields (data not shown). Compound **9** was purified by column chromatography and isolated as an oil. In order to achieve high specific activity in the radiolabeled product, it was neccessary to wash the oil with water to remove traces of iodide released from the silica gel. The precursor **9** was stable in absolute ethanol for over one year after which the radiochemical yields began to decrease to below 50%.

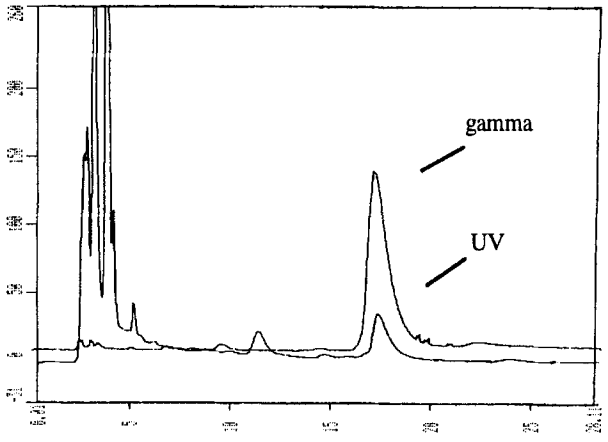


FIGURE 1. HPLC chromatogram of [^{125}I]-**3** reaction mixture

Treatment of **9** with chloramine-T and 5 mCi of sodium [^{125}I]iodide in dilute hydrochloric acid[16] gave 2.7 mCi of [^{125}I]-**3** after purification by reverse-phase HPLC in ethanol-phosphate buffer (Figure 1). Seven radiolabeling reactions gave an average of 2.58 ± 0.22 mCi (52%) of [^{125}I]DAIZAC with >98% radiochemical purity. Only small amounts of the 3-chloro derivative at 14.8 min were observed and were easily separated from the product at 17.5 min. Comparison of the UV peak at 235 nm with that of a standard solution of unlabeled **3** resulted in a specific activity of 1500 Ci/mmol. A possible reason for the specific activity being lower than the theoretical possible (2100 Ci/mmol) was the presence of traces of unlabeled **3** from unreacted starting material in the precursor preparation.

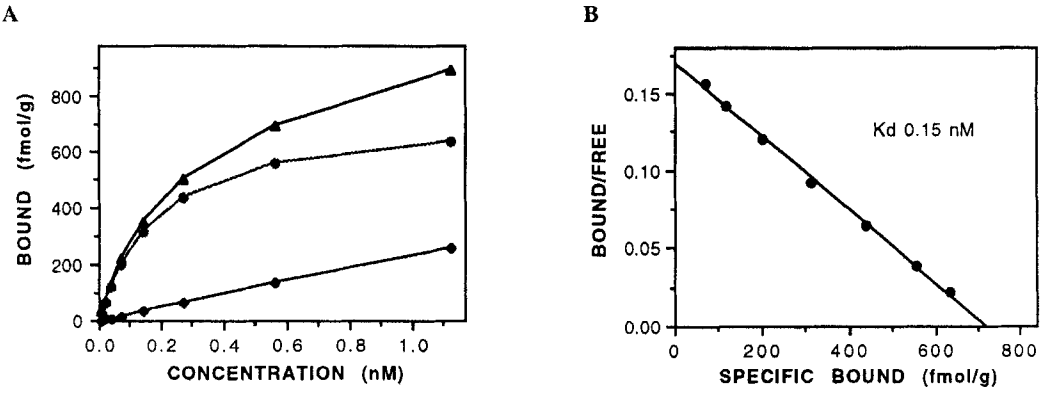


FIGURE 2. **A.** Total, specific, and nonspecific binding of [^{125}I]-**3** to rat brain excluding cerebellum. **B.** Scatchard analysis of the specific binding giving K_D 0.15 nM and B_{max} 0.66 pmol/g.

Analysis of saturation binding of [^{125}I]DAIZAC ([^{125}I]-**3**) to whole rat brain (minus the cerebellum) homogenates showed a single binding site with the receptor density B_{max} of 0.66 ± 0.03 pmol/g and a receptor affinity K_D of 0.15 ± 0.01 nM (Figure 2). This affinity is approximately twice that of (*S*)-[^{3}H]zacopride[4,18] and more than 20 times that of [^{125}I]iodozacopride.[3,19] When non-5-HT-3 receptor binding was defined with an excess of unlabeled DAIZAC, the same affinity and receptor density of [^{125}I]DAIZAC was observed. In consideration of the higher affinity of [^{125}I]DAIZAC as compared to [^{125}I]iodozacopride ([^{125}I]-**1**)[19] and convenient methods of preparation, we find [^{125}I]DAIZAC ([^{125}I]-**3**)[12] to be advantageous for identifying and characterizing 5-HT-3 receptors in the mammalian brain.

EXPERIMENTAL

Receptor binding was performed by a modification of the method of Kilpatrick et al.[18] Whole frozen rat brains (minus cerebellum) were homogenized, the membrane fraction isolated by centrifugation (2 x 12,000 *g*), and incubated in HEPES buffer (15 mg tissue/0.4 mL) with [^{125}I]-**3** for 1 h at 20 °C. Nonspecific binding was defined by co-incubation with 3 μM bemesetron (MDL-72222). Gamma emission spectrometry was performed with an ICN Isomatic Model 4/600 HE instrument. Biochemical reagents were purchased from Sigma, St Louis MO. Synthetic reagents were purchased from Aldrich, Milwaukee WI, and used without purification. Solvents were purchased from Fisher Scientific, Pittsburgh PA. Melting points were taken on a Haake-Buchler apparatus. NMR spectra were recorded on a Bruker 300 MHz instrument. Optical rotation were measured at the sodium D-line in a Rudolph Autopol instrument using 10 cm tubes. Elemental analysis were performed by Atlantic Microlab, Norcross GA, and were within ± 0.4% of the calculated values unless otherwise noted.

(*S*)-5-chloro-3-iodo-2-methoxy-*N*-(1-azabicyclo[2.2.2]oct-3-yl)benzamide (**3**). 5-Chloro-3-iodo-2-methoxybenzoyl chloride (**8**; 1.6 g, 5.0 mmol) was dissolved in MeCN (15 mL) and solid (*S*)-3-amino-quinuclidine (0.9 g, 7.1 mmol) was added with stirring at 25 °C for 2 h. Dilute NaOH (0.1 N, 50 mL) was added and the product was extracted with ether (2 x 50 mL). The combined organic layer was extracted with 0.2 N HCl (3 x 50 mL), neutralized with 5 N NaOH (3 mL), and extracted with ether (2 x 50 mL). Drying

(Na_2SO_4) and evaporation of the solvent gave 0.89 g (42%) of **3**. Mp 142-144 °C. NMR ($CDCl_3$) δ 7.99 (d, 1, *J*=2.6 Hz, C-6 H), 7.87 (d, 1, *J*=2.7 Hz, C-4 H), 7.85 (b, 1, NH), 4.18 (m, 1, C-3' H), 3.85 (s, 3, OCH_3), 3.44 (dd, 1, *J*=9.5, 14.2 Hz, C-2' H), 2.88 (m, 4, C-6' and C-7' H), 2.60 (dd, 1, *J*=4.6, 14.2 Hz, C-2' H), 2.00 (q, 1, *J*=2.9 Hz, C-4' H), 1.72 (dt, 3, C-5' and C-8' H), 1.58 ppm (m, 1, C-8' H). Rotation $[\alpha]_D^{20}$ -11.2° (*c* 0.42, EtOH). Analysis ($C_{15}H_{18}ClIN_2O_2$): C, H, N.

Methyl 5-chlorosalicylate (4). 5-Chlorosalicylic acid (17.3 g, 0.10 mol) was dissolved in MeOH (400 mL) and 98% H_2SO_4 (3.0 mL, 0.06 mol) was added dropwise at room temperature. The mixture was heated to refluxing conditions for 30 h. The solvent was evaporated to give the methyl ester **4** as a crystalline residue. Redissolving of the product in ether (500 mL) and washing with water (2 x 100 mL), drying (Na_2SO_4), and evaporation of the solvent gave 16.5 g (88%) of **4** after recrystallization from 90% aqueous MeOH (60 mL). Mp 48-50 °C. Lit[13] mp 48 °C (EtOH).

Methyl 5-chloro-3-iodosalicylate (5). Methyl 5-chlorosalicylate (**4**; 9.4 g, 0.05 mol) was dissolved in DMF (100 mL) and NaI (7.6 g, 0.05 mol) was added at 20 °C. Chloramine-T (11.4 g, 0.05 mol) was added in portions over 10 min. Stirring was continued for 1.5 h. The reaction mixture was poured into 1 L of ice-water containing $Na_2S_2O_5$ (0.5 g) and the solution was acidified by addition of 12 N HCl (0.5 mL). Extraction with ether (2 x 250 mL), washing of the combined etheral layers with water (100 mL), drying (Na_2SO_4), and evaporation of the solvent gave 16 g of a crystalline mixture of the product **5** and toluenesulfonamide. Recrystallization from MeOH (350 mL) gave 12.8 g (82%) of **5**. Mp 128-130 °C. Lit[15] mp 129-130 °C (EtOH). NMR ($CDCl_3$) δ 11.5 (s, 1, OH), 7.90 (d, 1, *J*=2.5 Hz, C-6 H), 7.82 (d, 1, *J*=2.5 Hz, C-4 H), 3.98 ppm (s, 3, OCH_3).

Methyl 5-chloro-3-iodo-2-methoxybenzoate (6). Methyl 5-chloro-3-iodosalicylate (**5**; 2.6 g, 8.3 mmol) was dissolved in acetone (100 mL) and K_2CO_3 (4.1 g, 30 mmol) was added, followed by dimethyl sulfate (2.0 g, 16 mmol). Heating to refluxing temperature for 6.5 h, evaporation of the solvent and extraction of the acidified residue with ether (2 x 75 mL), drying (Na_2SO_4), and evaporation of the solvent gave 3.2 g of crude **6**. Recrystallization from *i*-Pr_2O (50 mL) gave 2.41 g (86%) of **6**. Mp 52-54 °C. NMR ($CDCl_3$) δ 7.92 (d, 1, *J*=2.5 Hz, C-6 H), 7.77 (d, 1, *J*=2.5 Hz, C-4 H), 3.98 (s, 3, OCH_3), 3.93 ppm (s, 3, OCH_3). Analysis ($C_9H_8ClIO_3$): C, H, Cl, I.

5-Chloro-3-iodo-2-methoxybenzoic acid (7). Methyl 5-chloro-3-iodo-2-methoxybenzoate (**6**; 2.0 g, 6.4 mmol) was dissolved in EtOH (20 mL) and 2 N NaOH (20 mL) was added. The mixture was heated to refluxing temperature for 2.5 h, followed by addition of water (200 mL) and washing of the aqueous layer with ether (50 mL). Neutralization with 12 N HCl (4 mL) precipitated the acid **7**. Extraction with ether (2 x 75 mL), drying (Na_2SO_4), and evaporation of the solvent gave 1.8 g of crude **7**. Recrystallization from *i*-Pr_2O (60 mL) gave 1.38 g (69%). Mp 127-129 °C. NMR ($CDCl_3$) δ 8.02 (d, 1, C-6 H), 7.99 (d, 1, C-4 H), 3.96 ppm (s, 3, OCH_3). Analysis ($C_8H_6ClIO_3$): C, H, Cl, I.

5-Chloro-3-iodo-2-methoxybenzoyl chloride (8). 5-Chloro-3-iodo-2-methoxybenzoic acid (**7**; 1.6 g, 5.0 mmol) was dissolved in toluene (16 mL) to which was added 2 drops of DMF as catalyst. Thionyl chloride (1.5 mL, 20 mmol) was added and the mixture was heated to 70 °C for 1.5 h. The solvent was evaporated *in vacuo* and the crystalline residue of **8** was used without purification.

(*S*)-5-chloro-3-(tri-*n*-butyltin)-2-methoxy-*N*-(1-azabicyclo[2.2.2]oct-3-yl)benzamide (9). (*S*)-3-Iodo-5-chloro-2-methoxy-*N*-(3-quinuclidinyl)benzamide (**3**; 0.43 g, 1.0 mmol) was treated with bis(tributyltin) (1.16 g, 2.0 mmol) in the presence of $(Ph_3P)_4Pd^0$ (0.12 g, 0.1 mmol) in triethylamine (7.0 mL). After refluxing for 2 h the solvent was evaporated and the excess reagents removed by passing the residue through a silica gel column in hexane-EtOAc-EtOH (10:9:1). Fractions showing a single TLC spot at R_f 0.16 in EtOAc-EtOH-NH_4OH (20:10:1) were collected (starting material **3** had R_f 0.09) and the solvent was evaporated to give 0.47 g (80%) of **9** as an oil. NMR ($CDCl_3$) δ 7.91 (d, 1, C-6 H), 7.83 (bd, 1, NH), 7.41 (d, 1, C-4 H), 4.23 (m, 1, C-3' H), 3.74 (s, 3, OCH_3), 3.50 (dd, 1, C-2' H), 2.95 (m, 4, C-6' and C-7' H), 2.65 (dd, 1, C-2' H), 2.07 (q, 1, C-4' H), 1.6-1.8 (m, 4), 1.55 (m, 6, alkyl H), 1.36 (q, 6, alkyl H), 1.15 (t, 6, alkyl H), 0.92 ppm (t, 9, $(CH_3)_3$). The proton in the aromatic 4-position shows 16% as a doublet (J=18 Hz) from coupling with the natural abundance ^{117}Sn (7.7%) and ^{119}Sn (8.6%) isotopes with spin -1/2.[20]

(*S*)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-5-chloro-3-[^{125}I]iodo-2-methoxybenzamide ([^{125}I]-3).

The tributyltin precursor **9** was redissolved in absolute ethanol (1mg/mL). Ten μL of this solution (17 nmol) was added to 5 mCi of $Na^{125}I$ (2.3 nmol) in 20 μL of 0.2 N HCl followed by 10 μL of chloramine-T in water (1 mg/mL). After 2 min, 10 μL of 0.2 N sodium metabisulfite was added and the product was purified using reverse phase HPLC column (Waters Nova-Pak CN) in 30% ethanol - 0.1 M phosphate buffer at pH 6.7. The radioactive peak at 17.5 min was collected and the UV signal (235 nm) was showing 1.8 nmol of chemically pure [^{125}I]-**3** (2.7 mCi) in 3.6 mL buffer. Reinjection of 50 μL of the product showed 95% radiochemical purity. The presence of ethanol in the product was of no consequence for *in vitro* studies, since the material had to be diluted over 200-fold with buffer. For *in vivo* studies, the ethanol was removed by a stream of nitrogen and the solution was reformulated with sterile saline.

ACKNOWLEDGEMENT

This work was supported by the O. C. D. - Tourette Program, Department of Psychiatry, Vanderbilt University School of Medicine. The excellent technical assistance of Ms. Sofyia Fridman is gratefully acknowledged.

REFERENCES

1. Costall B. and Naylor R.J. - Pharmacol. Toxicol. **70**: 157-162 (1992).
2. Miller K, Weisberg E, Fletcher PW and Teitler M. - Synapse **11**: 58-66 (1992).
3. Laporte A.M., Koscielniak T., Ponchant M., Verge D., Hamon M. and Gozlan H. - Synapse **10**: 271-281 (1992).
4. Barnes J.M., Barnes N.M., Costall B., Ironside J.W. and Naylor R.J. - J. Neurochem. **53**: 1787-1793 (1989).
5. de Paulis T., Hewlett W.A., Schmidt D.E., Mason N.S., Trivedi B.L. and Ebert M.H. - J. Med. Chem. (submitted).
6. Abi-Darham A., Laurelle M., Wong D.T., Robertson D.W., Weinberger D.R. and Kleinman J.E. J. Neurochem. **60**: 730-737 (1993).
7. Kessler R.M., Mason S.M., Votaw J.R., de Paulis T., Clanton J.A., Ansari M.S., Schmidt D.E., Manning R.G. and Bell R.L. - Eur. J. Pharmacol. **223**: 105-107 (1992).

8. King F.D., Dabbs S., Bermudez J and Sanger G.J. - J. Med. Chem. 33: 2942-2944 (1990).
9. Hewlett W.A., de Paulis T., Schmidt D.E., Trivedi B.L., Mason N.S. and Ebert M.H. - Biol. Psychiat. **37**, 668 (1995).
10. Schmidt D.E., Votaw J.R., Kessler R.M. and de Paulis T. - J. Pharm. Sci. **83**: 305-315 (1994).
11. Kessler R.M., Ansari M.S., de Paulis T., Schmidt D.E., Clanton J.A., Ebert M.H., Smith H.E., Manning R.G. and Gillespie D. - J. Nucl. Med. **32**: 1593-1600 (1991).
12. Hewlett W.A., de Paulis T., Schmidt D.E., Trivedi B.L. and Ebert M.H. - Soc. Neurosci. Abstr. **21**: 776 (1995).
13. Varnholt, L. - J. Prakt. Chem. **36**: 17-31 (1887).
14. Yue E.W., Gerdes M. and Mathis C.A. - J. Org. Chem. **56**: 5451-5456 (1991).
15. Smith E.F., Knerr E.B. - Am. Chem. J. **8**: 95-101 (1886).
16. Clanton J.A., de Paulis T., Schmidt D.E., Ansari M.S., Manning R.G., Baldwin R.M., Kessler R.M. - J. Label. Compds. Radiopharm. **29**: 45-751 (1991).
17. de Paulis T. and Smith H.E. - Synth. Commun. **21**: 1091-1095 (1991).
18. Kilpatrick G.J., Jones B.J. and Tyers M.B. - Nature **330**: 746-748 (1987).
19. Ponchant M, Koscielniak T, Hamon M. and Gozlan H. - J. Label. Compds Radiopharm. **29**: 1147-1155 (1991).
20. Holden, N.E. - CRC Handbook of Chemistry and Physics, 74th Ed., CRC, Boca Raton FL, 1993.