A SIMPLE AND HIGH-YIELD SYNTHESIS OF (S)-BZM, (R)-BZM AND (S)-IBZM FOR THE PREPARATION OF (S)-^{123}I-IBZM

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SUMMARY

*(S)-2-Hydroxy-6-methoxy-N-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide ((S)-BZM; (S)-**5a**), the precursor of (S)-2-hydroxy-3-^{123}I-iodo-6-methoxy-N-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide ((S)-^{123}I-IBZM) was synthesized via a simple method and in high yield starting from 2,6-dimethoxybenzoic acid (**1a**). N-Hydroxysuccinimide/dicyclohexylcarbodiimide (DCC) was used as activating system in the reaction of **1a** with (S)-2-aminomethyl-1-ethylpyrrolidine (**3a**).*

Keywords: ^{123}I, Benzamides, Dopamine D-2 receptor imaging agent.

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INTRODUCTION

Recently, (S)-2-hydroxy-3-^{123}I-iodo-6-methoxy-N-[(1-ethyl-2-pyrrolidinyl)-methyl]benzamide ((S)-^{123}I-IBZM, Figure 1) has been introduced as a promising radio-ligand for in-vivo CNS dopamine D-2 receptor imaging.[1-6]

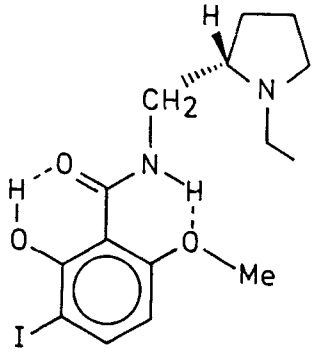


Figure 1. (S)-2-hydroxy-3-^{123}I-iodo-6-methoxy-N-[(1-ethyl-2-pyrrolidinyl)-methyl]benzamide ((S)-^{123}I-IBZM)

A synthetic route to a series of substituted benzamides has been described by de Paulis et al.[7,8] A synthesis of IBZM and BZM, basically via the same route, has been published by Kung et al.[9]

Our goal was to develop a simple synthesis of both (S)-BZM ((S)-**5a**), the precursor of (S)-^{123}I-IBZM, and (R)-BZM ((R)-**5a**). The preparation of benzamides as described by de Paulis et al.,[7,8] and the synthesis of BZM by Kung et al.[9] via acid chlorides appears less attractive because of the relatively low yields (15-50%, and 55% respectively), and the instability of the intermediate acid chlorides. Instead of activating 2,6-dimethoxybenzoic acid (**1a**) with thionyl chloride, the potency of N-hydroxysuccinimide/DCC in this respect was investigated.

Due to the low concentration of dopamine D-2 receptors present in brain tissue, a high specific activity is mandatory. Since this is not attainable by isotopic exchange labeling, direct oxidative iodination of (S)-BZM was

pursued. For the removal of the uniodinated substrate an alternative for the time consuming, preparative HPLC procedure was developed.

RESULTS AND DISCUSSION

Synthesis of (S)-BZM, (R)-BZM and (S)-IBZM

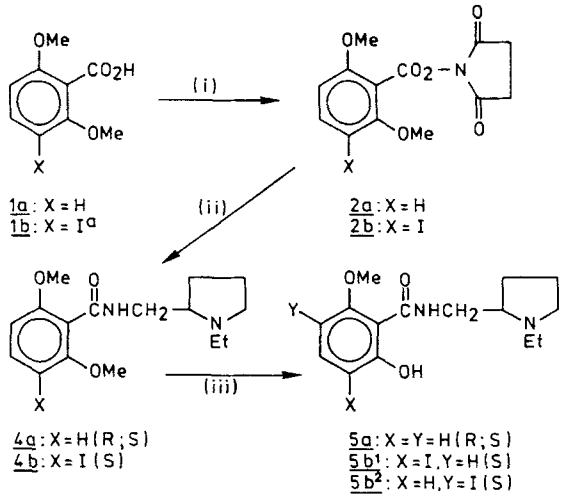
(S)-BZM was synthesized from its corresponding benzoic acid (**1a**) with the aid of N-hydroxysuccinimide[10] (Scheme I), which has been succesfully applied in peptide synthesis.[11] The resulting succinimate ester (**2a**) may be coupled with a primary amine of choice and represents a stable, well characterizable, common precursor for both (S)-BZM and (R)-BZM. The reaction of succinimate esters with an appropriate chiral amine is known to proceed in high yield and without racemization.[10,11]

Apart from the synthetic advantage of a common precursor, the overall yield following this procedure is signifantly higher: (S)-BZM and (R)-BZM were synthesized in an overall yield of approx. 70%, which compares favourably with the value of 26% reported by de Paulis et al.[7] and of 55% reported by Kung et al.[9] (S)-IBZM was prepared starting from 2,6-dimethoxy-3-iodobenzoic acid (**1b**), following an analogous reaction sequence (Scheme I). It was used for identification purposes.

Radiolabeling

The radiolabeling procedure applied is essentially the same as described by Kung et al.[12] Instead of using peracetic acid we generated this oxidant in-situ from acetic acid/ hydrogen peroxide added to the reaction mixture. The radiolabeling of (S)-BZM to (S)-^{123}I-IBZM, proceeded with a yield of 80-85%, which is slightly lower than the reported 95% by Kung et al.[12] The overall radiochemical yield amounts to 60-65%, mainly due to losses in the purification steps. The specific activity of ^{123}I-IBZM obtained, amounts to ≥3000 Ci/mmol (≥100 TBq/mmol).

A distinct feature of the procedure is the use of a disposable C-18 cartridge instead of a time-consuming, preparative HPLC separation. Thus,



Scheme I. The synthesis of (S)-BZM, (R)-BZM and (S)-IBZM

*(i): N-hydroxysuccinimide/DCC in 1,4-dioxane, (ii): 2-aminomethyl-1-ethylpyrrolidine in CH_2Cl_2, (iii): BBr_3 in CH_2Cl_2, (a): **Ia** was transformed into **Ib** in three steps: (1): HNO_3 in H_2SO_4; (2): H_2, Pd/C, HCl in EtOH; (3): $NaNO_2$, KI in H_2SO_4.*

drawbacks of preparative HPLC such as the recovery of the product from a relative large volume of solvent, high-level radioactive contamination of the system, and the interference with aseptic preparation are avoided, and HPLC is only used for analysis.

In-vitro saturation and displacement studies and in-vivo binding studies, with (S)-^{123}I-IBZM, (S)-BZM and (R)-BZM will be published in a separate article.[13]

EXPERIMENTAL

^{1}H NMR spectra were recorded on a Bruker AC 200 Spectrometer in $CDCl_3$ with TMS as internal standard. $[\alpha]_D^{20}$ Values were measured on an Optical Activity A-10 polarimeter. HPLC analysis was performed on a Waters system, equipped with a model 510 pump, U6k injector, RP-C18 column and

Lambda-Max-481 UV-absorbance detector. ^{123}I-NaI Was provided by Cygne B.V., Eindhoven, The Netherlands.

Synthesis of (S)-BZM, (R)-BZM and (S)-IBZM

2,6-Dimethoxy-3-iodo-benzoic acid (**1b**). -- 2,6-Dimethoxy-3-iodo-benzoic acid (**1b**) was prepared from 2,6-dimethoxybenzoic acid (**1a**) according to methods in the literature[14,15] (Scheme I). The iodination, however, was performed on the HCl-salt of 3-amino-2,6-dimethoxybenzoic acid.

Succinimate ester of 2,6-dimethoxybenzoic acid (**2a**).[10]

1a (5.47 g, 30 mmol) and N-hydroxysuccinimide (4.32 g, 37.5 mmol) were dissolved in dry 1,4-dioxane (45 ml). To this solution dicyclohexylcarbodiimide (9.20 g, 45 mmol) was added. A little rise of temperature was noted and after approx. five minutes a white solid (dicyclohexylurea) precipitated. The reaction mixture was stirred overnight, the insoluble material was filtered off and washed with dry 1,4-dioxane. The combined filtrates were evaporated to leave a solid residue which was triturated with dry diethyl ether. Crystallization from EtOAc gave **2a**. Yield: 7.25 g = 87%; mp: 201-202°C; ^{1}H NMR: δ: 7.40 (t, J = 8.4 Hz, 1H, H_4); 6.60 (d, J = 8.6 Hz, 2H, $H_3 + H_5$); 3.89 (s, 6H, OMe); 2.88 (s, 4H, CH_2).

Succinimate ester of 2,6-dimethoxy-3-iodo-benzoic acid (**2b**).[10]

2b was prepared in the same manner starting from **1b**. Crystallization from EtOAc:petroleum ether (1:1). Yield: 92%; mp: 126-127°C; ^{1}H NMR: δ: 7.84 (d, J = 8.8 Hz, 1H, H_4); 6.58 (d, J = 8.8 Hz, 1H, H_5); 3.97 (s, 3H, 2-OMe); 3.89 (s, 3H, 6-OMe); 2.90 (s, 4H, CH_2).

(S)-2,6-Dimethoxy-N-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide ((S)-**4a**).[10]

To a solution of **2a** (5.60 g, 20 mmol) in CH_2Cl_2 (30 ml) (S)-2-aminomethyl-1-ethylpyrrolidine (**3a**; 2.69 g, 21 mmol) was added dropwise. A little rise of temperature was noted. After two hours of stirring, 1N NaOH (40 ml) was added and the mixture was stirred for an additional hour. The layers were

separated and the aqueous layer was extracted with CH_2Cl_2 (3x 50 ml). The combined organic layer was dried ($MgSO_4$) and the solvent was evaporated. Crystallization from diisopropyl ether gave (S)-**4a**. Yield: 5.56 g = 95%; mp: 114°C (lit[7]: 100-102°C); ^{1}H NMR: δ: 7.26 (t, J = 8.4 Hz, 1H, H_4); 6.56 (d, J = 8.4 Hz, 2H, H_3 + H_5); 6.28 (bs, 1H, NH); 3.81 (s, 6H, OMe); 3.0-3.4 (m, 2H); 2.85 (dd, J_1 = 8.0 Hz, J_2 = 7.5 Hz, 1H); 2.5-2.7 (m, 1H); 2.0-2.3 (m, 3H); 1.5-2.0 (m, 4H); 1.07 (t, J = 7.2 Hz, 3H, N-CH_2-CH_3); $[\alpha]_D^{20}$: -58.8° (c = 2, acetone; lit[7]: -59°).

(R)-2,6-Dimethoxy-N-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide ((R)-**4a**).[10] (R)-**4a** was prepared in the same manner with (R)-2-aminomethyl-1-ethyl-pyrrolidine (**3b**). Yield 94%; mp: 114°C; $[\alpha]_D^{20}$: +59.3° (c = 2, acetone).

(S)-2,6-Dimethoxy-3-iodo-N-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide(**4b**).[10] **4b** Was prepared in the same manner from **2b**. Crystallization from diisopropyl ether. Yield 96%; mp: 112°C (lit[7]: 112-113°C); ^{1}H NMR: δ: 7.70 (d, J = 8.8 Hz, 1H, H_4); 6.52 (d, J = 8.8 Hz, 1H, H_5); 6.31 (bs, 1H, NH); 3.87 (s, 3H, 2-OMe); 3.81 (s, 3H, 6-OMe); 3.0-3.4 (m, 2H); 2.83 (dd, J_1 = 7.4 Hz, J_2 = 12.1 Hz, 1H); 2.5-2.8 (m, 1H); 1.5-2.4 (m, 7H); 1.07 (t, J = 7.2 Hz, 3H, N-CH_2-CH_3); $[\alpha]_D^{20}$: -66.8° (c = 2, acetone; lit[7]: -67°).

Resolution of the isomeric pyrrolidines.[16] -- (S)-2-amino-methyl-1-ethyl-pyrrolidine (**3a**): Yield: 48%; $[\alpha]_D^{20}$: -54.7° (c = 1, $CHCl_3$); (R)-2-amino-methyl-1-ethylpyrrolidine (**3b**): Yield: 47%; $[\alpha]_D^{20}$: +55.1° (c = 1, $CHCl_3$). ^{1}H NMR of racemic 2-aminomethyl-1-ethylpyrrolidine: δ: 3.16 (qui, J = 4.8 Hz, 1H); 2.7-2.9 (m, 3H); 2.35 (m, 1H) 2.1-2.3 (m, 2H); 1.6-1.9 (m, 4H); 1.3-1.5 (m, 2H); 1.10 (t, J = 7.2 Hz, 3H, N-CH_2-CH_3).

(S)-2-Hydroxy-6-methoxy-N-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide, ((S)-BZM; (S)-**5a**)).[7] -- A stirred solution of (S)-**4a** (3.0 g, 10.2 mmol) in CH_2Cl_2 (75 ml) was treated with 4N HCl in diethyl ether (3.2 ml). Subsequently a solution of BBr_3 (2.6 g, 10.2 mmol) in CH_2Cl_2 (17.5 ml) was added

dropwise. After one hour 1N ammonia (25 ml) was added and stirring was continued for an additional hour. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2x 50 ml). The combined organic layer was washed with water, dried (Na_2SO_4) and concentrated. The residue was dissolved in dry diethyl ether and the insoluble material was removed by filtration. Finally the filtrate was evaporated to give (S)-**5a**. Yield: 2.41 g = 85%; ^{1}H NMR: δ: 14.2 (s, 1H, OH); 8.93 (bs, 1H, NH); 7.25 (t, J = 8.3 Hz, 1H, H_4); 6.61 (dd, J_{ortho} = 8.3 Hz, J_{meta} = 0.9 Hz, 1H, H_3); 6.39 (dd, J_{ortho} = 8.3 Hz, J_{meta} = 0.8 Hz, 1H, H_5); 3.92 (s, 3H, OMe); 3.68 (m, 1H); 3.1-3.4 (m, 2H); 2.7-3.0 (m, 1H); 2.5-2.7 (m, 1H); 2.1-2.4 (m, 2H); 1.5-2.0 (m, 4H); 1.13 (t, J = 7.2 Hz, N-CH_2-$\underline{CH_3}$); $[\alpha]_D^{20}$: -55.7° (c = 2, acetone; lit[7]: -56°). To a solution of (S)-**5a** in diethyl ether (15 ml), 4N HCl in diethyl ether (3.0 ml) was added with stirring to precipitate (S)-**5a**•HCl. The solvent was decanted and the sticky solid was dried in vacuo at 50°C. Overall yield: 2.41 g = 75%; mpt: 139°C; ^{1}H NMR: δ: 13.6 (bs, 1H, OH); 12.2 (bs, 1H, pyrr-$\underline{NH^+}$-Et); 9.57 (bs, 1H, NH); 7.31 (t, J = 8.3 Hz, 1H, H_4); 6.59 (d, J = 8.3 Hz, 1H, H_3); 6.44 (d, J = 8.3 Hz, 1H, H_5); 4.12 (s, 3H, OMe); 3.7-4.1 (m, 4H); 3.1-3.4 (m, 1H); 2.8-3.1 (m, 2H); 1.8-2.4 (m, 4H); 1.13 (t, J = 7.2 Hz, 3H, pyrr-NH^+-CH_2-$\underline{CH_3}$).

(R)-2-Hydroxy-6-methoxy-N-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide, ((R)-BZM; (R)-**5a**)).[7] -- (R)-BZM was prepared in the same manner from (R)-**4a**. Yield: 76%; $[\alpha]_D^{20}$: +56.1° (c = 2, acetone).

(S)-2-hydroxy-3-iodo-6-methoxy-N-[(1-ethyl-2-pyrrolidinyl)methyl]-benzamide, ((S)-IBZM; **5b1**).[7] -- A stirred solution of **4b** (2.16 g, 5 mmol) in CH_2Cl_2 (50 ml) was treated with 4N HCl in diethyl ether (1.5 ml). Subsequently a solution of BBr_3 (1.45 g, 5.8 mmol) in CH_2Cl_2 (12.5 ml) was added dropwise. After one hour 1N ammonia (25 ml) was added and stirring was continued for an additional hour. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2x 30 ml). The combined organic layer was washed with water, dried (Na_2SO_4) and concentrated. The residue

was dissolved in dry diethyl ether (30 ml) and the insoluble material was removed by filtration. The filtrate was evaporated. Crude yield: 1.92 g = 92%. Column chromatography (silica gel; diisopropyl ether:methanol:ammonia (80:19:1 v/v)) yielded 1.3 g = 65% **5b1**: ^{1}H NMR: δ: 15.20 (bs, 1H, OH); 8.97 (bs, 1H, NH); 7.71 (d, J = 8.8 Hz, 1H, H_4); 6.27 (d, J = 8.8 Hz, 1H, H_5); 3.92 (s, 3H, OMe); 3.68 (m, 1H); 3.1-3.4 (m, 2H); 2.6-3.0 (m, 2H); 2.1-2.4 (m, 2H); 1.5-2.0 (m, 4H); 1.13 (t, J = 7.2 Hz, 3H, N-CH_2-$\underline{CH_3}$). Also 210 mg = 10% (S)-2-hydroxy-5-iodo-6-methoxy-N-[(1-ethyl-2-pyrrolidinyl)methyl]-benzamide (**5b2**) was isolated: ^{1}H-NMR: δ: 14.65 (1H, OH); 8.96 (bs, 1H, NH); 7.69 (d, J = 8.8 Hz, 1H, H_3); 6.26 (d, J = 8.8 Hz, 1H, H_4); 3.92 (s, 3H, OMe); 3.70 (m, 1H); 3.1-3.4 (m, 2H); 2.6-3.0 (m, 2H); 2.1-2.4 (m, 2H); 1.5-2.0 (m, 4H); 1.13 (t, J = 7.2 Hz, 3H, N-CH_2-$\underline{CH_3}$).

Radiolabeling

For the oxidative iodination of (S)-BZM, ^{123}I-NaI (Specific activity: ≥3000 Ci/mmol (≥100 TBq/mmol); radionuclidic purity: ≥99.99%), produced via the indirect proton reaction on 99.8% enriched ^{124}Xe, was used.[17] To a solution of (S)-BZM·HCl (0.13 μmol) in EtOH (20 μl) were added consecutively: ammonium acetate buffer (275 μl; pH = 4), H_2O_2 (30%):HOAc = 2:1 v/v (10μl) and a solution of ^{123}I-iodide in 0.05 M NaOH (<100 μl). After approx. 10 minutes at ambient temperature, unreacted ^{123}I-iodide was removed by passing the solution over a weakly basic anion exchange resin [Sephadex DEAE-A25, Pharmacia]. The radiochemical yield amounted to 80-85%. To remove unreacted BZM the reaction mixture was loaded on a C-18 cartridge [Bond Elute, 1 ml, Analytech]. This column was washed subsequently with water (1 ml) and with a mixture of 0.5 M ammonium acetate buffer (pH = 9):EtOH:water (v/v = 7:18:24; 20-25 ml). The removal of (S)-BZM from the column was checked with HPLC (UV-detection: wavelength = 254 nm). If necessary, this procedure was repeated. Finally (S)-^{123}I-IBZM was eluted from the cartridge with EtOH:HOAc (v/v = 200:1; 2-5 ml). The eluate was evaporated and the residue was dissolved in EtOH. HPLC-analysis demonstrated, that the specific activity

had not changed during the radiolabeling procedure and still amounted to ≥3000 Ci/mmol (≥100 TBq). The overall radiochemical yield amounted to approx. 60-65%. The product was formulated with isotonic phosphate buffer (pH=6) to a final solution containing < 10 % (v/v) EtOH at activity concentration 2 mCi/ml (75 MBq/ml).

CONCLUSIONS

(S)-BZM, the precursor of (S)-^{123}I-IBZM, can be synthesized in high overall yield (69%) using the method described. The standard way to convert the benzoic acids into their corresponding benzamides, via their acid chlorides suffers from two major disadvantages: the acid chlorides cannot be stored for longer periods and the yields are modest (approx 50%). Conversion of the benzoic acids **1a** and **1b** into their corresponding benzamides **4a** and **4b** with the aid of N-hydroxysuccinimide/DCC via their common stable succinimate precursor, results in higher yields (80-90%). The prepared (S)-BZM provided a suitable substrate for oxidative iodination, leading to (S)-^{123}I-IBZM.

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