SYNTHESIS OF ^{77}BR-LABELLED 2-(4-BROMO-2,5-DIMETHOXYPHENYL)-ISOPROPYLAMINE WITH HIGH SPECIFIC ACTIVITY

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SUMMARY

The tagging of 2,5-dimethoxyphenylisopropylamine (DPIA) with bromine-77 ($T_{1/2}$= 56 hrs) was reinvestigated in order to achieve high specific activities which are necessary for in-vivo application of this centrally acting drug. A fast one-pot synthesis was developed using N-chlorotetrafluorosuccinimide (NCTFS) and practically carrier-free bromide to introduce radiobromide into the aromatic ring. Trifluoroacetic anhydride (TFA) was applied both as solvent and as a reversible blocking agent of the free amino group. TFA was also shown to suppress chlorinating side-reactions. Rapid separation of 4-^{77}Br-2,5-dimethoxyphenylisopropylamine was achieved by reverse phase high pressure liquid chromatography. The total labelling and separation procedure takes about two hours, and 4-^{77}Br-DOB is obtained with an overall radiochemical yield of 25% and a specific activity of >8 Ci/μmole.

Key words: 4-bromo-2,5-dimethoxyphenylisopropylamine, bromine-77, N-chlorotetrafluorosuccinimide, psychotomimetics, HPLC-separation

INTRODUCTION

The amphetamine analogue 2-(4-bromo-2,5-dimethoxyphenyl)-isopropylamine (DOB) was found by Shulgin, Sargent and Naranjo to be a highly active psychotomimetic agent (1). Oral- and i.v.-application of the radiobromine labelled drug to man demonstrated its active take up by different organs, especially by the brain (2). It was therefore suggested as a potential brain scanning agent (3).

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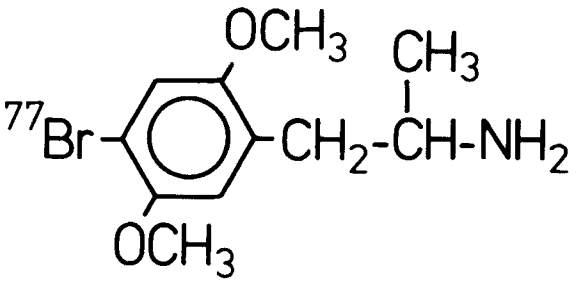


Labelling of the drug with bromine-77 is motivated by two reasons. It has been observed that substitution of hydrogen in the 4-position of 2,5-dimetoxyphenylisopropylamine (DPIA) by a methoxy group (TMA-2) or a methyl group (STP) causes an enhancement of central activity by a factor of 2 and 10, respectively (4). Substitution of the methyl group by the isosteric bromine intensifies the psychomimetic activity by a factor of hundred which means that it is 400 times more active than mescaline (1). The high activity is explained by the structural relation to 6-hydroxydopamine and the hindered metabolic attack at the 4-position. Secondly, maximum accumulation in the brain occurs 3 to 6 hours after application; thus among the potentially useful neutron deficient halogen isotopes the longer lived ^{77}Br ($T_{1/2}$= 56 hrs) is preferable. But also the shorter lived positron emitter ^{75}Br ($T_{1/2}$= 98 min) might still be useful for positron emission tomography (5).

Recently a labelling procedure was reported (2), using $^{77}Br\text{-}Br_2$ in relatively small specific activities of 57 mCi/mmol. This would necessitate the application of small activities and hence long time measurements when applied as a brain agent. The low psychotomimetic active dose of 0.2 to 2 mg per man and the desirable fast sequence of pictures for measurements of metabolic

turnover, however, requires high specific activities. A reinvestigation of the bromination of DPIA with the goal to obtain practically carrier-free products seemed therefore desirable. Theoretically carrier-free bromine labelled aromatic compounds can be obtained by a recently developed method using N-chlorotetrafluorosuccinimide (NCTFS) and carrier-free bromide-77 (6). NCTFS has been used for labelling of monosubstituted benzenes starting with ^{77}Br-bromide. High specific activities, relatively high radiochemical yields together with high para-selectivities (60-90%) had been observed. The application of the NCTFS-method to the bromination of DPIA was of special interest, since the amine acts as a scavenger for electrophilic species. Efficient labelling should be possible when using trifluoroacetic anhydride (TFA) as solvent which acted as reversible blocking agent of the amino group.

EXPERIMENTAL

Starting Materials

Carrier-free ^{77}Br-bromide was produced via the ^{75}As(α,2n)^{77}Br nuclear reaction bombarding thick Cu_3As-alloy targets at the Jülich Compact Cyclotron CV-28 (TCC) with 28 MeV α-particles. After isolation from the target material by a dry distillation technique (7) the bromide was dissolved in triple distilled water. The ^{77}Br-bromide-water solution was transferred to a reaction vessel and evaporated to dryness.

N-chlorotetrafluorosuccinimide (NCTFS) has been prepared via tetrafluorosuccinimide as described previously (8). This compound was also obtained by the reaction of ammonia with tetrafluorosuccinic anhydride (Merck), isolation of the free succinic acid-monoamide by HCl-treatment, and following cyclisation by condensation with P_2O_5 under reduced pressure (9).

2 5-Dimethoxyphenylisopropylamine (DPIA) was prepared by aldol condensation of 2,5-dimethoxybenzaldehyde and nitroethane using ammoniumacetate in acetic acid (10) and reduction of the resulting styrol to the amine (DPIA) by $LiAlH_4$ in dry THF (11). The free amine was purified by vacuum distillation [bp. (1 torr) = 120-122 ^{o}C] resulting in a colorless oil. Bromination of the amine gave rise to the 4-bromo-compound to be used as reference substrate for HPLC-separation (see below). Bromination was carried out with molecular bromine in acetic acid (12) similar to the procedure reported. The identity and purity of the amino compounds were checked by ^{1}H-NMR spectroscopy (Bruker, WP-80) and high pressure liquid chromatography.

High Pressure Liquid Chromatography

Separation and purification of the labelled compound has been performed by high pressure liquid chromatography. A reverse phase column packed with μ-Bondapak C-18 (Waters) or a polar column with silicagel (Latek) were used with different eluents. The columns were mounted to a Hewlett Packard liquid chromatograph 1010A with an UV detector from Waters Assoc.The identification of the labelled compound and the purity of the starting material (DPIA) have been tested using different columns and eluents. The conditions for the separations are listed in Table I.

For preparative separation of 4-^{77}Br-DOB from the starting material (DPIA) the reverse phase column using a 0.03 molar $(NH_4)_2CO_3$-solution of CH_3OH-H_2O (7:3) as eluent was best suited for this purpose. Earlier separations of amphetamine analogues (13) using 2N ammonia- 1N ammonium nitrate-methanol eluents on silicagel columns lead only to poor resolutions.

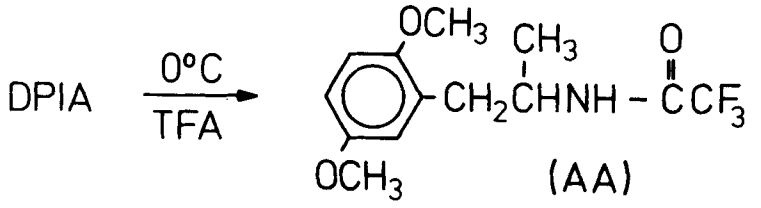
Table I. High Pressure Liquid Chromatography Separation of 2,5-Dimethoxyphenylisopropylamine (DPIA) from 4-Br-DOB

Column	Eluent	Flow (ml/min)	DPIA (k'-value)	4-Br-DOB (k'-value)
Waters, µ-Bondapak C_{18} 50 cm ø 0.4 cm	$CH_3OH:H_2O$ 8:2 0.05M $(NH_4)_2CO_3$	0.7	0.44	0.76
	$CH_3OH:H_2O$ 7:3 0.05M $(NH_4)_2CO_3$	0.6	0.86	1.86*
Waters, µ-Bondapak C_{18} 30 cm ø 0.4 cm	$CH_3OH:H_2O$ 19:1 0.025M $(NH_4)_2CO_3$	0.7	0.33	0.80
	CH_3OH:1N NH_4NO_3:2N NH_3 650:25:50	0.6	0.74	1.32
	$CH_3OH:H_2O$ 9:1 0.02M KH_2PO_4	0.7	0.35	0.62
Latek Spherisorb 55 W, 10 µm 50 cm ø 0.18 cm	$CH_3OH:H_2O:CH_3COOH$ 250:750:5	3.0	0.5	1.0
	CH_3OH:2N NH_3:1N $NaNO_3$ 9900:60:20	0.8	0.40	1.80

*) Retention of 4-Bromo-DPI-Trifluoroacetamide: k' = 2.13

4-^{77}Br-2,5-Dimethoxyphenylisopropylamine

The optimized reaction conditions of the NCTFS-method have been described previously (6). For the bromination of DPIA the corresponding trifluoroacetamide was employed, which was obtained by dissolving the amine in an excess of trifluoroacetic anhydride (TFA) while cooling (eq. I).



(I)

TFA serves as blocking agent for the amine group and as solvent. It was observed that a chlorinating side reaction (possibly via NCTFS) leading to non-isotopic carrier does not occur in pure anhydride solution (6).

0.5 ml of TFA containing 5 to 10 mg of the amine (DPIA) was given to a 2 ml reaction vessel with carrier-free, dry ^{77}Br-bromide and 2 mg NCTFS, weighed in a dry-box. The bromination reaction was allowed to take place in darkness at room temperature and gave rise to the 4-^{77}Br-acetamide compound (4-^{77}Br-AA) (eq. II).

$$AA + {}^{77}Br^{\ominus} + NCTFS \xrightarrow[TFA]{0.5h,\,20^{\circ}} 4-{}^{77}Br-AA \qquad (II)$$

After one hour the TFA was evaporated. The residue was dissolved in aqueous NaOH solution at pH 10 and warmed for 1 hour (or milder at pH 8 at room temperature for 12 hours) in order to hydrolise the acetamide (eq. III). The liberated ^{77}Br-amine was extracted with CCl_4 (3x0.5 ml), isolated by evaporation of the solvent and redissolved in methanol. The resulting ^{77}Br-4-bromo-amphetamine was separated by HPLC (see above). The whole labelling procedure took less than 2 hours and exclusively lead to the para-product. The overall radiochemical yield of 4-^{77}Br-DOB was

$$4\text{-}^{77}\text{Br-AA} \xrightarrow[\text{or } 12\text{h, pH 8, } 20^\circ]{\text{Hydrolysis}\ 1\text{h, pH 10, } 100^\circ} 4\text{-}^{77}\text{Br-DOB} \qquad \text{(III)}$$

25% after chromatographic separation.

During HPLC-analysis no UV-signal of 4-Br-DOB could be detected. Even if a bromide contamination from reagents in the order of 0.1 mg would be present, a specific activity of 8 Ci per µmole is obtained. This specific activity allows in-vivo application of higher total activities without toxicity problems.

The positive results suggest that the NCTFS-method can be applied to other complicated biomolecules, especially amino acids and oligopeptides which are of interest since they can be protected reversibly by TFA under mildest conditions (14).

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