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PREPARATION OF 4- AND 6-[^{76}Br] BROMOMETARAMINOL, TWO POTENTIAL RADIOTRACERS FOR THE STUDY OF THE MYOCARDIAL NOREPINEPHRINE NEURONAL REUPTAKE SYSTEM WITH PET

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Summary

Metaraminol is a norepinephrine analogue which is transported with high affinity by the uptake-1 mechanism of the sympathetic nerve terminal. Radiolabelled metaraminol and analogues are promising radiotracers to assess the integrity of the myocardial nerve system. The bromo analogues, 4-and 6-bromometaraminol, were synthesized from commercially available metaraminol bitartrate. Structural assignments were made by 2D-NMR experiments. 4- and 6-[^{76}Br]Bromometaraminol were prepared from *N*-Boc-metaraminol using [^{76}Br]NH_4Br and peracetic acid as the brominating agent. The total radiochemical yield based on starting [^{76}Br]NH_4Br was 17% and 38%, non decay-corrected, for derivative 4-[^{76}Br]bromometaraminol and 6-[^{76}Br]bromometaraminol, respectively, in a synthesis time of 3.5 hours including the preparation of [^{76}Br]NH_4Br. The specific radioactivity obtained for both radiotracers was 130 mCi/μmol (4.8 GBq/μmol). Tissue distribution studies were performed in rats and revealed a low cardiac uptake for both derivatives. These preliminary results suggest that neither 4- nor 6-[^{76}Br]bromometaraminol are suitable radiotracers to study the myocardial norepinephrine neuronal reuptake system with Positron Emission Tomography.

Key Words : positron emission tomography, bromine-76, metaraminol, myocardium.

Introduction

Metaraminol ((1R,2S) 1-(3-hydroxyphenyl)-2-aminopropanol, **1**) is a close analogue of norepinephrine, the endogenous neurotransmitter of the sympathetic nervous system. It shares with norepinephrine the same uptake, storage and release mechanisms, but it possesses higher metabolic

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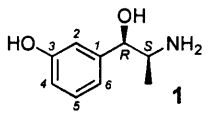
stability, as it is not metabolized by monoamino oxidase (MAO) and catechol O-methyl transferase (COMT) enzymes[1].

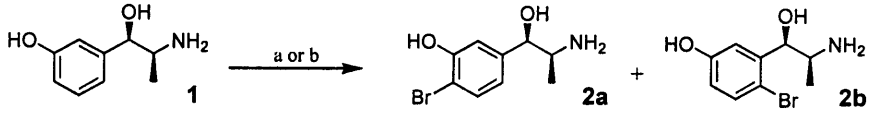


Radiolabelled metaraminol and derivatives are promising radiotracers to assess the integrity of the myocardial innervation with positron emission tomography (PET). [^{11}C]Metaraminol has recently been synthesized and PET studies have been performed in monkeys[2]. Its very high uptake as well as its prolonged retention in the heart confirmed it as a promising radiotracer for PET investigations. Labelled halo-analogues of metaraminol have also been synthesized and studied. First, 6-[^{18}F]fluorometaraminol has been prepared from [^{18}F]acetyl hypofluorite and has been studied in rats and dogs[3,4]. The radiotracer showed a rapid and high uptake and prolonged retention in the heart. However, due to the low specific radioactivity obtained by this electrophilic substitution reaction, the use of this radiotracer was not considered to be safe in man. Recently a synthetic pathway using nucleophilic substitution with [^{18}F]fluoride for obtaining 6-[^{18}F]fluorometaraminol with higher specific radioactivity has been investigated, but has not been completed yet[5]. Secondly, 4-[^{125}I]iodometaraminol and 6-[^{125}I]iodometaraminol have been synthesized and tissue concentrations have been measured in dog[4]. The cardiac uptake was estimated to be too low to continue any further investigation of these radiotracers. In order to compare the halo-derivatives of metaraminol, we decided to synthesize bromine-76 ($t_{1/2}$: 16.2 hours) labelled analogues of metaraminol (4- and 6-isomers) and to investigate their potentials as PET radiotracers.

This paper describes (I) : the synthesis of 4- and 6-bromometaraminol (**2a** and **2b**) from commercially available metaraminol bitartrate; (II) : the preparation of 4- and 6-[^{76}Br]bromometaraminol ([^{76}Br]**2a** and [^{76}Br]**2b**); (III) : the biodistribution of 4- and 6-[^{76}Br]bromometaraminol in rats.

Results and Discussion

Chemistry

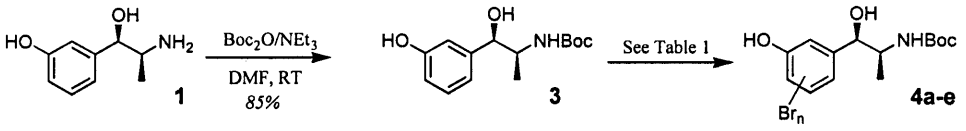


Conditions and reagents used	Ratio of both regioisomers	Yield
a. Br_2 (1 eq.), NH_4OH, EtOH/H_2O, RT	30 / 70	less than 5%
b. NBS (1 eq.), DMF, RT	cocktail of unidentified compounds	

Scheme 1 : Direct bromination of metaraminol (**1**).

Direct bromination of metaraminol (**1**) using either the Br_2/NH_4OH tandem or *N*-bromosuccinimide (NBS) in DMF[6] gave a poor and unreproducible yield (Scheme 1). Under the

former conditions, two monobrominated derivatives were observed with a ratio of 30/70 with yields of less than 5%. These two derivatives were identified as 4- and 6-bromometaraminol (**2a** and **2b**), respectively (see text below). The latter conditions gave a cocktail of unidentified compounds.



Scheme 2 : Synthesis of brominated derivatives of *N*-Boc-metaraminol (**4a-e**).

The free amino function of metaraminol (**1**) was protected using Boc_2O in a mixture of NEt_3 and DMF at room temperature to give the *N*-Boc derivative **3** in 85% non-optimized yield.

Bromination of **3** using either NBS in DMF[6] or NaBr and *N*-chlorosuccinimide (NCS) in EtOH[7] surprisingly yielded mono-, di- and tribrominated derivatives (Scheme 2). Table 1 gives an overview of the product composition for the use of different types of reagents and molar ratios.

Conditions and reagents used	Ratio of brominated isomers					Yield
	mono-		di-		tri-	
	4-Br **4a**	6-Br **4b**	4,6-Br **4c**	2,6-Br[a] **4d**	2,4,6-Br **4e**	
NBS (1 eq.), DMF, RT	0	39	31	21	9	49% [b]
NBS (0.15 eq.), DMF, RT	13	84	1	1	1	95% [c]
NaBr/NCS (1 eq.), EtOH, RT	19 [d]	49 [d]	8	3	21	95% [c]
NaBr/NCS (0.3 eq.), EtOH, RT	23	68	3	3	3	95% [c]
NaBr/NCS (0.15 eq.), EtOH, RT	26	71	1	1	1	95% [c]

[a] or 2,4-Br ; [b] isolated yield ; [c] based on converted starting material, calculated from the HPLC chromatograms ; [d] 65% isolated yield for the **4a/4b** mixture.

Table 1 : Product composition for the bromination of **3**.

The number of bromine atoms was easily determined by analysis of the aromatic region of the ^{1}H spectrum and then confirmed by mass spectrometry (Table 2). From the ^{1}H spectra and also based on a 2,4,6-preferred electrophilic aromatic substitution orientation due to the OH phenolic function, the position of the bromine atom for the mono-substituted derivatives **4a** and **4b** was assigned to be 4 or 6. A substitution pattern of 4,6-Br, 2,6-Br or 2,4-Br and 2,4,6-Br was attributed to derivatives **4c**, **4d** and **4e**, respectively.

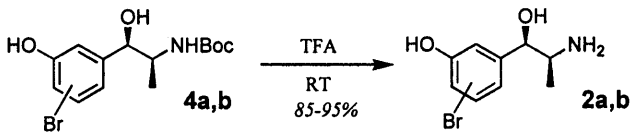
Discrimination between the 4-bromo and 6-bromo isomer was finally accomplished using 2D COSY & NOESY NMR experiments. Derivative **4a** was identified as the 4-substituted isomer (4-bromo-*N*-Boc-metaraminol) and derivative **4b** as the 6-substituted isomer (6-bromo-*N*-Boc-metaraminol). Structural evidence was based on the following facts : NOESY experiments run in DMSO-d_6 clearly showed two correlation peaks between the phenolic proton (OH, δ : 9.65 Hz) and

aromatic protons for compound **4b** (δ : 6.98 and 6.59) as it showed only one correlation peak for compound **4a** (δ : 6.95).

	NMR data					*MS data*
	δ_1	δ_2	δ_3	δ_{phenol}	notes	[M+H$^+$]
4a	6.80 (dd)	7.00 (d)	7.43 (d)	-	#	348, 346
	6.66 (dd)	6.95 (d)	7.36 (d)	9.65 (b)	£ §	
4b	6.66 (dd)	7.12 (d)	7.33 (d)	-	#	348, 346
	6.59 (dd)	6.98 (d)	7.27 (d)	9.65 (b)	£ §	
4c	7.23 (s)	7.62 (s)				428, 426, 424
4d	6.84 (d)	7.42 (d)				428, 426, 424
4e	7.73 (s)					508, 506, 504, 502

Table 2 : Compiled NMR and MS data for brominated derivatives of *N*-Boc-metaraminol (**4a-e**). ^{1}H NMR (Bruker AMX - 300 MHz) : δ_{1-3} (ppm) correspond to the aromatic region and are given in the order of appearance in the spectra ; Temperature : 298.0 K or # : 310.0 K or § : 318.0 K ; Solvent : CD_2Cl_2 or £ : DMSO-d_6 (s, d, dd, b, for singlet, doublet, doublet of doublet, and broad respectively). *J* values : see experimental section. Mass spectra (Nermag R10-10) : DCI/NH_4^+.

Bromination of *N*-Boc-metaraminol (**3**) with NBS in DMF in equimolar ratios afforded apart from the polybrominated compounds **4c**, **4d** and **4e** selectively the 6-bromo derivative **4b**. When the ratio of starting material to reagent was increased a mixture of both regio-isomers **4a** and **4b** was obtained. However, the formation of the 4-bromo derivative **4a** also seemed to depend on the type of brominating agent being used. The use of NaBr/NCS gave rise to **4a** even when equimolar ratios were used. As before, the percentage of **4a** increased when excess quantities of starting material **3** were used. The highest ratio of 7 to 3 for **4b**/**4a** was obtained when only 0.15 equivalent of NaBr/NCS with respect to starting *N*-Boc protected metaraminol (**3**) was used.



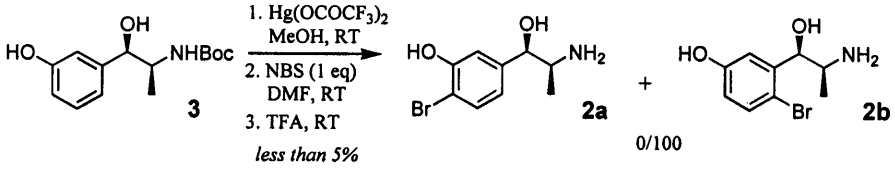
Scheme 3 : Synthesis of the brominated derivatives **2a** and **2b** from **4a** and **4b**.

Deprotection of the amino function of derivatives **4a** and **4b** (Scheme 3) using pure TFA gave 4-bromometaraminol (**2a**) and 6-bromometaraminol (**2b**) respectively, in excellent yields (85-95%).

As an additional approach for the structural determination of the two monobrominated derivatives of metaraminol (**2a,b**), regioselective halogenations of metaraminol (or its *N*-Boc protected derivative) were performed.

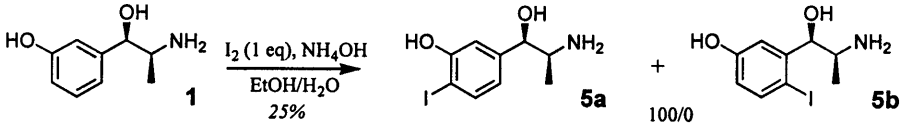
First, on an analytical scale, *N*-Boc-metaraminol (**3**) was transformed into its 6-trifluoroacetoxymercury derivative according to the literature[3,8] (Scheme 4). The mercury derivative was purified by semipreparative HPLC. Bromination with NBS in DMF, followed by TFA

deprotection of the amino function gave exclusively one monobrominated compound, which co-eluted with the above synthesized 6-bromo derivative **2b**, supporting the above described NMR attribution.



Scheme 4 : Synthesis of the brominated derivative **2b** *via* the trifluoroacetoxymercury route.

Secondly, iodination of metaraminol (**1**) using I_2 and aqueous NH_4OH in EtOH gave as described in the literature[4] only one iodinated product in 25% yield, which was described as the 4-iodo derivative **5a** (Scheme 5).



Scheme 5 : Direct iodination of metaraminol (**1**).

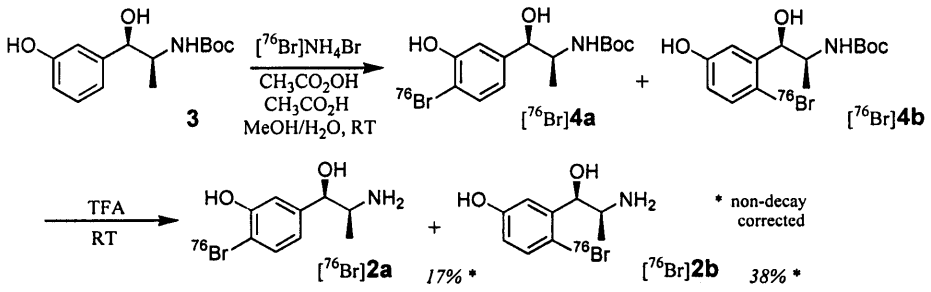
HPLC profiles of 4- and 6-iodometaraminol are described in the literature[4]. The elution order of 4- and 6-bromometaraminol (**2a** and **2b**, respectively) using the same HPLC conditions, strictly followed that of the former one and the retention time differences between the 4-bromo and 4-iodo derivative were consistent with all already synthesized bromo/iodo tandems in our laboratory. This also was in accordance with the attribution of the bromine atom position in our couple of monobrominated *N*-Boc protected derivatives **4a** and **4b**.

Radiochemistry

[^{76}Br]NH_4Br was used as the brominating agent and was prepared as described elsewhere[9]. In accordance with the "cold" direct bromination (see Scheme 1), radiobromination of unprotected metaraminol (**1**) was unsuccessful. [^{76}Br]NH_4Br, peracetic acid at room temperature or milder conditions such as [^{76}Br]NH_4Br, acetic acid and hydrogen peroxide at room temperature were used. According to the literature, a free amino function often acts as a scavenger in such electrophilic processes and has to be protected[10].

Radiobromination of *N*-Boc protected metaraminol (**3**) (Scheme 6) using [^{76}Br]NH_4Br and peracetic acid/acetic acid in a mixture of MeOH and water at room temperature afforded [^{76}Br]**4a** and [^{76}Br]**4b** as well as one other unidentified labelled side-product in a HPLC determined 3/7/0.5 ratio, respectively. Preparative separation of both *N*-Boc protected regio-isomers could not be

achieved at this stage of the synthesis. Treatment of the mixture with TFA quantitatively gave radiolabelled 4-bromo and 6-bromometaraminol ([^{76}Br]**2a** and [^{76}Br]**2b**) as well as the above mentioned, probably deprotected, side-product (Figure 1).



Scheme 6 : Radiobromination of *N*-Boc-metaraminol (**3**) and TFA deprotection.

Preparative HPLC gave chromatographically pure radiolabelled [^{76}Br]**2a** and [^{76}Br]**2b**, co-eluting with authentical non-labelled **2a** and **2b**, and with a specific radioactivity of 130 mCi/μmol (4.8 Gbq/μmol) (Figure 2). The total radiochemical yield based on starting [^{76}Br]NH_4Br was 17% and 38%, non decay-corrected, for derivatives [^{76}Br]**2a** and [^{76}Br]**2b**, respectively in a synthesis time of 3.5 hours including the preparation of [^{76}Br]NH_4Br.

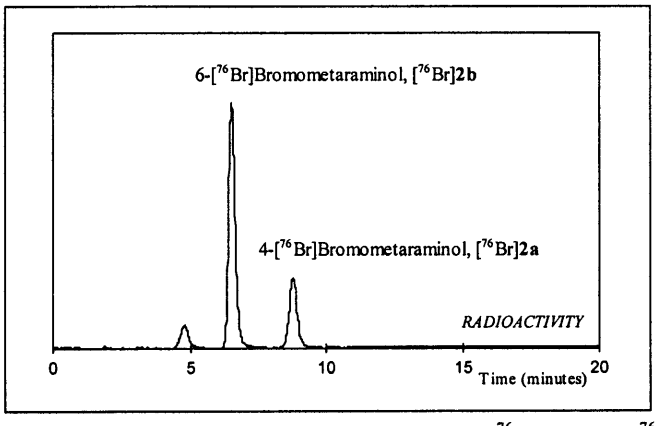


Figure 1 : Radiochromatogram of the mixture of [^{76}Br]**2a** and [^{76}Br]**2b** obtained after TFA deprotection (for HPLC conditions, see experimental section).

The [^{76}Br]**4a/4b** ratio of 30/70 obtained in the radiobromination just described is similar to the **4a/4b** ratio obtained in the "cold" bromination using NaBr/NCS. In both reactions, the electrophilic bromo species had to be formed by *in situ* oxidation of the bromide ion using peracetic acid as the oxidant in the first case and NCS in the second case. The use of NBS alone as described for the "cold" bromination, on the other hand, gave a different substitution pattern, as it may react more rapidly since it already represents the electrophilic bromo species itself.

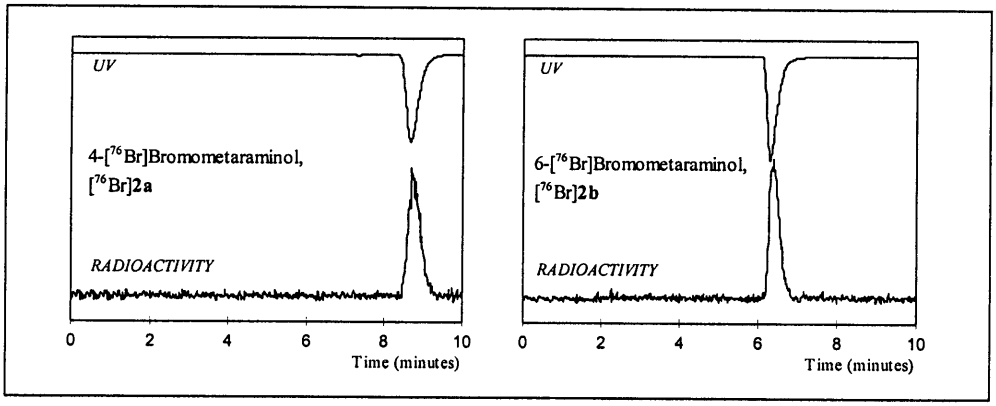


Figure 2 : Radiochromatograms of the co-injection of [^{76}Br]**2a** and [^{76}Br]**2b** with unlabelled **2a** and **2b**, respectively (for the HPLC conditions, see experimental section).

Biodistribution

Adult male Wistar rats were injected with 5.4 μCi (0.2 MBq, 42 pmol) of 4-[^{76}Br]bromo metaraminol ([^{76}Br]**2a**) or 10.8 μCi (0.4 MBq, 83 pmol) of 6-[^{76}Br]bromometaraminol ([^{76}Br]**2b**) in the tail vein. The rats were sacrificed, parts of heart, lung, liver, kidney, muscle and blood were removed, weighed and radioactivity was measured. Figure 3 summarizes the tissue radioactivity time-course distribution of [^{76}Br]**2a** and [^{76}Br]**2b** (expressed as percent of injected dose per gram of tissue, %ID/g).

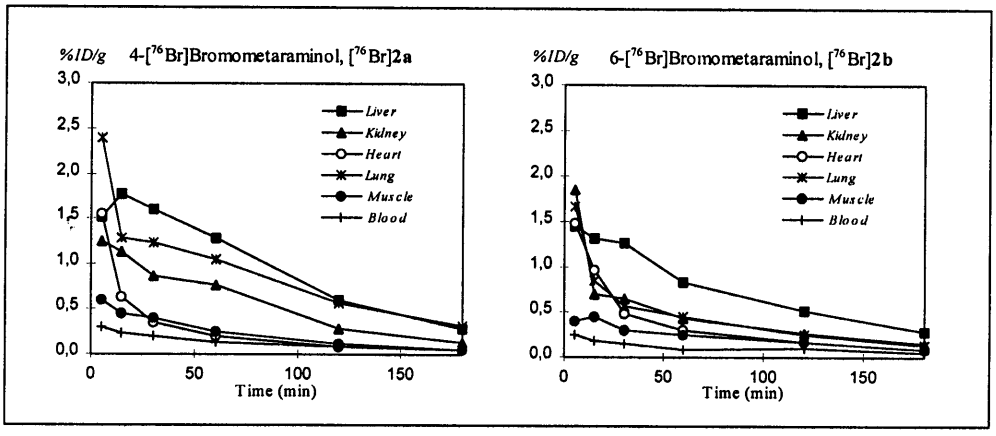


Figure 3 : Radioactivity time-course distribution of 4-[^{76}Br]bromometaraminol and 6-[^{76}Br]bromometaraminol in rats. %ID/g : mean of % of the injected dose per gram of tissue.

Both radiotracers showed a similar kinetic behaviour. In both cases the uptake in the heart was relatively low and the radiotracers were quickly eliminated from the heart tissue.

Experimental

General

Chemicals were purchased from Aldrich or Fluka France and were used without further purification. Metaraminol bitartrate was bought from Sigma France. TLC was run on pre-coated plates of silica gel 60F254 (Merck). The compounds were localized at 254 nm using a UV-lamp. Analytical HPLC was run on the following system: a HPLC Shimadzu LC-10AS pump, a Shimadzu SPD-10A UV detector and a Berthold LB 506 C-1 radioactivity detector. Semipreparative HPLC was carried out on a system equipped with a Waters 510 HPLC pump and a Waters 486 tunable UV absorbance detector. Radioactivity was detected with a Geiger-Müller counter. For both systems a Waters μBondapak C18 column (7.8 x 300 mm, 10 μm) eluted with a mixture of 50 mM aqueous sodium dihydrogen phosphate (a) and CH_3CN (b) or a mixture of 0.2 M aqueous ammonium dihydrogen phosphate (c) and THF (d) was used. UV absorption was detected at 254 nm. ^1H NMR spectra were recorded on a Bruker AMX (300 MHz) apparatus using TMS as an internal standard. The chemical shifts are reported in ppm, downfield from TMS (s, d, t, m, dd, b, bs for singlet, doublet, triplet, multiplet, doublet of doublet, broad and broad singlet respectively). 2D-NMR spectra were recorded in DMSO-d_6 at 318.0 K with an operating frequency of 300.13 MHz, a spectral width of 3247 Hz and a digital resolution of 0.40 Hz/pt. For COSY experiments, 256 increments were recorded; before Fourier transform and symetrisation, the data were multiplied with an unshifted sine bell function in each dimension. For NOESY experiments, 512 increments were recorded with a mixing time of one second; before Fourier transform and phase-sensitive treatment, the data were multiplied with a cosine bell squared function in each dimension. The mass spectra (MS) DCI/NH_4^+ were measured on a Nermag R10-10 apparatus (ionization potential 70 eV).

Chemistry

Direct bromination of metaraminol (**1**)

(a) with bromine : 100 mg of metaraminol (**1**) bitartrate (0.3 mmol, MW : 317.30) were dissolved in 17 mL of aqueous 10 M NH_4OH. To this solution were added dropwise 53 mg of Br_2 (0.3 mmol, 1 eq., MW : 159.82) dissolved in 9 mL of EtOH. The reaction mixture was stirred in the dark under nitrogen for 24 hours. The residue obtained after evaporation of the solvent was taken up in 10 mL of brine and extracted with EtOAc (3 x 100 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude mixture obtained after work-up was analyzed by HPLC (solvents a/b : 90/10, flow rate : 6.0 mL/min). Due to low yields (< 5 %) the products were not purified any further. Results are shown in Table 1.

(b) with NBS : 500 mg of metaraminol (**1**) bitartrate (1.6 mmol, MW : 317.30) were dissolved in 10 mL of water. The solution was alkalized with 28% aqueous NH_4OH and extracted with EtOAc (5 x 100 mL). The combined organic layers were dried over Na_2SO_4 and evaporated to dryness to give 250 mg (95% yield) of the corresponding free amine (1.5 mmol, MW : 167.21). The amine was treated as described for the synthesis of 6-bromo-*N*-Boc-metaraminol (**4b**) with 267 mg of NBS (1.5 mmol, 1 eq., MW : 177.99). The reaction mixture was analyzed by HPLC (solvents a/b : 90/10, flow rate : 6.0 mL/min). Results are shown in Table 1.

N-*Boc-metaraminol* (**3**)

1 g of metaraminol (**1**) bitartrate (3.2 mmol, MW : 317.30) was suspended in 3 mL of DMF and 1 mL of NEt_3 was added. The mixture was stirred until it became a yellow solution. To this solution were added 0.75 g of di-*tert*-butyl dicarbonate (3.4 mmol, MW : 218.25) dissolved in 1 mL of DMF. The reaction mixture was stirred overnight at room temperature. The solvent was removed under vacuum, the residue was taken up in 10 mL of water and was extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine and dried over Na_2SO_4. Removal of solvent gave 730 mg (85% yield) of *N*-Boc-metaraminol (**3**) as a glassy solid which was used without any further purification.

Rf ($CHCl_3$/MeOH/28% aq. NH_4OH : 30/70/10) : 0.87. ^{1}H NMR (CD_2Cl_2, 298.0 K) : δ : 0.96 (d, J = 6.9 Hz, 3H) ; 1.44 (s, 9H) ; 3.60 (b, $w_{1/2}$ = 18 Hz, 1H) ; 3.90 (b, $w_{1/2}$ = 24 Hz, 1H) ; 4.74 (s, 1H) ; 4.80 (bd, $w_{1/2}$ = 30 Hz, 1H) ; 6.45 (s, 1H) ; 6.73 (dd, J = 8.7 and 1.8 Hz, 1H) ; 6.81 (s, 1H) ; 6.91 (s, 1H) ; 7.18 (t, J = 8.1 Hz, 1H). MS : 285 [M + NH_4^+] ; 268 [M + H^+].

6-Bromo-N-*Boc-metaraminol* (**4b**) *and derivatives* **4c-e**

240 mg of *N*-Boc-metaraminol (**3**) (0.9 mmol, MW : 267.33) were dissolved in 4.5 mL of dry DMF. To this solution was added 154 mg of NBS (0.9 mmol, 1 eq., MW : 177.99) in 4.5 mL of DMF. The solution was stirred at room temperature for 30 min. The reaction mixture was poured into 50 mL of water and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were washed with water and dried over Na_2SO_4. Evaporation of solvent yielded 255 mg of an oily residue containing four different products (**4b-e**). This mixture was resolved by semipreparative HPLC (solvents a/b : 40/60, flow rate : 6.0 mL/min, 20 successive injections). Each of the combined HPLC fractions (containing either **4b**, **4c**, **4d** or **4e**) was then separately evaporated to dryness, taken up in 10 mL of water and extracted with CH_2Cl_2. The organic layers were washed with brine, dried over Na_2SO_4 and evaporated to dryness. 60 mg (20% yield) of 6-bromo-*N*-Boc-metaraminol (**4b**) in the form of white crystals were obtained as well as 35 mg (9% yield) of 2,6-dibromo-*N*-Boc-metaraminol (**4d**), 50 mg (14% yield) of 4,6-dibromo-*N*-Boc-metaraminol (**4c**) and 31 mg (7% yield) of 2,4,6-tribromo-*N*-Boc-metaraminol (**4e**).

4b : HPLC retention time (solvents a/b : 40/60, flow rate : 6.0 mL/min) : 6.5-7.0 min. ^{1}H NMR (DMSO-d_6, 298.0 K) : δ : 0.88 (d, J = 6.0 Hz, 3H) ; 1.35 (s, 9H) ; 3.80 (b, $w_{1/2}$ = 13 Hz, 1H) ; 4.72 (b, 1H) ; 5.45 (b, $w_{1/2}$ = 10 Hz, 1H) ; 6.34 (d, J = 9.0 Hz, 1H) ; 6.59 (dd, J = 8.6 and 2.7 Hz, 1H) ; 6.98 (d, J = 2.7 Hz, 1H) ; 7.27 (d, J = 8.7 Hz, 1H) ; 9.65 (b, ~1H) ; (DMSO-d_6, 310.0 K) : δ :

0.89 (d, J = 6.0 Hz, 3H) ; 1.36 (s, 9H) ; 3.80 (b, $w_{1/2}$ = 20 Hz, 1H) ; 4.72 (bd, $w_{1/2}$ = 10 Hz, 1H) ; 5.40 (b, $w_{1/2}$ = 25 Hz, 1H) ; 6.26 (b, J = 5.3 Hz, 1H) ; 6.59 (dd, J = 8.4 and 2.7 Hz, 1H) ; 6.98 (d, J = 2.7 Hz, 1H) ; 7.27 (d, J = 8.7 Hz, 1H) ; 9.60 (b, $w_{1/2}$ = 40 Hz, 1H) ; (DMSO-d_6, 318.0 K) : δ : 0.89 (d, J = 6.0 Hz, 3H) ; 1.36 (s, 9H) ; 3.80 (b, $w_{1/2}$ = 20 Hz, 1H) ; 4.72 (d, J = 3.9 Hz, 1H) ; 5.45 (b, $w_{1/2}$ = 25 Hz, 1H) ; 6.30 (b, $w_{1/2}$ = 26 Hz, 1H) ; 6.59 (dd, J = 8.6 and 2.7 Hz, 1H) ; 6.98 (d, J = 2.7 Hz, 1H) ; 7.27 (d, J = 8.7 Hz, 1H) ; 9.65 (b, $w_{1/2}$ = 40 Hz, 1H) ; (CD_2Cl_2, 310.0 K) : δ : 1.06 (d, J = 6.3 Hz, 3H) ; 1.40 (s, 9H) ; 4.05 (bm, $w_{1/2}$ = 19 Hz, 1H) ; 4.95 (bd, J = 8.4, 1H) ; 5.04 (d, J = 4.2 Hz, 1H) ; 6.66 (dd, J = 8.7 and 3.0 Hz, 1H) ; 7.12 (d, J = 3.0 Hz, 1H) ; 7.33 (d, J = 8.7 Hz, 1H) ; (CD_3OD, 298.0 K) : δ : 1.01 (d, J = 6.6 Hz, 3H) ; 1.40 (s, 9H) ; 3.30 (s, 1H) ; 3.35 (s, 1H) ; 3.96 (bm, $w_{1/2}$ = 18 Hz, 1H) ; 4.89 (b, 1H) ; 6.60 (bd, J = 8.4 Hz, 1H) ; 7.05 (s, 1H) ; 7.28 (d, J = 8.7 Hz, 1H) ; MS : 365, 363 [M + NH_4^+] ; 348, 346 [M + H^+].

4c : HPLC retention time (solvents a/b : 40/60, flow rate : 6.0 mL/min) : 11.5-12.0 min. ^{1}H NMR (CD_2Cl_2, 298.0 K) : δ : aromatic region : 2 peaks : 7.23 (s, 1H) ; 7.62 (s, 1H). MS : 445, 443, 441 [M + NH_4^+] ; 428, 426, 424 [M + H^+].

4d : HPLC retention time (solvents a/b : 40/60, flow rate : 6.0 mL/min) : 5.0-5.5 min. ^{1}H NMR (CD_2Cl_2, 298.0 K) : δ : aromatic region : 6.84 (d, J = 9.0 Hz, 1H) ; 7.42 (d, J = 9.0 Hz, 1H). MS : 445, 443, 441 [M + NH_4^+] ; 428, 426, 424 [M + H^+].

4e : HPLC retention time (solvents a/b : 40/60, flow rate : 6.0 mL/min) : 8.0-8.5 min. ^{1}H NMR (CD_2Cl_2, 298.0 K) : δ : aromatic region : 1 peak only : 7.73 (s, 1H). MS : 525, 523, 521, 519 [M + NH_4^+] ; 508, 506, 504, 502 [M + H^+].

4-Bromo-N-*Boc-metaraminol* (**4a**)

To a solution of 380 mg of *N*-Boc-metaraminol (**3**) (1.4 mmol, MW : 267.33) in 64 mL of absolute EtOH were added 144 mg of NaBr (1.4 mmol, 1 eq., MW : 102.90) and 187 mg of NCS (1.4 mmol, 1 eq., MW : 133.53) and the reaction mixture was stirred at room temperature for 1 hour. The mixture was evaporated to dryness, the residue was taken up in 30 mL of water and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated to dryness. The residue was purified by semipreparative HPLC (compare above: solvents a/b : 40/60, flow rate : 6.0 mL/min, 25 successive injections). The HPLC fractions were combined and worked up like described above: 317 mg (65% yield) of a 30/70 mixture of 4-bromo-*N*-Boc-metaraminol (**4a**) and 6-bromo-*N*-Boc-metaraminol (**4b**) were obtained in the form of a yellow oil. This mixture was used in the deprotection step without any further purification.

4a : HPLC retention time (solvents a/b : 40/60, flow rate : 6.0 mL/min) : 6.2-6.7 min. ^{1}H NMR (DMSO-d_6, 298.0 K) : δ : 0.86 (d, J = 6.0 Hz, 3H) ; 1.31 (s, 9H) ; 3.50 (b, $w_{1/2}$ = 20 Hz, 1H) ; 4.41 (bs, 1H) ; 5.32 (b, $w_{1/2}$ = 5 Hz, 1H) ; 6.56 (b, 1H) ; 6.65 (bd, J = 8.2 Hz, 1H) ; 6.90 (bd, J = 1.0 Hz, 1H) ; 7.34 (d, J = 8.2 Hz, 1H) ; 9.65 (b, ~1H) ; (DMSO-d_6, 310.0 K) : δ : 0.86 (d, J = 6.0 Hz, 3H) ; 1.32 (s, 9H) ; 3.50 (b, $w_{1/2}$ = 30 Hz, 1H) ; 4.41 (bd, $w_{1/2}$ = 10 Hz, 1H) ; 5.25 (bd, $w_{1/2}$ = 25 Hz, 1H) ; 6.50 (bd, J = 7.3 Hz, 1H) ; 6.67 (dd, J = 8.4 and < 1.0 Hz, 1H) ; 6.95 (d, J < 1.0 Hz, 1H) ; 7.36 (d, J = 8.1 Hz, 1H) ; 9.60 (b, ~1H) ; (DMSO-d_6, 318.0 K) : δ : 0.86 (d, J = 6.0 Hz, 3H) ; 1.32

(s, 9H) ; 3.50 (b, $w_{1/2}$ = 30 Hz, 1H) ; 4.41 (bd, $w_{1/2}$ = 10 Hz, 1H) ; 5.32 (b, $w_{1/2}$ = 25 Hz, 1H) ; 6.50 (b, $w_{1/2}$ = 20 Hz, 1H) ; 6.66 (dd, J = 8.4 and < 1.0 Hz, 1H) ; 6.95 (d, J < 1.0 Hz, 1H) ; 7.36 (d, J = 8.1 Hz, 1H) ; 9.65 (b, ~1H) ; (CD_2Cl_2, 310.0 K) : δ : 0.98 (d, J = 6.3 Hz, 3H) ; 1.35 (s, 9H) ; 3.95 (bm, $w_{1/2}$ = 25 Hz, 1H) ; 3.65-3.80 (b, 2H) ; 6.80 (dd, J = 7.5 and 1.0 Hz, 1H) ; 7.00 (d, J = 1.0 Hz, 1H) ; 7.43 (d, J = 7.8 Hz, 1H) ; MS : 365, 363 [M + NH_4^+] ; 348, 346 [M + H^+].

4b : HPLC retention time (solvents a/b : 40/60, flow rate : 6.0 mL/min) : 6.5-7.0 min. See above for ^{1}H NMR and MS characterizations.

6-Bromometaraminol (**2b**)

To 40 mg of 6-bromo-*N*-Boc-metaraminol (**4b**) (0.12 mmol, MW = 346.22) 2 mL of TFA were added and the solution was stirred at room temperature for 10 min. The reaction mixture was evaporated to dryness which afforded 28 mg (95% yield) of pure 6-bromometaraminol (**2b**) as a yellow oil.

HPLC retention time (solvents a/b : 90/10, flow rate : 6.0 mL/min) : 6.0-6.5 min ; (solvents c/d : 85/15, flow rate : 2.0 mL/min) : 7.0-7.5 min. ^{1}H NMR (CD_3OD, 293.0 K) : δ : 1.00 (d, J = 6.9 Hz, 3H) ; 3.25 (b, $w_{1/2}$ = 24 Hz, 1H) ; 4.92 (b, 1H) ; 6.62 (bd, J = 8.5 Hz, 1H) ; 7.04 (s, 1H) ; 7.29 (d, J = 8.6 Hz, 1H). MS : 265, 263 [M + NH_4^+] ; 248, 246 [M + H^+].

6-Bromometaraminol (**2b**) *via the trifluoroacetoxymercury route (analytical scale).*

170 mg of *N*-Boc-metaraminol (**3**) (0.64 mmol, MW : 267.33) were dissolved in 10 mL of MeOH and to this solution were added 137 mg of mercury (II) trifluoroacetate (0.32 mmol, 0.5 eq., MW : 426.60). The reaction mixture was stirred at room temperature for 2 hours. The residue obtained after removal of solvent was taken up in 15 mL of water and extracted with $CHCl_3$. The combined organic layers were washed with water and dried over Na_2SO_4. 156 mg of a white residue were obtained after evaporation of the solvent. Semipreparative HPLC purification (solvents a/b : 30/70, flow rate : 6.0 mL/min, 10 successive injections) afforded 42 mg of the trifluoroacetoxymercury derivative as white crystals. Bromination of an aliquot of this compound with NBS (1 eq.) and subsequent deprotection with TFA afforded 6-bromometaraminol (**2b**) as the only brominated product in low yield (less than 5%). No traces of 4-bromometaraminol (**2a**) could be found based on analytical HPLC analysis.

HPLC retention time (solvents a/b : 90/10, flow rate : 6.0 mL/min) : 6.0-6.5 min ; (solvents c/d : 85/15, flow rate : 2.0 mL/min) : 7.0-7.5 min. See above for ^{1}H NMR and MS characterizations.

4-Bromometaraminol (**2a**)

148 mg of the 30/70 mixture of 4-bromo-*N*-Boc-metaraminol (**4a**) and 6-bromo-*N*-Boc-metaraminol (**4b**) described above were dissolved in 2 mL of TFA and the resulting solution was stirred at room temperature for 10 min. The residue obtained after evaporation to dryness was resolved by semipreparative HPLC (solvents : a/b : 90/10, flow rate : 6.0 mL/min) in 10 successive injections. Each of the combined HPLC fractions (containing either 2a or 2b) was then separately evaporated to dryness, taken up in 15 mL of water, alkalized with 28% aqueous NH_4OH and

extracted with EtOAc (5 x 80 mL). The combined organic layers were dried over Na_2SO_4 and evaporated to dryness to give 68 mg (62% yield) of 6-bromometaraminol (**2b**) and 24 mg (23% yield) of 4-bromometaraminol (**2a**).

2a : HPLC retention time (solvents a/b : 90/10, flow rate : 6.0 mL/min) : 8.0-8.5 min ; (solvents c/d : 85/15, flow rate : 2.0 mL/min) : 18.5-19.0 min. ^{1}H NMR (CD_3OD, 298.0 K) : δ : 1.01 (d, J = 6.5 Hz, 3H) ; 3.15 (m, 1H) ; 4.53 (d, J = 4.5 Hz, 1H) ; 6.70 (d, J = 7.8 Hz, 1H) ; 6.92 (s, 1H) ; 7.40 (d, J = 8.2 Hz, 1H). MS : 265, 263 [M + NH_4^+] ; 248, 246 [M + H^+].

4-Iodometaraminol (**5a**)

100 mg of metaraminol (**1**) bitartrate (0.31 mmol, MW : 317.3) were dissolved in 17 mL of 10 M aqueous NH_4OH. To this solution were added dropwise 85 mg of I_2 (0.33 mmol, 1 eq., MW : 253.81) dissolved in 9 mL EtOH. The reaction mixture was stirred in the dark under nitrogen for 24 hours. The residue obtained after evaporation of solvent was taken up in 10 mL of brine and extracted with EtOAc (3 x 100 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by semipreparative HPLC (solvents a/b : 90/10, flow rate : 6.0 mL/min). The HPLC fractions were evaporated to dryness, taken up in 15 mL of water, alkalized with 28% aqueous NH_4OH and extracted with EtOAc (5 x 80 mL). The combined organic layers were dried over Na_2SO_4 and evaporated to dryness to give 27 mg (25% yield) of 4-iodometaraminol (**5a**).

HPLC retention time (solvents a/b : 90/10, flow rate : 6.0 mL/min) : 12.5-13.0 min ; (solvents c/d : 85/15, flow rate : 2.0 mL/min) : 21.0-21.5 min. ^{1}H NMR (CD_3OD, 295.0 K) : δ : 1.02 (d, J = 6.5 Hz, 3H) ; 3.12 (m,1H) ; 4.46 (d, J = 3.0 Hz, 1H) ; 6.56 (dd, J = 8.1 Hz and 1.7 Hz, 1H) ; 6.86 (d, J = 1.7 Hz, 1H) ; 7.62 (d, J = 8.1 Hz, 1H). MS : 311 [M + NH_4^+] ; 294 [M + H^+].

Radiochemistry

Preparation of [^{76}Br]NH_4Br

^{76}Br ($t_{1/2}$: 16.2 hours) was produced by irradiation of natural arsenic (1.8 g) with a 30 MeV ^{3}He beam. The target was kept for 15 hours before work up to allow for the decay of ^{75}Br ($t_{1/2}$: 1.6 hours). Then the target was dissolved in 40 mL of concentrated H_2SO_4 at 180°C. The solution was cooled (50°C) and treated with an aqueous solution of chromic acid (4.2 g in 12 mL water). The radioactive [^{76}Br]Br_2 was swept out with a nitrogen stream and trapped in 2 mL of 1.0 M aqueous NH_4OH in the form of [^{76}Br]NH_4Br. This solution was evaporated to dryness and the residue used in the next step.

4-[^{76}Br]Bromometaraminol ([^{76}Br]**2a**) *and 6-[^{76}Br]bromometaraminol* ([^{76}Br]**2b**)

[^{76}Br]NH_4Br (2.5 mCi or 92 MBq) was dissolved in 20 μL of water. To this solution were added 0.5 mg of *N*-Boc-metaraminol (**3**) (1.87 μmol, MW : 267.33) dissolved in 20 μL of MeOH, and 100 μL of a 1.6% (v/v) solution of peracetic acid in acetic acid. The reaction mixture was

allowed to stand at room temperature for 20 min. The mixture was then evaporated to dryness and 250 µL of TFA were added. The reaction was allowed to proceed for 5 min at room temperature. Afterwards the solution was evaporated to dryness, redissolved in HPLC solvent and purified by semipreparative HPLC (solvents a/b : 90/10, flow rate : 6.0 mL/min) to give 0.43 mCi (16 Mbq) of 4-[^{76}Br]bromometaraminol ([^{76}Br]**2a**) and 0.94 mCi (35 Mbq) of 6-[^{76}Br]bromometaraminol ([^{76}Br]**2b**) with 17% and 38% non decay-corrected radiochemical yield (based on starting [^{76}Br]NH_4Br). The specific radioactivity for both radiotracers was 130 mCi/µmol (5 GBq/µmol). Retention time: [^{76}Br]**2a** : 8.5-9.0 min, [^{76}Br]**2b** : 6.5-7.0 min, deprotected labelling precursor (metaraminol) : 2.0-2.5 min.

Formulation of both products for i.v. injection was effected as follows : (1) HPLC solvent removal by evaporation after addition of 20 µl of 1,2-propanediol; (2) taking up the residue in 0.9% saline; (3) filtration on a 0.22 µm Millipore filter.

The total synthesis time including the preparation of [^{76}Br]NH_4Br, semipreparative HPLC and final formulation was 3.5 hours.

Tissue distribution studies

All animals use procedures were in accordance with the recommendations of the EEC (86/609/CEE) and the French National Committee (decret 87/848) for the care and use of laboratory animals. Adult male Wistar rats of 200 g were injected with 5.4 µCi (0.2 MBq, 42 pmol) of 4-[^{76}Br]bromometaraminol ([^{76}Br]**2a**) or 10.8 µCi (0.4 MBq, 83 pmol) of 6-[^{76}Br]bromometaraminol ([^{76}Br]**2b**) in the tail vein. Four rats were sacrificed at 5, 15, 30, 60, 120 and 180 min after injection of the radiotracer. Parts of heart, lung, liver, kidney, muscle and blood were removed and weighed. Radioactivity of the samples was measured in a γ-counter (Packard Cobra Quantum) and tissue concentrations were expressed as percent of injected dose per gram of tissue (%ID/g).

Conclusion

The bromo analogues 4-and 6-bromometaraminol (**2a** and **2b**) were synthesized from commercially available metaraminol bitartrate. Structural assignments for both compounds were made by 2D-NMR experiments. An efficient radiosynthesis for 6-[^{76}Br]bromometaraminol and 4-[^{76}Br]bromometaraminol was found. 4- and 6-[^{76}Br]Bromometaraminol were prepared from *N*-Boc-metaraminol using [^{76}Br]NH_4Br and peracetic acid as the brominating agent. The total radiochemical yield based on starting [^{76}Br]NH_4Br was 17% and 38%, non decay-corrected, for 4-[^{76}Br]bromometaraminol and 6-[^{76}Br]bromometaraminol, respectively in a synthesis time of 3.5 hours including the preparation of [^{76}Br]NH_4Br and final semipreparative HPLC (specific

radioactivity : 130 mCi/μmol (4.8 GBq/μmol)). Tissue distribution in rats revealed a relatively low uptake and retention in the heart for both radiotracers, suggesting that neither 4- nor 6-[^{76}Br]bromometaraminol are suitable ligands to study the myocardial norepinephrine neuronal reuptake system with PET. However, further experiments are needed to determine the full pharmacological profile of both compounds and to explain the different *in vivo* behaviour of radiobrominated metaraminol in comparaison with the radiolabelled fluoro- and iodo-analogues.

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