Bromine-75-Labeled 1,4-Benzodiazepines: Potential Agents for the Mapping of Benzodiazepine Receptors In Vivo: Concise Communication

Hans Scholl, Gerd Kloster, and Gerhard Stöcklin

Institut für Chemie 1: Nuklearchemie der KFA Jülich GmbH, D-5170 Jülich, FRG

We have prepared four different 1,4-benzodiazepines, labeled at C-7 with the 1.6-hr positron emitter Br-75 or the 57-hr gamma emitter Br-77, as potential radiopharmaceuticals for the mapping of cerebral benzodiazepine receptor areas. The triazene method was used and optimized. Yields at the no-carrier-added level were 20%. 7-[^{75}Br]-5-(2-fluorophenyl)-1-methyl-1,3-dihydro-2H-1,4-benzodiazepine-2-one (Br-75 BFB) was isolated with a minimum specific activity of 20,000 Ci/mmole. Biodistribution in mice shows that BFB is taken up rapidly by the brain and is retained there at useful concentrations for significant periods of time. The maximum uptake is observed at 0.25 min. Brain-to-blood concentration ratios are larger than 2 during the interval (0.25 to 10 min) investigated.

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Due to their excellent anxiolytic, hypnotic, muscle-relaxant, and anticonvulsant properties, 1,4-benzodiazepines rank among the most widely used pharmaceuticals in the developed countries. During experiments to elucidate the mechanism of their action at the molecular level, specific binding sites for benzodiazepines—the so-called benzodiazepine receptors—were discovered in the brain (*1*). The present status of research on the benzodiazepine receptor has been reviewed recently (*2,3*). The anatomic sites and abundance of benzodiazepine receptors were determined in animal (*4,5*) and human (*6*) brains. Binding or inhibition constants have been determined for a large number of different 1,4-benzodiazepines (*4*) and a rank order for specific binding to the benzodiazepine receptor has been established. Most of these experiments, however, were performed in vitro; only a few in-vivo experiments have been carried out (*7–9*).

With one exception (*7*), only long-lived electron-emitting nuclides (H-3, C-14) have been used to label the 1,4-benzodiazepines. Since the radiation emitted by these nuclei cannot be detected by external measurements, at present few in-vivo data from humans are available (*7*).

In vivo data on the regional distribution and the behavior of benzodiazepines can be obtained by incorporating positron-emitting nuclides such as C-11, F-18, or Br-75 into benzodiazepines and recording their biodistribution in brain by positron emission tomography (PET). For in vivo mapping of benzodiazepine receptor areas, a ligand is needed with both high receptor affinity and high specific activity, in order to favor specific over nonspecific binding. Recently, synthesis and in vivo application of C-11 flunitrazepam have been reported (*7*). Since C-11 has a short half-life (20 min), the time available for its synthesis and application is rather limited. In addition, it is difficult to obtain very high specific activities using C-11. These difficulties can be avoided to some extent by using the halogen analog approach with Br-75 ($T_{1/2}$ = 98 min) as the tag. Compared with fluorine-18, the chemistry is much easier and the yields are higher. Bromine-75 has been produced in our laboratory via the $^{75}As(^{3}He, 3n)^{75}Br$ reaction in high specific activity and yields (*10,11*). A specific problem of Br-75 that has to be solved is the effect of the coincidence of the 286-keV gamma photon (91% abundance) with positron

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For reprints contact: Dr. Gerhard Stöcklin, Institut für Chemie 1, Nuklearchemie der KFA Jülich GmbH. D-5170 Jülich, FRG.

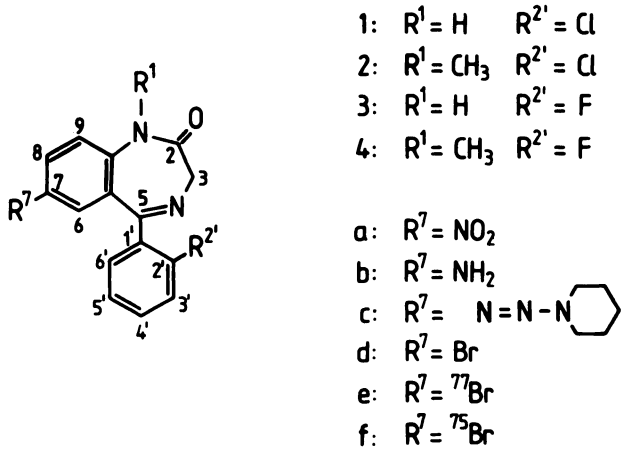

FIG. 1. Benzodiazepine derivatives investigated in this study (4d = BFB).

emission, leading to random coincidences in PET. Studies evaluating this effect are in progress.

Because receptor affinity is increased by halogens and other electron-withdrawing substituents at positions C-2′ and C-7, and it is decreased by halogen substitution at other aromatic positions (*5,12*), we chose benzodiazepines *1d–4d* (*4d* = BFB, Fig. 1) for radiobromination using the triazene method (*13,14*).

MATERIALS AND METHODS

Analytical methods. Analytical data were determined by mass spectrometry, proton nuclear magnetic resonance (NMR), and ir (from KBr) and uv (from methanol) spectroscopy. Melting points are uncorrected.

Synthesis of unlabeled benzodiazepines (see Fig. 2). Piperidyltriazenes *1c–4c* as well as 7-bromo-benzodiazepines *1d–4d* were prepared from the corresponding 7-amino derivatives *1b–4b*.

1b–4b were prepared by reduction of the commercial 7-nitro compounds clonazepam *1a*, methylclonazepam *2a*, nor-flunitrazepam *3a*, or flunitrazepam *4a* according to published procedures (*15*).

7-Amino-5-(2-chlorophenyl)-1,3-dihydro-2H-1,4-benzodiazepine-2-one (*1b*): MS: m/z 287/5 $[M]^+$; NMR (ppm): 4.35 (s, 2H); 6.43 (d, 2.5 Hz, 1H); 7.05 (dd, 2.5/9 Hz, 1H); 7.43 (d, 9 Hz, 1H); 7.68 (m, 4H); ir (cm^{-1}): 3435/3340/3220 (—NH st); uv (nm, log ε): 240 (4.39), 270 sh, 360 (3.30); mp 223 °C.

7-Amino-5-(2-chlorophenyl)-1,3-dihydro-1-methyl-2H-1,4-benzodiazepine-2-one (*2b*): MS: m/z 301/299 $[M]^+$; NMR: 3.32 (s, 3H); 3.90 (d, 11 Hz, 1H); 4.62 (d, 11 Hz, 1H); 6.42 (d, 2.5 Hz, 1H); 7.05 (dd, 2.5/9 Hz, 1H); 7.40 (d, 9 Hz, 1H); 7.55–7.8 (m, 4H); ir: 3405/3310/3190 (—NH, st); uv: 240 (4.39), 264 sh, 355 (3.30); mp 231–2°C.

7-Amino-1,3-dihydro-5-(2-fluorophenyl)-2H-1,4-benzodiazepine-2-one (*3b*): MS: m/z 269 $[M]^+$; NMR: 4.25 (s, 2H); 6.49 (d, 2.5 Hz, 1H); 6.8–7.9 (m, 6H); ir: 3455/3305/3195 (—NH, st); uv: 242 (4.50), 266 sh, 360 (3.30); mp 255–6°C.

7-Amino-1,3-dihydro-5-(2-fluorophenyl)-1-methyl-2H-1,4-benzodiazepine-2-one (*4b*): MS: m/z 283 $[M]^+$; NMR: 3.38 (s, 3H); 3.97 (d, 10 Hz, 1H); 4.83 (d, 10 Hz, 1H); 6.66 (d, 2.5 Hz, 1H); 7.19 (dd, 2.5/9 Hz, 1H); 7.3–8.0 (m, 5H); ir: 3420/3320/3210 (—NH, st); uv: 242 (4.40), 272 sh, 355 (3.34); mp 206°C.

Piperidyltriazenes *1c–4c*: Four hundred eighty mg (7 mmole) $NaNO_2$ in 5 ml H_2O were added dropwise to a suspension of 6.4 mmole of the 7-amino derivatives *1b–4b* in 13 ml 6 *N* HCl, keeping the mixture at 0°C. This mixture was poured into an ice-cold solution of 0.7 ml (7 mmole) piperidine and 3.5 g KOH in 30 ml H_2O to form a yellow precipitate. The solution was adjusted to pH 12 with ammonia and extracted exhaustively with CH_2Cl_2/ethyl acetate (2:1). After evaporation and separation by HPLC (LiChrosorb Si 60, 10 μm, with hexane/isopropanol 10:1 as eluent), pure piperidyltriazenes *1c–4c* were isolated.

5-(2-Chlorophenyl)-1,3-dihydro-7-piperidinylazo-2H-1,4-benzodiazepine-2-one (*1c*): MS: m/z (rel. int.); 381/3 (25/9) $[M]^+$; 297/9 (14/5) $[M-C_5H_{10}N]^+$; 269/71 (42/16) $[M-C_5H_{10}N-28]^+$; 241/3 (51/18) $[M-C_5H_{10}N-28-28]^+$; 206(60) $[M-C_5H_{10}N-28-28-Cl]^+$; 84 (100) $[C_5H_{10}N]^+$; NMR: 1.74 (m, 6H); 3.85 (m, 4H); 4.43 (s, 2H); 7.25 (d, 2.5 Hz, 1H); 7.4–7.95 (m, 6H); UV: 226 (4.57); 258 sh, 309 (4.47); mp 239–40°C (decomp.).

5-(2-Chlorophenyl)-1,3-dihydro-1-methyl-7-piperidinylazo-2H-1,4-benzodiazepine-2-one (*2c*): MS: 395/7 (44/15) $[M]^+$; 311/3 (30/10) $[M-C_5H_{10}N]^+$; 283/5 (46/18) $[M-C_5H_{10}N-28]^+$; 255/7 (100/36) $[M-C_5H_{10}N-28-28]^+$; 219(51) $[M-C_5H_{10}N-28-28-HCl]^+$; 205(42) $[M-C_5H_{10}N-28-28-CH_3-Cl]^+$; 84(46) $[C_5H_{10}N]^+$; NMR: 1.74 (m, 6H); 3.45 (s, 3H); 3.85 (m, 4H); 4.01 (d, 11 Hz, 1H); 4.80 (d, 11 Hz, 1H); 7.23 (d, 2.5 Hz, 1H); 7.5–8.0 (m, 6H); UV: 227 (4.40), 260 sh, 310 (4.43); mp 136–7°C.

1,3-Dihydro-5-(2-fluorophenyl)-7-piperidinylazo-2H-1,4-benzodiazepine-2-one (*3c*): MS: 365(25) $[M]^+$; 218(19) $[M-C_5H_{10}N]^+$; 253(45); 225(97); 84(100) $[C_5H_{10}N]^+$; NMR: 1.68 (m, 6H); 3.75 (m, 4H); 4.32 (s, 2H); 7.0–7.9 (m, 7H); UV: 231

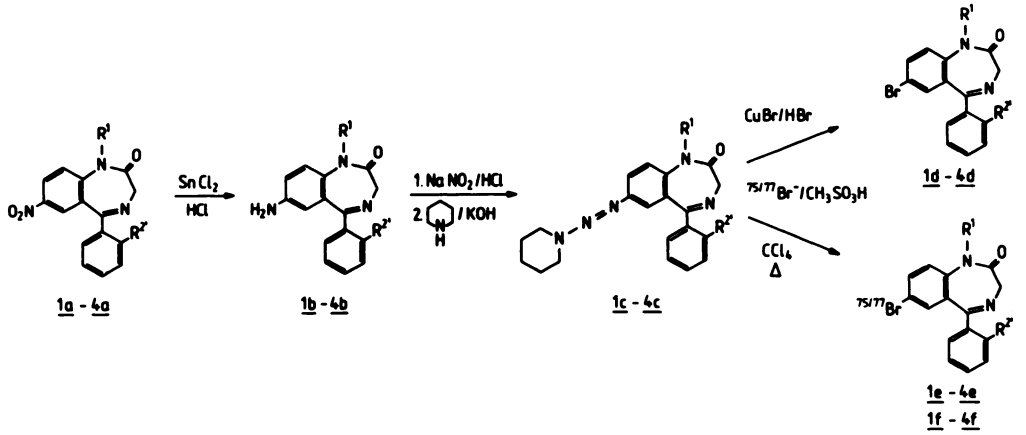

FIG. 2. Synthetic route to radiobrominated benzodiazepines via triazene precursors.

TABLE 1. INHIBITION (K_i) OF H-3 FLUNITRAZEPAM BINDING BY *1d–4d* (Fig. 1)

Compound	*1d*	*2d*	*3d*	*4d*
K_i (*nM*)	1.3	2.3	1.5	1.7

(4.35), 262 sh, 313 (4.33); mp 232–3 °C (decomp.).

1,3-Dihydro-5-(2-fluorophenyl)-1-methyl-7-piperidinylazo-2H-1,4-benzodiazepine-2-one (*4c*): MS: 379(29) $[M]^+$; 295(26) $[M-C_5H_{10}N]^+$; 267(36); 239(100); 84(31) $[C_5H_{10}N]^+$; NMR: 1.75 (m, 6H); 3.50 (s, 3H); 3.81 (m, 4H); 4.0 (d, 10 Hz, 1H); 4.82 (d, 10 Hz, 1H); 7.2–8.1 (m, 7H); UV: 228 (4.36), 257 sh, 308 (4.36); mp 178°C.

7-Bromobenzodiazepines *1d–4d*: One-half mmole of the corresponding piperidyltriazene *1c–4c* was added to a solution of 0.55 mmole copper bromide catalyst (prepared according to Ref. *16*) in 2 ml of 4.5 *N* HBr. This mixture was stirred for 10 min, then adjusted to pH 12 with ammonia and exhaustively extracted with CH_2Cl_2/ethyl acetate 2:1. After evaporation of the solvents and separation by HPLC (LiChrosorb RP-18, 7 μm, with methanol/water 3:2 as eluent), pure 7-bromobenzodiazepines *1d–4d* were isolated.

7-Bromo-5-(2-chlorophenyl)-1,3-dihydro-2H-1,4-benzodiazepine-2-one (*1d*): MS: 348/50/52 $[M]^+$; NMR: 4.45 (s, 2H); 7.40 (d, 2.5 Hz, 1H); 7.5–8.1 (m, 6H); UV: 230 (4.56), 255 sh, 320 (3.30); mp 218°C.

7-Bromo-5-(2-chlorophenyl)-1,3-dihydro-1-methyl-2H-1,4-benzodiazepine-2-one (*2d*): MS: 362/4/6 $[M]^+$; NMR: 3.55 (s, 3H); 4.05 (d, 10 Hz, 1H); 4.82 (d, 10 Hz, 1H); 7.45 (d, 2.5 Hz, 1H); 7.6–7.8 (m, 5H); 8.0 (dd, 2.5/9 Hz, 1H); UV: 232 (4.49); 256 sh, 318 (3.17); mp 136–7°C.

7-Bromo-1,3-dihydro-5-(2-fluorophenyl)-2H-1,4-benzodiazepine-2-one (*3d*): MS: 332/4 $[M]^+$; NMR: 4.18 (s, 2H); 6.8–7.9 (m, 7H); UV: 233 (4.50); 255 sh, 318 (3.25); mp 182–3°C.

7-Bromo-1,3-dihydro-5-(2-fluorophenyl)-1-methyl-2H-1,4-benzodiazepine-2-one (*4d*): MS: 346/8 $[M]^+$; NMR: 3.40 (s, 3H); 3.90 (d, 11 Hz, 1H); 4.84 (d, 11 Hz, 1H); 7.2–8.0 ppm (m, 7H); UV: 230 (4.48), 251 sh, 315 (3.30); mp 68°C.

Receptor affinity of 7-bromobenzodiazepines *1d–4d*: The affinity of *1d–4d* for the benzodiazepine receptor was determined by their competitive displacement of tritiated flunitrazepam from rat brain synaptic membranes, according to the method of Möhler and Okada (*4*). The data obtained are shown in Table 1.

Radiobromination. Bromine-75 and Br-77 were produced at the Jülich compact cyclotron CV 28 using the $^{75}As(^3He,3n)^{75}Br$ or the $^{75}As(\alpha,2n)^{77}Br$ reaction (10,11). It was isolated as no-carrier-added (n.c.a.) $^{75,77}Br$-bromide ion in aqueous solution.

For convenience, most of the experiments were performed using the longer-lived gamma emitter Br-77 ($T_{1/2}$ = 57 hr). The aqueous solution of the n.c.a. $^{77}Br^-$ and 7 μmole of the corresponding piperidyltriazenes *1c–4c* were mixed in a glass vial containing 1 ml acetonitrile. After removal of the solvent under vacuum, an additional 1 ml portion of acetonitrile was added and removed under vacuum. The vial was then closed under an argon atmosphere. The residue was dissolved in 200 μl dry CCl_4, then 21 μmole CH_3SO_3H was added. The emulsion was sonicated for 1 min and refluxed for 10 min. After evaporation of the solvent, the dry residue was dissolved in 200 μl CH_3OH/H_2O (3:2) and purified by HPLC (LiChrosorb RP-18, 7 μm, 25 × 1 cm, with CH_3OH/H_2O 3:2 as eluent). By this HPLC system, the three main side products of the reaction *4c* → *4e* could be quantitatively separated from *4e* (k′ = 6.9). They were characterized by MS as 1,3-dihydro-5-(2-fluorophenyl)-1-methyl-2H-1,4-benzodiazepine-2-one (m/z 268 $[M]^+$; k′ = 3.2), 7-chloro-1,3-dihydro-5-(2-fluorophenyl)-1-methyl-2H-1,4-benzodiazepine-2-one (m/z (rel. int.) 302/4 (81/28) $[M]^+$; k′ = 6.5), and the dimer 1,3-dihydro-7-[1,3-dihydro-5-(2-fluorophenyl)-1-methyl-2-H-1,4-benzodiazepine-2-onyl-7]-5-(2-fluorophenyl)-1-methyl-2H-1,4-benzodiazepine-2-one (m/z 534 $[M]^+$; k′ = 7.5). The products *1e–4e* were characterized by co-chromatography with authentic standards *1d–4d* (for conditions see Table 2).

The same reaction procedure was performed using $^{75}Br^-$ as starting material. In a heavily shielded apparatus, up to 14 mCi of *4f* have been produced at a specific activity ≥20 K Ci/mmole (^{77}Br: ≥17 K Ci/mmole) in a single batch for clinical studies. The specific activities were estimated from the absence of a carrier-UV peak in the chromatograms by determination of the detection limit of the system via calibration. The synthesis time required was about 1 hr.

The influence of experimental parameters on reaction yields was optimized using the reaction *4c–4e* as a model. The effect of carrier was determined from experiments with 8 μCi n.c.a. $^{77}Br^-$ and subsequent addition of various amounts of inactive KBr (see Results and Discussion). No difference was observed between yields of n.c.a. Br-75 and n.c.a. Br-77.

Animal experiments. Br-77 BFB (*4e*) was dissolved in saline to yield a radioactivity concentration of 20 μCi per ml. After 0.22-μ filtration, 100 μl of this solution were injected by tail vein into female NMRI mice. The mice were killed by cervical dislocation at various times after i.v. injection. The organs were removed, blotted dry, weighed, and counted in a NaI(Tl) well scintillation counter. Organ radioactivity was expressed as % mean body concentration 100 times cpm/g organ over cpm/g body weight in order to eliminate experimental variables such as body weight and to facilitate interspecies comparisons (*17*).

RESULTS AND DISCUSSION

Synthesis. Only a limited number of 1.4-benzodiazepines have a high degree of specific receptor-binding affinity as well as relatively low nonspecific binding. A high affinity for the benzodiazepine receptor is always observed with derivatives that have useful pharmacological effects at low doses (*15*). Prominent among these

TABLE 2. CHROMATOGRAPHIC SEPARATION OF *1d–4d*

	Compound	*1d*	*2d*	*3d*	*4d*
K′	HPLC LiChrosorb RP-18, 7 μM methanol/H_2O (3:2):	7.6	9.2	5.8	6.9
	CH_2Cl_2/acetone (2:1):	0.54	0.67	0.53	0.68
R_f TLC Si60	hexane/isopropanol (3:1):	0.56	0.48	0.57	0.54
	diethyl ether	0.45	0.58	0.42	0.65

TABLE 3. INFLUENCE OF SUBSTITUTION ON PHARMACOLOGICAL ACTIVITY

Substitution (Fig. 1)	Pharmacological activity
R^1	$CH_3 \geq H$
R^7	$NO_2 > Br > Cl > F$
$R^{2'}$	$Cl > F > Br$

is the 1.4-benzodiazepine-2-one group, all of which have strongly electron-withdrawing substituents (such as $-NO_2$ or halogen) at C-7, halogen at C-2', and either —H or $-CH_3$ at N-1 (*18*). Thus, these compounds can be labeled with positron emitters, either by halogenation with F-18 or Br-75 at C-7 or C-2', or by methylation with $^{11}CH_3I$ at N-1 (*7*). From the data shown in Table 3 (taken from Ref. *12*) we concluded that it would be suitable to introduce bromine-75 at C-7.

As expected, the 7-bromo-benzodiazepines *1d–4d* do indeed show high receptor affinity (see Table 1). Their receptor affinities are slightly higher than that of the potent drug flunitrazepam. Thus, the first requirement, namely, biological specificity, is met.

Synthesis of the radioactive analogs *1e–4e* requires halogenation methods that allow regioselective, if not regiospecific, introduction of bromine into the aromatic ring, since bromination at C-6, C-8, or C-9 leads to a complete loss of pharmacological activity (*18*). Thus, electrophilic brominations, using N-chlorotetrafluorosuccinimide (*19*) or chloramine-T (20) as oxidants for bromide ions, do not seem to be suitable. A useful alternative is the triazene decomposition, an approach originally proposed by Wallach (*13*) for the synthesis of aromatic fluorine derivatives. Recently this approach was applied to the radioiodination of benzodiazepines using I-123 (*21*). From the stable triazenes, diazonium salts can be generated in situ using nonnucleophilic acids such as methanesulfonic or *p*-toluene-sulfonic acid. These diazonium salts can then be regiospecifically substituted by adding nucleophiles such as halides. The ratio between substitution and other unwanted side reactions typical for diazonium salt decomposition (*22*) depends on reaction conditions, especially on the solvent used. In model experiments using the piperidyltriazene of 2-aminobenzoic acid, we found, in accordance with (*23*), that the combination of CCl_4 and CH_3SO_3H led to a high degree of substitution with fewer side reactions

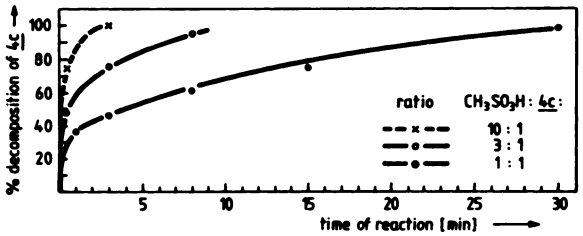

FIG. 3. Influence of CH_3SO_3H concentration on rate of decomposition of *4c*.

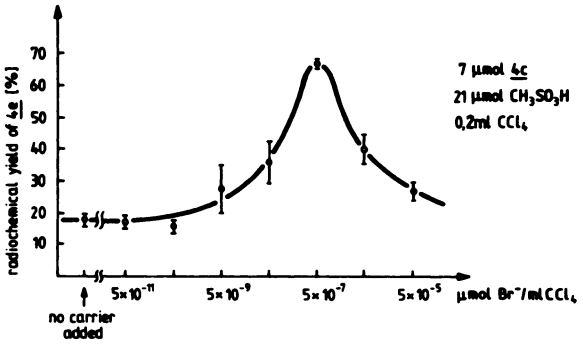

FIG. 4. Effect of bromine carrier on radiochemical yield of decomposition of *4c* to *4e* (mean ± s.d. of six individual experiments).

such as protodiazonization. Accordingly this combination was used for the sequence *1c–4c* → *1d–4d*.

As shown in Fig. 3, the rate of decomposition of the triazene *4c* depends on the ratio of CH_3SO_3H to *4c*. Equimolar amounts of acid to *4c* lead to complete decomposition after 30 min, whereas this time is reduced to 10 min for an acid-to-triazene ratio of three, and is reduced further to 1 min if the ratio is ten. Since CH_3SO_3H is insoluble and forms an emulsion in CCl_4, the optimum reaction conditions were found at a molar ratio of three for CH_3SO_3H to *4c*. Under these conditions side reactions were minimal.

Going from carrier-added to n.c.a. conditions leads to a reduction in yields, as shown in Fig. 4. At n.c.a. conditions the yields are about 20% for all four triazenes investigated (see Table 4). At higher carrier concentrations, the yields decrease again, because the solubility of the bromide is exceeded.

Summarizing the chemical aspects, by using a simple experimental procedure we were able to prepare large amounts of 7-bromo-1.4-benzodiazepines, labeled with either Br-77 (*1e–4e*) or Br-75 (*4f*), from stable precursor

TABLE 4. TRIAZENE DECOMPOSITION OF *1c–4c* TO *1e–4e*; RADIOCHEMICAL YIELDS ( ) = NUMPER OF EXPERIMENTAL POINTS

Compound	*1e* (6)	*2e* (6)	*3e* (8)	*4e* (12)
Radiochemical yield %	24.0 ± 2.1	23.8 ± 2.7	25.3 ± 0.5	17.9 ± 2

TABLE 5. ACCUMULATION OF Br-77 BFB (*4e*) IN MICE; ( ) = NUMBER OF EXPERIMENTAL POINTS; MEAN AND RANGE

min	0.25 (6)	0.5 (4)	1 (6)	3 (4)	5 (6)	10 (4)
Organ						
Brain	188(146–251)	161(141–188)	144(126–167)	117(113–127)	119(104–130)	86(76–97)
Intestine	82(38–119)	88(59–124)	90(55–118)	102(97–116)	124(101–177)	127(120–133)
Pancreas	154(69–216)	164(108–214)	166(88–213)	157(115–172)	162(141–177)	132(112–153)
Adipose tissue	15(7–31)	25(11–53)	30(21–67)	56(45–100)	72(56–96)	104(88–140)
Muscle	91(14–115)	73(44–91)	79(62–85)	68(66–74)	77(73–83)	70(61–78)
Liver	82(37–132)	112(72–253)	137(70–216)	234(207–265)	292(245–339)	310(296–335)
Blood	52(34–61)	45(39–59)	43(39–49)	41(39–44)	46(41–53)	46(42–48)
Kidney	499(238–693)	560(461–743)	512(373–590)	386(373–426)	336(320–348)	257(238–273)
Heart	317(217–445)	275(199–317)	189(87–238)	162(144–177)	163(149–171)	147(139–156)
Lung	550(448–760)	468(314–538)	400(314–510)	284(273–307)	292(260–342)	177(143–208)
Thyroid	151(112–204)	135(97–145)	115(97–138)	129(106–152)	126(108–143)	102(82–128)
Spleen	52(25–96)	65(31–138)	60(27–200)	139(122–148)	157(130–183)	133(124–142)
Uterus	49(23–83)	75(38–85)	60(40–100)	77(50–120)	99(78–111)	123(90–141)

Values in %MBC (100 times cpm/g organ divided by cpm/g body weight).

triazenes (*1c–4c*). Reaction times (overall 55 min) are convenient for the efficient introduction of Br-75.

Biodistribution in animals. The tissue distribution data obtained after i.v. injection of Br-77 BFB (*4e*) into female NMRI mice are shown in Table 5. Immediately after administration, maximum brain uptake is observed at 188% of mean body concentration (see Fig. 5). It then decreases with time to about 100% MBC at 10 min after injection. During this period, the blood radioactivity is nearly constant at the low level of 45% MBC. Thus, the brain-to-blood concentration ratio exceeds 2 at all time intervals investigated. Like other benzodiazepines (*2,24–27*), Br-77 BFB is rapidly taken up in the lung (max. 550% MBC), kidney (max. 560% MBC), and heart (max. 317% MBC). The course of each time-activity curve for these organs parallels that of the activity in the brain. In the liver, by contrast, the activity concentration rises steadily with time (from 82% MBC at 0.25 min to 310% MBC at 10 min), also in the intestine (from 82% MBC at 0.25 min to 127% MBC at 10 min) and adipose tissue (from 15% MBC at 0.25 min to 104% MBC at 10 min). This effect may be explained by biliary excretion (*24*) and enterohepatic circulation between liver and intestine, and the lipid solubility (*24*) of benzodiazepines may be responsible for their accumulation in adipose tissue.

1,4-Benzodiazepines are known (*25–27*) to be extensively metabolized to a number of pharmacologically active metabolites that usually have elimination half-times significantly longer than that of the parent drug. We expect that Br-75 BFB (*4f*) will be metabolized in a manner similar to flunitrazepam—namely by preferential N-demethylation (*25,26*)—leading to the active metabolite *3f* (see Table 4). In contrast to C-11 flunitrazepam (*7*), which on demethylation loses its C-11 label, Br-75 BFB still retains the radioactivity. Thus, all expected pharmacologically active metabolites will also be radioactively labeled in our case, whereas in the case of C-11 flunitrazepam (*7*) the major metabolite, norflunitrazepam, no longer contains radioactivity. For C-11 flunitrazepam, one therefore has to expect competition for specific receptor binding by unlabeled norflunitrazepam, whereas in the case of Br-75 BFB the competitor for specific receptor binding, *3f*, will not reduce the amount of radioactivity specifically bound. Further studies are needed to prove whether *3f* is indeed the main metabolite of *4f*.

Summarizing the biodistribution data, we have shown that Br-77 BFB is rapidly taken up by the brain and retained there at useful concentrations for significant periods of time; the brain-to-blood concentration ratio is larger than 2 at all times investigated.

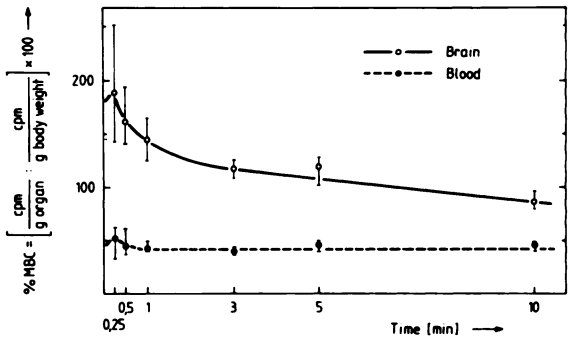

FIG. 5. Time course of radioactivity in brain and blood of mice after i.v. injection of BFB.

CONCLUSION

Since the physiological meaning of the benzodiazepine receptor in mammalian brain is not entirely clear, we feel that more in vivo data are needed to monitor their changes in different diseased states. Besides C-11 flunitrazepam (*7*), the Br-75-labeled benzodiazepine Br-75 BFB is the only other compound reported that allows dynamic three-dimensional in vivo mapping of brain benzodiazepine receptor areas in man with positron emission tomography. Compared with C-11 flunitrazepam, Br-75 BFB has the following advantages: (a) The label is in a metabolically stable position; (b) the half-life of Br-75 (98 min) is 5 times that of C-11, thus leaving significantly longer times for synthesis and imaging; and (c) multimillicurie amounts of Br-75 BFB can be prepared reproducibly at the n.c.a. level. Thus, Br-75 BFB should be an excellent radioagent for the in vivo mapping of benzodiazepine receptor areas if data from mice can be extrapolated to man.

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