A PRACTICAL SYNTHESIS OF ISOTOPICALLY LABELLED 1-(4-ISOTHIOCYANATOPHENYL)-4-(t-BUTYL)-2,6,7-TRIOXABICYCLO-[2.2.2]OCTANE, A PROBE FOR THE BENZODIAZEPINE RECEPTOR-COUPLED CHLORIDE IONOPHORE

Brian R. de Costa, Anita Lewin[a], Joyce A. Schoenheimer[b], Phil Skolnick[b], and Kenner C. Rice*

Laboratory of Medicinal Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892, U.S.A. [a]Present address: Research Triangle Institute, P.O. Box 12194, Research Triangle Park, North Carolina 27709, U.S.A.. [b]Laboratory of Neuroscience, National Institute of Digestive, Diabetes and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892, U.S.A.

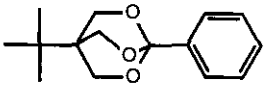
Abstract-An efficient synthesis of high specific activity [^{3}H]1-(4-isothiocyanato-3,5-ditritiophenyl)-4-(t-butyl)-2,6,7-trioxa-bicyclo[2.2.2]octane ([^{3}H]-1), an affinity ligand for the benzodiazepine (BZ)-coupled γ-aminobutyric acid (GABA)-gated chloride channel, was achieved starting with methyl p-aminobenzoate and 3-(t-butyl)-3-oxetanemethanol. A key step in the reaction sequence utilized the azide group as a latent aromatic amine allowing synthesis of 1-(4-amino-3,5-ditritiophenyl)-4-(t-butyl)-2,6,7-trioxabicyclo[2.2.2]octane ([^{3}H]-18) via boron trifluoride etherate catalysed isomerization of 3-(t-butyl)-3-(3,5-dibromo-4-azidobenzoyloxymethyl)-oxetane (15) to 1-(3,5-dibromo-4-azidophenyl)-4-(t-butyl)-2,6,7-trioxabicyclo[2.2.2]octane (17) Model experiments performed in an attempt to use unprotected or trifluoroacetamide protected aromatic amines in this sequence of reactions were unsuccessful.

The benzodiazepine (BZ)/γ-aminobutyric acid (GABA) receptor chloride ionophore complex ("supramolecular complex") mediates the neuropharmacological actions of structurally diverse classes of compounds such as the benzodiazepines, barbiturates, and β-carbolines.[1] During the past decade, both photo- and electrophilic affinity ligands have been effectively employed to characterize the structure and function of BZ receptors and a subpopulation of $GABA_A$ receptors that are constituents

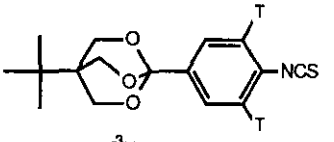
of this supramolecular complex.[2] Both electrophysiological[3] and neurochemical[4] evidence indicates that "cage" convulsants such as picrotoxin (PTX), t-butylbicycloorthobenzoate (TBOB) (2),[4] and t-butylbicyclophosphorothionate (TBPS, 3) act at sites on or near the GABA-gated chloride channel (ionophore). These sites are distinct from, but allosterically coupled to, benzodiazepine and GABA receptors.[5]

Recently, we described the synthesis and neurochemical properties of 1-(4-isothiocyanatophenyl)-4-(t-butyl)-2,6,7-trioxabicyclo[2.2.2]octane (1) the p-isothiocyanate derivative of 2[6] This ligand potently inhibited [^{35}S]-TBPS binding to rat cortical membranes with characteristics indicative of an irreversible ligand while the corresponding ortho and meta isomers were much less effective.[6] Compound (1) is the first described site-directed irreversible ligand for [^{35}S]-TBPS binding sites. In order to gain further insight into the structure and function of the proteins which constitute the supramolecular complex, we wished to develop an efficient synthesis of high specific activity [^{3}H]-1. We report here a practical synthesis of high specific activity [^{3}H]-1.

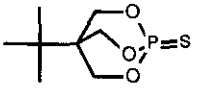
We selected the azide group as a latent aromatic amine in the synthesis of [^{3}H]-1 (Scheme 1), since this group has been widely used in the introduction of and protection of amino groups in carbohydrate chemistry. Initial model experiments were performed (Scheme 2) to investigate whether use of either unprotected amines (20) and (21) or trifluoroacetamide protected amine (25) would lead to the corresponding amino-2,6,7-trioxa[2.2.2]bicyclooctanes. These experiments indicated that in neither case could $BF_3 \cdot OEt_2$ catalysed rearrangement to the desired trioxabicyclo[2.2.2]octanes be achieved. In contrast, the azido group allowed efficient formation of the precursors (16) and (17) and provided for regeneration of the amine function of 18 or [^{3}H]-18.



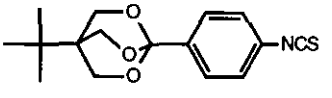
2 (TBOB)



[^{3}H]-1



3(TBPS)



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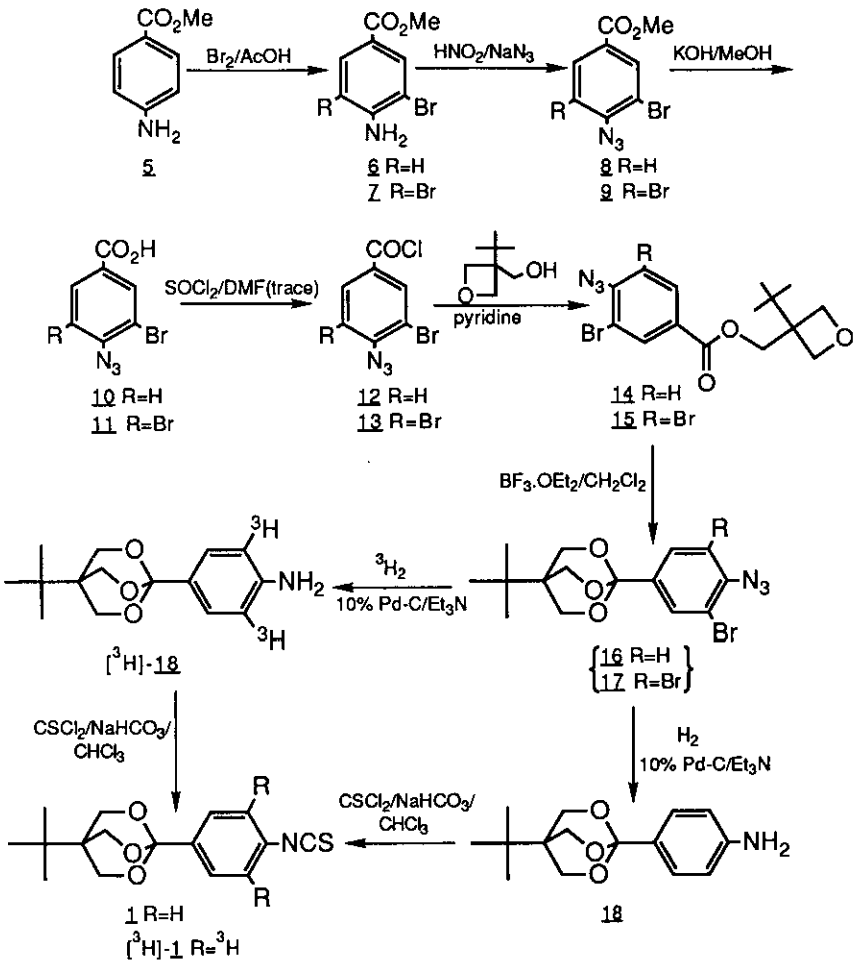
Thus (Scheme 1), bromination of methyl p-aminobenzoate (5) with either one or two molar equivalents of bromine gave the corresponding mono- and dibrominated esters (6,[7] 90% yield) and (7,[8] 99% yield). Compounds (6 and 7) served as precursors for the synthesis of either mono- or ditritiated 1, respectively (Scheme 1).

Scheme 1



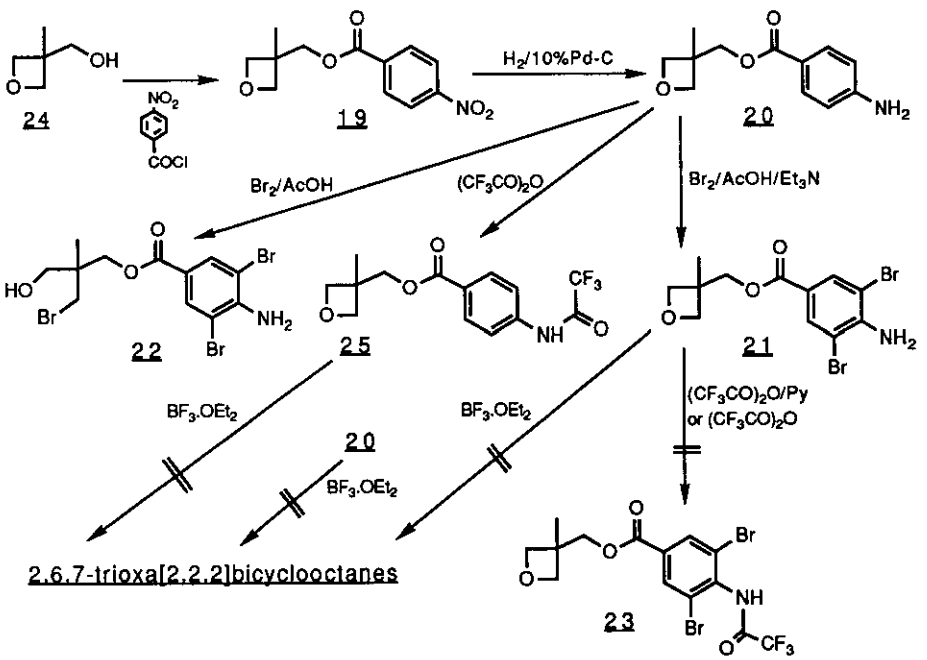
Protection of the amino group of 6 and 7 as an azido group (8 and 9) via diazotization, quenching with excess aqueous NaN_3 and finally hydrolysis with 10% KOH/MeOH during 1 h, afforded benzoic acids (10) and (11) in high yield. Coupling of their corresponding acid chlorides (12) and (13) with 3-(t-butyl)-3-oxetanemethanol (derived from 3,3-dimethylbutanol[4]) in the presence of pyridine afforded in both cases aryl azide esters (14) and (15) in 95-96% yield; these ester intermediates exhibited characteristic peaks in the ir spectrum: 2120 (N_3 str.) and 1720 (CO str.).

The ^{1}H-nmr spectrum displayed an AB quartet at 4.58 and 4.63 ppm (J_{gem}=6.4 Hz) as a consequence of the oxetane ring geminal protons. Isomerization of oxetane esters (14) and (15) to the isomeric trioxabicyclo[2.2.2]octanes (16) and (17) occurred smoothly after overnight treatment with an excess of BF_3·OEt_2 in dry CH_2Cl_2.[4,9] Attempted catalytic hydrogenation of 16 and 17 with 10% Pd/C, PtO_2, 5% Pd/$BaSO_4$, 10% Pd/C-K_2CO_3 in either EtOH or EtOAc failed to give amine (18). A mixture of products (including products resulting from benzylic cleavage of the 2,6,7-trioxabicyclo[2.2.2]octane ring system) were instead formed. However, an alternative approach using 10% Pd/C and neat triethylamine as solvent furnished pure 18 in quantitative yield.[10] The ^{1}H-nmr spectrum exhibited a broad D_2O exchangeable resonance at 3.69 ppm characteristic of the aromatic amine. An intense singlet at 4.16 (6 H) was confirmative of an intact 2,6,7-trioxabicyclo[2.2.2]octane system. Since we desired to maximize the specific activity of [^{3}H]-1, the dibrominated azide (17) was selected as the tritiation precursor. Thus, catalytic tritiolysis of 17 (0.045 mmol) over 10% Pd/C using the same conditions as for unlabelled 17 except for substituting hydrogen for carrier-free tritium gas (10 Ci, 0.172 mmol) afforded [^{3}H]-18 (407.7 mCi, specific activity 25.5 Ci/mmol, 15.7% radiochemical yield). The azido group proved instrumental in this reaction sequence in that firstly it protected the amino group to allow BF_3·OEt_2 catalyzed rearrangement of 14 and 15 to 16 and 17, respectively, and secondly it allowed simultaneous introduction of tritium and regeneration of amine ([^{3}H]-18). The target compound ([^{3}H]-1) was obtained in 53.5% yield by treatment of [^{3}H]-1 with thiophosgene in a two-phase aqueous $NaHCO_3$/$CHCl_3$ system. Tritium labelled 1 was identical chromatographically to an authentic sample of unlabelled 1 generated by treatment of unlabelled 18 with thiophosgene in the presence of $NaHCO_3$. All synthetic manipulations involving use of tritium labelled compounds were first performed with unlabelled compounds and the products identified spectroscopically.

In model experiments designed to test the feasibility of using either unprotected aromatic amines, or the easily deprotected trifluoroacetamides as alternatives to the azido masking group, the sequence of reactions depicted in Scheme 2 was followed. Thus, starting with commercially available 3-methyloxetanemethanol (24), coupling with 4-nitrobenzoyl chloride in the presence of Et_3N afforded the nitro ester (19) in quantitative yield. Catalytic hydrogenation of 19 in MeOH afforded amine (20) in 88% yield. Longer hydrogenation times resulted in considerably lowered yields as a result of hydrogenolysis of the oxetane ring.

Bromination of 20 in acetic acid with 2 eq. of Br_2 gave the tribromide (22) in 84% yield instead of the expected 21. However, bromination of 20 in acetic acid in the presence of triethylamine gave the desired 21 in 78% yield. Attempted protection of 21 by reaction with $(CF_3CO)_2O$ in pyridine or boiling under reflux with $(CF_3CO)_2O$ was unsuccessful; however, non-brominated aniline (20) proved sufficiently reactive to form the corresponding N-trifluoroacetamide (25) on treatment with $(CF_3CO)_2O$ at room temperature. Unfortunately, neither unprotected anilines (20) and (21) nor trifluoroacetamide (25) could be transformed to orthoesters in the presence of $BF_3{\cdot}OEt_2$. These observations underscore the utility of the azido group as a latent amine in this reaction sequence. The exact reason for the inability to isomerize trifluoroacetate (25) or unprotected amines (20) and (21) to orthoesters is unclear at the present time.

Scheme 2



EXPERIMENTAL

Materials

Spectra from nuclear magnetic resonance (^{1}H-nmr) (Varian XL300 spectrometer), infrared (ir) (Beckmann 3001 instrument), and chemical ionization mass spectra (CIms) (Finnegan Mat-311 spectrometer) and electron ionization mass spectra (EIms) (V.G. Micromass 7070F mass spectrometer) were in accord with the assigned structures. Specific activity measurements for radiolabelled compounds were determined by

ultraviolet (uv) analysis with a Hewlett-Packard HP 8450A UV/VIS spectrophotometer. Gas chromatographic analysis (gc) was performed on a Hewlett-Packard 5880A instrument with a carbowax capillary column and a flame ionization detector. Melting points (mp) were obtained using a Thomas-Hoover Unimelt apparatus, and are uncorrected. Thin layer chromatographic analysis (tlc) was performed on Analtech silica gel (GHF) plates; tlc system A refers to chloroform/methanol/conc. ammonium hydroxide (80:18:2); solvent system B refers to ethyl acetate-hexane (1:4); solvent system C refers to ethyl acetate-hexane (3:7). Catalytic tritiolysis of 17 was performed at Amersham Corporation, Illinois, U.S.A. and the crude reaction product was processed as described below. Precautions were taken to avoid exposure of all azido intermediates to light because of their inherent photolability.

Methyl 3,5-dibromo-4-aminobenzoate (7). To a vigorously stirred solution of methyl p-aminobenzoate (5) (20 g, 132 mmol) and triethylamine (55 ml, 339 mmol) in glacial acetic acid (200 ml) was added, dropwise at room temperature, freshly redistilled bromine (15.0 ml, 264 mmol) over a period of 30 min. The reaction mixture was diluted to 1000 ml with cold water, stirred briefly, and filtered. The crude product was washed with cold water and dried in vacuo at 60 °C, yield 40.6 g, 99.3%. Recrystallization from water/2-propanol (2:3) afforded 34.6 g (89.0%) of 7 as needles: mp 131.5-132 °C (lit.,[8] mp 131 °C); ir (KBr): 3440, 3340, 3090, 2970, 1735, 1615, 1440, 1305, 1270, 980, 765 cm^{-1}; ^{1}H-nmr ($CDCl_3$): δ 3.87 (s, OMe, 3H), 5.00 (br s, NH_2, 2H), 8.07 (s, H-2, H-6, 2H).

Methyl 3-bromo-4-aminobenzoate (6). The procedure above for 7 was adopted starting with methyl p-aminobenzoate·hydrochloride (5.HCl) (5.25 g, 28.0 mmol), triethylamine (8.58 ml, 61.6 mmol), glacial acetic acid (10 ml), and 1.44 ml (28 mmol) of bromine. Crystallization of the HCl salt from 2-propanol afforded 6.HCl (6.68 g, 89.6%) as needles: mp 174-175 °C; ^{1}H-nmr ($CDCl_3$) (base): δ 3.86 (s, OMe, 3H), 4.51 (br s, NH_2, 2H), 6.73 (d, J=8.4 Hz, 1H), 7.80 (dd, J=8.4, 1.9 Hz, H-2, 1H), 8.12 (d, J=1.9 Hz, 1H).

Methyl 3-bromo-4-azidobenzoate (8). To a stirred suspension of 6 (2.80 g, 10.51 mmol) in a mixture of distilled water (50 ml) and conc. HCl (1.57 ml, 18.84 mmol) was added, dropwise at 0-5 °C, a solution of $NaNO_2$ (0.84 g, 12.2 mmol) in 10 ml of distilled water. After 1 h, the reaction mixture was filtered (5 °C) to remove insolubles, and the filtrate was transferred to a large (200 ml) beaker. To the cooled and stirred solution was added, dropwise, a solution of sodium azide (0.79 g, 12.1 mmol) in 10 ml of distilled water. After the vigorous foaming had subsided, the solution was filtered, and the crystalline filter cake was washed with distilled water and dried overnight over P_2O_5. Recrystallization from cold hexane

afforded 8 (2.50 g, 93%) as feathery needles: mp 68-69 °C; ir (KBr): 2940, 2120, 1715, 1590, 1485, 1430, 1380, 1300, 1110, 750 cm^{-1}; ^{1}H-nmr ($CDCl_3$): δ 3.92 (s, OMe, 3H), 7.21 (d, J=8.4 Hz, H-5, 1H), 8.02 (dd, J=8.4, 1.9 Hz, H-6, 1H), 8.23 (d, J=1.9 Hz, H-2, 1H). Anal. Calcd for $C_8H_6N_3O_2Br$: C, 37.53; H, 2.36; N, 16.41. Found: C, 37.80; H, 2.30; N, 16.47.

Methyl 3,5-dibromo-4-azidobenzoate (9). To a stirred solution of 7 (5.00 g, 16.2 mmol) and $NaNO_2$ (1.23 g, 17.8 mmol) in distilled water was added dropwise at 0-5 °C, methanesulfonic acid (3.78 ml, 58.2 mmol). The solution was kept at 5 °C for 24 h and then filtered to remove unreacted starting material (81% recovered yield). The cold (5 °C) filtrate was transferred to a 200 ml beaker and treated dropwise with a solution of NaN_3 (1.00 g, 15.4 mmol) in distilled water (10 ml). The white precipitate (0.77 g, 75% based on amount of starting material recovered) was filtered, washed with distilled water (100 ml), and dried overnight at 60 °C in vacuo. Recrystallization from cold (0 °C) hexane afforded 9 (0.70 g) as feathery needles: mp 81-82 °C; ir (KBr): 3070, 2950, 2120, 1720, 1580, 1425, 1370, 1275, 1125, 750 cm^{-1}; ^{1}H-nmr ($CDCl_3$): δ 3.93 (s, OMe, 3H), 8.18 (s, H-2, H-6, 2H); EIms: M^+ (calcd for $C_8H_5N_3O_2{}^{79}Br_2$)=333. Found: 333 (M^+), 305 (M^+-N_2). Anal. Calcd for $C_8H_5N_3O_2Br_2$: C, 28.69; H, 1.50; N, 12.50. Found: C, 28.91; H, 1.49; N, 12.44.

3-Bromo-4-azidobenzoic acid (10). Compound (8) (2.20 g, 8.59 mmol) in 50 ml of MeOH at 40 °C was treated with 5.0 g (13.4 mmol) of KOH, and stirring was continued at room temperature. Tlc (solvent system B) indicated that the reaction was complete after 1 h. The excess MeOH was evaporated in vacuo and the residue was dissolved in distilled water (200 ml). The aqueous mixture was then neutralized by dropwise addition of conc. HCl (to pH=0). The flocculent suspension of product was cooled to 0 °C (with continued stirring), filtered (slow), and the filter cake was washed with cold (0 °C) distilled water and dried over P_2O_5 in vacuo. Recrystallization of the crude product from ethyl acetate-hexane afforded 10 (1.93 g, 93%) as a microcrystalline powder: mp 177-178 °C; ir (KBr): 3600-2300 (COOH), 2670, 2140, 1700, 1600, 1565, 1500, 1425, 1300, 1160, 1140, 915, 760 cm^{-1}; ^{1}H-nmr ($CDCl_3$): δ 7.25 (d, J=8.3 Hz, H-2, 1H), 8.08 (dd, J=1.9, 8.3 Hz, H-6, 1H), 8.31 (d, J=1.9 Hz, H-5); EIms: M^+ (calcd for $C_7H_4N_3O_2{}^{79}Br$)=241. Found: 241 (M^+), 213 (M^+-N_2). Anal. Calcd for $C_7H_4N_3O_2Br$: C, 34.74; H, 1.67; N, 17.36. Found: C, 34.98; H, 1.66; N, 17.43.

3,5-Dibromo-4-azidobenzoic acid (11). Compound (9) (0.70 g, 2.09 mmol) was treated as for 8 above and the product was recrystallized from ethyl acetate-hexane to afford 11 (0.60 g, 89%) as small needles: mp 160-161 °C; ir (KBr): 3680-2300 (COOH), 2660, 2540, 2140, 1690, 1585, 1410, 1285, 1140, 900, 740 cm^{-1}; ^{1}H-nmr ($CDCl_3$): δ 8.23 (s, H-2, H-6, 2H). Anal.

Calcd for $C_7H_3N_3O_2Br_2$: C, 26.20; H, 0.94; N, 13.09. Found: C, 26.29; H, 0.94; N, 13.03.

3-(t-Butyl)-3-(3-bromo-4-azidobenzoyloxymethyl)oxetane(14). Compound (10) (1.64 g, 6.78 mmol) in thionyl chloride (30 ml, 411 mmol) containing a trace (100 µl) of DMF was boiled under reflux for 5 h. The excess thionyl chloride was distilled at atmospheric pressure and the residue was azeotropically distilled in vacuo with 4 x 20 ml of dry toluene to remove traces of thionyl chloride. The acid chloride (12) was dissolved in dry CH_2Cl_2[11] (20 ml) and added dropwise at room temperature to a stirred solution of 3-(t-butyl)-3-oxetanemethanol (0.97 g, 6.78 mmol) (see ref 4, Casida et al, 1984 for preparation) and dry pyridine (4.0 ml, 49.4 mmol) in dry CH_2Cl_2[11] (20 ml). The reaction was complete (tlc; solvent system B) after stirring overnight at room temperature. The solvent was evaporated in vacuo at room temperature and the residue was partitioned between ether (200 ml) and water (100 ml). The aqueous layer was discarded and the ethereal layer was washed with 1 M HCl (4 x 50 ml), saturated aqueous $NaHCO_3$ (2 x 50 ml), water (50 ml) and the solvent was evaporated to give 14 (2.37 g, 95.5%) as a colorless oil: Ir (film): 2960, 2880, 2120, 1720, 1590, 1480, 1395, 1290, 1240 cm^{-1}; ^{1}H-nmr ($CDCl_3$): δ 1.07 (s, t-Bu, 9H), 4.46 (s, CH_2OCO, 2H), 4.58, 4.63 (ABq, J_{gem}=6.4 Hz, oxetane CH_2, 4H), 7.24 (d, J=8.35 Hz, H-5, 1H), 8.10 (dd, J=8.35, 1.8 Hz, H-6, 1H), 8.28 (d, J=1.8 Hz, H-2, 1H).

3-(t-Butyl)-3-(3,5-dibromo-4-azidobenzoyloxymethyl)oxetane (15). The same procedure as above for 14 starting instead with 11 (0.50 g, 1.55 mmol), DMF (100 µl), thionyl chloride (20 ml, 274 mmol), 3-(t-butyl)-3-oxetanemethanol (0.22 g, 1.55 mmol), pyridine (1.0 ml, 12.4 mmol) and CH_2Cl_2[11] (5.0 ml) afforded 15 (0.65 g, 95%) from hexane as a microcrystalline powder: mp 83-85 °C; ir (KBr): 2970, 2890, 2120, 1730, 1595, 1550, 1450, 1280, 1150, 980, 760, 740 cm^{-1}; ^{1}H-nmr ($CDCl_3$): δ 1.06 (s, t-Bu, 9H), 4.47 (s, CH_2OCO, 2H), 4.56, 4.63 (ABq, J_{gem}=6.4 Hz, oxetane CH_2, 4H), 8.21 (s, H-2, H-6, 2H); EIms: M^+ (calcd for $C_{15}H_{17}N_3O_3{}^{79}Br_2$)=446. Found: 446 ($M^+$), 418 ($M^+$-$N_2$). Anal. Calcd for $C_{15}H_{17}N_3O_3Br_2$: C, 40.29; H, 3.83; N, 9.40. Found: C, 40.31; H, 3.84; N, 9.34.

1-(3-Bromo-4-azidophenyl)-4-(t-butyl)-2,6,7-trioxabicyclo[2.2.2]octane (16). To a stirred solution of 14 (2.34 g, 6.36 mmol) in dry CH_2Cl_2[11] (40 ml) at -78 °C was added freshly redistilled boron trifuoride etherate (2.0 ml, 16.3 mmol) and the solution was stirred for 1 h at - 78 °C and then overnight at room temperature. Triethylamine (2.3 ml, 16.3 mmol) was added to quench the reaction, and the reaction mixture was diluted to 200 ml with ether and washed with water (4 x 100 ml). Drying (Na_2SO_4) and evaporation of the solvent afforded 2.24 g (96%) of crude 16.

Recrystallization from 30 ml of MeOH afforded 1.30 g (55.6%) of 16 as colorless laminae: mp 186-187 °C; ir (KBr): 2940, 2880, 2080, 1590, 1460, 1325, 1295, 1235, 1120, 985 cm^{-1}; ^{1}H-nmr ($CDCl_3$): δ 0.92 (s, t-Bu, 9H), 4.17 (s, CH_2, 6H), 7.13 (d, J=8.4 Hz, H-5, 1H), 7.57 (dd, J=8.3, 1.9 Hz, H-6, 1H), 7.81 (d, J=1.9 Hz, H-2, 1H); EIms: M^+ (calcd for $C_{15}H_{18}N_3O_3{}^{79}Br$)=367. Found: 339 ($M^+$-$N_2$), 341 ($M^+$-$N_2$). Anal. Calcd for $C_{15}H_{18}N_3O_3Br$: C, 48.93; H, 4.93; N, 11.41. Found: C, 49.02; H, 4.96; N, 11.43.

1-(3,5-Dibromo-4-azidophenyl)-4-(t-butyl)-2,6,7-trioxabicyclo[2.2.2]-octane (17). Treatment of 15 (0.59 g, 1.32 mmol) in 20 ml of dry CH_2Cl_2[11] at -78 °C with 1.0 ml (8.1 mmol) of freshly redistilled boron trifluoride etherate as described above followed by quenching of the reaction with triethylamine (1.13 ml, 8.1 mmol) afforded 17 (0.30 g, 51%) as colorless plates from EtOH: mp 129-130 °C; ir (KBr): 2960, 2880, 2120, 1450, 1400, 1345, 1305, 1245, 1125, 9090, 880 cm^{-1}; ^{1}H-nmr ($CDCl_3$): δ 0.91 (s, t-Bu, 9H), 4.16 (s, CH_2, 6H), 7.76 (s, H-3, H-5, 2H); EIms: M^+ (calcd for $C_{15}H_{17}N_3O_3{}^{79}Br_2$)=445. Found: 417 ($M^+$-$N_2$), 419 ($M^+$-$N_2$), 421 ($M^+$-$N_2$). Anal. Calcd for $C_{15}H_{17}N_3O_3Br_2$: C, 40.29; H, 3.83; N, 9.40. Found: C, 40.17; H, 3.87; N, 9.36.

1-(4-Aminophenyl)-4-(t-butyl)-2,6,7-trioxabicyclo[2.2.2]octane (18). A solution of 16 (0.30 g, 0.81 mmol) in triethylamine (30 ml) containing 10% Pd/C (30 mg) was stirred under an atmosphere of hydrogen for 12 h at room temperature when tlc (solvent system C) indicated the reaction to be complete. The reaction mixture was filtered through celite and the solvent was evaporated to give a white crystalline residue. The residue was partitioned between 10% aqueous NaOH (20 ml) and $CHCl_3$ (30 ml). The aqueous layer was extracted with a further 30 ml of $CHCl_3$ and the combined organic layer was dried over Na_2SO_4 and evaporated to give a crystalline residue. Recrystallization from hot 2-propanol (3 ml) afforded 18 (0.18 g, quantitative) as small colorless needles: mp 244-245 °C; ir (KBr): 3420, 3350, 2960, 2890, 1625, 1610, 1515, 1330, 1280, 1240, 1180, 1120, 1075, 1005, 830 cm^{-1}; ^{1}H-nmr ($CDCl_3$): δ 0.91 (s, t-Bu, 9H), 3.69 (br s, NH_2, 2H), 4.16 (s, CH_2, 6H), 6.63, 7.38 (ABq, J=8.6 Hz, H-2,3,5,6, 4H); uv (THF): λ_{max} 291 nm (ε=1745 liter $mol^{-1}cm^{-1}$); CIms: MH^+ (calcd for $C_{15}H_{21}NO_3$)=264. Found: 264 (MH^+). Anal. Calcd for $C_{15}H_{21}NO_3$: C, 68.42; H, 8.04; N, 5.32. Found: C, 68.50; H, 8.10; N, 5.32.

Hydrogenation of 17 (9.8 mg, 0.022 mmol) in triethylamine (1.0 ml) containing 10% Pd/C (9.8 mg) for 2 h and isolation of the product as above afforded 18 (5 mg), identical in all respects to the above, mp 244-245 °C.

1-(4-Isothiocyanatophenyl)-4-(t-butyl)-2,6,7-trioxabicyclo[2.2.2]octane (1). To a rapidly stirred solution of 18 (0.20 g, 0.76 mmol) in a mixture of saturated $NaHCO_3$ (5 ml) and $CHCl_3$ (5 ml) was added (in one portion), a

solution of freshly redistilled thiophosgene 69.6 µl (0.91 mmol) in $CHCl_3$ (1.0 ml). Tlc (solvent system C) indicated the reaction to be complete after 20 min at ambient temperature. The $CHCl_3$ layer was separated, diluted with a further amount of $CHCl_3$ (25 ml) and washed with saturated aqueous $NaHCO_3$ (10 ml), water (10 ml) and dried (Na_2SO_4). Evaporation of the solvent afforded pure crystalline 1 in quantitative yield: recrystallization from hot EtOH (3.0 ml) afforded analytically pure 1 (0.16 g, 71%) as colorless plates: mp 191-192 °C (lit.,[6] mp 191-192 °C); ^{1}H-nmr ($CDCl_3$): δ 0.92 (s, 9H, t-Bu), 4.18 (s, 6H, CH_2), 7.19, 7.58 (ABq, J=8.5 Hz, 4H).

1-(4-Amino-3,5-ditritiophenyl)-4-(t-butyl)-2,6,7-trioxabicyclo[2.2.2]-octane ([^{3}H]-18). A solution of 17 (20.0 mg, 0.045 mmol) in 2.0 ml of triethylamine containing 20 mg of 10% Pd/C was stirred for 2 h at room temperature under an atmosphere of carrier-free tritium gas (10.0 Ci, 0.017 mmol). The solution was filtered to remove catalyst and evaporated under a stream of argon to remove labile tritium. The residue (1.74 Ci) was reconstituted to a final volume of 10 ml with toluene-triethylamine (9:1) for storage prior to purification. Evaporation of the solvent under a stream of argon afforded the crude product. This was taken up in CH_2Cl_2 and applied to one 20 cm x 20 cm x 0.25 mm (Woelm Alumina Basic F; Analtech) tlc plate. The plate was eluted to a height of 15 cm with CH_2Cl_2 and the band that comigrated with reference unlabelled 18 was removed and extracted for 1 h at room temperature with a mixture of CH_2Cl_2/Et_2O (1:1) (50 ml). The solution was filtered through a plug of glass wool (to remove particles of alumina) and evaporated under a stream of nitrogen to afford a crystalline residue. The residue was diluted to a volume of 50 ml with toluene for storage: radiochemical yield = 407.7 mCi (15.7%) (>99% radiochemically pure); specific activity=25.5 Ci/mmol (calculated from uv absorbance value in THF at λ=291 nm where ε=1745 liter $mol^{-1}cm^{-1}$).

1-(4-Isothiocyanato-3,5-ditritiophenyl)-4-(t-butyl)-2,6,7-trioxabicyclo-[2.2.2]octane ([^{3}H]-1). To a stirred solution of [^{3}H]-18 (50 mCi, 1.96 x 10^{-3} mmol) in a mixture of $CHCl_3$ (1 ml) and saturated aqueous $NaHCO_3$ (1 ml) was added 2.24 µl (1.5 eq.) of a solution of 22.4 µl of freshly redistilled thiophosgene diluted to a volume of 0.224 ml with hydrocarbon stabilized $CHCl_3$. The reaction was allowed to proceed for 1 h, and then the $CHCl_3$ layer was diluted to 5 ml with $CHCl_3$ and separated. The $CHCl_3$ extract was evaporated under a stream of nitrogen and applied to one 20 cm x 20 cm x 0.5 mm preparative tlc plate (silica gel GF). The plate was eluted with ethyl acetate-hexane (1:9) and the band running at the same rf value as unlabelled 1 was removed and eluted with ether (50 ml). The extract was filtered and the solvent was evaporated under a stream of argon and the residue reconstituted to a volume of 5.00 ml with toluene: yield = 26.7

mCi (53.5%); radiochemical purity >99%. The toluene solution was adjusted to a final concentration of 1 mCi/ml of toluene for storage.

3-Methyl-3-(4-nitrobenzoyloxymethyl)oxetane (19). To a stirred solution of 3-methyl-3-oxetanemethanol (24) (8.56 g, 83.8 mmol) and triethylamine (TEA) (35 ml, 251 mmol) in dry THF (100 ml) at 0°C was added, dropwise, a solution of p-nitrobenzoyl chloride (17.1 g, 92.1 mmol) in dry THF (50 ml) and the reaction mixture allowed to warm to room temperature. The reaction was complete after 10 min. at 20 °C (tlc solvent system B). The reaction mixture was filtered and the filter cake of TEA·HCl was washed with THF (20 ml) and the combined filtrate and washings were evaporated to give 21.0 g (quantitative) of crystalline 19. Purification by recrystallization from hot isooctane afforded pure 19: mp 77-78 °C; ir (KBr) 2960, 2870, 1725, 1710, 1600, 1520, 1340, 1280, 1110, 975, 845 cm^{-1}; ^{1}H-nmr ($CDCl_3$): δ 1.44 (s, 3H), 4.46 (s, 2H), 4.49, 4.63 (ABq, J=6.1 Hz, 4H), 8.23, 8.30 (ABq, J=8.9 Hz, 4H); CIms: MH^+(calcd for $C_{12}H_{13}NO_5$)=252. Found: 252 (MH^+). Anal. Calcd for $C_{12}H_{13}NO_5$: C, 57.37; H, 5.22; N, 5.58. Found: C, 57.26; H, 5.20; N 5.59.

3-Methyl-3-(4-aminobenzoyloxymethyl)oxetane (20). A solution of 19 (10 g, 39.8 mmol) in 100 ml of MeOH containing 10% Pd/C (1 g) was hydrogenated at 50 p.s.i. for 1 h or until complete by tlc (solvent system B). The hydrogenation mixture was purged with N_2 and filtered through a pad of celite and the celite was washed with MeOH (20 ml). Evaporation of the solvent afforded a crystalline residue which on recrystallization from aqueous MeOH (1:1) afforded 20 (7.77 g, 88%): mp 102-103 °C; ir (KBr) 3460, 3360, 3230, 2960, 2880, 1700, 1650, 1610, 1520, 1385, 1290, 1175, 1120, 980 cm^{-1}; ^{1}H-nmr ($CDCl_3$): δ 1.41 (s, 3H), 4.11 (br s, 2H, NH_2), 4.32 (s, 2H, $COOCH_2$), 4.43, 4.64 (ABq, J=5.9 Hz, 4H), 6.64 (d, J=8.7 Hz, 2H), 8.67 (d, J=8.7 Hz, 2H); CIms: MH^+ (calcd for $C_{12}H_{15}NO_3$)=222. Found: 222 (MH^+). Anal. Calcd for $C_{12}H_{15}NO_3$: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.05; H, 6.85; N, 6.28.

3-Methyl-3-(4-amino-3,5-dibromobenzoyloxymethyl)oxetane (21). To a stirred solution of 20 (4.50 g, 20.4 mmol) in acetic acid (50 ml) was added triethylamine (8.51 ml, 61.1 mmol) and the solution was treated dropwise at 20 °C with a solution of bromine (2.09 ml, 40.6 mmol) in acetic acid (20 ml). Reaction was complete by tlc (solvent system C) after 1 h. The reaction was quenched into 184 g of K_2CO_3 dissolved in 1000 ml of water and the resulting mixture was extracted with ether (2 x 300 ml). The combined organic layer was back-extracted with water (100 ml) and evaporated to an orange oil. The oil was dissolved in 30 ml of ethyl acetate and diluted with isooctane until the solution became turbid. Crystallization occurred spontaneously on allowing the solution to stand at

room temperature. The crystals were filtered, washed with cold (0°C) ethyl acetate/isooctane (1:3) and oven dried to give pure 21 (6.00 g, 77.7%): mp 99-100 °C; ir (KBr) 3490, 3380, 2960, 2880, 1710, 1600, 1480, 1380, 1310, 1260, 1230, 1120, 970, 750 cm^{-1}; ^{1}H-nmr ($CDCl_3$): δ 1.40 (s, 3H, CH_3), 4.36 (s, 2H, $COOCH_2$), 4.44, 4.60 (ABq, J=6.0 Hz, 4H, oxetane CH_2), 5.03 (br s, 2H, NH_2), 8.05 (s, 2H); CIms: MH^+ (calcd for $C_{12}H_{13}NO_3{}^{79}Br_2$)=378. Found: 378 ($MH^+$). Anal. Calcd for $C_{12}H_{13}NO_3Br_2$: C, 38.02; H, 3.46; N, 3.70. Found: C, 38.10; H, 3.48; N, 3.66.

3-Bromo-2-(hydroxymethyl)-2-methyl-n-propyl-(4-amino-3,5-dibromo-benzoate (22). To a stirred solution of 20 (2.00 g, 9.05 mmol) in glacial acetic acid (50 ml) was added, dropwise during 20 min, a solution of bromine (2.89 g, 18.1 mmol) in acetic acid (20 ml). The reaction mixture was stirred for a further 10 min after the addition was complete, and then poured into 500 ml of ice water and filtered (slow) to afford 22 as a crystalline solid after air drying overnight. Recrystallization from EtOAc-isooctane (1:3) afforded 22 (3.49 g, 83.8 %): mp 158-160 °C; ir (KBr) 3470, 3360, 2950, 2870, 1700, 1610, 1400, 1315, 1250, 1121, 1035 cm^{-1}; ^{1}H-*nmr* ($CDCl_3$): δ 1.11 (s, 3H), 3.45, 3.50 (ABq, J=10.3 Hz, 2H), 3.52 (d, J=6.4 Hz, 2H), 4.30 (s, 2H), 5.06 (br s, 2H, NH_2), 8.04 (s, 2H); EIms: M^+(calcd for $C_{12}H_{14}NO_3{}^{79}Br{}^{81}Br_2$)=461. Found: 461 (M^+). Anal. Calcd for $C_{12}H_{14}NO_3Br_3$: C, 31.34; H, 3.07; N, 3.05. Found: C, 31.90; H, 2.94; N, 2.89.

3-Methyl-3-(4-trifluoroacetamidobenzoyloxymethyl)oxetane (25). To aniline (20) (1.0 g, 4.52 mmol) under an argon atmosphere was added trifluoroacetic anhydride (10 ml) and the solution was stirred at ambient temperature for 20 min when tlc (1:1 EtOAc/hexane) indicated completion of the reaction. The excess trifluoroacetic anhydride was removed by evaporation in vacuo. Traces of anhydride were removed by addition and evaporation of $CHCl_3$ (3 x 5 ml) to give 25 in quantitative yield as a clear colorless oil (1 spot on tlc) which crystallized on standing; an analytically pure sample was obtained by recrystallization from EtOAc/hexane (1:4): mp 117-120 °C; ir (KBr) 3430, 3250, 3200, 3060, 2960, 2890, 1715 (broad), 1605, 1545, 1410, 1280, 1150 cm^{-1}; ^{1}H-nmr ($CDCl_3$): δ 1.44 (s, 3H), 4.40 (s, 2H, $COOCH_2$), 4.51, 4.69 (ABq, J=6.0 Hz, 4H), 7.70 (d, J=8.7 Hz, 2H). 8.10 (d, J=8.7 Hz, 2H), 8.24 (br s, $NHCOOCF_3$); CIms: MH^+(calcd for $C_{14}H_{14}NO_4F_3$)=318. Found: 318 (MH^+). Anal. Calcd for $C_{14}H_{14}NO_4F_3 \cdot 0.25H_2O$: C, 52.26; H, 4.54; N, 4.35. Found: C, 52.25; H, 4.47; N 4.36.

ACKNOWLEDGEMENT

The authors would like to offer their sincere appreciation to Wesley White and Noel Whittaker for providing ^{1}H-nmr and mass spectral analysis of all intermediates and final compounds described in this paper.

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11. The CH_2Cl_2 was dried and freed of EtOH stabilizer by filtration through a column of Brockmann I basic alumina prior to use.

Received, 18th July, 1991