Synthesis of Tritiated 1-[2-(diphenylmethoxy)ethyl]-4-[3-(3-azidophenyl)propyl]piperazine ([^{3}H]-meta azido GBR-12935), a photoaffinity ligand for the dopamine reuptake site.

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SUMMARY

A tritiated photoaffinity ligand for the dopamine reuptake transporter protein, 1-[2-(diphenylmethoxy) ethyl]-4-[3-(3-azidophenyl)-2,3-ditritiopropyl]piperazine, of specific activity 41.8 Ci/mmol, was prepared in six steps starting from 1-[2-(diphenylmethoxy)ethyl] piperazine.

Key words: Cocaine, dopamine reuptake, GBR-12935, 1-[2-(diphenylmethoxy)ethyl]-4-[3-phenylpropyl]piperazine, 1-[2-(diphenylmethoxy)ethyl]-4-[3-(3-azidophenyl)propyl]piperazine, tritium.

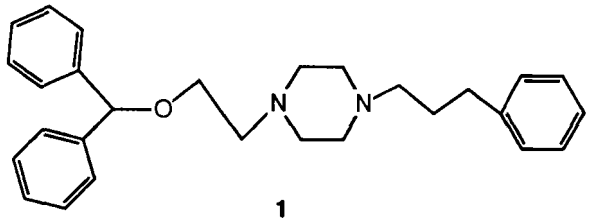
INTRODUCTION

The widely abused stimulant cocaine, has been shown to bind to the dopamine transporter protein.[1] It has been suggested that some of the psychological effects of the drug are a consequence of its ability to inhibit dopamine reuptake.[2]

In addition to cocaine, a number of other ligands including nomifensine, mazindol, methylphenidate and GBR-12935 have been shown to bind to this transporter protein.[3] Within this group, the piperazine derivative GBR-12935 (**1**) binds with the highest affinity and unlike the other ligands does not bind to the catecholaminergic or serotonergic transporter proteins.[4, 5] In connection with ongoing research directed toward the purification of the dopamine reuptake carrier, we required a radiolabelled photoaffinity ligand for the protein. We herein report the synthesis of 1-[2-(diphenylmethoxy)ethyl]-4-[3-(3-azidophenyl)-2,3-ditritiopropyl]piperazine (ditritio-*m*-azido GBR-12935, **7**).

0362–4803/91/020125–05$05.00

Received May 16, 1990
Revised September 21, 1990



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CHEMISTRY

The starting material for the synthesis was 1-[2-(diphenylmethoxy)ethyl]piperazine (**2**), prepared as previously described.[6] This compound was condensed with 3-nitrocinnamoyl chloride in the presence of triethylamine to provide the cinnamide derivative **3** in 88% yield. Reduction of the nitro function using ruthenium dodecacarbonyl under an atmosphere of carbon monoxide using the method of Alper *et al*[7] gave the meta amino cinnamide **4** in 95% yield. The amide carbonyl was reduced with lithium aluminum hydride[8] to provide 1-[2-(diphenylmethoxy)ethyl]-4-[3-(3-aminophenyl)-2-propenyl]piperazine(**5**) in 43% yield after chromatographic purification. Compound **5** was reductively tritiated using 10% palladium on carbon under an atmosphere of tritium to give **6**. The desired compound **7** was then prepared by the conversion of the aromatic amine of **6** to an azide via the displacement of an intermediate diazonium salt[9].

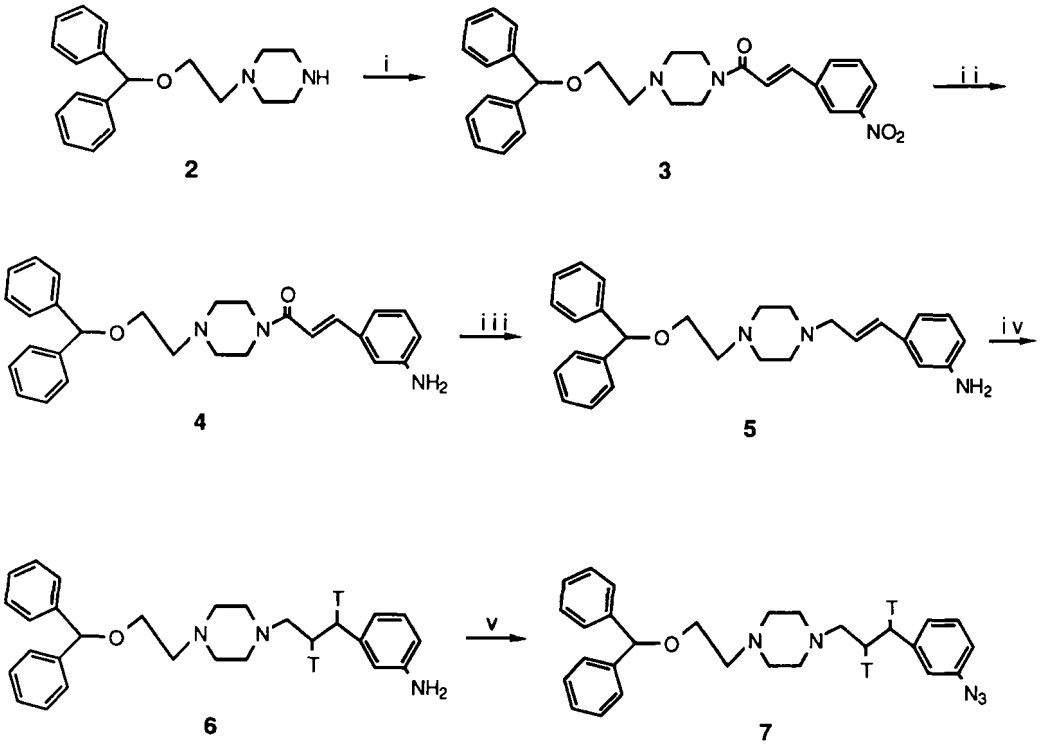
EXPERIMENTAL

1-[2-(diphenylmethoxy)ethyl]-4-[3-(3-nitrophenyl)-1-oxo-2-propenyl]piperazine (3). To a solution of1-[2-(diphenylmethoxy)ethyl]piperazine (**2**, 7.2 g, 24 mmol) in 220 mL of chloroform containing 10 mL of triethylamine was added a solution of 3-nitrocinnamoyl chloride (4.15 g, 1.05 equiv) in 20 ml of chloroform. After 20 min, the reaction was carefully poured into a separatory funnel containing 100 mL of 1 N NH_4OH solution. The organic layer was dried ($MgSO_4$) and concentrated to give **3** as an oil (10.15 g, 88.6%). ^1H NMR ($CDCl_3$) 8.32 (s, 1H), 8.13 (dd, J = 8,1 Hz, 1H), 7.70 (d, J = 7 Hz, 1H) 7.62 (d, J =16, Hz, 1H), 7.5 (dd, J = 7, 7 Hz, 1H), 7.1-7.3 (m, 11 H), 5.3 (s, 1H), 3.55-3.75 (m, 4H), 3.55 (t, J= 6 Hz, 2H), 2.65 (t, J = 6 Hz, 2H), 2.5 (m, 4H).

1-[2-(diphenylmethoxy)ethyl]-4-[3-(3-aminophenyl)-1-oxo-2-propenyl]piperazine (4). To $Ru_3(CO)_{12}$ (15 mmol) in benzene (100 mL) containing 2-methoxyethanol (5 mL) was added benzyltriethylammonium chloride (1.25 mmol) in 5N NaOH (50 mL). The reaction mixture was stirred for one hour under an atmosphere of carbon monoxide. The nitro compound **3** (20 mmol) in benzene (15 mL) was added and stirring was continued until the reaction was compete as judged by thin-layer chromatography. The organic layer

Scheme 1

Synthesis of Tritiated *m*-azido GBR-12935



Reagents: i) 3-nitrocinnamyl chloride, $N(Et)_3$; ii) CO, $Ru_3(CO)_{12}$, 5N NaOH, $PhCH_2N(Et)_3^+Cl^-$, $CH_3OCH_2CH_2OH$; iii) $LiAlH_4$ iv) 10% Pd/C, T_2; v) HONO, NaN_3

was concentrated, the aqueous later was acidified to pH 6 with dilute HCl, and the amine was extracted with ether, and the ether extract was dried ($MgSO_4$). The reaction mixture was concentrated to give the aromatic amine **4**. (8.41 g, 95%). ^1H NMR ($CDCl_3$) 7.50 (d, J = 13 Hz, 1H), 7.15 -7.35 (m, 10 H), 7.08 (dd, J = 7,7 Hz, 1H), 6.83 (d, J = 8 Hz, 1H), 6.75 (m, 2H), 6.61 (d, J = 7 Hz, 1H), 5.30 (s, 1H), 3.6-3.8 (m, 6H), 2.64 (t, J = 6, Hz, 2H), 2.5 (m, 4H).

1-[2-(diphenylmethoxy)ethyl]-4-[3-(3-aminophenyl)-2-propenyl]piperazine (5). A solution of lithium aluminum hydride (5 mL, 1.0 M) was added dropwise to a solution of **4** (1 g, 2.27 mmol) in 30 mL of ether at 0 °C. After 20 min, 1 mL of 2.0 N NaOH solution was added followed by 3 mL of water. After stirring for 30 min the

reaction was filtered through celite and concentrated. Purification by chromatography on silica eluting with 2% methanol in chloroform provided 416 mg (43%) of **5**. ^{1}H NMR ($CDCl_3$) 7.50 (d, J = 13 Hz, 1H), 7.15 -7.35 (m, 10 H), 7.08 (dd, J = 7,7 Hz, 1H), 6.83 (d, J = 8 Hz, 1H), 6.75 (m, 2H), 6.61 (d, J = 7 Hz, 1H), 5.30 (s, 1H), 3.6-3.8 (m, 6H), 2.64 (t, J = 6, Hz, 2H), 2.5 (m, 4H). Mass spectrum (CI, NH_3) 428 (M+1). Anal. (Calculated for the dimaleate salt, $C_{36}H_{41}N_3O_9$) C, 65.54; H, 6.26; N, 6.37%. Anal. (Found) C, 65.83; H, 6.31; N, 6.43%.

1-[2-(diphenylmethoxy)ethyl]-4-[3-(3-aminophenyl)-2,3-ditritiopropyl]piperazine (6). A solution of **5** (10.0 mg, 0.023 mmol) in methanol (2 mL) containing 10% Pd-C (10 mg) was stirred overnight at room temperature under an atmosphere of carrier free tritium gas (30 Ci, 0.52 mmol). The solution was filtered, evaporated under a stream of argon. Purification by reverse phase HPLC (using a XXX column eluting with 90/10 water methanol at 1ml/min) afforded 64 mCi (5.1 % radiochemical yield) of **6** in greater than 99% radiochemical purity. The product was dissolved in 30 mL of methanol for UV analysis (UV_{max} = 282 nM, ε_{max} = 640 cm.liter.mol^{-1} in MeOH). Specific activity = 41.8 Ci/mmol. Incorporation of tritium = 72%.

1-[2-(diphenylmethoxy)ethyl]-4-[3-(3-azidophenyl)-2,3-ditritiopropyl]piperazine (7). To a solution of **6** (64 mCi, .016 mmol) in 1 mL of 0.2 N HCl solution at 0 °C was slowly added 20 mg of powdered sodium nitrite. After 10 min, 40 mg of sodium azide was added. Chloroform (1 mL) was added to the stirred solution and NH_4OH was added by micropipette until the aqueous solution was basic to pH paper (pH > 9). The stirring was stopped and the organic layer was removed by pipette. The aqueous layer was extracted with a further 1 mL of chloroform and the combined organic layers were evaporated under a stream of argon. Purification by TLC using one 20 cm X 20 cm X 0.5 mm TLC plate eluting with 2% methanol in chlroform and extraction (10 mL of ethyl acetate) of the band comigrating with unlabelled m-azido GBR 12935 afforded 49 mCi (74%) of **7** in >99% radiochemical purity as determined by TLC scanner.

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