Super High Specific Activity Tritium Labelled Receptor Ligands - I. The Use of Polybromodiphenylacetic Acid as a Tritiation Substrate. Synthesis of a Tritium Labelled Sodium-Channel Blocker and Angiotensin II Antagonist with Super High Specific Activity

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Summary

We describe herein the synthesis of polybromodiphenylacetic acid (4), a fragment common to the tritiation substrates (8) and (11) of the sodium channel blocker, PD-85639 (1) and the angiotensin II inhibitor EXP-655 (2) respectively. Preparation of (8) and (11) followed by reductive debromonation with carrier free tritium gas furnished the corresponding receptor ligands (1) and (2) having specific activities of **180 Ci/mmole** and **209 Ci/mmole,** respectively.

Key words: Polybromodiphenylacetic acid, diphenylacetic-^{3}H, reductive dehalogenation, tritiated angiotension II antagonist, tritiated sodium channel blocker, EXP-665^{3}H, PD-85639-^{3}H

Introduction

N-[3-(2,6-dimethyl)-1-piperidinyl)propyl]-a-phenylbenzeneacetamide (1) and (S)-1-[(4-amino-3-methylphenyl)methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid, (2) have been studied [1,2] as a voltage-dependent sodium channel blocker and as a non-peptide angiotensin II (Ang II) receptor binding inhibitor, respectively. Sodium channels are proposed

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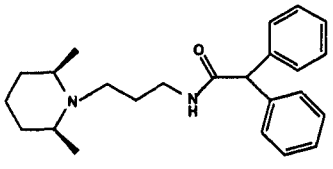
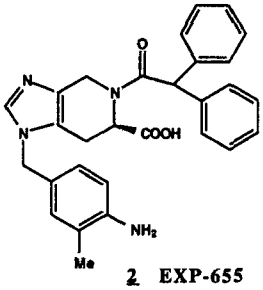
to be the site of action for a number of important drugs such as local anaesthetics, antiarrhythmics, and anticonvulsant agents[1]. Compound (1) was found to bind specifically to a Na^+ channel local anaesthetic receptor site and is an ideal representative of a novel class of local anaesthetic-like, therapeutically important drugs[3]. Receptors for the peptide hormone angiotensin II (ANG II) mediate a large variety of physiological effects regulating circulatory functions[2,4,5,6]. It was found that some Ang II inhibitors were very efficacious in treating hypertension and congestive heart failure[2]. Recently, Ang II receptors have been found to have other functions such as hormone release, fluid transport and neurotransmission[2,4,5]. It was therefore very important to thoroughly study the different binding sites associated with Ang II receptors. Compound (2) has emerged as a novel non-peptide Ang II receptor binding inhibitor that interacts with a newly discovered Ang II binding site[2]. It has also been proven to be of use in further studies of Ang II receptor pharmacology and physiology [2].



1 PD-85639



2 EXP-655

Therefore, high specific activity tritiated analogs of both compounds were required as standard ligands for receptor binding/mapping studies. Since both compounds have the diphenylacetyl component in common, a synthetic route incorporating polybromodiphenylacetyl group as a sub-unit and subsequent tritiation was planned. Described herein are the synthesis of polybromodiphenylacetic acid by direct electrophilic bromination, the coupling of the brominated unit, and the tritiation of precursors to give the title compounds.

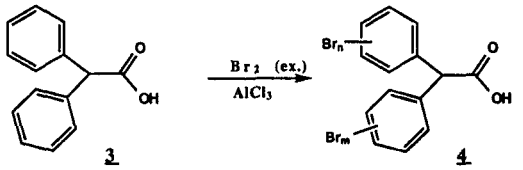
Discussion

Super high specific activity tritiated ligands are essential for the characterization of receptors which are present in low concentration. The tritiated analog of compound (1), (PD-85639-^{3}H) has been previously prepared having a specific

actiivty of 45 Ci/mmole[3] by reductive debromination with tritium of the bis-p-dibromophenacyl analog. However, this level of enrichment was insufficient to achieve the required sensitivity for binding/whole body autoradiography (wba) studies. We focused our efforts, therefore, on introducing as many bromines as possible into the diphenylacetyl group in order to increase tiritium enrichment by subsequent catalytic reduction with tritium gas.

Initial bromination attempts using benzyltriethylammonium tribromide and DBU tribromide, failed. We then turned our attention to a more reactive system consisting of $Br_2/AlCl_3$. This reagent had been reported to furnish a 65% yield of 3,5-dibromo-4-methylbenzoic acid methyl ester[7] when combined in an equimolar ratio with neat 4-carboxymethytoluene. Since diphenylacetic acid is a solid, we repeated the literature reaction using carbon tetrachloride as solvent. This reaction was unsuccessful. However, when bromine was used as solvent, highly brominated products (4) were obtained in 72% yield as shown below.

Scheme 1: Synthesis of polybromodiphenylacetic acid

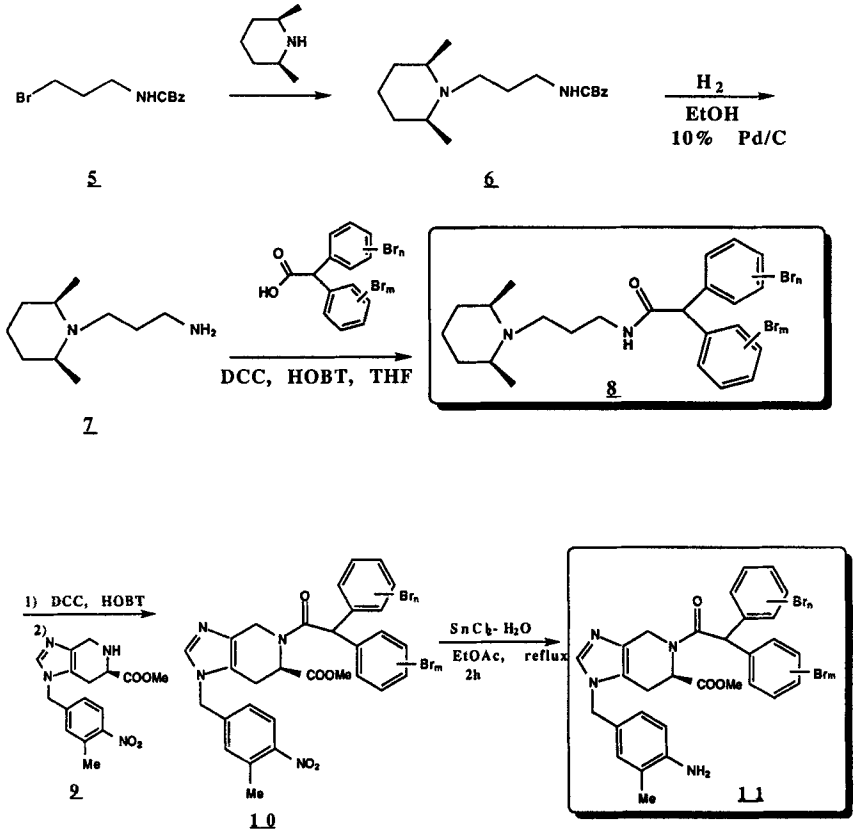


3 4

^{1}H NMR and Mass spectral analysis showed this product to be a mixture consisting of 47% octabromodiphenylacetic acid and 57% nonabromodiphenyacetic acid. The average bromine content being 8.57 bromines per molecule. This key intermediate was then used to construct the polybrominated tritiation precursors to both the sodium channel (8) and angiotensin II antagonists (11) respectively as shown in **Scheme 2**.

Thus, treatment of cis-2,6-dimethylpiperidine with N-protected 3-bromopropanamine (5) afforded the addition product (6) in 53% yield. Catalytic reduction of (6) produced the desired primary amine (7) in 93% yield. Activation of the carboxyl group of (4) with 1,3-dicyclohexylcarbodiimide (DCC) in the presence of 3-hydroxybenzotriazole (HOBT) followed by addition of amine (7) in THF gave the amide (8) in 35% yield.

Scheme 2: Synthesis of tritiation precursors

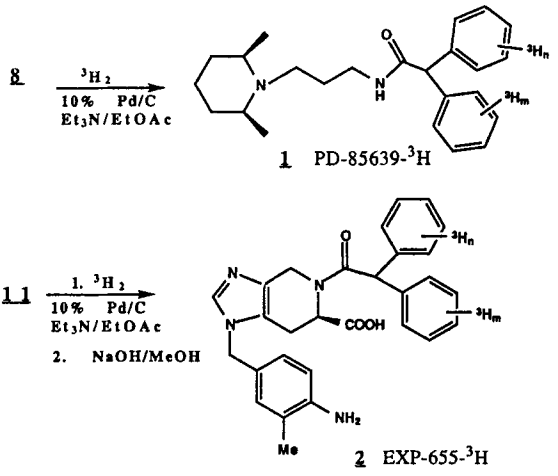


Polybromodiphenylacetic acid was then condensed in a similar fashion with amine (9) to give nitroamide (10) in 47% yield. The nitro group was then reduced with $SnCl_2$ to give the requisite tritiation substrate (11) in 76% yield.

Catalytic reduction of (8) with carrier free tritium over 10% Pd/C in THF/Et_3N provided the sodium channel blocker PD-85639-^{3}H (1) in 96% purity. Further purification by column chromatography followed by HPLC analysis showed a peak with retention time identical to that of standard (1) having a purity of >99%. The specific activity determined by HPLC using the external standard method was 180 Ci/mmol.

In a similar manner, reductive debromination of (11) with tritium gas, followed by basic ester hydrolysis, afforded the desired angiotensin II antagonist EXP-655-^{3}H (2) having specific activity of 209 Ci/mmole.

Scheme 3: Tritiation of polybrominated substrates



Conclusion

Utilization of $AlCl_3$ in neat Br_2 with diphenylacetic acid afforded polybromoacetic acid (4) containing >8 bromine atoms per molecule. Reductive dehalogenation with tritium gas of substrates prepared from (4) furnished receptor ligands (1) and (2) having specific acitivities of 180 Ci/mmole and 209 Ci/mmole, respectively, an unprecedented level of enrichment for such compounds.

Experimental

Carrier free tritium gas was purchashed from New England Nuclear. Unlabelled reagents were purchased from Aldrich Chemical Co. and were used without further purification. Radiochromatography was performed on a Bioscan 200 scanner. Radioassays were performed on a Packard 4000 liquid scintillation counter. NMR spectra were recorded using a Varian EM 390 spectrometer. Chemical shifts (δ) were reported downfield from tetramethylsilane. Mass spectra were obtained on a Finnigan-MAT 8230 spectrometer and for multibromination compounds, M/Z values were reported as that of the highest isotope distribution peak. HPLC analyses of final products were performed on a Beckman System Gold chromatograph. Specific Activity of tritium labelled products was determined by injecting known radioactivity concentration of labelled compound followed by known concentration of unlabelled compound, and comparing both uv absorption peak areas.

Polybromodiphenylacetic acid (4)

Diphenylacetic acid (3) (0.50 g, 2.4 mmol) was dissolved in 2.5 mL of Br_2 (48.4 mmol) and $AlCl_3$ (anhydrous, 4.00 g, 30 mmol) was added portion-wise slowly. The mixture was stirred at rt for 1h, at 80° for 2h, and was then quenched by slow addition of H_2O. The product was extracted with EtOAc . The EtOAc layer was washed with H_2O, NaCl (sat.), and dried over Na_2SO_4. Evaporation of the solvent gave 1.95 g of a white solid (yield: 72%).

MS (LSIMS): (M^+-CO_2H) n+m=9, 876; n+m=8, 798.

^1H NMR ($CDCl_3$): δ n+m=9, 7.29 (s, 1H-Ph), 6.23 (s, 1H-CHPh); n+m=8, 7.32 (s, 2H-Ph), 5.72 (s, 1H-CHPh).

N-(3-Bromopropyl) Benzylcarbamate (5)

3-Bromopropylamine hydrobromide (4.38 g, 0.02 mol) was dissolved in 10 mL of NaOH (4N) and cooled to 0 °C. To this solution was added benzylchloroformate dropwise. The mixture was then allowed to warm to ambient temperature and stirring for 1h. The product was extracted with EtOAc three times and the EtOAc layer was washed with NH_4Cl (aq.), NaCl (sat.), and dried over Na_2SO_4. Evaporation of the solvent gave 5.4 g of a colorless oil (99%).

^1H NMR ($CDCl_3$): δ 7.35 (m, 5H), 5.10 (s, 2H), 4.89 (br.s. 1H), 3.44 (t, 2H, j=6.4), 3.35 (q, 2H, j=6.4), 2.08 (t, 2H, j=6.4).

MS (EI): M/Z 272 (Br, 79), 274 (Br, 81).

N-[3-(2,6-Dimethyl-1-piperidinyl)propyl] Benzylcarbamate (6)

Compound (5) (2.00 g, 7.4 mmol) and 2,6-dimethylpiperidine (2.50 g, 22 mmol) in 10 mL of DMF was heated at 80° for 6h at which time the reaction was judged to be complete by TLC. The reaction was quenched with H_2O and extracted with EtOAc. The EtOAc layer was then washed with H_2O (4x), NaCl (sat.), and dried over Na_2SO_4. Concentration gave 2.7 g of crude oil. Purification by column chromatography (10% MeOH-CH_2Cl_2) gave 1.20 g of brown oil (53%).

TLC: NH_4OH-MeOH-CH_2Cl_2 (1:9:90); Rf 0.32.

^1H NMR ($CDCl_3$): δ 7.35 (m, 5H), 5.10 (s, 3H), 3.16 (q, 2H, J=6.3), 2.76 (t, 2H, J=8.0), 2.41 (br.s. 2H), 1.60 (m, 6H), 1.28 (m, 2H), 1.09 (d, 6H, J=6.3).

MS (EI): M/Z 304.

3-(2,6-Dimethyl-1-piperidinyl)propyl Amine (7)

A solution of (6) (0.70 g, 2.3 mmol) in 10 mL of EtOH over 10% Pd/C (10 mg) was treated with H_2. After stirring for 7 h, the reaction mixture was filterred through a celite bed and concentrated to give 0.39 g of yellow oil (99%).
TLC: NH_4OH-MeOH-CH_2Cl_2 (2:18:80); Rf 0.25.
^1H NMR ($CDCl_3$): δ 2.82 (t, 2H, J=8.2), 2.65 (t, 2H, J=7.1), 2.47 (br.s, 2H), 1.65 (m, 6H), 1.27 (m, 2H), 1.13 (d, 6H, J=6.3). MS (EI): M/Z 170.

N-[3-(2,6-Dimethyl-1-piperidenyl)propyl] Polybromodiphenyl-acetamide (8)

Compound (4) (600 mg, 0.69 mmol) was dissolved in 7 mL of THF and DCC (0.14 g, 0.69 mmol), HOBT-H_2O (0.093 g, 0.69 mmol) were added. The mixture was stirred at ambient temperature for 1h before amine (7) (0.12 g, 0.71 mmol) in 3 mL of THF was added. The resulting mixture was stirred at ambient temperature for 48h. It was then filterred through celite. The filtrate was diluted with EtOAc, washed with $NaHCO_3$ (sat.), NaCl (sat.), and dried over Na_2SO_4. Evaporation to dryness gave 0.85 g of crude (8). Purification by column chromatography (0.5% NH_4OH, 4.5% MeOH in CH_2Cl_2) gave 0.25 g of white solid (35%).
TLC: NH_4OH-MeOH-CH_2Cl_2 (1:9:90); Rf 0.55.
MS (LSIMS): M/Z 1066 (n+m of Br=9), 996 (n+m=8)

N-[3-(2,6-Dimethyl)piperidinyl)propyl]-a-phenylbenzene-acetamide (1)

Compound (8) (5 mg, 0.0049 mmol) and 10% Pd/C (5 mg) were put under vacuum for 1h and were then dissolved in 1 mL of THF and 0.3 mL of Et_3N. After degassing, $^3H_2$ (10 Ci, 0.172 mmol, 58 Ci/mmol) was introduced. The mixture was stirred at ambient temperature overnight. Labile radioctivity was vacuum transferred into a cold trap, and the catalyst was removed by filtration. The filtrate was diluted with MeOH and eveporated to dryness three times to ensure removal of volatile radioactivity. The residue was dissolved in MeOH and assayed to give 667 mCi of crude (1) (96% purity by TLC). Column purification with 1% NH_4OH, 9% MeOH in CH_2Cl_2 gave 510 mCi of pure 1 (98% pure by HPLC), 50 mCi of which was further purified by chromatotron with 1% NH_4OH, 9% MeOH in CH_2Cl_2 to give 42 mCi of (1) at >99.9% purity.
Radio-tlc: NH_4OH-MeOH-CH_2Cl_2 (1:9:90); Rf 0.45.
Radio-HPLC: Beckman-C18, ultrasphere; 28% ACN in TEAP (PH=3, 0.03M), 1 mL/min; 210 nm; Rt 14.5 min.

Methyl (S)-1-[(3-Methyl-4-nitrophenyl)methyl]-5-(polybromodipheyl-acetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylate (10)

To a solution of compound (4) (0.36 g, 0.41 mmol) in 3 mL of THF were added DCC (0.08 g, 0.41 mmol) and HOBT.H_2O (0.055 g, 0.41 mmol). After the resulting suspension was stirred at ambient temperature for 15 min, compound (9) in 1 mL of THF was added and the mixture was stirred at ambient temperature for 48h. The resulting solid was filtered and the filtrate was concentrated. The residue was then partitioned between 10% Na_2CO_3 (aq.) and CH_2Cl_2. The organic layer was washed with H_2O, NaCl (sat.), and dried over Na_2SO_4. Purification with 2% MeOH in CH_2Cl_2 gave 0.20 g of solid (yield: 47%).
MS (LSIMS): 1156 (n+m=8); 1234 (n+m=9).
^{1}H NMR ($CDCl_3$): δ 7.97 (d, 1H, 5-Ar1), 7.48 (s, 1.5H, Ar-$Br_{8.57}$), 7.14 (s, 1H, H-2), 7.02 (d, 2H, 2+6-Ar1), 6.07 (s, 1H, $CHPh_2$), 5.91 (dd, 1H, H-6), 5.11 (s, 2H, CH_2Ar1), 4.57 (d, 1H, H-4), 4.33 (d, 1H, H-4'), 3.68 (s, 3H, CO_2Me), 3.18 (d, 1H, H-7), 2.73 (dd, 1H, H-7'), 2.58 (s, 3H, CH_3Ar).

Methyl (S)-1-[(4-Amino-3-Methylphenyl)methyl]-5-(polybromo-dipheylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylate (11)

A solution of (10) (0.05 g, 0.04 mmol) in 2 mL of EtOAc was treated with $SnCl_2$•$2H_2O$ (0.08 g, 0.35 mmol) and heated to reflux for 4h. It was then cooled, quenched with $NaHCO_3$ (sat.), and diluted with EtOAc. The EtOAc layer was separated and the aqueous layer was extracted with EtOAc twice. EtOAc layers were combined, washed with NaCl (sat.), and dried over Na_2SO_4. Concentration and column purification with 2% MeOH in CH_2Cl_2 gave 0.03 g of white solid, (11) (yield: 76%).
MS (LSIMS): M/Z 1126 (n+m), 1204 (n+m).
^{1}H NMR ($CDCl_3$): δ 7.46 (s, 1H, H-2), 7.40 (s, 1H, 2-Ar1), 7.14 (s, 1H, 6-Ar1), 6.81 (s, 1.5H, Ar-$Br_{8.57}$), 6.63 (d, 1H, 5-Ar1), 6.05 (s, 1H, $CHPh_2$), 5.86 (dd, 1H, H-6), 4.88 (d, 2H, CH_2Ph), 4.52 (d, 1H, H-4), 3.25 (d, 1H, H-7), 2.74 (d, 1H, H-7'), 2.14 (s, 3H, CH_3Ar).

(S)-1-[(4-Amino-3-Methylphenyl)methyl]-5-(polytritiated dipheylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid methyl ester (12)

A side arm septum flask was charged with (11) (11.7 mg, 0.01 mmol) and 10% Pd/C (20 mg) the system was evacuated for 0.5h. EtOAc (1 mL) and Et_3N (0.3 mL) were then added *via* syringe and degassed. The reaction flask was frozen in liquid nitrogen and tritium gas (10 Ci, 0.17 mmol) was introduced. After the mixture was stirred for 22h at ambient temperature, volatile radioactivity was vacuum transferred to a waste bulb and the catalyst was removed by filtration. The filtrate was evaporated from MeOH three times to remove labile radioactiivty. An assay of the residue dissolved in MeOH showed it to contain 1.57 Ci crude (12) of about 88% purity. A 240 mCi portion of the latter was purified by column chromatography (3% MeOH in CH_2Cl_2) to give 165 mCi of >99% pure (12).

Radio-tlc: MeOH-CH_2Cl_2 (3:97); Rf 0.32

(S)-1-[(4-Amino-3-Methylphenyl)methyl]-5-(polytritiated dipheylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic Acid (2)

To a solution of 12 (53 mCi) in 0.5 mL of THF and 0.15 mL of MeOH was added 0.02 mL of 1N NaOH (0.02 mmol). After the mixture was stirred at ambient temperature for 2h, TLC showed the reaction to be complete. HCl (0.02 mL, 1N) was added and solvents were removed by rotary evaporation. The mixture was purified by column chromatography eluting with NH_4OH-MeOH-CH_2Cl_2 (1.5:13.5:85) to give 37 mCi of pure (2) having a specific activity of 209 Ci/mmole.

Radio-tlc: NH_4OH-MeOH-CH_2Cl_2 (2:18:80); Rf 0.33

Radio-HPLC: Beckman-Ultrasphere C18 (4.6x250); 25% acetonitrile in triethylammonium phosphate (PH=3, 0.03 M); 1 mL/min, 220nm, Rt 16.07).

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