Synthesis of [^{3}H]DIPPA: a Potent Irreversible Antagonist Selective for the κ Opioid Receptor

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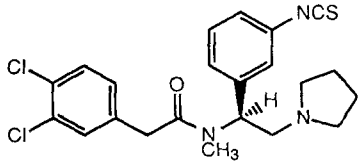
Summary

2-(3,4-Dichlorophenyl)-N-methyl-N-[(1S)-1-(3-isothiocyanatophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide (**1**, DIPPA) has been previously reported to be an opioid receptor affinity label that produces selective and long-lasting κ opioid receptor antagonism in mice. High specific activity [^{3}H]DIPPA (39.7 Ci/mmol) was prepared by bromination and catalytic tritiation of the amino precursor of DIPPA followed by conversion to the isothiocyanate with thiophosgene.

Key words: DIPPA, antagonist, kappa, radiolabeled, affinity label, tritium.

Introduction

Opioid ligands exert their pharmacologic actions through at least three types of opioid receptors: μ, κ, and δ.[1] While activation of each class of receptors produces analgesia, κ opioid receptors have been of special interest because their activation produces analgesia without the undesirable side-effects (physical dependence, respiratory depression) associated with μ receptor activation by morphine. In addition to analgesia, κ selective ligands can be used for eating disorders,[2] motion sickness,[3] and neuroprotection.[4] In order to improve our understanding of the κ receptor's mechanism of action, both κ selective agonists and antagonists are needed as pharmacologic tools.



1, DIPPA

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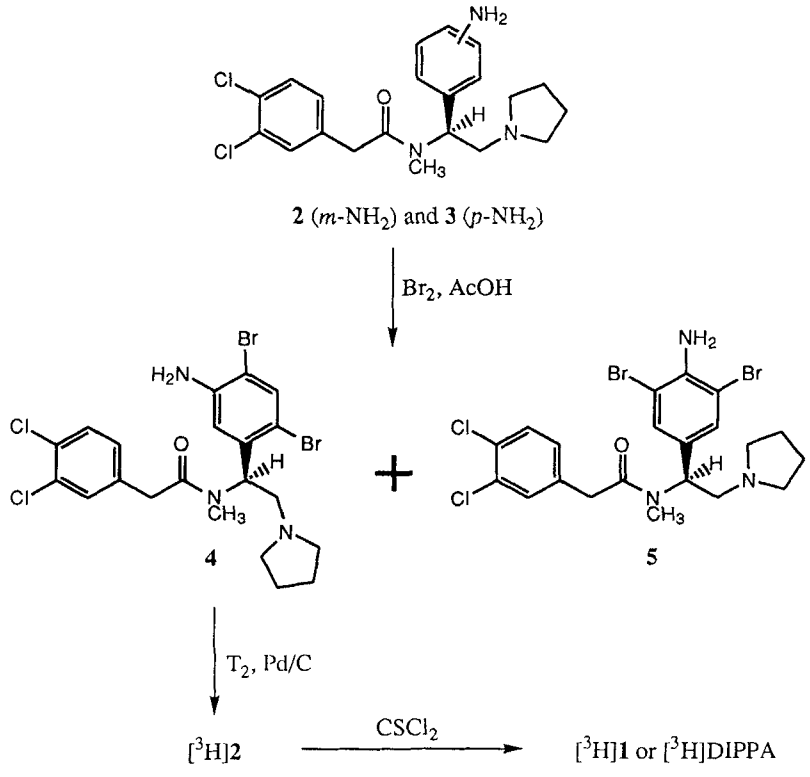
Scheme 1



Previously, we reported 2-(3,4-dichlorophenyl)-N-methyl-N-[(1S)-1-(3-isothiocyanatophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide (**1**, DIPPA) to be an opioid receptor affinity label that produces selective and long-lasting κ antagonism in mice.[5,6] In addition, DIPPA exhibits biological activities that are superior to previously reported κ selective affinity labels.[7-12] While DIPPA is useful in pharmacologic studies to delineate the functions of κ receptors, [^{3}H]DIPPA can be employed for determining the amino acid residues at the κ opioid receptor recognition site. Here we report the synthesis of high specific activity [^{3}H]DIPPA.

Results and Discussion

The preparation of [^{3}H]DIPPA began with the chromatographic separation of the bromination products of a mixture of regioisomers **2** and **3** (Scheme 1). Previously, **2** was obtained by crystallization from a mixture of the nitro precursors of **2** and **3** while the nitro precursor of **3** could not be obtained in regio- and enantiomerically pure form.[5] The bromination products **4** and **5**, however, were readily separable by column chromatography.

After treating a mixture of **2** and **3** with 2-4 equivalents of bromine in glacial acetic acid, the major components of the crude mixture were di-bromination products, **4** and **5**, which were obtained in 38-52% and 11.5% yields, respectively (Scheme 1).

The structural assignments were confirmed by catalytic hydrogenation of **4** and **5** to yield **2** and **3** in 96% and 60% yields, respectively, after chromatography. The ^{1}H NMR spectra of the hydrogenation products **2** and **3** are identical to those of authentic material previously prepared by other methods.[6]

Thus, the bromination products of **2** and **3** can be completely separated by normal chromatographic methods and then can be readily converted back to the starting material by catalytic hydrogenation. Furthermore, it was possible to selectively brominate only one of the phenyl groups as well as to selectively hydrogenolyze the aromatic bromides without affecting the aromatic chlorides. Although not yet fully investigated, this separation technique may find general application in the purification of activated aromatic regioisomers when the classical techniques fail to achieve separation.

Once the catalytic hydrogenation conditions were determined, **4** was tritiated to produce 98% radiochemically pure [^{3}H]**2** (specific activity = 39.7 Ci/mmol) in 40% yield after HPLC purification. Treatment of [^{3}H]**2** with thiophosgene produced 98% radiochemically pure [^{3}H]DIPPA in 18% yield after purification on preparative TLC.

Experimental

All NMR spectra were recorded on a GE 300MHz spectrometer at room temperature. Mass spectra were recorded by the Chemistry Mass Spec Labs at the University of Minnesota's Chemistry Department.

2-(3,4-Dichlorophenyl)-N-methyl-N-[(1S)-1-(5-amino-2,4-dibromophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide (4) and 2-(3,4-Dichlorophenyl)-N-methyl-N-[(1S)-1-(4-amino-3,5-dibromophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide (5). With stirring at 25 °C under Ar (g), a solution of Br_2 (0.63 mL, 12.23 mmol) in 20 mL of AcOH was added dropwise over 30 min to the solution of a mixture of **2** and **3** (1.6565 g, 4.0766 mmol) in 110 mL of AcOH and 1.14 mL of Et_3N. After 30 min, the reaction mixture was poured into a mixture of crushed ice and conc. NH_4OH (aq) (300 mL), and the aqueous fraction was extracted with $CHCl_3$ (600 mL), which was dried (Na_2SO_4), filtered through celite, and evaporated. The crude product was then gravity-column chromatographed eluting with $CHCl_3$: 2% NH_3: 2% MeOH to yield 0.8729 g of **4** (38%). The

fractions containing **5** were combined and gravity-column chromatographed again eluting with $CHCl_3$: 2% MeOH to yield 0.2648 g of **5** (11.5%). The yield of **5** is based on pure fractions obtained from column and is not optimized.

4·2HCl: ^1H NMR (DMSO-d_6-2 dr D_2O) δ 1.8-2.0 (m, 4H), 2.6 (s, 3H), 3.0-4.0 (complex, 8H), 5.80 (m, 1H), 6.81 (s, 1H), 7.17 (m, 1H), 7.41 (d, 1H, J = 1.8 Hz), 7.46 (d, 1H, J = 7.8 Hz), 7.56 (s, 0.6H), 8.10 (s, 0.18H). MS (FAB) m/z 564.0.

5 (free base): ^1H NMR ($CDCl_3$) δ 1.70 (br s, 4H), 2.65 (s, 3H), 2.4-2.7 (complex, 4H), 3.02 (m, 1H), 3.60-3.78 (m, 3H), 4.53 (br s, 2H), 5.90 (m, 1H), 7.10-7.12 (m, 1H), 7.25 (s, 2H), 7.30-7.36 (m, 2H). MS (FAB) m/z 564.1.

2-(3,4-Dichlorophenyl)-N-methyl-N-[(1S)-1-(5-amino-2,4-ditritiophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide ([^{3}H]2). A mixture of **4** (10 mg, 0.018 mmol) and 5 mg of 10% Pd/C in 2 mL of a 3% Et_3N/MeOH solution was treated with tritium gas at 25 °C and atmospheric pressure for 24 h. The reaction mixture was filtered, and the Pd/C was rinsed with EtOH. The crude product was purified by HPLC on a Zorbax RX-C8 column using 1% triethylammonium acetate pH 4/methanol (1:1) as the mobile phase. The mobile phase was removed under reduced pressure, and the product was redissolved in a 1% 0.1N HCl/EtOH solution. The purity of [^{3}H]**2** (285 mCi, 40%)was determined to be 98% pure when checked by HPLC using the above solvent system and by TLC on silica gel using $CHCl_3$:MeOH:NH_4OH (30:3:0.2) as elution solvent. Specific activity was determined by mass spec and found to be 39.7 Ci/mmol.

2-(3,4-Dichlorophenyl)-N-methyl-N-[(1S)-1-(5-isothiocyanato-2,4-ditritiophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide ([^{3}H]DIPPA or [^{3}H]1). To a solution of [^{3}H]**2** (85 mCi, 0.002 mmol) in 4 mL of $CHCl_3$ was added a solution of $NaHCO_3$ (2 mg) in 0.5 mL of H_2O. After stirring at 25 °C for 10 min, 0.2 mL (0.013 mmol) of a 65 mM solution of $CSCl_2$ in $CHCl_3$ was added, and the resulting mixture was stirred at 25 °C for 4 h. The mixture was diluted with 10 mL of $CHCl_3$ and stirred vigorously for 10 min before the phases were allowed to separate and the organic layer was drawn off. After drying (Na_2SO_4) and filtering through celite, the solvent was removed under reduced pressure, and the crude product was purified by preparative TLC on 1 x 250 μm silica gel plate using $CHCl_3$:acetone (85:15) as the elution solvent. The band corresponding to product was scraped off and eluted with acetone to recover the product, which was converted to the HCl salt with

Et_2O:HCl and stored as a solution in CH_3CN. [^{3}H]DIPPA (15 mCi, 18%) was determined to be 98% pure when checked by HPLC on a Zorbax ODS column with 1% triethylammonium acetate pH4/CH_3CN (1:1) as the mobile phase and by TLC on silica gel eluting with $CHCl_3$:acetone (85:15).

References

1. Simon, E. J.; Gioannini, T. L.- Opioid Receptor Multiplicity: Isolation, Purification, and Chemical Characterization of Binding Sites. In *Opioids I*; Herz, A., Ed.; Springer-Verlag: Berlin, 1993; pp 3- 26.

2. Cooper, S. J.; Jackson, A.; Kirkham, T. C.- Pharmacol. Biochem. Behavior 23: 889 (1985)

3. Kohl, R. L.; MacDonald, S.- J. Clin. Pharmacol. 31: 934 (1991)

4. Rees, D. C.- Prog. Med. Chem. 29: 109 (1992)

5. Chang, A.-C.; Takemori, A. E.; Portoghese, P. S.- J. Med. Chem. 37: 1547 (1994)

6. Chang, A.-C.; Takemori, A. E.; Ojala, W. H.; Gleason, W. B.; Portoghese, P. S.- J. Med. Chem.: in press.

7. de Costa, B. R.; Rothman, R. B.; Bykov, V.; Jacobson, A. E.; Rice, K. C.- J. Med. Chem. 32: 281 (1989)

8. de Costa, B. R.; Rothman, R. B.; Bykov, V.; Band, L.; Pert, A.; Jacobson, A. E.; Rice, K. C.- J. Med. Chem. 33: 1171 (1990)

9. de Costa, B. R.; Band, L.; Rothman, R. B.; Jacobson, A. E.; Bykov, V.; Pert, A.; Rice, K. C.- FEBS Lett. 249: 178 (1989)

10. Cheng, C.-Y.; Wu, S.-C.; Hsin, L.-W.; Tam, S. W.- J. Med. Chem. 35: 2243 (1992)

11. Weerawarna, S. A.; Davis, R. D.; Nelson, W. L.- J. Med. Chem. 37: 2856 (1994)

12. Horan, P.; de Costa, B. R.; Rice, K. C.; Porreca, F.- J. Pharmacol. Exp. Ther. 257: 1154 (1991).