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Research Article

Synthesis of ^{14}C-labeled 4-hydroxyindole as an intermediate for the preparation of (*S*)-2-[4-[2-[3-(indol-2-[^{14}C]-4-yloxy)-2-hydroxypropylamino]-2-methylpropyl]-phenoxy]pyridine-5-carboxamide (LY368842-[indole-^{14}C]) glycolate

Boris A. Czeskis*, Dean K. Clodfelter and William J. Wheeler
Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285, USA

Summary

Synthesis of 4-hydroxyindole labeled with ^{14}C at the 2-position was accomplished based on the vicarious nucleophilic substitution reaction of benzyl-protected 3-nitrophenol with *p*-chlorophenoxyacetonitrile-[1-^{14}C]. This was followed by the reductive cyclization of *o*-nitrocyanomethyl derivative by palladium catalyzed hydrogenation. *p*-Chlorophenoxyacetonitrile-[1-^{14}C] was prepared from commercially available *p*-chlorophenoxymethyl chloride and sodium cyanide-[^{14}C]. 4-Hydroxyindole-[2-^{14}C] was used for the synthesis of ^{14}C-labeled β_3 adrenergic agonist LY368842. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: 4-hydroxyindole; β_3 adrenergic agonist; LY368842 glycolate; carbon-14-labeled

*Correspondence to: B. A. Czeskis, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285, USA. E-mail: czeskis@lilly.com

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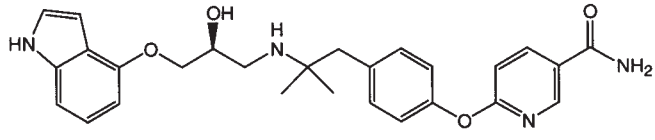
Introduction

4-Hydroxyindole is a key intermediate in the synthesis of a number physiologically active compounds[1] including pindolol[2] and psilocin.[3] We were interested in the preparation of ^{14}C-labeled 4-hydroxyindole to be used in the synthesis of radiolabeled β_3 adrenergic agonist LY368842, a compound with a potential for the treatment of obesity and diabetes.[4]

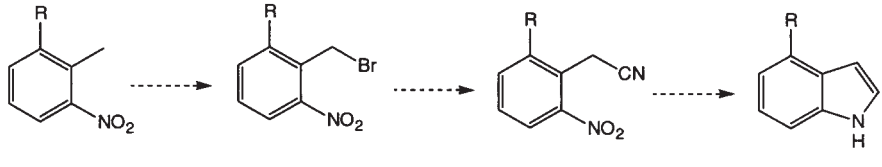


LY368842

There are different methods of the synthesis of 4-hydroxyindole presented in literature. Among them are electrochemical couplings of 1,3-cyclohexadione with ethyl vinyl ether,[5] and 2,6-dinitrotoluene with formaldehyde,[6] the Leimgruber-Batcho procedure,[7] and palladium catalyzed *N*-heteroannulation of styrenes.[8] However, most of these methods are not suitable for the preparation of the radiolabeled compound. We report herein an efficient way for the synthesis of 4-hydroxyindole-[2-^{14}C] which was used for the preparation of β_3 adrenergic agonist LY368842.

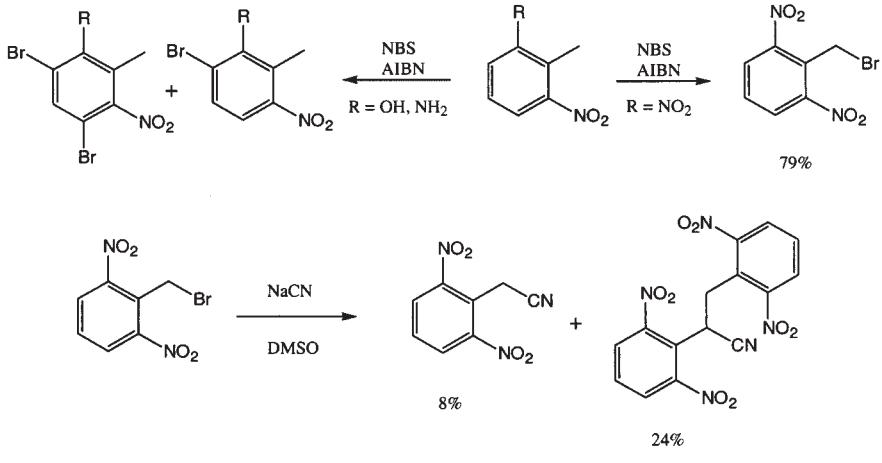
Results and discussion

Our initial attempt to prepare ^{14}C-labeled 4-hydroxyindole consisted of the attractive possibility of allylic bromination followed by cyanation of 6-substituted 2-nitrotoluenes. The resulting *o*-nitrosubstituted benzyl cyanides can be converted into 4-substituted indole by reductive cyclization.[9]



We found that the results of bromination of 2-nitrotoluenes were dependent on the nature of the substituent in the 6-position. Thus,

hydroxy and aminotoluenes formed aromatic bromination products only, whereas 2,6-dinitrotoluene was selectively converted into the desired bromomethyl derivative. The next step of cyanation, however, proved to be problematic because the starting benzyl bromide reacted with initially formed benzyl cyanide faster than with sodium cyanide. This resulted in preferable formation of the dimer (Scheme 1). Lowering the pH of the reaction with trifluoroacetic acid, previously used for the preparation of other 2-nitrobenzyl cyanides,[10] gave a mixture of the above compounds and trifluoroacetyl derivatives.

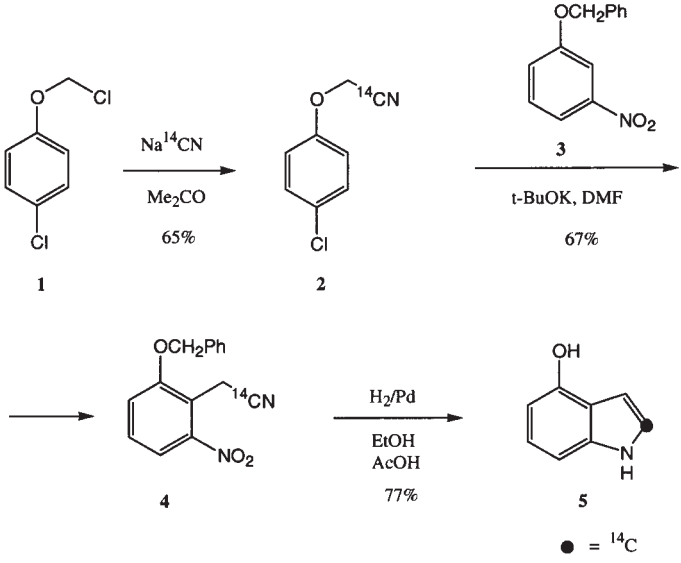


Scheme 1.

Another way to obtain 2-nitrobenzyl cyanides is the introduction of a cyanomethyl group into nitrobenzenes using vicarious nucleophilic substitution of an *ortho*-hydrogen with the anion generated from *p*-chlorophenoxyacetonitrile.[11] *p*-Chlorophenoxyacetonitrile can be obtained by the reaction of *p*-chlorophenol with chloroacetonitrile,[12] an expensive ^{14}C-labeled material. We found that *p*-chlorophenoxyacetonitrile-[1-^{14}C] (**2**) can be prepared from commercially available *p*-chlorophenoxymethyl chloride (**1**) and sodium cyanide-[^{14}C] in acetone. Surprisingly, the addition of sodium iodide[13] lowers the yield of **2** by leading to the partial formation of *p*-chlorophenol. This was the only product formed when the reaction was conducted in dimethylsulfoxide. Alkylation of benzyl-protected 3-nitrophenol (**3**) (prepared straightforwardly from 3-nitrophenol, benzyl bromide and potassium carbonate in acetone) with the anion derived from nitrile **2** in the presence of potassium *tert*-butoxide in dimethylformamide, afforded 2-(2-benzy-

loxy-6-nitrophenyl)acetonitrile-[1-^{14}C] (**4**). Reductive cyclization of **4** and simultaneous deprotection of the hydroxy-group, using hydrogenation on palladium catalyst in the presence of acetic acid,[11b] proceeded smoothly to give 4-hydroxyindole-[2-^{14}C] (**5**) in good yield (Scheme 2).

The synthesis of LY368842-[indole-^{14}C] glycolate from 4-hydroxyindole-[2-^{14}C] (**5**) was accomplished based on the previously developed procedure.[4] Thus, reaction of **5** with (*S*)-glycidyl nosylate (**6**) in the presence of potassium carbonate gave (*S*)-glycidyloxyindole (**7**), which was coupled with amine **8** to afford the target radiolabeled compound LY368842-[indole-^{14}C], and subsequently its glycolate (Scheme 3).

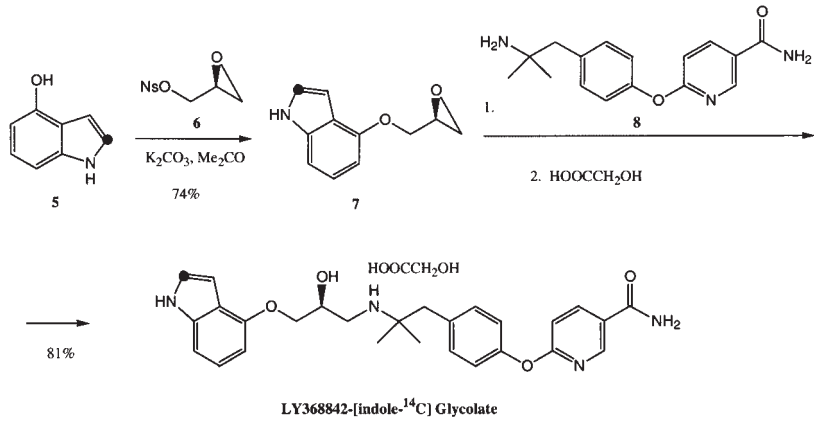


Scheme 2.

The method described above allowed us to obtain LY368842-[indole-^{14}C] glycolate with 98.4% radiochemical purity and a specific activity of 60.4 μCi/mg in six steps from commercially available *p*-chlorophenoxymethyl chloride and sodium cyanide-[^{14}C] in total 20% chemical and 17.4% radiochemical yields.

Experimental

The sodium cyanide-[^{14}C] was purchased from American Radiolabeled Chemicals Inc. The NMR spectra were obtained on a Varian



Scheme 3.

Mercury−400 at 400 (^{1}H) and 100 (^{13}C) MHz. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane. Flash chromatography was performed as described by Still *et al.*,[14] using silica gel 60 (230–400 mesh). Unless otherwise noted, HPLC was conducted on a Hitachi instrument with UV detection at 230 nm; a Zorbax C8 column (4.6 mm × 25 cm); isocratic elution with a mobile phase consisting of 50% aqueous 0.5% monobasic ammonium phosphate and 50% acetonitrile at a flow rate of 1 ml/min. TLC was performed on precoated plates of silica gel 60 F_{254}.

2-(4-Chlorophenoxy)acetonitrile-[1-^{14}C], 2

To a solution of α,4-dichloroanisole (**1**) (685 mg, 3.87 mmol) in acetone (4 ml) was added sodium cyanide-[^{14}C] (150 mCi, 55 mCi/mmol, 2.73 mmol). The reaction mixture was heated at 60–65°C (bath) for 30 min whereupon sodium cyanide (56 mg, 1.14 mmol) was added, and the mixture was further heated at the same temperature for 5.5 h. After cooling to room temperature the mixture was evaporated under vacuum. The residue was diluted with water (3 ml) and extracted with ethyl ether (30 ml). The extract was washed with saturated aqueous sodium chloride (2 × 5 ml). The combined aqueous layer was re-extracted with ethyl ether (10 ml). The combined organic extract was dried over sodium sulfate, and evaporated under vacuum. The residue was subjected to flash chromatography, eluting with ethyl acetate/hexanes (15:85) to give nitrile **2** (428 mg, 65%) as a colorless oil. TLC:

R_f = 0.39 (20% of ethyl acetate in hexanes). The compound co-eluted with an authentic sample of 2-(4-chlorophenoxy)acetonitrile using the above TLC conditions. For non-radioactive compound (prepared in model experiment): ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.71 (s, 2 H), 6.88 (d, J = 8.8 Hz, 2 H), 7.27 (d, J = 8.8 Hz, 2 H).

O-Benzyl-3-nitrophenol, 3

A mixture of 3-nitrophenol (4.0 g, 28.8 mmol), benzyl bromide (3.8 ml, 31.9 mmol), and potassium carbonate (12.0 g, 86.8 mmol) in acetone (55 ml) was stirred at room temperature for 16 h, and evaporated under vacuum. The residue was diluted with water (20 ml) and extracted with ethyl ether (50 ml). The extract was washed with saturated aqueous sodium chloride (2 × 10 ml), dried over sodium sulfate, and evaporated under vacuum. The residue was subjected to flash chromatography, eluting with ethyl acetate/hexanes (10:90) to give benzyl ether **3** (6.29 g, 95%) as a light yellow solid. TLC: R_f = 0.40 (10% of ethyl acetate in hexanes). ^{1}H-NMR ($CDCl_3$, δ, ppm): 5.10 (s, 2 H), 7.22–7.42 (m, 7 H), 7.77–7.81 (m, 2 H).

2-(2-Benzyloxy-6-nitrophenyl)acetonitrile-[1-^{14}C], 4

To a solution of potassium *tert*-butoxide (597 mg, 5.32 mmol) in dimethylformamide (5 ml) at 0–5°C (ice bath) was added a solution of phenoxyacetonitrile **2** (428 mg, 2.53 mmol) and nitroarene **3** (610 mg, 2.66 mmol) in dimethylformamide (2.3 ml). The dark blue reaction mixture was stirred for 1 h in an ice bath, poured onto a mixture of aqueous hydrochloric acid (1N, 11 ml) and ice (ca. 2 g), and extracted with ethyl acetate (20 ml × 3). The extract was washed with saturated aqueous sodium chloride (2 × 10 ml), and the combined aqueous layer was re-extracted with ethyl acetate (15 ml). The combined organic extract was dried over sodium sulfate, and evaporated under vacuum. The residue was subjected to flash chromatography, eluting with ethyl acetate/hexanes (30:70) to give arylacetonitrile-**4** (459 mg, 67%) as a light yellow solid. TLC: R_f = 0.34 (30% of ethyl acetate in hexanes). The compound co-eluted with an authentic sample of 2-(2-benzyloxy-6-nitrophenyl)acetonitrile by HPLC and TLC under the above conditions. For non-radioactive compound[11b] (prepared in model experiment): ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.00 (s, 2 H), 5.19 (s, 2 H), 7.21–7.44 (m, 7 H), 7.62 (dd, J = 8.3 and 1.0 Hz, 1 H).

4-Hydroxyindole-[2-^{14}C], 5

To a solution of nitrophenylacetonitrile **4** (459 mg, 1.7 mmol) in a mixture of ethanol (9.0 ml), acetic acid (1.4 ml) and ethyl acetate (2.5 ml) was added 10% palladium on carbon (46 mg). The resulting suspension was evacuated and re-filled with hydrogen gas 3 times, followed by stirring under an atmosphere of hydrogen (balloon) for 18 h. The reaction mixture was diluted with ethyl acetate (15 ml), filtered through Hyflo, and evaporated under vacuum. The residue was subjected to flash chromatography, eluting with ethyl acetate/hexanes (30:70) to give 4-hydroxyindole-[2-^{14}C] (**5**) (177 mg, 77%) as a light yellow solid. TLC: R_f = 0.32 (30% of ethyl acetate in hexanes). The compound co-eluted with an authentic sample of 4-hydroxyindole by HPLC and TLC under the above conditions. For non-radioactive compound (prepared in model experiment): ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.97 (br. s, 1 H), 6.48 (dd, J = 7.3 and 1.0 Hz, 1 H), 6.56 (m, 1 H), 6.95–7.03 (m, 2 H), 7.11 (m, 1 H), 8.13 (br. s, 1 H).

(S)-4-(2,3-Epoxypropyloxy)indole-[2-^{14}C], 7

To a solution of 4-hydroxyindole-[2-^{14}C] (**5**) (177 mg, 1.31 mmol) in acetone (12 ml) were added nosylate **6** (400 mg, 1.54 mmol) and potassium carbonate (530 mg, 3.83 mmol). The reaction mixture was heated at 60–65°C (bath) for 20 h, diluted with ethyl acetate (15 ml), filtered through Hyflo, and evaporated under vacuum. The residue was subjected to flash chromatography, eluting with ethyl acetate/hexanes (30:70) to give epoxide **7** (186 mg, 74%) as a white solid. TLC: R_f = 0.43 (30% of ethyl acetate in hexanes). The compound co-eluted with an authentic sample of (*S*)-4-(2,3-epoxypropyloxy)indole by HPLC and TLC under the above conditions. For non-radioactive compound (prepared in model experiment): ^{1}H-NMR ($CDCl_3$, δ, ppm): 2.78 (dd, J = 4.9 and 2.9 Hz, 1 H), 2.90 (m, 1 H), 3.42 (m, 1 H), 4.12 (dd, J = 11.2 and 5.6 Hz, 1 H), 4.31 (dd, J = 11.2 and 3.4 Hz, 1 H), 6.49 (m, 1 H), 6.65 (m, 1 H), 6.97–7.11 (m, 3 H), 8.14 (br. s, 1 H).

(S)-2-[4-[2-[3-(Indol-2-[^{14}C]-4-yloxy)-2-hydroxypropylamino]-2-methylpropyl]phenoxy]pyridine-5-carboxamide, (LY368842-[indole-^{14}C])

To a mixture of epoxide **7** (186 mg, 0.97 mmol) in 2-propanol (9 ml) was added 2-[4-(2-amino-2-methylpropyl)phenoxy]pyridine-5-carboxamide

(**8**)[4] (330 mg, 1.16 mmol) in one portion. The reaction mixture was heated at 80–83°C for 16 h and evaporated under vacuum. The residue was subjected to flash chromatography, eluting with one column volume of chloroform/methanol (90:10) following by three column volumes of chloroform/methanol/ammonium hydroxide (90:10:1) to give LY368842-[indole-^{14}C] (378 mg, 82%) as a pale solid. TLC: R_f = 0.31 (chloroform/methanol/ammonium hydroxide 90:10:1). The compound co-eluted with an authentic sample of LY368842 by HPLC and TLC under the above conditions. For non-radioactive compound (prepared in model experiment): ^{1}H-NMR (CD_3OD, δ, ppm): 1.10 (d, J = 3.4 Hz, 6 H), 2.75 (d, J = 5.4 Hz, 2 H), 2.85 (m, 1 H), 2.99 (m, 1 H), 4.09 (m, 3 H), 6.45 (dd, J = 4.9 and 3.4 Hz, 1 H), 6.49 (d, J = 3.4 Hz, 1 H), 6.88–6.94 (m, 5 H), 7.05 (d, J = 2.9 Hz, 1 H), 7.23 (d, J = 8.8 Hz, 2 H), 8.18 (dd, J = 8.8 and 2.4 Hz, 1 H), 8.57 (d, J = 2.4 Hz).

(S)-2-[4-[2-[3-(Indol-2-[^{14}C]-4-yloxy)-2-hydroxypropylamino]-2-methylpropyl]phenoxy]pyridine-5-carboxamide glycolate, (LY368842-[indole-^{14}C]) glycolate

To a solution of free base LY368842-[indole-^{14}C] (215 mg, 0.45 mmol) in ethanol (1.4 ml) was added a solution of glycolic acid (35 mg, 0.46 mmol) in ethanol (1.0 ml). The reaction mixture was stirred for 30 min at room temperature and evaporated under vacuum. The residue was triturated with ethyl ether (3 × 10 ml), and the supernatant liquid was decanted each time. After drying under vacuum, LY368842-[indole-^{14}C] glycolate (246 mg, 99%) was obtained as a white solid. The radiochemical purity was determined to be 98.4% by radio-HPLC: Zorbax SB-Phenyl column (4.6 × 250 mm); flow rate: 1.0 ml/min; column temperature: 40°C; detection: UV 260 nm; mobile phase: methanol/0.1% trifluoroacetic acid. The specific activity of the product was 60.4 μCi/mg. The compound co-eluted with an authentic sample of LY368842 glycolate by HPLC under the above conditions. For non-radioactive compound (prepared in model experiment): ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.01 (d, J = 3.4 Hz, 6 H), 2.70 (s, 2 H), 2.82 (m, 1 H), 2.95 (m, 1 H), 3.72 (s, 2 H), 4.01 (m, 3 H), 6.38–6.43 (m, 2 H), 6.88–6.99 (m, 5 H), 7.14 (m, 1 H), 7.18 (d, J = 8.8 Hz, 2 H), 7.42 (s, 1 H), 7.97 (s, 1 H), 8.18 (dd, J = 8.3 and 2.4 Hz, 1 H), 8.56 (d, J = 2.4 Hz, 1 H), 11.00 (s, 1 H).

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