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Research Article

Synthesis of β_3 adrenergic receptor agonist LY377604 and its metabolite 4-hydroxycarbazole, labeled with carbon-14 and deuterium

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Summary

Synthesis of ^{14}C-radiolabeled 4-hydroxycarbazole was accomplished starting from aniline-[U-^{14}C], based on zinc chloride initiated Fischer cyclization of the phenylhydrazone prepared from phenylhydrazine-[U-^{14}C] and cyclohexane-1,3-dione. The resulting tetrahydrooxocarbazole was subjected to dehydrogenation–aromatization using palladium on carbon. The aromatized 4-hydroxycarbazole-[4b,5,6,7,8,8a-^{14}C] was then used for the synthesis of ^{14}C-labeled β_3 adrenergic receptor agonist LY377604. The introduction of four deuteria in the carbazole fragment of LY377604 accomplished by its initial bromination and subsequent catalytic deuteration of the resulting tetrabromide. A similar approach was used for the conversion of 4-hydroxycarbazole into its tetradeutero-isotopomer.

Key Words: 4-hydroxycarbazole; β_3 adrenergic agonist; LY377604 succinate; carbon-14-labeled; deuterium-labeled

Introduction

Compound **1**, (4-hydroxycarbazole) is a key intermediate in the synthesis of a number of physiologically active compounds[1] including carvedilol[2] and carazolol.[3] We were interested in the preparation of ^{14}C-labeled 4-hydroxycarbazole to be used in the synthesis of radiolabeled β_3 adrenergic agonist **LY377604**, a compound with a potential for the treatment of obesity and diabetes[4] (Figure 1). Stable labeled isotopomers of **LY377604** and its putative metabolite 4-hydroxycarbazole (**1**) were also required as internal standards for bioanalytical studies.

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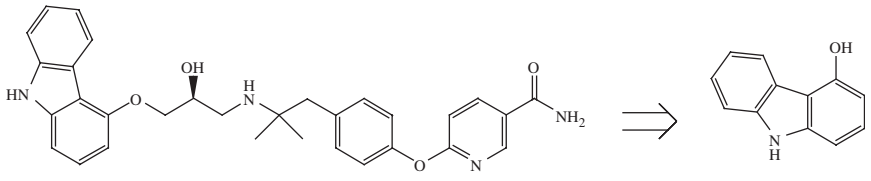


LY377604 **1**

Figure 1.

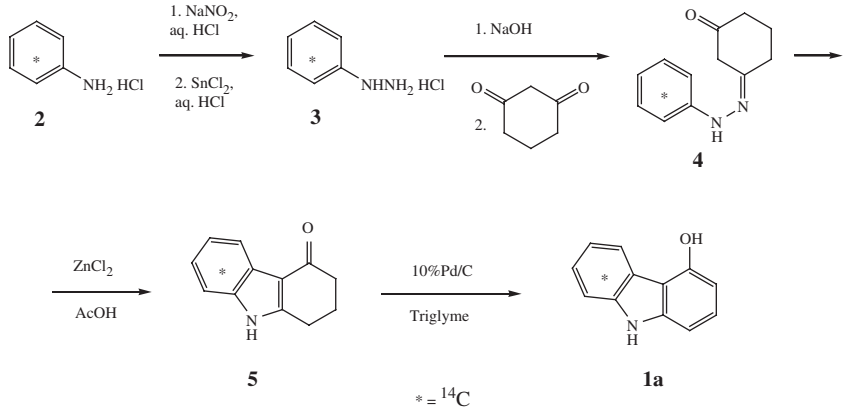
We describe herein the syntheses of radiolabeled and deuterium-labeled **LY377604** and 4-hydroxycarbazole.

Results and discussion

Synthesis of ^{14}C-labeled compounds

There are few methods for the synthesis of 4-hydroxycarbazole (**1**) presented in the literature, including the coupling of 2-bromonitrobenzene with 2-iodoanisole in the presence of copper,[5] bacterial oxygenation of heteroaromatic precursors,[6] and cyclocarbonylation of indole derivatives.[7] However, the most convenient approach for the preparation of 4-hydroxycarbazole seems to be the classical Fischer indole synthesis.[3a,8] Some challenges and findings during the application of this method for the synthesis of ^{14}C-labeled 4-hydroxycarbazole, and following preparation of **LY377604**-[**^{14}C**] are presented below.

The synthesis of ^{14}C-labeled 4-hydroxycarbazole (**1a**) is shown in Scheme 1. Diazotization of aniline-[U-^{14}C] hydrochloride (**2**) and subsequent reduction of the resulting diazonium salt smoothly gave ^{14}C-labeled phenylhydrazine hydrochloride (**3**) when tin (II) chloride was used as a reducing agent.[9] Use of sodium sulfite[10] resulted in significantly lower yield of **3**. The condensation of hydrochloride **3** with cyclohexane-1,3-dione in the presence of acetic acid[11] or in aqueous media was not successful suggesting that the generation of a free base from **3** was necessary. Indeed, when phenylhydrazine obtained by the alkaline treatment of hydrochloride **3**, was reacted with cyclohexane-1,3-dione, the hydrazone **4** was obtained in satisfactory yield. Initial attempt at the cyclization of the phenylhydrazone **4** using sulfuric acid[11,12] gave just trace quantities of the desired tetrahydrooxocarbazole **5**. A somewhat better result was obtained using 85% phosphoric acid. A considerably higher yield of **5** was achieved when zinc chloride in acetic acid[3a,8a] was used to initiate the cyclization. The last step in the preparation of 4-hydroxycarbazole-[^{14}C] (**1a**) was dehydrogenation–aromatization of the tetrahydroprecursor **5**. We investigated three reagents known from the literature for achieving such a goal: 2,3-dichloro-1,3-dicyano-1,4-benzoquinone (DDQ),[13] Raney nickel,[3a,8a]



Scheme 1.

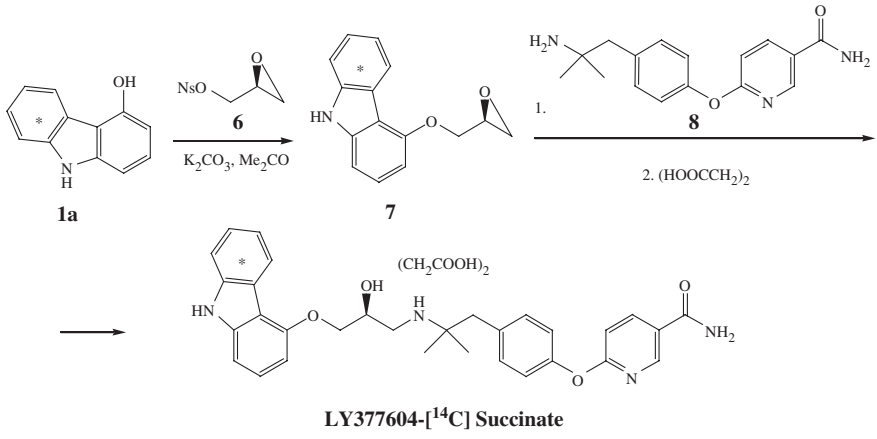
and palladium on carbon.[14] In our hands the best, although not very reproducible, results were achieved using 10% palladium on carbon in triglyme at 200–220°C. In model experiments non-radiolabeled 4-hydroxycarbazole was obtained in up to 90% yields, whereas the dehydrogenation of ^{14}C-labeled compound **5** resulted in poor yields of **1a** (10 and 35% after two attempts, correspondingly).

The synthesis of **LY377604**-[**^{14}C**] succinate from 4-hydroxycarbazole-[^{14}C] (**1a**) was accomplished based on the previously developed procedure.[4] Thus, reaction of **1a** with (*S*)-glycidyl nosylate (**6**) in the presence of potassium carbonate gave (*S*)-glycidyloxycarbazole (**7**). The epoxide **7** was coupled with amine **8** to afford the target radiolabeled compound **LY377604**-[**^{14}C**], and subsequently its succinate (Scheme 2).

The method described above allowed us to obtain 4-hydroxycarbazole-[4b,5,6,7,8,8a-^{14}C] (**5**) and then convert it into **LY377604**-[**^{14}C**] succinate with 97.5% radiochemical purity in six steps from commercially available aniline-[U-^{14}C] hydrochloride.

Synthesis of deuterium-labeled compounds

In order for an isotopomer to be a good internal standard in mass spectrometry it should have a mass of at least four mass units higher than the parent compound. Our goal was to introduce four deuteria into the molecule of **LY377604** through initial aromatic bromination of this compound, followed by catalytic reduction of the resulting polybromide with deuterium. The known examples of halogenation of structurally similar compounds include carvedilol bromination on microscale using bromine and potassium carbonate in chloroform to give tribromide isolated by preparative HPLC,[15] and the bromination of carbazole itself with *N*-bromosuccinimide on silica gel to provide dibromide as a main product.[16]

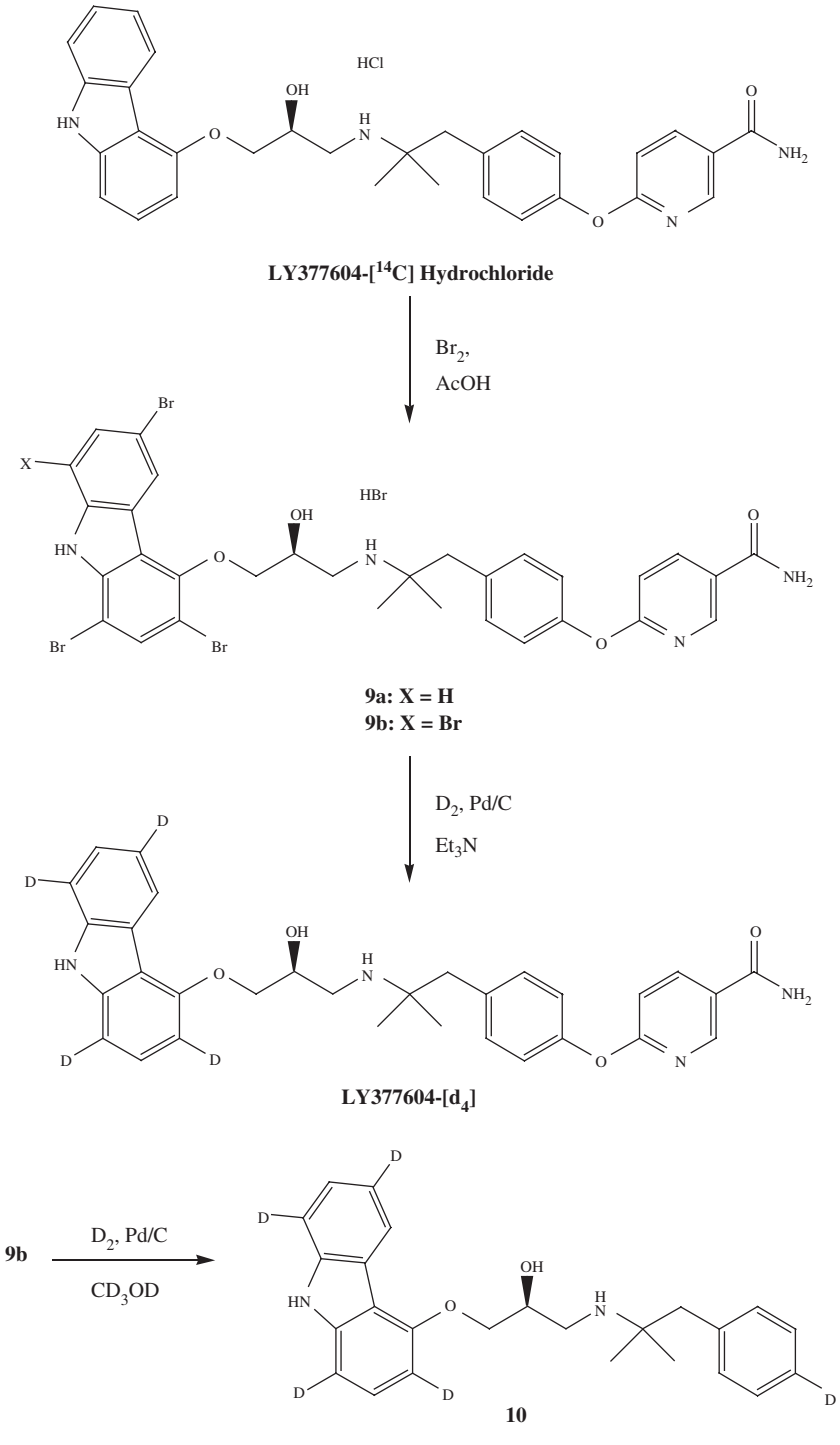


LY377604-[^{14}C] Succinate

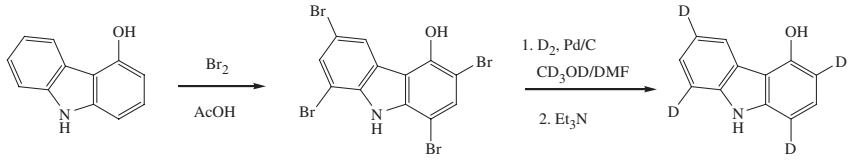
Scheme 2.

In an attempt to reach a high level of bromine incorporation in the molecule of **LY377604**, we investigated its bromination with excess of bromine under acidic conditions. When hydrochloride salt of **LY377604** was treated with four equivalents of bromine at room temperature the reaction provided mainly 1,3,6-tribromosubstituted carbazole derivative **9a**. It took almost 10 equivalents of bromine to push the reaction further to form 1,3,6,8-tetrabromo-derivative **9b** (Scheme 3). Surprisingly, the bromination occurred exclusively onto the carbazole fragment even though two other aromatic rings were present in the molecule.

The next step, catalytic reduction of tetrabromide **9b**, was performed using atmospheric pressure of deuterium in the presence of palladium on carbon and triethylamine in dimethylformamide. According to mass spectral data (ES+), the reaction provided a mixture of D_4, D_3, D_2, and D_1 isotopomers of **LY377604** in a ratio 7:15:4:1. Although the reason for the isotopic exchange remains unclear, the obvious approach to overcome this problem was the elimination of any possible proton donors. Generation of free base (**9c**) from the hydrobromide salt **9b** and following its re-evaporation with methanol-d_4 would exclude internal proton source (HBr, NH, NH_2); however deuteration of the resulting material did not provide better results. The ratio of D_4, D_3, D_2, D_1 isotopomers was 3:9:4:1. Considerable improvement in the isotopic ratio was achieved when we turned our attention to the catalyst as a possible external proton source. To remove hydrogen, possibly contained in the palladium on carbon, the catalyst was pre-activated by triple evacuating and re-filling with deuterium. As a result, the deuteration reaction gave D_4, D_3, D_2 isotopomers in a ratio 46:9:1, and no D_1 isotopomer was detected. Surprisingly, changing the solvent from dimethylformamide to methanol-d_4 led to the formation of D_5-compound **10** resulting from the reductive cleavage *O*-aromatic bond, an unprecedented reaction to the best of our knowledge.



Scheme 3.



1 **11** **1b**

Scheme 4.

The bromination of 4-hydroxycarbazole (**1**) was performed under conditions similar to those described above for **LY377604** (Scheme 4). The resulting 1,3,6,8-tetrabromo-4-hydroxycarbazole (**11**) appeared to be very unstable under the basic conditions required for the catalytic reduction. The addition of triethylamine to a solution of **11** caused formation of a dark mixture, and no desired product was detected after the deuteration. We found that the reaction could be accomplished successfully if triethylamine was added after a few hours of deuteration in the presence of 10% palladium on carbon. The best isotopic ratio of the resulting product **1b** ($D_4/D_3/D_2$, 6:4:1) was achieved when the mixture of methanol-d_4 and dimethylformamide was used as a solvent. In dimethylformamide itself this ratio was 2.7:2.4:1.

Experimental

The aniline-[U-^{14}C] hydrochloride was purchased from American Radiolabeled Chemicals, Inc. The NMR spectra were obtained on a General Electric QE-300 at 300 (^{1}H) and 75 (^{13}C) MHz, and on Varian mercury-400 at 400 (^{1}H) and 100 (^{13}C) MHz. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane. Mass spectra were obtained on Agilent 1100 spectrometer. Microanalytical, IR, and UV data were provided by Physical Chemistry Department of Lilly Research Laboratories. Flash chromatography was performed using silica gel 60 (230–400 mesh) or Biotage Flash System. TLC was performed on precoated plates of silica gel 60 F_{254}. HPLC was conducted on a Hitachi instrument with UV detection at 220 nm; a Zorbax C8 column (4.6 mm × 25 cm); isocratic elution with a mobile phase consisting of aqueous 0.5% monobasic ammonium phosphate and acetonitrile with ratio 65:35 (conditions A), or 55:45 (conditions B), or 45:55 (conditions C), or UV detection at 230 nm; a Zorbax RX-C8 column (4.6 mm × 25 cm); gradient elution (time, ratio 0.013 N trifluoroacetic acid to acetonitrile): 0 min, 60:40; 6 min, 60:40; 7 min, 50:50; 20 min, 50:50; 20.1 min, 60:40; 30 min, 60:40 (conditions D) at a flow rate of 1 ml/min.

Phenylhydrazine-[U-^{14}C] hydrochloride, **3**

To a solution of aniline-[U-^{14}C] hydrochloride (**2**) (55.3 mCi/mmol, 250 mCi, 4.52 mmol) and aniline hydrochloride (710 mg, 5.48 mmol) in water (3 ml) and

concentrated hydrochloric acid (3 ml) at 0–5°C (ice bath) was added a solution of sodium nitrite (800 mg, 11.6 mmol) in water (2.3 ml) dropwise over the period of 15 min. The reaction mixture was stirred for 1 h, and a solution of tin(II) chloride (4.0 g, 21.1 mmol) in conc. hydrochloric acid (5 ml) was added dropwise. After 2 h a bulky precipitate was collected, washed with water (5 ml), ethanol (3 ml), ethyl ether (10 ml), and dried under vacuum to give hydrochloride **3** (1.165 g, 80%) as a reddish solid. For the non-radioactive compound (prepared in a model experiment): ^{1}H-NMR (DMSO-d_6 + D_2O, δ, ppm): 6.99 (br. d, $J = 7$ Hz, 3H), 7.30 (t, $J = 7$ Hz, 2H).

Cyclohexane-1,3-dione monophenylhydrazone-[phenyl-^{14}C], **4**

To a suspension of hydrochloride **3** (1.165 g, 7.98 mmol) in water (12 ml) was added aqueous sodium hydroxide (5 N, 1.65 ml, 8.25 mmol). The resulting mixture was added to a solution of cyclohexane-1,3-dione (900 mg, 8.0 mmol) in water (12 ml) dropwise over the period of 35 min. The reaction mixture was stirred for 2 h at room temperature. The precipitate was filtered off and dried under vacuum at 35°C to give hydrazone **4** (666 mg, 41%) as a brown solid. TLC: $R_f = 0.41$ (ethyl acetate/ethanol, 9:1). HPLC (conditions A): $R_t = 6$ min. The compound co-eluted with an authentic sample of cyclohexane-1,3-dione monophenylhydrazone by HPLC and TLC under the above conditions. For the non-radioactive compound (prepared in a model experiment): ^{1}H-NMR ($CDCl_3$, δ, ppm): 2.0 (m, 2H), 2.3–2.7 (m, 6H), 6.80 (d, $J = 7$ Hz, 2H), 6.95 (t, $J = 7$ Hz, 1H), 7.22 (d, $J = 7$ Hz, 2H).

1,2,3,4-Tetrahydro-9H-carbazol-4-one-[4b,5,6,7,8,8a-^{14}C], **5**

To a solution of zinc chloride (3.29 g, 24.1 mmol) in acetic acid (3.3 ml) at 70°C (bath) was added hydrazone **4** (666 mg, 3.26 mmol) in one portion. The reaction mixture was heated at 105°C (bath) for 4.5 h, then cooled to 70°C and diluted with water (3.7 ml). After further cooling to room temperature, the reaction mixture was poured into water (16 ml). The precipitate was collected by filtration, washed with water (5 ml), and dried under vacuum to give ketone **5** (317 mg, 52%) as a dark solid. TLC: $R_f = 0.79$ (ethyl acetate/ethanol, 9:1), 0.21 (hexane/ethyl acetate, 1:1). HPLC (conditions A): $R_t = 8$ min, HPLC (conditions B): $R_t = 5$ min. The compound co-eluted with an authentic sample of 1,2,3,4-tetrahydro-9H-carbazol-4-one by HPLC and TLC under the above conditions. For the non-radioactive compound (prepared in a model experiment): ^{1}H-NMR ($CDCl_3$, δ, ppm): 2.13 (t, $J = 4$ Hz, 2H), 2.50 (m, 2H), 2.97 (t, $J = 6$ Hz, 2H), 7.16 (m, 2H), 7.39 (d, $J = 7$ Hz, 1H), 7.95 (d, $J = 7$ Hz, 2H).

4-hydroxy-9H-carbazole-[4b,5,6,7,8,8a-^{14}C], **1a**

A mixture of ketone **5** (317 mg, 1.7 mmol), 10% palladium on carbon (150 mg) and 1-dodecene (286 mg, 1.7 mmol) in triglyme (6.7 ml) was evacuated under vacuum and refilled with argon twice. The reaction mixture was stirred at 220°C (bath) for 21 h. HPLC showed that the ratio of starting material to product was 10:1. The mixture was filtered through Hyflo super cel. To the filtrate was added 10% palladium on carbon (125 mg), and the suspension was evacuated under vacuum and refilled with argon twice. The reaction mixture was heated at 220°C (bath) for additional 13 h. HPLC showed that the reaction was still incomplete. The mixture was filtered, fresh catalyst (100 mg) was added as before, and the heating was continued for 11 h. Most of the triglyme was evaporated under vacuum. The residue was diluted with ethyl acetate (50 ml), filtered, concentrated, and subjected to flash chromatography (eluting with hexane/ethyl acetate, 7:3) to obtain hydroxycarbazole **5** (32 mg, 10%) as light-yellow solid. Subsequent elution with ethyl acetate gave starting material (129 mg). For hydroxycarbazole **1a**, TLC: $R_f = 0.39$ (hexane/ethyl acetate, 7:3). HPLC (conditions B): $R_t = 8$ min. The compound co-eluted with an authentic sample of 4-hydroxy-9H-carbazole by HPLC and TLC under the above conditions. For the non-radioactive compound (prepared in a model experiment): ^{1}H-NMR ($CDCl_3$, δ, ppm): 5.39 (br. s, 1H), 6.63 (d, $J = 8$ Hz, 1H), 7.06 (d, $J = 8$ Hz, 1H), 7.30 (m, 2H), 7.45 (d, $J = 3.7$ Hz, 2H), 8.08 (br. s, 1H), 8.33 (d, $J = 8$ Hz, 1H).

In a separate experiment, starting from ketone **5** (410 mg, 2.19 mmol), 10% palladium on carbon (205 mg and then 120 mg) and 1-dodecene (369 mg, 2.19 mmol) in triglyme (8.6 ml), hydroxycarbazole **1a** (142 mg, 35%) was prepared.

(S)-3-(9H-carbazol-4-yloxy)-1,2-epoxypropane-[carbazole-4b,5,6,7,8,8a-^{14}C], **7**

A mixture of hydroxycarbazole **5** (32 mg, 0.173 mmol), (*S*)-glycidyl 3-nitrobenzenesulfonate (**6**) (51 mg, 0.197 mmol) and potassium carbonate (72 mg, 0.521 mmol) in acetone (1.5 ml) was refluxed for 9 h, and evaporated under vacuum. The residue was diluted with a warm solution of non-radioactive (*S*)-3-(9H-carbazol-4-yloxy)-1,2-epoxypropane (25 mg) in dichloromethane (0.5 ml). Flash chromatography of the resulting mixture (eluting with hexane/ethyl acetate, 7:3) gave epoxide **7** (59 mg, 82%) as a pale solid. TLC: $R_f = 0.39$ (hexane/ethyl acetate, 7:3). HPLC (conditions C): $R_t = 9$ min. The compound co-eluted with an authentic sample of (*S*)-3-(9H-carbazol-4-yloxy)-1,2-epoxypropane by HPLC and TLC under the above conditions. For the non-radioactive compound (prepared in a model experiment): ^{1}H-NMR ($CDCl_3$, δ, ppm): 2.9–3.1 (m, 2H), 3.62 (m, 1H), 4.32 (dd, $J = 11.0$ and 5.4 Hz, 1H), 4.52 (dd, $J = 11.0$ and 3.3 Hz, 1H), 6.71 (d, $J = 8$ Hz, 1H), 7.12 (d, $J = 8$ Hz, 1H), 7.3–7.5 (m, 4H), 8.12 (br. s, 1H), 8.39 (d, $J = 8$ Hz, 1H).

(S)-6-[4-[2-[3-(9H-carbazol-4-yloxy)-2-hydroxypropylamino]-2-methylpropyl] phenoxy]pyridine-3-carboxamide-[carbazole-4b,5,6,7,8,8a-^{14}C], ***LY377604-[^{14}C]***

A mixture of epoxide **7** (59 mg, 0.245 mmol) and 6-[4-(2-amino-2-methylpropyl)-phenoxy]-pyridine-3-carboxamide (**8**) (140 mg, 0.49 mmol) in 2-propanol (3 ml) was heated at 75–80°C (bath) for 18 h, and evaporated under vacuum. Flash chromatography of the residue was performed on a column 25 × 2 cm, eluting with one column volume each of hexane/ethyl acetate (1:1) and ethyl acetate. Elution was continued in a gradient fashion starting with chloroform/methanol (95:5) and progressing successively to chloroform/methanol (90:10), chloroform/methanol/ammonium hydroxide (90:10:1), chloroform/methanol/ammonium hydroxide (85:15:1.5), and finally chloroform/methanol/ammonium hydroxide (80:20:2) yielding **LY377604-[^{14}C]** (115 mg, 89%) as a white solid. HPLC (conditions C): $R_t = 6$ min. The compound co-eluted with an authentic sample of **LY377604** by HPLC under the above conditions. For the non-radioactive compound (prepared in a model experiment): ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.05 (s, 6H), 2.70 (s, 2H), 2.9–3.1 (m, 2H), 3.35 (s, 2H), 4.1–4.3 (m, 3H), 6.70 (d, $J = 8$ Hz, 1H), 6.9–7.1 (m, 5H), 7.2–7.4 (m, 4H), 7.45 (d, $J = 8$ Hz, 1H), 7.51 (s, 1H), 8.05 (br. s, 1H), 8.25 (d, $J = 8$ Hz, 2H), 8.63 (s, 1H).

(S)-6-[4-[2-[3-(9H-carbazol-4-yloxy)-2-hydroxypropylamino]-2-methylpropyl] phenoxy]pyridine-3-carboxamide-[carbazole-4b,5,6,7,8,8a-^{14}C] succinate, ***LY 377604-[^{14}C] succinate***

To a suspension of **LY377604-[^{14}C]** (115 mg, 0.219 mmol) in ethyl acetate (0.8 ml) and ethanol (0.2 ml) at 50–60°C (bath) was added a solution of succinic acid (13 mg, 0.11 mmol) in ethanol (0.35 ml). The reaction mixture was stirred at 80°C (bath) for 30 min, and evaporated under vacuum. The residue was triturated with ethyl ether, and the resulting solid was collected by filtration and dried under vacuum to give **LY377604-[^{14}C] succinate** (117 mg, 91%) as a white solid. Radiochemical purity: 97.5% (radio-HPLC). Specific activity: 22.8 μCi/mg. The compound co-eluted with an authentic sample of LY377604 succinate by HPLC under the above conditions. For the non-radioactive compound (prepared in a model experiment): ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.09 (s, 6H), 2.35 (s, 2H), 2.77 (s, 2H), 2.9–3.2 (m, 2H), 3.35 (s, 2H), 4.1–4.3 (m, 3H), 6.71 (d, $J = 8$ Hz, 1H), 6.9–7.1 (m, 5H), 7.2–7.4 (m, 4H), 7.45 (d, $J = 8$ Hz, 1H), 7.51 (s, 1H), 8.05 (br. s, 1H), 8.25 (br. d, $J = 8$ Hz, 2H), 8.63 (s, 1H).

(S)-6-[4-[2-[3-(9H-1,3,6,8-tetrabromocarbazol-4-yloxy)-2-hydroxypropylamino]-2-methylpropyl]phenoxy]pyridine-3-carboxamide hydrobromide, **9b**

To a solution of **LY377604 hydrochloride** (561 mg, 1.0 mmol) in acetic acid (25 ml) was added bromine (500 μl, 9.7 mmol) dropwise. The reaction mixture

was stirred at room temperature for 20 h and evaporated under vacuum. The residue was re-evaporated with toluene (2 × 3 ml) to leave crude hydrobromide **9b** (1.4 g). HPLC (conditions D): $R_t = 14$ min. ^{1}H-NMR (CD_3OD, δ, ppm): 1.50 (s, 3H), 1.51 (s, 3H), 3.15 (AB q, $J_{AB} = 13.6$ Hz, 2H), 3.49 (dd, $J = 12.3$ and 8.8 Hz, 1H), 3.59 (dd, $J = 12.3$ and 3.5 Hz, 1H), 4.23 (dd, $J = 9.7$ and 4.0 Hz, 1H), 4.37 (dd, $J = 9.7$ and 4.8 Hz, 1H), 4.49 (m, 1H), 7.08 (d, $J = 7.9$ Hz, 1H), 7.10 (d, $J = 7.5$ Hz, 2H), 7.41 (d, $J = 7.5$ Hz, 2H), 7.86 (d, $J = 1.4$ Hz, 1H), 7.88 (s, 1H), 8.34 (dt, $J = 7.9$ and 2.6 Hz, 1H), 8.63 (t, $J = 2.6$ Hz, 1H), 8.71 (d, $J = 1.4$ Hz, 1H). IR (KBr, ν, cm^{-1}): 595, 738, 764, 851, 1016, 1155, 1191, 1294, 1415, 1451, 1502, 1638, 1676, 1709, 3170, 3390. UV (EtOH, λ_{max}, nm): 296 (ε 11842), 252 (ε 45217).

(S)-6-[4-[2-[3-(9H-1,3,6,8-tetrabromocarbazol-4-yloxy)-2-hydroxypropylamino]-2-methylpropyl]phenoxy]pyridine-3-carboxamide, **9c**

To a solution of hydrobromide **9b** (350 mg) in methanol (5 ml) was added 10% aqueous potassium carbonate (4 ml). The reaction mixture was stirred for 10 min, and extracted with dichloromethane (20 ml). The extract was washed with brine (2 ml), dried over sodium sulfate, and evaporated under vacuum. Biotage chromatography of the residue (column 40S, eluting with dichloromethane/methanol/ammonium hydroxide, 92:8:0.8) gave **9c** (111 mg) as a colorless solid. TLC: $R_f = 0.42$ (dichloromethane/methanol/ammonium hydroxide, 90:10:1). HPLC (conditions D): $R_t = 14$ min. ^{1}H-NMR (CD_3OD, δ, ppm): 1.23 (s, 3H), 1.25 (s, 3H), 2.88 (AB q, $J_{AB} = 13.2$ Hz, 2H), 3.11 (m, 2H), 4.22 (m, 2H), 4.29 (m, 1H), 6.99 (d, $J = 8.8$ Hz, 1H), 7.05 (d, $J = 8.3$ Hz, 2H), 7.35 (d, $J = 8.3$ Hz, 2H), 7.83 (d, $J = 1.4$ Hz, 1H), 7.85 (s, 1H), 8.27 (dd, $J = 8.8$ and 2.4 Hz, 1H), 8.64 (d, $J = 2.4$ Hz, 1H), 8.68 (d, $J = 1.4$ Hz, 1H). IR (KBr, ν, cm^{-1}): 541, 741, 851, 1071, 1165, 1202, 1257, 1373, 1415, 1451, 1480, 1594, 1668, 3439. UV (EtOH, λ_{max}, nm): 295 (ε 14759), 252 (ε 57052), 224 (ε 53653). Analysis calculated for $C_{31}H_{28}Br_4N_4O_4$: C, 44.31; H, 3.36; N, 6.67. Found: C, 44.13; H, 3.40; N, 6.31.

(S)-6-[4-[2-[3-(9H-1,3,6,8-tetradeuteriocarbazol-4-yloxy)-2-hydroxypropylamino]-2-methylpropyl]phenoxy]pyridine-3-carboxamide, ***LY377604-[d$_4$]***

A flask containing 10% palladium on carbon (20 mg) was placed under vacuum and refilled with deuterium 3 times. To the resulting catalyst suspended in dimethylformamide (0.5 ml), a solution of tetrabromide **9b** (150 mg) and triethylamine (300 µl, 2.15 mmol) in dimethylformamide (3.5 ml) was added. The reaction mixture was placed under vacuum and refilled with deuterium 3 times, and then vigorously stirred under balloon pressure of deuterium for 48 h. The catalyst was filtered off, rinsed with ethyl acetate (10 ml). The filtrate was washed with water (2 ml), and brine (2 ml), dried over sodium sulfate, and evaporated under vacuum. Biotage chromatography of

the residue (column 12 M, eluting with dichloromethane/methanol/ammonium hydroxide, 92:8:0.8) gave **LY377604-[d_4]** (45 mg, 80% over two steps) as a white solid. TLC: $R_f = 0.33$ (dichloromethane/methanol/ammonium hydroxide, 90:10:1). HPLC (conditions D): $R_t = 6$ min. ^{1}H-NMR (CD_3OD, δ, ppm): 1.17 (s, 3H), 1.18 (s, 3H), 2.88 (AB q, $J_{AB} = 13.2$ Hz, 2H), 3.06 (m, 1H), 3.18 (m, 1H), 4.28 (m, 2H), 4.33 (m, 1H), 6.95 (d, $J = 8.5$ Hz, 3H), 7.29 (d, $J = 8.5$ Hz, 2H), 7.30 (s, 1H), 7.33 (s, 1H), 8.26 (dd, $J = 8.5$ and 2.5 Hz, 1H), 8.33 (s, 1H), 8.65 (d, $J = 2.5$ Hz, 1H). IR (KBr, ν, cm^{-1}): 594, 886, 1097, 1203, 1258, 1281, 1373, 1418, 1482, 1595, 1668, 3409. UV (EtOH, λ_{max}, nm): 319 (ε 6647), 285 (ε 19735), 242 (ε 59507). MS (ES+, m/z, %): 529 (100, M + 1), 528 (19), 527 (2). HRMS (AP+): calculated for $C_{31}H_{28}D_4N_4O_4$: 529.2749. Found: 529.2723. The compound co-eluted with an authentic sample of non-labeled **LY377604** by TLC and HPLC under the above conditions.

(S)-4-[2-hydroxy-3-[3-(4-deuteriophenyl)-2-methylprop-2-ylamino]propyloxy] -9H-1,3,6,8-tetradeuteriocarbazole, **10**

A flask containing 10% palladium on carbon (20 mg) was placed under vacuum and refilled with deuterium 3 times. To the resulting catalyst suspended in methanol-D_4 (0.5 ml), a solution of tetrabromide **9b** (150 mg) in methanol-d_4 (2.5 ml), and triethylamine (300 µl, 2.15 mmol) were successively added. The reaction mixture was placed under vacuum and refilled with deuterium 3 times, and then vigorously stirred under balloon pressure of deuterium for 16 h. The catalyst was filtered off, and rinsed with ethyl acetate (10 ml). The filtrate was evaporated under vacuum. The residue was diluted with ethyl acetate, washed with water (2 ml), and brine (2 ml), dried over sodium sulfate, and evaporated under vacuum. Biotage chromatography of the residue (column 12 M, eluting with dichloromethane/methanol/ammonium hydroxide, 93:7:0.7) gave **10** (33 mg, 78% over two steps). TLC: $R_f = 0.50$ (dichloromethane/methanol/ammonium hydroxide, 90:10:1). HPLC (conditions D): $R_t = 11$ min. ^{1}H-NMR (CD_3OD, δ, ppm): 1.13 (s, 3H), 1.14 (s, 3H), 2.80 (AB q, $J_{AB} = 12.3$ Hz, 2H), 3.03 (m, 1H), 3.13 (m, 1H), 4.26 (m, 2H), 4.29 (m, 1H), 7.23 (s, 4H), 7.32 (s, 1H), 7.33 (s, 1H), 8.32 (s, 1H). MS (ES+, m/z, %): 393 (100, M + 1).

9H-4-hydroxy-1,3,6,8-tetrabromocarbazole, **11**

To a solution of 4-hydroxycarbazole (**1**) (183 mg, 1.0 mmol) in acetic acid (15 ml) was added bromine (420 µl, 8.15 mmol) dropwise. The reaction mixture was stirred at room temperature for 20 h and evaporated under vacuum to give crude bromide **11** (485 mg, 97%) as a grayish solid. ^{1}H-NMR (CD_3OD, δ, ppm): 7.70 (s, 1H), 7.76 (d, $J = 1.8$ Hz, 1H), 8.39 (d, $J = 1.8$ Hz, 1H). IR (KBr, ν, cm^{-1}): 536, 735, 848, 1053, 1198, 1241, 1267, 1282, 1327, 1419, 1457, 1480, 1556, 1603, 1630,

3443, 3492. UV (EtOH, λ_{max}, nm): 291, 252, 232. Analysis calculated for $C_{12}H_5Br_4NO$: C, 28.89; H, 1.00; N, 2.81. Found: C, 29.45; H, 1.09; N, 2.69.

9H-4-hydroxy-1,3,6,8-tetradeuteriocarbazole, **1b**

To a solution of bromide **11** (140 mg, 0.28 mmol) in methanol-d_4 (1.5 ml) and dimethylformamide (1.0 ml) was added a suspension of 10% palladium on carbon (30 mg) in dimethylformamide (0.5 ml). The reaction mixture was placed under vacuum and refilled with deuterium 3 times, and then vigorously stirred under balloon pressure of deuterium for 2.5 h. Triethylamine (300 µl, 2.15 mmol) was added dropwise. The resulting mixture was placed under vacuum and refilled with deuterium 3 times, and then vigorously stirred under balloon pressure of deuterium for 4 h. The catalyst was filtered off, rinsed with ethyl acetate (10 ml). The filtrate was washed with water (1 ml), and brine (1 ml), dried over sodium sulfate, and evaporated under vacuum. Biotage chromatography of the residue (column 12 M, eluting with hexane/ethyl acetate, 75:25) gave **1b** (43 mg, 82%) as a white solid. TLC: $R_f = 0.34$ (hexane/ethyl acetate, 70:30). NMR (CD_3OD, δ, ppm): 7.17 (s, 1H), 7.31 (s, 1H), 8.24 (s, 1H). IR (KBr, ν, cm^{-1}): 462, 597, 756, 806, 902, 974, 1038, 1172, 1203, 1258, 1303, 1337, 1420, 1477, 1576, 1601, 1631, 3223, 3399. UV (EtOH, λ_{max}, nm): 333, 284, 244, 224. MS (ES+, *m/z*, %): 188 (100, M + 1), 187 (66), 186 (16). Analysis calculated for $C_{12}H_9NO$: C, 76.97; H, 4.85; N, 7.48. Found: C, 76.33; H, 5.09; N, 7.39.

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