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Research Article

Synthesis of ^{14}C-labelled EM-800 (SCH 57050) and EM-652 · HCl (SCH 57068 · HCl, acolbifene), pure selective estrogen receptor modulators

Jean-Yves Sancéau, Fernand Labrie and Sylvain Gauthier*
Oncology and Molecular Endocrinology Research Center, Laval University Medical Center (CHUL), Québec, Canada G1V 4G2

Summary

EM-800 (SCH 57050) and EM-652 · HCl (SCH 57068 · HCl, acolbifene) are orally active pure selective estrogen receptor modulators. The corresponding $^{14}C_2$-radiolabelled compounds **1** and **2** were synthesized for metabolic studies with uniform labelling of two carbons within the benzene ring of the 2H-1-benzopyran moiety by optical resolution of racemic (±)-[$^{14}C_2$]EM-343 **4**. This pivotal intermediate amine was prepared in 6 steps with 38% yield from commercially available [U-$^{14}C_2$]resorcinol (**3**). Resolution by selective crystallization of the diastereomeric mixture of (*S*)-(+)-camphorsulfonates salts gave the desired (+)-[$^{14}C_2$]EM-652 · (+)-CSA **13**. Moreover, the racemic amine **4** was recovered from mother liquors by basic treatment, and resolved again. We obtained salt **13**, at a 52% yield with 97% diastereomeric excess by repeating the resolution–racemization process. Finally, the corresponding dipivaloate (+)-[$^{14}C_2$]EM-800 **1** and hydrochloride salt (+)-[$^{14}C_2$]EM-652 · HCl **2** were prepared at respective specific activities of 19.7 and 24.5 μCi/mg with 96.3% radiochemical purity. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: EM-800; EM-652 · HCl; SERM; carbon-14; radiochemical resolution

Introduction

EM-800 (SCH 57050) and EM-652 · HCl (SCH 57068 · HCl, acolbifene) (Figure 1) are pure and orally active selective estrogen receptor modulators (SERMs).[1–3] Preclinical and clinical data indicate that they have positive effects on the skeletal and cardiovascular system while blocking the effect of estrogens on breast and uterine cancer. Acolbifene is currently in advanced

*Correspondence to: S. Gauthier, Oncology and Molecular Endocrinology Research Center, Laval University Medical Center (CHUL), Québec, Canada G1V 4G2. E-mail: sylvain.gauthier@crchul.ulaval.ca

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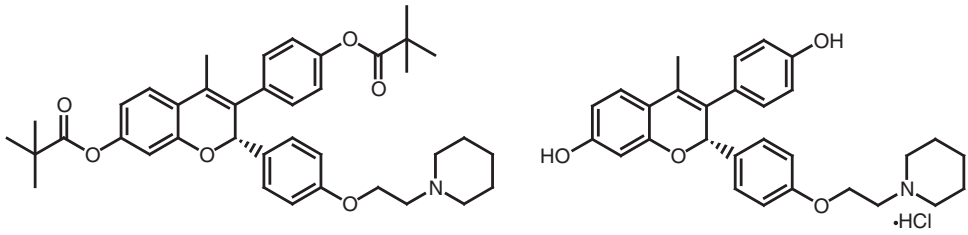


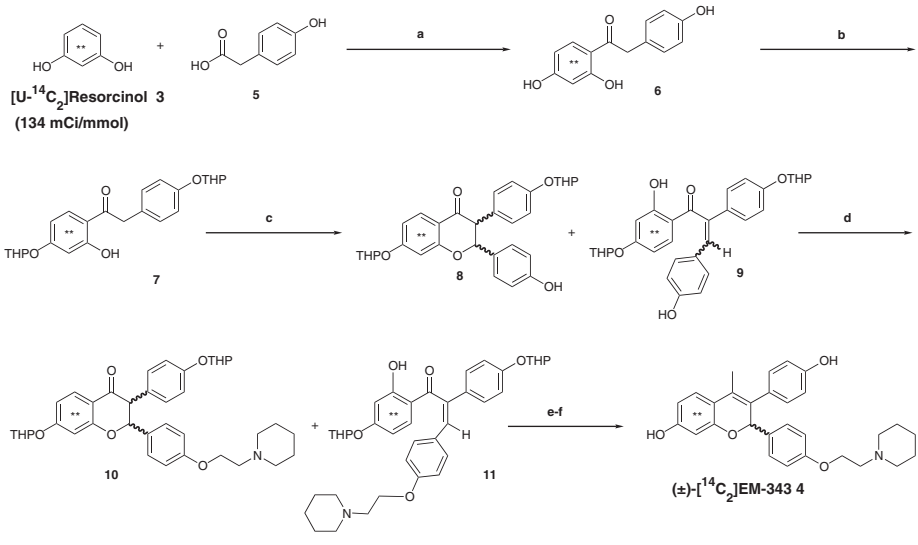
EM-800 (SCH 57050) EM-652·HCl (SCH 57068·HCl, acolbifene)

Figure 1. Structures of EM-800 and EM-652 · HCl (acolbifene)

clinical trials for treatment of estrogen-dependent breast cancer.[4] In support of our preclinical program, the corresponding $^{14}C_2$-radiolabelled EM-800 **1** and EM-652 · HCl **2** were synthesized for pharmacokinetic studies and metabolite profiling work (for recent results on metabolites studies of compounds **1** and **2**, see Reference)[5], and described in this paper.[6]

Results and discussion

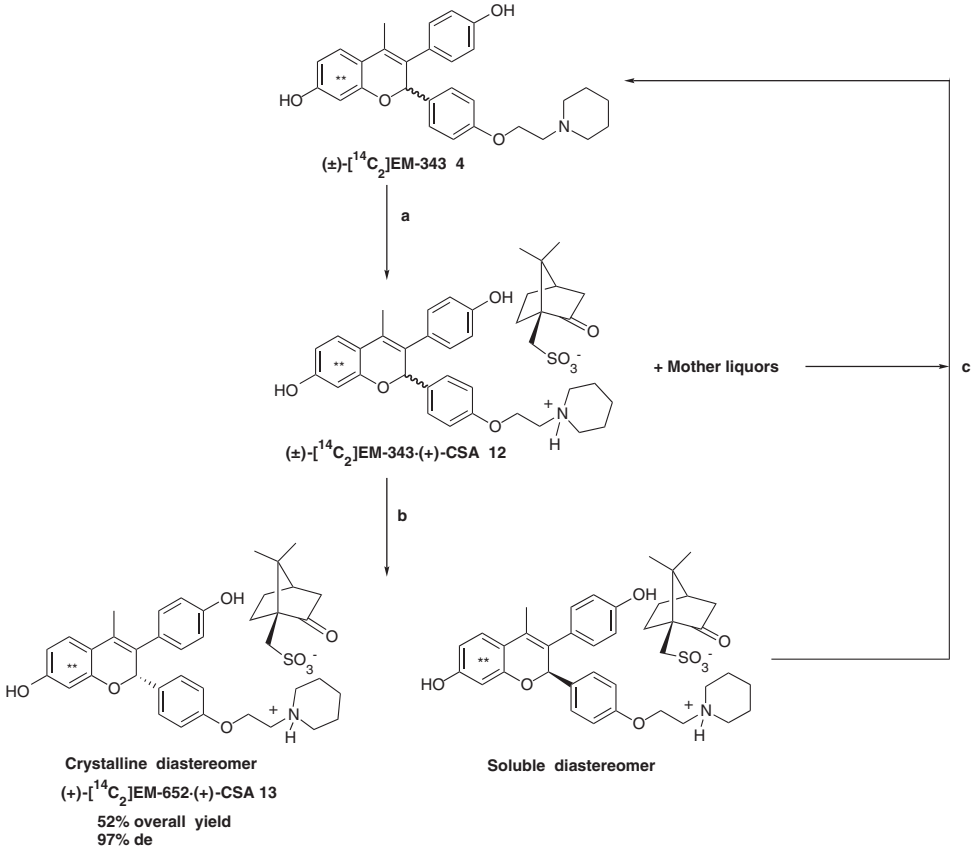
Carbon atoms of the benzene ring in the 2H-1-benzopyran moiety were chosen for ^{14}C-labelling because they were expected to be metabolically stable. Incorporation of these two radiolabelled carbons was achieved using commercially available [U-$^{14}C_2$]resorcinol (**3**) (134 mCi/mmol) as starting material, which was converted to racemic (±)-[$^{14}C_2$]EM-343 **4** in the same manner as in the preparation of unlabelled EM-800, EM-652[7] and its analogs[8] as shown in Scheme 1. Firstly, Friedel-Crafts acylation of [U-$^{14}C_2$]resorcinol (**3**) with 4-hydroxyphenylacetic acid (**5**) and $BF_3 \cdot OEt_2$ at 100°C in toluene furnished [$^{14}C_2$]trihydroxydeoxybenzoin **6** in 93% yield. Phenols were selectively protected with 3,4-dihydro-2H-pyran in the presence of *p*-toluenesulfonic acid monohydrate to afford the radiolabelled bis-THP ether **7** as a white solid in 88% yield. Knoevenagel reaction of ether **7** with 4-hydroxybenzaldehyde, in the presence of piperidine in refluxing benzene gave quantitatively a mixture of [$^{14}C_2$]chromanones **8** and [$^{14}C_2$]chalcones **9**. The crude intermediaires were alkylated with 1-(2-chloroethyl)piperidine monohydrochloride in the presence of Cs_2CO_3 in refluxing 99:1 acetone–water, to yield [$^{14}C_2$]chromanones **10** (3:1 *trans*/*cis* ratio) contaminated with *Z*-[$^{14}C_2$]chalcone **11** (3:1 molar ratio) in 76% yield. Alkylation of **10** with methyllithium at −78°C to room temperature gave a mixture of tertiary alcohols which upon acid treatment with 19:1 acetic acid–water at 90°C gave racemic (±)-[$^{14}C_2$]EM-343 **4** in 62% yield. The 2H-1-benzopyran **4** was difficult to purify and only obtained in 90% purity as an amorphous solid (light pink to red) containing a large amount of residual solvents (5–10% by weight).



Scheme 1. Synthesis of racemic 4. Reagents and conditions: (a) $BF_3.OEt_2$ (2.5 equiv.), toluene, 100°C, 3 h, 93%; (b) 3,4-dihydro-2H-pyran (8 equiv.), TsOH (cat.), dichloromethane, 0°C to room temperature, 55 min, 88%; (c) 4-hydroxybenzaldehyde (1.04 equiv.), piperidine (0.3 equiv.), benzene, reflux, 48 h; (d) 1-(2-chloroethyl)piperidine monohydrochloride (1.2 equiv.), Cs_2CO_3 (2.4 equiv.), 99:1 acetone–water, reflux, 19 h, 76% from 7; (e) MeLi (3 equiv.), THF, −78°C to room temperature, 3 h; (f) 19:1 acetic acid–water, 90°C, 0.5 h, 62% from 10

We next turned our attention to the chiral separation of racemic (±)-[$^{14}C_2$]EM-343 **4**. Initially, radiolabelled racemate **4** was resolved into its enantiomers by chiral preparative HPLC using a Chiralpak AD column. However, we decided to use an alternative method which resolved the racemate **4** with (+)-CSA as previously described by us for the resolution of unlabelled (±)-EM-343.[7] To the best of our knowledge, this is the first report of chemical resolution of racemic radiolabelled amine by chiral acid. Initially, the (±)-EM-343·(+)-CSA salt was prepared by adding (+)-CSA to a solution of amine in methanol, then the isolated salt was recrystallized from a mixture of DMF-dichloromethane to give crystalline (+)-EM-652·(+)-CSA (34% yield, 95% diastereomeric excess (de)). We found later that a solution of (±)-EM-343 and (+)-CSA in DMF diluted in dichloromethane gave directly the desired (+)-EM-652·(+)-CSA crystals (41% yield, 92% de). We found that the obtained results (yield and de) are very dependent on (±)-EM-343 quality. Then, the Chemical Process Research & Development Division of Schering-Plough Research Institute have improved this resolution process. The (±)-EM-343·(+)-CSA salt was firstly preformed in 95% aqueous

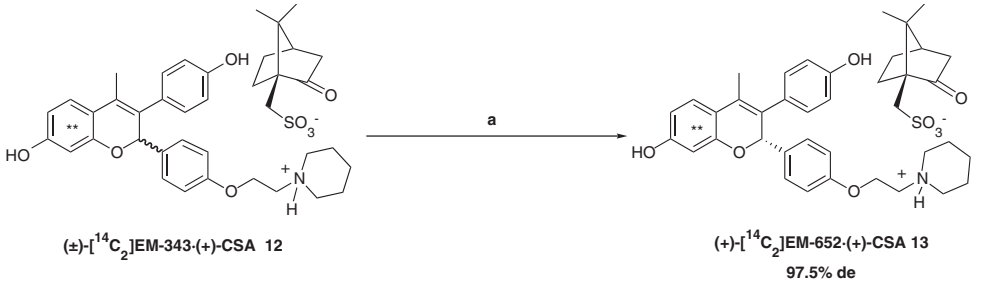
acetone then recrystallized in 95% EtOH.[9] Secondly, they perfected an efficient kinetic procedure for the large scale production of (+)-EM-800 and acolbifene, by heating a suspension of (±)-EM-343 · (+)-CSA in the presence of a catalytic amount of (+)-CSA (acid racemization mechanism). Based on these results, we have started recrystallization trials of cold (±)-EM-343 · (+)-CSA from 95% EtOH on the radiosynthesis scale in order to optimize yield and de. We found that the optimal salt concentration was 6 g/l. Scheme 2 summarizes our work on the chemical resolution of (±)-[$^{14}C_2$]EM-343 **4** with (+)-CSA. The amine **4** was mixed with (+)-CSA in 20:1 acetone–water to furnish after filtration (±)-[$^{14}C_2$]EM-343 · (+)-CSA **12** in 81% yield. The resulting salt was filtered and dissolved in hot 95% EtOH. After standing for 2 days at room temperature, we obtained crystalline (+)-[$^{14}C_2$]EM-652 · (+)-CSA **13** in 29% yield with a 97% radiodiastereomeric



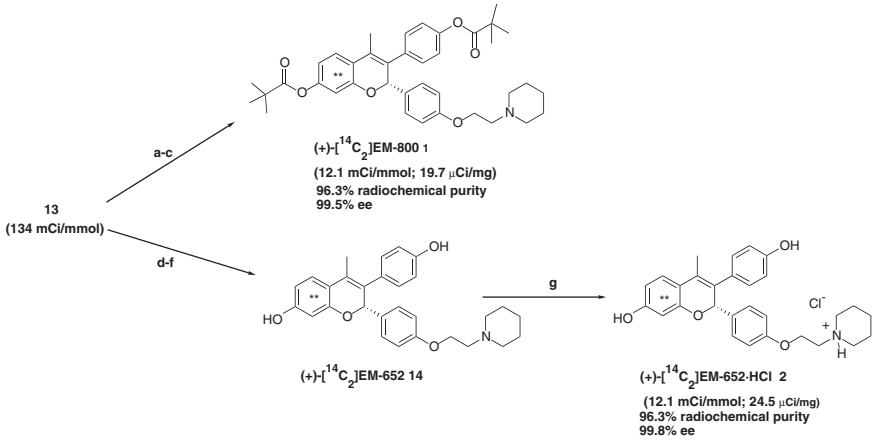
Scheme 2. Chemical resolution of 4 with (+)-CSA and recycling the mother liquors. Reagents and conditions: (a) (+)-CSA (1 equiv.), 20:1 acetone–water, 1 h, 81%; (b) dissolution in hot 95% EtOH then cooled to room temperature, 2 days, 29% (first crop); (c) 10% NaOH, EtOH, 80°C, 82%

purity. As expected, treatment of the mother liquors with a 10% NaOH solution at 80°C gave radiolabelled amine **4** (82% recovery). The recovered (±)-EM-343 was resolved by the same procedure described above to furnish a second crop of salt **13**. By repeating this resolution–racemization cycle two more times, we obtained two additional crops of salt **13** improving the overall yield of this process to 52% (conversion of racemic **4** to salt **13**). Each crop of salts gave 97% radiodiastereomeric purity and could be stored at −80°C for several months without appreciable decomposition. Similar yield and de were obtained when applying the kinetic process to our scale of experiment. Thus, heating a suspension of **12** in the presence of a catalytic amount of (+)-CSA in 95% EtOH at 75°C for 36 h gave **13** in 53% yield (Scheme 3). Unfortunately, recovered radiolabelled amine **4** was only obtained in 38% yield. Prolonged heating probably resulted in decomposition of the mother liquors (inherent chemical instability and/or self-radiolysis). Additional experiments on cold material revealed that the kinetic resolution process is inefficient when using the recovered (±)-EM-343 (38% yield and 74% de were obtained).

Finally, chiral salt (+)-[$^{14}C_2$]EM-652 · CSA **13** was converted into **1** and **2** as reported[7,9] (Scheme 4). Pivaloylation of radiolabelled salt **13** with trimethylacetylchloride in the presence of triethylamine yielded the desired (+)-[$^{14}C_2$]EM-800 **1** in 80% yield. In order to adjust the specific activity for biological tests, this hot material was diluted with cold (+)-EM-800 and recrystallized from hot isopropanol (80% yield). Thus, (+)-[$^{14}C_2$]EM-800 **1** was obtained with a specific activity of 19.7 μCi/mg, a 96.3% radiochemical purity, and a 99.5% radioenantiomeric purity. On the other hand, cold (+)-EM-652 · (+)-CSA was mixed with **13** and recrystallized from a mixture of 94:6 dichloromethane-DMF in 80% yield. This procedure allowed us to adjust the final specific activity and increase de to >99%. Neutralization with aqueous Na_2CO_3 afforded the free amine (+)-[$^{14}C_2$]EM-652 **14** in 94% yield.



Scheme 3. Kinetic resolution of 12. Reagents and conditions: (a) (+)-CSA (0.35 equiv.), 95% EtOH, 75°C, 36 h, 53%



Scheme 4. Conversion of 13 to 1 and 2. Reagents and conditions: (a) trimethylacetylchloride (2.5 equiv.), triethylamine (2.8 equiv.), dichloromethane, 0°C to room temperature, 2 h, 80%; (b) dilution with cold EM-800; (c) recrystallization from hot isopropanol, 80%; (d) dilution with cold (+)-EM652 · (+)-CSA; (e) recrystallization from 94:6 dichloromethane-DMF, 80% from 13; (f) aqueous Na_2CO_3, EtOAc, 1 h, 94%; (g) 2 N HCl, EtOH, room temperature, 45 min, 81%

After dissolution with a minimum amount of EtOH, 2 N HCl was slowly added. Ten minutes later, the hydrochloride salt started to precipitate. Precipitation was completed by addition of water. After filtration and drying, (+)-[$^{14}C_2$]EM-652 · HCl **2** was obtained in 81% yield with a specific activity of 24.5 μCi/mg, a 96.3% radiochemical, and a 99.8% radioenantiomeric purity.

Conclusion

We have prepared (+)-[$^{14}C_2$]EM-800 **1** and (+)-[$^{14}C_2$]EM-652 · HCl **2** with two radiolabelled carbons in the metabolically stable benzene ring of the 2H-1-benzopyran skeleton with specific activities of 19.7 and 24.5 μCi/mg, respectively. These radiosyntheses were achieved from commercially available [U-$^{14}C_2$]resorcinol (**3**) via optical resolution of the pivotal racemic amine **4** with 14.9 and 12.2% overall yield, respectively.

Experimental section

All reagents were purchased from Aldrich Chemical Co. All reactions were carried out in flame-dried glassware under a positive atmosphere of dry Ar. Column chromatography was carried out using a silica gel (230–400 mesh) (EM Science). [U-$^{14}C_2$]Resorcinol (**3**) (400 mCi, lot No CFQ 10752) was

purchased from Amersham UK and purified by flash chromatography (dichloromethane-MeOH 95:5) before use. All labelled compounds were identified by TLC and ^{1}H NMR spectral data comparison with the corresponding unlabelled authentic sample. ^{1}H NMR spectra were recorded at 300 MHz on a Bruker WH300 spectrometer. Radiochemical and radiochiral purities were determined by high performance liquid chromatography (HPLC) on a Waters system equipped with a radioactivity flow monitor (Packard Ultima Flo AP cocktail liquid scintillation). The specific activity of compounds **1** and **2** was determined by gravimetry (mean value of two determinations).

2,4-Dihydroxy-2′-(4″-hydroxyphenyl)-[$^{14}C_2$]acetophenone (**6**)

A suspension of [U-$^{14}C_2$]resorcinol (**3**) (352 mCi, 301 mg, 2.63 mmol, 134 mCi/mmol) and 4-hydroxyphenylacetic acid (**5**) (480 mg, 3.16 mmol) in toluene was treated with $BF_3 \cdot OEt_2$ (1.0 ml, 7.9 mmol) and heated at 100°C for 3 h. After cooling to room temperature, 15% NaOAc (20 ml) was added and the resulting suspension was vigorously stirred overnight. The resulting precipitate was collected, washed with distilled water (100 ml) to yield, after drying, the triphenol **6** (327 mCi, 607 mg, 93% yield) as a creamy solid. ^{1}H NMR (acetone-d_6) δ 4.17 (s, 2H, H-2'), 6.32 (d, $J = 2.2$ Hz, 1H, Ar), 6.44 (dd, $J = 2.4$ and 8.8 Hz, 1H, Ar), 6.78 (d, $J = 8.4$ Hz, 2H, Ar), 7.16 (d, $J = 8.5$ Hz, 2H, Ar), 7.95 (d, $J = 8.8$ Hz, 1H, Ar), 12.76 (s, 1H, OH).

2-Hydroxy-4-tetrahydropyranyloxy-2′-(4″-tetrahydropyranyloxyphenyl)-[$^{14}C_2$] acetophenone (*7*)

A suspension of [$^{14}C_2$]triphenol **6** (327 mCi, 607 mg, 2.44 mmol, 134 mCi/mmol) and *p*-toluenesulfonic acid monohydrate (4 mg) in dry dichloromethane (10 ml) was treated with 3,4-dihydro-2H-pyran (1.78 ml, 19.5 mmol) at 0°C. The reaction mixture was stirred for 10 min, and after removing the ice bath for 45 min, three drops of triethylamine were added and the solution was concentrated. Flash chromatography (hexanes-EtOAc 99:1) afforded pure bis-tetrahydropyranyl ether **7** (289 mCi, 901 mg, 88% yield) as a white solid with a 99% radiochemical purity. HPLC conditions: YMC C_4 column (4.6 × 250 mm) eluted with 10 mM ammonium acetate in methanol–water 30:70, flow rate 1.0 ml/min, UV detector at 275 nm, Rt 24.47 min (24.42 min for unlabelled material); ^{1}H NMR ($CDCl_3$) δ 1.55–2.10 (m, 12H, CH_2), 3.60 (m, 2H, CH_2O), 3.85 (m, 2H, CH_2O), 4.15 (s, 2H, H-2′), 5.39 (t, $J = 3$ Hz, 1H, CH-O), 5.47 (t, $J = 3$ Hz, 1H, CH-O), 6.55 (dd, $J = 2.3$ and 8.9 Hz, 1H, Ar), 6.61 (d, $J = 2.4$ Hz, 1H, Ar), 7.01 (d, $J = 8.4$ Hz, 2H, Ar), 7.17 (d, $J = 8.5$ Hz, 2H, Ar), 7.75 (d, $J = 8.8$ Hz, 1H, Ar).

2-(4″-[2‴-(1-Piperidino)ethoxy]phenyl)-3-(4′-tetrahydropyranyloxyphenyl)-7-tetrahydropyranyloxy-2,3-dihydro-4H-1-[$^{14}C_2$]benzopyran-4-one (**10**) *and 2-hydroxy-4′-(2‴-[1-piperidino]ethoxy)-α-(4″-tetrahydropyranyloxyphenyl)-4-tetrahydropyranyloxy-[$^{14}C_2$]chalcone* (**11**)

A solution of *bis*-tetrahydropyranyl ether **7** (289 mCi, 899 mg, 2.16 mmol, 134 mCi/mmol), 4-hydroxybenzaldehyde (277 mg, 2.27 mmol) and piperidine (2 drops) in benzene (2.5 ml) was refluxed with a Dean-Stark apparatus for 48 h. After cooling at room temperature, the reaction mixture was concentrated under vacuum to yield a mixture of chromanones **8** and chalcones **9**. These crude intermediates, 1-(2-chloroethyl)piperidine monohydrochloride (477 mg, 2.59 mmol) and cesium carbonate (1.69 g, 5.18 mmol) in 99:1 acetone–water (50 ml) were refluxed for 19 h, and then cooled to room temperature. The cesium salts were filtered off and washed with acetone (50 ml). After concentration under reduced pressure, the residue was purified by flash chromatography (EtOAc-methanol 9:1) to give in 76% yield the amine **10** (3:1 *trans*/*cis* ratio) (221 mCi, 1.04 g) which contains (*Z*)-chalcone **11** (3:1 molar ratio). ^{1}H NMR ($CDCl_3$) for **10**-(*trans*) δ 1.43 (m, 2H, CH_2), 1.59 (m, 10H, CH_2), 1.81 (m, 4H, CH_2), 1.87 (m, 2H, CH_2), 2.48 (m, 4H, CH_2N), 2.73 (t, $J = 6$ Hz, 2H, NCH_2), 3.58 (m, 2H, OCH_2), 3.84 (m, 2H, OCH_2), 4.04 (t, $J = 6$ Hz, 2H, OCH_2), 5.32 (m, 1H, OCH), 5.46 (m, 2H, OCH and H-2), 6.71 (s, 1H, Ar), 6.73 (m, 1H, Ar), 6.76 (d, $J = 8.5$ Hz, 2H, Ar), 6.88 (d, $J = 4.4$ Hz, 4H, Ar), 7.13 (dd, $J = 2.2$ and 8.7 Hz, 2H, Ar), 7.93 (dd, $J = 2.2$ and 8.7 Hz, 1H, Ar); selected data for **10**-(*cis*) δ 5.6 (br s, 1H, H-2), 7.94 (dd, $J = 2.2$ and 8.9 Hz, 1H, Ar); selected data for (*Z*)-chalcone **11** δ 6.33 (d, $J = 8.9$ Hz, 1H, Ar), 6.62 (d, $J = 2.2$ Hz, 1H, Ar), 6.97 (s, 1H, H-β), 7.01 (d, $J = 8.8$ Hz, 2H, Ar), 7.20 (d, $J = 8.7$ Hz, 2H, Ar), 7.49 (d, $J = 8.9$ Hz, 1H, Ar), 12.6 (s, 1H, OH).

(2R,S)-7-hydroxy-3-(4′-hydroxyphenyl)-4-methyl-2-(4″-[2‴-(1-piperidino)ethoxy]phenyl)-2H-1-[$^{14}C_2$]benzopyran (**4**)

To a solution of the above mixture of amines **10** (221 mCi, 1.04 g, 1.65 mmol, 134 mCi/mmol) in dry THF (20 ml) was added dropwise at −78°C methylithium (1.4 M solution in ether, 3.53 ml, 4.95 mmol). After 10 min, the cooling bath was removed and the reaction allowed to warm to room temperature over 75 min. The chilled reaction mixture was quenched with saturated NH_4Cl (10 ml). The aqueous solution was extracted with EtOAc (2×20 ml). The combined organic phase was washed with brine (10 ml), dried (Na_2SO_4), and evaporated under reduced pressure. The residue was dissolved in 19:1 acetic acid–water (20 ml) and heated at 90°C for 30 min under a stream of argon. The deep-red solution was cooled to room temperature, carefully basified with 5% Na_2CO_3 (200 ml), and vigorously stirred overnight with EtOAc (100 ml). After decantation, the aqueous phase was extracted with

EtOAc (2 × 50 ml). The combined organic phase was washed with 5% Na_2CO_3 (50 ml) and brine (50 ml) and evaporated under reduced pressure. The crude red product was purified by flash chromatography (dichloromethane-EtOH 9:1) to yield almost pure (±)-[$^{14}C_2$]EM-343 **4** as a pink amorphous solid (138 mCi, 472 mg, 62% yield). A 90% radiochemical purity was determined by HPLC: YMC C_4 column (4.6 × 250 mm) eluted with 20 mM ammonium acetate in methanol–water 55:45, flow rate 1.0 ml/min, UV detector at 240 nm, Rt 12.4 min (11.7 min for unlabelled material). ^{1}H NMR (CD_3OD) δ 1.47(m, 2H, CH_2), 1.63 (m, 4H, CH_2), 2.05 (s, 3H, CH_3), 2.54 (br s, 4H, NCH_2), 2.75 (t, J = 5.6 Hz, 2H, CH_2N), 4.06 (t, J = 5.6 Hz, 2H, OCH_2), 5.80 (s, 1H, H-2), 6.15 (d, J = 2.4 Hz, 1H, Ar), 6.34 (dd, J = 2.3 and 8.9 Hz, 1H, Ar), 6.73 (d, J = 8.4 Hz, 2H, Ar), 6.74 (d, J = 8.4 Hz, 2H, Ar), 7.00 (d, J = 8.4 Hz, 2H, Ar), 7.13 (d, J = 8.5 Hz, 1H, Ar), 7.20 (d, J = 8.8 Hz, 2H, Ar).

*Chemical resolution of **4** with (+)-(1S)-10-camphorsulfonic acid and recycling the mother liquors. (2S)-7-hydroxy-3-(4′-hydroxyphenyl)-4-methyl-2-(4″-[2‴-(1-piperidino)ethoxy]phenyl)-2H-1-[$^{14}C_2$]benzopyran (1S)-10-camphorsulphonic acid salt (**13**)*

To a solution of **4** (73.7 mCi, 256 mg, 0.55 mmol, 134 mCi/mmol) in 20:1 acetone–water (1.5 ml) was added (+)-(1*S*)-10-camphorsulfonic acid (129 mg, 0.55 mmol). After 5 min, the salt started to precipitate. Acetone (3 ml) was added and after 1 h the crystals were filtered, washed with acetone (5 ml), and dried to give racemic (±)-[$^{14}C_2$]EM-343 · CSA **12** (59.7 mCi, 312 mg) in 81% yield. The above radiolabelled salt **12** was dissolved in hot 95% EtOH (50 ml). After standing for 2 days at room temperature in a capped Erlenmeyer flask, the white crystals were filtered, washed with 95% EtOH, and dried to give (+)-[$^{14}C_2$]EM-652 · CSA **13** (17.4 mCi, 90 mg, 29% yield). A 97% radiodiastereomeric purity for salt **13** was determined by chiral HPLC: Chiralpak AD column (4.6 × 250 mm) eluted with Hexanes-Reagent alcohol-Diethylamine 89.5:10:0.5; flow rate 1.0 ml/min, detector UV at 240 nm, Rt 27.63 min (27.32 min for unlabelled material). All filtrates obtained from the preparation of racemic salt and the optical resolution mentioned above were combined (50 ml) and heated at 80°C under a stream of argon for 3 h in the presence of 10% NaOH (5 ml). The reaction mixture was cooled to room temperature. Ethanol was then evaporated under reduced pressure. The residue was diluted with water (20 ml) and extracted with EtOAc (3 × 50 ml). The combined organic phase was washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure. Flash chromatography of the residue afforded racemic (±)-[$^{14}C_2$]EM-343 **4** (46.1 mCi, 160 mg) in 82% yield. The recovered amine **4** was again resolved to give a second crop of (+)-[$^{14}C_2$]EM-652 · CSA **13** (11.1 mCi, 58 mg) in 34% yield. This racemization–resolution process was repeated two times more to furnish a third crop (6.5 mCi, 34 mg) and then a

fourth crop of salt **13** (4.2 mCi, 22 mg). The overall yield of the resolution was 52% (39.2 mCi, 204 mg, 97% radiodiastereomeric purity). Each crop was stored at −80°C for several months without appreciable decomposition. ^{1}H NMR (DMSO-d_6) δ 0.73 (s, 3H, CSA), 1.04 (s, 3H, CSA), 1.26 (m, 2H, CSA), 1.31 (m, 1H, CH_2), 1.66 (m, 3H, CH_2), 1.79 (d, $J = 18.1$ Hz, 1H, CSA), 1.80 (m, 2H, CH_2), 1.84 (m, 1H, CSA), 1.93 (t, $J = 4.6$ Hz, 1H, CSA), 2.03 (s, 3H, CH_3), 2.21 (dt, $J = 3.6$ and 18.1 Hz, 1H, CSA), 2.35 (d, $J = 14.7$ Hz, 1H, CSA), 2.69 (t, $J = 10.3$ Hz, 1H, CSA), 2.85 (d, $J = 14.7$ Hz, 1H, CSA), 2.96 (m, 2H, CH_2N), 3.44 (m, 4H, NCH_2), 4.25 (t, $J = 4.9$ Hz, 2H, OCH_2), 5.94 (s, 1H, H-2), 6.06 (s, 1H, Ar), 6.33 (d, $J = 8.3$ Hz, 1H, Ar), 6.71 (d, $J = 8.1$ Hz, 2H, Ar), 6.87 (d, $J = 8.7$ Hz, 2H, Ar), 7.08 (d, $J = 8.5$ Hz, 2H, Ar), 7.12 (d, $J = 8.4$ Hz, 1H, Ar), 7.25 (d, $J = 8.7$ Hz, 2H, Ar), 9.15 (br s, 1H, NH^+), 9.47 (s, 1H, OH), 9.49 (s, 1H, OH).

Kinetic resolution using a catalytic amount of (+)-(1S)-10-camphorsulfonic acid

A suspension of (±)-[$^{14}C_2$]EM-343 · CSA **12** (157 mCi, 900 mg, 1.29 mmol, 134 mCi/mmol) and (+)-(1*S*)-10-camphorsulfonic acid (181 mg, 0.46 mmol) in 95% EtOH (10 ml) was heated at 75°C under argon for 36 h and allowed to stir at room temperature overnight. The radiolabelled salt **13** was filtered, washed with cold 95% EtOH, and dried (83 mCi, 480 mg, 53% yield, 97.5% radiodiastereomeric purity). Recycling mother liquors, as described above, gave racemic **4** (45 mCi, 180 mg) in 38% yield.

(2S)-7-trimethylacetoxy-3-(4′-trimethylacetoxyphenyl)-4-methyl-2-(4″-[2‴-(1-piperidino)ethoxy]phenyl)-2H-1-[$^{14}C_2$]benzopyran (***1***)

A cooled suspension of (+)-[$^{14}C_2$]EM-652 · CSA **13** (39.4 mCi, 204 mg, 0.29 mmol, 134 mCi/mmol) in dry dichloromethane (5 ml) was treated with triethylamine (113 µl, 0.81 mmol) and stirred for 15 min (or until complete dissolution). The reaction mixture was treated with trimethylacetylchloride (89 µl, 0.73 mmol) and stirred for 5 min. The cooling bath was removed and the reaction mixture was stirred for 2 h. The solution was diluted with dichloromethane (10 ml), washed with saturated $NaHCO_3$ (2 × 10 ml) and brine (10 ml), dried over Na_2SO_4, and then evaporated under reduced pressure. The crude product was purified by flash chromatography (dichloromethane-EtOH 99:1) to yield **1** (32.7 mCi, 155 mg, 213 µCi/mg) in 80% yield. In order to adjust the specific radioactivity of the final compound, cold (+)-EM-800 (1.40 g) was added, and then the mixture was dissolved in hot isopropanol (20 ml). After standing overnight at room temperature, the white crystals were filtered to afford almost pure (+)-[$^{14}C_2$]EM-800 **1** (29.4 mCi, 1.40 g, 80% yield, specific activity: 19.7 µCi/mg, 12.1 mCi/mmol). A 96.3% radiochemical purity was determined by HPLC using a Waters NovaPak C18

column (3.9 × 150 mm) eluted with 10 mM ammonium acetate in methanol–water 50:50, flow rate 1.0 ml/min, UV detector at 240 nm. A 99.5% radioenantiomeric purity was determined by chiral HPLC: Chiralpak AD column (4.6 × 250 mm) eluted with Hexanes-Reagent alcohol-Diethylamine 94.8:5:0.2, flow rate 1.0 ml/min, UV detector at 240 nm.^{1}H NMR ($CDCl_3$) δ 1.31 (s, 9H, CH_3), 1.33 (s, 9H, CH_3), 1.42 (m, 2H, CH_2), 1.66 (m, 4H, CH_2), 2.07 (s, 3H, CH_3), 2.46 (m, 4H, NCH_2), 2.72 (t, J = 4.9 Hz, 2H, CH_2N), 4.02 (t, J = 4.9 Hz, 2H, OCH_2), 5.85 (s, 1H, H-2), 6.47 (d, J = 2.3 Hz, 1H, Ar), 6.64 (dd, J = 2.5 and 8.4 Hz, 1H, Ar), 6.74 (d, J = 8.5 Hz, 2H, Ar), 6.99 (d, J = 8.5 Hz, 2H, Ar), 7.14 (d, J = 8.6 Hz, 2H, Ar), 7.20 (d, J = 8.6 Hz, 2H, Ar), 7.27 (d, J = 7.8 Hz, 1H, Ar).

(2S)-7-hydroxy-3-(4′-hydroxyphenyl)-4-methyl-2-(4″-[2‴-(1-piperidino) ethoxy]phenyl)-2H-1-[$^{14}C_2$]benzopyran hydrochloride (**2**)

In order to adjust the specific activity, cold (+)-EM-652·(+)-CSA (540 mg, 0.783 mmol) was added to **13** (11.4 mCi, 59 mg, 0.085 mmol, 134 mCi/mmol) and the mixture solubilized in 94:6 dichloromethane–DMF (10.6 ml). After standing for 24 h at room temperature, the crystals were collected by filtration to give 480 mg of diluted salt **13** with a 99% radiodiastereomeric purity in 80% yield. To a suspension of this salt in EtOAc (7 ml) was added Na_2CO_3 (295.2 mg, 2.78 mmol) and water (7 ml). After stirring for 1 h at room temperature, the organic phase was washed with saturated $NaHCO_3$ (10 ml), brine (10 ml) and dried (Na_2SO_4). Concentration *in vacuo* gave a foamy residue which was purified by flash chromatography (dichloromethane-EtOH 95:5) to yield 298 mg of (+)-[$^{14}C_2$]EM-652 **14** as a pink foam (94% yield). To a solution of the above amine in EtOH (2 ml) was added slowly 2 N HCl (0.39 ml, 0.78 mmol). The hydrochloride salt **2** start to precipitate after 10 min. Stirring was continued for 35 min before addition of water (5 ml). After 30 min, the precipitate was filtered and washed with water (10 ml). Drying under high vacuum at 35–40°C afforded almost pure **2** (6.9 mCi, 262 mg, 81% yield, specific activity: 24.5 μCi/mg, 12.1 mCi/mmol). A 96.3% radiochemical purity was determined by HPLC using a Waters NovaPak C18 column (3.9 × 150 mm) eluted with 20 mM ammonium acetate in methanol–water 50:50, flow rate 1.0 ml/min, UV detector at 240 nm. A 99.8% radioenantiomeric purity was determined by chiral HPLC using a Chiralpak AD column (4.6 × 250 mm) eluted with Hexanes-Reagent alcohol-Diethylamine 89.5:10:0.5, flow rate 1.0 ml/min, UV detector at 240 nm.^{1}H NMR (DMSO-d_6) δ 1.34 (m, 1H, CH_2), 1.75 (m, 5H, CH_2), 2.01 (s, 3H, CH_3), 2.94 (t, J = 4.9 Hz, 2H, CH_2N), 3.41 (m, 4H, NCH_2), 4.28 (t, J = 4.9 Hz, 2H, OCH_2), 5.91 (s, 1H, H-2), 6.07 (d, J = 2.5 Hz, 1H, Ar), 6.32 (dd, J = 2.3 and 8.5 Hz, 1H, Ar), 6.70 (d, J = 8.6 Hz, 2H, Ar), 6.84 (d, J = 8.7 Hz, 2H, Ar),

7.05 (d, $J = 8.5$ Hz, 2H, Ar), 7.09 (d, $J = 8.3$ Hz, 1H, Ar), 7.25 (d, $J = 8.7$ Hz, 2H, Ar), 9.5 (s, 2H, OH), 10.29 (bs, 1H, HCl).

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