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Research Article

[^{14}C] and [^{3}H]-labelling of Ragaglitazar: A dual acting PPARα and PPARγ agonist with hypolipidemic and anti-diabetic activity

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Summary

Currently, Ragaglitazar is being developed as a drug for the treatment of hyperglycaemia and hyperlipidemia in patients with type 2 diabetes. Here, we report the labelling of Ragaglitazar with carbon-14 and tritium for in vivo and in vitro investigations. Two different carbon-14 labelled as well as two different tritium labelled tracers of Ragaglitazar were synthesised. The carbon-14 label was introduced from either ethyl bromo[2-^{14}C]acetate (5 steps/33% overall yield) or [U-^{14}C]phenoxazine (4 steps/48% overall yield). Tritium was incorporated either by catalytic tritiation of an alkene precursor followed by chiral HPLC separation (2 steps/17% overall yield) or by catalytic tritium–halogen exchange of an aryl bromide precursor (2 steps/68% overall yield). Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: carbon-14; tritium; type 2 diabetes; PPARα and PPARγ agonist; Ragaglitazar

Introduction

Ragaglitazar is being developed as a new anti-diabetic drug for the treatment of type 2 diabetes. Ragaglitazar acts by increasing the sensitivity towards insulin, thus normalizing blood glucose levels.

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On the molecular level, the target is the peroxisome proliferator-activator receptors (PPAR), for which Ragaglitazar is an agonist targeting both the PPARγ and the PPARα receptor subtypes. These are linked to the plasma glucose and plasma triglycerides lowering effect, respectively.[1] Ragaglitazar (Figure 1) is a phenoxazine analogue of phenyl propionic acid and belongs to a new class of compounds, which possess this unique dual acting ability.[2]

Drug candidates in development must be submitted for a series of metabolism studies, for which a tracer labelled with carbon-14 is the preferred choice. The labelling position is of paramount importance, since the amount of information, that can be obtained, will depend on this decision. In order to achieve as detailed information as possible, it was decided to synthesize a tracer labelled with carbon-14 in the ethoxy spacer as well as a tracer labelled in the phenoxazine moiety.

Tritium has less metabolic stability than carbon-14 *in vivo*, but it offers the advantage of high specific radioactivity and is very useful for exploratory *in vitro* investigations. In most cases, tritium also offers the added advantage of shorter and faster synthetic routes compared to carbon-14. Two tritium labelled tracers were prepared: one labelled in the phenyl propionic acid part and one labelled in the phenoxazine moiety.

Results and discussion

[Ethoxy-2-^{14}C]Ragaglitazar

For the carbon-14 labelling of Ragaglitazar in the ethoxy spacer, we decided to introduce the label starting from ethyl bromo[2-^{14}C]acetate, which is a commercially available starting material. Reaction of

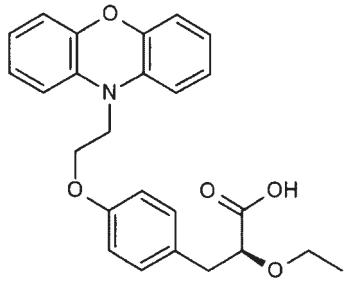
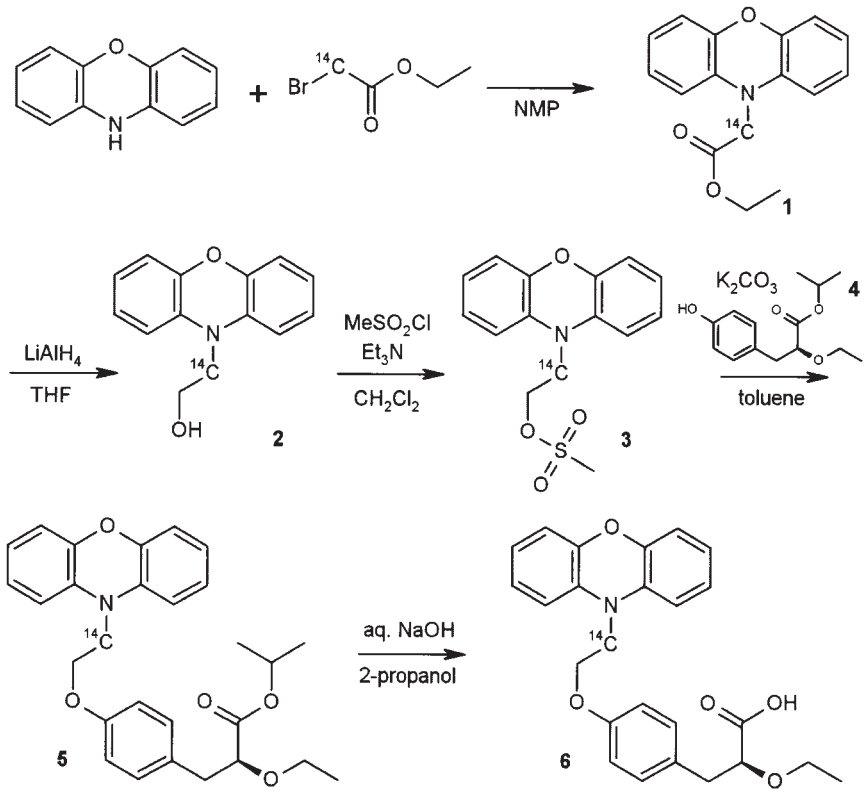


Figure 1. Structure of Ragaglitazar



Scheme 1. Synthesis of [ethoxy-2-^{14}C]Ragaglitazar

phenoxazine with ethyl bromo[2-^{14}C]acetate proceeded to give the ester **1** in high yield (84%) (Scheme 1). It was necessary to run the reaction with an excess of phenoxazine (5 eq.) in order to achieve full conversion of ethyl bromo[2-^{14}C]acetate. However, this necessitated a purification step by HPLC.

The ester **1** was reduced to the alcohol **2** with lithium aluminium hydride. It was necessary to add excess lithium aluminium hydride (4 eq.) in order to achieve complete radiochemical conversion. Reaction of **2** with methanesulfonyl chloride gave the activated sulfonate **3**. The reaction proceeded smoothly and quickly when using an excess (5 eq.) of methanesulfonyl chloride and triethylamine and provided **3** in 84% yield (two steps).

The sulfonate **3** was coupled to isopropyl (*S*)-3-(4-hydroxyphenyl)-2-ethoxypropionate (**4**) in the presence of K_2CO_3 (toluene, 100°C, 16 h) to give the ester **5** in 93% yield. Finally, the ester **5** was hydrolysed in

aqueous NaOH. Purification by semi-preparative HPLC gave [ethoxy-2-^{14}C]Ragaglitazar (**6**) (51%) in an overall yield of 33% and with a radiochemical purity of >98%. Analysis of the enantiomeric purity (ee >97%) showed that no racemization had taken place during the synthesis, neither in the coupling nor in the hydrolysis step. The specific activity was determined by mass spectroscopy to be 55 mCi/mmol.

[Phenoxazine-U-^{14}C]Ragaglitazar

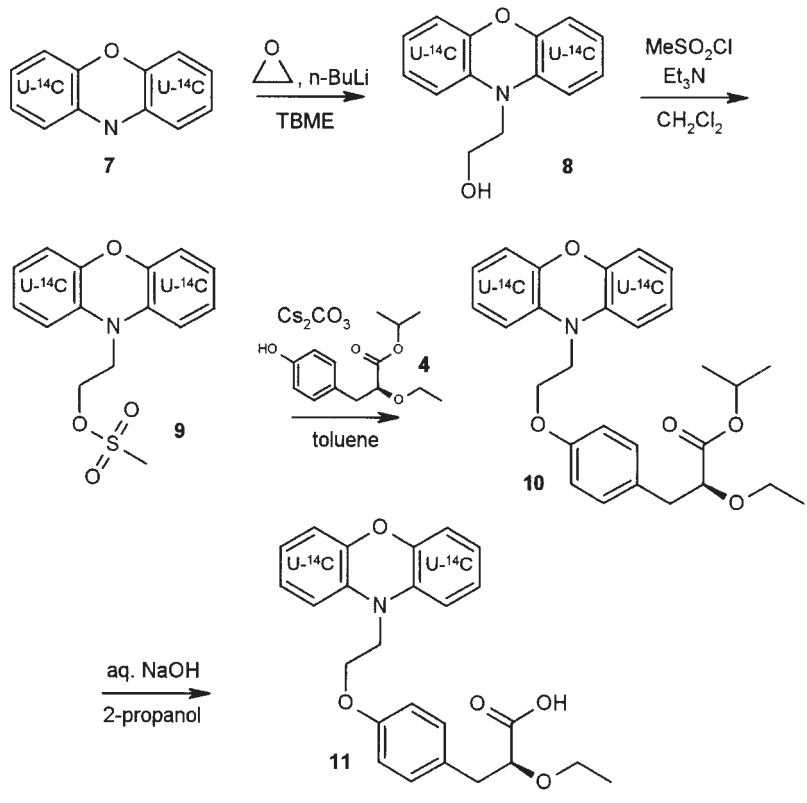
The labelling of Ragaglitazar with carbon-14 in the phenoxazine moiety started from [U-^{14}C]phenoxazine (**7**), which was acquired from Amersham Biosciences. Although it would be possible to use the same synthetic strategy as for labelling in the ethoxy spacer, this would not be practical, as the first step requires the use of five equivalents of phenoxazine. Thus, a new synthetic strategy was needed for this synthesis.

The reaction between [U-^{14}C]phenoxazine (**7**) and ethylene oxide offered the opportunity of a simple one step route to 2-([phenoxazine-U-^{14}C]-10-yl)ethanol (**8**) (Scheme 2). However, it was difficult to control the addition of the ethylene oxide gas and excess ethylene oxide was found to produce a large amount of impurities. We found that by employing a large excess of *n*-butyllithium (25 eq.) that this sensitivity towards ethylene oxide was negated, and **8** was easily produced in high yield (100% crude yield). Excess n-butyllithium most likely acts by quenching excess ethylene oxide, thus preventing it from reacting with **8**.

Conversion of the alcohol **8** to the sulfonate **9** was carried out in quantitative yield similar to the procedure for the preparation of **3**.

Although the coupling reaction using K_2CO_3 proceeded without major problems, we felt that room for improvement still existed. Especially, we wanted to shorten the reaction time, if this could be performed without compromising yield and purity. For this reason, we decided to investigate the use of a different base in the coupling step. Winters *et al.* have compared the alkylation of potassium and cesium phenolates with different alkylating agents.[3] They found the use of Cs_2CO_3 as the base superior to K_2CO_3, as it gave accelerated reaction rates, higher yields, and also provided for the introduction of a wider range of alkyl groups. This is attributed to the greater polarizability and reduced solvation of the cesium cation.[3]

This proved to be true in our case as well. The sulfonate **9** was coupled with **4** using Cs_2CO_3 in toluene at 100°C. These conditions



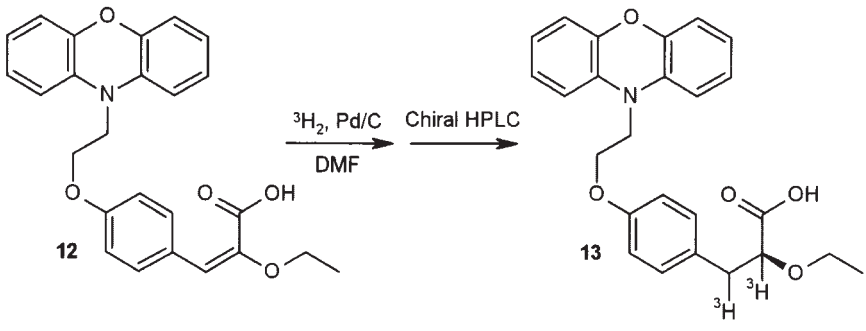
Scheme 2. Synthesis of [phenoxazine-U-^{14}C]Ragaglitazar

proceeded to give **10** in quantitative yield within a relatively short reaction time (2–4 h). Whilst the crude yield proved higher (100% vs. 93%), the purity was slightly lower (76% vs 87%) making the two reactions comparable, when the two factors were combined.

The final hydrolytic step toward [phenoxazine-U-^{14}C]Ragaglitazar (**11**) was carried out as described above to give **11** in an overall yield of 48% and with a radiochemical purity of >98%. The enantiomeric purity was found to be >97%, which again showed that no racemization had taken place during synthesis. The specific activity was determined by mass spectroscopy to be 81 mCi/mmol.

[Propionic-2,3-$^{3}H_2$]Ragaglitazar

Whereas labelling with carbon-14 often requires lengthy syntheses in order to incorporate the label in a metabolically stable position, tritium



Scheme 3. Synthesis of [propionic-2,3-$^3H_2$]Ragaglitazar

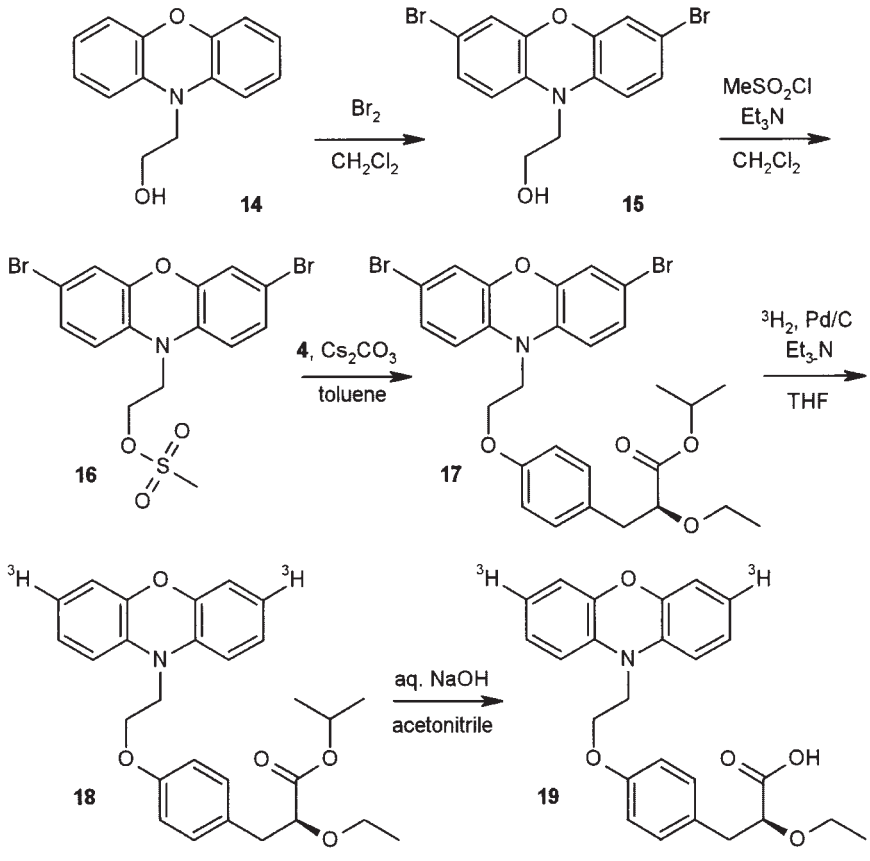
labelling more often relies on the availability of a suitable precursor, which in a few steps can lead to the desired molecule.

Our first strategy for the synthesis of tritium labelled Ragaglitazar was based on the catalytic tritiation of acrylic acid **12**, which in one step would lead to tritium labelled Ragaglitazar, albeit in the racemic form. Reaction of **12** with tritium gas and Pd/C in DMF proceeded smoothly to give crude racemic **13** (66% — based on **12**) (Scheme 3). Separation by chiral HPLC followed by purification by reverse phase HPLC provided the desired enantiomer of [propionic-2,3-$^3H_2$]Ragaglitazar (13) in 17% overall yield with a radiochemical purity $>98\%$ and an enantiomeric purity $>97\%$. The specific radioactivity was determined to be 27 Ci/mmol (MS).

[Phenoxazine-6,14-$^3H_2$]Ragaglitazar

The relatively low specific activity obtained in the synthesis of **13** combined with the need for chiral separation prompted us to develop a second method based on catalytic debromination. In our experience, this routinely gives high specific activities (25–30 Ci/mmol per atom incorporated), whereas tritiation of double bonds often gives lower incorporation of tritium (10–15 Ci/mmol per atom incorporated).

The synthetic strategy was to obtain a brominated precursor of the Ragaglitazar isopropyl ester with the correct chiral configuration using much of the chemistry described above. Tritium would be introduced by catalytic dehalogenation followed by hydrolysis of the ester to complete the synthesis.



Scheme 4. Synthesis of [phenoxazine-6,14-$^{3}H_2$]Ragaglitazar

Experimentation showed that direct bromination of the phenoxazine ring system in **14** was a feasible approach and with a little optimization it was possible to isolate the dibrominated compound **15** in one simple step in high yield (88%) (Scheme 4). Assignment of structure was performed by 2D NMR (^{1}H-^{13}C HSQC and HMBC), which clearly demonstrated the incorporation of bromine at positions 6 and 14. The ester **17** was synthesized using the same methodology employed in the syntheses of **5** and **10** and provided **17** in 77% yield (two steps). Catalytic dehalogenation using tritium gas and Pd/C in THF proceeded smoothly to give crude **18** (75%), which was hydrolysed directly to provide [phenoxazine-6,14-$^{3}H_2$]Ragaglitazar (**19**) (90%) in 68% overall yield for the steps employing radioactivity. The radiochemical purity was $>98\%$ with the specific radioactivity determined to be 53 Ci/mmol (MS).

Conclusion

In summary, we have developed synthetic methods for the labelling of the dual acting PPARα and PPARγ agonist Ragaglitazar with carbon-14 and tritium. Ragaglitazar was labelled with carbon-14 in the ethoxy spacer part of the molecule as well in the phenoxazine moiety, whereas the tritium label was introduced in the phenyl propionic acid part as well as in the phenoxazine ring. The same methodology could be employed in most of the synthetic sequences, thus ensuring a high degree of synergy. Some improvements in the individual synthetic steps were also developed in the process. In all cases, Ragaglitazar was obtained in high radiochemical and enantiomeric purity as well as with high specific activity.

Experimental

General

Ethyl bromo[2-^{14}C]acetate (specific activity: 55 mCi/mmol) and [U-^{14}C]phenoxazine (specific activity: 84 mCi/mmol) were supplied by Amersham Biosciences, UK. Reactions using tritium gas were performed on a custom built tritium handling unit from RC Tritec AG, Switzerland, who also supplied the tritium gas. Tritium gas was stored in the form of U^3H_3 and released in situ by heating the uranium bed. Phenoxazine, 2-(phenoxazine-10-yl)ethanol, and isopropyl (*S*)-3-(4-hydroxyphenyl)-2-ethoxypropionate were all supplied by Novo Nordisk A/S. 3-[4-[2-(Phenoxazine-10-yl)ethoxy]phenyl]-2-ethoxypropeonic acid was supplied by Dr Reddy's Research Foundation, India. All reagents and solvents were of analytical grade and used without further purification.

HPLC was performed using a Merck Hitachi Intelligent pump L6200 equipped with a Merck Hitachi Column Thermostat T6300 with a Rheodyne Injector and Merck Hitachi UV detector L4000 (detection at 245 nm). Detection of [^{14}C] and [^{3}H] was performed on a Canbarra Packard Flow Detector 500 TR. Reactions were monitored using the following system: RP C18 column (4.6 × 250 mm, 5 µm, OdDMeSi 120 Å, Novo Nordisk) with a gradient running from 60:40 to 20:80 A/B over 30 min followed by 0:100 A/B for 10 min (A: 10% acetonitrile in 0.1% aqueous TFA, B: 90% acetonitrile in 0.1% aqueous TFA).

Enantiomeric purity was analysed using the following system: Chiralcel OJ column (4.6 × 250 mm, Diacel Chemical Industries) with isocratic elution (hexane:ethanol:AcOH 90:10:0.3; 1.2 ml/min). Radioactivity measurements were performed on a Packard Tri-Carb 1000 liquid scintillation analyzer using Ultima Flo^{TM} M (Packard Bioscience) as liquid scintillation cocktail. Specific activities were determined on a Sciex API 300 mass spectrometer equipped with an ionspray interface. ^{1}H NMR spectra were recorded on a Bruker DRX 300 spectrometer. Flash chromatography was performed with silica gel 60 Å (Merck, 230–400 mesh), and TLC was performed on Merck F_{254} precoated silica plates.

Ethyl (phenoxazine-10-yl)[2-^{14}C]acetate (**1**)

Phenoxazine (332 mg, 1.8 mmol) was dissolved in NMP (0.6 ml) in a 1 ml reaction vial. Ethyl bromo[2-^{14}C]acetate in NMP (175 µl, 20 mCi, 0.36 mmol) was added, and the vial was filled with NMP and sealed tightly to prevent evaporation. The reaction mixture was stirred overnight at 80°C. The crude product was purified by semi-preparative HPLC using a RP C18 column (10 × 250 mm, 10 µm, OdDMeSi 120 Å, Novo Nordisk) and a gradient running from 100:0 to 20:80 A/B over 30 min (A: 10% acetonitrile in 0.1% aqueous TFA, B: 90% acetonitrile in 0.1% aqueous TFA) to provide **1** (12.6 mCi). A total of 12 repetitions of this procedure provided **1** in a total yield of 219 mCi (84%) with a radiochemical purity of > 98%.

2-(Phenoxazine-10-yl)[2-^{14}C]ethanol (**2**)

Ethyl (phenoxazine-10-yl)[2-^{14}C]acetate (**1**) (219 mCi, 4.0 mmol) in THF (50 ml) was added to a slurry of lithium aluminium hydride (0.20 g, 5.3 mmol) in THF (10 ml). The reaction mixture was stirred at room temperature, and additional lithium aluminium hydride (0.20 g, 5.3 mmol) was added after 1 and 2 h. The reaction was quenched after 2.5 h by addition of water (200 ml) and aqueous HCl (2N, 15 ml), and the mixture was extracted with EtOAc (2 × 100 ml). The combined organic layers provided **2** (185 mCi, 84%) with a radiochemical purity of > 98%.

2-(Phenoxazine-10-yl)[2-^{14}C]ethanol methanesulfonate (**3**)

Triethylamine (0.6 ml, 4.3 mmol) and methanesulfonyl chloride (0.3 ml, 3.8 mmol) were added to a solution of 2-(phenoxazine-10-yl)[2-^{14}C]

ethanol (**2**) (42 mCi, 0.77 mmol) in dichloromethane (25 ml). The reaction mixture was stirred for 3 h at room temperature and quenched by addition of water (25 ml). The mixture was extracted with dichloromethane (2×25 ml), the combined organic layers were concentrated to dryness *in vacuo* and the residue dissolved in toluene (25 ml). This provided **3** (42 mCi, 100%) with a radiochemical purity of 95%.

Isopropyl (S)-3-[4-[2-(phenoxazine-10-yl)[2-^{14}C]ethoxy]phenyl]-2-ethoxypropionate (**5**)

Isopropyl (*S*)-3-(4-hydroxyphenyl)-2-ethoxypropionate (**4**) (544 mg, 2.2 mmol) and K_2CO_3 (372 mg, 2.7 mmol) were added to a solution of 2-(phenoxazine-10-yl)[2-^{14}C]ethanol methanesulfonate (**3**) (15 mCi, 0.27 mmol) in toluene (10 ml). The reaction mixture was stirred overnight at 100°C. The reaction was quenched by addition of water (50 ml) and EtOAc (50 ml) followed by careful addition of aqueous HCl (2N), until pH 2–3 was reached. The mixture was extracted with EtOAc (2×50 ml), the combined organic layers were concentrated *in vacuo,* and the residue was dissolved in 2-propanol (15 ml). This gave **5** (14 mCi, 93%) with a radiochemical purity of 87%.

(S)-3-[4-[2-(Phenoxazine-10-yl)[2-^{14}C]ethoxy]phenyl]-2-ethoxypropionic acid (**6**)

Aqueous NaOH (5 N, 20 ml) was added to a solution of isopropyl (*S*)-3-[4-[2-(phenoxazine-10-yl)[2-14C]ethoxy]phenyl]-2-ethoxypropionate (**5**) (14 mCi, 0.26 mmol) in 2-propanol (15 ml), and the reaction mixture stirred for 5 h at 30°C. The reaction was quenched by addition of water (50 ml) and EtOAc (50 ml) followed by addition of aqueous HCl (2N) until pH 2. The mixture was extracted with EtOAc (3×50 ml), and the combined organic layers were concentrated to dryness *in vacuo*. The crude product was purified by semi-preparative HPLC using a RP C18 column (10×250 mm, 10 μm, OdDMeSi 120 Å, Novo Nordisk) and isocratic elution (acetonitrile:water:AcOH 58:42:0.1, 5 ml/min). This provided [ethoxy-2-^{14}C]Ragaglitazar (**6**) (7.1 mCi, 51%) in 33% overall yield with a radiochemical purity $>98\%$. The enantiomeric purity was determined to be $>97\%$ (HPLC), and the specific radioactivity was determined to be 55 mCi/mmol (MS).

2-([U-^{14}C]Phenoxazine-10-yl)ethanol (**8**)

[U-^{14}C]Phenoxazine (**7**) (15 mCi, 0.18 mmol) was dissolved in TBME (8 ml) under a N_2 atmosphere and cooled to -10°C to -20C°. n-Butyllithium (1.6 M in hexane, 2.8 ml, 4.5 mmol) was slowly added and the mixture stirred for 7 minutes. Then, ethylene oxide gas was bubbled directly into the reaction mixture for 30 seconds. The reaction mixture was stirred for 1 hour at room temperature and quenched by addition of aqueous HCl (1N, 3 ml) and water (45 ml). The mixture was extracted with toluene (30 ml), and the organic layer was concentrated *in vacuo* and the residue dissolved in dichloromethane (15 ml). This gave **8** (15 mCi, 100%) with a radiochemical purity of 80%.

2-([U-^{14}C] Phenoxazine-10-yl)ethanol methanesulfonate (**9**)

2-([U-^{14}C]Phenoxazine-10-yl)ethanol (**8**) (15 mCi, 0.18 mmol) was reacted according to the procedure for **3**. This provided **9** (15 mCi, 100%) with a radiochemical purity of 81%.

Isopropyl (S)-3-[4-[2-([U-^{14}C]phenoxazine)-10-yl)ethoxy]phenyl]-2-ethoxypropionate (**10**)

2-([U-^{14}C]Phenoxazine-10-yl)ethanol methanesulfonate (**9**) (15 mCi, 0.18 mmol) was reacted for 4 h at 100°C according to the procedure for **5** using Cs_2CO_3 instead of K_2CO_3. This gave **10** (15 mCi, 100%) with a radiochemical purity of 76%.

(S)-3-[4-[2-([U-^{14}C]Phenoxazine-10-yl)ethoxy]phenyl]-2-ethoxypropionic acid (**11**)

Isopropyl (*S*)-3-[4-[2-([U-^{14}C]phenoxazine-10-yl)ethoxy]phenyl]-2-ethoxypropionate (**10**) (15 mCi, 0.18 mmol) was hydrolysed for 1 h at 60°C according to the procedure for **6**. The crude product was purified by semi-preparative HPLC using a RP C18 column (10 × 250 mm, 10 μm, OdDMeSi 120 Å, Novo Nordisk) and isocratic elution (acetonitrile:water:AcOH 44:56:0.1; 5 ml/min). This provided [phenoxazine-U-^{14}C]Ragaglitazar (**11**) (7.2 mCi, 48%) in 48% overall yield with a radiochemical purity > 98%. The enantiomeric purity was determined to be > 97% (HPLC), and the specific radioactivity was determined to be 81 mCi/mmol (MS).

(S)-3-[4-[2-(Phenoxazine-10-yl)ethoxy]phenyl]-2-[2,3-di-^{3}H]-ethoxy-propionic acid (**13**)

Pd/C (10%, 1.2 mg) was added to a solution of 3-[4-[2-(phenoxazine-10-yl)ethoxy]phenyl]-2-ethoxypropeonic acid (**12**) (2.3 mg, 5.5 μmol) in DMF (0.55 ml). The reaction mixture was degassed by three freeze/thaw cycles and then stirred overnight with tritium gas (7.9 Ci) at room temperature. Excess tritium gas was re-absorbed on to an uranium waste bed followed by filtration of the reaction mixture and lyophilisation with ethanol (3 × 1 ml) in order to remove labile tritium. Finally, the residue was dissolved in ethanol (5 ml) to give crude **13** (97 mCi, 66%) with a radiochemical purity of 81%.

Purification by chiral HPLC using a Chiralcel OJ column (4.6 × 250 mm, Diacel Chemical Industries) and isocratic elution (hexane:ethanol:AcOH 90:10:0.3; 1.2 ml/min) gave the (*S*)-enantiomer of **13** (27 mCi) in 96% radiochemical purity. 3.4 mCi of this was further purified by HPLC using a RP C18 column (4.6 × 250 mm, 5 μm, OdDMeSi 120 Å, Novo Nordisk) and isocratic elution (ethanol:water 57:43; 0.5 ml/min). This provided [propionic-2,3-$^3H_2$]Ragaglitazar (**13**) (3.2 mCi, 17%) with a radiochemical purity > 98%. The compound was formulated in the HPLC pool, as it is unstable towards concentration. The enantiomeric purity was determined to be > 97% (HPLC), and the specific radioactivity was determined to be 27 Ci/mmol (MS).

2-(6,14-Di-bromo-phenoxazine-10-yl)ethanol (**15**)

A solution of Br_2 in dichloromethane (0.18 g/ml, 1.0 ml, 1.1 mmol) was added to a solution of 2-(phenoxazine-10-yl)ethanol (**14**) (0.12 g, 0.53 mmol) in dichloromethane (10 ml). The reaction mixture was stirred for 1 hour at room temperature and concentrated *in vacuo*. Purification by flash chromatography (EtAOc/heptane 4:6) gave **15** as colourless crystals (0.18 g, 88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.67 (2 H, t, *J* 6 Hz), 3.88 (2 H, t, *J* 6 Hz), 5.29 (1 H, s), 6.45 (2 H, t, *J* 8.5 Hz), 6.74 (2 H, d, *J* 2 Hz), 6.89 (2 H, dd, *J* 2.0, 8.5 Hz).

2-(6,14-Di-bromo-phenoxazine-10-yl)ethanol methanesulfonate (**16**)

2-(6,14-Di-bromo-phenoxazine-10-yl)ethanol (**15**) (97 mg, 0.25 mmol) was reacted according to the procedure for **3**. This provided **16** as off-white crystals (0.13 g, 100%), which were used directly in the following step.

Isopropyl (S)-3-[4-[2-(6,14-dibromo-phenoxazine-10-yl)ethoxy] phenyl]-2-ethoxypropionate (**17**)

Isopropyl (*S*)-3-(4-hydroxyphenyl)-2-ethoxypropionate (**4**) (129 mg, 0.50 mmol) and Cs_2CO_3 (230 mg, 0.71 mmol) were added to a solution of 2-(6,14-di-bromo-phenoxazine-10-yl)ethanol methanesulfonate (**16**) (112 mg, 0.25 mmol) in toluene (10 ml). The reaction mixture was stirred for 2 h at 100°C. Then, the reaction was quenched by addition of water (10 ml) and toluene (15 ml), and the mixture extracted with toluene (2 × 25 ml). The combined organic layers were washed with brine (10 ml), dried ($MgSO_4$), and concentrated *in vacuo*. Purification by flash chromatography (EtOAc/heptane 1:4) gave **17** as a clear oil (0.12 g, 77%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15 (3 H, t, *J* 7.0 Hz), 1.18 (3 H, d, *J* 6.5 Hz), 1.24 (3 H, d, *J* 6.5 Hz), 2.93 (2 H, m), 3.33 (1 H, m), 3.58 (1 H, m), 3.86 (2 H, t, *J* 6.5 Hz), 3.92 (1 H, dd, *J* 6.0, 7.0 Hz), 4.12 (2 H, t, *J* 6.5 Hz), 5.04 (1 H, m), 6.47 (2 H, d, *J* 8.5 Hz), 6.73 (2 H, d, *J* 2.0 Hz), 6.77 (2 H, d, *J* 8.5 Hz), 6.89 (2 H, dd, *J* 2.0, 8.5 Hz), 7.16 (2 H, d, *J* 8.5 Hz).

Isopropyl (S)-3-[4-[2-([6,14-di-^{3}H-phenoxazine]-10-yl)ethoxy]phenyl]-2-ethoxypropionate (**18**)

Pd/C (10%, 7.0 mg) and Et_3N (10 µl, 72 µmol) were added to a solution of isopropyl (*S*)-3-[4-[2-(6,14-dibromo-phenoxazine-10-yl)ethoxy]phenyl]-2-ethoxypropionate (**17**) (7.7 mg, 12 µmol) in THF (0.4 ml). The reaction mixture was degassed by three freeze/thaw cycles and stirred overnight with tritium gas (9.5 Ci). Excess tritium gas was re-absorbed on to an uranium waste bed followed by filtration of the reaction mixture and lyophilization with ethanol (3 × 1 ml) in order to remove labile tritium. Finally, the residue was dissolved in acetonitrile (5 ml) to give **18** (470 mCi, 75%) with a radiochemical purity of 88%.

(S)-3-[4-[2-([6,14-Di-^{3}H]-phenoxazine-10-yl)ethoxy]phenyl]-2-ethoxypropionic acid (**19**)

Aqueous NaOH (1 N, 0.3 ml) was added to a solution of isopropyl (*S*)-3-[4-[2-([6,14-di-^{3}H]-phenoxazine-10-yl)ethoxy]phenyl]-2-ethoxypropionate (**18**) (70.5 mCi, 1.3 mmol) in acetonitrile (1.5 ml), and the reaction mixture was stirred for 5 hours at 60°C. The reaction was quenched by addition of aqueous HCl (2N, 0.3 ml). Approximately 20 mCi of this solution was purified by HPLC using a RP C18 column (4.6 × 250 mm,

5 μm, OdDMeSi 120 Å, Novo Nordisk) and isocratic elution (acetonitrile:water:AcOH 42:58:0.1; 1 ml/min). This provided [phenoxazine-6,14-$^{3}H_2$]Ragaglitazar (**19**) (18 mCi, 90%) in 46% overall yield with a radiochemical purity >98%. The specific radioactivity was determined to be 53 Ci/mmol (MS).

Acknowledgements

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References

1. Lohray BB, Lohray VB, Bajji AC, *et al*. *J Med Chem* 2001; **44**: 2675–2678.
2. Sauerberg, P, Pettersson I, Jeppesen L, *et al*. *J Med Chem* 2002; **45**: 789–804.
3. Winters RT, Sercel AD, Showalter HDH. *Synthesis* 1988; 712–714.

