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Asymmetric [^{3}H]-labeling using ruthenium catalyzed transfer hydrogenation

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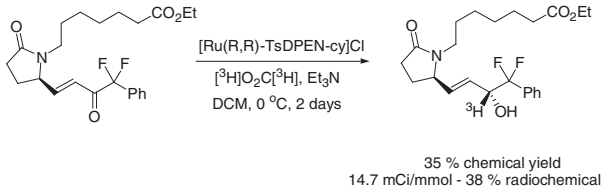
The first general method for asymmetric tritiation using ruthenium catalyzed transfer hydrogenation and its application to the radiosynthesis of an exemplary prostaglandin EP4 receptor-selective Prostaglandin E_2 analog is described. The methodology should be of general utility for the enantioselective preparation of α-tritiated chiral secondary alcohols.

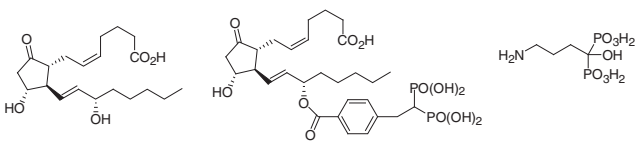


Keywords: radiosynthesis; tritium labeling; asymmetric reduction; transfer hydrogenation; prostaglandin E_2; PGE_2

Introduction

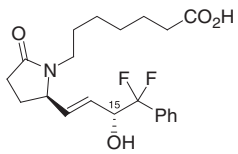
Prostaglandin E_2 (PGE_2, **1**) shows efficacy in promoting bone growth through activation of the EP4 G-linked protein receptor.[1,2] Unfortunately at therapeutic doses PGE_2 induces unacceptable side effects and cannot be used as a stand-alone treatment for osteoporosis. In previous attempts to avoid these unwanted side effects, PGE_2 was conjugated to bone-targeting bisphosphonates (for example, **2**) for site-selective delivery to bone.[3–5] In these studies the uptake of the conjugate into bone and subsequent release of PGE_2 was quantified by using radiolabeled PGE_2.[3] Tissue distribution of bioactive molecules is often monitored by using radiolabeled compounds and in this case was the only practical way to derive the needed proof of uptake and release.

Although some *in vivo* efficacy was observed for these conjugates, PGE_2 proved unsuitable for coupling with other more potent anti-resorptive bisphosphonates, such as alendronic acid **3**. Subsequently we have developed a series of potent, EP4-selective and more chemically stable agonists such as **4**,[6–8] and we are now in the process of preparing a series of conjugates of **4** with alendronic acid for *in vivo* evaluation. These studies will be the subject of future publications.



1 2 3

In order to measure the uptake of these new conjugates into bone and the subsequent release of the EP4 agonist over time it is necessary to prepare the radiolabeled EP4 agonist. This article describes the preparation of tritium labeled **4** *via* an unprecedented ruthenium catalyzed asymmetric transfer tritiation.



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Results and discussion

The obvious solution to insert tritium by reduction of precursor enone **5** with sodium borotritiide is not viable as this reaction strongly favors the undesired stereoisomer at C15, which is essentially inactive as an EP4 agonist. Because the stereochemistry at C15 is critical to the activity of **4** and wanting a general method to introduce tritium into this series of EP4 agonists, we turned our attention to methods for the asymmetric introduction of tritium. Such labeling strategies have not been described. The current protocol for the synthesis of unlabeled **4** is *via* the asymmetric reduction of enone **5** using ruthenium catalyzed transfer hydrogenation (Scheme 1).[9]

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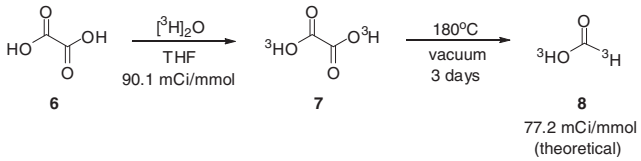
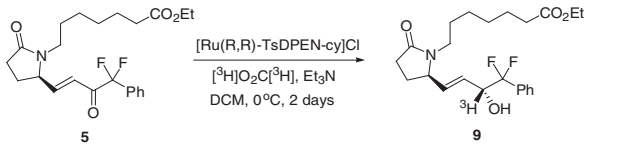
Scheme 1.



6 7 8

77.2 mCi/mmol
(theoretical)

Scheme 2.



5 9

35 % chemical yield
14.7 mCi/mmol - 38 % radiochemical yield
42 % returned starting material (not labelled)

Scheme 3.

Our approach was to adapt the known asymmetric transfer hydrogenation to accommodate the use of a tritium source in place of the current proton source, formic acid. Unfortunately the preferred hydrogen donors formic acid and 2-propanol are not available in tritiated form. Of the two, formic acid is the more attractive tritium donor in this procedure as the reduction proceeds with kinetic enantioselection as opposed to being under thermodynamic control as is the case with 2-propanol. The use of formic acid therefore normally results in increased yields. This is particularly important in a tritiated version of this reduction as it in theory assures that maximum tritium will be consumed should we use a slight excess of the enone **5**.

Literature reports for the generation of $[^2H]_2$-formic acid describe its synthesis *via* the thermolysis of $[^2H]_2$-oxalic acid, with the deuterium being introduced by proton–deuterium exchange between $[^1H]_2$-oxalic acid and $[^2H]_2O$.[10] Thus, replacing $[^2H]_2O$ with $[^3H]_2O$ allowed for the synthesis of $[^3H]_2$-formic acid, with special care taken in the handling and disposal of excess $[^3H]_2O$ as described in the experimental details (Scheme 2).

The hydrogenation itself proceeds smoothly using an adapted procedure as described below by performing the reaction in the same vessel where the $[^3H]_2$-formic acid was generated, giving the desired product **9** with a radiochemical yield of 38% (Scheme 3).

Experimental

The initial sample of radioactive material was purchased as 200 μL of 5 Ci/mL (90.1 mCi/mmol) tritiated water. This sample was divided into 10 × 20 μL aliquots by transfer into 200 μL glass ampules using a 250-μL gas-tight syringe. Each ampule upon addition of the tritiated water was immediately sealed using a propylene/oxygen torch.

Anhydrous oxalic acid (16.7 mg, 0.185 mmol) was transferred to a 2.5-mL thick-walled glass pressure vessel. One ampule of tritiated water (20 uL, 90.1 mCi/mmol, 1.11 mmol) was carefully opened and the contents were transferred to the pressure vessel as a solution in THF (100 μL). The ampule was rinsed with a further 100 μL of THF, which was also placed in the pressure vessel. This mixture was shaken to dissolve the oxalic acid. After dissolution the pressure vessel was slowly placed under vacuum with gentle shaking to minimize bumping of the solvent, and then was left under vacuum for 1 h. This produced a white solid with a nominal specific activity of 77.2 mCi/mmol.

Because the oxalic acid will not exchange with all of the tritium present in the tritiated water, a special collection system was needed to assure none of the excess radioactive material was able to escape to the vacuum pump or the atmosphere. An in-line system of a −78°C cold finger, a calcium chloride trap and a second cold finger was adequate to trap the volatiles resulting from the evaporation.

While maintaining the vacuum, the pressure vessel was pull sealed using a propylene/oxygen torch. The resulting sealed pressure vessel was heated in an oven to assure uniform heating of the vessel at 180°C for 72 h. Upon cooling a colorless liquid condensed in the sealed tube which assuming complete conversion is the desired tritiated formic acid, with a theoretical mass of 9.3 mg (0.185 mmol) and a specific activity of 77.2 mCi/mmol.

The sealed tube was opened by scoring with a file and breaking near the top of the tube. On this scale there is little pressure build-up from the CO_2 produced in the generation of the formic acid. Any condensed formic acid in the upper portion of the tube was rinsed into the lower portion with DCM (2 × 50 μL). The tube was then sealed with a rubber septum under an argon atmosphere. To the tritiated formic acid solution was added a solution of the enone **5** (82.5 mg, 0.196 mmol) and Et_3N (27.3 μL, 0.196 mmol) in DCM (100 uL, rinse with 50 μL), which was shaken gently to mix the solutions and immediately placed in a 0°C cooling bath. After 10 min, the Noyori catalyst [Ru-(*R*,*R*)-TsDPEN-cymene]Cl (3.7 mg, 0.00582 mmol) was added as a solution in DCM (100 μL, 50 μL rinse). After gentle shaking to mix the solutions, the reaction was left to stand at 0°C for 48 h. No stirring was necessary after the initial mixing of the solutions.

After 48 h, the reaction mixture was transferred from the reaction vessel to a 20-mL glass vial using a pipette. The mixture was diluted with tert-butyldimethylether (TBDME, 4 mL) and the reaction was rinsed with TBDME (1 mL), with the rinses also being transferred to the glass vial. This organic phase was washed with saturated $NaHCO_3$, brine and water by adding the solutions to the vial, then pipetting out the aqueous phase. The organic phase was dried with $MgSO_4$, filtered through a filter pipette into a flask and evaporated to give a dark brown oil.

The product was isolated by flash chromatography (80% EtOAc/hexanes), giving returned starting material **5** (34.9 mg, 0.0828 mmol, 42%, $R_f = 0.40$) as a colorless oil and the desired product (27.8 mg, 0.0655 mmol, 35%, $R_f = 0.25$) **9** as a brown oil. The specific activity of the product **9** was determined to be 14.7 mCi/mmol, corresponding to a radiochemical yield of 38% based on the nominal specific activity of formic acid used in the reduction. The product is isolated as a single detectable

diastereoisomer and is radiochemically pure. The returned starting material was not radioactive.

The purity of the product was analyzed using a HPLC system consisting of a Waters 2695 Separations Module, a Waters 996 Photodiode Array Detector, a Perkin-Elmer Radiometric 150TR Flow Scintillation Analyzer and an Agilent Eclipse XDB-C18 column (5 μm, 4.6 × 150 mm). With a mobile phase of 65% MeOH+0.1% formic acid and 35% H_2O+0.1% formic acid the undesired diastereoisomer elutes with a retention time of 8.62 min and the desired diastereoisomer elutes with a retention time of 9.61 min, as determined from the mixture of products from the reduction of enone **5** with $NaBH_4$. Analysis of the transfer hydrogenation product **9** shows only one peak (retention time = 9.60 minutes) using both the photodiode array and the radiodetector. None of the unwanted diastereoisomer was detected.

Conclusion

We have successfully synthesized a [^{3}H]-labeled EP4 agonist **9** from an easily prepared tritium source with a specific activity of 14. 7 mCi/mmol and a radiochemical yield of 38%. The successful adaptation of ruthenium catalyzed transfer hydrogenation for asymmetric tritium labeling is the first example of a chiral tritium labeling reaction, and it should be broadly applicable to situations where stereodefined tritium labels are required at a secondary alcohol. *In vivo* experiments with our labeled EP4 agonist **9** and its alendronate conjugate are currently underway.

Acknowledgement

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