Supplement Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 5 April 2010, Revised 17 May 2010, Accepted 28 May 2010 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.1801

Direct metal-catalyzed tritiation of organic compounds

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The metal catalyzed tritium labelling of diverse biologically relevant compounds at PerkinElmer Health Sciences is described.

Keywords: tritium; metal catalysis; tritium NMR

Introduction

Tritium is perhaps the ideal radionuclide for labelling valuable compounds of interest to life science investigators. With a host of installation methods, tritium provides the desired high specific activity and best preserves the substance's structural integrity. Over the past fifty years, we at PerkinElmer Health Sciences have joined other global radiochemists to develop novel and efficient methods to selectively place tritium at high specific activity in important target compounds. Recently, the diverse subject of tritiation strategy was comprehensively reviewed,[1] and the use of heterogeneous and homogeneous metal catalysis for the direct tritiation of useful organic substrates has obvious and distinct advantages over many other methods. This appealing method obviates the need for challenging precursor preparation and often facilitates product synthesis and purification. This discussion will relate our experience with the technique of direct metal-catalyzed tritiations.

Heterogeneous metal-catalyzed tritiations

Our initial exploration of this area began with heterogeneous metal-catalyzed tritium labelling. Early on, such catalysts were widely available and offered the ease of removal by simple filtration. The heterogeneous catalyst system that we have had the most experience and success with for tritiations has been 5% rhodium on alumina. Others have previously reported the use of this catalyst for hydrogen exchange chemistry,[2–5] including the most recent and intriguing paper by Jones and Lockley.[6] No doubt, the support structure and its chemistry are important to this catalyst's performance and earlier mechanistic studies with 5% rhodium on alumina have also examined the possible participation of the surface hydroxyl groups in the hydrogen exchange process.[2] We have had experience with tritiations using 5% rhodium on alumina, employing both tritiated water and tritium gas as radiolabelling reagents. Our earliest work with this tritiation method was in the steroid field and, in particular, the cardiac glycoside class of compounds. Digitoxin (**1**), isolated from the purple Foxglove (*Digitalis purpurea*), was one of the first in this structurally complex steroid series to interest us. We discovered that treatment of **1** with 5% rhodium on alumina and high specific activity tritiated water at elevated temperature for several days would afford significant amounts of [^{3}H] digitoxin (**2**) at specific activities of 20–40 Ci/mmol (Scheme 1). Not surprisingly, the closely related cardiac glycoside [^{3}H] digoxin (**3**) was similarly prepared with equal ease. The structurally less complex toxin ouabain, isolated from the seeds of the African plant *Strophanthus gratus* and historically used as an arrow poison, could also be tritiated in the same fashion, affording [^{3}H] ouabain (**4**). The proton decoupled tritium NMR spectra for these latter two products can be seen in Figures 1 and 2 respectively. These spectra reveal that the catalyst has regiospecifically introduced tritium almost equally into the butenolide lactone olefin (the most downfield peak) as well as both the alpha and beta positions of the butenolide lactone allylic oxymethylene. This tritium exchange process was indeed robust, with little batch-to-batch product variation observed when analyzed by tritium NMR. From time-to-time, a small radiochemical impurity was detected in these tritiations. After isolating and analyzing it, our speculation is that it was the result of catalyst-mediated isomerization of the butenolide lactone ring from the beta to the alpha position, resulting in an allylic 17-beta tritium resonance as seen at 3.1 ppm. Since the butenolide ring structural motif is so ubiquitous in numerous natural products of diverse biological activity,[7] it is likely that the use of 5% rhodium on alumina with high specific activity water would be an effective method to tritium label these valuable substances as well.

This same tritium labelling technique was applied to other compounds of interest as well. For instance, we were able to prepare the steroid bile acid [2,4-^{3}H] cholic acid (**6**) in a stepwise process from 3-keto precursor **5** (Scheme 2). Initially, exchange labelling with 5% rhodium on alumina and tritiated water easily inserted tritium into the 2 and 4 A-ring positions. Finally, sodium borohydride reduction of the intermediate 3-ketone followed by basic hydrolysis afforded the desired product with the tritium labels conveniently secured in the now non-exchangeable

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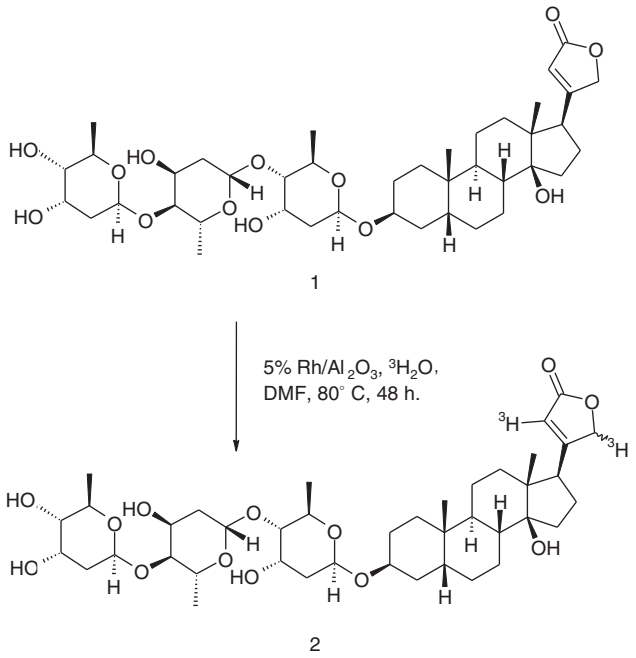


1

2

Scheme 1. Synthesis of [^{3}H] digitoxin **2**.

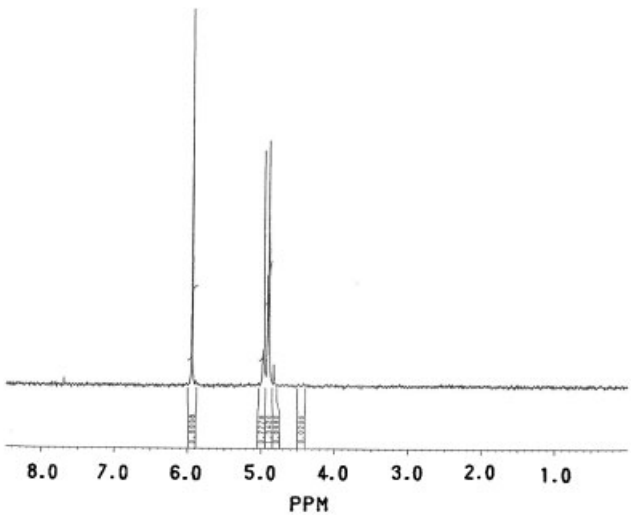


Figure 1. Proton decoupled tritium NMR (CD_3OD) of **3**.

A ring locations. The complex proton decoupled tritium NMR for product **6** is seen in Figure 3 and reveals each of the four exchanged tritons of the 2 and 4 positions as sharp singlets. Our interpretation of the tritium chemical shift assignments for this spectrum is as follows: 1.38 ppm (2-alpha, 16%); 1.68 ppm (2-beta, 28%); 1.72 ppm (4-beta, 41%); 2.23 ppm (4-alpha, 15%). We have also used this exact technique to directly tritiate other substances of varied structure and application, including 4-hydroxyacetanilide, the anti-psychotic [^{3}H] haloperidol (**7**),[8] the anthracycline antibiotic daunomycin and the photoactivated anti-tumor agent hematoporphyrin derivative.[9–12] The proton decoupled tritium NMR (CD_3OD) of **7** showed a sharp multiplet at 3.20 ppm, corroborating the exclusive tritium labelling of the methylene position adjacent to the carbonyl group. Another interesting example of this versatile method is that of [^{3}H]

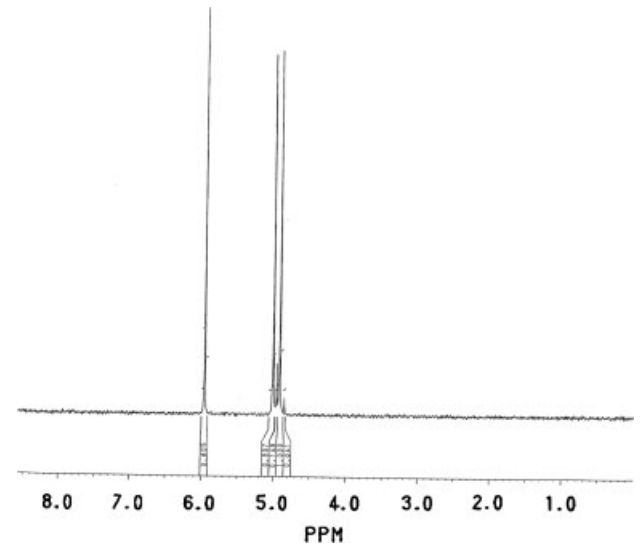


Figure 2. Proton decoupled tritium NMR (CD_3OD) of **4**.

3

4

5

1. 5% Rh/Al2O3, 3H2O, TEA, DMF, 100° C, 16 h.
2. NaBH4, KOH

6

Scheme 2. Synthesis of [2,4-^{3}H] cholic acid **6**.

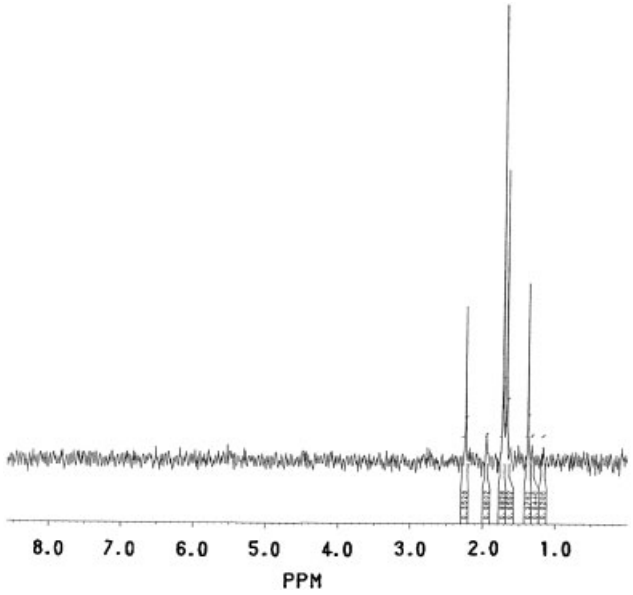


Figure 3. Proton decoupled tritium NMR ($CDCl_3$) of **6**.

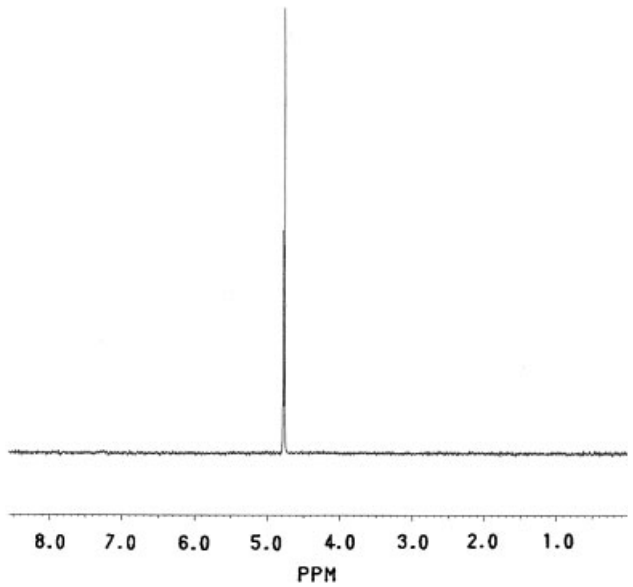
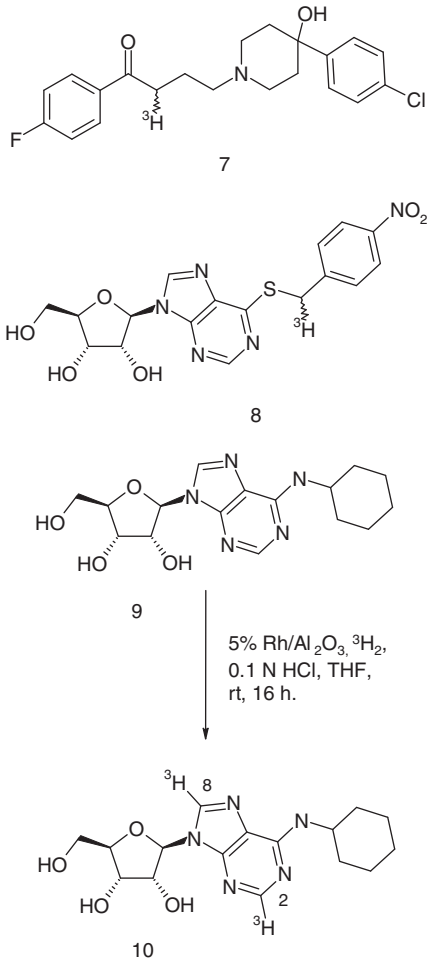


Figure 4. Proton decoupled tritium NMR (CD_3OD) of **8**.



Scheme 3. Synthesis of [2,8-^{3}H] N^6-cyclohexyladenosine **10**.

nitrobenzylthioinosine (**8**) which has been successfully employed as a photoaffinity label[13] for the nucleoside transport protein. Radioligand **8** was also tritium labelled by employing 5% rhodium on alumina with tritiated water. Its tritium NMR in Figure 4 indicates that the installation of tritium had occurred exclusively at the benzyl methylene position with no concomitant reduction of the aromatic nitro group. It also revealed the presence of two isotopologues for the product, with the peaks at 4.76 and 4.78 ppm attributed to the ditritiated and monotritiated species respectively. Clearly, with products **6**, **7** and **8**, besides direct metal-catalyzed tritiation, it is possible that base-catalyzed tritium exchange played a role in these radiolabellings as well.

While the use of tritiated water has dominated our efforts using 5% rhodium on alumina, we have also had success with tritium gas. Bufalin is a cardiotonic steroid isolated from the venomous Chinese toad *Bufo bufo gargarizans* and we tritium labelled it using 5% rhodium on alumina with tritium gas to a specific activity of 4.1 Ci/mmol. Although no tritium NMR was obtained, we suspect by analogy with other cardiac glycosides that tritium was added predominantly to the dienolide lactone ring. The nucleoside N^6-cyclohexyladenosine (**9**), a selective A_1 adenosine receptor agonist, was similarly tritiated by this method (Scheme 3). A proton decoupled tritium NMR (CD_3OD) of product **10** (Figure 5) clearly revealed regiospecific exclusive adenine ring tritium incorporation at the 2 (8.22 ppm) and 8 (8.28 ppm) positions in a ratio of approximately 40:60. This positional tritium distribution ratio was observed to be essentially constant in batch-to-batch runs. The corresponding proton NMR (CD_3OD) of product **10** also showed the expected and complimentary proton deficiency for the adenine 2 and 8 ring positions. Furthermore, by integration of the residual proton resonances, it easily allowed the calculation of specific activity for **10**, which routinely exceeded 25 Ci/mmol. The neurochemicals amphetamine and histamine were also tritium labelled in this same manner at high specific activity.

Along with 5% rhodium on alumina, we have had some success with various other direct metal-assisted tritiations. For example, Pounds reported the use of 5% palladium on calcium carbonate and tritium gas to tritium label the aminoglycoside amikacin (**11**).[14] In this procedure the antibiotic

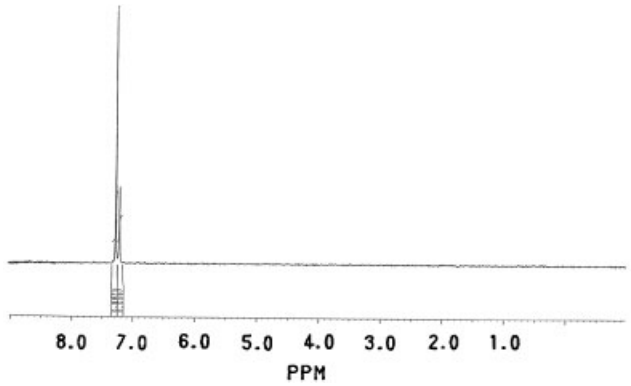


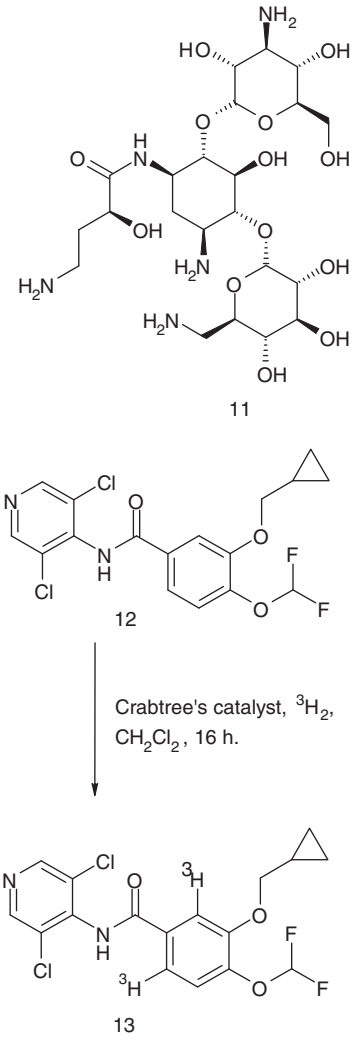
Figure 5. Proton decoupled tritium NMR (CD_3OD) of **10**.

was absorbed directly onto the catalyst and heated at 160°C with tritium for 0.5 h with a specific activity outcome of 3.4 Ci/mmol. Finally, we have employed 5% palladium on barium sulfate with tritium gas to radiolabel the bis-indole alkaloid staurosporine at 17.2 Ci/mmol as well as platinum oxide with tritiated water to tritium label both 3-methylcholanthrene at 2.4 Ci/mmol and tyramine at 13.6 Ci/mmol.

Homogeneous metal-catalyzed tritiations

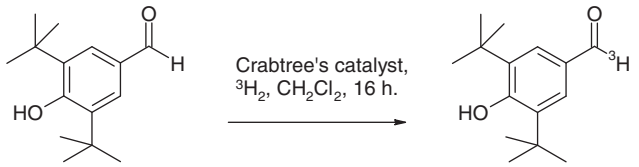
The concept of using homogeneous metal catalysts for olefin tritiation was something quite familiar to us and for decades we had employed Wilkinson's catalyst[15] for this purpose. This catalyst was especially useful in the steroid area based on the pioneering work of Djerassi regarding its deuteration regioselectivity.[16] The thought that homogeneous metal catalysis could also promote tritium exchange was quite surprising and for that keen insight we must be grateful to the first report by Heys in 1992 of iridium-mediated hydrogen exchange.[17] Additionally, the key paper by Hesk and Schering-Plough co-workers[18] employing commercially available and stable Crabtree's catalyst for hydrogen exchange reactions prompted our increased attention to this methodology. Another important contribution that guided us in this technique was the thorough exploration of the scope and limitations of Crabtree's catalyst as described by Herbert and Sanofi-Aventis co-workers.[19]

One of the most intriguing papers in this area was the contribution of Lockley along with AstraZeneca colleagues, describing the preparation of polystyrene bead impregnated Crabtree's catalyst for the effective *ortho* exchange of many aromatic substrates.[20] Like many others, we had observed the annoying catalyst promoted tritiated impurities that often arise during these exchange tritiations, greatly complicating purification. An explanation suggested for this observation was the formation of catalyst deactivating clusters, originally reported and characterized by Crabtree.[21] Solid supported Crabtree's catalyst, minimizing iridium catalyst-to-catalyst interaction and cluster formation, would seem to be a logical remedy, but its practical synthesis had been elusive for several decades. The fact that the preparation of a CODIr(I) catalyst anchored to diphenylphosphinylated polystyrene beads is as simple and straightforward as described in footnote 6 of Lockley's paper is indeed remarkable, and we ourselves have successfully prepared this useful reagent exactly as technically detailed. Perhaps even more surprising is the fact that the newly constrained iridium catalyst is as substrate accessible and active as it is.



Scheme 4. Synthesis of [^{3}H] roflumilast **13**.

We have tritium labelled a number of substances with Crabtree's catalyst, but for confidentiality reasons we can only disclose a few examples at this time. Roflumilast (**12**) is an anti-inflammatory drug that acts as an enzyme inhibitor for the phospho-diesterase enzyme PDE-4 and we have long been interested in the tritiation of such suicide enzyme substrates.[22,23] To the best of our knowledge the tritiation of **12** has not yet been reported and its structure presented limited options for high specific activity radiolabelling. One could possibly employ the rhodium black tritium exchange method as reported by Jones and Lockley,[6] targeting the available 2-position pyridine hydrogens. However, based on the work of Hesk,[18] we reasoned that compound **12** might also be a suitable substrate for Crabtree's catalyst tritiation. As seen in Scheme 4 this method worked very smoothly to provide [^{3}H] roflumilast (**13**) at 77.7 Ci/mmol as determined by mass spectral analysis. Although no tritium NMR was obtained for **13**, the large cluster of peaks centered around 409 *m/z* (the MW of **12** is 403) documented the incorporation of tritium in at least 2, and to some extent, 3 positions. Based on mass spectral fragmentation evidence and earlier precedent, we infer that tritium had been installed as depicted in Scheme 4. However, as pointed out by a reviewer, other precedented[19] sites of tritium incorporation for **13** are the available 2-positions of the pyridine ring and its high

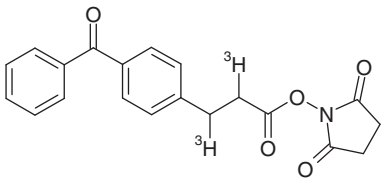


14 15

Scheme 5. Synthesis of [^{3}H] aldehyde **15**.

specific activity could have been caused by the elevated tritium gas pressure we chose for the reaction.

As observed by others, Pounds also reported the surprising result that the aldehyde proton of **14** could be easily exchanged with tritium and Crabtree's catalyst, yielding **15**[14] (Scheme 5). No *ortho* tritium insertion was noted and it is likely that the bulky *t*-butyl groups blocked catalyst access to these sites. Using this methodology one can easily imagine further reduction of such tritiated aldehydes with sodium borotritide to obtain high specific activity dual labelled alcohols as well. Armed with this relatively new homogeneous catalyst technology, it is also inviting to now muse and revisit alternative ways to tritium label compounds that were of interest to us earlier. For instance, the useful tritiated photoaffinity reagent **16** was first radiolabelled in the positions shown by means of an olefin precursor.[24] Almost certainly, it could have been directly tritiated on the benzophenone ring at high specific activity by means of homogenous iridium-based catalysis.



16

Clearly, methylene chloride is the solvent of choice for optimum catalyst performance, but it is also a widely recognized disadvantage with regard to limited solubility for many interesting substrates. Besides solubility, however, a further significant cause for concern limiting this technology is the increasing waste disposal cost of methylene chloride as a halogenated solvent. These issues make even more valuable the reported alternative use of ionic liquids by Salter[25] for such iridium-based tritiations.

Mixed heterogeneous and homogeneous metal-catalyzed tritiations

One final intriguing and puzzling topic to discuss is the very recent and serendipitous discovery by Schou[26] that Crabtree's catalyst appeared to impart a synergistic hydrogen exchange activation of some metals including rhodium black. His extensive paper was a careful study of the scope of this interesting result. Out of sheer desperation to tritium label several recent recalcitrant substrates, we recently tried this new process with some degree of success. The exact structures cannot be revealed at this time, but in one particular case we observed by tritium NMR the regiospecific insertion of tritium on a methylene adjacent to a heteroatom with a specific activity of 3.2 Ci/mmol. Although only a modest incorporation of tritium to be sure, it must be appreciated that all other attempts to tritiate this particular molecule had failed. No doubt it will be interesting to watch for other reports that employ this unique hybrid catalyst tritiation method and perhaps provide more insight into its exact mechanism.

Experimental

General

All chemicals used were reagent grade. Evaporations were carried out on a Buchi rotary evaporator at bath temperatures less than 40°C. Analytical TLC was done on Analtech 5 × 15 cm glass plates coated with silica gel. Autoradiography was performed at 0°C after spraying with PPO and exposing plates to X-ray film. TLC plates were also scanned for applied radioactivity. Preparative and analytical HPLC were accomplished on a PerkinElmer instrument and peak detection was done simultaneously by UV and a IN/US Systems Beta RAM Model 3 radioactivity detector. Solution assays were performed with a PerkinElmer Tri-Carb 3100TR instrument. NMR spectra were obtained on a Bruker 300 MHz instrument and chemical shift values are expressed in parts per million (ppm) downfield from TMS. Mass spectra were obtained on a Kratos Model MS25 RF instrument with direct injection.

[^{3}H] Digitoxin (2)

Proper glove usage and caution should be exercised in this synthesis due to the cardiotoxicity of digitoxin. Digitoxin (**1**, 76 mg, 0.1 mmol, Sigma D5878) in 0.5 mL of DMF with 300 mg of 5% Rh/Al_2O_3 catalyst and 100 Ci of tritiated water (58 Ci/mmol) was rapidly stirred and heated at 80°C for 2 days. After this time, the catalyst was filtered off and volatile tritium was removed with several evaporations of ethanol to afford 1988 mCi of crude product. It was purified by reverse phase HPLC eluted with water:methanol (25:75). Pooling of appropriate fractions, solvent evaporation under reduced pressure and reconstitution in ethanol afforded 683 mCi (a 20.6% yield based on precursor **1**) of product **2** which was >98% radiochemically pure on reverse phase HPLC (same system as above) and TLC eluted with toluene:ethanol (6:1). Furthermore, **2** completely co-chromatographed with authentic **1** in both chromatographic systems. The specific activity of product **2** was measured to be 33.1 Ci/mmol by mass spectrometry analysis. Alternatively, the product specific activity could also be measured by simultaneous solution assay and mass determination of **2**, employing UV spectrometry at a wavelength of 216 nm where **1** has an extinction coefficient value of 18 182.

[2, 8−^{3}H] N^6-Cyclohexyladenosine (10)

N^6-Cyclohexyladenosine (**9**, 18 mg, 0.05 mmol, RBI A-002) in a solution of 1.5 mL THF and 0.5 mL of 0.1 N HCl with 32 mg of 5% Rh/Al_2O_3 catalyst and 60 Ci of tritium gas was rapidly stirred at ambient temperature overnight. After this time, the catalyst was filtered off and volatile tritium was removed with several evaporations of 1 N ammonium hydroxide to afford 538 mCi of crude product. It was purified by reverse phase HPLC eluted with water:acetonitrile (70:30). Pooling of appropriate fractions, solvent evaporation under reduced pressure and reconstitution in ethanol afforded 49 mCi (a 3.2% yield based on precursor **9**) of product **10** which was >97% radiochemically pure on reverse

phase HPLC (same system as above) and TLC eluted with methylene chloride:methanol (9:1). Furthermore, **10** completely co-chromatographed with authentic **9** in both chromatographic systems. The specific activity of product **10** was measured to be 30.2 Ci/mmol by proton NMR (CD_3OD) integration of the aromatic region, noting proton deficiency. Alternatively, the product specific activity could also be measured by simultaneous solution assay and mass determination of **10** employing UV spectrometry at a wavelength of 265 nm where **9** has an extinction coefficient value of 19 400.

[^{3}H] Roflumilast (13)

Roflumilast (**12**, 6 mg, 0.015 mmol) in a solution of 2.5 mL methylene chloride with 24 mg (0.03 mmol) of Crabtree's catalyst and 113 Ci of tritium gas at a pressure of 40 psi was rapidly stirred at ambient temperature overnight. After this time, solvent and volatile tritium were removed with several evaporations of ethanol to afford 294 mCi of crude product. It was purified by reverse phase HPLC eluted with a gradient of 0.2% of aqueous TFA:methanol (40:60) to pure methanol over the course of 30 min. Pooling of appropriate fractions, solvent evaporation under reduced pressure and reconstitution in ethanol afforded 47 mCi (a 4.0% yield based on precursor **12**) of product **13** which was $>$98% radiochemically pure on reverse phase HPLC (same system as above). Furthermore, **13** completely co-chromatographed with authentic **12** in this HPLC system. The specific activity of product **13** was measured to be 77.7 Ci/mmol by mass spectrometry with the following prominent ions (*m/z*) and relative intensities observed 403 (10.55), 405 (32.54), 407 (66.59), 409 (116.45) 411 (73.69).

Acknowledgements

We thank Drs Puliyer Srinivasan and Mahendra Suryan of PerkinElmer Health Sciences, Inc. for obtaining the proton, tritium NMR and mass spectra respectively.

The skilled technical contribution of John Seaburg in the tritiation of roflumilast is also recognized.

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