JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2004; **47**: 407–414.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.827

Research Article

Synthesis of tritiated Cyclosporin A and FK-506 by metal-catalyzed hydrogen isotope exchange

V. P. Shevchenko[1], I. Yu. Nagaev[1], N. F. Myasoedov[1,*], H. Andres[2], T. Moenius[2] and A. Susan[3]

[1] *Institute of Molecular Genetics, Russian Academy of Sciences, pl. Akademika Kurchatova 2, Moscow 123182, Russia*

[2] *Preclinical Safety, Novartis Pharma AG, CH-4002 Basel, Switzerland*

[3] *International Isotopes Clearing House, Inc., 9110 Lee Blvd./P.O. Box 6986, Leawood, KS66206, USA*

Summary

Tritium-labeled Cyclosporin A (**I**) and FK-506 (**II**) have been prepared using a metal-catalysed hydrogen isotope exchange procedure and high specific activity tritiated water. Specific activities of the labeled compounds were 0.15 and 0.59 TBq/mmol, respectively. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: tritium; labeling; H/T-exchange; FK506; cyclosporin A

Introduction

The introduction of the label by isotope exchange with tritiated water can be carried out efficiently even in the absence of heterogeneous or homogeneous catalysts if the substance is stable in media with pH > 11 or < 2.[1] This is the case, for example, with camptothecin, which is resistant to heating at 90°C in 98% sulfuric acid for many days.[1]

Labeling of compounds that can exchange tritium for protons in α-positions to the keto group[2] requires milder conditions (a solution in dimethylformamide in the presence of triethylamine, 64 h, 80°C). In this manner, a number of labeled steroids have been obtained with specific activities in the range 57–130 MBq/mmol. To label primary alcohols, tris(triphenylphosphine)ruthenium(**II**) chloride has been shown to be an efficient catalyst.[3]

*Correspondence to: N. F. Myasoedov, Institute of Molecular Genetics, Russian Academy of Sciences, pl. Akademika Kurchatova 2, Moscow 123182, Russia. E-mail: nagaev@img.ras.ru

Contract/grant sponsor: Research Program 'Physico-Chemical Biology'
Contract/grant sponsor: RFFR grant 'Leading Scientific School'

Copyright © 2004 John Wiley & Sons, Ltd.

Received 6 January 2004
Revised 18 March 2004
Accepted 19 March 2004

A significant increase in the specific activity of labeled compounds has been achieved by means of *in situ* reduction of palladium or platinum oxides with gaseous tritium. The tritiated water formed has the maximum possible specific activity and is suitable for use in the form of solutions in aprotic solvents, which minimize degradation of the target product due to radiolysis and prevent isotopic dilution.

Heterogeneous catalysts that are stable to radiolysis and create no additional difficulties in the isolation of labeled preparations from reaction mixtures are usually used for work with 100% tritiated water. Several procedures have been developed for labeling of aromatic compounds by isotope exchange with tritiated water.[4]

The traditional procedure consists in stirring at room temperature a mixture of catalyst, substrate, tritiated water, and dioxane in the reaction ampoule. Under these conditions the specific activity can be as high as 65 GBq/mmol.

The most successful procedure was the one in which the substrate, PdO, and catalyst were kept in the gaseous tritium atmosphere for 10–15 min at 70°C. The resulting tritiated water and activated catalysts were frozen in liquid nitrogen and the ampoule was evacuated and filled with argon. Triethylamine solution in dioxane was injected into the ampoule, and it was sealed and kept in a thermostat at 150–200°C. The specific activity of the resulting labeled preparation reached 900–1000 GBq/mmol, which was by an order of magnitude higher than that achieved by using traditional procedures.

The application of this procedure towards various biologically active compounds, such as aromatic, sulfur containing, and alicyclic, has been studied.[5,6] The specific activities of the resulting preparations varied from 67 to 670 GBq/mmol. The dependence of the degree of isotope exchange on the nature of the catalyst, the ratio of components of the reaction mixture (PdO as tritiated water source, compound to be labeled, catalyst, dioxane, and triethylamine), temperature, and the reaction time were studied. Such reactions are normally completed within 30–180 min, the optimal catalysts for exchange are 5% PdO/Al_2O_3 and 5% $Pd/BaSO_4$, the optimal ratios of palladium oxide-catalyst-substance are (25–30) : (30–35) : (10–20), optimal solvent is a 10:1 dioxane-triethylamine mixture, and the optimal temperatures are in the range 60–200°C.[5,6] This approach was shown to be applicable for labeling of various compounds (Table 1).

However, as has already mentioned, the reaction conditions need to be related to the stability of the substrates. This is especially the case for compounds with unsaturated carbon–carbon bonds.

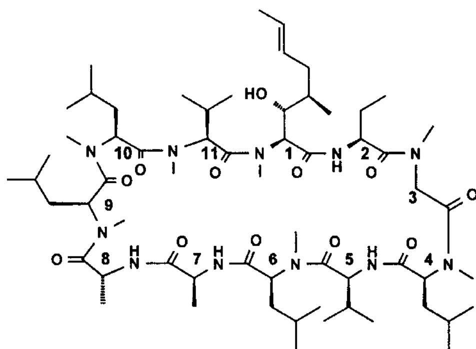
The object of the present study was to apply this approach to the tritium labeling of unsaturated, chemically highly complex compounds such as Cyclosporin A (**I**) ($C_{62}H_{111}N_{11}O_{12}$) and FK-506 (**II**) ($C_{44}H_{69}NO_{12}$) in order

Copyright © 2004 John Wiley & Sons, Ltd.

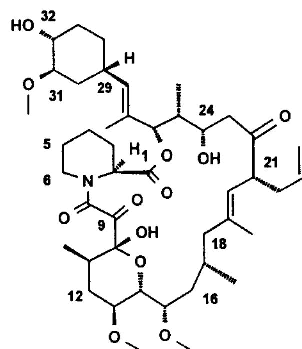
Table 1. Isotopic exchange with tritiated water[7]

Compound	Specific activity (TBq/mmol)
Coenzyme A (CoASH)	0.13–0.15
Coenzyme A acetate (CoASAc)	0.33–0.35
Thiazofurine	0.26–0.30
Alprazolam	0.93–1.00

to provide an easy and straightforward alternative to existing labeling strategies.[8]



Cyclosporin A (I)



FK506 (II)

Experimental

A 5% $Pd/BaSO_4$, PdO, and 5% PdO/Al_2O_3 catalysts were supplied by Aldrich (Milw, WI, US), Cyclosporin A and FK-506 – by Novartis International AO (Basel, Switzerland). The labeled products were analyzed and purified by thin-layer (TLC) and high-performance liquid (HPLC) chromatography. Details of the TLC systems used for Cyclosporin A and FK-506 analysis are given in Table 2, and the HPLC systems are listed in Table 3.

Multi-step TLC and HPLC purifications using different systems were needed in order to reach a radiochemical purity of 98% for both compounds (**I**) and (**II**).

The multi-channel chromatographic data were collected and processed with a Chrom&Spec v.l.5 Data Station (Ampersand Ltd., Russia).

The radioactivity was measured using a scintillation counter with the 30% tritium registration efficiency in a dioxane scintillator and with a Berthold LB506 HPLC device equipped with a cell with a solid scintillator.

The reaction conditions were optimized in experiments performed with 0.1% tritium–protium mixtures.[4,9–12] The specific activity was studied in relation to the reaction time (15–120 min), nature of the catalyst, catalyst-to-substrate ratio, and temperature (100–220°C).

Copyright © 2004 John Wiley & Sons, Ltd.

Table 2. R_f values for Cyclosporin A and FK-506 in different TLC systems on silica gel – (Sorbfil, Russia)

Compound	System	Mobile phase	R_f
(I)	**A**	Acetone-ethyl acetate-50% acetic acid (2:1:5)	0.82
(II)	**A**	Acetone-ethyl acetate-50% acetic acid (2:1:5)	0.78
(I)	**B**	*t*-butanol-methyl ethyl ketone-water-NH_4OH(c) (6.6:5.0:2.5:2.5)	0.91
(II)	**B**	*t*-butanol-methyl ethyl ketone-water-NH_4OH(c) (6.6:5.0:2.5:2.5)	0.88
(I)	**C**	Benzene-dioxane (4:1)	0.36
(II)	**C**	Benzene-dioxane (4:1)	0.31
(I)	**D**	Chloroform-dioxane (4:1)	0.65
(II)	**D**	Chloroform-dioxane (4:1)	0.44
(I)	**E**	Hexane-*i*-propanol (4:1)	0.53
(II)	**E**	Hexane-*i*-propanol (4:1)	0.44

The identity of the material was determined by co-chromatography with unlabeled reference material as well as by ^{1}H- and ^{3}H-NMR spectroscopy (Bruker AM- 500.13 and 533.46 MHz, $CDCl_3$ as solvent and TMS as internal standard). The specific activity was determined by mass spectroscopy (ionization mode: ESI).

*Procedure 1. Preparation of Dihydrocyclosporin A (**dihydro-I**)*

A reaction ampoule was charged with 0.2 ml of a 10 mg/ml solution of compound (**I**) in methanol and 4 mg of 5% Pd/BaSO4, frozen with liquid nitrogen, evacuated to 0.1 Pa, filled with 0.1% tritium to a pressure of 400 hPa, and warmed to room temperature. The reaction was performed for 30 min under stirring. Then the ampoule was frozen with liquid nitrogen, excess tritium was pumped off, the catalyst was filtered off, and labile tritium was removed by re-exchange with methanol (3 × 2 ml). The yield of (**dihydro-I**) after purification by HPLC was 70–80%.

Procedure 2. Preparation of [^{3}H]Cyclosporin A ([^{3}***H***]***I***)

A 12.1 mg of Cyclosporin A, 33.0 mg of 5% PdO/Al_2O_3, and 50.7 mg of PdO hydrate (Aldrich) were placed in the first part of a 2-section reaction vial, and 50 μl of dioxane-triethylamine mixture was placed in the second part. The reaction mixture was frozen with liquid nitrogen, the vial evacuated, and tritium gas up to a pressure of 400 hPa was introduced. The section of the vial containing the catalyst was heated up to 70–80°C for 10–15 min while the other part of the vial containing solvents was kept frozen. High specific activity tritiated water formed during the PdO reduction was condensed in the frozen part. Excess tritium was removed and the tritiated water redistilled under vacuum into the section with the substrate and catalyst. The vial was filled with argon and sealed. The reaction was carried out at 180°C for 40 min.

Copyright © 2004 John Wiley & Sons, Ltd.

Table 3. HPLC systems and retention times for Cyclosporin A and FK-506

Compound	System	Conditions	Ret. time (min)
(II)	**F**	Separon SGX CN, 7 μm, 4.6 × 250 mm, hexane-*i*-propanol (4:1), flow 1 ml/min, 40°C	11.26
(I)	**G**	Separon SGX, 5 μm, 3.3 × 150 mm, Methanol-50 mM ammonium phosphate buffer (pH 4.5) (85:15), flow 2 ml/min, 60°C	15.41
Dihydro-(I)	**G**	Separon SGX, 5 μm, 3.3 × 150 mm, Methanol-50 mM ammonium phosphate buffer (pH 4.5) (85:15), flow 2 ml/min, 60°C	16.87
(II)	**H**	Silasorb C_{18}, 13 μm, 10 × 250 mm, acetonitril-water-*o*-phosphoric acid (80:20:0.1), flow 2 ml/min, 50°C	11.31
(I)	**I**	Silasorb C_{18}, 13 μm, 10 × 250 mm, methanol-water-acetic acid (85:15:0.1), flow 2 ml/min, 60°C	15.85
Dihydro-(I)	**I**	Silasorb C_{18}, 13 μm, 10 × 250 mm, methanol-water-acetic acid (85:15:0.1), flow 2 ml/min, 60°C	17.54
(I)	**J**	Nucleosil C_{18}, 5 μm, 2 × 75 mm, methanol-50 mM ammonium phosphate buffer pH2.7 (82:18), flow 0.1 ml/min, 50°C	9.05
Dihydro-(I)	**J**	Nucleosil C_{18}, 5 μm, 2 × 75 mm, methanol-50 mM ammonium phosphate buffer pH2.7 (82:18), flow 0.1 ml/min, 50°C	10.65
(II)	**J**	Nucleosil C_{18}, 5 μm, 2 × 75 mm, methanol-50 mM ammonium phosphate buffer pH2.7 (82:18), flow 0.1 ml/min, 50°C	3.95
(II)	**K**	Nucleosil C_{18}, 5 μm, 2 × 75 mm, methanol-50 mM ammonium phosphate buffer pH 4.5 (82:18), flow 0.1 ml/min, 50°C	4.24
(I)	**L**	Nucleosil C_{18}, 5 μm, 2 × 75 mm, methanol-50 mM ammonium phosphate buffer pH 4.5 (87:13), flow 0.1 ml/min, 60°C	5.52
Dihydro-(I)	**L**	Nucleosil C_{18}, 5 μm, 2 × 75 mm, methanol-50 mM ammonium phosphate buffer pH 4.5 (87:13), flow 0.1 ml/min, 60°C	6.18
(II)	**M**	Separon SGX CN, 5 μm, 3.3 × 150 mm, hexane-*i*-propanol (4:1), flow 0.5 ml/min	13.10
(II)	**N**	Silasorb C_{18}, 13 μm, 10 × 250 mm, methanol-50 mM ammonium phosphate buffer pH 4.5 (80:20), flow 2 ml/min, 50°C	31.06
(I)	**O**	RP18 (Brownlee Labs), 5 μ, 4.6 × 250 mm, acetonitrile-water-*tret*-butylmethyl ether (TBME) (325:525:100), flow 2 ml/min, 70°C, UV(210 nm)	39.92
(II)	**P**	MN Nucleosil 100-5, C18 AB 5 μm, 250 × 3 mm A:ACN-water-TBME-*o*-phosphoric acid (240:650:70:0.2) B:ACN-water-TBME-*o*-phosphoric acid (660:200:70:0.2) 1.5 ml/min, 60°C, gradient from system A to system B	11.85

Copyright © 2004 John Wiley & Sons, Ltd.

After the reaction the ampoule was frozen again and opened, the catalyst was filtered off and washed with methanol. Labile tritium was removed by evaporation with methanol (5×3 ml). The labeled product was purified by TLC (in system **D** or **E**) and HPLC (in system **G**, **I** or **O**); yield of **[^{3}H]I** was about 80% (10.1 mg), specific activity – 30 GBq/mmol.

Procedure 3. Preparation of [^{3}H]Cyclosporin A (*[^{3}**H**]**I***) *using activated PdO*

The reaction was performed as described above in Procedure 2 using 12.4 mg of Cyclosporin A, 31.0 mg of 5% PdO/Al_2O_3, and 51.7 mg of PdO pre-activated at 200°C. The labeled product was purified by chromatography as described above; the isolated yield of **[^{3}H]I** was about 65% (8 mg), specific activity – 148 GBq/mmol.

Procedure 4. Preparation of [^{3}H]FK-506 (*[^{3}**H**]**II***)

The reaction was carried out at 180°C for 40 min as described for Cyclosporin A, using 16.8 mg of FK-506, 31.2 mg of 5% PdO/Al_2O_3, and 50.0 mg of PdO hydrate (Aldrich). The labeled product was purified by chromatographic methods (TLC in system **D** or **E**; HPLC in system **H**, **F** or **M**, and **P**); yield of **[^{3}H]II** – about 5% (0.8 mg), specific activity – 370 GBq/mmol.

Procedure 5. Preparation of [^{3}H]FK-506 (*[^{3}**H**]**II***) *using activated PdO*

The reaction was performed as in Procedure 3 but using 16.4 mg of FK-506, 30.6 mg of 5% $PdO/A1_2O_3$, and 50.3 mg of PdO (pre-activated). The labeled product was purified by chromatography as described above; the isolated yield of **[^{3}H]II** was about 10% (1.5 mg), specific activity – 592 GBq/mmol.

Results and discussion

Labeling of biologically active compounds has been the subject of numerous reviews.[8,9] Unspecific labeling of unsaturated compounds with specific activities up to 30 GBq/mmol is usually performed by liquid-phase isotopic exchange with tritium gas. Compounds of higher specific activities could be prepared by solid-phase isotope exchange with tritium gas at temperatures above 100°C, however, mono- and di-substituted C=C bonds proved to be incompatible with this approach. So, isotopic exchange with tritiated water was assumed to be a practical alternative for these kinds of substrates.

Originally, an exchange procedure was applied allowing isotopic exchange with tritiated water at elevated temperatures. When aromatic compounds were treated, any residual tritium was removed by evacuation at liquid nitrogen temperature. In the case of compounds (**I**) and (**II**) an additional catalyst desorption step was essential in order to prevent double bond hydrogenation.

Copyright © 2004 John Wiley & Sons, Ltd.

Desorption was carried out for 15 min at temperatures of 20–80°C and pressure of 0.1 Pa.

Experience showed that commercial PdO hydrate (Aldrich Chem. Ltd.) was rather inert towards reduction by tritium gas (10% only was reduced). Therefore, the catalyst was pre-activated for 4 h at 200°C in air. As a result, a quick quantitative reduction of PdO to Pd* and ^3H_2O took place. Moreover, specific activities increased for **[^{3}H]I** and **[^{3}H]II** by a factor of 4–5 and 1.5–2, respectively.

Tritium labeling of unsaturated compounds (**I**) and (**II**) on the activated catalyst at 100°C and higher in a mixture of triethylamine and dioxane produce a number of by-products. Isolation of the desired labeled product from such multi-component reaction mixtures is a demanding problem. In case of (**I**) and (**II**) this difficulty was solved by using the purification procedure described above. After final purification of Cyclosporin A and FK-506 the radiochemical purities determined by HPLC were 96 and 98%, respectively. ^{3}H-NMR spectroscopy identified tritium incorporation in about 10–15 different positions. When administered to rats ($n = 3$) HTO-formation for [^{3}H]FK-506 and [^{3}H]Cyclosporin A turned out to be less than 6% of the dose indicating a reasonable biological stability.[13]

Thus, the developed procedure allowed preparation of labeled Cyclosporin A and FK-506 of the required purity and specific activity of 148 and 592 GBq/mmol, respectively.

This work was supported by research program ‘Physico-Chemical Biology’ and RFFR grant ‘Leading Scientific School’.

References

1. Hinz HR, Harris NJ, Giovanella BC, Ezell EL, Liehr JG. *J Label Compd Radiopharm* 1996; **38**: 733–742.
2. Kolbe A, Schneider B, Voigt B, Adam G. *J Label Compd Radiopharm* 1998; **41**: 131–139.
3. Leseticky L, Sedlak K, Fiser B. *Radioisotopy* 1990; **31**: 163–169.
4. Shevchenko VP, Nagaev IYu, Potapova AV, Myasoedov NF. *Radiochemistry* 1998; **40**: 74–78.
5. Shevchenko VP, Nagaev IYu, Myasoedov NF, Susan A. *Abstract of Sixth International Symposium on The Synthesis and Applications of Isotopes and Isotopically Labeled Compounds* (*ISSAILC6*). Philadelphia, Pennsylvania, 1997; 73.
6. Shevchenko VP, Nagaev IYu, Myasoedov NF. *Radiochemistry* 1998; **40**: 83–87.
7. Shevchenko VP, Nagaev IYu, Myasoedov NF. Isotopic exchange with tritiated water. In *Lipophilic Compounds Labelled with Tritium*, Fedoseev VM (ed.). Nauka: Moscow, 2003: 38–42 (in Russian).
8. Moenius Th, Andres H, Acemoglu M, Kohler B, Schnelli P, Zueger C. *J Label Compd Radiopharm* 2000; **43**: 113.

Copyright © 2004 John Wiley & Sons, Ltd.

9. Shevchenko VP, Myasoedov NF. In *Isotopes in the Physical and Biochemical Sciences*, Buncel E, Jones JR (eds), vol. 1. Labelled Compounds (Part A). Elsevier: NY, USA, 1987; 237.
10. Shevchenko VP, Myasoedov NF. In *Isotopes in the Physical and Biochemical Sciences*, Buncel E, Jones JR (eds), vol. 1. Labelled Compounds (Part B). Elsevier: NY, USA, 1991; 179.
11. Shevchenko VP, Nagaev IYu. Potapova AV, Myasoedov NF. *Radiochemistry* 1994; **36**: 486–489.
12. Shevchenko VP, Nagaev IYu, Potapova AV, Myasoedov NF. *Radiochemistry* 1995; **37(3)**: 244–247.
13. Bruelisauer A. Unpublished results.

Copyright © 2004 John Wiley & Sons, Ltd.