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Direct Tritium Labeling of Multifunctional Compounds using Organoiridium Catalysis

W. Chen, K.T. Garnes, S.H. Levinson, D. Saunders,
S.G. Senderoff, A.Y.L. Shu, A.J. Villani and J.R. Heys*
Radiochemistry, SmithKline Beecham Pharmaceuticals
P.O. Box 1539, King of Prussia, PA 19406

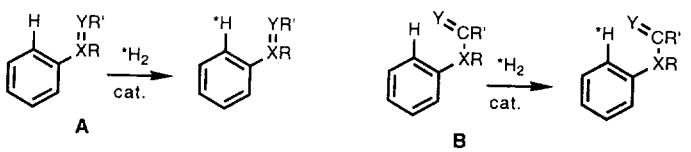
Abstract

The tritium exchange labeling of a variety of complex compounds is achieved in the presence of catalyst precursor $[(cod)Ir(PPh_3)_2]BF_4$ and limited amounts of tritium gas. The regioselectivity of exchange is high and consistent with empirical rules previously observed. High specific activity levels are often achieved, usually with specific aryl C-H bonds. However, remarkably efficient exchange occurs in certain N-alkyl groups. Studies of intermolecular inhibition of catalytic exchange suggest reasons why larger amounts of complex are sometimes required to label complex molecules; nevertheless, significant amounts of label incorporation into substrates can be achieved even starting with small amounts of labeling gas.

Keywords: Tritium exchange, deuterium exchange, exchange labeling, iridium catalysis, C-H activation, alkyl C-H, aryl C-H

Introduction

Recent communications from these laboratories[1-4] have described the exchange labeling of a variety of compounds with deuterium (and in a few cases tritium), catalyzed by several organoiridium complexes. The exchange is generally rapid, efficient and highly regioselective, and proceeds under very mild conditions (room temperature, less than one atmosphere of deuterium) which are suitable for use with tritium gas. The regioselectivity observed has usually involved aryl C-H bonds, with several functional groups capable of directing the labeling to specific positions following presumed initial coordination of the metal center to a functional heteroatom **Y** (**Y** = N or O; **X** can be C, N or S, and R,R' may be nothing, H, C, N, O, and either/both may be part of a cyclic subsystem, including a fused ring). Selectivity in labeling of either functional type **A** or **B** (below) can be achieved by choice of organoiridium complex and reaction conditions[4]. For example, labeling of type **A** compounds was shown to be mediated best by catalysts such as $[IrH_2(acetone)_2(PPh_3)_2]BF_4$, which contains monodentate phosphine ligands, and type **B** complexes are labeled by catalysts related to $[(cod)Ir(dppe)]BF_4$, whose phosphine ligand is bidentate[4].



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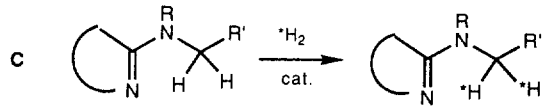
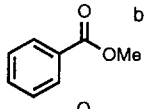
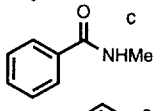
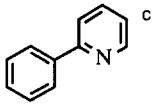
Our work stems from previous reports by Crabtree on $[IrH_2(acetone)_2(PPh_3)_2]BF_4$, which described alkyl C-H activation[5] and deuterium exchange[6] (in 8-methylquinoline and caffeine). Our results have until now included only one further example of alkyl labeling, namely the N-methyl labeling of N,N-dimethylbenzamide and ring-substituted derivatives[2,4]; new examples are discussed below, including the very efficient labeling of certain methyl and methylene groups in systems illustrated by **C**.



Results and Discussion

This report describes tritium exchange labeling of some complex, multifunctional compounds by use of the catalyst precursor $[(cod)Ir(PPh_3)_2]BF_4$ (**1**). The results of some deuterium trials are also described. We have also conducted tritiations of complex compounds using other, related, complexes, which show promise for modifying and controlling the regioselectivity of exchange. These results will be reported on elsewhere. Recent results with model systems indicated that the use of **1** sometimes gives more efficient exchange than $[IrH_2(acetone)_2(PPh_3)_2]BF_4$, used previously[1], in addition to being accessible in one simple step from commercially available $[ClIr(cod)]_2$. Table 1 shows the results of direct comparison of the activities of the two complexes on some representative model compounds.

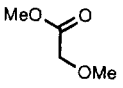
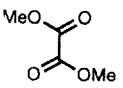
Table 1
Dependence of Labeling Efficiency on Catalyst[a]

	$[IrH_2(acetone)_2(PPh_3)_2]BF_4$	$[(cod)Ir(PPh_3)_2]BF_4$
	0.55	1.33
	1.02	1.04
	0.32	0.29

[a]Mol deuterium per mol incorporated into substrate; [b]100 mg substrate, 10 mg catalyst in 5 mL CH_2Cl_2 stirred under 1 atm D_2 at rt for 18 h; [c]same, except for 30 min reaction time.

It was found in many cases that larger amounts of iridium complex must be used for successful labeling of compounds containing additional functional groups, compared to monofunctional model compounds. This is probably the result of competitive coordination of the metal center by the other functional groups, slowing turnovers at the catalytically active site. In model studies we have observed such effects in an *intermolecular* sense, including the inhibition of *ortho*-labeling of benzoate esters by added N,N'-dimethylbenzamide and N,N-dimethylbenzylamine[1], and by 1,4-dioxa compounds such as 1,2-dimethoxyethane ~ methyl methoxyacetate > dimethyl oxalate (see Table 2).

Table 2
Inhibition of Ethyl Benzoate Deuteration by 1,4-Dioxa Compounds[a]

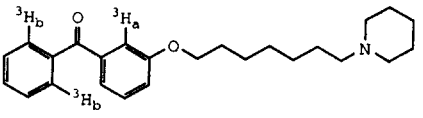
Inhibitor/ Substrate Ratio (mol/mol)			
	Degree	**of**	**Inhibition**
0.03:1	<5%	<5%	<5%
0.06:1	19%	23%	9%
0.12:1	49%	56%	23%
1:1	>95%	>95%	>95%

[a]0.133M ethyl benzoate in CH_2Cl_2 with 2.66 mM $[IrH_2(acetone)_2(PPh_3)_2]BF_4$ under 1 atm D_2, rt, 18 h. Level of ethyl benzoate deuteration in absence of inhibitor ~1.2 mol D/mol.

For some studies, products of lower specific radioactivities were required and it was not necessary to achieve the highest possible level of tritium incorporation. In these cases, we used tritium gas recovered from previous tritiation experiments and accumulated on a secondary uranium storage bed. This recycled tritium contained an unknown proportion of hydrogen, which varied according to the history of the mixture. Therefore, the specific activity of products obtained through use of recycled tritium is less (sometimes much less) than would have been obtained had pure tritium gas been used.

Benzophenones:

Some of our previous model work explored the influence of single substituents on the rates and regioselectivities of deuteration of benzophenone derivatives.[2,7] Whereas in these cases simple *meta*- or *para*-monosubstituted benzophenones (methoxy, methyl, chloro, trifluoromethyl) could be labeled to complete equilibrium within about 50 min under an excess of deuterium gas (rt, 1 atm) using only 2-3 mol% of catalyst precursor **1**, compounds **2** and **3** (below) were labeled to only a small extent in the presence of 30 mol% of **1** overnight.



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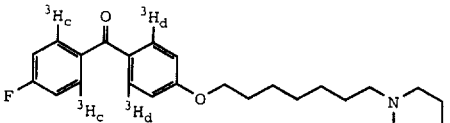

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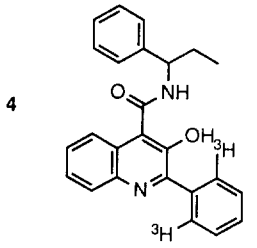
However, when the proportion of complex **1** was increased to 200 mol%, stirring of the exchange mixture under deuterium gas for 18 h led to the incorporation of 1.9 mol D/mol in **2** (H_a:H_b deuterium ratio ~3:2) and 2.9 mol D/mol in **3** (H_c:H_d deuterium ratio ~1:1), as measured by diminution of the respective *ortho*-proton signals in ^1H NMR. When this reaction was repeated with recycled tritium, **2** was labeled to the extent of 2 Ci/mmol and **3** to 56 Ci/mmol. Distribution of tritium within the two compounds as measured by ^3H NMR was shown to be 7:1 ^3H_a to ^3H_b in **2** (the C6-H was not exchanged) and 1:1 ^3H_c to ^3H_d in **3**. The reason for the lower incorporation of tritium into 2 is unknown; however, it shows more clearly the selectivity of the catalyst for labeling of H_a compared to H_b. The regioselectivities of isotopic labeling observed here are consistent with the results of the previous model experiments with deuterium,[7] which showed that 3-methoxybenzophenone (comparator to **2**) was deuterated at C2 at a faster rate than at C6 or C2' (*ortho*

position of the unsubstituted ring); likewise, exchanges at C2 of 4-methoxybenzophenone and 4-chlorobenzophenone (comparators to **3**) were faster than at C2'. The higher level of tritium incorporation in **3** compared to **2** could be attributed to the activation of both rings by their respective substituents.

Quinolines:

Given the previous observations of efficient labeling of 2-phenylpyridine and 2-phenylimidazole[1], it was anticipated that the quinolines **4** and **5** might be successful substrates for exchange as well. Indeed, deuteration of **4** for 20 h with 1.20 equivalents of iridium complex in the presence of 36 equivalents of D_2 resulted in the introduction of 1.57 mol D/mol into the compound, according to mass spectrometric measurements. A similar deuteration of **5** with 1.10 equivalents of catalyst gave an incorporation of 1.70 mol D/mol. The location of deuterium in the molecules was not determined. Subsequent tritiation of **4** (1.16 mg) under the same conditions except with 23 equivalents (4.1 Ci) of tritium gas gave [^{3}H]**4** (25 mCi at >99.5% radiochemical purity after HPLC purification) at a specific activity of 38 Ci/mmol. Tritium NMR analysis showed the label to reside exclusively in the indicated positions.



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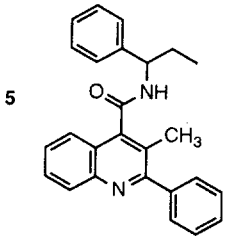

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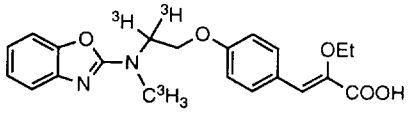
2-Aminopyridine and 2-Aminobenzoxazole Derivatives:

Compounds **6** and **7**, when stirred under approximately 17 equivalents of tritium gas in the presence of approximately 1.5 equivalents of **1**, were labeled to a high degree. Mass spectrometric analyses of the purified products showed both to contain up to five tritium atoms, and the specific activities calculated from these spectra were 126 Ci/mmol (**6**) and 99 Ci/mmol (**7**). Tritium NMR analyses showed that the labels reside exclusively at the positions indicated, and clearly resolved the separate isotopomers (i.e., $-CH_2T$, $-CHT_2$, $-CT_3$ and -CHT- and $-CT_2-$ species). The integrated intensities of these peaks were consistent with the MS-derived specific activities. Partial reduction of both **6** and **7** occurred during the labeling to give some of the hydrocinnamate analogs (ca. 34% and ca. 26% by radioactivity, relative to recovered **6** and **7**, respectively) containing additional tritium at the positions of the reduced double bond. The specific activities of these products were 154 Ci/mmol and 128 Ci/mmol, respectively. Observation of partial reduction is not surprising, as complexes derived from **1** are known[8] to catalyze olefin hydrogenation.



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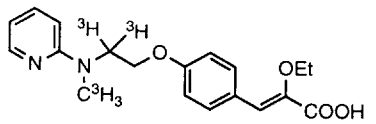

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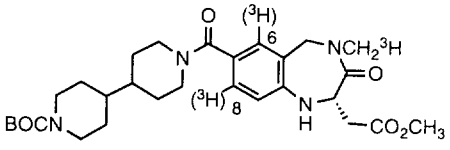
These results indicate remarkably efficient alkyl exchange labeling, reaching ≥80% of the theoretical level in both the N-methyl and N-methylene groups, taking into account the amount of available tritium and degree of dilution by exchanged hydrogens. The labeling of higher N-alkyl groups is significant not only because it indicates the lack of an adverse steric effect, but because such products should have wider utility in biological studies than N-methyl labels.

Benzazepinone Amides:

Compounds **8** and **9** contain benzamide functions analogous to the model compounds such as N,N-dimethylbenzamide which was observed[3] to be labeled in the *ortho* positions via iridium catalysis, and in some instances in the N-methyl groups.



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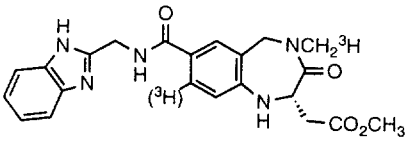


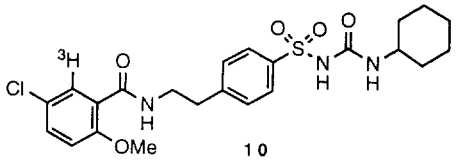
9

When **8** was stirred in the presence of 30 mol% of **1** under 30 molar equivalents of deuterium gas, the product was found to contain 0.42 mol D/mol, located mainly in the benzo ring (^{1}H NMR). Repeated with the same amount of tritium gas, the exchange provided **8** at a specific activity of only 1.5 Ci/mmol (equivalent to 0.05 mol ^{3}H/mol). However, when the amount of **1** was increased to 120 mol% and the exchange conducted under 20 equivalents of tritium gas, the resulting compound had a specific activity of 22.2 Ci/mmol. Tritium NMR of this product showed that 72% of the tritium was at C6 and/or C8 (*ortho* to the amide function; their chemical shifts were indistinguishable), and 28% was in the methyl group of the diazepinone ring. In contrast to **8**, compound **9** was not labeled to a significant extent in deuterium trials utilizing 110 mol% of complex **1**; however, when 2.9 molar equivalents was used, along with 10.7 equivalents of deuterium gas, 1.42 mol D/mol were incorporated into **9**. When repeated with 13.3 equivalents of tritium gas and 3.9 equivalents of catalyst, **9** was labeled to a specific activity of 42 Ci/mmol. Tritium NMR showed that ≥90% of the tritium was in the N-methyl group, and the remainder at C8.

It is not obvious why these two compounds should differ in the ease and regioselectivity of their labeling; however, a reasonable explanation for both could involve chelation of one mole of iridium in **9** by a benzimidazole nitrogen and the amide function, rendering the latter incapable of mediating aryl H exchange.

A Complex Benzamide:

A 2.5 mg portion of unlabeled compound **10** was labeled in methylene chloride solution, starting with 2 equivalents of complex **1**. However, this time the complex was prereduced by exposure to one atm of hydrogen gas for a short time. This served to avoid consumption of tritium gas in this initial stage. The mixture was then frozen by liquid nitrogen cooling, the excess hydrogen pumped off, then 6 Ci of tritium gas was introduced and the reaction continued at room temperature for 16 h. Workup provided directly 478 mCi (85% radiochemical purity) of [^{3}H]**10**. Purification of a portion by HPLC provided material at 99% radiochemical purity and 21 Ci/mmol specific activity. Tritium NMR showed the compound to be labeled exclusively in the position shown, which is consistent with previous observations that a benzamide carbonyl is better at directing exchange than a benzenesulfonyl group, and that a *meta*-chloro substituent enhances the efficiency of labeling between itself and a directing group.



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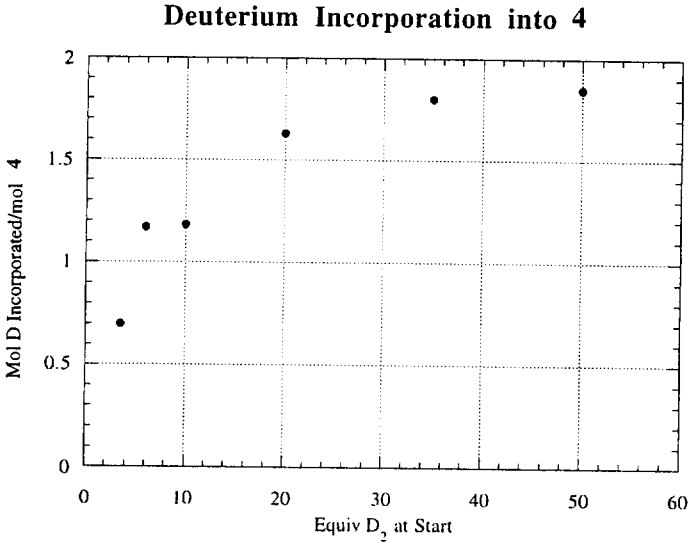
Kinetics:

Because of concerns about radiation safety and disposal costs for radioactive waste, it is of interest to reduce the amount of tritium gas used in labeling reactions. We investigated the issue by conducting deuterium exchange labeling of a representative substrate

(compound **4**) beginning with various amounts of deuterium gas. The results are shown in the following graph, which displays the amount of deuterium incorporated into **4** (determined by mass spectrometry) as a function of the amount of deuterium gas introduced at the beginning of the reaction. Two features of these results are of interest. First, the data fit well a first-order dependence of the label incorporation on the amount of deuterium gas [$\log(D_{max}/(D_{max}-D_{exp})$, $r = 0.996$; $D_0 = 0.27$] ; and second, a significant level (0.7 mol/mol) of deuterium incorporation into **4** was achieved starting with only 3.5 equivalents of deterium gas. These points are especially interesting because a significant amount of deuterium would be expected to be consumed in the activation of the catalyst by reduction of the cyclooctadiene (cod) ligand, which occurs within a few minutes. This may not be exactly the expected two moles of deuterium per mole of cod, as Siedle, et al.[9], have observed that cyclooctane produced by the reduction of $[(cod)Ir(PPh_3)_2]PF_6$ in acetone solution contained only 3.08 mol D/mol (a range of isotopomers from D_0 to D_{10} was observed). Nonetheless, this initial consumption of deuterium would be expected to reveal itself in an increasing deviation of the log curve from linearity as the initial amount of deuterium is decreased. However, this was not observed. It would be of interest to extend this series of experiments to even smaller amounts of deuterium gas in order to determine whether the data eventually deviate from the first-order curve. Nevertheless, the data here indicate that the amount of deuterium gas (and by extension tritium gas) used in an iridium-catalyzed exchange can be quite small while still achieving significant exchange labeling, even when the initial complex is not prereduced with hydrogen. When it is, however, results are often even better, as will be described in a subsequent report.



(5 mg compound **4**, 120 mol% $[(cod)Ir(PPh_3)_4]BF_4$, 1 mL CH_2Cl_2, rt, 20 h)

Conclusions

The complex $[(cod)Ir(PPh_3)_2]BF_4$ is an effective precatalyst for the exchange labeling of a variety of complex, multifunctional compounds using tritium or deuterium gas even in limited amounts. The regioselectivity of labeling is very high and predictable in most cases. As such, this method can in many cases supplant multistep synthetic and semisynthetic approaches *via* unsaturated or halogenated analogs of target compounds, and enable direct labeling of compounds of interest. The compatibility of the method with nitro groups and aryl halogens[1] further broadens the applicability of the method to compounds for which traditional catalytic processes are inapplicable.

Experimental

$[(cod)Ir(PPh_3)_2]BF_4$ was prepared from $[Ir(cod)Cl]_2$ (Johnson Matthey) by treatment with triphenylphosphine according to the procedure of Haines and Singleton[10]. Compounds **2** - **10** were obtained from various SmithKline Beecham Pharmaceuticals Research and Development programs. Other chemicals and reagents were purchased from the Aldrich Chemicals Co. Dichloromethane was from J.T. Baker, HPLC grade, and was used without prior purification. Tritium and deuterium gases were handled on a purpose-built stainless steel manifold which allowed accurate measurement of the gases used. Tritium gas was stored on a stainless steel storage trap containing uranium (Cerac, Inc.), and a secondary uranium bed was used to take up tritium/hydrogen at the end of each reaction. Tritium gas was purchased from RC Tritec, Teufen, Switzerland. Proton NMR spectra were recorded on a Bruker AM400 or AM300 instrument in suitable deuterated solvents that could resolve signals of interest. Tritium NMR spectra were recorded at 426 MHz. Mass spectra were run on a Finnegan model 4610 mass spectrometer, in chemical ionization mode using NH_3 as reagent gas.

General labeling procedure: A solution of the substrate (generally 1-3 mg) and the appropriate amount of $[(cod)Ir(PPh_3)_2]BF_4$ in dichloromethane (ca. 1 mL) was attached to the manifold, and the desired amount of tritium was introduced. The mixture was stirred vigorously at rt for 18-24 h, the tritium gas was removed, and the labile tritium was separated from the nonvolatile reaction residue by several stages of dissolution in MeOH or EtOH and removal by static vacuum transfer. The resulting residue was then subjected to chromatography (generally reverse-phase HPLC) to isolate the substrate. All products were purified to the >95% radiochemical purity level and compared to authentic reference materials by HPLC, MS and proton NMR.

Compound **2**:
HPLC [Beckman Octyl column (5 μm, 4.6 mm I.D. x 25 cm), 50/50/0.1 (v/v/v) water/acetonitrile/trifluoroacetic acid, 1.0 mL/min, UV at 254 nm, Rt = 11.5 min] radiochemical purity: 99.5%.
^3H NMR (proton-coupled mode, CD_2Cl_2): 7.30 (87% T, s, **Ta**), 7.78 (13% T, d, **Tb**).
CI-MS: t_0 (93%), t_1 (7%); specific activity: 2 Ci/mmol.

Compound **3**:
HPLC [Beckman Octyl column (5 μm, 4.6 mm I.D. x 25 cm), 50/50/0.1 (v/v/v) water/acetonitrile/trifluoroacetic acid, 1.0 mL/min, UV at 254 nm, Rt = 11.5 min] radiochemical purity: 99.5%.
^3H NMR (proton-decoupled mode, C_6D_6): 7.55 (48% T, s, **Tc**), 7.75 (52% T, s, **Td**).
CI-MS: t_0 (8%), t_1 (27%), t_2 (36%), t_3 (23%), t_4 (6%); specific activity: 56 Ci/mmol.

Compound **4**:
HPLC [Zorbax C-18 column (5 μm, 4.6 mm I.D. x 25 cm), 80/20/1(v/v/v) water/acetonitrile/triethylamine, 1.0 mL/min, UV at 250 nm, Rt = 10.7 min] radiochemical purity: 99.6%.
^3H NMR (proton-decoupled mode, CD_3OD):8.00 (s).
CI-MS: t_0 (12%), t_1 (45%), t_2 (43%), t_3 (23%); specific activity: 38 Ci/mmol.

Compound **6**:
HPLC [Zorbax C-18 column (5 μm, 4.6 mm I.D. x 25 cm), 60/40/0.1 (v/v/v) water/acetonitrile/trifluoroacetic acid, 1.0 mL/min, UV at 229 nm, Rt = 7.8 min] radiochemical purity: 95%.
^3H NMR (proton-decoupled mode, CD_3OD):3.15-3.25 (58%T, m, N-C**T**$_3$), 3.85-3.95 (42%T, m, N-C**T**$_2$-).
CI-MS: t_2 (3%), t_3 (14%), t_4 (38%), t_5 (43%), t_6 (2%); specific activity: 126 Ci/mmol.

Compound **7**:
HPLC [Zorbax C-18 column (5 μm, 4.6 mm I.D. x 25 cm), 75/25/0.1 (v/v/v) water/acetonitrile/trifluoroacetic acid, 1.0 mL/min, UV at 200 nm, Rt = 13.9 min] radiochemical purity: 97%.
^3H NMR (proton-decoupled mode, CD_3OD): 3.02 (43% T, s, N-C**T**$_3$), 3.05 (17% T, s, C-CH**T**$_2$), 3.08 (2%T, s, N-CH$_2$**T**), 3.85 (32%T, s, N-C**T**$_2$-), 3.88 (6%T, s, N-CH**T**-).
CI-MS: t_0 (18%), t_1 (1%), t_2 (3%), t_3 (12%), t_4 (30%), t_5 (36%); specific activity: 99 Ci/mmol.

Compound **8** (high specific activity analog):
HPLC [Beckman ODS C-18 column (5 µm, 4.6 mm I.D. x 25 cm), 87.5/12.5/0.1 (v/v/v) water/ethanol/trifluoroacetic acid, 1.0 mL/min, UV at 220 nm, Rt = 12.6 min] radiochemical purity: 99%.
^{3}H NMR (proton-coupled mode, CD_2Cl_2): 2.95-3.02 (28%T, m, N-C**T**$_3$), 7.10 (22%T, s, 6-**T**), 7.11 (50%T, d, J = 3.7 Hz, 8-**T**).
Specific activity determined by mass concentration (HPLC weight based assay) and radioactive concentration (scintillination counting): 22.2 Ci/mmol.

Compound **9** (acid derivative):
The exchange was carried out on ester **9**, which was then hydrolyzed to the corresponding acid with aqueous LiOH.
HPLC [Beckman ODS C-18 column (5 µm, 4.6 mm I.D. x 25 cm), 85/15/0.1 (v/v/v) water/acetonitrile/trifluoroacetic acid, 1.0 mL/min, UV at 220 nm, Rt = 11.4 min] radiochemical purity: 98.8%.
^{3}H NMR (proton-decoupled mode, CD_3OD): 2.99 (9% T, m, N-C**T**$_3$), 7.29 (91% T, s, Ar-**T**).
CI-MS: t_0 (33%), t_1 (25%), t_2 (20%), t_3 (12%), t_4 (7%), t_5 (3%); specific activity: 42 Ci/mmol.

Compound **10**:
HPLC [Beckman ODS C-18 column (5 µm, 4.6 mm I.D. x 25 cm), 40/60 (v/v) aq. KH_2PO_4 (0.05 M, pH=3)/acetonitrile, 1.0 mL/min, UV at 254 nm, Rt = 6.8 min] radiochemical purity: 99.8%.
^{3}H NMR (proton-coupled mode, CD_3OD):7.77 (d, J = 3.0 Hz).
CI-MS: t_0 (27%), t_1 (73%); specific activity: 21 Ci/mmol.

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