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Direct Tritium Labeling of Multifunctional Compounds using Organoiridium Catalysis. 2.

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Abstract

A variety of complex compounds have been labeled with tritium gas by catalytic exchange in the presence of catalyst precursors [(cod)Ir(dppe)]BF_4 or [(cod)Ir(py)(PCy_3)]BF_4. In most cases, predictable regioselectivity and high specific activities are achieved. These results are compared in some cases to the results of labeling related compounds with [(cod)Ir(PPh_3)$_2$]BF_4. Prereduction of the catalyst precursors *in situ* with hydrogen allows the use of smaller quantities of tritium gas and reduces the amount of radioactive waste. Two or more compounds can be labeled simultaneously as mixtures then separated in the HPLC purification step to increase compound throughput.

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

Introduction

We recently described[1] the direct tritium labeling of a number of complex compounds mediated by the catalyst precursor [(cod)Ir(PPh_3)$_2$]BF_4 (**1**). The regioselectivity of labeling was consistent with the intermediacy of 5-membered metallacycles, formed upon iridium coordination with a suitable heteroatom followed by oxidative addition into a proximal C-H bond. Thus, substrates successfully tritiated include aryl amides, 2-aryl quinolines and benzophenones.

Two related complexes, the bidentate phosphine [(cod)Ir(dppe)]BF_4 (**2**) (dppe = bis-1,2-diphenylphosphinoethane) and [(cod)Ir(py)(PCy_3)]BF_4 ("Crabtree's Catalyst", **3**) (py = pyridine; Cy = cyclohexyl), have been shown to exert a different labeling regioselectivity, via both 5- and 6-membered metallacycles, both in surveys of model compounds using deuterium gas[2] and in tritium labeling[3].

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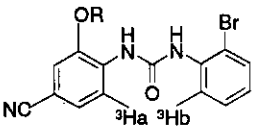
In this paper we describe some of our recent tritiation work, mainly with the latter two complexes, and compare these in a few cases with the results obtained with the monodentate bisphosphine complex. In addition, we describe one example of the successful application of the method to a mixture of substrates. Three compounds were labeled simultaneously then separated/purified by HPLC in a process which was nearly as quick as that for a single component labeling and purification.

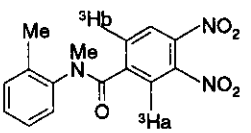
Another noteworthy feature of the experiments described here is that the catalyst precursors were generally prereduced *in situ* with hydrogen, which was then removed and replaced with tritium gas for the labeling process (see Experimental). This is a simple modification with significant benefits—it allows for the use of smaller quantities of tritium gas without detracting from the labeling results, and a significant reduction in the amount of radioactive waste generated, since tritiated cyclooctane is not produced.

Results and Discussion

Compound **4** (R = *t*-butyldimethylsilyl) was labeled in both open positions *ortho* to the urea nitrogens (29 Ci/mmol, 60% at Ha and 40% at Hb) by complex **2** (1.9 equiv., 6.1 equiv. of tritium gas), consistent with iridium coordination to the urea carbonyl and formation of a 6-membered metallacycle upon oxidative addition to an *ortho* C-H bond. In contrast, **5** (1 mg) is labeled equally in the indicated positions *ortho* to the amide carbonyl but not in the aminocarbonyl ring (20 Ci/mmol, 40 mCi purified) through the use of **1** (2.1 equiv., 1.78 Ci of tritium gas). The silyl group of **4** was partially removed during the exchange reaction; the labeled product was isolated and purified as the phenol. In addition to the compatibility of these processes with nitro and cyano functions, and halo groups in general, it is notable that the presumed ligation which enables selective cleavage of the *ortho* C-H bond in **4** does not likewise facilitate oxidative addition to the C-Br bond: no detectable reduction of the aryl bromide function occured.



4 (R = H or TBS)



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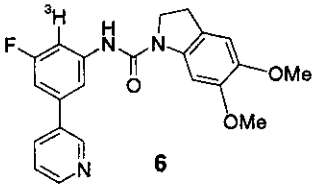
Compound **6** was labeled efficiently and exclusively at the indicated position (14 Ci/mmol) using [(cod)Ir(dppe)]BF_4 (**2**) (3.5 equiv., 3.7 equiv. of tritium gas), consistent again with mediation by the urea oxygen via a 6-membered metallacycle intermediate. Labeling in the other *ortho* position was presumably inhibited by the steric bulk of the pyridyl group. Interestingly, compound **7** (3 mg) was found to be labeled by the monodentate complex **1** (4.8 equivalents, in the presence of 25 equivalents of tritium gas) equally in all three indicated positions (44 Ci/mmol), both *ortho* to the carboxamide function and *para* to it, the latter site labeling presumably being mediated by the urea carbonyl.



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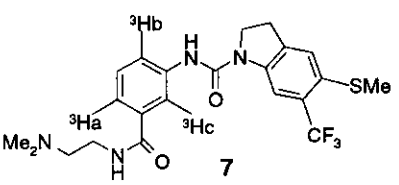

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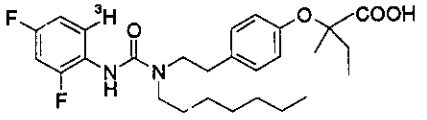
The utility of Crabtree's Catalyst is illustrated in the tritiation of compound **8** (2.34 equiv. of **3**, 7.3 equiv. of tritium gas) exclusively in the open *ortho* position of the anilide ring (9.6 Ci/mmol).



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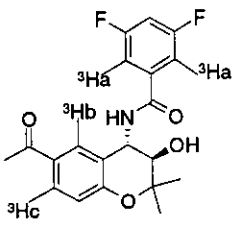
Again, PPh_3 catalyst **1** is suited to labeling all four positions *ortho* to the carbonyl functions in compound **9** below (1 mg; 1.25 equiv of **1** and 1.7 Ci tritium gas) to a specific activity of 41 Ci/mmol (48 mCi pure; ~1:1 Ha/Hb+Hc; Hc>Hb). However, complex **2** labels compound **10** not only at the positions (Ha) *ortho* to the aryl carbonyl group but at Hb as well (ratio 2.6:1 Ha/Hb; 48.7 Ci/mmol, 137 mCi pure from 3 mg of unlabeled **10**). The latter outcome may have been facilitated by the adjacent chloro substituent, as previously observed[4] in other structures. And treatment of compound **11** with 18 equiv. (2.06 Ci) of tritium gas in the presence of 6.7 equiv. of complex **2** results in labeling at both Ha and Hb (ratio a:b 2.1:1; no tritium adjacent to the piperazine substituents, 70 Ci/mmol).



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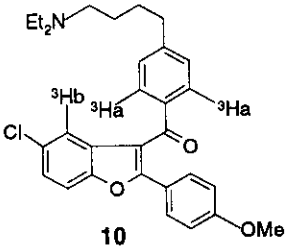

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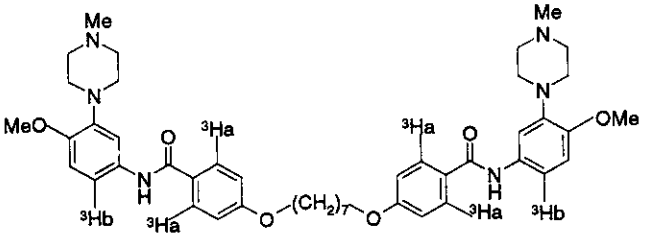

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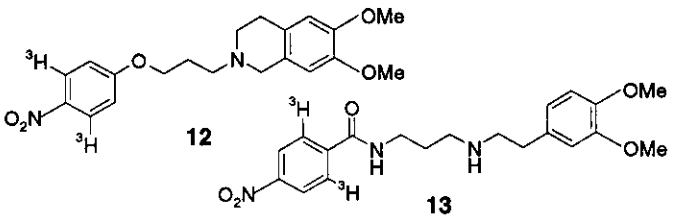
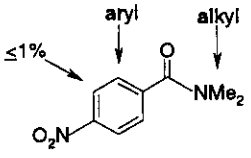
Aryl nitro functions were earlier shown to mediate labeling with the complex related to **1** in simple model compounds[5]. In the case of compound **12**, labeling was also successful (4.35 equiv of **1**, 34 equiv. of tritium gas, 32 Ci/mmol). However, the compound **13**, when tritiated using complex **2**, was shown to be labeled only *ortho* to the amide carbonyl and not also adjacent to the nitro function (2.25 equiv. of **2**, 5.3 equiv. of tritium gas, 8.7 Ci/mmol).



Was this difference in regioselectivity the result of using different catalysts? To explore this question, we conducted a model study using deuterium gas (35 equiv.) to label N,N-dimethyl-4-nitrobenzamide using various catalysts in 18-hour reactions. As shown in the table below, in no case was any labeling (~1% detection limit) evident in positions adjacent to the nitro function (C3, C5); only the positions *ortho* to the amide function (C2, C6) were labeled in the ring. This suppression of one group's (nitro) ability to mediate exchange by the presence of another (the amide) is an effect not observed in the labeling of compound **9** above, nor of some of those following. One explanation is that, whereas in amide **13** and N,N-dimethyl-4-nitrobenzamide both functions are on the same ring, in the other compounds the functions are on the different rings. It may be that when both functions are on the same ring, the inductive effect of the amide group reduces the nitro group's already weak ability to coordinate with iridium; this effect would be compounded by coordination of iridium to the amide function.



Catalyst	equiv.	mole D/ mole	aryl:alkyl
1 (PPh_3)	0.04	2.08	80:20
1 ($PMePh_2$)	0.04	0.14	84:16
1 "	0.14	0.63	84:16
1 "	0.46	3.12	58:41
2	0.05	1.76	>95:5
3	0.04	0.14	>95:5
3	0.14	0.86	>95:5

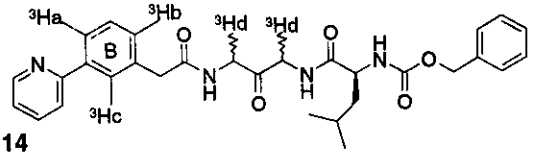
The catalysts in the table above differed in their activities and in their abilities to label the N-methyl groups, as well as the aryl positions. Catalyst **1**, prepared with triphenylphosphine[6], was a more efficient catalyst for incorporation of deuterium than the commercial analog (**1**, $PMePh_2$) containing methyldiphenylphosphine. Both of them labeled the N-methyl groups as well as the ring, but at a rate at least 15 times slower than that of the aryl positions (taking into account the number of available hydrogens). The dppe containing catalyst (**2**) showed high activity similar to that of **1**, but Crabtree's Catalyst (**3**) was less active. However, in contrast to the behavior of **1**, catalysts **2** and **3** were completely selective for the aryl positions and did not label the alkyl positions to a detectable degree.

Polyamide compounds **14** – **16** were tritium labeled using catalyst **3** (1 mg substrate, 3-4.2 equiv. of 3, 1.5-2.2 Ci tritium gas), with the results shown in Table 2.

Table 2. Label Distribution in Compounds **14 - 16**

Compound	mCi obtained pure	specific activity (Ci/mmol)	Percent of label at position			
			^{3}Ha	^{3}Hb	^{3}Hc/ ^{3}He	^{3}Hd
14	64	78	25	24	24	27
15	39	73	21	21	21	37
16*	29	49	18	25	50	7

*^{3}H NMR assignments are tentative



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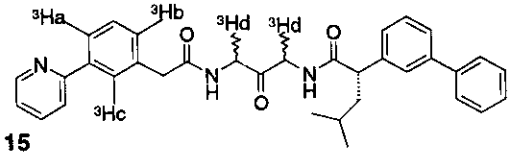

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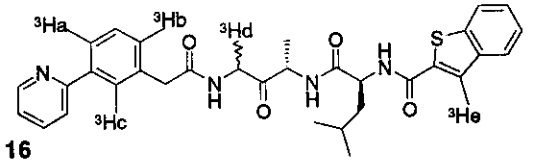

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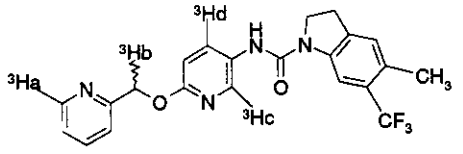
Most of the tritium in these three compounds is, as expected, in ring B, owing to the presence of the pyrid-2-yl function (labeling Ha and Hc) and the carbonylmethyl function (labeling Hb and Hc). In compound **16**, the chemical shift of the C3-H of the benzthiophen-2-oyl group was nearly coincident with Hc, so that any labeling in the former location, which might be expected as well, was not resolved. Certainly, the higher proportion of label associated with the Hc/He signal suggests that labeling has occurred in both sites.

In addition to aryl site labeling, each compound bears some label on one or both sides of the diaminoacetone moiety (Hd). It is not clear whether this labeling is the result of direct mediation by the iridium center, or simple acid/base catalysis. In support of the latter is the previous finding[7] that in the absence of stabilizing ligands (which would be the situation in our experiments with large amounts of complex), complexes such as **1** form the clusters $[Ir_2H_5(PPh_3)_4]^+$ and $[(Ir(PPh_3)_2H_2)_3(\eta^3\text{-}H)]^{2+}$ *with release of* H^+. Organoiridium complexes such as **1 – 3** are known to efficiently catalyze $H_2 - H_2O$ exchange, so that $^3H_2$ would be expected to rapidly equilibrate with adventitious water and labile heteroatom hydrogens. This source of 'protonic' tritium could be involved with the alkyl labeling in **14 – 16** (as well as the partial desilylation of **7**). In contrast, the N,N-dimethyl-4-nitrobenzamide labeling above, which is not subject to acid/base exchange with protons, must certainly be the result of mediation by iridium.

Lastly, compounds **17** - **19** were labeled as a mixture. In this experiment, 1 mg quantities of all three compounds and 3.13 equiv. of complex **3** were dissolved in methylene chloride and stirred under 4.6 Ci of tritium gas overnight. Workup and separation/purification by HPLC provided each labeled compound in >97% radiochemical purity.



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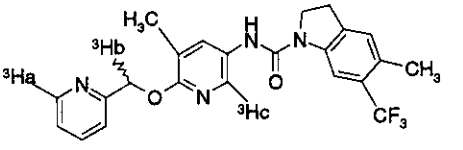

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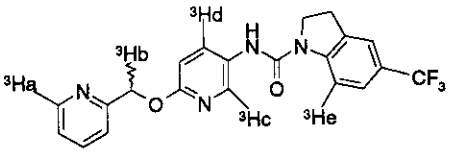

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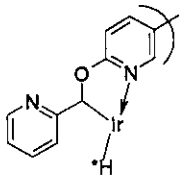
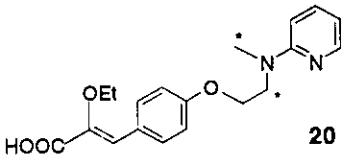
Table 3. Label Distribution in Compounds **17** - **19**

Compound	mCi obtained pure	specific activity (Ci/mmol)	Percent of label at position				
			^{3}Ha	^{3}Hb	^{3}Hc	^{3}Hd	^{3}He
17	23	64	18	42	18	22	
18	34	49	25	50	25		
19	33	69	20	28	20	10	20

In this experiment, we see the expected labeling at C4 and C6 of the middle pyridine ring of **17** and **19**, mediated by the aminocarbonyl function, but the corresponding C4 of **18** is completely unlabeled, a result of steric blockage by the C3-methyl group. For a similar reason, C7 of the dihydroindole moiety (labeled ^{3}He of **19**) is not exchanged in **17** and **18**. Less clear are the reasons for the labeling of all three compounds at sites Ha and Hb. Iridium complex-catalyzed C2 labeling of pyridines has not been previously observed; however, benzo[*h*]quinoline is deuterated at C2 as well as C10, possibly by a reduction-reoxidation process[5]. The Hb exchange in the three compounds could occur by acid-catalyzed exchange, as proposed for Hd in **14** – **16**, or alternatively via mediation by the nitrogen of the middle pyridine as shown below, in analogy with the efficient tritiation of **20** in the N-methyl and methylene sites as previously reported[1]. The proton-coupled ^{3}H NMR signals for ^{3}Hb in each of the compounds **17** – **19** consist of a doublet (J = ~15 Hz; -CH^3**H**-) and a singlet 0.03 ppm upfield(-C^3**H$_2$**-) in the ratio of approximately 1:1.





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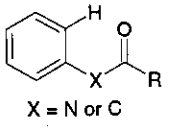
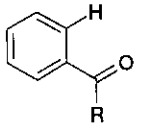
We have similarly applied this labeling procedure to other mixtures of compounds, either structurally similar to or very different from one another. In some cases the approach has produced useful amounts of labeling of all components, and in other cases one or more component was produced in a much smaller quantity than the others, either because of a slower labeling rate or greater loss during labeling.

In summary, we have discussed the labeling of a number of compounds with the regioselectivity illustrated in partial structure **A** (**4**, **6**, **8**, **17**, **18** and **19**) using the dppe-containing complex **2** or Crabtree's Catalyst (**14**, **15** and **16**), and the labeling of substrates in both type **A** and type **B** substructures (**10** and **11**) with complex **2**. In contrast, only type **B** substructures were labeled in compounds **5** and **9** using the monodentate phosphine complex **1**, even though compound **5** also contains a type **A** substructure. These results are consistent with previous findings[2,4,5] in which differences in regioselectivity of exchange exerted by different catalysts were elucidated using simple model substrates.



X = N or C

Type A



Type B

New or anomalous findings presented here include the label pattern produced by complex **1** in compound **7**, which included the C4 position (type **A**) when only C2 and C6 (type **B** sites) were expected to be exchanged. Other unanticipated results were the labeling of $\mathbf{H_d}$ in compounds **14** – **16** and $\mathbf{H_b}$ in compounds **17** – **19**, both of which could be acid catalyzed, and the $\mathbf{H_a}$ labeling in the latter set of compounds.

In addition, our study with the model substrate N,N-dimethyl-4-nitrobenzamide gave insight into intramolecular competition for labeling, aryl-versus-alkyl selectivity, and relative activity of the different catalysts under the same conditions.

Experimental

[(cod)Ir(dppe)]BF_4 and [(cod)Ir(PPh_3)$_2$]BF_4 were prepared from [Ir(cod)Cl]$_2$ (Johnson Matthey) by treatment with bis(1,2-diphenylphosphinoethane) or triphenylphosphine according to the procedure of Haines and Singleton.[5] [(cod)Ir(py)(PCy_3)]BF_4 was purchased from the Aldrich Chemicals Co. Compounds **4** – **18** were obtained from various SmithKline Beecham Pharmaceuticals Research and Development programs. Other chemicals and reagents were pruchased from Aldrich. 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. 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 dichloromethane solution of the substrate and iridium complex in dichloromethane in a small round-bottom flask was attached to the tritium manifold, and frozen with lN_2 cooling. Hydrogen was introduced and the flask was allowed to warm with stirring until the deep burgundy color of the initial complex was discharged (<30 min). The solution was refrozen, the hydrogen removed and then 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/or proton NMR.

Compound **4**: HPLC [YMC ODS-AM column (5 um, 4.6 mm I.D. x 25 cm), A = 50/50/0.1 (v/v/v) acetonitrile/water/trifluoroacetic acid; B = 90/10/0.1 (v/v/v) acetonitrile/water/trifluoroacetic acid; 100% A 0–15 min, linear gradient over 10 min to 100% B, 100% B for 5 min; 1.0 mL/min; UV at 215nm; R_t = 13.7 min] radiochemical purity: 97.9%. ^{3}H NMR (proton-coupled mode, CD_3OD): 8.19 (60%, d, J = 8.8 Hz, **^{3}Ha**), 7.87 (40% of T, dd, J = 8.5, 1.7 Hz, **^{3}Hb**). CI-MS (NH_3 reagent gas): t_0 (25%), t_1 (46%), t_2 (29%); specific activity: 29 Ci/mmol

Compound **5**: HPLC [Beckman ODS (5 um, 4.6 mm I.D x 25 cm), A = acetonitrile:water:trifluoroacetic acid, 50:50:0.1 (v/v/v), B = acetonitrile:water:trifluoroacetic acid, 90:10:0.1 (v/v/v), 100% A 0-15 min, linear gradient over 10 min to 100% B, 100% B for 5 min; 1.0 mL/min; UV at 210 nm; R_t = 11.7 min] radiochemical purity: 99.5%. ^{3}H NMR (proton-coupled mode, CD_3OD): 7.92 (50%, d, J = 1.7 Hz, **^{3}Ha**), 7.69 (50%, dd, J = 8.7, 1.7 Hz, **^{3}Hb**). CI/MS: t_0 (44%), t_1 (42%), t_2 (14%); specific activity 20.0 Ci/mmol.

Compound **6**: HPLC [Beckman ODS (5um, 4.6 mm I.D x 25 cm), A = acetonitrile/water/trifluoroacetic acid, 40:60:0.1 (v/v/v), B = acetonitrile/water/trifluoroacetic acid, 90:10:0.1 (v/v/v), 100% A 0-15 min, linear gradient over 10 min to 100% B, 100% B for 5 min; 1.0 mL/min; UV at 230 nm; R_t = 8.2 min] radiochemical purity: 98.7%. ^{3}H NMR (proton-coupled mode, DMSO): 2.39 (anilino-C2-**^{3}H**). CI/MS: t_0 (52%), t_1 (48%); specific activity 14.0 Ci/mmol.

Compound **7**: HPLC [Zorbax SB-C18 column (5 um, 4.6 mm I.D. x 25 cm), 79:21 (v/v/v): acetonitrile/0.1M NaH_2PO_4 buffer at pH = 3, 1.0 mL/min, UV at 220 nm, R_t = 18.4 min] radiochemical purity: 98.2%. ^{3}H NMR (proton-coupled mode, CD_3OD): 7.55 (67%, apparent d, $J_{H\text{-}T}$ = 9 Hz, **^3H_a** + **^3H_b**), 8.06 (33%, s, **^3H_c**). CI-MS: t_0 (5%), t_1 (20%), t_2 (43%), t_3 (32%); specific activity: 58 Ci/mmol.

Compound **8**: HPLC [Zorbax SB-C18 column (5 um, 4.6 mm I.D. x 25 cm), 66:34:0.1 (v/v/v) acetonitrile/water/trifluoroacetic acid, 1.0 mL/min, UV at 220 nm, R_t = 18.6 min] radiochemical purity: 97.4%. ^{3}H NMR (proton-decoupled mode, $CDCl_3$): 8.04 (dd, $J_{F\text{-}H}$ = 6.5, 9.6 Hz, anilino-C6-**^{3}H**). CI-MS: t_0 (67%), t_1 (33%); specific activity: 9.6 Ci/mmol.

Compound **9**: HPLC [Beckman ODS (5um, 4.6 mm I.D x 25 cm, A = acetonitrile:water:trifluoroacetic acid, 35:65:0.1 (v/v/v), B = acetonitrile:water:trifluoroacetic acid, 90:10:0.1 (v/v/v); 100% A 0-1 min, linear gradient over 18 min to 100% B, 100% B for 5 min; 1.0 mL/min; UV at 230 nm; R_t = 9.2 min] radichemical purity: 99.5%. ^{3}H NMR (proton-coupled mode, MeOD): 7.52 (~50%, d, J_{F-H} = 9.4 Hz, **^{3}Ha**), 7.82 (~50%, m, **^{3}Hb** + **^{3}Hc**). CI/MS: t_0 (24%), t_1 (33%), t_2 (30%), t_3 (12%), t_4 (2%); specific activity 41 Ci/mmol.

Compound **10**: HPLC [Beckman ODS C-18 (5 um, 4.6 mm I.D. x 250 mm) 90:10:0.2 (v/v/v) methanol/water/diethylamine, 1 mL/min, UV at 248 nm, R_t = 8.2 min] radiochemical purity: 95.7%. ^{3}H-NMR (proton-coupled mode, MeOD): 7.44 (28%, d, J = 2.0 Hz, **^{3}Hb**), 7.76 (72%, d, J = 9.2 Hz, **^{3}Ha**). Specific activity determined by mass concentration (HPLC weight based assay) and radioactive counting (scintillation counting): 48.7 Ci/mmol

Compound **11**: HPLC [Vydac Protein and Peptide C18 (5 um, 4.6 mm I.D x 25 cm), A = acetonitrile/water/trifluoroacetic acid, 10:9 0:0.1 (v/v/v), B = acetonitrile/water/trifluoroacetic acid, 90:10:0.1 (v/v/v); 100% A 0-30 min, linear gradient to 100% B, 100% B for 10 min; 1.0 mL/min; UV at 220 nm; R_t = 16.3 min] radiochemical purity: 99.5%. ^{3}H NMR (proton-coupled mode, DMSO): 7.94 (66%, d, J = 9.7 Hz, **^{3}Ha**), 2.56 (34%, dd, J = 10.2, 2.4 Hz, **^{3}Hb**). Specfic activity determined by mass concentration (HPLC weight based assay) and radioactive concentration (scintillation counting): 70.0 Ci/mmol,

Compound **12**: HPLC [Beckman Ultrasphere ODS (5 um, 4.6 mm I.D. x 25 cm), 52:48 (v/v) 0.05 M ammonium acetate (pH 8.0)/acetonitrile, 1.0 mL/min, UV at 314 nm, R_t = 13.2 min] radiochemical purity: 93.6%. ^{3}H NMR (proton-coupled mode, MeOD): 8.20 (100%, d, J=10 Hz). CI-MS (NH_3): t_0 (19%), t_1 (48%), t_2 (33%); specific activity 32 Ci/mmol.

Compound **13**: HPLC [Beckman ODS C-18 column (5 um, 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 254 nm, R_t = 16.9 min] radiochemical purity: 99.5%. ^{3}H NMR (proton-coupled mode, MeOD): 8.00 (100%, d, J = 8.0 Hz). Specfic activity determined by mass concentration (HPLC weight based assay) and radioactive concentration (scintillation counting): 8.7 Ci/mmol.

Compound **14**: HPLC [Beckman ODS C-18 column (5 um, 4.6 mm I.D. x 25 cm), Solution A: 10:90:0.1 (v/v/v) acetonitrile/water/trifluoroacetic acid, Solution B: 90:10:0.1 (v/v/v) acetonitrile/water/trifluoroacetic acid; linear gradient 100% A to 100% B over 30 min; 1.0 mL/min; UV at 220 nm; R_t = 15.6 min] radiochemical purity: 99.5%. ^{3}H NMR (proton-coupled mode, C_6D_6): 8.08 (25%, s, **^{3}H$_c$**), 7.90 (24%, d, **^{3}H$_a$**), 7.16 (24%, d, **^{3}H$_b$**), 3.68 (27%, br m, **^{3}H$_d$**). CI-MS: t_1 (8%), t_2 (36%), t_3 (37%), t_4 (19%); specific activity: 78 Ci/mmol.

Compound **15**: HPLC [Beckman ODS C-18 column (5 um, 4.6 mm I.D. x 25 cm), 50:50:0.1 (v/v/v) acetonitrile/water/trifluoroacetic acid, 1.0 mL/min, UV at 220 nm, R_t = 7.4 min] radiochemical purity: 99.5%. ^{3}H NMR (proton-coupled mode, CD_3COCD_3-C_6D_6): 8.06 (21%, s, **^{3}H$_c$**), 7.94 (21%, d, J = 7.4 Hz, **^{3}H$_a$**), 7.15 (21%, d, J = 7.9 Hz, **^{3}H$_b$**), 3.59-3.63 (37%, m, **^{3}H$_d$**). CI-MS: t_0 (5%), t_1 (16%), t_2 (31%), t_3 (30%), t_4 (13%), t_5 (5%); specific activity: 73 Ci/mmol.

Compound **16**: HPLC [Beckman ODS C-18 column (5 um, 4.6 mm I.D. x 25 cm), 35:65:0.1 (v/v/v) acetonitrile/water/trifluoroacetic acid, 1.0 mL/min, UV at 220 nm, R_t = 11.0 min] radiochemical purity: 99.4%. ^{3}H NMR (proton-coupled mode, $CDCl_3$-C_6D_6): 7.93 (18%, br s, $^3\mathbf{H_a}$), 7.63 (50%, br s, $^3\mathbf{H_{c/e}}$), 7.30 (25%, br m, $^3\mathbf{H_b}$), 3.94 (7%, br s, $^3\mathbf{H_d}$). CI-MS: t_0 (12%), t_1 (31%), t_2 (34%), t_3 (18%), t_4 (5%); specific activity: 49 Ci/mmol.

Compound **17**: HPLC [Beckman ODS C-18 column (5 um, 4.6 mm I.D. x 25 cm), Solution A: 10:90:0.2 (v/v/v) acetonitrile/water/trifluoroacetic acid, Solution B: 90:10:0.2 (v/v/v) acetonitrile/water/trifluoroacetic acid, linear gradient from 60% A to 100% B over 30 min; 1.0 mL/min; UV at 254 nm; R_t = 9.8 min] radiochemical purity: 97.6%. ^{3}H NMR (proton-coupled mode, CD_3SOCD_3-$CDCl_3$): 8.43 (18%, br s, $^3\mathbf{H_a}$), 8.00 (18%, br s, $^3\mathbf{H_c}$), 7.75 (22%, d, J = 9.5 Hz, $^3\mathbf{H_d}$), 5.23 and 5.26 (42%, s and d , $J_{T\text{-}T}$ = 15 Hz, $^3\mathbf{H_b}$ singly and doubly labeled). CI-MS: t_0 (6%), t_1 (20%), t_2 (33%), t_3 (28%), t_4 (11%), t_5 (2%); specific activity: 64 Ci/mmol.

Compound **18**: HPLC [Beckman ODS C-18 column (5 um, 4.6 mm I.D. x 25 cm), Solution A: 10:90:0.2 (v/v/v) acetonitrile/water/trifluoroacetic acid, Solution B: 90:10:0.2 (v/v/v) acetonitrile/water/trifluoroacetic acid, linear gradient from 60% A to 100% B over 30 min; 1.0 mL/min; UV at 254 nm; R_t = 11.4 min] radiochemical purity: 98.5%. ^{3}H NMR (proton-coupled mode, CD_3SOCD_3-$CDCl_3$): 8.50 (25%, br s, $^3\mathbf{H_a}$), 7.85 (25%, br s, $^3\mathbf{H_c}$), 5.34 and 5.37 (50%, s and d , $J_{T\text{-}T}$ = 15 Hz, $^3\mathbf{H_b}$ singly and doubly labeled). CI-MS: t_0 (12%), t_1 (32%), t_2 (34%), t_3 (18%), t_4 (4%); specific activity: 49 Ci/mmol.

Compound **19**: HPLC [Beckman Ultrasphere ODS (5 um, 4.6 mm x 25 cm), Solution A: 91:90:0.2 (v/v/v) acetonitrile/water/trifluoroacetic acid, Solution B: 90:10:0.2 (v/v/v) acetonitrile/water/trifluoroacetic acid, 70% Soln A at 0 min, ending at 100% Soln B at 30 min, 1.0 mL/min, UV at 254 nm, Rt = 9.4 min] radiochemical purity: 95.7%. ^{3}H NMR (proton-coupled mode, CD_3SOCD_3-$CDCl_3$): 8.55 (21%, br s, $^3\mathbf{H_a}$), 8.12 (21%, br s, $^3\mathbf{H_c}$), 7.99 (10%, d, J = 8.9 Hz, $^3\mathbf{H_d}$), 7.87 (21%, d, J = 9.6 Hz, $^3\mathbf{H_e}$), 5.35 and 5.38 (27% of T, s and d, $J_{T\text{-}T}$ = 14.4 Hz, $^3\mathbf{H_b}$ singly and doubly labeled). CI-MS: t_0 (6%), t_1 (18%), t_2 (30%), t_3 (28%), t_4 (14%), t_5 (4%); specific activity 69 Ci/mmol.

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