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Research Article

Convenient and efficient deuteration of functionalized aromatics with deuterium oxide: catalysis by cycloocta-1,5-dienyliridium(I) 1,3-dionates

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Summary

Aromatic compounds bearing an *ortho*-directing substituent may be deuterated by exchange with deuterium oxide in the presence of a range of cycloocta-1,5-dienyliridium(I)1,3-dionate catalysts. The exchange takes place in several dipolar aprotic solvents and is directly applicable to the deuteration of polar compounds. Isotope incorporation is efficient and regiospecific. The method is applicable to a wide range of *ortho*-directing groups some of which are only weak directors for alternative *ortho*-labelling approaches. In addition, the application of microwaves enables labelling within minutes even with sub-stituents which are poor directors. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: *ortho*-labelling; cycloocta-1,5-dienyliridium(I)pentan-2,4-dionate; cycloocta-1,5-dienyliridium(I)-1,1,1,5,5,5-hexafluoropentan-2,4-dionate; deuteration; CH-activation; isotope-exchange; COD.Ir.F_6Acac; microwave

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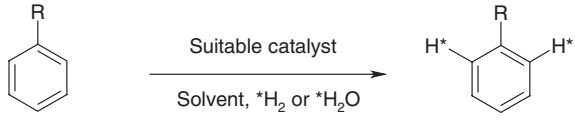
Introduction

The preparation of deuterium labelled compounds is of great importance in the biological and life sciences and amongst the most powerful methods is that of *ortho*-directed hydrogen–deuterium exchange. The isotope donor is usually deuterated water[1] or deuterium gas,[2] whilst $RhCl_3 \cdot 3H_2O$, $Ru(Acac)_3$ and Crabtree's catalyst[3] or variants thereof, are the most widely used catalysts for the process as well as for the corresponding tritiation reactions (for tritium oxide donor applications at high and low specific activity see[4]; for tritium gas applications see[5]).



Nevertheless there is still a great need to improve the performance of such catalysts so that they can operate in a larger number of solvents and in the process make labelling amenable to a more diverse range of compounds. This is particularly the case since the, otherwise excellent, isotopic hydrogen gas procedures pioneered by Heys and Shu[2a–c,5a,b] and Hesk[5c] are applicable only to compounds soluble in non-polar solvents, or which are easily recovered from ionic liquid matrices.[6] This excludes many polar and ionic compounds of interest.

Unfortunately the current state of knowledge allows only broad predictions as to which metals and ligands will provide appropriate catalytic activity. Fortunately, the development of parallel chemistry procedures has made it possible to adopt a partially empirical approach to identifying catalytic activity.[2d,f,7a,b,8] In this paper, we capitalize on this opportunity to develop a new generation of efficient catalysts and in the process we have been able to assign the optimal reaction conditions, study the regiochemistry of the exchange process, assess solvent/substrate compatibility and investigate the benefits of using microwave irradiation. Finally, detailed methods for catalyst synthesis and typical large-scale deuteration procedures are presented. A preliminary account of some of these studies has been published.[8]

Results and discussion

To identify new catalysts we chose a panel of model substrates with acidic (4-phenylbenzoic acid, **1**), neutral (4-phenylbenzamide, **2**), and basic (2-phenyl-pyridine, **3**) functionalities. All yielded strong LC–MS pseudomolecular ions and therefore provided clear indications of catalytic activity following exposure to deuteration conditions in the presence of a range of potential catalysts (Figure 1).

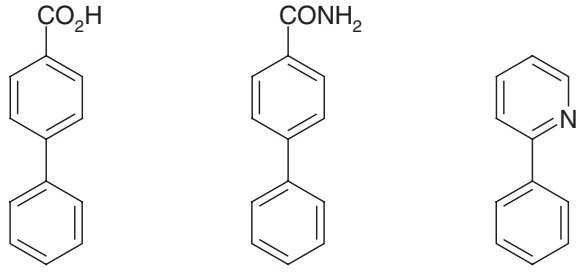
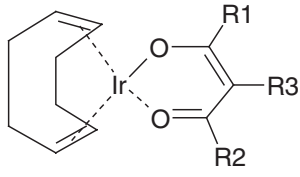


Figure 1. Screening substrates

A wide range of salts and complexes of transition metals were then screened in batches of 80. Nearly all the transition metals were represented in the screens, most by several salts or complexes selected on the basis of availability and solubility. Heterogeneous metal preparations were excluded from the study, the intent being to identify homogeneous catalysts. Positive controls of $RhCl_3 \cdot 3H_2O$ and ruthenium (III) acetylacetonate[9] were included in the batches.

Screening results

No activity was identified from any first transition series metal derivatives, the most active hits being provided by salts and complexes of rhodium, ruthenium and iridium. Later screens therefore tested an expanded range of derivatives of these metals. From these latter screens, the most active catalyst identified was 1,5-cyclooctadienyl-iridium(I)pentane-2,4-dionate, COD.Ir.Acac (Figure 2, **4**) a commercially available compound with excellent stability and solubility properties.



4 (R1,R2=CH_3, R3=H)
5 (R1,R2,R3=CH_3)
6 (R1,R2=CF_3, R3=H)

Figure 2. Initial catalyst hit and subsequent improved catalysts

Assessment of catalytic activity

The catalytic activity of **4** was shown to follow predictable patterns by a study of the deuteration of **1** using the VAST parallel NMR technique[10] to observe both the disappearance of the ^{1}H-*ortho* proton resonance of **1** in the ^{1}H-NMR spectrum, and the appearance of the corresponding ^{2}H-resonance in the ^{2}H-NMR spectrum (Figure 3).

Here the degree of deuteration achieved under various conditions of temperature and catalyst:substrate molar ratio is shown by the size of the filled circles. These correspond to the integration of the ^{1}H- or ^{2}H-resonance of the positions *ortho* to the carboxyl group.

Within the precision of the technique, it is clear that deuteration using **4** as a catalyst behaves predictably, proceeding more rapidly with increasing temperature and increasing catalyst:substrate ratio. In view

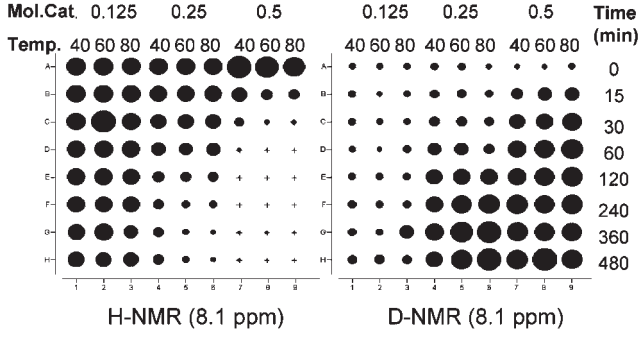


Figure 3. Degree of deuteration of 4-phenylbenzoic acid in DMA/D_2O using various temperatures and catalyst loadings of COD.Ir.Acac, 4, monitored by VAST-NMR

of this predictability, procedures involving comparison of the relative extent of deuteration achieved with a fixed panel of substrates seems a reasonable approach to evaluating the catalytic activity of complexes prepared during the ligand optimization programme.

Studies with homogeneous iridium systems[2a,e–g] suggest that the catalytic activity of **4** may be mediated by an iridium dionate species resulting from replacement of the cyclooctadiene ligand by solvent or substrate. Modulation of the catalytic activity via alteration of the dionate ligand was therefore investigated by the preparation of analogues with dionates of varying steric and electronic properties.

Subsequently, substrates **1**, **2** and **3** were incubated with the newly prepared catalysts and the relative deuteration achieved per micromole of catalyst compared with that achieved by the initial hit compound (**4**). The results are summarized in Table 1. Two complexes in which the ligand bite-angle was varied were also prepared; however, these resulted in reduced catalytic activity, as did several further electronic and steric modifications (Table 2).

In addition, the rhodium analogues of **4** and **6** were screened. The former was commercially available and the latter was prepared from commercial $(COD.Rh.Cl)_2$ dimer by a procedure analogous to that described for **6** in the Experimental Section. These compounds had no activity in the screens with the exception of the rhodium analogue of **6**,

Table 1. Catalytic activity of cycloocta-1,5-dienyliridium(1)dionate complexes

Ligand, R1.CO.CH(R3).CO.R2			Relative deuteration for substrate		
R1	R2	R3	1	2	3
Me	Me	H	1.00	1.00	1.00
t-Bu	*t*-Bu	H	1.19	0.79	0.67
CF_3	CF_3	H	1.26	1.50	2.61
CF_3	CF_3	F	1.11	1.07	2.67
CF_3	Me	H	0.96	0.79	2.33
Ph	Me	H	0.52	0.21	0.56
4-NO_2-Ph	Me	H	0.26	0.14	0.17
Me	Me	Me	1.44	2.64	1.94
Me	Et	Me	1.48	2.71	1.67
Me	*n*-Pr	Me	1.52	2.78	1.89
Me	*i*-Pr	Me	1.55	2.93	1.39
Me	*n*-Bu	Me	1.55	2.93	1.78
Me	CH_2Ph	Me	1.67	2.93	1.33
Me	Ph	Me	1.59	2.36	2.00

Data is normalized to the deuteration achieved with catalyst **4** (for which the atom% *ortho*-D_2 was 68, 34 and 44% for substrates **1**, **2** and **3**, respectively) and is corrected for the molar quantity of catalyst. Conditions: substrate (5 mg)/catalyst (1 mg)/DMF (400 µl)/D_2O (200 µl)/75°C/1 h.

Table 2. Further variations of catalyst structure

Ligand			Relative deuteration
R1.CO.CH(R3).CO.R2			
R1	R2	R3	
Me	Me	H	1.00
Me	CO_2Me	H	0.27
iPr	CF_3	H	0.11
Et	Et	H	0.30
Tropolone			0.61
2-Acetylcyclopentanone			0.06

Data is normalized, as in Table 1, to the deuteration achieved with catalyst **4** (45 atom% *ortho*-D_2). Substrate **2** (6.7 mg)/catalyst (1 mg)/DMA(334 µl)/D_2O (167 µl)/90°C/2.25 h.

which did demonstrate a minor degree of activity, though solely with substrate **3**. These studies identified excellent activity for several catalysts and subsequently two easily prepared, and stable representatives of the 3-substituted and unsubstituted dionate classes were selected to check their efficacy with a wider range of substrates (Table 3).

Clearly both the new catalysts, **5** and **6,** are active with a range of substrates. Moreover, the labelling reaction seems to be reasonably insensitive to the electronic activation or deactivation state of the substrate, a behaviour previously noted for other metal-catalysed isotope exchanges.[2g,9a,b,11] The hexafluoro-acetoacetonate catalyst, **6**, showed somewhat better activity in general, particularly with pyridines and other nitrogen heterocyclics. Both the catalysts showed low activity with disubstituted benzamides, as expected on steric grounds, but good activity with the sulphonamide, a directing group for which previous systems have shown little efficacy.

The conditions described in Table 3 were chosen to yield only moderate levels of labelling, allowing the maximum discrimination between substrates. With higher catalyst:substrate ratios, higher temperatures and/or the application of microwave heating,[12] the reaction is facile and even the more resistant substrates can be induced to undergo exchange.

The application of microwave heating to the reaction is also included in Table 3. Using both a higher catalyst:substrate ratio and a higher temperature, achieved rapidly via microwave heating, provided a rate improvement of around 70-fold in deuteration rate over the standard thermal conditions. Moreover, with disubstituted amides and anilides, which are only moderate directors, increasing catalyst:substrate ratios (finally up to 1:1) combined with further increases in microwave

Table 3. Comparison of the activities of COD.Ir.MeAcac (5) and COD.Ir.F6Acac (6) with a selected range of substrates

Substrate	COD.Ir. MeAcac (5) Thermal %[a]	COD.Ir. F_6Acac (6) Thermal %[a]	COD.Ir. F_6Acac (6) Microwave %[b]
Acetanilide*	1	2	2
4-Aminobenzoic acid	56	46	77
Antipyrine*	25	27	41
Benzanilide***	58, 0	32, 0	55, 8
7,8-Benzoquinoline**	58	75	70
N-Benzylbenzamide*	7	9	15
2-Benzylpyridine*	4	18	40
N,N-Di-*iso*-propylbenzamide*	9	10	13
N,N-Dimethylbenzamide*	4	4	2
α,α-Dimethylbenzylamine	48	63	92
4-Fluorocinnamic acid**	26	19	30
4-Methylbenzenesulphonamide	50	50	66
4-Methylbenzylamine	65	65	75
4-Phenylbenzamide	79	50	50
4-Phenylbenzoic acid	78	72	93
2-Phenylimidazole****	68, 50	100, 12	50, 100
2-Phenylpyridine	54	100	100
Sodium 4-phenylbenzoate	27	62	77
4-Trifluoromethylbenzylamine	28	57	75

[a] Substrate (0.034 mmol) heated at 90°C for 2.25 h in 2:1 v/v DMA/D_2O (0.5 ml) with catalyst (0.002 mmol).
[b] Substrate (0.05 mmol) microwaved at 130°C for 2 min in 2:1 v/v DMA/D_2O (1.2 ml) with catalyst (0.01 mmol).
*Labelling regiochemistry not assigned due to low deuterium abundance or low spectroscopic resolution.
**MS shows ca. 10% labelling at other sites.
***% *ortho* to amide, % *ortho* to anilide.
****% *ortho*, % imidazole.

temperatures led to labelling (Table 4) even of the highly hindered di-*iso*-propyl amide.

In contrast to the conditions required for such weak directors, catalyst **5** proved sufficiently active to promote labelling at room temperature in compounds with more active directing groups such as **1** or its corresponding sodium salt. This could prove useful in labelling thermally unstable compounds.

Co-solvent

Although DMF was used as co-solvent throughout the initial phases of these studies and is a co-solvent of choice for the reaction, other co-solvents may also be used (Table 5). DMA in particular has proved useful for NMR studies, since in this case the aromatic region of the spectrum is clear of solvent resonances.

Table 4. Labelling of substrates bearing weak directors using COD.Ir.F_6Acac (6) under microwave conditions

Substrate	Thermal[a]	Microwave[b]	Microwave[c]	Microwave[d]
Acetanilide	2%	2%	75%	n.d.
N,N-Dimethylbenzamide	0%	0%	52%	n.d.
N,N-Di-*iso*-propylbenzamide	0%	0%	10%	30%
Benzanilide(*ortho* to amide)	32%	55%	100%	94%
Benzanilide(*ortho* to anilide)	0%	8%	31%	96%
4-Fluorocinnamic acid	19%	30%	92%	n.d.

[a] Substrate (0.034 mmol) heated at 90°C for 2.25 h in 2:1 v/v DMA/D_2O (0.5 ml) with catalyst (0.002 mmol).
[b] Substrate (0.05 mmol) microwaved at 130°C for 2 min in 2:1 v/v DMA/D_2O (1.2 ml) with catalyst (0.01 mmol).
[c] Substrate (0.025 mmol) microwaved at 160°C for 2 min in 2:1 v/v DMA/D_2O (0.6 ml) with catalyst (0.04 mmol).
[d] Substrate (0.025 mmol) microwaved at 200°C for 5 min in 2:1 v/v DMA/D_2O (0.6 ml) with catalyst (0.025 mmol).
n.d. = Not done.
Low degrees of labelling were determined from LC–MS, higher degrees from MS and ^{1}H-NMR.

Table 5. Deuteration of 4-phenylbenzamide catalyzed by COD.Ir.F_6Acac (6) and D_2O in various polar solvents*

Co-solvent	Atom% D_2	Co-solvent	Atom% D_2
N,N-Dimethylformamide	91	Propylene carbonate	19
N,N-Dimethylacetamide	60	**1-Et-3MeImid$^+$ BF4$^-$	13
N-Methylpyrrolidine	35	Dioxan	2
N-Methylpyrrolidinone	23	Acetonitrile	2
Dimethylsulphoxide	21	Sulpholan	1

*COD.Ir.F_6Acac (**6**,1 mg) and 4-phenylbenzamide (5 mg) were heated at 90°C with D_2O (150 µl) and co-solvent (300 µl) for 1.5 h **1-Ethyl-3-methylimidazolium tetrafluoroborate.

Experimental

[Di-μ-chlorobis(1,2,5,6-η)-1,5-cyclooctadiene]diiridium (Fluka item), deuterium oxide (99.8%D), DMF and DMA (water content $<0.03\%$ and 0.05%, respectively) were obtained from Sigma-Aldrich. All other solvents and reagents were obtained from recognized chemical suppliers, including the above, and were used as received. Microwave experiments were carried out using a C.E.M. Discover microwave system.

Screening procedure and analysis methods

The potential catalysts to be screened (ca. 2 mg of each) were dispensed into deep-well reaction boxes each capable of holding up to 96

polypropylene tubes of 1 ml capacity and then the appropriate substrate (5 mg/tube) dissolved in DMF (400 µl/tube) and deuterium oxide (200 µl/tube) was added. The tubes were then sealed and heated at the appropriate temperature (95°C in the initial screens, 50°C for the more active catalysts prepared later in the programme). After an appropriate time (18 h in the initial screens, 1 h later in the programme) 20 µl aliquots from each of the wells were taken, diluted 10-fold with 1:1 DMSO/H_2O and analysed by high-throughput HPLC/MS (5 µm C_8 silica stationary phase using acetonitrile/aqueous ammonium acetate gradients with positive and negative ion modes) to determine the degree of deuteration achieved. Later in the programme the *ortho*-regioselectivity of the labelling was confirmed for the active catalysts using the automated multi-sample VAST-NMR technique.[10] This enabled automated analysis of parallel reactions performed in 96 well arrays and yielded data on the extent of reaction and the regiospecificity of the process. The parallel reaction wells were sampled, filtered and spiked with an internal standard ([$^2H_6$]DMSO) to yield a 10% v/v ^2H solution and then subjected to VAST analysis via a Gilson 215 autosampler in conjunction with a Varian Unity INOVA 500 MHz NMR spectrometer. Aliquots (170 µl) were analysed using a wash solvent of [$^2H_6$]DMSO (200 µl) and a 60 µl flow cell. Both ^1H- and ^2H-spectra were recorded and the data analysed using the SPADEZ spectra analysis program.[13]

Preparation of the catalysts

The cycloocta-1,5-dienyliridium(1) dionate complexes were prepared using modifications of a literature procedure.[14] The detailed methods used for the preparation of two of the most active species are given below.

Cycloocta-1,5-dienyliridium(I) 1,1,1,5,5,5-hexafluoropentan-2,4-dionate, 6

Commercial [di-μ-chlorobis(1,2,5,6-η)-1,5-cyclooctadiene]diiridium (300 mg) is stirred under nitrogen in degassed ether (6.0 ml) at which point it is only partly dissolved. Commercial 1,1,1,5,5,5-hexafluoro-2,4-pentanedione (0.3 ml) in ether (0.6 ml) is then added via a syringe and the reaction stirred for 10 min. Sodium hydroxide solution (1 M, 1.25 ml) is then added, dropwise via syringe. During this phase the remaining crystals of precursor dissolve and the mixture becomes a deep claret colour. Water (3.0 ml) is then added, again via a syringe, and the

biphasic mixture stirred under nitrogen for a further 20 min. The ether layer is evaporated under a slow stream of nitrogen leaving suspended clumps of fine claret-coloured crystals. The aqueous supernatant is decanted and the crystals washed twice more by decantation with 2.0 ml portions of water. Finally, the crystals are filtered and dried over silicagel overnight to leave cycloocta-1,5-dienyliridium(I) 1,1,1,5,5,5-hexafluoropentan-2,4-dionate (412.5 mg, 91%) as a fine free-flowing stable red-purple solid. ^{1}H-NMR: δ ($CDCl_3$) 1.75 (4H, q, $J = 7.5$ Hz), 2.30 (4H, m), 4.31 (4H, s), 6.32 (1H, s) p.p.m. ^{13}C-NMR: δ ($CDCl_3$) 31.1, 62.6, 93.1, 118.5 (quartet), 174.3 (quartet) p.p.m. MS (EI mode) m/z 508/506 (mol. ion), 301-292 (cluster) a.m.u. Found C 30.55%, H 2.91%, Required C 30.67%, H 2.91%.

This catalyst, and also catalyst **4**, retain activity when stored at −20°C under nitrogen for three years. The catalyst retains full activity for at least six months when stored at room temperature in the dark under nitrogen and shows no loss of activity when stored for several weeks at room temperature without exclusion of air.

Cycloocta-1,5-dienyliridium(I) 3-methylpentan-2,4-dionate, 5

Commercial [di-μ-chlorobis(1,2,5,6-η)-1,5-cyclooctadiene]diiridium (500 mg) is stirred under nitrogen in degassed ether (8.5 ml) until nearly all dissolved. 3-Methylpentane-2,4-dionate (200 mg) is then added to the mixture, followed by 1 M potassium hydroxide solution (2.5 ml) and water (2.0 ml) and the biphasic mixture stirred under nitrogen for 45 min. Removal of the ether under reduced pressure leaves yellow crystals in the aqueous layer. The crystals are filtered and dried over silicagel overnight to yield cycloocta-1,5-dienyliridium(I) 3-methylpentan-2,4-dionate (498 mg, 81%). ^{1}H-NMR: δ ($CDCl_3$) 1.65 (4H, q, $J = 8$ Hz), 1.95 (3H, s), 2.10 (6H, s), 2.25 (4H, m), 3.95 (4H, s). ^{13}C-NMR: δ ($CDCl_3$) 16.6, 27.8, 31.4, 59.5, 105.2, 185.3 p.p.m. MS (EI mode), m/z 414/412 (mol. ion), 301-292 (cluster) a.m.u.

Typical large-scale deuterations

[2,6-$^{2}H_2$]-4-Phenylbenzoic acid. 4-Phenylbenzoic acid (100 mg) is heated with commercial cyclooctadienyliridium(I) pentan-1,3-dionate (**4**, 20 mg) in a mixture of DMF (6.6 ml) and deuterium oxide (3.3 ml) at 90°C for 2 h. The resulting solution is cooled, partitioned between ethyl acetate (30 ml) and 5% w/v aqueous sodium hydrogen carbonate solution

(10 ml). The aqueous layer is separated, acidified with dilute hydrochloric acid to pH < 3, and the precipitated product re-extracted into ethyl acetate (10 ml). After the removal of the solvent under reduced pressure, crystallization of the resulting solid from hot methanol (2.0 ml) yields [2,6-$^2H_2$]-4-phenylbenzoic acid (75 mg, 74%, m.p. 223–225°C). Characterization by ^{2}H-NMR (61.4 MHz in [$^1H_6$]DMSO) shows a single resonance at δ 8.1 ppm (*ortho* to carboxyl) with no other resonances detectable. The atom% abundance of deuterium by MS is 97%, calculated for two exchangeable positions.

[2,6-$^2H_2$]-4-Phenylbenzamide. 4-Phenylbenzamide (99.5 mg) is heated with cyclooctadienyliridium(I) pentan-1,3-dionate (**4,** 20 mg) in a mixture of DMF (6.6 ml) and deuterium oxide (3.3 ml) at 91°C for 2 h. The resulting solution is cooled, partitioned between ethyl acetate (30 ml) and 5% w/v aqueous sodium hydrogen carbonate solution (10 ml). The ethyl acetate layer is separated and washed with water acidified to pH 4 with a few drops of dilute hydrochloric acid. After removal of the solvent under reduced pressure, crystallization of the resulting solid from hot methanol (1.5 ml) yields [2,6-$^2H_2$]-4-phenylbenzamide (65 mg, 65%, m.p. 230–231°C). The ^{2}H-NMR (61.4 MHz in [$^1H_6$]acetone) shows a single resonance at δ 8.15 ppm (*ortho* to amide) with no other resonances detectable. The atom% abundance of deuterium by MS is 94.4%, calculated for the two exchangeable positions.

[2,6-$^2H_2$]-4-Methylbenzenesulphonamide. 4-Methylbenzenesulphonamide (50 mg) is dissolved in DMA (2 ml) containing cycloocta-1,5-dienyliridium(I) 1,1,1,5,5,5-hexafluoropentan-2,4-dionate (**6**, 29 mg) and D_2O (1 ml) added; the vial is then heated at 95°C for 5 h. The resulting reaction mixture is partitioned between sodium hydroxide solution (1 M, 3 ml) and diethyl ether (4 ml) and the aqueous layer separated. The ether is washed with water (2 × 0.5 ml) and the aqueous layers combined. After acidification with 10% v/v hydrochloric acid solution, the resulting cloudy solution is extracted twice with ether (8 ml), the ether extracts combined and washed with a minimum volume of water. The ether is blown to dryness under a stream of nitrogen and recrystallized from chloroform/hexane to yield the labelled compound (27 mg, 50% unoptimized, m.p. 135°C). The ^{1}H-NMR ([$^2H_6$]DMSO) shows only a singlet resonance at δ 7.36 ppm and no detectable residual proton intensity at δ 7.7 ppm. The ^{2}H-NMR

(61.4 MHz, [$^1H_6$]DMSO) shows a single deuteron resonance at δ 7.7 ppm (*ortho* to sulphonamide).

([2,6-$^2H_2$]Benzoyl)aniline, [$^2H_2$]benzanilide. Benzanilide (100 mg) is heated with cyclooctadienyliridium(I) pentan-1,3-dionate (**4,** 20 mg) in a mixture of DMF (6.6 ml) and deuterium oxide (3.3 ml) at 95°C for 4 h. The solution is cooled and ethyl acetate (30 ml) added. The ethyl acetate solution is then washed with 5% w/v sodium hydrogen carbonate solution (10 ml) followed by careful washing with water acidified to pH 4 with dilute hydrochloric acid. The solvent is then removed under reduced pressure, and the product recrystallized from methanol (87 mg, 86%, m.p. 163–164°C). The atom% abundance of deuterium is 98.7%, based on the two benzoyl *ortho* positions which exchange under these conditions. The ^{2}H-NMR of the benzanilide shows a single resonance at δ 8.06 ppm (*ortho* to the C=O), with no other detectable resonances.

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