Supporting information

SI_NMR

Parahydrogen Hyperpolarized NMR Detection of Underivatized Short Oligopeptides

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Sample preparation for A_2 and A_3 nhPHIP complex characterization by ^{1}H, COSY, NOESY, HSQC and HMBC

Oligopeptide, Ir-catalyst and cosubstrate were dissolved, mixed and heated as described for nhPHIP samples in SI_nhPHIP. As the only difference, methanol-d_3 was used as the solvent to avoid deuteration of exchangeable NH protons by methnanol-d_4. The sample consisted of 1.2 mM [Ir(Cl)(COD)(IMes)], 18-fold excess of 3F4MePy and 1 mM oligopeptide in a total sample volume of 600 μL.

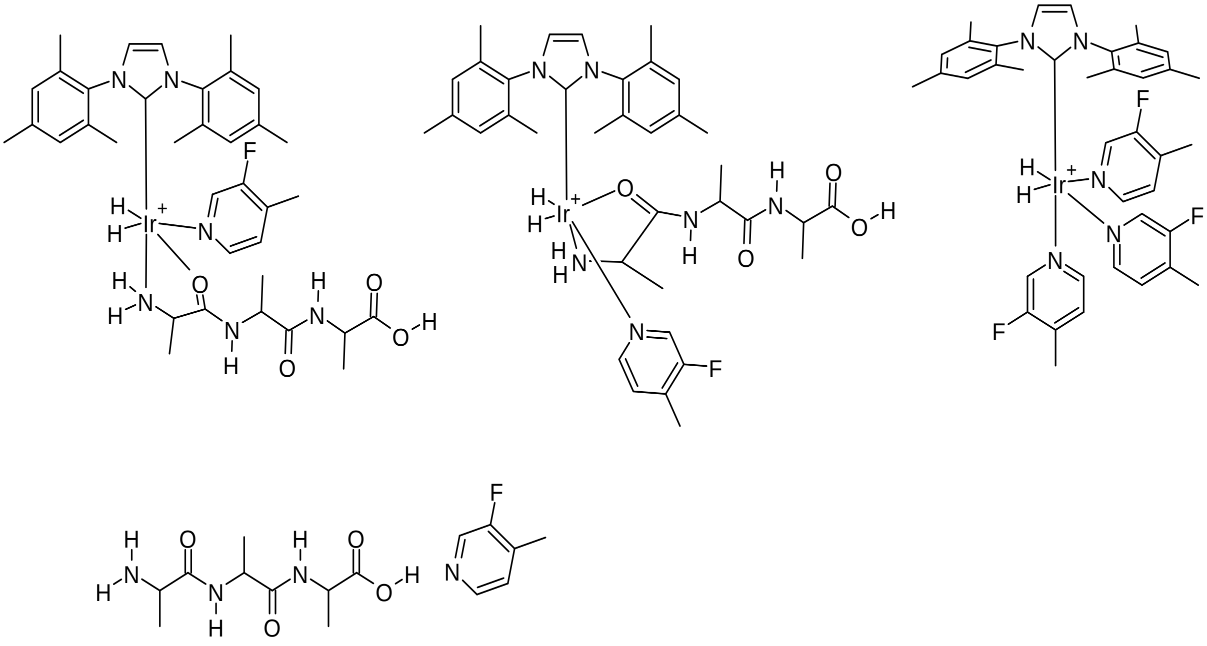


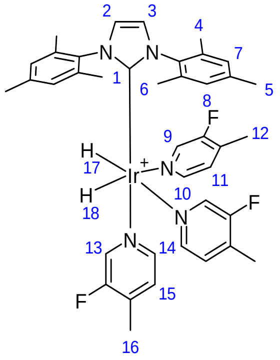
Figure SA1. Typical sample contains all of the depicted structures. The two A_3 containing Iridium complexes are diastereomers.

NMR experimental conditions

Spectra were acquired on a cryoprobe equipped 800 MHz Bruker Avance III spectrometer. ^{1}H, COSY and NOESY spectra of samples in methanol-d_3 were acquired with single solvent signal (-OH) suppression by excitation sculpting to remove the non-deuterated methanol-d_3 signal without suppressing exchangeable peptide NH signals. Homonuclear 2D experiments were acquired in 7200 Hz spectral width in both dimensions and the indirect dimension was sampled in 256 increments. NOESY spectra were recorded with 700 ms mixing time for optimal observation of both NOE and exchange effects.

HSQC and HMBC spectra were acquired with standard Bruker Topspin parameter set conditions, using adiabatic pulses for ^{13}C.

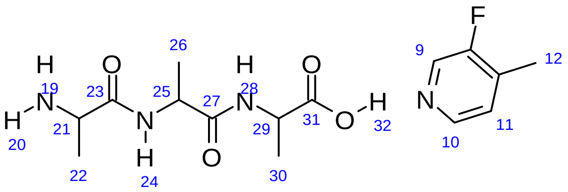
Table SA1. NMR data for the symmetric chemosensor complex without an analyte, measured at 10ºC.



Atom label	^{1}H	^{13}C
1	-	137.2
2 and 3	7.11 (s, 2H)	128.2
4 and 6	2.04 (s, 12H)	17.5
5	2.31 (s, 6H)	NA
7 and 8	7.13 (s, 2H)	122.6
9	7.86 (d, *J* = 3.5 Hz, 2H)	1423.0
10	8.18 (d, *J* = 5.4 Hz, 2H)	148.5
11	7.20-7.16 (m, 2H)	127.6
12	2.22 (s, 6H)	19.7
13	7.97 (d, *J* = 3.9 Hz, 1H)	143.0
14	7.73 (d, *J* = 5.8 Hz, 1H)	150.7
15	6.94-6.90 (m, 1H)	128.2
16	2.13 (s, 3H)	NA
17 and 18	-23.08 (s, 2H)	-

^{13}C chemical shifts obtained from HSQC and HMBC measurements for the A_2-Ir-complex.

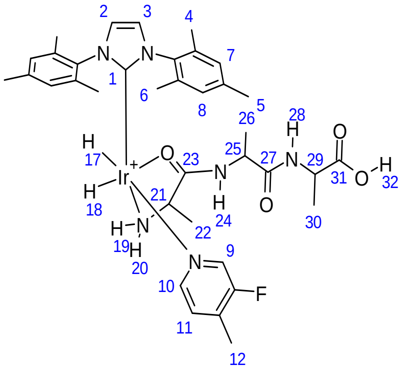
Table SA2. ^{1}H NMR data for A_3 and 3F4MePy solution in ratio of 1:8 at 25ºC.

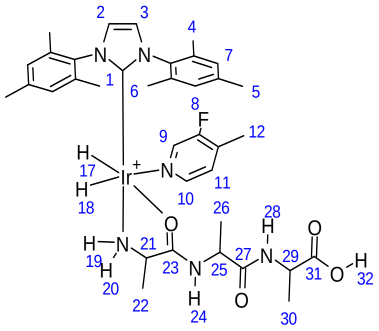


Atom label	1H	^{13}C
9	8.32 (d, *J* = 1.9 Hz, 1H)	145.7
10	8.23 (dd, *J* = 4.9, 0.9 Hz, 1H)	145.7
11	7.41 – 7.32 (m, 1H)	127.6
12	2.35 (d, *J* = 0.9 Hz, 3H)	13.4
19	-	-
20	-	-
21	3.89 (q, *J* = 7.1 Hz, 1H)	49.9
22	1.55 (d, *J* = 7.1 Hz, 3H)	NA
23	-	171.0
24	8.61 (d, *J* = 7.1 Hz, 1H)	-
25	4.36 (q, *J* = 7.2 Hz,1H)	50.7
26	1.39 (d, *J* = 7.2 Hz, 3H)	NA
27	-	173.1
28	7.95 (d, *J* = 6.4 Hz, 1H)	-
29	4.14 (q, *J* = 7.0 Hz, 1H)	51.2
30	1.34 (d, *J* = 7.1 Hz, 3H)	NA
31	-	178.7
32	-	-

NA - Not clearly resolved in HMBC

Table SA3. NMR data for the two diastereomeric A_3-Ir complexes, measured at 10ºC





Atom label	Diastereomer 1	Diastereomer 2	Comment
1	-	-	
2 and 3	6.97 (s, 1H)	7.01 (s, 1H)	
4 and 6	1.96 (s, 6H) 1.83 (s, 6H)	2.13 (s, 6H) 2.04 (s, 6H)	Could not be fully assigned
5	2.34 (s, 6H)	2.34 (s, 6H)	Diastereomers overlap
7 and 8	7.04 (s, 2H) 6.91 (s, 2H)	6.89 (s, 2H) 6.80 (s, 2H)	Could not be fully assigned
9	7.55 (s, 1H)	7.60 (s, 1H)	
10	7.74 (d, *J* = 4.1 Hz, 1H)	7.90 (d, *J* = 5.7 Hz, 1H)	
11	7.06 – 7.04 (m, 1H)	7.11 – 7.08 (m, 1H)	
12	2.29 (s, 3H)	2.30 (s, 3H)	
17	-23.39 (d, *J* = 9.2 Hz, 1H)	-30.46 (d, *J* = 9.2 Hz, 1H)	
18	-30.74 (d, *J* = 9.2 Hz, 1H)	-23.28 (d, *J* = 9.2 Hz, 1H)	
19 and 20	5.16	5.16	Overlaps with OH; chemical shifts obtained from COSY
21	3.47 (p, *J* = 7.6 Hz, 1H)	3.59 – 3.52 (m, 1H)	
22	1.11 (d, *J* = 7.6 Hz, 3H)	1.26 (d, *J* = 7.4 Hz, 3H)	
23	-	-	
24	8.58 (d, *J* = 7.7 Hz, 1H)	8.97 (d, *J* = 8.8 Hz, 1H)	
25	4.33 (p, *J* = 7.7 Hz, 1H)	4.55-4.52 (m, 1H)	Diastereomer 2 overlaps with H_2 signal
26	1.44 (d, *J* = 7.7 Hz, 3H)	~1.40	Diastereomer 2 overlaps with unbound A_3
27	-	-	
28	~7.57	~7.90	Multiples could not be resolved
29	4.21 – 4.17 (m, 1H)	4.30 (p, *J* = 6.9 Hz, 1H)	Diastereomer 1 overlaps with unbound A_3
30	~1.40	1.47 (d, *J* = 7.4 Hz, 3H)	Diastereomer 1 overlaps with unbound A_3
31	-	-	
32	-	-	

Diastereomer 1 and 2 signals could not be explicitly assigned because they form in equal amounts. Therefore, all left-hand signals were tentatively assigned to diastereomer 2 and right-hand signals to diastereomer 1.

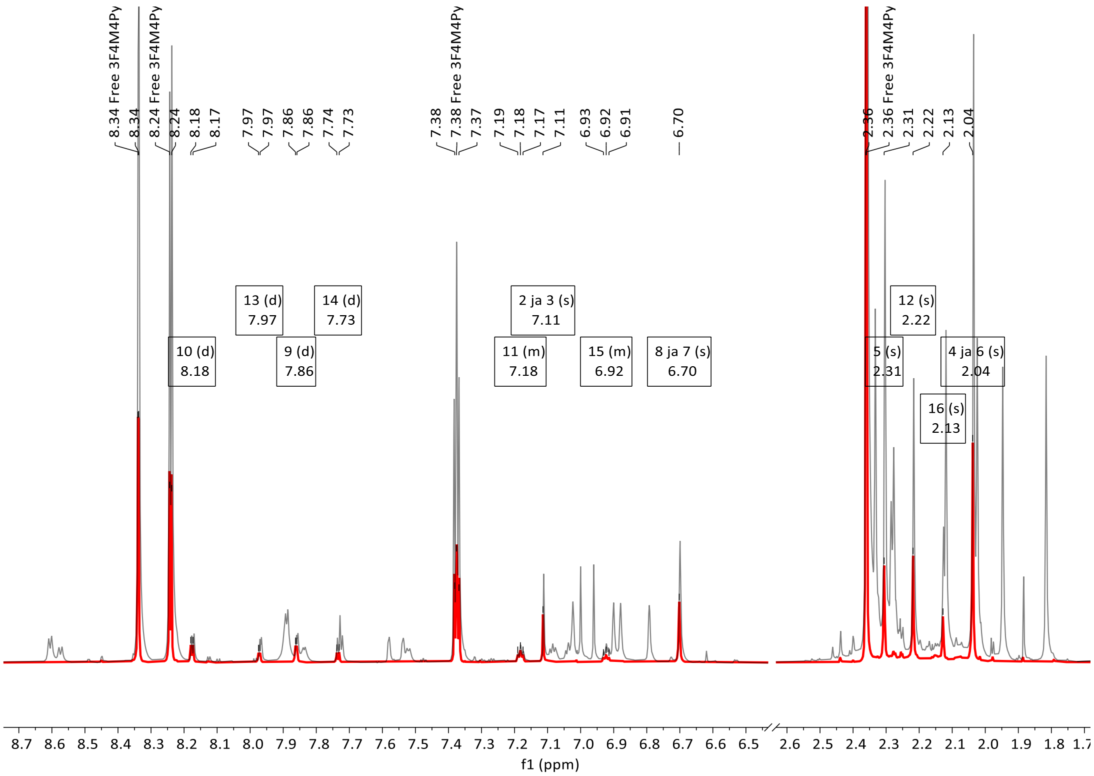


Figure SA2. Symmetric complex (red spectrum, Ir:3F4MePy in 1:18 ratio in CD_3OD) overlayed with A_3 containing hyperpolarization mixture (dark grey, Ir:3F4MePy:A_3 in 1:18:0.8 ratio in CD_3OH). The spectra were used to identify the non-analyte associated complex in the spectrum. See page 14 of this document for NMR spectra of individual components.

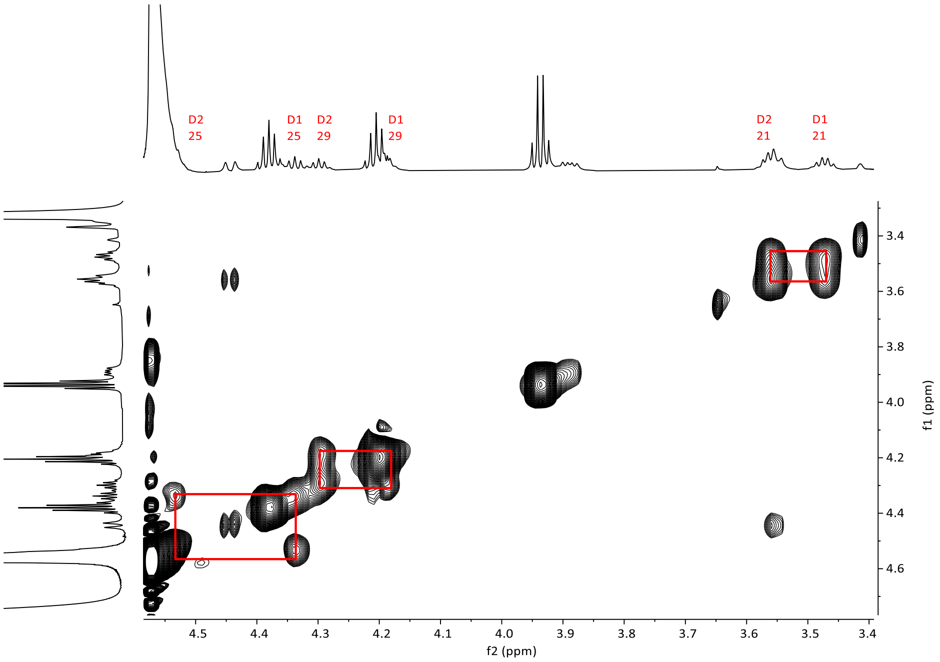


Figure SA3. Expansion of in-phase exchange peaks in NOESY experiment in the region of A_3 α-CH protons, showing the exchange between nhPHIP complex diastereomers.

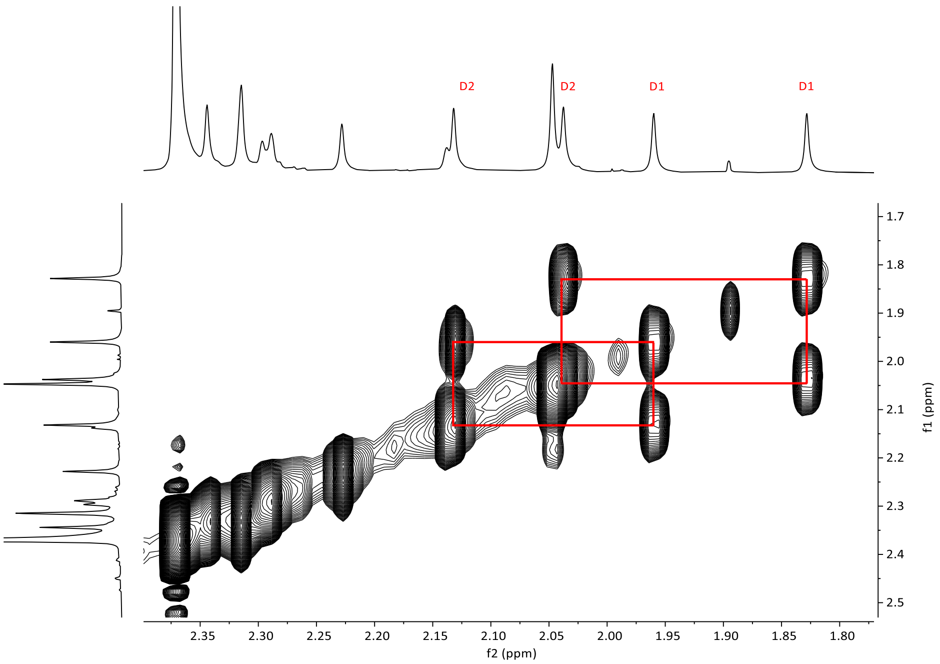


Figure SA4. Expansion of in-phase exchange peaks in NOESY experiment in the region of IMes CH_3 protons 4 and 6, showing exchange between diastereomers.

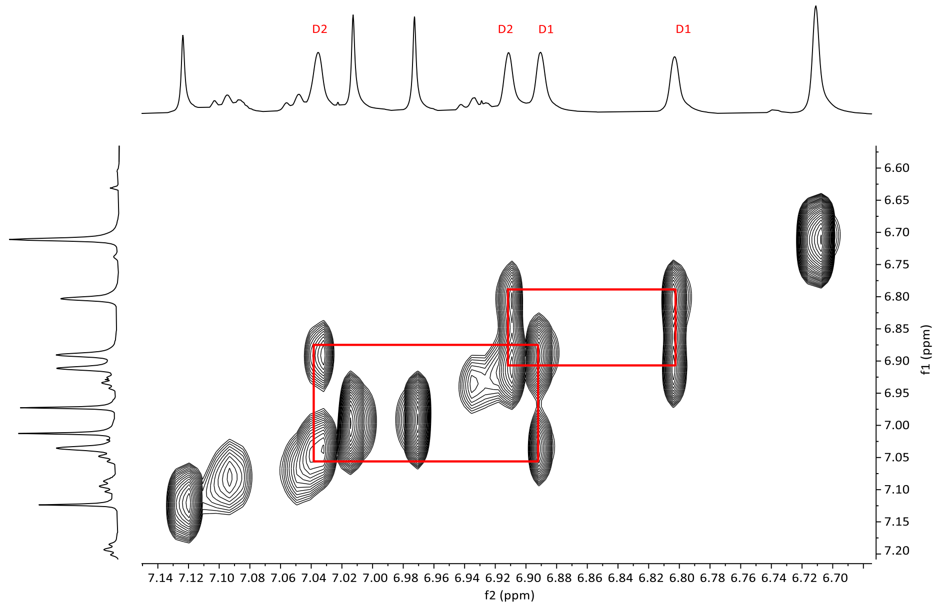


Figure SA5. Expansion of in-phase exchange peaks in NOESY experiment in the region of IMes aromatic CH protons 7 and 8, showing the exchange between diastereomers.

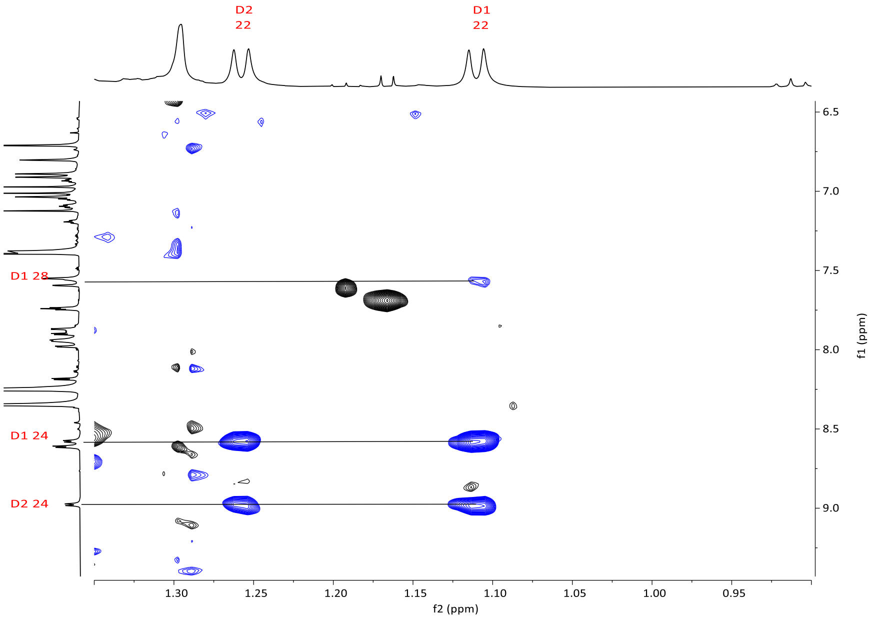


Figure SA6. Expansion of NOESY spectrum depicting NOE responses between A_3 CH_3 (22) and NH protons 24 and 28. NOE responses between 22 and 28 can only appear in the case of a 5-membered Ir-A_3 complex (see SI_DFT for calculated geometries).

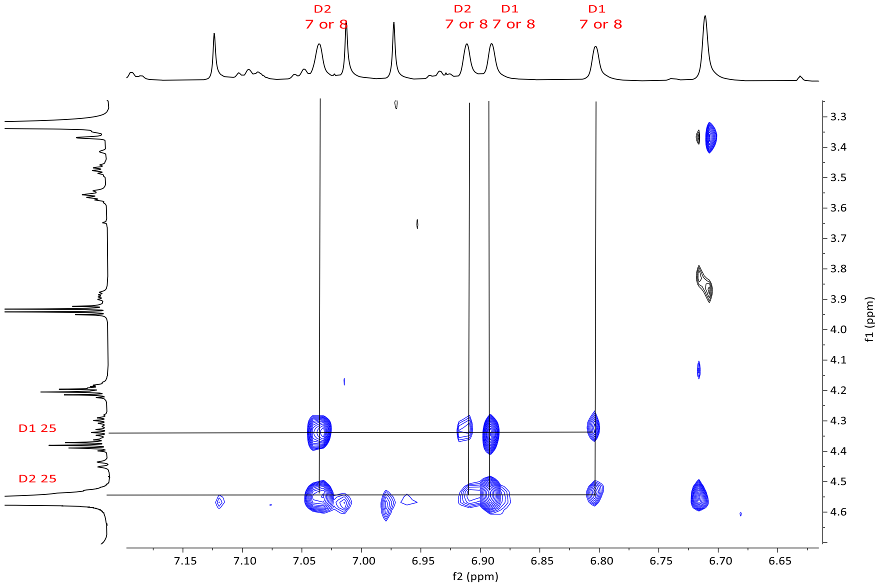


Figure SA7. Expansion of NOESY spectrum depicting NOE responses between A_3 -CH (25) and IMes aromatic protons 7 and 8. NOE responses between 25 and IMes aromatic 7 and 8 can only appear in the case of a 5-membered Ir-A_3 complex (see SI_DFT for calculated geometries).

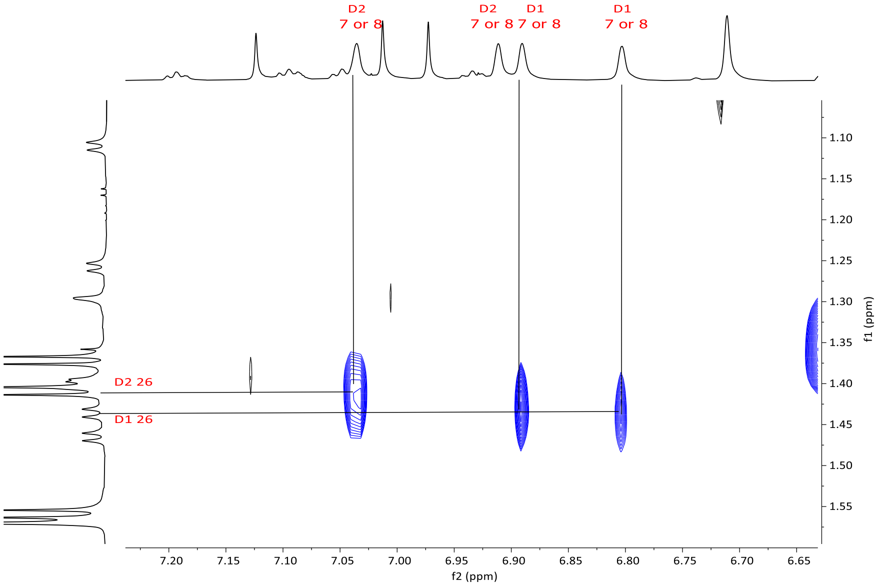


Figure SA8. Expansion of NOESY spectrum depicting NOE responses between A_3 -CH_3 (26) and IMes aromatic protons 7 and 8. NOE responses between 26 and IMes aromatic 7 and 8 can only appear in the case of a 5-membered Ir-A_3 complex (see SI_DFT for calculated geometries).

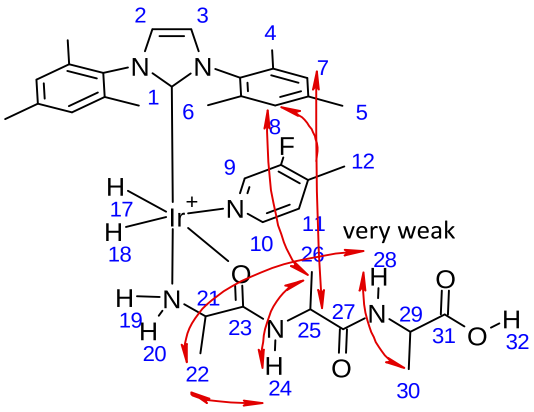


Figure SA9. All NOE responses in A_3-Ir complex diastereomers.

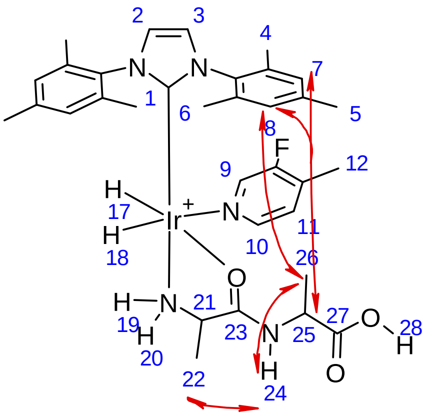
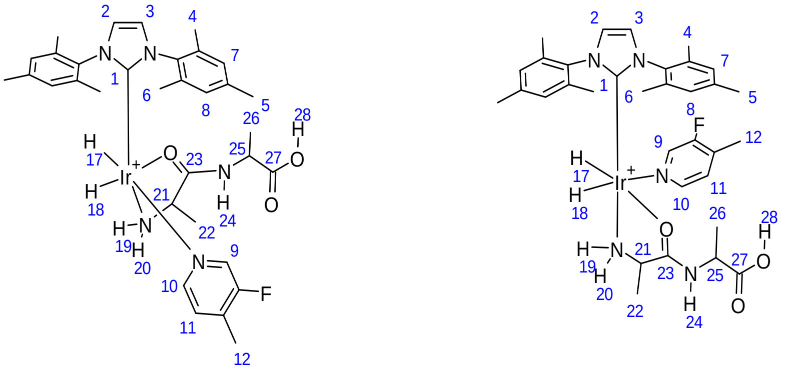


Figure SA10. All NOE responses in A_2-Ir complex diastereomers.

Table SA4. NMR data for the two diastereomeric A$_2$-Ir complexes, measured at 10ºC.



Atom label	Diastereomer 1 (minor)		Diastereomer 2 (major)		Comment
	^{1}H	^{13}C	^{1}H	^{13}C	
1	-	137.6	-	137.6	
2 and 3	6.95 (s, 1H)	121.5	6.99 (s, 1H)	121.5	Diastereomer 2 overlaps with Diastereomer 2 8/7 signals in ^{1}H
4 and 6	1.84 (s, 6H) 2.13 (s, 6H)	17.0 19.7	2.03 (s, 6H) 1.94 (s, 6H)	17.5 17.1	Could not be fully assigned
5	2.31 (s, 6H)	19.9	2.31 (s, 6H)	19.9	Diastereomers overlap ^{1}H
7 and 8	6.99 (s, 2H) 6.79 (s, 2H)	128.2 128.0	6.88 (s, 2H) 6.95 (s, 2H)	128.4 128.2	Could not be fully assigned. Diastereomer 2 overlaps with diastereomer 2 2/3 signals in ^{1}H
9	7.61 (s, 1H)	142.7	7.56 (d, *J* = 3.2 Hz, 1H)	140.7	
10	7.94 (d, *J* = 5.7 Hz, 1H)	148.3	7.91 (d, *J* = 5.8 Hz, 1H)	149.1	
11	7.10 – 7.06 (m, 1H)	126.9	7.10 – 7.06 (m, 1H)	126.9	The two diastereomers overlap in ^{1}H
12	2.26 (s, 3H)	12.6	2.28 (s, 3H)	12.6	
17	-23.39 (d, *J* = 9.6 Hz, 1H)	-	-30.46 (d, *J* = 9.1 Hz, 1H)	-	
18	-30.03 (d, *J* = 9.6 Hz, 1H)	-	-23.33 (d, *J* = 9.1 Hz, 1H)	-	
19 and 20	5.05	-	5.05	-	Overlaps with OH, chemical obtained shifts from NOESY
21	3.45 – 3.39 (m, 1H)	56.0	3.52 (p, *J* = 7.3 Hz, 1H)	54.6	
22	0.97 (d, *J* = 7.5 Hz, 3H)	18.1	1.20 (d, *J* = 7.3 Hz, 3H)	16.7	
23	-	179.7	-	180.1	
24	7.99	-	8.25	-	Diastereomer 1 and 2 overlap with other signals in ^{1}H, chemical shifts obtained from NOESY
25	4.34 – 4.28 (m, 1H)	NA	4.37 (p, *J* = 7.3 Hz, 1H)	50.3	Deastereomer 1 overlaps with an impurity in the sample
26	1.39 (d, *J* = 7.8 Hz, 3H)	17.3	1.36 (d, *J* = 7.3 Hz, 3H)	18.8	
27	-	176.9	-	176.2	
28	-	-	-	-	

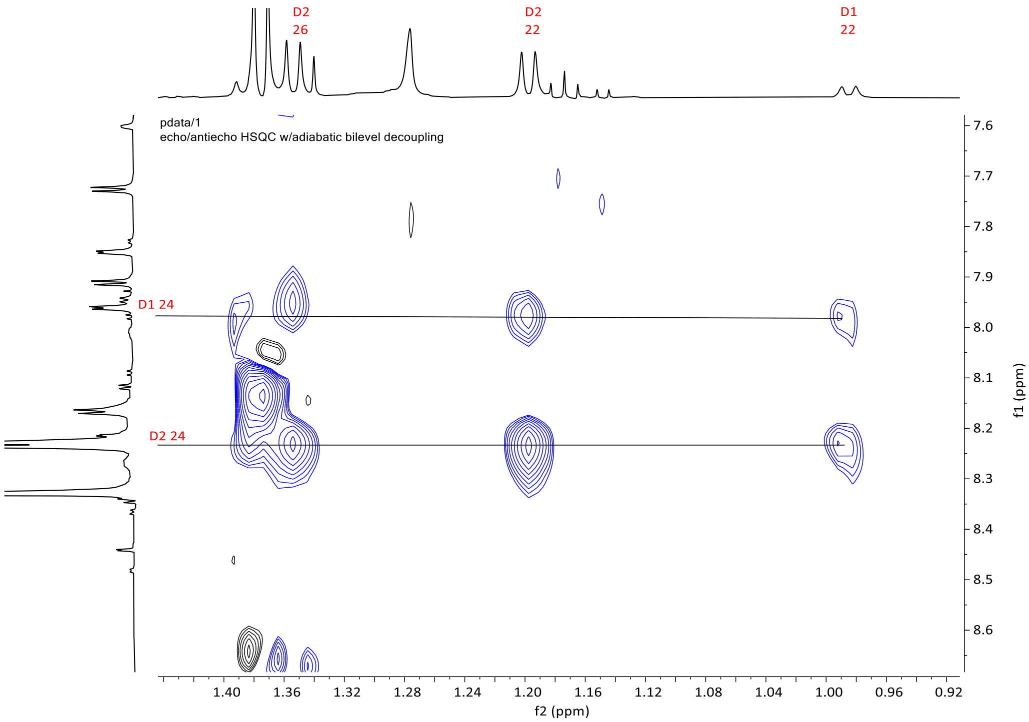


Figure SA11. Expansion of NOESY spectrum depicting NOE responses between A_2 NH (25) and CH3 protons of 22 and 26. NOE responses between 24 and 22 and 26 can only appear in the case of a 5-membered Ir-A_2 complex (see SI_DFT for calculated geometries).

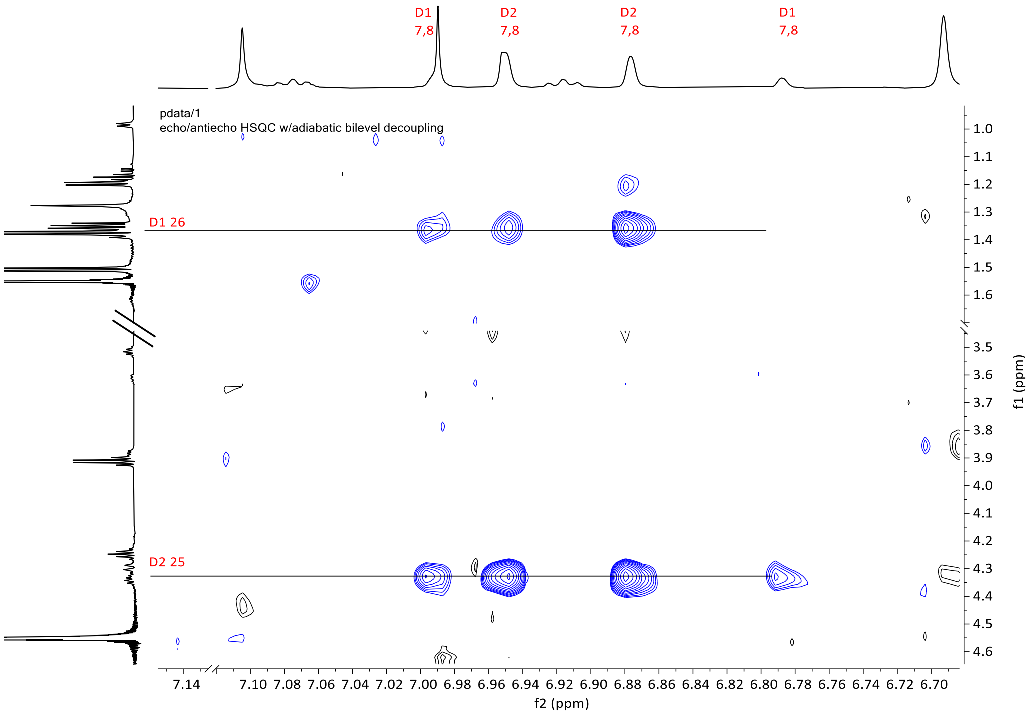


Figure SA12. Expansion of NOESY spectrum depicting NOE responses between A_2 25, 26 and IMes 8 and 7 signals.

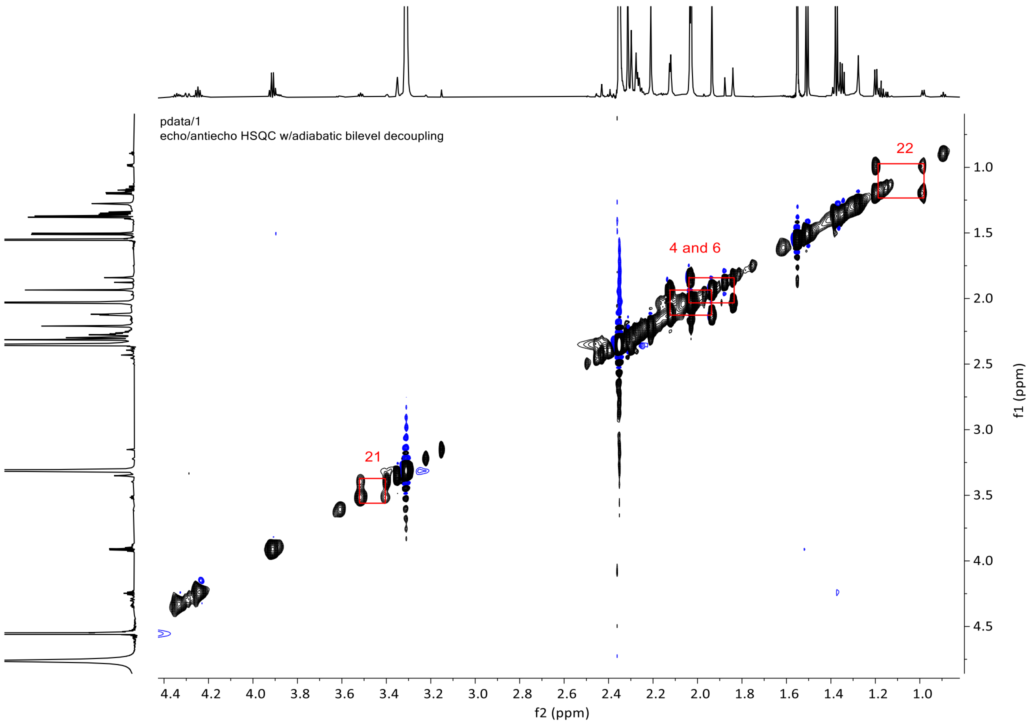
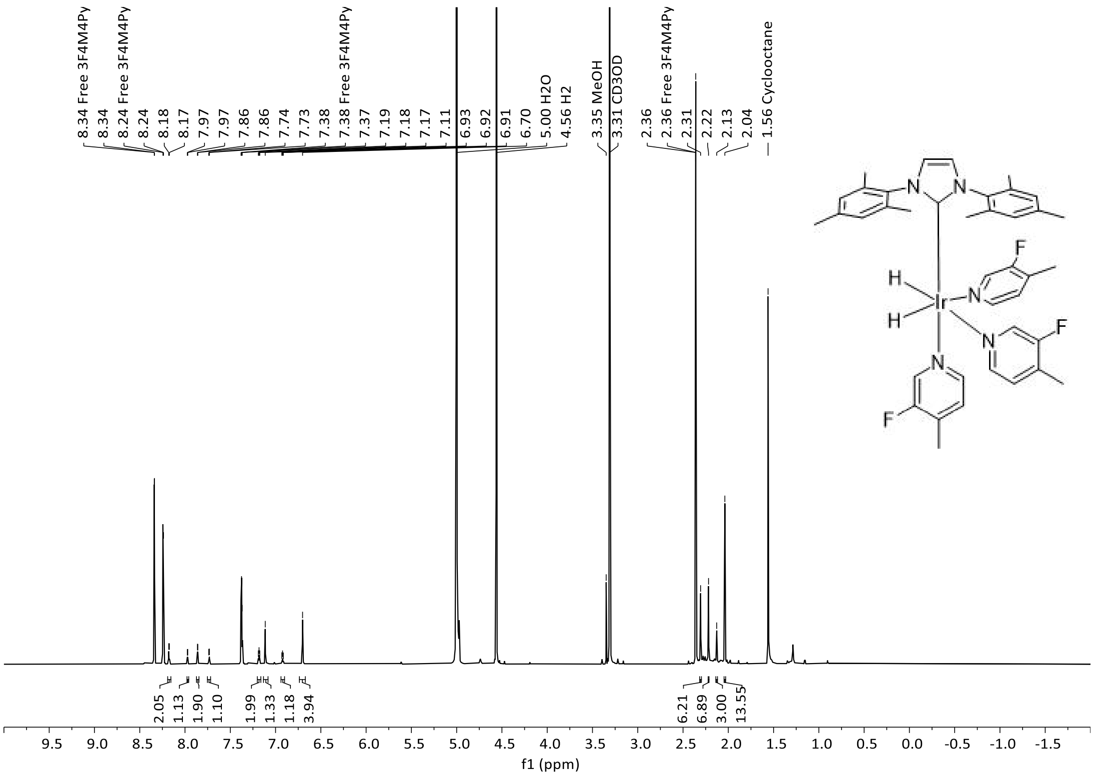


Figure SA13. Expansion of in-phase exchange peaks in NOESY experiment in the region of IMes CH_3 protons 4 and 6, and A_2 21 and 22, showing exchange between diastereomers

^{1}H NMR (800 MHz,CD$_3$OD) for the symmetric complex at 10^{o}C



^{1}H NMR (800 MHz,CD$_3$OD) for the A$_3$:3F4MePy 1:8 mixture at 25^{o}C