An Efficient Route to Asymmetrically Diconjugated tris(heteroleptic) Complexes of Ru(II)

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Supplementary Information
Structural characterisation of the Ru(II) complexes and conjugates 
[Ru(dppz)(DMSO)_2Cl_2] (1)

Figure S1 – ^1H NMR Spectrum (400 MHz) of (1) in CDCl_3.

Figure S2 – ^13C NMR Spectrum of (1) in CDCl_3.
Figure S3 – HR-MS (ESI-QTOF): Single Mass Analysis of (1) indicating [M]+.

[Ru(dppz)(bpy)(ox)] (2)

Figure S4 – $^1$H NMR Spectrum (400 MHz) of (2) in DMSO-$d_6$. 
**Single Mass Analysis**
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 1000.0
Isotope cluster parameters: Separation = 1.0  Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
106 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

DCU_TK_CBTK-022 13 (0.43) AM (C6n4, 80.00; Ar; 1,0,556.29,70.70,LS 1); Sm (Mn, 2x5.00); Sb (16,15.00); Cn (13.23)
1 TOP MS ESI+

Minimum: 200.0 5.0 -1.5
Maximum: 651.0358 1.0000

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<th>Calc. Mass</th>
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<td>24.5</td>
<td>1</td>
<td>C30 H18 N6 O4 Na Ru</td>
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Figure S5 – HR-MS (ESI-QTOF): Single Mass Analysis of (2) indicating [M + Na]+.

[Ru(dppz)(bpyArCOOH)(ox)] (3)

Figure S6 – 1H NMR Spectrum (400 MHz) of (3) in DMSO-d6.
Figure S7 – $^1$H NMR Spectrum (600 MHz) of (4) in CD$_3$CN.

Figure S8 – $^{13}$C NMR Spectrum of (4) in CD$_3$CN.
Figure S9 – COSY Spectrum (600 MHz) of (4) in CD$_3$CN.

**Single Mass Analysis**
Tolerance = 5.0 PPM  /  DBE: min = -1.5, max = 1000.0  
Isotope cluster parameters: Separation = 1.0  Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
392 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

[Ru(dppz)(bpy)(bpyArCOOH)](PF_6)_2 (5)

Figure S11 – ^1^H NMR Spectrum (600 MHz) of (5) in CD_3CN.

Figure S12 – ^1^3C NMR Spectrum of (5) in CD_3CN.
Figure S13 – COSY NMR Spectrum of (5) in CD$_3$CN (aromatic region).

Figure S14 – HRMS (ESI-QTOF): Mass spectrum of (5) indicating [M – PF$_6$]$^+$. 
Figure S15 – $^1$H NMR Spectrum (600 MHz) of (6) in CD$_3$CN. Peaks at 1.1 and 3.4 are assigned to residual diethyl ether.

Figure S16 – $^{13}$C NMR Spectrum of (6) in CD$_3$CN.
Figure S17 – COSY NMR Spectrum (600 MHz) of (6) in CD$_3$CN (aromatic region).

Single Mass Analysis
Tolerance = 6.0 PPM / DBE: min = -1.5, max = 1000.0
Isotope cluster parameters: Separation = 1.0  Abundance = 1.0%

Monoisotopic Mass. Odd and Even Electron Ions
469 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Minimum: 1105.1757
Maximum: 1105.1701

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Figure S18 – HR-MS (ESI-QTOF): Single Mass Analysis of (6) indicating [M – PF$_6$]$^+$. 
[Ru(dppz)(bpy-PEG)(bpyArCOOEt)][PF$_6$]$_2$ (7)

Figure S19 – $^1$H NMR Spectrum (600 MHz) of (7) in acetone-d$_6$. The peaks at 2.09 and 3.10 are assigned to residual acetone and water respectively.

**Single Mass Analysis**

| Tolerance | 100.0 PPM / | DBE: min = -1.5, max = 500.0 |
| Element prediction | Off |
| Number of isotope peaks used for i-FIT | 3 |

Monoisotopic Mass, Odd and Even Electron Ions
5250 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-85  H: 0-101  N: 0-9  O: 0-18  F: 0-6  P: 0-1  Ru: 0-1

Figure S20 – HR-MS (MALDI-QTOF): Single Mass Analysis of (7) indicating [M – PF$_6$]$^+$. 

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Figure S21 – $^1$H NMR Spectrum (600 MHz) of (8) in acetone-$d_6$. The peaks at 5.63, 2.84 and 1.79 are assigned to dichloromethane, water and THF residual solvents from reaction work-up.

Figure S22 – HR-MS (MALDI-QTOF): Single Mass Analysis of (8) indicating $[\text{M} – \text{PF}_6]^+$. 

Single Mass Analysis
Tolerance = 100.0 PPM / DBE: min = -1.5, max = 500.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Odd and Even Electron Ions
5250 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-83  H: 0-97  N: 0-9  O: 0-6  F: 0-6  P: 0-1  Ru: 0-1

Christopher Burke (TX), CBTX-033
Q-TOF20160108MF002 95 (1.020) AM (Cen:4.60.00, Ar:10000 0.1570 66.0.70); Sm (50, 1x5.00); Sb (15, 10.00); Cm (27:122:00.97+119); TOF MS LD+ 2.96e+004

Minimum:
Mass: 1754.5691
Calc. Mass: 1754.5697
nDa: 5.4
PPM: 3.1
DBE: 37.5
i-FIT: 12.2
i-FIT (Norm): 0.0
C93  H97  N9  O18  P6  P

Maximum:
Mass: 1754.5691
Calc. Mass: 1754.5697
nDa: 5.4
PPM: 3.1
DBE: 37.5
i-FIT: 12.2
i-FIT (Norm): 0.0
C93  H97  N9  O18  P6  P
Figure S23 – $^1$H NMR Spectrum (600 MHz) of (9) in methanol-d$_4$. The peaks at 4.79 and 2.15 ppm are due to residual water and acetone solvent from reaction work-up.

Figure S24 – COSY NMR Spectrum of (9) in methanol-d$_4$. 

$[\text{Ru(dppz)(bpy-PEG)(bpy-NFkB)](PF_6)_6}$ (9)
Figure S25 – HR-MS (MALDI-QTOF): Mass spectrum of (9).

Figure S26 – Spectral overlay of the mass spectra of (8) and (9).
HPLC Analysis of the Conjugates

Figure S27 – HPLC trace (RP-C18, MeCN/Water (0.1 % TFA) Gradient; PDAD) for (6). Indicative Purity (vs Ru(II) precursors; 450 nm) = 100 %.

Figure S28 – HPLC trace (RP-C18, MeCN/Water (0.1 % TFA) Gradient; PDAD) for (7). Indicative Purity (vs Ru(II) precursors; 450 nm) = 99.2 %.
Figure S29 – HPLC trace (RP-C18, MeCN/Water (0.1 % TFA) Gradient; PDAD) for (8). Indicative Purity (vs Ru(II) precursors; 450 nm) = 99.7 %.

Figure S30 – HPLC trace (RP-C18, MeCN/Water (0.1 % TFA) Gradient; PDAD) for (9). Early eluting peaks are due to sample solvent.
Additional Photophysical Data

General Information
For compounds (4) – (6), solutions were prepared in MeCN using the PF$_6^-$ salt of the complex whereas the Cl$^-$ salt was preferred for the aqueous samples. Samples (7) and (9) were prepared from their PF$_6^-$ salts and required ca. 0.5 % v/v DMSO as an initial solubilising agent. All scans and lifetime measurements were performed using 10 μM solutions. The extinction coefficients were calculated from standard curves (5 – 30 μM). Standard deviations are calculated from triplicate analyses. All lifetime curve fitting conformed to chi-squared tail-fit criteria of $0.9 < \chi^2 < 1.10$. Slit widths set to 5 nm for emission and excitation runs. Deaeration was performed by bubbling N$_2$ through the analytical sample for 15 mins.

Charts

Figure S31 – Absorbance (full lines) and emission (dashed lines) spectra in water for the complexes (4) – (9).