ORGANOMETALLICS

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Introducing a New Family of Biotinylated Ir(III)-Pyridyltriazole Lumophores: Synthesis, Photophysics, and Preliminary Study of Avidin-Binding Properties

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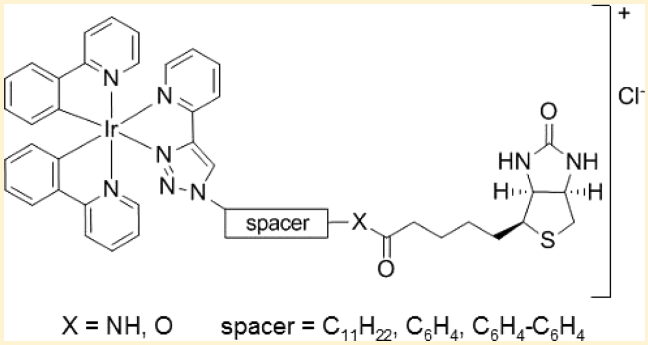
Supporting Information

ABSTRACT: Six new biotinylated reagents derived from monocationic pyridyl-1,2,3-triazole-based Ir(III) complexes of the general formula $[(C^N)_2Ir(N^N\text{-spacer-X-CO-biotin})]^+$, where HC^N is 2-phenylpyridine, N^N is the neutral chelating (2-pyridyl)-1,2,3-triazole ligand, the term "spacer" refers to alkyl ($-C_{11}H_{22}$) or aromatic (*p*-phenyl- or 4,4′-biphenyl-) chains, and X is NH or O, have been prepared and characterized. Upon photoexcitation, all six complexes in fluid CH_2Cl_2 and aqueous solutions at room temperature displayed intense—with quantum yields as high as 0.60 (complex **4b**)—and quite long-lived blue-green luminescence, corresponding in each case to a vibronically structured emission profile peaking at ca. 480 and ca. 508 nm. These features, in combination with the reduced rigidochromic shift that was observed from the same samples frozen at 77 K, suggested the ^{3}LC-type excited states as the prevalent contributors to the emission. The interactions of these new biotinylated complexes with avidin have been studied by 4′-hydroxyazobenzene-2-carboxylic acid (HABA) assays and emission titrations. In general, the amplification of the emission intensity of the Ir(III) complexes occurring upon their interaction with avidin was observed. It is also worth mentioning how a similar or better response was displayed by $[(C^N)_2Ir(N^N\text{-spacer-O-CO-biotin})]^+$-type complexes, in which biotin is appended to the Ir(III) lumophore through an ester moiety.



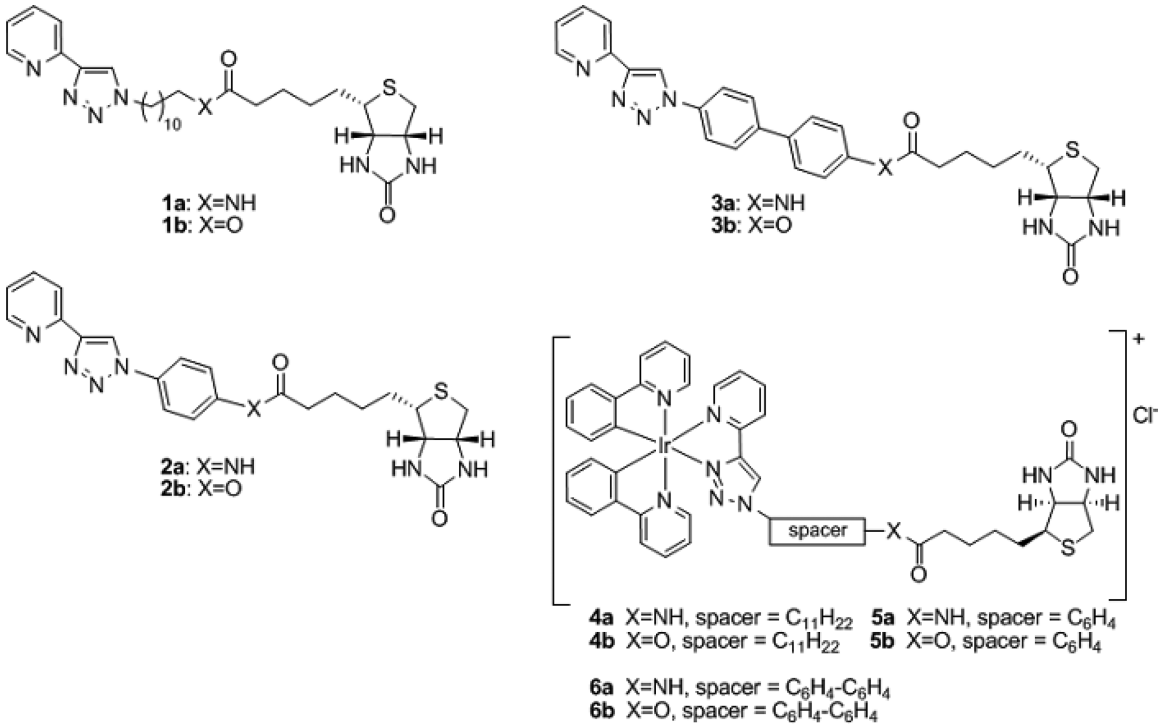
INTRODUCTION

Environment-responsive luminescence is one of the features that has driven some classes of luminescent metal complexes to huge scientific popularity.[1] In this context, Ru(II) polypyridyls,[2] Ir(III) cyclometalates,[3] and tris-carbonyl Re(I) diimine complexes[4] are probably the most emblematic examples. The emission output of these metal-containing molecules can be readily perturbed following their interactions with a wide range of analytes including cations and anions, O_2, and biological targets such as proteins and DNA.[5] The reasons for such pronounced sensitivity are usually found in their displaying radiative processes that originate from excited states of "pure" metal to ligand charge-transfer (MLCT) nature, as for the Ru(II) complexes, or from emissive levels that can be described as admixtures of MLCT and ligand-centered (LC)-type states, as in the cases of third-row transition metals such as Re(I) and, to a greater extent, Ir(III). Taken together, these aspects have prompted the scientific community to design and prepare an incredibly vast number of ligands to be employed for building new families of luminescent metal complexes with continuously improved emission performances and remarkable sensing capabilities.[1−3] In particular, truly decisive progress in the use of such classes of d^6 metal complexes as luminescent probes for bioimaging applications has been achieved through the peripheral decoration of the ligand set with biologically relevant functional groups.[6] Following this approach, Lo and co-workers reported numerous examples of Re(I)- and Ir(III)-based luminescent complexes[7] in which one or more coordinated ligands were derivatized with a wide variety of end groups—some examples of which are represented by biotin, indole, estradiol,[5a,6−8] and sugars[5a,7,9]—in order to enable the optical detection of the corresponding bioconjugation products and, in general, to endow the modified lumophores with increased lipophilicity. Among the multitude of different options, the one dealing with the conjugation of Re(I)- and Ir(III)-based lumophores with biotin has been extensively explored in light of the extremely high affinity that is displayed by biotin with respect to the tetrameric glycoprotein avidin. It has to be noted that, while the introduction of biotin in the backbone of such molecules does not cause any change in the intrinsic photophysical features of the metal-based fluorophores, the luminescence output of the

Received: August 2, 2014
Published: October 7, 2014

   dx.doi.org/10.1021/om5007962 | *Organometallics* 2014, 33, 6154−6164

Scheme 1. Ligands and Complexes Studied in This Work



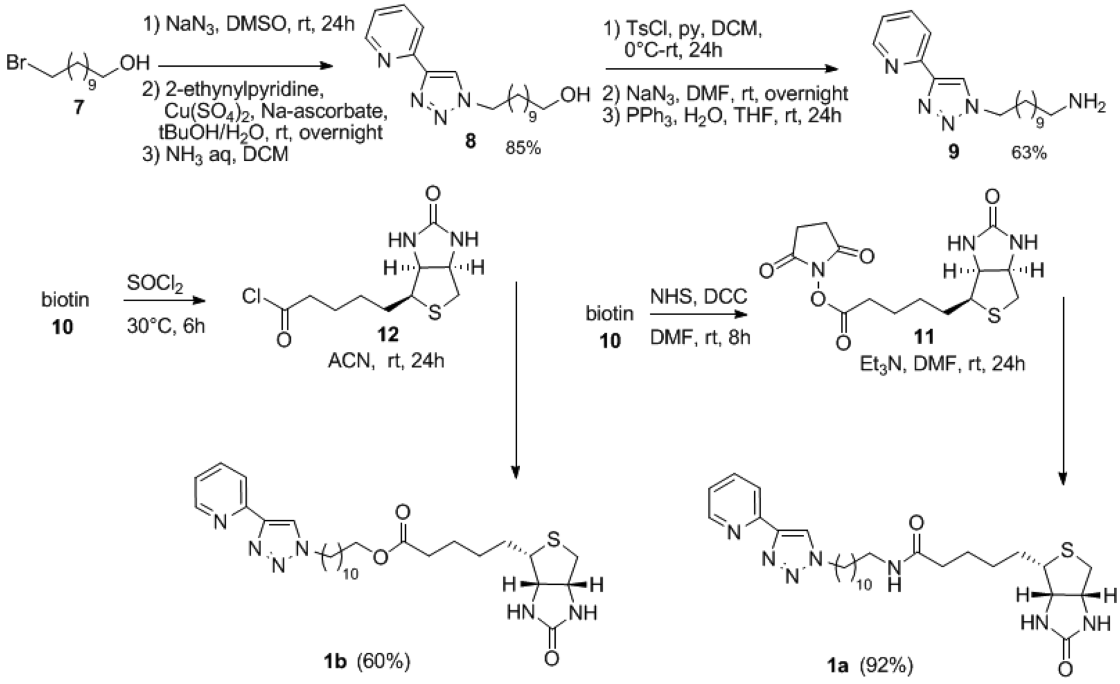
biotinylated species is avidin-sensitive.[5a,6−8] This feature, in conjunction with the improvement of the cellular uptake performance that derives from the presence of biotin itself, has led to the use of similar biotin−avidin arrays as the core system of many assays and probes.[10] In this context, apart from the examples of neutral Ir(III) complexes described by Hong and co-workers in 2008,[11] the biotinylated Ir(III)-based species that have been reported in the literature usually deal with cationic Ir(III) complexes of the general formula $[(C^N)_2Ir(N^N)]^+$, which consist of two identical cyclometalating ligands (C^N) and one neutral ancillary ligand (N^N).[5a,6,7] The site for biotinylation may vary from the cyclometalating C^N units to the N^N neutral one, and, in general, the biotin-containing aliphatic or aromatic pendant arms have been appended to the structure of such lumophores through the formation of the corresponding amines or amides. In this regard, Lo and co-workers have demonstrated how the use of a similar synthetic strategy might also lead to the production of Ir(III) multibiotinylated complexes as attractive examples of cross-linkers for avidin.[8b,c] However, within the framework of the design of biotinylated Ir(III) complexes, a great deal of attention has been paid to the modification and/or the variation of the cyclometalating C^N ligands from the archetypal cyclometalated phenylpyridine (ppy), while the structure of the neutral N^N ligand usually consists of 2.2′-bipyridine (bpy) or 1,10-phenanthroline (phen) type backbones.[5a,6,7] These latter considerations introduce the scope of this present work. Here, we report the synthesis, the study of the photophysical features, and the preliminary account of the avidin-binding properties of a series of six new biotinylated Ir(III) complexes (Scheme 1) of the general formula $[(C^N)_2Ir(N^N\text{-spacer-X-CO-biotin})]^+$ (Scheme 1), where HC^N is 2-phenylpyridine and X is NH or O, that introduce some elements of novelty for this class of Ir(III)-based lumophores. First, the architecture of the N^N ligands described herein differs from those that have been reported so far since, in all of the six new complexes, it is represented by a metal-chelating pyridyl-1,2,3-triazole unit. Also, relative to the design of the biotinylated N^N ligands, the biotin moiety has been appended to aromatic (*para* phenyl and 4,4′-biphenyl) or aliphatic ($C_{11}H_{22}$-) pendant chains through the formation of the "traditional" amide-type linkage as well as the first reported examples of ester-type ones (Scheme 1).

RESULTS AND DISCUSSION

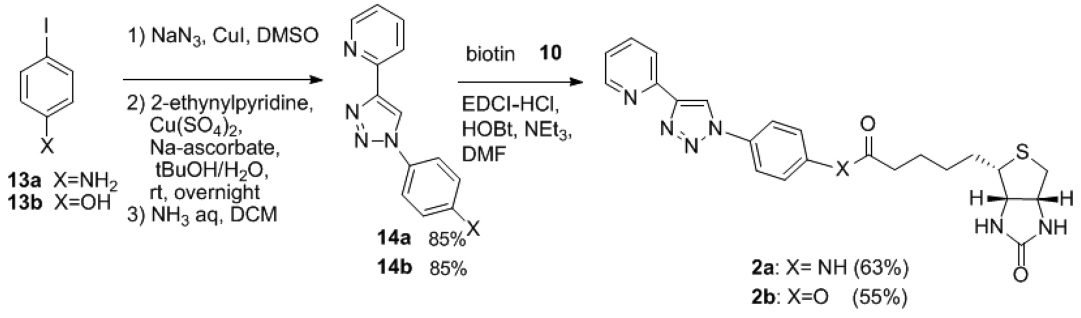
Ligand Design and Synthesis. We have designed a set of neutral ligands, that might be schematized as [(N^N)-spacer-X-CO-biotin], in order to provide molecules that can coordinate to the Ir(III) metal center through the N^N moiety and, at the same time, are capable of interaction with the avidin target with the biotin end unit. In particular, all the ligands reported herein have been prepared in good yield by following a convergent procedure that entailed the synthesis of the two different subunits—the Ir(III)-coordinating pyridyl triazole and the biotin arm—which were finally combined through the formation of the desired ester or amide (see Schemes 2, 3, and 4 and Figures S1−S14, Supporting Information). In detail, all the pyridyl triazole intermediates **8**, **14a**,**b**, and **16**[12] were obtained by following the well-known CuI-catalyzed azide−alkyne cycloaddition (CuAAC)[13] involving use of 2-ethynylpyridine and the appropriate alkyl or aryl azides.[14] Then, the synthesis of the ligand **1a** was carried out by a two-step procedure involving the further transformation of the hydroxylpyridine-triazole **8** into the amine **9**, followed by its reaction with biotin-succinimino derivative **11**. On the other hand, the key intermediate **8** was employed with no further transformation to give ligand **1b** in 60% yield by reaction with the biotin acyl chloride **12** (Scheme 2). The syntheses of ligands containing aromatic spacers **2a**, **2b**, **3a**, and **3b** are reported in Schemes 3 and 4. In particular, the amide **2a** and the ester **2b** were obtained in 63% and 55% yields, respectively, upon the condensation of the pyridyl triazole

dx.doi.org/10.1021/om5007962 | *Organometallics* 2014, 33, 6154−6164

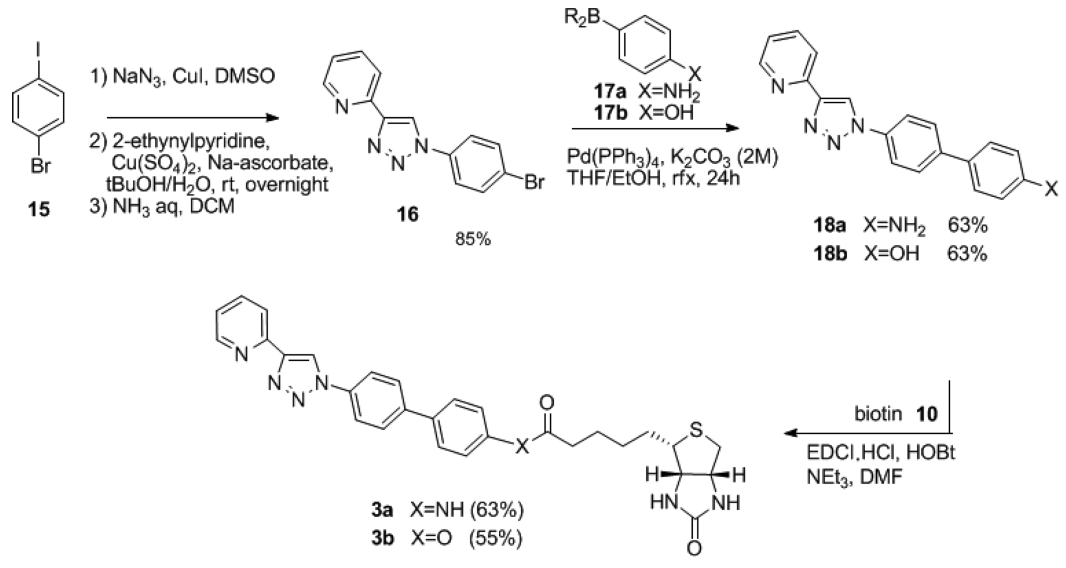
Scheme 2. Synthetic Route to Ligands 1a and 1b



Scheme 3. Synthetic Route to Ligands 2a and 2b



Scheme 4. Synthetic Route to Ligands 3a and 3b



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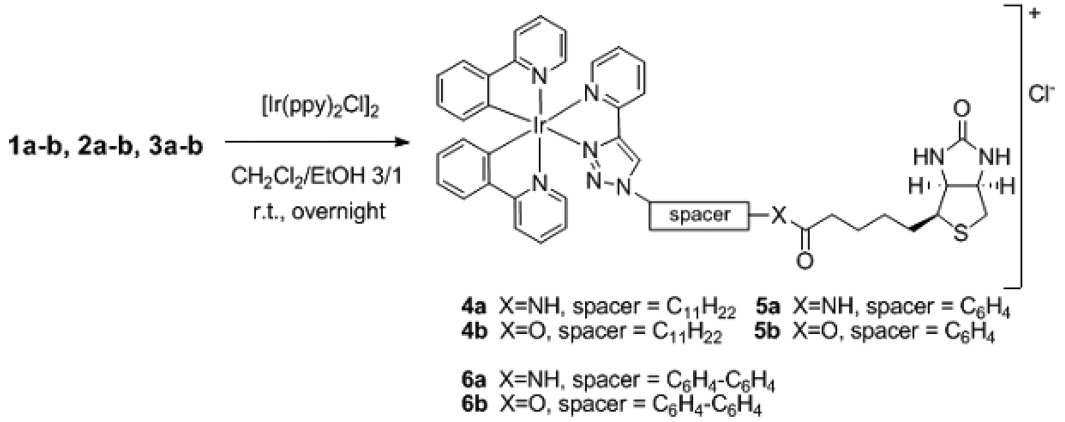
Scheme 5. Synthesis of Ir(III) Complexes 4a,b, 5a,b, and 6a,b



intermediates **14a** and **14b** with biotin **10**, in accordance with a previously reported procedure for similar aromatic substrates.[15]

Relative to the preparation of the ligands **3a,b** (Scheme 4), the pyridyl triazole **16**, following a Suzuki coupling procedure, was first treated with the appropriate phenylboronic derivative **17a,b** in order to obtain the corresponding biphenyl derivatives **18a** and **18b**.[16] Then, the latter compounds were finally converted into the biotin ligands **3a** (63% yield) and **3b** (63% yield).

The target Ir(III) complexes **4**−**6** were synthesized through a well-established procedure[16] that involved the reaction of the dimer **19** with a slight molar excess of the proper ligand in a 3:1 dichloromethane/ethanol (v/v) mixture at room temperature (Scheme 5).

The formation of the desired cationic Ir(III) complexes was first confirmed by electrospray ionization mass spectrometry (ESI-MS) with the observation of the patterns of signals whose (m/z) values matched those of the expected positive molecular ions. Despite its being complicated from the presence of at least 25 magnetically inequivalent hydrogens or carbons all resonating in the aromatic region, the analysis of the NMR spectra (^{1}H; ^{13}C) was also congruent with the formation of the target Ir(III) species (Supporting Information, Figures S15−S19).

Photophysical Properties. The relevant absorption and emission features of the Ir(III) complexes are summarized in Table 1.

In dichloromethane solutions maintained at room temperature, all the Ir(III) complexes show quite typical and similar absorption profiles. Up to 300 nm, each spectrum is dominated by intense and spin-allowed ligand-centered (^{1}LC) $\pi-\pi^*$ transitions involving both the cyclometalating and the ancillary ligands. Weaker and broader spin-allowed ^{1}MLCT and spin-forbidden ^{3}MLCT bands are found at longer wavelength (300 to 380 nm) (Figure 1). Apart from some slight hypsochromic shifts of the absorption maxima, this picture (see Table 1, Figure 1, and Figures S21−S25, Supporting Information) does not vary to a significant extent when the absorption spectra of the same complexes were recorded from the corresponding aqueous (50 mM potassium phosphate buffer at pH 7.4/DMSO (95:5, v/v)) solutions.

The luminescence properties of the new biotinylated Ir(III) complexes are rather similar to those of the parent and biotin-free cationic Ir(III) triazole complexes that have been described by De Cola and co-workers.[17a] This similarity provides an initial indication for the not particularly important influence played by the different types of biotin pendant arms, as well as by the presence of the biotin moiety itself, in determining the luminescence performances of the various biotinylated Ir(III) triazole complexes described herein. At room temperature, upon excitation of the ^{1}MLCT feature (λ = 380 nm) of the corresponding diluted dichloromethane solution (ca. 10^{-5} M), each of the new Ir(III) complexes displays bright blue-green luminescence originating from excited states of triplet character, as witnessed by the increase of emission quantum yield and the concomitant elongation of the emission lifetimes that took place on passing from air-equilibrated to deoxygenated solutions (Table 1). The emission profiles were found substantially coincident for all complexes and always consisted of an intense band centered at ca. 480 nm followed by an almost equally intense vibronic progression peaking at ca. 508 nm. The occurrence of such similarly structured emission spectra from Ir(III) cyclometalated complexes is usually representative for the interplay of ^{3}LC/^{3}MLCT type emissive excited states.[3] In our cases, even though the determination of the radiative rate constants (k_r) for the air-equilibrated dichloromethane solutions provided values (see Table 1) consistent with the ^{3}MLCT nature of the emissions,[3] the very small rigidochromic blue-shift of the emission maxima that is observed at 77 K indicates the likely prevalence of the ^{3}LC excited states over the ^{3}MLCT ones (Table 1, Figure 2, and Figures S18−S22, Supporting Information). The minor contribution coming from such polar CT states could also be deduced from observing how all the biotinylated complexes **4**−**6a,b** retained their luminescence features in an aqueous environment, with steady-state emission spectra that were found almost exactly superimposable to the ones obtained from dichloromethane solutions (Table 1, Figure 2, and Figures S26−S30, Supporting Information) and radiative rate constants (k_r) lower than those calculated in organic medium.[3] Conversely, as previously reported by Lo and co-workers for biotinylated Ir(III) substrates,[8e] a significant elongation of the emission lifetimes was observed upon passing from organic to aqueous solutions, an effect that might likely be explained by considering the mostly apolar character of the emissive excited states and the lipophilic environment that is brought by the spacer arms. However, as these trends are general for the whole series of complexes **4**−**6a,b**, no obvious correlations of the emissive performances of the new Ir(III) complexes with respect to the different type of linkers (ester or amide) and/or the nature (aliphatic or aromatic) of the spacer arms can be made.

Interaction with Avidin. According to the approach reported by Lo and co-workers in their pioneering works,[18] the avidin-binding properties of the biotinylated Ir(III) complexes

dx.doi.org/10.1021/om5007962 | Organometallics 2014, 33, 6154−6164

Table 1. Photophysical Properties of the Obtained Complexes[a]

	absorption	emission, 298 K[b,c,d]							emission, 77 K[b,e]	
complex (solvent)	λ_{abs} (nm): ($10^{-4}\varepsilon$) (M^{-1} cm^{-1})	λ_{em} (nm)	τ (μs) air	τ (μs) under Ar	Φ_{air}	Φ under Ar	k_r (10^5 s^{-1})	k_{nr} (10^6 s^{-1})	λ_{em} (nm)	τ (μs)
4a (CH_2Cl_2)	255 (4.5)	476, 508	0.14 (90)	0.42	0.048	0.148	3.4	6.8	474, 506	4.2
	381 (0.66)		0.055 (10)							
4a (buffer)	248 (4.0)	478, 504	0.57		0.13		2.3	1.5		
	374 (3.8)									
4b (CH_2Cl_2)	255 (4.5)	476, 508	0.15	1.1	0.064	0.60	4.3	2.7	470, 505	2.1 (20)
	381 (0.55)									4.9 (80)
4b (buffer)	268 (4.0)	478, 508	0.62 (60)		0.070		1.1	1.5		
	376 (3.8)		0.090 (40)							
5a (CH_2Cl_2)	269 (4.6)	478, 506	0.13	0.51	0.12	0.55	9.2	6.8	472, 506	4.7 (90)
	380 (0.16)									1.7 (10)
5a (buffer)	270 (4.0)	478, 506	0.30 (70)		0.10		3.2	2.9		
	285 (3.8)		0.041 (30)							
	385 (0.63)									
5b (CH_2Cl_2)	268 (4.7)	476, 508	0.13	0.50	0.10	0.25	7.7	6.9	470, 506	3.9
	383 (0.37)									
5b (buffer)	255 (2.9)	478, 506	0.42		0.22		5.2	1.9		
	263 (2.9)									
	378 (0.27)									
6a (CH_2Cl_2)	270 (6.1)	476, 508	0.23	1.2 (88)	0.080	0.34	3.5	4.0	470, 506	6.0 (84)
	294 (5.3)			0.36 (12)						1.7 (16)
	382 (0.69)									
6a (buffer)	266 (4.1)	478, 506	0.47		0.020		0.42	2.0		
	303 (3.8)									
	406 (0.63)									
6b (CH_2Cl_2)	272 (5.5)	476, 506	0.13	0.82	0.12	0.41	9.2	6.8	472, 506	4.9 (67)
	288 (5.1)									2.9 (33)
	382 (0.39)									
6b (buffer)	272 (3.3)	478, 504	0.39		0.060		1.5	2.4		
	286 (3.1)									
	387 (0.49									

[a]"Buffer" means 50 mM potassium phosphate buffer at pH 7.4/DMSO (95:5, v/v); "air" denotes air-equilibrated samples, "under Ar" means degassed (O_2-free) samples. [b]For the biexponential excited-state lifetimes (τ), the relative weights of the exponential curve are reported in parentheses. [c]Quantum yields (Φ) are measured versus air-equilibrated $[Ru(bpy)_3)]Cl_2$ aqueous solution ($\Phi = 0.028$). [d]$k_r = (\Phi/\tau)$, $k_{nr} = (1 - \Phi)/\tau$ with reference to air-equilibrated samples. [e]In frozen CH_2Cl_2 matrices.

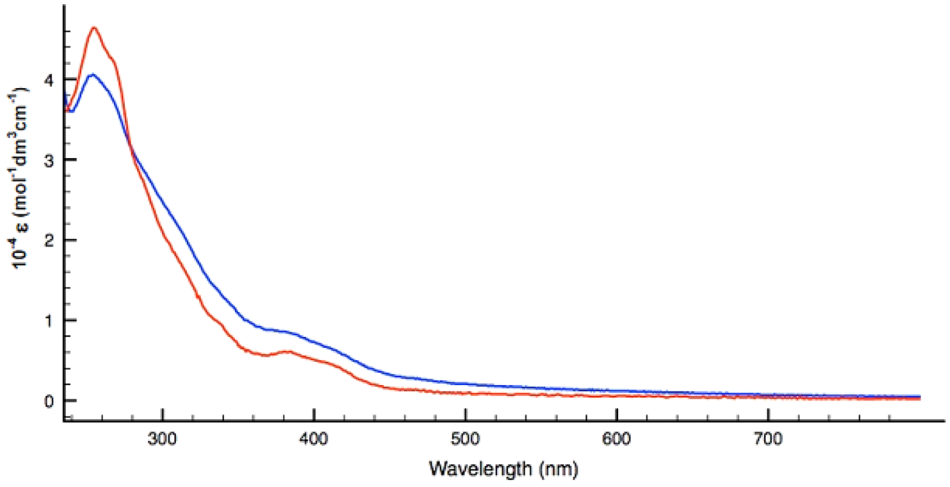


Figure 1. Absorption profiles of complex **4a** in CH_2Cl_2 solution at 298 K (continuous line) and in 50 mM potassium phosphate buffer at pH 7.4/DMSO (95:5, v/v) (dashed line).

4–**6** have been investigated by the standard HABA (4,4′-hydroxyazobenzene-2-carboxylic acid) assay followed by luminescence titrations.

In the former instance, the HABA assay relies on the higher affinity for avidin that is displayed by biotin (K_d = ca. 6×10^{-6} M) with respect to that of HABA (K_d = ca. 10^{-15} M),[10] the binding of

dx.doi.org/10.1021/om5007962 | Organometallics 2014, 33, 6154–6164

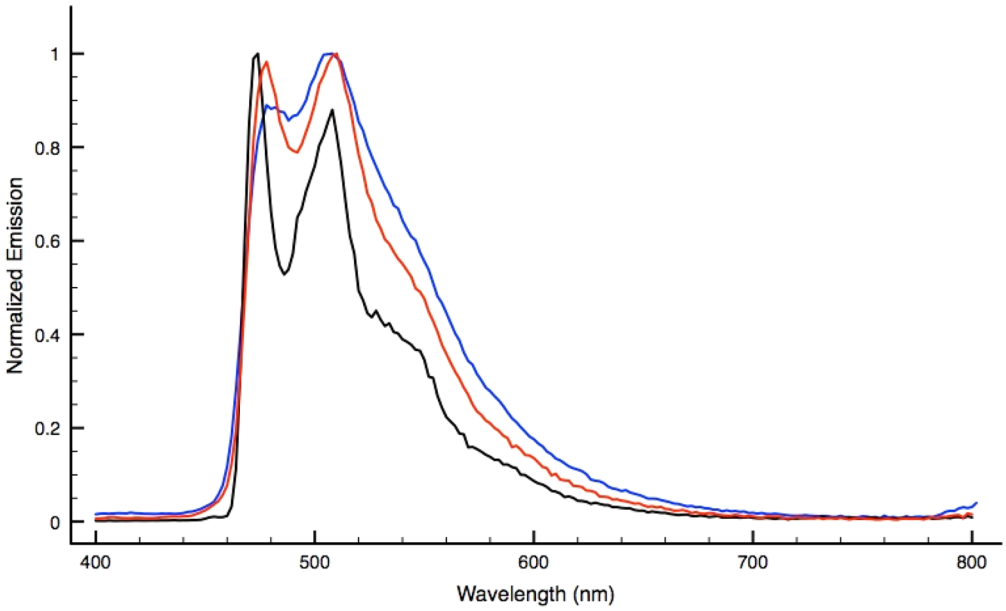


Figure 2. Emission profiles of complex **4a** obtained from a CH_2Cl_2 solution at 298 K (red trace) and 77 K (black trace) and from a 50 mM potassium phosphate buffer at pH 7.4/DMSO (95:5, v/v) (blue trace).

HABA to avidin being witnessed by an intense absorption feature centered at 500 nm. As a consequence, the displacement of HABA from the corresponding $(HABA)_4$-avidin adducts, as the result of addition of unmodified biotin or biotinylated molecules, results in the decrease of the absorbance at 500 nm. This effect is also clearly evident from the addition of the biotinylated Ir(III) complexes **4**–**6** to solutions of avidin presaturated with HABA. In each case, the results of the HABA assay indicate that all of the new Ir(III) complexes bind avidin with the same stoichiometry ([Ir]/[avidin] = 4:1) as that deduced by carrying out the same experiments with unmodified biotin. The results of such spectrophotometric titrations are reported in Supporting Information Figures S31 to S33. The behavior of our new biotinylated Ir(III) complexes **4**–**6** was studied by performing luminescence titrations in which avidin, as the analyte, was titrated with the appropriate Ir(III) complex (Figure 3). In particular, avidin (3.8 μM) was dissolved in a phosphate buffer solution (pH = 7.4), and titrations were carried out by adding aliquots of 5 μL of a solution of the appropriate Ir(III) complex (0.55 mM). Each avidin-complex titration ("main experiment") was compared to those obtained from two different control experiments, the first one consisting in the titration of avidin-free buffer solution and the second being represented by the titration of a buffer solution containing avidin presaturated with an excess of unmodified biotin. In all cases the comparative analysis of the three emission titrations unequivocally indicated how the emission intensity—which was always monitored by considering the higher energy emission feature (λ_{max} = ca. 480 nm)—and the corresponding emission lifetime of the Ir(III) complexes were significantly enhanced upon interaction with avidin. The occurrence of this effect is likely due to the ensuing increase of rigidity and lipophilicity in the local environment of the biotinylated ligand. In particular, as can be observed in the emission titration curves reported in Figure 3, the enhancement of the emission intensity reached its maximum in correspondence with what can be considered as a proper equivalence point that occurred, in all cases, at [Ir]:[avidin] ca. 4:1. After this point, the slope of the curves became almost identical to that displayed by each of the control experiments, the only exceptions being complexes **4a** and, to a lower extent, **6a**, where the intervention of nonspecific interaction between the free Ir(III) probes and the saturated protein cannot be confidently excluded.

In general, at the equivalence point, each of the Ir(III) complexes showed a different response in terms of increase of emission intensity and elongation of lifetimes. In particular, among the amide-linked derivatives **4a**, **5a**, and **6a**, the highest emission amplification factor (I/I_0) (see Table 2) was displayed by complex **6a**, while the most relevant elongation of the emission lifetimes was observed for the complex **5a**. Relative to the ester-linked complexes **4b**, **5b**, and **6b**, the most pronounced amplification of emission intensity was exhibited by complex **4b** (I/I_0 = 8.1), whereas the extent of the lifetime elongation was found essentially constant along the series. In general, the magnitude of such amplication factors is quite in line with those reported by Lo and co-workers.[5a,7b] However, from the comparison of the results obtained for the amide-containing complexes $[(C^\wedge N)_2Ir(N^\wedge N\text{-}C_{11}H_{22}\text{-}NH\text{-}CO\text{-}biotin)]^+$ **4a**, **5a**, and **6a** with those relative to ester-based ones $[(C^\wedge N)_2Ir(N^\wedge N\text{-}C_{11}H_{22}\text{-}O\text{-}CO\text{-}biotin)]^+$ **4b**,

Table 2. Relative Emission Intensities[a] and Emission Lifetimes of the Biotin Complexes 4–6 in the Absence[b] and in the Presence of Avidin[c] and in the Presence of Avidin and Excess Biotin[d] in Aerated 50 mM Potassium Phosphate Buffer pH 7.4/DMSO (9:1, v/v) at 298 K

complex	I_0 (τ/μs)[b]	I/I_0 (τ/μs)[c]	I/I_0 (τ/μs)[d]
4a	1.0 (0.61)	1.4 (0.81)	1.0 (0.64)
5a	1.0 (0.30)	6.0 (1.0)	1.0 (0.30)
6a	1.0 (0.47)	13 (0.80)	1.5 (0.42)
4b	1.0 (0.62)	8.1 (1.1)	1.4 (0.64)
5b	1.0 (0.42)	2.6 (0.94)	1.0 (0.42)
6b	1.0 (0.39)	5.3 (0.66)	0.9 (0.41)

[a]Emission intensities were measured in correspondence with the maximum at higher energy (λ = 480 nm); emission lifetimes (τ) are referred to air-equilibrated samples. [b][Ir] = 15.5 μM; [avidin] = 0 M; [biotin] = 0 M. [c][Ir] = 15.5 μM; [avidin] = 3.8 μM; [biotin] = 0 M. [d][Ir] = 15.5 μM; [avidin] = 3.8 μM; [biotin] = 380 μM.

dx.doi.org/10.1021/om5007962 | *Organometallics* 2014, 33, 6154–6164

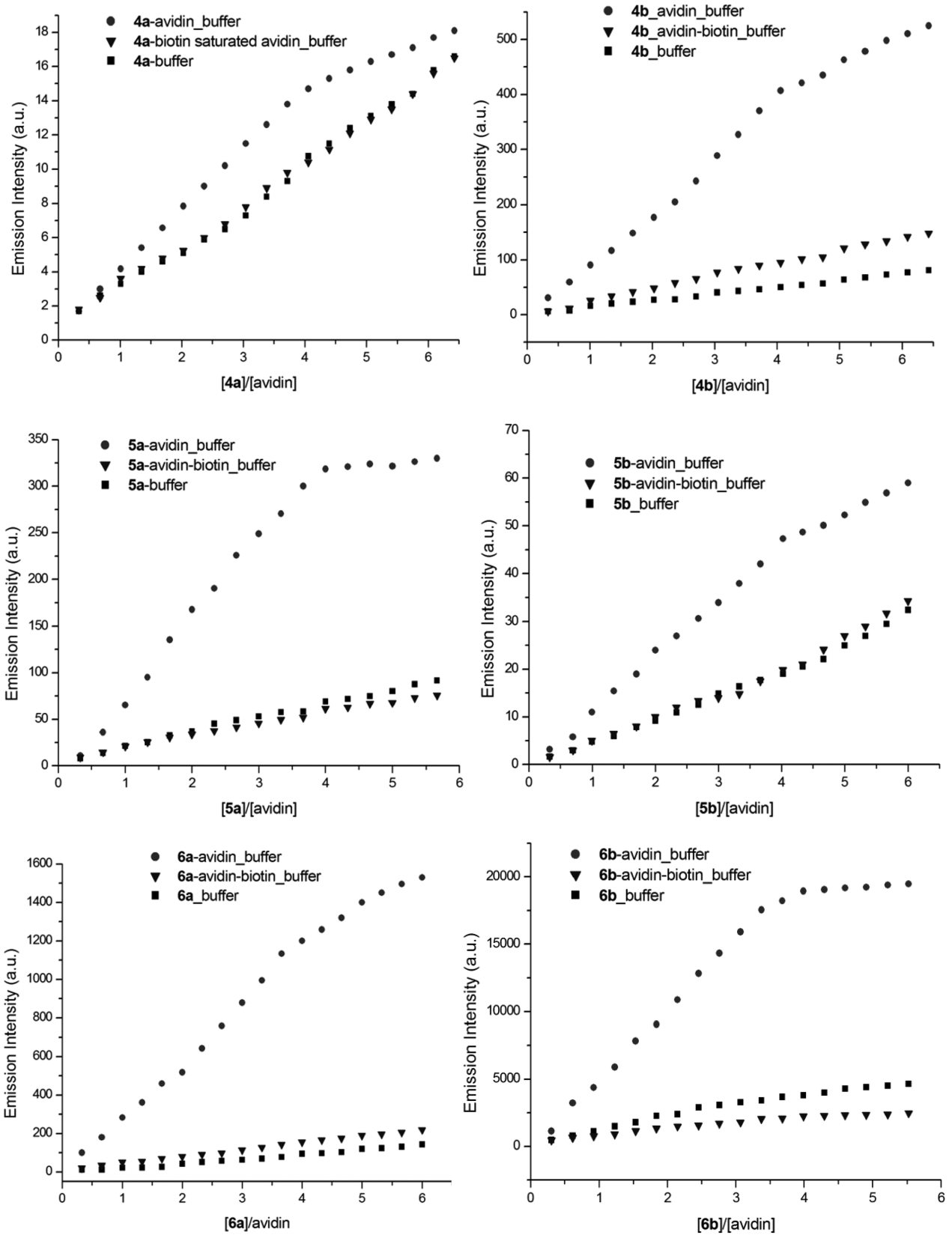


Figure 3. Emission titration curves for the titrations of (i) 3.80 μM avidin (●)), (ii) 3.80 μM avidin and 3.80 μM unmodified biotin (▼), and (iii) a blank phosphate buffer solution (■) with complexes **4a**, **4b** (top), **5a**, **5b** (middle), and **6a**, **6b** (bottom).

5b, and **6b**, it is possible to observe how the replacement of the amide group with an ester moiety did not affect the avidin-binding properties of the Ir(III) biotinylated complexes. On the contrary, in some cases, such modification seemed to improve the avidin affinity of the amide-based complexes. As can be deduced from Figure 3, the ester complex **4b** displays much higher emission

dx.doi.org/10.1021/om5007962 | *Organometallics* 2014, 33, 6154−6164

amplification than that exhibited by the amide analogue **4a**. Also, the interaction of avidin with complex **6b**, $[(C^N)_2Ir$-(4,4′-biphenyl)-O-CO-biotin$)]^+$, is characterized by a more evident turning point than that displayed by the amide complex **6a**, $[(C^N)_2Ir$-(4,4′-biphenyl)-NH-CO-biotin$)]^+$. Finally, on the basis of the analysis of these data, no clear rationalization can be done in consideration of the aliphatic (as for complexes **4a** and **4b**) or aromatic (such as the species **5a**, **5b**, **6a**, and **6b**) nature of the spacer arm.

CONCLUSIONS

In this work, we have reported the synthesis of a series of six new Ir(III) complexes containing a biotin-appended pyridyl-1,2,3-triazole derivative as the neutral ligand, their photophysical characterization, and the preliminary study of their avidin-binding features. These molecules have been designed in order to provide luminescent Ir(III) complexes capable of binding avidin through a biotinylated arm that is carried by a Ir(III)-coordinated ligand different from the "traditionally" employed bpy- or phen-type derivatives. In this regard, the presence of the biotin moiety did not affect the photoemissive performances of the Ir(III) complexes, since all the new species displayed intense phosphorescent emission with quantum yield values up to 0.60 in dichloromethane solutions. It is important to note how the emissive features of the biotinylated Ir(III) complexes were substantially retained in an aqueous environment. In addition, a significant elongation of the phosphorescence lifetimes was observed upon passing from dichloromethane to water solvent, as the result of the hydrophobicity of the spacer arms that connect the biotin unit to the Ir(III) phosphors. Taken together, these features constitute the basis for the study of the avidin-binding properties of the new complexes. Indeed, the emission titrations of avidin with the biotinylated Ir(III) complexes as the titrants suggested the occurrence of the interaction between the complexes and the protein. In particular, in almost all cases, the new iridium(III) complexes displayed a relevant emission intensity enhancement accompanied by emission lifetime elongation upon their binding to avidin. This effect was observed both for the "traditional" amide-linked biotin complexes and, in particular, for the newly reported examples of biotinylated compounds in which the biotin group was appended to the Ir(III)-complex structure through esterification. However, even though these interactions need to be further studied by many aspects such as cytotoxicity and cellular uptake ability of the new complexes, the analysis of the results reported herein provided an important indication about the feasibility of the replacement of bpy-based biotin carriers with another type of diimine ligand, such as pyridyl-1,2,3-triazole derivatives, in the structure of biotinylated Ir(III) phosphors. Finally, it is important to note how the design strategy we have adopted in this work might be applied for developing new libraries of luminescent Ir(III) complexes decorated with a variety of biologically relevant groups or, more in general, with moieties that might endow the Ir(III) complexes with the very delicate balance of factors (lipophilicity, cellular uptake ability, and cellular localization) that might determine their use as imaging reagents.

EXPERIMENTAL SECTION

The solvents and chemicals were used as received from sellers, unless otherwise mentioned. The synthetic procedures for the preparation of the precursor compounds **8**, **9**, **11**, **14a**,**b**, **16**, and **18a**,**b** and the corresponding characterization are reported in the Supporting Information, pp S3−S7. NMR spectra were recorded by using a Varian Mercury 400 MHz or a Varian Inova 600 MHz spectrometer with tetramethylsilane as the internal standard. ESI-MS analyses were performed by direct injection of acetonitrile solutions of the compounds using a Waters ZQ 4000 mass spectrometer. Elemental analyses were performed on a ThermoQuest Flash 1112 series EA instrument. HABA assays and emission titrations have been performed according to the procedures reported by Lo and co-workers.[18b]

Photophysics. Absorption spectra were recorded at room temperature using a PerkinElmer Lambda 35 UV/vis spectrometer. The buffer solutions (50 mM potassium phosphate buffer at pH 7.4/DMSO (95:5, v/v)) containing the Ir(III)-based species were prepared at first by dissolving the solid samples in DMSO, then diluting with the aqueous buffer. The obtained solutions were then submitted to sonication (ca. 5 min). In some cases, as for complexes **5a** and, to a lesser extent, **6a**, the solutions became a bit opaque within 2 min, therefore determining a not optimal background subtraction. Uncorrected steady-state emission and excitation spectra were recorded on an Edinburgh FLSP920 spectrometer equipped with a 450 W xenon arc lamp, double excitation and single emission monochromators, and a Peltier-cooled Hamamatsu R928P photomultiplier tube (185−850 nm). Emission and excitation spectra were corrected for source intensity (lamp and grating) and emission spectral response (detector and grating) by a calibration curve supplied with the instrument. The wavelengths for the emission and excitation spectra were determined using the absorption maxima of the MLCT transition bands (emission spectra) and at the maxima of the emission bands (excitation spectra). According to the approach described by Demas and Crosby,[19] luminescence quantum yields (Φ_{em}) were measured in optically dilute solutions (OD < 0.1 at excitation wavelength) obtained from absorption spectra on a wavelength scale [nm] and compared to the reference emitter by the following equation:[20]

$$\Phi_x = \Phi_r\left[\frac{A_r(\lambda_r)}{A_x(\lambda_x)}\right]\left[\frac{I_r(\lambda_r)}{I_x(\lambda_x)}\right]\left[\frac{n_x^2}{n_r^2}\right]\left[\frac{D_x}{D_r}\right]$$

where A is the absorbance at the excitation wavelength (λ), I is the intensity of the excitation light at the excitation wavelength (λ), n is the refractive index of the solvent, D is the integrated intensity of the luminescence, and Φ is the quantum yield. The subscripts r and x refer to the reference and the sample, respectively. The quantum yield determinations were performed at identical excitation wavelengths for the sample and the reference, therefore canceling the $I(\lambda_r)/I(\lambda_x)$ term in the equation. All the Ir(III) complexes were measured against an aqueous solution of $[Ru(bpy)_3]Cl_2$ ($\Phi_r = 0.028$).[21] Emission lifetimes (τ) were determined with the single photon counting technique (TCSPC) with the same Edinburgh FLSP920 spectrometer using pulsed picosecond LEDs (EPLED 295 or EPLED 360, fhwm < 800 ps) as the excitation source, with repetition rates between 1 kHz and 1 MHz, and the above-mentioned R928P PMT as detector. The goodness of fit was assessed by minimizing the reduced χ^2 function and by visual inspection of the weighted residuals. To record the 77 K luminescence spectra, the samples were put in quartz tubes (2 mm diameter) and inserted in a special quartz dewar filled with liquid nitrogen. The solvent (dichloromethane) used in the preparation of the solutions for the photophysical investigations was of spectrometric grade. Degassed solutions were prepared by gently bubbling argon gas into the prepared sample for 15 min before measurement. Experimental uncertainties are estimated to be ±8% for lifetime determinations, ±20% for quantum yields, and ±2 nm and ±5 nm for absorption and emission peaks, respectively.

Synthesis of Ligands. *Synthesis of Ligand 1a.* Compounds **9** (208 mg, 0.61 mmol) and **11** (250 mg, 0.79 mmol) were dissolved in anhydrous DMF (10 mL). Et_3N (250 μL, 1.84 mmol) was added to the solution, and the reaction mixture was stirred at room temperature for 24 h. Then the solvent was removed by vacuum, and the crude was washed with Et_2O (3 × 15 mL). The solid was dissolved in CH_2Cl_2 (2 mL) and then precipitated with Et_2O to give pure **1a** (306 mg) in 92% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.58 (bs, 1H, J_{HH} = 5.1), 8.18 (dt, 1H, J_{HH} = 8.0, J_{HH} = 1.0), 8.16 (s, 1H), 7.78 (td, 1H, J_{HH} = 7.6, J_{HH} = 1.8), 7.23 (ddd, 1H, J_{HH} = 7.6, J_{HH} = 5.0, J_{HH} = 1.3), 6.0 (bs, 1H), 5.9 (bs, 1H),

dx.doi.org/10.1021/om5007962 | *Organometallics* 2014, 33, 6154−6164

5.3 (bs, 1H), 4.51 (dd, 1H, J_{HH} = 7.6, J_{HH} = 5.1), 4.28 (t, 2H, J_{HH} = 7.0), 4.32 (dd, 1H, J_{HH} = 7.6, J_{HH} = 5.1), 3.21 (q, 2H, J_{HH} = 6.9), 3.10−3.15 (m, 1H), 2.91 (dd, 1H, J_{HH} = 12.7, J_{HH} = 5.0), 2.73 (d, 1H, J_{HH} = 12.7), 2.25−2.15 (m, 2H), 2.00−1.90 (m, 2H), 1.80−1.20 (m, 22H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.1 (C), 163.4 (C), 150.6 (C), 149.6 (CH), 148.5 (C), 137.2 (CH), 123.0 (CH), 122.1 (CH), 120.5 (CH), 62.0 (CH), 60.4 (CH), 55.6 (CH), 50.7 (CH_2), 40.8 (CH_2), 39.7 (CH_2), 36.3 (CH_2), 30.4 (CH_2), 29.9 (CH_2), 29.8 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.47 (CH_2), 29.4 (CH_2), 29.1 (CH_2), 28.3 (CH_2), 27.1 (CH_2), 26.6 (CH_2), 25.9 (CH_2) ppm. ESI-MS (m/z): 564 [M + Na^+]. Anal. Calcd for $C_{28}H_{43}N_7O_2S$ (541.76): C, 62.08; H, 8.00; N, 18.10. Found: C, 61.87; H, 8.10; N, 17.93.

Synthesis of Ligand ***1b***. Biotin **10** (185 mg, 0.76 mmol) was dissolved in $SOCl_2$ (6 mL), and the reaction mixture was stirred at room temperature for 2 h under a nitrogen atmosphere. Then the excess of $SOCl_2$ was removed by distillation and the crude was dissolved in anhydrous acetonitrile (10 mL). A solution of **8** (120 mg, 0.38 mmol) in anhydrous acetonitrile (5 mL) was then added dropwise. The mixture was stirred at room temperature for 24 h under a nitrogen atmosphere. Then the solvent was removed by vacuum and the crude was dissolved with a 1:1 mixture of EtOAc and aqueous $NaHCO_3$. The aqueous phase was extracted with ethyl acetate (3 × 10 mL) and DCM (2 × 10 mL). The combined organic layers were dried over $MgSO_4$ and filtered, and the solvent was evaporated. The solid was dissolved in $CHCl_3$ (1.5 mL) and precipitated with hexane to give pure **1b** (124 mg) in 60% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.58 (d, 1H, J_{HH} = 4.3), 8.19 (d, 1H, J_{HH} = 8.6), 8.16 (s, 1H), 7.79 (t, 1H, J_{HH} = 8.0), 7.25−7.20 (m, 1H), 5.3 (bs, 1H), 5.1 (bs, 1H), 4.52 (dd, 1H, J_{HH} = 7.2, J_{HH} = 5.4), 4.42 (t, 2H, J_{HH} = 7.2), 4.32 (dd, 1H, J_{HH} = 8.0, J_{HH} = 5.4), 4.05 (t, 2H, J_{HH} = 6.7), 3.20−3.15 (m, 1H), 2.92 (dd, 1H, J_{HH} = 12.7, J_{HH} = 5.0), 2.74 (d, 1H, J_{HH} = 12.7), 2.32 (t, 2H, J_{HH} = 7.3), 2.00−1.90 (m, 2H), 1.75−1.55 (m, 6H), 1.50−1.20 (m, 16H) ppm. ^{13}C NMR ($CDCl_3$, 101 MHz): δ 173.6 (C), 163.1 (C), 150.4 (C), 149.3 (CH), 148.2 (C), 137.0 (CH), 122.8 (CH), 121.9 (CH), 120.3 (CH), 64.5 (CH_2), 61.9 (CH), 60.1 (CH), 55.3 (CH), 50.5 (CH_2), 40.5 (CH_2), 33.9 (CH_2), 30.2 (CH_2), 29.4 (CH_2), 29.36 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.0 (CH_2), 28.6 (CH_2), 28.31 (CH_2), 28.26 (CH_2), 26.4 (CH_2), 25.9 (CH_2), 24.8 (CH_2) ppm. ESI−MS (m/z): 565 [M + Na^+]. Anal. Calcd for $C_{28}H_{42}N_6O_3S$ (542.74): C, 61.96; H, 7.80; N, 15.48. Found: C, 62.17; H, 7.68; N, 15.73.

Synthesis of Ligand ***2a***. In a 50 mL two-necked round-bottom flask equipped with a stirring bar, biotin **10** (220 mg, 0.90 mmol), EDCI·HCl (207 mg, 1.1 mmol), HOBt (146 mg, 1.1 mmol), and Et_3N (173 mg, 238 μL, 1.7 mmol) were dissolved in anhydrous DMF (6 mL) under a nitrogen atmosphere. After 10 min, **14a** (214 mg, 0.9 mmol) was added and the reaction was left to stir 72 h at room temperature. Then the solvent was removed by vacuum, and the crude was purified by column chromatography on neutral alumina (DCM/MeOH = 94:6) to give **2a** (263 mg, 0.57 mmol) in 63% yield. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.30 (s, 1H), 9.28 (s, 1H), 8.72 (bd, 1H, J_{HH} = 4.6), 8.17 (d, 1H, J_{HH} = 8.1), 8.05−7.95 (m, 3H), 7.95−7.85 (m, 2H), 7.45 (ddd, 1H, J_{HH} = 1.1, J_{HH} = 4.8, J_{HH} = 7.4), 6.5 (bs, 1H), 6.4 (bs, 1H), 4.40−4.35 (m, 1H), 4.25−4.15 (m, 1H), 3.25−3.15 (m,1H), 2.89 (dd, 1H,, J_{HH} = 4.8, J_{HH} = 12.0), 2.64 (d, 1H, J_{HH} = 12.0), 2.42 (t, 2H, J_{HH} = 7.5), 1.80−1.40 (m, 6H) ppm. ^{13}C NMR (DMSO-d_6, 101 MHz): δ 172.0 (C), 163.2 (C), 150.1 (CH), 150.0 (C), 148.5 (C), 140.2 (C), 137.8 (CH), 131.9 (C), 123.7 (CH), 121.4 (CH), 121.2 (CH), 120.2 (CH), 120.1 (CH), 61.5 (CH), 60.0 (CH), 55.8 (CH), 40.3 (CH_2), 36.7 (CH_2), 28.7 (CH_2), 28.6 (CH_2), 25.5 (CH_2) ppm. ESI-MS (m/z): 488 [M + Na^+]. Anal. Calcd for $C_{23}H_{25}N_7O_2S$ (463.56): C, 59.59; H, 5.44; N, 21.15. Found: C, 59.27; H, 5.58; N, 20.93.

Synthesis of Ligand ***2b***. In a 50 mL two-necked round-bottom flask equipped with a stirring bar, biotin **10** (37 mg, 0.15 mmol), **14b** (71 mg, 0.30 mmol), and HOBt (41 mg, 0.30 mmol) were added under N_2 to anhydrous DMF (3.5 mL). After bubbling N_2 in the solution for 15 min, Et_3N (84 μL, 0.60 mmol) and EDCI·HCl (43 mg, 0.23 mmol) were added, and the reaction mixture was stirred under N_2 at room temperature for 24 h. Then further EDCI·HCl (43 mg, 0.23 mmol) was added, and the reaction mixture was stirred under N_2 at room temperature overnight. Then the solvent was removed by vacuum, and the crude was washed with MeOH (3 × 5 mL) to give **2b** (38 mg) in 55% yield. ^{1}H NMR (DMSO, 400 MHz): δ 9.34 (s, 1H), 8.67 (d, 1H, J_{HH} =4.4), 8.13 (d, 1H, J_{HH} = 7.4), 8.08 (d, 2H, J_{HH} = 8.3), 7.96 (dt, 1H, J_{HH} = 1.9, J_{HH} =7.7), 7.45−7.35 (m, 3H), 6.5 (bs, 1H), 6.4 (bs, 1H), 4.35−4.30 (m, 1H), 4.20−4.15 (m, 1H), 3.20−3.10 (m, 1H), 2.85 (dd, 1H, J_{HH} = 4.8, J_{HH} = 12.8), 2.70−2.55 (m, 3H), 1.75−1.40 (m, 6H) ppm. ^{13}C NMR (DMSO-d_6, 101 MHz): δ 172.1 (C), 163.2 (C), 150.9 (C), 150.1 (CH), 150.0 (C), 148.7 (C), 137.8 (CH), 134.5 (C), 123.8 (CH), 123.7 (CH), 122.0 (CH), 121.9 (CH), 120.3 (CH), 61.5 (CH), 59.7 (CH), 55.8 (CH), 40.3 (CH_2), 33.7 (CH_2), 28.5 (CH_2), 28.4 (CH_2), 24.8 (CH_2) ppm. ESI-MS (m/z): 487 [M + Na^+]. Anal. Calcd for $C_{23}H_{24}N_6O_3S$ (464.54): C, 59.47; H, 5.21; N, 18.09. Found: C, 59.68; H, 5.04; N, 18.35.

Synthesis of Ligand ***3a***. In a 50 mL two-necked round-bottom flask equipped with a stirring bar, biotin **10** (220 mg, 0.90 mmol), EDCI·HCl (207 mg, 1.1 mmol), HOBt (146 mg, 1.1 mmol), and Et_3N (173 mg, 238 μL, 1.7 mmol) were dissolved in anhydrous DMF (6 mL) under a N_2 atmosphere. After 10 min **18a** (232 mg, 0.9 mmol) was added, and the reaction was left to stir 24 h at room temperature. Then MeOH was added to the solution, and the precipitate was filtered and washed with MeOH (3 × 5 mL) to give pure **3a** (305 mg) in 63% yield. ^{1}H NMR (DMSO, 300 MHz): δ 10.0 (bs, 1H), 9.38 (s, 1H), 8.67 (ddd, 1H, J_{HH} = 1.0, J_{HH} = 1.5, J_{HH} = 4.8), 8.15−8.05 (m, 3H), 7.96 (td, 1H, J_{HH} =2.0, J_{HH} = 7.8), 7.95−7.85 (m, 2H), 7.65 (s, 4H), 7.42 (ddd, 1H, J_{HH} = 1.2, J_{HH} = 4.9, J_{HH} = 7.6), 6.5 (s, 1H), 6.4 (s, 1H), 4.35−4.30 (m, 1H), 4.15−4.10 (m, 1H), 3.20−3.10 (m, 1H), 2.84 (dd, 1H, J_{HH} = 5.0, J_{HH} = 12.2), 2.59 (d, 1H, J_{HH} = 12.2), 2.35 (t, 2H, J_{HH} = 7.3), 1.70−1.40 (m, 6H) ppm. ^{13}C NMR (DMSO-d_6, 101 MHz): δ 171.8 (C), 163.2 (C), 150.1 (CH), 150.0 (C), 148.7 (C), 140.5 (C), 139.8 (C), 137.8 (CH), 135.8 (C), 133.5 (C), 127.9 (CH), 127.5 (CH), 123.8 (CH), 121.6 (CH), 121.0 (CH), 120.3 (CH), 119.9 (CH), 61.5 (CH), 59.7 (CH), 55.9 (CH), 40.3 (CH_2), 36.7 (CH_2), 28.7 (CH_2), 28.6 (CH_2), 25.6 (CH_2) ppm. ESI-MS (m/z): 562 [M + Na^+]. Anal. Calcd for $C_{29}H_{29}N_7O_2S$ (539.66): C, 64.54; H, 5.42; N, 18.17. Found: C, 64.28; H, 5.64; N, 18.49.

Synthesis of Ligand ***3b***. In a 50 mL two-necked round-bottom flask equipped with a stirring bar, biotin (37 mg, 0.15 mmol), EDCI·HCl (74 mg, 0.39 mmol), HOBt (42 mg, 0.31 mmol), and Et_3N (84 μL, 0.60 mmol) were dissolved in anhydrous DMF (6 mL) under a nitrogen atmosphere. After 10 min, **18b** (32 mg, 0.10 mmol) was added, and the reaction was left to stir 24 h at room temperature. Then further EDCI·HCl (43 mg, 0.23 mmol) was added, and the reaction mixture was stirred under N_2 at room temperature overnight. Then the solvent was removed by vacuum, and the crude was washed with MeOH to give **3b** in 55% yield. ^{1}H NMR (DMSO, 400 MHz): δ 9.40 (s, 1H), 8.68 (d, 1H, J_{HH} = 4.2), 8.20−8.10 (m, 3H), 8.00−7.90 (m, 3H), 7.85−7.80 (m, 2H), 7.42 (dd, 1H, J_{HH} = 7.1, J_{HH} = 5.1), 7.30−7.20 (m, 2H), 6.5 (bs, 1H), 6.4 (bs, 1H), 4.35−4.30 (m, 1H), 4.20−4.15 (m, 1H), 3.20−3.10 (m, 1H), 2.85 (dd, 1H, J_{HH} = 12.4, J_{HH} = 5.0), 2.65−2.55 (m, 3H), 1.75−1.40 (m, 6H) ppm. ^{13}C NMR (DMSO-d_6, 101 MHz): δ 172.2 (C), 163.2 (C), 150.9 (C), 150.1 (CH), 150.0 (C), 148.7 (C), 140.1 (C), 137.8 (CH), 136.8 (C), 136.2 (C), 128.5 (CH), 128.4 (CH), 128.8 (CH), 122.9 (CH), 121.7 (CH), 121.1 (CH), 120.3 (CH), 61.5 (CH), 59.7 (CH), 55.8 (CH), 40.3 (CH_2), 33.8 (CH_2), 28.5 (CH_2), 28.4 (CH_2), 24.9 (CH_2) ppm. ESI-MS (m/z): 563 [M + Na^+]. Anal. Calcd for $C_{29}H_{28}N_6O_3S$ (540.64): C, 64.43; H, 5.22; N, 15.54. Found: C, 64.65; H, 5.50; N, 15.79.

Synthesis of Complexes. *Synthesis of Complex* ***4a***. In a 50 mL round-bottom flask equipped with a stirring bar, the ligand **1a** (54 mg, 0.10 mmol) was dissolved in a 3:1 mixture of CH_2Cl_2/EtOH (8 mL), and the dimer **19** (54 mg, 0.05 mmol) was added. The mixture was left to stir at rt for 24 h. The solvent was removed, and the crude was dissolved in DCM (1 mL) and precipitated with Et_2O to give **4a** (63 mg) in 60% yield. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.66 (s, 1H), 9.13 (d, 1H, J_{HH} = 8.2), 8.04 (t, 1H, J_{HH} = 7.7), 7.95−7.85 (m, 2H), 7.80−7.70 (m, 3H), 7.70−7.60 (m, 3H), 7.47 (d, 1H, J_{HH} = 5.7), 7.25−7.20 (m, 1H), 7.05−6.85 (m, 6H), 6.45−6.35 (m, 1H), 6.35−6.25 (m, 2H), 5.8 (bs, 1H), 5.0 (bs, 1H), 4.55−4.40 (m, 3H), 4.35−4.30 (m, 1H), 3.30−3.10 (m, 3H), 2.92 (dd, 1H, J_{HH} = 5.6, J_{HH} = 12.9), 2.75 (d, 1H, J_{HH} = 12.9), 2.30−2.20 (m, 2H), 2.00−1.15 (m, 24H) ppm. ^{13}C NMR ($CDCl_3$, 150 MHz): δ 173.4 (C), 168.3 (C), 167.6 (C), 163.8 (C), 150.1 (C), 149.5 (CH), 149.3 (CH), 148.5 (C), 148.4 (CH), 146.6 (C), 144.6 (C),

143.7 (C), 143.6 (C), 139.9 (CH), 137.9 (CH), 137.8 (CH), 131.8 (CH), 131.7 (CH), 130.6 (CH), 130.0 (CH), 128.5 (CH), 125.9 (CH), 124.6 (2CH), 124.3 (CH), 123.2 (CH), 122.8 (CH), 122.6 (CH), 122.1 (CH), 119.4 (CH), 119.3 (CH), 61.8 (CH), 60.2 (CH), 55.6 (CH), 52.3 (CH_2), 40.6 (CH_2), 39.4 (CH_2), 35.8 (CH_2), 29.7 (CH_2), 29.3 (CH_2), 29.02 ($2CH_2$), 28.99 ($2CH_2$), 28.5 (CH_2), 28.04 (CH_2), 27.99 (CH_2), 26.7 (CH_2), 26.1 (CH_2), 25.7 (CH_2) ppm. ESI-MS (m/z): 1042 [M^+]. Anal. Calcd for $C_{50}H_{59}ClIrN_9O_2S$ (1077.81): C, 55.72; H, 5.52; N, 11.70. Found: C, 55.49; H, 5.73; N, 12.07.

Synthesis of Complex ***4b***. In a 10 mL round-bottom flask equipped with a stirring bar, the ligand **1b** (54 mg, 0.10 mmol) was dissolved in a 3:1 mixture of CH_2Cl_2/EtOH (4 mL), and the dimer **19** (54 mg, 0.05 mmol) was added. The mixture was left to stir at rt for 24 h. The solvent was removed, and the crude was dissolved in DCM (1.0 mL) and precipitated with Et_2O to give **4b** (83 mg) in 80% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.66 (s, 1H), 9.15 (d, 1H, J_{HH} = 7.7), 8.03 (t, 1H, J_{HH} = 7.2), 7.95−7.85 (m, 2H), 7.80−7.60 (m, 6H), 7.47 (d, 1H, J_{HH} = 5.2), 7.20 (dd, 1H, J_{HH} = 7.5, J_{HH} = 6.0), 7.05−6.85 (m, 6H), 6.31 (t, 2H, J_{HH} = 7.5), 5.5 (bs, 1H), 5.4 (bs, 1H), 4.55−4.40 (m, 3H), 4.35−4.25 (m, 1H), 4.06 (t, 2H, J_{HH} = 6.7), 3.20−3.10 (m, 1H), 2.88 (dd, 1H, J_{HH} = 12.8, J_{HH} = 5.3), 2.78 (dd, 1H, J_{HH} = 12.8, J_{HH} = 3.0), 2.31 (t, 2H, J_{HH} = 7.1), 1.95−1.90 (m, 2H), 1.75−1.55 (m, 6H), 1.45−1.15 (m, 16H) ppm. ^{13}C NMR ($CDCl_3$, 150 MHz): δ 173.8 (C), 167.2 (C), 166.7 (C), 162.6 (C), 149.4 (C), 149.1 (C), 149.0 (CH), 148.8 (CH), 148.0 (CH), 146.0 (2C), 143.3 (C), 143.2 (C), 139.2 (CH), 137.3 (CH), 137.2 (CH), 131.2 (CH), 130.9 (CH), 129.7 (CH), 129.0 (CH), 127.85 (CH), 125.3 (CH), 124.0 (CH), 123.8 (CH), 123.6 (CH), 122.6 (CH), 122.3 (CH), 121.8 (CH), 121.3 (CH), 118.8 (CH), 118.7 (CH), 63.7 (CH_2), 61.1 (CH), 59.5 (CH), 54.7 (CH), 51.7 (CH_2), 39.9 (CH_2), 33.2 (CH_2), 29.3 (CH_2), 28.9 (CH_2), 28.63 (CH_2), 28.57 (CH_2), 28.4 (CH_2), 28.1 (CH_2), 27.9 (CH_2), 27.6 (CH_2), 27.5 (CH_2), 25.6 (CH_2), 25.2 (CH_2), 24.1 (CH_2) ppm. ESI-MS (m/z): 1043 [M^+] Anal. Calcd for $C_{50}H_{58}ClIrN_8O_3S$ (1078.79): C, 55.67; H, 5.42; N, 10.39. Found: C, 55.89; H, 5.19; N, 10.67.

Synthesis of Complex ***5a***. In a 10 mL round-bottom flask equipped with a stirring bar, the ligand **2a** (28 mg, 0.06 mmol) was dissolved in a 3:1 mixture of CH_2Cl_2/EtOH (8 mL), and the dimer **19** (32 mg, 0.03 mmol) was added. The mixture was left to stir at rt for 48 h. The solvent was removed, and the crude was purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 9:1) to give **5a** (41 mg) in 70% yield. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.40 (s, 1H), 10.15 (s, 1H), 8.47 (d, 1H, J_{HH} = 7.9), 8.30−8.20 (m, 3H), 8.00−7.95 (m, 3H), 7.90 (d, 1H, J_{HH} = 7.2), 7.90−7.80 (m, 3H), 7.75−7.70 (m, 3H), 7.69 (d, 1H, J_{HH} = 5.7), 7.59 (t, 1H, J_{HH} = 6.6), 7.25−7.15 (m, 2H), 7.03 (t, 1H, J_{HH} = 7.5), 6.95−6.90 (m, 2H), 6.89 (t, 1H, J_{HH} = 7.5), 6.43 (s, 1H), 6.37 (s, 1H), 6.20 (d, 2H, J_{HH} = 7.6), 6.17 (d, 2H, J_{HH} = 7.6), 4.35−4.30 (m, 1H), 4.15−4.10 (m, 1H), 3.15−3.10 (m, 1H), 2.83 (dd, 1H, J_{HH} = 5.5, J_{HH} = 12.6), 2.59 (d, 1H, J_{HH} = 12.6), 2.40−2.30 (m, 2H), 1.65−1.30 (m, 6H) ppm. ^{13}C NMR (DMSO-d_6, 150 MHz): δ 172.2 (C), 167.7 (C), 166.9 (C), 163.2 (C), 152.8 (C), 150.4 (CH), 150.0 (CH), 149.9 (C), 149.5 (CH), 149.4 (C), 149.1 (C), 146.8 (C), 144.5 (C), 144.4 (C), 141.4 (C), 140.6 (CH), 139.3 (CH), 139.2 (CH), 131.9 (CH), 131.3 (CH), 130.7 (CH), 129.9 (CH), 127.6 (CH), 125.7 (CH), 125.4 (CH), 125.0 (CH), 124.4 (CH), 124.2 (CH), 123.4 (CH), 122.9 (CH), 122.3 (CH), 122.1 (CH), 120.3 (CH), 120.1 (CH), 61.5 (CH), 59.7 (CH), 55.8 (CH), 40.3 (CH_2), 36.6 (CH_2), 28.7 (CH_2), 28.5 (CH_2), 25.4 (CH_2) ppm. ESI-MS (m/z): 964 [M^+]. Anal. Calcd for $C_{45}H_{41}ClIrN_9O_2S$ (999.61): C, 54.07; H, 4.13; N, 12.61. Found: C, 54.32; H, 4.30; N, 12.42.

Synthesis of Complex ***5b***. In a 50 mL round-bottom flask equipped with a stirring bar, the ligand **2b** (28 mg, 0.06 mmol) was dissolved in a 3:1 mixture of CH_2Cl_2/EtOH (8 mL), and the dimer **19** (32 mg, 0.03 mmol) was added. The mixture was left to stir at rt for 48 h. The solvent was removed and the crude was purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 9:1) to give pure complex **5b** (29 mg) in 50% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.6 (s, 1H), 9.24 (d, 1H, J_{HH} = 7.6), 8.04 (d, 1H, J_{HH} = 8.0), 7.98 (t, 1H, J_{HH} = 8.0), 7.84 (d, 2H, J_{HH} = 8.4), 7.75−7.65 (m, 4H), 7.65−7.55 (m, 2H), 7.43 (d, 1H, J_{HH} = 5.8), 7.20−7.10 (m, 3H), 7.00−6.70 (m, 6H), 6.30−6.25 (m, 2H), 5.8 (bs, 1H), 5.5 (bs, 1H), 4.45−4.40 (m, 1H), 4.25−4.20 (m, 1H), 3.15−3.05 (m, 1H), 2.82 (dd, 1H, J_{HH} = 4.7, J_{HH} = 12.6), 2.56 (d, 1H, J_{HH} = 12.6), 2.49 (d, 2H, J_{HH} = 7.5 Hz), 1.75−1.35 (m, 6H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.6 (C), 169.3 (C), 167.4 (C), 166.5 (C), 162.6 (C), 150.4 (C), 149.9 (C), 148.9 (C), 148.51 (CH), 148.47 (CH), 148.45 (CH), 147.4 (CH), 145.2 (C), 142.7 (C), 142.6 (C), 139.0 (CH), 137.04 (CH), 137.00 (CH), 132.5 (C), 130.9 (CH), 130.7 (CH), 129.7 (CH), 129.0 (CH), 125.8 (CH), 125.2 (CH), 124.3 (CH), 123.7 (CH), 123.3 (CH), 122.3 (CH), 122.2 (CH), 121.8 (CH), 121.7 (CH), 121.2 (CH), 120.6 (CH), 118.6 (CH), 118.4 (CH), 60.9 (CH), 59.2 (CH_2), 54.4 (CH), 39.6 (CH_2), 32.9 (CH_2), 27.30 (CH_2), 27.27 (CH_2), 23.6 (CH_2) ppm. ESI-MS (m/z): 965 [M^+]. Anal. Calcd for $C_{45}H_{40}ClIrN_8O_3S$ (1000.60): C, 54.02; H, 4.03; N, 11.20. Found: C, 53.79; H, 4.25; N, 10.89.

Synthesis of Complex ***6a***. In a 10 mL round-bottom flask equipped with a stirring bar, the ligand **3a** (47 mg, 0.09 mmol) was dissolved in a 3:1 mixture of CH_2Cl_2/EtOH (10 mL), and the iridium dimer **19** (48 mg, 0.045 mmol) was added. The mixture was left to stir at rt for 48 h. The solvent was removed, and the resulting residue was dissolved in MeOH (0.5 mL) and precipitated with Et_2O. The solid was filtered to give pure complex **6a** (56 mg) in 60% yield. ^{1}H NMR (CD_3OD, 400 MHz): δ 9.62 (s, 1H), 8.27 (d, 1H, J_{HH} = 8.0), 8.10−8.00 (m, 3H), 7.85−7.65 (m, 10H), 7.65−7.50 (m, 5H), 7.40−7.35 (m, 1H), 7.05−6.90 (m, 3H), 6.88 (dt, 1H, J_{HH} = 1.1, J_{HH} = 7.5), 6.81 (dt, 1H, J_{HH} = 1.1, J_{HH} = 7.5), 6.73 (dt, 1H, J_{HH} = 1.1, J_{HH} = 7.5), 6.21 (dd, 2H, J_{HH} = 7.4 Hz, J_{HH} = 2.6), 4.60−4.55 (m, 1H), 4.55−4.45 (m, 1H), 3.45−3.35 (m, 1H), 3.10−3.00 (m, 1H), 3.00−2.95 (m, 1H), 2.36 (t, 2H, J_{HH} = 7.2), 1.90−1.50 (m, 6H) ppm. ^{13}C NMR (DMSO-d_6, 101 MHz): δ 171.8 (C), 167.4 (C), 167.0 (C), 161.7 (C), 150.4 (CH), 150.1 (CH), 150.0 (C), 149.5 (CH), 149.34 (C), 149.27 (C), 146.8 (C), 144.52 (C), 144.47 (C), 141.7 (C), 140.7 (CH), 140.1 (C), 139.3 (CH), 139.2 (CH), 135.0 (C), 133.0 (C), 131.9 (CH), 131.3 (CH), 130.7 (CH), 129.9 (CH), 127.9 (CH), 127.7 (CH), 127.5 (CH), 125.7 (CH), 125.5 (CH), 125.5 (CH), 124.4 (CH), 124.2 (CH), 123.4 (CH), 122.9 (CH), 122.3 (CH), 121.7 (CH), 120.3 (2CH), 119.9 (CH), 70.4 (CH), 59.1 (CH_2), 56.3 (CH), 53.2 (CH), 36.6 (CH_2), 25.6 (CH_2), 25.4 (CH_2) ppm. ESI-MS (m/z): 1040 [M^+]. Anal. Calcd for $C_{51}H_{45}ClIrN_9O_2S$ (1075.71): C, 56.94; H, 4.22; N, 11.72. Found: C, 56.67; H, 4.50; N, 12.00.

Synthesis of Complex ***6b***. In a 10 mL round-bottom flask equipped with a stirring bar, the ligand **3b** (32 mg, 0.06 mmol) was dissolved in a 3:1 mixture of CH_2Cl_2/EtOH (8 mL), and the iridium dimer **19** (32 mg, 0.03 mmol) was added. The mixture was left to stir at rt for 48 h. The solvent was removed, and the crude was purified by column chromatography on silica gel (CH_2Cl_2/ MeOH = 9:1) to give **6b** (19 mg) in 30% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.79 (s, 1H), 9.38 (d, 1H, J_{HH} = 8.0), 8.15 (d, 2H, J_{HH} = 7.8), 8.06 (t, 1H, J_{HH} = 7.8), 7.92 (dd, 2H, J_{HH} = 7.8, J_{HH} = 3.5), 7.80−7.75 (m, 4H), 7.70−7.65 (m, 4H), 7.55−7.50 (m, 3H), 7.25−7.20 (m, 1H), 7.15 (d, 2H, J_{HH} = 8.5), 7.05−6.95 (m, 4H), 6.95−6.85 (m, 2H), 6.34 (dd, 2H, J_{HH} = 2.5, J_{HH} = 7.4), 5.55 (s, 1H), 5.18 (s, 1H), 4.55−4.45 (m, 1H), 4.35−4.30 (m, 1H), 3.20−3.15 (m, 1H), 2.92 (dd, 1H, J_{HH} = 5.2, J_{HH} = 13.2), 2.75 (d, 1H, J_{HH} = 13.2), 2.60 (, t, 2H, J_{HH} = 7.2), 1.90−1.40 (m, 6H) ppm. ^{13}C NMR ($CDCl_3$, 101 MHz): δ 171.1 (C), 167.5 (C), 166.6 (C), 162.3 (C), 149.6 (C), 149.0 (C),148.9 (C), 148.50 (C), 148.47 (CH), 148.4 (CH), 147.4 (CH), 145.3 (C), 142.7 (C), 142.6 (C), 140.6 (C), 139.0 (CH), 137.0 (CH), 136.9 (CH), 136.1 (C), 134.3 (C), 130.9 (CH), 130.7 (CH), 129.7 (CH), 129.0 (CH), 127.4 (CH), 127.1 (CH), 125.9 (CH), 125.0 (CH), 125.4 (CH), 123.7 (CH), 123.3 (CH), 122.3 (CH), 121.8 (CH), 121.7 (CH), 121.2 (CH), 121.1 (CH), 119.7 (CH), 118.5 (CH), 118.4 (CH), 60.9 (CH), 59.1 (CH), 54.3 (CH), 39.6 (CH_2), 33.0 (CH_2), 29.7 (CH_2), 27.3 (CH_2), 23.7 (CH_2) ppm. ESI-MS (m/z): 1041 [M^+]. Anal. Calcd for $C_{51}H_{44}ClIrN_8O_3S$ (1076.69): C, 56.89; H, 4.12; N, 10.41. Found: C, 57.12; H, 4.35; N, 10.07.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthesis, chatacterization, and ^{1}H and ^{13}C NMR spectra of the precursor compounds **8**, **9**, **11**, **14a**,**b**, **16**, and **18a**,**b**. ^{1}H and ^{13}C NMR spectra of all ligands (**1**−**3a**,**b**) and complexes (**4**−**6a**,**b**). Absorption spectra of all Ir(III) complexes. Emission spectra in

dx.doi.org/10.1021/om5007962 | *Organometallics* 2014, 33, 6154−6164

dicholoromethane (rt and 77 K) and aqueous solutions (rt) and HABA assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors wish to thank the Toso Montanari Foundation for financial support.

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