Electronic Supplementary Information

A dual–functional molecular strategy for in–situ suppressing and visualizing of neuraminidase in aqueous solution using iridium(III) complexes

Chun Wu,‡[a] Ke-Jia Wu,‡[b] Jin-Biao Liu,‡[a,c] Xiao-Ming Zhou,[d] Chung-Hang Leung*[b] and Dik-Lung Ma*[a]

[a] *Department of Chemistry, Hong Kong Baptist University, Kowloon, Hong Kong, China.*
[b] *State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macao, China.*
[c] *School of Metallurgical and Chemical Engineering, Jiangxi University of Science and Technology, Ganzhou, China.*
[d] *MOE Key Laboratory of Laser Life Science and Institute of Laser Life Science, College of Biophotonics, South China Normal University, Guangzhou, China.*
‡ The authors contribute equally in this work.
** Corresponding authors.*
E-mail addresses: edmondma@hkbu.edu.hk (Dik-Lung Ma) and duncanleung@um.edu.mo (Chung-Hang Leung).

Experimental

Material

Unless specified, all the reagents were purchased from Sigma Aldrich (St. Louis, MO) and used as received without further purification, and all aqueous solutions were prepared with Milli-Q water (18.2 MΩ cm^{-1}) unless specified. Iridium chloride hydrate ($IrCl_3 \cdot xH_2O$) was purchased from Precious Metals Online (Australia).

General Experiment

Mass spectrometry was performed in the Mass Spectroscopy Unit at Department of Chemistry, Hong Kong Baptist University, Hong Kong (China). Deuterated solvents for NMR purposes were obtained from Armar and used as received. ^{1}H and ^{13}C NMR were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz (^{1}H) and 100 MHz (^{13}C). ^{1}H and ^{13}C chemical shifts were referenced internally to solvent shift (Acetonitrile-*d*3: ^{1}H, 1.94, ^{13}C, 1.32 and 118.26). Chemical shifts are quoted in ppm, the downfield direction being defined as positive. Uncertainties in chemical shifts are typically ±0.01 ppm for ^{1}H and ±0.05 for ^{13}C. Coupling constants are typically ±0.1 Hz for ^{1}H-^{1}H and ±0.5 Hz for ^{1}H-^{13}C couplings. The following abbreviations are used for convenience in reporting the multiplicity of NMR resonances: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. All NMR data was acquired and processed using standard Bruker software (Topspin).

Synthesis of complexes 11a–11d

Complex **11a** were prepared according to modified literature methods.[1,2,3] Briefly, 2.1 equivalents of 1-phenylisoquinoline and 1 equivalent of $IrCl_3 \cdot xH_2O$ were mixed together and further heated overnight at 120 °C in 12 mL of 2-methoxyethanol/H_2O (3/1). Afterwards, the mixture was filtered and washed by excessive deionized water

and then diethyl ether for three times respectively to generate the dichloro-bridged dimer $[Ir(piq)_2Cl]_2$. The oven-dried dimer was treated with 2.1 equivalents of 1,10-phenanthroline-5-carboxylic acid in DCM (4 mL) and methanol (4 mL) at ambient temperature for 10 h. Then, an excess of solid ammonium hexafluorophosphate (NH_4PF_6) was added and the reaction was stirred for another 20 min. The brown powder thus obtained was isolated and filtrated by removing the solvent under reduced pressure, and the residue was purified by silica gel column chromatography employing DCM and methanol as solvent. Yield: 48%. Complexes **11b–11d** were synthesized by the same method as that of complex **11a** via the modification of the auxiliary C^N and N^N ligands in parent complex **11a**.

Synthesis of complexes 1a–1d

Complex **1a** was synthesized according to a reported literature[4]. Compound **11a** (0.45 g, 1 eq.) was dissolved in distilled DCM (30 mL) at 0 °C. Compound **10** (0.24 g, 1.2 eq.) was added, followed by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (0.17 g, 2 eq.), HOBt (0.10 g, 1.5 eq.), and, finally, Et_3N (0.20 g, 4 eq.). After complete addition of reagents, the reaction mixture was stirred at 0 °C for a further 1 h and allowed to complete for 24 h. Completion of reaction was monitored by TLC using MeOH/DCM (5/95, v/v). The solvent was removed under reduced pressure, and the red colour residue was dissolved in EtOAc. The organic layer was washed with $NaHCO_3$ (saturated) solution and dried over Na_2SO_4. The organic layer was evaporated and purified by column chromatography to get compound **1a**. Compound **1b–1d** was synthesized by the same method as that of compound **1a** by the replacement of **11a** into **11b–11d**.

Complex **1a** Yield: 62%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.03 (s, 1H), 8.87 (s, 2H), 8.62 (s, 1H), 8.36 (s, 1H), 8.23 (s, 2H), 8.00 (s, 2H), 7.78 (s, 2H), 7.69 (s, 5H), 7.09 (s, 4H), 6.88

(s, 2H), 6.76 (s, 1H), 6.60 (d, *J* = 21.2 Hz, 1H), 6.31 (s, 2H), 5.33 (d, *J* = 43.4 Hz, 1H), 4.68 (d, *J* = 44.0 Hz, 1H), 4.14 (s, 2H), 3.70 (s, 1H), 3.32 (s, 1H), 3.16 (s, 1H), 2.38 (s, 1H), 2.14 (s, 1H), 1.94 (s, 2H), 1.81 (s, 1H), 1.67 (s, 3H), 1.45 (s, 4H), 0.82 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.00, 149.92, 145.91, 144.61, 139.43, 138.54, 137.61, 131.55, 131.37, 131.25, 131.13, 130.70, 130.07, 129.84, 128.96, 128.78, 127.74, 126.56, 125.87, 121.51, 121.31, 81.27, 59.82, 28.68, 26.19, 25.37, 24.81, 22.63, 21.67, 13.20, 8.52. HRMS: Calcd. for $C_{59}H_{54}IrN_6O_5PF_6$ $[M\text{-}PF_6]^+$: 1119.3785 Found: 1119.3805.

Complex **1b** Yield: 58%. ^{1}H NMR (400 MHz, DMSO) δ 9.05 (ddd, *J* = 29.2, 19.3, 8.0 Hz, 3H), 8.50 (d, *J* = 9.3 Hz, 1H), 8.33 (s, 4H), 8.27 (d, *J* = 10.2 Hz, 1H), 8.15 (d, *J* = 11.1 Hz, 2H), 8.04 (d, *J* = 7.1 Hz, 2H), 7.96 (s, 2H), 7.55 (t, *J* = 19.2 Hz, 2H), 7.14 (t, *J* = 7.4 Hz, 2H), 7.10 – 6.98 (m, 4H), 6.79 (s, 1H), 6.36 (s, 2H), 4.38 (s, 1H), 4.32 (s, 1H), 4.24 (d, *J* = 6.8 Hz, 2H), 4.04 (s, 1H), 3.54 (s, 1H), 2.80 (d, *J* = 18.1 Hz, 1H), 2.06 (s, 1H), 1.92 (d, *J* = 13.0 Hz, 3H), 1.53 (s, 4H), 0.93 (s, 3H), 0.87 (d, *J* = 4.8 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 170.25, 167.26, 165.91, 165.76, 152.10, 151.52, 150.32, 149.97, 149.69, 146.82, 146.70, 144.47, 139.67, 139.24, 139.01, 135.13, 135.00, 131.71, 130.73, 130.28, 129.12, 128.85, 128.34, 127.77, 125.59, 124.37, 122.92, 120.50, 81.73, 75.51, 61.03, 54.59, 48.86, 30.33, 29.53, 26.29, 25.70, 23.53, 14.59, 9.96, 9.50. HRMS: Calcd. for $C_{51}H_{50}IrN_6O_5PF_6$ $[M\text{-}PF_6]^+$: 1019.3472 Found: 1019.3436.

Complex **1c** Yield: 56%. ^{1}H NMR (400 MHz, DMSO) δ 8.92 (t, *J* = 6.0 Hz, 2H), 8.76 (d, *J* = 8.9 Hz, 1H), 8.56 (s, 1H), 8.44 (d, *J* = 7.0 Hz, 2H), 8.39 (d, *J* = 10.9 Hz, 1H), 8.23 (t, *J* = 5.5 Hz, 1H), 8.17 – 8.10 (m, 2H), 8.04 (d, *J* = 9.9 Hz, 1H), 7.98 (d, *J* = 12.2 Hz, 1H), 7.90 (dd, *J* = 18.8, 5.3 Hz, 1H), 7.61 (d, *J* = 9.2 Hz, 2H), 7.58 (s, 1H), 7.37 – 7.31 (m, 1H), 7.25 (d, *J* = 6.2 Hz, 2H), 7.22 – 7.16 (m, 3H), 6.88 (s, 2H), 6.68 (s, 1H), 6.49 (t, *J* = 8.8 Hz, 2H), 4.17 (s, 3H), 4.12 – 4.08 (m, 1H), 3.86 (s, 1H), 3.43 (s, 1H), 2.47 (s, 3H), 2.06 – 1.95 (m, 2H), 1.60 (s, 2H), 1.51 (s, 2H), 1.47 – 1.39 (m, 3H), 0.84 (t, *J* = 7.2 Hz, 6H), 0.72 (d, *J* = 4.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.46, 169.69, 166.20, 162.34, 156.51,

155.26, 154.86, 152.50, 151.87, 150.95, 147.86, 146.74, 144.70, 143.83, 138.64, 134.54, 132.38, 131.46, 129.89, 128.66, 127.87, 127.28, 125.79, 125.44, 125.01, 123.36, 122.13, 117.72, 60.41, 29.68, 26.36, 25.61, 23.66, 21.27. HRMS: Calcd. for $C_{58}H_{54}IrN_6O_5Cl_2PF_6$ $[M\text{-}PF_6]^+$: 1177.3162 Found: 1177.3200.

Complex **1d** Yield: 64%. ^{1}H NMR (400 MHz, DMSO) δ 9.35 (d, *J* = 8.6 Hz, 1H), 8.76 (d, *J* = 8.8 Hz, 1H), 8.55 (s, 4H), 8.37 (s, 1H), 8.29 (d, *J* = 7.1 Hz, 2H), 8.22 (d, *J* = 5.5 Hz, 1H), 8.07 – 8.00 (m, 1H), 7.94 (dd, *J* = 12.6, 5.9 Hz, 3H), 7.81 (d, *J* = 5.7 Hz, 1H), 7.55 (d, *J* = 4.3 Hz, 1H), 7.41 (d, *J* = 7.1 Hz, 2H), 7.24 (d, *J* = 6.7 Hz, 1H), 7.13 (dd, *J* = 16.2, 8.8 Hz, 2H), 7.11 – 7.04 (m, 2H), 6.80 (t, *J* = 6.7 Hz, 2H), 6.66 (s, 2H), 6.39 (d, *J* = 7.4 Hz, 2H), 4.20 – 4.13 (m, 3H), 4.11 (d, *J* = 5.6 Hz, 1H), 3.85 (s, 1H), 2.56 (d, *J* = 15.7 Hz, 1H), 2.42 (s, 3H), 1.75 (s, 2H), 1.58 (s, 2H), 1.51 (s, 2H), 1.39 (d, *J* = 4.7 Hz, 3H), 0.80 (s, 3H), 0.71 (dd, *J* = 16.5, 9.2 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 174.73, 170.11, 165.84, 163.50, 162.81, 151.76, 150.61, 147.19, 140.71, 139.94, 134.25, 131.11, 130.12, 127.59, 123.25, 118.73, 81.67, 74.97, 61.01, 31.78, 29.13, 27.04, 25.60, 21.94, 21.29, 14.51. HRMS: Calcd. for $C_{58}H_{56}IrN_6O_5PF_6$ $[M\text{-}PF_6]^+$: 1109.3941 Found: 1109.3962.

Photophysical measurement

Emission spectra and lifetime measurements for complexes **1a**–**1d** were performed on a PTI TimeMaster C720 Spectrometer (Nitrogen laser: pulse output 335 nm) fitted with a 395 nm filter. Error limits were estimated: λ (±1 nm); τ (±10 %); ϕ (±10 %). All solvents used for the lifetime measurements were degassed using three cycles of freeze-vac-thaw. Luminescence quantum yields were determined using the method of Demas and Crosby with $[Ru(bpy)_3][PF_6]_2$ in degassed acetonitrile (ACN) as a standard reference solution (Φ_r = 0.062) and were calculated according to the following reported equation (1):

$$\Phi_s = \Phi_r(B_r/B_s)(n_s/n_r)^2(D_s/D_r) \quad (1)$$

Where the subscripts s and r refer to the sample and reference standard solution respectively, n is the refractive index of the solvents, D is the integrated intensity, and Φ is the luminescence quantum yield. The quantity B was calculated by $B = 1 - 10^{-AL}$, where A is the absorbance at excitation wavelength and L is the optical path length.

Inhibition assay of complexes 1a–1d

After mixing neuraminidase with buffer solution, the inhibitors (oseltamivir and complexes **1a**–**1d**) were added into the mixtures for 30 min at 100 μM. After then, the chemiluminescent substrate was added into the mixed solution for 30min. The neuraminidase activity was detected at ex/em = 330nm/450nm.

Detection of neuraminidase using complexes 1a–1d as probes

After mixing neuraminidase into buffer solution, the inhibitor (oseltamivir) was added into the mixtures for 30 min at indicated concentrations. After then, complexes **1a**–**1d** as probes were added into the mixed solution for 30 min. The luminescence was detected at ex/em = 330nm/600nm.

Dose-dependent assay using complex 1d as an inhibitor

After mixing neuraminidase and buffer solution, complex **1d** (0–200 μM) was added into the mixtures for 30 min. After then, the chemiluminescent substrate was added into the mixed solution for 30 min. The neuraminidase activity was detected at ex/em = 330nm/450nm.

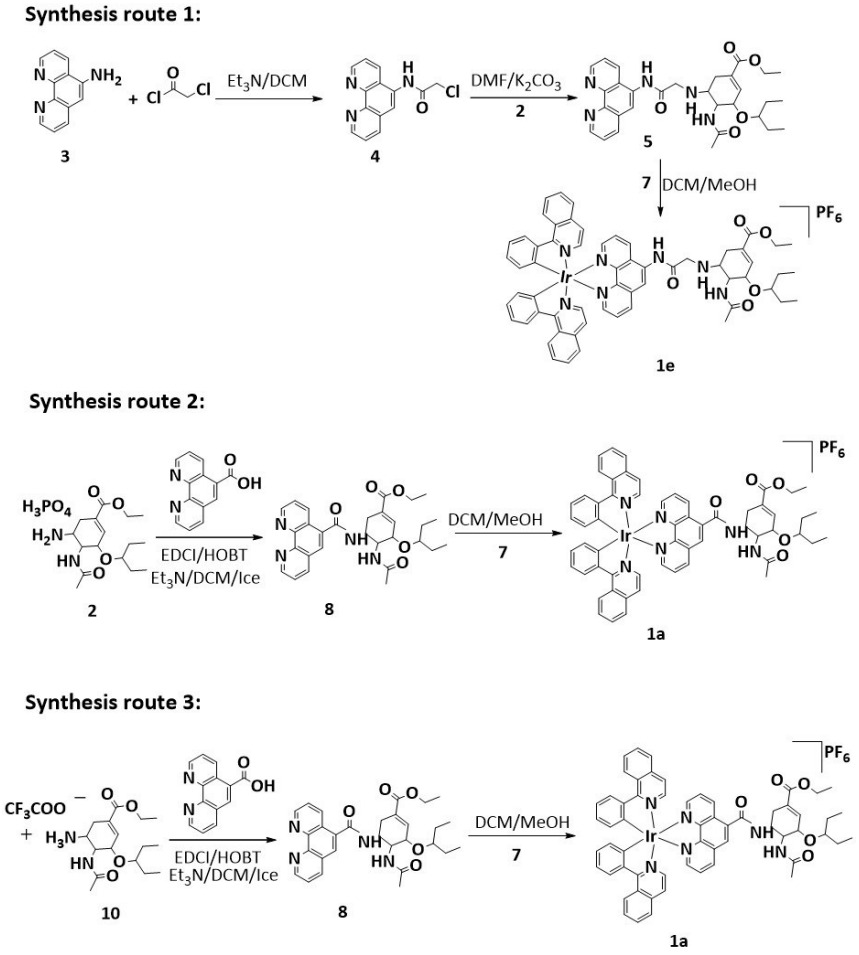
Protein thermal shift assay

To evaluate binding affinity of complex **1d** with neuraminidase, a protein thermal shift assay was performed using the GloMelt™ Thermal Shift Protein Stability Kit. Briefly, after incubation neuraminidase with different concentrations of complex **1d** for 30 min, GloMelt dye was added. The reaction of each sample in triplicate was run in the

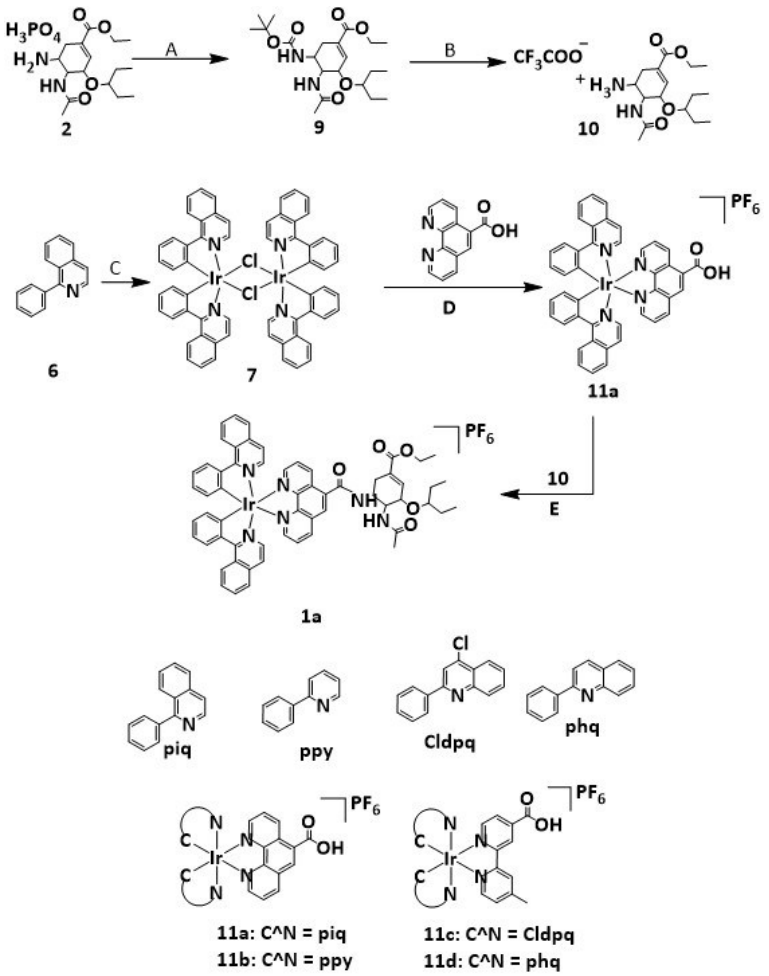
Applied Biosystems™ ViiA™ 7 system. The data were exported to Excel and fluorescence signal of each samples was plotted and a significant increase in slope corresponds to the melting temperature of the protein.

Table S1 Photophysical properties of complexes **1a–1d** (10 μM).

Complex	Quantum yield	λ_{ex} / nm	λ_{em} / nm	Lifetime / μs	UV-Vis absorption λ_{abs} / nm (ε / $dm^3\,mol^{-1}\,cm^{-1}$)
1a	0.293	364	601	0.565	230 (1.42×10^4), 288 (0.85×10^4)
1b	0.409	381	606	0.277	232 (1.16×10^4), 263 (1.12×10^4)
1c	0.963	358	585	0.517	210(1.77×10^4), 282 (1.06×10^4)
1d	0.550	354	603	0.283	212 (1.55×10^4), 269 (0.91×10^4)



Scheme S1 Synthesis route of iridium(III) complexes **1a** and **1e**.



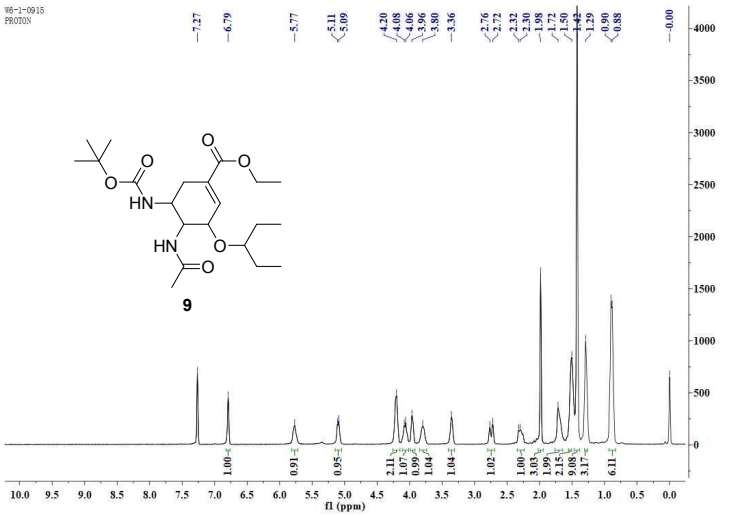
Scheme S2 Synthesis route of iridium(III) complexes **1a–1d**. Conditions: (A) 10% Na_2CO_3, 1,4-dioxane, di-*tert*-butyl decarbonate, 0 °C to r.t., yield = 93%; (B) trifluoroacetic acid, DCM, 0 °C to r.t., yield = 90%; (C) 2-methoxyethanol/H_2O (3:1, v/v), yield = 80%; (D) DCM/methanol (1:1, v/v), yield = 70%; (E) EDCI/HOBT, Et_3N, DCM, 0 °C to r.t., yield = 60%.

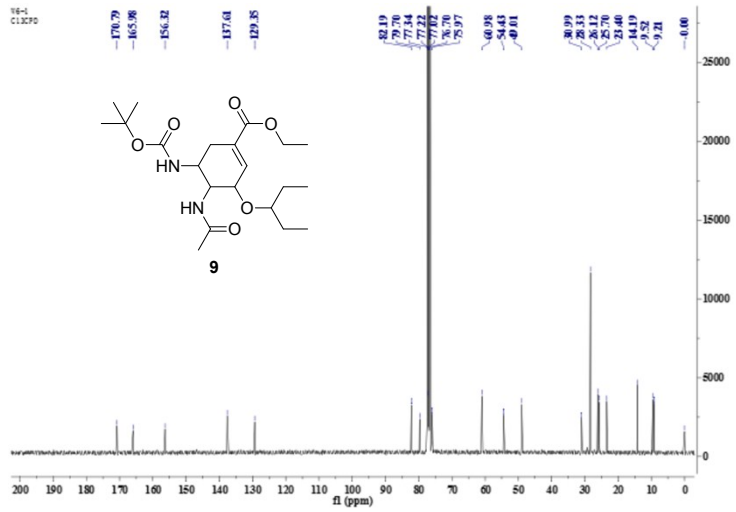
In **Route 1**, 1,10-phenanthrolin-5-amine **3** was first reacted with 2-chloroacetyl chloride in dichloromethane (DCM) using Et_3N as a catalyst to generate 2-chloro-*N*-(1,10-phenanthrolin-5-yl)acetamide **4**. The water-soluble oseltamivir was then directly mixed with **4** in DMF with K_2CO_3, however, only a low yield (12%) of **5** was produced. Moreover, further optimization of the solvent (THF or ACN), base (Cs_2CO_3), or temperature (reflux or ambient) failed to increase the yield of **5**.

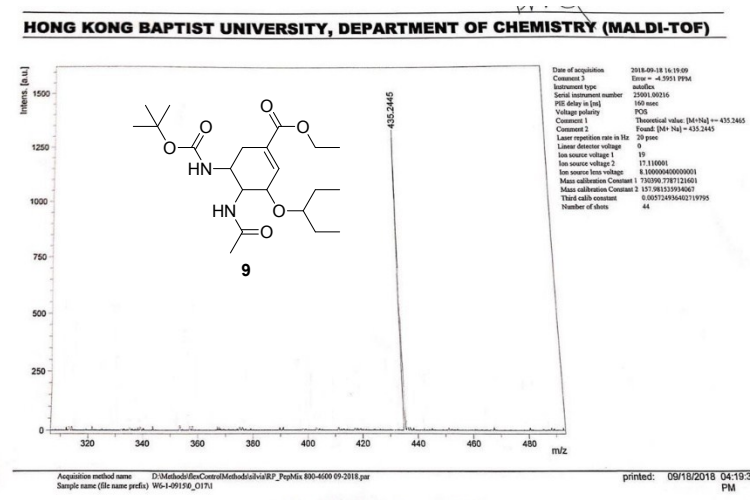
In **Route 2**, we used the more reactive 1,10-phenanthroline-5-carboxylic acid) to

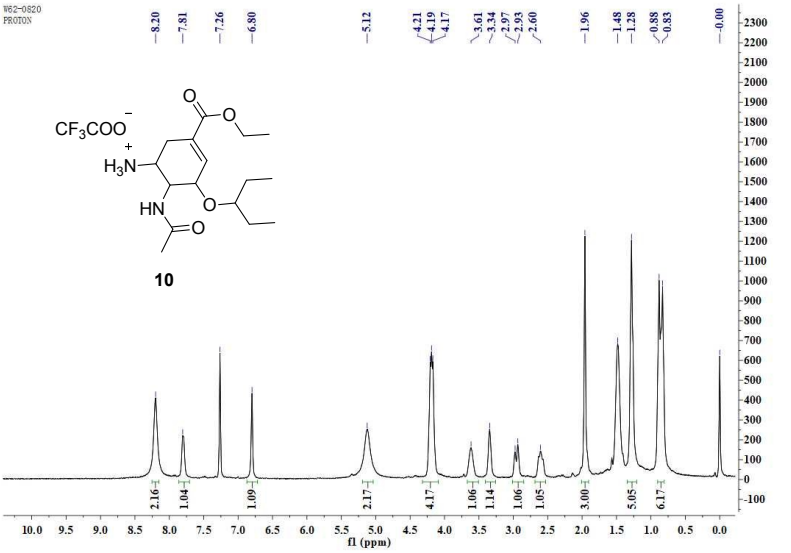
directly conjugate to oseltamivir phosphate **2** under standard EDCI/HOBT coupling conditions. Unfortunately, the yield of the target oseltamivir conjugate **8** also turned out to be disappointing (15%) owing to the poor solubility of oseltamivir phosphate **2** in organic solvent.

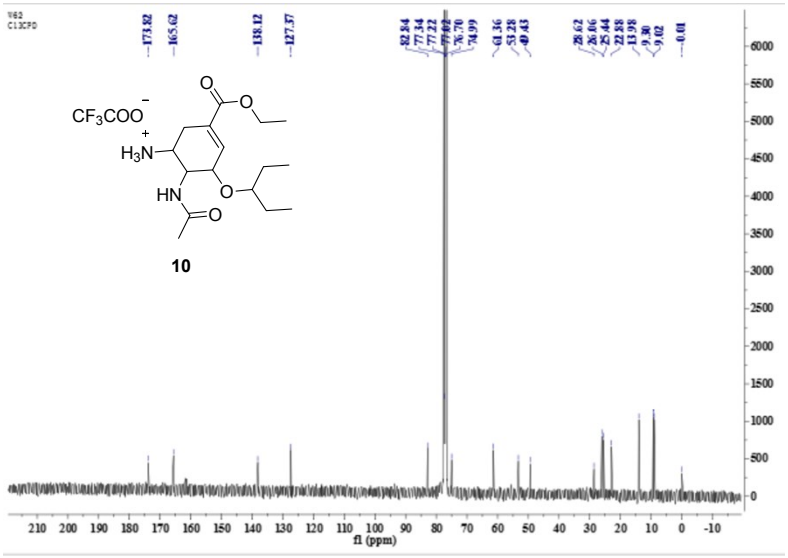
To overcome the solubility issue, oseltamivir phosphate **2** was first reacted with di-*tert*-butyl dicarbonate to give the amine-protected compound **9** (**Scheme S2**). Afterwards, compound **9** was deprotected using trifluoroacetic acid in DCM at 0 °C, giving the organic solvent-soluble oseltamivir trifluoroacetate compound **10**. However, conjugation of oseltamivir trifluoroacetate **10** to the carboxyl-containing N^N ligand 1,10-phenanthroline-5-carboxylic acid proceeded poorly (**Synthesis Route 3**, **Scheme S1**).

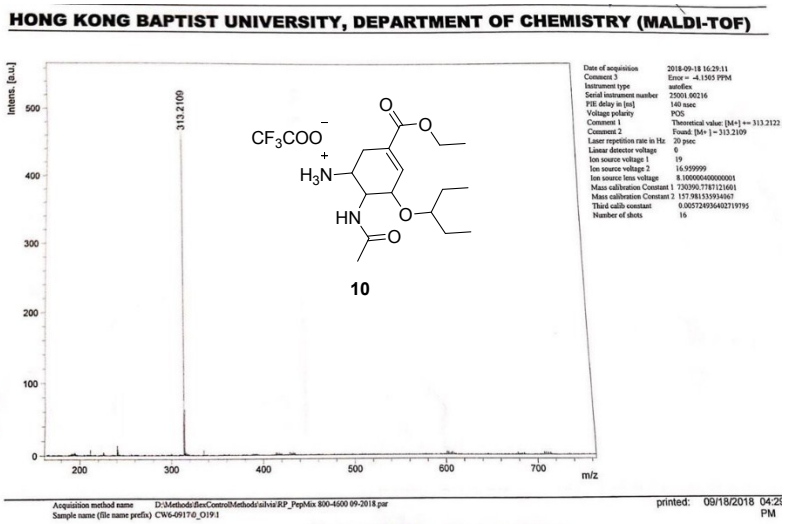

W6-1-0915
PROTON
7.27
6.79
5.77
5.11
5.09
4.20
4.08
4.06
3.96
3.80
3.36
2.76
2.72
2.32
2.30
1.98
1.72
1.50
1.42
1.29
0.90
0.88
0.00
1.00
0.91
0.95
2.11
2.05
0.99
1.04
1.04
1.02
1.00
3.05
1.99
2.15
9.05
3.17
6.11
f1 (ppm)
9

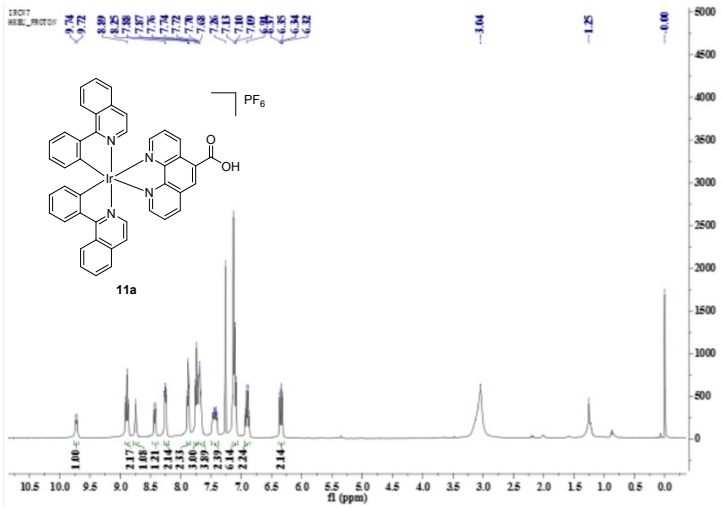

Y6-1
C13CPD
170.79
165.98
156.82
137.61
129.35
82.19
79.70
77.54
77.22
77.02
76.70
75.97
60.98
54.43
40.01
30.99
28.33
26.12
25.70
23.40
14.19
9.52
9.21
0.00
f1 (ppm)
9

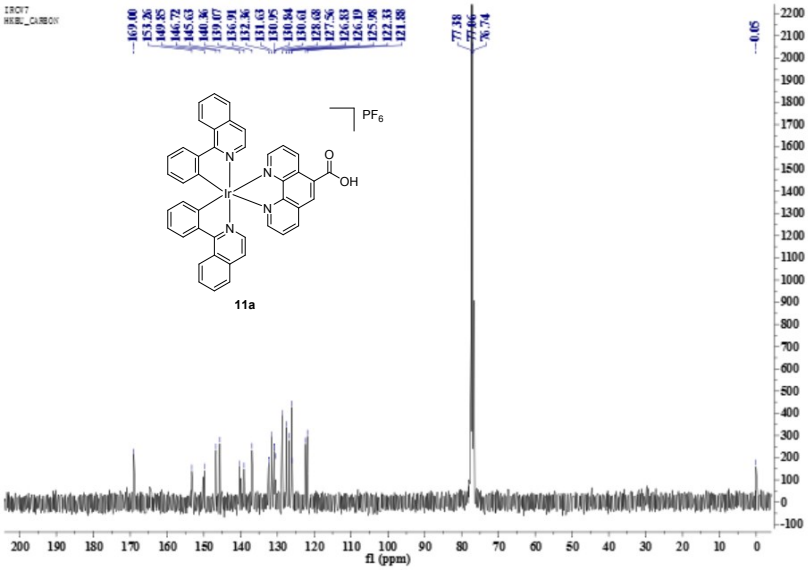

HONG KONG BAPTIST UNIVERSITY, DEPARTMENT OF CHEMISTRY (MALDI-TOF)
Intens. [a.u.]
435.2445
m/z
9
Date of acquisition 2018-09-18 16:19:09
Comment 3 Error = -4.5951 PPM
Instrument type autoflex
Serial instrument number 25001.00216
PIE delay in [ns] 160 nsec
Voltage polarity POS
Comment 1 Theoretical value: [M+Na]+= 435.2465
Comment 2 Found: [M+ Na] = 435.2445
Laser repetition rate in Hz 20 psec
Linear detector voltage 0
Ion source voltage 1 19
Ion source voltage 2 17.110001
Ion source lens voltage 8.100000400009001
Mass calibration Constant 1 730390.7787121601
Mass calibration Constant 2 157.98153993404367
Third calib constant 0.005724936402719795
Number of shots 44
Acquisition method name D:\Methods\flexControlMethods\silvia\RP_PepMix 800-4600 09-2018.par
Sample name (file name prefix) W6-1-09150_O17\1
printed: 09/18/2018 04:19:3
PM

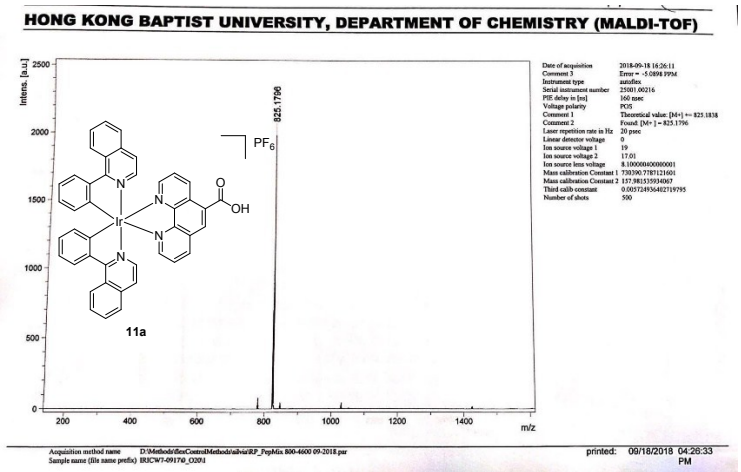

W62-0820
PROTON
8.20
7.81
7.26
6.80
5.12
4.21
4.19
4.17
3.61
3.34
2.97
2.93
2.60
1.96
1.48
1.28
0.88
0.83
0.00
2.16
1.04
1.09
2.17
4.17
1.06
1.14
1.06
1.05
3.00
5.05
6.17
f1 (ppm)
CF3COO
H3N
HN
O
10

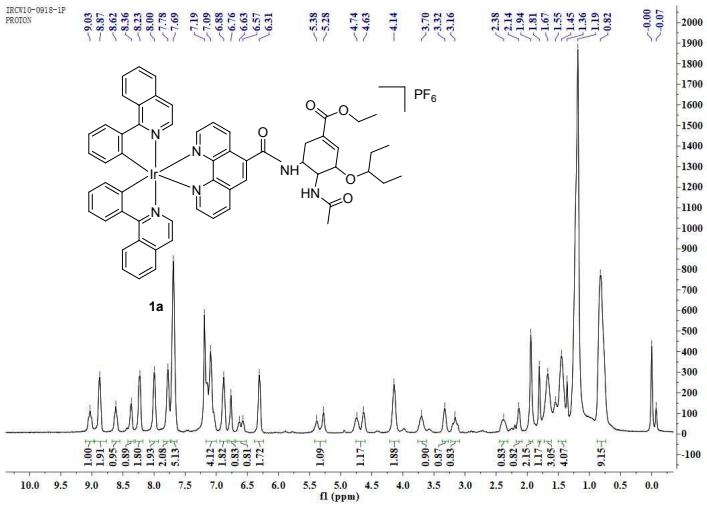

W62
C13CPD
173.82
165.62
138.12
127.57
82.84
77.34
77.22
77.02
76.70
74.99
61.36
53.28
49.43
25.62
25.06
25.44
22.58
13.98
9.30
9.02
0.01
f1 (ppm)
CF3COO
H3N
HN
O
10

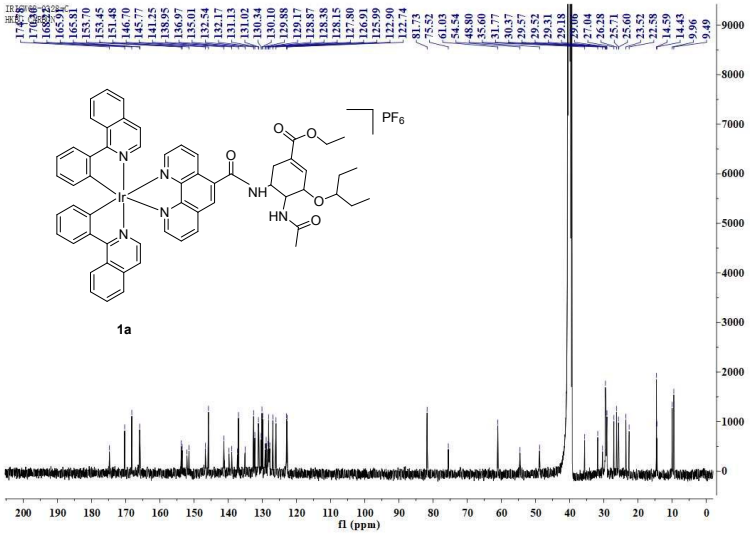

HONG KONG BAPTIST UNIVERSITY, DEPARTMENT OF CHEMISTRY (MALDI-TOF)
Intens. [a.u.]
313.2100
m/z
Date of acquisition 2018-09-18 16:29:11
Comment 3 Error= -4.1505 PPM
Instrument type autoflex
Serial instrument number 25001.00216
PIE delay in [ns] 140 nsec
Voltage polarity POS
Comment 1 Theoretical value: [M+] += 313.2122
Comment 2 Found: [M+]= 313.2109
Laser repetition rate in Hz 20 psec
Linear detector voltage 0
Ion source voltage 1 19
Ion source voltage 2 16.959999
Ion source lens voltage 8.100004000000001
Mass calibration Constant 1 730390.7787123681
Mass calibration Constant 2 157.9815359344067
Third calib constant 0.005724936402719795
Number of shots 16
CF3COO
H3N
HN
O
10
Acquisition method name D:\Methods\flexControl\Methods\silvia\RP_PepMix 800-4600 09-2018.par
Sample name (file name prefix) CW6-0917\0_O19:1
printed: 09/18/2018 04:28 PM


PF6
OH
11a
f1 (ppm)

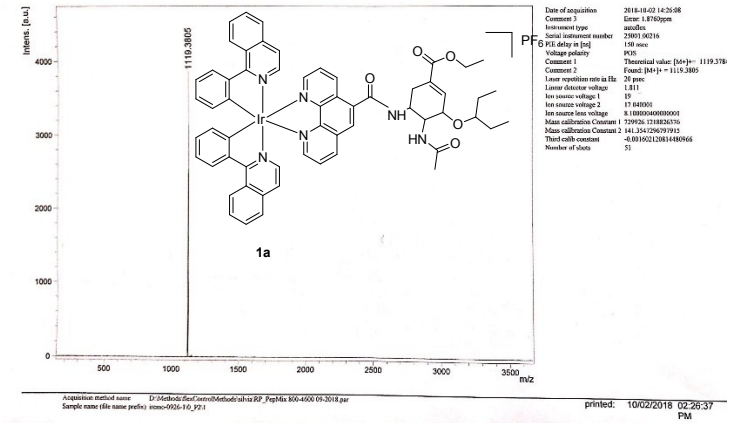

PF6
OH
11a
f1 (ppm)

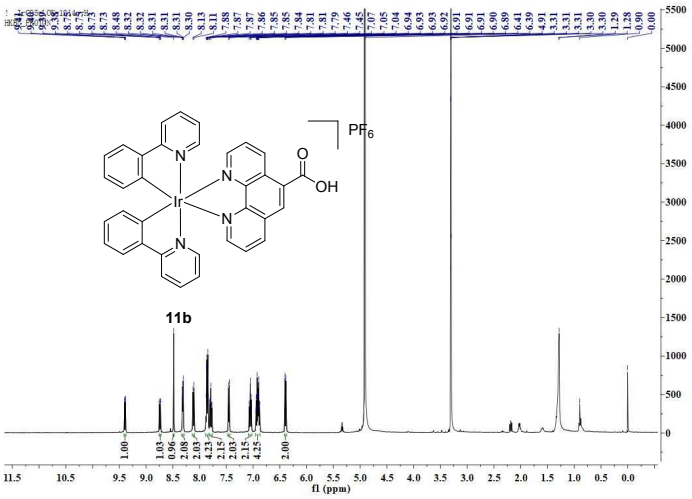

HONG KONG BAPTIST UNIVERSITY, DEPARTMENT OF CHEMISTRY (MALDI-TOF)
825.1796
PF6
OH
11a
m/z
printed: 09/18/2018 04:26:33 PM

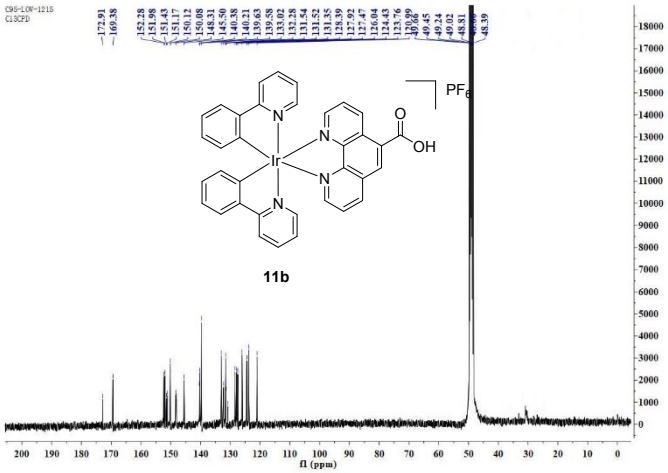


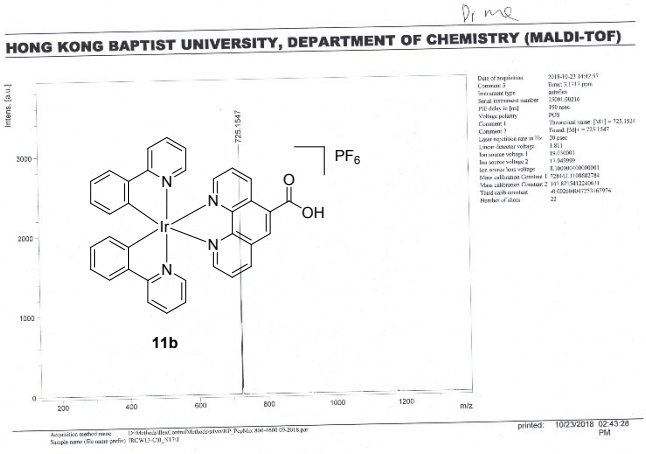


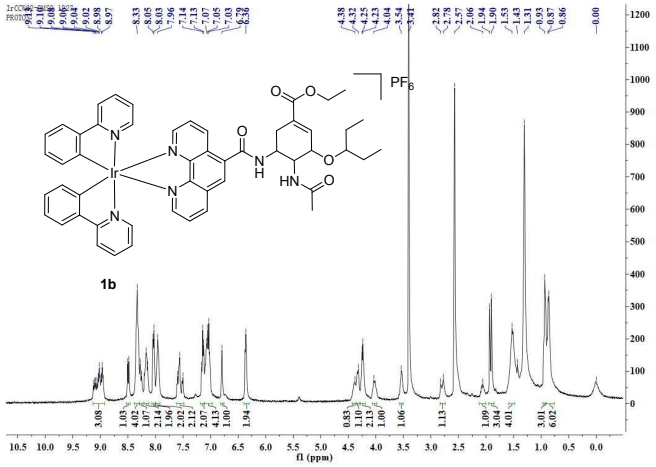
HONG KONG BAPTIST UNIVERSITY, DEPARTMENT OF CHEMISTRY (MALDI-TOF)

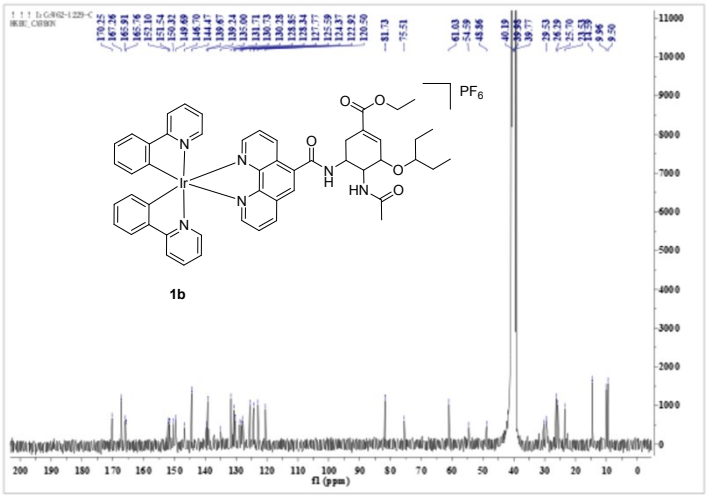



PF6
N
Ir
N
N
O
OH
11b
1.00
1.03
0.96
2.08
2.03
4.23
2.15
2.10
2.15
4.25
2.00
f1 (ppm)

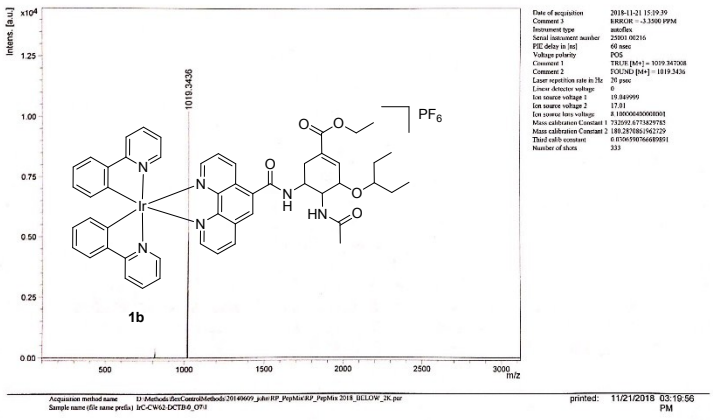

C95-LOF-1215
C13CPD
PF6
N
Ir
N
N
O
OH
11b
f1 (ppm)

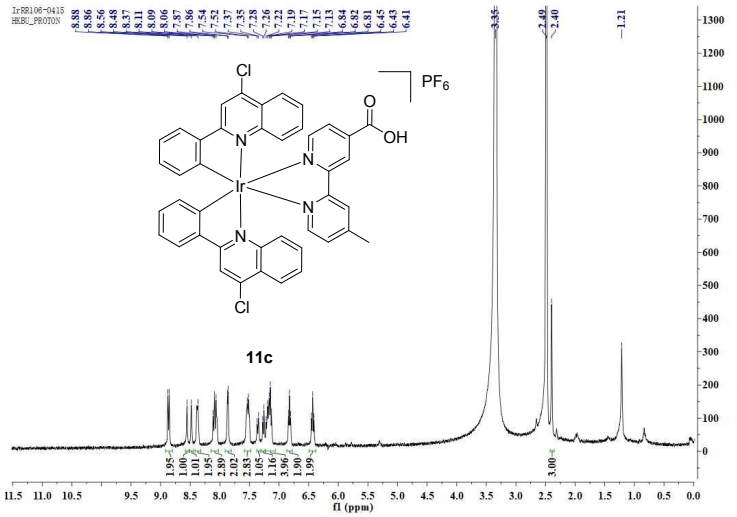

HONG KONG BAPTIST UNIVERSITY, DEPARTMENT OF CHEMISTRY (MALDI-TOF)
Intens. [a.u.]
725.1547
PF6
N
Ir
N
N
O
OH
11b
m/z
printed: 10/23/2018 02:43:28 PM

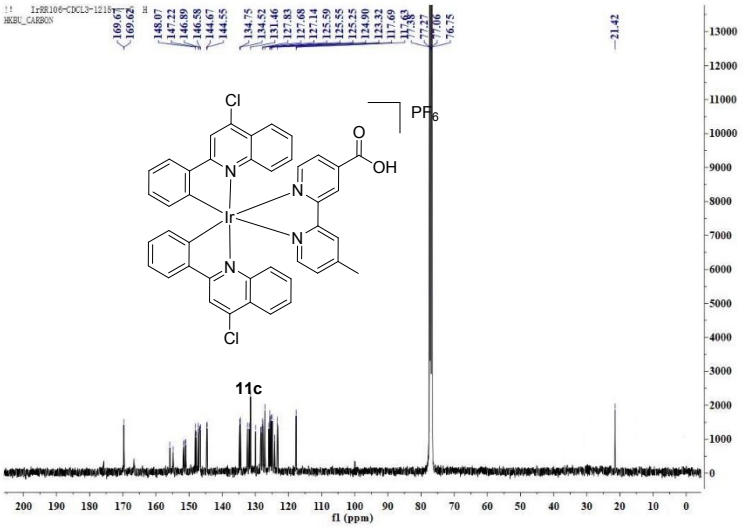


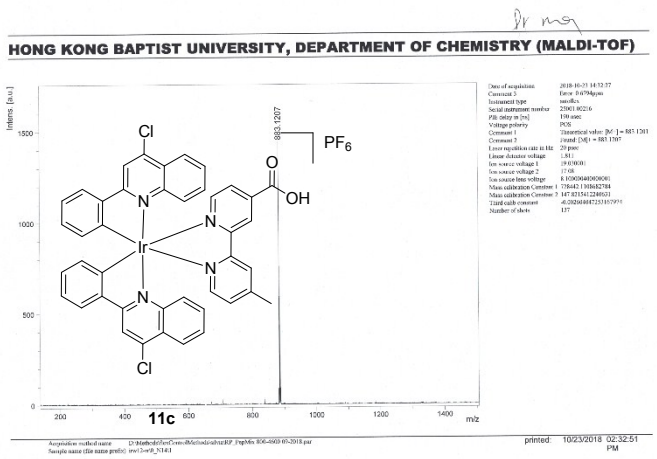


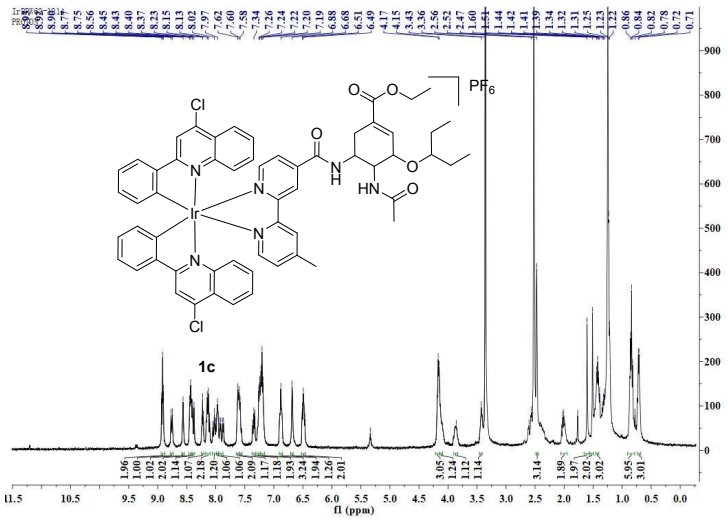
HONG KONG BAPTIST UNIVERSITY, DEPARTMENT OF CHEMISTRY (MALDI-TOF)

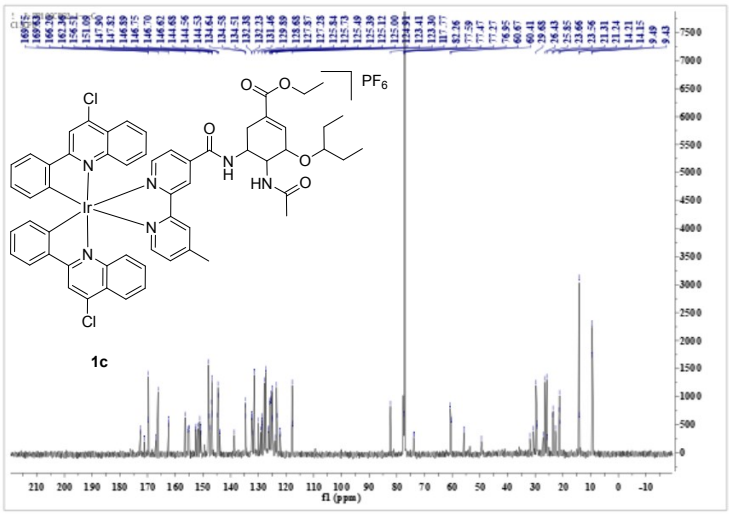



11c
PF6
fl (ppm)

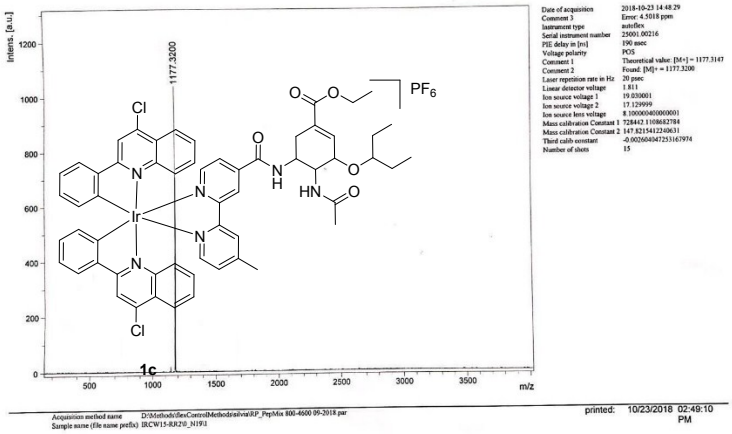

11c
PF6
fl (ppm)

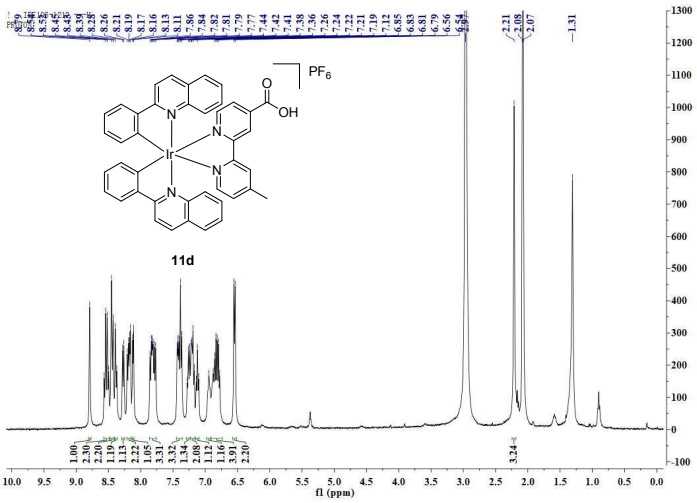

HONG KONG BAPTIST UNIVERSITY, DEPARTMENT OF CHEMISTRY (MALDI-TOF)
11c
PF6
m/z

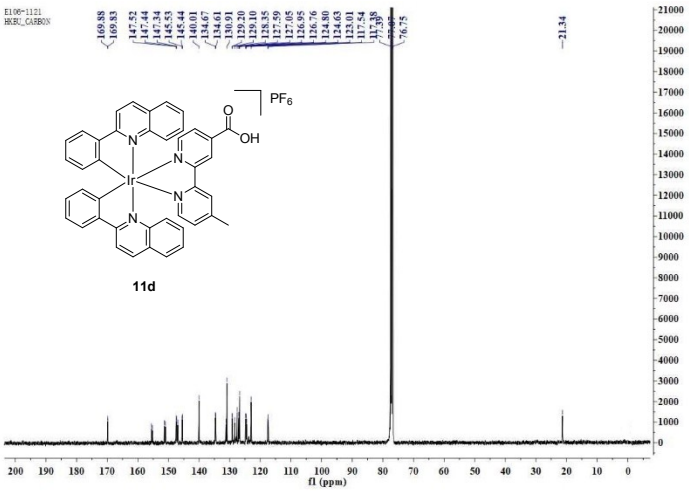




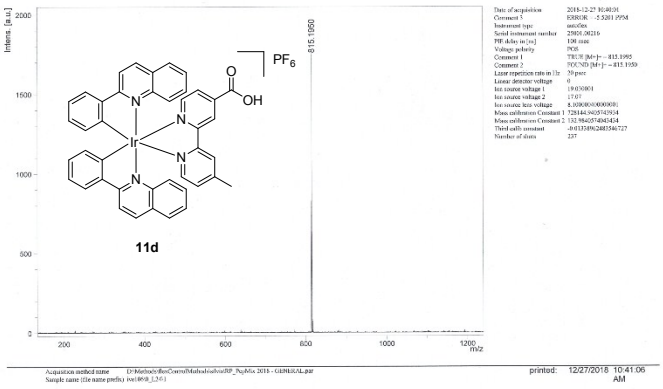
HONG KONG BAPTIST UNIVERSITY, DEPARTMENT OF CHEMISTRY (MALDI-TOF)

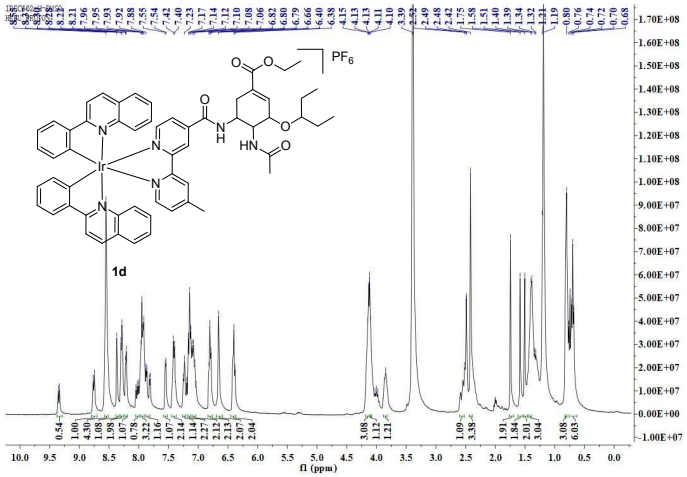


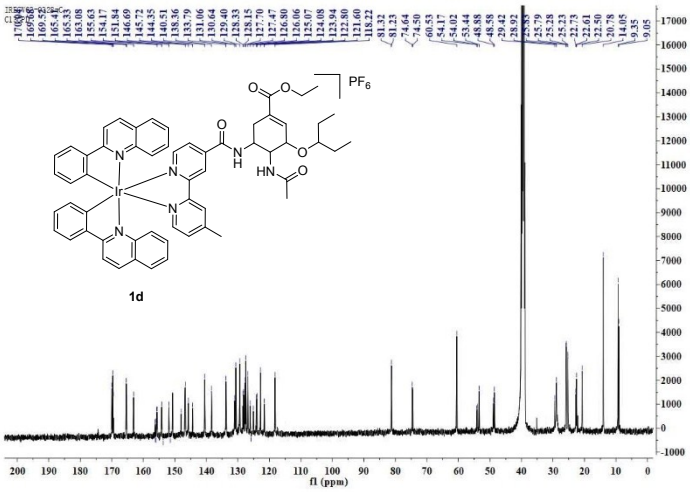




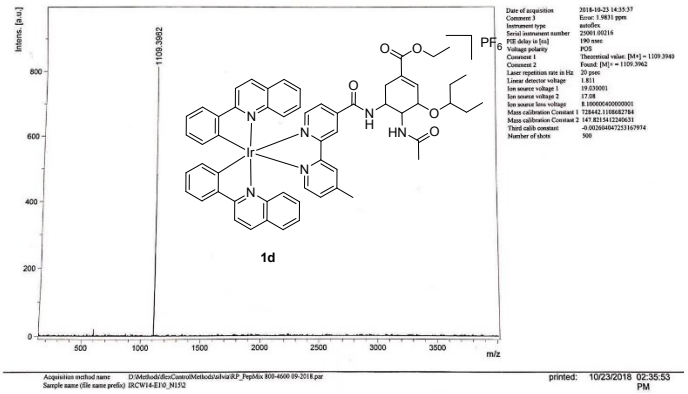
HONG KONG BAPTIST UNIVERSITY, DEPARTMENT OF CHEMISTRY (MALDI-TOF)







HONG KONG BAPTIST UNIVERSITY, DEPARTMENT OF CHEMISTRY (MALDI-TOF)



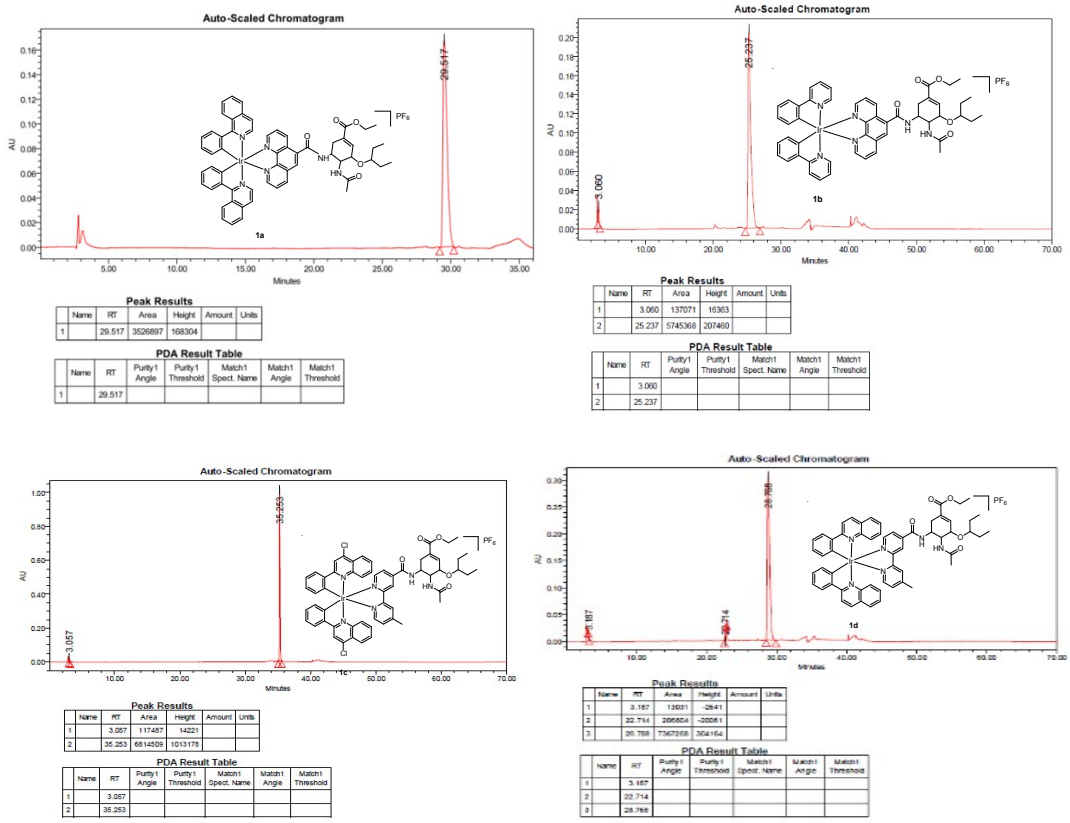


Fig. S1 ^{1}H NMR, ^{13}C NMR, HRMS and HPLC spectra for compounds **9**, **10**, **11a–11d** and **1a–1d**.

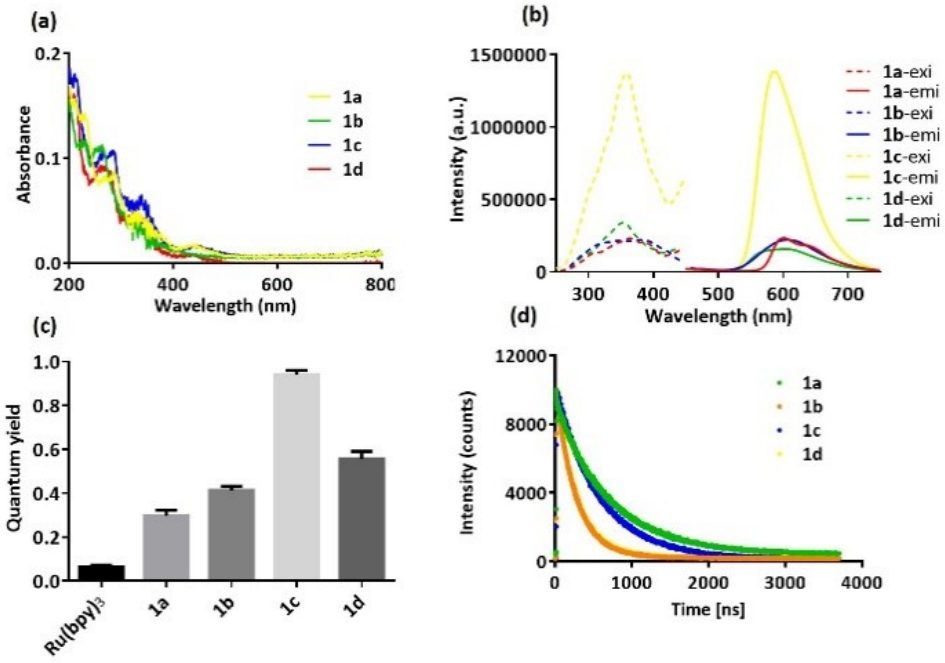


Fig. S2 (a) Absorbance spectra of complexes **1a–1d** (10 μM) in ACN. **(b)** Excitation and emission spectra of complexes **1a–1d** (5 μM) in PBS buffer containing 0.5% ACN. **(c)**

Quantum yields of complexes **1a–1d** (10 μM). **(d)** Emission decay curves of complexes **1a–1d** (10 μM) in DMSO.

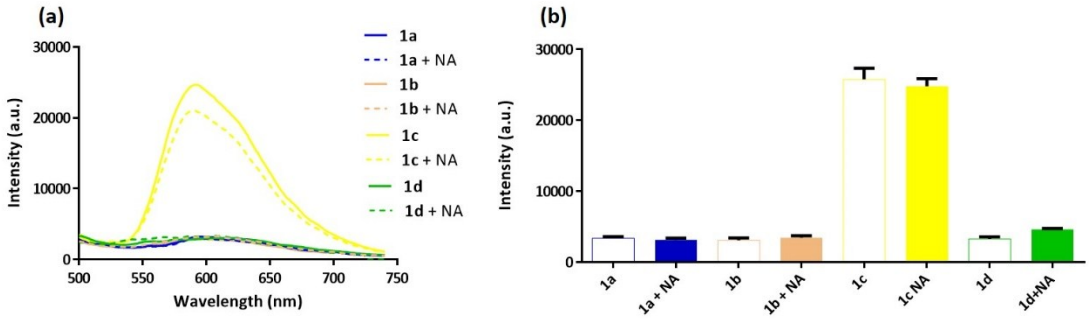


Fig. S3 (a) Emission spectra of the response of complexes **1a–1d** (100 μM) as a luminescent probe for NA (10 μg/mL) in PBS buffer containing 0.5% ACN. **(b)** Column diagram of the response of complexes **1a–1d** (100 μM) as a luminescent probe for NA (10 μg/mL). The luminescence intensity was detected at ex/em = 330 nm/600 nm.

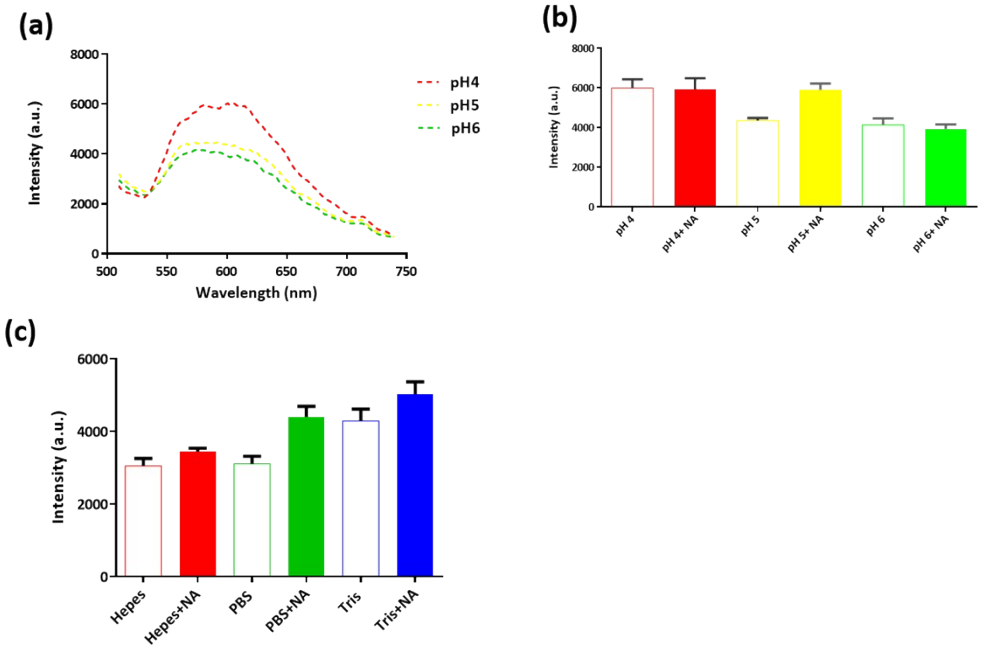


Fig. S4 (a) Luminescence spectra of complex **1d** (5 μM) in PBS buffer at various pH values; **(b)** Luminescence intensity of complex **1d** (5 μM) at the absence and presence of NA (10 μM) in PBS buffer with various pH values; **(c)** Luminescence intensity of complex **1d** (5 μM) at the absence and presence of NA (10 μM) in different buffer

solutions containing 0.5% ACN.

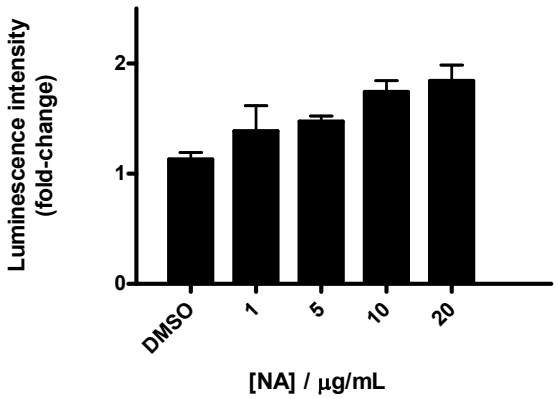


Fig. S5 Dose-dependent assay using complex **1d** (100 μM) as a luminescent probe for NA (0–20 μg/mL). The luminescence intensity was detected at ex/em = 330 nm/600 nm.

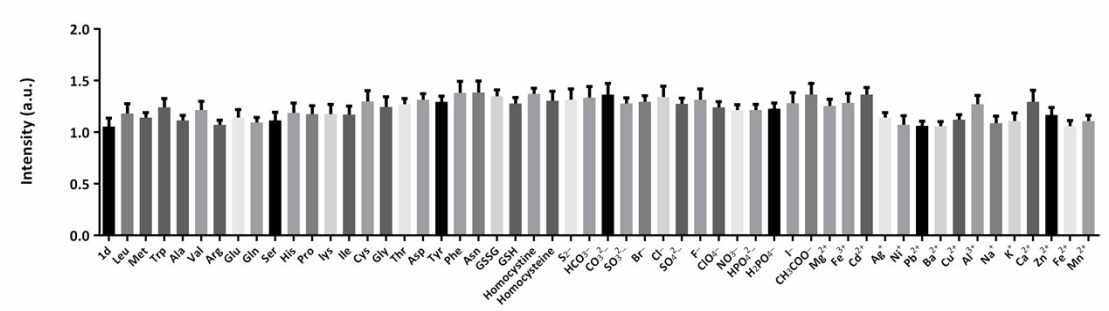


Fig. S6 Luminescence intensity of complex **1d** (5 μM) in the absence and presence of various potentially interfering species including amino acids, anions or cations (20 μM).

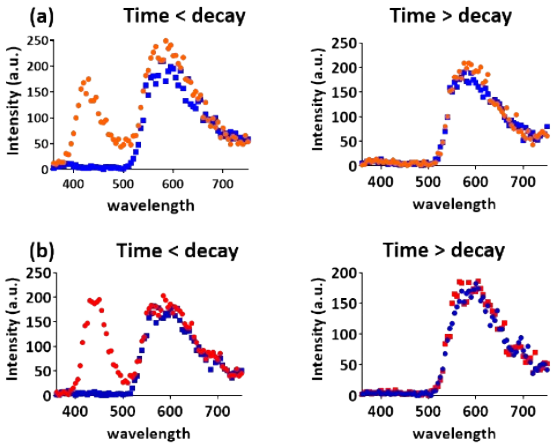


Fig. S7 Time-resolved spectra of complex **1d** (10 μM) with (red dots) or without (blue dots) the presence of fluorescent organic dyes **(a)** thioflavin S (THS) and **(b)** coumarin 460 (Cm460) as two model matrix interferences in DMSO.

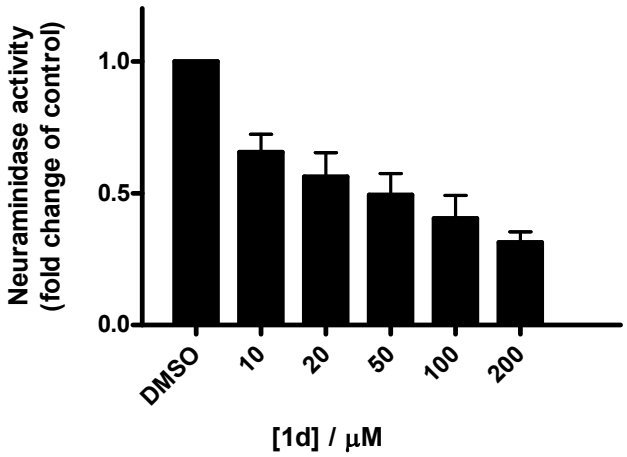


Fig. S8 Dose-dependent assay using complex **1d** (0–200 μM) as an inhibitor for NA (10 μg/mL). The luminescence intensity was detected at ex/em=330 nm/450 nm.

References

1. M. Wang, Z. Mao, T. S. Kang, C. Y. Wong, J. L. Mergny, C.-H. Leung, D.-L. Ma, *Chem. Sci.*, 2016, **7**, 2516.
2. D.-L. Ma, T. Xu, D. S.-H. Chan, B. Y.-W. Man, C.-H. Leung, *Nucleic Acids Res.*, 2011, **39**, e67.
3. W. Zhang, F. Zhang, Y.-L. Wang, B. Song, R. Zhang and J. Yuan, *Inorg. Chem.*, 2017, **56**, 1309-1318.
4. K. Vellaisamy, G. Li, C.-N. Ko, H.-J. Zhong, S. Fatima, H.-Y. Kwan, C.-Y. Wong, W.-J. Kwong, W. Tan, C.-H. Leung, D.-L. Ma, *Chem. Sci.*, 2018, **9**, 1119.

5. H.-J. Zhong, L. Lu, K.-H. Leung, C. C. Wong, C. Peng, S.-C. Yan, D.-L. Ma, Z. Cai, H.-M. D. Wang and C.-H. Leung, *Chem. Sci.*, 2015, **6**, 5400-5408.
6. L.-J. Liu, L. Lu, H.-J. Zhong, B. He, D. W. J. Kwong, D.-L. Ma and C.-H. Leung, *J. Med. Chem.*, 2015, **58**, 6697-6703.
7. L. Lu, H.-J. Zhong, S.-L. H. Modi Wang, H.-W. Li, C.-H. Leung and D.-L. Ma, *Sci. Rep.*, 2015, **5**, 14619-14623.
8. D. L. Ma, L. J. Liu, K. H. Leung, Y. T. Chen, H. J. Zhong, D. S. H. Chan, H. M. D. Wang and C. H. Leung, *Angew. Chem. Int. Ed. Engl.,* 2014, **53**, 9178-9182.
9. M. Wang, K.-H. Leung, S. Lin, D. S.-H. Chan, C.-H. Leung and D.-L. Ma, *J. Mater. Chem. B*, 2014, **2**, 6467-6471.
10. K.-H. Leung, L. Lu, M. Wang, T.-Y. Mak, D. S.-H. Chan, F.-K. Tang, C.-H. Leung, H.-Y. Kwan, Z. Yu and D.-L. Ma, *PloS One*, 2013, **8**, e77021.
11. L. Lu, D. S.-H. Chan, D. W. Kwong, H.-Z. He, C.-H. Leung and D.-L. Ma, *Chem. Sci.*, 2014, **5**, 4561-4568.
12. L. Lu, M. Wang, L.-J. Liu, C.-H. Leung and D.-L. Ma, *ACS Appl. Mater. Interfaces*, 2015, **7**, 8313-8318.