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# Luminescent rhenium(I) polypyridine complexes with an isothiocyanate moiety–versatile labelling reagents for biomolecules

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The synthesis, characterisation and photophysical properties of a series of new rhenium(I) polypyridine complexes, $[Re(N–N)(CO)_3(py\text{-}3\text{-}NCS)]^+$, are reported; the isothiocyanate moiety enables these complexes to react with primary amine groups of modified oligonucleotides and proteins.

## Introduction

Design of biological labels for various therapeutic, diagnostic and mechanistic applications is currently attracting much interest. Transition metal complexes, by virtue of their variable oxidation states, unique photophysical and photochemical properties, and variable coordination geometries, have been covalently linked to different biomolecules in electron-transfer studies and other analytical applications.[1] In this context, rhenium(I) polypyridine complexes show a great potential to be developed in bioanalytical applications in view of their remarkable photophysical and photochemical properties.[2–7] Recently, studies on the utilisation of luminescent rhenium(I) polypyridine complexes as a DNA probe using extensively planar diimine ligands,[4] as well as from a strategy based on intramolecular energy-transfer quenching,[5] have been reported. These complexes have also been used as photo-oxidants in the studies of electron tunnelling in proteins,[6] and as anisotropy probes for protein hydrodynamics.[7] However, to date, a systematic design of luminescent rhenium(I) polypyridine complexes as a label for biological substrates, such as DNA and proteins, has not been realised.

On the other hand, although labelling of biological substrates such as nucleic acids and proteins with fluorescent organic dyes is a routine procedure nowadays,[8] the short fluorescence lifetimes, self-quenching effects, photobleaching and pH-dependence of these compounds are still significant disadvantages. We envisage that the intense and long-lived photoluminescence properties of rhenium(I) polypyridine complexes can be exploited in the development of luminescent labelling reagents for biomolecules because (1) the use of various diimine ligands for the rhenium(I) complexes can alter the MLCT emission energy and give rise to a series of multi-colour labelling reagents; (2) the large Stokes' shifts could minimise self-quenching effects which are commonly observed in multiple labelling of biomolecules with fluorescent organic dyes such as fluorescein;[9] and (3) the long emission lifetimes of rhenium(I) polypyridine complexes could be applied in time-resolved detection techniques that can offer higher sensitivity.[10]

The isothiocyanate functional group has captured our attention as it has been commonly used in the labelling of biological molecules with fluorescent organic dyes.[11] In this paper, we report the synthesis, characterisation and photophysical properties of a series of new luminescent rhenium(I) polypyridine complexes containing an isothiocyanate moiety, $[Re(N–N)(CO)_3(py\text{-}3\text{-}NCS)](CF_3SO_3)$ [N–N = 2,2′-bipyridine, bpy **1**; 4,4′-dimethyl-2,2′-bipyridine, 4,4′-$Me_2$-bpy **2**; 4,4′-bis(*tert*-butyl)-2,2′-bipyridine, 4,4′-$Bu^t_2$-bpy **3**; 1,10-phenanthroline, phen **4**; 2,9-dimethyl-1,10-phenanthroline, 2,9-$Me_2$-phen **5**; 4,7-dimethyl-1,10-phenanthroline, 4,7-$Me_2$-phen **6**; 3,4,7,8-tetramethyl-1,10-phenanthroline, 3,4,7,8-$Me_4$-phen **7**; 5-phenyl-1,10-phenanthroline, 5-Ph-phen **8**; 5-chloro-1,10-phenanthroline, 5-Cl-phen **9**; 4,7-diphenyl-1,10-phenanthroline, 4,7-$Ph_2$-phen **10**; 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline, 2,9-$Me_2$-4,7-$Ph_2$-phen **11**; 6,7-dihydro-5,8-dimethyldibenzo-[*b*, *j*][1,10]phenanthroline, HM-phen **12** and 2,2′-biquinoline, biq **13**; py-3-NCS = 3-pyridyl isothiocyanate]. The X-ray crystal structure of **1** has also been determined. The tunability of the luminescence properties of these complexes by variation of the diimine ligands has been studied. Different biomolecules have been labelled with **4** and the photophysical properties of the bioconjugates have also been examined.

## Experimental

### Materials and reagents

All solvents were of analytical reagent grade. $Re(CO)_5Cl$, 3-aminopyridine (py-3-$NH_2$), thiophosgene and all the diimine ligands (N–N) were purchased from Aldrich and were used without purification. The oligonucleotides **M13–R**, **M13–20**, **M13–50** and **M13–80** (Scheme 1) were purchased from Sigma-Genosys. Human serum albumin (HSA) fraction V was obtained from Calbiochem and was used as received. All buffer components were of molecular biology grade and used without purification.

**M13-R**: 5′-$H_2N$-$(CH_2)_6$-AACAGCTATGACCATG-3′
**M13-20**: 5′-TAATCATGGTCATAGCTGTT-3′
**M13-50**: 5′-GAGTCGACCTGCAGGCATGCAAGCTTGGCG TAATCATGGTCATAGCTGTT-3′
**M13-80**: 5′-AATTCGAGCTCGGTACCCGGGGATCCTCTA GAGTCGACCTGCAGGCATGCAAGCTTGGCGTAATCATGGT CATAGCTGTT-3′

**Scheme 1** DNA sequences of **M13-R**, **M13–20**, **M13–50** and **M13–80**. The underlined sequence is complementary to that of **M13–R**.

### Synthesis of complexes

The precursor complexes $[Re(N–N)(CO)_3(py\text{-}3\text{-}NH_2)](CF_3SO_3)$ were prepared from the reactions of $[Re(N–N)(CO)_3(CH_3CN)]$-

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($CF_3SO_3$) and py-3-$NH_2$ according to a related literature procedure.[2b]

**[Re(bpy)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$) 1.** The synthetic procedure of **1** was based on a reported method for the preparation of a ruthenium(II) isothiocyanate complex.[12] In a typical reaction, thiophosgene (27 μl, 0.36 mmol) was added to a mixture of [Re(bpy)(CO)$_3$(py-3-$NH_2$)](CF$_3$SO$_3$) (121 mg, 0.18 mmol) and finely crushed $CaCO_3$ (73 mg, 0.72 mmol) in 10 ml acetone under an inert atmosphere of nitrogen. The suspension was stirred in the dark for two hours at room temperature. The suspension was then filtered and evaporated to dryness. Subsequent recrystallisation of the complex from acetone–diethyl ether–petroleum ether afforded **1** as air-stable yellow crystals. Yield: 109 mg (85%). $^1$H NMR (300 MHz, acetone-d$_6$, 298 K, relative to TMS): $\delta$ 9.51 (d, 2H, $J$ = 4.9 Hz, H6 and H6′ of bpy), 8.79 (d, 2H, $J$ = 8.2 Hz, H3 and H3′ of bpy), 8.66 (d, 1H, $J$ = 1.9 Hz, H2 of py-3-NCS), 8.55 (d, 1H, $J$ = 5.5 Hz, H6 of py-3-NCS), 8.49 (m, 2H, H4 and H4′ of bpy), 8.07–8.00 (m, 3H, H5 and H5′ of bpy, and H4 of py-3-NCS), 7.58 (dd, 1H, $J$ = 8.2 and 5.5 Hz, H5 of py-3-NCS). Anal. calc. for [Re(bpy)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$)·1/4 (Me$_2$CO): C, 34.32; H, 1.87; N, 7.72. Found: C, 34.38; H, 1.78; N, 7.44%. IR (KBr) $\nu$/cm$^{-1}$: 2061 (m, N=C=S), 2033 (s, C≡O), 1911 (s, C≡O). Positive-ion ESI-MS: $m/z$ at 562 {[Re(bpy)(CO)$_3$(py-3-NCS)]}$^+$.

**[Re(4,4′-Me$_2$-bpy)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$) 2.** The procedure was similar to that described for the preparation of complex **1**, except that [Re(4,4′-Me$_2$-bpy)(CO)$_3$(py-3-$NH_2$)](CF$_3$SO$_3$) (126 mg, 0.18 mmol) was used instead of [Re(bpy)(CO)$_3$(py-3-$NH_2$)](CF$_3$SO$_3$). **2** was isolated as air-stable yellow crystals. Yield: 114 mg (86%). $^1$H NMR (300 MHz, acetone-d$_6$, 298 K, relative to TMS): $\delta$ 9.31 (d, 2H, $J$ = 5.8 Hz, H6 and H6′ of 4,4′-Me$_2$-bpy), 8.65 (s, 2H, H3 and H3′ of 4,4′-Me$_2$-bpy), 8.63 (d, 1H, $J$ = 2.2 Hz, H2 of py-3-NCS), 8.53 (d, 1H, $J$ = 5.5 Hz, H6 of py-3-NCS), 8.09–8.05 (m, 1H, H4 of py-3-NCS), 7.84 (d, 2H, $J$ = 5.8 Hz, H5 and H5′ of 4,4′-Me$_2$-bpy), 7.58 (dd, 1H, $J$ = 8.5 and 5.5 Hz, H5 of py-3-NCS), 2.64 (s, 6H, Me of 4,4′-Me$_2$-bpy). Anal. calc. for [Re(4,4′-Me$_2$-bpy)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$)·1/2(H$_2$O): C, 35.29; H, 2.29; N, 7.48. Found: C, 35.35: H, 2.26; N, 7.47%. IR (KBr) $\nu$/cm$^{-1}$: 2111 sh (m, N=C=S), 2031 (s, C≡O), 1917 (s, C≡O). Positive-ion ESI-MS: $m/z$ at 590 {[Re(4,4′-Me$_2$-bpy)(CO)$_3$(py-3-NCS)]}$^+$, 454 {[Re(4,4′-Me$_2$-bpy)(CO)$_3$]}$^+$.

**[Re(4,4′-Bu$^t_2$-bpy)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$) 3.** The procedure was similar to that described for the preparation of complex **1**, except that [Re(4,4′-Bu$^t_2$-bpy)(CO)$_3$(py-3-$NH_2$)](CF$_3$SO$_3$) (141 mg, 0.18 mmol) was used instead of [Re(bpy)(CO)$_3$(py-3-$NH_2$)](CF$_3$SO$_3$). **3** was isolated as air-stable yellow crystals. Yield: 123 mg (83%). $^1$H NMR (300 MHz, acetone-d$_6$, 298 K, relative to TMS): $\delta$ 9.37 (d, 2H, $J$ = 6.0 Hz, H6 and H6′ of 4,4′-Bu$^t_2$-bpy), 8.78 (d, 2H, $J$ = 1.6 Hz, H3 and H3′ of 4,4′-Bu$^t_2$-bpy), 8.57–8.53 (m, 2H, H2 and H6 of py-3-NCS), 8.06–7.99 (m, 3H, H5 and H5′ of 4,4′-Bu$^t_2$-bpy, and H4 of py-3-NCS), 7.58 (dd, 1H, $J$ = 8.5 and 5.8 Hz, H5 of py-3-NCS), 1.45 (s, 18H, Bu$^t$ of 4,4′-Bu$^t_2$-bpy). Anal. calc. for [Re(4,4′-Bu$^t_2$-bpy)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$)·1/4(Me$_2$CO)·1/2(H$_2$O): C, 40.75; H, 3.63; N, 6.61. Found: C, 40.76; H, 3.50; N, 6.46%. IR (KBr) $\nu$/cm$^{-1}$: 2100 sh (w, N=C=S), 2031 (s, C≡O), 2022 (s, C≡O), 1918 (s, C≡O). Positive-ion ESI-MS: $m/z$ at 674 {[Re(4,4′-Bu$^t_2$-bpy)(CO)$_3$(py-3-NCS)]}$^+$.

**[Re(phen)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$) 4.** The procedure was similar to that described for the preparation of complex **1**, except that [Re(phen)(CO)$_3$(py-3-$NH_2$)](CF$_3$SO$_3$) (125 mg, 0.18 mmol) was used instead of [Re(bpy)(CO)$_3$(py-3-$NH_2$)](CF$_3$SO$_3$). **4** was isolated as air-stable yellow crystals. Yield: 116 mg (88%). $^1$H NMR (300 MHz, acetone-d$_6$, 298 K, relative to TMS): $\delta$ 9.93 (d, 2H, $J$ = 5.2 Hz, H2 and H9 of phen), 9.11 (d, 2H, $J$ = 8.5 Hz, H4 and H7 of phen), 8.70 (d, 1H, $J$ = 2.2 Hz, H2 of py-3-NCS), 8.62 (d, 1H, $J$ = 5.5 Hz, H6 of py-3-NCS), 8.37–8.33 (m, 4H, H3, H5, H6 and H8 of phen), 7.95–7.91 (m, 1H, H4 of py-3-NCS), 7.46 (dd, 1H, $J$ = 8.2 and 5.5 Hz, H5 of py-3-NCS). Anal. calc. for [Re(phen)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$)·3(H$_2$O): C, 33.46; H, 2.30; N, 7.09. Found: C, 33.56; H, 2.33; N, 7.15%. IR (KBr) $\nu$/cm$^{-1}$: 2111 (m, N=C=S), 2033 (s, C≡O), 1928 (s, C≡O), 1905 (s, C≡O). Positive-ion ESI-MS: $m/z$ at 586 {[Re(phen)(CO)$_3$(py-3-NCS)]}$^+$, 450 {[Re(phen)(CO)$_3$]}$^+$.

**[Re(2,9-Me$_2$-phen)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$) 5.** The procedure was similar to that described for the preparation of complex **1**, except that [Re(2,9-Me$_2$-phen)(CO)$_3$(py-3-$NH_2$)](CF$_3$SO$_3$) (130 mg, 0.18 mmol) was used instead of [Re(bpy)(CO)$_3$(py-3-$NH_2$)](CF$_3$SO$_3$). **5** was isolated as air-stable yellow crystals. Yield: 123 mg (89%). $^1$H NMR (300 MHz, acetone-d$_6$, 298 K, relative to TMS): $\delta$ 8.87 (d, 2H, $J$ = 8.5 Hz, H4 and H7 of 2,9-Me$_2$-phen), 8.27 (d, 2H, $J$ = 8.5 Hz, H3 and H8 of 2,9-Me$_2$-phen), 8.20–8.17 (m, 4H, H5 and H6 of 2,9-Me$_2$-phen, and H2 and H6 of py-3-NCS), 7.94–7.91 (m, 1H, H4 of py-3-NCS), 7.37 (dd, 1H, $J$ = 8.2 and 5.8 Hz, H5 of py-3-NCS), 3.48 (s, 6H, Me of 2,9-Me$_2$-phen). Anal. calc. for [Re(2,9-Me$_2$-phen)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$)·1/4(Me$_2$CO): C, 38.20; H, 2.27; N, 7.20. Found: C, 38.06; H, 2.22; N, 7.05%. IR (KBr) $\nu$/cm$^{-1}$: 2127 (m, N=C=S), 2030 (s, C≡O), 1917 (s, C≡O). Positive-ion ESI-MS: $m/z$ at 614 {[Re(2,9-Me$_2$-phen)(CO)$_3$(py-3-NCS)]}$^+$, 478 {[Re(2,9-Me$_2$-phen)(CO)$_3$]}$^+$.

**[Re(4,7-Me$_2$-phen)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$) 6.** The procedure was similar to that described for the preparation of complex **1**, except that [Re(4,7-Me$_2$-phen)(CO)$_3$(py-3-$NH_2$)](CF$_3$SO$_3$) (130 mg, 0.18 mmol) was used instead of [Re(bpy)(CO)$_3$(py-3-$NH_2$)](CF$_3$SO$_3$). **6** was isolated as air-stable yellow crystals. Yield: 117 mg (85%). $^1$H NMR (300 MHz, acetone-d$_6$, 298 K, relative to TMS): $\delta$ 9.78 (d, 2H, $J$ = 5.3 Hz, H2 and H9 of 4,7-Me$_2$-phen), 8.70 (d, 1H, $J$ = 2.2 Hz, H2 of py-3-NCS), 8.61 (dd, 1H, $J$ = 5.6 and 0.9 Hz, H6 of py-3-NCS), 8.52 (s, 2H, H5 and H6 of 4,7-Me$_2$-phen), 8.22 (dd, 2H, $J$ = 5.3 and 0.8 Hz, H3 and H8 of 4,7-Me$_2$-phen), 7.99–7.95 (m, 1H, H4 of py-3-NCS), 7.49 (dd, 1H, $J$ = 8.3 and 5.6 Hz, H5 of py-3-NCS), 3.09 (s, 6H, Me of 4,7-Me$_2$-phen). Anal. calc. for [Re(4,7-Me$_2$-phen)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$)·(H$_2$O): C, 36.87; H, 2.32; N, 7.17. Found: C, 37.00, H, 2.23; N, 7.08%. IR (KBr) $\nu$/cm$^{-1}$: 2115 sh (w, N=C=S), 2032 (s, C≡O), 1930 (s, C≡O). Positive-ion ESI-MS: $m/z$ at 614 {[Re(4,7-Me$_2$-phen)(CO)$_3$(py-3-NCS)]}$^+$.

**[Re(3,4,7,8-Me$_4$-phen)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$) 7.** The procedure was similar to that described for the preparation of complex **1**, except that [Re(3,4,7,8-Me$_4$-phen)(CO)$_3$(py-3-$NH_2$)](CF$_3$SO$_3$) (135 mg, 0.18 mmol) was used instead of [Re(bpy)(CO)$_3$(py-3-$NH_2$)](CF$_3$SO$_3$). **7** was isolated as air-stable yellow crystals. Yield: 132 mg (92%). $^1$H NMR (300 MHz, acetonitrile-d$_3$, 298 K, relative to TMS): $\delta$ 9.32 (s, 2H, H2 and H9 of 3,4,7,8-Me$_4$-phen), 8.29 (s, 2H, H5 and H6 of 3,4,7,8-Me$_4$-phen), 8.27–8.20 (m, 2H, H2 and H6 of py-3-NCS), 7.68–7.64 (m, 1H, H4 of py-3-NCS), 7.20 (dd, 1H, $J$ = 8.2 and 5.5 Hz, H5 of py-3-NCS), 2.82 (s, 6H, Me at C4 and C7 of 3,4,7,8-Me$_4$-phen), 2.68 (s, 6H, Me at C3 and C8 of 3,4,7,8-Me$_4$-phen). Anal. calc. for [Re(3,4,7,8-Me$_4$-phen)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$)·2(H$_2$O): C, 37.72; H, 2.92; N, 6.77. Found: C, 37.99; H, 3.10; N, 6.65%. IR (KBr) $\nu$/cm$^{-1}$: 2107 sh (w, N=C=S), 2033 (s, C≡O), 1910 (s, C≡O). Positive-ion ESI-MS: $m/z$ at 642 {[Re(3,4,7,8-Me$_4$-phen)(CO)$_3$(py-3-NCS)]}$^+$.

**[Re(5-Ph-phen)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$) 8.** The procedure was similar to that described for the preparation of complex **1**, except that [Re(5-Ph-phen)(CO)$_3$(py-3-$NH_2$)](CF$_3$SO$_3$) (139 mg, 0.18 mmol) was used instead of [Re(bpy)(CO)$_3$(py-3-$NH_2$)](CF$_3$SO$_3$). **8** was isolated as air-stable yellow crystals.

Yield: 139 mg (95%). $^1$H NMR (300 MHz, acetone-$d_6$, 298 K, relative to TMS): $\delta$ 9.99 (d, 1H, $J$ = 5.2 Hz, H2 of 5-Ph-phen), 9.95 (d, 1H, $J$ = 5.2 Hz, H9 of 5-Ph-phen), 9.13 (d, 1H, $J$ = 8.5 Hz, H4 of 5-Ph-phen), 8.88 (d, 1H, $J$ = 8.5 Hz, H7 of 5-Ph-phen), 8.76 (d, 1H, $J$ = 2.2 Hz, H2 of py-3-NCS), 8.67 (d, 1H, $J$ = 5.2 Hz, H6 of py-3-NCS), 8.41–8.31 (m, 3H, H3, H6 and H8 of 5-Ph-phen), 7.99–7.96 (m, 1H, H4 of py-3-NCS), 7.64–7.59 (m, 5H, Ph of 5-Ph-phen), 7.50 (dd, 1H, $J$ = 8.2 and 5.5 Hz, H5 of py-3-NCS). Anal. calc. for [Re(5-Ph-phen)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$)·(Me$_2$CO)·(H$_2$O): C, 41.94; H, 2.72; N, 6.31. Found: C, 42.15; H, 2.63; N, 6.19%. IR (KBr) $\nu$/cm$^{-1}$: 2100 sh (w, N=C=S), 2033 (s, C≡O), 1918 (s, C≡O). Positive-ion ESI-MS: $m/z$ at 662 {[Re(5-Ph-phen)(CO)$_3$(py-3-NCS)]}$^+$.

**[Re(5-Cl-phen)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$) 9.** The procedure was similar to that described for the preparation of complex **1**, except that [Re(5-Cl-phen)(CO)$_3$(py-3-NH$_2$)](CF$_3$SO$_3$) (131 mg, 0.18 mmol) was used instead of [Re(bpy)(CO)$_3$(py-3-NH$_2$)](CF$_3$SO$_3$). **9** was isolated as air-stable yellow crystals. Yield: 124 mg (90%). $^1$H NMR (300 MHz, acetone-$d_6$, 298 K, relative to TMS): $\delta$ 10.02 (d, 1H, $J$ = 5.2 Hz, H2 of 5-Cl-phen), 9.93 (d, 1H, $J$ = 5.2 Hz, H9 of 5-Cl-phen), 9.25 (d, 1H, $J$ = 8.5 Hz, H4 of 5-Cl-phen), 9.07 (d, 1H, $J$ = 8.5 Hz, H7 of 5-Cl-phen), 8.70 (d, 1H, $J$ = 2.2 Hz, H2 of py-3-NCS), 8.63–8.61 (m, 2H, H6 of 5-Cl-phen and H6 of py-3-NCS), 8.48 (dd, 1H, $J$ = 8.5 and 5.2 Hz, H3 of 5-Cl-phen), 8.37 (dd, 1H, $J$ = 8.2 and 5.0 Hz, H8 of 5-Cl-phen), 7.96–7.92 (m, 1H, H4 of py-3-NCS), 7.46 (dd, 1H, $J$ = 8.5 and 5.8 Hz, H5 of py-3-NCS). Anal. calc. for [Re(5-Cl-phen)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$)·1/4(Me$_2$CO)·(H$_2$O): C, 34.04; H, 1.82; N, 6.98. Found: C, 34.13; H, 2.10; N, 6.97%. IR (KBr) $\nu$/cm$^{-1}$: 2092 sh (m, N=C=S), 2034 (s, C≡O), 1918 (s, C≡O). Positive-ion ESI-MS: $m/z$ at 620 {[Re(5-Cl-phen)(CO)$_3$(py-3-NCS)]}$^+$.

**[Re(4,7-Ph$_2$-phen)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$) 10.** The procedure was similar to that described for the preparation of complex **1**, except that [Re(4,7-Ph$_2$-phen)(CO)$_3$(py-3-NH$_2$)](CF$_3$SO$_3$) (152 mg, 0.18 mmol) was used instead of [Re(bpy)(CO)$_3$(py-3-NH$_2$)](CF$_3$SO$_3$). **10** was isolated as air-stable orange–yellow crystals. Yield: 147 mg (92%). $^1$H NMR (300 MHz, acetone-$d_6$, 298 K, relative to TMS): $\delta$ 10.01 (d, 2H, $J$ = 5.5 Hz, H2 and H9 of 4,7-Ph$_2$-phen), 8.81 (d, 1H, $J$ = 2.2 Hz, H2 of py-3-NCS), 8.72 (d, 1H, $J$ = 5.8 Hz, H6 of py-3-NCS), 8.31 (d, 2H, $J$ = 5.5 Hz, H3 and H8 of 4,7-Ph$_2$-phen), 8.26 (s, 2H, H5 and H6 of 4,7-Ph$_2$-phen), 8.01–7.97 (m, 1H, H4 of py-3-NCS), 7.74–7.69 (m, 10H, Ph of 4,7-Ph$_2$-phen), 7.51 (dd, 1H, $J$ = 8.2 and 5.8 Hz, H5 of py-3-NCS). Anal. calc. for [Re(4,7-Ph$_2$-phen)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$)·7/4(Me$_2$CO): C, 47.64; H, 3.11; N, 5.66. Found: C, 47.35; H, 2.80; N, 5.38%. IR (KBr) $\nu$/cm$^{-1}$: 2092 sh (w, N=C=S), 2032 (s, C≡O), 1918 (s, C≡O). Positive-ion ESI-MS: $m/z$ at 738 {[Re(4,7-Ph$_2$-phen)(CO)$_3$(py-3-NCS)]}$^+$.

**[Re(2,9-Me$_2$-4,7-Ph$_2$-phen)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$) 11.** The procedure was similar to that described for the preparation of complex **1**, except that [Re(2,9-Me$_2$-4,7-Ph$_2$-phen)(CO)$_3$(py-3-NH$_2$)](CF$_3$SO$_3$) (157 mg, 0.18 mmol) was used instead of [Re(bpy)(CO)$_3$(py-3-NH$_2$)](CF$_3$SO$_3$). **11** was isolated as air-stable yellow crystals. Yield: 138 mg (84%). $^1$H NMR (300 MHz, acetone-$d_6$, 298 K, relative to TMS): $\delta$ 8.29–8.25 (m, 4H, H3 and H8 of 2,9-Me$_2$-4,7-Ph$_2$-phen, and H2 and H6 of py-3-NCS), 8.03 (s, 2H, H5 and H6 of 2,9-Me$_2$-4,7-Ph$_2$-phen), 7.98–7.94 (m, 1H, H4 of py-3-NCS), 7.71–7.65 (m, 10H, Ph of 2,9-Me$_2$-4,7-Ph$_2$-phen), 7.43 (dd, 1H, $J$ = 8.2 and 5.5 Hz, H5 of py-3-NCS), 3.52 (s, 6H, Me of 2,9-Me$_2$-4,7-Ph$_2$-phen). Anal. calc. for [Re(2,9-Me$_2$-4,7-Ph$_2$-phen)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$)·1/4(Me$_2$CO)·2(H$_2$O): C, 45.67; H, 3.08; N, 5.80. Found: C, 45.41; H, 3.33; N, 6.00%. IR (KBr) $\nu$/cm$^{-1}$: 2092 sh (w, N=C=S), 2030 (s, C≡O), 1909 (s, C≡O). Positive-ion ESI-MS: $m/z$ at 766 {[Re(2,9-Me$_2$-4,7-Ph$_2$-phen)(CO)$_3$(py-3-NCS)]}$^+$.

**[Re(HM-phen)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$) 12.** The procedure was similar to that described for the preparation of complex **1**, except that [Re(HM-phen)(CO)$_3$(py-3-NH$_2$)](CF$_3$SO$_3$) (148 mg, 0.18 mmol) was used instead of [Re(bpy)(CO)$_3$(py-3-NH$_2$)](CF$_3$SO$_3$). **12** was isolated as air-stable orange crystals. Yield: 139 mg (89%). $^1$H NMR (300 MHz, acetone-$d_6$, 298 K, relative to TMS): $\delta$ 8.88 (d, 2H, $J$ = 8.8 Hz, H1 and H12 of HM-phen), 8.54 (dd, 2H, $J$ = 8.5 and 1.4 Hz, H4 and H9 of HM-phen), 8.20 (ddd, 2H, $J$ = 8.8, 6.9 and 1.4 Hz, H2 and H11 of HM-phen), 8.04–7.95 (m, 3H, H3 and H10 of HM-phen, and H4 of py-3-NCS), 7.74 (d, 1H, $J$ = 2.5 Hz, H2 of py-3-NCS), 7.70 (d, 1H, $J$ = 5.2 Hz, H6 of py-3-NCS), 7.36 (dd, 1H, $J$ = 8.5 and 5.8 Hz, H5 of py-3-NCS), 3.50–3.37 (m, 2H, CH$_2$ of HM-phen), 3.33–3.18 (m, 2H, CH$_2$ of HM-phen), 2.92 (s, 6H, Me of HM-phen). Anal. calc. for [Re(HM-phen)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$)·1/2(Me$_2$CO)·3(H$_2$O): C, 42.40; H, 3.29; N, 5.90. Found: C, 42.35; H, 3.07; N, 5.61%. IR (KBr) $\nu$/cm$^{-1}$: 2100 sh (w, N=C=S), 2030 (s, C≡O), 1911 (s, C≡O). Positive-ion ESI-MS: $m/z$ at 716 {[Re(HM-phen)(CO)$_3$(py-3-NCS)]}$^+$.

**[Re(biq)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$) 13.** The procedure was similar to that described for the preparation of complex **1**, except that [Re(biq)(CO)$_3$(py-3-NH$_2$)](CF$_3$SO$_3$) (139 mg, 0.18 mmol) was used instead of [Re(bpy)(CO)$_3$(py-3-NH$_2$)](CF$_3$SO$_3$). **13** was isolated as air-stable orange crystals. Yield: 116 mg (80%). $^1$H NMR (300 MHz, acetone-$d_6$, 298 K, relative to TMS): $\delta$ 9.12 (d, 2H, $J$ = 8.5 Hz, H4 and H4′ of biq), 8.97 (d, 2H, $J$ = 9.1 Hz, H8 and H8′ of biq), 8.88 (d, 2H, $J$ = 8.5 Hz, H3 and H3′ of biq), 8.40 (d, 2H, $J$ = 8.0 Hz, H5 and H5′ of biq), 8.30 (t, 2H, $J$ = 7.1 Hz, H7 and H7′ of biq), 8.05 (t, 2H, $J$ = 7.1 Hz, H6 and H6′ of biq), 7.97–7.95 (m, 1H, H4 of py-3-NCS), 7.81 (d, 1H, $J$ = 2.2 Hz, H2 of py-3-NCS), 7.74–7.72 (m, 1H, H6 of py-3-NCS), 7.34 (dd, 1H, $J$ = 7.7 and 6.3 Hz, H5 of py-3-NCS). Anal. calc. for [Re(biq)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$)·6(H$_2$O): C, 36.56; H, 3.07; N, 6.09. Found: C, 36.52; H, 3.02; N, 6.36%. IR (KBr) $\nu$/cm$^{-1}$: 2076 sh (w, N=C=S), 2030 (s, C≡O), 1936 (s, C≡O). Positive-ion ESI-MS: $m/z$ at 662 {[Re(biq)(CO)$_3$(py-3-NCS)]}$^+$.

### Labelling of biomolecules

**Labelling of M13-R with complex 4.** In a typical labelling reaction, **4** (1.0 mg, 1.36 μmol) in 50 μl anhydrous DMSO was added slowly to **M13-R** (10 nmol) in 500 μl of 50 mM carbonate buffer at pH 9.0. The mixture was incubated in the dark at room temperature for 12 h. The solid residue was removed by centrifugation. To the supernatant, 50 μl of NaOAc (3.0 M, pH 5.2) and 1 ml of isopropanol were added. The labelled DNA was collected by centrifugation and the pellet was washed with 70% aqueous EtOH, twice with absolute EtOH and then dried *in vacuo*. The labelled oligonucleotide was further purified by RP-HPLC with MeCN (5–50% over 50 min) and 0.1 M triethylammonium acetate pH 7.0 as the mobile phase at a flow rate of 1 ml min$^{-1}$.

**Labelling of HSA with complex 4.** In a typical reaction, **4** (1.4 mg, 1.90 μmol) in 20 μl anhydrous DMSO was added slowly to 180 μl of a HSA (1.3 mg, 19.7 nmol) solution in 50 mM carbonate buffer pH 9.0. The mixture was incubated in the dark at room temperature for 12 h. The solid residue was removed by centrifugation. The supernatant was diluted to 1 ml with 50 mM Tris·HCl pH 7.4 and loaded onto a PD-10 column (Pharmacia) that had been equilibrated with the same buffer. The first band coming out from the column with intense yellow luminescence was collected and the solution was concentrated with a YM-30 centricon (Amicon). The labelled protein was further purified by HPLC equipped with a size-exclusion column. The mobile phase was 50 mM Tris·HCl pH 7.4 at a flow rate of 1 ml min$^{-1}$.

### Physical measurements and instrumentation

$^1$H NMR spectra were recorded on a Varian Mercury 300 MHz NMR spectrometer at 298 K. Positive-ion ESI mass spectra were recorded on a Perkin Elmer Sciex API 365 mass spectrometer. IR spectra were recorded on a Perkin Elmer 1600 series FT-IR spectrophotometer. Elemental analyses were carried out on an Elementar Analysensysteme GmbH Vario EL elemental analyser. Electronic absorption and steady-state emission/excitation spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer and a Spex Fluorolog-2 Model F 111 fluorescence spectrophotometer, respectively. All solutions for photophysical studies were degassed with no fewer than four successive freeze–pump–thaw cycles and stored in a 10 cm$^3$ round-bottomed flask equipped with a side-arm 1 cm fluorescence cuvette and sealed from the atmosphere by a Rotaflo HP6/6 quick-release Teflon stopper. Luminescence quantum yields were measured by the optical dilution method[13] using an aerated aqueous solution of $[Ru(bpy)_3]Cl_2$ ($\varphi$ = 0.028, excitation wavelength at 455 nm)[14] as the standard solution. Concentrations of the standard and sample solutions were adjusted to such a point that the absorbance at 455 nm was less than 0.1. The excitation source for emission lifetime measurements was the 355 nm output (third harmonic) of a Quanta-Ray Q-switched GCR-150–10 pulsed Nd–YAG laser. Luminescence decay signals from a Hamamatsu R928 photomultiplier tube were converted to potential changes by a 50 Ω load resistor and then recorded on a Tektronix Model TDS 620A digital oscilloscope.

### Crystal structure determination

**Crystal data for complex 1.** $[(C_{19}H_{12}N_4O_3SRe)^+(CF_3SO_3^-)]$; formula weight = 711.66, monoclinic, space group $P2_1/c$ (no. 14), $a$ = 11.572(1), $b$ = 15.864(1), $c$ = 13.054(1) Å, $\beta$ = 91.508(7)°, $V$ = 2395.6(3) Å$^3$, $Z$ = 4, $D_c$ = 1.973 g cm$^{-3}$, $\mu$(Mo-Kα) = 53.17 cm$^{-1}$, $F(000)$ = 1368, $T$ = 301 K. A yellow crystal of dimensions 0.4 × 0.2 × 0.1 mm in a glass capillary was used for data collection at 28 °C on a Rigaku AFC7R diffractometer with graphite monochromatised Mo-Kα radiation ($\lambda$ = 0.71073 Å) using $\omega$–$2\theta$ scans with $\omega$-scan angle (0.73 + 0.35 tan $\theta$)° at a scan speed of 8.0° min$^{-1}$ [up to 6 scans for reflection with $I < 15\sigma(I)$]. Unit-cell dimensions were determined based on the setting angles of 25 reflections in the $2\theta$ range of 36.3–38.8°. Intensity data (in the range of $2\theta_{max}$ = 50°; $h$: 0 to 13; $k$: 0 to 18; $l$: −15 to 15 and 3 standard reflections measured after every 300 reflections showed decay of 2.51%) were corrected for Lorentz and polarisation effects, and empirical absorption corrections based on the $\psi$-scan of five strong reflections (minimum and maximum transmission factors 0.721 and 1.000). A total of 4624 reflections was measured, of which 4397 were unique and $R_{int}$ = 0.018; 3278 reflections with $I > 3\sigma(I)$ were considered observed and used in the structural analysis. The space group was uniquely determined based on systematic absences and the structure was solved by Patterson methods and expanded by Fourier methods (PATTY[15]) and refinement by full-matrix, least squares using the software package TEXSAN[16] on a Silicon Graphics Indy computer. One crystallographic asymmetric unit consists of one formula unit. In the least squares refinement, all 36 non-H atoms were refined anisotropically and 12 H atoms at calculated positions with thermal parameters equal to 1.3 times that of the attached C atoms were not refined. Convergence for 325 variable parameters by least squares refinement on $F$ with $w = 4F_o^2/\sigma^2(F_o^2)$, where $\sigma^2(F_o^2) = [\sigma^2(I) + (0.030\ F_o^2)^2]$ for 3278 reflections with $I > 3\sigma(I)$ was reached at $R$ = 0.036 and $wR$ = 0.054 with a goodness-of-fit of 2.20. $(\Delta/\sigma)_{max}$ = 0.05. The final difference Fourier map was featureless, with maximum positive and negative peaks of 1.03 and 0.86 e Å$^{-3}$, respectively.

CCDC reference number 154472.

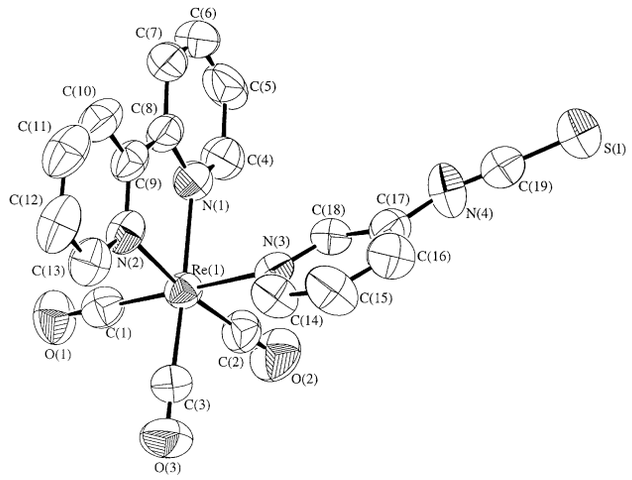


**Fig. 1** Perspective view of the complex cation of **1** with atomic numbering. Hydrogen atoms have been omitted for clarity and thermal ellipsoids are shown at the 50% probability level.

See http://www.rsc.org/suppdata/dt/b1/b103371a/ for crystallographic data in CIF or other electronic format.

## Results and discussion

The rhenium(I) polypyridine complexes containing an isothiocyanate moiety, **1**–**13**, were prepared, in good yields, from the reactions of the precursor complexes $[Re(N–N)(CO)_3(py\text{-}3\text{-}NH_2)](CF_3SO_3)$ and $S{=}CCl_2$ in the presence of $CaCO_3$ in acetone. A similar procedure was adopted for the synthesis of a ruthenium(II) isothiocyanate complex $[Ru(bpy)_2(phen\text{-}5\text{-}NCS)]^{2+}$ (phen-5-NCS = 5-isothiocyanato-1,10-phenanthroline).[12] In this work, 3-aminopyridine was used instead of 4-aminopyridine as we noticed that the amine group of 4-aminopyridine coordinated to a rhenium(I) centre was insufficiently nucleophilic to react with $S{=}CCl_2$. The choice of 3-pyridyl isothiocyanate as a ligand also provides the advantage that the photophysical properties of the complexes would not be drastically different before and after their conjugation to biomolecules because a substituent at the *meta*-position of the pyridine ligand would only cause minimal electronic effects to the rhenium(I) centre.

All the new rhenium(I) complexes were characterised by $^1$H NMR, positive-ion ESI-mass spectrometry, IR and gave satisfactory elemental analyses. The crystal structure of **1** has also been studied by X-ray crystallography.

### Crystal structure

Single crystals of **1** were obtained by layering a concentrated acetone solution of the complex with a mixture of diethyl ether and petroleum ether. The perspective drawing of the complex cation of **1** with atomic numbering is depicted in Fig. 1. Selected bond distances and angles are summarised in Table 1. The rhenium(I) centre adopts a distorted octahedral coordination geometry and the carbonyl groups are arranged in a *facial* orientation. The Re–N bond lengths [Re(1)–N(1) = 2.171(6), Re(1)–N(2) = 2.167(6), Re(1)–N(3) = 2.225(6)] are similar to those observed in related systems.[3,4a,b,6a] While the bond angle of C(17)–N(4)–(C19) [149.7(10)°] is significantly large for an sp$^2$ hybridised nitrogen atom, the isothiocyanate functional group is essentially linear [S(1)–C(19)–N(4) = 176.8(10)°], typical of a carbon atom under sp hybridisation.

### Electronic absorption and emission properties

The electronic absorption spectral data of the complexes are summarised in Table 2. The electronic absorption spectra of **1** reveal strong absorption bands at *ca.* 252–322 nm, with absorption coefficients in the order of $10^4$ dm$^3$ mol$^{-1}$ cm$^{-1}$, and a less

intense absorption shoulder at *ca.* 348–362 nm. With reference to previous photophysical studies on related rhenium(I) diimine systems,[2–7] these transitions are assigned to ligand-centred (bpy and py-3-NCS) and metal-to-ligand charge-transfer (MLCT) [dπ(Re) ⟶ π*(bpy)] transitions, respectively. Similar spectroscopic assignments are made for the other complexes. However, owing to the extended π-conjugation of 4,7-$Ph_2$-phen, 2,9-$Me_2$-4,7-$Ph_2$-phen, HM-phen and biq, the intraligand (diimine) absorptions of **10**–**13** are extended to a lower energy region (*ca.* 328–396 nm) and the $^1$MLCT [dπ(Re) ⟶ π*(diimine)] transitions of these complexes occur at lower energy yet (*ca.* 378–446 nm).

Excitation of the complexes in the solid state and in fluid solutions gives rise to intense and long-lived green to orange–red luminescence (Table 3). As an example, the emission spectrum of **8**, together with its UV-vis absorption spectra in $CH_2Cl_2$ at 298 K are shown in Fig. 2. The sub-microsecond to microsecond luminescence lifetimes of the complexes, together with large Stokes' shifts (for example, for **8** in $CH_2Cl_2$ at 298 K, Stokes' shift = 0.67 eV), suggest the phosphorescence nature of the emission. The emission energies of the complexes essentially depend on the energy levels of the empty π* (diimine) molecular orbitals. In general, complexes with electron-donating substituents on the diimine ligands emit at a higher energy than those with electron-withdrawing substituents or with a more conjugated diimine ligand. For example, the four

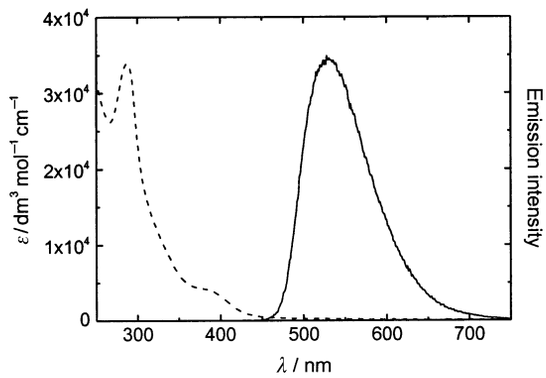


**Fig. 2** Emission (—) and UV–vis absorption (- - -) spectra of **8** in degassed $CH_2Cl_2$ at 298 K.

electron-donating methyl groups on the 3,4,7,8-$Me_4$-phen ligand of **7** increase its emission energy whereas the more conjugated biq ligand renders **13** to emit at the lowest energy among the complexes. Based on this observation, together with previous photophysical studies on related Re(I)–diimine systems,[2–7] the photoluminescence is assigned to originate from a $^3$MLCT [dπ(Re) ⟶ π*(diimine)] excited state. The luminescence quantum yields of the complexes are comparable to those of related $[Re(N–N)(CO)_3(py)]^+$ complexes.[2b,e,g,7a]

**Table 1** Selected geometric data (bond distances in Å, angles in °) for complex **1**

| | | | |
|---|---|---|---|
| Re(1)–N(1) | 2.171(6) | Re(1)–N(2) | 2.167(6) |
| Re(1)–N(3) | 2.225(6) | Re(1)–C(1) | 1.923(10) |
| Re(1)–C(2) | 1.916(9) | Re(1)–C(3) | 1.941(10) |
| C(17)–N(4) | 1.39(1) | N(4)–C(19) | 1.15(1) |
| S(1)–C(19) | 1.58(1) | | |
| | | | |
| N(1)–Re(1)–N(2) | 74.8(3) | N(1)–Re(1)–N(3) | 86.3(2) |
| N(1)–Re(1)–C(1) | 93.8(3) | N(1)–Re(1)–C(2) | 99.0(3) |
| N(1)–Re(1)–C(3) | 173.3(3) | N(2)–Re(1)–N(3) | 83.7(3) |
| N(2)–Re(1)–C(1) | 94.8(3) | N(2)–Re(1)–C(2) | 172.9(3) |
| N(2)–Re(1)–C(3) | 98.7(3) | N(3)–Re(1)–C(1) | 178.5(3) |
| N(3)–Re(1)–C(2) | 92.4(3) | N(3)–Re(1)–C(3) | 91.1(3) |
| C(17)–N(4)–C(19) | 149.7(10) | S(1)–C(19)–N(4) | 176.8(10) |

## Labelling of biomolecules

Since the isothiocyanate group can react readily with primary amines to form stable thiourea derivatives,[8,10,11] we have employed these luminescent rhenium(I) polypyridine complexes to label amine-modified oligonucleotides. For example, the universal **M13** reverse sequencing primer (16 mer) modified with an aminohexyl group at the 5′-end, **M13-R** (Scheme 1) was labelled with **4**. The successful conjugation of **4** and **M13-R** was confirmed by polyacrylamide gel electrophoresis using **M13-R** labelled with fluorescein isothiocyanate as a reference. The labelled primer, **4-M13-R**, exhibits yellow emission ($\lambda_{em}$ = 548 nm, $\tau_o$ = 0.52 μs) in degassed 50 mM Tris·Cl pH 7.4 at 298 K. It is likely that the isothiocyanate group of **4** and the thiourea moiety of **4-M13-R** would only have minor effects on the Re(I)–diimine luminophore because they are located at the *meta*-

**Table 2** Electronic absorption spectral data for complexes **1**–**13** at 298 K

| Complex | Medium | $\lambda_{abs}$/nm ($\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$) |
|---|---|---|
| **1** | $CH_2Cl_2$ | 254 (20 530), 280 (28 380), 308 sh (18 080), 322 (13 565), 362 sh (4075) |
| | MeCN | 252 sh (17 855), 276 (21 600), 308 sh (13 185), 320 (12 215), 348 sh (4295) |
| **2** | $CH_2Cl_2$ | 256 sh (20 395), 280 (26 885), 316 (13 675), 354 sh (4480) |
| | MeCN | 276 (26 875), 304 sh (17 235), 318 (14 720), 334 sh (6295) |
| **3** | $CH_2Cl_2$ | 256 sh (23 715), 280 (30 535), 318 (16 210), 354 sh (5140) |
| | MeCN | 256 sh (17 665), 274 (21 045), 304 sh (13 080), 318 (11 545), 340 sh (4310) |
| **4** | $CH_2Cl_2$ | 258 sh (22 880), 278 (32 425), 336 sh (6190), 374 sh (3780) |
| | MeCN | 276 (33 780), 328 sh (6365), 364 sh (3465) |
| **5** | $CH_2Cl_2$ | 284 (35 025), 374 sh (2760) |
| | MeCN | 282 (28 425), 366 sh (2415) |
| **6** | $CH_2Cl_2$ | 260 sh (23 660), 276 (33 520), 332 sh (7610), 372 sh (3885) |
| | MeCN | 274 (33 225), 316 sh (9680), 360 sh (4075) |
| **7** | $CH_2Cl_2$ | 254 (23 975), 282 (35 455), 334 sh (8890), 372 sh (3310) |
| | MeCN | 246 sh (25 150), 280 (36 280), 316 sh (12 775), 368 sh (3170) |
| **8** | $CH_2Cl_2$ | 288 (33 925), 388 sh (3955) |
| | MeCN | 238 sh (34 615), 282 (28 990), 322 sh (8855), 370 sh (3214) |
| **9** | $CH_2Cl_2$ | 260 sh (19 105), 282 (28 810), 314 sh (9480), 378 sh (3070) |
| | MeCN | 262 sh (19 030), 280 (25 275), 368 sh (3080) |
| **10** | $CH_2Cl_2$ | 290 (41 920), 342 sh (13 980), 388 sh (7095) |
| | MeCN | 290 (40 945), 330 sh (15 295), 382 sh (6600) |
| **11** | $CH_2Cl_2$ | 262 sh (22 120), 298 (35 115), 336 sh (12 270), 388 sh (4035) |
| | MeCN | 260 sh (26 655), 296 (39 335), 328 sh (15 670), 378 sh (4695) |
| **12** | $CH_2Cl_2$ | 274 (43 940), 304 sh (20 620), 378 sh (15 365), 396 (18 030), 446 sh (2980) |
| | MeCN | 274 (41 670), 298 sh (16 735), 378 sh (14 680), 394 (16 800), 428 sh (4315) |
| **13** | $CH_2Cl_2$ | 272 (38 735), 306 sh (15 400), 364 (14 820), 382 (20 205), 420 sh (3050) |
| | MeCN | 270 (42 550), 300 sh (17 070), 362 (15 940), 378 (21 095), 414 sh (3350) |

**Table 3** Photoluminescence wavelengths and lifetimes for complexes **1**–**13** at 298 K

| Complex | Medium | Emission $\lambda$/nm (lifetime $\tau_o$/μs) | Luminescence quantum yield $\varphi$ |
|---|---|---|---|
| **1** | $CH_2Cl_2$ | 542 (0.66) | 0.16 |
| | MeCN | 558 (0.36) | |
| | Solid | 530 (0.31) | |
| **2** | $CH_2Cl_2$ | 532 (0.82) | 0.32 |
| | MeCN | 546 (0.42) | |
| | Solid | 508 (0.34) | |
| **3** | $CH_2Cl_2$ | 530 (0.90) | 0.29 |
| | MeCN | 546 (0.49) | |
| | Solid | 522 (0.45) | |
| **4** | $CH_2Cl_2$ | 530 (2.51) | 0.54 |
| | MeCN | 546 (1.35) | |
| | Solid | 534 (0.32) | |
| **5** | $CH_2Cl_2$ | 518 (2.20) | 0.45 |
| | MeCN | 530 (1.79) | |
| | Solid | 502 (0.50) | |
| **6** | $CH_2Cl_2$ | 516 (4.63) | 0.48 |
| | MeCN | 530 (3.59) | |
| | Solid | 526 (1.06) | |
| **7** | $CH_2Cl_2$ | 492 sh, 510 (3.74) | 0.17 |
| | MeCN | 514 (2.32) | |
| | Solid | 480, 504, 544 sh (0.32) | |
| **8** | $CH_2Cl_2$ | 530 (4.75) | 0.42 |
| | MeCN | 548 (4.06) | |
| | Solid | 550 (2.43) | |
| **9** | $CH_2Cl_2$ | 544 (1.81) | 0.41 |
| | MeCN | 562 (0.82) | |
| | Solid | 562 (0.40) | |
| **10** | $CH_2Cl_2$ | 544 (6.54) | 0.59 |
| | MeCN | 558 (3.74) | |
| | Solid | 568 (2.02) | |
| **11** | $CH_2Cl_2$ | 538 (9.15) | 0.32 |
| | MeCN | 546 (4.74) | |
| | Solid | 556 (4.33) | |
| **12** | $CH_2Cl_2$ | 636 (0.21) | 0.0056 |
| | MeCN | 636 (0.12) | |
| | Solid | 636 (0.17) | |
| **13** | $CH_2Cl_2$ | 642 (0.10) | 0.0024 |
| | MeCN | 650 (0.05) | |
| | Solid | 613, 644 sh (0.32) | |

position of the pyridine ligand. Therefore, the red shift of the emission wavelength of **4** in $CH_2Cl_2$ (530 nm) to MeCN (546 nm) and **4-M13-R** in Tris buffer (548 nm) appears to result from the increase in polarity of solvents. Similar solvatochromic properties are commonly observed for Re(I)–diimine MLCT emitters.[2a,c–f,3b]

On the other hand, the labelled primer, **4-M13-R**, has been used as a luminescent probe to target unmodified oligonucleotides. Three different single-stranded DNA oligonucleotides [**M13–20** (20 mer), **M13–50** (50 mer) and **M13–80** (80 mer)] (Scheme 1), all containing a region that is complementary to **4-M13-R**, were hybridised with **4-M13-R**. A mixture of **4-M13-R**, **M13–20**, **M13–50** and **M13–80** was heated to 95 °C for 5 min and cooled to 25 °C over 50 min before being analysed by PAGE. The polyacrylamide gel (Fig. 3) shows three yellow luminescent bands, indicating the presence of three heteroduplexes, (**4-M13-R**)·(**M13–20**), (**4-M13-R**)·(**M13–50**), and (**4-M13-R**)·(**M13–80**). This reveals that the hybridisation abilities of **M13-R** are retained in the presence of the luminescent label.

Since the isothiocyanate moiety is also known to react with the amine groups of lysine and arginine residues,[8,10,11] we have labelled HSA with **4**. The conjugate was purified by size-exclusion chromatography and the conjugation was investigated by polyacrylamide gel electrophoresis. The gel displays an intense yellow luminescent band corresponding to a molecular weight of 66 kDa, indicating that the protein has been successfully labelled with **4**. Irradiation of **4-HSA** in degassed 50 mM Tris·Cl pH 7.4 at 298 K results in intense and long-lived yellow emission ($\lambda_{em}$ = 534 nm, bi-exponential decay: $\tau_1$ = 0.79 μs, $\tau_2$ = 0.13 μs). The emission of the conjugate is assigned to arise from a $^3$MLCT [dπ(Re) ⟶ π*(phen)] excited state. In the presence of oxygen, the emission intensity exhibits only a small decrease ($I/I_o$ = 0.89). The reduction in the emission lifetimes was also small ($\tau_1$ = 0.68 μs, $\tau_2$ = 0.10 μs). It is likely that the labels are well shielded within the protein matrix and a low exposure to the solvent environment results in inefficient quenching by the oxygen molecules.[7] The observation of emission lifetimes in the microsecond timescale for the bioconjugates **4-M13-R** and **4-HSA** indicates that these rhenium(I) labels are promising candidates for different time-resolved bioassays.

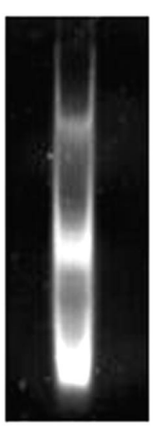

**Fig. 3** Polyacrylamide gel electrophoresis result of a hybridisation mixture of **4-M13-R**, **M13–20**, **M13–50** and **M13–80**.

## Summary

We have synthesised and characterised a new series of rhenium(I) polypyridine complexes with an isothiocyanate moiety. The X-ray crystal structure of **1** has also been studied. These complexes display intense and long-lived emission under ambient conditions. An amine-modified oligonucleotide and HSA have been labelled with one of the complexes, **4**. The bioconjugates exhibit intense and long-lived $^3$MLCT [d$\pi$(Re) $\longrightarrow$ $\pi^*$(N–N)] emission, characteristic of luminescent rhenium(I) diimine systems. Applications of these luminescent rhenium(I) labelling reagents in DNA hybridisation assays, DNA sequencing experiments, polymerase chain reactions and immunoassays are currently under investigation.

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