Supporting Information

Embedding a Ruthenium-based Structural Mimic of the [Fe]-Hydrogenase Cofactor into Papain

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Figure S15. Deconvoluted and deisotoped ESI-HRMS of (A) papain and (B) bioconjugate **5**, both at 2.15 μM in water/acetonitrile/formic acid (49:50:1, v/v/v).

Figure S16. Graph of % activity of papain vs incubation time.

Figure S17. Overlay of the UV-visible spectra of **2b**, papain and **5**.

Table S1. Crystal and refinement data for **2a**, **2b** and **3bB**.

Table S2. Crystal and refinement data for **4aA**, **4aB** and **4bA**.

Table S3. Common atom labelling scheme and selected bond parameters for **2a**, **2b** and **3bB**.

Table S4. Common atom labelling scheme and selected bond parameters for **4aA**, **4aB** and **4bA**.

Table S5. Experimental and computational CO vibrational frequencies for **2b, 2b.H_2O**, **[2b.2H_2O]$^+$**, **3bB, 3bB.H_2O**, **2b.Im**, **3bB.Im** and **5**.

Table S6. Computed free energy and CO vibrational frequencies for the isomers of **2a**.

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Table S8. Computed free energies (kJ/mol) for the isomers of **2a** and **2b** (relative to isomer 1).

Table S9. Selected interatomic distances (Å) for the computationally optimized isomers of **2a**.

Table S10. Selected interatomic distances (Å) for the computationally optimized isomers of **2b**.

Figure S1: ORTEP plot of **2a**. All hydrogen atoms have been omitted for clarity. Thermal ellipsoids are drawn at the 50% probability level.

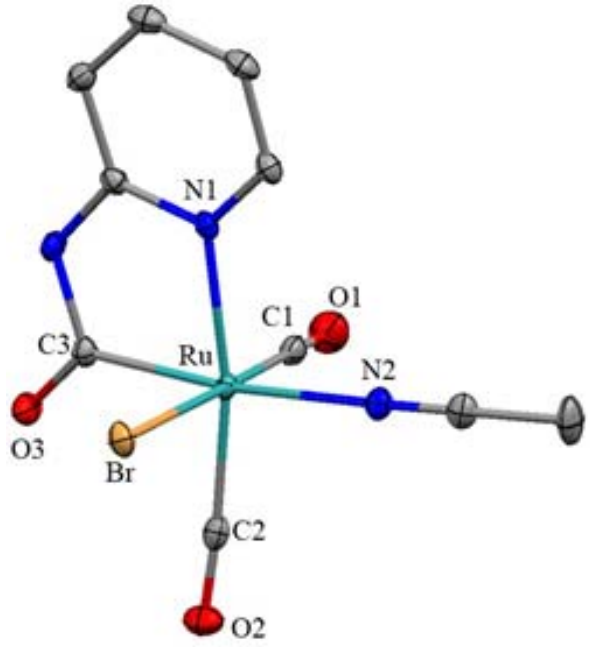


Figure S2. ORTEP plot of **4aA**. All hydrogen atoms have been omitted for clarity. Thermal ellipsoids are drawn at the 50% probability level.

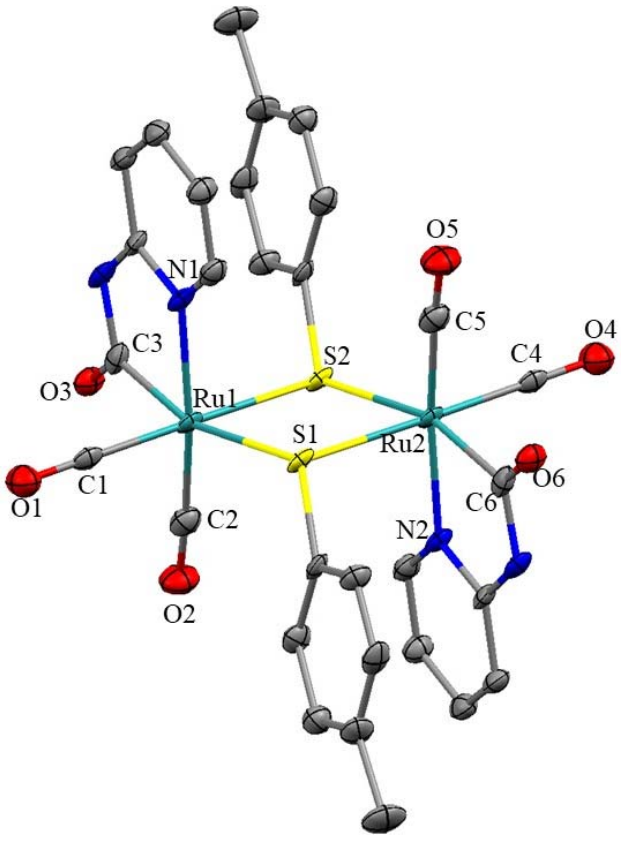


Figure S3. FT-IR spectra of **2a** (left) and **2b** (right) in acetonitrile.

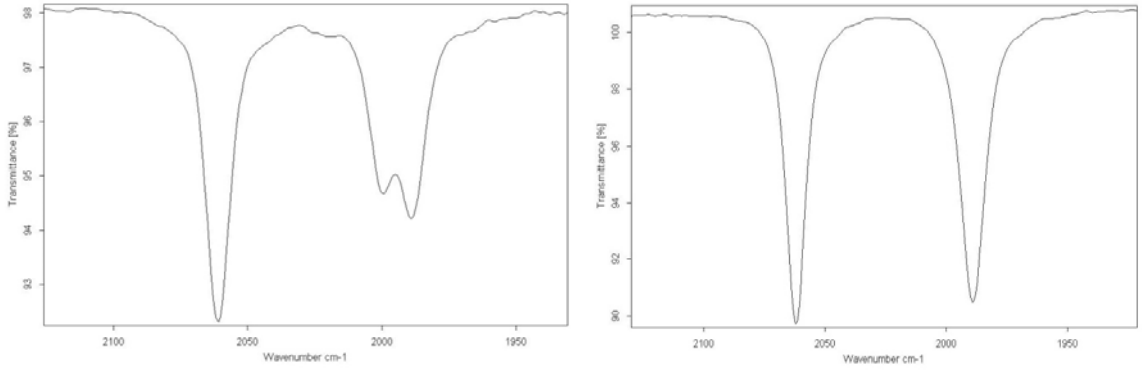


Figure S4. FT-IR spectra of **2b** in water/acetone mixtures.

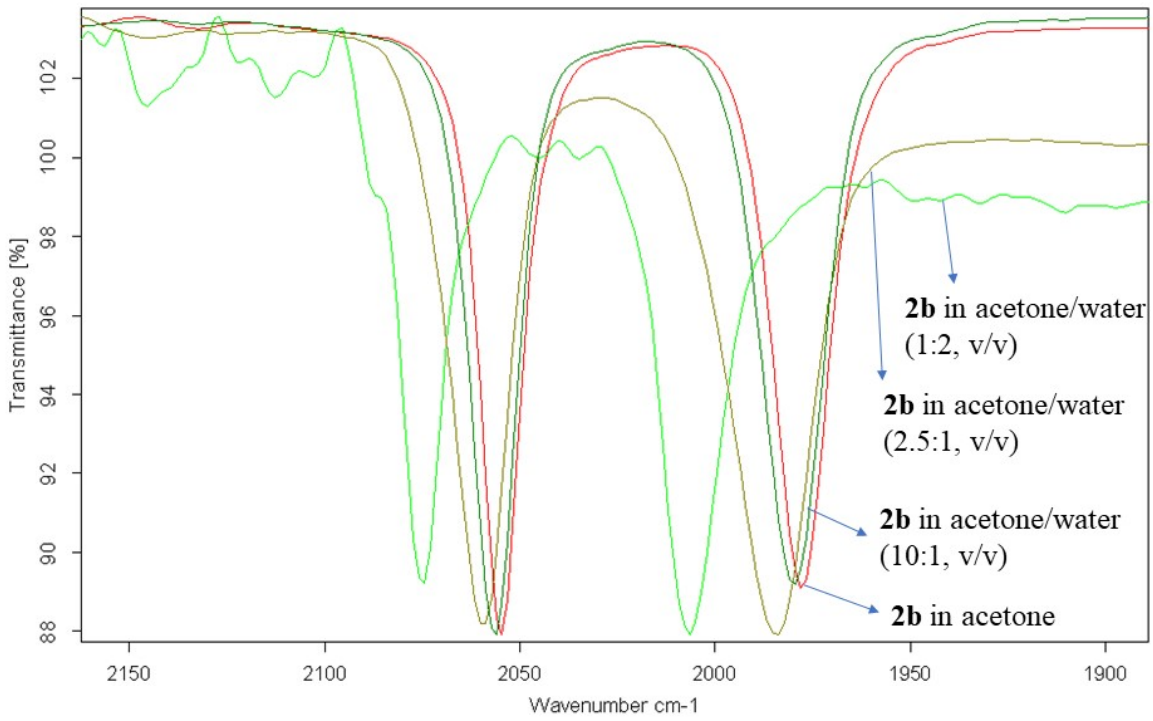


Figure S5. FT-IR spectra of **3bB** in water/acetonitrile mixtures.

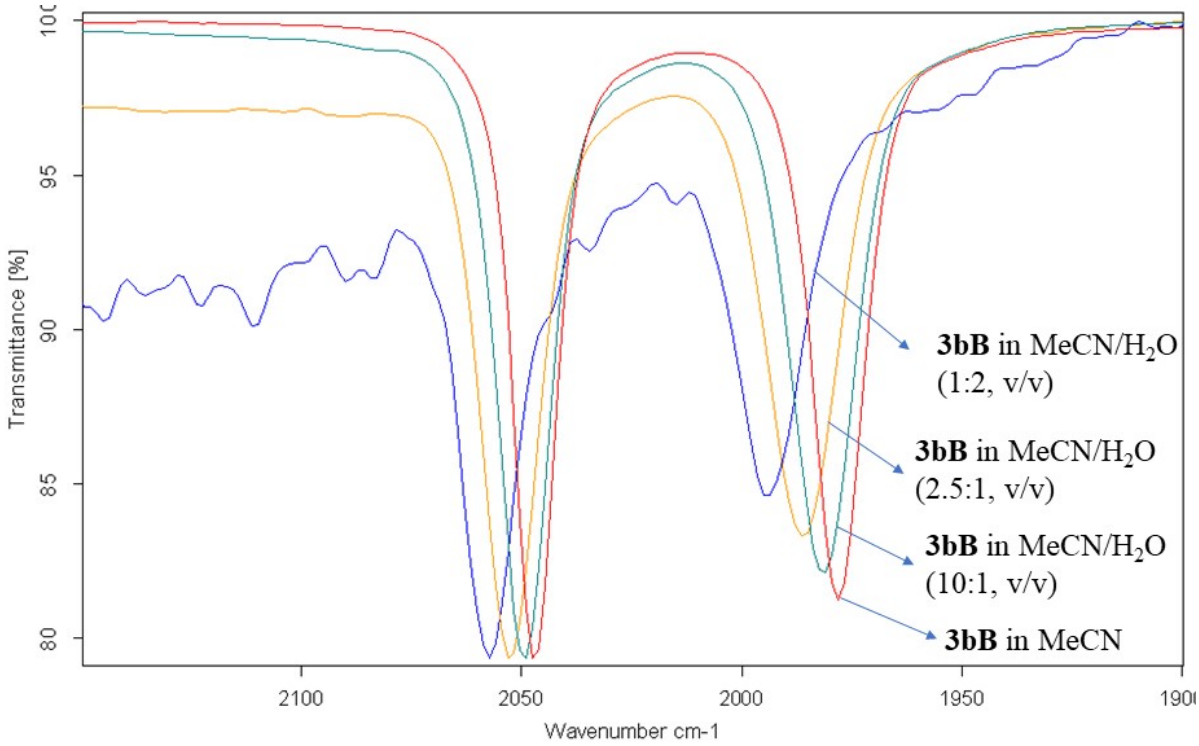


Figure S6. FT-IR study for the reaction of **2b** with imidazole in methanol.

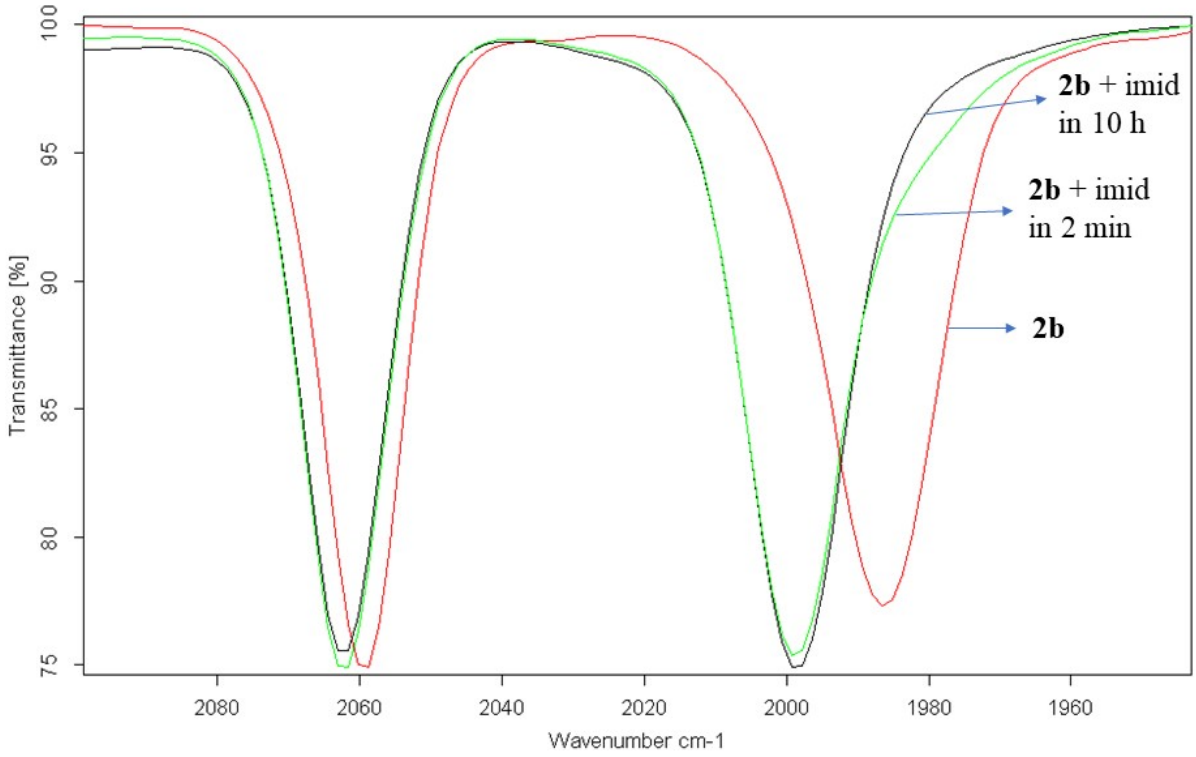


Figure S7. FT-IR study for the reaction of **2b** with imidazole in acetonitrile.

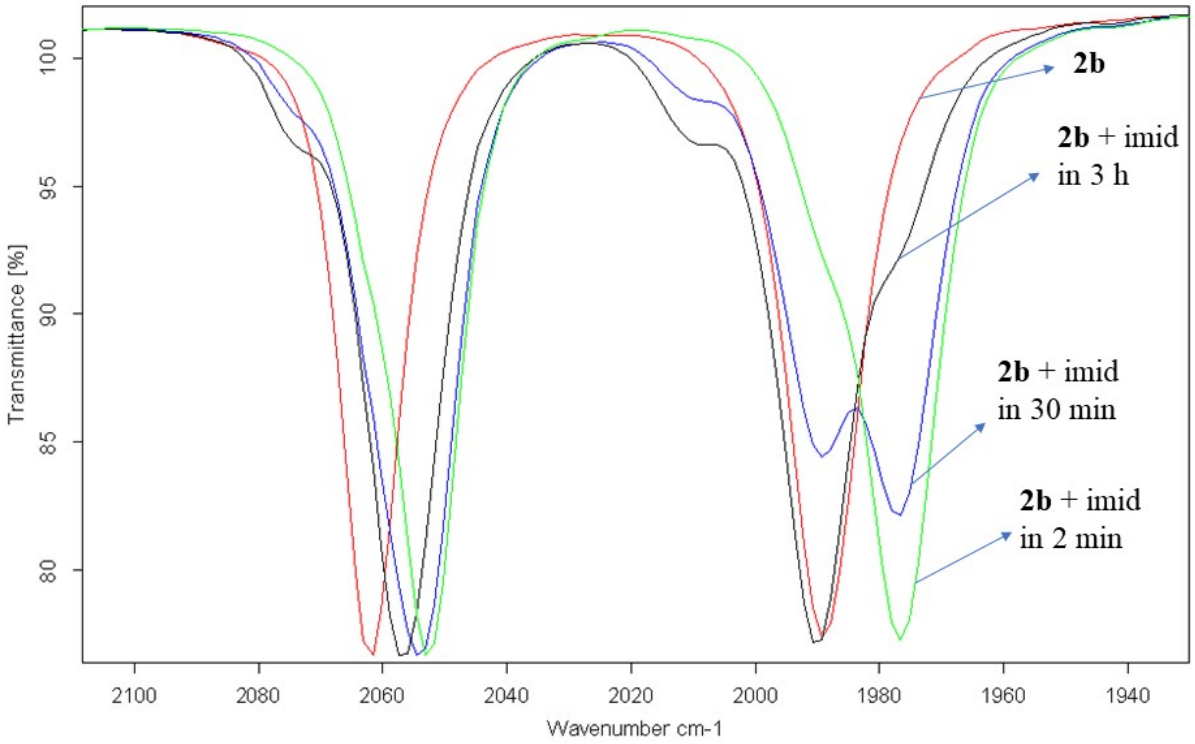


Figure S8. FT-IR study for the reaction of **3bB** with imidazole in acetonitrile.

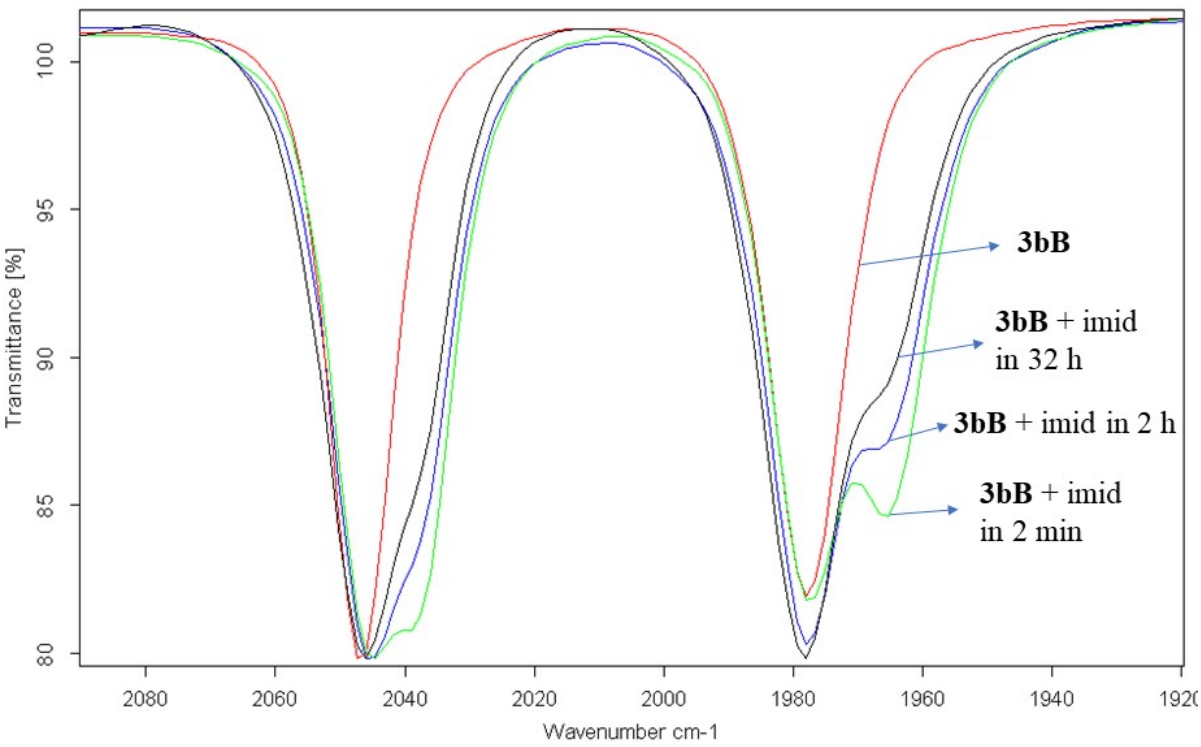


Figure S9. VT ^{1}H NMR spectra of **2b**.

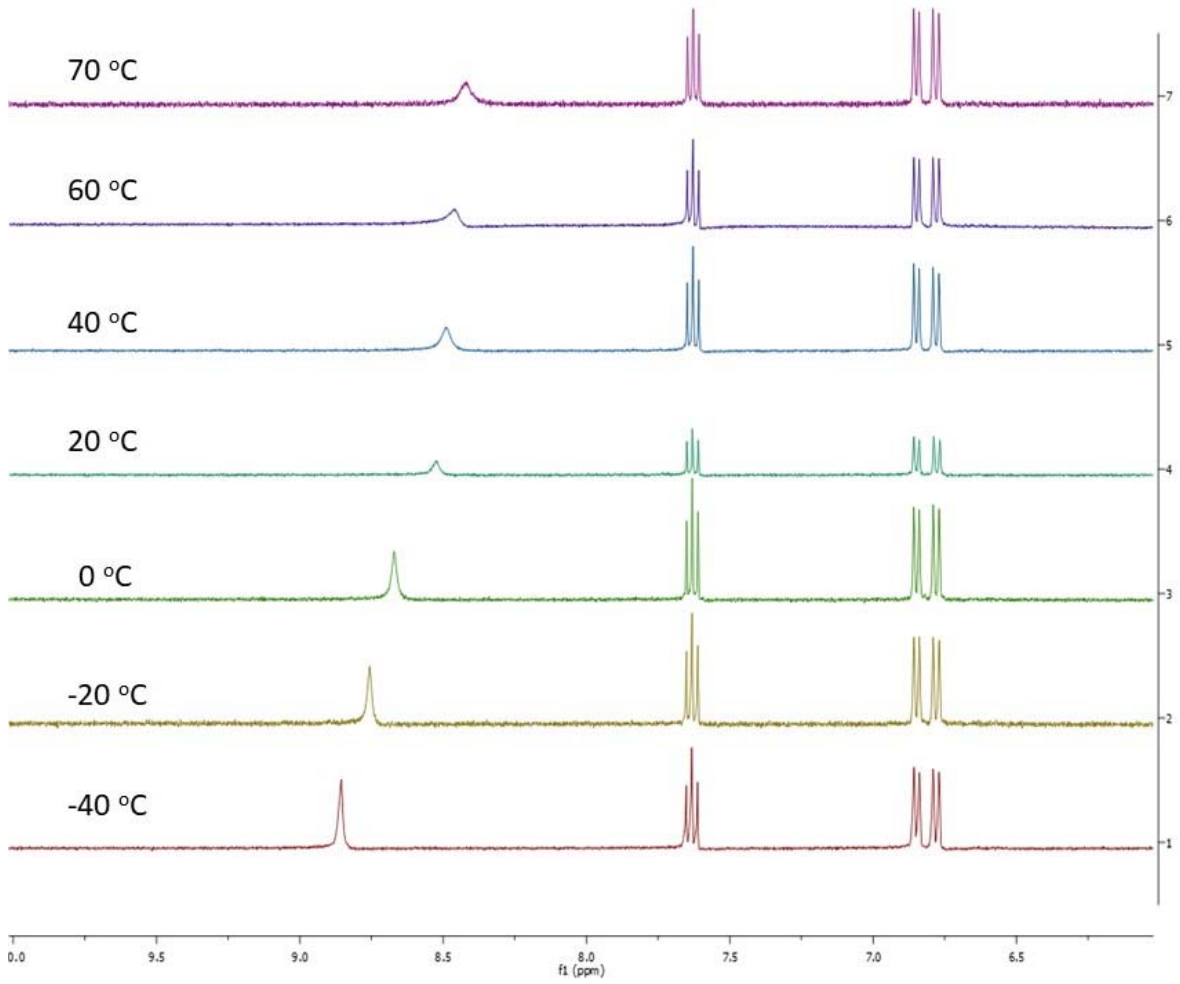


Figure S10. ^{1}H NMR spectra (aromatic region) of **2b** in water/acetonitrile-d_3 mixtures.

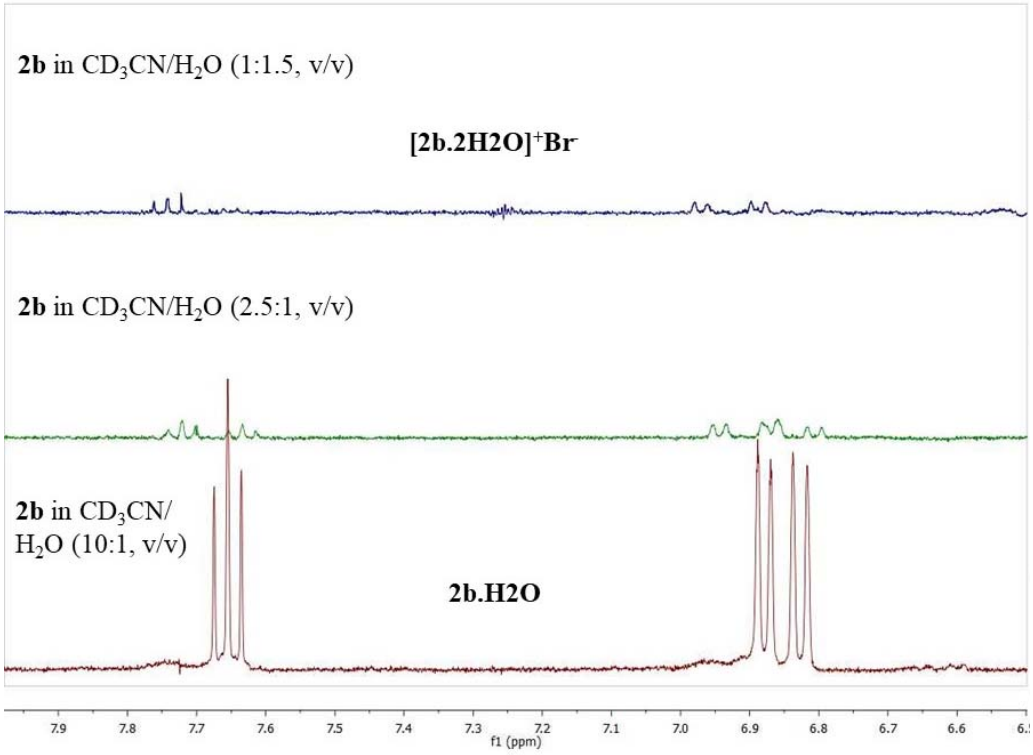


Figure S11. ^{1}H NMR spectra (aromatic region) of **3bB** in water/acetonitrile-d_3 mixtures.

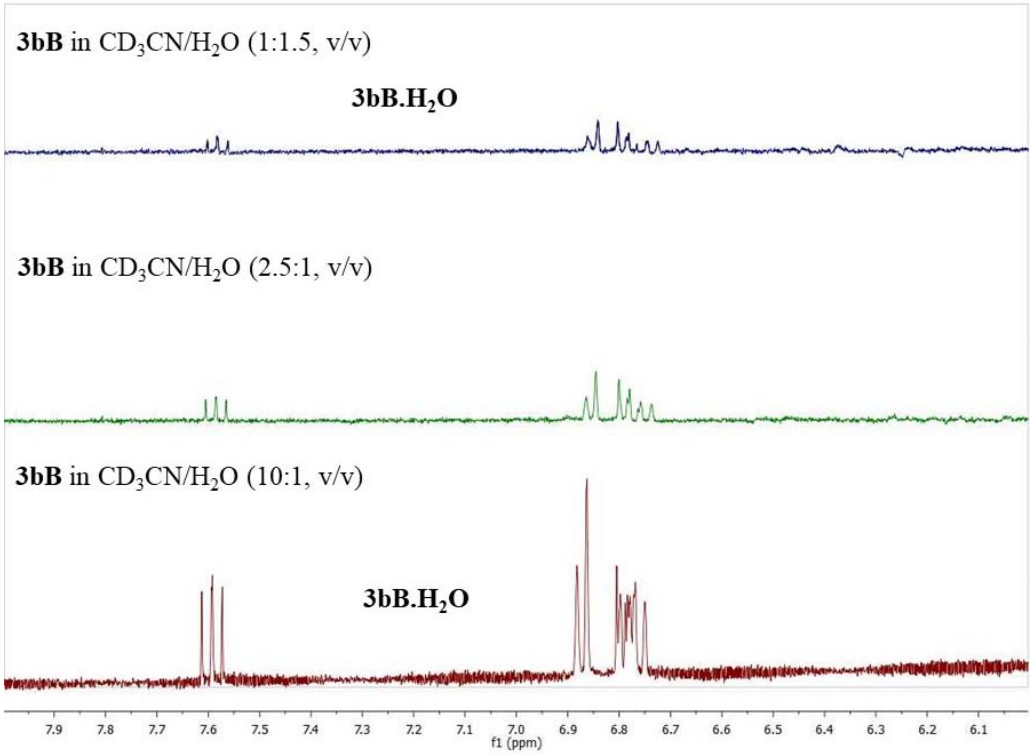


Figure S12. ^{1}H NMR spectra of **2b** in water/acetone-d$_6$ mixtures.

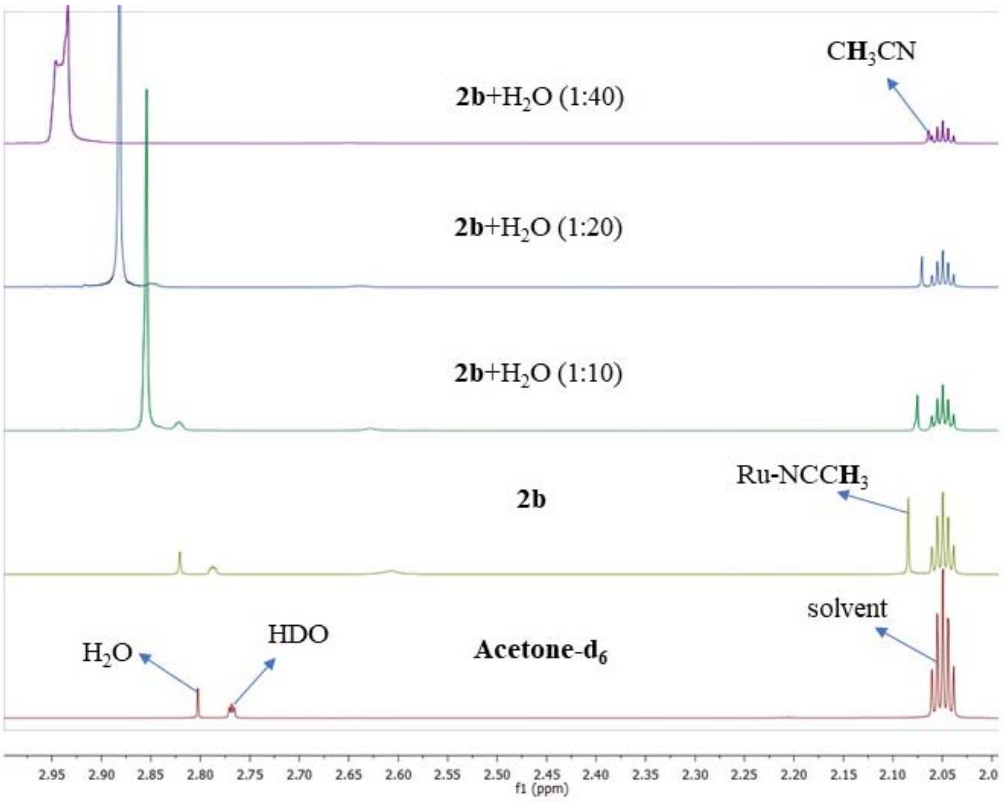


Figure S13. Mass spectrum of **2b** in acetonitrile at room temperature.

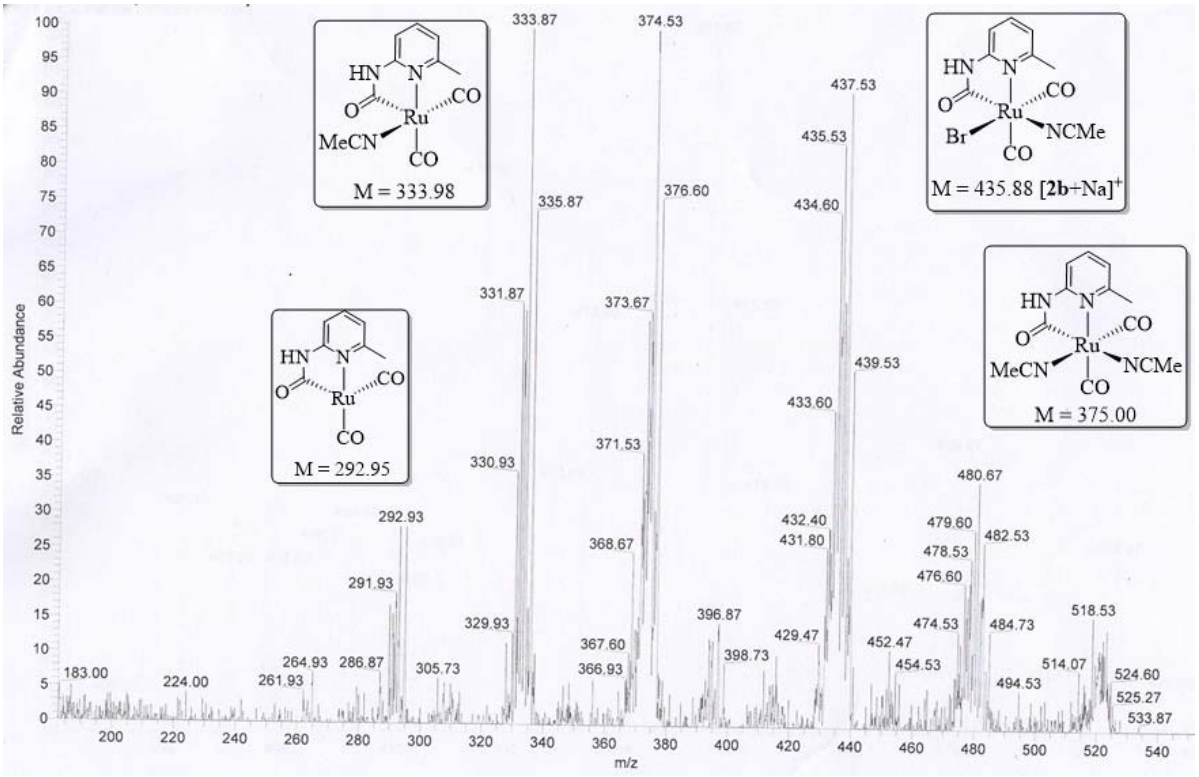


Figure S14. Mass spectrum of **3bB** in acetonitrile at room temperature.

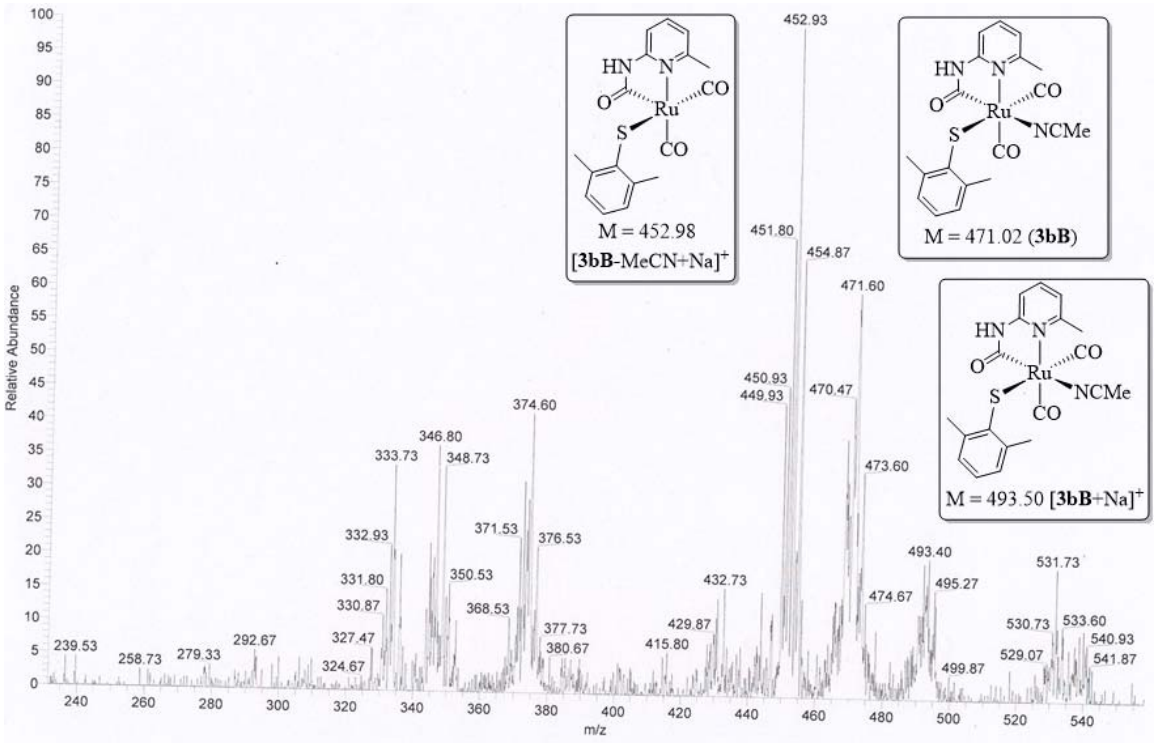


Figure S15. Deconvoluted and deisotoped ESI-HRMS of (A) papain and (B) bioconjugate **5**, both at 2.15 μM in water/acetonitrile/formic acid (49:50:1, v/v/v).

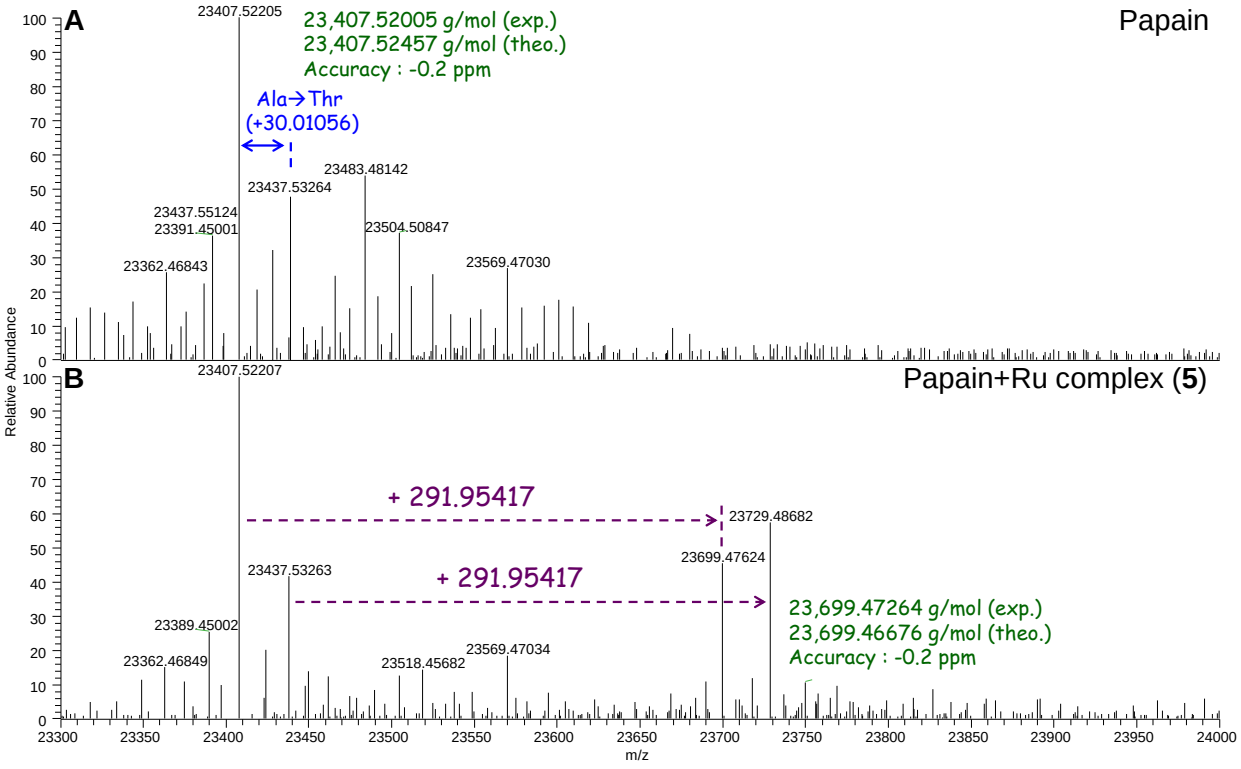


Figure S16. Graph of % activity of papain vs incubation time.

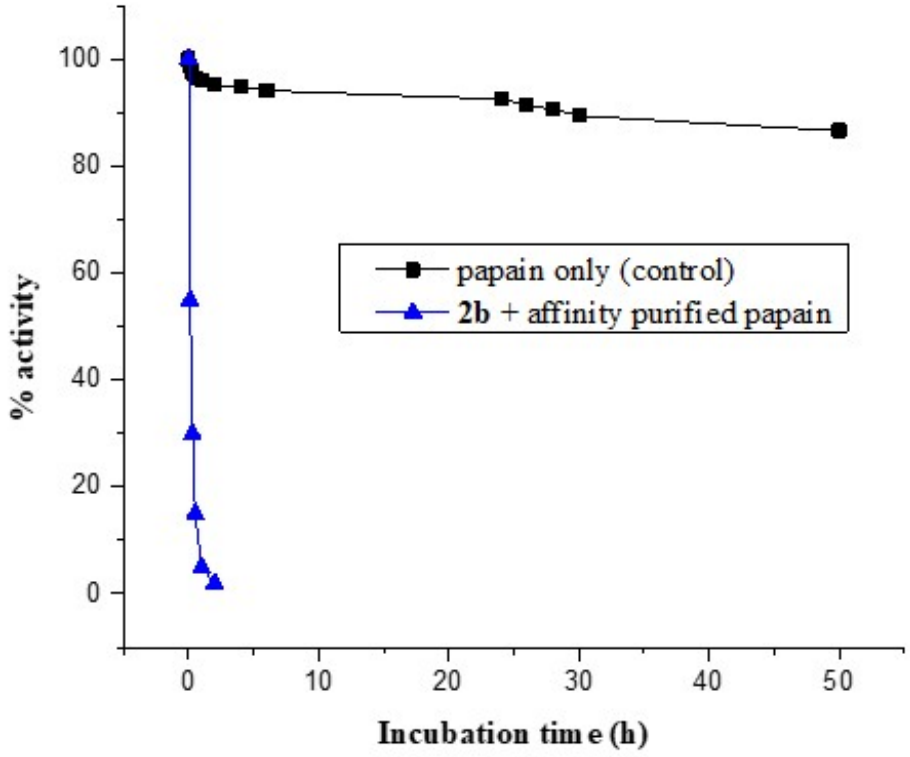


Figure S17. Overlay of the UV-visible spectra of **2b**, papain and **5.**

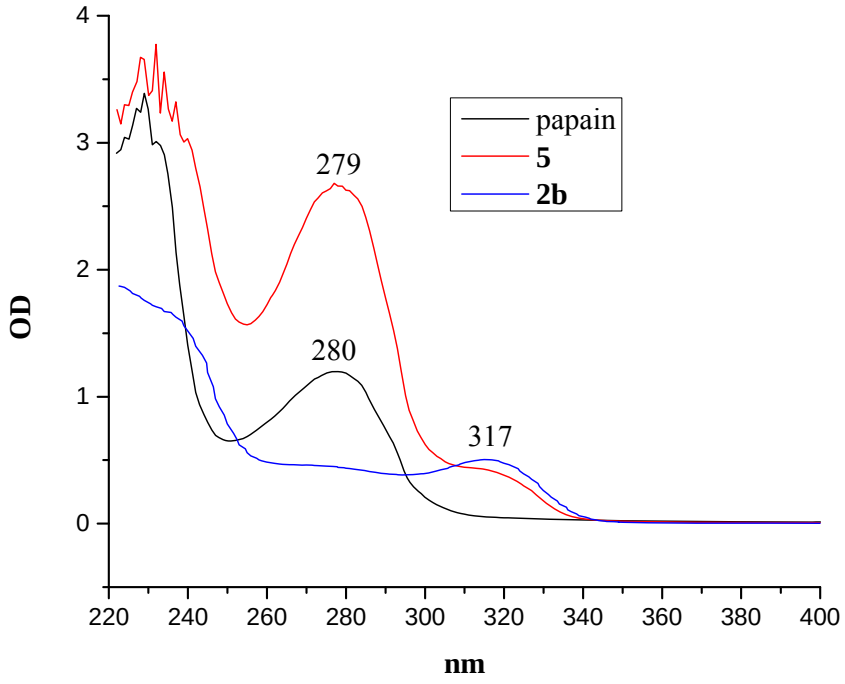


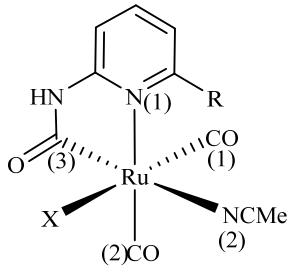
Table S1. Crystal and refinement data for **2a**, **2b** and **3bB**.

Complex	**2a**	**2b**	**3bB**
Empirical formula	$C_{12}H_{11}BrN_4O_3Ru$	$C_{11}H_{10}BrN_3O_3Ru$	$C_{19}H_{19}N_3O_3RuS$
Formula weight	440.23	413.20	470.50
Crystal system	Triclinic	Monoclinic	Monoclinic
Space group	P $\bar{1}$	P $2_1/n$	P $2_1/n$
a, Å	7.0469(2)	11.3743(7)	7.4279(2)
b, Å	9.9374(3)	9.1237(5)	16.2154(4)
c, Å	12.2509(4)	14.6542(9)	15.9289(4)
α, deg	108.6197(9)	90	90
β, deg	100.6851(9)	110.109(4)	90.8020(9)
γ, deg	101.0785(10)	90	90
Volume, $Å^3$	769.25(4)	1428.05(15)	1918.39(9)
Z	2	4	4
ρ_c, Mg/m^3	1.901	1.922	1.629
μ, mm^{-1}	3.630	3.902	0.951
F(000)	428	800	952
Crystal size, mm^3	0.36 x 0.36 x 0.30	0.200 x 0.220 x 0.240	0.310 x 0.100 x 0.100
Reflections collected	32811	29734	58011
Independent reflections	4928 [R(int) = 0.0352]	6340 [R(int) = 0.1123]	4752 [R(int) = 0.0619]
Max. and min. transmission	0.4089 and 0.3549	0.5090 and 0.4540	0.91 and 0.78
Data / restraints / parameters	4928 / 0 / 215	6340 / 83 / 205	4752 / 0 / 248
Goodness-of-fit on F^2	1.276	1.079	1.172
Final R indices [I>2sigma(I)]	R1 = 0.0201, wR2 = 0.0585	R1 = 0.0447, wR2 = 0.0662	R1 = 0.0303, wR2 = 0.0633
R indices (all data)	R1 = 0.0255, wR2 = 0.0754	R1 = 0.0764, wR2 = 0.0769	R1 = 0.0361, wR2 = 0.0652
Largest diff. peak and hole, $e.Å^{-3}$	0.927 and -0.685	0.875 and -0.800	0.616 and -0.817

Table S2. Crystal and refinement data for **4aA**, **4aB** and **4bA**.

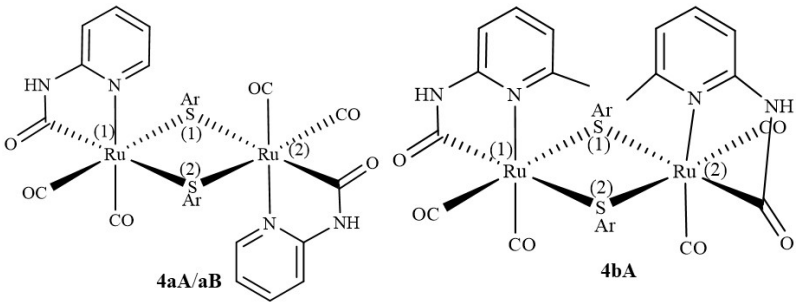
Complex	**4aA**	**4aB**	**4bA**
Empirical formula	$C_{30}H_{24}N_4O_6Ru_2S_2$	$C_{32}H_{28}N_4O_6Ru_2S_2$	$C_{32}H_{28}N_4O_6Ru_2S_2$
Formula weight	802.79	830.84	830.84
Crystal system	Monoclinic	Triclinic	orthorhombic
Space group	P $2_1/c$	P $\bar{1}$	P $2_12_12_1$
a, Å	10.7820(9)	8.1126(3)	10.6574(3)
b, Å	11.0144(12)	9.0321(3)	16.0828(4)
c, Å	13.0500(11)	10.9347(3)	19.4466(4)
α, deg	90	80.1023(12)	90
β, deg	101.800(5)	85.2609(8)	90
γ, deg	90	81.0634(12)	90
Volume, $Å^3$	1517.0(2)	778.43(5)	3333.16(14)
Z	2	1	4
ρ_c, Mg/m^3	1.757	1.772	1.656
μ, mm^{-1}	1.184	1.157	1.080
F(000)	800	416	1664
Crystal size, mm^3	0.040 x 0.040 x 0.060	0.126 x 0.131 x 0.238	0.080 x 0.120 x 0.320
Reflections collected	25244	35954	36227
Independent reflections	3115 [R(int) = 0.1019]	7522 [R(int) = 0.0452]	10049 [R(int) = 0.1003]
Max. and min. transmission	0.9540 and 0.9320	0.8680 and 0.7700	0.9190 and 0.7240
Data / restraints / parameters	3115 / 0 / 204	7522 / 0 / 210	10049 / 0 / 420
Goodness-of-fit on F^2	1.088	1.111	1.127
Final R indices [I>2sigma(I)]	R1 = 0.0440, wR2 = 0.1115	R1 = 0.0427, wR2 = 0.0806	R1 = 0.0636, wR2 = 0.0802
R indices (all data)	R1 = 0.0712, wR2 = 0.1360	R1 =0.0605, wR2 = 0.0889	R1 = 0.0954, wR2 = 0.0896
Largest diff. peak and hole, $e.Å^{-3}$	1.210 and -1.489	1.814 and -1.206	1.381 and -1.282

Table S3. Common atom labelling scheme and selected bond parameters for **2a**, **2b** and **3bB**.



Complex	**2a**	**2b**	**3bB**
	X = Br		X = $SC_6H_3Me_2$
Bond parameter	R = H	R = Me	R = Me
Ru-N1	2.110(2)	2.163(3)	2.194(2)
Ru-N2	2.182(2)	2.185(3)	2.171(2)
Ru-X	2.560(3)	2.537(5)	2.4564(7)
Ru-C1	1.893(3)	1.872(5)	1.886(3)
Ru-C2	1.886(2)	1.898(3)	1.876(3)
Ru-C3	2.015(2)	2.004(5)	2.022(2)
N1-Ru-N2	92.73(8)	101.6(1)	99.00(8)
N2-Ru-C2	95.52(8)	89.2(1)	91.9(1)
N1-Ru-C3	80.07(8)	79.8(1)	79.80(9)

Table S4. Common atom labelling Scheme and selected bond parameters for **4aA**, **4aB** and **4bA**.



Complex	**4aA**	**4aB**	**4bA***
Ar	*p*-tolyl	2,6-xylyl	*p*-tolyl
Ru-N	2.146(4)	2.148(2)	2.198(5); 2.175(5)
Ru1-S1	2.471(1)	2.4596(6)	2.446(2); 2.447(2)
Ru1-S2	2.517(1)	2.5240(6)	2.504(2); 2.550(2)
Ru-CO	1.888(6); 1.878(5)	1.884(3); 1.878(2)	1.866(8) - 1.891(7)
Ru-C(O)NH	2.044(5)	2.029(2)	2.017(8); 2.006(6)
N-Ru-C(O)NH	79.5(2)	79.2(1)	78.9(2); 79.5(2)
S-Ru-S	80.71(4)	77.12(2)	80.56(6); 79.66(6)

*exhibits racemic twinning.

Table S5. Experimental and computational CO vibrational frequencies for **2b**, **2b.H$_2$O**, **[2b.2H$_2$O]$^+$**, **3bB**, **3bB.H$_2$O**, **2b.Im**, **3bB.Im** and **5**.

Compounds	Experimental frequencies		Computed frequencies	
	ν_{CO}	$\Delta\nu$	ν_{CO}	$\Delta\nu$
2b	MeCN: 2062,1989	73	2057, 1981	76
2b.H$_2$O	[MeCN+H$_2$O(10:1,v/v)]: 2085 (w), **2065 (s)**, 2026 (w), **1993 (s)** [MeCN+H$_2$O(5:1,v/v)]: 2086 (w), 2066 (s), 2027 (w), 1995 (s) [MeCN+H$_2$O(3:1,v/v)]: 2086 (m), 2066 (s), 2027 (m), 1995 (s) [MeCN+H$_2$O(2.5:1,v/v)]: 2087 (s), 2067 (s), 2028 (s), 1997 (s) [MeCN+H$_2$O(2:1,v/v)]: 2087 (s), 2068 (s), 2029 (s), 1999 (s) [MeCN+H$_2$O(1.5:1,v/v)]: 2087 (s), 2070 (m), 2030 (s), 2001 (m) [MeCN+H$_2$O(1:1,v/v)]: 2089 (s), 2071 (w), 2031 (s), 2005 (w)	**72**	2055, 1984	71
[2b.2H$_2$O]$^+$	[MeCN+H$_2$O(1:2,v/v)]: **2089 (s), 2033 (s)** [MeCN+H$_2$O(1:3,v/v)]: 2090 (s), 2034 (s) [MeCN+H$_2$O(1:4,v/v)]: 2090 (s), 2034 (s) [MeCN+H$_2$O(1:5,v/v)]: 2090 (s), 2034 (s) [MeCN+H$_2$O(1:6,v/v)]: 2090 (s), 2034 (s) [MeCN+H$_2$O(1:8,v/v)]: 2090 (s), 2034 (s) [MeCN+H$_2$O(1:10,v/v)]: 2090 (s), 2034 (s)	**56**	2089, 2028	61
3bB	MeCN: 2047,1978	69	2043, 1975	68
3bB.H$_2$O	[MeCN+H$_2$O(10:1,v/v)]: 2049 (s), 1981 (s) [MeCN+H$_2$O(5:1,v/v)]: 2051 (s), 1984 (s) [MeCN+H$_2$O(2.5:1,v/v)]: 2053 (s), 1986 (s) [MeCN+H$_2$O(1:1,v/v)]: 2055 (s), 1989 (s) [MeCN+H$_2$O(1:2,v/v)]: **2057 (s), 1994 (s)** [MeCN+H$_2$O(1:3,v/v)]: 2059 (s), 1998 (s)	**63**	2043, 1979	64
2b.Im	-	-	2051, 1962	89
3bB.Im	-	-	2036, 1956	80
Bioconjugate (**5**)	ATR: 2064 (s), 2000 (s)	64		

Table S6. Computed free energy and CO vibrational frequencies for the isomers of **2a**.

Isomer	$\Delta G^{\ominus}$/kJ mol^{-1}	$\nu_{(C=O)}$/cm^{-1}	$\nu_{(CO)}$/cm^{-1}
1	0.00	1714	2120, 2038
2	1.1	1715	2123, 2049
3	38.2	1718	2126, 2040
4	46.6	1700	2124, 2050
5	45.3	1722	2119, 2044
6	46.3	1715	2169, 2068
7	57.9	1717	2159, 2055

*$\Delta G^{\ominus}$ are with respect to the lowest energy isomer.

Table S7. Computed free energy and CO vibrational frequencies for the isomers of **2b**.

Isomer	$\Delta G^{\ominus}$/kJ mol^{-1}	$\nu_{(C=O)}$/cm^{-1}	$\nu_{(CO)}$/cm^{-1}
1	0.00	1706	2123, 2039
2	7.8	1708	2127, 2053
3	35.0	1713	2125, 2038
4	43.9	1693	2122, 2049
5	44.8	1716	2116, 2041
6	52.6	1711	2168, 2067
7	62.7	1711	2161, 2055

*$\Delta G^{\ominus}$ are with respect to the lowest energy isomer.

Table S8. Computed free energies (kJ/mol) for the isomers of **2a** and **2b** (relative to isomer 1).

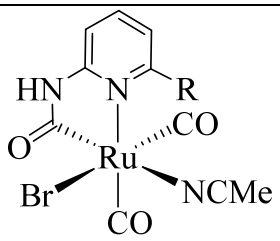
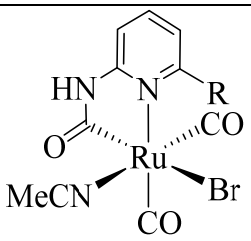
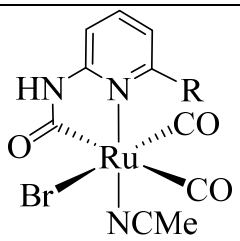
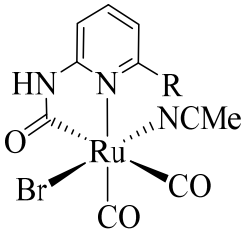
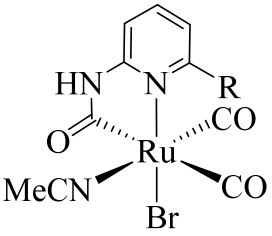
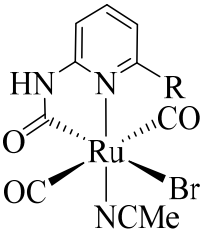
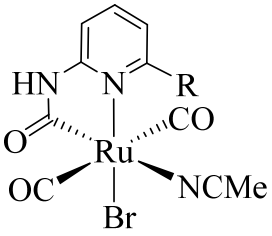
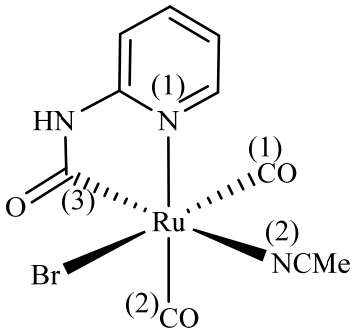
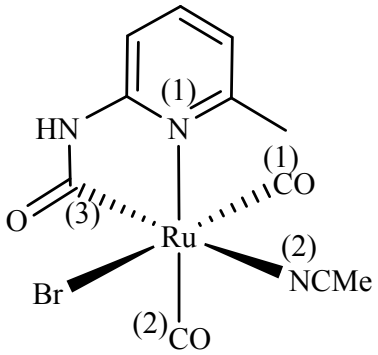
Isomer#		*1*	*2*	*3*
Structure				
Free energy (kJ/mol)	**2a**	0	1	38
	2b	0	8	35
4		*5*	*6*	*7*
				
47		45	46	58
44		45	53	63

Table S9. Selected interatomic distances (Å) for the computationally optimized isomers of **2a**.



Isomer / Distance	1	2	3	4	5	6	7
Ru–Br	2.601	2.685	2.613	2.567	2.590	2.699	2.599
Ru–N1	2.136	2.149	2.092	2.145	2.104	2.098	2.096
Ru–C1	1.872	1.874	1.863	2.003	1.860	1.961	1.954
Ru–C2	1.889	1.885	2.007	1.876	1.985	1.961	1.954
Ru–C3	2.033	2.044	2.079	2.078	2.102	2.034	2.044
Ru–N2	2.205	2.114	2.060	2.052	2.120	2.055	2.177
C3-Ru-N1	80.00	79.68	79.56	79.06	79.25	80.20	80.15
N1-Ru–N2	93.09	93.55	97.13	97.03	99.00	96.05	95.67

Table S10. Selected interatomic distances (Å) for the computationally optimized isomers of **2b**.



Isomer / Distance	1	2	3	4	5	6	7
Ru–Br	2.596	2.747	2.613	2.566	2.583	2.746	2.597
Ru–N1	2.197	2.212	2.138	2.199	2.149	2.161	2.156
Ru–C1	1.873	1.876	1.861	2.008	1.859	1.960	1.955
Ru–C2	1.882	1.880	2.014	1.864	1.991	1.960	1.955
Ru–C3	2.026	2.032	2.071	2.073	2.093	2.025	2.039
Ru–N2	2.233	2.111	2.052	2.052	2.120	2.050	2.192
C3–Ru-N1	79.62	79.31	79.72	78.91	79.43	79.83	79.85
N1-Ru-N2	102.01	106.65	103.50	102.92	104.70	108.16	103.48