

Supporting Information for

Solution equilibrium studies on salicylidene aminoguanidine Schiff base metal complexes: impact of the hybridization with L-proline on stability, redox activity and cytotoxicity

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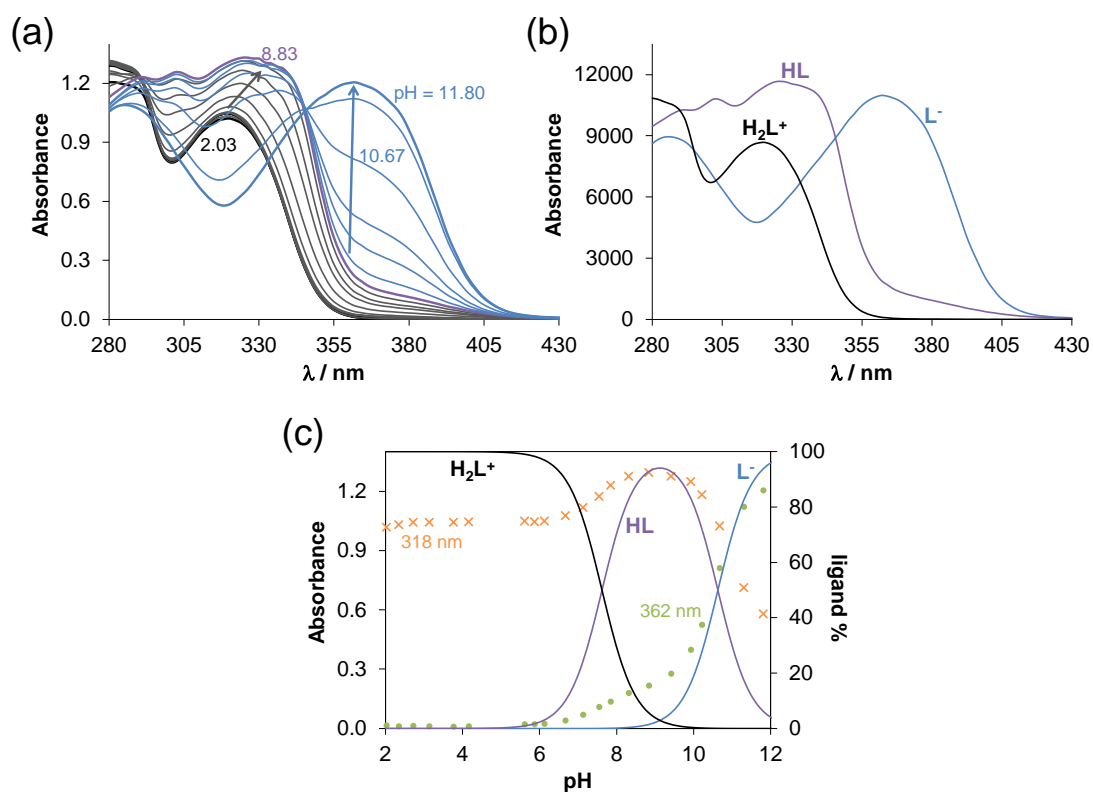


Figure S1. (a) UV-vis absorption spectra of SISC recorded in 30% (v/v) DMSO/H₂O at various pH values, and (b) individual molar absorption spectra of the ligand species in the different protonation states. (c) Concentration distribution curves with absorbance values measured at 318 nm (x) and 362 nm (•). { $C_{\text{ligand}} = 120 \mu\text{M}$; $T = 25 \text{ }^\circ\text{C}$; $I = 0.10 \text{ M (KCl)}$; $\ell = 1 \text{ cm}$ }

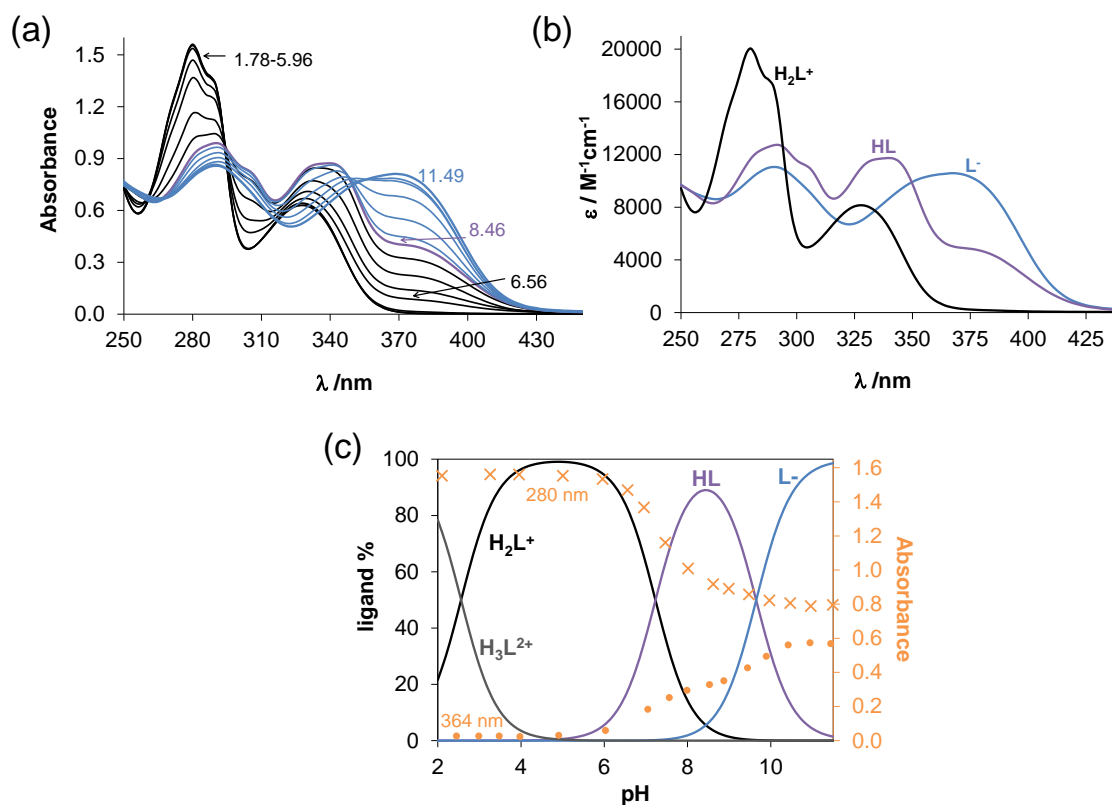


Figure S2. (a) UV-vis absorption spectra of Pro-SISC-Me recorded at various pH values in water. (b) Individual molar absorptivity spectra of the ligand species in the different protonation states. (c) Concentration distribution curves with absorbance values measured at 280 nm (\times) and 364 nm (\bullet). $\{C_{\text{ligand}} = 73 \mu\text{M}; T = 25^\circ\text{C}; I = 0.10 \text{ M (KCl)}; \ell = 1 \text{ cm}\}$

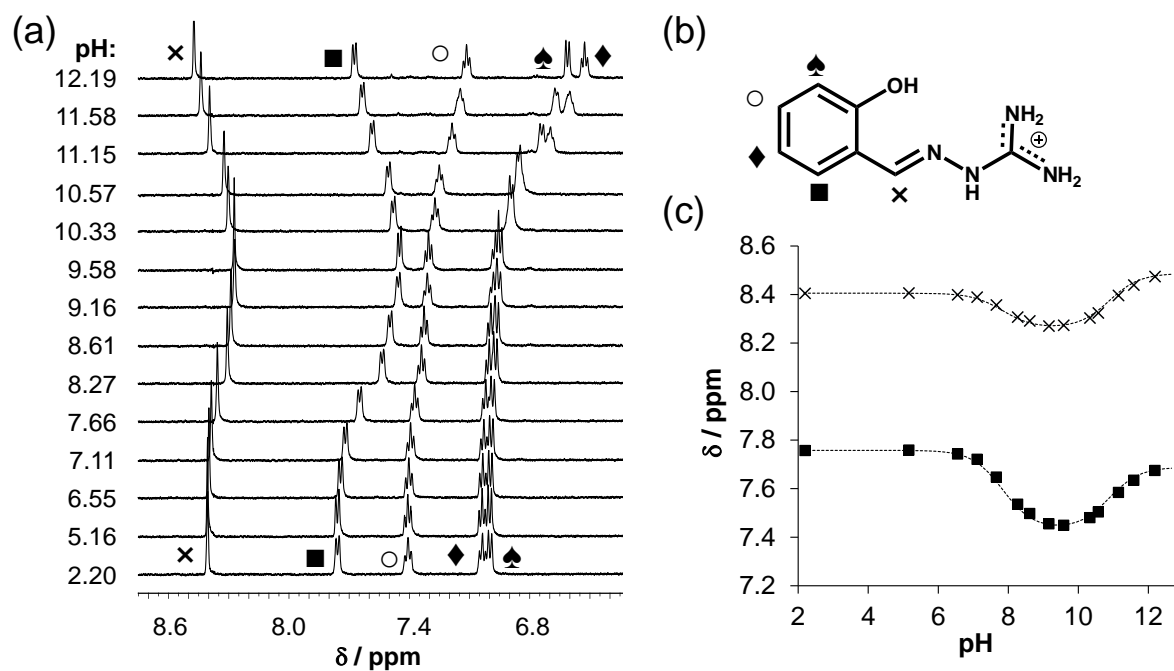


Figure S3. (a) ¹H NMR spectra of SISC recorded at the indicated pH values, (b) notation of the symbols at the various peaks, and (c) pH-dependent chemical shifts of two selected CH protons (x, ■) with the fitted curves (dashed lines). {*c*_{ligand} = 1.0 mM; 30% (*v/v*) d₆-DMSO/H₂O; pH = 2.2 – 12.2; *T* = 25 °C; *I* = 0.10 M (KCl)}.

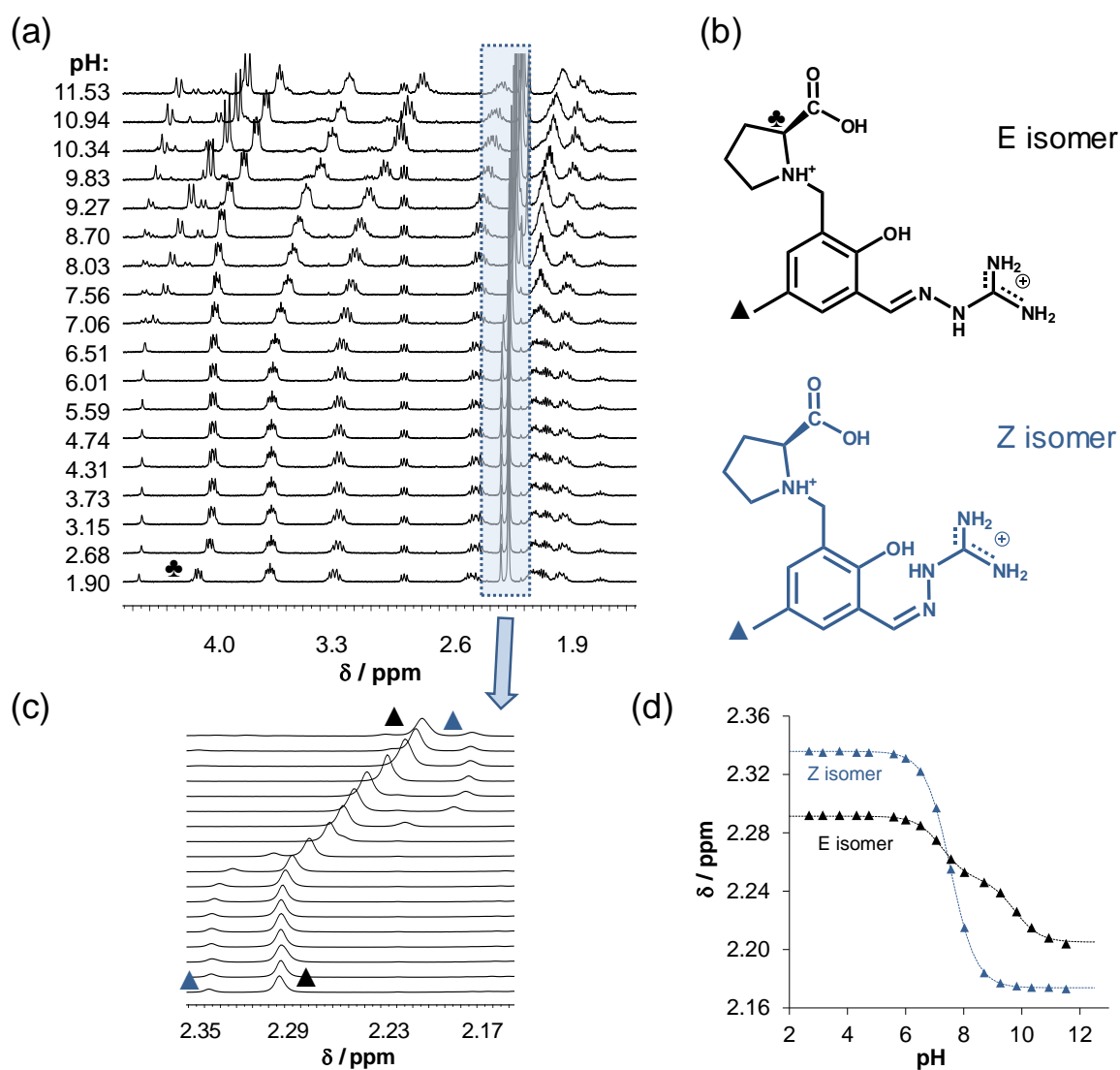


Figure S4. (a) ^1H NMR spectra of Pro-SISC-Me in the high field region recorded at the indicated pH values, (b) structure of the E and the Z isomers with the notation of the symbols of the CH_3 protons ($\blacktriangle, \blacktriangleleft$), and (c) ^1H NMR spectra in the chemical shift range of the CH_3 protons, and (d) the chemical shifts of the CH_3 protons plotted against the pH for the E isomer (\blacktriangle) and the Z isomer (\blacktriangleleft) with the fitted curves (dashed lines). $\{C_{\text{ligand}} = 1.0 \text{ mM}; 10\% (v/v) \text{ D}_2\text{O}/\text{H}_2\text{O}; \text{pH} = 2.2 - 12.2; T = 25 \text{ }^\circ\text{C}; I = 0.10 \text{ M (KCl)}\}$.

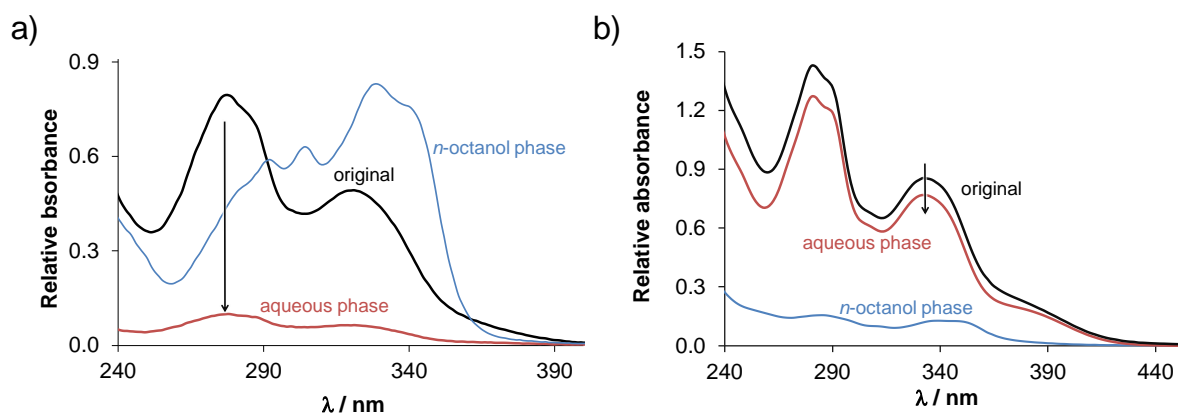


Figure S5. UV-vis absorption spectra of (a) SISC and (b) Pro-SISC-Me recorded for the original solution (black line) and for the aqueous (red line) and *n*-octanol (blue line) phases after partitioning. {pH = 7.4 (10 mM HEPES); *I* = 0.1 M (KCl); *T* = 25 °C}.

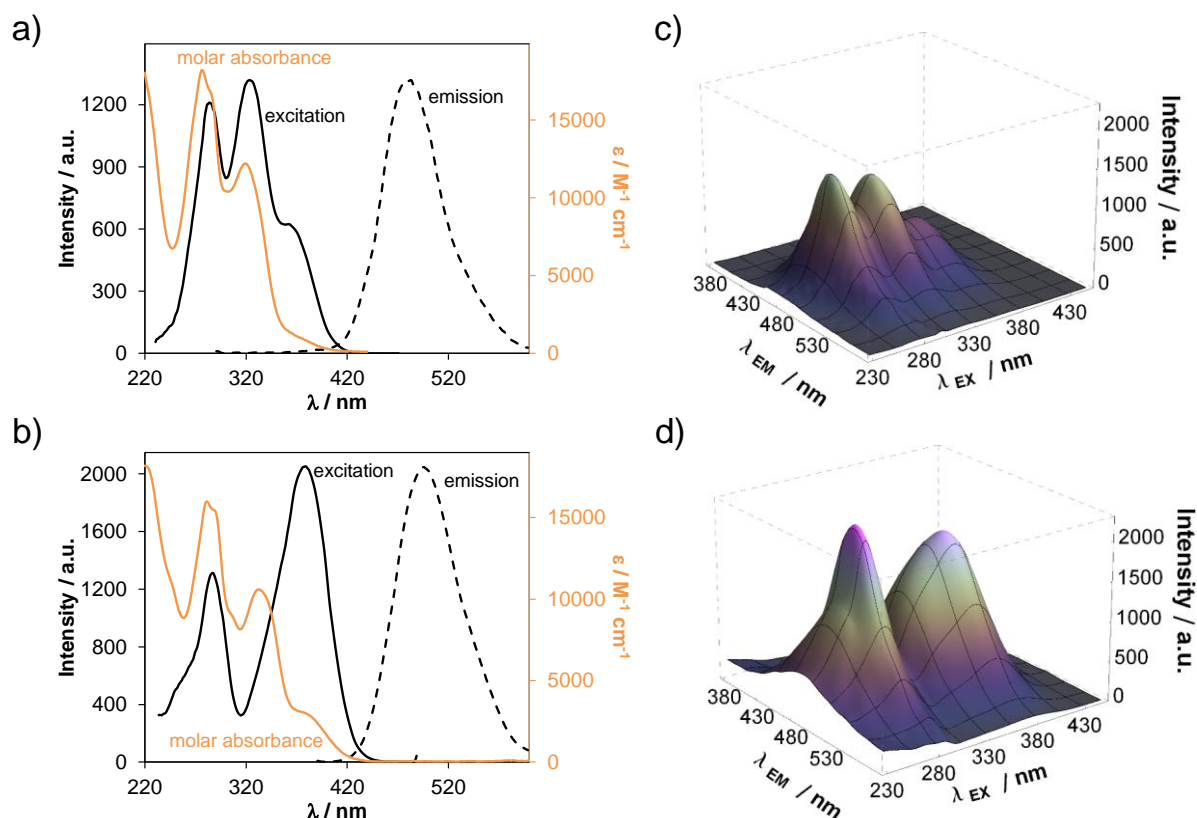


Figure S6. Molar UV-vis absorbance (orange line), excitation (black solid line) and emission (black dashed line) spectra of (a) SISC, and (b) Pro-SISC-Me, and the 3-dimensional fluorescence spectrum of (c) SISC and (d) Pro-SISC-Me at pH 7.40 in water. { C_{ligand} = 10 μM ; *T* = 25 °C; *I* = 0.10 M (KCl); ℓ = 1 cm}

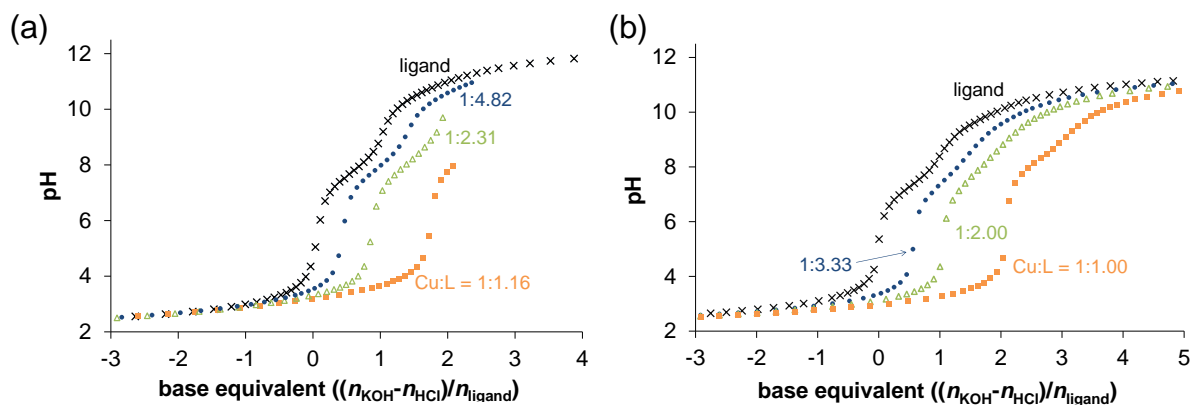


Figure S7. pH-potentiometric titration curves obtained for the (a) Cu(II) – SISC system in 30% (v/v) DMSO/H₂O and (b) Cu(II) – Pro-SISC-Me system in water at various metal-to-ligand ratios (indicated in the figure). $\{C_{\text{ligand}} = 1 \text{ mM}; T = 25 \text{ }^{\circ}\text{C}; I = 0.10 \text{ M (KCl)}\}$

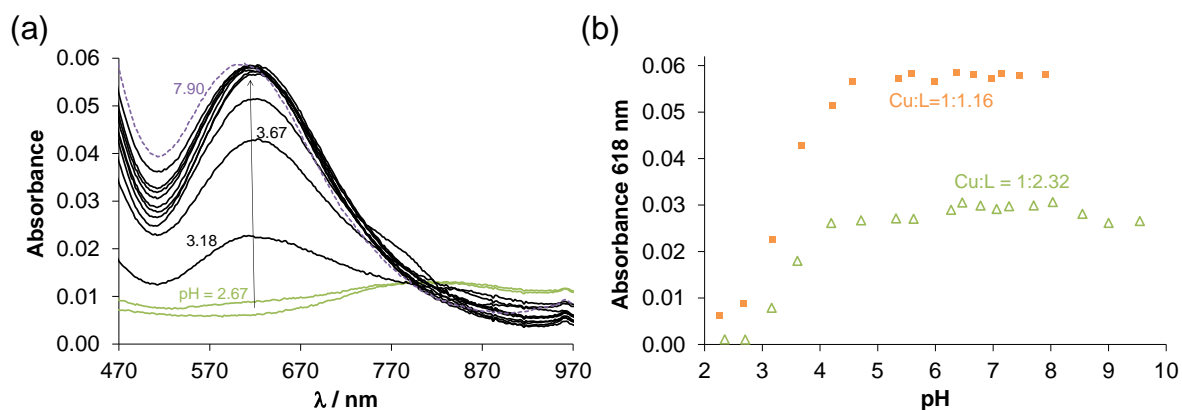


Figure S8. (a) UV-vis absorption spectra of the Cu(II) – SISC (1:1.16) system recorded at various pH values and (b) changes of the absorbance at 618 nm at 1:1.16 and 1:2.32 Cu(II)-to-ligand ratios. $\{30\% \text{ (v/v) DMSO/H}_2\text{O}; C_{\text{ligand}} = 564 \text{ } \mu\text{M}; T = 25 \text{ }^{\circ}\text{C}; I = 0.10 \text{ M (KCl)}; \ell = 2 \text{ cm}\}$

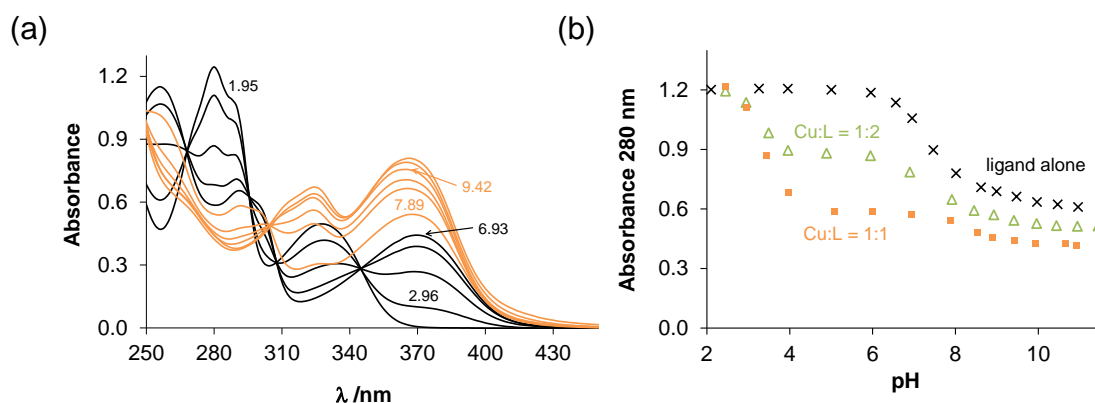


Figure S9. (a) UV-vis absorption spectra of the Cu(II) – Pro-SISC-Me (1:1) system recorded in water at various pH values, and (b) changes of the absorbance at 280 nm at 1:1 and 1:2 Cu(II)-to-ligand ratios and for the ligand alone. $\{C_{\text{ligand}} = 50 \text{ } \mu\text{M}; T = 25 \text{ }^{\circ}\text{C}; I = 0.10 \text{ M (KCl)}; \ell = 1 \text{ cm}\}$

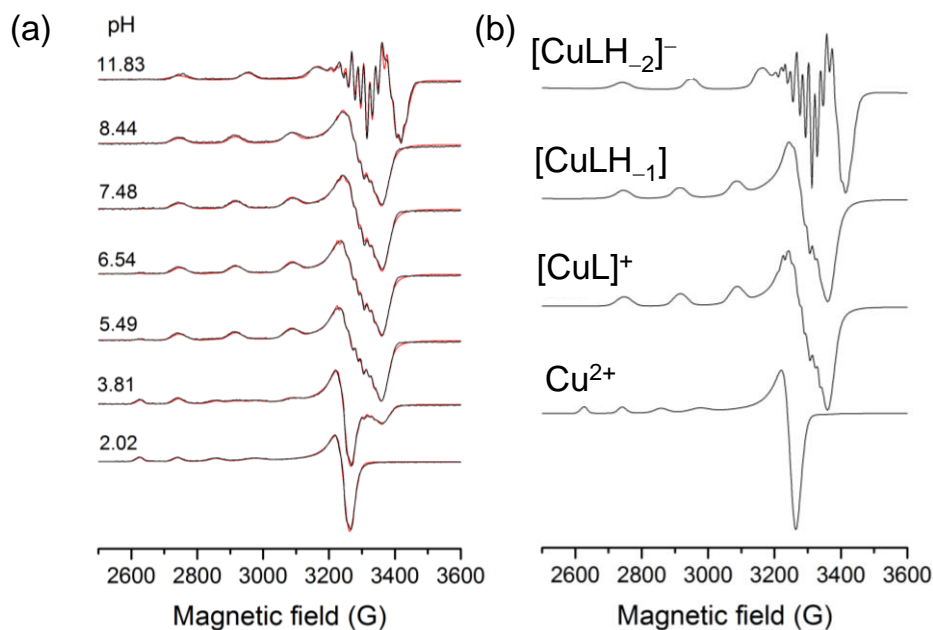


Figure S10. (a) Experimental (black) and simulated (red) anisotropic EPR spectra recorded for the Cu(II) – SISC (1 : 1) system in 30% (*v/v*) DMSO/H₂O at 77 K at various pH values, and (b) the calculated component frozen EPR spectra. {*c*_{ligand} = *c*_{Cu(II)} = 250 μM; *I* = 0.10 M (KCl)}.

Table S1. Isotropic EPR parameters calculated for the various species in the Cu(II) – Pro-SISC-Me complexes in water, and the overall stability constants of the complexes calculated from the deconvolution of the spectra. {*I* = 0.10 M (KCl)}^a

	Cu^{2+}	$[\text{CuL}]^+$	$[\text{CuLH}_{-1}]$	$[\text{CuLH}_2]^-$
g_0	2.192	2.119	2.106	2.100
A_0 / G	36.6	61.6	66.2	86.6
a^{N1} / G	–	16.9	17.2	15.5
a^{N2} / G	–	12.1	12.2	14.1
α / G	48	25	25	23.4
β / G	–1	–13.3	–12.7	2.8
γ / G	1.5	2.7	2.8	3.0
$\log \beta$	–	14.78	6.50	–4.32

^a The experimental errors were ± 0.001 for g_0 and ± 1 G for couplings and linewidth parameters.

Table S2. Anisotropic EPR parameters calculated for the various species in the Cu(II) – SISC complexes. {30% (v/v) DMSO/H₂O; *I* = 0.10 M (KCl)}^a

	Cu ²⁺	[CuL] ⁺	[CuLH ₁]	[CuLH ₂] ⁺
g_x	2.080	2.039	2.045	2.033
g_y	2.080	2.059	2.048	2.045
g_z	2.410	2.248	2.245	2.206
g_0 , calc. ^b	2.190	2.115	2.113	2.095
A_x / G	8	20	14	24
A_y / G	8	22	22	33
A_z / G	113	166	168	205
a_x^{N1} / G	–	9	10	18
a_y^{N1} / G	–	14	14	16
a_z^{N1} / G	–	11	10	14
a_x^{N2} / G	–	16	15	14
a_y^{N2} / G	–	8	16	22
a_z^{N2} / G	–	15	11	6

^a The experimental errors were ± 0.002 for g_x and g_y , ± 0.001 for g_z , ± 2 G for A_x and A_y , and ± 1 G for A_z and nitrogen couplings. ^b Isotropic values of the *g* tensor are calculated via equation: $g_0 = (g_x + g_y + g_z)/3$.

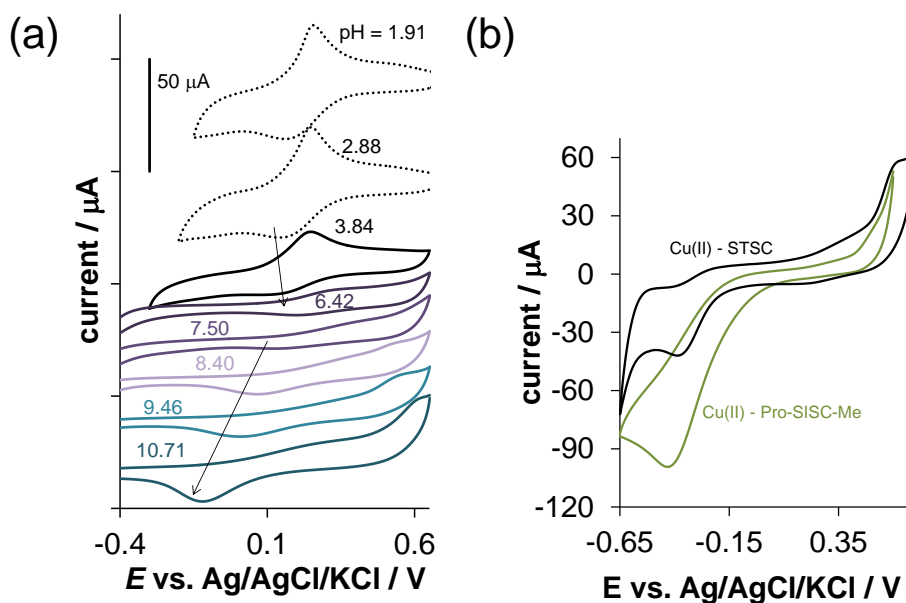


Figure S11. Cyclic voltammograms recorded for the (a) Cu(II) – SISC system at various pH values and for (b) Cu(II) – Pro-SISC-Me and Cu(II) – STSC systems at pH 7.4 at 1:1 metal-to-ligand ratio. {30% (v/v) DMSO/H₂O; *c*_{ligand} = *c*_{Cu(II)} = 1 mM; *T* = 25 °C; *I* = 0.10 M (TBAN); scan rate: 20 mV/s}

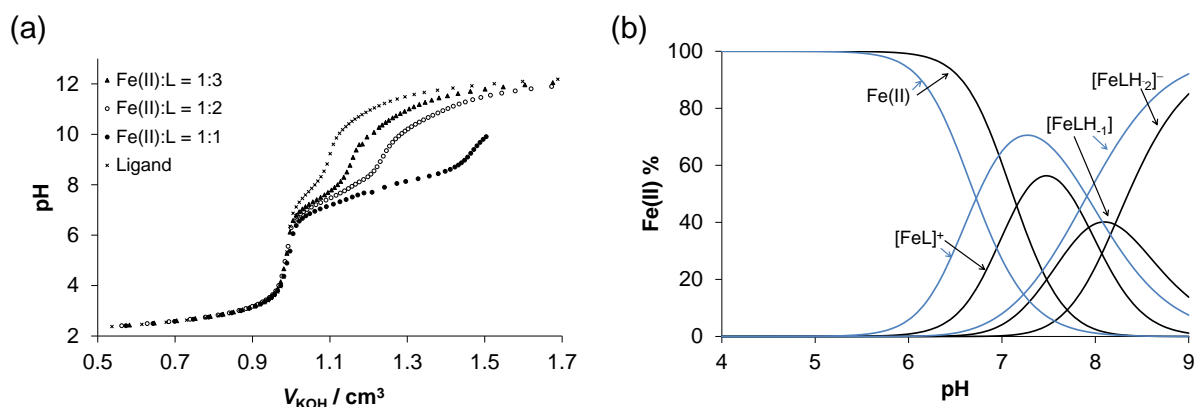


Figure S12. pH-potentiometric titration curves obtained for the (a) Fe(II) – SISC system in 30% (v/v) DMSO/H₂O at various metal-to-ligand ratios (indicated in the figure). (b) Concentration distribution curves for the Fe(II) – SISC (black lines) and Fe(II) – Pro-SISC-Me (blue lines) systems at 1:2 metal-to-ligand ratio. { $C_{\text{ligand}} = 1 \text{ mM}$; $T = 25 \text{ }^\circ\text{C}$; $I = 0.10 \text{ M (KCl)}$ }

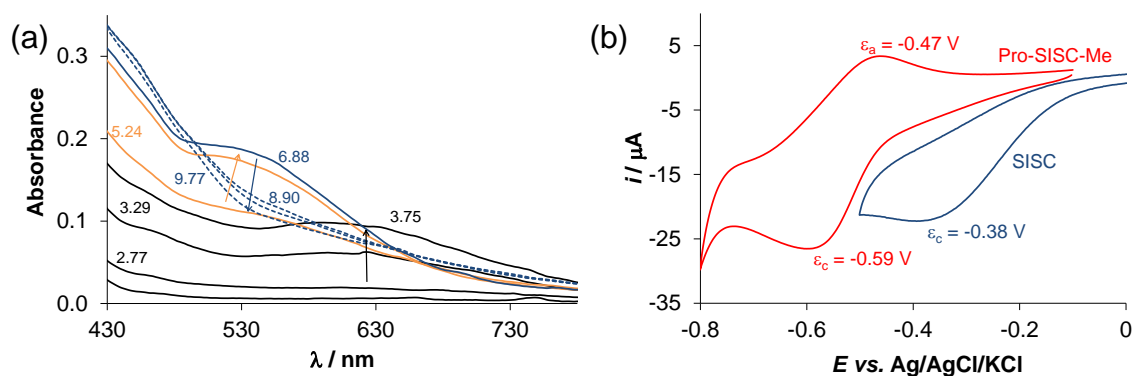


Figure S13. (a) UV-vis spectra recorded for the Fe(III) – SISC (1:2) system in 30% (v/v) DMSO/H₂O at various pH values (indicated in the figure). { $C_{\text{ligand}} = 0.5 \text{ mM}$; $T = 25 \text{ }^\circ\text{C}$; $I = 0.10 \text{ M (KCl)}$; $\ell = 1 \text{ cm}$ } (b) Cyclic voltammograms for the iron complexes of SISC (blue line) and Pro-SISC-Me (red line) at 1:2 metal-to-ligand ratio at pH 7.6 in 30% (v/v) DMSO/H₂O. { $C_{\text{ligand}} = 1 \text{ mM}$; $T = 25 \text{ }^\circ\text{C}$; $I = 0.10 \text{ M (TBAN)}$ }

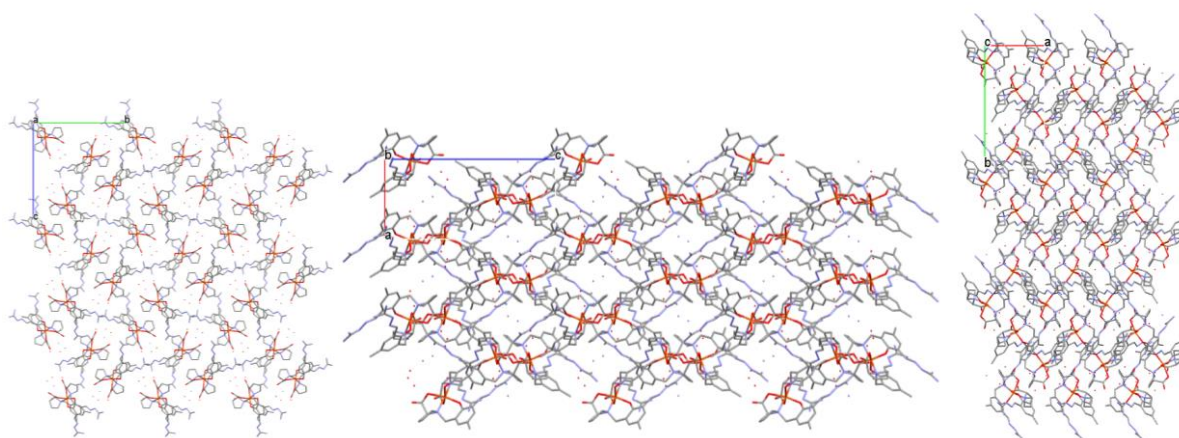


Figure S14. Packing arrangement of crystal (2) showing some selected hydrogen bonds and C-H... π interactions.

Table S3. Crystal data and details of data collection for Pro-SISC-Me·HBr·4H₂O (**1**)

Compound	1
Empirical formula	C ₁₅ H ₃₀ BrN ₅ O ₇
Formula weight	472.35
Space group	orthorhombic, <i>P</i> 2 ₁ 2 ₁ 2 ₁
<i>Unit cell dimensions</i>	
<i>a</i> (Å)	6.7067(2)
<i>b</i> (Å)	14.9720(4)
<i>c</i> (Å)	21.2674(5)
<i>V</i> (Å ³)	2135.52(10)
<i>Z</i>	4
λ (Å)	1.54178
Density (calculated) (mg/m ³)	1.469
Crystal size (mm)	0.28 × 0.03 × 0.02
Temperature (K)	100(2)
Absorption coefficient, μ (mm ⁻¹)	3.027
<i>R</i> ₁ ^a	0.0382
<i>wR</i> ₂ ^b	0.1003
Goodness-of-fit ^c	1.052

^a $R_1 = \Sigma ||F_o| - |F_c|| / \Sigma |F_o|$, ^b $wR_2 = \{\Sigma[w(F_o^2 - F_c^2)^2] / \Sigma[w(F_o^2)^2]\}^{1/2}$, ^c Goodness-of-fit (GOF) = $\{\Sigma[w(F_o^2 - F_c^2)^2] / (n - p)\}^{1/2}$, where *n* is the number of reflections and *p* is the total number of parameters refined.

Table S4. Crystal data and structure refinement of Na[Fe(III)(Pro-SISC-MeH-₂)₂](H₂O)₄ (**2**)

Compound	2
Colour/shape	Red/Chunk
Empirical formula	C ₃₀ H ₄₆ N ₁₀ O ₁₀ FeNa
Moiety formula	[Fe(C ₃₀ H ₃₈ N ₁₀ O ₆)], 4(H ₂ O), Na
Formula weight	785.61
Radiation and wavelength (Å)	Mo-Kα, λ =0.71073
Crystal system	orthorhombic
Space group	<i>P</i> 2 ₁ 2 ₁ 2 ₁
<i>Unit cell dimensions</i>	
a (Å)	9.361(2)
b (Å)	18.993(4)
c (Å)	20.041(4)
Volume (Å ³)	3562.9(14)
Z/Z'	4/1
Density (calculated) (mg/m ³)	1.465
Absorption coefficient, μ (mm ⁻¹)	0.505
Crystal size (mm)	0.60 x 0.10 x 0.05
Temperature (K)	103(2)
Absorption correction	Numerical
Data / restraints / parameters	6003 /12 /495
<i>R</i> ₁ ^a	0.1051
<i>wR</i> ₂ ^b	0.2268
Goodness-of-fit on <i>F</i> ² ^c	1.092

^a $R_1 = \sum ||F_o| - |F_c|| / \sum |F_o|$. ^b $wR_2 = \{\sum [w(F_o^2 - F_c^2)^2] / \sum [w(F_o^2)^2]\}^{1/2}$. ^c Goodness-of-fit (GOF) = $\{\sum [w(F_o^2 - F_c^2)^2] / (n - p)\}^{1/2}$, where *n* is the number of reflections and *p* is the total number of parameters refined.