Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

for

TriAzaCyclophane (TAC)-Scaffolded Histidine and Aspartic Acid Residues as Structural Mimics of non-heme Metalloenzyme Active Sites

by

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Contents:

Detailed Experimental Information

Chemicals were obtained from commercial sources and used without further purification. Reactions were performed at room temperature. Solution phase reactions were monitored by TLC analysis and R_f-values were determined on Merck pre-coated silica gel 60 F-254 (0.25 mm) plates. Spots were visualized with UV-light. Solid-phase synthesis was carried out in plastic syringes with PE frit (20 μm), Applied Separations Inc., distributed by Alltech Applied Science Group (Hoogeveen, The Netherlands). Column chromatography was carried out using Silica-P Flash silica gel (60 Å; particle size 40-63 μm; Silicycle). Electrospray Ionisation mass spectrometry (ESI–MS) was performed on a Finnigan LCQ Deca XP MAX LC/MS system. HPLC was performed on a Shimadzu Class-VP automated high performance liquid system, using an analytical reverse-phase column (Alltima, C8, 300 Å, 5 μm, 250 × 4.6 mm) and a UV-detector (operating at 220 and 254 nm) and an ELSD-detector. Elution was realized using a gradient from water/MeCN/TFA – 95/5/0.1 % (v/v) to MeCN/water/TFA – 95/5/0.1 % (v/v) in 20 min and at a flow rate of 1 mL/min.

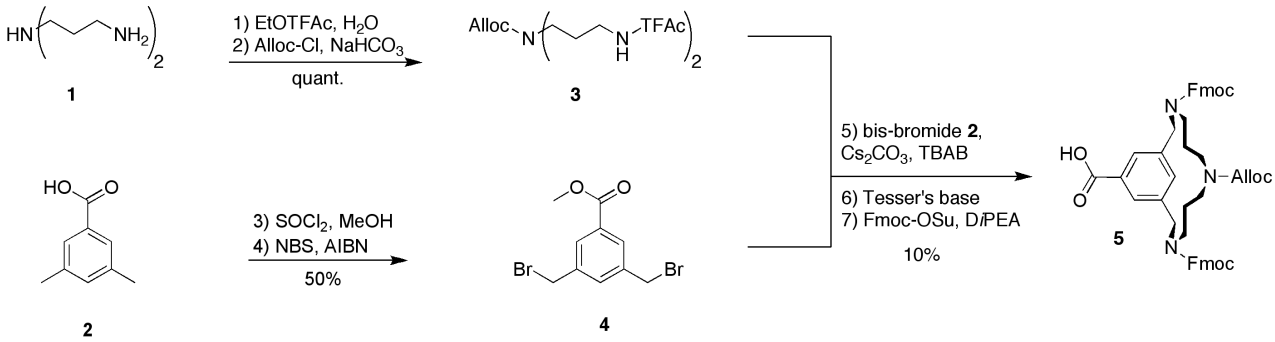
A PHM120 standard pH-meter from Meterlab was used in the titration experiment. Microtiterplate reader was from BioTek mQuant (Beun De Ronde, Abcoude, The Netherlands). The UV-vis microtiterplate was from Greiner bio-one (Alphen aan de Rijn, The Netherlands). Software used for data analysis was the Full Mode-KC4 (Version 3.4 (Rev 21)) software (BioTek instruments) and data point resolution was set at 2 nm. ESI-MS spectra were recorded on a Micromass LCT mass spectrometer calibrated with CsI using nano-ESI at 1200V capillairy voltage and 50V at the sample cone. IR spectra were recorded on a Bruker Tensor 37 FT-IR spectrometer with a DTGS detector using KBr pellets, at a data-point resolution of 4 cm^{-1}. Raman spectra of all copper-complexes were recorded on a Kaiser RXN spectrometer equipped with a 785 nm diode laser and coupled to a Hololab 5000 Raman microscope with a pixel resolution of 2 cm^{-1}. A 10× objective was used for beam focusing and collection of scattered radiation and the laser output power was 18 mW. Mimics **2** and **3** were measured on a Perkin Elmer-2000 Fourier-Transform Raman spectrometer equipped with a 1064 nm laser using 30 mW laser output. The data point resolution was 8 cm^{-1} and 10 scans were accumulated with an exposure time of 30 s for each spectrum. The setup for UV-vis diffuse reflection measurements was an Olympus BX41 upright research microscope with a 50×, 0.5 NA high working distance microscope objective. A 75 W Xenon lamp is used for illumination. The microscope was equipped with a 50/50 double viewport tube, which accommodates CD video camera (ColorViewIIIu, Soft Imaging System GmbH) and an optical fiber mount. A 200 micrometer-core fiber connects the microscope to a CCD UV-vis

spectrometer (AvaSpec-2048TEC, Avantes BV). 10 scans were collected with an integration time of 50 ms per scan. For measuring ESI-MS spectra of the complexes, the end-stage complexes obtained in the UV-vis measurement were lyophilized. Dissolved in water and diluted with water to a final concentration of 100 μM. A cone voltage of 20 V was used during the measurement.

In silico complexes between the TAC-ligands and copper(II) were prepared in DS Viewer Pro 6.0.[1] Ligands around the copper centre were fixed in a square-pyramidal (for the mononuclear Cu(II)-complexes) or square-pyramidal (for the dinuclear bis(μ-hydroxo) dicopper(II)-complexes) geometry with the coordinating side-chain functionalities positioned according to empirical findings of the described spectroscopic studies. Structure optimization was performed using the 'clean structure' command until no change in the structures was observed. These structures were then loaded into YASARA in order to generate the pictures.[2]

Detailed Synthetic Procedures

For this, protected tri-amine **3** and bis-bromide **4** had to be prepared (scheme S1). The synthesis of bis-bromide **4** from dimethylbenzoic acid **2** has been described elsewhere.[3] Synthesis of tri-amine **3**, cyclization to bis-bromide **4** and protecting-group manipulations to afford scaffold **5** are described in detail below.



Scheme S1. Synthesis of the Fmoc and Alloc-protected TriAzaCyclophane (TAC)-scaffold **5**.

Synthesis of bis(3-(TFAc)aminopropyl)(Alloc)amine 3:

Bis(3-aminopropyl)amine **1** (5 mL; 35 mmol) and water (630 μL; 35 mmol) were added to 100 mL MeCN (stored on 3Å molsieves). To this solution, ethyl trifluoroacetate (13.2 mL; 105 mmol) was added and the mixture was refluxed for 2 days. The mixture was concentrated under reduced pressure, which resulted in the formation of a white solid. The solid was dissolved in 60 mL dioxane and 60 mL water and $NaHCO_3$ (11.8 g; 140 mmol) was added. To this mixture, a solution of Alloc-Cl (4.5 mL; 42 mmol; 1.2 equiv) in dioxane (30 mL) was added

slowly. The reaction was allowed to proceed overnight, after which the mixture was concentrated *in vacuo*. To the residue water was added and the product was extracted using DCM. The DCM extract was dried over Na_2SO_4, filtered and concentrated under reduced pressure. Colorless oil was obtained that crystallized under vacuum to a white solid. Yield: 14.3 g (35 mmol; quantitative). R_f = 0.45 (5% MeOH in DCM). ^{1}H-NMR (300 MHz, $CDCl_3$, TMS): δ 1.76 (s, 4H, $CH_2CH_2CH_2$), 3.30-3.32 (s, 8H, NCH_2), 4.56-4.58 (d, 2H, OCH_2), 5.20-5.31 (m, 2H, $=CH_2$), 5.82-5.93 (m, 1H, $=CH_2$-), 7.18 and 8.03 (ds, 2H, N*H*-TFAc). ^{13}C-NMR (75 MHz, $CDCl_3$, APT): δ 27.5 ($CH_2CH_2CH_2$), 36.1 (NCH_2), 43.8 ($NHCH_2$), 66.7 and 67.0 (OCH_2), 113.9 and 114.6 and 117.7 ($C(O)CF_3$), 118.5 ($=CH_2$), 132.1 (=*C*H-), 156.6-158.1 (*C*=O). ^{19}F-NMR (282 MHz, $CDCl_3$): δ -76.56, -76.34 (d, $C(O)CF_3$).

Synthesis of MeO-TAC(TFAc/Alloc/TFAc):

Protected triamine **3** (4.1 g; 11 mmol), bis-bromide **4** (3.2 g; 11 mmol), TBAB (3.2 g; 11 mmol) and Cs_2CO_3 (14.3 g; 44 mmol) were added to 500 mL MeCN (3Å). The mixture was refluxed until completion of the reaction and then condensed to half of its original volume. $KHSO_4$ (9.0 g; 66 mmol) in water (250 mL) was added and the products were extracted using DCM (2 × 250 mL). This organic phase was washed with brine and dried over Na_2SO_4. After filtration and condensing the filtrate, the obtained product was purified by column chromatography (eluent gradient: DCM:hexanes:EtOAc – 80:1:9 to 80:9:1 (v/v/v)) and obtained as off-white foam. Yield: 3.1 g (5.4 mmol; 49%). R_f = 0.32 (5% MeOH in DCM). ESI-MS: *m/z* 568.20 (calculated: 568.18 for $[M+H]^+$). ^{1}H-NMR (300 MHz, $CDCl_3$, TMS): δ 1.26-1.63 (m, 4H, $CH_2CH_2CH_2$), 2.87-3.49 (m, 8H, NCH_2CH_2), 3.91-3.96 (t, 3H, OCH_3), 4.49-4.51 (m, 2H, OCH_2), 4.68-4.79 (m, 4H, Ar-CH_2N), 5.17-5.28 (m, 2H, $=CH_2$), 5.81-5.92 (m, 1H, =C*H*-), 7.50-8.07 (m, 3H, $C^{Ar}H$).

A dimeric species was also collected: Yield: 1.9 g (1.7 mmol; 31%). R_f = 0.30 (5% MeOH/DCM). ESI-MS: *m/z* 568.50 (calculated: 568.68 for $[M+2H]^{2+}$); *m/z* 1135.20 (calculated: 1135.36 for $[M+H]^+$). ^{1}H-NMR (300 MHz, $CDCl_3$, TMS): δ 1.79-1.83 (d, 4H, $CH_2CH_2CH_2$), 3.19-3.32 (m, 8H, NCH_2CH_2), 3.91-3.95 (t, 3H, OCH_3), 4.43-4.76 (m, 12H, 2 x OCH_2 and 4 x Ar-CH_2N), 5.14-5.30 (m, 2H, $=CH_2$), 5.78-5.86 (m, 1H, =C*H*-), 7.47-7.92 (m, 3H, $C^{Ar}H$).

Synthesis of Fmoc/Alloc-protected TAC-scaffold

Fully protected monomeric TAC-scaffold (3.1 g; 5.4 mmol; see ESI for synthesis) was dissolved in Tesser's base (177 mL; dioxane:methanol:4N NaOH – 14:5:1 (v/v))[4] and allowed to react overnight at room temperature. The resulting solution was neutralized using 1N HCl

and concentrated under reduced pressure. The crude product was dissolved in 100 mL acetonitril:water – 1:1 (v/v) and the pH of the solution was adjusted to 9.5 using Et_3N. A solution of Fmoc-OSu (3.8 g; 11.3 mmol) in acetonitril (10 mL) was added and the pH of the mixture was maintained between 8.5-9.0 using Et_3N. After approximately 90 min, the reaction was complete and acidified using 1N HCl. Water (350 mL) was added and the product was extracted with EtOAc. The collected organic layers were dried over Na_2SO_4, concentrated *in vacuo* and the product was purified by column chromatography (eluent: EtOAc:hexanes – 2:1 (v/v) and a trace of AcOH). After concentration of the fractions, the product was obtained as off-white foam. The desired product was obtained as a white solid by precipitation from THF using Et_2O. Yield: 2.9 g (3.6 mmol; 20%). HPLC (C_8): t_R = 24.98 min. ESI-MS: *m/z* 806.60 (calc. 806.34 for $[M+H]^+$). HR-MS: 806.3441 (calc. 806.3436 for $[M+H]^+$). ^{1}H-NMR (300 MHz, $CDCl_3$, TMS): *d* 1.05-1.35 (m, 4H, $CH_2CH_2CH_2$), 2.5-3.4 (m, 8H, NCH_2CH_2), 4.24-4.63 (m, 12H, Ar-CH_2N and OCH_2 and Fmoc-C*H*), 5.16-5.25 (m, 2H, =CH_2), 5.83-5.92 (m, 1H, =C*H*-), 7.3-7.9 (m, 19H, $C^{Ar}H$). ^{13}C-NMR (75 MHz, THF-d_8, APT): *d* 29.6 ($CH_2CH_2CH_2$), 46.0 and 47.7 (NCH_2CH_2), 48.6 (Fmoc-*C*H), 53.5 (Ar-*C*H_2N), 66.2 (Alloc-O*C*H_2 and Fmoc-O*C*H_2), 117.1 (=*C*H_2), 120.9-145.6 (C^{Ar} and =*C*H-), 155.9 and 157.0 (N*C*(O)O), 167.4 (*C*(O)OH).

Solid-phase synthesis of the TAC-based mimics:

For the solid-phase synthesis of the mimics, several standard procedures were applied. These are described in detail below. In general, for each gram of resin roughly 6 mL of solvent/solution (stored on 4 Å molsieves) was used. The reactions were carried out in special solid-phase reaction syringes equipped with filtering frit above the outlet.

- Fmoc-removal: 20% piperidine/NMP was used (2 × 6 mL each 8 min), after which the resin was washed with NMP (3 × 6 mL each 2 min) and DCM (3 × 6 mL each 2 min).
- Alloc-removal: to the resin were subsequently added anilinium *p*-toluenesulfinate (PTSA) (20 eq) and $Pd(PPh_3)_4$ (0.25 eq). The reaction was allowed to proceed for 16 h, after which the reaction mixture was removed by filtration. Remaining Pd was removed by extensive washing with a 20 mM solution of sodium *N*,*N*-diethyldithiocarbamate (3 × 6 mL each 5 min). After this, the resin was washed with NMP (3 × 6 mL each 2 min) and DCM (3 × 6 mL each 2 min).
- Coupling of acids: 1 eq of scaffold **5** or 4 eq Fmoc-AA(PG)-OH was used together with 1 or 4 eq BOP and 2 or 8 eq D*i*PEA, respectively, in NMP. Coupling was performed overnight in case of scaffold **5** and for 3 h in case of Fmoc-AA(PG)-OH and. The resin was washed with NMP (3 × 6 mL each 2 min) and DCM (3 × 6 mL each 2 min). Coupling was monitored using the

Kaisertest[5] in case of coupling to primary amines and the Chloranil test[6] in case of coupling to secondary amines.

- Acetylation: A freshly prepared capping reagent solution was applied in order to acetylate the *N*-terminal amine. This solution consists of Ac_2O (14.2 mL), D*i*PEA (6.5 mL) and HOBt (608 mg). After acetylation the resin was washed with NMP (3 × 6 mL each 2 min) and DCM (3 × 6 mL each 2 min).

- Deprotection and cleavage: The ligand was deprotected and simultaneously removed from the resin using 3 mL TFA/TIS/H_2O – 92.5/5/2.5 (v/v/v).

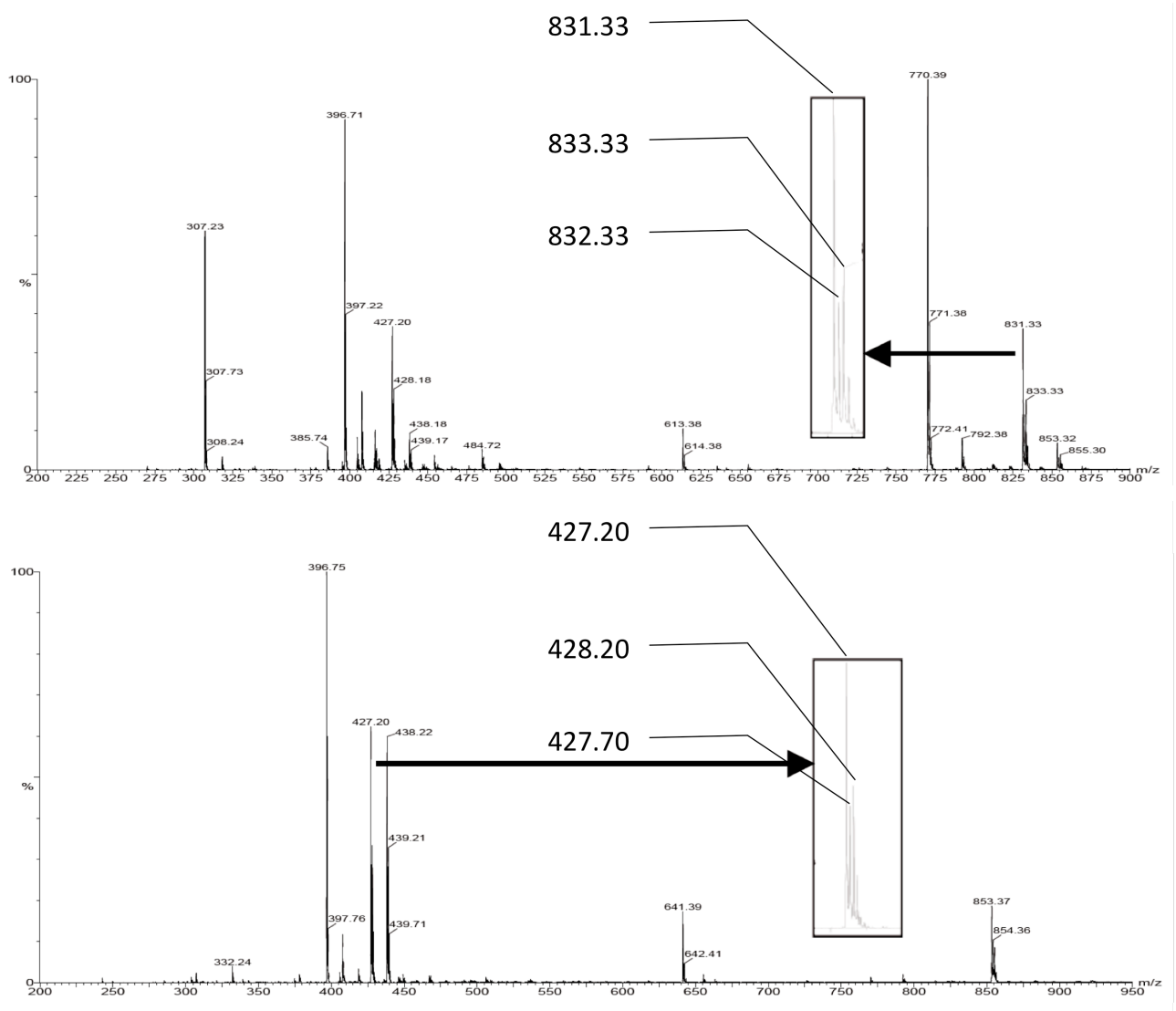
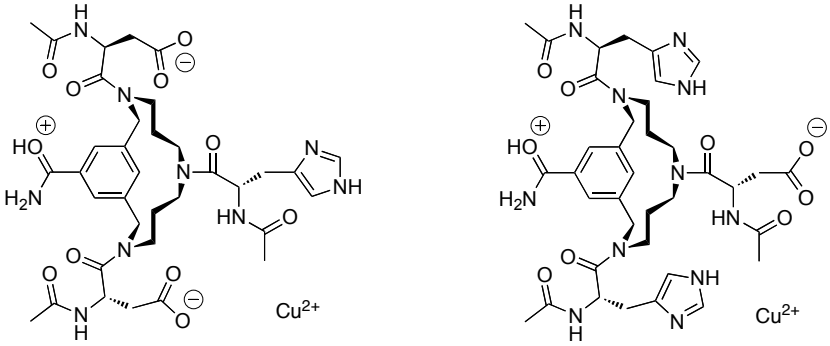


***Figure S1**. ESI-MS spectra of Cu(II)-complexes of the mimics: "1-His-2-Asp" or DHD (top) and "2-His-1-Asp" or HDH (bottom) and the Cu-isotope pattern in the enlargements (see below).*

Assignments (**DHD** = dianion; **HDH** = monoanion): Top: *m/z* 396.71 [**DHD**+3H+Na]$^{2+}$, 770.39 [**DHD**+3H]$^{+}$, 831.33 [**DHD**+Cu]$^{+}$; Bottom: *m/z* 396.75 [**HDH**+3H]$^{2+}$, 427.20 [**HDH**+H+Cu]$^{2+}$, 438.22 [**HDH**+Na+Cu]$^{2+}$, 853.37 [**HDH**+Cu]$^{+}$.



Chemical Formula: $C_{35}H_{46}CuN_9O_{11}^{+}$
Exact Mass: 831.26
m/z: 831.26 (100.0%), 833.26 (45.9%), 832.26 (41.2%), 834.26 (18.6%)

Chemical Formula: $C_{37}H_{49}CuN_{11}O_9^{2+}$
Exact Mass: 854.30
m/z: 427.15 (100.0%), 428.15 (46.3%), 427.65 (44.1%), 428.65 (20.2%)

***Figure S2.** ESI-MS values as calculated for the complexes and their calculated isotope distribution for comparison with the enlargements in Figure S2a.*

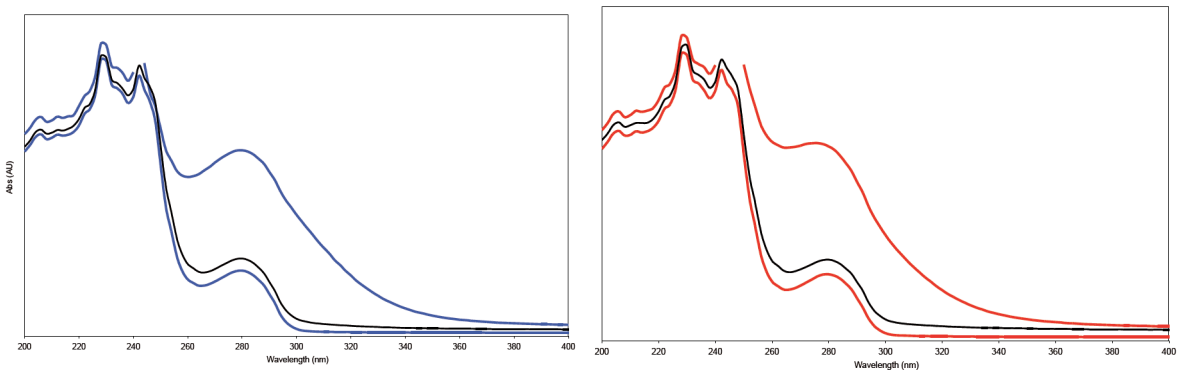


Chart S1. Charger-transfer bands observed in the Cu(II)-complexes of the "2-His-1-Asp" (left) and "1-His-2-Asp" (right) mimics. At 280 nm imidazole-to-copper(II) charge transfer bands can be observed, they increased with increasing pH of the solution. The black trace shows this part of the spectrum corresponding to the sodium-salt of the mimics (pH ~ 7).[7]

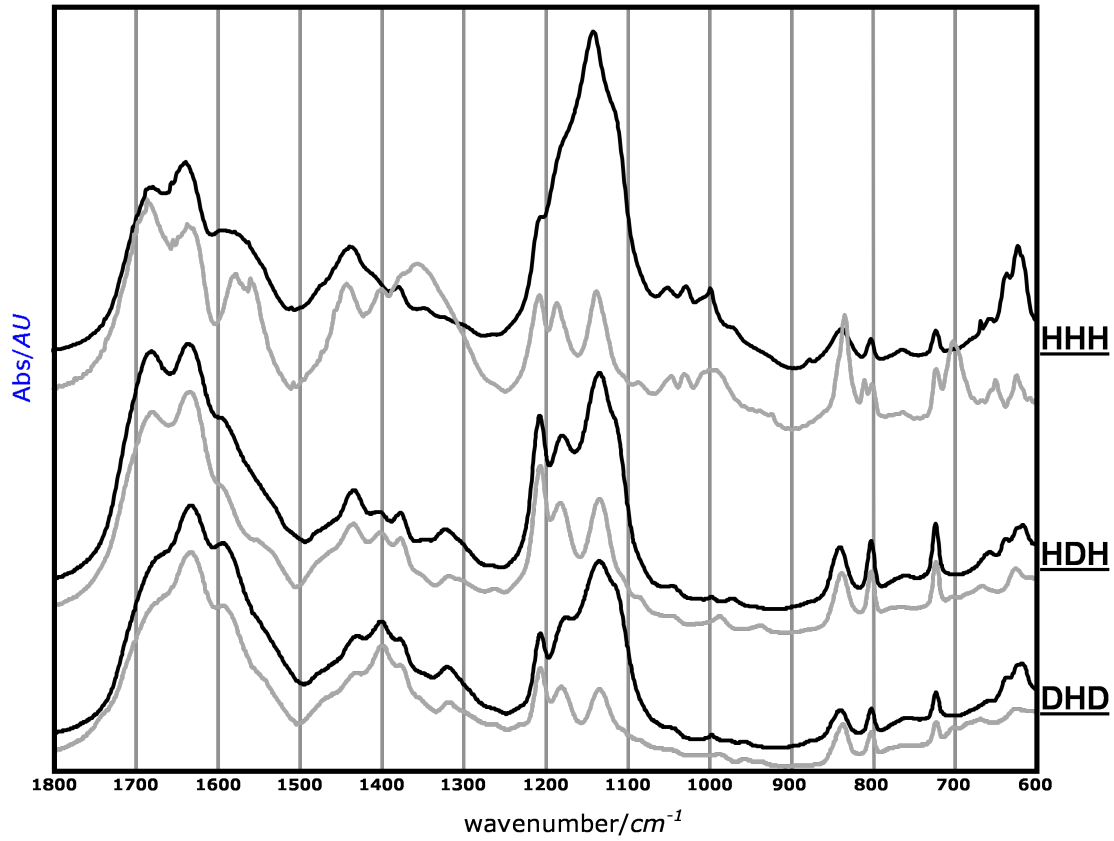


Chart S2. Overlay of the IR-spectra of the "3-His" (top), "2-His-1-Asp" (middle) and "1-His-2-Asp" (bottom) mimics (grey traces) and their Cu(II)-complexes (black traces). The traces of the "3-His" mimic, published before in Chemical Communications, have been added to facilitate comparison. For full description of assignments of that mimic, reference 7 has to be consulted.

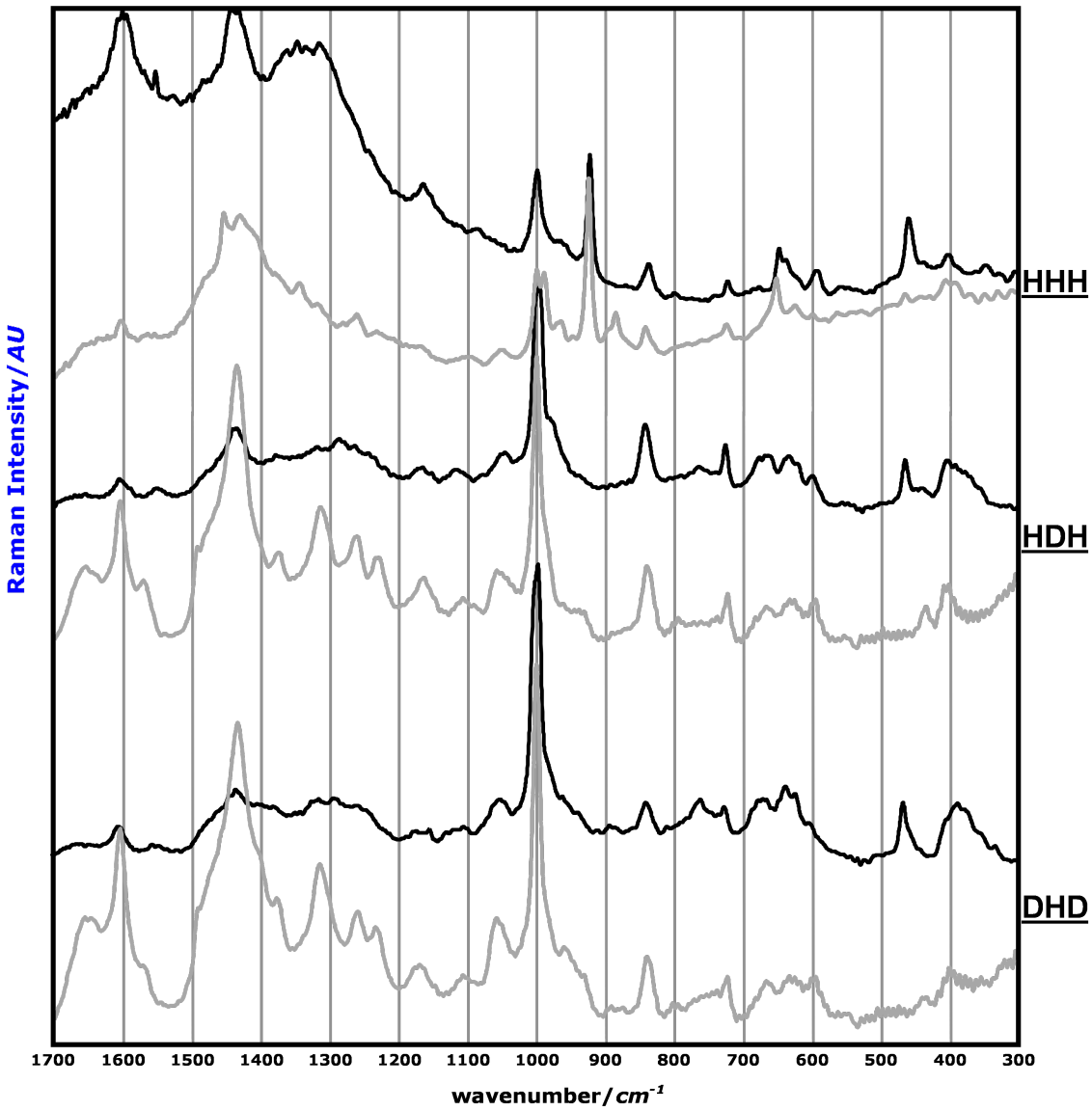


Chart S3. Overlay of the Raman-spectra of the "3-His" (top), "2-His-1-Asp" (middle) and "1-His-2-Asp" (bottom) mimics (grey traces) and their Cu(II)-complexes (black traces). The traces of the "3-His" mimic, published before in Chemical Communications, have been added to facilitate comparison. For full description of assignments of that mimic, reference 7 has to be consulted.

Table S1. Proposed assignments for both Infrared and Raman spectra of the mimics and their Cu(II)-complexes.

"2-His-1-Asp"				"1-His-2-Asp"				
Infrared		Raman		Infrared		Raman		
Mimic	Complex	Mimic	Complex	Mimic	Complex	Mimic	Complex	
3400 (m br)				3400 (m br)				ν N-H (1° and 2°) [a]
3280 (m br)				3280 (m br)				ν N-H (1° and 2°) [a]
3070 (w br)		3110 (w)		3070 (w br)		3110 (w)		ν N-H (2°) [a]
2983 (w)				2979 (w)				ν_a CH_3 [a]
2928 (w)		2932 (vs)	2945 (vs)	2916 (w)		2932 (vs)	2941 (vs)	ν_a CH_2 [a]
2847 (w)				2847 (w)				ν_s CH_2 + CH_3 [a]
2600 (w br)				2600 (w br)				ν Im^+-H [a]
1676 (s)	1675 (s)			1670 (s)	1670 (s)			ν C=O (A-I: 1°, 2° and 3°) [a]
1630 (s)	1629 (s)			1627 (s)	1627 (s)			δ N-H + ν C-N (A-II: 1°) [a]
		1645 (w)				1645 (*m*)		ν C=C* + ν C=N* [d]
		1601 (m)	1606 (w)			1601 (m)	1606 (w)	ν C=C* + ν C=N* (*N*·) [d]
1591 (*m*)	1593 (*m*)		1591 (*w*)	1587 (m)	1588 (m)			ν C=O (carboxylate) [b,d]
								δ H_2O [c]
								δ N-H ν C-N (A-II 2°) [a]
		1564 (w)				1565 (*w*)		ν C=C* + ν C=N* (*N*·) [d]
1430 (w)	1430 (w)	1431 (s)	1437 (m)	1426 (w)	1426 (w)	1430 (s)	1435 (m)	ν ring* [d] / δ CH_2 [d]
								ν ring* [d]
1397 (*w*)	1398 (*w*)			1396 (m)	1396 (m)	1400 (*m*)		ν C-O (carboxylate) [b,d]
								ν ring* [d]
1374 (w)	1371 (w)	1369 (w)		1373 (w)	1374 (w)	1372 (w)		ν C-N (A-III: 1°) [a]
								ν C(O)-N [a] / ν ring*
								ν C(O)-N [a] / ν ring*
								ν C(O)-N [a] / ν ring*
1312 (w)	1316 (w)	1308 (m)		1312 (w)	1317 (w)	1310 (m)	1312 (w)	δ CH_2 [d] / δ =C-H [d]
			1290 (brw)				1290 (brw)	ν ring* (*N*·) [d] / A-III: 2° [a]
		1256 (w)				1255 (w)		ν ring* (*N*·) [d] / A-III: 2° [a]
1205 (s)	1205 (s)	1225 (w)		1202 (m)	1205 (m)	1227 (w)		ν ring* (*N*·) [d] / τ CH_2 [a]
1179 (s)	1176 (s)		1167 (w)	1176 (w)	1172 (m)	1165 (w)	1153 (w)	ν N-C=N + δ_{ip} N-H* [d]
1132 (m)	1130 (s)	1157 (w)		1129 (m)	1129 (s)			ν =C-N* + δ N-H* [d]
	1114 (*s*)		1120 (w)		1116 (*s*)			ν =C-N=* + δ N-H* [d]
1084 (w)		1099 (vw)				1099 (w)	1104 (vw)	ν =C-N=* / δ =C-N (*N*·) [d]
1041 (vw)	1041 (vw)	1050 (w)	1049 (w)	1042 (vw)	1043 (vw)	1050 (m)	1052 (m)	ν C(O)-N [a]
								δ C-H* / δ_{ip} C^{Ar}-H
	994 (vw)	998 (s)	998 (s)		993 (vw)	998 (s)	998 (s)	ν =C-NH* (*N*·) / δ_{ip} C^{BA}-H [d]
983 (vw)			984 (*m*)	980 (vw)			980 (*m*)	ν =C-NH* (*N*·) [d]
	968 (vw)			955 (vw)		954 (w)		ν =C-N* + δ ring [d]
								δ_{ip} C^3-H* [d]
								δ ring* [a]
836 (m)	837 (m)	836 (m)	843 (m)	834 (m)	836 (m)	836 (m)	839 (w)	δ_{oop} =C-H* [d]
801 (m)	801 (m)			801 (m)	801 (m)			δ_{oop} C^{Ar}-H [a]
							761 (w)	δ_{oop} C-H*
722 (m)	722 (m)	720 (w)	727 (m)	722 (w)	722 (w)	720 (w)	726 (w)	δ_{oop} N-H [a] / ρ_{ip} CH_2 [a]
								ω N-H (2°) [a]
	663 (w)		668 (w)	667(w)			673 (w)	δ ring* [d]
	654 (w)	658 (w)				658 (w)		δ ring* [d]
	635 (w)	628 (w)	638 (w)	631 (w)			638 (m)	δ ring* [d]
622 (w)	615 (w)		624 (w)	622 (w)	615 (w)	625 (w)	623 (w)	δ ring* [d]
								ν Cu-OH_2^{eq} [d, e]
		593 (w)	601 (w)			592 (w)	604 (w)	δ_{oop} CO_2 [d]
			467 (m)				466 (m)	ν Cu-N [d]
			406 (m)				404 (*w*)	ν Cu-O
		399 (w)	394 (*w*)			394 (w)	389 (w)	ν Cu-O
			355 (*w*)				359 (*w*)	ν Cu-O
							334 (*w*)	ν Cu-O

Used abbreviations In the peak section (*italic* refers to a shoulder): s = strong, m = medium, w = weak, v = very, br = broad. In the assignment section: ν = stretching, δ = bending, ρ = rocking, τ = twisting, ω = wagging; subscript s = symmetric; subscript a = anti-symmetric; * = imidazole ring vibrations; N_τ = tautomer; N_π = tautomer; ip = in-plane; oop = out-of-plane; C^{BA} = aromatic ring (from the benzamide-core of the TAC-scaffold); 1^o, 2^o or 3^o refer to the type of amide involved (primary, secondary or tertiary, respectively); + = coupled vibrations; / = isolated vibrations; Im = imidazole; A-I = Amide I; A-II = Amide II; A-III = Amide III.

Specified references: a) R.M. Silverstein and F.X. Webster, *Spectrometric Identification of Organic Compounds*, 6th edition, **1998**, Wiley, USA, ch. 3, pp. 71-143; b) G.B. Deacon, R.J. Philips, *Coord. Chem. Rev.* **1980**, *33*, 227-250; V. Zelenák, Z. Vargová, K. Györyová, *Spectrochimica Acta Part A* **2007**, *66*, 262-272; c) K. Nakamoto, *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, 5th edition, **1997**, Wiley, USA, pp. 227-230; P.A. Thiel, T.E. Madey, *Surface Sceince Reports* **1987**, *7*, 211; d) J.G. Mesu, T. Visser, F. Soulimani, E.E. van Faassen, P. de Peinder, A.M. Baele, B.M. Weckhuysen, *Inorg. Chem.* **2006**, *45*, 1960 and J.G. Mesu, T. Visser, F. Soulimani, B.M. Weckhuysen, *Vibr. Spectrosc.* **2005**, *39*, 114; e) C.R. Andrew, J. Han, T. Den Blaauwen, G. Van Pouderoyen, E. Vijgenboom, G.W. Canters, T.M. Loehr, J. Sanders-Loehr, *J. Biol. Inorg. Chem.* **1997**, *2*, 98.

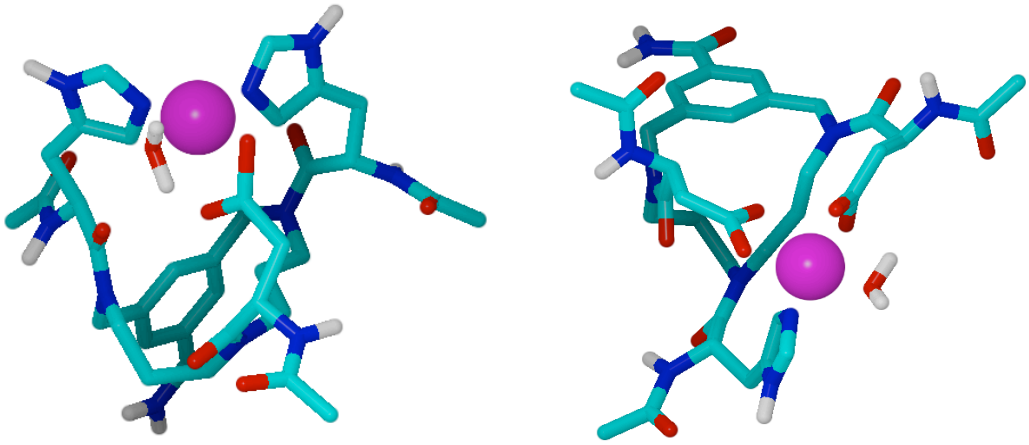


Figure S3. Computer models of the Cu(II)-complexes of the "2-His-1-Asp" (left) and "1-His-2-Asp" (right) mimics. One water molecule is included in order to complete the four positions of the square-planar geometry.

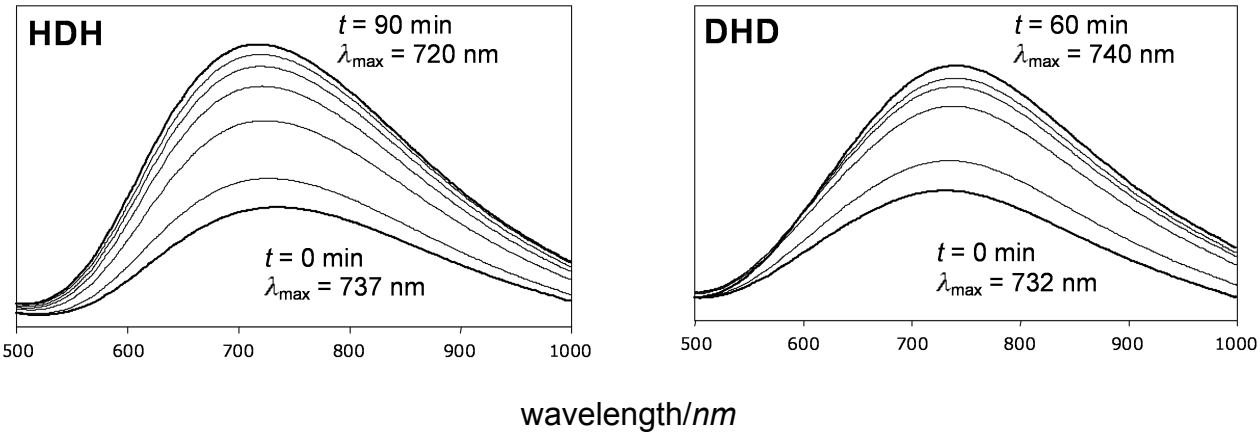


Chart S4. Changes in d-d absorption bands as result of the oxygen-binding by the Cu(I)-complexes (top, one letter-code of the amino acid residues that were attached to the scaffold are given in the graph).

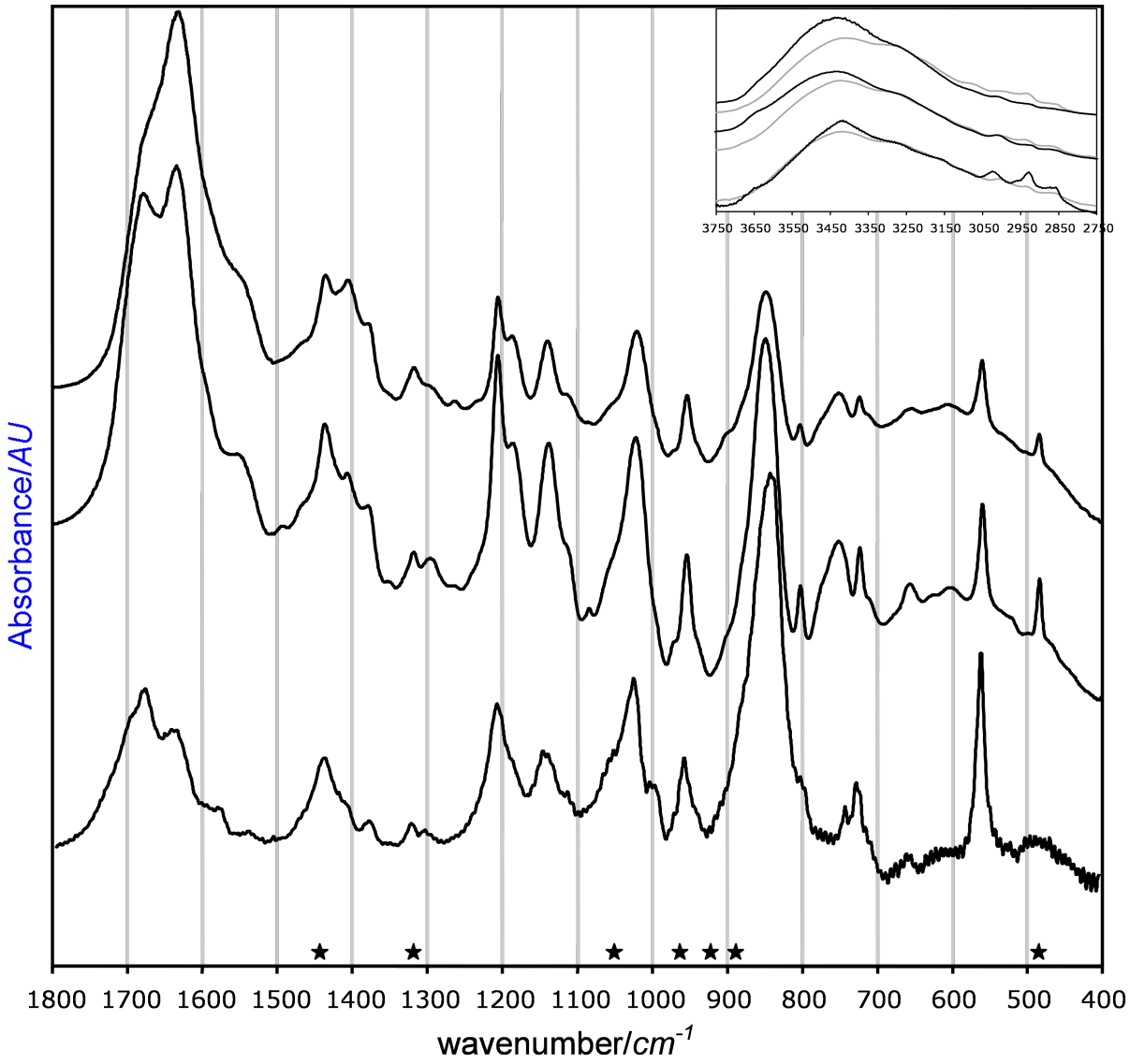


Chart S5. Overlay of the IR-spectra of the dinuclear bis(μ-hydroxo) dicopper(II)-complexes of the "1-His-2-Asp" (top), "2-His-1-Asp" (middle) and "3-His" (bottom) mimics. Insert shows the area where O-H stretching vibrations can be observed, grey traces are from the mononuclear Cu(II)-mimic complexes. Asterisks mark the positions where DMSO vibrations are usually observed. The traces of the "3-His" mimic, published before in Chemical Communications, have been added to facilitate comparison; for full assignments of that mimic, reference 7 has to be consulted.

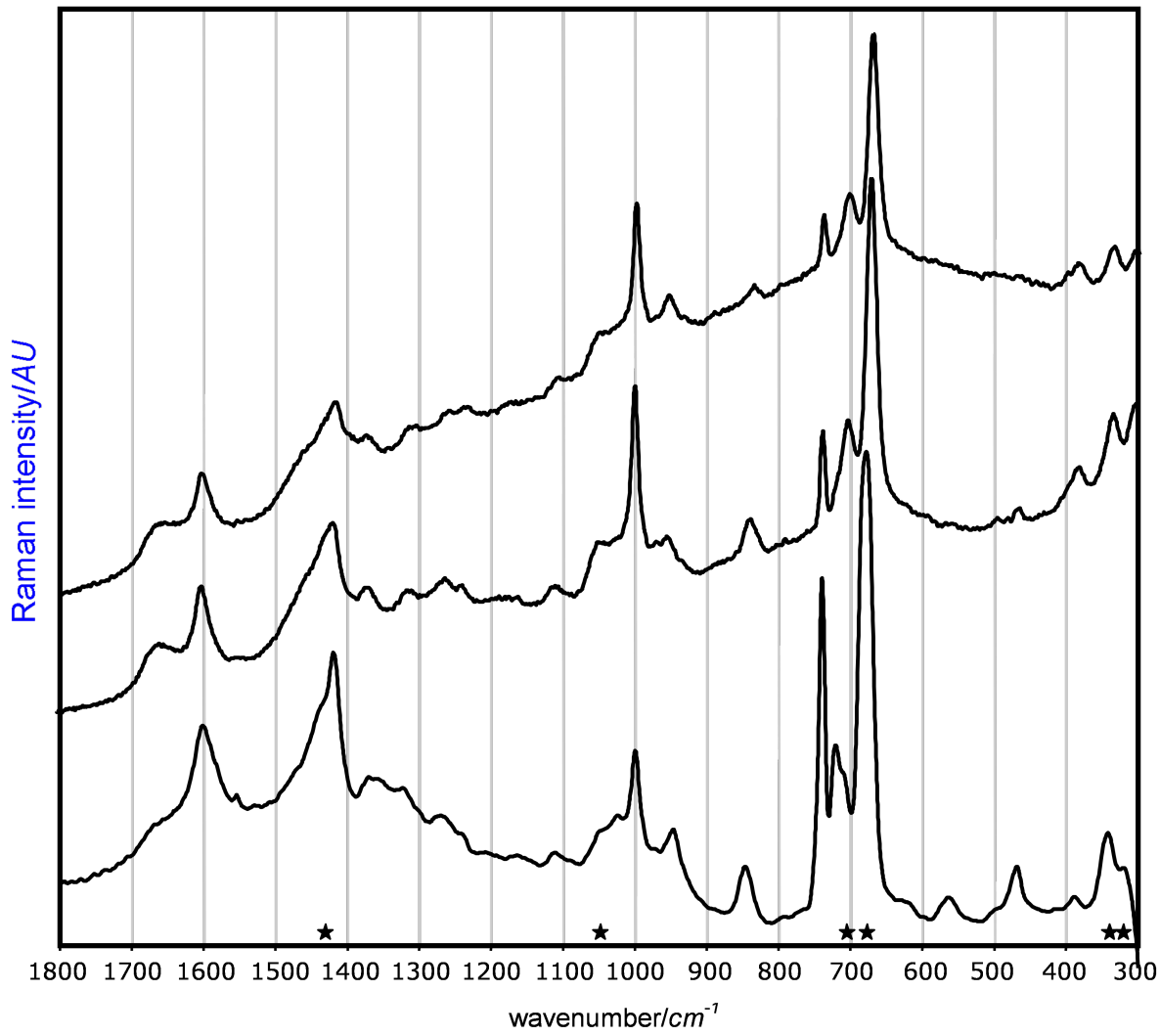


Chart S6. Overlay of the Raman-spectra of the dinuclear bis(μ-hydroxo) dicopper(II)-complexes of the "1-His-2-Asp" (top), "2-His-1-Asp" (middle) and "3-His" (bottom) mimics. Asterisks mark the positions where DMSO vibrations are usually observed. The traces of the "3-His" mimic, published before in Chemical Communications, have been added to facilitate comparison. For full assignments of that mimic, reference 7 has to be consulted.

Table S2. Proposed assignments for the Infrared and Raman spectra of the bis(μ-hydroxo) dicopper(II) complexes based on the three mimics.

2-His-1-Asp		1-His-2-Asp		
Infrared	Raman	Infrared	Raman	
3420 (br)		3430 (br)		ν C(O)N-H (1^o and 2^o) / ν O-H (H-bonded) [a]
2923 (*w*)		2915 (*w*)		ν_a CH_2
1674 (s)	1660 (w br)	1669 (*s*)	1663 (w br)	ν C=O (A-I: 1^o, 2^o and 3^o) [a]
1629 (s)		1626 (vs)		δ N-H + ν C-N (A-II: 1^o) [a]
	1603 (w)		1604 (w)	ν C=C* + ν C=N* (*N*-) [d]
1541 (*m*)		1548 (*m*)		ν C=O (carboxylate) [b),d]
1432 (m)		1431 (m)		δ N-H* / ν C=C* / δ_a CH_3
	1421 (m)		1419 (m)	δ N-H* / ν C=C* / δ CH_2
1402 (m)		1403 (m)		ν C-O (carboxylate) [b),d]
1376 (w)	1375 (w)	1375 (w)		ν C-N (A-III: 1^o) [a]
1315 (w)	1316 (w)	1312 (w)	1314 (w)	δ =C-H [d] / ν C(O)-N [a] / ν ring*
	1265 (brw)		1263 (brw)	ν ring* (*N*-) [d] / A-III: 2^o [a]
1203 (s)		1204 (m)		ν ring* (*N*-) [d] / τ CH_2 [a]
1182 (m)		1182 (m)		ν N-C=N + δ_{ip} N-H* [d]
1136 (m)		1136 (s)		ν =C-N* + δ N-H* [d]
1113 (*m*)	1112 (w)	1109 (*w*)		ν =C-N=* + δ N-H* [d]
	1052 (w)		1053 (m)	ν C(O)-N [a]
1020 (m)		1016 (m)		δ C-H* / δ_{ip} C^{Ar}-H
	999 (s)		999 (s)	ν =C-NH* (*N*-) / δ_{ip} C^{BA}-H [d]
953 (m)	958 (w)	950 (w)	955 (w)	δ CuO-H (ip) / ν =C-N* + δ ring*
848 (s)		844 (s)		PF_6-ion
	843 (m)		838 (w)	δ_{oop} =C-H* [d]
801 (w)		800 (w)		δ_{oop} C^{Ar}-H [a]
				δ CuO-D (ip)
	739 (m)		739 (m)	$Cu_2(OH)_2$ core breathing
722 (m)		719 (w)		δ_{oop} N-H [a] / ρ_{ip} CH_2 [a]
	705 (m)		705 (m)	DMSO
	671 (vs)		672 (vs)	DMSO
				ν Cu-OH_2^{eq} [d), e]
560 (m)		559 (m)		δ_{oop} CO_2 [d]
	470 (w)			ν Cu-N^{eq} [d]
	409 (*vw*)		404 (*w*)	ν Cu-N^{ax}
	386 (m)		390 (w)	ν Cu-O
	339 (m)		339 (m)	DMSO
	307 (m)		307 (m)	DMSO

See Table S1 for used abbreviations and references.

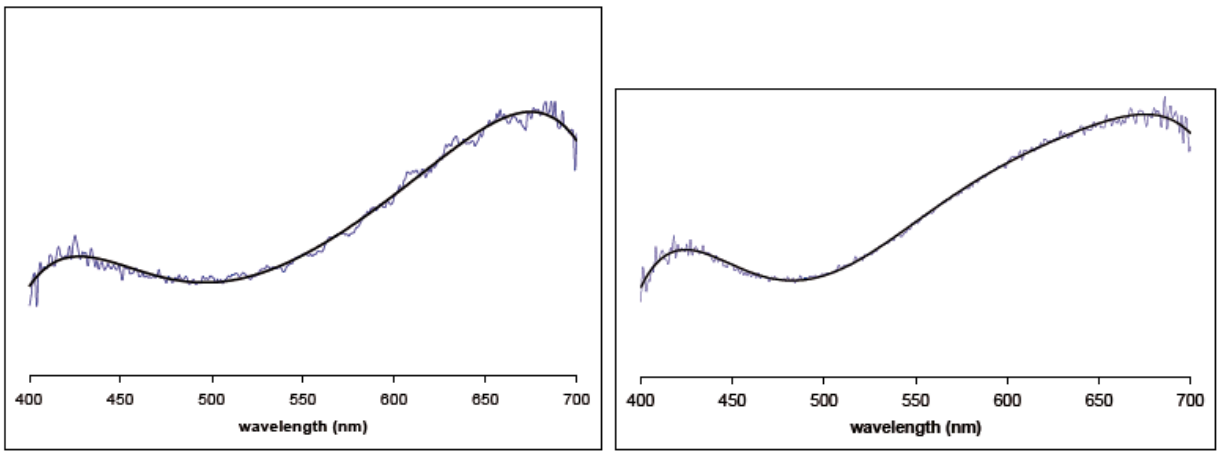


Chart S7. *Hydroxo-to-copper(II) c*harger-transfer bands around 425 nm as observed in the dinuclear bis(μ-hydroxo) dicopper(II)-complexes of the "2-His-1-Asp" (left) and "1-His-2-Asp" (right) mimics.

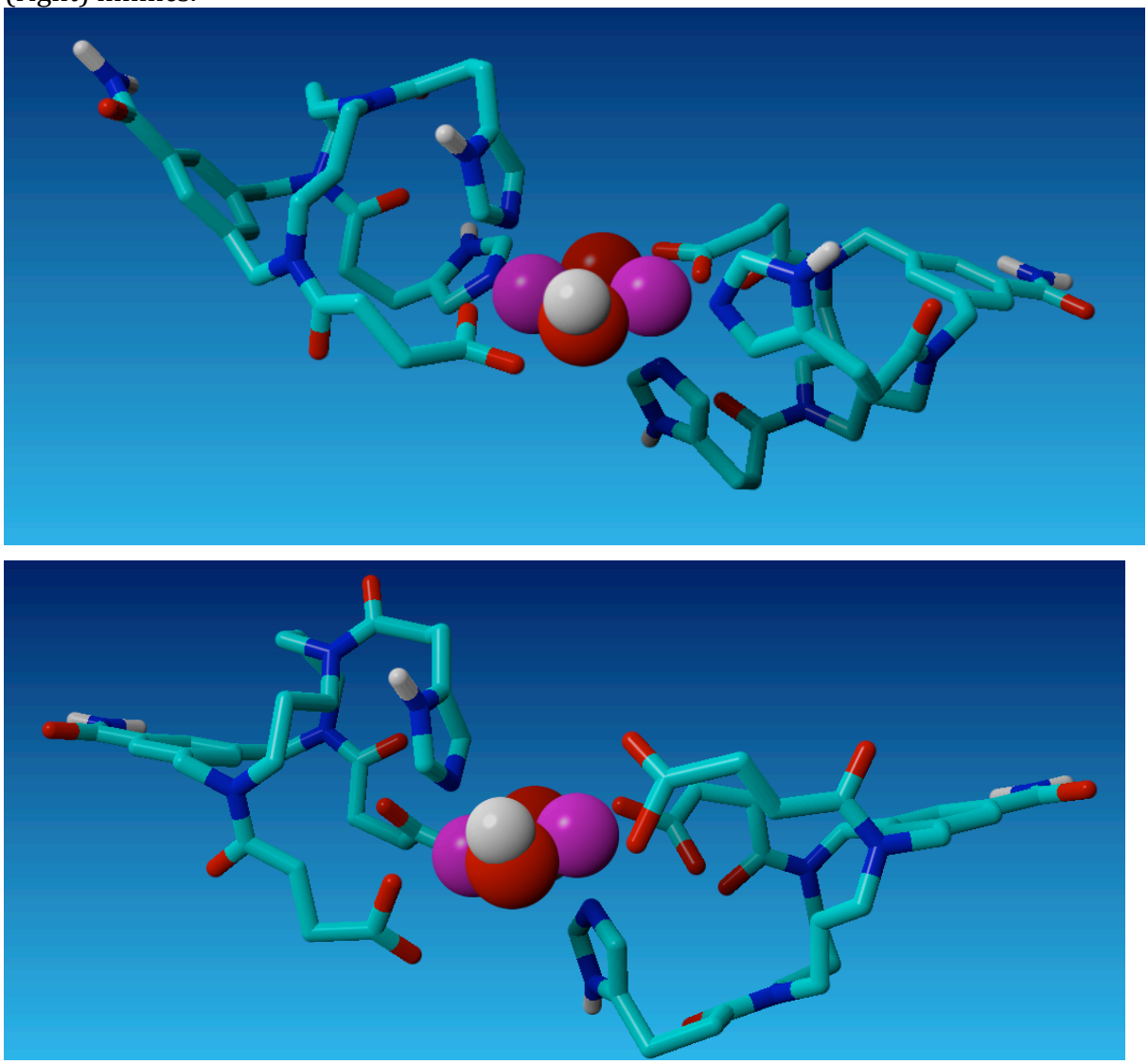


Figure S4. Computer models of bis(μ-hydroxo) dicopper(II)-complexes of the "2-His-1-Asp" (top) and "1-His-2-Asp" (bottom) mimics. Acetylated α-amine functionalities have been omitted for clarity.

Notes and References

[1] Obtained as trial-version from *Accelrys*® (see http://accelrys.com/products/discovery-studio/visualization.html).
[2] E. Krieger, G. Koraimann, G. Vriend, *Proteins: Structure, Function, and Genetics* **2002**, *47*, 393.
[3] T. Opatz, R.M.J. Liskamp, *Org. Lett.* **2001**, *3*, 3499.
[4] G.I. Tesser and I.C. Balvert-Geers, *Int. J. Pept. Protein Res.* 1975, **7**, 295.
[5] E. Kaiser, R.L. Colescott, C.D. Bossinger, P.I. Cook, *Anal. Biochem.* **1970**, *34*, 595; V.K. Sarin, S.B.H. Kent, J.P. Tam, R.B. Merrifield, *Anal. Biochem.* **1981**, *117*, 147.
[6] T. Vojkovsky, *Pept. Res.* **1995**, *8*, 236.
[7] H.B. Albada, F. Soulimani, B.M. Weckhuysen, R.M.J. Liskamp, *Chem. Commun.* **2007**, 4895.