Electronic Supplementary Information

Disaccharide recognition by binuclear copper(II) complexes

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1. Instruments and Methods

Isothermal calorimetry titration (ITC) experiments. All investigations were conducted in 50 mM CHES buffer at pH 10 at 27 °C on a VP isothermal titration calorimeter from Microcal® Inc. (Northampton, MA). The reference cell was loaded with nanopure water.
Typically, the sample cell was filled with a 1-2 mM copper(II) complex solution and titrated in 5-10 µl portions with a 20 mM carbohydrate solution. Dilution effects are determined by titration of the buffer solution into the metal complex solutions, and then subtracted from the initial carbohydrate titration curve to obtain the final binding curve. The obtained data were fitted using the subsequential binding site model implemented into the data acquiring Origin® software, version 7.0.

CD experiments. All experiments were recorded on a Jasco J-810 spectropolarimeter at ambient temperature, with a scanning speed of 100 nm/min and a bandwidth set to 1 nm from 200-800nm in 1.5 ml disposable semi-micro Brandtech UV cuvettes with a 1 cm path length. The dinuclear metal complexes were used at 1-2 mM concentrations, while the final carbohydrates concentration was adjusted to 10 mM. Each spectrum was signal-averaged at least three times and corrected for signal contributions from the metal complex in CHES buffer as baseline. CD measurements in mdeg (θ) were converted into molar dichroic absorption units ($\Delta\varepsilon$ in M^{-1} cm^{-1}) using the relationship $\Delta\varepsilon = \theta/(32980\times l\times c)$ with θ as ellipticity in millidegrees (mdeg), l as pathlength in cm and c as molar concentration.

UV/Vis experiments. The UV/vis spectra were recorded on Cary WinUV, Vers. 3.0, Analysis Suite with suprasil standard cells (200-2000 nm) of 1 cm thickness and 4.5 ml volume at 30 °C over a range of 200-900 nm. The pH value was measured using a Beckman Φ 250 pH meter equipped with refillable long Futura pH electrode of 0.7 mm thickness. The pH meter was calibrated before each set of readings.
All experiments were performed in thermostated 4 ml UV/vis cuvettes in aqueous solution at constant ionic strength (0.1 M $NaClO_4$) at 30 ± 0.1°C. Typically, 10 µl portions of a 0.025–1M aqueous NaOH solution were added into a 2 ml aliquot of a solution that is 1 mM in $CuCl_2$, 0.5 mM in NaOAc and 0.5 mM in the ligand concentration. After mixing the solution with a pipette and equilibration, UV/Vis spectra were recorded as a function of the pH; the spectral data of complex formation were then computed from the data by the fitting procedures implemented by the program SPECFIT.

2. Binding constants of the complexes 1-3 with the saccharides 4-12.

Table 1 Binding constants (K_{app}/M^{-1}) and thermodynamic data of the complexes formed from selected disaccharides and complexes **1-3** at 300 K in CHES buffer at pH 10.

Entry	Complex	$K_{1,app}$/ M^{-1}	ΔH_1/ kcal mol^{-1}	ΔS_1/ cal mol^{-1} K^{-1}	$K_{2,app}$/ M^{-1}	ΔH_2/ kcal mol^{-1}	ΔS_2/ cal mol^{-1} K^{-1}	$K_{3,app}$/ M^{-1}	ΔH_3/ kcal mol^{-1}	ΔS_3/ cal mol^{-1} K^{-1}	K_{app}/M^{-1}
α-D-galp(1→4)fru, Lactulose											
22	**1–4**	1.6×10^3 ($\pm 0.5 \times 10^3$)	-3.1×10^2 ($\pm 0.5 \times 10^2$)	13.7	5.0×10^3 ($\pm 1.4 \times 10^3$)	2.5×10^2 ($\pm 0.7 \times 10^3$)	17.8	1.6×10^4 ($\pm 0.5 \times 10^4$)	-1.1×10^2 ($\pm 0.8 \times 10^2$)	18.8	2.3×10^4
23	**2–4**	1.4×10^3 ($\pm 0.5 \times 10^3$)	-3.9×10^2 ($\pm 0.4 \times 10^2$)	13.0	6.0×10^2 ($\pm 3.9 \times 10^2$)	9.2×10^2 ($\pm 3.5 \times 10^3$)	15.8	1.1×10^4 ($\pm 0.9 \times 10^4$)	-6.5×10^2 ($\pm 3.4 \times 10^2$)	16.2	1.3×10^4
24	**3–4**	1.2×10^3 ($\pm 0.4 \times 10^3$)	-3.3×10^2 ($\pm 0.4 \times 10^2$)	13.0	2.7×10^3 ($\pm 1.0 \times 10^3$)	1.1×10^2 ($\pm 0.4 \times 10^3$)	16.0	5.1×10^2 ($\pm 3.6 \times 10^2$)	3.6×10^2 ($\pm 1.1 \times 10^2$)	11.2	4.4×10^3
Fru, Fructose											
1	**1–5**	9.3×10^2 ($\pm 4.9\times 10^2$)	-5.3×10^2 ($\pm 1.2 \times 10^2$)	11.8	7.6×10^2 ($\pm 4.3 \times 10^2$)	8.4×10^2 ($\pm 4.2 \times 10^2$)	16.0	2.0×10^3 ($\pm 0.9 \times 10^2$)	-7.4×10^2 ($\pm 3.2 \times 10^2$)	12.6	3.7×10^3
2	**2–5**	6.2×10^2 ($\pm 1.8\times 10^2$)	-4.8×10^2 ($\pm 0.7 \times 10^2$)	11.2	1.0×10^3 ($\pm 0.3 \times 10^3$)	7.2×10^2 ($\pm 1.5 \times 10^2$)	16.2	7.6×10^2 ($\pm 4.6 \times 10^2$)	-8.1×10^2 ($\pm 1.4 \times 10^2$)	10.5	2.4×10^3
3	**3–5**	4.0×10^2 ($\pm 1.2\times 10^2$)	-8.2×10^2 ($\pm 1.5 \times 10^2$)	9.1	3.1×10^3 ($\pm 0.7 \times 10^3$)	7.1×10^2 ($\pm 1.5 \times 10^2$)	18.4	2.1×10^2 ($\pm 0.7\times 10^2$)	-6.4×10^2 ($\pm 1.4 \times 10^2$)	8.5	3.4×10^3
α-D-glcp(1→4)fru, Maltulose											
13	**1–6**	2.3×10^3 ($\pm 0.8 \times 10^3$)	-7.3×10^2 ($\pm 0.9 \times 10^2$)	13.0	4.3×10^3 ($\pm 1.7 \times 10^3$)	9.8×10^2 ($\pm 1.0 \times 10^2$)	19.9	1.3×10^4 ($\pm 0.6 \times 10^4$)	-5.2×10^2 ($\pm 1.0 \times 10^2$)	17.1	2.0×10^4
14	**2–6**	1.6×10^3 ($\pm 0.6 \times 10^3$)	-4.9×10^2 ($\pm 0.4 \times 10^2$)	13.1	1.0×10^3 ($\pm 0.5 \times 10^3$)	1.4×10^3 ($\pm 0.4 \times 10^3$)	18.4	1.3×10^4 ($\pm 0.7 \times 10^4$)	-1.1×10^3 ($\pm 0.4 \times 10^3$)	15.0	1.6×10^4
15	**3–6**	2.4×10^2 ($\pm 0.7 \times 10^2$)	-1.8×10^3 ($\pm 0.4 \times 10^3$)	4.8	2.0×10^3 ($\pm 0.5 \times 10^3$)	1.8×10^3 ($\pm 0.4 \times 10^3$)	21.3	7.4×10^2 ($\pm 2.1 \times 10^2$)	-1.1×10^3 ($\pm 0.1 \times 10^3$)	9.3	3.0×10^3
α-D-glcp(1→6)fru, Palatinose											
19	**1–7**	4.7×10^3 ($\pm 0.9 \times 10^3$)	-1.4×10^3 ($\pm 0.1 \times 10^3$)	12.0	4.5×10^2 ($\pm 2.1 \times 10^2$)	4.9×10^3 ($\pm 1.6 \times 10^3$)	28.6	1.8×10^4 ($\pm 0.5 \times 10^4$)	4.9×10^3 ($\pm 1.5 \times 10^3$)	3.1	2.3×10^4
20	**2–7**	8.9×10^3 ($\pm 1.6 \times 10^3$)	-6.0×10^2 ($\pm 0.2 \times 10^2$)	16.1	1.1×10^4 ($\pm 0.3 \times 10^4$)	4.3×10^2 ($\pm 0.2 \times 10^3$)	20.0	1.9×10^3 ($\pm 0.5 \times 10^3$)	-5.4×10^2 ($\pm 0.5 \times 10^2$)	13.2	2.2×10^4
21	**3–7**	1.4×10^3 ($\pm 0.5 \times 10^3$)	-1.1×10^3 ($\pm 0.1 \times 10^3$)	10.7	5.3×10^3 ($\pm 1.1 \times 10^3$)	3.5×10^2 ($\pm 1.3 \times 10^2$)	15.9	6.1×10^2 ($\pm 2.1 \times 10^2$)	4.5×10^3 ($\pm 0.7 \times 10^3$)	27.9	7.3×10^3
Me-α-D-glcp											
4	**1–8**	n.b.[a]									
5	**2–8**	n.b.[a]									
6	**3–8**	n.b.[a]									
α-D-glcp(1→3)fru, Turanose											
10	**1–9**	8.6×10^2 ($\pm 1.4 \times 10^2$)	-1.1×10^2 ($\pm 0.1 \times 10^2$)	15.4							8.6×10^2
11	**2–9**	3.9×10^1 ($\pm 1.7 \times 10^1$)	-7.6×10^2 ($\pm 2.9 \times 10^2$)	20.1							3.9×10^1
12	**3–9**	6.9×10^1 ($\pm 1.6 \times 10^1$)	-9.3×10^2 ($\pm 1.7 \times 10^2$)	16.6							6.9×10^1
α-D-glcp(1→2)fruf, Sucrose											
7	**1–10**	n.b.[a]									
8	**2–10**	n.b.[a]									
9	**3–10**	n.b.[a]									
α-D-glcp(1→3)-β-D-fruf(2→1)-α-D-glcp, Melizitose											
7	**1–11**	n.b.[a]									
8	**2–11**	n.b.[a]									
9	**3–11**	n.b.[a]									
α-D-glcp(1→5)fru, Leucose											
16	**1–12**	n.b.[a]									
17	**2–12**	n.b.[a]									
18	**3–12**	1.2×10^2 ($\pm 0.1 \times 10^2$)	-7.0×10^2 ($\pm 0.4 \times 10^2$)	16.9							1.2×10^2

[a] n. b. = not binding

3. Synthesis of binuclear copper(II) complexes 1a and 3.

N,N-1,3-Bis{2-hydroxy-4-[2-(methoxyethoxy)]}-benzylideneamino]propan-2-ol dicopper complex (1a).

The backbone ligand EGbsdpo was prepared following the synthetic procedure to derivatize 4-hydroxysalicylaldehydes with ethylene glycol derivatives as described previously (M. G. Gichinga and S. Striegler, *J. Am. Chem. Soc.*, **2008**, *130*, 5150-5156). Accordingly, a solution of 1,3-diamino-2-propanol (115 mg, 1.24 mmol) in ethanol (10 mL) was added to a solution of 2-hydroxy-4-(2-methoxy-ethoxy)benzaldehyde in 150 mL of ethanol/methanol (1/1, v/v). The resulting solution was stirred for 48 h at ambient temperature, and then concentrated to give a yellow residue that was recrystallized from MeOH/EtOH (1/1, v/v) to yield the EGbsdpo backbone ligand as a yellow solid. Yield 23% (230 mg, 0.52 mmol), mp 120-121 ºC; δ_H ($CDCl_3$, 400.2 MHz) 8.16 (s, 2H –CH=N–), 7.06 (d, 2H, 8.34, ArH), 6.37 (m, 4H, ArH), 4.15 (m, 1H, –CHOH–), 4.06 (t, 4H, 4.67, $ArOCH_2$–), 3.70 (m, 4H, –CH_2–), 3.59 (m, 2H, –CH_2–); δ_C ($CDCl_3$, 100.6 MHz) 166.1, 165.1, 163.2, 132.9, 112.2, 107.0, 101.8, 70.7, 70.2, 67.2, 61.3, 59.2; positive ion ESI MS, calcd for $(C_{23}H_{30}N_2O_7 +H)^+$ 446.21, found 446.16.

To prepare the metal complex, a solution of copper(II) acetate monohydrate (100 mg, 0.5 mmol) in 10 ml DMF was added dropwise to a solution of the ligand EGbsdpo (110 mg, 0.25 mmol) in 30 ml DMF. The resulting solution was stirred at 60 ºC for 24 h after the addition of triethyl amine (76 mg, 0.75 mmol). The solution was then concentrated to half of its volume in vacuum at 60-70°C, and the remaining solution allowed to evaporate slowly in an open flask at ambient temperature. A green powder was isolated by filtration after 7 days and recrystallized from ethanol to obtain a dark green crystalline solid. Yield 44 % (70 mg, 0.11 mmol). Found: C 47.63, H 4.80, N 4.40 %; $C_{25}H_{31}Cu_2N_2O_9$ requires: C 47.62, H 4.95, N 4.44 %.

N,N'-bis(2-pyridylmethyl)-1,4-diaminobutan-2-ol dicopper complex (3).

The diamine 1,4-diaminobutan-2-ol was prepared following the procedure described by I. Murase, S. Ueno and S. Kida, *Inorg. Chim. Acta* **1984**, *87*, 155-7. The asymmetric backbone ligand bpdbo was prepared using 1,4-diaminobutan-2-ol and pyridine-2-carbaldehyde in analogy to a synthetic procedure described previously (S. Striegler and M. Dittel, *J. Am. Chem. Soc.*, **2003**, *125*, 11518-11524).

Copper(II) acetate monohydrate (1.4 g, 7 mmol) was dissolved in 10 ml nanopure water and added to a solution of sodium perchlorate (2.8 g, 22.8 mmol) in 5 ml water. The resulting solution was diluted with 140 ml methanol and added to the ligand bpdbo (716 mg, 2.5 mmol) dissolved in 20 ml ethanol at ambient temperature. The resulting solution turned immediately deep blue, and was concentrated to 30 ml under vacuum at 40°C. The remaining solution was allowed to stand at ambient temperature for 4 days. A blue crystalline solid formed that was isolated by filtration, air dried and not further purified. Yield 28 % (480 mg, 0.7 mmol) ***Caution: perchlorate salts are potentially explosive. We did, however, not experience any difficulty in handling or drying the compound below 40 °C.*** Found: C 31.52, H 3.70, N 8.09 %; $C_{18}H_{26}Cl_2Cu_2N_4O_{12}$ requires C 31.36, H 3.95, N 8.13 %.

4. Speciation curves for binuclear copper(II) complexes 1 and 3.

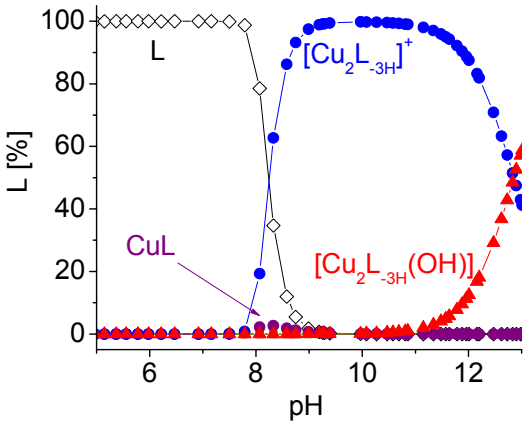


Fig. 1, speciation curve for complex **1**, Cu_2(TEGbsdpo), in water; derived from the TEGbsdpo ligand (L) in the presence of $Cu(OAc)_2$ in dependence of the pH at constant ionic strength (0.1 M $NaClO_4$) at 30 ± 0.1°C.

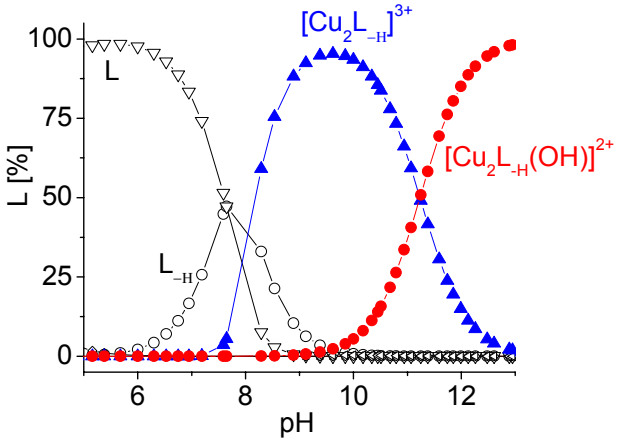
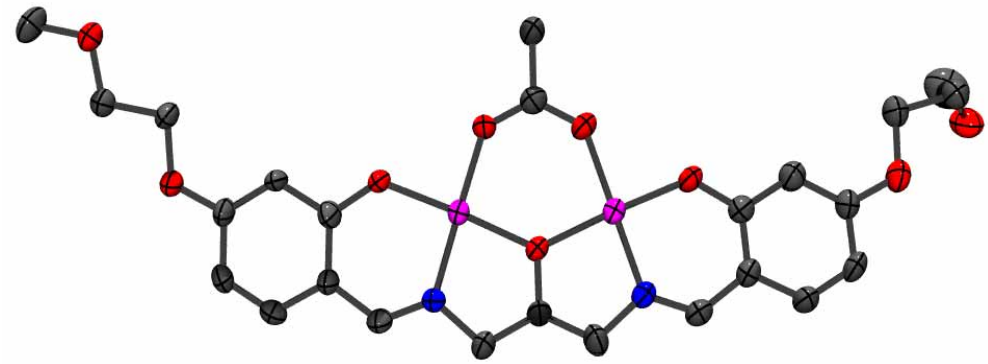


Fig. 2, speciation curve for complex **3**, Cu_2(bpdbo), in water; derived from the bpdbo ligand (L) in the presence of $Cu(OAc)_2$ in dependence of the pH at constant ionic strength (0.1 M $NaClO_4$) at 30 ± 0.1°C.

5. Figures for the structural analysis of complexes 1a and 3.

(a)



(b)

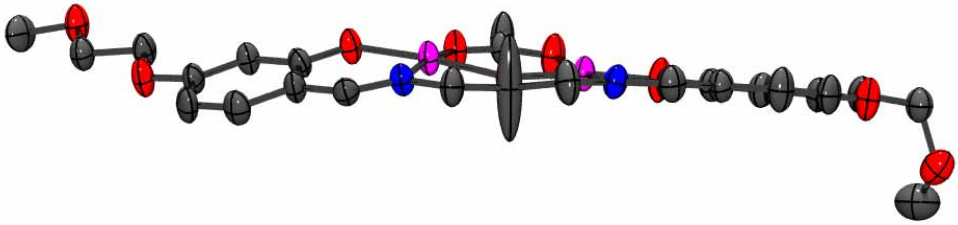


Fig. 3, Structure of complex **1a**, Cu_2(EGbsdpo), depicted in ellipsoids with 70% probability in (a) front and (b) side view; C atoms are shown in gray, copper in margenta, nitrogen in blue, oxygen in red, chlorine in green. One weakly coordinated perchlorate ion and all hydrogen atoms are omitted for clarity. The figure was prepared using Ortep-3, Vers. 1.08 and POV-Ray, Vers. 3.06.

(a) **(b)**

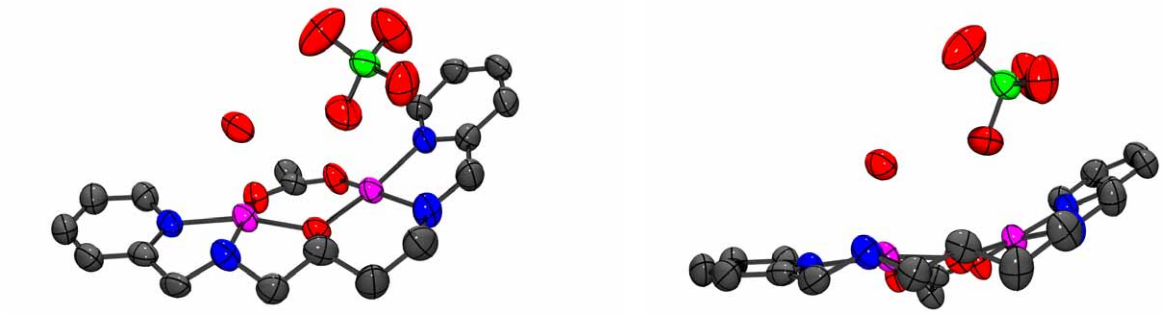


Fig. 4, Structure of complex **3**, Cu_2(bpdbo), depicted in ellipsoids with 70% probability in (a) front and (b) side view; C atoms are shown in gray, copper in margenta, nitrogen in blue, oxygen in red, chlorine in green. One weakly coordinated perchlorate ion and all hydrogen atoms are omitted for clarity. The figure was prepared using Ortep-3, Vers. 1.08 and POV-Ray, Vers. 3.06.