Binuclear copper(II) complexes discriminating epimeric glycosides and α - and β -glycosidic bonds in aqueous solution

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Abstract.

Two chiral binuclear copper(II) complexes were synthesized and characterized for the first time as efficient chemoselective catalysts for the hydrolysis of aryl glycosides and disaccharides in aqueous solution at near neutral pH. Under these conditions, discrimination of epimeric aryl α -glycopyranosides was observed both by 29-fold different reaction rates and 3-fold different proficiency of the catalyst. Additionally, large differentiation of the nature of α - and β - glycosidic bond in aryl glycosides as model compounds is apparent, but also noted in selected disaccharides. The influence of the chirality of the complexes and the role of the configuration of the carbohydrate upon interaction with the catalyst is discussed in detail. Lastly, a putative mechanism for the metal complex-catalyzed hydrolysis is derived from the experimental evidence pointing at deprotonation of the hydroxyl group at C-2 as a pre-requisite for glycoside hydrolysis.

Introduction.

Chiral discrimination is displayed by many enzyme classes, such as glycosidases, lipases, and esterases during bond formation and cleavage.[1] While much progress has been made in the synthesis and evaluation of selective catalysts mimicking such features,[2-6] most synthetic entities do not show sufficient diastereoselective or even chiral discrimination ability in aqueous solution. Man-made stereoselective catalysts for the discrimination of epimeric glycosides in aqueous solution or the selective hydrolysis of α - and β -glycosidic bonds are not known yet.

We previously disclosed a symmetric binuclear copper(II) complex, Cu₂bpdpo (**1**), that interacts differently with glycopyranosides upon binding in alkaline solution resulting in a 30-fold stronger binding to mannose over glucose.[7] Similar observations were made by others thereafter using related metal complexes confirming our early results on carbohydrate-metal complex binding in alkaline aqueous solution.[7-12]

These combined results imply that binuclear copper(II) complexes may also be able to discriminate epimeric glycosides during hydrolysis enabling chemoselective catalysis. We report here the synthesis, characterization and evaluation of chiral binuclear copper(II) complexes that discriminate epimeric glycosides and α - and β -glycosidic bonds upon hydrolysis in aqueous solution at pH 7.5 for the first time.

Chart 1. Binuclear copper(II) complexes **1** and **2**

Results and Discussion.

Synthesis of the chiral binuclear copper(II) complexes.

Symmetric complex Cu₂bpdpo (**1**) was derived from a reduced Schiff-base ligand bpdpo that was obtained from 1,3-diaminopropanol and pyridinecarbaldehyde as described.[7] The same strategy was employed to prepare chiral complexes *S*-Cu₂bpdbo (*S*-**2**) and *R*-Cu₂bpdbo (*R*-**2**) by using enantiopure *S*- and *R*-malic acid **3** as inexpensive starting material for the synthesis of chiral 1,4-diaminobutanols (**4**).

Scheme 1. Synthesis of chiral copper(II) complexes *S*-**2** and *R*-**2**; reagents and conditions: (i) AcBr, MeOH, 0°C → r.t., 18h, 59%; (ii) 7N NH₃/MeOH, r.t., 24h, 43-48%; (iii) BH₃/THF, HCl/EtOH, 77°C, 1h, 29-74%; (iv) NaOH, r.t., 5h; C₅H₄NCHO, r.t. 22h; NaBH₄, r.t., 48h, 59-83%; (v) Cu(OAc)₂, MeOH, r.t., 12h, 25-32%.

In short, the chiral acids **3** were converted into methyl malates **5** using methanol in the presence of acetyl bromide.[13-15] Treatment of esters **5** with excess ammonia in methanol yielded malamides **6**. [16] The hydrochlorides of *S*-**4** and *R*-**4** were obtained after reduction of **6** with borane in THF and treatment of the reaction products with hydrogen chloride in absolute ethanol.[17] In analogy to the synthesis of bpdpo described above,[18] condensation of the free diaminoalcohols with pyridinecarbaldehyde afforded the chiral ligands *S*-bpdbo (*S*-**7**), and *R*-bpdbo (*R*-**7**), respectively, after reduction of the initially formed Schiff bases with sodium borohydride in methanol. Enantiopure binuclear copper(II) complexes *S*-**2** and *R*-**2** were prepared from the pentadentate ligands **7** and copper(II) acetate in methanol following our synthetic protocols for the synthesis of complex **2**. [18] **Caution:** Perchlorate salts are potentially explosive. However, we did not experience any difficulty in handling or drying the complexes below 50 °C.

Characterization of the chiral complexes in the solid state. Blue needles suitable for X-ray crystallography were obtained by diffusion of hexane into solutions of *S*-**2**, or *R*-**2**, in methanol. X-ray diffraction studies of the single crystals revealed bond angles and bond lengths for the central Cu1-O-Cu2 geometry similar to those of previously studied racemic **2**. [18] The coordinating methanol in *S*-**2**, the perchlorate ion coordinated to Cu1, and the methylene C-chain C7-C10 are disordered in both complexes, yet the anticipated absolute configurations are confirmed by the solid state data (Flack X=

0.021(5) and 0.020(3) for S and R respectively). As *S*-**2** and *R*-**2** crystallize in the same space group ($P2_12_12_1$), the structures have opposite coordinates. Absolute structure determination was carried out using Parson's method.[19]

The Cu...Cu distance was deduced from the structural data as 3.368(1) Å on average. The intermetallic Cu(II) distances in the chiral complexes is shorter than in **1** (3.499(1) Å) confirming previous observations for a comparison between **2** and **1**. [18] The average Cu1-O1-Cu2 angle (121.7°) is smaller in the chiral complexes than in **1** (132.9°) due to distortion in the ligand backbone caused by the additional methylene group. A full description and all details of the crystal structure determination are given in the supporting information.

(A)

(B)

Chart 2. Structures of (A) *S*-**2** and (B) *R*-**2** in the solid state; atoms are shown as ellipsoids with 60 % probability; carbon (gray), oxygen (red), nitrogen (blue), copper (magenta); H-atoms, disordered C-atoms, and perchlorate ions are omitted for clarity.

Characterization of the binuclear complexes in solution. To evaluate the complexes for their ability to discriminate glycosidic bonds, two pH values for kinetic evaluations were selected reflecting different complex compositions. The composition of chiral *S*-**2** and *R*-**2** in aqueous solution is identical to the speciation of racemic complex **2** and deduced from data previously determined using spectrophotometric titration methods.[20, 21] At pH 10.5, the predominant species of *S*-**2** and *R*-**2** is still a $[\text{Cu}_2\text{L}_{\text{-H}}]^{3+}$ species (57.5 %), while only one other additional $[\text{Cu}_2\text{L}_{\text{-H}}(\text{OH})]^{2+}$ species (42.5%) is formed (L = *S*- and *R*-bpdbo, respectively) (**Scheme 2**). By contrast, complex **1** forms under these conditions $[\text{Cu}_2\text{L}_{\text{-H}}(\text{OH})_2]^+$ as the main species (88.5%) in equilibrium with a minor $[\text{Cu}_2\text{L}_{\text{-H}}(\text{OH})]^{2+}$ species (11.5 %) as previously discussed.[7, 22] Mononuclear complexes formed from remaining ligand or free metal ions can be neglected for catalysis under the applied conditions or were demonstrated to be inactive.[18] At pH 7.5, complexes *S*-**2** and *R*-**2** exist predominantly as a binuclear $[\text{Cu}_2\text{L}_{\text{-H}}]^{3+}$ species (94 %, L = *S*- and *R*-bpdbo, respectively), while complex **1** exists as $[\text{Cu}_2\text{L}_{\text{-H}}(\text{OH})]^{2+}$ species (98.6 %, L = bpdpo) (**Scheme 3**).

Scheme 2. Predominant species of complex **1** and **S-2** at pH 10.5; **R-2** likewise; X = H₂O.

Scheme 3. Predominant species of complex **1** and **S-2** at pH 7.5; **R-2** likewise; X = H₂O.

To allow a comparison between all complexes and species derived therefrom during the hydrolysis of glycosidic bonds, the catalyst amounts used for the determination of kinetic parameters are corrected to reflect the different amounts of the respective catalytically active species.

Differentiation of 4-nitrophenyl glycosides during catalytic hydrolysis in alkaline solution.

Based on previously established assays using UV/Vis spectroscopy to evaluate the catalytic activity of complexes **1** and **2** during glycoside hydrolysis,[18] we employed chiral catalysts **S-2** and **R-2**, extended the substrate scope by employing six commercially available 4-nitrophenylglycosides, and transferred the previous assay from 1 mL standard cuvettes into 96-well plate format. The adjusted procedure allows considerably faster catalyst screening using smaller compound amounts and volumes, and thereby circumvents previously observed limitations caused by low substrate solubility.

Along these lines, the catalytic hydrolysis of 4-nitrophenyl- α -D-mannopyranoside (**8a**), 4-nitrophenyl- β -D-mannopyranoside (**8b**), 4-nitrophenyl- α -D-galactopyranoside (**8c**), 4-nitrophenyl- β -D-galactopyranoside (**8d**), 4-nitrophenyl- α -D-glucopyranoside (**8e**), and 4-nitrophenyl- β -D-

glucopyranoside (**8f**) (Chart 3) was studied by UV/Vis spectroscopy recording the product formation at 405 nm over time (**Scheme 4**).

Chart 3. Structures of substrates **8a-f**

Scheme 4. Catalytic hydrolysis of **8e** as representative example for the hydrolysis of substrates **8a-f**

The adjusted assay has a total volume of 200 μ L and lowered the catalyst concentration from 0.1 mM to 0.03 mM, while the substrates were used between 6-10 mM. The substrate hydrolysis depends linearly on the catalyst concentration under these conditions.[18] The measured absorbance was converted into concentration using the apparent extinction coefficient ϵ_{app} , corrected for the catalyst concentration, its relative speciation amount, and the uncatalyzed reaction, and then plotted versus the substrate concentration. By applying a non-linear fit to the resulting hyperbolic data, the catalytic rate constant k_{cat} [min^{-1}] and the substrate affinity K_M [mM] were determined utilizing the Michaelis-Menten model (Table 1)

Table 1. Kinetic parameters for the hydrolysis of 4-nitrophenylglycosides **8a-f** at pH 10.5 and 30°C

Entry	S	cat	$k_{cat} \times 10^{-3}$ [min^{-1}]	K_M [mM]	$k_{cat}/K_M \times 10^{-3}$ [$\text{min}^{-1} \text{M}^{-1}$]	$k_{cat}/k_{non} \times 10^3$ [M]	$k_{cat}/(K_M \times k_{non})$
1	8a	1	3.09	107.5	28.7	20.7	193,000
2		S-2	6.32	107.7	58.7	42.4	394,000
3		R-2	4.42	57.7	76.6	29.7	514,000
4	8b	1	0.30	21.0	14.3	1.5	74,000
5		S-2	0.76	26.6	28.6	3.9	147,000
6		R-2	0.64	23.9	26.7	3.3	138,000
7	8c	1	0.65	70.5	9.2	18.2	258,000
8		S-2	1.07	42.1	25.4	29.9	710,000
9		R-2	1.15	18.7	61.7	32.1	1,720,000
10	8d	1	0.12	12.1	9.9	0.9	74,000
11		S-2	0.28	10.2	27.5	2.1	206,000
12		R-2	0.26	16.8	15.5	1.9	116,000
13	8e	1	0.27	7.5	35.8	2.5	335,000
14		S-2	0.83	9.1	91.5	7.8	857,000
15		R-2	0.81	6.3	129.0	7.6	1,210,000

16	8f	1	0.15	53.2	2.8	0.3	13,000
17		S-2	0.81	72.2	11.2	1.4	51,000
18		R-2	1.17	42.8	27.3	20.0	124,000

$k_{\text{non},8a} = 1.5 \times 10^{-7} [\text{min}^{-1} \text{M}^{-1}]$, $k_{\text{non},8b} = 1.9 \times 10^{-7} [\text{min}^{-1} \text{M}^{-1}]$, $k_{\text{non},8c} = 0.4 \times 10^{-7} [\text{min}^{-1} \text{M}^{-1}]$, $k_{\text{non},8d} = 1.3 \times 10^{-7} [\text{min}^{-1} \text{M}^{-1}]$; $k_{\text{non},8e} = 1.1 \times 10^{-7} [\text{min}^{-1} \text{M}^{-1}]$, $k_{\text{non},8f} = 2.2 \times 10^{-7} [\text{min}^{-1} \text{M}^{-1}]$

The uncatalyzed reactions (k_{non}) of all substrates remain in the same order of magnitude as previously determined ($k_{\text{non}} = 0.4\text{--}2.2 \times 10^{-7} \text{ min}^{-1} \text{ M}^{-1}$). [18] For comparison of different substrates, only the proficiency ($k_{\text{cat}}/(K_{\text{M}} \times k_{\text{non}})$) of the catalysts is discussed to account for the different strengths of glycosidic bonds and the resulting different hydrolysis rates in absence and presence of catalysts.

The catalytic proficiency of the symmetric complex **1** under the employed conditions is very modest and shows only limited differentiation of substrates with α -glycosidic bond (**8a**, **8c** and **8e**) from substrates with β -glycosidic bond (**8b**, **8d** and **8f**) that are typically hydrolyzed even less efficiently, if at all (**Figure 1**). The substrates are not significantly discriminated by complex **1** for their epimeric sugar moiety reflecting previously stated observations.[18]

Figure 1. Catalytic proficiency of binuclear complexes **1**, **S-2** and **R-2** in aqueous solution at pH 10.5 during the hydrolysis of 4-nitrophenylglycosides; the data are grouped by (A) catalysts promoting the hydrolysis of glycosides as **8a-f**, and (B) glycosides **8a-f** hydrolyzed by catalysts **1**, **S-2** and **R-2**.

By contrast, both chiral complexes are more proficient for cleaving the glycosides **8a-f** than **1** (Figure 1A). In addition, **S-2** and **R-2** show higher proficiency than **1** to hydrolyze α -over β -glycosidic bonds. Complex **R-2** hydrolyzes **8c** with a 6.7-fold, and **8e** with a 3.6-fold higher proficiency than **1**, and shows a 1.3-fold, and 2.4-fold, respectively, higher proficiency for the hydrolysis of the same substrates than **S-2**. Interestingly, while **R-2** shows the overall highest proficiency for hydrolyzing 4-nitrophenyl- α -D-galactopyranoside (**8c**) ($k_{\text{cat}}/K_{\text{M}} \times k_{\text{non}} = 1,720,000$), the same complex shows an almost 10-fold higher proficiency for hydrolyzing the β -glucosidic bond in **8f** (**R-2**: $k_{\text{cat}}/K_{\text{M}} \times k_{\text{non}} = 124,000$) than **1** (**1**: $k_{\text{cat}}/K_{\text{M}} \times k_{\text{non}} = 12,800$).

While the results encourage further investigation of binuclear Cu(II) complexes for their ability to discriminate glycosidic bonds in natural systems, including disaccharides, initial attempts to use these substrates under the above described assay conditions were futile and led to catalyst destruction. Visibly to the naked eye, the originally blue solutions will turn green and then orange within 1-2 h indicating the formation of Cu(I) oxide without evidence for a significant hydrolysis of the disaccharide at 30 or 40 °C. Decreasing the pH of the solution to pH 7 or 8 was found to increase catalyst stability, but resulted in catalyst inactivation below 40°C (see below). Selective oxidation of the primary hydroxyl group at C-6 in methyl glycosides was previously observed for complex **1** after activation with TEMPO in alkaline solution.[23]

Following the hydrolysis of glycosides and natural saccharides at near physiological pH appears more relevant for the synthesis of functional enzyme models and modelling of enzyme activity. Unfortunately, the commercially available 4-nitrophenyl glycopyranosides have a low molar extinction coefficient under these conditions that hamper their use as model compounds at pH values below 9.[18] As a consequence, rapid catalyst screening in 96-well plate format using such substrates is limited and results in high uncertainty for the evaluation of the catalyst performance due to the resulting small apparent absorbance changes, small extinction coefficients, and large errors of the associated data. To overcome this obstacle, two approaches come to mind: the use of derivatized nitrophenyl glycosides with large extinction coefficients suitable to follow hydrolysis reactions at near physiological pH by UV/Vis spectroscopy, and/or the use of ‘real’ saccharides instead of phenylglycoside model compounds after further modification of the current assay. Toward the development of functional enzyme models, both approaches were followed and the results are summarized below.

Synthesis of 2'-chloro-4'-nitrophenyl glycosides

As noted above, the commercially available 4-nitrophenyl glycopyranosides **8** hydrolyze into in saccharide and 4-nitrophenolate (**9**) that both have fairly low molar extinction coefficients at pH values below 9.[18] However, a significantly higher apparent extinction coefficient of 2-chloro-4-nitrophenolate (**10**) at near neutral pH was noted in this and other laboratories prompting us to synthesize a small library of 2'-chloro-4'-nitrophenylglycosides (**11a-i**) as substrates for this study (**Chart 4**).[24-28][29][27, 28, 30]

Chart 4. Structures of substrates **11a-i**

The aryl α -glycopyranosides **11a**, **11c** and **11e** were obtained in two steps after treatment of peracetylated glycosides with 2-chloro-4-nitrophenol in the presence of Lewis acids yielding peracetylated aryl α -glycopyranosides **12a**, **12c**, and **12e**, and global deacetylation.[26, 27, 31, 32] The aryl β -D-glycopyranosides **11b**, **11d** and **11f** were prepared via peracetylated glucosyl bromides (**13**) as described.[26-28]

Scheme 5. Synthesis of aryl α - and β -galactoside **11g** and **11h** from **14**; conditions and reagents: (i) Ac_2O , $\text{C}_6\text{H}_5\text{N}$, r.t., 16 h, 95 %; (ii) 2-chloro-4-nitrophenol, CH_2Cl_2 , *N*-iodosuccinimide, AgOTf , 0 °C, 30 min, 53 %; (iii) IBr , CH_2Cl_2 , 0 °C, 10 min, 81%; (iv) 2-chloro-4-nitrophenol, NaOH , CH_2Cl_2 , Bu_4NBr , 35 °C, 3 h, 80 %; (v) 7N NH_3/MeOH , r.t., 24h, 79-87%.

For mechanistic studies, we synthesized aryl 2-*O*-methyl- α -galactopyranoside (**11g**), and its β -isomer **11h** from galactoside (**14**) (**Scheme 5**), which was obtained as an intermediate during an unrelated study.[33] Here, galactoside **14** was peracetylated with acetic anhydride in pyridine yielding galactoside **15**,[34] and treated with 2-chloro-4-nitrophenol in the presence of *N*-iodosuccinimide and silver triflate to afford aryl α -galactoside **12g**. By contrast, the transformation of **15** with iodine monobromide yielded bromide (**16**), which then allowed the coupling with 2-chloro-4-nitrophenol affording aryl β -galactoside **12h** via phase transfer catalysis as described for other β -glycosides.[27] The target compounds **11h** and **11g** were finally obtained after global deacetylation.[35]

Lastly, allopypyranoside **11i** was obtained from substrate **11f** as readily accessible starting material (**Scheme 6**). Initially, the free hydroxyl groups at C-4 and C-6 were protected as benzyl acetal yielding glucoside (**17**). After treatment of **17** with dibutyltin oxide, selective benzylation of the hydroxyl group at C-2 yielded aryl glucoside (**18**). The reaction of **18** with trifluoromethanesulfonic anhydride afforded triflyl β -glucoside (**19**) that was immediately treated with tetrabutylammonium acetate to give β -allopypyranoside (**20**) as a result of the inversion of the configuration at C-3 in the glycon.[36] Treatment

of **20** with diluted acetic acid afforded aryl β -allopyranoside (**21**), and the target compound **11i** after global deprotection.

Scheme 6. Synthesis of peracetylated 2-chloro-4-nitrophenyl β -D-allopyranoside (**11i**); conditions and reagents: (i) benzaldehyde dimethyl acetal, CSA, CH_3CN , r.t., 2 h, 87 %; (ii) Bu_2SnO , MeOH, reflux, 1.5 h; benzoyl chloride, $\text{C}_6\text{H}_5\text{CH}_3$, $0^\circ\text{C} \rightarrow \text{r.t.}$, 16h; (iii) Tf_2O , CH_2Cl_2 , $\text{C}_6\text{H}_5\text{N}$, 0°C , 30 min, quantitative; (iv) TBA·OAc, DMF, 0°C , 30 min, 91%; (v) HOAc/ H_2O (4/1, v/v), 50°C , 15h, 97%; (vi) 7N NH_3/MeOH , r.t., 16 h, 83%.

Discrimination of 2'-chloro-4'-nitrophenyl glycopyranosides during catalytic hydrolysis at physiological pH.

With substrates **11a-i** on hand, their hydrolysis was initially studied under the same conditions as outlined above for the hydrolysis of *p*-nitrophenyl glycopyranosides, i.e. at alkaline pH. While the catalytic rate constants are of similar order of magnitude, the uncatalyzed hydrolyses of 2'-chloro-4'-nitrophenyl glycopyranosides (e.g. $k_{\text{non}} = 0.5\text{-}3.3 \times 10^{-4} [\text{min}^{-1} \text{M}^{-1}]$, 50 mM CAPS buffer, pH 10.5, 30 °C) are considerably faster than the uncatalyzed hydrolysis reactions of *p*-nitrophenyl glycopyranosides (Table 1) rendering all complexes less efficient as catalysts and the differences in catalyst proficiency smaller. This observation accounts for the decreased stability of the glycosidic bond after introduction of the chloro-substituent in the ortho position of the aglycon. However, the decreased stability of the glycosidic bond in the 2'-chloro-4'-nitrophenyl glycopyranosides does allow the investigation of the catalyst performance at near neutral pH values as hypothesized above.

Along these lines, the proficiency of the selected complexes toward glycoside hydrolysis was evaluated in 50 mM HEPES buffer at pH 7.50 and 30 °C. Typically, lag times around 200 min were observed prior to the start of the catalytic hydrolyses that may indicate substrate deprotonation, distortion or inversion of the glycon upon interaction with the catalyst. Data collection over 9-12 h allowed the determination of all kinetic parameters after conversion of the observed absorbance data into product concentrations and application of the Michaelis-Menten model (**Table 2**).

Table 2. Kinetic parameters for the catalytic hydrolysis of **11a-f, i** at pH 7.5 and 30°C

Entry	S	cat	$k_{\text{cat}} \times 10^{-3} [\text{min}^{-1}]$	$K_{\text{M}} [\text{mM}]$	$k_{\text{cat}}/(K_{\text{M}} \times k_{\text{non}})$
1	11a	1	1.51	25.7	43,000
2		S-2	1.74	30.0	42,000
3		R-2	1.98	38.3	38,000
4	11b	1	0.42	25.6	62,000
5		S-2	0.33	22.8	55,000
6		R-2	0.26	17.3	57,000
7	11c	1	0.37	15.6	51,000
8		S-2	0.28	8.8	68,000
9		R-2	0.71	31.6	48,000
10	11d	1	5.49	73.0	35,400
11		S-2	4.55	58.4	37,000
12		R-2	3.28	43.2	36,000
13	11e	1	0.07	4.5	66,000
14		S-2	0.06	2.1	121,000
15		R-2	0.06	2.2	118,000

16	11f	1	1.10	30.7	50,000
17		S-2	1.67	47.7	49,000
18		R-2	1.26	45.0	39,000
19	11i	1	0.53	4.0	60,000
20		S-2	0.49	4.8	47,000
21		R-2	0.24	1.9	58,000

$k_{\text{non},11a} = 1.3 \times 10^{-6} [\text{min}^{-1} \text{M}^{-1}]$; $k_{\text{non},11b} = 2.6 \times 10^{-7} [\text{min}^{-1} \text{M}^{-1}]$; $k_{\text{non},11c} = 6.7 \times 10^{-7} [\text{min}^{-1} \text{M}^{-1}]$; $k_{\text{non},11d} = 2.1 \times 10^{-6} [\text{min}^{-1} \text{M}^{-1}]$; $k_{\text{non},11e} = 2.4 \times 10^{-7} [\text{min}^{-1} \text{M}^{-1}]$; $k_{\text{non},11f} = 7.1 \times 10^{-7} [\text{min}^{-1} \text{M}^{-1}]$; $k_{\text{non},11i} = 2.2 \times 10^{-6} [\text{min}^{-1} \text{M}^{-1}]$

Figure 2. Product formation over time, catalyst and substrate concentration for the hydrolysis of **11a**, **11c**, and **11e** by **S-2** in 50 mM HEPES buffer at 7.50 ± 0.05 and 30.0 ± 0.1 °C.

The following discussion of the obtained data highlights the most predominant discrimination trends by focusing on the performance of catalyst **S-2** for (i) the hydrolysis of epimeric α -glycosides in comparison to symmetric **1**, (ii) the hydrolysis of α - and β -glycosides in comparison to **R-2**, and (iii) deducing a mechanism for the observed glycoside hydrolysis.

Performance of S-2 during hydrolysis of epimeric α -glycopyranosides

Catalyst **S-2** discriminates epimeric α -glycosides **11a**, **11c** and **11e** by catalyzing their hydrolysis with significantly different rates (**Figure 2**). The rate for the catalyzed hydrolysis of **11a** is more than 6-fold higher than for **11c** and 29-fold higher than for **11e** pointing at a significant influence of the hydroxyl groups at C-2 and C-3 in the glycon of the substrates during metal complex-catalyzed hydrolysis. Substrates with hydroxyl groups *trans* to each other (**11c**, **11e**) promote slower hydrolysis than a substrate with hydroxyl groups *cis* to each other (**11c**). This observation correlates with known metal complex coordination abilities to *trans*-diols (weak) and *cis*-diols (strong) in pyranosides.[37] To account for uncatalyzed background reactions and different substrate affinity toward a complex, the catalyst proficiency ($k_{\text{cat}}/K_{\text{M}} \times k_{\text{non}}$) was calculated (**Figure 3**).[38]

Figure 3. Proficiency of *S-2* for the catalytic hydrolysis of α -glycosides **11a**, **11c**, and **11e** in 50 mM HEPES buffer at pH 7.50 ± 0.05 and 30.0 ± 0.1 °C.

The known lability of the α -glycosidic bond in **11a** causes its uncatalyzed hydrolysis (k_{non}) to be about an order of magnitude faster than for **11c** and **11e**. Thus, the catalytic proficiency of *S-2* decreases in the order of **11e** > **11c** > **11a** showing nevertheless for the first time a distinct discrimination of epimeric glycosides by a metal complex at near neutral pH. For comparison, symmetric complex **1** shows an overall lower proficiency to hydrolyze the epimeric substrates with negligible differences (1.5-fold or less).

Performance of *S-2* during hydrolysis of α - and β -glycopyranosides

The rate of the *S-2* catalyzed hydrolysis of α -mannoside **11a** is about 5-fold higher than for β -mannoside **11b**, while the reverse trend is observed for gluco- and galactosides where the β -glycosides are hydrolyzed 16-fold (**11d**) and 28-fold (**11f**) faster than their corresponding α -glycosides (Table 2, entries 2 & 5; 8 & 11; 14 & 17). As the uncatalyzed hydrolyses of the manno- and galactosides differ by an order of magnitude within each α -/ β -pyranoside pair (Table 2, footnote), the catalytic proficiency of the catalyst for the hydrolysis of the respective α - and β -glycosides do not reflect the discrimination its ability, but reveal a rather equivalent catalytic proficiency near 50,000 instead. Substrate **11i** behaves likewise.

By contrast, the uncatalyzed reactions of the α - and β -glucosides **11e** and **11f** are of the same order of magnitude and the *S-2*-catalyzed hydrolyses differ by almost 1.5 orders of magnitude or 28-fold (Figure 4; Table 2, entries 14 & 17). This observation translates into a 3-fold higher proficiency of *S-2* to hydrolyze **11e** over **11f** due to higher substrate affinity of **11f** over **11e** for *S-2*, and demonstrates for the first time the ability of a metal complex to discriminate α - and β -glycosidic bonds notably close to physiological pH. This finding is of particular significance for catalyst development, use of biomass or its transformation into fine chemicals and fuel due to the abundance of α - and β -glucopyranosyl moieties as building blocks in natural products and oligosaccharides including cellulose and starch.

Figure 4. Product formation during the hydrolysis of **11e** and **11f** by **S-2** in 50 mM HEPES buffer at pH 7.50 ± 0.05 and 30.0 ± 0.1 °C.

Performance of **R-2** during hydrolysis of glucopyranosides

As elaborated previously, the overall catalyst performance correlates to the intramolecular Cu...Cu distance in the metal complex core.[18] As similar rates of the catalyzed substrate hydrolyses are observed for **S-2** and **R-2** (Table 2), the chirality of the complexes is, however, unlikely to have a profound contribution to the observed discrimination of the epimeric or anomeric model compounds by **S-2** and is echoed by **R-2**. Instead, the configuration of the hydroxyl groups in the glycon of the substrate presents itself as a rationale for the observed glycoside discrimination. For simplicity, the following discussion on the mechanistic insights is consequently limited to complex **S-2**.

Putative mechanism of the α -glycoside hydrolysis

The high catalytic proficiency of **S-2** for the hydrolysis of glucopyranosides **11e-f** over those of **11a-d** indicates again different interactions of the catalyst with the glycon of the substrates. As the epimeric substrates are overall only different in their configuration at C-2 and C-4, respectively, we proposed that the higher acidity of the hydroxyl group at C-2 over that of the hydroxyl group at C-4 and its proximity to the anomeric center promote substrate deprotonation and coordination to the metal complex as prerequisite for catalytic hydrolysis to occur. The coordination is consequently stronger when a deprotonated *cis*-diol structure (**11a**) is participating in metal complex chelation and weaker when a *trans*-diol structure (**11c** or **11e**) is present accounting for above described observations confirming previous observations by others[37].

For experimental evidence, the catalytic hydrolysis of substrates **11g** and **11h** was evaluated (Chart 4). Both substrates are methylated in the glycon at the hydroxyl group at C-2 preventing deprotonation at this position upon interaction with the catalyst, while weaker hydrogen-bonding interactions are still enabled. The hydrolysis of **11g** and **11h** is unsuccessful with any studied catalyst both at pH 7.5 and in alkaline solution at pH 10.5 indicating that all substrates indeed coordinate to the catalysts over a deprotonated hydroxyl group at C-2. A putative mechanism for the hydrolysis of α -glycoside by **S-2** at

pH 7.5 is deduced from the described experimental observations, and depicted for the hydrolysis of **11a** (Scheme 7).

Scheme 7. Putative mechanism for the metal complex-catalyzed hydrolysis of α -glycopyranosides at pH 7.5 and 30°C using **S-2** and **11a** as representative example

Upon dissolving complexes **S-2** and **R-2** in solution, a binuclear species I is formed that coordinates the glycoside substrate under release of water and protons resulting in half-acetal formation (Scheme 7, species II). Distortion of the configuration may then yield a structure similar to the transition state proposed for enzymatic glycoside hydrolyses encompassing substrate distortion to a half-chair conformation, sp^2 -character of the anomeric C-atom, partially positively charged endocyclic O-atom and lengthening of the glycosidic bond (Scheme 7, species III). Models suggest a twisted boat-like structure for a similar species derived from β -glycosides requiring further computational analyses in future efforts. Hydration of the binuclear copper species and protonation of the sugar by solvent molecules may release the coordinated hydrolysis products to reform species I and close the catalytic cycle.

Discrimination of disaccharides during catalytic hydrolysis

In order to apply the catalysts to natural carbohydrates, all complexes were explored in their ability to hydrolyze the disaccharides maltose (**22**), cellobiose (**23**), and lactose (**24**) (**Figure 5a**). Disaccharides **22** and **23** differ in the nature of their glycosidic bonds, and **23** and **24** are epimers in their non-reducing sugar moiety. All disaccharides were hydrolyzed at 60 °C at pH 8 over 24 h in presence and absence of 10 mol % of complex **1**, **S-2**, and **R-2**, respectively. The amount of remaining starting material was then quantified by HPLC analysis (**Figure 5b**).^[39] Evidence for catalyst destruction or sugar oxidation were

not apparent under the elaborated conditions. The composition of the complexes is similar to that at pH 7.5 with slightly different amounts of the major catalytically active species.

Figure 5a, Disaccharide structures

Figure 5b, Catalyzed disaccharide hydrolysis

The data reveal indifference of **1** to hydrolyze the selected disaccharides in any significantly higher amount than buffer solution alone. By contrast, **S-2** hydrolyzed twice as much of α -glycoside **22** as buffer, **1**, or **R-2**. Likewise, **R-2** hydrolyzed up to 2.5-fold more of β -glycosides **23** and **24** than buffer solution or another catalyst. The finding indicates stereoselective discrimination of the glycosidic bonds in disaccharides by the chiral catalysts with a preference of **S-2** for α -glycosidic bonds and of **R-2** for β -glycosidic bonds. This preliminary finding is promising for future applications of the developed catalysts and prompted an in-depth study that is topic of current investigation.

Conclusions

In conclusions, chiral binuclear copper(II) complexes **S-2** and **R-2** were synthesized and fully characterized including analysis by X-ray diffraction to confirm their stereochemistry. Subsequent evaluations of the complexes as catalysts for the cleavage of glycosidic bonds in aqueous alkaline solution showed moderate proficiency during the hydrolysis of 4-nitrophenyl glycosides and small discrimination ability among the selected epimeric substrates.

However, at near physiological pH, complex **S-2** shows distinct discrimination of epimeric aryl α -glycopyranosides. Discrimination of α - and β -glycosidic bonds in manno- and galactopyranosides by the same complex is apparent in their reaction rates, but masked in the catalytic proficiency by the different rates of the uncatalyzed reaction. By contrast, a 28-fold faster hydrolysis of aryl β - over α -glucopyranoside is noted translating into 3-fold higher proficiency of **S-2** for the hydrolysis of β -glucopyranoside, while the uncatalyzed reactions are of the same order of magnitude for both substrates. The discrimination is not related to the chirality of the complexes, but rather due to the configuration of

the glycosides promoting *cis*- or *trans*-configured diol binding sites for catalyst coordination. Mechanistic studies reveal deprotonation of the hydroxyl group at C-2 as pre-requisite for catalysis. An initial catalyst evaluation toward the hydrolysis of representative disaccharides revealed a preference of ***S*-2** for the cleavage of α -glycosidic bonds, and of ***R*-2** for the hydrolysis of β -glycosidic bonds. The study identifies for the first time catalysts that are able to discriminate epimeric and anomeric model glycosides promoting a stereoselective hydrolysis of glycosidic bonds in disaccharides in aqueous solution at near neutral pH. The results point at exciting possibilities for further catalyst development that may include biomass transformation into valuable chemical synthons and fuels, applications in pharmaceutical industry, or the development of functional enzyme mimics.

Experimental section

Instrumentation.

^1H and ^{13}C NMR spectra were recorded on a 400 MHz Bruker spectrometer (Bruker Biospin) with Z gradient and broadband probe (400.2 MHz for ^1H , and 100.6 MHz for ^{13}C). Chemical shifts (δ) in ^1H NMR are expressed in parts per million (ppm) and coupling constants (J) in Hz. Signal multiplicities are denoted as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). CDCl_3 , CD_2Cl_2 , MeOH-d_4 , DMSO-d_6 and D_2O were used as solvents, and chemical shift values are reported relative to the residual signals of these solvents (CDCl_3 : $\delta_{\text{H}} = 7.29$, $\delta_{\text{C}} = 77.0$; $(\text{CD}_3)_2\text{SO}$: $\delta_{\text{H}} = 2.50$, $\delta_{\text{C}} = 39.5$; D_2O : $\delta_{\text{H}} = 4.80$, $\delta_{\text{C}} = 49.0$ for ^{13}C after addition of a few drops of CD_3OD or $\delta_{\text{C}} = 39.5$ for ^{13}C after addition of a few drops of DMSO-d_6). IR spectra were obtained on a Perkin-Elmer spectrum 100 series FT-IR spectrophotometer with a resolution 0.5 cm^{-1} as thin films on KBr plates or as KBr pellets (ν in cm^{-1}). The software used to record the spectrum is PerkinElmer Spectrum Express version 1.01.00. High resolution mass spectrometry data were obtained in the state-wide mass spectrometry facility at Arkansas University on a Bruker ultrTOF-Q quadrupole time-of-flight (qQ-TOF) mass spectrometer equipped with an electrospray ionization source or the Mass Spectrometry Facility at Georgia State University, Atlanta, GA. Elemental analyses were obtained from Atlantic Microlab, Atlanta, GA. X-ray diffraction data were collected in the X-ray diffraction laboratory of the University of Missouri at St. Louis on a Bruker X8 diffractometer.

The hydrolysis of saccharides was monitored on an HPLC system from Shimadzu equipped with SCL-10Avp system controller, 2 LC-20AD analytical pumps, DGU-20A3R three channel online degassers, SIL-20A UFLC autosampler with 96 well capability, CTO-20A/ prominence column oven and ELSD-90LT light scattering and LC solution software, version 1.25 from Shimadzu for data recording and analysis. Lyophilization was performed on a FreeZone 1 liter benchtop freeze dry system from Labconco. Melting points (uncorrected) were recorded on a Mel-Temp melting point apparatus. Apparent optical rotations were measured at 589 nm on an Autopol III polarimeter from Rudolph Research Analytical at ambient temperature in a 1 mL cell glass center fill stainless steel jacketed cell with an optical path length l of 100 mm. The specific optical rotation was calculated as $[\alpha]_D^T = \alpha \times 100 / c \times l$, where the value for the concentration c is expressed in g/100 mL solvent and l is 1 dm; the values are not temperature-corrected. UV/Vis absorbances in 96 well, non-binding microtiter plates from Greiner Bio-one were recorded on a FilterMax F5 Multi-Mode Microplate Reader from Molecular Devices at 405 nm and $30 \pm 0.1^\circ\text{C}$.

Column chromatography was carried out using silica gel 60 from Silicycle[®] (40–63 μm , 230–240 mesh) or basic aluminum oxide (pH range 9.7 ± 0.4 , activity I), 32–63 μm , surface area $150\text{ m}^2\text{ g}^{-1}$ from Sorbent Technologies as stationary phase. Thin layer chromatography (TLC) was performed using silica gel TLC plates from SORBENT Technologies, 200 μm , $4 \times 8\text{ cm}$, aluminum backed, with fluorescence indicator F_{254} and detection by UV light or by charring with an aqueous vanillin-sulfuric acid reagent and subsequent heating of the TLC plate. The pH values were measured using a Beckman Φ 250 pH meter equipped with refillable ROSS Orion combination pH electrode with a 165 mm long epoxy body, a 95 mm long semi-micro tip and an 8 mm diameter. The pH meter was calibrated before each set of readings. Nanopure water at a resistance of $18.2\text{ m}\Omega$ was obtained from a ThermoScientific Barnstead E-pureTM water purification system.

Chemicals.

Pyridinecarboxaldehyde was distilled in vacuum prior to use; p-tolyl 2-*O*-methyl-1-thio- β -D-galactopyranoside (**14**),^[33] 2-chloro-4-nitrophenyl β -D-mannopyranoside (**11b**),^[28] 2-chloro-4-nitrophenyl β -D-galactopyranoside (**11d**),^[26] *N,N'*-[1,4-bis[(pyridin-2-ylmethyl)amino]propan-2-ol]atodicopper(II) (μ -acetato) diperchlorate, Cu_2bpdpo (**1**),^[7] and (*S*)-methyl malate, (**S-5**)^[40-42] were prepared as described; all other chemicals were used as received from commercial suppliers.

Metal complex syntheses.

The intermediates and copper(II) complexes were prepared following protocols by this laboratory for the synthesis of racemic complex Cu₂bpdbo (**2**).[18]

(R)-methyl malate (R-5).[43, 44] Acetyl bromide (2.2 mL, 0.031 mol) was added dropwise to 20 mL cold methanol. The solution was stirred for 30 minutes in ice. Then *R*-malic acid, **R-3**, (12.5 g, 93.28 mmol) was added. The acid dissolved in about 5 min, and the resulting solution was stirred at ambient temperature. After 18 h, sodium bicarbonate (4.00 g, 0.048 mol) was added to the pale yellow solution. After 15 min of stirring, the mixture was filtered, the filtrate collected, and all volatile compounds evaporated in vacuum, yielding a sticky, oily raw material containing a white precipitate. The desired ester was distilled from this mixture in vacuum yielding 8.88 g of **R-5** (54.77 mmol, 59 %) of a colorless liquid; bp 65-80°C (0.9 torr); $[\alpha]_D^{23.5} = +9.7$ (c 0.288, EtOH) (lit.[44] $[\alpha]_D^{25} = +9.6$ (c 2.3, EtOH); δ_H (CDCl₃) 4.51 (dd, 6.0, 4.3, 1H), 3.81 (s, 3H), 3.71 (s, 3H), 3.28 (br. s, 1H), 2.87 (dd, 16.6, 4.5, 1H), 2.79 (dd, 16.3, 6.0, 1H); δ_C (CDCl₃) 173.6, 170.9, 67.1, 52.6, 51.9, 38.3; ν_{max}/cm^{-1} (thin film on KBr) 3470 (br), 2958, 1737 (s), 1442, 1220. The NMR, IR and optical rotation data agree well with literature data.[43, 44]

(S)-malamide (S-6).[16, 45, 46] (*S*)-methyl malate **S-5** (6.20 g, 38.27 mmol) was dissolved in 45 mL of 7 N ammonia in methanol under inert atmosphere and stirred at ambient temperature. After 24 h, the formed precipitate was separated by filtration and washed with methanol. The raw material was recrystallized from methanol and dried in vacuum yielding 2.20 g of **S-6** (16.54 mmol, 43 %) as a colorless solid; 154-155 °C (lit.[16] 157-158°C; lit.[46] 156-158°C); $[\alpha]_D^{22} = -36.6$ (c 0.333, H₂O) (lit.[16] $[\alpha]_D^{23} = -34.8$ (c 1.5, H₂O); lit.[46] $[\alpha]_D^{18} = -36.7$ (c 3.1, H₂O)); δ_H (D₂O) 4.45 (dd, 8.5, 4.0, 1H), 2.72 (dd, 15.3, 4.0, 1H), 2.57 (dd, 15.3, 8.8, 1H); δ_C (D₂O+DMSO-d₆) 179.9, 176.7, 69.7, 41.0; ν_{max}/cm^{-1} (KBr) 3388, 1677, 1440. The NMR, IR and optical rotation data agree well with literature data.[16, 46]

(R)-malamide (R-6).[47-49] The title compound was prepared from 6.84 g (42.22 mmol) of *R*-methyl malate, **R-5**, as described for *S*-malamide from **S-5**, yielding 2.68 g (20.15 mmol, 48 %) of **R-6** as a colorless solid; 154-156 °C; $[\alpha]_D^{22} = +33.7$ (c 0.309, H₂O); δ_H (D₂O) 4.44 (dd, 8.7, 3.9, 1H), 2.72 (dd, 15.3, 4.0, 1H), 2.56 (dd, 15.3, 8.8, 1H); δ_C (D₂O+DMSO-d₆) 180.0, 176.7, 69.7, 41.1; ν_{max}/cm^{-1} (KBr) 3388, 1677, 1440.

(S)-1,4-diamino-2-butanol hydrochloride S-4.[48, 49] Under an inert atmosphere, 100 mL of 1 M borane in THF were added to ice-cooled **S-6** (1.50 g, 11.28 mmol), and the resulting solution was heated to 77 °C. After 11 h, the solution was cooled in an ice bath, and 40 mL of methanol were added in small portions to control the gas development. The solution was then heated to reflux for 1 h. After cooling, all volatile components were removed by rotary evaporation leaving the crude material as yellowish oil. The oil was dried in vacuum yielding a gummy-like off-white solid that was triturated with 200 mL water-free ethanol and filtered. The filtrate was subjected to gaseous HCl in the cold yielding a precipitate. The precipitate was isolated, washed once with 2 mL ice-cold ethanol and dried in vacuum over drierite yielding 0.575 g of **S-4** (3.248 mmol, 29 %); mp 233-240 °C (lit.[49] 249-254°C); δ_H (D₂O) 3.94 (tdd, 9.5, 6.5, 3.3, 1 H), 3.02 - 3.20 (m, 3 H), 2.92 (dd, 13.1, 9.8, 1 H), 1.82 - 1.93 (m, 1 H), 1.70 - 1.82 (m, 1 H); δ_C (D₂O+MeOH-d₄) 66.0, 44.6, 36.8, 31.5.

(R)-1,4-diamino-2-butanol hydrochloride, R-4.[49] The title compound was obtained as colorless solid in 74 % yield (5.96 g, 33.67 mmol) from 6.0 g (44.8 mmol) (*R*)-malamide **R-6** as described above for the synthesis of **S-4** from **S-6**; 244-246 °C (lit.[49] 232-236 °C); δ_H (D₂O) 3.96 (tt, 3.3, 9.5, 1 H), 3.21 - 3.03 (m, 3 H), 2.92 (dd, 9.5, 13.1, 1 H), 1.88 - 1.88 (m, 1 H), 1.98 - 1.84 (m, 1 H), 1.83 - 1.68 (m, 1 H); δ_C (D₂O+ MeOH-d₄) 66.6, 45.4, 37.6, 32.3.

2S, N, N'-bis(2-pyridylmethyl)-1,4-diaminobutan-2-ol, S-bpdbo, S-7. Sodium hydroxide (1.53 g, 38.25 mmol) was added to a solution of **S-4** (1.50 g, 8.475 mmol) in 80 mL methanol at ambient temperature. The initially turbid solution became clear and after 5 min turbid again. After 5 h, 2.27 g (21.19 mmol) of distilled 2-pyridinecarbaldehyde were added. After additional 22 h, the solution was diluted with 80 mL methanol prior to the addition of 3.04 g (0.080 mol) of sodium borohydride. After further 48 h, all volatile material was removed in vacuum to yield a residue that was taken up in 50 mL chloroform and 15 mL ice water. The organic layer was separated and extracted two times with 15 mL of ice water each. The combined organic layer was dried over sodium sulfate, filtered and concentrated to dryness yielding the raw product as yellowish oil (1.42 g, 4.958 mmol, 59 %). Typically, the ligand obtained by this procedure was diluted in an appropriate amount of ethanol to yield a 1 M stock solution, which was then used without further purification or characterization for the synthesis of copper(II) complexes.

To obtain analytical data, 1.0 g (3.491 mmol) of the raw material were dissolved in dichloromethane and purified by column chromatography over silica gel (dichloromethane/methanol, 20/1-1/1, v/v) yielding 0.52 g (1.815 mmol, 52 %) of **S-7** as a pale yellowish oil; $[\alpha]^{23.4}_D + 3.7$ (c 0.782, EtOH); δ_H (CD₂Cl₂) 8.57 - 8.46 (m, 2 H), 7.62 (tt, 1.6, 7.7, 2 H), 7.33 - 7.24 (m, 2 H), 7.17 - 7.10 (m, 2 H), 3.96 - 3.78 (m, 5 H), 3.34 (br. s, 3 H), 2.94 - 2.86 (m, 1 H), 2.83 - 2.72 (m, 1 H), 2.64 (dd, 3.8, 11.5, 1 H), 2.57 (dd, 7.8, 12.0, 1 H), 1.68 - 1.51 (m, 2 H); δ_C (CD₂Cl₂) 160.7, 159.9, 149.6, 149.6, 136.8, 136.8, 122.6, 122.5, 122.4, 122.2, 71.2, 56.1, 55.5, 55.3, 48.0, 34.3; $\nu_{\max}/\text{cm}^{-1}$ (KBr) 3304, 2927, 2843, 1593, 1597, 1476, 1117; HR ESI-MS calcd for (C₁₆H₂₂N₄O+H)⁺ 287.1866; found 287.1858.

2R, N, N'-bis(2-pyridylmethyl)-1,4-diaminobutan-2-ol, R-bpdbo, R-7. The title compound was prepared from **R-6** using the same procedure as described for the synthesis of **S-7** from **S-6** above yielding 2.42 g (8.450, quantitative) of **R-7** as raw material. Purification of the raw material by column chromatography over silica gel (dichloromethane/methanol, 20/1-1/1, v/v) yielded 2.01 g (7.0189 mmol, 83 %) of **R-7** as a pale yellowish oil; R_f 0.12 (SiO₂, CH₂Cl₂/MeOH, 4/1, v/v); $[\alpha]^{23.4}_D - 3.8$ (c 0.603, EtOH); δ_H (CD₂Cl₂) 8.50 (qd, 1.4, 4.7, 1 H), 7.63 (tt, 1.8, 7.7, 1 H), 7.35 - 7.23 (m, 1 H), 7.18 - 7.08 (m, 1 H), 3.95 - 3.78 (m, 5 H), 3.50 - 3.26 (m, 3 H), 2.96 - 2.87 (m, 1 H), 2.84 - 2.74 (m, 1 H), 2.65 (dd, 5.0, 11.8, 1 H), 2.57 (dd, 7.8, 11.8, 1 H), 1.66 - 1.55 (m, 2 H); δ_C (CD₂Cl₂) 160.7, 159.9, 149.7, 149.6, 136.9, 136.8, 122.7, 122.5, 122.4, 122.3, 71.3, 56.1, 55.5, 55.2, 48.0, 34.3; $\nu_{\max}/\text{cm}^{-1}$ (KBr) 3304, 2927, 2843, 1593, 1597, 1476, 1117; HR ESI-MS calcd for C₁₆H₂₃N₄O (M+H)⁺ 287.1866; found 287.1860.

2S, N, N'-[1,4-bis[(pyridin-2-ylmethyl)amino]butan-2-ol]ato dicopper(II) (μ -acetato) diperchlorate, (S)-Cu₂bpdbo, S-2. Copper(II) acetate monohydrate (2.00 g, 10.00 mmol) were dissolved in 20 mL water and 400 mL methanol at ambient temperature. To the greenish-blue solution, 4.5 mL of the 1 M stock solution of **S-7** in ethanol was added followed by a solution of 4.00 g (32.68 mmol) of sodium perchlorate in 10 mL water and 40 mL ethanol. The resulting dark blue solution was stirred for 12 h, filtered and concentrated below 50 °C to about 40 mL. *Caution: Perchlorate salts are potentially explosive. However, we did not experience any difficulty in handling or drying the complex below 45 °C.* Upon standing at ambient temperature, a precipitate formed that was isolated by filtration and dried at ambient temperature in air yielding 2.85 g (4.155 mmol) of a blue raw material. The raw material was recrystallized from aqueous methanol yielding 2.19 g (3.193 mmol, 32 %) of **S-2** as a blue solid; $\tilde{\nu}/\text{cm}^{-1}$ 1611, 1569, 1085; calcd for C₁₈H₂₆Cl₂Cu₂N₄O₁₂ [Cu₂L-H(OAc)(ClO₄)₂+H₂O, L = **S-7**] C 31.40, H 3.81, N 8.14; found: C 31.66, H 3.66, N 8.07; $[\alpha]^{22.4}_D + 23.6$ (c 0.1116, MeOH); n-hexane was diffused into a solution prepared from 20 mg of **S-2** in 1 mL of methanol to give dark blue needles suitable for X-ray diffraction within 24h at ambient temperature

2R, N, N'-[1,4-bis[(pyridin-2-ylmethyl)amino]butan-2-ol]ato dicopper(II) (μ -acetato) diperchlorate, R-Cu₂bpdbo, R-2. The title compound was prepared from 4.5 mL of a 1 M stock solution of **R-7** as described for the preparation of S-Cu₂bpdbo from **S-7** yielding 1.70 g (2.478 mmol,

25 %) of **R-2** as a blue solid; $\tilde{\nu}/\text{cm}^{-1}$ 1611, 1569, 1085; calcd for $\text{C}_{18}\text{H}_{26}\text{Cl}_2\text{Cu}_2\text{N}_4\text{O}_{12}$ [$\text{Cu}_2\text{L}_\text{H}(\text{OAc})(\text{ClO}_4)_2 \cdot \text{H}_2\text{O}$, $\text{L} = \text{R-7}$] C 31.40, H 3.81, N 8.14; found: C 31.77, H 3.70, N 8.07; $[\alpha]^{22.4}_\text{D} - 23.5$ (c 0.0895, MeOH); n-hexane was diffused into a solution prepared from 20 mg of **S-2** in 1 mL methanol to give dark blue needles suitable for X-ray diffraction within 48 h at ambient temperature

X-ray crystallography.

Crystals of **S-2** and **R-2** were mounted on MiTeGen cryoloops in random orientations. Preliminary examination and data collection were performed using a Bruker X8 Kappa Apex II Charge Coupled Device (CCD) Detector system single crystal X-Ray diffractometer equipped with an Oxford Cryostream LT device. All data were collected using graphite monochromated Mo $K\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$) from a fine focus sealed tube X-Ray source. Preliminary unit cell constants were determined with a set of 36 narrow frame scans. Typical data sets consist of combinations of ω and ϕ scan frames with a scan width of 0.5° and counting time of 15 seconds/frame at a crystal to detector distance of 4.0 cm. The collected frames were integrated using an orientation matrix determined from the narrow frame scans. Apex II and SAINT software packages were used for data collection and data integration.[50] Analysis of the integrated data did not show any decay. Final cell constants were determined by global refinement of reflections harvested from the complete data set. Collected data were corrected for systematic errors using SADABS[50] based on the Laue symmetry using equivalent reflections. The disorder was modeled with partial occupancy atoms and geometrical restraints for both structures. Structure solution and refinement were carried out using the SHELXTL- PLUS software package.[51] The structures were solved by direct methods and refined successfully in the space group $P2_12_12_1$. Full matrix least-squares refinements were carried out by minimizing $\Sigma w(F_o^2 - F_c^2)^2$. The non-hydrogen atoms were refined anisotropically to convergence. All hydrogen atoms were treated using appropriate riding models.

X-ray data for S-2 ($\text{C}_{19}\text{H}_{28}\text{Cl}_2\text{Cu}_2\text{N}_4\text{O}_{12}$), blue needles ($0.567 \times 0.149 \times 0.102 \text{ mm}^3$, V 2640.4(3) $\text{\AA}^3$), were collected at 100 K. The crystals are orthorhombic, space group $P2_12_12_1$ with $a = 7.2680(5) \text{ \AA}$, $b = 14.5809(10) \text{ \AA}$ and $c = 24.9155(17) \text{ \AA}$, and $Z = 4$. The θ -range for data collection was 1.618 to 30.743° . The number of reflections collected was 61147, with 8157 unique reflections ($R_\text{int} = 0.0402$). Refinement by full-matrix least-squares on F^2 , 392 parameters, gave final R indices ($I > 2\sigma_I$) $R_1 = 0.0290$, weighted $R_2 = 0.0706$; R indices on all data were $R_1 = 0.0335$ and weighted $R_2 = 0.0721$. The absolute structure parameter x was $-0.020(3)$; CCDC 1431768.

X-ray data for R-2 ($\text{C}_{19}\text{H}_{28}\text{Cl}_2\text{Cu}_2\text{N}_4\text{O}_{12}$), blue needles ($0.516 \times 0.104 \times 0.066 \text{ mm}^3$, V 2650.10(13) $\text{\AA}^3$), were collected at 100 K. The crystals are orthorhombic, space group $P2_12_12_1$ with $a = 7.2854(2) \text{ \AA}$, $b = 14.5841(4) \text{ \AA}$ and $c = 24.9419(7) \text{ \AA}$, and $Z = 4$. The θ -range for data collection was 1.633 to 34.970° . The number of reflections collected was 88769, with 11457 unique reflections ($R_\text{int} = 0.0662$). Refinement by full-matrix least-squares on F^2 , 385 parameters, gave final R indices ($I > 2\sigma_I$) $R_1 = 0.0398$, weighted $R_2 = 0.0832$; R indices on all data were $R_1 = 0.0570$ and weighted $R_2 = 0.0891$. The absolute structure parameter x was $-0.021(5)$; CCDC 1431767.

Crystal data and intensity data collection parameters, the final residual values and structure refinement parameters, complete listings of positional and isotropic displacement coefficients for hydrogen atoms, anisotropic displacement coefficients for the non-hydrogen atoms and tables of calculated and observed structure factors are available in the Supplementary Information.

Substrate syntheses

2-chloro-4-nitrophenyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (12a). The title compound was obtained using a general protocol for the synthesis of related aryl α -D-mannopyranosides as described by Han et al.[32] Boron trifluoride diethyl etherate (2.28 mL, 18.14 mmol, 3.0 equiv.) was added in nitrogen atmosphere to a solution of peracetylated mannose (2.34 g, 6.0 mmol) and 2-chloro-4-nitrophenol (2.08 g, 12.0 mmol, 2.0 equiv.) in 40 mL of dry dichloromethane. The solution was then brought to reflux. After 96 h, the solution was cooled to ambient temperature, diluted with 100 mL dichloromethane and 50 mL of water. The separated aqueous layer was extracted twice with 60 mL of dichloromethane each. The combined organic layers were washed with 40 mL of saturated bicarbonate solution, 40 mL water, and 40 mL brine, and dried over anhydrous sodium sulfate. After filtration, the filtrate was concentrated under reduced pressure to dryness. The obtained residue was purified on silica gel by column chromatography (hexane/ethyl acetate = 6/1-4/1, v/v) to give **12a** as a colorless solid (1.45 g, 2.883 mmol, 48%); mp: 144-145 °C; R_f 0.36 (SiO₂, cyclohexane/ethyl acetate = 3/2, v/v); δ_H (CDCl₃) 8.35 (d, J = 2.8 Hz, 1 H), 8.15 (dd, J = 2.8, 9.3 Hz, 1 H), 7.32 (d, J = 9.0 Hz, 1 H), 5.70 (d, J = 1.8 Hz, 1 H), 5.59 (dd, J = 3.5, 10.3 Hz, 1 H), 5.41 (t, J = 10.0 Hz, 1 H), 4.33 - 4.23 (m, 1 H), 4.12 - 4.00 (m, 2 H), 2.23 (s, 3 H), 2.08 (s, 3 H), 2.06 (s, 3 H), 2.04 (s, 3 H); δ_C (CDCl₃) 170.3, 169.9, 169.7, 169.6, 155.9, 142.9, 126.3, 124.8, 123.6, 115.3, 96.3, 70.3, 68.9, 68.4, 65.4, 61.8, 20.8, 20.6, 20.6; Calcd for C₂₀H₂₂ClNO₁₂ C, 47.68; H, 4.40; N, 2.78; found: C, 47.65, H, 4.50; N, 2.68.

General description for the synthesis of aryl 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranosides and -galactopyranosides.[31] A 75 mL aliquot of 1 M anhydrous tin(IV) chloride in dichloromethane was added to 15.0 g (38.46 mmol) peracetylated monosaccharide and 9.76 g (56.24 mmol) 2-chloro-4-nitrophenol in 50 mL dichloromethane. The resulting reaction mixture was refluxed for 5 days and then poured into 200 mL of a saturated aqueous sodium bicarbonate solution. The solution was neutralized by further addition of sodium bicarbonate. The suspension was filtered on celite, and the recovered solid was extracted with three 150-mL portions of ethyl acetate. The combined organic layers were extracted with 450 mL of 1 M aqueous sodium hydroxide solution, dried over sodium sulfate, filtered and concentrated to dryness. The residue was purified by column chromatography on silica gel using hexane/ethyl acetate = 3/1 (v/v) as eluent, yielding the target compound.

2-Chloro-4-nitrophenyl 2,3,4,6-tetra-*O*-acetyl- α -D-galactopyranoside (12c). Off-white solid; 6.25 g (12.40 mmol, 32 %); mp 175-176°C; R_f 0.15 (SiO₂, hexane/ethyl acetate = 3/1 (v/v); δ_H (CDCl₃) 8.33 (d, 2.4 , 1H), 8.15 (dd, 9.0, 2.7 , 1H), 7.32 (d, 9.3 , 1H), 5.95 (d, 3.4 , 1H), 5.55 - 5.62 (m, 2H), 5.30 (ddd, 11.7, 3.7, 1.2 , 1H), 4.33 (t, 6.8 , 1H), 4.11 (ddd, 17.1, 11.7, 6.8 , 2H), 2.19 (s, 3H), 2.11 (s, 3H), 2.05 (s, 3H), 1.98 (s, 3H); δ_C (CDCl₃) 170.5, 170.1, 169.9, 169.8, 156.9, 142.9, 126.2, 125.0, 123.6, 115.8, 96.0, 68.1, 67.4, 67.4, 67.2, 61.3, 20.7, 20.6, 20.5, 20.5; Calcd for C₂₀H₂₂ClNO₁₂ C, 47.68; H, 4.40; found: C, 47.90, H, 4.47; HRMS, ESI-TOF⁺ m/z calcd. for C₂₀H₂₂ClNO₁₂Na [M+Na]⁺ 526.0722; found 526.0712.

2-Chloro-4-nitrophenyl 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranoside (12e). Off-white solid; 2.75 g (5.458 mmol, 14 %); An analytical sample (0.5 g, 1.0 mmol) was purified by precipitation of the target compound from a 30 mL solution in cyclohexane/ethyl acetate (4/1, v/v) by cyclohexane addition. The recovered material was subjected to the precipitation procedure two more times, and the recovered solid was dried in vacuum; mp 117-118 °C; R_f 0.19 (SiO₂, hexane/ethyl acetate 3/1, v/v); δ_H (CDCl₃) 8.34 (d, 2.5, 1H), 8.15 (dd, 9.0, 2.8, 1 H), 7.32 (d, 9.0, 1 H), 5.91 (d, 3.8, 1 H), 5.74 (t, 9.8, 1 H), 5.20 (t, 9.9, 1 H), 5.06 (dd, 10.2, 3.6, 1 H), 4.29 - 4.24 (m, 1 H), 4.17 - 4.07 (m, 2 H), 2.10 (s, 3 H), 2.08 (s, 3 H), 2.07 (s, 6 H); δ_C (CDCl₃) 170.3, 170.2, 169.9, 169.5, 156.7, 143.0, 126.2, 125.0, 123.6, 115.7, 95.4, 70.2, 69.5, 69.0, 67.8, 61.3, 20.6, 20.6, 20.5, 20.5; HRMS, ESI-TOF⁺ m/z calcd. for C₂₀H₂₂ClNO₁₂Na (M+Na)⁺: 526.0722; found 526.0718.

2-Chloro-4-nitrophenyl 3,4,6-tri-*O*-acetyl-2-*O*-methyl- α -D-galactopyranoside (12g). The synthesis was performed according to a general protocol described by Olsson et al for the preparation of related compounds with α -glycosidic bonds.[52] A solution of p-tolyl 3,4,6-tri-*O*-acetyl-2-*O*-methyl-1-thio- β -D-galactopyranoside **15** (2.70 g, 6.308 mmol) and 2-chloro-4-nitrophenol (1.64 g, 9.474 mmol, 1.50 equiv.) in 50 mL of dry dichloromethane was stirred at 0 °C. After 5 min, 2.84 g (12.622 mmol, 2.0 equiv.) of *N*-iodosuccinimide and 0.65 g (2.519 mmol, 0.40 equiv.) of silver trifluoromethanesulfonate were added and stirring continued at 0°C. After 30 min, 3 mL of triethylamine were added, and the resulting mixture was filtered through a pad of celite. The filtrate was diluted with 200 mL dichloromethane and washed with 100 mL of 10 % aqueous sodium disulfite solution, 50 mL water, 50 mL brine and dried over anhydrous sodium sulfate. After filtration and concentration to dryness, the residue obtained was purified by column chromatography over silica gel (cyclohexane/ethyl acetate, 5/1 – 3/1, v/v) yielding compound **12g** as a colorless solid (1.60g, 3.361 mmol, 53%); R_f 0.25 (SiO₂, cyclohexane/ethyl acetate, 2/1, v/v); mp: 87-89 °C; δ_H (CDCl₃) 8.34 (d, 2.8, 1 H), 8.15 (dd, 2.8, 9.3, 1 H), 7.31 (d, 9.3, 1 H), 5.87 (d, 3.5, 1 H), 5.56 (dd, 1.4, 3.4, 1 H), 5.49 (dd, 3.3, 10.5, 1 H), 4.26 (dt, 1.5, 6.8, 1 H), 4.13 - 4.02 (m, 2 H), 3.90 (dd, 3.5, 10.5, 1 H), 3.54 (s, 3 H), 2.19 (s, 3 H), 2.08 (s, 3 H), 1.96 (s, 3 H); δ_C (CDCl₃) 170.1, 169.9, 157.0, 142.6, 126.2, 125.0, 123.5, 115.4, 96.5, 74.8, 69.4, 68.3, 67.7, 61.3, 59.4, 20.8, 20.6, 20.5; calcd for C₁₉H₂₂ClNO₁₁ C, 47.96; H, 4.66; found: C, 48.12, H, 4.64.

General procedure for the synthesis of aryl 2,3,4,6-tetra-*O*-acetyl- β -D-glycopyranosides.[26] The syntheses were achieved by phase-transfer-catalyzed glycosylation of phenols with peracetylated glycopyranosyl bromide as described by Kroger et al and us earlier.[53, 54] Typically, 15 mL of a 1N aqueous sodium hydroxide solution were added to a solution of the peracetylated glycosyl bromide (3.5 mmol), tetrabutylammonium bromide (1.45 equiv.) and 2-chloro-4-nitrophenol (2 equiv.) in 25 mL dichloromethane. The resulting two phase system was vigorously stirred at 35 °C for 3h. After cooling to ambient temperature, the mixture was diluted with 100 mL ethyl acetate. After separation of the organic layer, the aqueous layer was extracted two times with 50 mL ethyl acetate each. The combined organic layers were washed with 1N aqueous sodium hydroxide solution, 20 mL water, 20 mL brine and dried over anhydrous sodium sulfate. After filtration, the filtrate was concentrated to dryness yielding a residue that was purified by column chromatography over silica gel affording the target compounds.

2-Chloro-4-nitrophenyl 2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranoside (12f). Off-white solid, 4.40 g (8.733 mmol, 23 %); mp 152-153 °C; R_f 0.29 (SiO₂, hexane/ethyl acetate = 2/1 (v/v); δ_H (CDCl₃) 8.32 (d, 2.6, 1H), 8.14 (dd, 9.0, 2.6, 1H), 7.24 (s, 1H), 5.12 - 5.46 (m, 4H), 4.30 (dd, 12.4, 5.3, 2H), 4.22 (dd, 12.4, 2.6, 2H), 3.90 - 3.98 (m, 2H), 2.10 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H); δ_C (CDCl₃) 170.3, 170.0, 169.2, 168.9, 157.1, 143.0, 126.0, 124.8, 123.5, 116.3, 99.1, 72.4, 71.9, 70.3, 67.8, 61.6, 20.6, 20.5, 20.5, 20.5; calcd for C₂₀H₂₂ClNO₁₂ C, 47.68; H, 4.40; found: C, 47.83, H, 4.43.

2-Chloro-4-nitrophenyl 3,4,6-tri-*O*-acetyl-2-*O*-methyl- β -D-galactopyranoside (12h). Purification by column chromatography over silica gel (hexane/ethyl acetate, 3/1-1/1, v/v) as colorless solid (1.30 g, 2.731 mmol, 80 %); mp 148-149 °C; R_f 0.40 (SiO₂, hexane/ ethyl acetate, 1/1, v/v); δ_H (CDCl₃) 8.34 (d, 2.5, 1 H), 8.15 (dd, 2.8, 9.0, 1 H), 7.20 (d, 9.3, 1 H), 5.46 (dd, 0.9, 3.4, 1 H), 5.10 (d, 7.5, 1 H), 5.02 (dd, 3.5, 10.3, 1 H), 4.26 - 4.02 (m, 3 H), 3.76 (dd, 7.5, 10.3, 1 H), 3.69 (s, 3 H), 2.20 (s, 3 H), 2.09 (s, 3 H), 2.08 (s, 3 H); δ_C (CDCl₃); 170.2, 170.0, 157.4, 142.7, 126.2, 124.1, 123.6, 115.0, 101.1, 77.5, 72.0, 71.4, 66.9, 61.5, 61.5, 20.7, 20.7, 20.6; calcd for C₁₉H₂₂ClNO₁₁ C, 47.96; H, 4.66; found: C, 47.96, H, 4.64.

General procedure for deacetylation reactions. Typically, the peracetylated aryl glycopyranosides **12** were suspended in 12-15 mL 7 M ammonia in methanol and stirred at ambient temperature. After 5-24 h, all volatile material was evaporated. The resulting residue was recrystallized from ethyl acetate or purified by column chromatography on silica gel as noted yielding the target compounds.[26]

2-Chloro-4-nitrophenyl α -D-mannopyranoside (11a).[24, 25] Colorless solid; 0.64 g (0.192 mmol, 71%) after column chromatography on silica gel (ethyl acetate/methanol, 30/1-20/1, v/v); mp 156-157 °C; R_f 0.35 (SiO₂, ethyl acetate/methanol, 10/1, v/v); δ_H (DMSO-d₆) 8.34 (dd, 2.8, 0.8, 1H), 8.20 (ddd, 9.2, 2.8, 0.9, 1H), 7.56 (d, 8.8, 1H), 5.74 (d, 1.3, 1H), 5.24 (dd, 4.5, 0.8, 1H), 4.96 (dd, 5.6, 0.6, 1H), 4.92 (dd, 5.9, 0.6, 1H), 4.46 (dd, 6.3, 5.8, 1H), 3.90 (t, 4.5, 1H), 3.70 - 3.77 (m, 1H), 3.48 - 3.62 (m, 2H), 3.39 - 3.47 (m, 1H), 3.33 (s, 3H), 3.22 - 3.30 (m, 1H); δ_C (DMSO-d₆) 156.6, 141.5, 125.4, 124.2, 122.6, 116.2, 99.2, 75.9, 70.5, 69.6, 66.3, 60.8; HRMS, FTMS⁺ m/z calcd. for C₁₂H₁₄ClNNaO₈ [M+Na]⁺: 358.0306; found 358.0304.

2-Chloro-4-nitrophenyl α -D-galactopyranoside (11c). Off-white solid, 1.50 g (4.468 mol, 31 %); mp 90-115 °C; R_f 0.89 (SiO₂, MeOH); δ_H (DMSO-d₆) 8.33 (d, 2.8, 1H), 8.20 (dd, 9.2, 2.9, 1H), 7.53 (d, 9.3, 1H), 5.86 (d, 3.3, 1H), 5.16 (d, 5.5, 1H), 4.97 (d, 5.3, 1H), 4.68 (d, 3.8, 1H), 4.51 (dd, 6.1, 5.4, 1H), 3.88 (dd, 5.1, 3.4, 1H), 3.76 - 3.86 (m, 2H), 3.56 - 3.65 (m, 1H), 3.46 - 3.55 (m, 1H), 3.35 - 3.42 (m, 1H); δ_C (DMSO-d₆) 157.6, 141.2, 125.4, 124.2, 122.8, 115.8, 98.5, 73.5, 69.1, 68.3, 67.4, 60.1; Calcd for C₁₂H₁₄ClNO₈: C, 42.93; H, 4.20; found: C, 42.89, H, 4.29; HRMS, ESI-TOF⁺ m/z calcd. for C₁₂H₁₄ClNO₈Na [M+Na]⁺: 358.0300; found 358.0291.

2-Chloro-4-nitrophenyl β -D-galactopyranoside (11d). The compound was prepared as described by us previously.[26]

2-Chloro-4-nitrophenyl α -D-glucopyranoside (11e). Off-white solid; 1.20 g (3.575 mmol, 25 %); mp 157-159 °C; R_f 0.66 (SiO₂, MeOH); δ_H (DMSO-d₆) 8.33 (d, 2.8, 1H), 8.21 (dd, 9.3, 2.8, 1H), 7.54 (d, 9.5, 1H), 5.83 (d, 3.5, 1H), 5.33 (d, 5.3, 1H), 5.13 (t, 5.4, 2H), 4.48 (t, 5.9, 1H), 3.70 (td, 9.2, 5.0, 1H), 3.53 (ddd, 12.0, 5.8, 2.5, 1H), 3.41 - 3.50 (m, 2H), 3.35 - 3.40 (m, 1H), 3.22 (ddd, 10.0, 8.8, 5.3, 1H); δ_C (DMSO-d₆) 157.5, 141.3, 125.5, 124.2, 122.8, 115.8, 98.2, 74.9, 72.6, 71.2, 69.4, 60.5; Calcd for C₁₂H₁₄ClNO₈: C, 42.93; H, 4.20; found: C, 43.05, H, 4.23; ESI-TOF⁺ m/z calcd. for C₁₂H₁₄ClNO₈Na [M+Na]⁺: 358.0300; found 358.0302.

2-chloro-4-nitrophenyl β -D-glucopyranoside (11f). Off-white solid; 1.94 (5.779 mmol, 40%); mp 181-183 °C; R_f 0.66 (SiO₂, MeOH); δ_H (DMSO-d₆) 8.15 (t, 9.0, 1 H), 7.21 (dd, 13.3, 2.5, 1 H), 7.04 (dd, 9.3, 2.3, 1 H), 5.48 (d, 4.5, 1 H), 5.18 (d, 4.5, 1 H), 5.06 - 5.14 (m, 2 H), 4.61 (t, 5.6, 1 H), 3.69 (dd, 9.8, 5.3, 1 H), 3.40 - 3.52 (m, 2 H), 3.10 - 3.33 (m, 3 H); δ_C (DMSO-d₆) 157.8, 141.5, 125.5, 124.3, 122.2, 115.7, 99.9, 77.3, 76.6, 73.0, 69.3, 60.5; calcd for C₁₂H₁₄ClNO₈, C 42.93, H 4.20; found: C 42.96, H 4.19.

2-Chloro-4-nitrophenyl 2-O-methyl- α -D-galacto-pyranoside (11g). Chromatographic purification over silica gel (hexane/ethyl acetate 2/1 – 0/1, v/v), colorless solid; 0.81 g (2.314 mmol, 79 %); mp 130-132 °C; δ_H (DMSO-d₆) 8.33 (d, 2.8, 1 H), 8.22 (dd, 2.8, 9.3, 1 H), 7.55 (d, 9.3, 1 H), 6.15 (d, 3.5, 1 H), 5.15 (d, 6.0, 1 H), 4.81 (d, 4.3, 1 H), 4.52 (t, 5.8, 1 H), 3.91 (ddd, 3.1, 6.1, 9.8, 1 H), 3.81 (t, 3.5, 1 H), 3.64 - 3.45 (m, 3 H), 3.42 (s, 3 H), 3.39 - 3.30 (m, 2 H); δ_C (DMSO-d₆) 157.3, 141.3, 125.5, 124.2, 122.8, 115.8, 95.7, 77.3, 73.3, 68.5, 68.5, 60.0, 58.4; HRMS, ESI-TOF⁺ m/z calcd. for C₁₃H₁₆ClNNaO₈ [M+Na]⁺: 372.0462; found 372.0456.

2-Chloro-4-nitrophenyl 2-O-methyl- β -D-galactopyranoside (11h). Chromatographic purification over silica gel (hexane/ethyl acetate 4/1 – 0/1, v/v), colorless solid; 0.77g (2.200 mmol, 87 %); R_f 0.17 (SiO₂, ethyl acetate); mp 193-195 °C; δ_H (DMSO-d₆) 8.36 (d, 2.8, 1 H), 8.22 (dd, 2.8, 9.3, 2 H), 7.44 (d, 9.3, 2 H), 5.24 (d, 7.5, 2 H), 5.14 (d, 6.0, 2 H), 4.76 (d, 4.8, 2 H), 4.71 (t, 5.5, 1 H), 3.77 - 3.66 (m, 2 H), 3.63 - 3.45 (m, 6 H), 3.41 (dd, 7.5, 9.5, 1 H); δ_C (DMSO-d₆) 157.5, 141.6, 125.6, 124.4, 122.0, 115.5, 100.3, 80.5, 75.8, 72.3, 68.1, 60.4, 60.1; HRMS, ESI-TOF⁺ m/z calcd. for C₁₃H₁₆ClNNaO₈ [M+Na]⁺: 372.0462; found 358.0451.

2'-chloro-4'-nitrophenyl β -D-allopyranoside (11i). Chromatographic purification over silica gel (ethyl acetate/methanol, 1/0-10/1, v/v); colorless solid; 0.42 g, 1.254 mmol, 83%; R_f 0.27 (SiO₂, ethyl acetate/methanol = 10/1, v/v); mp 153-154 °C; δ_H (CD₃OD) 8.30 (d, 2.5, 1H), 8.19 (dd, 9.3, 2.8, 1H), 7.43 (d, 9.3, 1H), 5.48 (d, 7.8, 1H), 4.16 (t, 2.9, 1H), 3.91 - 3.97 (m, 1H), 3.88 (dd, 12.0, 2.3, 1H), 3.66 - 3.77 (m, 2H), 3.63 (dd, 9.8, 3.0, 1H); δ_C (CD₃OD) 159.6, 143.4, 126.6, 124.9, 124.6, 116.7, 100.1, 76.1, 73.1, 71.7, 68.4, 62.7. The spectral data match those reported by others.[28]

***p*-Tolyl 3,4,6-tri-*O*-acetyl-2-*O*-methyl-1-thio- β -D-galactopyranoside (15).** The synthesis was performed using a general protocol by Qin Li et al. for related compounds.[34] A solution of **14** (0.86 g, 2.85 mmol) in 8 mL pyridine and 5 mL acetic anhydride was stirred at ambient temperature for 3 h. The solution was then diluted with 100 mL ethyl acetate and neutralized with saturated sodium bicarbonate solution. The separated organic layer was extracted with 20 mL water, 20 mL brine, and dried over anhydrous sodium sulfate. After filtration and concentration, a residue was obtained and purified by column chromatography over silica gel (hexane/ethyl acetate = 6/1 - 4/1, v/v) to give compound **15** (1.20 g, 94%) as a colorless foam; R_f 0.27 (SiO₂, cyclohexane/ethyl acetate = 3/1, v/v); δ_H (CDCl₃) 7.48 - 7.52 (m, 2H), 7.15 (dd, 8.5, 0.5, 2H), 5.40 (dd, 3.5, 1.0, 1H), 4.96 (dd, 9.7, 3.4, 1H), 4.58 (d, 9.8, 1H), 4.18 (dd, 11.5, 7.0, 1H), 4.11 (dd, 11.3, 6.3, 1H), 3.87 (td, 6.5, 1.0, 1H), 3.55 (s, 3H), 3.44 (t, 9.7, 1H), 2.37 (s, 3H), 2.15 (s, 3H), 2.05 (s, 6H); δ_C (CDCl₃) 170.3, 170.1, 169.9, 138.0, 132.8, 129.6, 129.1, 87.8, 77.2, 76.5, 74.2, 74.0, 67.6, 61.7, 61.0, 21.1, 20.7, 20.6; HRMS (ESI-TOF⁺) m/z calcd. for C₂₀H₂₆NaO₈S [M+Na]⁺ 449.1246; found 449.1238.

3,4,6-tri-*O*-acetyl-2-*O*-methyl- α -D-galactopyranosyl bromide 16. The synthesis was performed according to a protocol described for related compounds.[55] Iodine monobromide (0.12 g, 0.580 mmol, 1.13 equiv.) was added to a solution of **15** (0.22 g, 0.514 mmol) in 5 mL dichloromethane at 0 °C. After 10 min, the solution was diluted with 100 mL dichloromethane and neutralized with 5 mL saturated aqueous sodium bicarbonate solution. The organic layer was separated and washed with 5 mL saturated aqueous sodium bisulfite solution, 20 mL water, 20 mL brine and dried over anhydrous sodium sulfate. After filtration and concentration of the filtrate to dryness, a residue was obtained that was purified by column chromatography over silica gel (hexane/ethyl acetate, 4:1 - 3:1, v/v) to give compound **16** (0.16 g, 0.419 mmol, 81%) as a colorless foam; R_f 0.20 (SiO₂, cyclohexane/ethyl acetate = 2/1, v/v); δ_H (CDCl₃) 6.65 (d, 3.8, 1H), 5.49 (dd, 3.3, 1.3, 1H), 5.27 (dd, 10.3, 3.3, 1H), 4.46 - 4.48 (m, 1H), 4.17 (dd, 12.0, 4.0, 1H), 4.11 (dd, 12.0, 4.0, 1H), 3.63 (dd, 10.2, 3.9, 1H), 3.48 (s, 3H), 2.16 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H); δ_C (CDCl₃) 170.3, 169.8, 169.8, 90.1, 75.1, 71.1, 70.0, 67.1, 60.8, 58.3, 20.7, 20.6, 20.6; HRMS (ESI-TOF⁺) m/z calcd. for C₁₃H₁₉BrNaO₈ [M+Na]⁺ 405.0161 and 407.0141; found 405.0157 and 407.0137.

2'-chloro-4'-nitrophenyl 4,6-benzylidene- β -D-glucopyranoside (17). The synthesis was performed according to a general protocol for related compounds.[56] Benzaldehyde dimethyl acetal (0.43 g, 2.799 mmol, 1.25 equiv.) and camphorsulfonic acid (0.10 g, 0.431 mmol, 0.14 equiv.) were added to the solution of **11f** (0.75 g, 2.239 mmol) in 15 mL acetonitrile at ambient temperature. After 2h, the solution was neutralized with 0.3 mL trimethylamine, concentrated to yield a residue that was purified by column chromatography over silica gel (hexane/ethyl acetate = 4/1-1/1, v/v) to give **17** (0.82 g, 1.953 mmol, 87%) as a colorless solid; R_f 0.17 (SiO₂, cyclohexane/ethyl acetate = 2/1, v/v); mp 222-224 °C ; δ_H (CD₃OD) 8.33 (d, 2.8, 1H), 8.20 (dd, 9.0, 2.8, 1H), 7.49 - 7.56 (m, 2H), 7.46 (d, 9.3, 1H), 7.31 - 7.40 (m, 2H), 5.62 (s, 1H), 5.34 (d, 7.8, 1H), 4.33 (dd, 9.8, 4.5, 1H), 3.66 - 3.84 (m, 4H), 3.61 (t, 9.2, 1H); δ_C (CD₃OD) 159.1, 143.8, 139.0, 130.0, 129.1, 127.5, 126.8, 124.9, 124.9, 116.8, 103.0, 102.1, 81.7, 75.4, 74.7, 69.4, 67.9; HRMS, ESI-TOF⁺ m/z calcd. for C₁₉H₁₈ClNNaO₈ [M+Na]⁺: 446.0619; found 446.0622.

2'-chloro-4'-nitrophenyl 2-benzoyl-4,6-benzylidene- β -D-glucopyranoside (18) The synthesis was performed according to the general protocol for related compounds.[36] A suspension of **17** (1.50 g, 3.538 mmol) and dibutyltin oxide (0.97 g, 3.891 mmol, 1.1 equiv.) in 50 mL absolute methanol was kept under reflux. After 1.5 h, the homogenous solution was concentrated and co-evaporated with toluene twice. The residue was suspended in 30 mL toluene and cooled in ice. A solution of benzoyl chloride (0.55 g, 3.891 mmol) in 2 mL toluene was then added dropwise at 0 °C. Lastly, the solution was allowed to warm gradually to ambient temperature. After 16h, the reaction mixture was cooled to 0 °C, diluted with 150 mL ethyl acetate and filtered through a short pad of Celite. The pad was washed with cold ethyl acetate. The organic layers were combined, all volatile material was evaporated in vacuum, and the resulting residue was purified by column chromatography over silica gel (hexane/ethyl acetate = 8/1-3/1, v/v) to give compound **18** (1.30 g, 2.675 mmol, 76%) as an off-white solid; R_f 0.14 (SiO₂, cyclohexane/ethyl acetate = 4/1, v/v); mp 190-192 °C; δ_H (CD₂Cl₂) 8.21 (d, 2.8, 1H), 8.13 (dd, 9.0, 2.8, 1H), 8.02 - 8.09 (m, 2H), 7.57 - 7.65 (m, 1H), 7.36 - 7.55 (m, 7H), 7.31 (d, 9.3, 1H), 5.64 (s, 1H), 5.57 (dd, 9.0, 7.8, 1H), 5.38 (d, 7.8, 1H), 4.46 (dd, 10.3, 4.8, 1H), 4.19 (t, 9.2, 1H), 3.90 (t, 9.8, 1H), 3.84 (t, 9.2, 1H), 3.72 - 3.80 (m, 1H), 2.76 (br. s., 1H); δ_C (CD₂Cl₂) 165.9, 157.9, 143.6, 137.5, 134.1, 130.3, 129.9, 129.0, 128.8, 126.8, 126.6, 125.2, 124.2, 116.9, 102.5, 100.5, 80.8, 74.2, 72.6, 68.9, 67.4; HRMS (ESI-TOF⁺) m/z calcd. for C₂₆H₂₃ClNO₉ [M+H]⁺ 528.1061; found 528.1050.

2'-chloro-4'-nitrophenyl 2-O-benzoyl-4,6-benzylidene-3-O-triflyl- β -D-glucopyranoside (19). The synthesis was performed according to the general protocol.[36] A solution of **18** (1.20 g, 2.469 mmol) in 20 mL dry dichloromethane and dry pyridine (0.78 g, 0.988 mmol, 4.0 equiv.) was cooled to -5 °C. Then, a solution of trifluoromethanesulfonic anhydride (1.39 g, 4.938 mmol, 2.0 equiv.) in 2 mL dry dichloromethane was added dropwise. The yellow solution was stirred at 0 °C for 0.5 h and then poured into 50 mL of iced water. The resulting mixture was extracted with 80 mL dichloromethane three times each. The combined organic layer was washed with 50 mL water, 40 mL brine, separated, and dried over anhydrous sodium sulfate. After filtration and concentration, **19** was obtained as a yellowish foam (1.63 g, 2.469 mmol, quantitative) and used immediately in the next step; HRMS (ESI-TOF⁺) m/z calcd. for C₂₇H₂₁ClF₃NNaO₁₁S [M+Na]⁺ 682.0374; found 682.0378.

2'-chloro-4'-nitrophenyl 3-O-acetyl-2-O-benzoyl-4,6-benzylidene- β -D-allopyranoside (20). The synthesis was performed according to a general protocol for related compounds.[36] Tetrabutylammonium acetate (1.92 g, 6.383 mmol, 3.0 equiv.) was added to a solution of **19** (1.40 g, 2.128 mmol) in 15 mL dry DMF at 0 °C. After 15 min, the solution was poured into 50 mL of ice-water. The resulting mixture was extracted with 100 mL ethyl acetate three times each. The combined organic layer was washed with 50 mL water, 40 mL brine, separated, and dried over anhydrous sodium sulfate. After filtration and concentration, a residue was obtained that was purified by column chromatography over silica gel column (hexane/ethyl acetate = 8/1-6/1, v/v) to give compound **20** (1.10 g, 1.941 mmol, 91%) as a colorless foam; R_f 0.33 (SiO₂, cyclohexane/ethyl acetate = 4/1, v/v); δ_H (CD₂Cl₂) 8.24 (d, 2.8, 1H), 8.17 (dd, 9.0, 2.8, 1H), 7.92 - 7.98 (m, 2H), 7.56 - 7.64 (m, 1H), 7.41 - 7.48 (m, 4H), 7.34 - 7.40 (m, 4H), 6.00 (t, 2.9, 1H), 5.68 (d, 8.3, 1H), 5.63 (s, 1H), 5.55 (dd, 8.2, 3.1, 1H), 4.49 (dd, 10.5, 5.0, 1H), 4.27 (td, 9.9, 5.1, 1H), 4.00 (dd, 9.5, 2.5, 1H), 3.89 (t, 10.3, 1H), 2.16 (s, 3H); δ_C (CD₂Cl₂) 170.0, 165.2, 158.1, 143.6, 137.4, 134.1, 130.2, 129.7, 129.7, 129.1, 128.8, 126.7, 126.6, 125.2, 124.2, 116.8, 102.3, 98.9, 76.5, 70.3, 69.3, 68.7, 65.6, 21.1; HRMS (ESI-TOF⁺) m/z calcd. for C₂₈H₂₅ClNO₁₀ [M+H]⁺ 570.1167; found 570.1152.

2'-chloro-4'-nitrophenyl 3-O-acetyl-2-O-benzoyl- β -D-allopyranoside (21). The synthesis was performed according to protocol for related compounds.[57] A suspension of **20** (0.90 g, 1.579 mmol) in 25 mL aqueous acetic acid/water (4/1, v/v) was stirred at 50 °C. After 15h, the suspension was cooled to ambient temperature and concentrated to dryness. The obtained residue was purified by column chromatography over silica gel (hexane/ethyl acetate = 1/2- 2/1, v/v) to give **21** as a colorless foam (0.74 g, 1.535 mmol, 97%); R_f 0.12 (SiO₂, cyclohexane/ethyl acetate = 1/1, v/v); δ_H (CDCl₃) 8.25 (d,

2.8, 1H), 8.16 (dd, 9.0, 2.8, 1H), 7.93 - 8.00 (m, 2H), 7.56 - 7.63 (m, 1H), 7.41 - 7.48 (m, 2H), 7.32 (d, 9.0, 1H), 5.89 (t, 3.0, 3H), 5.62 (d, 8.0, 1H), 5.48 (dd, 8.0, 3.0, 1H), 4.18 (dd, 9.7, 2.9, 1H), 3.99 - 4.11 (m, 2H), 3.92 (dd, 12.5, 5.0, 1H), 2.20 (s, 3H), 1.32 - 2.02 (br s, 2H); δ_C (CDCl₃) 170.7, 165.0, 157.6, 142.9, 133.6, 129.7, 129.0, 128.5, 126.1, 124.8, 123.7, 116.0, 97.8, 77.2, 75.0, 71.2, 69.4, 66.1, 62.0, 20.8; HRMS (ESI-TOF⁺) m/z calcd. for C₂₁H₂₀ClNNaO₁₀ [M+Na]⁺ 504.0673; found 504.0664.

Kinetic assays

All experiments were conducted in triplicate in 50 mM HEPES buffer at pH 7.50 $\pm$ 0.05 and 30.0 $\pm$ 0.1 °C, 100 mM TES buffer at pH 8.00 $\pm$ 0.05 and 60 $\pm$ 0.1 °C, or 50 mM CAPS buffer at pH 10.50 $\pm$ 0.05 and 30 $\pm$ 0.1 °C.

Substrate hydrolysis followed by UV/Vis spectroscopy

Substrate stock solution. Typically, 25-30 mg (75-90 μ mol) of the aryl-D-glycopyranoside substrates were dissolved in 500 μ L DMSO and diluted in a total volume of 10 mL with buffer solution yielding 7.5-10.0 mM substrate stock solutions. The substrate stock solutions were then used in 25 μ L aliquot increments between 0 and 175 μ L for all experiments.

Catalyst stock solution. Typically, 3-4 mg of the binuclear metal complexes were dissolved in 25 mL of buffer solution yielding 0.27 mM stock solutions. The catalyst stock solutions were then used in constant 25 μ L aliquots for all experiments resulting in 0.03 mM catalyst concentrations.

Substrate hydrolysis assay. All experiments were performed in triplicate in 96-well plate format with a 200 μ L volume. The substrate stock solution was distributed in the plate in aliquots with regular increments between 0-175 μ L. The overall volume of the solutions in each well was then adjusted to 175 μ L by addition of appropriate aliquots of buffer solution followed by addition of 25 μ L of the respective metal complex stock solutions. The covered plate was shaken and equilibrated at 30 °C for 30 min. The hydrolysis of the substrates was followed by monitoring the formation of 4-nitrophenolate and 2-chloro-4-nitrophenolate, respectively, using UV/Vis spectroscopy at 405 nm over 1 and 12h, respectively. Control experiments were conducted in similar fashion by substituting the aliquots of the metal complex stock solution with buffer solution.

Data analysis. The absorbance recorded was plotted versus time in minutes. The rate of the reaction was determined at each substrate concentration from the slope of the linear fit of the data after conversion of the absorbance into product concentration using the apparent extinction coefficients as described below. The rate of the reaction was corrected for the catalyst concentration and the uncatalyzed reaction, and then plotted versus the substrate concentration. By applying a non-linear fit of the resulting hyperbolic data, the catalytic rate constant k_{cat} [min⁻¹] and the substrate affinity K_M [mM] were determined utilizing the Michaelis-Menten model. All experiments were conducted in triplicate and the data were averaged.

Determination of the apparent extinction coefficient. Typically, 5 mg of the respective nitrophenol were dissolved in 250 μ L DMSO and diluted into 5 mL with buffer solution. Then, 100 μ L of this solution were further diluted into 5 mL with buffer solution yielding the phenol stock solution. Subsequently, 25 μ L aliquot increments of this stock solution were diluted into 200 μ L with buffer solution in a 96-well plate. The solutions were thoroughly mixed and then equilibrated at 30.0 $\pm$ 0.1 °C for 30 min prior to determination of their absorbance at 405 nm.

The obtained absorbance values were plotted versus the phenol concentrations. The linear fit of the data equals the product of extinction coefficient ϵ_{405} times the unknown path length d for product absorbance in 96-well plates containing 200 μ L solutions in the respective buffer, i.e. for 4-nitrophenolate in 50 mM CAPS buffer at pH 10.5 and 30°C, $\epsilon_{app} \times d = 8120 \text{ M}^{-1}$, for 2-chloro-4-nitrophenolate in 50 mM HEPES

buffer at pH 7.5 and 30°C, $\epsilon_{\text{app}} \times d = 7580 \text{ M}^{-1}$. The values were obtained from three independent experiments by averaging the data.

Substrate hydrolysis followed by HPLC

All experiments were conducted in 100 mM TES buffer at pH 8.0 and 60°C in triplicate, and the obtained data were averaged. The substrate stock solutions were prepared from maltose (**22**), cellobiose (**23**) or lactose (**24**). The metal complex stock solutions were prepared from Cu₂bpdpo (**1**), *S*-Cu₂bpdbo (**S-2**) or *R*-Cu₂bpdbo (**R-2**).

Substrate stock solution. Typically, 25-30 mg (73-83 mmol) of the disaccharides were dissolved in 1 mL nanopure water yielding 70-83 mM substrate stock solutions. A constant 50 μL aliquot of each stock solution was then used for all experiments.

Catalyst stock solution. Typically, 5 mg of the binuclear metal complexes were dissolved in 10 mL of buffer solution yielding 0.73 mM stock solutions. The catalyst stock solutions were then used in constant 450 μL aliquots for all experiments.

Substrate hydrolysis assay. The 50 μL aliquot of the substrate stock solutions were diluted with 450 μL nanopure water, buffer or catalyst stock solutions and heated to 60°C. After 24h, the solutions were cooled in ice, and 100 μL aliquot was taken and 30 mM sodium sulfide solution added. After centrifugation for 5 min at 8.5g, the supernatant was filtered and subjected to HPLC analysis.

HPLC assay and data analysis. All experiments were conducted on a Shimadzu HPLC with a Rezex-Carbohydrate Na⁺ (8%) column 300 $\times$ 7.8 mm and 50 $\times$ 7.8 mm guard column (Phenomenex) using nanopure water as eluent isocratic with a flow rate of 0.4 mL/min at 80°C and ELS detection. The filtered samples were diluted with an equal volume of 50 mM acetic acid buffer at pH 5.0. The resulting solution was subjected to analysis in 25 μL aliquots, and the elution was monitored for 30 min. Disaccharides elute under these conditions between 14-18 min, monosaccharides elute between 20-24 min and the TES buffer elutes at 22.5 min. The area of the peaks in the chromatograms was integrated using the software supplied by Shimadzu. The percentage of hydrolysis for each sugar aliquot was determined by correlation of the area in the respective sample to a reference samples in water.

Supporting Information. ¹H and ¹³C NMR spectra of all new compounds **4**, **5-7**, **11a**, **11c-h**, **12a**, **12c-h**, **15**, **16**, cif-files and crystallographic data for **S-2** and **R-2**.

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Abbreviations

HEPES, N-(2-Hydroxyethyl)piperazine-N'-2-ethanesulfonic acid; TES, N-[Tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid

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Table of Content Graphic

Chiral binuclear copper(II) complexes were synthesized and shown to discriminate epimeric glycosides and glycosidic bonds of disaccharides in aqueous solution at near neutral pH.