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Synthesis, crystal structure and investigation of mononuclear copper(II) and zinc(II) complexes of a new carboxylate rich tripodal ligand and their interaction with carbohydrates in alkaline aqueous solution

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Abstract

A new carboxylate rich asymmetric tripodal ligand, *N*-[2-carboxybenzomethyl]-*N*-[carboxymethyl]- β -alanine (**H₃camp**), and its *di*-copper(II), (NH₄)₂[**1**]₂, and *di*-zinc(II), ((CH₃)₄N)₂[**2**]₂, complexes have been synthesized as carbohydrate binding models in aqueous solutions. The ligand and complexes have been fully characterized using several techniques, including single crystal X-ray diffraction. The interactions of (NH₄)₂[**1**]₂ and ((CH₃)₄N)₂[**2**]₂ with D-glucose, D-mannose, D-xylose and xylitol in aqueous alkaline media were investigated using UV–Vis and ¹³C-NMR spectroscopic techniques, respectively. The molar conductance, NMR and ESI–MS studies indicate that the complexes dissociate in solution to produce the respective complex anions, **1**[–] and **2**[–]. Complexes **1**[–] and **2**[–] showed chelating ability towards the naturally abundant and biologically relevant sugars, D-glucose, D-mannose, D-xylose, and xylitol. The complex ions bind to one molar equivalent of the sugars, even in the presence of stoichiometric excess of the substrates, in solution. Experimentally obtained spectroscopic data and computational results suggest that the substrates bind to the metal center in a bidentate fashion. Apparent binding constant values, p*K*_{app}, between the complexes and the substrates were determined and a specific mode of substrate binding is proposed. The p*K*_{app} and relativistic density functional theory (DFT) calculated Gibbs free energy values indicate that D-mannose displayed the strongest interaction with the complexes. Syntheses, characterizations, detailed substrate binding studies using spectroscopic techniques, single crystal X-ray diffraction and geometry optimizations of the complex-substrates with DFT calculations are also reported.

Keywords

Metal complex; Monosaccharides; Sugar–metal complex interaction; Binding constants; Mutarotation

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1. Introduction

Sugar–metal ion interactions are not only ubiquitous in biological processes but are also vital in cell functions [1–5]. Although some effort has been directed towards understanding carbohydrate recognition, unlike various other metalloenzymes, the study of carbohydrates in biological systems using synthetic model complexes is largely unexplored. One of the contributing factors for the absence of such studies has been the lack of technological advances in the realm of high magnetic field NMR and other quantitative spectroscopic methods to handle the complicated equilibrium and polyfunctional nature of carbohydrates and other natural products in solution. However, NMR spectroscopy and several other techniques have developed significantly in recent times. As the consequence of the developments, the study of complicated systems including carbohydrates has been attracting considerable interest. Recently, some synthetic receptors have been used to investigate carbohydrate recognition in relation to the important roles they play in biological processes [3,4]. One of such recognition which is being investigated with ever increasing interest includes simple metal ions in carbohydrate interactions [6–13]. This particular interaction has important implications in a variety of biological systems such as support in membrane systems, cell–cell adhesion, intercellular recognition, signal transduction, fertilization, and as targets of bacterial or viral infections of cells [1–5]. Although understanding carbohydrate–metal ion coordination chemistry is of fundamental importance to these systems, structural and functional investigations of metal complexes with carbohydrates has been limited to complexes derived from amino sugars [14–16]. Aside from the biological relevance, carbohydrate interaction with metal ions has also been a subject of intense research in the field of enantioselective catalysis of organic reactions [17–24].

To understand the carbohydrate–metal ion interactions, some model complexes have been prepared and their interactions reported in the literature [25–28]. In the past several years, the study of carbohydrate–transition metal ion interactions in chemistry and biology has been pursued by several research groups [2,14–16,24, 29–32]. For example, synthetic strategies for VO^{2+} , Cr^{3+} , Mn^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , and MoO_2^{2+} carbohydrate complexes have been developed [6,7,11–14,28,31–36]. Furthermore, the biologically relevant aspects of carbohydrate complexes of Fe^{3+} , Cr^{3+} , VO^{2+} , and Zn^{2+} , have also been reported [32–35,37–43]. It has also been elucidated that carboxylato-bridged dinuclear complexes of Mg^{2+} , Mn^{2+} , Co^{2+} , Ni^{2+} , and Zn^{2+} , are involved in non-redox active enzymatic processes [33,44–51].

In an attempt to contribute to the understanding of carbohydrate–metal complex chemistry, recently, we reported new *polynuclear* iron(III) and copper(II) complexes and their interaction with several monosaccharides in aqueous media [34]. Thus, the main focus of the current manuscript is on the interaction of newly synthesized *mononuclear* copper(II) and zinc(II) complexes of a carboxylate rich asymmetric tripodal ligand, *N*-[2-carboxybenzomethyl]-*N*-[carboxymethyl]- β -alanine (H_3camb) with the monosaccharides in alkaline aqueous media. Herein, we report the syntheses and characterizations of H_3camb , $(\text{NH}_4)_2[\mathbf{1}]_2$ and $((\text{CH}_3)_4\text{N})_2[\mathbf{2}]_2$ complexes, solution behavior and the level and mode of their interaction with D-glucose, D-mannose, D-xylose and xylitol in alkaline aqueous

media. Also reported is relativistic density functional theory (DFT) modeling study on the complexes and their interaction with the substrates.

2. Experimental

2.1. General remarks

All starting materials were purchased from commercial sources and were used without further purification. Elemental analyses were determined by Atlantic Microlab, 6180 Atlantic Blvd. Suite M, Norcross, GA. FTIR spectra were recorded on solid samples using a Bruker Vector 22 FTIR-ATR spectrometer. Molar conductivities were measured in aqueous solution of the complexes (10^{-3} M) using a Corning Model 441 conductivity meter.

2.2. UV-Vis spectroscopy

All experiments were performed on an Agilent 8453 diode array UV-visible (UV-Vis) spectrophotometer with 1 cm quartz cell at room temperature over a range of 200–900 nm. An Eppendorf Research micropipette was used to measure volumes. All experiments were carried out in degassed nanopure water, in which pH of the solutions was adjusted using NaOH solution. Each concentration was made and measured three times and the data points were averaged. Standard deviation was applied to these averages. The detailed experimental procedure used for the sugar titrations was adopted from our earlier published work [34].

2.3. NMR spectroscopy

^1H and ^{13}C NMR spectra of H_3camb and $((\text{CH}_3)_4\text{N})_2[\mathbf{2}]$ were obtained in D_2O solution with a Varian Inova 500 NMR spectrophotometer. All ^{13}C NMR experiments for the determination of binding interactions were performed on the same NMR spectrophotometer at room temperature. The experiments were done in D_2O solution, for which pH was adjusted to pH 12.5 with NaOH prior to the use for each set of NMR titration. The pH of the resulting solution was measured immediately after mixing.

2.4. Mass spectrometry

Electrospray ionization time-of-flight mass-spectrometry (ESI-TOF-MS) spectrometry data was collected using a Bruker Daltonics micrOTOF instrument at the RCMI program. Data was collected for a m/z range of 100–2000 in negative ion mode. Samples were delivered as either dilute (1–2 mg/mL) aqueous or methanol solutions with a relatively moderate flow rate of 0.90 mL/h. In all the measurements, the setting of the nebulization gas, N_2 , was 45.0 psi, the capillary potential was 4 kV, the drying gas was 5.0 L/min, the skimmer was set to –33 V, and the hexapole RF was set to 300 Vpp. Data was analyzed and the spectra plots were generated using the Mass software package [52,53]. Simulations of the stable isotope patterns were made using Molecular Weight Calculator (Matthew Monroe, PNNL, Richland WA, U.S.A.) software.

2.5. Potentiometric titration

The potentiometric studies were carried out in doubly distilled and boiled water solution with a Metrohm 848 Titrino plus automatic titrator combined with an Ag/AgCl electrode

calibrated to read $-\log[\text{H}^+]$ directly, designated as pH. The electrode was calibrated using the data obtained from a potentiometric titration of a known volume of a standard 0.0100 mol L⁻¹ HCl solution with standard 0.100 M KOH. The experiments were carried out in a 50 mL sealed thermostated cell at 25 °C and bubbled with argon to ensure an inert atmosphere and the ionic strength adjusted to 0.100 M by addition of KCl. In a typical experiment the mass equivalent to 0.05 mmol of the H₃camb ligand was weighted in an analytical balance and dissolved in 30 mL of water and 20 mL of 0.0100 M HCl solution to ensure the complete protonation and an initial pH value near 2. The titration was carried out by the addition of 0.05 mL aliquots of a standard CO₂-free 0.100 M KOH solution pH 12 was reached. Computation of results was carried out with BEST7 program and species distribution calculations with SPECIES program [54].

2.6. Computational studies

The theoretical calculations were carried out using the Gaussian 09 implementation of B3LYP DFT [55–57]. The geometry optimization was carried out using a 6–31G(d) basis set on C, N, O and H atoms where LANL's double zeta basis set (LANL2DZ) with effective core potentials (ECP) were used for metals [58–60]. All structures were fully optimized with polarizable continuum model (PCM) and frequency analyses were performed to ensure a minimum state was achieved [61,62]. To test the robustness and reliability of the B3LYP functional used in the simulations, all geometries were also optimized under parameterized DFT functional M06-2X, which have shown promise for noncovalent interactions [63]. The thermodynamic functions, including enthalpies, entropies and free energies, were calculated at 298.15 K and 1 atm within the harmonic potential approximation at optimized structures. In order to examine basis set effect, Dunning's correlation consistent triple zeta basis set cc-pVTZ for all atoms was implemented with B3LYP functions to perform the single point energy (SPE) calculation upon the aforementioned optimized geometries [64].

2.7. Syntheses

2.7.1. Synthesis of N-[2-carboxybenzomethyl]-β-alanine, H₂cbal—The ligand has been prepared according to our previously published procedure [65]. The product was collected by filtration, washed with water, methanol and dried at 80 °C. The product was confirmed by elemental analysis and ¹H NMR spectroscopy. Yield: 8.5 g (76%). *Anal. Calcd.* for C₁₁H₁₃NO₄: C, 59.19%; H, 5.87%; N, 6.27%. *Found:* C, 59.08%; H, 5.93%; N, 6.20%. ¹H NMR for the sodium salt of the compound (500 MHz, D₂O, 25 °C, δ): 7.66 (d, 1H, *J* = 7.0 Hz), 7.48 (m, 2H), 7.45 (t, 1H, *J* = 7.0 Hz), 4.11 (s, 2H), 3.05 (t, 2H, *J* = 7.5 Hz), 2.52 (t, 2H, *J* = 7.5 Hz).

2.7.2. Synthesis of N-[2-carboxybenzomethyl]-N-[carboxymethyl]-β-alanine, H₃camb—An aqueous solution containing H₂cbal (3.50 g, 13.5 mmol), NaOH (1.62 g, 40.5 mmol), and *N,N*-Diisopropylethylamine (2.82 mL, 16.2 mmol) in 10 mL deionized water was prepared. In a separate container an aqueous solution of bromoacetic acid (2.25 g, 16.2 mmol) in 10 mL deionized water was similarly prepared. The above two solutions were combined (pH = 11 after mixing) in a 100 mL round bottom flask and refluxed for 4 h with stirring. The pH of the solution had decreased to 9 over the 4 hour reflux. The resulting solution was acidified with 6 M HCl to pH = 3 while being kept at 90 °C, after which a

white precipitate formed over a period of 10 min. The product was collected by filtration, washed with water, methanol and dried at 80 °C. The product was characterized as H₃camb by elemental analysis, ESI-MS, ¹H and ¹³C NMR spectroscopy. Yield: 2.81 g (74%). *Anal. Calcd.* for C₁₃H₁₅NO₆: C, 55.51%; H, 5.38%; N, 4.98%. *Found*: C, 55.39%; H, 5.43%; N, 5.03%. ESI-MS *m/z* (%): 280.0867 (80) [C₁₃H₁₄NO₆]⁻; 302.0624 (95) [C₁₃H₁₃NO₆Na]⁻; 324.0535 (100) [C₁₃H₁₂NO₆Na₂]⁻. ¹H NMR for the sodium salt of the compound (500 MHz, D₂O, 25 °C, δ): 7.51 (d, 1H, *J* = 8.1 Hz), 7.34 (m, 3H), 3.80 (s, 2H, *J* = 7.6 Hz), 3.06 (s, 2H), 2.75 (t, 2H, *J* = 7.5 Hz), 2.36 (t, 2H, *J* = 7.5 Hz).

2.7.3. Synthesis of (NH₄)₂[Cu₂(camb)₂], (NH₄)₂[1]₂—An aqueous solution (1.0 mL) containing [Cu(NH₃)₄]SO₄ · H₂O (0.1880 g, 0.77 mmol) was added drop wise, at ambient temperature, to a stirring 1.0 mL aqueous suspension of the ligand H₃camb (0.2150 g, 0.76 mmol). After complete addition the resulting dark blue solution was allowed to stir for 24 h at ambient temperature, after which the volume of solvent was removed by heating to 70 °C and the resulting blue powder was redissolved in 2.0 mL of water. The solution was filtered to remove any undissolved solids and the product was precipitated using acetone to afford the blue complex characterized as (NH₄)₂[1]₂ after filtering and drying over calcium sulfate. Na₂[1]₂ complex was synthesized in similar manner and X-ray quality single crystals were grown by acetone diffusion into the concentrated solution in 30 days. Yield: 0.2640 g (48%). *Anal. Calc.* for C₂₆H₃₂N₄O₁₂Cu₂: C, 43.39; H, 4.48; N, 7.79. *Found*: C, 43.21; H, 4.47; N, 7.88%. ESI-MS *m/z* (%): 341.0016 (100) [C₁₃H₁₂CuNO₆]⁻; 683.0242 (29) [C₂₆H₂₅Cu₂N₂O₁₂]⁻; 705.0004 (7) [C₂₆H₂₄Cu₂N₂NaO₁₂]⁻. IR (Solid on ATR): ν 3234 (w) 3038 (w) 2900, 2866 (vw) 2793 (br), 1631, 1598, 1571 (s), 1464, 1441 (m), 1368, 1361, 1334 (s), 1239, 1156, 1112, 1083, 1011 (m), 975 (w), 942, 921 (m), 866 (s), 807 (m), 767, 717, 673, 626 (s) 596, 572 (m) (cm⁻¹). UV-Vis (H₂O) λ_{max}/nm (ε/L mol⁻¹ cm⁻¹): 725 (161); Molar conductance, *A*_M: (H₂O) = 128 Ω⁻¹cm²mol⁻¹.

2.7.4. Synthesis of ((CH₃)₄N)₂[Zn₂(camb)₂], ((CH₃)₄N)₂[2]₂—A methanol solution (5.0 mL) containing Zn(NO₃)₂ · 6H₂O (0.1058 g, 0.36 mmol) was added drop wise, at ambient temperature, to a stirring 5.0 mL methanol solution of the ligand H₃camb (0.1000 g, 0.36 mmol) and (CH₃)₄NOH (0.2255 g, 1.25 mmol). After complete addition the resulting colorless solution was allowed to stir at ambient temperature for 2 h. X-ray quality single crystals of [((CH₃)₄N)₂[2]₂] were grown in 3 days by slow acetone vapor diffusion into the methanoic solution of the complex. Yield: 0.2633 g (88%). *Anal. Calc.* for C₃₄H₆₀N₆O₁₅Zn₂: C, 44.21; H, 6.55; N, 9.10. *Found*: C, 44.83; H, 6.43; N, 9.09%. ESI-MS *m/z* (%): 342.0021 (100) [C₁₃H₁₂NO₆Zn]⁻; 760.1113 (2) [C₃₀H₃₆N₃O₁₂Zn₂]⁻. IR (Solid on ATR): ν 3031 (w) 2955 (w) 1644 (m), 1615, 1583 (s) 1489, 1468, 1447, 1421, 1396 (m) 1346, 1330 (s), 1258, 1232, 1148, 1118, 989 (m), 954, 855, 759, 721, 666 (s) (cm⁻¹); Molar conductance, *A*_M: (H₂O) = 118 Ω⁻¹cm²mol⁻¹.

2.8. X-ray crystallography and data analysis

The data was collected at 98(2) K using a Rigaku AFC12/Saturn 724 CCD fitted with Mo Kα radiation (λ = 0.71073 Å). Data collection and unit cell refinement were performed using CRYSTAL CLEAR software [66]. The total number of data was measured in the range 3.09° < θ < 27.5° using ω scans. Data processing and absorption correction, giving minimum

and maximum transmission factors, was accomplished with CRYSTAL CLEAR and ABSCOR, respectively [66,67]. The structure was refined with Olex2 [68] using SHELX-2014 by direct methods and refined (on F^2) using full-matrix, least-squares techniques [69]. All non-hydrogen atoms, for all structures, were refined with anisotropic displacement parameters. All carbon bound hydrogen atom positions were determined by geometry and refined by a riding model. In the case of $(\text{Na})_2[\mathbf{1}]_2$ complex, electron density peaks were used to identify oxygen bound hydrogen atom for the O9 and the hydrogen atom on O7 was placed on a calculated position, and the displacement parameters were set to 1.5 times the displacement parameters of the bonded atoms.

3. Results and discussion

3.1. Synthesis of the ligand and metal complexes

The asymmetric tri-carboxylate amine tripodal ligand, H_3camb , has been synthesized in two reaction steps, Scheme 1. The synthesis of the precursor ligand, H_2cbal , was accomplished by the condensation of stoichiometric amounts of 2-carboxybenzaldehyde and β -alanine in the presence of NaOH in methanol under refluxing conditions for 4 h, followed by subsequent reduction using NaBH_4 [65]. Alkylation of the secondary amine group of H_2cbal with stoichiometric quantities of bromoacetic acid in the presence of NaOH/*N,N*-Diisopropylethylamine followed by acidification of the solution with HCl yielded a pure H_3camb as white powder.

The H_3camb ligand has been fully characterized using various analytical techniques such as elemental analysis, ESI-MS, FTIR, ^1H and ^{13}C NMR spectroscopies. Additionally, the pK_a values for the ligand have been determined using potentiometric titration methods. The titration curve for H_3camb ligand has two observable buffer regions. One region involves the consumption of three equivalents of the base and is attributed to the protonation/deprotonation equilibria of the three carboxylic groups is at 2.5 to 4.5 pH range. The second buffer region which involves the consumption of one equivalent of the base due to the protonation/deprotonation of the amino group is observed after pH 5. The calculated equilibrium constant values for each of the protonation/deprotonation steps involved are shown in Scheme 2. The calculated species distribution as a function of pH indicates that HL^{2-} as the main species in the pH values above 4.3 and less than 10.2, the completely deprotonated species, L^{3-} , predominates at pH values 10.2 while the *di*-, *tri*- and *tetra*-protonated species have a maximum formations at pH values of 3.8, 2.8 and 2.0, respectively.

The ligand, H_3camb , was specifically designed for the investigation for two reasons. Firstly, its carboxylate rich coordination environment mimics the coordination environment of an active site of the metalloprotein that involves carbohydrates, xylose/glucose isomerase (XGI), Fig. 1.

Secondly, H_3camb as a *mono*-nucleating ligand gives a structural complementarity to our reported *di*-nucleating ligand, H_5ccdp ; and thus, it presents an opportunity to study and contrast the effect of metal nuclearity on the complex-carbohydrate interaction without

significantly altering the molecular structure of the ligands [33,34]. For comparison reasons, the molecular structures of H₅ccdp and H₃camb ligands are depicted in Fig. 2.

The direct synthetic routes to (NH₄)₂[1]₂ and ((CH₃)₄N)₂[2]₂ are described in Scheme 3. The synthesis of (NH₄)₂[1]₂ in aqueous solution is accomplished by reacting [Cu(NH₃)₄]SO₄ · H₂O and H₃camb in 1:1 mole ratio in ambient conditions. Similarly, a reaction of equimolar amounts of Cu(NO₃)₂ salt and H₃camb in the presence of four equivalents of NaOH in water produced Na₂[1]₂. Both the (NH₄)₂[1]₂ and Na₂[1]₂ complexes are stable under standard conditions, soluble in water and insoluble in most common organic solvents. While single crystals of the sodium salt of the complex suitable for X-ray analysis were grown by slow acetone diffusion into an aqueous solution of the reaction mixture, similar crystallization effort on the ammonium salt has not produced crystals suitable for diffraction. Correspondingly, the synthesis of ((CH₃)₄N)₂[2]₂ was carried by the reaction of Zn(NO₃)₂ · 6H₂O and H₃camb in a 1:1 molar ratio in the presence of an 3.5 equivalents of (CH₃)₄NOH as a base in methanol under ambient conditions. Slow acetone diffusion into the reaction mixture produced X-ray quality single crystals characterized as ((CH₃)₄N)₂[2]₂. The product is stable under ambient conditions, exceedingly soluble in water, and moderately soluble in most common organic solvents. Characterization of (Na)₂[1]₂, (NH₄)₂[1]₂ and ((CH₃)₄N)₂[2]₂ has been carried out using a variety of techniques including elemental analysis, FTIR, UV–Vis, ¹H NMR, ¹³C NMR, ESI–MS, and single crystal X-ray diffraction.

3.2. Spectroscopic characterizations

The UV–Vis spectra of (NH₄)₂[1]₂ (2.50 mM) were recorded in H₂O at pH 7.0 and 12.5. The spectrum in water at pH = 7 has only one *d*–*d* transition band with the λ_{max} centered at 725 nm, while in pH = 12.5 the λ_{max} is blue-shifted to 704 nm. Solution characterizations of ((CH₃)₄N)₂[2]₂ dissolved in D₂O was performed using ¹³C-NMR spectroscopy. The spectrum displayed fourteen well resolved NMR signals corresponding to the 14 different sets of carbons in the complex, including the carbon from the (CH₃)₄N⁺ counter ion which appears at 55 ppm. The chemical shifts for the aromatic, methylene and carboxylates carbons positioned around 138–129, 60–33, and 182–178 ppm, respectively.

In order to gain further insight into the behavior of the complexes in aqueous solution, the ESI–MS spectra of complexes (NH₄)₂[1]₂ and ((CH₃)₄N)₂[2]₂ were recorded and analyzed. The negative ion mode ESI–MS spectra of the complexes in aqueous media are shown in Fig. 3. Besides to the value of the *m/z* ratio, the distinct copper and zinc nuclei isotope distribution patterns have been used in the identification and determination of the number of metal ions present in the molecular ion peaks. The ESI–MS spectrum of (NH₄)₂[1]₂ dissolved in nanopure water with the pH adjusted to 12.5 with NaOH, is shown in Fig. 3(a). The spectrum contains signals corresponding to [Cu(camb)][–], [Cu(C₁₂H₁₂NO₄)][–] and [Cu(camb)(CH₃COOH)][–] at *m/z* = 341 (100%), 297 (26%) and 401 (15%), respectively. The data indicates that the ion at *m/z* = 401 losses a mass that corresponds to CH₃CO₂H to produce [Cu(camb)][–] at *m/z* = 341, subsequently followed by a loss of CO₂ from the ligand to from the [Cu(C₁₂H₁₂NO₄)][–] at *m/z* = 297. The experimentally obtained and the simulated

isotope distribution patterns for the molecular ion $[\text{Cu}(\text{camb})]^-$ at $m/z = 341$ are shown in Fig. 3(c).

The comparison of the distribution patterns for the experimentally obtained and the simulated data shows an excellent agreement to one another. Similarly, the ESI-MS spectrum for $((\text{CH}_3)_4\text{N})_2[\mathbf{2}]_2$ under the same experimental conditions was obtained and is presented in Fig. 1(b). The spectrum contains signals corresponding to molecular ions $[\text{Zn}(\text{camb})]^-$ at $m/z = 342$ (100%), $[\text{Zn}(\text{C}_{12}\text{H}_{12}\text{NO}_4)]^-$ at $m/z = 342$ (16%), and $[\text{Zn}_2(\text{camb})_2 + ((\text{CH}_3)_4\text{N})]^-$ at $m/z = 760$ (2%). The ESI-MS data indicates that the ion at $m/z = 760$ dissociates to give $[\text{Zn}(\text{camb})]^-$ at $m/z = 342$, which subsequently loses a CO_2 from the ligand to produce $[\text{Zn}(\text{C}_{12}\text{H}_{12}\text{NO}_4)]^-$ at $m/z = 298$. The experimentally obtained and simulated isotope distribution patterns for $[\text{Zn}(\text{camb})]^-$ at $m/z = 342$ are shown in Fig. 3(d). Once again, the two distribution patterns are in agreement to one another. The data obtained in this ESI-MS study not only helps to establish the m/z ratio corresponding species in solution, but also exposes the stability of the ions at pH 12.5. Furthermore, molar conductance of 10^{-3} M aqueous solutions of complexes $(\text{NH}_4)_2[\mathbf{1}]_2$ and $((\text{CH}_3)_4\text{N})_2[\mathbf{2}]_2$ at different temperatures were measured. The values obtained for the complexes in water (25 °C) were 118 and $128 \Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$, respectively. The molar conductance values in aqueous solutions fall in the range $118\text{--}131 \Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$, suggesting that these are 1:1 electrolytes [70]. The solution behavior and dissociation of the complexes into their respective mono-anionic complexes are depicted on Scheme 4.

The FTIR spectra of solid samples of complexes $(\text{Na})_2[\mathbf{1}]_2$, $(\text{NH}_4)_2[\mathbf{1}]_2$ and $((\text{CH}_3)_4\text{N})_2[\mathbf{2}]_2$ were recorded and analyzed, Supporting Information Fig. S1. When comparing the IR spectrum of $(\text{Na})_2[\mathbf{1}]_2$ and $(\text{NH}_4)_2[\mathbf{1}]_2$, the spectra for $\text{Na}_2[\mathbf{1}]_2$ notably lacks the asymmetric $\nu_{\text{as}}(\text{N-H})$ vibration at 3234 cm^{-1} whereas the spectra for $(\text{NH}_4)_2[\mathbf{1}]_2$ contains a broad O-H stretching vibration at 3370 cm^{-1} . This difference is attributed to the absence of ammonium cations and the presence of water molecules in the solid sample $\text{Na}_2[\mathbf{1}]_2$. Deacon and Phillips had previously examined the FTIR spectra of many metal-carboxylate complexes with known X-ray crystal structures and were able to draw useful conclusions for the correlations between carboxylate stretching frequencies and their geometries [71]. Similarly, FTIR analysis of zinc(II) carboxylates has been extensively studied as a means to correlate their spectra with their known crystal structures [72]. The IR spectra of $(\text{NH}_4)_2[\mathbf{1}]_2$ (Supporting Information, Fig. S1(b)) shows three strong asymmetric $\nu_{\text{as}}(\text{COO}^-)$ vibrations at 1631, 1598, and 1571 cm^{-1} , and three strong symmetric $\nu_{\text{s}}(\text{COO}^-)$ vibrations at 1368, 1361, and 1334 cm^{-1} . The difference, $(\Delta = \nu_{\text{as}}(\text{COO}^-) - \nu_{\text{s}}(\text{COO}^-))$, between the carboxylate stretches of 203, 264, and 270 cm^{-1} is attributed to the difference in binding modes between the carboxylates. The value of 203 is characteristic of a monodentate *monatomic* bridging carboxylate, while the values of 264 and 270 cm^{-1} are characteristic of a monodentate carboxylate binding mode [71,72]. In the IR spectra obtained for $((\text{CH}_3)_4\text{N})_2[\mathbf{2}]_2$ (Supporting Information, Fig. S1(c)), three strong asymmetric $\nu_{\text{as}}(\text{COO}^-)$ vibrations at 1617, 1585, and 1569 cm^{-1} , and three strong symmetric $\nu_{\text{s}}(\text{COO}^-)$ vibrations at 1423, 1348, and 1332 cm^{-1} were observed. The significant difference in the magnitude of the separation between the carboxylate stretches, Δ , of 146, 269, and 253 cm^{-1} is attributed to the two different binding modes of the carboxylates in the complex. The lower value of

146 cm⁻¹ is characteristic of *syn-anti* bridging coordination while the larger values of 269 and 253 cm⁻¹ are characteristic of monodentate carboxylate binding [71,72].

3.3. X-ray molecular structure characterization

Detailed crystal structure analysis of complexes is described herein. The crystal structural data and selected metric data for the complexes are given in Tables 1 and 2, respectively. The thermal ellipsoid representations of the single crystal X-ray molecular structures for Na₂[1]₂ and ((CH₃)₄N)₂[2]₂ are depicted in Figs. 4 and 5, respectively.

3.4. Crystal structure of Na₂[1]₂

The complex crystallized in the monoclinic P 2₁/n space group with two mononuclear [1]⁻ anions bridged by the O(5) and O(5A) of the β-alanine arms of the ligands in a μ₂:η¹η¹(O,O) fashion forming a centrosymmetric dimer. Two Na⁺ ions are present as counter ions to balance the charge and link adjacent [1]⁻ units together forming a continuous coordination chain, [Na₂Cu₂(camb)₂(H₂O)₅]_n [73]. The continuous coordination chain of the complex is shown in the Supporting Information Fig. S2(A). The thermal ellipsoid diagram for the dicopper complex anion, [1]₂²⁻, with atomic numbering scheme is shown in Fig. 4 the B3YLP generated electrostatic potential map is shown in the Supporting Information Fig. S2(B). Each copper(II) ion is in a penta-coordinated environment with a [NO₄] donor set. The Addison parameter, defined as $\tau = (\beta - \alpha) / 60$, is a structural index parameter used to systematize the deviation between square pyramidal and trigonal bi-pyramidal geometries in penta-coordinate species [74].

When applied to the complex, the calculated Addison parameter of 0.13 indicates a distorted square pyramidal geometry around the copper center. The O(3) atom of the acetate arm of the ligand opens at 10.5° out of the square plane of the base defined by O(1), O(5A), N(1), O(3) and the Cu(1). This distortion most likely arises from the steric hindrance imposed by the shorter acetate arm of the ligand. The long Cu(1)–O(5) bond length appears longer than normal at 2.222 Å; while the remaining bond lengths and angles appear typical for coordination complexes of this nature [75]. The dinuclear copper(II) core of the complex anion shown in two views in Fig. 6(a) and (b) exists as a planar four membered ring with alternating copper and oxygen atoms forming a corners of a rhomboid geometry. The Cu(1)–Cu(1A) and O(5)–O(5A) distances across the core are 3.188 and 2.719 Å, respectively. The rest of the metric data is present in Table 2.

3.5. Crystal structure of ((CH₃)₄N)₂[2]₂

The single crystal X-ray structure analysis reveals that the complex consists two zinc(II) ions, two camb³⁻ ligands and two tetramethylammonium as counter cations, where the methyl groups of the ammonium cations are disordered. A structural view of the anion of complex [2]₂²⁻ is depicted in Fig. 5 and the B3YLP generated electrostatic potential map is shown in the Supporting Information Fig. S3. Using the Addison parameter, the coordination geometry around each of the zinc(II) centers is best described as a slightly distorted trigonal bipyramidal geometry [75]. While the trigonal plane of the bipyramidal geometry is defined

by O(1), O(3) and O(5) atoms of one camb^{3-} ligand, the axial positions are taken by N(1) of the same ligand and O(6) of the second camb^{3-} of the complex.

Whereas the benzoate and the acetate groups are bound as a terminal ligands, the propionate arm of the ligand bridges between the two zinc(II) centers in a rare *syn,anti*- $\mu_2:\eta^1\eta^1$ fashion. Structural search performed on the core of complex as shown in Fig. 6(c) in the Cambridge Structural Database (CSD) returned with no crystal structure match. Although no coordination mode of this nature in *di*-zinc(II) complexes has been reported in the literature, Lalioti and coworkers have observed four *syn,syn*- $\mu_2:\eta^1\eta^1$ and two *syn,anti*- $\mu_2:\eta^1\eta^1$ coordination modes of a bridging CH_3CO_2^- ions in the crystal structure of the *hexa*-zinc(II) complex obtained by the reaction of $\text{Zn}(\text{CH}_3\text{CO}_2)_2 \cdot 2\text{H}_2\text{O}$ and D-2-pyridyl ketone (C_5NH_4)₂CO [76]. The two zinc centers and the bridging propionate arms in crystal structure of $((\text{CH}_3)_4\text{N})_2[\mathbf{2}]_2$ form a unique octagonal cavity core as shown in Fig. 6(c) and (d). The Zn(II)---Zn(II) and O---O distances across the cavity are 4.353 and 4.372 Å, respectively. The Zn–O (bridging carboxylate) bond length of 2.0514(17) Å is slightly longer than the 1.999(2) Å observed for Zn–O (terminal carboxylate).

However, the Zn–O (benzoate) bond length at 1.9773(17) Å is comparable to the Zn–O (terminal carboxylate) distance and shorter than the Zn–N bond length, 2.185(2). The distortion from the ideal trigonal bipyramidal around the zinc centers is particularly reflected in the bond angles of N(1)–Zn–O(6), O(3)–Zn(1)–N(1) and O(1)–Zn(1)–O(5) at 171.57(7)°, 81.04(7)°, and 101.00(7)°, respectively. As presented in Table 2, most of the observed metric values for the complex are well within the range of previously reported similar *di*-zinc(II) complexes in the literature [44,77]. However, the observed Zn(II)---Zn(II) distance in the complex is slightly longer than bond distances reported for similar *di*-nuclear zinc(II) complex in the literature [33,45,78–81].

3.6. Carbohydrate/metal complex binding studies

Room temperature molecular interactions between D-glucose, D-mannose, D-xylose, and xylitol with $(\text{NH}_4)_2[\mathbf{1}]_2$ and $((\text{CH}_3)_4\text{N})_2[\mathbf{2}]_2$ at pH 12.5 were studied using UV–Vis and NMR spectrometry, respectively. The percentage distribution of the main equilibrium structures of the substrates under investigation are illustrated in Supporting Information, Scheme S1. In an effort to obtain further insights into the stability of the complexes in solution, UV–Vis and ^1H NMR techniques were applied to study $(\text{NH}_4)_2[\mathbf{1}]_2$ and $[((\text{CH}_3)_4\text{N})_2[\mathbf{2}]]_2$ at pH 12.5, respectively. The UV–Vis absorbance values at wavelengths 680, 704 and 720 nm for $(\text{NH}_4)_2[\mathbf{1}]_2$ vs time for the respective bands were monitored. The plots of the data are shown in the Supporting Information Fig. S4. The data shows no decay of absorption bands for extended period of time under the experimental conditions. Similarly, the stability of $((\text{CH}_3)_4\text{N})_2[\mathbf{2}]_2$ in the experimental conditions were studied by monitoring the ^1H NMR signal intensity of the aromatic protons at 7.31 ppm of the complex. The plot of the signal intensity values vs time showing a straight line with a zero slope is presented in the Supporting Information, Fig. S5. Therefore, the data establishes the stabilities of $(\text{NH}_4)_2[\mathbf{1}]_2$ and $((\text{CH}_3)_4\text{N})_2[\mathbf{2}]_2$ in solution under the operative conditions. Several copper and zinc complexes found in literature with similar structural features to $(\text{NH}_4)_2[\mathbf{1}]_2$ and $((\text{CH}_3)_4\text{N})_2[\mathbf{2}]_2$ have also been shown to be stable over a wide pH range in

aqueous solutions [12,13]. In an effort to assess the stability of the complexes towards displacement of the camb^{3-} ligand from the complexes by any of the substrates under the experimental conditions, several control studies were carried out in aqueous solutions and their UV–Vis spectroscopy, NMR and electrospray ionization time-of-flight mass spectrometry were studied. In a typical experiment, UV–Vis in case of the copper(II) and ^{13}C NMR in case of the zinc(II), a spectrum of a known concentration of a complex in the presence of stoichiometric excess of a substrate in one hand and a spectrum of a free metal ion and a substrate under the same experimental conditions in the other hand were recorded and analyzed. The results of this exercise clearly indicate that the camb^{3-} ligand remains firmly bound to the metal centers in the presence of the substrates under the experimental conditions.

Systematic addition of substoichiometric amounts of the substrates into aqueous solutions of $(\text{NH}_4)_2[\mathbf{1}]_2$ at pH 12.5 resulted in a significant reduction in the absorbance value at $\lambda_{\text{max}} = 704 \text{ nm}$. The reductions in the absorption value continued with each aliquot added until a 1:1 molar ratio of $[\mathbf{1}]^-$ to substrate was reached, at which point further additions of substrate did not bring change to the absorbance value. The decrease in absorbance was accompanied by a blue shift in the spectra with all of the substrates investigated. A typical change in the λ_{max} and absorbance values with the addition of substoichiometric amounts of the substrates into $[\mathbf{1}]^-$ solution is shown in Fig. 7. This representative particular set of data was obtained for the titration of a 2.5 mM $(\text{NH}_4)_2[\mathbf{1}]_2$ solution with aliquots of xylitol at 25 °C and pH of 12.5. Similar data for the rest of the systems is provided in the Supplementary Information, Fig. S6. Treatment of the data using the Rose–Drago method allowed determination of the binding stoichiometry between substrates and metal complex [82,83].

Representative binding isotherms, shown in Fig. 8, are plots of the change in absorbance at 704 nm ($A_{704\text{nm}}$) versus the substrate equivalents added, $\text{SE} = [\text{S}] / [\text{M}_1]$, where $[\text{S}]$ is the moles of the substrate and $[\text{M}_1]$ is the moles of $(\text{NH}_4)_2[\mathbf{1}]_2$. The graphs shows a large change in absorbance with each aliquot of substrate added up to a 1.0 equivalent of substrate, followed by a plateau where additional aliquots of substrate do not significantly affect the absorbance values even up to 5.0 equivalents of substrate to the $(\text{NH}_4)_2[\mathbf{1}]_2$ solution. The binding isotherms provide further spectroscopic evidence of a 1:1 binding interaction between the substrate and the complex.

The method established by Rose & Drago for determining the number of spectroscopic states, and hence the number of species present in solution, has been used with great success in previously reported studies [11,13,33,34,82]. Full mathematical justification of the Rose–Drago method as it relates to the present study is provided in the Supplementary Information section. Connors relates the method to a chemical equilibrium (Eq. (1)) in which the apparent binding constant is given by the equation $\text{p}K_{\text{app}} = \log(K_{\text{app}}^{-1})$, where K_{app}^{-1} is defined in Eq. (2) [82].

$$K_{\text{app}}^{-1} = \frac{[MS]}{([M] \cdot [S])} \quad (2)$$

When applied to a system in which the only absorbing species present in solution are the metal complex [M] and the substrate-bound metal complex [MS], a two-state system can be observed by plotting the change in absorbance (ΔA) at a specific wavelength ($n = 1$) of two different concentrations (j and k) versus the ΔA at a different wavelength ($n = 2$) of concentrations j and k ; $\{(A_{1j} - A_{1k}) \text{ versus } (A_{2j} - A_{2k}), \text{ where } j \neq k\}$. If only one absorbing species is present in solution the graph will contain two overlapping lines with identical slopes. In the case of a two-state system, the graph will contain two different lines with unique slopes both of which intersect at the origin. The method also provides insight into systems in which there are more than two spectroscopic states. When a single substrate binds in different modes to a metal complex in solution then multiple spectroscopic states could exist. When this situation arises it can be clearly seen in the Rose–Drago plot by the deviation from linearity of the data to a non-linear quadratic slope [83].

When the UV–Vis data obtained from the titration of $(\text{NH}_4)_2[\mathbf{1}]_2$ with xylitol in aqueous solution at pH = 12.5 was treated with the Rose–Drago method of analysis, a linear plot was observed, shown in Fig. 9. The presence of two linear functions with different slopes which pass through the origin indicates a 1:1 binding interaction between $[\mathbf{1}]^-$ and xylitol as expected for two-state systems. When the data for the titration of $(\text{NH}_4)_2[\mathbf{1}]_2$ with D-glucose, D-mannose, and D-xylose were subjected to the same treatment, similar results were obtained, Supporting Information Fig. S7. For each graph it can be seen that there are two plots with different linear slopes which both pass through the origin, again suggesting the presence of only one substrate-bound species in solution for D-glucose, D-mannose, and D-xylose. Evidence of multiple equilibria in solution is not present and a 1:1 [substrate]/[complex] ratio persists even when up to a five-fold excess of substrate is added.

The apparent binding constant, pK_{app} , values between $(\text{NH}_4)_2[\mathbf{1}]_2$ and the substrates were calculated and are presented in Table 3. Also present in Table 3, for comparison purposes, are pK_{app} values for other copper(II) complexes found in the literature. Based on the value of the binding constants, the most tightly bound substrate to $(\text{NH}_4)_2[\mathbf{1}]_2$ is D-mannose with a pK_{app} value of 2.71 ± 0.15 . Subsequent to the D-mannose are the pK_{app} values for D-xylose, D-glucose, and xylitol. When the binding constant values for $(\text{NH}_4)_2[\mathbf{1}]_2$ with D-glucose and D-mannose are compared with other *mono*-nuclear copper(II) complexes values reported in the literature (Table 3), they are consistently smaller. The binding constant values reported in literature for *mono*-nuclear copper(II) complexes with D-mannose are 3.44 ± 0.04 for $[\text{Cu}(\text{bpdpo})]^{2+}$, 3.07 ± 0.03 for $[\text{Cu}(\text{paeo})]^{2+}$, 3.68 ± 0.12 for $[\text{Cu}(\text{DIEN})]^{2+}$, 3.38 ± 0.19 for $[\text{Cu}(\text{HEN})]^{2+}$, and 3.05 ± 0.41 for $[\text{Cu}(\text{styDIEN})]^{2+}$ [12,13].

Similarly, the binding constant values reported in literature for *mono*-nuclear copper(II) complexes with D-glucose are 3.66 ± 0.02 [12] for $[\text{Cu}(\text{bpdpo})]^{2+}$, 3.15 ± 0.02 for $[\text{Cu}(\text{paeo})]^{2+}$, 3.73 ± 0.12 for $[\text{Cu}(\text{DIEN})]^{2+}$, 3.61 ± 0.17 for $[\text{Cu}(\text{HEN})]^{2+}$, and 3.37 ± 0.31 for $[\text{Cu}(\text{styDIEN})]^{2+}$ [12,13]. While the complex reported herein is *mono*-anionic in solution, all the previously reported studies on *mono*-nuclear copper(II) complexes involved

are *di*-cationic copper(II) species. Hence, the main cause for the observed lower binding affinity of the complex in this study could well be the charge difference between the complex ions. Direct comparison of the pK_{app} values for D-xylose and xylitol with other *mono*-nuclear copper(II) complexes reported in literature was not possible because of the lack of reported data. However, the binding constant values for $(NH_4)_2[1]_2$ with D-xylose and xylitol are in the range of several *di*-nuclear first row transition metal complexes reported in recent literature. The list of these data is presented in the Supporting Information, Table S1 and Fig. S8.

3.7. ^{13}C NMR spectroscopy and substrate binding with $((CH_3)_4N)_2[2]_2$

To get insight into specific binding to the metal centers using NMR spectroscopy, we have synthesized the new zinc(II) complex of the H_3camb ligand, and investigated its interaction with the substrates. A representative ^{13}C NMR spectra for $((CH_3)_4N)_2[2]_2$, D-glucose, and the reaction mixture of $((CH_3)_4N)_2[2]_2$ and D-glucose are shown in Fig. 10. The best spectrum resolution for the reaction mixture was obtained when using 1:2 molar ratio of $((CH_3)_4N)_2[2]_2$ to D-glucose. The assigned chemical shifts in Fig. 10(a) are typical for ^{13}C NMR spectra of α - and β -D-glucopyranose in aqueous alkaline medium [33]. The most informative feature in the spectra can be easily identified when comparing the spectra of the free D-glucose and $((CH_3)_4N)_2[2]_2$ /D-glucose reaction mixture, Fig. 10(a) and (c) respectively.

Firstly, the intrinsic equilibrium between α and β forms at the anomeric carbon is disrupted, which results in α/β population inversion upon interaction with the metal complex. A proposed metal complex induced mutarotation of D-glucose responsible for the α/β population inversion is shown in Scheme 5.

Similar mutarotation of D-glucose induced by its interaction with MCl_2 ($M = Cu$ and Cr) in methylimidazolium based ionic liquids have been reported in the literature [84]. Secondly, the binding sites of D-glucose to the complex are indicated by a characteristic “coordination-induced shift” (CIS), about 2 ppm up-field chemical shift, by the carbons bearing the coordinating oxygen atoms to the zinc(II) center. The complete resonance shifts for all the substrates are present in Table 4. While the most change in the CIS is observed for the anomeric carbon (C^1) and C^2 , the rest show insignificant change during the substrate metal complex interaction. The NMR study clearly indicates that $((CH_3)_4N)_2[2]_2$ competitively binds to the substrates in solution at pH 12.5. Recent literature has examples of some metal ions interacting with D-glucose and D-xylose [14,16,31,85]. Klüfer and co-workers reported an X-ray single crystal structure and ^{13}C NMR spectral characterization of a D-glucose bound dipalladium(II) complex [8]. Their study revealed that C^1-O to C^4-O are deprotonated and the α -D-glucopyranose tetraanion is bridging in a bis(chelate) fashion between two different $Pd(en)^{2+}$ units.

3.8. DFT calculations

To get insight into the solution coordination chemistry and energetics involved, we performed comparative theoretical studies using DFT on our system. Calculations have been performed on complex ions, 1^- and 2^- , with the substrates in different coordination modes at

the B3LYP and M06-02 functional with 6-31G*/LANL2DZ, cc-pVTZ, and 6-311G** basis set. When $(\text{NH}_4)_2[\mathbf{1}]_2$ and $[(\text{CH}_3)_4\text{N}]_2[\mathbf{2}]_2$ dissociate to their respective monomeric complex ions in solution, as shown in Scheme 5, the metal centers take a pseudo-trigonal bipyramidal geometry. As depicted in Fig. 11, the metal centers of the complexes are coordinated to the oxygen atoms of the three carboxylate arms of camb^{3-} to form the trigonal bases. The optimized geometry parameters for complex $\mathbf{2}^-$, for example, gave the N–Zn–O bond angles at 85.32° , 100.5° , 101.4° , and O–Zn–O bond angles at 114.2° , 121.0° and 122.0° . Similar optimization was carried out for complex $\mathbf{1}^-$ and the metric data is presented in the Supporting Information Table S2. The metric data is consistent with pseudo-trigonal planar geometry around the metal centers. As the result of the geometry around the metal centers, in the presence of the coordinating sugars, two possible products are expected in solution. The first possibility is where the substrate acts as a monodentate ligand and binds through the anomeric oxygen to take the axial position to form the trigonal bipyramidal geometry around the metal center. The second scenario is where a substrate behaves as a bidentate ligand coordinating via $\text{C}^1\text{--O}$ and $\text{C}^2\text{--O}$, as observed in our NMR studies, to produce an octahedral geometry where the oxygen atoms of the substrate take one equatorial and one axial position around the metal center. Due to high flexibility and reorganization ability of the tripodal camb^{3-} ligand around the metal centers, the formation of *penta*- and *hexa*-coordination geometries upon binding to the substrates is expected. Recently, Martin and coworkers reported the flexibility of cobalt complexes with a related tripodal aminotrisphenolate ligand scaffold which easily accommodated the two geometries when reacted with mono- and bidentate exogenous ligands [86].

The *penta*- and *hexa*-coordinated $\mathbf{1}^-$ and $\mathbf{2}^-$ conformation with substrate were optimized, Fig. 11, and their thermodynamic parameters and the binding energy with the substrate were determined. Whereas the Gibbs free energy for the trigonal bipyramidal [$\mathbf{1}$ - α -D-mannose] is only -3.05 kcal/mol, the octahedral complex is -76.42 kcal/mol. The observed difference in energy is partly due to the different reorganization and adaptability of the camb^{3-} ligand around the metal center when binding the substrate. For example, while the arrangement and thus metric values of the camb^{3-} ligand in the *penta*-coordination product reassembles that of $\mathbf{1}^-$, major rearrangement was required to accommodate the bidentate substrate. The Metal–O bond lengths increase by $0.07\text{--}0.31$ Å where metal–N bond length increase by up to 0.29 Å for the trigonal bipyramidal and the octahedral complexes, Supporting Information Table S2. The most significant changes for the octahedral geometry comes from the bond angles, for example, N–Zn–O bond angle change from $\sim 100^\circ$ to 154° , and 170° , and 174° respectively. The results of the DFT study corroborate the experimentally observed ^{13}C -NMR chemical shifts which suggested the C^1 and C^2 substrate coordination mode to the metal centers of the complexes, *vide supra*. The calculated binding energy values in the form of solvated Gibbs free energy for the hexa-coordinated anomeric substrate bound complexes are presented Table 5. A representative optimized structure between $\mathbf{2}^-$ and α -D-glucopyranose and between $\mathbf{2}^-$ and β -D-glucopyranose is shown in Fig. 10. While the optimized structure involving the β -D-glucopyranose shows a H-bonding interaction between the $\text{C}^6\text{--OH}$ and the carboxylate arm of the camb^{3-} ligand at a 1.928 Å distance, such interaction was absent in the case of α -D-glucopyranose, Fig. 12. Instead, a long range interaction at 2.325 Å between $\text{C}^4\text{--OH}$ and $\text{C}^3\text{--OH}$ was observed. Due to steric congestions

around the metal centers, coordination of two different sugar molecules in a monodentate fashion at the same time is highly unlikely. The structural optimization and energy minimizations for the rest possible interactions are presented in Table 5. The study indicates that, generally, the β -anomers of the substrate bind to the metal centers of $\mathbf{1}^-$ and $\mathbf{2}^-$ at lower energy than their α -anomer counterparts.

For example, the binding energy of β -mannose with $\mathbf{2}^-$ is -31.49 kcal/mol while the α -mannose bound at -22.84 kcal/mol with the same complex. With the exception of xylose, the trend observed in the calculated binding energy values are in accordance to the trend observed in the experimentally determined K_{app} values between $\mathbf{2}^-$ and the substrates. Upon close examination of the optimized geometries involving xylose, the M–O(substrate) bond distances were found to be slightly longer than the bond lengths for the other substrates. Furthermore, the C–C bond lengths of the coordinated xylose reassemble to those of free xylose substrate. The effect of having less σ -induction due to the missing C⁶ group on the ring of the pentose is factor to the observed calculated free energy value.

4. Conclusions

The rapidly increasing number of the publications dealing with coordination chemistry of the carbohydrates in the last few decades is associated with the extensive advancement of technological methods such as high field multinuclear NMR, X-ray, CD, FTIR, Raman spectroscopy and molecular modeling calculation programs. Such technological advances has made possible to assign the coordinating hydroxides and conformational changes due to metal coordination of carbohydrates. To take advantage of such prospects, we synthesized and investigated new copper(II) and zinc(II) complexes, $(\text{NH}_4)_2[\mathbf{1}]_2$ and $((\text{CH}_3)_4\text{N})_2[\mathbf{2}]_2$, for potential interaction with biologically important carbohydrates in aqueous alkaline solution. Both $(\text{NH}_4)_2[\mathbf{1}]_2$ and $((\text{CH}_3)_4\text{N})_2[\mathbf{2}]_2$ are very soluble in water and stable in a wide range of pHs. Solution studies using NMR and ESI–MS techniques indicate that the complexes dissolve and dissociate to produce complex anions $\mathbf{1}^-$ and $\mathbf{2}^-$. In aqueous alkaline media, complexes $\mathbf{1}^-$ and $\mathbf{2}^-$ showed chelating ability towards the abundant and biologically relevant sugars, D-glucose, D-mannose, D-xylose, and xylitol. Our investigation into the interaction of the substrates with the complexes revealed that only 1:1 substrate/complex molar ratio is formed and sustained in solution, even in the presence of stoichiometric excess of substrate. The determined binding constant, pK_{app} , values ranging from 2.33 to 2.71 are 1–2 orders of magnitude lower than the values reported for the multinuclear copper(II), cobalt(II) and iron(III) complexes in the literature. Based on the pK_{app} values, the most tightly bound substrate to the complexes is D-mannose. This observation was also supported by the Gibbs free energy results obtained from the Density Functional Theory calculations on the system. Although direct structural evidence, from single crystal X-ray structure, for these complexes is presently unavailable, the NMR study on the zinc(II) complex suggested that binding of the carbohydrates with these types of complexes most likely occurs through the hydroxyl groups attached to the anomeric carbon, C¹, and C². The results of this investigation will positively contribute to the field of carbohydrate recognition in aqueous media. Additionally, the present study provides important structural and functional information relevant to various sugar-metabolizing metalloenzymes and catalysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

bpdpo	<i>N,N</i> -1,3-bis[(pyridin-2-ylmethyl)amino]propan-2-ol
CIS	Coordination Induced Shift
DFT	Density Functional Theory
DIEN	Diethylenetriamine
ECP	Effective Core Potentials
ESI-MS	Electron-Spray Ionization Mass Spectroscopy
H₂cbal	<i>N</i> -[2-carboxybenzomethyl]- β -alanine
H₃camb	<i>N</i> -[2-carboxybenzomethyl]- <i>N</i> -[carboxymethyl]- β -alanine
H₅ccdP	<i>N,N</i> -Bis[2-carboxybenzomethyl]- <i>N,N</i> -Bis[carboxymethyl]-1,3-diaminopropan-2-ol
HEN	<i>N</i> -(2-hydroxyethyl)ethylenediamine
hpnbpda	<i>N,N</i> -bis(2-pyridylmethyl)-2-hydroxy-1,3-propanediamine- <i>N,N</i> -diacetic acid
Paeo	<i>N</i> -2-[(pyridin-2-ylmethyl)amino]ethanol
PCM	Polarizable Continuum Model
SPE	Single Point Energy
styDIEN	4-(<i>N</i> -vinylbenzyl)-diethylenetriamine
tedc	<i>N,N,N,N</i> -tetrakis(sodium carboxylatemetethyl)-2,6-diami nocresolate

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Appendix A. Supplementary data

The X-ray crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as CCDC 1048114 and CCDC 1048115 for $(\text{NH}_4)_2[\mathbf{1}]_2$ and $((\text{CH}_3)_4\text{N})_2[\mathbf{2}]_2$, respectively. Copies of the data can be obtained free of charge from the CCDC (12 Union Road, Cambridge, respectively.ge CB2 1EZ, UK; tel: (+44) 1223-336-408; fax: (+44) 1223-336-003; e-mail: deposit@ccdc.cam.ac.uk; website www.ccdc.cam.ac.uk). UV–Vis, IR data, ESI–MS data, figures of the substrate/complex interactions, binding isotherms, DFT calculation results and optimized structures, partial views of molecular structures of $(\text{NH}_4)_2[\mathbf{1}]_2$ and $((\text{CH}_3)_4\text{N})_2[\mathbf{2}]_2$, and Rose–Drago graphs are also included. This material is available free of charge via the Internet at doi: <http://dx.doi.org/10.1016/j.jinorgbio.2015.04.012>.

Fig. 1.
The active site of xylose/glucose isomerase, XGI.

Fig. 2. Comparison of the previously reported di-nucleating ligand, H₅ccdp (a), with the new mono-nucleating ligand, H₃camb (b).

Fig. 3.

Negative ion mode ESI-MS spectra of 1 mg/mL aqueous solution of $(\text{NH}_4)_2[\mathbf{1}]_2$ (a) and $((\text{CH}_3)_4\text{N})_2[\mathbf{2}]_2$ (b); with the expanded regions to show the isotope distribution patterns (showing in red is a simulated pattern) for $m/z = 341$ $[\text{CuC}_{13}\text{H}_{12}\text{NO}_6]^-$ (c); and $m/z = 342$ $[\text{ZnC}_{13}\text{H}_{12}\text{NO}_6]^-$ (d). Simulated isotope distribution patterns generated using Molecular Weight Calculator (Matthew Monroe, PNNL, Richland WA, U.S.A.) for the fragments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 4. ORTEP drawing (50% probability) with atomic numbering scheme of the structure of Na₂[1]₂ including symmetry generated atoms. Hydrogen atoms, counter cations and any water molecules are omitted for clarity.

Fig. 5. ORTEP drawing (50% probability) with atomic numbering scheme of the structure of $((\text{CH}_3)_4\text{N})_2[\mathbf{2}]_2$ including symmetry generated atoms. Hydrogen atoms, counter cations and water of crystallizations are omitted for clarity.

Fig. 6.

(a) Front view of the binuclear Cu(II) core displaying the carboxylates (O(5) and O(5A)) in $\mu_2\text{:}\eta^1\eta^1$ (O,O) coordination mode; (b) side view of the core of $\text{Na}_2[\mathbf{1}]_2$; (c) front view of the binuclear Zn(II) core displaying the carboxylates (O(5), O(5A) and O(6), O(6A)) in the *syn,anti*- $\mu_2\text{:}\eta^1\eta^1$ coordination mode; (d) side view of the core of $((\text{CH}_3)_4\text{N})_2[\mathbf{2}]_2$.

Fig. 7.

Selected UV–Vis spectra observed during titration of $(\text{NH}_4)_2[\mathbf{1}]_2$ (1.25 mM) with xylitol at 25 °C in unbuffered, aqueous solution at pH = 12.5; the concentration of xylitol was varied from 0.0 to 12.5 mM.

Fig. 8. Binding isotherm plot observed during the titration of $(\text{NH}_4)_2[1]_2$ (1.25 mM) with D-glucose (▲), D-mannose (■), D-xylose (●), and xylitol (◆) (0.00–12.50 mM) at 704 nm. Data collected at pH = 12.5 at 25 °C.

Fig. 9.

Plot of differences in absorbance $A_n = (A_{n,j} - A_{n,k})$ over $A_{704\text{nm}} = (A_{704\text{nm},j} - A_{704\text{nm},k})$ from titration of 1^- with xylitol where $n = 680$ (●) and 720 nm (▲) at pH = 12.5, 25 °C.

Fig. 10.

^{13}C NMR of D-glucose (a), $((\text{CH}_3)_4\text{N})_2[\mathbf{2}]_2$ (b), and a 1:2 molar ratio of $((\text{CH}_3)_4\text{N})_2[\mathbf{2}]_2$ to D-glucose (c) in D_2O , pH = 12.5. Chemical shifts in the 100–65 ppm region expanded for clarity (top).

Fig. 11. DFT optimized electrostatic potential maps showing the pseudo-trigonal planar geometries and the open coordination sites on the metal centers for **1**⁻ (a) and **2**⁻ (b).

Fig. 12.

DFT optimized structures for α -D-glucopyranose (a) and β -D-glucopyranose (b) bound to 2^- through the C₁ and C₂ hydroxyl groups of the substrate. Hydrogen bonding interactions are shown as yellow dashed lines and the zinc(II) center in green color. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Scheme 1.

Schematic description of the synthesis procedure for H₃camb.

Scheme 2.

Proposed equilibrium showing the protonation/deprotonation steps of the ligand.

Scheme 3.

Schematic description of the synthesis route for (NH₄)₂[1]₂ and ((CH₃)₄N)₂[2]₂.

Scheme 4.

Schematic description illustrating the dissociation of $(\text{NH}_4)_2[\mathbf{1}]_2$ (a) and $((\text{CH}_3)_4\text{N})_2[\mathbf{2}]_2$ (b) in aqueous solution.

Scheme 5.

Proposed metal complex catalyzed mutarotation leading to the α/β -anomer interconversion.

Table 1Crystal data and structure refinement for Na₂[**1**]₂(H₂O)₅ and ((CH₃)₄N)₂[**2**]₂.^a

	Na ₂ [1] ₂ (H ₂ O) ₅	[((CH ₃) ₄ N) ₂ [2] ₂]
Empirical formula	C ₂₆ H ₂₇ Cu ₂ N ₂ Na ₂ O ₁₇	C ₃₄ H ₄₈ N ₄ O ₁₂ Zn ₂
Formula weight	812.55	835.5
Crystal system	Monoclinic	Monoclinic
Space group	P 2 ₁ /n	P 2 ₁ /c
<i>a</i> , Å	10.748(3)	13.237(3)
<i>b</i> , Å	6.945(2)	9.4047(19)
<i>c</i> , Å	21.355(6)	16.799(6)
<i>α</i> , deg	90.00	90.00
<i>β</i> , deg	103.695(4)	121.10(2)
<i>γ</i> , deg	90.00	90.00
Vol., Å ³	1548.7(8)	1790.7
<i>Z</i>	2	2
<i>D</i> _(cal) , g/cm ³	1.742	1.550
<i>μ</i> (Mo–K _α , mm ^{−1})	1.485	1.408
<i>R</i> (000)	826	872
2θ range for data collection (°)	3.92 to 53.00	5.18 to 55.00
Index ranges	−13 <i>h</i> 13, −8 <i>k</i> 7, −26 <i>l</i> 26	−17 <i>h</i> 17, −10 <i>k</i> 12, −21 <i>l</i> 21
Reflections collected	9385	12,494
Independent reflections	3186[R(int) = 0.0475]	4083[R(int) = 0.0371]
Max. and min. transmission	1.000, 0.791	1.000, 0.853
Data//parameters/restraints	3186/229/1	4083/235/0
<i>wR</i> (<i>F</i> ² all data)	<i>R</i> 1 = 0.0529, <i>wR</i> 2 = 0.1336	<i>R</i> 1 = 0.0417, <i>wR</i> 2 = 0.1013
<i>R</i> (<i>F</i> obsd data) [<i>I</i> > 2σ(<i>I</i>)]	<i>R</i> 1 = 0.0502, <i>wR</i> 2 = 0.1308	<i>R</i> 1 = 0.0394, <i>wR</i> 2 = 0.0990
Goodness-of-fit on <i>F</i> ²	1.031	1.031
Largest diff. peak and hole, e Å ^{−3}	1.643/−0.839	0.813/−0.771

$$^a wR2 = \left\{ \sum [w(F_o^2 - F_c^2)^2] / \sum [w(F_o^2)^2] \right\}^{1/2}, R1 = \Sigma ||F_o| - |F_c|| / \Sigma |F_o|.$$

Table 2Selected bond lengths and angles in Na₂[**1**]₂ and ((CH₃)₄N)₂[**2**]₂.

Na ₂ [1] ₂		[(CH ₃) ₄ N] ₂ [2] ₂	
Bond lengths [Å]			
Cu(1)–O(5)	2.222(2)	Zn(1)–O(1)	1.9773(17)
Cu(1)–O(3)	1.942(2)	Zn(1)–O(3)	1.9993(18)
Cu(1)–O(1)	1.956(3)	Zn(1)–O(5)	2.0514(17)
Cu(1)–N(1)	2.022(3)	Zn(1)–N(1)	2.1849(18)
Cu(1)–O(5A)	1.960(2)	Zn(1)–O(6)	2.0598(18)
Cu(1)–Cu(1A)	3.188(1)	Zn(1)–Zn(1A)	4.353(1)
Bond angles [deg]			
O(5)–Cu(1)–O(3)	100.50(10)	O(1)–Zn(1)–O(3)	128.24(7)
O(5)–Cu(1)–O(1)	93.65(10)	O(1)–Zn(1)–O(5)	101.00(7)
O(5)–Cu(1)–N(1)	93.92(10)	O(1)–Zn(1)–N(1)	96.84(7)
O(5)–Cu(1)–O(5A)	80.85(10)	O(1)–Zn(1)–O(6)	91.48(7)
O(3)–Cu(1)–O(1)	165.84(11)	O(3)–Zn(1)–O(5)	130.41(7)
O(3)–Cu(1)–N(1)	85.83(11)	O(3)–Zn(1)–N(1)	81.04(7)
O(3A)–Cu(1A)–O(5)	91.18(10)	O(3)–Zn(1)–O(6)	92.80(7)
O(1)–Cu(1)–N(1)	92.89(11)	O(5)–Zn(1)–N(1)	88.48(7)
O(1)–Cu(1)–O(5)	91.42(10)	O(5)–Zn(1)–O(6)	91.21(7)
Cu(1)–O(5)–Cu(1A)	99.15(10)	N(1)–Zn(1)–O(6)	171.57(7)

Table 3

Apparent binding constants ($\text{p}k_{\text{app}} = \log(k_{\text{app}}^{-1})$) between the substrate and the complexes.

Complex	D-mannose	D-glucose	D-xylose	Xylitol	Ref.
$1^-, [\text{Cu}(\text{camb})]^-$	2.71 ± 0.15	2.58 ± 0.07	2.62 ± 0.05	2.33 ± 0.12	This work, ^a
$[\text{Cu}_2(\text{cdp})(\mu\text{-CO}_3)]^{3-}$	2.62 ± 0.25	1.94 ± 0.43	2.25 ± 0.11	2.43 ± 0.08	[34]
$[\text{Cu}(\text{bpdpo})]^{2+}$	3.44 ± 0.04	3.66 ± 0.02	–	–	[12]
$[\text{Cu}(\text{paeo})]^{2+}$	3.07 ± 0.03	3.15 ± 0.02	–	–	[12]
$[\text{Cu}(\text{DIEN})]^{2+}$	3.68 ± 0.12	3.73 ± 0.12	–	–	[13]
$[\text{Cu}(\text{HEN})]^{2+}$	3.38 ± 0.19	3.61 ± 0.17	–	–	[13]
$[\text{Cu}(\text{styDIEN})]^{2+}$	3.05 ± 0.41	3.37 ± 0.31	–	–	[13]

^a 10^{-2} at pH = 12.5.

¹³C NMR spectral data for D-glucose, D-mannose, D-xylose, and xylitol in the free state at pH 12.5 and in the mononuclear zinc(II) complex, **2**⁻.**Table 4**

	C ₁	C ₂	C ₃	C ₄	C ₅	C ₆
Free α-D-glucopyranose	92.04	72.68	75.67	71.32	69.52	60.51
α-D-glucopyranose and 2 ⁻	92.84	72.90	75.65	71.07	69.76	60.61
CIS	-0.81	-0.22	0.02	0.25	-0.24	-0.10
Free β-D-glucopyranose	95.90	74.09	75.83	71.41	69.56	60.67
β-D-glucopyranose and 2 ⁻	97.35	74.94	75.87	71.94	69.85	60.84
CIS	-1.45	-0.85	-0.04	-0.53	-0.29	-0.18
Free α-D-mannopyranose	95.04	71.31	70.32	66.92	71.96	60.96
α-D-mannopyranose and 2 ⁻	96.67	71.47	70.62	67.17	72.41	61.08
CIS	-1.63	-0.16	-0.30	-0.25	-0.45	-0.12
Free β-D-mannopyranose	94.21	71.49	73.05	66.62	75.98	-
β-D-mannopyranose and 2 ⁻	95.50	72.30	73.33	66.80	75.84	-
CIS	-1.29	-0.81	-0.28	-0.18	0.14	-
Free α-D-xylopyranose	92.234	71.439	74.05	69.18	60.83	-
α-D-xylopyranose and 2 ⁻	93.22	72.04	74.97	69.5	60.60	-
CIS	-0.99	-0.60	-0.92	-0.32	0.23	-
Free β-D-xylopyranose	96.73	72.76	75.75	69.34	65.08	-
β-D-xylopyranose and 2 ⁻	98.46	72.97	75.92	69.54	64.88	-
CIS	-1.73	-0.21	-0.17	-0.20	0.20	-
Free xylitol	71.87	70.73	62.56	70.73	71.87	-
Xylitol and 2 ⁻	71.93	70.70	62.70	70.70	71.93	-
CIS	-0.06	0.03	-0.14	0.03	-0.06	-

Table 5

Difference in energies between the α - and β -substrate bound complexes of **1**[−] and **2**[−].

Substrate	<i>E</i> (kcal/mol)	
	α -anomer	β -anomer
1 — mannose	−22.84	−38.08
1 — xylose	−24.84	−18.50
1 — glucose	−31.33	−32.76
1 — xylitol	−34.37	
2 — mannose	−16.30	−31.49
2 — xylose	−19.49	−18.95
2 — glucose	−25.20	−27.69
2 — xylitol	−25.19	