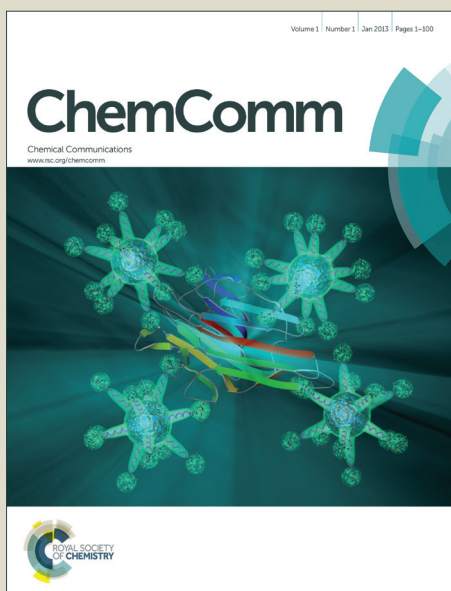This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

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Journal Name

ARTICLE TYPE

Bismuth(III) benzohydroxamates: powerful anti-bacterial activity against *Helicobacter pylori* and hydrolysis to a unique Bi$_{34}$ oxido-cluster [Bi$_{34}$O$_{22}$(BHA)$_{22}$(*H*-BHA)$_{14}$(DMSO)$_6$]

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Reaction of BiPh$_3$ or Bi(O^{t}Bu)$_3$ with benzohydroxamic acid (*H$_2$*-BHA) results in formation of novel mono- and di-anionic hydroxamato complexes; [Bi$_2$(BHA)$_3$]$_\infty$ 1, [Bi(*H*-BHA)$_3$] 2,
10 **[Bi(BHA)(*H*-BHA)] 3, all of which display nM activity against *Helicobacter pylori*. Subsequent dissolution of [Bi$_2$(BHA)$_3$]$_\infty$ in DMSO/toluene results in hydrolysis to the first structurally authenticated {Bi$_{34}$} oxido-cluster [Bi$_{34}$O$_{22}$(BHA)$_{22}$(*H*-BHA)$_{14}$ (DMSO)$_6$]**

15 In conjunction with other antibiotics bismuth subsalicylate (BSS) and colloidal bismuth subcitrate (CBS) are taken therapeutically in the treatment and eradication of the ulcer and cancer causing bacterium *Helicobacter pylori*.[1,2] Recent studies have begun to uncover mechanisms by which bismuth is toxic to the bacterium,
20 including disrupting the enzymes urease and alcohol dehydrogenase, and interfering with Fe^{3+} regulating proteins.[3] While having a strong affinity for S-based ligands, such as glutathione and metallothionein,[4] Bi^{3+} is also known to compete with ferric ions *in vivo* with predominantly N- and O-binding
25 biomolecules.[5] In human serum this involves strong complexation with transferrin and lactoferrin, and it has been speculated that this is an important factor in the unusually low toxicity of bismuth in humans.[6] In *H. pylori,* Fe^{3+} limitation mimics the antimicrobial effects of bismuth, though the mode of action
30 appears unrelated, while increasing Fe^{3+} concentration acts to protect the bacterium against low concentrations of Bi^{3+} by outcompeting the heavy metal for transport proteins.[7]

To survive many bacteria rely on the ability to sequester and transport ferric ions by use of strong metal chelators, known as
35 siderophores.[8] These are predominantly polydentate O-binding molecules with carboxylate, catecholate and hydroxamate functional groups. Evidence suggests, however, that *H. pylori* does not generate siderophores but obtains iron from host transferrin and lactoferrin,[9] and indeed *H. pylori* infection is
40 associated with iron deficiency anaemia.[10]

While the chemistry of bismuth carboxylates has been relatively well studied,[11,12] there have been no reports on the synthesis and structural chemistry of bismuth(III) complexes derived from
45 hydroxamic acids. Carboxylates are known to form poorly soluble polymeric species, and can be hydrolytically unstable. As a result polynuclear oxido-clusters can form, as occurs in the formation of bismuth *sub*salicylate, [Bi$_{38}$O$_{44}$(HSal)$_{26}$(Me$_2$C=O)$_{16}$(H$_2$O)$_2$],[13] and bismuth *sub*citrate,
50 (NH$_4$)$_6$[Bi$_6$O$_4$OH(cit)$_3$(H$_2$O)$_3$](H$_2$O)$_2$,[14] (NH$_4$)$_{12}$[Bi$_{12}$O$_8$(cit)$_8$](H$_2$O)$_{10}$.[15]

Antibiotic resistance, particularly to clarithromycin and metronidazole, is limiting the effectiveness of non-bismuth
55 therapies for the treatment of *H. pylori*.[16] Hydroxamic acids, particularly acetohydroxamic acid, are also known as urease inhibitors and so have been proposed as drug candidates for the treatment of *H. pylori*, though no compounds are yet clinically available.[17] Taking this in conjunction with our knowledge of the
60 close relationship of Bi^{3+} and Fe^{3+} in the lifecycle and killing of *H. pylori*, it is clear that bismuth(III) hydroxamates deserve closer examination as possible combination drugs for the treatment and eradication of *H. pylori*. In fact the role of hydroxamates as siderophores means they could have much
65 broader application as novel anti-bacterial compounds.

In this paper we report the synthesis of the first bismuth(III) hydroxamate complexes: [Bi$_2$(BHA)$_3$] 1, [Bi(*H*-BHA)$_3$] 2, [Bi(BHA)(*H*-BHA)] 3, as derived from benzohydroxamic acid (*H$_2$*-BHA), and demonstrate its excellent *in vitro* activity against
70 *H. pylori*. We also show that complex 1 undergoes slow hydrolysis in DMSO solution to give crystals of the unique Bi$_{34}$ oxido-cluster [Bi$_{34}$O$_{22}$(BHA)$_{22}$(*H*-BHA)$_{14}$(DMSO)$_6$] 4.
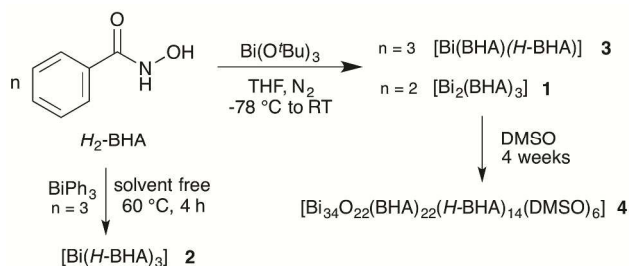
Scheme 1 Synthesis of bismuth hydroxamate complexes [Bi$_2$(BHA)$_3$]$_\infty$ 1, [Bi(*H*-BHA)$_3$] 2, [Bi(BHA)(*H*-BHA)] 3, and the oxido-cluster
75 [Bi$_{34}$O$_{22}$(BHA)$_{22}$(*H*-BHA)$_{14}$DMSO$_6$] 4.

The 2:1 stoichiometric reaction of H$_2$-BHA with Bi(O^{t}Bu)$_3$ in dry THF results in the clean generation of [Bi$_2$(BHA)$_3$] 1 in 66 % yield. The low temperature addition of Bi(O^{t}Bu)$_3$ prevents a rapid reduction of Bi(III) to Bi(0) as indicated by the appearance of a
80 grey solid. Complex 1, comprises only hydroxamate di-anions.

The solid was found to be only soluble in the highly polar solvents DMSO and DMF.

In an attempt to obtain crystals of the complex the solid was dissolved in DMSO/toluene. However, rather than crystals of **1**
5 the pale yellow crystals which formed are of a unique bismuth oxido-cluster; [$Bi_{34}O_{22}(BHA)_{22}(H\text{-}BHA)_{14}DMSO_6$] **4**. Formed slowly through hydrolysis this oxido-cluster contains both the *H*-BHA mono-anion, PhC(=O)-N(H)-O⁻, as well as the expected BHA di-anion, PhC(-O⁻)=N-O⁻.

10 Interestingly, the 3:1 stoichiometric reaction of H_2-BHA with Bi(O^{t}Bu)$_3$ in dry THF resulted only in the isolation of the mixed anion species; [Bi(BHA)(*H*-BHA)] **3** in 75 % yield (based on Bi). To obtain the fully substituted product [Bi(*H*-BHA)$_3$], the weaker base BiPh$_3$ was necessary. This allows for only a single
15 deprotonation to give the *H*-BHA mono-anion. Complex **3** was formed by solvent-mediated reactions, in toluene (24 h, yield 58 %) or ethanol (24 h, yield 55 %) at reflux, but in higher yield under solvent free conditions (60 °C, 4 h, yield 65 %).

Full synthetic details and compound characterization for **1 - 4** are
20 provided in the Supporting Information. NMR spectra were obtained on **1 - 4** in dry d$_6$-DMSO. In the ^{1}H NMR spectrum of [Bi$_2$(BHA)$_3$] **1**, the resonances assigned to the N*H* and O*H* in benzohydroxamic acid, at 11.19 and 9.05 ppm respectively are absent (Fig S5). While the *o*-H and *p*-H protons shift to a lower
25 frequency on complexation with Bi (7.43 to 7.33 and 7.50 to 7.33 ppm), the m-*H* protons shift to higher frequency (7.76 to 7.70 ppm). Suggesting electronic and structural fluxionality in the *H*-BHA ligand, the spectrum of **2** (Fig S8) shows resonances for both N*H* (0.5 H) and O*H* (0.5 H) at 11.14 and 9.02 ppm
30 respectively. In contrast, complex **3** shows only a broad N*H* resonance at the higher frequency value of 12.48 ppm (Fig S11). The spectrum of the oxido-cluster **4** has the most acidic N*H* with the proton resonating at 13.66 ppm (Figure S13).

The sensitivity of complex **1** to hydrolysis is apparent in the ^{1}H
35 NMR spectrum collected in d$_6$-DMSO, wherein the relative integral value for the N*H* signal at 13.60 ppm, when it becomes apparent, increases slowly over time. A similar behaviour of hydrolysis was recently reported for various bismuth oxido-nitrates and carboxylates, mainly on the basis of electrospray
40 mass spectrometry and isolation of crystalline intermediates.[18-20] So far, single crystal X-ray structure analyses exist for the most prominent cluster nuclearities {Bi$_6$}, {Bi$_{22}$} and {Bi$_{38}$}. Cluster **4** represents a novel and unique intermediate of {Bi$_{34}$} within this series.

45 Its molecular structure (Figure 1) is composed of 34 bismuth atoms, 22 O^{2-} anions, 22 di-anionic and 14 mono-anionic hydroxamate ligands, as well as 6 coordinated DMSO molecules. To the best of our knowledge this is the first structurally characterised Bi$_{34}$-oxido cluster to date, with the heterometallic
50 Bi/Na cluster [Bi$_{33}$NaO$_{38}$(OSiMe$_3$)$_{24}$] the closest comparison with regard to nuclearity.[21]

The cluster has a central Bi$_{24}$ unit [Bi(1)- Bi(12)], held together by 16 μ$_4$-oxido and 6 μ$_3$-oxido ligands, which can be easily distinguished with a Bi-O bond range of 2.098(10)- 2.747(11)Å.
55 The Bi$_{24}$O$_{22}$ core contains six edge sharing polyhedral units, of which two in the center, are constructed of [Bi$_6$O$_7$]$^{4+}$ units (Figure 2 red polyhedro) while the remaining four consist of [Bi$_6$O$_6$]$^{6+}$
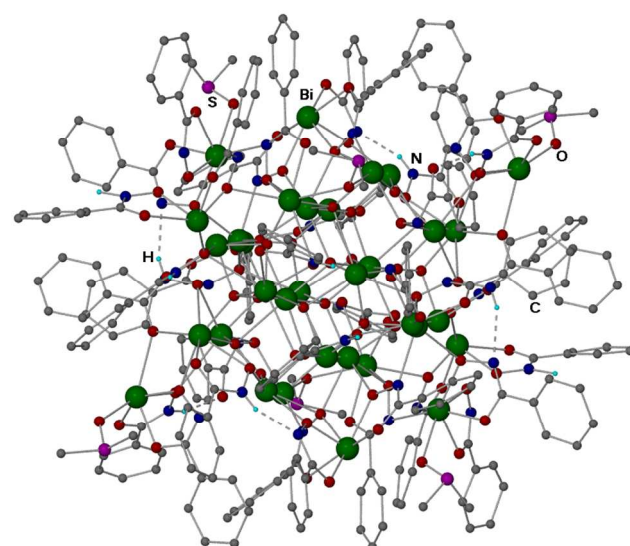

Figure 1 Molecular structure of [Bi$_{34}$O$_{22}$(BHA)$_{22}$(*H*-BHA)$_{14}$DMSO$_6$] **4**.
60 Hydrogen atoms (except N-H) have been omitted for clarity. Selected bond lengths (Å) and angles (°) listed in the SI. Colour scheme: Bi, green; O, red; N, blue; S, purple; C, grey.

units (Figure 2, blue polyhedro), all connected through their Bi-Bi edges. The two different {Bi$_6$} units in **4** can be considered to
65 be oxygen deficient and unique in its composition when compared to the more commonly seen hexameric unit [Bi$_6$O$_8$]$^{2+}$ present in a wide variety of previously reported molecular Bi-oxido clusters.[22] Making up the final Bi$_{34}$ composition, the core of **4** is then encapsulated by a further 10 Bi atoms, each of which
70 is bound to the Bi$_{24}$-oxido core by either mono- or di-anionic HBA ligands, or a mixture of both.
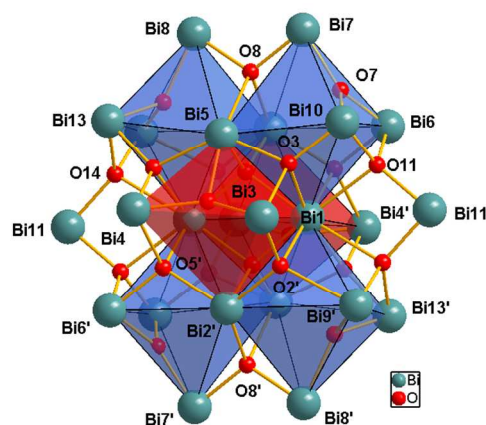

Figure 2 View of the [Bi$_{24}$O$_{22}$] central core in **2** with octahedral [Bi$_6$O$_7$]$^{4+}$ and [Bi$_6$O$_6$]$^{5+}$ units highlighted in red and blue respectively.

90 The Bi$_{24}$-oxido core of **4** can be envisaged as a Bi$_{22}$-oxido core, having three bismuth containing layers, with two additionally attached bismuth atoms to the central layer. Both {Bi$_{22}$} and {Bi$_{24}$} each represent a cut-out of high-nuclearity {Bi$_{38}$} oxido clusters, *eg* [Bi$_{38}$O$_{44}$(HSal)$_{26}$(Me$_2$C=O)$_{16}$(H$_2$O)$_2$][13] and
95 [Bi$_{38}$O$_{45}$(HSal)$_{22}$(OH)$_2$(DMSO)$_{16.5}$].[23] It might be assumed that clusters with such a {Bi$_{24}$}-oxido core are representative of the hydrolysis step directly following the prominent {Bi$_{22}$} clusters

(Figs S1-3).[21] Additionally, two bismuth atoms (Bi14 and Bi17 in Fig S4) could be regarded as being on their way to attach to the cluster core upon additional hydrolysis. However, not all of the peripheral 10 bismuth atoms of **4** are found in positions close to those in the highly condensed $\{Bi_{38}O_{45}\}$ clusters and it is assumed that dynamic coordination behaviour is crucial to further cluster growth processes. Most probably, coordination of single bismuth atoms (rather than larger moieties) prior to hydrolysis/condensation at the periphery of the cluster is an important step upon cluster growth, demonstrated here for the first time by a snapshot. It is assumed that ligands bound to the surface will control the kinetics of cluster growth, as it is reported for example for the growth of nanoparticles using surfactants.[24]

The bactericidal activity of benzohydroxamic acid and complexes **1 - 4** was assessed against three laboratory strains of *H. pylori*: B128, 251 and 26695, using compound concentrations ranging from 25 to 0.024 μg mL^{-1}. The Minimum Inhibitory Concentration (MIC) of each was determined by the Agar Diffusion method. The benzohydroxamic acid showed no activity against any of the *H. pylori* strains > 25 μg mL^{-1}. In contrast, as shown in Table 1, the bismuth complexes display excellent anti-bacterial activity against all three strains. Interestingly, any slow hydrolysis that takes the di-anion BHA (**1**) to the mono-anion *H*-BHA (**2**, **3**) within a complex appears to have little or no impact on the bacterical properties.

Table 1 Anti-bacterial activities of bismuth(III) benzohydroxamates **1 - 4** against *H. pylori* in ug mL^{-1}(μM).

Compound	*H. pylori* strain		
	26695	B128	251
Benzohydroxamic acid	> 25	> 25	> 25
[Bi$_2$(BHA)$_3$] **1**	0.19 (0.23)	0.19 (0.23)	1.56 (1.89)
[Bi(*H*-BHA)$_3$] **2**	0.09 (0.14)	0.05 (0.08)	0.78 (1.26)
[Bi(BHA)(*H*-BHA)] **3**	0.19 (0.39)	0.09 (0.19)	1.56 (3.24)
[Bi$_{34}$O$_{22}$(BHA)$_{22}$(*H*-BHA)$_{14}$(DMSO)$_6$] **4**	3.12 (0.24)	6.25 (0.48)	3.12 (0.24)

These are remarkably low MIC values, with the only close comparison being with some amino-arenesulfonates which showed activities as low as MIC 0.05 μg mL^{-1} (0.06 μM), eg [Bi(O$_3$S-(*o*-AB))$_3$] from *ortho*-aminobenzenesulfonic acid ((o-AB)SO$_3$H).[25] As expected, the cluster is not so active, yet MIC values are all significantly lower than for the clinically available compounds: BSS and CBS, 12.5 μg mL^{-1}; and RBC 8 μg mL^{-1}.[26] The bismuth benxohydroxamates are by far more effective than other Bi(III) complexes we have studied. For example, mono-nuclear carboxylates[27] and thiobenzoates, [BiL$_3$], generally gave MIC values of 6.25 μg mL^{-1},[28] and thioxoketones 3.12 μg mL^{-1}.[29] These results indicate that both the structural chemistry and the anti-bacterial activity of bismuth(III) hydroxamates requires further investigation. As such, we are now currently expanding the breadth of complexes being studied.

Notes and references

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Graphical Abstract

The first mono- and di-anionic bismuth(III) benzohydroxamates; [Bi$_2$(HBA)$_3$], [Bi(H-BHA)$_3$] and [Bi(HBA)(H-HBA)], display very low inhibitory concentrations against *Helicobacter pylori* (0.08 – 3.24 μM), as does the unique mixed-anion {Bi$_{34}$} oxido-cluster, [Bi$_{34}$O$_{22}$(BHA)$_{22}$(H-BHA)$_{14}$(DMSO)$_6$] (MIC 0.48 μM), derived from hydrolysis of [Bi$_2$(HBA)$_3$] in DMSO.

[Bi$_2$(HBA)$_3$]

1

Ph

Ph

Ph

Activity against *H. pylori*

MIC μg mL^{-1}

26695   B128   251

H. pylori strain

[Bi$_{34}$O$_{22}$(BHA)$_{22}$(H-BHA)$_{14}$(DMSO)$_6$]

4

DMSO
slow hydrolysis