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CITATION

Keoghan, Donal M.; Fagan, Lauren E.; Passos, Thayse Marques; Müller-Bunz, Helge; Quilty, Bríd; Griffith, Darren M. (2016): Synthesis of polymeric bismuth chlorido hydroxamato complexes; X-ray crystal structure and antibacterial activity of a novel Bi(III) salicylhydroxamato complex. figshare. Journal contribution. <https://hdl.handle.net/10779/rcsi.10991525.v1>

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Synthesis of polymeric bismuth chlorido hydroxamato complexes; X-ray crystal structure and antibacterial activity of a novel Bi(III) salicylhydroxamato complex

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Abstract

Reaction of salicylhydroxamic acid (Sha) and 2-aminophenyl hydroxamic acid (2-NH₂-pha) with BiCl₃ affords the corresponding novel polymeric bismuth chlorido hydroxamato complexes; [BiCl₂(μ-Sha_{1H})]_∞ and [BiCl₃(2-NH₃-Pha_{1H})]_∞ respectively. The X-ray crystal structure of the THF-solvated polymeric bismuth chlorido salicylhydroxamato complex, [BiCl₂(μ-Sha_{1H})(THF)]_∞ was solved, confirming the polymeric structure of this class of compounds and the (O,μ-O')-bidentate bridging coordination mode of the hydroxamato ligand. The antibacterial activity of the THF-free polymeric bismuth chlorido salicylhydroxamato complex, [BiCl₂(μ-Sha_{1H})]_∞, was investigated against a broad panel of bacteria, further highlighting the antibacterial properties of Bi-based compounds against Gram-positive and Gram-negative pathogenic and environmental strains of bacteria.

Keywords: Bismuth; hydroxamic acid; polymer, antibacterial.

1. Introduction

Hydroxamic acids, of general formula $RCONHOH$, represent a very important family of bioligands, Figure 1(a).[1] One of the first roles attributed to hydroxamic acids is their use as siderophores, a class of low molecular weight iron (Fe)-sequestering agents involved in microbial iron transport. Desferal for example, a natural trihydroxamic acid siderophore, which forms an Fe(III) trishydroxamato complex, Ferrioxamine-B, with $\log \beta \sim 30$, is clinically used to treat Fe overload, Figure 1(b).[1]

Hydroxamic acids are therefore effective metal chelators and as ligands, classically coordinate metal centres in an (O,O') bidentate chelating fashion, coordinating through the carbonyl oxygen atom and the deprotonated hydroxyl group, Figure 1(c), to form very stable five-membered hydroxamato chelates.[2, 3] Other binding modes are possible, including monodentate binding through the deprotonated nitrogen or oxygen atoms and bidentate binding of two metal ions has also been observed in an (O, μ -O') chelating mode, Figure 1(d).[2-4] Significantly the complexation behaviour of hydroxamic acids can be enriched by the incorporation of secondary coordinating groups, such as a hydroxy or an amino group, at neighbouring sites in the molecule.[2, 3] In turn hydroxamic acids have been employed to develop a diverse range of interesting complexes including metallacrowns, coordination polymers and cluster complexes.[2, 3]

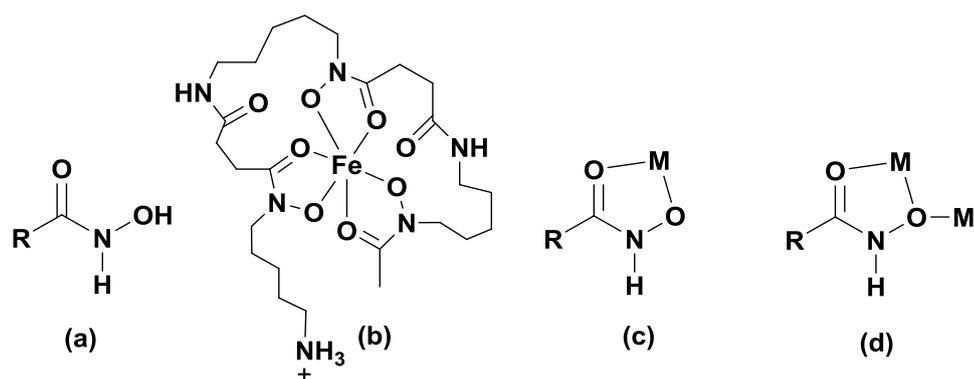


Figure 1. (a) General structure for hydroxamic acids, (b) structure of Ferrioxamine-B, (c) representation of hydroxamato (O,O') chelating mode and (d) representation of hydroxamato (O, μ -O') chelating mode.

More recently the versatile coordination chemistry of hydroxamic acids has facilitated the development of important inhibitors of metalloenzymes such as GSK-1322322, a peptide deformylase inhibitor and antibacterial agent, Figure 2 (a),[5] and suberoylanilide hydroxamic acid, SAHA, a potent histone deacetylase (HDAC) inhibitor and anti-cancer agent, Figure 2 (b).[6-8]

We are currently interested in the reactivity of hydroxamic acids with bismuth, of which there has been few reports in the literature.[9-11] We recently, for example, reported a novel class of Bi(III) hydroxamato nitrate complexes and the urease inhibitory activity and antibacterial activity against *H. pylori* of the Bi benzohydroxamato complex, $[\text{Bi}_2(\text{Bha}_{-1\text{H}})_2(\mu\text{-Bha}_{-1\text{H}})_2(\eta^2\text{-NO}_3)_2]$, Figure 2 (c).[11]

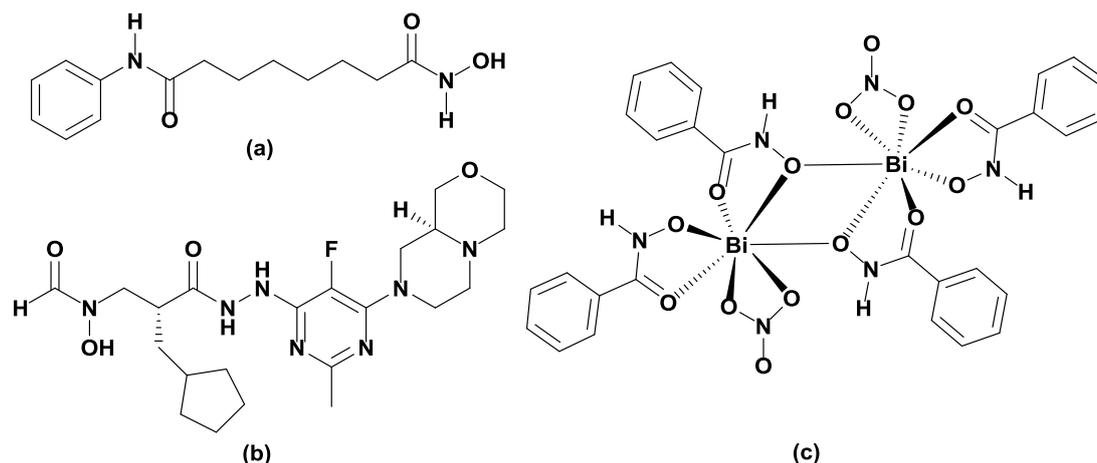


Figure 2. Structures of (a) SAHA, (b) GSK1322322 and (c) $[\text{Bi}_2(\text{Bha}_{-1\text{H}})_2(\mu\text{-Bha}_{-1\text{H}})_2(\eta^2\text{-NO}_3)_2]$.

Prior to the discovery of antibiotics numerous different Bi preparations were used as treatments for bacterial infections associated with wounds, cholera and gastroenteritis.[12] The development of antibiotics in the 1940's drastically reduced the use of Bi-based antibacterial medications until the discovery of the gastric pathogen *H. pylori* in the 1980s.[12] Currently Bi-containing quadruple therapies for *H. pylori* represent a successful strategy for overcoming multidrug bacterial resistance.[13, 14]

Metallomic and metalloproteomic approaches have been employed to investigate the potential antibacterial mechanism of action of Bi against *H. pylori* for example and strong evidence suggests that Bi interference with metal homeostasis and oxidation reduction processes may be important.[15-19]

Considering Bi strong association with effective treatment for *H. pylori* it is very surprising that there are so few reports in the literature on the investigation of the activity of Bi compounds against bacteria in addition to *H. pylori*.[20] Furthermore given infection involving multidrug bacterial resistance represents an emergent global

disease and a major global health challenge there is an urgent need to develop new classes of effective antibacterial agents.[21]

We hypothesised that reaction of Bismuth chloride, BiCl_3 , with salicylhydroxamic acid (Sha) and 2-aminophenyl hydroxamic acid (2-apha) would generate interesting Bi(III) hydroxamato complexes, particularly as the availability of the hydroxyl group, amino groups or pyridine nitrogen at the 2-position respectively, might act as anchors and encourage (O_{OH}, O) or (N_{NH_2}, O) hydroxamato coordination respectively to Bi(III).

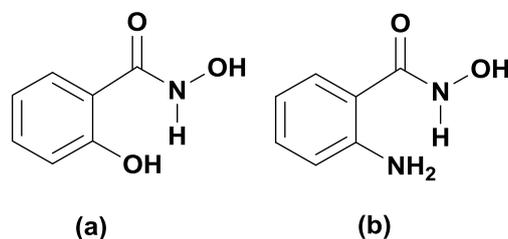


Figure 3. Structures of (a) Salicylhydroxamic acid and (b) 2-aminophenylhydroxamic acid.

Herein, we report the synthesis and characterisation of novel polymeric Bi(III) chlorido complexes of salicylhydroxamic acid and 2-aminophenylhydroxamic acid. The X-ray crystal structure of the THF-solvated polymeric complex, $[\text{BiCl}_2(\mu\text{-Sha}_{1\text{H}})(\text{THF})]_{\infty}$ was determined and the antibacterial activity of the THF-free complex, $[\text{BiCl}_2(\mu\text{-Sha}_{1\text{H}})]_{\infty}$, against a broad panel of bacteria investigated.

2. Experimental

2.1. Materials

Salicylhydroxamic acid, BiCl₃, Ampicillin and deuterated solvents were purchased from Sigma Aldrich and used without further purification. 2-NH₂-phawas synthesised as previously reported.[22]

2.2. Synthetic methods

IR spectra were recorded using a Bruker VERTEX 70/70v FT-IR spectrometer using KBr discs (4000-400 cm⁻¹). The spectra were analysed using OPUS software. ¹H NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer. The spectra were analysed using MestReNova software. The residual undeuterated d⁴-Methanol signal at 3.31 ppm was used as an internal reference. Mass spectrometry experiments were performed on an Advion Expression Compact Mass Spectrometer where 10 μL of the samples were injected in 300 μL of methanol:water:formic acid (90:9:1 v/v). The mass spectrometry data were acquired both in positive and negative ion modes and the spectra analysed using the Advion Mass Express software programme.

Elemental analysis (C, H and N) was performed at the Microanalytical Laboratory, School of Chemistry and Chemical Biology, University College Dublin, Ireland.

2.3. Syntheses

2.3.1. Synthesis of [BiCl₂(μ-Sha-_{1H})]_∞

Anhydrous THF (25 mL) was added to BiCl₃ (470 mg, 1.49 mmol) and Sha (265 mg, 1.7 mmol) in a dry round bottomed flask under Argon. The reaction was stirred at room temperature for three days. The reaction mixture was concentrated *in vacuo* to c. 5 mL. H₂O (5 mL) was added resulting in precipitation of a light yellow

solid, which was filtered and dried, 370 mg (88 %). Elemental analysis: (C₇H₆BiCl₂NO₃) Calc. (Found): C 19.46 (19.4), H 1.4 (1.14), N 3.24 (2.84), Cl 16.41 (13.58) %; ¹H NMR (400 MHz, CD₃OD, 25 °C, d = doublet, t = triplet, s = singlet, br = broad, m = multiplet) δ_H: 7.91 (d, *J* = 8.1 Hz, 1H, Aromatic H), 7.40 (t, *J* = 7.0 Hz, 1H, Aromatic H), 6.93 (m, 2H, aromatic H); IR (KBr disc, br = broad, s = strong, vs = very strong) ν_{max}/cm⁻¹: 3230 (s, O-H), 1604 (s, C=O); MS (ESI+) *m/z*: 396.1 BiCl(Sha-_{1H}), 512.9 Bi(Sha-_{1H})₂.

Single crystals of [BiCl₂(μ-Sha-_{1H})(THF)]_∞ were formed upon standing of the filtrate. Elemental analysis; (C₁₅H₂₂BiCl₂NO₄) Calc. (Found): C 31.27 (30.85), H 3.85 (3.63), N 2.43 (2.20), Cl 12.30 (10.26) %.

2.3.2. [BiCl₃(2-NH₃-Pha-_{1H})]_∞

Anhydrous THF (35 mL) was added to BiCl₃ (490 mg, 1.55 mmol) and 2-NH₂-Pha (269 mg, 1.76 mmol) in a dry round bottomed flask under Argon. The reaction was stirred at room temperature for three days. A yellow precipitate was filtered and dried, 360 mg (49 %). Elemental analysis; (C₇H₇BiCl₂N₂O₂·0.1C₄H₈O) Calc. (Found): C 18.72 (18.84), H 1.87 (1.72), N 5.90 (5.92), Cl 22.40 (22.46) %; ¹H NMR (400 MHz, CD₃OD), δ_H: 7.70 (d, ³*J* = 8 Hz, 1H, Aromatic H), 7.60 (t, ³*J* = 8 Hz, 1H, Aromatic H), 7.38 (m, 2H, Aromatic H); IR (KBr disc) ν_{max}/cm⁻¹: 1614 (s, C=O); MS (ESI+) *m/z*: 430.9 BiCl₂(2-NH₃-pha-_{1H}) and 619 BiCl₃(2-NH₃-pha-_{1H})₂.

2.4. X-Ray data collection and structure determination for [BiCl₂(μ-Sha-_{1H})(THF)]_∞.

Crystal data for [BiCl₂(THF)(μ-Sha-_{1H})]_∞ were collected with Mo-K_α radiation (0.71073 Å) using an Rigaku Oxford Diffraction (former Agilent Technologies, former Oxford Diffraction) SuperNova A diffractometer fitted with an Atlas detector. A five times redundant dataset was collected, assuming that the Friedel pairs are not

equivalent. An analytical absorption correction based on the shape of the crystal was performed.[24] The structures were solved by direct methods using SHELXS-97[25] and refined by full matrix least-squares on F^2 for all data using SHELXL-97.[25] Hydrogen atoms were added at calculated positions and refined using a riding model. Their isotropic temperature factors were fixed to 1.2 times (1.5 times for methyl groups) the equivalent isotropic displacement parameters of the carbon atom the H-atom is attached to. Anisotropic thermal displacement parameters were used for all non-hydrogen atoms.

Table 1.

Crystallographic data collection and structure refinement for $[BiCl_2(\mu\text{-Sha}_{1H})(THF)] \cdot THF_\infty$

Empirical formula	$C_{15} H_{22} N O_5 Cl_2 Bi$
Molecular formula	$C_{11} H_{14} N O_4 Cl_2 Bi \times C_4 H_8 O$
Formula weight	576.22
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	$Pna2_1$ (#33)
Unit cell dimensions	$a = 16.6571(1)$ Å $\alpha = 90^\circ$. $b = 33.0779(3)$ Å $\beta = 90^\circ$. $c = 20.4220(2)$ Å $\gamma = 90^\circ$.
Volume	11252.15(16) Å ³
Z	24
Density (calculated)	2.041 Mg/m ³
Absorption coefficient	9.710 mm ⁻¹
F(000)	6624
Crystal size	0.3408 x 0.1964 x 0.1525 mm ³
Theta range for data collection	2.93 to 29.69°.
Index ranges	$-22 \leq h \leq 20$, $-43 \leq k \leq 45$, $-27 \leq l \leq 27$
Reflections collected	122294

Independent reflections	27266 [R(int) = 0.0361]
Completeness to theta = 28.00°	99.0 %
Absorption correction	Analytical
Max. and min. transmission	0.319 and 0.102
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	27266 / 23 / 1304 ^{a)}
Goodness-of-fit on F ²	1.059
Final R indices [I>2sigma(I)]	R1 = 0.0342, wR2 = 0.0842
R indices (all data)	R1 = 0.0355, wR2 = 0.0859
Absolute structure parameter	0.325(3) ^{b)}
Largest diff. peak and hole	1.999 and -1.650 e.Å ⁻³

^{a)} ISOR and DELU restraints were applied to O(2) and O(5) in order to stop them from going NPD.

^{b)} Hence refined as an inversion twin

2.5. Antibacterial testing

2.5.1 Bacterial strains and culture conditions

The bacterial strains investigated include *Bacillus subtilis* DSM10, *Enterobacter aerogenes* DSM30053, *Escherichia coli* DSM1103, *Escherichia coli* DSM498, *Pseudomonas fluorescens* DSM50090, *Pseudomonas putida* DSM6125 (KT2440) and *Staphylococcus aureus* DSM799 obtained from The Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures GmbH and the in-house strains *Escherichia coli* T37-1 and *Pseudomonas putida* CPI. The organisms were maintained on nutrient agar at 4⁰C. Three of the bacteria are pathogenic or biohazard class II bacterial species; *Enterobacter aerogenes* DSM30053, *Escherichia coli* DSM1103 and *Staphylococcus aureus* DSM799.

2.5.2 Determination of LC_{50}

Stock solutions of investigated compounds were freshly prepared and diluted with Mueller-Hinton broth to various working concentrations with a final DMSO concentration of $\leq 1\%$.

LC_{50} values for the compounds were determined using a modification of the broth microdilution method described by Amsterdam.[26] Strains were grown in nutrient broth overnight, washed with phosphate buffered saline (PBS) and the cell number adjusted to give an optical density reading of 0.07 or 0.09, dependent on the bacterial strain, at 660 nm. Each well was inoculated with 5 μ L of bacterial culture.

Qualitative agar incorporation tests indicated that the LC_{50} of $[BiCl_2(\mu\text{-Sha}_{1H})]_{\infty}$ and BSS would be found in the 0-10 μ g/mL range against all bacteria and that Ampicillin had varying activities across the 0-500 μ g/mL range against all bacteria. In turn the LC_{50} of $[BiCl_2(\mu\text{-Sha}_{1H})]_{\infty}$ and BSS were investigated using two fold dilutions in the 0.3125 -10 μ g/mL range and Ampicillin was tested using two fold dilutions ranging from 0.977 - 500 μ g/mL. Wells containing only the stock solutions of investigated compounds in Mueller-Hinton broth were used as blanks and wells containing only 1% DMSO in Mueller-Hinton broth and culture were used as positive controls.

The microplates were incubated overnight at 30 °C for all bacteria except *Enterobacter aerogenes* DSM30053, *Escherichia coli* DSM1103 and *Staphylococcus aureus* DSM799 which were incubated at 37°C. The presence or absence of growth was determined by measuring the optical density of the wells at a wavelength of

660 nm using a plate reader. The LC₅₀ values were determined as the concentration or range of concentrations that caused a 50 % reduction in cell growth.

3. Results and discussion

3.1. Synthesis and characterisation of polymeric Bi chlorido hydroxamato complexes

BiCl₃ was reacted with a slight excess of hydroxamaic acids (Sha, 2-NH₂-pha and 2-pyha) in anhydrous THF and stirred at RT for 72 hours. Solids were collected in reasonably good yields by filtration post stirring or post addition of H₂O and their structures characterised by elemental analysis IR, ¹H NMR spectroscopy and mass spectrometry and in the case of [BiCl₂(μ-Sha-_{1H})(THF)]_∞ by X-ray crystallography.

3.1.1 [BiCl₂(μ-Sha-_{1H})]_∞

Elemental analyses indicates the formation of a 1:2:1 Bi(III) chlorido hydroxamic acid complex. The salicylhydroxamic acid ligand is therefore monodeprotonated.

IR spectroscopy is a particularly useful technique for characterisation of metal hydroxamato complexes. A shift of the intense carbonyl stretching band in the free ligand, Sha (1619 cm⁻¹), to lower wavenumber in the corresponding complex (1604 cm⁻¹) was observed. This observation confirms the involvement of the carbonyl O in coordination of the metal ion and is consistent with previous reports, where hydroxamato coordination is (O,O')-bidentate chelating, with coordination through the carbonyl oxygen atom and the deprotonated hydroxamic acid hydroxy group to form five-membered chelates.[22, 27] We have also previously observed similar shifts in Bi hydroxamato complexes which feature

(O,O')-bidentate chelating and (O, μ -O')-bidentate bridging coordination modes.[11]

^1H NMR spectra were carried out in methanol- d_4 given all ligands and Bi complexes were readily soluble in methanol. No signals associated with the hydroxamic acid protons (N-H and O-H) or the 2-OH proton were observed as expected. Nonetheless characteristic shifts in aromatic protons in the ligands compared to the corresponding Bi complexes were observed. For instance we observe a significant shift in the doublet associated with the proton at the 6 position (H^6) on the aromatic ring, which is closest to the carbonyl group, from 7.63 in the free Sha ligand to 7.90 ppm in $[\text{BiCl}_2(\mu\text{-Sha}_{\text{-1H}})]$. The remaining two signals associated with free Sha at 7.34 and 6.9 ppm were found at 7.34 and 6.9 ppm respectively in $[\text{BiCl}_2(\mu\text{-Sha}_{\text{-1H}})]$.

The absence of a shift in the signal associated with the proton at the 3-position on the aromatic ring (H^3) which is found within a multiplet at 6.9 ppm, the elemental analysis indicating that salicylhydroxamic acid is monodeprotonated and the typical $\nu(\text{C}=\text{O})$ shift associated with (O,O) chelating mode in the IR spectrum, strongly suggests the hydroxyl group at the 2-position does not coordinate to the Bi(III) centre.

ESI-MS in the positive mode was used to identify the product fragments $\text{BiCl}(\text{Sha}_{\text{-1H}})$ and $\text{Bi}(\text{Sha}_{\text{-1H}})_2$ with mass peaks at 396.1 and 512.9 a.m.u respectively.

The proposed polymeric structure for $[\text{BiCl}_2(\mu\text{-Sha}_{\text{-1H}})]_\infty$ is shown in Figure 6 and is supported by the X-ray crystal structure of the THF-solvated $[\text{BiCl}_2(\mu\text{-Sha}_{\text{-1H}})(\text{THF})]_n$ described below.

3.1.2. X-ray structural characterisation of $[\text{BiCl}_2(\mu\text{-Sha}_{-1\text{H}})(\text{THF})]\cdot\text{THF}_\infty$

Single crystals of THF-solvated $[\text{BiCl}_2(\mu\text{-Sha}_{-1\text{H}})(\text{THF})]\cdot\text{THF}_\infty$ were formed upon standing of the filtrate in 2.3.1. - synthesis of $[\text{BiCl}_2(\mu\text{-Sha}_{-1\text{H}})]_\infty$.

The atomic numbering scheme and atom connectivity for $[\text{BiCl}_2(\mu\text{-Sha}_{-1\text{H}})(\text{THF})]\cdot\text{THF}_\infty$ are shown in Figures 4 and 5 and a selection of bond lengths and angles reported in Table 2. The structure of $[\text{BiCl}_2(\mu\text{-Sha}_{-1\text{H}})(\text{THF})]\cdot\text{THF}_\infty$ consists of a charge neutral Bi(III) polymer where each Bi(III) centre has a coordination of 7 and possesses distorted pentagonal bipyramidal geometry. Each Bi(III) centre is coordinated to three chlorido ligands two of which are bridging with different but adjacent Bi(III) centres and a salicylhydroxamato ligand with an (O, μ -O') chelating mode, where the deprotonated hydroxyl oxygen bridges with one adjacent Bi(III) centre. It is noteworthy that the oxygen bridge is asymmetric, where the bridging oxygen is significantly closer in length to the hydroxamato chelated Bi(III) centre (c. 2.25 Å) as compared to the adjacent Bi(III) centre (c. 2.8 Å; see Table 2). We previously reported a Bi(III) nitrate salicylhydroxamato complex, $[\text{Bi}_6(\text{CH}_3\text{OH})_2(\eta^1\text{-NO}_3)_2(\eta^2\text{-NO}_3)(\text{OH}_2)_2(\text{Sha}_{-1\text{H}})_{12}](\text{NO}_3)_2$, where the bismuth atoms are bridged by salicylhydroxamato ligands to form terminal $\text{Bi}_2\text{-}\mu\text{-O}_3$ (Bi-O range = 2.226-2.82Å).[11]

The coordination sphere around each Bi(III) is completed by a coordinated THF molecule and each hydroxyl group at the 2-position on the aromatic ring of Sha forms a hydrogen bond to one uncoordinated THF molecule in the lattice.

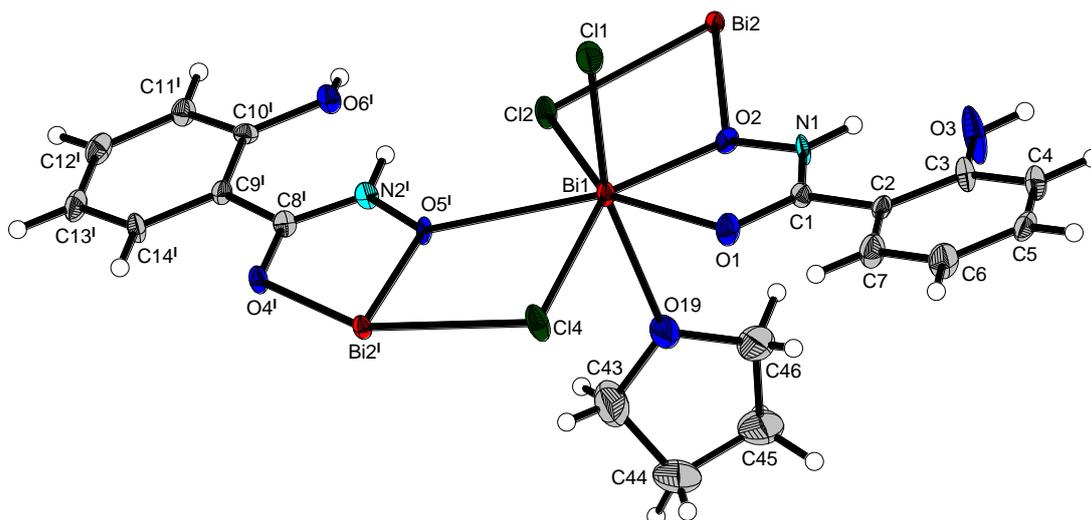


Figure 4. ORTEP diagram of $[\text{BiCl}_2(\mu\text{-Sha}_{.1\text{H}})(\text{THF})]\cdot\text{THF}_\infty$, featuring neighbouring environment of one Bi centre with adjacent Bi atoms continuing the chain; thermal ellipsoids are drawn on the 50% probability level; symmetry operation: $^1 -0.5+x, 0.5-y, z$.

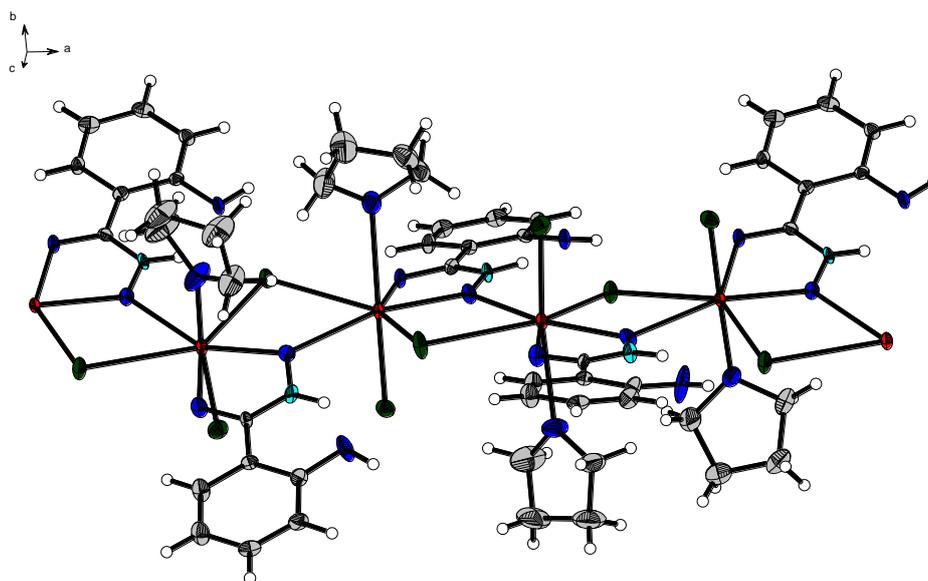


Figure 5. ORTEP diagram of $[\text{BiCl}_2(\mu\text{-Sha}_{.1\text{H}})(\text{THF})]\cdot\text{THF}_\infty$, featuring repeating units of the chain.

Table 2. Selected bond lengths (Å) and angles (°) for [BiCl₂(μ-Sha-_{1H})(THF)].THF_∞.

	Lengths		Angles		Angles
Bi(1)–O(2)	2.237(4)	O(2)–Bi(1)–O(1)	70.88(14)	O(1)–Bi(1)–O(5)#1	138.31(13)
Bi(1)–O(1)	2.290(4)	O(2)–Bi(1)–Cl(1)	92.25(11)	Cl(1)–Bi(1)–O(5)#1	94.21(9)
Bi(1)–Cl(1)	2.5190(17)	O(1)–Bi(1)–Cl(1)	87.62(13)	O(19)–Bi(1)–O(5)#1	99.76(13)
Bi(1)–O(19)	2.641(5)	O(2)–Bi(1)–O(19)	77.15(14)	Cl(2)–Bi(1)–O(5)#1	75.99(9)
Bi(1)–Cl(2)	2.7272(14)	O(1)–Bi(1)–O(19)	79.86(17)	O(2)–Bi(1)–Cl(4)#1	144.89(10)
Bi(1)–O(5)#1	2.797(4)	Cl(1)–Bi(1)–O(19)	165.68(11)	O(1)–Bi(1)–Cl(4)#1	75.49(10)
Bi(1)–Cl(4)#1	3.0159(15)	O(2)–Bi(1)–Cl(2)	74.64(10)	Cl(1)–Bi(1)–Cl(4)#1	96.19(5)
Cl(2)–Bi(2)	3.0059(15)	O(1)–Bi(1)–Cl(2)	145.51(10)	O(19)–Bi(1)–Cl(4)#1	87.44(12)
O(2)–Bi(2)	2.750(4)	Cl(1)–Bi(1)–Cl(2)	94.01(5)	Cl(2)–Bi(1)–Cl(4)#1	138.18(4)
Bi(2)–O(5)	2.225(4)	O(19)–Bi(1)–Cl(2)	92.43(13)	O(5)#1–Bi(1)– Cl(4)#1	62.90(9)
Bi(2)–O(4)	2.277(4)	O(2)–Bi(1)–O(5)#1	150.28(13)	Bi(1)–Cl(2)–Bi(2)	98.78(4)
Bi(2)–Cl(3)	2.5255(16)				
Bi(2)–O(20)	2.709(5)				
Bi(2)–Cl(4)	2.7356(14)				
Cl(4)–Bi(1)#2	3.0160(15)				

Symmetry transformations used to generate equivalent atoms:
 #1 $x-1/2, -y+1/2, z$ #2 $x+1/2, -y+1/2, z$ #3 $x+1, y, z$

3.1.3 [BiCl₃(2-NH₃-Pha-_{1H})]

Elemental analyses indicates the formation of a 1:3:1 Bi(III) chlorido hydroxamic acid complex. The 2-aminophenylhydroxamic acid ligand is therefore charge neutral.

In the IR spectrum a shift of the intense carbonyl stretching band in the free ligand, 2-NH₂-pha (1639 cm⁻¹), to lower wavenumber in the corresponding complex was observed (1614 cm⁻¹). This observation again confirms hydroxamato coordination through the carbonyl oxygen atom and the deprotonated hydroxy group, (O,O'). [11, 22, 27]

Regarding the ^1H NMR data in methanol- d_4 for $[\text{BiCl}_3(2\text{-NH}_3\text{-Pha}_{1\text{H}})]$ we observe significant shifts in all four of the aromatic signals found at 7.30, 7.16, 6.74 (doublet, 1 H^3) and 6.58 (triplet, H^4) ppm for the free ligand to three signals at 7.70, 7.6 and 7.38 (multiplet, $\text{H}^{3\&4}$) ppm in the Bi(III) complex. Though the signal for the 2-amino protons are found at 4.59 ppm in the 2- NH_2 -Pha, no amino proton signals are observed in the Bi(III) complex.

Given the dramatic shift in the signal associated with aromatic H found at the 6-position together with the loss or broadening of the amino signals it is likely that the amino group is either coordinated to Bi(III) or protonated.

Significantly though given the elemental analysis indicates that 2-aminophenylhydroxamic acid is charge neutral in the corresponding Bi(III) complex and the typical $\nu(\text{C}=\text{O})$ shift associated with (O,O) chelating mode is observed in its IR spectrum, it is likely that the amino group at the 2-position is protonated while the hydroxamic acid functional group is monodeprotonated, 2- $\text{NH}_3\text{-Pha}_{1\text{H}}$. We have solved the X-ray crystal structure of a separate Group V 2- $\text{NH}_3\text{-Pha}_{1\text{H}}$ compound, which features the suggested (O,O') hydroxamato coordination mode and protonated amino group at the 2-position. {Griffith, #480}

ESI-MS in the positive mode was used to identify the product fragments $\text{BiCl}_2(2\text{-NH}_3\text{-pha}_{1\text{H}})$ and $\text{BiCl}_3(2\text{-NH}_3\text{-pha}_{1\text{H}})_2$ with mass peaks at 430.9 and 619 a.m.u respectively.

The proposed polymeric structure for $[\text{BiCl}_3(2\text{-NH}_3\text{-Pha}_{1\text{H}})]_\infty$ is shown in Figure 6 and is also supported by the X-ray crystal structure of the tetrahydrofuran (THF)-solvated $[\text{BiCl}_2(\mu\text{-Sha}_{1\text{H}})(\text{THF})]_n \cdot \text{THF}_m$.

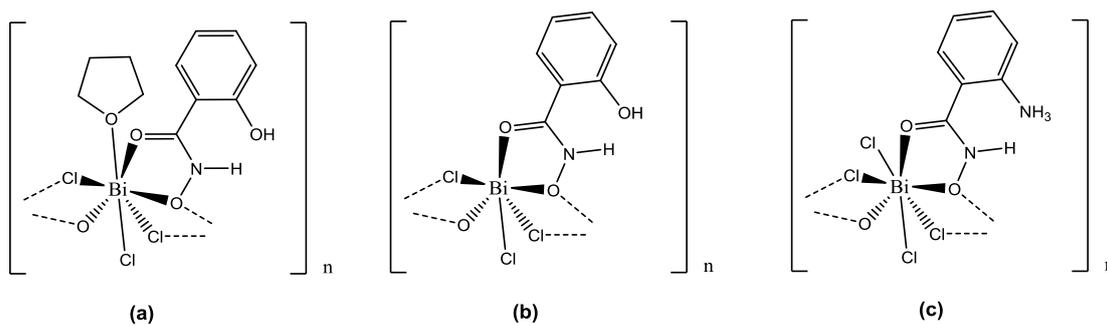


Figure 6. Structure of $[\text{BiCl}_2(\mu\text{-Sha-1H})(\text{THF})]_\infty$ (a) and proposed structures of $[\text{BiCl}_2(\mu\text{-Sha-1H})]_\infty$ (b) and $[\text{BiCl}_3(2\text{-NH}_3\text{-Pha-1H})]$ (c).

3.2. Antibacterial Activity of $[\text{BiCl}_2(\mu\text{-Sha-1H})]_\infty$, Bismuth Subsalicylate and Ampicillin

The antibacterial activity of $[\text{BiCl}_2(\mu\text{-Sha-1H})]_\infty$ and the commercially available drug bismuth subsalicylate (BSS) were investigated in six environmental bacterial strains; *E. coli* DSM498, *E. coli* T37-1, *P. putida* DSM6125 (KT2440), *P. putida* CP1, *P. fluorescens* DSM50090 and *B. subtilis* DSM10 and three biohazard class II pathogenic strains; *E. coli* DSM1103, *E. aerogenes* DSM30053 and *S. aureus* DSM799. Ampicillin, a beta lactam broad spectrum antibiotic, which is on the WHO's List of Essential Medicines, was chosen as a control compound. LC_{50} values, where LC_{50} is lethal concentration which inhibits the growth of 50% of viable cells present, were determined using the broth microdilution method, Table 3.[26]

Table 3 – LC₅₀ values (µg/mL) for [BiCl₂(µ-Sha_{1H})], BSS and Ampicillin against nine bacterial strains (± values = standard deviation).

Bacteria	Ampicillin	[BiCl ₂ (µ-Sha _{1H})]	BSS
<i>E. coli</i> DSM 498	≤ 0.98	5.7 ± 0.04	8.7 ± 0.032
<i>E. coli</i> T37-1	≤ 0.98	3.5 ± 0.036	7.4 ± 0.018
<i>E. coli</i> DSM 1103*	≤ 0.98	6 ± 0.026	6.7 ± 0.01
<i>E. aerogenes</i> DSM 30053*	7 ± 0.016	6.9 ± 0.024	7 ± 0.01
<i>P. putida</i> DSM 6125	62.5 ± 0.02	4.7 ± 0.039	4.1 ± 0.09
<i>P. putida</i> CP1	98 ± 0.019	3.9 ± 0.022	6 ± 0.05
<i>P. fluorescens</i> DSM50090	> 500	2.3 ± 0.029	4.4 ± 0.03
<i>S. aureus</i> DSM 799*	7.81 ± 0.012	5.8 ± 0.03	6.8 ± 0.028
<i>B. subtilis</i> DSM 10	> 500	2.5 ± 0.032	4.6 ± 0.03

The bismuth compounds [BiCl₂(µ-Sha_{1H})] and BSS, exhibit similar antibacterial activity against all of the strains tested, although [BiCl₂(µ-Sha_{1H})] is slightly more active against every strains apart from *P. putida* DSM 6125. The bismuth compounds do not display preferential activity for the Gram-positive (*S. aureus* and *B. subtilis*) over the Gram-negative bacteria or vice versa.

In contrast Ampicillin exhibits diverse activity against the panel of bacteria. This antibiotic exhibits significantly superior activity (≤ 0.98 µg/ mL) against all three strains of *E. coli* including the pathogenic strain, *E. coli* DSM 1103, as compared to the bismuth compounds (3.5-8.7 µg/mL). It is noteworthy though that both bismuth compounds exhibit similar activity to Ampicillin against the remaining two pathogenic strains *E. aerogenes* DSM 30053 and *S. aureus* DSM 799.

The bismuth compounds have superior activity with respect to Ampicillin in four out of the six environmental strains, two of which are resistant to Ampicillin (*P. fluorescens* DSM50090 and *B. subtilis* DSM 10) with $LC_{50} \geq 500 \mu\text{g/mL}$.

4. Conclusion

The polymeric bismuth(III) chlorido hydroxamato complexes $[\text{BiCl}_2(\mu\text{-Sha}_{1\text{H}})]$ and $[\text{BiCl}_3(2\text{-NH}_3\text{-Pha}_{1\text{H}})]$ were successfully synthesised and fully characterised. The structure of the THF solvated $[\text{BiCl}_2(\mu\text{-Sha}_{1\text{H}})(\text{THF})] \cdot \text{THF}_\infty$ was solved by X-ray crystallography, featuring the (O, μ -O') chelating mode of the salicylhydroxamato ligand where the deprotonated hydroxyl oxygen bridges with one adjacent Bi(III) centre. There is no evidence to suggest that the secondary groups associated with Sha, and 2- α -pha, i.e. the hydroxyl group and amino group at the 2-position respectively, act as anchors and encourage coordination.

An investigation of the antibacterial activity of $[\text{BiCl}_2(\mu\text{-Sha}_{1\text{H}})]$, BSS, and ampicillin against nine bacterial strains revealed that both bismuth compounds exhibited broad antibacterial activity in the experiments described including noteworthy activity against two pathogenic strains, *E. aerogenes* DSM 30053 and *S. aureus* DSM 799. Furthermore both bismuth compounds exhibited appreciable activity against six environmental bacterial strains and in four cases far superior activity than Ampicillin. This preliminary antibacterial study suggest that bismuth compounds should be investigated alone or in combination with antibiotics against multidrug resistant strains of antibiotics and could with further enquiry have a role to play in water disinfection.

5. Supplementary material

Crystallographic data (excluding structure factors) reported in this paper have been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 832349. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: ++44-1223-336-033; e-mail: deposit@ccdc.cam.ac.uk or [www: http://www.ccdc.cam.ac.uk/products/csd/request/](http://www.ccdc.cam.ac.uk/products/csd/request/)). Atomic coordinates and equivalent isotropic displacement parameters, bond lengths and angles, anisotropic displacement parameters, hydrogen coordinates and isotropic displacement parameters, torsion angles and hydrogen bonds for $[\text{BiCl}_2(\mu\text{-Sha}_{1\text{H}})(\text{THF})]\cdot\text{THF}_\infty$ are provided.

6. Acknowledgements

We sincerely thank the Irish Research Council (GOIPG/2014/693) and the Science Without Borders Programme (CAPES/BEX/13405-13-6) for financial support.

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