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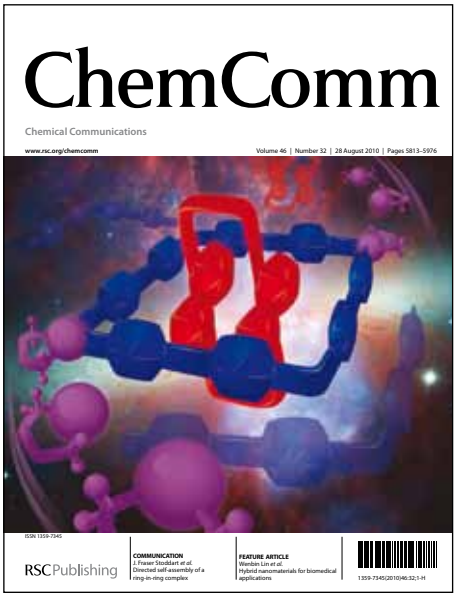
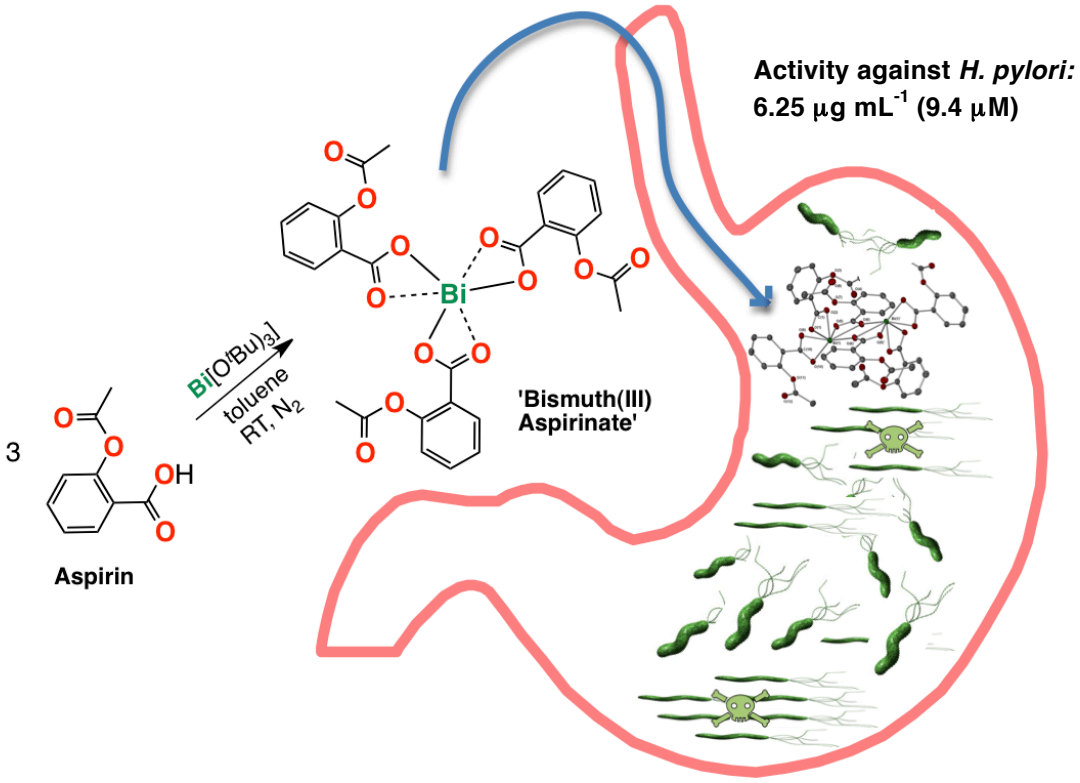


Graphical Abstract

The bismuth(III) complexes of acetylsalicylate (aspirinate), [Bi(asp)$_3$] and [KBi(asp)$_4$], are formed and crystallised without deacetylation or hydrolysis from treatment of aspirin with Bi(O^{t}Bu)$_3$ and Bi(O^{t}Bu)$_3$/KO^{t}Bu respectively, with [Bi(asp)$_3$], showing a minimum inhibitory concentration of 6.25 ug/mL against *H. pylori*.



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ARTICLE TYPE

Making Bispirin: Synthesis, structure and activity against *Helicobacter pylori* of bismuth(III) acetylsalicylate

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Reaction of $Bi(O^tBu)_3$ with aspirin (acetylsalicylic acid = aspH) in dry toluene results in the bismuth(III) complex, $[Bi(O_2C(C_6H_4)OAc)_3]_\infty$ 1 ($O_2C(C_6H_4)OAc$ = asp), minimum inhibitory concentration (MIC) against *Helicobacter pylori* ≥ 6.25 μg mL^{-1}, while the inclusion of a stoichiometric equivalent of KO^tBu leads to crystals of the bismuthate salt $[KBi(O_2C(C_6H_4)OAc)_4]_\infty$ 2.

Acetylsalicylic acid (or Aspirin) is one of the worlds most widely used drugs, providing anti-inflammatory, anti-pyretic and analgesic action.[1, 2] In contrast to other common non-steroidal anti-inflammatory drugs (NSAIDs) it acts irreversibly by deactivating both COX-1 and COX-2 enzymes. In low doses (< 300 mg per day) it functions as an anti-platelet agent, preventing heart attack and blood-clotting and thus lowering the risk of adverse cardiovascular and brain-bleeding events.[3,4] It has also been demonstrated to reduce and delay incidences of various types of cancer when taken on a regular basis.[3-6] As with other NSAIDs, the major drawback in the consistent ingestion of aspirin is a greater risk of upper-gastrointestinal bleeding, affecting 1 - 2 % of patients, though this can be higher is some populations.[7, 8] The associated risk factors include; old age, a history of peptic ulcer disease, co-administration of other NSAIDs, antithrombotic drugs, and infection by *Helicobacter pylori*.[7] While there have been contradictory reports dealing with the relationship of NSAIDs, *H. pylori* and peptic ulcer disease,[7] it is now generally accepted that *H. pylori* infection should be treated to minimise the possibility of NSAID induced gastrointestinal injury, ulceration and prolonged bleeding.[9, 10] Attempts to minimise aspirin related gastrointestinal injury have primarily focused on dose lowering and modified release formulations. This appears to have little effect.[8]

Bismuth compounds; bismuth *sub*salicylate and potassium bismuth citrate, are commonly used in triple and quadruple therapies in the treatment and eradication of *H. pylori* infection.[11-13] They also have a positive effect on reducing bleeding and in healing stomach ulcers. It is feasible that a single drug; bismuth(III) acetylsalicylate $[Bi(O_2C(C_6H_4)OAc)_3]$, could provide simultaneously the desirable therapeutic and preventative effects of aspirin while providing the gastrointestinal protection of chemotherapeutic bismuth(III) carboxylates.

Metal complexes of aspirinate (acetylsalicylate) and its derivatives can show improved anti-inflammatory action when compared with the parent acids.[14 - 16] They have also been shown to have potential in a wider range of biological and therapeutic activities; for example, the organometallic cobalt(II) complex [(μ^4-η^2)-(prop-2-ynyl)-2-acetoxybenzoate] dicobalthexacarbonyl is cytotoxic towards tumorous cells,[17] and *bis*-substituted $[Zn(asp)_2]$ complexes are described as promising candidates for treating type-2 diabetes and metabolic disorders.[18] Other examples for which crystal structures of the 'active compounds' are known are: $[Cu_2(asp)_4(DMF)_2]$[19] and $[Zn(asp)(H_2O)_2]$[20] as anticonvulsants; $[Cu_2(asp)_4(DMSO)_2]$ as an antioxidant;[21] and $[Ag(asp)(PPh_3)_3]$•DMF as an anti-tumour agent.[22]

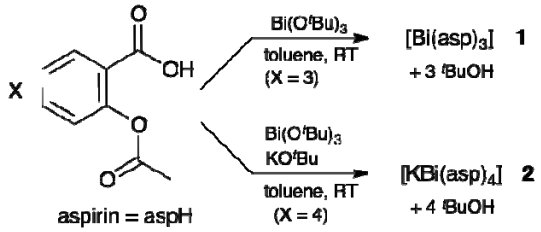
In this vein, we recently described the synthesis, characterisation and activity against *H. pylori* of bismuth(III) carboxylates derived from a series of common NSAIDs,[23] as well as the polynuclear oxido-cluster structure of bismuth *sub*salicylate.[24] However, the aspirin derivative remained elusive due to facile deacetylation during deprotonation and complexation, a common problem since bases frequently increase the rate of hydrolysis and/or deacetylation. The only previous report in this arena coming from a solution study on the 2:1 complex formed between acetylsalicylic acid and bismuth nitrate.[25]

Having explored various synthetic routes and reaction conditions for the formation of bismuth aspirinate complexes, we can now describe the formation, stability, solubility and crystal structures of $[Bi(asp)_3]_\infty$ **1** and $[KBi(asp)_4]_\infty$ **2**, and the bactericidal activity of **1** against three strains of *H. pylori.*

The protolysis reaction of aspirin with $BiPh_3$ (3:1) under both solvent-mediated and solvent-free conditions, our typical routes into bismuth carboxylates, proved problematic providing a mixture of complexes, including those formed through deacetylation. The DSC for the solvent-free reaction shows an exotherm (73.5 °C) immediately after the melting of $BiPh_3$ (71.3 °C). The corresponding weight-loss in the TGA (17 %) is indicative of the removal of two equivalents of benzene or three equivalents of acetic acid. The pungent aroma of acetic acid from the solvent-free reaction indicates that it is most likely a mixture of both. Up to 250 °C the total weight-loss is *ca*. 46 %, representing formation of *tris*-salicylato bismuth(III), $[Bi(Sal)_3]$, through the loss of three equivalents each of benzene and acetic acid. The ^{1}H NMR spectrum of the solid recovered from the solvent-mediated reaction conducted in dry toluene (to avoid aspirin hydrolysis) at reflux showed a complex mixture of products. Alongside residual aspirin (*ca* 15 %) and $[Bi(asp)_3]$ (*ca* 47%), was evidence of other bismuth complexes incorporating

anions of aspirinate, *o*-salicylate and acetate (*ca* 11 %). Most notable from the NMR data is a *bis*-phenyl complex (or complexes) of form [Ph_2BiL]. These data are presented as Supplementary Information (SI).

To overcome these problems $Bi(O^tBu)_3$, as a stronger and more labile base, was employed under dry, inert conditions. The stoichiometric 1:3 reaction of $Bi(O^tBu)_3$ with aspirin in dry toluene produced the desired *tris*-substituted bismuth(III) complex $[Bi(asp)_3]_\infty$ **1** in 70 % yield after stirring at room temperature for 6 hr and subsequent washing with dry ethanol (Scheme 1). Crystals suitable for single crystal X-ray diffraction were obtained from the toluene or ethanol mother liquors after approximately 1-2 days. Similarly, reacting a 1:1 stoichiometry of $Bi(O^tBu)_3$ and KO^tBu with four equivalents of aspirin gave the heterometallic aspirinate polymer $[KBi(asp)_4]_\infty$ **2** as a white solid in a 71% yield. Crystals suitable for X-ray analysis were grown from an acetone or ethanol solution over four days (Scheme 1).



Scheme 1 Synthesis of bismuth carboxylate complexes $[Bi(asp)_3]_\infty$ **1** and $[KBi(asp)_4]_\infty$ **2**.

Figures 1 and 2 show dimeric sections of complexes of the crystal structures of **1** and **2.** Corresponding figures S1 and S2 in the SI show a longer section of their extended polymeric chain structure of each.

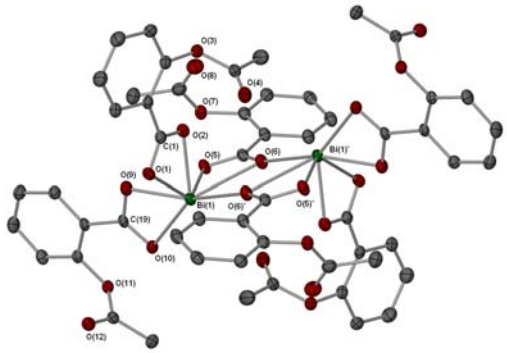


Figure 1 Molecular structure of $[Bi(asp)_3]_\infty$ **1** with thermal ellipsoids at 50% probability. Hydrogen atoms have been omitted for clarity. Symmetry operator: ' = (-x, 1-y, 2-z). Selected bond lengths (Å) and angles (°): Bi(1)-O(1), 2.748(3); Bi(1)-O(2), 2.274(2); Bi(1)-O(5), 2.260(2); Bi(1)-O(6), 2.666(2); Bi(1)-O(6)', 2.786(2) Bi(1)-O(9), 2.223(2); Bi(1)-O(10), 2.547(2); Bi(1)-O(12)', 2.833(2); O(2)-Bi(1)-O(1), 54.79(7); O(9)-Bi(1)-O(10), 54.52(7); O(5)-Bi(1)-O(6), 52.33(6); O(6)-Bi(1)-O(6)', 68.37(7).

Complex **1** crystallises in the monoclinic space group $P2_1/c$, with the asymmetric unit comprising one half of the centrosymmetric dimer $[Bi(asp)_3]_2$. The dimer is composed of two $[Bi(asp)_3]$ units joined through O atoms from unsymmetrical bridging carboxylate moieties [Bi(1)-O(6) 2.666(2), Bi(1)-O(6)', 2.786(2) Å] while the remaining carboxylate ligands surrounding the Bi(III) centre in **1** adopt the more usual bidentate chelating bonding mode (Figure 1). The dimers polymerise through formation of longer intermolecular Bi-O bonds belonging to the carbonyl function of one of the acetoxy moieties on the aspirin ligand [Bi(1)-O(12)', 2.833(2) Å] to give rise to a new three element 16-membered '$Bi_2O_6C_8$' ring. Thus, the polymer results from a series of alternating conjoined 4-membered planar Bi_2O_2 and 16-membered '$Bi_2O_6C_8$' rings (Figure S1) bringing the overall coordination number of each bismuth centre to seven in the dimer and increased to eight through polymerization.

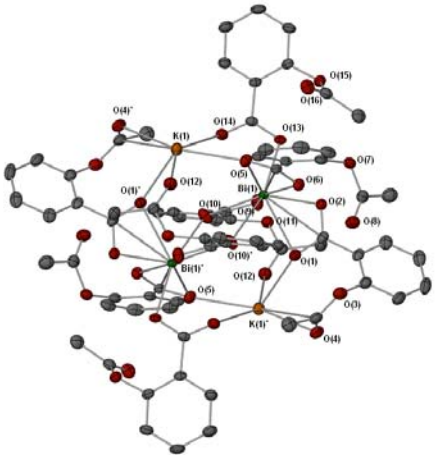


Figure 2 Molecular structure of $[KBi(asp)_4]_\infty$ **2** with thermal ellipsoids at 50% probability. Hydrogen atoms have been omitted for clarity. Symmetry operator: ' = (1-x, 1-y, 1-z). Selected bond lengths (Å) and angles (°):Bi(1)-O(1), 2.572(3); Bi(1)-O(2), 2.300(2); Bi(1)-O(5), 2.664(2); Bi(1)-O(6), 2.348(2); Bi(1)-O(9), 2.407(3); Bi(1)-O(10), 2.799(3); Bi(1)-O(10)', 2.711(3); Bi(1)-O(13), 2.243(2); Bi(1)-O(14), 2.800(3); K(1)-O(5), 2.713(2); K(1)-O(14), 2.744(3); K(1)-O(1)', 2.798(3); K(1)-O(4)', 2.845(3); K(1)-O(12)', 2.704(3); Bi(1)-O(10)-Bi(1)', 102.69(9); K(1)-O(4)-K(1)', 114.15(9).

Similar to **1**, **2** adopts a polymeric structure in the solid state. Crystallising in the triclinic space group *P*-1, it is composed of centrosymmetric dimers containing two Bi(III) atoms, two K atoms and eight aspirinate ligands. The central planar Bi_2O_2 ring is similar to that in complex **1**, were O atoms belonging to the carboxylate moiety [Bi(1)-O(10), 2.799(3); Bi(1)-O(10)', 2.711(2) Å] bridge the two Bi atoms via a tridentate coordinative bonding mode. Three more carboxylate groups surround each Bi centre in a chelating bidentate bonding mode [Bi(1)-O(1), 2.572(3); Bi(1)-O(2), 2.300(2); Bi(1)-O(5), 2.664(2); Bi(1)-O(6), 2.348(2); Bi(1)-O(13) 2.407(3), Bi(1)-O(14), 2.800(3) Å] with the overall coordination number of each bismuth being nine (Figure 2). Perpendicular to the central Bi_2O_2 ring a K atom lies above and below the plane coordinated to five aspirinate ligands in a range of bonding modes. One aspirinate ligand is bound through both its carboxylate [K(1)-O(1)', 2.798(3) Å] and acetoxy functional groups [K(1)-O(4)', 2.845(3) Å]; two are bound via traditional bidentate carboxylate bonding modes [K(1)-O(5), 2.713(2); K(1)-O(14), 2.744(3) Å]; and one *via* its acetoxy moiety alone [K(1)-O(12)', 2.704(3) Å] to give a formal five-coordinate K atom. These $[BiK(asp)_4]_2$ dimers polymerise through formation of longer K-O bonds [K(1)-O(4)', 2.826(3) Å] from a bridging acetoxy group, increasing the coordination number of each K atom to six (Figure S2). The final polymeric structural arrangement is best viewed as alternating adjoining 8-

membered $(KOBiO)_2$ and K_2O_2 ring systems.

Complexes **1** and **2** can best be compared to two other similar bismuth carboxylate complexes, $[Bi(O_2C\text{-}C_6H_4\text{-}2\text{-}OEt)_3]_2$[26] **3** and $[Bi(O_2C\text{-}C_6H_4\text{-}2\text{-}OMe)_3]_\infty$[27] **4**. Both **3** and **4** share a common planar Bi_2O_2 central motif with both **1** and **2** where the carboxylate moiety bridges the two bismuth centers together via a tri-dentate bonding mode. In **1**, **3** and **4** the carboxylate bridges are asymmetrically bound, with **1** having a an elongated 'short bond' [2.666(2) and 2.786(2) Å in **1**; 2.325(1) and 2.758(1) Å in **3**; and 2.3302(2) and 2.804(3) Å in **4**], while complex **2** adopts a more symmetrically bonded arrangement [Bi(1)-O(10), 2.799(3); Bi(1)-O(10)', 2.711(2) Å]. Similar to both **1** and **2**, **4** forms an overall polymeric structure although all three complexes do not share a common Bi-O polymeric growth pattern.

The ^1H and ^{13}C NMR spectra of both **1** and **2** (in D_6-DMSO) show a downfield shift for the H and C resonances in comparison to the NMR spectrum of aspirin in its free acid form. Disappearance of the CO_2H proton supports the formation of *tris*-substituted complexes, as established in the solid-state structures of **1** and **2**. Both **1** and **2** display similar aromatic proton signal resonances with the biggest difference being the greater downfield shift of the CH_3 group; 2.17 ppm in **1** and 2.34 ppm in **2**. Both complexes show no signs of immediate decomposition or deacetylation in solution, even on the addition of a few drops of D_2O. However, both complexes decompose quickly in weakly acidic media, a behaviour similar to other bismuth(III)-NSAID complexes,[23] and decompose slowly in polar and non-polar media over several weeks, forming salicylic acid and bismuth oxido-salicylate.

In the infrared spectra of **1** and **2** the acidic OH absorption is absent and the C=O absorption band of the ester group is observed at 1754 cm^{-1} for **1** and 1767 cm^{-1} for **2** (*versus* 1670-1700 cm^{-1} in aspirin). The value of $\Delta\nu$ ($\nu\ CO_2^-{}_{(asymm)}$ - $\nu\ CO_2^-{}_{(symm)}$) in both **1** and **2** is less than 200 cm^{-1} indicating a bidentate chelating mode of the ligand consistent with the solid state structural analysis.[28]

An assessment of the antibacterial activity of **1** and aspirin in its free acid form was carried out against three laboratory strains of *H. pylori*: B128, 251 and 26695, using compound concentrations ranging from 25 to 6.25 μg mL^{-1}. The Minimum Inhibitory Concentration (MIC) of each was determined by the Agar Diffusion method.[29] The activity of the complex was found to be 6.25 μg mL^{-1}, which is typical of carboxylato-based Bi-NSAID complexes.[23] Below this confluent growth of the bacteria was observed. In comparison, we found aspirin to be inactive (> 25 μg mL^{-1}), supporting a previous report of its activity (MIC_{50}) against *H. pylori* to be 256 μg mL^{-1}.[30] This indicates that the bismuth complex shows far greater bactericidal activity and is at least comparable, if not better than, standard bismuth-based treatments for *H. pylori* infection.[23] The anti-inflammatory and anti-ulcerative activity of **1** and **2** is the focus of ongoing research.

Notes and references

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† Electronic Supplementary Information (ESI) available: full experimental and analytical details, including crystallographic data and tables. See DOI: 10.1039/b000000x/

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