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Stability and toxicity of tris-tolyl bismuth(v) dicarboxylates and their biological activity towards *Leishmania major*⁺

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A series of 29 tris-tolyl bismuth(v) di-carboxylato complexes of composition [Bi(Tol)₃(O₂CR)₂] involving either ortho, meta or para substituted tolyl ligands have been synthesized and characterised. Of these 15 were assessed for their toxicity towards Leishmania promastigotes and human fibroblast cells, with ten then being subsequently assessed against parasite amastigotes. The carboxylate ligands are drawn from a series of substituted and biologically relevant benzoic acids which allow a comparison with earlier studies on $[BiPh_3(O_2CR)_2]$ and analogous Sb(v) $[SbAr_3(O_2CR)_2]$ (Ar = Ph and Tol) complexes. Twelve complexes have been structurally characterized by single crystal X-ray diffraction and shown to adopt a typical trigonal bipyramidal geometry in which the three tolyl ligands occupy the equatorial plane. NMR studies on two illustrative examples indicate that the complexes are stable in D₂O and DMSO but only have a half-life of 1.2 hours in culture medium, with glucose being a contributing factor in decomposition and reduction to Bi(Tol)₃. Despite their short lifetime many complexes show significant toxicity towards promastigotes at low concentration (<6 µM) and at that concentration provide for good selectivity indices (parasite vs. mammalian cells), for example 114 for [Bi(o-Tol)₃(O₂CC₆H₃(2-OH,5-C₆H₃(2,4-F₂)))₂] and 838 for $[Bi(m-Tol)_3(O_2CC_6H_4(2-OAc))_2]$. Best activity and selectivity is observed with complexes containing o- and m-tolyl ligands, and it appears the primary influence on fibroblast toxicity is the Ar ligand while the carboxylate influences promastigote toxicity. The complexes are less effective in vitro against the parasite amastigotes, where longer incubation times and harsher chemical and biological environments are encountered in the assay. Nevertheless, there were some statistically relevant differences at $1 \, \mu M$ against the positive controls with the best performing complexes being $[Bi(o-Tol)_3(O_2CC_6H_4(2-EtO))_2]$ and [Bi(m-Tol)₃(O₂CC₆H₄(2-OAc))₂].

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Introduction

Leishmaniasis is a group of diseases caused by the *Leishmania* parasite and transmitted *via* infected female sandflies. Visceral leishmaniasis (VL), the most serious form of the disease, affects the internal organs and without treatment is normally fatal within two years.¹ The World Health Organisation estimates that 300 000 cases of VL have been reported in the past

5 years with over 20 000 deaths annually, and 310 million people are currently at risk of infection. $^{\rm 2}$

For more than 70 years the frontline treatment for leishmaniasis has been pentavalent antimonals; sodium stibogluconate (Pentostam; SSG)³ and meglumine antimoniate (Glucantime),⁴ which are readily available and cost-effective. However, they have severe and toxic side effects, and due to their parenteral mode of administration over a 28-day period compliance can be low⁵ and areas of drug resistance have appeared, such as Bihar in India.^{6,7} Alternative treatments such as liposomal Amphotericin B8 and Miltefosine9 are available, however their prohibitive cost makes access to the drug difficult for the majority of patients. Furthermore, liposomal Amphotericin B is still administered intravenously, and Miltefosine, the only orally available drug has a narrow therapeutic window and is teratogenic.9 These challenges mean that there is a need for alternative, low cost, safe oral treatments with reduced toxicity and side effects.



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Fig. 1 Tris-tolylbismuth(v) dicarboxylato complexes 1–3, 5–11 and Bi(Tol)₃, 4 and 12 studied for anti-leishmanial activity on promastigotes and amastigotes.

Pentavalent antimony itself is relatively non-toxic and serves as a pro-drug which is reduced by trypanothione, gluthathione or thiol-dependent reductase, TDR1 at pH 5 to the active Sb(m) species within the macrophage.^{10,11} This leads to a decrease in buffering ability and imbalance in thiol redox potential as Sb(m) induces the efflux of trypanothione and glutathione out of the cell and their respective disulfides accumulate inside cells.¹² It is suggested that the redistribution of the Sb(m) species out of the parasite is responsible for the toxic effects.¹⁰

In seeking more lipophilic alternatives to SSG we have shown that several organometallic Sb(v) dicarboxylato complexes of general form [SbAr₃(O₂CR)₂] are promising leads, being highly effective against both promastigotes and amastigotes at very low concentrations (0.5-3.5 µM) while maintaining low toxicity towards human fibroblasts.¹³ Considering the periodic relationship of antimony and bismuth, and the low systemic toxicity of bismuth in humans,¹⁴ we extended our explorations to both $Bi(m)^{15-17}$ and $Bi(v)^{18}$ based compounds as promising candidates for future anti-leishmanial drugs, targeting decreased toxicity, reduced side effects, and greater potential for oral delivery. To that end we recently reported the chemistry and biological activity of a series of [BiPh₃(O₂CR)₂] complexes towards L. major promastigotes and human fibroblasts,¹⁸ showing that while [BiPh₃(O₂CR)₂] complexes show good activity against promastigotes, they were much less stable than their Sb(v) analogues,¹³ and were also non-selectively toxic against the fibroblasts, even at low concentrations. Using one complex, $[BiPh_3(O_2CC_6H_3(m-OH)_2)_2]$ as an illustrative example, we showed the complexes to be stable in water and

DMSO but to be unstable in culture medium, undergoing oxidative decomposition to reform and precipitate BiPh₃.¹⁸ However, the decomposition process was slow enough that the complex was still able to exert substantial toxic effects.

In our extensive study on $[SbAr_3(O_2CR)_2]$ complexes we found that it was not those derived from SbPh₃ which proved to be the most promising, but complexes in which Ar = *m*- or *p*-tolyl.¹³ Therefore, to establish a much closer comparison we undertook an assessment of the tolyl based complexes and here report on the synthesis and characterization of 29 organometallic tris-tolylbismuth(v) dicarboxylate complexes $[Bi(Tol)_3(O_2CR)_2]$ (where Ar = *o*-, *m*-, *p*-Tol) and their subsequent solubility and biological activity against *Leishmania major* promastigotes and human fibroblast cells. The most promising complexes were subsequently assessed on *L. major* amastigotes. Ten complexes and two starting compounds, shown in Fig. 1 below, were found to have good anti-leishmanial activity with low fibroblast toxicity.

Results and discussion

The target $[Bi(Tol)_3(O_2CR)_2]$ (*o*-, *p*- or *m*-tolyl) complexes were formed through an oxidative addition reaction between $Bi(Tol)_3$ and the chosen benzoic acid promoted by hydrogen peroxide in a 1:2:1 stoichiometric ratio in diethyl ether at room temperature.¹⁹ After 30 minutes each reaction mixture was filtered and the resulting colourless solutions allowed to stand overnight. This resulted in the isolation of a suite of

BiAr₃ + 2 RCO₂H
$$\xrightarrow{H_2O_2}$$
 [BiAr₃(O₂CR)₂]
Ar = Tol (o-, m-, p-)

 $[Bi(Tol)_3(O_2CR)_2]$ complexes as microcrystalline solids (Scheme 1).

The composition of the final products as $[Bi(Tol)_3(O_2CR)_2]$ was confirmed through ¹H and ¹³C NMR spectroscopy, melting point, FT-IR spectrometry, mass spectrometry and elemental analysis. In addition, the diflunisal (difH) derived complex, $[Bi(o-Tol)_3(dif)_2]$, **3**, was analysed by single crystal X-ray diffraction. Full analytical details for **1–3** and **5–11** are given in the Experimental section while for all other complexes are provided in the ESI† (Table 1).

Depending on the solubilities of the final products, NMR spectra were collected in either $CDCl_3$ or D_6 -DMSO at room temperature. The chemical shifts and integrals all corresponded with formation of the target $[Bi(Tol)_3(O_2CR)_2]$ complexes. All complexes showed low frequency shifts in the *ortho*, *meta* and *para* proton resonances of the tolyl ligand compared to the corresponding protons in $Bi(Tol)_3$. The -OH signal corresponding to the -COOH of the carboxylic acid, observed in the ¹H NMR spectra of the parent acids between 11.61–13.67 ppm, was also absent indicating deprotonation and complexation of the carboxylates to the Bi atom.

Compared to the parent acids which have their carboxylic acid carbonyl stretches in the range of $1650-1700 \text{ cm}^{-1}$, all the complexes show a shift to a lower wavenumber for their carboxylate carbonyl asymmetric and symmetric stretches to $1540-1650 \text{ cm}^{-1}$ and $1300-1420 \text{ cm}^{-1}$. This indicates the deprotonation of the –COOH functionality to form the [Bi(Tol)₃-(O₂CR)₂] complex. The difference between the symmetric and asymmetric shifts are less than 200 cm⁻¹ for all the complexes. According to Deacon and Phillips,²⁰ this indicates that the carboxylate ligands adopt a bidentate chelating mode to the bismuth centre, and this trend was observed also in the solid state structures of the [BiPh₃(O₂CR)₂] complexes.¹⁸



Fig. 2 Molecular structure of $[Bi(o-Tol)_3(dif)_2]$, 3 showing thermal ellipsoids at 50% probability. Hydrogen atoms have been omitted for clarity. Selected bond lengths(Å) and bond angles (°): Bi(1)–C(15) 2.208(2); Bi(1)–C(1) 2.216(2); Bi(1)–C(8) 2.223(2); Bi(1)–O(4) 2.2764(15); Bi(1)–O(1) 2.2936(15); Bi(1)–O(5) 3.0740(16); Bi(1)–O(2) 3.0813(17); C(15)–Bi(1)–C(1) 110.62(8);C(15)–Bi(1)–C(8) 116.29(8);C(1)–Bi(1)–C(8) 132.94(8); O(4)–Bi(1)–O(5) 46.22(5); O(1)–Bi(1)–O(2) 46.02(5).

X-Ray crystallography

Of the 29 complexes synthesised crystals suitable for single crystal X-ray diffraction studies were obtained for 11 of them: $[Bi(o-Tol)_3(dif)_2]$ **3**, $[Bi(m-Tol)_3(O_2CC_6H_3(2,5-OH))_2]$ **6**, $[Bi(m-Tol)_3(O_2CC_6H_4(2-EtO))_2]$ **8**, $[Bi(m-Tol)_3(O_2CC_6H_4(4-NO_2))_2]$ **9**, $[Bi(m-Tol)_3(O_2CC_6H_3(2-OH,5-Cl))_2]$ **19**, $[Bi(m-Tol)_3(O_2CC_6H_4(2-NHC_6H_3(2-Me,3-Cl)))_2]$ **22**, $[Bi(p-Tol)_3(O_2CC_6H_4(2-OME))_2]$ **25**, $[Bi(p-Tol)_3(O_2CC_6H_4(2-OEt))_2]$ **26**, $[Bi(p-Tol)_3(O_2CC_6H_4(2-NHC_6H_3(2-Me,3-Cl)))_2]$ **27**, $[Bi(p-Tol)_3(O_2CC_6H_3(2-OH,5-Cl))_2]$ **28** and $[Bi(p-Tol)_3(O_2CC_6H_4(2-NHC_6H_3(2-Me,3-Cl)))_2]$ **31**. The complexes are isostructural and discussion on **3** is provided here as an illustrative example. Figures depicting the solid-state structures of the other complexes along with a summary of their bond lengths and crystallographic data are provided as ESI.[†]

Suitable single crystals of $[Bi(o-Tol)_3(dif)_2]$, 3 were obtained from a THF/*n*-hexane mixture through slow evaporation. As shown in Fig. 2 the complex adopts a typical distorted trigonal bipyramidal geometry. The tolyl ligands sit in the equatorial plane with a propeller-like orientation, while the carboxylates sit in the axial positions. All the Bi–C bond lengths are similar; Bi(1)–C(15) 2.208(2), Bi(1)–C(1) 2.216(2), Bi(1)–C(8) 2.223(2). However, one of the C–Bi–C trigonal angles is more obtuse

Complex	Carboxylic acid (code)	Ar	R	Mp (°C)	Yield (%) 39.0
1	2-Ethoxybenzoic acid	<i>o-</i> Tol	$2\text{-EtOC}_6\text{H}_4$	126-128	
2	4-Nitrobenzoic acid	o-Tol	$4-NO_2C_6H_4$	146-148	44.6
3	Diflunisal (difH)	o-Tol	2-OH,5-C ₆ H ₃ (2,4-F ₂)C ₆ H ₃	153-154	75.1
5	3,5-Dimethylbenzoic acid	<i>m</i> -Tol	$3,5-Me_2C_6H_3$	173-176	51.5
6	3,5-Dihydroxybenzoic acid	<i>m</i> -Tol	$3,5-(OH)_2C_6H_3$	170 (dec.)	64.6
7	2-Methoxybenzoic acid	<i>m</i> -Tol	$2-MeOC_6H_4$	145-146	47.6
8	2-Ethoxybenzoic acid	<i>m</i> -Tol	$2-EtOC_6H_4$	144-145	46.2
9	4-Nitrobenzoic acid	<i>m</i> -Tol	$4-NO_2C_6H_4$	170-172	41.9
10	Flufenamic acid	<i>m</i> -Tol	2-NH(C ₆ H ₄ -3-CF ₃)C ₆ H ₄	143-147	66.4
11	Aspirin (aspH)	<i>m</i> -Tol	$2\text{-OAcC}_6\text{H}_4$	129-132	76.9

Table 1 Synthesis of [Bi(Tol)₃(O₂CR)₂] complexes 1-3, 5-11 with their melting points and yields

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than the rest (*cf.* 132.94(8) *vs.* 110.62(8) and 116.29(8)), leading to a distortion towards square pyramidal based geometry. The oxygen atoms of the carboxylate moieties are bound to the bismuth centre in a bidentate fashion confirming observation in the IR spectrum. The second carboxyl (C=O) oxygen atoms, O(2) and O(5) of the carboxylate ligands, have longer Bi–O distances, 3.0740(16) Å and 3.0813(17) Å, as compared to the two shorter Bi–O bonds 2.2764(15) Å and 2.2936(15) Å. For this complex, the presence of an *ortho*-OH functionality on the phenyl ring of the carboxylate ligand forms a hydrogen bond to the oxygen of the carboxylate, and may contribute to the longer Bi–O bond length.

This structure for the [Bi(Tol)₃(O₂CR)₂] analogues are similar to that observed for the [BiPh₃(O₂CR)₂] complexes.¹⁸ For example, the analogous $[BiPh_3(dif)_2]$ complex also shows a distorted trigonal bi-pyramidal structure. A comparison between the phenyl and tolyl analogues shows that the bond lengths; Bi-C (2.204(8) Å, 2.217(12) Å) and Bi-O (2.253(6) Å, 3.041(7) Å), are similar and are only slightly shorter than those in [Bi(Tol)₃(dif)₂]; Bi-C (2.208(3), 2.2218(3), 2.227(3) Å) and Bi-O (2.227(2), 2.293(2), 3.073(2), 2.081(2) Å). This indicates that the presence of an additional o-Me group on the phenyl ring has little influence on the structure of the complex. Conversely, the analogous Sb-C bonds in the Sb(v) complex $[SbPh_3(O_2CCH_2C_6H_4(m-CH_3))_2]$ are 2.0949(14), 2.0974(14) and 2.1105(13) Å, and the Sb-O bonds are 2.0880(9) Å and 2.1108 (10) Å, with the carboxylate ligands binding in a monodentate manner.

Stability studies

To determine whether the complexes are stable to atmospheric moisture and gases, all complexes were exposed to air and screened by melting point over a period of six months. There was no change in the observed melting points over this time, indicating the complexes have a high degree of stability in the solid-state. Short-term stability in solution was evaluated through the ¹H NMR spectrum of each complex being collected and compared at t = 0 and 48 h in either CDCl₃ or D₆-DMSO. No change in the chemical shifts was observed.

In our earlier studies on $[BiPh_3(O_2CR)_2]$ complexes we used $[BiPh_3(O_2CC_6H_3(2,5\text{-}OH))_2]$ as an example to study and under-

stand the stability of the complex in culture medium.¹⁸ While the complex was found to be stable in water and DMSO, decomposition was found to occur in DMEM culture media to precipitate the starting reagent $BiPh_3$ as a white precipitate. A subsequent NMR study showed that the complex was stable to phosphate (or other anion) exchange and did not react with the amino acids in solution, noting that the key amino acid primed for oxidation, cysteine, is already present in DMEM in its oxidised state.¹⁸

Similar studies were therefore conducted on [Bi(o- $Tol_{3}(dif_{2})$, 3 as a representative example of the Bi(v)-tolyl series of complexes. To assess stability in cell culture medium, ¹H NMR spectral data was collected at t = 0 h and 48 h. Conditions similar to those used in the biological assay were used, however the signal-to-noise ratio for the highest concentration used in the in vitro biological assay (100 µM) was too low to provide any meaningful data and, as such, a sample concentration of 1.0 mM was used. The culture medium was freezedried and reconstituted in D₂O to minimise the H₂O signal. Complex 3 was added to the culture medium and the resulting mixture allowed to stand for 24 hours, resulting in the formation of a white precipitate. The supernatant was then decanted off, and the white precipitate dissolved in d₆-DMSO and analysed by ¹H NMR. By comparing the chemical shifts produced by the unknown white precipitate, it was deduced that the precipitate was the starting reagent $Bi(o-Tol)_3$. (Fig. 3) This was confirmed by a comparison of the melting point which showed the unknown white precipitate (mp. 130 °C) was consistent with Bi(o-Tol)₃ (mp. 132 °C), as opposed to 3 (mp. 154 °C). This indicated that over time the Bi(v) complex 3 was being reduced.

To determine the rate of decomposition, a ¹H NMR spectrum of **3** in culture medium was recorded every 30 min for 10 hours. Using the integration of one chemical shift from the culture medium at 4.4 ppm as a standard, the decrease in integration of the *ortho* proton in **3** was measured. It was found that the integration fell from 1.000 (t = 0 h) to 0.0245 after 4.5 hours and then to 0.000 after 5 hours. The plot, shown in Fig. 4, followed a one-phase exponential decay ($R^2 = 0.9955$) with a half-life of 1.2 hours. This indicated that even though the complexes have a short half-life, they have potent anti-



Fig. 3 1 H NMR spectra comparison of unknown white precipitate with [Bi(o-Tol)₃(dif)₂], 3 & Bi(o-Tol)₃ in the aromatic region. Coloured dots indicate the chemical shifts for the respective tolyl protons.



Fig. 4 Decomposition curve of $[Bi(o-Tol)_3(dif)_2]$, 3 in DMEM culture media at 25 °C with time.

parasitic activity while being non-toxic towards human fibroblast cells.

It is still not clear what is oxidised as the Bi(v) complex reduces to $BiAr_3$. One of the principle constituents of culture medium (DMEM and M199) is glucose, second only to NaCl in concentration. Initial NMR spectra looking at the interaction of **3** with glucose proved complicated, so to simplify the data the aspirin (aspH) derivative $[Bi(m-Tol)_3(asp)_2]$, **11** was chosen as the illustrative Bi(v) complex for the study. Glucose was added to $[Bi(m-Tol)_3(asp)_2]$ **11** in d₆-DMSO in a stoichiometric ratio of 2:1, and the ¹H NMR spectrum recorded at t = 0, 48 h, and 1 week, at room temperature (20 °C). Control studies showed that when the individual species were left to stand in d₆-DMSO, there were no changes in the spectrum. However, when both **11** and glucose were added together, after 48 h significant changes in the chemical shifts of **11** and glucose were observed (Fig. 5). Integrations of the initial signals of **11** and glucose decreased, while signals matching that of starting reagent Bi(m-Tol)₃ appeared. In the range of the aromatic region at t = 48 h, both complex **11** and Bi(m-Tol)₃ are present in solution. However after 1 week, only the Bi(m-Tol)₃ could be observed.

This experiment was repeated, this time with **11** and glucose stirred at 40 °C over 18 hours. The resulting ¹H NMR spectrum resembled that of t = 48 h at 25 °C, where both **11** and Bi(*m*-Tol)₃ were present, indicating that the rate of decomposition of **11** increases at higher temperatures. However, the rate of decomposition is not comparable with that observed in the culture medium (5 h in culture medium vs. 1 week in DMSO), suggesting that while glucose is a component of the decomposition process, other, as yet unknown, factors contribute to an accelerated rate of decay.

Biological activity

L. major promastigotes and human fibroblast cells. All complexes were first screened for solubility in DMSO (see ESI†). A total of fifteen Bi(v) complexes 1–3, 5–11, 25–29, their parent



Fig. 5 ¹H NMR spectroscopic studies of $[Bi(m-Tol)_3(asp)_2]$ **11** and glucose in d₆-DMSO, 25 °C at t = 0 h, t = 48 h, and t = 1 week. Dotted boxes are drawn around the chemical signals of $Bi(m-Tol)_3$ and solid boxes are drawn around the corresponding tolyl chemical signals of **11**.

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benzoic acids along with $Bi(o-Tol)_3$, **4**, $Bi(m-Tol)_3$, **36** and $Bi(p-Tol)_3$, **12** were assessed for their anti-parasitic activity on *L. major* promastigotes and their toxicity towards human fibroblast cells. Insoluble complexes were excluded from the assay. The complexes were tested over concentration range of 48 nM to 100 μ M in culture media from a 10 mM DMSO stock. The resulting IC₅₀ values are presented in Table 2. DMSO and Amphotericin B controls were set up at concentrations equivalent to that of the compound concentrations (48 nM to 100 μ M). DMSO showed no apparent effect on any of the promastigotes or human fibroblast cells. Amphotericin B was the reference anti-leishmanial reagent. Complexes with a suitable selectivity index (parasites *vs.* human cells) were taken forward into a study on their effectiveness towards amastigotes.

After 48 hours, the parent carboxylic acids showed no toxicity towards the parasites or the fibroblasts. In contrast, all of the bismuth complexes showed anti-promastigote activity. Ideally, Bi(v) complexes would show excellent bioactivity towards the promastigotes (low percentage viabilities) while maintaining non-toxicity towards human fibroblasts (high percentage viability). This would be an indication that the complex would be less toxic and so would have decreased harmful side effects. Most of the complexes tested also showed toxicity to human fibroblasts at concentrations above 12.5 µM. However, at lower concentrations, below 10 µM, compounds 1-12 showed distinct discrimination between the promastigotes and fibroblasts. Some compounds in this group performed well at one concentration, higher and lower concentrations tended not to give as desirable fibroblast/promastigote viabilities. Other compounds were able to perform well over a larger range of concentrations.

Compounds 2, 3, 9, 10 and 11 belong to this latter category (Fig. 6). At 6.12 μ M, promastigote viability ranged from 0.0–6.3% and fibroblast viability of 83.8–100%. At a complex

concentration of 3.12 μ M, promastigote viability was slightly higher, ranging from 0.4–21.9%, with 100% fibroblast viability.

Compounds 1, 5, 7 and 8 on the other hand belong to the first category (Fig. 6). They had promastigote viabilities and fibroblast viabilities of 3.9-9% and 82-100% at 6.12μ M respectively, and 11.9-24% and 92.7-100% at 3.12μ M respectively. At a lower concentration of 3.12μ M, the lowest threshold reached by the complex in eradicating promastigotes was 11.9%, while still maintaining a respectable >92% viability for the fibroblasts. In comparison, the lower viability for the promastigotes at 6.12μ M also indicated that the fibroblasts were affected by the complex, giving the lowest fibroblast viability to be 82%. In this case, compound 1 sustained a constant 100% fibroblast viability while killing up to 91% of the promastigotes at 6.12μ M, and 76% promastigotes at 3.12μ M (Fig. 6).

Selectivity

Our previous studies focused on two areas: the Sb(v) compounds derived from carboxylates of both non-NSAID and NSAID classes,¹³ and the Bi(m) compounds which were derived from NSAID and non-NSAID classes.¹⁵ With the Sb(v) compounds, it was determined that there was a trend in which *p*-Tol and *m*-Tol analogues were more promising than the *o*-Tol and Ph analogues by being more bioactive against both *L. major* promastigotes and amastigotes.¹³ With the Bi(v) compounds however, the trend was found to differ from that of the Sb(v) complexes.

Complexes derived from 4-nitrobenzoic acid were available for comparison across the entire tolyl series while compounds derived from 2-ethoxybenozic acid were available for comparison across the aryl series of *o*-, *m*-, *p*-Tol and Ph.

Based on duplicate tests on fibroblasts and promastigotes, it became apparent that the primary influence on the nontoxicity of the complex was based on the aryl group while the

Table 2 IC_{50} and selectivity ratios of Bi(Tol)₃ 4, 36, 12 and their derived complexes 1–3, 5–11, 25–29 respectively with *L. major* promastigotes and human fibroblasts

~ 1		L	$IC_{50} (\pm SEM) (\mu M)$)		Selectivity index at 6.12 μM
[Bi(Tol) ₃ L ₂]	Ar		Promastigotes	Fibroblasts	Selectivity ratio:	
1	o-Tol	$O_2CC_6H_4(2-EtO)$	0.93 (0.04)	17.13 (0.05)	18.4	11.3
2		$O_2C C_6H_4$ (4-NO ₂)	0.69 (0.03)	14.21 (0.03)	20.6	42.4
3		$O_2CC_6H_3(2-OH,5-C_6H_3(2,4-F_2))$	0.57 (0.02)	16.13 (0.04)	28.3	114.1
4		_	0.85 (0.03)	11.02 (0.03)	13.0	1.2
5	<i>m</i> -Tol	$O_2CC_6H_3(3,5-Me_2)$	1.10 (0.04)	9.86 (0.02)	9.0	10.8
6		$O_2CC_6H_3(3,5-(OH)_2)$	1.11 (0.11)	11.19 (0.02)	10.0	2.0
7		$O_2CC_6H_4(2-MeO)$	0.93 (0.04)	12.02 (0.01)	12.9	20.7
8		$O_2CC_6H_4(2-EtO)$	0.89 (0.03)	11.52 (0.02)	12.9	21.0
9		$O_2CC_6H_4(4-NO_2)$	0.75 (0.05)	13.77 (0.02)	18.4	39.9
10		$O_2CC_6H_4(2-NH(C_6H_4(3-CF_3)))$	0.76 (0.02)	19.81 (0.04)	26.1	60.0
11		$O_2CC_6H_4(2-OAc)$	0.76 (0.02)	11.96 (0.01)	15.7	838.0
32			20.99 (0.05)	460.20 (0.29)	21.9	1.3
12	<i>p</i> -Tol	_	0.98 (0.02)	28.38 (0.04)	29.0	2.1
25	1	$O_2CC_6H_3(3,5-Me_2)$	0.84(0.02)	5.55(0.04)	6.6	6.0
26		$O_2CC_6H_3(3,5-(OH)_2)$	1.04(0.02)	5.76 (0.05)	5.5	3.5
27		$O_2CC_6H_4(2-MeO)$	1.04(0.02)	5.82(0.05)	5.6	4.6
28		$O_2CC_6H_4(2-EtO)$	1.77 (0.02)	7.82 (0.03)	4.4	3.8
29		$O_2CC_6H_4(4-NO_2)$	0.89 (0.02)	4.48 (0.04)	5.0	3.7
		= (-)				



Fig. 6 Activity of 1–12 on *L. major* promastigotes (●) and human fibroblasts (○) after 48 hours.

activity against the promastigote was based on the carboxylate ligand. By looking at their IC₅₀ values, the general trend of selectivity observed across the series was identified as *o*-Tol \geq *m*-Tol \geq *p*-Tol \geq Ph (Table 2).

For the promastigotes, $[Bi(o-Tol)_3(O_2CC_6H_4(4-NO_2))_2]$ had the lowest IC_{50} (0.69 ± 0.03 µM), followed closely by $[Bi(m-Tol)_3(O_2CC_6H_4(4-NO_2))_2]$ (0.75 ± 0.04 µM), and then $[Bi(p-Tol)_3(O_2CC_6H_4(4-NO_2))_2]$ (0.89 ± 0.02 µM).

For the fibroblasts, the same trend was followed, with the *ortho* analogue having the highest IC_{50} (14.21 ± 0.03 µM), followed very closely by *meta* (14.04 ± 0.02 µM) and *para* (4.48 ± 0.04 µM). This trend was also followed by compounds derived from 2-ethoxybenzoic acid, [Bi(Tol)₃(O₂CC₆H₄(2-EtO))₂].

For promastigotes, $[Bi(o-Tol)_3(O_2CC_6H_4(2-EtO))_2]$ and $[Bi(m-Tol)_3(O_2CC_6H_4(2-EtO))_2]$ had similar IC₅₀ values; (0.93 ± 0.04 µM) and (0.89 ± 0.03 µM) respectively, followed by $[Bi(p-Tol)_3(O_2CC_6H_4(2-EtO))_2]$ at (1.77 ± 0.02 µM), and lastly $[BiPh_3(O_2CC_6H_4(2-EtO))_2]$ at (2.29 ± 0.04 µM).¹⁸

For fibroblasts, a similar trend was followed as for the first set: the IC₅₀ values are highest for the *ortho* analogues (17.13 \pm 0.05 μ M), followed by the *meta* (11.63 \pm 0.02 μ M), *para* (0.89 \pm 0.02 μ M) and finally phenyl (3.84 \pm 0.03 μ M).¹⁸

From this comparison of IC_{50} values across different aryl groups, it can be seen that the *ortho* and *meta* analogues were more selective and promising, while the *p*-Tol and the Ph series were relatively similar in terms of activity on both parasites and fibroblasts, and were not as selective. These complexes were therefore not included for future testing.

From the individual percentage viabilities at certain complex concentrations, we analysed the data to obtain the IC_{50} for each complex based on their activity against promastigotes and fibroblasts. A selectivity index was calculated from

the ratio of IC_{50} of the fibroblasts to the IC_{50} of the promastigotes. Compounds with a score ≥ 9.0 proceeded to further biological testing against *L. major* amastigotes. This excluded $[Bi(m\text{-Tol})_3]$ **36** and the $[Bi(p\text{-Tol})_3L_2]$ complexes **25–29**.

L. major **amastigotes.** Based on their selectivity index, compounds **1–12** were assessed for their activity against *L. major* amastigotes, the clinically relevant form of the parasite. Amastigote-infected macrophages were incubated for 48 hours in the presence 1 μ M complex concentration, selected based on IC₅₀ values obtained from the promastigote assay (Fig. 7).

The macrophage invasion assay presents a more challenging biological environment and demands more from the complexes than the promastigote assay. Compounds have to traverse two membranes and be active in the acidic environment of phagolysosomes. Compounds 1, 2, 3, 9, and 11 showed anti-amastigote activity that resulted in statistically significant differences when compared to the positive control, while compounds 5, 6, 7, 8 and 10 displayed no anti-amastigote activity. Differences although significant, were small and do not appear physiologically relevant. Nevertheless, these data provide information regarding the structure-activity relationship. The more active and selective compounds were mostly the $[Bi(o-Tol)_3L_2]$ and $[Bi(m-Tol)_3L_2]$ complexes, though the m-Tol complexes displayed a greater range of toxicities based on their ligand combination. Compared to earlier assays performed with tris-arylantimony(v) dicarboxylates, the bismuth analogues were not as effective. Future complexes have to be designed to be more stable. The Bi(III) compounds $Bi(o-Tol)_3$ 4 and $Bi(p-Tol)_3$ 12, products of the *in situ* reduction of the Bi(v) complexes were tested at a 10-fold higher concentration than the bismuth(v) complexes and showed some antiparasitic activity.



Fig. 7 Infected macrophages after 48 hours. Number of macrophages infected with at least one amastigote was determined by microscopy of fixed specimens in duplicates. Amphotericin B (AmpB) was used as a reference compound at 0.5 μ g mL⁻¹. DMSO control was at 1%. Error bars indicate SEM, one-way ANOVA with Dunnett's multiple comparison test was used to determine statistical significance between test samples and non-treated control (+ve control).

It is not surprising to see low or no activity in the macrophage invasion assay. In the majority of cases promising results from promastigote testing cannot be extrapolated to amastigote setting.²¹ The short half-life of compounds could be responsible for relatively low levels of observed activity, particularly if they can only exert most of their toxicity towards the amastigotes during the first few hours in culture medium.

It is also possible that the concentration tested (the IC_{50} value) was too low. Despite being sufficient to kill 50% of promastigotes in culture that concentration was too low to be was effective against amastigotes. It is reasonable to assume that this is due to a number of coincident factors: an inability of the compounds to cross macrophage membranes because of permeability constraints; inefficient uptake by parasites; rapid intracellular degradation; or inactivity in the more acidic environment. Moreover, non-optimized compounds were tested and it is unlikely that they would exhibit potent anti-leishmanial activities without further iterative rounds of medicinal chemistry optimisation. As such our ongoing efforts are aimed at structural modification to improve permeability and stability, and to focus on compounds encapsulation as a delivery system to the amastigotes.

Conclusion

A series of twenty-nine organometallic triarylbismuth(v) dicarboxylates of the form $[Bi(Tol)_3(O_2CR)_2]$ were synthesized and fully characterized. Eleven solid-state structures were determined by single crystal X-ray diffraction (3, 6, 8, 9, 19, 22, 25–28, 31) showing the complexes adopt a typical trigonal bipyramidal geometry. The complexes are monomeric in the solid state with a seven coordinate Bi(v) centre. The carboxylate ligands adopt a bidentate binding mode to the Bi(v) atom, a general feature across the series of compounds as confirmed by FT-IR analysis.

Fifteen of the complexes were assessed for their anti-parasitic activity against *L. major* promastigotes and for cytotoxicity against human fibroblasts. All complexes were highly active against the promastigotes while the free acids proved to be non-toxic at the highest tested concentration of 100 μ M. The primary influence on the non-toxicity of the complexes appears to be based mainly on the aryl group while the activity against the promastigote is based on the carboxylate ligand. IC₅₀ values showed the general trend of selectivity observed across the series to be *o*-Tol \geq *m*-Tol \geq Ph.

To assess the stability of the Bi(v) complexes, a representative example, complex $[Bi(o-Tol)_3(dif)_2]$, 3 was studied in D₂O and DMSO and in DMEM cell culture medium. Resulting ¹H NMR spectra showed the complex to be stable in D_2O and DMSO, however in cell culture medium, $[Bi(o-Tol)_3(dif)_2]$ was observed to reduce to its starting compound Bi(o-Tol)₃ which precipitates out as an off-white solid. Further solution state studies found the half-life of complex 3 to be 1.2 hours. While our previous studies have shown little impact of amino acids, phosphate or other anions on the decomposition of the Bi(v) complexes, ¹H NMR solution studies on the structurally simpler complex $[Bi(m-Tol)_3(asp)_2]$, 11, indicated that glucose, present in high concentration in DMEM and M199, does plays a role in the decomposition though the rate is not as fast as that observed when the complex is in culture medium meaning other, as yet unknown factors, contribute to the reduction of the Bi compounds.

Despite the rapid decomposition rate of the Bi(v) complexes in culture medium they are still active long enough to exert a significant, and somewhat selective, toxic effect on the promastigotes and fibroblasts. The decomposition products Bi(Tol)₃ are relatively non-toxic to both.

Based on their selectivity index, ten complexes derived mainly from $Bi(o-Tol)_3$ and $Bi(m-Tol)_3$ were further screened for amastigote and macrophage toxicity, representing the clinical form of the disease. In this assay the compounds did not perform as well reflecting the significantly more challenging biological environment, as well the inherent instability of the complexes under such conditions.

Experimental section

All solvents used were purchased from Merck. For synthesis of BiPh₃, solvents were dried prior to use *via* the MBraun-SPS-800 and stored over molecular sieves (4 Å) in a Schlenk flask under N₂. Tris-*o*-tolylbismuth, 4 and tris-*p*-tolylbismuth, 12 were synthesized *via* literature procedure.²² All other required chemicals were purchased from Sigma-Aldrich and used as received. NMR spectra were recorded on a Bruker Avance DRX400 spectrometer (400 MHz) with chemical shifts referenced to the

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appropriate deuterated solvents. Infrared spectra were recorded on an Agilent Technologies Cary 360 FTIR spectrometer in the range 4000–500 cm⁻¹. Melting points were determined in soda glass tubes on a digital Stuart Scientific melting point apparatus SMP10. Mass spectrometry (ESI) was performed on a Micromass Platform QMS spectrometer with an electrospray source and a cone voltage of 35 eV. CHN elemental analysis was performed by The Campbell Microanalytical Laboratory, Department of Chemistry, University of Otago in Dunedin, New Zealand.

Biological assays

Cell viability assay. The Celltiter Blue Cell Viability Assay (Promega, Madison, WI, USA) was used for screening for antileishmanial activity and toxicity. Compounds were dissolved in DMSO at 10 mmol L⁻¹ working stock and diluted out in appropriate culture media. The assay was set up in duplicates in 96well plates according to the manufacturer's instructions. 10⁶ promastigotes per mL and 105 mL-1 primary human fibroblasts were used. Cell viability was assessed by measuring fluorescence at 550 nm excitation and 590 nm emission as per manufacturers' instructions.²³ The Celltiter Blue dye was added to samples at the time of setting up the assay and the negative control (no cells) value was subtracted from all subsequent readings as a background value. The mean was calculated from duplicate readings. All readings were compared to the no-drug control and the percentage growth inhibition was calculated. DMSO controls were included. All plates were assessed microscopically. The graphs shown in this paper give the percentage of positive control versus concentration. Macrophage invasion assay was performed as previously described.24

Cell culture. *Leishmania major* virulent clone V121 was derived from the *L. major* isolate LRC-L137 and maintained at 26 °C in M199 medium supplemented with 10% (v/v) heat inactivated FBS (Trace Biosciences, NSW, Australia).²⁵ The human primary fibroblast cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Life Technologies) supplemented with 10% HI-FBS at 37 °C in 5% CO₂.

Data analysis. Statistical analysis IC50 values were calculated in Graphpad Prism by non-linear regression (curve fit), dose–response inhibition, log(inhibitor) *vs.* normalised response, variable slope.

Crystallography

Crystallographic data of compounds **6**, **22**, **25**, **26**, **28** were collected at the MX1 beamline and compound **31** was collected on the MX2 beamline at the Australian Synchrotron, Melbourne, Victoria, Australia, operating at 17.4 keV ($\lambda = 0.71080$ Å) using an open flow of N₂ cryostream and cooled to 173(2) K for MX1 and 100(2) K for MX2. The software used for data collection and reduction of the data were BluIce²⁶ and XDS.²⁷ Crystallographic data for compounds **3**, **8**, **9**, **19** was collected on an OXFORD Gemini Ultra equipped with an OXFORD Cryosystems 700 Cryostream and cooled to 123(2) K. Data was collected with monochromatic (graphite) MoK_α

radiation ($\lambda = 0.71070$ Å) and processed using the CrysAlisProv 1.171.34.36 software;²⁸ Lorentz, polarization and absorption corrections (multi-scan) were applied. Crystallographic data for compound 27 was obtained on a Bruker X8 APEXII CCD diffractometer equipped with an OXFORD Cryosystems 700 Cryostream and cooled to 123(2) K. Data was collected with monochromatic (graphite) MoK_{α} radiation ($\lambda = 0.71065$ Å) and processed using the Bruker Apex2 v2014.7-1 software;29 Lorentz, polarization and absorption corrections (multi-scan -SADABS)³⁰ were applied. All compounds were solved and refined with SHELX-97.³¹ All non-hydrogen atoms were refined with anisotropic thermal parameters unless otherwise indicated and hydrogen atoms were placed in calculated positions using a riding model with C-H = 0.95–0.98 Å and $U_{iso}(H)$ = $xU_{iso}(C)$, x = 1.2 or 1.5 unless otherwise indicated. A summary of crystallographic data for 3 (CCDC 1420787) is provided below in the Experimental section while for compounds 6, 8, 9, 19, 22, 25-28, 31 (CCDC 1420788-1420797) it is provided in the ESI.†

General procedure

General synthetic procedure (GP1): stoichiometric equivalents of Bi(Tol)₃ and benzoic acid (1 : 2) were each dissolved in 5 mL of warm solvent and mixed. An excess of 30% H₂O₂ was added and the mixture was stirred for 10 minutes and filtered. Crystals were subsequently obtained on allowing the filtrate to stand at room temperature overnight. Grignard reagents were synthesized under Schlenk conditions in Et₂O by the reaction of stoichiometric ratios of Mg turnings with 2-bromotoluene, 3-bromotoluene and 4-bromotoluene respectively. The resulting Grignard reagent was reacted with BiCl₃ in a 3 : 1 stoichiometric ratio to obtain the Bi(Tol)₃ product as a crystalline solid.

Synthesis of tris-o-tolylbismuth bis(2-ethoxybenzoate), 1. Bi-(o-Tol)₃ (0.200 g, 0.41 mmol), 2-ethoxybenzoic acid (125 µL, 0.83 mmol) and 100 μL 30% H_2O_2 were reacted in warm diethyl ether according to GP. Yield: 39.0% (0.130 g); mp. 126–128 °C; ¹H NMR (400 MHz, (CD₃)₂SO, 25 °C): δ = 8.34 (3H, dd, J = 7.9 Hz, 1.1 Hz, Tol CH), 7.58 (6H, m, Tol CH), 7.47 (3H, td, J = 7.4 Hz, 1.0 Hz, Tol CH), 7.25 (2H, m, CH), 7.07 (2H, dd, J = 7.6 Hz, 1.8 Hz, CH), 6.92 (2H, d, J = 7.9 Hz, CH), 6.78 (2H, td, J = 7.4 Hz, 0.8 Hz, CH), 3.90 (4H, q, J = 6.9 Hz,OCH₂CH₃), 2.60 (9H, s, Tol CH₃), 1.13 (6H, t, J = 7.0 Hz, OCH₂CH₃); ${}^{13}C{}^{1}H{}$ (100 MHz, (CD₃)₂SO, 25 °C): δ = 170.9 (COO), 163.0 (BiC), 156.3 (COCH₂CH₃), 141.5 (CCH₃), 134.0 (CHar), 133.2 (CHar), 130.9 (CHar), 130.7 (CHar), 129.5 (CHar), 128.3(CHar), 125.4 (CHar), 119.7 (CHar), 113.2 (CHar), 63.6 (CH₂), 22.9 (Tol CH₃), 14.4 (CH₃); MS ESI⁺ 208.9 [Bi], 391.0 [Bi- $(o-\text{Tol})_2$ ⁺, 647.1 [Bi $(o-\text{Tol})_3$ L]⁺; ESI⁻ 165.1 [L]⁻, 721.2 [Bi $(o-\text{Tol})_3$ L]⁺; Tol)₂L₂⁻; IR 2980 (w), 2931 (w), 2897 (w), 2115 (w). 1742 (w), 1719 (w), 1646 (w), 1597 (sh), 1475 (m), 1447 (sh), 1382 (sh), 1326 (s), 1297 (sh), 1269 (sh), 1232 (s), 1163 (sh), 1143 (sh), 1111 (sh), 1041 (sh), 988 (sh), 922 (sh), 794 (m), 742 (s), 709 (sh), 663 (sh); Elemental analysis $[C_{39}H_{39}BiO_6 \cdot 2H_2O]$ (848.28) Calculated C 55.19 H 5.11 Found C 55.20 H 4.69.

Synthesis of tris-o-tolylbismuth bis(4-nitrobenzoate), 2. Bi(o-Tol)₃ (0.200 g, 0.41 mmol), 4-nitrobenzoic acid (0.139 g, 0.83 mmol) and 100 µL 30% H₂O₂ were reacted in warm diethyl ether according to GP. Yield: 44.6% (0.149 g); mp. 146–148 °C; ¹H NMR (400 MHz, CDCl₃, 25 °C): δ = 8.44 (3H, dd, J = 1.1 Hz, 8.0 Hz, Tol CH), 8.11 (4H, d, J = 8.9 Hz, CH), 7.93 (4H, d, CH), 7.56 (3H, dd, J = 1.0 Hz, 7.5 Hz, Tol CH), 7.53 (3H, t, J = 7.0 Hz, Tol CH), 2.68 (9H, s, CH₃); ${}^{13}C{}^{1}H{}$ $(100 \text{ MHz}, (CD_3)_2 \text{SO}, 25 \text{ °C}): \delta = 168.6 (COO), 161.9 (BiC), 149.6$ (CCH₃), 142.1 (CNO₂), 140.0 (CCOO), 134.7 (CH_{ar}), 133.7 (CH_{ar}), 131.6 (CH_{ar}), 130.7 (CH_{ar}), 129.0 (CH_{ar}), 123.2 (CH_{ar}), 23.8 (CH₃); MS ESI⁺ 208.9 [Bi], 391.1 $[Bi(o-Tol)_2]^+$, 648.1 $[Bi(o-Tol)_3L]^+$; 166.0 $[L]^-$, 723.2 $[Bi(o-Tol)_2L_2]^-$; IR 2929 (br), 2861 (br), 1646 (sh), 1601 (sh), 1519 (sh), 1474 (m), 1449 (m), 1405 (w), 1349 (m), 1292 (m), 1206 (sh), 1163 (sh), 1123 (sh), 1099 (sh), 999 (sh), 874 (sh), 819 (sh), 791 (sh), 742 (sh), 719 (sh), 669 (m); Elemental analysis $[C_{35}H_{29}BiN_2O_8]$ (814.60) Calculated C 51.61 H 3.59 N 3.44 Found C 51.47 H 3.63 N 3.31.

Synthesis of tris-o-tolylbismuth bis(2',4'-difluoro-4-hydroxybiphenyl-3-carboxylate), 3. Bi(o-Tol)₃ (0.200 g, 0.41 mmol), Diflunisal (0.208 g, 0.83 mmol) and 100 µL 30% H₂O₂ were reacted in warm diethyl ether according to GP. Yield: 75.1% (0.302 g); mp. 153–154 °C; ¹H NMR (400 MHz, (CD₃)₂SO, 25 °C): δ = 11.69 (2H, s, OH), 8.36 (3H, d, J = 7.9 Hz, o-CH_{ar}), 7.72 (5H, m, CH_{ar}), 7.66 (3H, t, J = 7.4 Hz, m- CH_{ar}), 7.57 (3H, t, J = 7.4 Hz, p-CH_{ar}), 7.53 (2H, d, J = 8.9 Hz, CH_{ar}), 7.46 (2H, q, J = 6.9 Hz, CH_{ar}), 7.31 (2H, t, J = 10.4 Hz, CH_{ar}), 7.16 (2H, td, J = 8.1 Hz, 1.8 Hz, CH_{ar}), 6.89 (2H, d, J = 8.6 Hz, CH_{ar}), 2.60 (9H, s, CH₃); ${}^{13}C{}^{1}H{}$ (100 MHz, (CD₃)₂SO, 25 °C): δ = 172.6 (COO), 161.2 (BiC), 160.4 (CF), 159.8 (CF), 158.1 (CCOO), 141.7 (CCH₃), 134.8 (CH_{ar}), 134.3 (CH_{ar}), 134.0 (CH_{ar}), 132.0 (CH_{ar}), 131.3 ((F)CCHC(F)), 130.6 (CHar), 129.3 (CHar), 124.6 (i-Car), 123.8 (i-Car), 117.1 (CHar), 115.2 (COH), 112.1 (CHar), 104.5 (CH_{ar}), 23.0 (CH₃); MS ESI⁺ 208.9 [Bi], 391.1 [Bi(o-Tol)₂]⁺, 731.2 [Bi(o-Tol)₃L]⁺; ESI⁻ 249.0 [L]⁻; IR 3074 (br), 2951 (w), 1634 (m), 1593 (m) 1561 (sh), 1509 (w), 1481 (sh), 1435 (sh), 1381 (sh), 1352 (sh), 1292 (m), 1245 (s), 1223 (sh), 1140 (sh), 1102 (sh), 1033 (w), 967 (sh), 917 (w), 889 (sh), 851 (sh), 833 (sh), 743 (sh), 726 (sh), 703 (sh), 662 (sh); Elemental Analysis $\left[C_{47}H_{35}BiF_4O_6\right]$ (980.77) Calculated C 57.56 H 3.60 Found C 57.67 H 3.88.

Crystal data for 3. $C_{51}H_{45}BiF_4O_7$; $M_r = 1054.85$; triclinic; space group: $P\bar{1}$; a = 12.2901(3), b = 12.4486(3), c = 15.1716(4); a = 76.296(2); $\beta = 76.185(2)$; $\gamma = 84.494(2)$; V = 2187.95(9) Å³; Z = 2, reflections collected/unique: 56 966/14 625 ($R_{int} = 0.0449$); R_1 values ($I > 2\sigma(I)$) = 0.0265; $wR(F^2)$ values ($I > 2\sigma(I)$) = 0.0503; R_1 values (all data) = 0.0355; $wR(F^2)$ values (all data) = 0.0539; GOF = 1.033; temperature = 123(2) K.

Synthesis of tris-*m*-tolylbismuth bis(3,5-dimethylbenzoate), 5. Bi(*m*-Tol)₃ (0.200 g, 0.41 mmol), 3,5-dimethylbenzoic acid (0.125 g, 0.83 mmol) and 100 µL 30% H₂O₂ were reacted in warm diethyl ether according to **GP**. Yield: 51.5% (0.165 g); mp. 173–176 °C; ¹H NMR (400 MHz, (CD₃)₂SO, 25 °C): δ = 7.99 (3H, s, Tol *o*-CH), 7.96 (3H, d, ³J = 8.1 Hz, Tol *o*-CH), 7.61 (3H, t, ³J = 7.7 Hz, Tol *m*-CH), 7.47 (4H, s, *o*-CH), 7.36 (3H, d, ³J = 7.5 Hz, Tol *p*-C*H*), 7.13 (2H, s, *p*-C*H*), 2.35 (9H, s, Tol *CH*₃), 2.27 (12H, s, *CH*₃); ${}^{13}C{}^{1}H{}$ (100 MHz, (CD₃)₂SO, 25 °C): δ = 171.8 (COO), 160.2 (BiC), 141.2 (CCH₃), 137.4 (CCH₃), 133.4 (*o*-CH₃), 132.4 (CCCO), 131.8 (CH_{ar}), 131.3 (CH_{ar}), 130.5 (CH_{ar}), 127.3 (CH_{ar}), 21.5 (CH₃), 20.7 (CH₃); MS ESI⁺ 208.97 [Bi], 346.18 [2Bi-(*m*-Tol) + (*m*-Tol)H]²⁺, 391.03 [Bi(*m*-Tol)₂]⁺, 631.18 [Bi(*m*-Tol)₃(L)]⁺; ESI⁻ 149.10 [L]⁻; IR 3503 (w), 3377 (br), 3051 (w), 2917 (w), 2862 (w), 1574 (sh), 1553 (sh), 1472 (w), 1447 (w), 1385 (sh), 1347 (s), 1309 (m), 1259 (m), 1208 (w), 1168 (w), 1092 (w), 1040 (w), 999 (w), 980 (sh), 869 (w), 811 (sh), 771 (sh), 675 (sh); Elemental analysis [C₃₉H₃₉BiO₄·H₂O] (798.28) Calculated C 58.65 H 5.17 Found C 58.31 H 5.18.

Synthesis of tris-m-tolylbismuth bis(3,5-dihydroxybenzoate), 6. Bi(*m*-Tol)₃ (0.400 g, 0.83 mmol), 3,5-dihydroxybenzoic acid (0.256 g, 1.66 mmol) and 100 μ L 30% H₂O₂ were reacted in warm diethyl ether according to GP. Yield: 64.6% (0.377 g); mp. 170 °C (decomp.); ¹H NMR (400 MHz, (CD₃)₂SO, 25 °C): δ = 9.40 (4H, s, OH), 7.96 (3H, s, Tol o-CH), 7.91 (3H, d, ³J = 7.6 Hz, Tol *o*-CH), 7.61 (3H, t, ³J = 7.7 Hz, Tol *m*-CH), 7.37 (3H, d, ³*J* = 7.5 Hz, Tol *p*-C*H*), 6.72 (3H, d, ⁴*J* = 2 Hz, *o*-C*H*), 6.31 (2H, t, ${}^{4}J = 2$ Hz, p-CH), 2.36 (9H, s, Tol CH₃); ${}^{13}C{}^{1}H{}$ (100 MHz, $(CD_3)_2$ SO, 25 °C): δ = 171.7 (COO), 160.1 (BiC), 158.2 (COH), 141.2 (CCOO), 134.4 (CCH₃), 133.4 (CH_{ar}), 131.8 (CH_{ar}), 131.2 (CHar), 130.3 (CHar), 107.6 (CHar), 106.0 (CHar), 21.5 (CH3); MS ESI^+ 208.99 [Bi], 391.00 [Bi(*m*-Tol)₂]⁺, 635.10 [Bi(*m*-Tol)₃(L)]⁺, 725.13 [BiL]²⁺; ESI⁻ 153.08 [L]⁻; IR 3511 (w), 3394 (w), 3157 (br), 2980 (w), 1569 (s), 1465 (m), 1465 (w), 1341 (sh), 1298 (s), 1282 (m), 1202 (w), 1155 (sh), 1097 (w), 1005 (sh), 979 (sh), 954 (w), 858 (sh), 811 (sh), 811 (sh), 785 (sh), 678 (sh); Elemental analysis [C35H31BiO8·2H2O·Et2O] (898.28) Calculated C 52.12 H 5.05 Found C 52.01 H 5.04.

Synthesis of tris-m-tolylbismuth bis(2-methoxybenzoate), 7. Bi(m-Tol)₃ (0.200 g, 0.41 mmol), 2-methoxybenzoic acid (0.126 g, 0.83 mmol) and 100 μ L 30% H₂O₂ were reacted in warm diethyl ether according to GP. Yield: 47.6% (0.153 g); mp. 145–146 °C; ¹H NMR (400 MHz, $(CD_3)_2SO$, 25 °C): $\delta = 8.08$ (3H, s, Tol *o*-C*H*), 8.00 (3H, d, ${}^{3}J$ = 8 Hz, Tol *o*-C*H*), 7.61 (3H, t, ³J = 7.7 Hz, Tol *m*-CH), 7.39 (7H, *m*, Tol *p*-CH + CH), 7.04 (2H, d, ${}^{3}J = 8$ Hz, CH), 6.91 (2H, t, ${}^{3}J = 7.4$ Hz, p-CH), 3.80 (6H, s, OCH₃), 2.37 (9H, s, Tol CH₃); ${}^{13}C{}^{1}H{}$ (100 MHz, (CD₃)₂SO, 25 °C): δ = 172.1 (COO), 159.6 (BiC), 157.6 (COCH₃), 149.7 (CCH3), 141.2 (CHar), 133.6 (CHar), 131.8 (CHar), 131.7 (CHar), 131.1 (CH_{ar}), 130.4 (CH_{ar}), 130.1 (CH_{ar}), 123.9 (CCOO), 120.0 (CH_{ar}), 112.5 (CH_{ar}), 55.8 (OCH₃), 21.5 (CH₃); MS ESI⁺ 208.99 [Bi], 391.00 $[Bi(m-Tol)_2]^+$, 633.14 $[Bi(m-Tol)_3(L)]^+$; ESI⁻ 151.10 [L]⁻, 693.00 [Bi(*m*-Tol)₂L₂]⁻; IR 3045 (w), 3001 (w), 2915 (w), 2835 (w), 1601 (sh), 1581 (sh), 1542 (sh), 1457 (m), 1435 (sh), 1352 (s), 1300 (w), 1268 (sh), 1249 (m), 1177 (sh), 1162 (sh), 1142 (sh), 1097 (w), 1021 (w), 981 (sh), 857 (sh), 809 (sh), 774 (sh), 750 (sh), 704 (sh), 659 (sh); Elemental analysis [C₃₇H₃₅BiO₆] (784.66) Calculated C 56.64 H 4.50 Found C 56.22 H 4.53.

Synthesis of tris-*m*-tolylbismuth bis(2-ethoxybenzoate), 8. Bi(m-Tol)₃ (0.200 g, 0.41 mmol), 2-ethoxybenzoic acid (0.125 µL, 0.83 mmol) and 100 µL 30% H₂O₂ were reacted in warm diethyl ether according to **GP**. Yield: 46.2% (0.154 g);

mp. 144–145 °C; ¹H NMR (400 MHz, (CD₃)₂SO, 25 °C): δ = 8.05 (3H, s, Tol *o*-C*H*), 8.01 (3H, d, ³*J* = 7.7 Hz, Tol *o*-C*H*), 7.60 (3H, t, ${}^{3}J = 7.8$ Hz, Tol *m*-CH),7.36 (7H, m, Tol *p*-CH + CH), 7.02 (2H, d, ${}^{3}J$ = 8.3 Hz, CH), 6.90 (2H, t, ${}^{3}J$ = 7.4 Hz, p-CH), 4.02 (4H, q, ${}^{3}J$ = 6.9 Hz, OCH₂CH₃), 2.35 (9H, s, Tol CH₃), 1.28 (6H, t, OCH₂CH₃); ${}^{13}C{}^{1}H{}$ (100 MHz, (CD₃)₂SO, 25 °C): δ = 172.4 (COO), 160.0 (BiC), 156.9 (i-Car), 141.0 (CCH₃), 133.5 (CHar), 131.7 (CHar), 131.6 (CHar), 131.0 (CHar), 130.4 (CHar), 130.1 (CH_{ar}), 124.2 (CCOO), 120.0 (CH_{ar}), 113.6 (CH_{ar}), 63.9 (OCH₂CH₃), 21.4 (CH₃), 14.7 (OCH₂CH₃); MS ESI⁺ 391.2 [Bi(m- Tol_{2}^{+} , 647.3 $[\text{Bi}(m\text{-Tol}_{3}(\text{L})]^{+}$, 835.4 $[\text{Bi}(m\text{-Tol}_{3}(\text{L})_{2} + \text{Na}]^{+}$; ESI⁻ 165.0 [L]⁻, 721.2 [Bi(*m*-Tol)₂L₂]⁻; IR 3052 (w), 2979 (w), 2921 (w), 1583 (sh), 1554 (sh), 1532 (w), 1472 (w), 1450 (sh), 1356 (s), 1292 (w), 1269 (sh), 1238 (s), 1164 (sh), 1146 (sh), 1113 (sh), 1035 (sh), 980 (sh), 925 (sh), 853 (sh), 811 (sh), 795 (sh), 754 (sh), 704 (m), 667(sh); Elemental analysis $[C_{39}H_{39}BiO_6]$ (812.72) Calculated C 57.64 H 4.84 Found C 57.11 H 4.92.

Synthesis of tris-m-tolyl-bismuth bis(4-nitrobenzoate), 9. $Bi(m-Tol)_3$ (0.200 g, 0.41 mmol), 4-nitrobenzoic acid (0.138 g, 0.83 mmol) and 100 μL 30% H_2O_2 were reacted in warm diethyl ether according to GP. Yield: 41.9% (0.140 g); mp. 170–172 °C; ¹H NMR (400 MHz, $(CD_3)_2$ SO, 25 °C): $\delta = 8.15$ (8H, br. dd, ³*J* = 8.6 Hz, ⁴*J* = 3.0 Hz, *o*-CH, *m*-CH), 8.02 (3H, s, Tol *o*-C*H*), 7.98 (3H, d, ${}^{3}J$ = 8.0 Hz, Tol *o*-C*H*), 7.65 (3H, t, ${}^{3}J$ = 7.7 Hz, Tol *m*-CH), 7.40 (3H, d, ³J = 7.5 Hz, Tol *p*-CH), 2.37 (9H, s, Tol CH₃); ${}^{13}C{}^{1}H{}$ (100 MHz, (CD₃)₂SO, 25 °C): δ = 158.3 (BiC), 149.5 (CNO₂), 147.6 (CCH₃), 141.7 (CCOO), 133.6 (CH_{ar}), 132.2 (CHar), 131.6 (CHar), 130.7 (CHar), 130.6 (CHar), 123.5 (CH_{ar}) , 21.5 (CH_3) ; MS ESI⁺ 209.0 [Bi], 391.1 $[Bi(m-Tol)_2]^+$, 648.3 $[Bi(m-Tol)_3(L)]^+$; ESI⁻ 166.0 $[L]^-$, 723.1 $[Bi(m-Tol)_2L_2]^-$; IR 2920 (w), 1622 (m), 1583 (sh), 1523 (sh), 1467 (w), 1406 (sh), 1331 (s), 1162 (w), 1130 (sh), 1099 (sh), 1011 (sh), 981 (sh), 876 (sh), 831 (sh), 777 (sh), 720 (sh), 676 (sh); Elemental analysis [C35H29BiN2O8] (814.60) Calculated C 51.61 H 3.59 N 3.44 Found C 51.18 H 3.31 N 3.29.

Synthesis of tris-m-tolylbismuth bis(2-{[3-trifluoromethyl]phenyl]amino}benzoate), 10. Bi(*m*-Tol)₃ (0.200 g, 0.41 mmol), flufenamic acid (0.233 g, 0.83 mmol) and 100 µL 30% H₂O₂ were reacted in warm diethyl ether according to GP. Yield: 66.4% (0.284 g); mp. 143-147 °C; ¹H NMR (400 MHz, (CD₃)₂SO, 25 °C): δ = 9.57 (2H, s, NH), 8.03 (3H, s, o-CH_{ar}), 7.97 (3H, d, J = 7.9 Hz, o-CH_{ar}), 7.88 (2H, d, J = 7.5 Hz, CH_{ar}), 7.59 $(3H, t, J = 7.7 \text{ Hz}, CH_{ar}), 7.49 (2H, t, J = 7.7 \text{ Hz}, CH_{ar}), 7.35$ (10H, m, CH_{ar}), 7.26 (5H, m, CH_{ar}), 6.85 (2H, t, CH_{ar}), 2.26 (9H, s, CH₃); ${}^{13}C{}^{1}H{}$ (100 MHz, (CD₃)₂SO, 25 °C): δ = 159.4 (BiC), 142.4 (C(NH), 141.6 (C(NH)), 133.6 (CH_{ar}), 132.5 (CH_{ar}), 132.0 (CHar), 131.4 (CHar), 130.5 (CHar), 130.4 (CHar), 130.3 (CHar), 130.1 (CH_{ar}), 124.9 (i- C_{ar}), 123.1 (i- C_{ar}), 122.5 (CH_{ar}), 119.2 (CH_{ar}), 117.9 (CH_{ar}), 115.2 (CH_{ar}), 21.2 (CH₃); MS ESI⁺ 209.0 [Bi], 391.2 $[Bi(m-Tol)_2]^+$, 762.3 $[Bi(m-Tol)_3L]^+$; ESI⁻ 236.0 [L-COO]⁻, 280.0 [L]⁻; IR 3256 (br), 3058 (w), 2922 (w), 1583 (sh), 1561 (m), 1513 (m), 1462 (sh), 1462 (w), 1352 (sh), 1333 (sh), 1272 (s), 1214 (m), 1160 (sh), 1116 (sh), 1067 (sh), 998 (sh), 979 (sh), 931 (sh), 902 (w), 860 (sh), 796 (sh), 748 (sh), 696 (sh), 665 (sh); Elemental analysis [C49H39BiF6N2O4·2H2O] (1078.28) Calculated C 54.55 H 4.02 N 2.60 Found C 54.72 H 3.65 N 2.71.

Synthesis of tris-m-tolylbismuth bis(2-(acetoxy)benzoate), 11. Bi(*m*-Tol)₃ (0.200 g, 0.41 mmol), aspirin (0.150 g, 0.83 mmol) and 100 µL 30% H₂O₂ were reacted in warm diethyl ether according to GP. Yield: 76.9% (0.265 g); mp. 129–132 °C; ¹H NMR (400 MHz, (CD₃)₂SO, 25 °C): δ = 7.92 (3H, s, Tol *o*-C*H*), 7.88 (3H, d, ³*I* = 7.8 Hz, Tol *o*-C*H*), 7.75 (2H, d, ${}^{3}J$ = 7.6 Hz, o-CH), 7.63 (3H, t, ${}^{3}J$ = 7.6 Hz, Tol m-CH), 7.52 $(2H, t, {}^{3}J = 7.7 \text{ Hz}, p\text{-}CH), 7.41 (3H, d, {}^{3}J = 7.6 \text{ Hz}, \text{Tol } p\text{-}CH),$ 7.29 (2H, t, ${}^{3}J$ = 7.3 Hz, asp *m*-C*H*), 7.08 (2H, d, ${}^{3}J$ = 7.9 Hz, *m*-CH), 2.38 (9H, s, Tol CH₃), 2.00 (6H, s, asp CH₃); ${}^{13}C_{1}^{1}H_{1}^{1}$ (150 MHz, (CD₃)₂SO, 25 °C): δ = 170.3 (COO), 169.1 (BiC), 159.3 (i-C_{ar}), 149.7 (i-C_{ar}), 141.6 (CCH₃), 133.4 (CH_{ar}), 132.7 (CH_{ar}), 131.9 (CHar), 131.4 (CHar), 131.3 (CHar), 130.3 (CHar), 126.6 (i-C_{ar}), 125.9 (CH_{ar}), 123.5 (CH_{ar}), 21.5 (CH₃), 20.7 (CH₃); MS ESI⁺ 209.0 [Bi], 391.2 $[Bi(m-Tol)_2]^+$, 661.3 $[Bi(m-Tol)_3L]^+$, 863.2 [Bi- $(m\text{-Tol})_{3}L_{2} + \text{Na}^{+}$; IR 2920 (w), 1755 (sh), 1624 (sh), 1592 (sh), 1553 (m), 1473 (m), 1448 (m), 1355 (sh), 1324 (sh), 1220 (sh), 1192 (sh), 1089 (sh), 1040 (w), 1012 (w), 980 (m), 954 (w), 919 (m), 878 (w), 850 (w), 809 (sh), 768 (sh), 748 (sh), 703 (sh), 672 (sh); Elemental Analysis [C39H35BiO8·2H2O] (876.23) Calculated C 53.43 H 4.48 Found C 53.00 H 4.04.

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