Electronic Supplementary Information

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

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Contents:

Experimental Details

General: Triphenylbismuth ($BiPh_3$) was synthesized through a standard Grignard metathesis reaction from the treatment of $BiCl_3$ with PhMgBr in dried THF (tetrahydrofuran) at 0 °C and subsequently recrystallized from ethanol. $Bi(O^tBu)_3$ was prepared according to a literature procedure in 75% yield.[1] The reaction with $Bi(O^tBu)_3$ was carried out in a Schlenk flask under inert N_2 atmosphere following standard Schlenk protocols. Toluene (obtained from solvent purification system) was removed under reduced pressure after the reaction had stirred overnight and the resulting solid residues were dried *in vacuo* for few hours. The products were isolated in an open atmosphere. Aspirin was purchased from Aldrich Chemical Co. and used without further purification.

Horse blood agar (HBA) and brain heart infusion broth (BHI) were obtained from Oxoid Australia Pty. Fetal calf serum (FCS) was purchased from Invitrogen. Polymyxin B, Vancomycin, Trimethoprim and Amphotericin B were purchased from Sigma, MO, USA. The physical properties of the complex prepared have been analysed using a variety of techniques to characterise its identity and purity. ^1H NMR spectra were recorded using either a Bruker DRX 400 MHz or Bruker DPX 300 MHz spectrometer as solutions in d_6-DMSO. Multiplicities are denoted as singlet (s), doublet (d), triplet (t), multiplet (m) and broad (b), or a combination Bruker where necessary. Coupling constants, J, are expressed in Hertz. ^{13}C NMR spectra were recorded using either a Bruker DRX 400 MHz or DPX 300 MHz spectrometer as solutions in d_6- DMSO. Infrared Spectra (IR) as pure solid were recorded on a Agilent Technologies Cary 630 FTIR. IR absorptions (ν_{max}) are reported in units of wavenumbers (cm^{-1}). Mass spectra were recorded on a Micromass Platform Electrospray mass spectrometer at cone voltages as specified using a DMSO/methanol or methanol solution as the mobile phase. The ion peaks (*m/z*) and their assignments are listed. Elemental Analysis was performed by the Campbell Microanalytical Laboratory, Department of Chemistry, University of Otago, New Zealand. Differential Scanning Calorimetry (DSC) was carried out using a TA Q100 DSC in a nitrogen atmosphere (50 ml/min) between 10 and 350°C with a temperature ramp rate of 5°C/min.

Bacterial Strains and culture conditions

H. pylori strains 251, B128 and 26695 were routinely cultured on HBA or in BHI, supplemented with either 7.5% (v/v) fresh horse blood or 10% (v/v) FCS, respectively.[2] Culture media were further supplemented with 155 mg L^{-1} polymyxin B, 6.25 mg L^{-1} vancomycin, 3.125 mg L^{-1} trimethoprim, and 1.25 mg L^{-1} amphotericin B.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the bismuth complexes was determined by the agar dilution technique. For this, *H. pylori*

cultures were incubated in BHI for 18 hours shaking at 140 rpm at 37°C under microaerobic conditions. Bacteria were pelleted, washed in phosphate-buffered saline, then re-suspended in BHI.[3] Each suspension was adjusted to give an approximate density of 106 bacteria ml^{-1}. Aliquots (10 ml) of these suspensions were then streaked onto HBA plates containing doubling dilutions of the different concentrations of bismuth compounds, ranging in concentration from 25 to 6.25 μg ml^{-1}. The compound was tested alongside the free ligand, aspirin, in comparable concentrations. The MICs were determined by examination of the plates after incubation for 3–5 days at 37 °C.

Synthesis

Synthesis of $[Bi(asp)_3]_\infty$ 1: Reaction of $Bi(O^tBu)_3$ (1.0 mmol, 0.48 g) with aspirin (*o*-acetoxy benzoic acid) (3.0 mmol, 0.54 g) was carried out in an inert atmosphere under Schlenk conditions in dry toluene as the solvent. The homogeneous reaction mixture was stirred at room temperature for 6-7 hours. Toluene was removed under reduced pressure or via cannulation and the solid residue washed with a small amount of dry ethanol to remove excess of ^tBuOH. The white solid was further dried under vacuum to give the title product **1** 0.52 g, 70%. Crystals suitable for X-ray diffraction were grown from the toluene mother liquor or an ethanol solution. M.p: 170-172°C. ^1H NMR (300 MHz, d_6-DMSO) δ (ppm) = 7.92 (3H, d, J = 7.8 Hz, Ar), 7.58 (3H, t, Ar), 7.36 (3H, t, Ar), 7.14 (3H, d, J = 8.1 Hz, Ar), 2.17 (9H, s, CH_3). ^{13}C NMR (75 MHz, d_6-DMSO) δ (ppm) = 171.0 (q, $COCH_3$) 169.2 (q, CO_2^-), 150.0 (q, $COCOCH_3$), 133.2 (Ar), 131.3 (Ar), 128.4 (q, CCO_2^-), 125.7 (Ar), 123.7 (Ar), 20.8 (CH_3). *m/z* (ESI+) 616.9 $[BiL_2(H_2O)(CH_3OH)]^+$, 634.8 $[BiL_3(H_2O)_2(CH_3OH)]^+$, 768.7 $[BiL_3Na]^+$, 818.7 $[BiL_3Na(H_2O)(CH_3OH)]^+$ (where L = $C_8H_7O_2CO_2^-$). ν_{max} (cm^{-1}): 1754 m, 1734 s, 1609 m, 1590 m, 1536 s, 1389 s, 1357 s, 1199 s, 1162 s, 1093 m, 1080 m, 1045 m, 1011 m. Anal. found; C 43.3, H 2.8; Calculated(%): C 43.4, H 2.8 .

Synthesis of $K[Bi(asp)_4]_\infty$ 2: To a solution of $Bi(OtBu)_3$ (0.48 g, 1mM) and $KOtBu$ (0.11 g, 1 mM) in dry toluene (8 mL) aspirin (*o*-acetoxy benzoic acid) (0.72 g, 4 mM) was added. The homogeneous light yellow solution was allowed to stir at room temperature for 16 hours after which a white precipitate was seen. Toluene was subsequently removed via cannulation and the white solid washed with a small amount of dry ethanol. The white solid was further dried under vacuum to give the title product **2** 0.68 g, 71%. Crystals suitable for X-ray diffraction were grown from an acetone, toluene or ethanol solution. M.p. 190-192 °C. ^1H MNR (400 MHz, d_6-DMSO) δ (ppm); 8.08 (4H, d, J = 8.0 Hz, Ar), 7.67 (4H, t, J = 8.0 Hz, Ar), 7.44 (4H, m, J = 8.0 Hz, Ar), 7.26 (4H, d, J = 8.0 Hz, Ar) 2.34 (12H, s, CH_3). ^{13}C NMR (75 MHz, d_6-DMSO) δ (ppm); 170.1 (q, $COCH_3$), 169.9 (q, CO_2^-), 150.0 (q, $COCOCH_3$), 131.6 (Ar), 130.9 (Ar), 129.2 (q, CCO_2^-), 124.8 (Ar), 123.1 (Ar), 20.4 (CH_3). *m/z* (ESI^+) 241.1

$[(LK)Na]^+$, 257.0 $[(LNa)NaMeOH]^+$, 297.1 $[(MK)(H_2O)(MeOH)]^+$, 661.1 $[(BiM_3)Na(H_2O)]^+$, 677.2 $[(BiM_3)K(H_2O)]^+$, 693.1 $[(BiM_3)Na(MeOH)(H_2O)]^+$, 857.2 $[(BiL_3)K(H_2O)_4]^+$, 1059.3 $[(BiL_3)(KL)Na(H_2O)_4]^+$, 1091.2 $[(BiL_3)(KL)Na(H_2O)_4MeOH]^+$ (where L= $C_8H_7O_2CO_2^-$; M= $C_6OH_5CO_2^-$) ν_{max} (cm^{-1}): 1767 m, 1734 s, 1609 m, 1598 m, 1529 m, 1389 s, 1369 s, 1246 s, 1238 s, 1197 s. Anal. Found(%); C 45.04, H 2.97; Calculated(%): C 44.82, H 2.93.

Crystallographic data:

Crystallographic data of compounds **1** and **2** were collected at the MX1 (**2**) and MX2 (**1**) beamlines at the Australian Synchrotron, Melbourne, Victoria, Australia with silicon monochromated MoK_α radiation (λ = 0.71070 Å). All data was collected at 100 K, maintained using an open flow of nitrogen. The software used for data collection and reduction of the data were BluIce[4] and XDS.[5] Compounds **1** and **2** were solved and refined with SHELX-97.[6] 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. For **2**, due to degrading crystals in the beam line the final data was a result of two individual data sets merged to give adequate completeness. The structures have been deposited with the Cambridge Crystallographic Database with reference numbers CCDC 920801 and 920802.

Table 1

Compound	**1**	**2**
Chemical formula	$C_{54}H_{42}Bi_2O_{24}$	$C_{36}H_{28}BiKO_{16}$
Formula Mass	1492.84	964.66
Crystal system	Monoclinic	Triclinic
a/Å	12.631(3)	11.243(2)
b/Å	18.890(4)	11.341(2)
c/Å	11.552(2)	16.047(3)
α/°	90.00	106.16(3)
β/°	109.82(3)	104.71(3)
γ/°	90.00	103.86(3)
Unit cell volume/$Å^3$	2592.9(9)	1791.8(6)
Temperature/K	173(2)	173(2)
Space group	*P2(1)/c*	*P*_1
No. of formula units per unit cell, *Z*	2	2
No. of reflections measured	48984	58724
No. of independent reflections	6938	8732
R_{int}	0.0421	0.0543
Final R_1 values ($I > 2\sigma(I)$)	0.0283	0.0256
Final $wR(F^2)$ values ($I > 2\sigma(I)$)	0.0700	0.0624
Final R_1 values (all data)	0.0303	0.0267
Final $wR(F^2)$ values (all data)	0.0712	0.0633

References

1. D. Mansfeld, *PhD thesis*, Technische Universität Chemnitz (Germany), 2009.

2. R. L. Ferrero, J.-M. Thiberge, M. Huerre and A. Labigne, *Infect. Immun.* 1998, **66**, 1349-1355.

3. J. Viala, F. G. Boneca, A. Cardona, S. E. Girardin, A. P. Moran, R. Athman, S. M'emet, M. R. Huerre, A. J. Coyle, P. S. DiStefano, P. J. Sansonetti, A. Labigne, J. Bertin, D. J. Philpott and R. L. Ferrero, *Nat. Immunol.* **2004**, *5*, 1166-1174.

4. T. M. McPhillips, S. E. McPhillips, H. J. Chiu, A. E. Cohen, A. M. Deacon, P. J. Allis, E, Garman, A. Gonzales, N. K. Sauter, R. P. Phizackerley, S. M. Soltis, and P. Kuhn, *Synchrotron Rad.* 2002, **9**, 401.

5. W. Kabsch, *J. Appl. Crystallogr.,* 1993, **26**, 112.

6. G. M. Sheldrick, *Acta. Cryst. Sect. A,* 2008, **64**, 112.

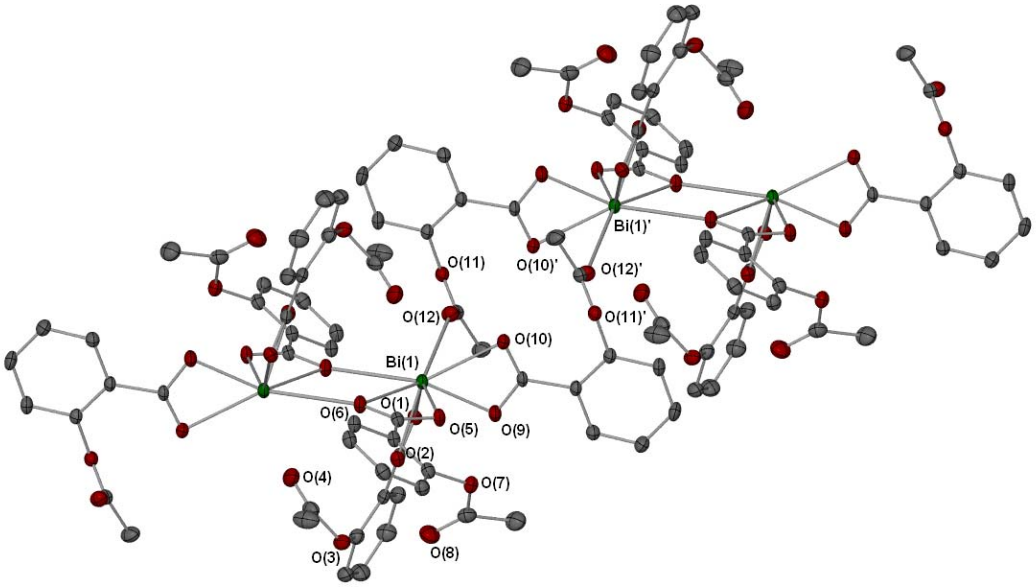


Figure S1 Extended polymeric structure of $[Bi(asp)_3]_\infty$ **1** with thermal ellipsoids at 50% probability. Hydrogen atoms have been omitted for clarity. Symmetry operations: ' = (-x, 1-y, 1-z).

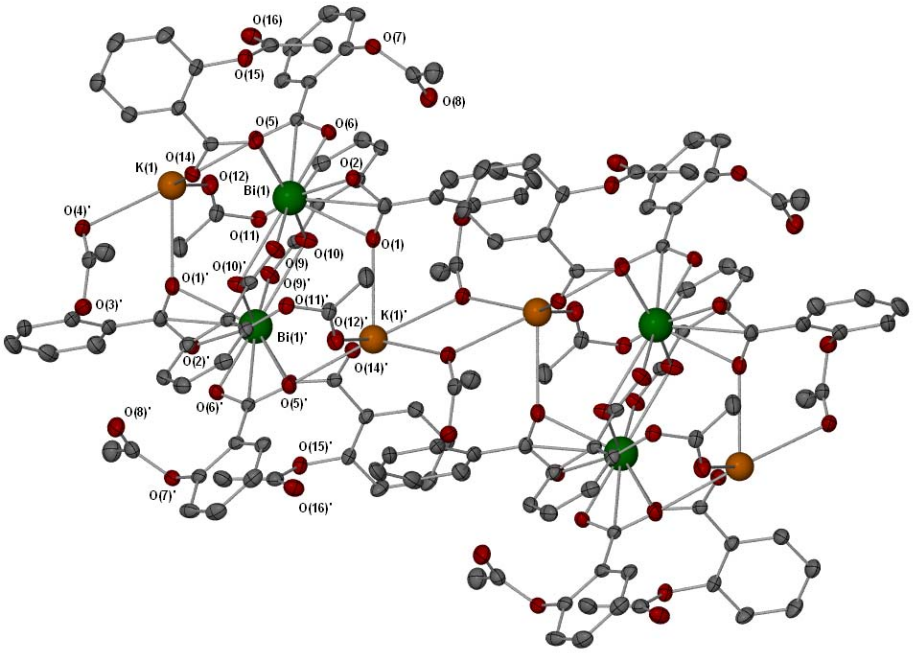


Figure S2 Extended polymeric structure of $[KBi(asp)_4]_\infty$ **2** with thermal ellipsoids at 50% probability (except bismuth and potassium). Hydrogen atoms have been omitted for clarity. Symmetry operation: ' = (1-x, 1-y, 1-z).

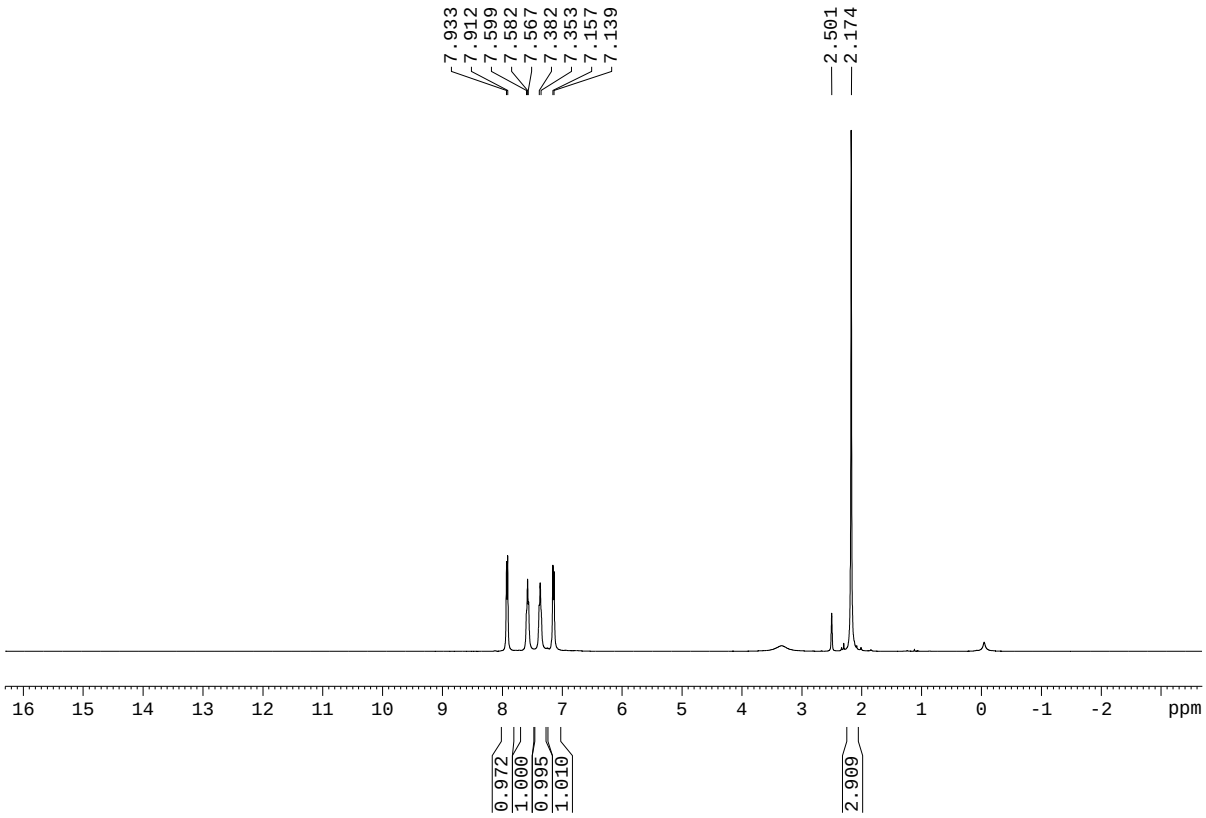


Figure S3 ^{1}H NMR spectrum of complex **1** $[Bi(asp)_3]_\infty$, ^{1}H NMR (300 MHz, d_6-DMSO) δ (ppm) = 7.92 (3H, d, J = 7.8 Hz, Ar), 7.58 (3H, t, Ar), 7.36 (3H, t, Ar), 7.14 (3H, d, J = 8.1 Hz, Ar), 2.17 (9H, s, CH_3).

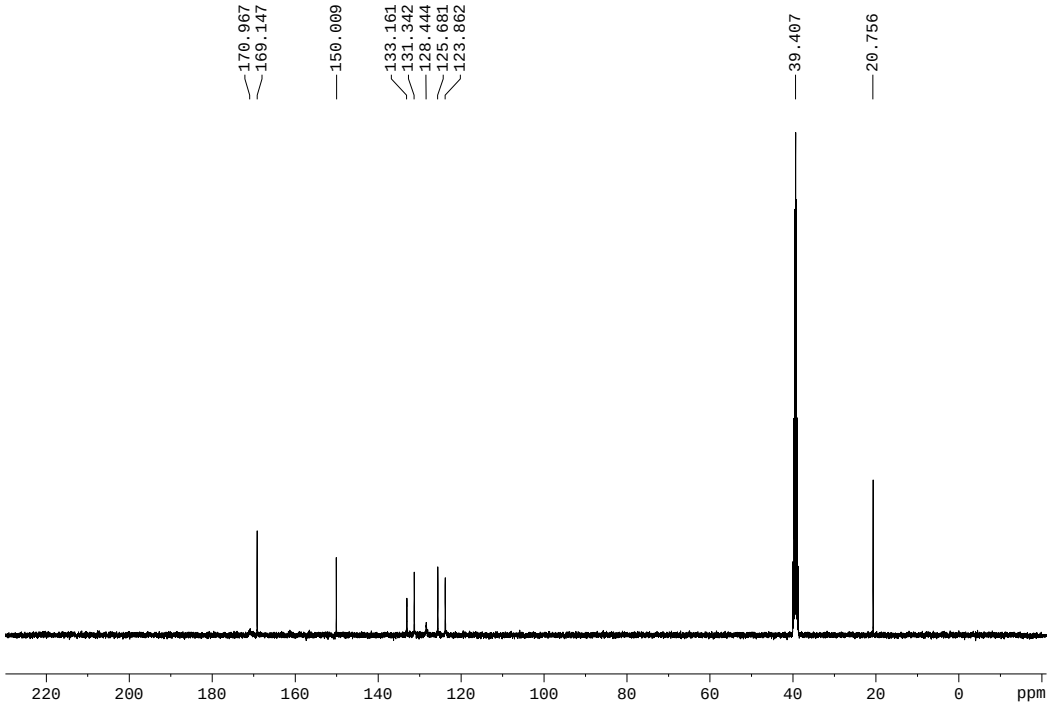


Figure S4 ^{13}C NMR spectrum of complex **1** $[Bi(asp)_3]_\infty$, ^{13}C NMR (75 MHz, d_6-DMSO) δ (ppm) = 171.0 (q, *C*OCH$_3$) 169.2 (q, *C*O$_2^-$), 150.0 (q, *C*OCOCH$_3$), 133.2 (Ar), 131.3 (Ar), 128.4 (q, *C*CO$_2^-$), 125.7 (Ar), 123.7 (Ar), 20.8 (*C*H$_3$).

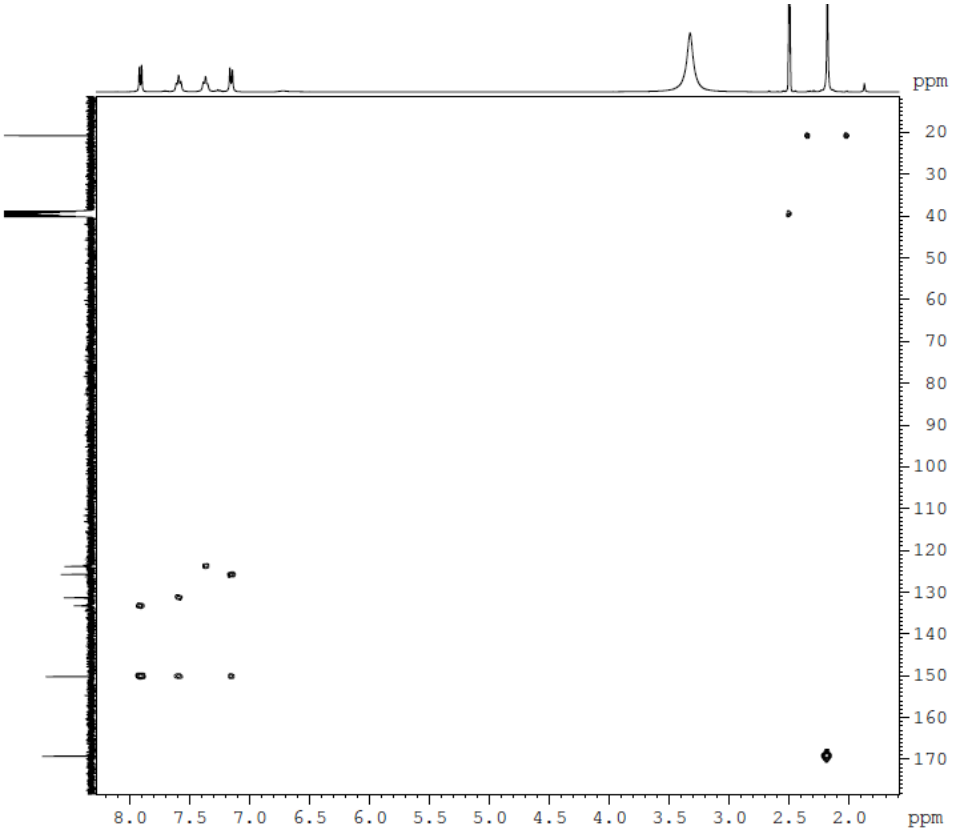


Figure S5 HMBC ^{1}H-^{13}C correlation spectrum of complex **1,** $[Bi(asp)_3]_\infty$

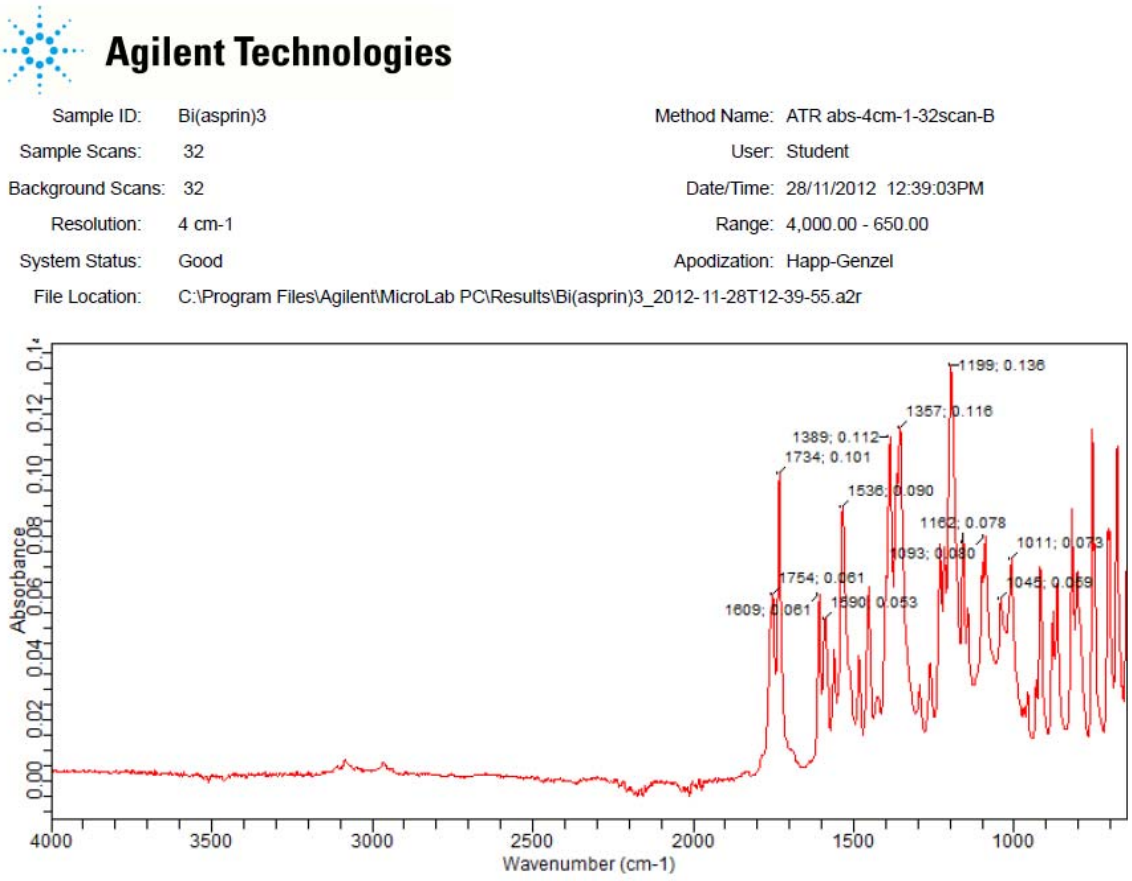


Figure S6 FT-IR spectrum of complex **1** $[Bi(asp)_3]_\infty$, ν_{max} (cm^{-1}): 1754 m, 1734 s, 1609 m, 1590 m, 1536 s, 1389 s, 1357 s, 1199 s, 1162 s, 1093 m, 1080 m, 1045 m, 1011 m.

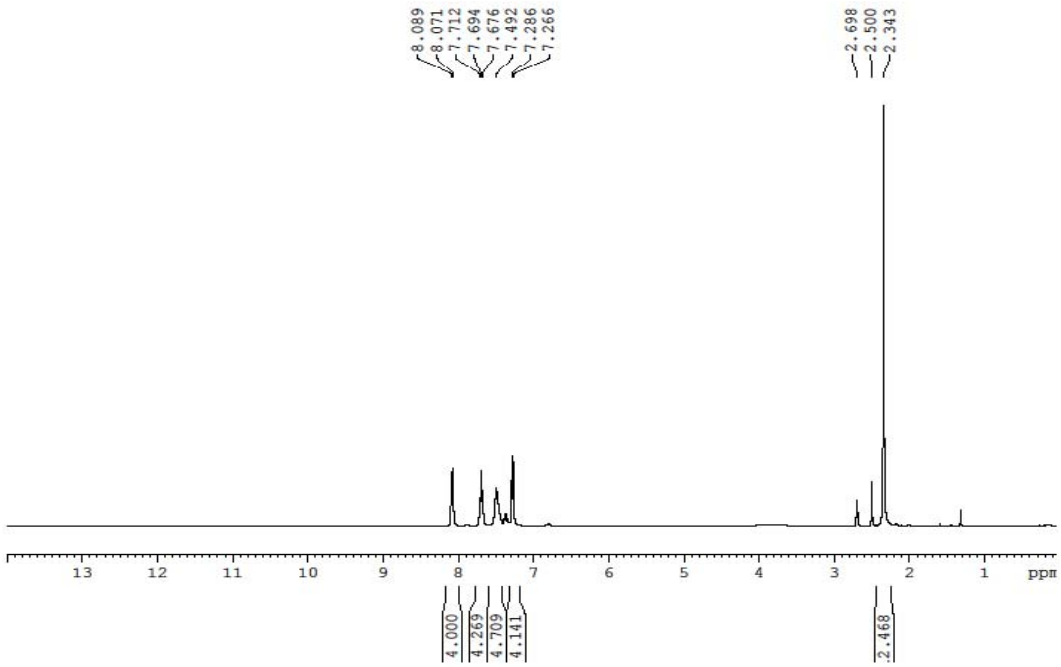


Figure S7 ^{1}H NMR spectrum of complex **2** $[KBi(asp)_4]_\infty$, trace toluene solvent present. ^{1}H MNR (400 MHz, d_6-DMSO) δ (ppm); 8.08 (4H, d, J = 8.0 Hz, Ar), 7.67 (4H, t, J = 8.0 Hz, Ar), 7.44 (4H, m, J = 8.0 Hz, Ar), 7.26 (4H, d, J = 8.0 Hz, Ar) 2.34 (12H, s, CH_3).

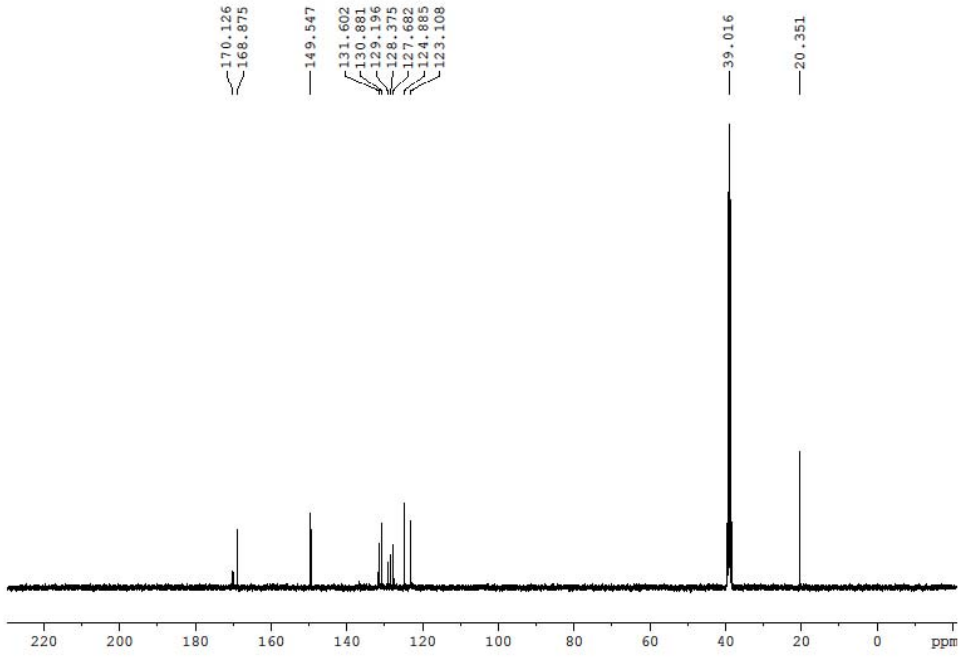


Figure S8 ^{13}C NMR spectrum of complex **2** $[KBi(asp)_4]_\infty$, trace toluene solvent present. ^{13}C NMR (75 MHz, d_6-DMSO) δ (ppm); 170.1 (q, *C*OCH_3), 169.9 (q, *C*O_2^-), 150.0 (q, *C*$OCOCH_3$), 131.6 (Ar), 130.9 (Ar), 129.2 (q, *C*CO_2^-), 124.8 (Ar), 123.1 (Ar), 20.4 (CH_3).

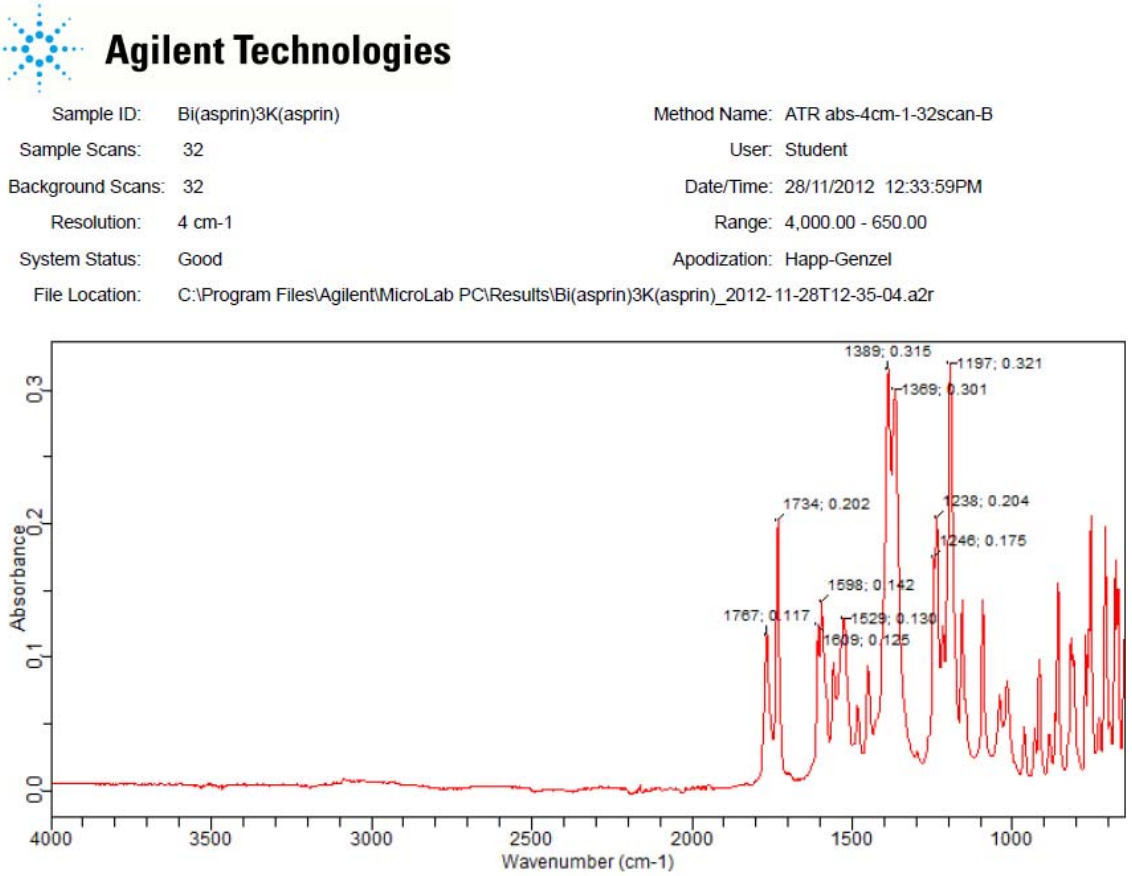


Figure S10 FT-IR spectrum of complex **2** $[KBi(asp)_4]_\infty$, ν_{max} (cm^{-1}): 1767 m, 1734 s, 1609 m, 1598 m, 1529 m, 1389 s, 1369 s, 1246 s, 1238 s, 1197 s.

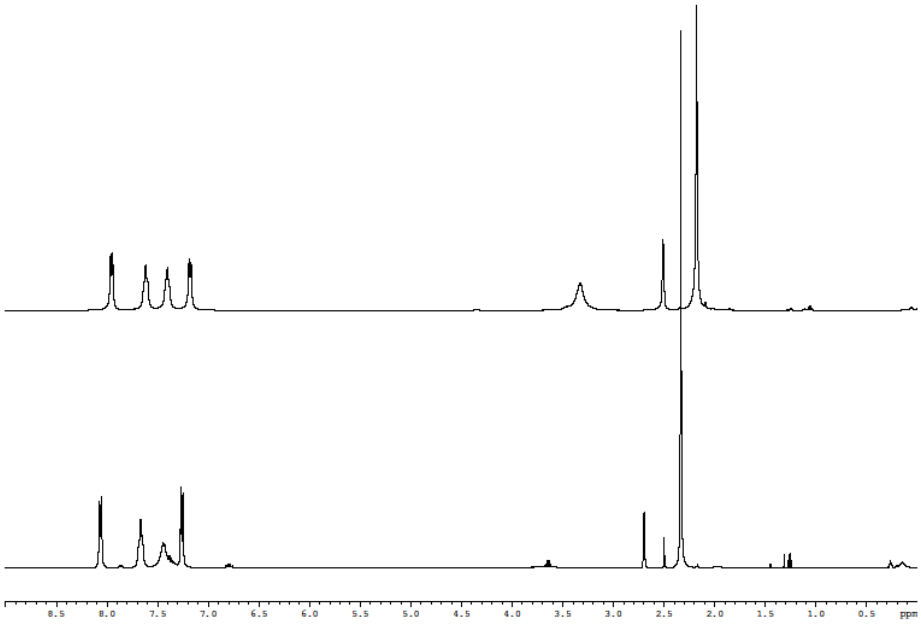


Figure S11 ^{1}H NMR comparison of complex **1** (top spectra) and complex **2** (bottom spectra).

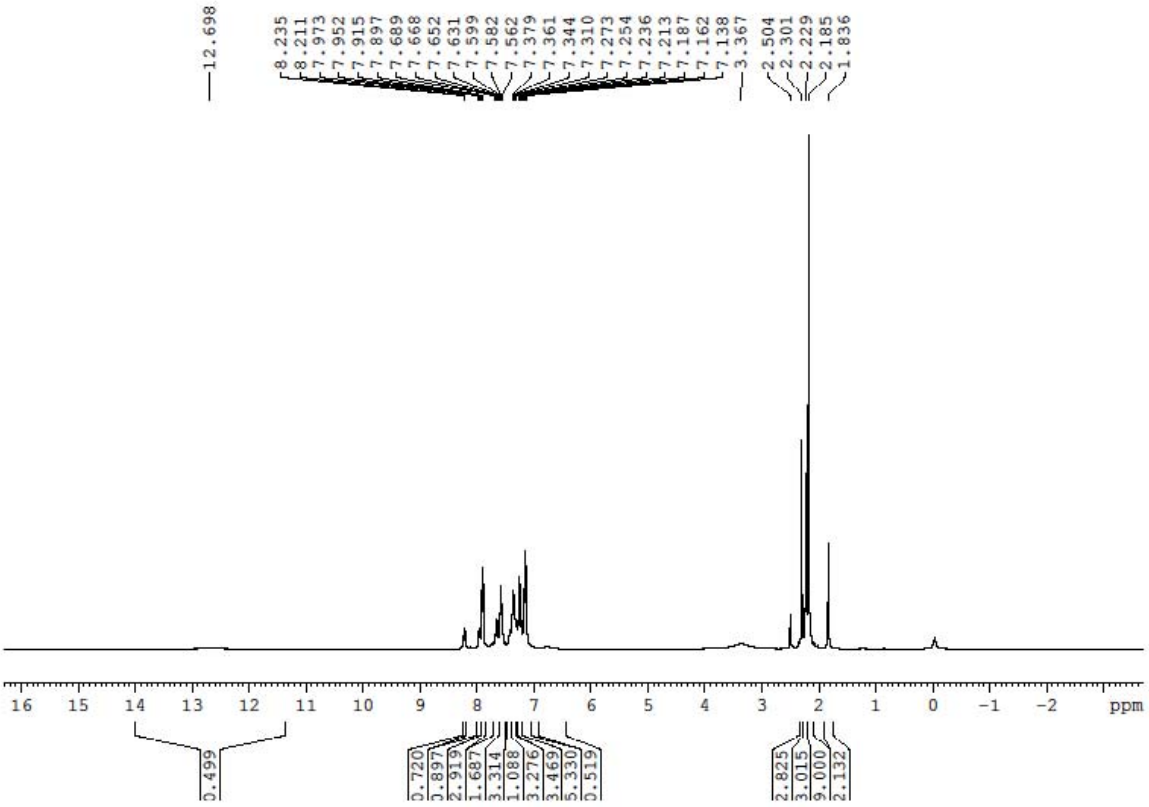


Figure S12 ^{1}H NMR spectrum of white solid from $BiPh_3$ + 3 aspirin under inert conditions (dry toluene, N_2 gas)

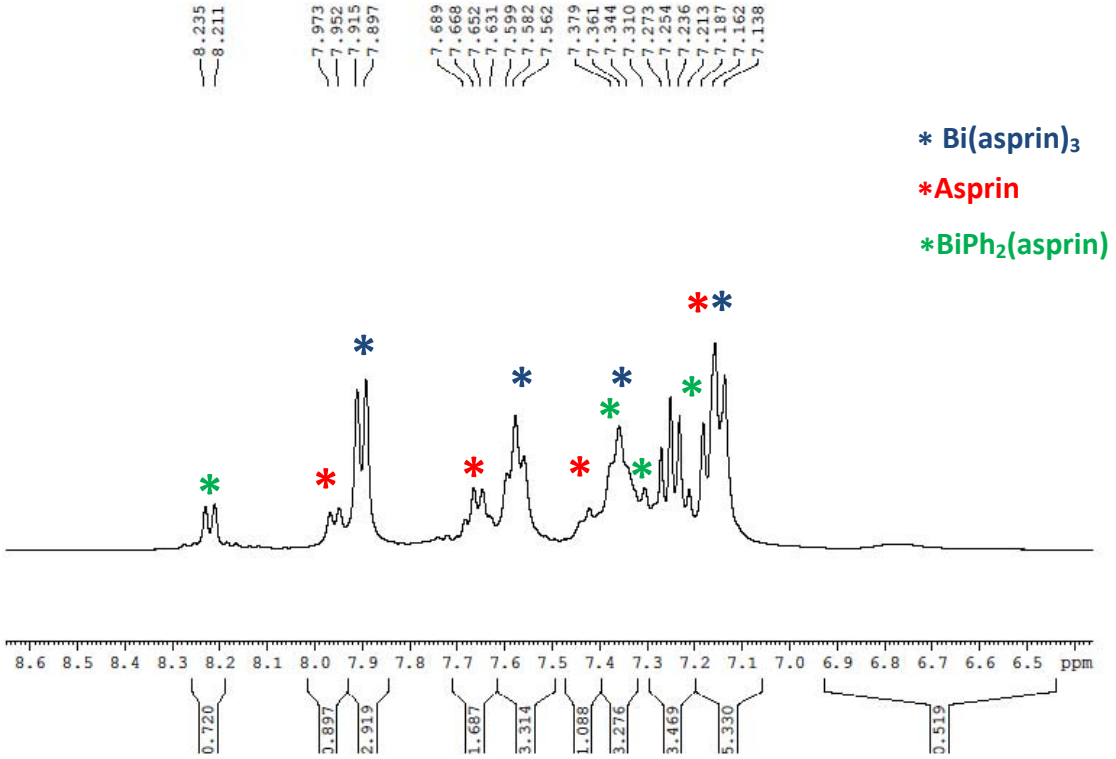
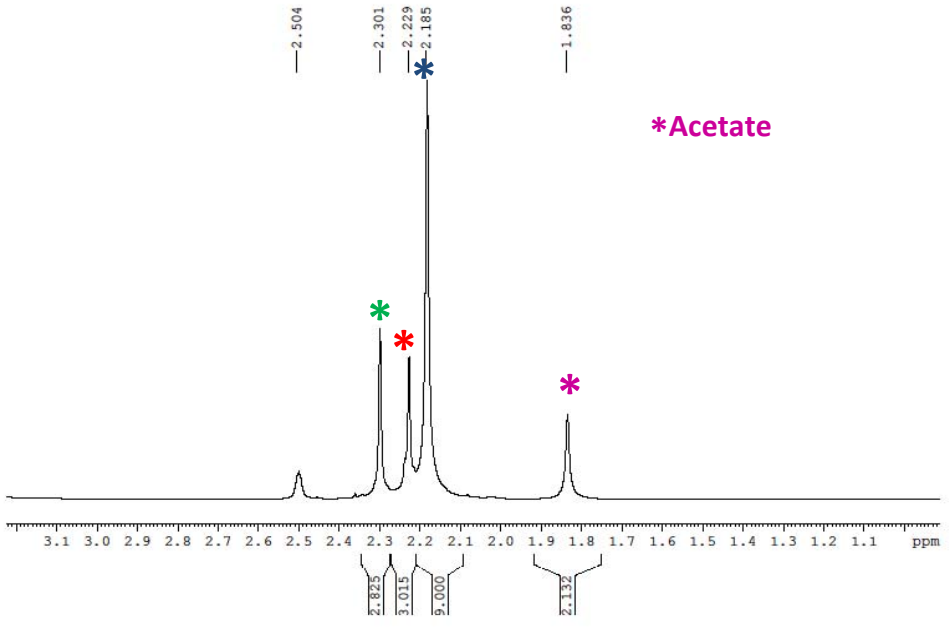


Figure S13 ^{1}H NMR spectrum of white solid from $BiPh_3$ + 3 aspirin in inert conditions enhanced aromatic region



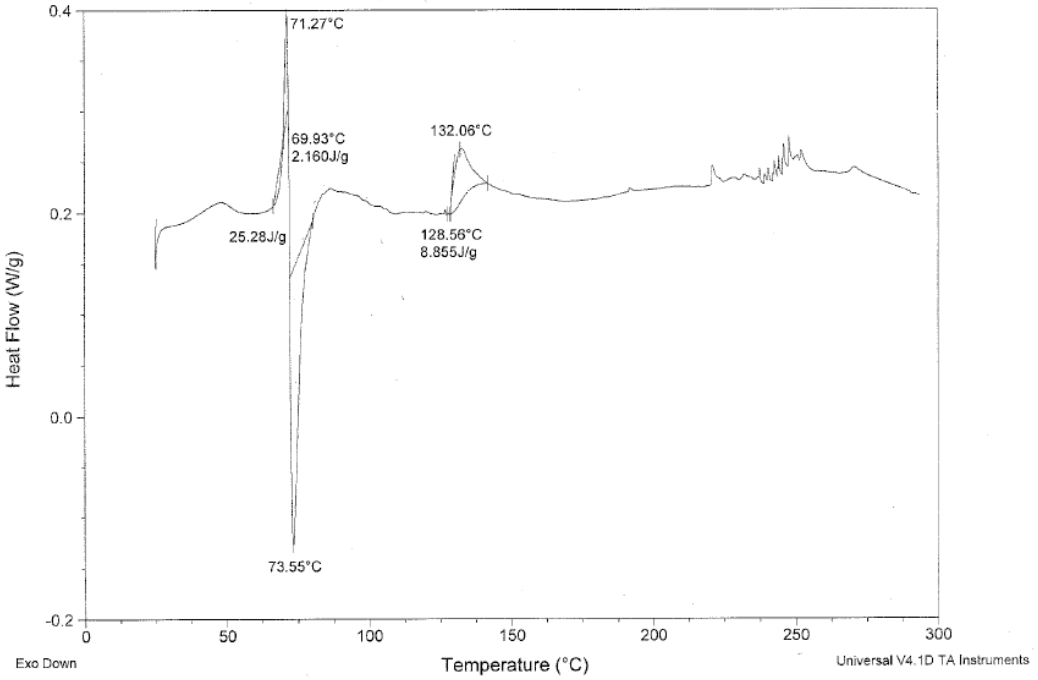


Figure S14 ^{1}H NMR spectrum of white solid from $BiPh_3$ + 3 aspirin under inert condition enhanced aliphatic region

Figure S15 DSC analysis of the solvent-free reaction of $BiPh_3$ with three equivalents of aspirin

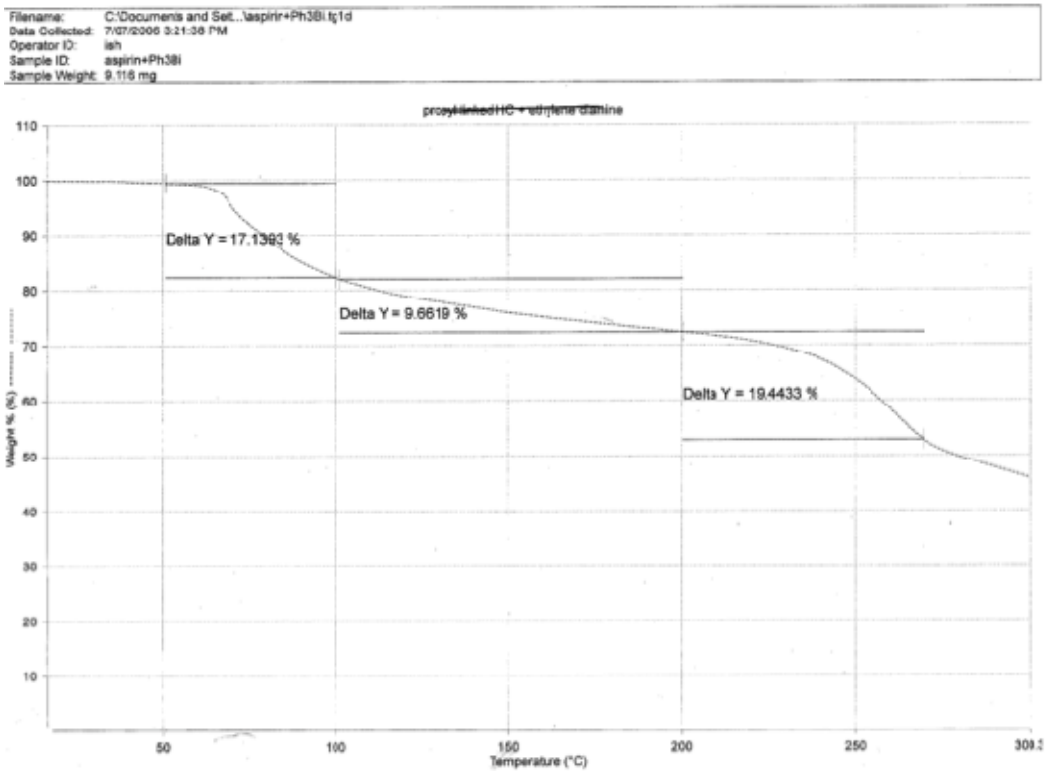


Figure S16 TGA analysis of the solvent-free reaction of $BiPh_3$ with three equivalents of aspirin