Tripodal molecules for the promotion of phosphoester hydrolysis

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Electronic Supplementary Information

Experimental

General remarks: All reactions were performed under slight positive pressure of nitrogen using oven-dried glassware. ^{1}H NMR (300 MHz) and ^{13}C{^{1}H} NMR (75 MHz) were determined on a Bruker AV300 spectrometer with the chemical shifts reported in parts per million (ppm), calibrated to the centre of the solvent peak set. The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Infrared (IR) spectra were recorded on a Matterson Satellite (ATR), and reported in wavenumbers (cm^{-1}). All solvents and starting materials were purchased from chemical stores where available. Low resolution mass spectra were recorded on a Micromass Platform II Single Quadrupole mass spectrometer. High resolution mass spectra were recorded on a VG 70-250-SE normal geometry double focusing mass spectrometer by the mass spectrometry service at the University of Southampton. Melting points were recorded in open capillaries on a Gallenkamp melting point apparatus and are uncorrected.

(2S,2'S,2''S)-N,N',N''-(2,2',2''-nitrilotris(ethane-2,1-diyl))tris(2-acetamido-3-(1H-imidazol-4-yl)propanamide) (1) A dry solution of 2-(1H-Benzotriazole-1-yl)-1,1,3,3-Tetramethyluronium hexafluorophosphate (HBTU) (1.22 g, 3.22 mM), *N*-acetyl-L-histidine monohydrate (0.63 g, 2.93 mM) and *N,N*-diisopropylethylamine (DIPEA) (0.85 mL, 4.88 mM) in DMF (8 mL) was stirred at 40 ºC for 2.5 h. Tris(2-aminoethyl)amine (TREN) (0.14 mL, 0.98 mM) was then added dropwise and the solution allowed to stir overnight at 40 ºC. The resultant solution was then taken to dryness and the resultant oil dissolved in methanol (10 mL) and purified by SCX-2 flash chromatography, firstly with methanol, and secondly with a 7 mol/l ammonia in methanol solution. This basic portion was taken to dryness. The crude product was purified by flash chromatography (1:1) methanol:chloroform followed by (97:3) methanol:ammonia. This resulted in a white solid. Yield 61 % (0.41 g, 0.60 mM); mp: 122 ºC; ^{1}H NMR (300 MHz, DMSO-d_6): δ: 1.80 (s, 9H), 2.36-2.41 (br m, 6H), 2.72 (dd, J_1 = 8.67 Hz, J_2 = 14.31 Hz, 3H), 2.89 (dd, J_1 = 4.89 Hz, J_2 = 14.31 Hz, 3H), 3.03-3.05 (br m, 6H), 4.42 (dd, J_1 = 8.64 Hz, J_2 = 13.92 Hz, 3H), 6.76 (s, 3H), 7.53 (s, 3H), 7.80 (s, 3H, amide NH), 8.13 (d, J_1 = 7.92 Hz, 3H, amide NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ: 22.6 (CH_3), 29.5 (CH_2), 37.0 (CH_2), 53.0 (CH), 53.2 (CH_2), 117.2 (ArCH), 133.3 (ArC), 134.6 (ArCH), 169.3 (CO), 171.3 (CO); IR (film): ν = broad 3260, 3170, 3080 (amide NH stretching), 1620, 1540 (amide CO stretching); LRMS (ESI$^+$): m/z: 706 [M+Na]$^+$; HRMS (ESI$^+$): m/z: act: 706.3502 [M+Na]$^+$ cal: 706.3508 [M+Na]$^+$.

Tert-butyl 2-(bis(2-(2-methoxyacetamido)ethyl)amino)ethylcarbamate Methoxyacetic acid (0.58 g, 6.42 mM) was heated at reflux with carbonyldiimidazole (CDI) (1.15 g, 7.06 mM) in chloroform (40 mL) for 2 hrs. Tert-butyl 2-(bis(2-aminoethyl)amino)ethylcarbamate[1] (0.79 g, 3.21 mM) in chloroform (2 mL) was then added to the solution and the mixture heated at reflux overnight. The solution was then allowed to cool to room temperature and washed with water (2 x 50 mL). The organic phase was dried with $MgSO_4$ and taken to dryness. The crude oil was then dissolved in methanol (3 mL) and further purified by SCX-2 flash chromatography with methanol followed by ammonia in methanol (7 mol/l). The ammonia methanol fraction was taken to dryness to give the product as a viscous light yellow oil. Yield 83 % (1.05 g, 2.68 mM); ^{1}H NMR (300 MHz, DMSO-d_6): δ: 1.36 (s, 9H), 2.44-2.52 (m, 6H), 2.94 (dd, J_1 = 6.21 Hz, J_2 = 12.45 Hz, 2H), 3.13 (dd, J_1 = 6.24 Hz, J_2 = 12.45 Hz, 4H), 3.29 (s, 6H), 3.77 (s, 4H), 6.57 (t, J_1 = 5.10 Hz, 1H, amide NH), 7.64 (t, J_1 = 5.13 Hz, 2H, amide NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ: 28.2 (CH_3), 36.4 (CH_2), 38.3 (CH_2), 53.1 (CH_2), 53.4 (CH_2), 58.6 (CH_3), 71.5 (CH_2), 77.5 (C), 155.6 (CO), 168.8 (CO); IR (film): ν = broad 3320 (amide NH stretching), 1660, 1530 (amide CO stretching); LRMS (ESI$^+$): m/z: 413 [M+Na]$^+$; HRMS (ESI$^+$): m/z: act: 413.2365 [M+Na]$^+$ cal: 413.2371 [M+Na]$^+$.

(S)-N,N'-(2,2'-(2-(2-acetamido-3-(1H-imidazol-4-yl)propanamido)ethylazanediyl)bis(ethane-2,1-diyl))bis(2-methoxyacetamide) (2) A solution of *tert*-butyl 2-(bis(2-(2-methoxyacetamido)ethyl)amino)ethylcarbamate (1.05 g, 2.68 mM) in dioxane/HCl (4 M) (5 mL) for 5 mins at

room temperature, the dioxane/HCl was then removed under reduced pressure and the process repeated with the solution left to stir overnight at room temperature. The resulting amine was taken straight on to the next step of the reaction without any further purification.
A dry solution of HBTU (1.12 g, 2.95 mM), *N*-acetyl-L-histidine monohydrate (0.58 g, 2.68 mM) and DIPEA (0.93 mL, 5.36 mM) in DMF (8 mL) was stirred at 40 ºC for two and a half hours. Tert-butyl 2-(bis(2-aminoethyl)amino)ethylcarbamate (0.66 g, 2.68 mM) in dry DMF (1 mL) was then added drop wise and the solution allowed to stir overnight at 40 ºC. The resultant solution was then taken to dryness and the resultant oil dissolved in methanol (10 mL) and purified by SCX-2 flash chromatography, firstly with methanol, and secondly with a 7 mol/l ammonia in methanol solution. This basic portion was taken to dryness. The crude product was purified by flash chromatography (1:1) methanol:chloroform. This resulted in a viscous light yellow semi-solid. Yield 56 % (0.70 g, 1.50 mM); ^{1}H NMR (300 MHz, DMSO-d_6): δ: 1.81 (s, 3H), 2.43-2.53 (m, 6H), 2.72 (dd, J_1 = 8.40 Hz, J_2 = 14.61 Hz, 1H), 2.89 (dd, J_1 = 5.10 Hz, J_2 = 14.61 Hz, 1H), 3.07-3.17 (m, 6H), 3.29 (s, 6H), 3.79 (s, 4H), 4.40 (, J_1 = 5.49 Hz, J_2 = 8.07 Hz, 1H), 6.74 (s, 1H), 7.50 (s, 1H), 7.66-7.44 (m, 3H, amide NH), 8.02 (d, J_1 = 8.04 Hz, 1H, amide NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ: 22.6 (CH_3), 29.6 (CH_2), 36.5 (CH_2), 37.0 (CH_2), 52.9 (CH), 53.0 (CH_2), 58.6 (CH_3), 71.5 (CH_2), 116.8 (ArCH), 133.9 (ArC), 134.6 (ArCH), 169.0 (CO), 169.2 (CO), 171.2 (CO) (missing CH_2 due to overlapping signals); IR (film): ν = broad 3280, 3090 (amide NH stretching), 1640, 1520 (amide CO stretching); LRMS (ESI$^+$): m/z: 492 $[M+Na]^+$; HRMS (ESI$^+$): m/z: act: 492.2537 $[M+Na]^+$ cal: 492.2541 $[M+Na]^+$.

N,N',N''-(2,2',2''-nitrilotris(ethane-2,1-diyl))tris(2-methoxyacetamide) (3) A solution of CDI (1.46 g, 9.00 mM) and methoxyacetic acid (0.64 mL, 8.25 mM) was heated to reflux in chloroform (20 mL) for 1 hr. TREN (0.38 mL, 2.5 mM) was then added to the solution and the mixture heated at reflux over night. The solution was then taken to dryness and the crude oil purified by flash chromatography methanol:dichloromethane (1:49) and further purified by SCX-2 flash chromatography with methanol followed by ammonia in methanol (7 mol/l). Ethanol was then added to the ammonia in methanol fractions, the white solid that formed was removed by filtration and the filtrate that formed taken to dryness. This resulted in a viscous light yellow semi-solid. Yield 22% (0.20 g, 0.54 mM); ^{1}H NMR (400 MHz, DMSO-d_6): δ: 2.47-2.52 (m, 6H), 3.12 (dd, J_1 = 6.04 Hz, J_2 = 12.64 Hz, 6H), 3.27 (s, 9H), 3.76 (s, 6H), 7.59 (t, J_1 = 5.56 Hz, 3H, amide NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ: 36.3 (CH_2), 52.9 (CH_2), 58.5 (CH_3), 71.5 (CH_2), 168.8 (CO); IR (film): ν = broad 3304 (amide NH stretching), 1650, 1528 (amide CO stretching); LRMS (ESI$^+$): m/z: 385 $[M+Na]^+$; HRMS (ESI$^+$): m/z: act: 363.2235 $[M+H]^+$ cal: 363.2235 $[M+H]^+$.

UV-Vis studies: Kinetic data was obtained using a Varian Cary 50 Bio UV-Visible spectrophotometer. Stock Bis-Tris (20 mM) buffered aqueous solutions were prepared, calibrated to either pH 6.1 or 7.1 with solutions of HCl or NaOH. 5 mM substrate solutions were then prepared using the 20 mM buffered aqueous stock solutions. Compounds **1-3** were then added to 3 mL aliquots of the substrate (5 mM), buffered aqueous solutions in 20, 50 and 100 mol-% ratios to the substrate. Imidazole (**4**) was added to separate 3 mL aliquots of buffered substrate solutions in 60, 150 and 300 mol-% ratio to the substrate. Aliquots of 250 µL were then taken from the individual reaction mixtures at specific time points and a UV-Vis spectrum recorded.

^{31}P NMR studies: The chemical warfare agent (CWA) soman (GD) was synthesised in-house at Dstl and purity confirmed using standard methods. Deuterated solvents were purchased from Goss Scientific (UK) and used as received. Buffer solutions (20 mM) were prepared by dissolving bis-tris (0.042 g) in D_2O (9 ml) and adjusting the pD to 6.5 using DCl (10%) in D_2O. The final solution volume was made up to 10 ml using D_2O. Reaction solutions were prepared by addition of GD (2 µl, 1.1 x 10^{-5} mols) to a sample of the buffer solution (2 ml, [GD] = 5.6 mM), and a subsequent aliquot (600 µl) of this solution was transferred to an NMR tube for analysis. For hydrolytic reaction solutions, compound **1** (7.7 mg, 1.1 x 10-5 mols) was fully dissolved in the buffer solution (2ml, [compound **1**] = 5.6 mM) prior to addition of GD.

^{1}H NMR titrations: 1.5 mL of a 0.01 M solution of compound **3** was prepared. Of this solution, 0.5 mL was added to the NMR tube, which was then sealed with an air tight suba seal. The remaining 1 mL of the receptor solution was used to make a 0.15 M solution of the phosphonate. This mixture was then added in aliquots to the NMR tube, with a ^{1}H NMR taken after the addition of each aliquot of phosphonate solution. Chemical shifts for each ^{1}H NMR spectra were recorded in ppm and calibrated to the solvent peak set. The computer program WINEQNMR2[2] was then used to interpret the data to determine binding constants.

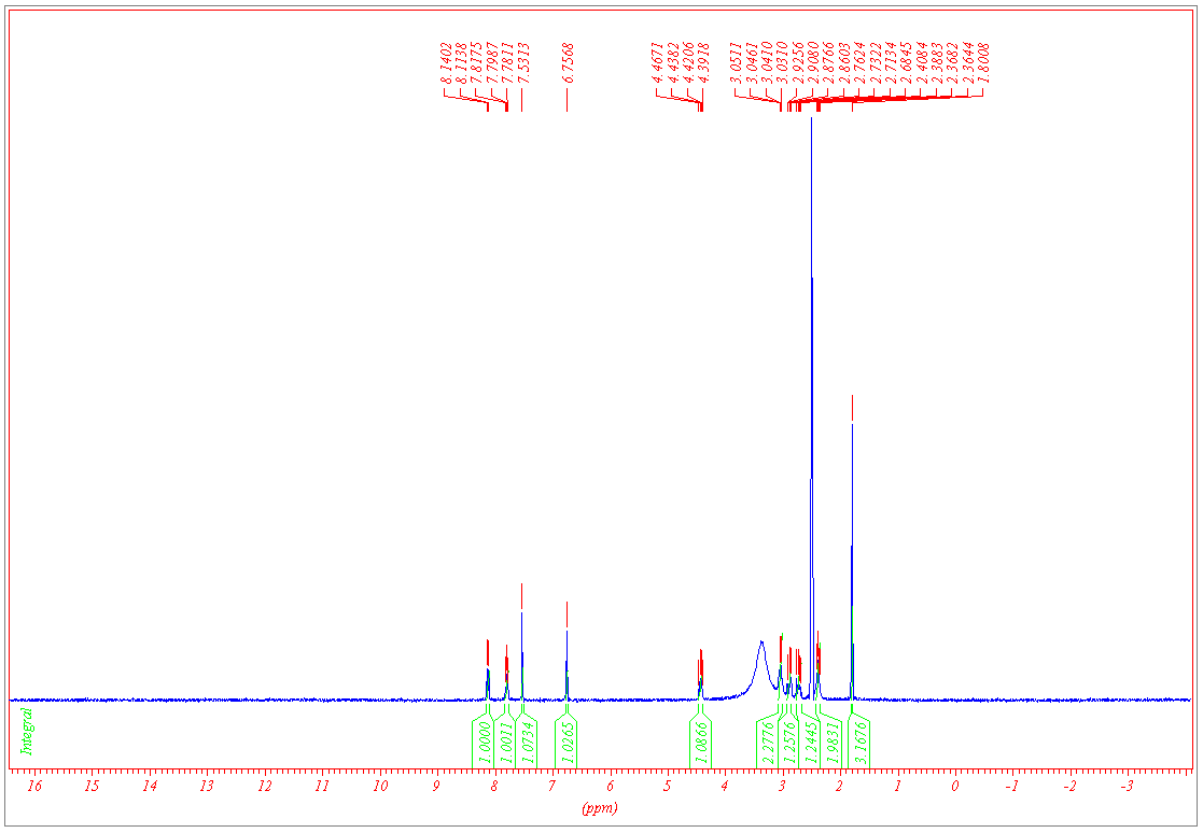


Figure S1 ^{1}H NMR spectrum of compound **1** in DMSO-d_6.

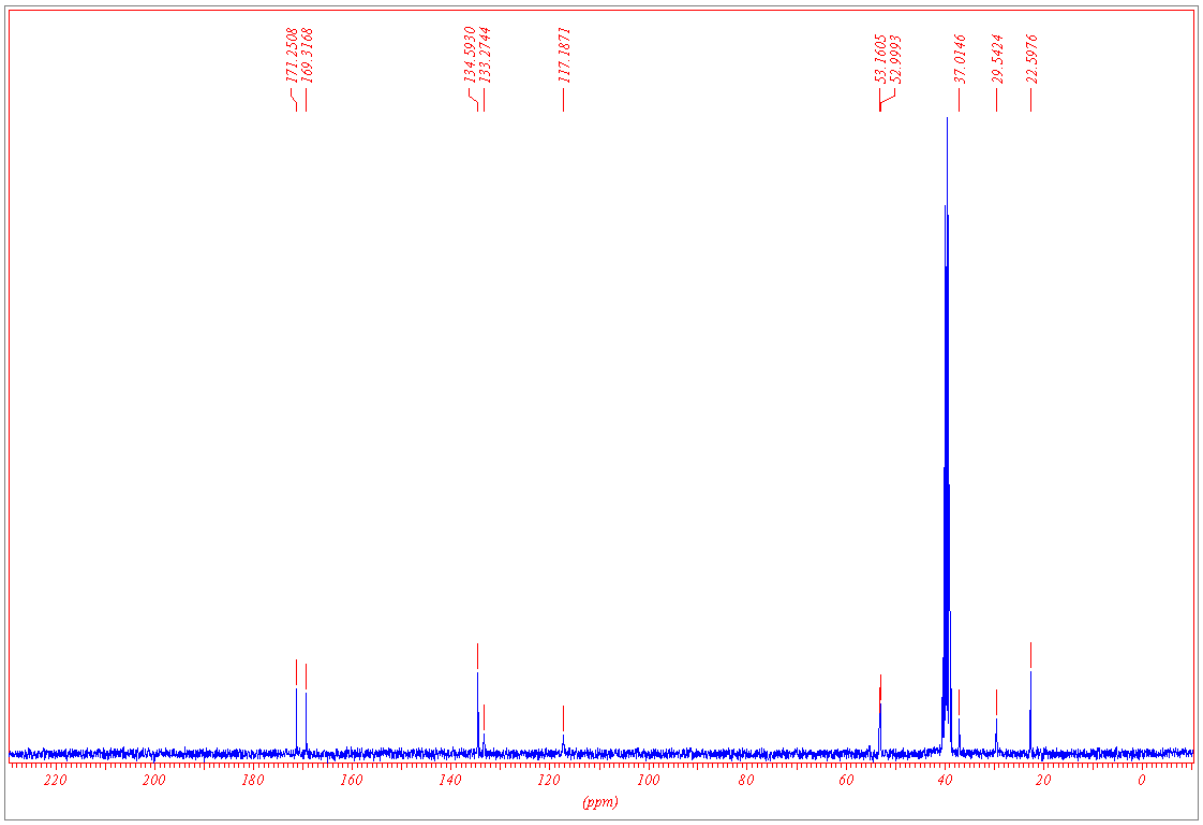


Figure S2 ^{13}C NMR spectrum of compound **1** in DMSO-d_6.

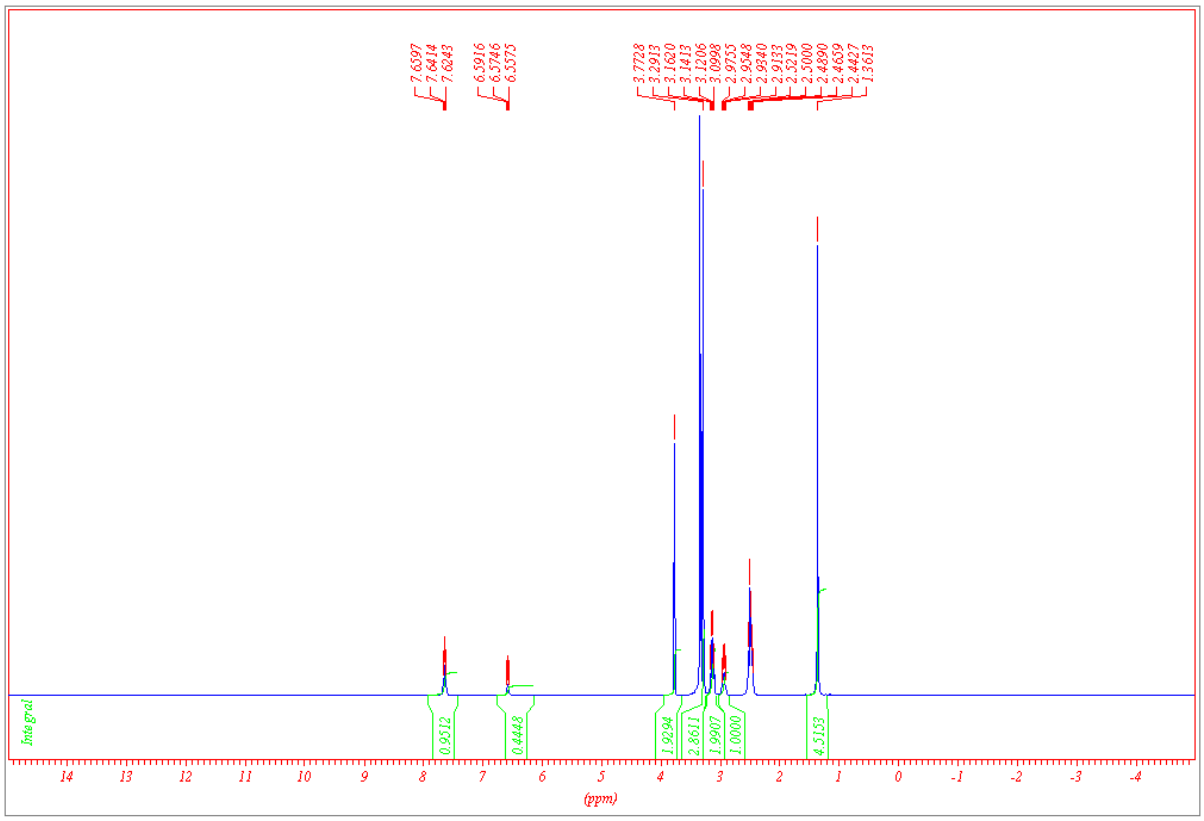


Figure S3 ^{1}H NMR spectrum of compound Tert-butyl 2-(bis(2-(2-methoxyacetamido)ethyl)amino) ethylcarbamate in DMSO-d_6.

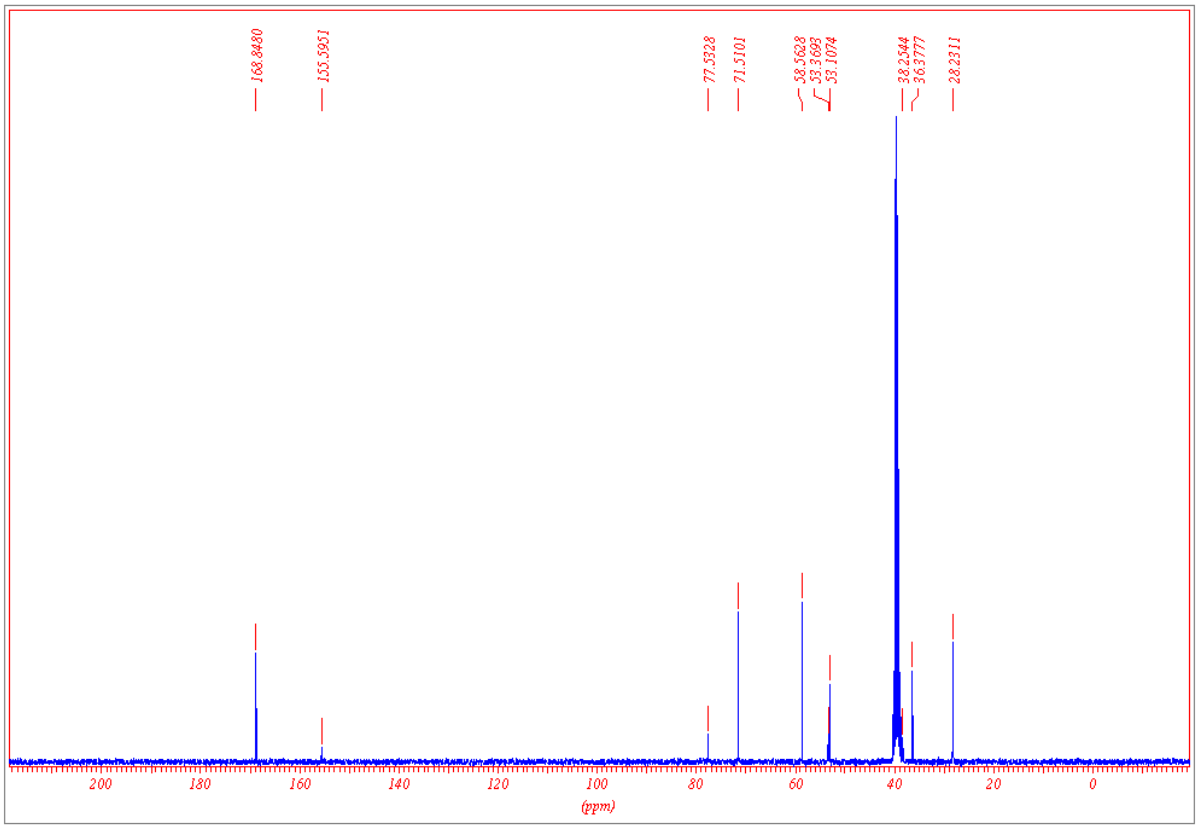


Figure S4 ^{13}C NMR spectrum of compound tert-butyl 2-(bis(2-(2-methoxyacetamido)ethyl)amino) ethylcarbamate in DMSO-d_6.

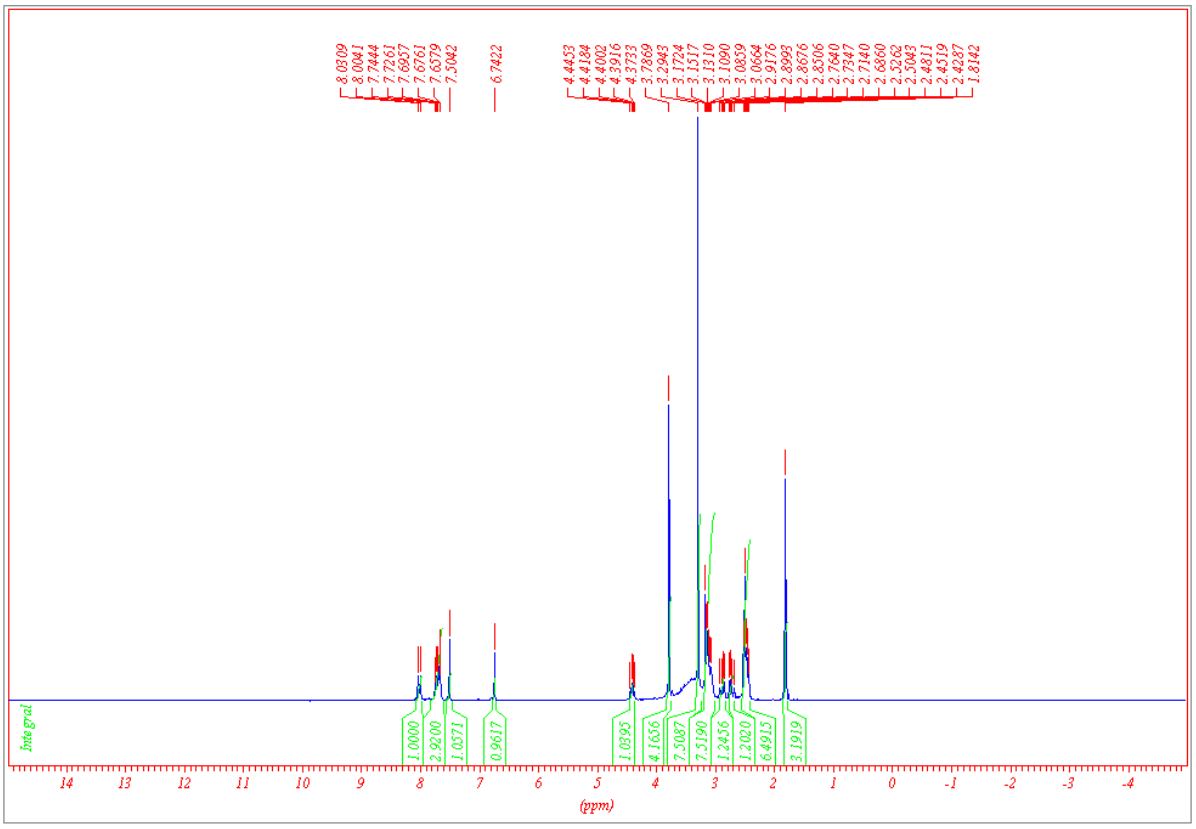


Figure S5 ^{1}H NMR spectrum of compound **2** in DMSO-d_6.

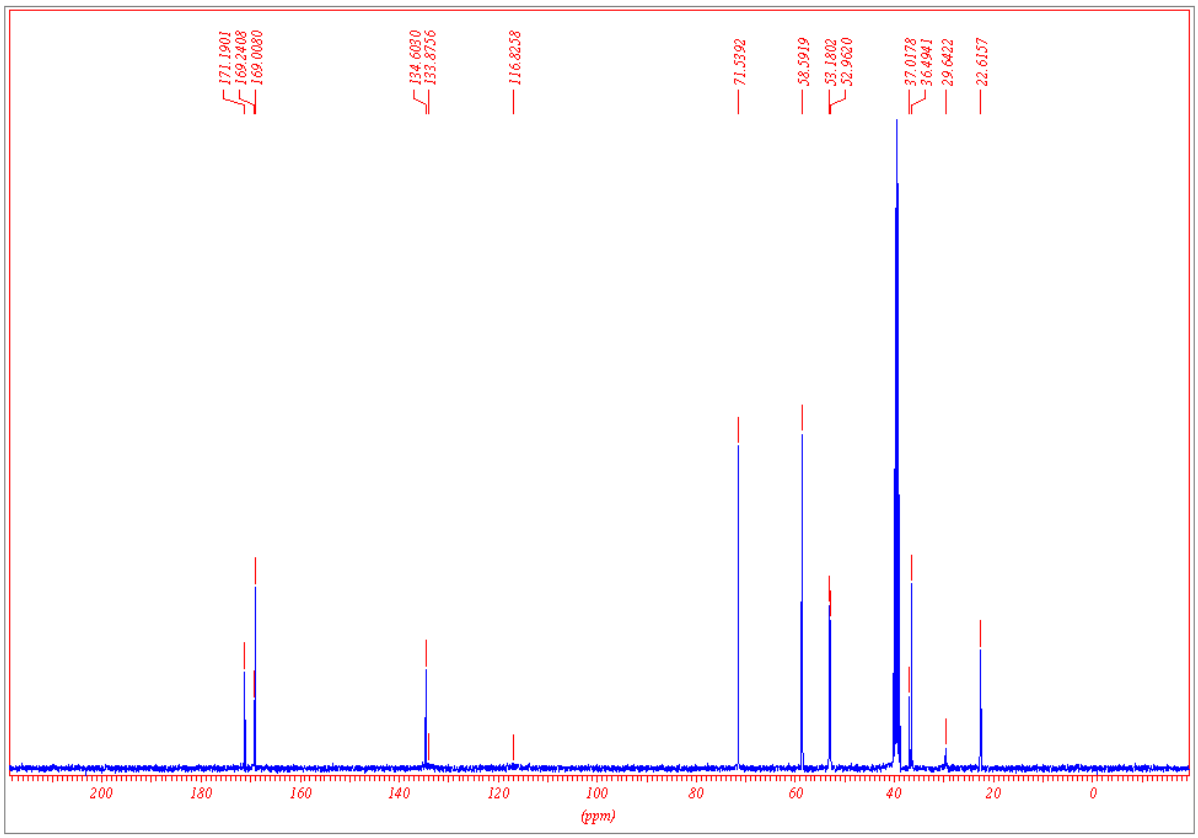


Figure S6 ^{13}C NMR spectrum of compound **2** in DMSO-d_6.

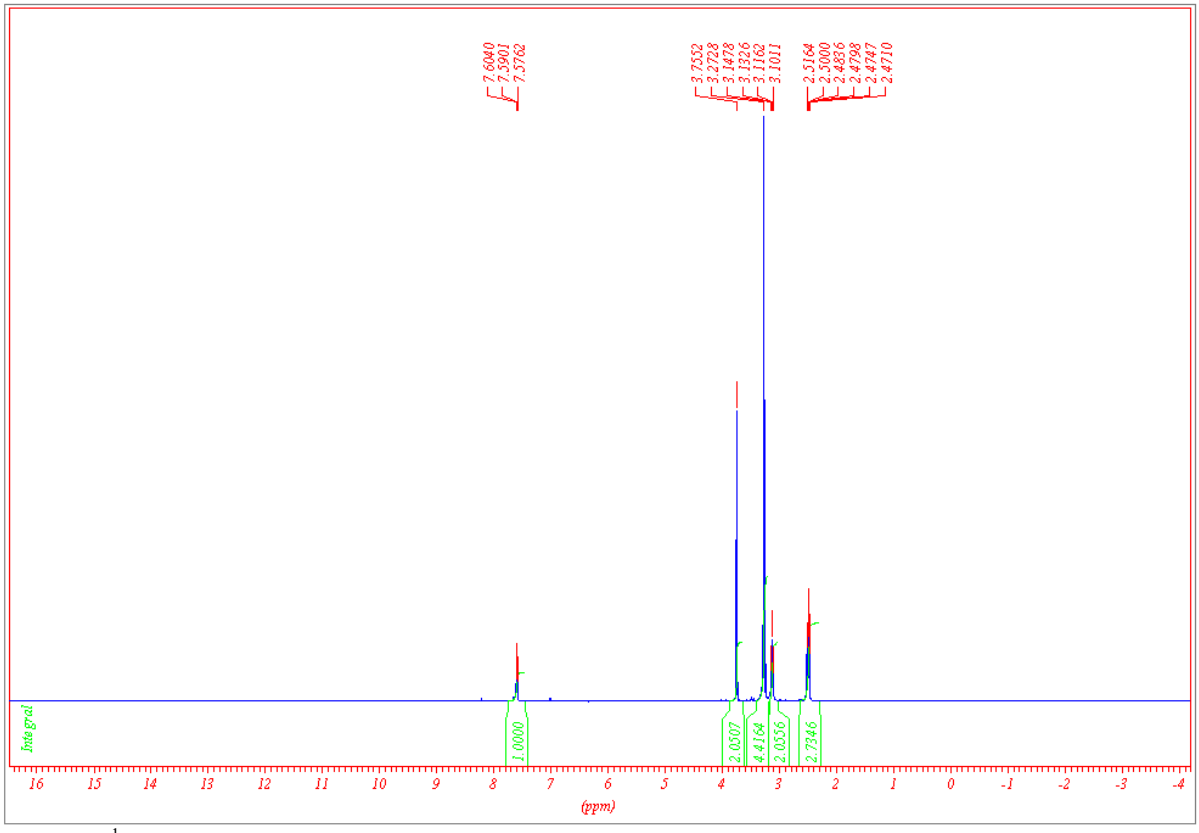


Figure S7 ^{1}H NMR spectrum of compound **3** in DMSO-d_6.

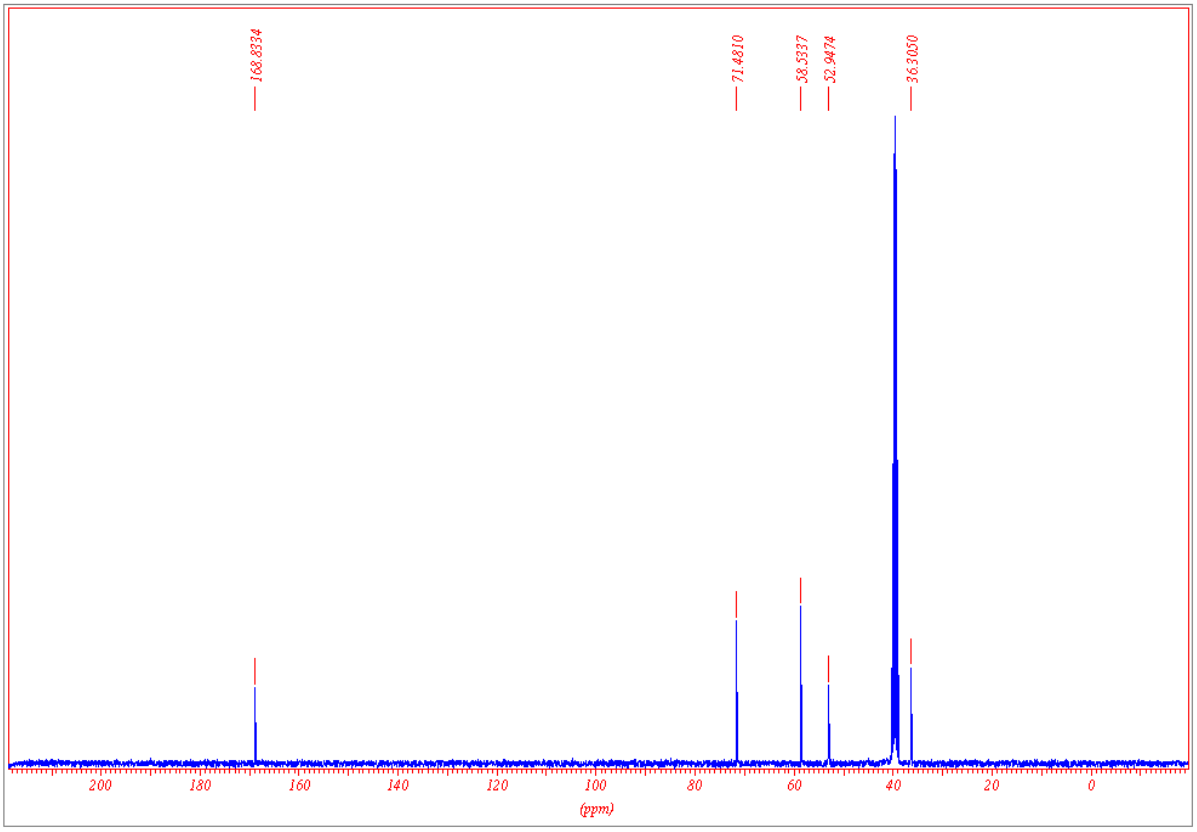


Figure S8 ^{13}C NMR spectrum of compound **3** in DMSO-d_6.

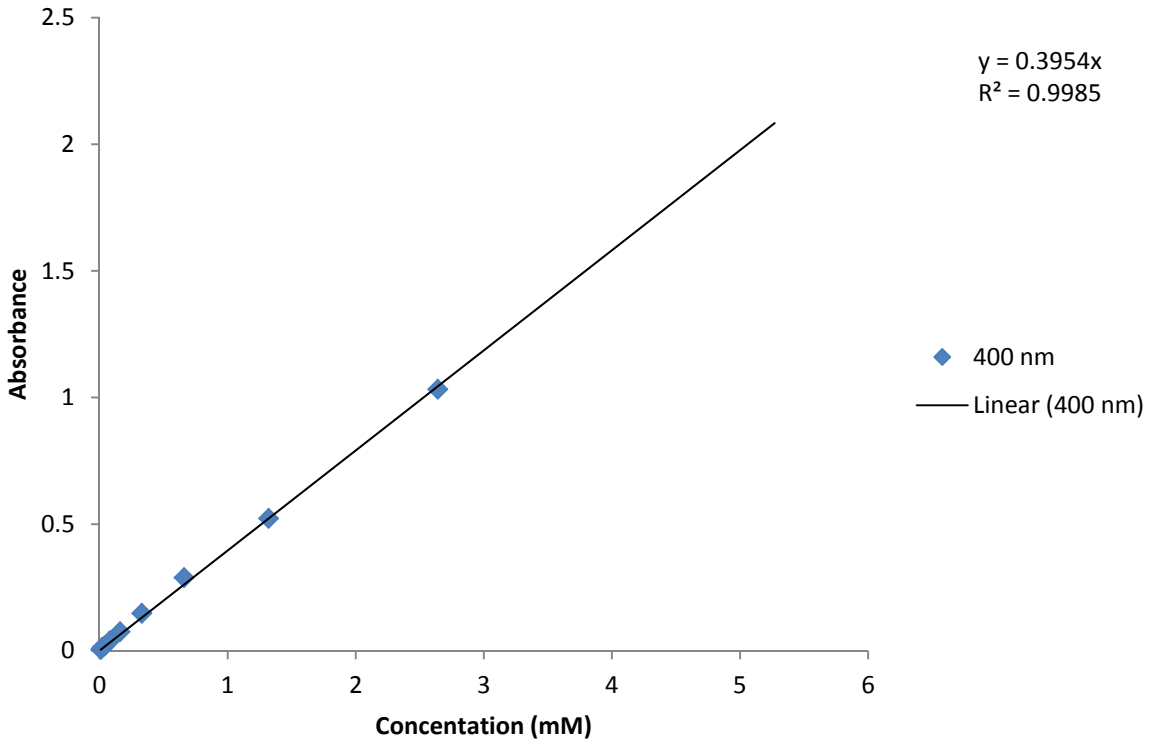


Figure S9 Calibration curve for the determining the concentration of *p*-nitrophenolate at 400 nm in a 20 mM Bis-Tris buffered aqueous solution set to pH 6.1.

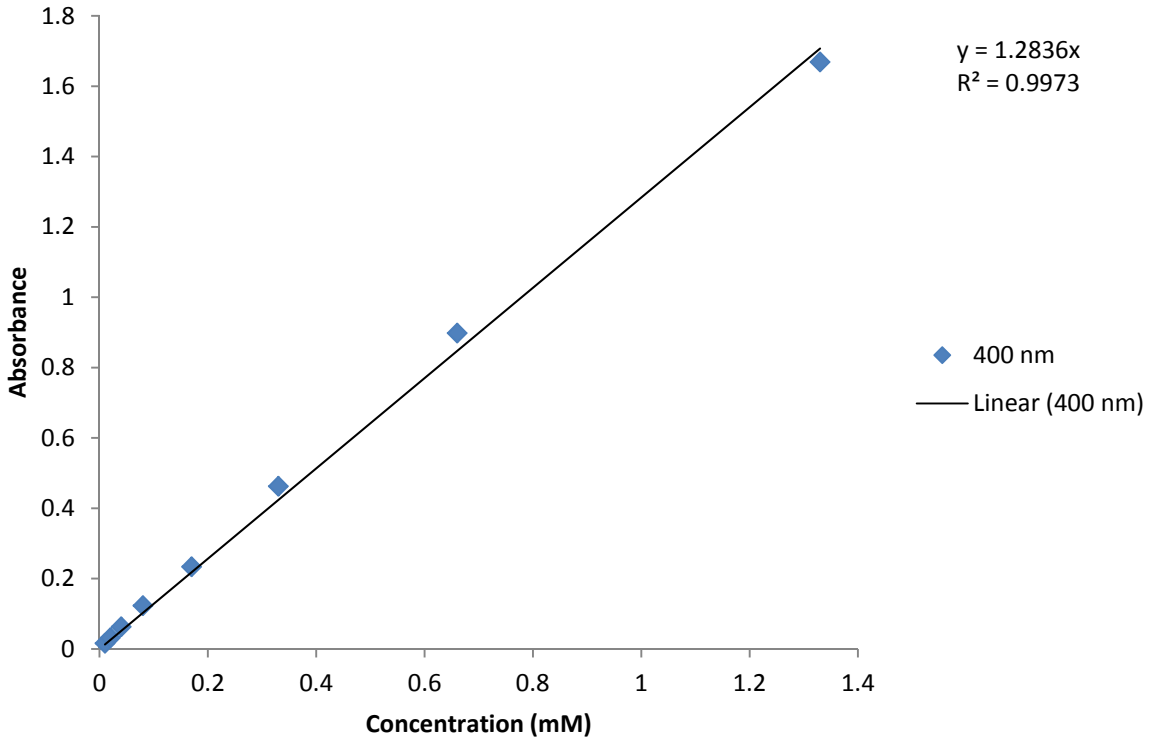


Figure S10 Calibration curve for the determining the concentration of *p*-nitrophenolate at 400 nm in a 20 mM Bis-Tris buffered aqueous solution set to pH 7.1.

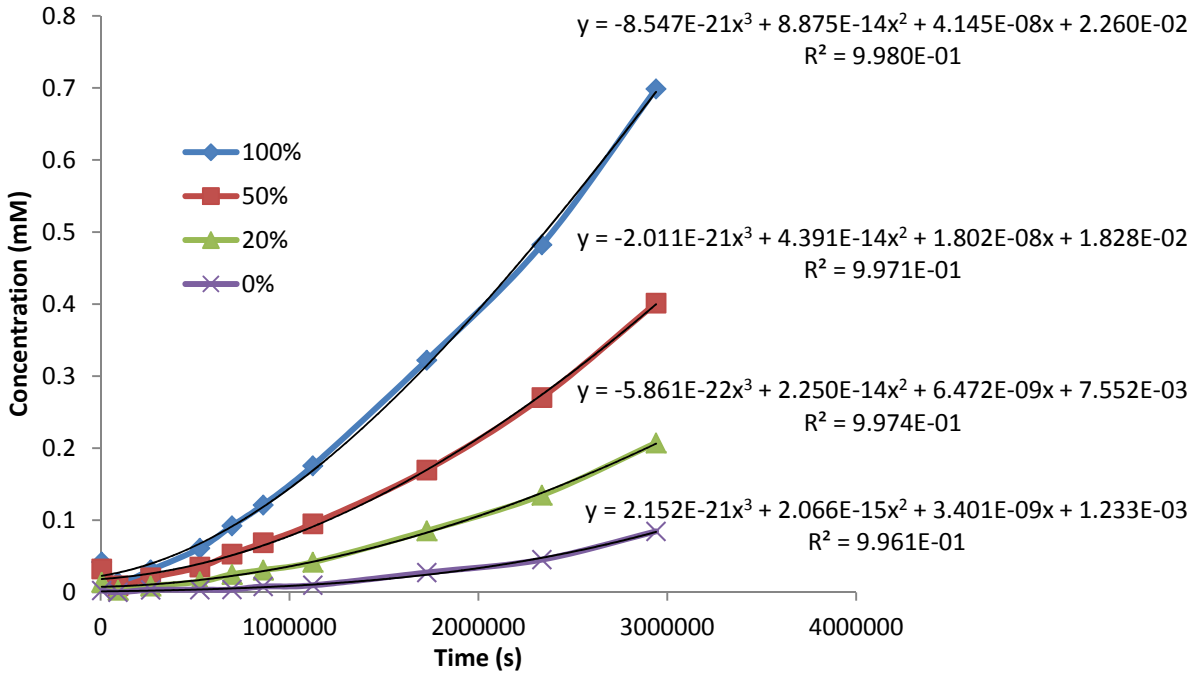


Figure S11 Graph illustrating the formation *p*-nitrophenolate (mM) due to the hydrolysis of BNPP (5 mM) in a 400 nm 20 mM Bis-Tris buffered aqueous solution set to pH 6.1, with compound **1** added in various mol-% with respect to BNPP concentration. Results have been normalised by the concentration of *p*-nitrophenolate at first time point of 0 % receptor taken as 0 % conversion of BNPP to *p*-nitrophenolate. The data has also been fitted to a line of best fit with R^2 values illustrating the error of the fit.

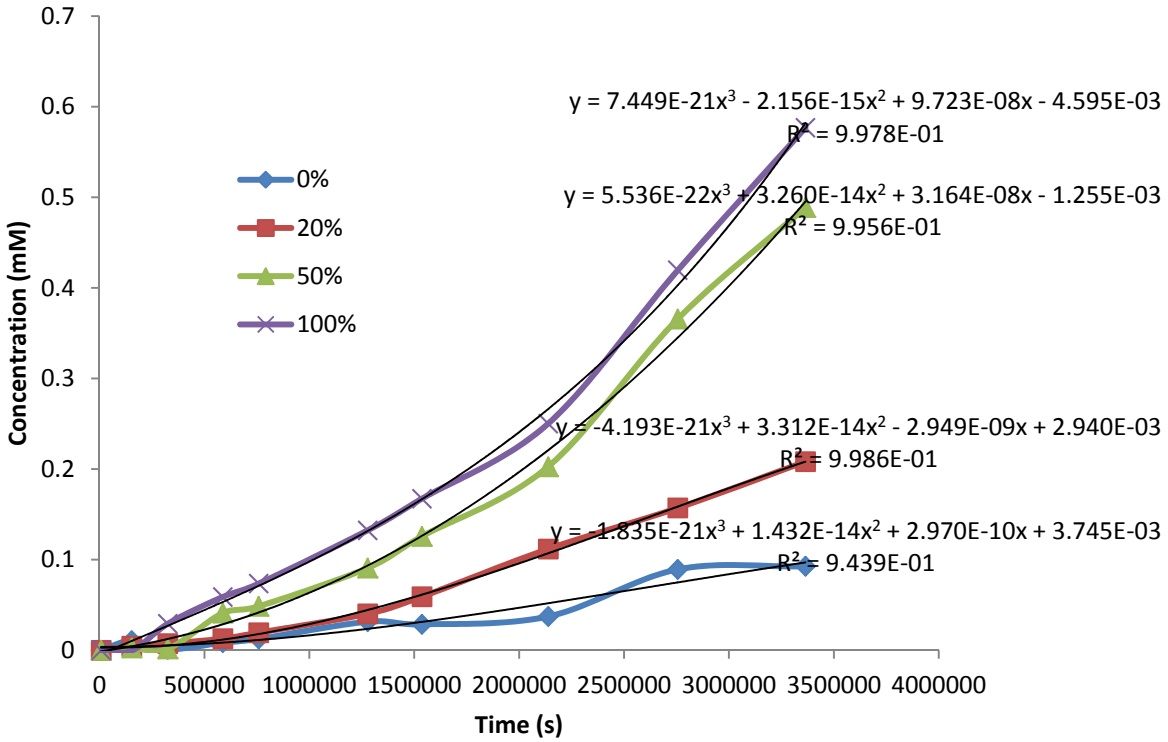


Figure S12 Graph illustrating the formation *p*-nitrophenolate (mM) due to the hydrolysis of BNPP (5 mM) in a 400 nm 20 mM Bis-Tris buffered aqueous solution set to pH 6.1, with compound **2** added in various mol-% with respect to BNPP concentration. Results have been normalised by the concentration of *p*-nitrophenolate at first time point of 0 % receptor taken as 0 % conversion of BNPP to *p*-nitrophenolate. The data has also been fitted to a line of best fit with R^2 values illustrating the error of the fit.

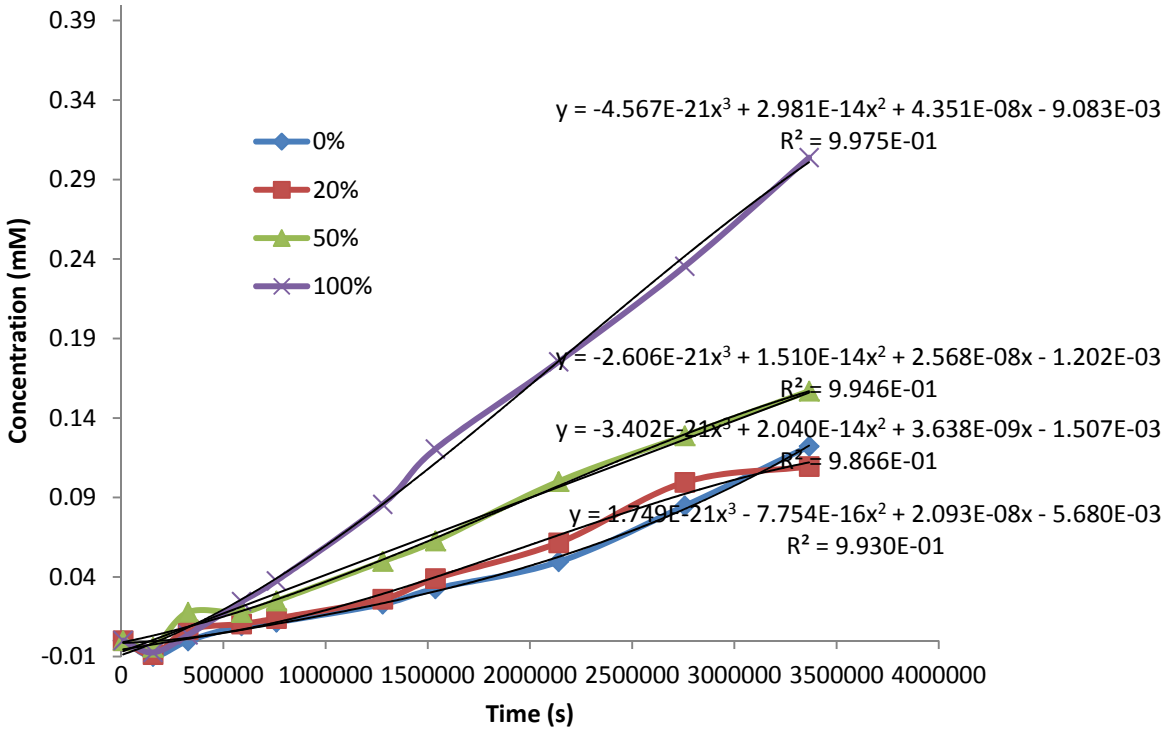


Figure S13 Graph illustrating the formation *p*-nitrophenolate (mM) due to the hydrolysis of BNPP (5 mM) in a 400 nm 20 mM Bis-Tris buffered aqueous solution set to pH 6.1, with compound **3** added in various mol-% with respect to BNPP concentration. Results have been normalised by the concentration of *p*-nitrophenolate at first time point of 0 % receptor taken as 0 % conversion of BNPP to *p*-nitrophenolate. The data has also been fitted to a line of best fit with R^2 values illustrating the error of the fit.

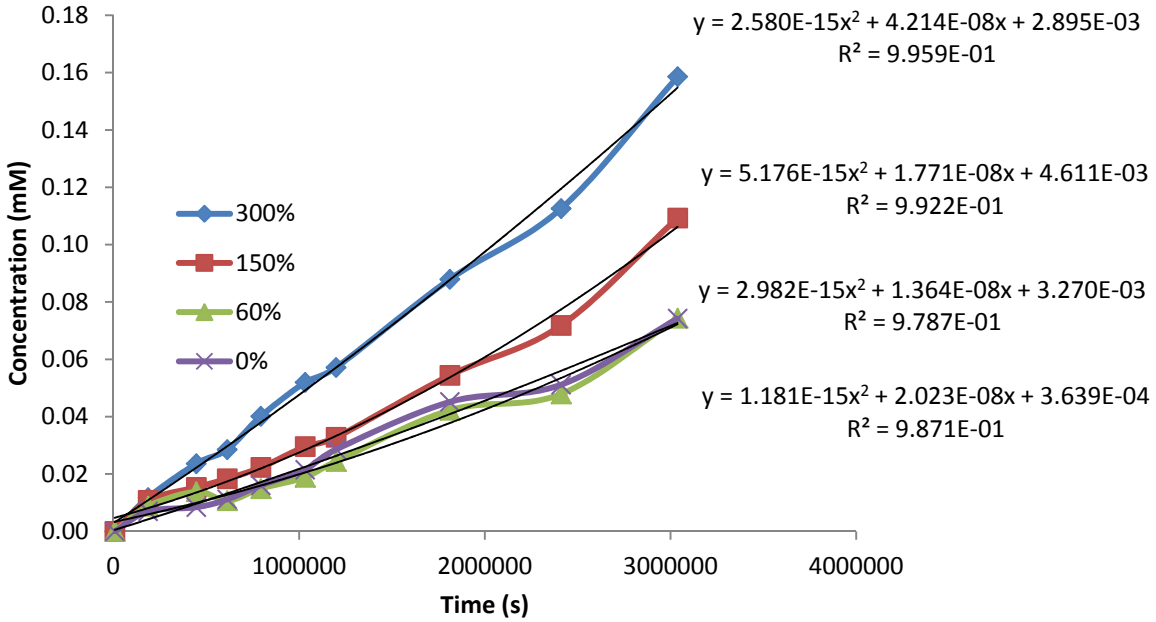


Figure S14 Graph illustrating the formation *p*-nitrophenolate (mM) due to the hydrolysis of BNPP (5 mM) in a 400 nm 20 mM Bis-Tris buffered aqueous solution set to pH 6.1, with imidazole added in various mol-% with respect to BNPP concentration. Results have been normalised by the concentration of *p*-nitrophenolate at first time point of 0 % receptor taken as 0 % conversion of BNPP to *p*-nitrophenolate. The data has also been fitted to a line of best fit with R^2 values illustrating the error of the fit.

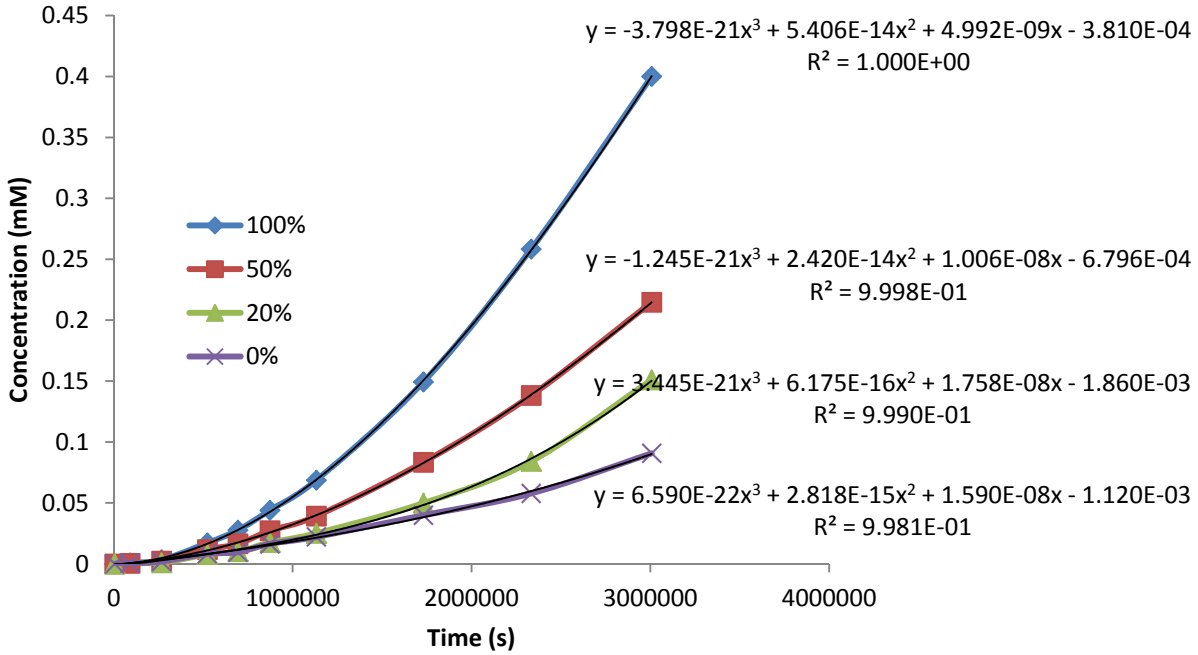


Figure S15 Graph illustrating the formation *p*-nitrophenolate (mM) due to the hydrolysis of BNPP (5 mM) in a 400 nm 20 mM Bis-Tris buffered aqueous solution set to pH 7.1, with compound **1** added in various mol-% with respect to BNPP concentration. Results have been normalised by the concentration of *p*-nitrophenolate at first time point of 0 % receptor taken as 0 % conversion of BNPP to *p*-nitrophenolate. The data has also been fitted to a line of best fit with R^2 values illustrating the error of the fit.

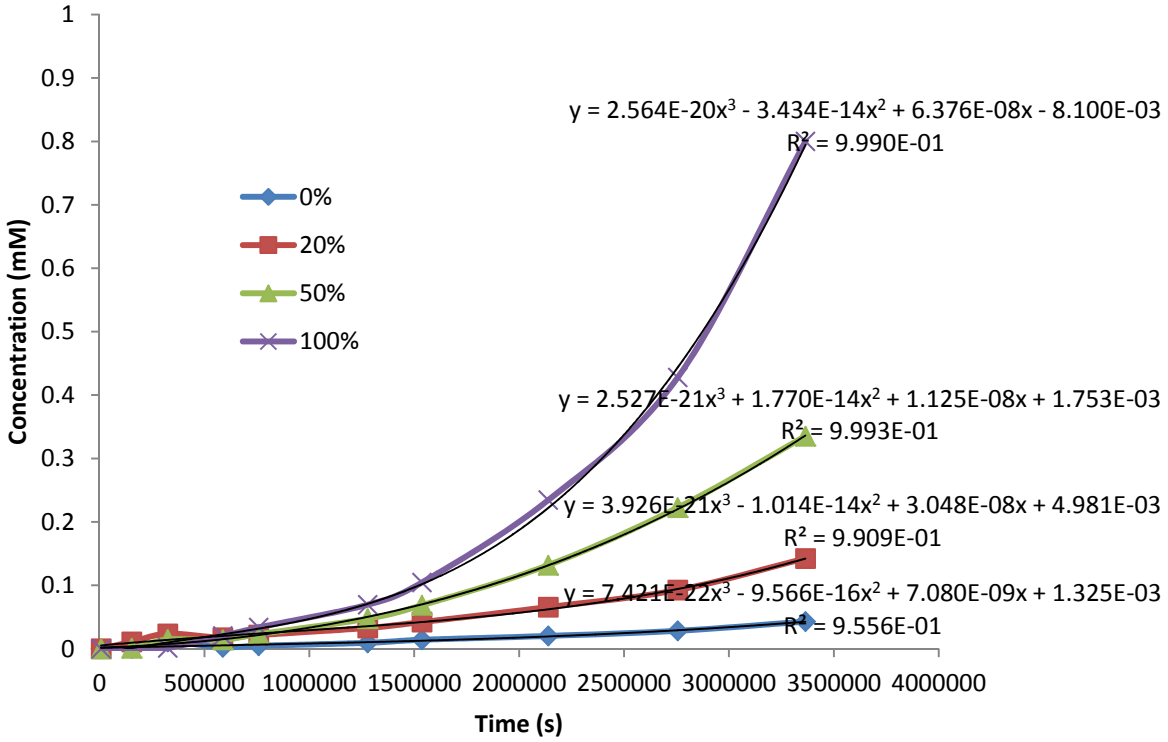


Figure S16 Graph illustrating the formation *p*-nitrophenolate (mM) due to the hydrolysis of BNPP (5 mM) in a 400 nm 20 mM Bis-Tris buffered aqueous solution set to pH 7.1, with compound **2** added in various mol-% with respect to BNPP concentration. Results have been normalised by the concentration of *p*-nitrophenolate at first time point of 0 % receptor taken as 0 % conversion of BNPP to *p*-nitrophenolate. The data has also been fitted to a line of best fit with R^2 values illustrating the error of the fit.

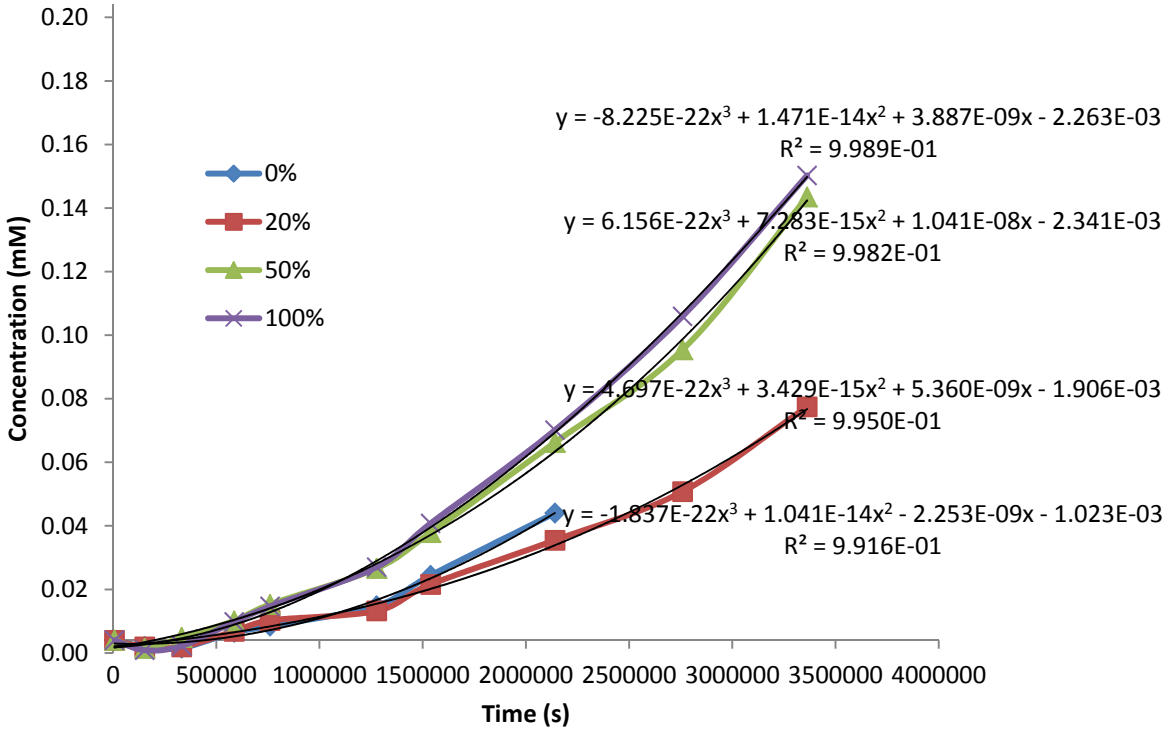


Figure S17 Graph illustrating the formation *p*-nitrophenolate (mM) due to the hydrolysis of BNPP (5 mM) in a 400 nm 20 mM Bis-Tris buffered aqueous solution set to pH 7.1, with compound **3** added in various mol-% with respect to BNPP concentration. Results have been normalised by the concentration of *p*-nitrophenolate at first time point of 0 % receptor taken as 0 % conversion of BNPP to *p*-nitrophenolate. The data has also been fitted to a line of best fit with R^2 values illustrating the error of the fit.

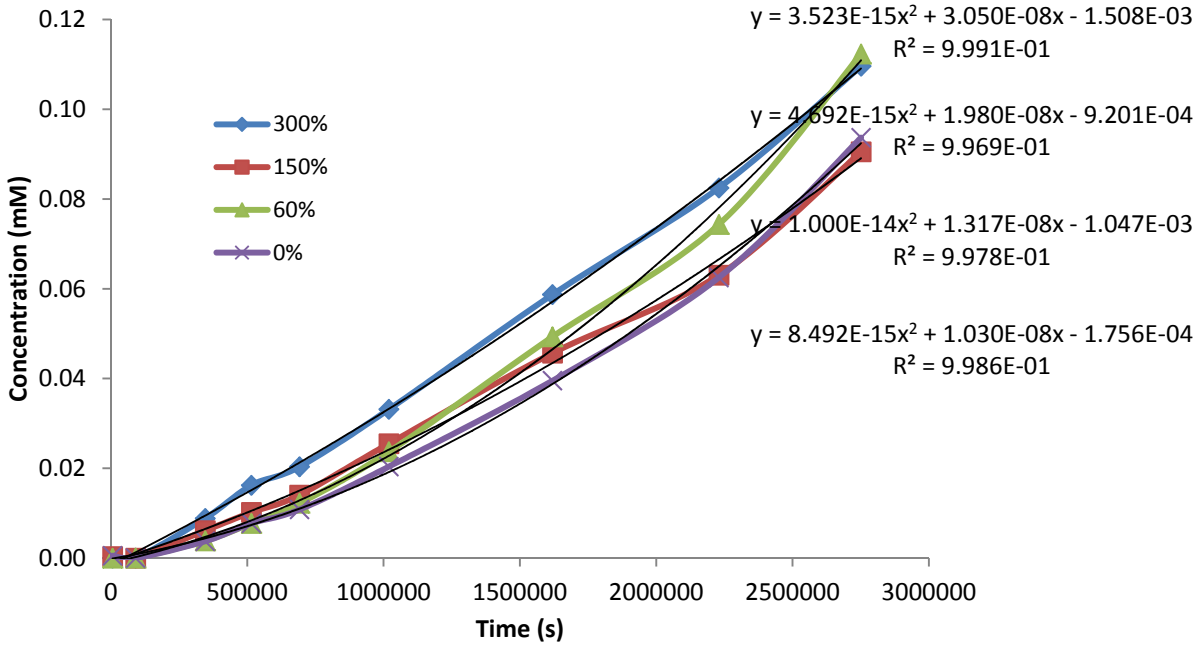


Figure S18 Graph illustrating the formation *p*-nitrophenolate (mM) due to the hydrolysis of BNPP (5 mM) in a 400 nm 20 mM Bis-Tris buffered aqueous solution set to pH 7.1, with imidazole added in various mol-% with respect to BNPP concentration. Results have been normalised by the concentration of *p*-nitrophenolate at first time point of 0 % receptor taken as 0 % conversion of BNPP to *p*-nitrophenolate. The data has also been fitted to a line of best fit with R^2 values illustrating the error of the fit.

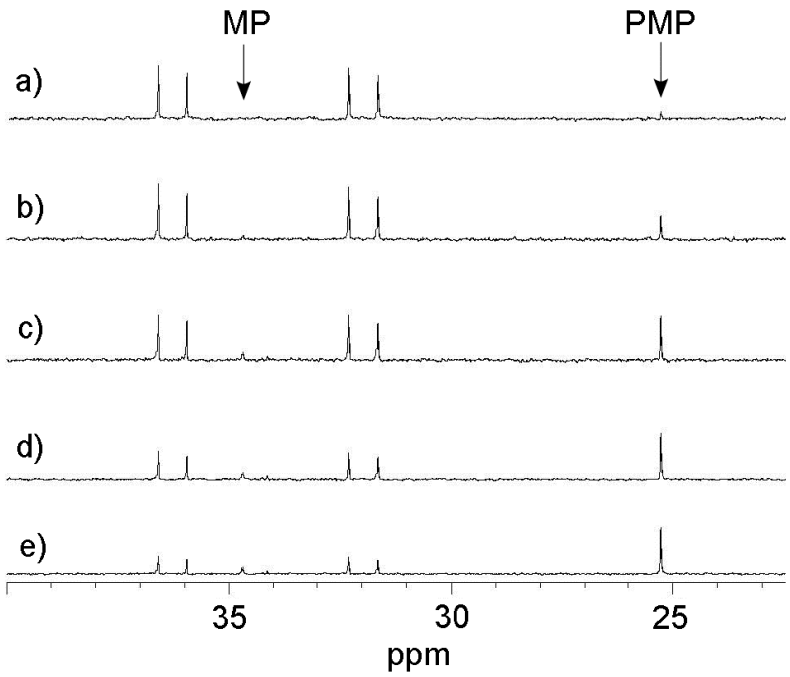


Figure S19 ^{31}P NMR stack plot of the breakdown of GD in bis-tris (20 mM) buffered solutions at pD 6.5 in the presence of 100 mol-percent of compounds **1** and **3**. Time = a) 0 hrs b) 3 hrs c) 7 hrs d) 12 hrs and e) 18 hrs. © Crown Copyright, Dstl.

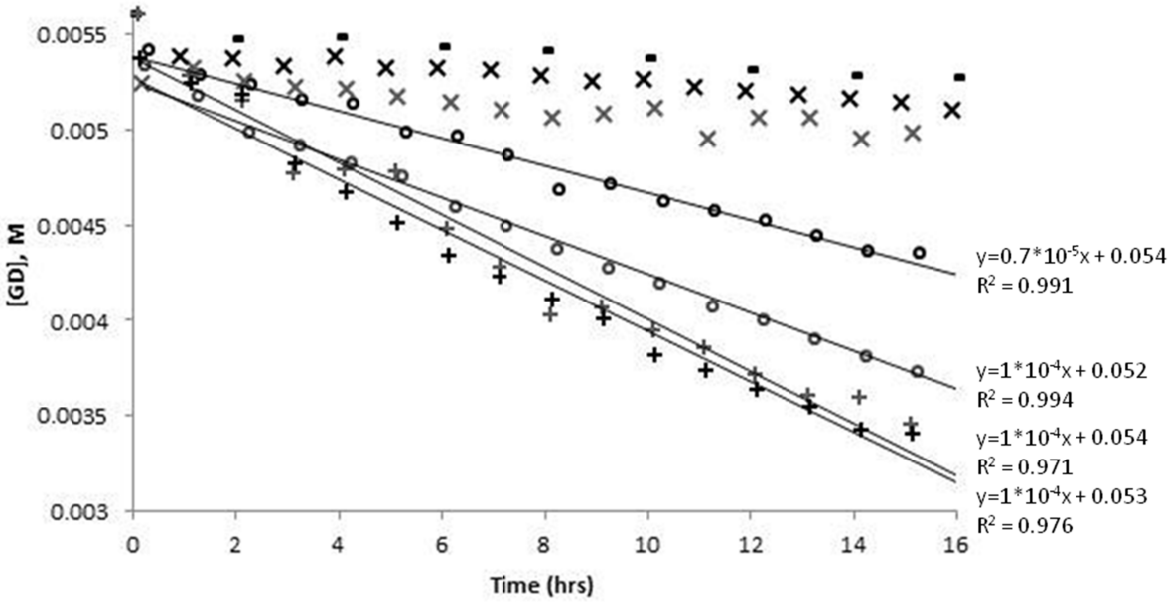
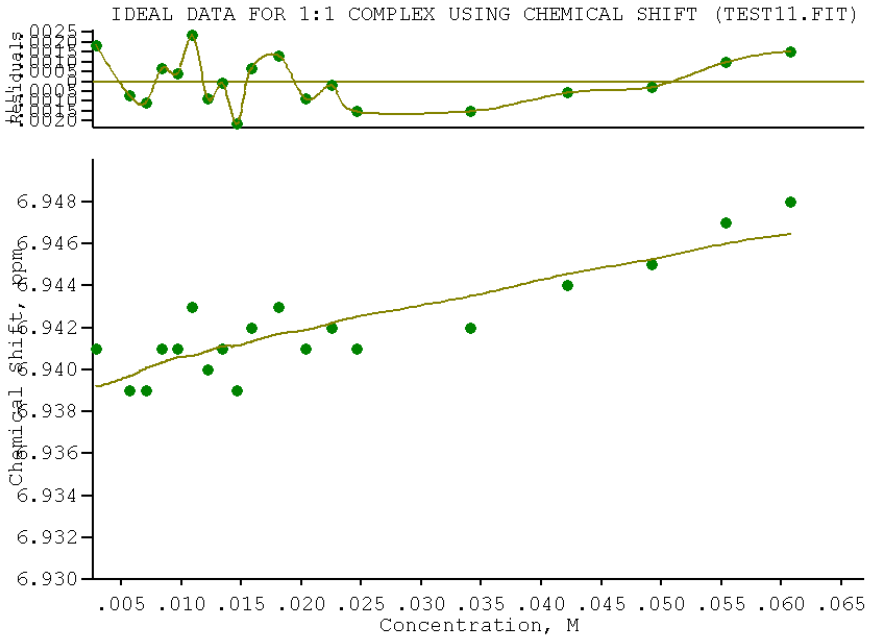
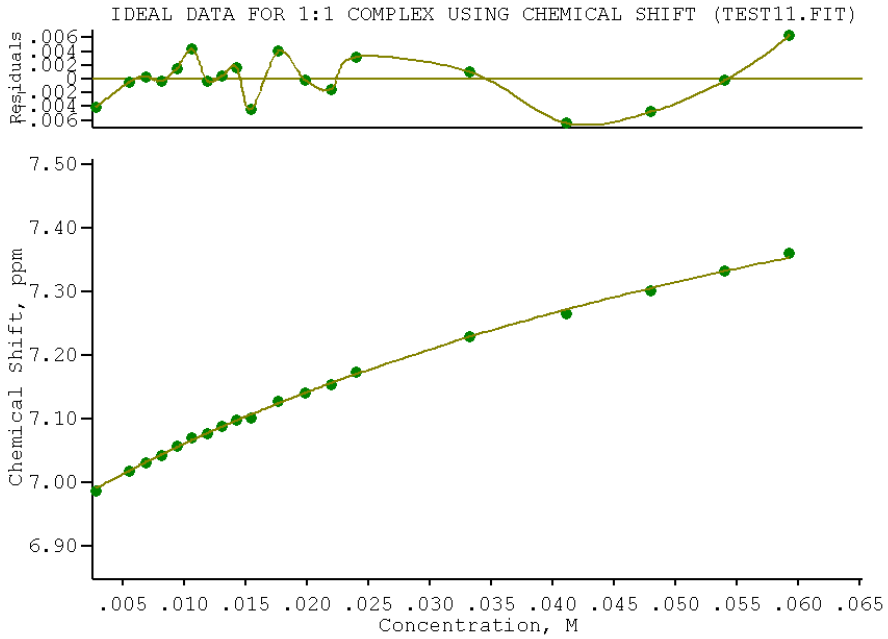


Figure S20 Concentration of GD present as a function of time in bis-tris buffered solutions (20 mM) at pD 6.5 in the absence (-) and presence of compound **1** at 100 (+) and 50 (○) mol-% and compound **3** at 100 mol-% (x) as determined by ^{1}H NMR analysis. © Crown Copyright, Dstl.



K = < 5 M^{-1} Error = NA

Figure S21 ^{1}H NMR titration of compound **3** *vs*. DMMP in MeCN-d_3. Following the amide NH.



K = 14 M^{-1} Error = ± 9 %

Figure S22 ^{1}H NMR titration of compound **3** *vs*. PMP in MeCN-d_3. Following the amide NH.

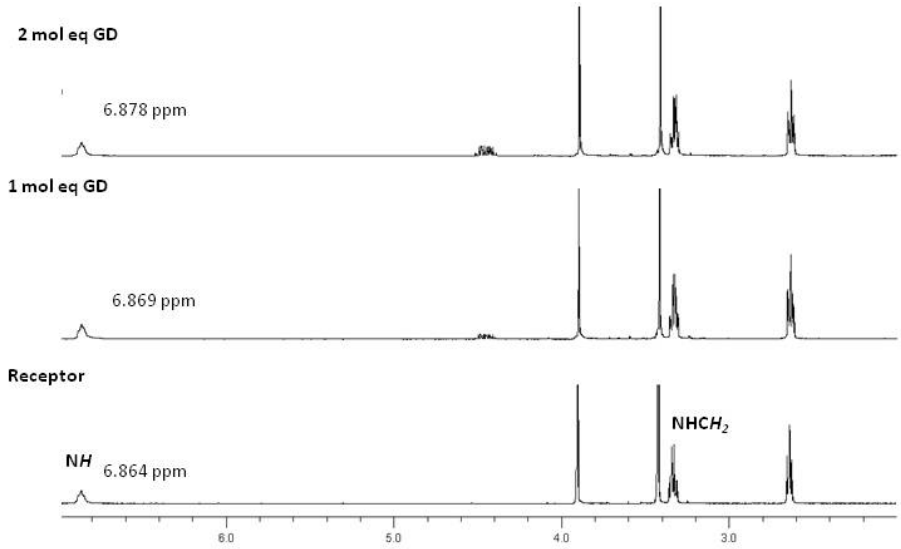


Figure S23 ^{1}H NMR single point study of compound **3** *vs*. GD in $CDCl_3$. Following the amide NH.

The percentage conversion of BNPP to *p*-nitrophenolate at 750 hrs, shown in Tables S1 and S2, was calculated though fitting the data to a polynomial 3rd or 2nd order line of best fit (see Figures S11-S18), allowing direct comparison of the different data sets.

Table S1 Conversion of BNPP to *p*-nitrophenolate with compounds **1**-**3** and imidazole in % after 750 hrs at pH 6.1. The mol-% of the compound added is with respect to the concentration of BNPP.

Compound (mol-%)	**1**	**2**	**3**	*Compound (mol-%)*	**Imidazole**
0	**1.3**[a]	**1.6**[a]	**1.6**[a]	0	**1.3**[a]
20	**3.6**	**3.0**	**1.8**	60	**1.2**
50	**6.7**	**6.6**	**2.6**	150	**1.8**
100	**12.3**	**7.8**	**4.8**	300	**2.7**

[a] average of two repeat experiments.

Table S2 Conversion of BNPP to *p*-nitrophenolate with compounds **1**-**3** and imidazole in % after 750 hrs at pH 7.1. The mol-% of the compound added is with respect to the concentration of BNPP.

Compound (mol-%)	**1**	**2**	**3**	*Compound (mol-%)*	**Imidazole**
0	**1.7**[a]	**1.0**[a]	**1.0**[a]	0	**1.7**[a]
20	**2.4**	**1.8**	**1.0**	60	**2.1**
50	**3.6**	**4.2**	**1.8**	150	**1.7**
100	**6.7**	**8.4**	**2.0**	300	**2.1**

[a] average of two repeat experiments.

Reference

1. J. M. Benito, M. Gomez-Garcia, C. O. Mellet, I. Baussanne, J. Defaye and J. M. G. Fernandez, *Journal of the American Chemical Society*, 2004, **126**, 10355-10363.
2. M. J. Hynes, *Journal of the Chemical Society-Dalton Transactions*, 1993, 311-312.