Electronic Supplementary Material (ESI) for Chemical Communications

Reagents and Assay Strategies for Quantifying Active Enzyme Analytes Using a Personal Glucose Meter

*Hemakesh Mohapatra and Scott T. Phillips**

Department of Chemistry,

The Pennsylvania State University,

University Park, PA 16802

*Corresponding author E-mail: sphillips@psu.edu

Supporting Information

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General experimental procedures

All reactions requiring inert atmosphere were performed in flame-dried glassware under a positive pressure of argon. Air- and moisture-sensitive liquids were transferred by syringe or stainless steel cannula. Organic solutions were concentrated by rotary evaporation (25–40 mmHg) at ambient temperature, unless otherwise noted. D-Glucose, D-lactose (**1a**), D-glucose-6-phosphate (**1c**), β-D-galactosidase, alkaline phosphatase, horse blood serum and all other reagents were purchased commercially and were used as received. Tetrahydrofuran and methylene chloride were purified by the method developed by Pangborn et al.[1] Flash-column chromatography was performed as described by Still et al.,[2] employing silica gel (60-Å pore size, 32–63 μm, standard grade, Dynamic Adsobents). Thin layer chromatography was carried out on Dynamic Adsorbants silica gel TLC (20 × 20 cm w/h, F-254, 250 μm). Deionized water was purified with a Millipore-purification system (Barnstead EASYpure® II UV/UF). All assays were performed in VWR microcentrifuge tubes (1.6 mL), unless otherwise noted.

Instrumentation

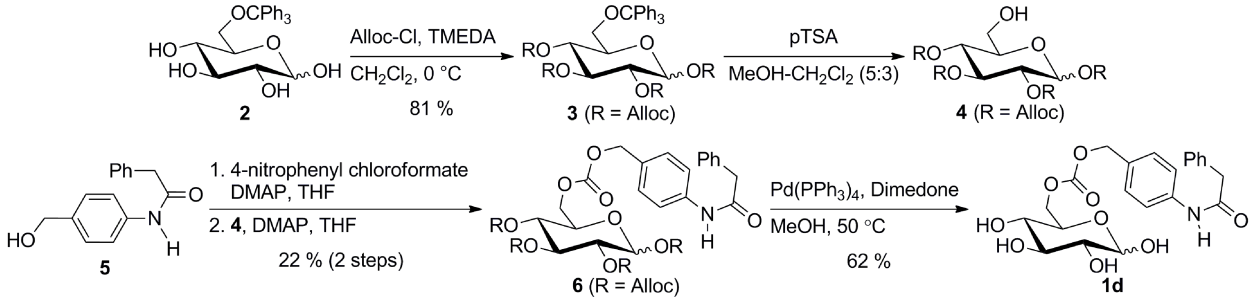
Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded using a Bruker AV-360 (360 MHz) at 25 °C. Proton chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to tetramethylsilane ($(CH_3)_4Si$, 0.00 ppm) or residual protium in the nmr solvent ($CDCl_3$, δ 7.26 ppm; CD_3OD, δ 3.33). Data are represented as follows: Integration, chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances, br s = broad singlet, dd = doublet of doublet), and coupling constant (J) in hertz. Carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded using a Bruker AV-360 (90 MHz) at 25 °C. Carbon chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to the carbon resonance of the NMR solvent ($CDCl_3$, δ 77.16 ppm; CD_3OD, 49.00). LC-MS data were obtained on an Agilent Technologies 1200 series analytical reverse-phase HPLC coupled to an Agilent Technologies 6120 quadrupole mass spectrometer. The column used was BETASIL Phenyl-Hexyl column (150 mm × 2.1 mm, 5 μm particle size). The column was equilibrated in 9:1 0.5 mM aqueous ammonium formate (A)–acetonitrile (B) at 0.4 mL min^{-1} flow rate. After injection of the sample, a solvent gradient was run as follows:

Time (min.)	A (%)	B (%)
0	90	10
10	10	90
16	10	90
18	90	10
20	90	10

A portion of the HPLC stream was automatically injected into the mass spectrometer. The mass spectrometer (APCI) settings were as follows: gas temperature = 350 °C, drying gas flow = 11 L min^{-1},

nebulizer pressure = 35 psig, and voltage = 3000 V. Preparatory HPLC was carried out on Agilent Technologies 1200 Series preparatory reverse-phase HPLC. The column used was Agilent Prep-C18 column(150 mm × 30 mm, 10 μm particle size). A True Track™ personal glucometer was used for all measurements of glucose levels.

Scheme 1: Synthesis of detection reagent **1d**



Tetraallyl ((3R,4S,5R,6R)-6-((trityloxy)methyl)tetrahydro-2H-pyran-2,3,4,5-tetrayl) tetracarbonate (3)

Allyl chloroformate (1.4 mL, 13 mmol, 4.5 equiv) was added to a solution of 6-*O*-(triphenylmethyl)- D-glucopyranose **2**[3] (1.3 g, 3.0 mmol, 1 equiv) and TMEDA (1.4 mL, 6.7 mmol, 2.3 equiv) in dry CH_2Cl_2 (25 mL) at 0 °C. The resulting solution was stirred at 0 °C for 1 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL). The resulting solution was washed with saturated aqueous $NaHCO_3$ (20 mL). The aqueous layer was collected and was extracted using CH_2Cl_2 (1 × 100 mL). The combined organic layers were washed with brine (1 × 20 mL), and dried over anhydrous sodium sulfate. The sodium sulfate was removed by filtration and the resulting solution was concentrated under reduced pressure. The resulting oil was purified using column chromatography (elution with 30% ethyl acetate/hexanes) to obtain tetraallyl ((3R,4S,5R,6R)-6-((trityloxy)methyl)tetrahydro-2H-pyran-2,3,4,5-tetrayl) tetracarbonate **3** (1.8 g, 2.4 mmol, 81%). IR (cm^{-1}) 2948, 2360, 1756, 1230; ^{1}H-NMR (360 MHz, $CDCl_3$, anomeric pair): δ 7.43–7.41 (6H, m), 7.30–7.19 (9H, m), 6.39 (0.6 H, d, J = 3.5), 5.94–5.85 (4H, m), 5.64 (0.4 H, d, J = 7.6), 5.39–5.21 (8H, m), 5.18–5.15 (1H, m), 5.15–5.01 (2H, m), 4.73–4.59 (6H, m) 4.52–4.37 (2H, m), 4.13–4.10 (0.6 H, m), 3.77–3.45 (0.4 H, m), 3.39–3.34 (1H, m), 3.20–3.12 (1H, m); ^{13}C-NMR (90 MHz, $CDCl_3$): δ 154.1, 153.8, 153.6, 153.5, 153.4, 153.1, 153.1, 143.5, 143.5, 131.6, 131.2, 131.2, 131.2, 131.1, 130.9, 128.7, 128.7, 128.6, 128.0, 127.9, 127.3, 127.1, 119.6, 119.2, 119.2, 119.1, 118.9, 94.9, 92.6, 86.8, 86.7, 74.0, 73.6, 72.9, 72.1, 72.0, 71.1, 69.3, 69.2, 69.0, 68.9, 68.9, 68.8, 68.5, 61.8, 61.4; MS (TOF MS ES+, *m/z*): 781.4 (M + Na^+); HRMS (TOF MS ES+, *m/z*) Calculated for $C_{41}H_{42}O_{14}Na$ (M + Na^+): 781.2472; Found: 781.2485.

Tetraallyl ((3R,4S,5R,6R)-6-(hydroxymethyl)tetrahydro-2H-pyran-2,3,4,5-tetrayl) tetracarbonate (4)

p-Toluenesulfonic acid monohydrate (110 mg, 0.58 mmol, 0.25 equiv) was added to a solution of **3** (1.7 g, 2.3 mmol, 1 equiv) in 5:3 MeOH-CH_2Cl_2 (24 mL). The resulting solution was stirred at 20 °C for 6 h. Triethylamine (0.3 mL) was added to the reaction mixture and the resulting solution was concentrated. The resulting crude product was partially purified by passing it through a plug of silica gel (elution with 40% ethyl acetate/hexanes) to obtain tetraallyl ((3R,4S,5R,6R)-6-(hydroxymethyl)tetrahydro-2H-pyran-2,3,4,5-tetrayl) tetracarbonate **4**, which was used for subsequent reactions without further purification.

Tetraallyl ((3R,4S,5R,6R)-6-((4'-(2''-phenylacetamido)carboxybenzoxymethyl)tetrahydro-2H-pyran-2,3,4,5-tetrayl) tetracarbonate (6)

4-Nitrophenyl chloroformate (210 mg, 1.0 mmol, 1.0 equiv) was added to a solution of N-[4-(hydroxymethyl)phenyl]-2-phenylacetamide **5**[4] (245 mg, 1.0 mmol, 1 equiv) and DMAP (130 mg, 1.0 mmol, 1.0 equiv) in dry THF (3.5 mL). The resulting solution was stirred at 20 °C for 8 h. The resulting solution of was treated with a dry THF (6 mL) solution of **4** (480 mg, 0.93 mmol, 0.93 equiv) containing DMAP (130 mg, 1.0 mmol, 1.0 equiv). The resulting solution was stirred at 20 °C for 24 h. The reaction mixture was diluted with ethyl acetate (15 mL). The resulting solution was washed with saturated aqueous NH_4Cl (10 mL). The aqueous layer was collected and was extracted using ethyl acetate (2 × 15 mL). The combined organic layers were successively washed with water (1 × 15 mL) and brine (1 × 15 mL), and dried over anhydrous sodium sulfate. The sodium sulfate was removed by filtration and the resulting solution was concentrated under reduced pressure. The resulting oil was purified using column chromatography (elution with 30% ethyl acetate/hexanes) to obtain tetraallyl ((3R,4S,5R,6R)-6-((4'-(2''-phenylacetamido)carboxybenzoxymethyl)tetrahydro-2H-pyran-2,3,4,5-tetrayl) tetracarbonate **6** (160 mg, 0.20 mmol, 22%). IR (cm^{-1}) 2960, 1752, 1603, 1225; ^{1}H-NMR (360 MHz, $CDCl_3$, anomeric pair): δ 7.44–7.29 (9H, m), 7.18 (1H, s), 6.28 (0.8 H, d, J = 3.5), 5.98–5.82 (4H, m), 5.62 (0.2 H, d, J = 8.3), 5.43–5.26 (8H, m), 5.25–5.23 (1H, m), 5.18–5.05 (2H, m), 5.03–4.98 (1H, m), 4.94–4.90 (1H, m), 4.65–4.57 (8H, m) 4.35–4.27 (2H, m), 4.25–4.19 (2 H, m), 3.74 (1H, s); ^{13}C-NMR (90 MHz, $CDCl_3$): δ 169.1, 154.6, 154.0, 153.7, 153.7, 152.9, 138.0, 134.3, 131.1, 131.0, 130.9, 130.8, 129.5, 129.4, 129.3, 127.8, 119.7, 119.3, 119.2, 119.0, 100.0, 92.2, 73.4, 72.5, 71.6, 69.7, 69.6, 69.3, 69.2, 69.0, 64.8, 44.9; MS (TOF MS ES+, *m/z*): 784.2 ($M + H^+$); HRMS (TOF MS ES+, *m/z*) Calculated for $C_{38}H_{42}NO_{17}$ ($M + H^+$): 784.2453; Found: 784.2469.

Tetraallyl ((3R,4S,5R,6R)-6-(hydroxymethyl)tetrahydro-2H-pyran-2,3,4,5-tetrayl) tetracarbonate (1d)

A suspension of **6** (100 mg, 0.13 mmol, 1 equiv), $Pd(PPh_3)_4$ (30 mg, 0.025 mmol, 0.20 equiv), and dimedone (140 mg, 1.0 mmol, 8.0 equiv) in dry MeOH (1.2 mL) was stirred at 50 °C for 1 h. The reaction mixture was cooled to room temperature and concentrated. The resulting crude product was purified using column chromatography (elution with 20% MeOH/ethyl aceate). The product obtained was further purified by preparatory HPLC to obtain tetraallyl ((3R,4S,5R,6R)-6-(hydroxymethyl)tetrahydro-2H-pyran-2,3,4,5-tetrayl) tetracarbonate **1d** (35 mg, 0.078 mmol, 62%). IR (cm^{-1}) 3297, 2956, 1741, 1660, 1606, 1215; ^{1}H-NMR (360 MHz, CD_3OD, anomeric pair): δ 7.57 (2H, d, J = 8.4), 7.37–7.30 (6H, m), 7.27–7.25 (1H, m), 5.09 (2H, s), 5.07 (0.6H, d, J = 3.6), 5.09 (2H, s), 4.48–4.42 (1H, m), 4.39–4.38 (0.4H, m), 4.30–4.22 (1H, m), 3.98–3.94 (1H, m), 3.68 (2H, s), 3.68–3.63 (0.4H, m), 3.50–3.45 (0.6H, m), 3.36–3.27 (1H, m), 3.26–3.25 (0.4H, m), 3.15–3.10 (0.6H, m); ^{13}C-NMR (90 MHz, $CDCl_3$): δ 172.4, 156.7, 140.1, 136.8, 132.7, 130.1, 129.6, 128.0, 121.1, 98.2, 94.0, 77.89, 94.0, 77.9, 76.1, 75.2, 74.7, 73.7, 71.7, 71.5, 70.6, 70.2, 68.4, 68.3, 44.7; MS (TOF MS ES+, *m/z*): 448.2 ($M + H^+$); HRMS (TOF MS ES+, *m/z*) Calculated for $C_{22}H_{26}NO_9$ ($M + H^+$): 448.1608; Found: 448.1602.

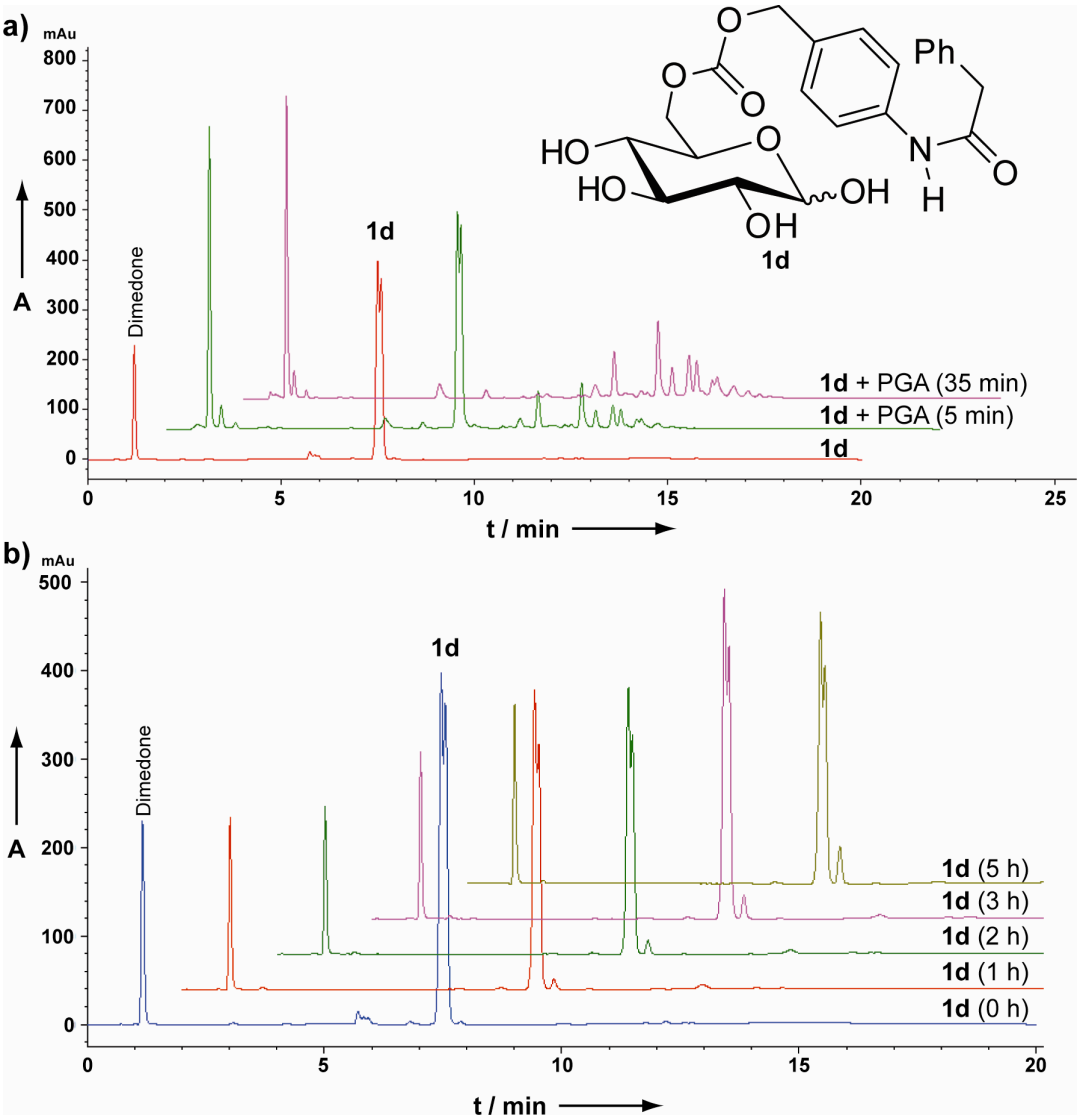


Figure S1 (a) Time-dependent LC-MS analysis of the reaction of **1d** with penicillin-G-amidase (PGA) (0.5 U mL^{-1}) in 0.1 M phosphate buffer (pH 7.5, 0.5% (v/v) Tween 20, 20 °C). The absorbance value (A) was recorded at 254 nm. The peaks appearing between 10 and 15 min (t) after exposure to PGA most likely are byproducts of quinone methide that is generated during the release reaction. (b) Time-dependent LC-MS analysis of the stability of **1d** in 0.1 M phosphate buffer (pH 7.5, 0.5% (v/v) Tween 20, 20 °C).

Experimental procedure corresponding to Figure S1

A solution of PGA (100 µL, 1 U mL^{-1} or 0 U mL^{-1} in 0.1 M phosphate buffer, pH 7.5) was added to a solution of **1d** (100 µL, 20 mM in 0.1 M phosphate buffer, pH 7.5, 1% (v/v) Tween 20). The mixture was agitated using a vortex mixer for 2 s. An aliquot of the resulting mixture (20 µL) was diluted with 0.1 M

phosphate buffer (60 µL, pH 7.5) and the resulting solution was injected into an analytical reversed-phase HPLC. Additional aliquots were diluted and injected at regular intervals.

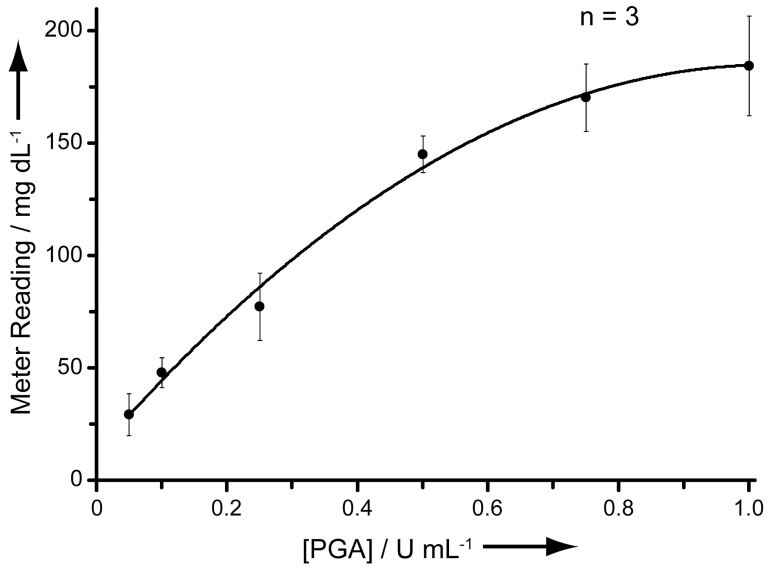


Figure S2 Calibration curve for measuring the level of penicillin-G-amidase (PGA) using **1d**. The data points are the average of three measurements and the error bars represent the standard deviations from these averages. The duration of the assay was 1 h in 0.1 M phosphate buffer (pH 7.5, 0.5% (v/v) Tween 20, 20 °C).

Experimental procedure corresponding to Figure S2

A solution of PGA (10 µL, 0.05–1.0 U mL^{-1} in 0.1 M phosphate buffer, pH 7.5) was added to a solution of **1d** (10 µL, 20 mM in 0.1 M phosphate buffer, pH 7.5, 1% (v/v) Tween 20). The mixture was agitated using a vortex mixer for 2 s. The resulting mixture was incubated at 20 °C for 1 h. The glucose concentration in the assay mixture was determined using a personal glucose meter.

Table S1: Final meter readings corresponding to Figure S2.

	Meter Reading / mg dL^{-1}		
[PGA] / U mL^{-1}	Trial 1	Trial 2	Trial 3
1	200	194	159
0.75	166	187	158
0.5	138	154	143
0.25	76	63	93
0.1	42	47	55
0.05	23	40	25

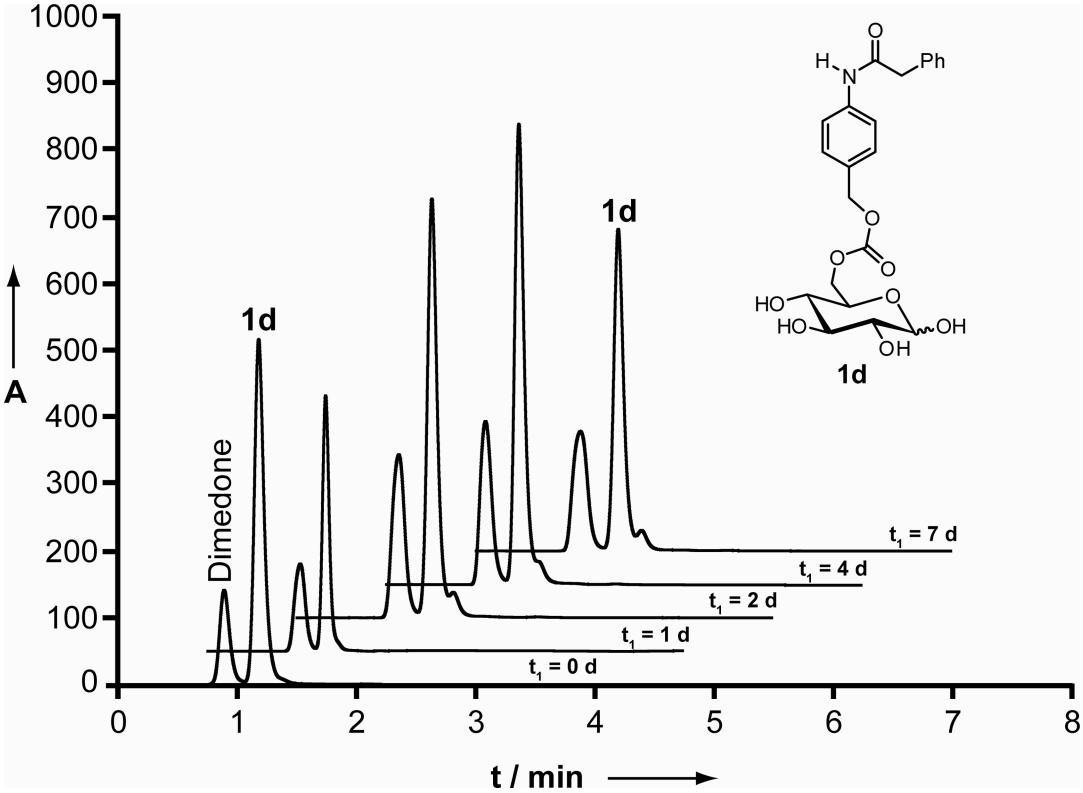


Figure S3 Study of the thermal stability of detection reagent **1d**. Samples of **1d** were held at 40 °C open to air for 7 days. At intervals, the samples were dissolved in 10 mM phosphate buffer (pH 7.5, 0.5% (v/v) Tween 20, 20 °C) and analyzed by LC-MS. The absorbance value (A) was recorded at 254 nm and dimedone (t = 0.8 min) was used as an internal standard to calculate peak areas.

Experimental procedure corresponding to Figure S3

Aliquots of detection reagent (~ 1 mg) in micro-centrifuge tubes were held at 40 °C open to air. At intervals, the samples were dissolved in 1 mL of 10 mM phosphate buffer (pH 7.5, 0.5% (v/v) Tween 20, 20 °C) and the resulting solution was injected into an analytical reversed-phase HPLC.

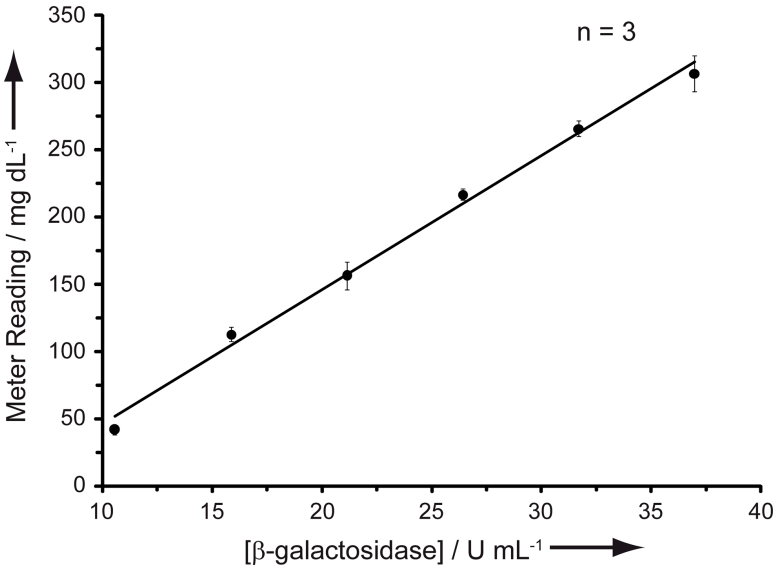


Figure S4 Calibration curve for detection of β-galactosidase using **1a**. The data points are the average of three measurements and the error bars reflect the standard deviations from these averages.

Experimental procedure corresponding to Figure S4

A solution of β-galactosidase (100 µL, 10.6–37.0 U mL^{-1} in 10 mM phosphate buffer, pH 7.4) was added to a solution of lactose **1a** (100 µL, 20 mM in 10 mM phosphate buffer, pH 7.4). The mixture was agitated using a vortex mixer for 2 s. The resulting mixture was incubated at 20 °C for 2 h. The glucose concentration in the assay mixture was determined using a personal glucose meter.

Table S2: Final meter readings corresponding to Figure S4.

	Meter Reading / mg dL^{-1}		
[β-galactosidase] / U mL^{-1}	Trial 1	Trial 2	Trial 3
10.6	38	44	44
15.9	119	110	110
21.1	145	160	164
26.4	212	220	218
31.7	269	269	259
37.0	322	300	298

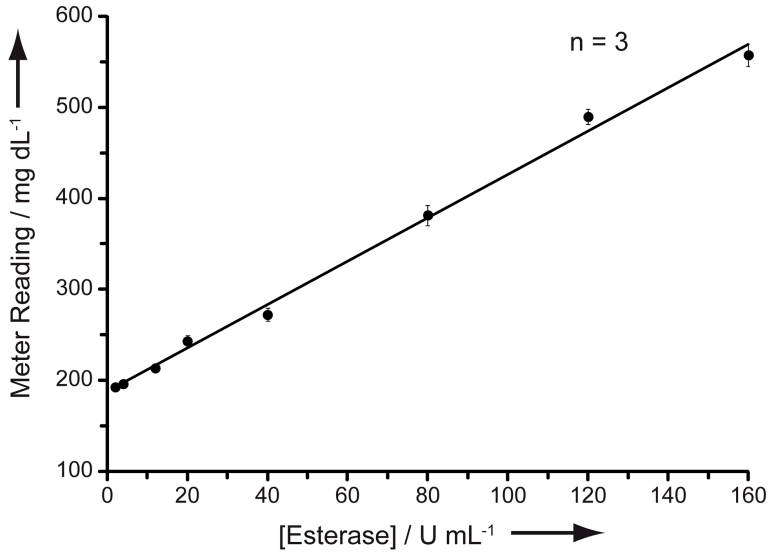


Figure S5 Calibration curve for detection of esterase using **1b**. The data points are the average of three measurements and the error bars reflect the standard deviations from these averages.

Experimental procedure corresponding to Figure S5

A solution of esterase (20 µL, 2–160 U mL^{-1} in 50 mM phosphate buffer, pH 8.0) was added to a solution of esterase **1b**[5] (20 µL, 100 mM in 0.1 M phosphate buffer, pH 8.0, 0.85% (v/v) Triton X-100). The mixture was agitated using a vortex mixer for 2 s. The resulting mixture was incubated at 40 °C for 1 h. The glucose concentration in the assay mixture was determined using a personal glucose meter.

Table S3: Final meter readings corresponding to Figure S5.

	Meter Reading / mg dL^{-1}		
[esterase] / U mL^{-1}	Trial 1	Trial 2	Trial 3
2	195	194	190
4	199	199	191
12	218	212	211
20	237	243	250
40	277	276	264
80	383	392	370
120	481	491	497
160	559	569	544

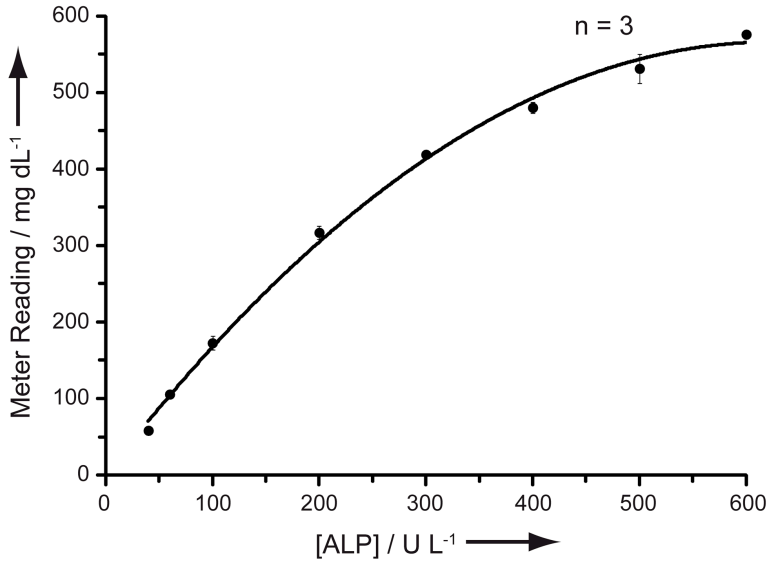


Figure S6 Calibration curve for detection of alkaline phosphatase using **1c**. The data points are the average of three measurements and the error bars reflect the standard deviations from these averages.

Experimental procedure corresponding to Figure S6

A solution of alkaline phosphatase (250 µL, 40–600 U L^{-1} in 0.1 M diethanolamine buffer, pH 7.4, 0.5 mM $MgCl_2$) was added to a solution of glucose-6-phosphate **1c** (25 µL, 220 mM in 0.1 M diethanolamine buffer, pH 7.4, 0.5 mM $MgCl_2$). The mixture was agitated using a vortex mixer for 2 s. The resulting mixture was incubated at 37 °C for 1 h. The glucose concentration in the assay mixture was determined using a personal glucose meter.

Table S4: Final meter readings corresponding to Figure S6.

	Meter Reading / mg dL^{-1}		
[alkaline phosphatase] / U L^{-1}	Trial 1	Trial 2	Trial 3
40	62	56	57
60	105	102	110
100	172	182	164
200	310	327	314
300	416	425	416
400	481	487	473
500	528	552	514
600	578	575	575

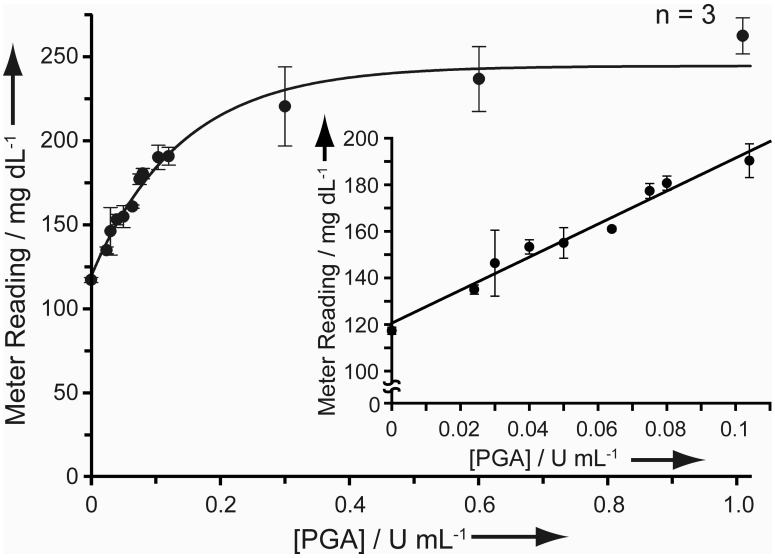


Figure S7 Calibration curves for measuring the levels of PGA (using **1d**) when 0.35 mg of glucose per mL of assay solution is included with the activity-based detection reagent prior to the assay. The data points are the average of three measurements and the error bars represent the standard deviations from these averages. The duration of the assay was 30 min in 0.1 M phosphate buffer (pH 7.5, 0.5% (v/v) Tween 20, 20 °C).

Experimental procedure corresponding to Figure S7

A solution of PGA (10 µL, 0–1 U mL^{-1} in 0.1 M phosphate buffer, pH 7.5) was added to a solution of **1d** (10 µL, 20 mM in 0.1 M phosphate buffer, pH 7.5, 1% (v/v) Tween 20, 20 °C) containing 7.2 µg D-glucose. The mixture was agitated using a vortex mixer for 2 s. The resulting mixture was incubated at 20 °C for 30 min. The glucose concentration in the assay mixture was determined using a personal glucose meter.

Table S5: Final meter readings corresponding to Figure S7.

	Meter Reading / mg dL^{-1}		
[PGA] / U mL^{-1}	Trial 1	Trial 2	Trial 3
0.00	116	119	117
0.02	137	135	133
0.03	154	130	155
0.04	154	150	156
0.05	154	149	162
0.06	160	162	161
0.08	175	176	181
0.08	184	178	180
0.10	182	194	195
0.12	187	189	197
0.30	219	198	245
0.60	251	245	215
1.01	251	272	265

Determination of the effect of decomposition of 1d on the PGA detection assay

Table S6: Meter readings for determining the effect of decomposition of **1d** on the PGA detection assay.

		Meter Reading / mg dL^{-1}			
Time of assay	[PGA] / U mL^{-1}	Trial 1	Trial 2	Trial 3	*Calculated [PGA] / U mL^{-1}
Day 0	0.06	169	176	167	0.07±0.01
Day 7 (heating to 40 °C)	0.06	155	154	151	0.05±0.00

* Calculated using the equation from the calibration curve in Figure S7 (inset) (y = 121 + 709x)

Experimental procedure corresponding to Table S6

A freshly prepared sample of **1d** was used to assay a solution PGA (10 µL, 0.06 U mL^{-1} in 0.1 M phosphate buffer, pH 7.5) using the standard assay procedure. An aliquot of **1d** (~ 1 mg) in micro-centrifuge tubes was held at 40 °C open to air for 7 days. This old sample of **1d** was used in an assay for detecting PGA (10 µL, 0.06 U mL^{-1} in 0.1 M phosphate buffer, pH 7.5) using the standard assay procedure. The concentrations of PGA in these assays were determined using the calibration in Figure S7. All assays were performed in triplicate.

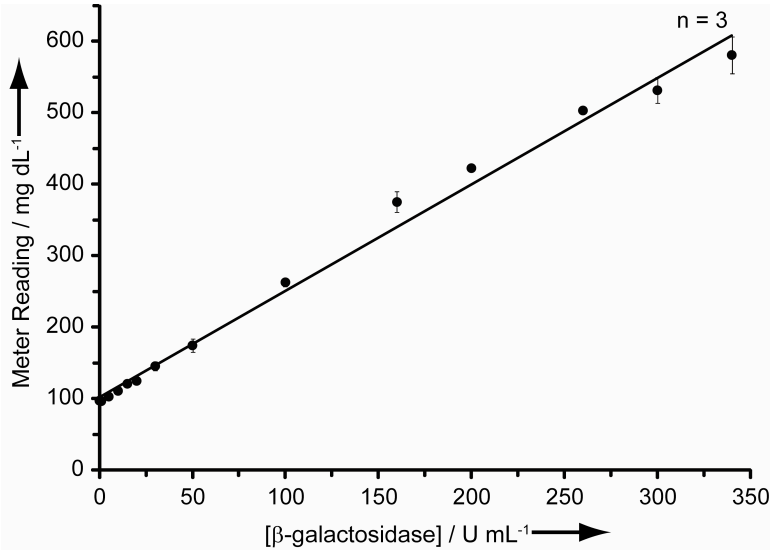


Figure S8 Calibration curves for measuring the levels of β-D-galactosidase (using **1a**) when 0.3 mg of glucose per mL of assay solution is included with the activity-based detection reagent prior to the assay. The data points are the average of three measurements and the error bars represent the standard deviations from these averages. The duration of the assay for β-D-galactosidase was 15 min in 10 mM phosphate buffer (pH 7.4, 20 °C).

Experimental procedure corresponding to Figure S8

A solution of β-galactosidase (100 μL, 0–340 U mL^{-1} in 10 mM phosphate buffer, pH 7.4) was added to a solution of lactose **1a** (100 μL, 20 mM in 10 mM phosphate buffer, pH 7.4) containing 62 μg D-glucose. The mixture was agitated using a vortex mixer for 2 s. The resulting mixture was incubated at 20 °C for 15 min. The glucose concentration in the assay mixture was determined using a personal glucose meter.

Table S7: Final meter readings corresponding to Figure S8.

	Meter Reading / mg dL^{-1}		
[β-galactosidase] / U mL^{-1}	Trial 1	Trial 2	Trial 3
0	99	96	96
1	96	98	95
5	107	101	100
10	107	112	113
15	124	121	117
20	125	123	126
30	143	152	141
50	178	181	164
100	259	260	269
160	390	374	361
200	420	419	428
260	502	504	503
300	534	548	512
340	593	551	598

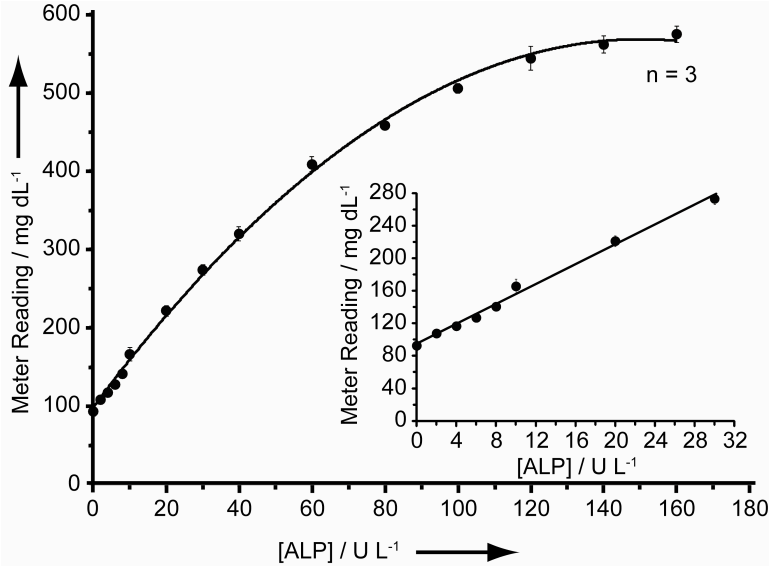


Figure S9 Calibration curves for measuring the levels of alkaline phosphatase (using **1a**) when 0.3 mg of glucose per mL of assay solution is included with the activity-based detection reagent prior to the assay. The data points are the average of three measurements and the error bars represent the standard deviations from these averages. The duration of the assay for alkaline phosphatase was 5 min in 0.1 M HEPES buffer (pH 8.0, 37 °C).

Experimental procedure corresponding to Figure S9

A solution of alkaline phosphatase (100 µL, 0–160 U L^{-1} in 0.1 M HEPES buffer, pH 8.0, 0.5 mM $MgCl_2$) was added to a solution of glucose-6-phosphate **1c** (10 µL, 220 mM in 0.1 M HEPES buffer, pH 8.0, 0.5 mM $MgCl_2$) containing 62 µg D-glucose. The mixture was agitated using a vortex mixer for 2 s. The resulting mixture was incubated at 37 °C for 5 min. The glucose concentration in the assay mixture was determined using a personal glucose meter.

Table S8: Final meter readings corresponding to Figure S9.

	Meter Reading / mg dL^{-1}		
[alkaline phosphatase] / U L^{-1}	Trial 1	Trial 2	Trial 3
0	92	87	99
2	109	110	104
4	118	115	117
6	125	130	126
8	144	141	137
10	157	175	165
20	217	229	218
30	281	271	268
40	320	328	310
60	418	407	399
80	456	462	455
100	511	505	499
120	535	534	561
140	559	573	551
160	564	585	574

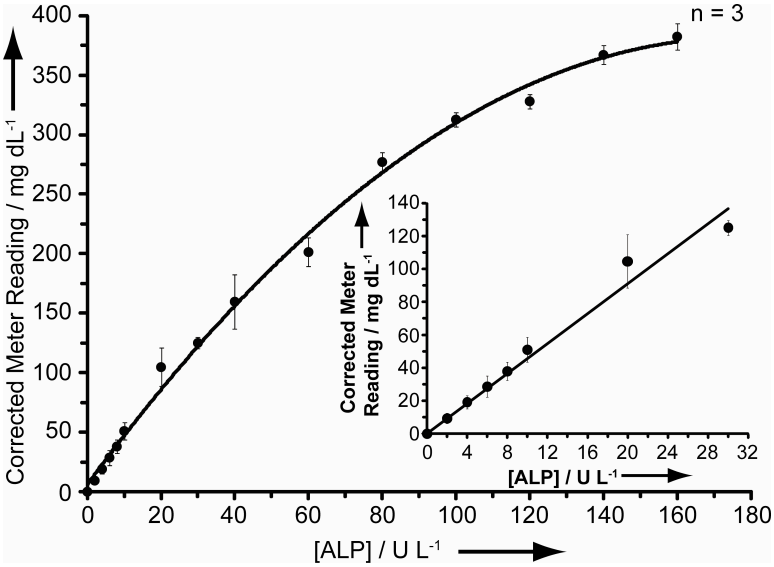


Figure S10 Calibration curve for detection of ALP in serum using **1c**. Corrected Meter Reading refers to the difference between the glucose concentration in the assay mixture and the glucose concentration in a negative control. The data points are the average of three measurements and the error bars reflect the standard deviations from these averages. The duration of the assay for alkaline phosphatase was 5 min in 0.1 M HEPES buffer (pH 8.0, 37 °C).

Experimental procedure corresponding to Figure S10

A 90 µL aliquot of horse blood serum was spiked with a solution of alkaline phosphatase (10 µL, 0–1600 U L^{-1} in 0.1 M HEPES buffer, pH 8.0, 0.5 mM $MgCl_2$). The resulting mixture was added to a solution of glucose-6-phosphate **1c** (10 µL, 220 mM in 0.1 M HEPES buffer, pH 8.0, 0.5 mM $MgCl_2$). The mixture was agitated using a vortex mixer for 2 s. The resulting mixture was incubated at 37 °C for 5 min. The glucose concentration in the assay mixture was determined using a personal glucose meter.

Table S9: Final meter readings corresponding to Figure S10.

	Meter Reading / mg dL^{-1}		
[ALP]* / U L^{-1}	Trial 1	Trial 2	Trial 3
0	144	137	140
2	154	149	146
4	159	156	163
6	168	173	166
8	176	176	183
10	187	195	192
20	231	256	248
30	274	258	264
40	277	311	312
60	336	334	355
80	418	423	411
100	450	454	455
120	466	471	468
140	502	509	511
160	539	512	517

* [ALP] refers to the concentration of ALP in spiked serum sample.

Determination of concentration ALP in various samples of blood serum

A 90 µL aliquot of a serum sample was spiked with a solution of alkaline phosphatase (10 µL, 200 U L^{-1} in 0.1 M HEPES buffer, pH 8.0, 0.5 mM $MgCl_2$). The resulting mixture was added to a solution of glucose-6-phosphate **1c** (10 µL, 220 mM in 0.1 M HEPES buffer, pH 8.0, 0.5 mM $MgCl_2$). The mixture was agitated using a vortex mixer for 2 s. The resulting mixture was incubated at 37 °C for 5 min. The glucose concentration in the assay mixture was determined using a personal glucose meter. The serum samples used were commercial horse blood serum spiked with various amounts of D-glucose. The negative control was identical to the assay except buffer (10 µL, 220 mM in 0.1 M HEPES buffer, pH 8.0, 0.5 mM $MgCl_2$) was used instead of the solution of **1c**.

Table S10: Detection of the concentration of ALP in various samples of blood Serum

Serum sample*	Meter Reading / mg dL^{-1}		*Calculated [ALP] / U L^{-1}
	Negative control	Test sample	
1	142±2	242±12	22±3
2	351±2	439±5	19±1
3	270±8	351±3	18±1

* Calculated using the equation of the calibration curve in Figure S10 (inset) (y = 4.555x)

Table S11: Final meter readings for the assay mixtures corresponding to Table S10

Serum Sample*	Meter Reading / mg dL^{-1}					
	Negative Control			Test Sample		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
1	140	141	144	242	230	253
2	350	354	350	437	436	445
3	263	269	279	348	354	351

* Serum 1 refers to commercial horse blood serum. Serum samples 2 and 3 were prepared by adding 0.72 and 0.36 mg D-glucose, respectively, to 600 µL of commercial horse blood serum.

Calculation of the Limit of Detection (LOD) of the assays

The LOD for the assays were calculated as follows:

LOD = (3 × "standard deviation of meter reading for the assays containing the lowest concentration of the analyte")/"the slope of calibration curve".

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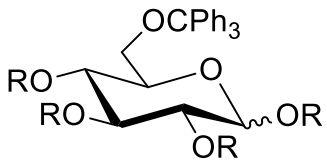

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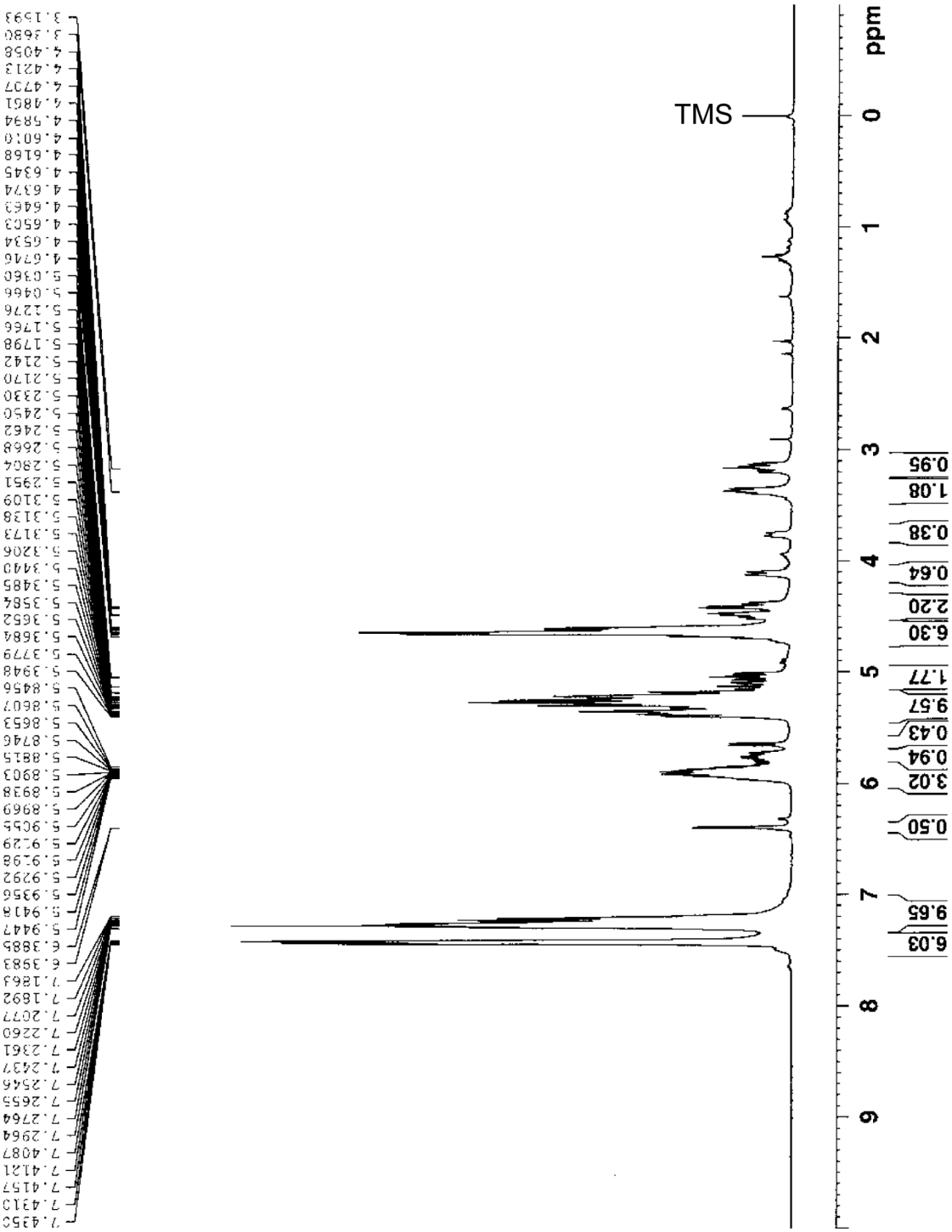

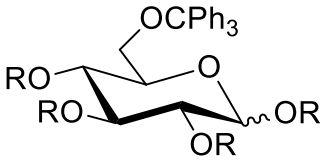

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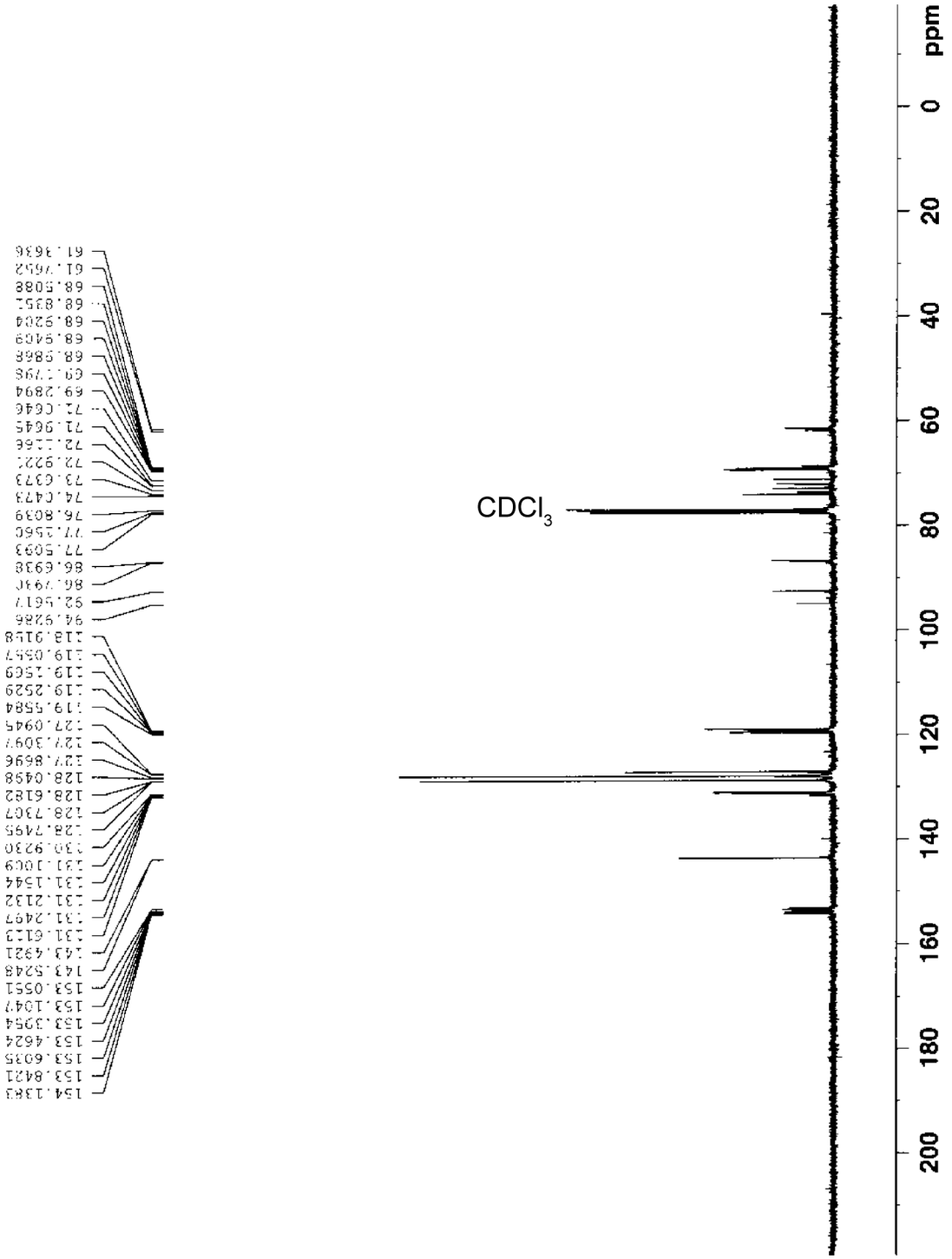

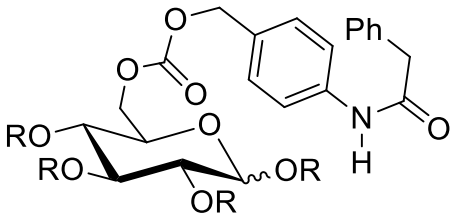
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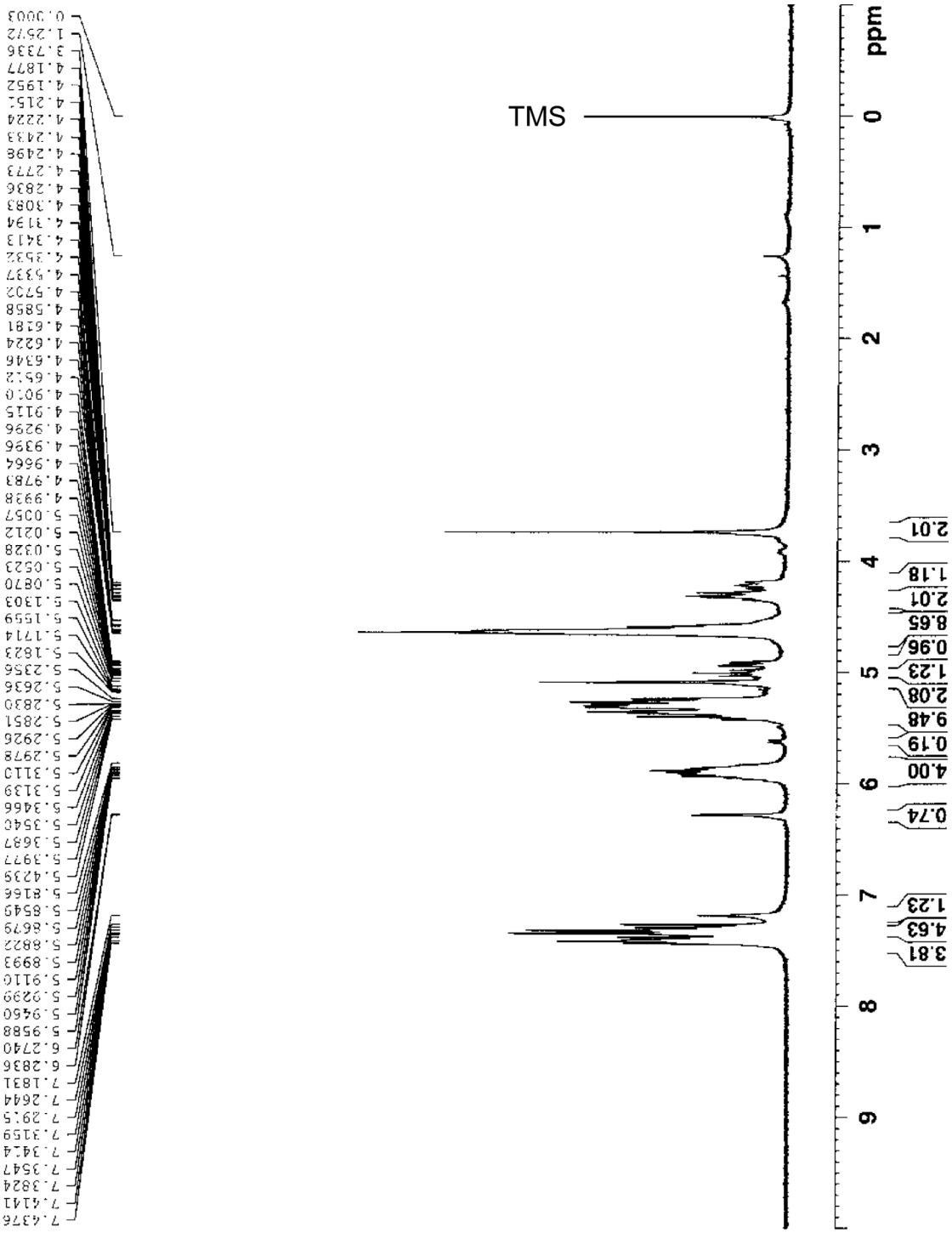
6 (R = Alloc)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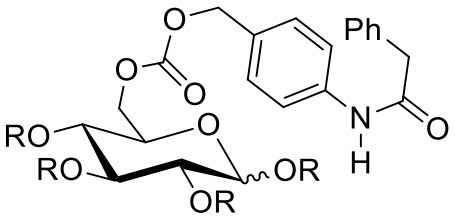

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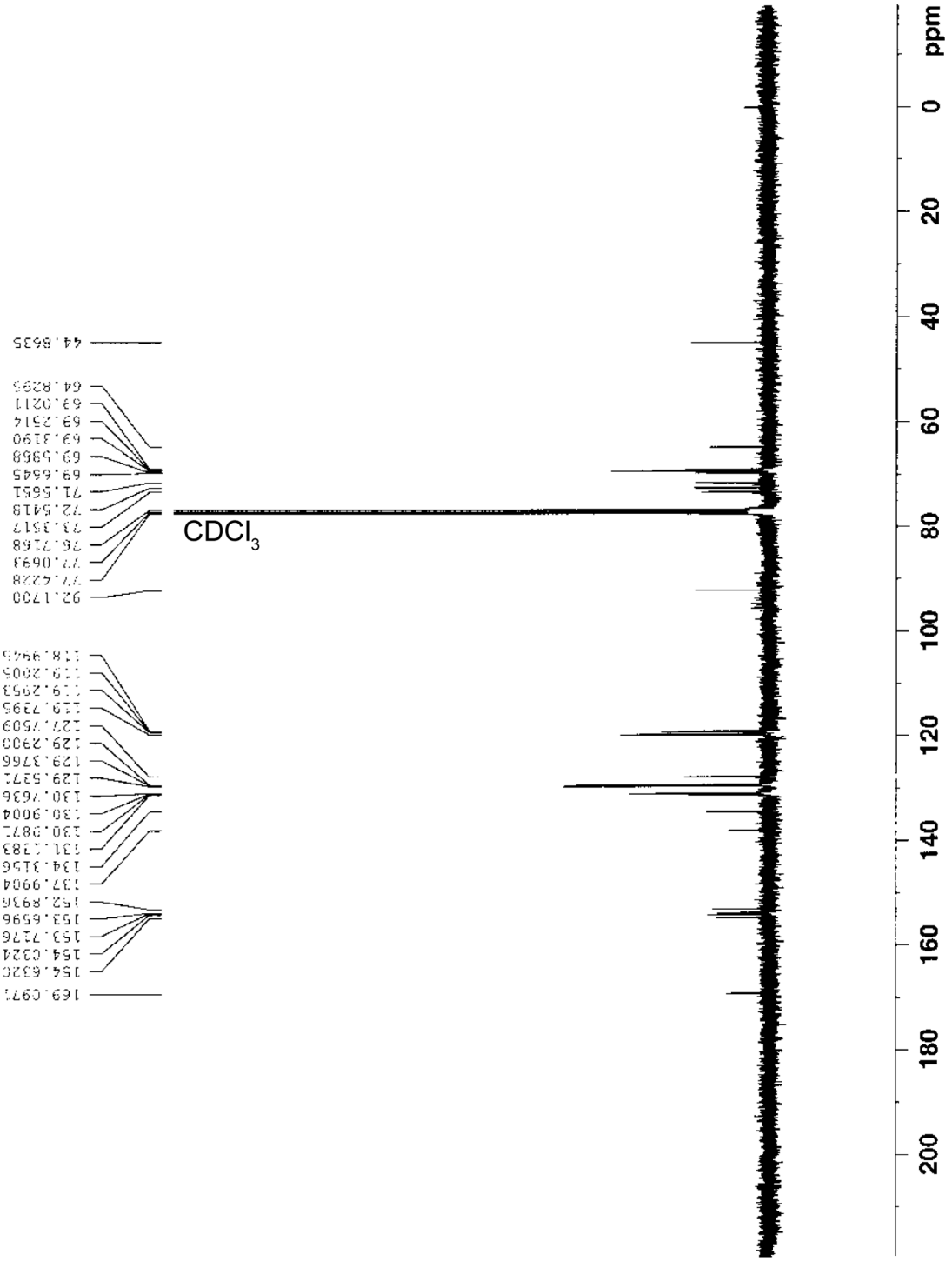

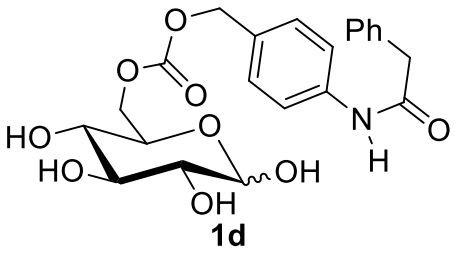

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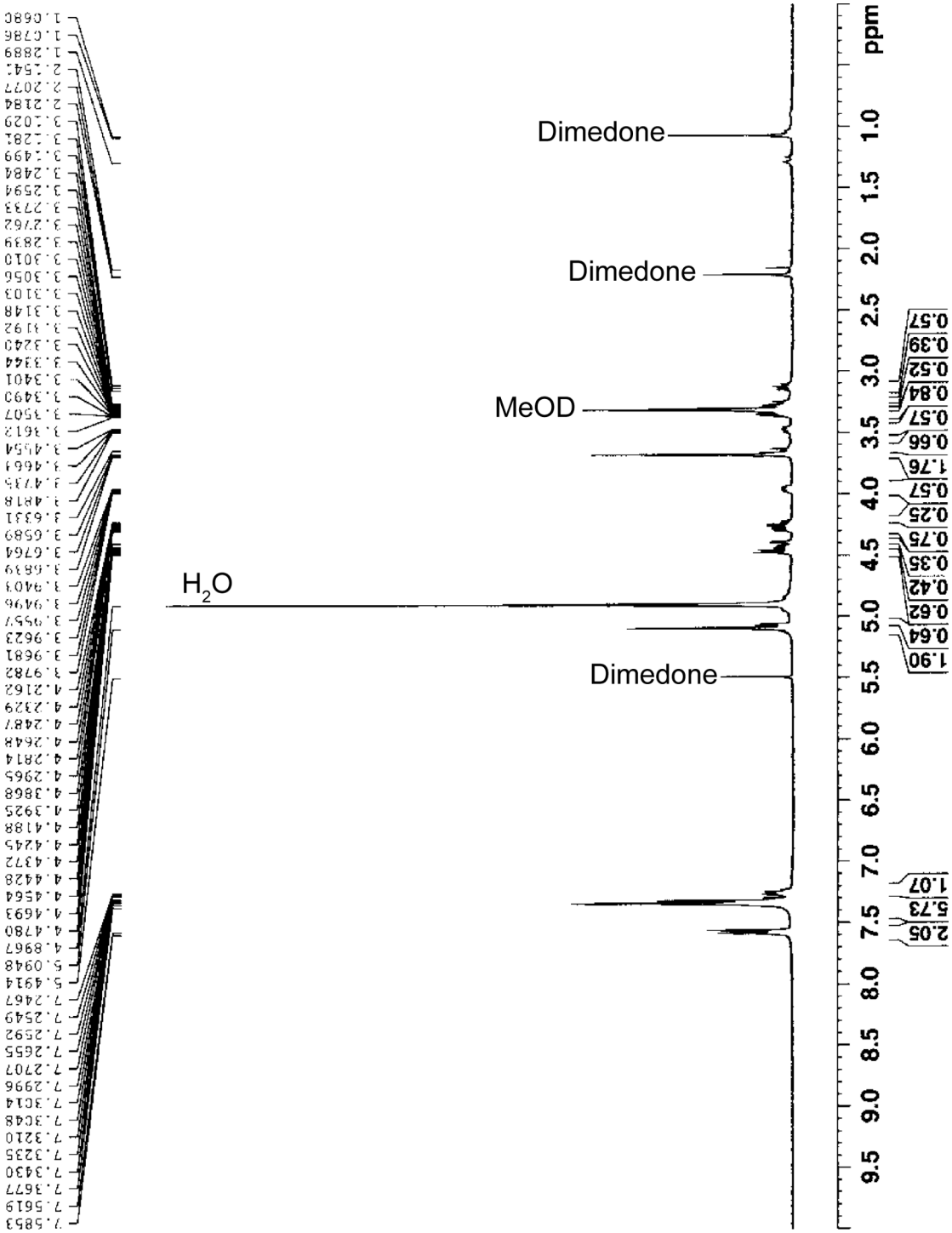

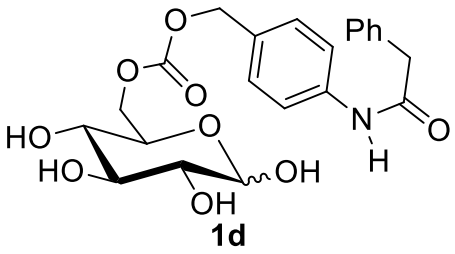

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