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Supporting Information

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Supporting Information for

Detection of Enzymatic Activity by PARACEST MRI: A General Approach to Target a Large Variety of Enzymes

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Synthesis and Enzymatic tests

Commercially available reagents were used without further purification. Solvents were of analytical grade. Reactions were monitored by thin-layer chromatography carried out on 0.25 mm SDS silica gel coated glass plates (60F254) or RP-18 (F254, Merck) plates using UV light as visualizing agent, ethanolic sulfuric acid solution (20%) or ethanolic molybdic acid solution (20%) and heat or Dragendorff reagent as staining agents. Silica gel 60 (particle size 40-63 μm) was used for flash column chromatography. ^{1}H and ^{13}C NMR spectra were recorded at 300 MHz and 75MHz respectively and calibrated using tetramethylsilane as an internal reference. The following abbreviations are used to designate the signal multiplicities: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad). Mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded using electro spray ionization (ESI) conditions. Melting points (mp) are uncorrected and were recorded on a capillary melting point apparatus.

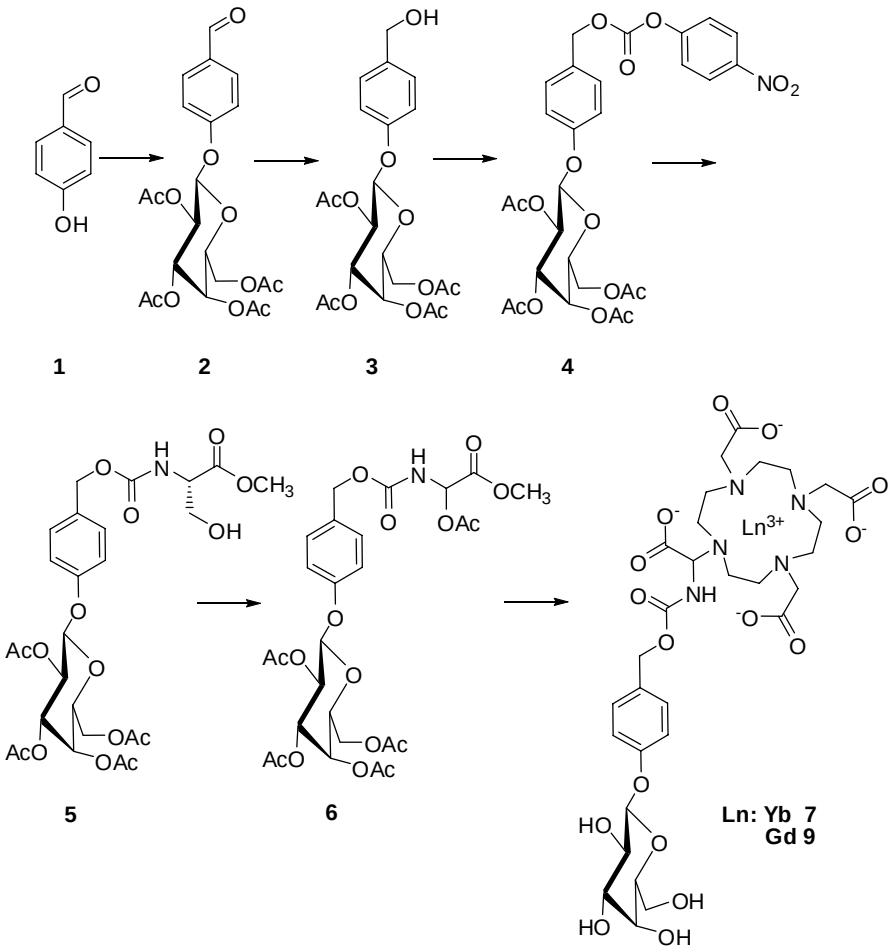
Semi preparative HPLC was done with Water pump 600, detection was done with photodiode array detector (200-400nm, Water 2496).

β−galactosidase from *E. coli* grade VIII (EC 3. 2. 1. 23) was purchased from Sigma (ref. G-5635). The lyophilized powder contains 80% of protein (743 units/mg and 826 units /mg).

Centrifugal filtration through membrane was done with Nanosep® system (PALL, Molecular Weight cutoff: 10 K.)

TBD-methyl polystyrene, the polymer-bound version of the organic base 7-methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene (MTBD) was purchased from Novabiochem (A29720, loading: 2.9 mmol/g).

Scheme 1: Synthesis of **7**



Synthesis of 4-O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-4-oxybenzaldehyde 2.

Silver oxide (5.64 g, 24.0 mmol) and 4-hydroxybenzaldehyde **1** (1.63 g, 13.0 mmol) were added to a solution of α-D-galactopyranosyl bromide (5g, 12.0 mmol) in acetonitrile (125 mL). The mixture was stirred at room temperature for 16h, filtered on celite and the pad washed with EtOAc. The filtrate was concentrated under vacuum and purified by flash chromatography on silica gel (EtOAc-Heptane 3:7) to give **2** as a white powder (3.27 g, 60%). *R*f = 0.68 (Silica, EtOAc/heptanes 7:3); mp: 115-117°C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ= 2.02 (s, 3H, C$\underline{H}_3$CO), 2.062 (s, 3H, C$\underline{H}_3$CO), 2.064 (s, 3H, C$\underline{H}_3$CO), 2.18 (s, 3H, C$\underline{H}_3$CO), 4.08-4.26 (m, 3H, H6',H5'), 5.14 (dd, $J_{3'4'}$=3.4 Hz; $J_{2'3'}$=10.5Hz, 1H, H'3); 5.17 (d, $J_{1'2'}$= 7.9 Hz, 1H, H'1); 5.48 (dd, $J_{3'4'}$=3.4Hz, $J_{4'5'}$= 0.63Hz, 1H, H'4); 5.53 (dd, $J_{2'3'}$=10.5 Hz, $J_{1'2'}$= 7.9 Hz,1H, H'2); 7.12 (d, J= 8.0 Hz, 2H, C$\underline{H}_{Ar}$); 7.86 (d, J= 8.7Hz, 2H, C$\underline{H}_{Ar}$); 9.9 (s, 1H, C$\underline{H}$O); ^{13}C-NMR (75 MHz, $CDCl_3$): δ= 20.54 (C$\underline{H}_3$CO), 20.61(C$\underline{H}_3$CO), 20.63 (C$\underline{H}_3$CO), 20.70 (C$\underline{H}_3$CO), 61.34 (C6'), 66.74 (C4'), 68.40 (C'2), 70.66 (C3'), 71.34 (C5'), 98.61 (C1'), 116.74 (C_{Ar}), -116,73 (2), 131,78 (2) (C_{Ar}), 131.82 (C_{qAr}), 161.26 (C_{qAr}), 169.25 (CH$_3$C$\underline{O}$), 170.02 (CH$_3$C$\underline{O}$), 170.11(CH$_3$C$\underline{O}$), 170.27 (CH$_3$C$\underline{O}$), 190.63 (C$\underline{H}$O).; FT-IR (cm^{-1}) : 2969.7, 2828.3, 1731.9, 1698.1, 1219.8, 1043.4; MS (ESI+): 475 $(M+Na)^+$, 491 $(M+K)^+$; HRMS calcd for $C_{21}H_{24}O_{11}Na$: 475.1216, found: 475.1206.

Synthesis of [4-(hydroxymethyl)phenyl]-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside) 3

Sodium borohydride (1.85 g, 48.6 mmol) was added portion-wise to a cooled solution of aldehyde **2** (10.1 g, 22.2 mmol) in $CHCl_3$-iPrOH (250 mL: 80 mL) at 0°C. The solution was allowed to reach room temperature and stirred for 3h. An aqueous solution of citric acid (10% (w/w), 250 mL) was added. After washing with a $NaHCO_3$ solution (10% (w/w), 3x 150 mL) and water (150mL) the organic phase was dried over $MgSO_4$. The solvent was evaporated under vacuum and the residue purified by flash chromatography on silica gel (EtOAc-heptane 5:5) to give **3** as a white solid. (7.93 g, 78 %). *R*f = 0.15 (Silica, EtOAc/heptanes 5:5); Mp: 100-112°C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.01(s, 3H, C$\underline{H}_3$CO), 2.05 (s,3H, C$\underline{H}_3$CO), 2.06 (s, 3H, C$\underline{H}_3$CO), 2.18 (s, 3H, C$\underline{H}_3$CO), 4.05 (ddd, 1H, $J_{4'5'}$ = 0.9 Hz, $J_{5'6A}$ = 6.2 Hz, $J_{5'6B}$ = 7.0Hz, H'5), 4.16 (dd, $J_{6'A6'B}$ = 11.2 Hz, $J_{5'6'A}$ = 6.2 Hz, 1H, H'6$_A$), 4.23 (dd, $J_{6'A6'B}$ = 11.2 Hz, $J_{5'6'B}$ = 7.0 Hz, 1H, H'6$_B$), 4.64 (sl, 2H, C$\underline{H}_2$-OH), 5.03(d, $J_{1'2'}$=7.9 Hz, 1H, H1'), 5.11 (dd, $J_{3'4'}$=3.0 Hz, $J_{2'3'}$=10.4Hz, 1H, H3'), 5.45 (dd, $J_{3'4'}$=3.0 Hz, $J_{4'5'}$= 0.9 Hz, 1H, H4'), 5.48 (dd, $J_{1',2'}$=7.9 Hz, $J_{2',3'}$=10,4Hz, 1H, H2'), 7.0 (d, J=8.6Hz, 2H, H_{Ar}), 7.3 (d, J=8,6Hz, 2H, H_{Ar}); ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 20.56 (C$\underline{H}_3$CO), 20.64 (2) (C$\underline{H}_3$CO), 20.71 (C$\underline{H}_3$CO), 61.34 (C'6), 64.76 (C$\underline{H}_2$OH), 66.86 (C'4), 68.65 (C'2), 70.81 (C'3), 71.03 (C'5), 99.76 (C'1), 117.04 (2) (C_{Ar}), 128.45(2) (C_{Ar}), 135.90 (C_{Ar}), 156.47 (C_{Ar}), 169.37 (CH$_3$C$\underline{O}$) 170.10 (CH$_3$C$\underline{O}$), 170.21 (CH$_3$C$\underline{O}$), 170.32 (CH$_3$C$\underline{O}$); FT-IR (cm^{-1}) : 3557.4, 2925.1, 1730.82, 1212.0, 1038.2; MS (ESI+): 477 $(M+Na)^+$, 493 $(M+K)^+$, HRMS calcd for $C_{21}H_{26}O_{11}Na$: 477.1373, found: 477,1357. Anal. calcd. for $C_{21}H_{26}O_{11}$: C;55.50, H; 5.86. found: C; 55.35, H; 5.86.

Synthesis of [4-(4-nitro-phenoxycarbonyloxymethyl)phenyl]-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside) 4

p-nitrophenyl chloroformate (9.96 g, 49.5 mmol) was added at room temperature to a mixture of alcohol **3** (7.5g, 16.5 mmol) and pyridine (3.80 mL, 49.5 mmol) in EtOAC (330 mL). The mixture was stirred for 20h and diluted with CH_2Cl_2 (200 mL). After washing with $NaHCO_3$ solution (5%w/w, 200mL) and water (200 mL) the organic phase was dried over $MgSO_4$. The solvent were evaporated under vacuum and the residue purified by flash chromatography on silica gel (EtOAc-heptane 15:5 then 5:5) to give **4** as yellow oil (9.50g, 93%). *R*f = 0.35 (Silica, EtOAc/heptanes 5:5); ^{1}H-NMR (300 MHz, $CDCl_3$): δ= 2.01 (s, 3H, C$\underline{H_3}$CO), 2.06 (s, 6H, C$\underline{H_3}$CO), 2.18 (s, 3H, C$\underline{H_3}$CO); 4,04-4.11 (m, 1H, H'5), 4.14-4.27(m,2H, H'6), 5.07 (d, $J_{1',2'}$=7,9Hz, 1H, H'1), 5.12 (dd, $J_{3',4'}$=3,4 Hz, $J_{2',3'}$=10,3 Hz, 1H, H'3), 5.24 (s, 2H, C$\underline{H_2}$O), 5.46 (d, $J_{3',4'}$=3,4Hz, 1H, H'4), 5.49 (dd, $J_{3',4'}$=7,9 Hz, $J_{2',3'}$=10,3Hz, 1H, H'2), 7,03 (d, J=8,3 Hz, 2H, H_{Ar}), 7.36 (d, J=9,1Hz, 2H, H_{Ar}), 7.41 (d, J=8,3 Hz, 2H, H_{Ar}), 8.27 (d, J=9,1Hz, 2H, H_{Ar}); ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 20.55 (C$\underline{H_3}$CO), 20.63 (2) (C$\underline{H_3}$CO), 20.70 (C$\underline{H_3}$CO), 61.3 (C'6), 66.80 (C'4), 68.57 (C'2), 70.46 (C3'), 70.75 (O-CH2), 71.11 (C5'), 99.40 (C'1), 117.06(2) (A_R), 121.71(2) (A_R), 125.27(2) (A_R), 129.07 (AR,q), 130.51(2) (A_R), 145.40 (A_R, q), 152.40 (O$\underline{C}$(O)O), 155.46 (A_R, q); 157.40 (A_R, q), 169.30 ($CH_3\underline{C}O$), 170.06 ($CH_3\underline{C}O$), 170.16 ($CH_3\underline{C}O$), 170.29 ($CH_3\underline{C}O$). FT-IR (cm^{-1}) : 1742, 1521, 1202, 1058; MS (ESI+): 642 $(M+Na)^+$, 658 $(M+K)^+$; HRMS calcd for $C_{28}H_{29}NO_{15}Na$: 642.1435, found: 642.1436; Anal. calcd. for $C_{28}H_{29}NO_{15}$: C; 54.28, H; 4.72, N; 2.26, found: C; 54.28, H; 4.63, N; 2.03.

Synthesis of N-[4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyloxy)-benzyloxycarbonyl]-(S)-Serine methyl ester. 5

L-serine methyl ester (3.09, 19.9 mmol) and triethyl amine (6.4 mL, 46 mmol) were successively added to a solution of carbonate **4** (9.5g, 15.3 mmol) in DMF (280mL). The solution was stirred at room temperature for 18h. The solvent was then evaporated under vacuum and the residue taken up with a mixture of EtOAc-water (400mL, 1/1 v/v). After washing with water the organic phase was dried over $MgSO_4$ and the solvent evaporated under vacuum. The residue was purified by flash chromatography on silica gel (EtOAc-CH_2Cl_2 3:7) to give **5** as colorless oil (7.6 g, 84%). *R*f = 0.44 (Silica, EtOAc/heptanes 5:5); ^{1}H-NMR (300 MHz, $CDCl_3$): δ= 2.00 (s, 3H, C$\underline{H_3}$CO), 2.05 (s, 6H, C$\underline{H_3}$CO), 2.18 (s, 3H, C$\underline{H_3}$CO), 3.77 (s, 3H, OCH_3), 3.90 (dd, $J_{HAH\alpha}$ =3.3Hz, J_{HAHB} =11.2 Hz , 1H, C$\underline{H_{2A}}$OH), 3.99 (dd, $J_{HBH\alpha}$ =3.5Hz, J_{HAHB} =11.2 Hz , 1H, C$\underline{H_{2A}}$OH), 4.02-4.08 (m, 1H, H5'), 4.09-4.25 (m, 2H, $H_{6A',6B'}$), 4.38-4.47 (m, 1H, Hα), 5.03 (d, $J_{1',2'}$=7.9 Hz, 1H, H1'), 5.06 (s, 2H, C$\underline{H_2}$-OCO), 5.10 (dd, $J_{2',3'}$=10.4 Hz, $J_{3',4'}$=3.4 Hz, 1H, H3'), 5.44 (dd, $J_{3',4'}$=3.4Hz, $J_{4',5'}$=0.9Hz 1H, H4'), 5.47 (dd, $J_{2',3'}$=10,4 Hz, $J_{1',2'}$=7.9Hz, 1H, H2'), 5.7 (d, $J_{H\alpha NH}$= 7.4 Hz, 1H,NH), 7.00 (d, J= 8,6Hz, 2H, H_{Ar}), 7.30(2) (d, J= 8,6Hz, 2H, H_{Ar}); ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 20.55 (C$\underline{H_3}$CO), 20.63 (C$\underline{H_3}$CO), 20.70 (C$\underline{H_3}$CO), 52.72 (C$\underline{H_3}$O), 55.95 (NHC$\underline{H}\alpha$), 61.33 (C6'), 63.22 (C$\underline{H_2}$OH), 66.33 (C$\underline{H_2}$O), 66.83 (C4'), 68.57 (C2'), 70.77 (C5'), 71.02 (C3'), 99.54 (C1'), 116.91(2) (Ar), 129.83(2) (Ar), 131.03 (Ar q), 156.85() (Ar q), 169.37 (NHC$\underline{O}$), 170.11 ($CH_3\underline{C}O$), 170.22 ($CH_3\underline{C}O$), 170.35 ($CH_3\underline{C}O$), 170.9 ($CH_3\underline{C}O$); FT-IR (cm^{-1}) : 1741, 1512, 1209, 1041; MS (ESI+): 622 $(M+Na)^+$, 638 $(M+K)^+$; HRMS calcd for $C_{26}H_{33}NO_{15}Na$: 622.1748, found: 622.1732; Anal. calcd. for $C_{26}H_{33}NO_{15}$: C; 52.09, H; 5.55, N; 2.34, found: C; 51.69, H; 5.46, N; 2.34.

Synthesis of 2-Acetoxy-N-[4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyloxy)-benzyloxycarbonyl] glycine methyl ester. 6

Lead tetraacetate (2.20g, 5.00 mmol) was added to a suspension of serine derivative **5** (1g, 1.70 mmol) and molecular sieves 4Å (10g) in EtOAc (20mL) placed under argon. The mixture was reflux for 2h, cooled to room temperature and filtered on a pad of celite. After washing of the pad with EtOAc, the combined filtrate were successively washed with a 20% (w/w) citric acid aqueous solution, brine, dried over $MgSO_4$ and evaporated under vacuum to give **6** as a yellow oil (0.96 g, 90%). This product was not further purified and was used as it in the next step. *R*f = 0.44 (Silica, CH_2Cl_2-EtOH 19:1); ^{1}H-NMR (300 MHz, $CDCl_3$): δ= 2.02 (s, 3H, C$\underline{H_3}$CO), 2.07 (s, 6H, C$\underline{H_3}$CO), 2.10 (d, 3H, C$\underline{H_3}$CO), 2.19 (s, 3H, C$\underline{H_3}$CO), 3.8 (s, 3H, OC$\underline{H_3}$), 4.05-4.27 (m, 3H, H5', H6'), 5.04 (d, $J_{1',2'}$=7.9 Hz, 1H, H1'), 5.10 (s, 2H, OC$\underline{H_2}$), 5.13 (dd, $J_{2',3'}$=10.3Hz; $J_{3',4'}$=3.4Hz, 1H, H3'), 5.46 (d, $J_{3',4'}$=3.4Hz, 1H, H4'), 5.50 (dd, $J_{2',3'}$=10.4 Hz, $J_{1',2'}$=7.9Hz, 1H, H2'), 6.15 (d, $J_{H\text{-}NH}$=9.5Hz, 0.5 H, C$\underline{H}$OAc), 6.28 (d, $J_{H\text{-}NH}$=9.5Hz, 0.5H, C$\underline{H}$OAc), 7.00 (d, J=8.6 Hz, 2H, H_{Ar}), 7.30 (d, J=8.6Hz, 2H, H_{Ar}).

General procedure for the synthesis of [[({[4-(β-D-galactopyranosyloxy) benzyl]oxy}carbonyl)amino]{4,7,10-tris[(carboxy-κO)methyl]-1,4,7,10-tetraazacyclododecan-1-yl}acetato(4-)-κO]ytterbium. 7

TBD resin (1.10g, 3.20 mmol) and acetoxy compound **6** (1.00 g, 1.60 mmol) were successively added to a solution of the triethyl ester derivative of DO3A (0.342 mg, 0.80 mmol) in dichloromethane (15 mL). The suspension was agitated on an orbital shaker for 30 min at room temperature and filtered. After washing of the resin with dichloromethane, the combined filtrates were evaporated under vacuum. The obtained yellow oil was taken up in a mixture of EtOH/H_2O (15mL, 1/2 v/v). The pH of the solution was adjusted to 12 and maintained at this value by continuous addition of 1N NaOH until it remains constant. The solution was then neutralized with 1N HCl and Ytterbium trichloride hexahydrate added (0.31g; 0.80 mmol), the pH was adjusted to pH 7.0 with NaOH 0.5N. After stirring for 72 h at room temperature, the solution was lyophilized. The obtained white powder was dissolved in water (20 mg/mL) and purified by semi-preparative HPLC by repeated injections (1.5 mL) on a hypersil C18 column (250x21.2mm, 5μ) with a flow rate of 21 mL.min^{-1}. Detection was at 210 nm using a photodiode array detector. An isocratic solvent system consisting of CH_3CN/aqueous NH_4OAc (25 mM), 95/5 (v/v) was used. The fractions containing the product (Rt: 13.53 min) were combined and lyophilized. The obtained powder was taken up in water (100mL) and lyophilized again. This operation was repeated one more time in order to eliminate completely the ammonium salt and to give **7** as a white powder (0.15g; 22% for the 3 steps). MS (ESI$^-$): 901 $(M(^{174}Y)\text{-}H)^-$; HRMS calcd for $C_{30}H_{41}N_5O_{16}$ ^{174}Yb: 901.1937, found: 901.1909.

Synthesis of [[({[4-(β-D-galactopyranosyloxy) benzyl]oxy}carbonyl)amino]{4,7,10-tris[(carboxy-κO)methyl]-1,4,7,10-tetraazacyclododecan-1-yl}acetato(4-)-κO]Gadolinium (9)

The product was obtained with the same yield by substituting Gadolinium trichloride hydrate for Ytterbium trichloride hexahydrate in the previous procedure.

MS (ESI+): 909 (M+Na); HRMS calcd for $C_{30}H_{42}N_5NaO_{16}{}^{158}Gd$: 909,1765. found: 909,1813.

Synthesis of [amino{4,7,10-tris[(carboxy-κO)methyl]-1,4,7,10-tetraazacyclododecan-1-yl}acetato(4-)-κO]gadolinate(1-)Ytterbium (8)

TBD resin (0.05g, 0.14 mmol) and α–acetoxy N-benzyloxycarbonyl glycine methyl ester (0.04 g, 0.14 mmol) were successively added to a solution of the triethyl ester derivative of DO3A (0.03 mg, 0.07 mmol) in dichloromethane (5 mL). The suspension was agitated on an orbital shaker for 1h at room temperature and filtered. After washing of the resin with dichloromethane, the combined filtrates were evaporated under vacuum. The obtained yellow oil was taken up in a mixture of EtOH/H_2O (5mL, 1/5 v/v). The pH of the solution was adjusted to 12 and maintained at this value by continuous addition of 1N NaOH until it remains constant. The solution was then neutralized with 1N HCl and Ytterbium trichloride hexahydrate added (0.03g; 0.07 mmol), the pH was adjusted to pH 7.0 with NaOH 0.5N. After stirring for 72 h at room temperature, the solution was filtered and freeze dried. The obtained white powder was dissolved in water (20 mg/mL) and purified by semi-preparative HPLC by repeated injections (1.5 mL) on a hypersil C18 column (250x21.2mm, 5µ) with a flow rate of 21 $mL.min^{-1}$. Detection was at 210 nm using a photodiode array detector. An isocratic solvent system consisting of CH_3CN/aqueous NH_4OAc (25 mM), 95/5 (v/v) was used. The fractions containing the product were combined and lyophilized. The obtained powder was taken up in water (100mL) and lyophilized again. This operation was repeated one more time in order to eliminate completely the ammonium salt and to give the N-benzyloxycarbonyl intermediate as a white powder (0.01g; 22% for the 3 steps). Pd/C (10%, 0.005g) was added to an aqueous solution of this product (1mL) and the mixture was stirred for 3h at room temperature under slight pressure of hydrogen (4psi). Filtration through Nylon membrane (0.45µm) followed by freeze drying give the product as a white powder (0.006g, 16%).

β galactosidase catalyzed hydrolysis of **9**: A solution of β galactosidase (10µL, 0.1mg/mL) in phosphate buffer (pH 7.0, 20mM) was added to a solution of the agent (250 µL, 2.7mM) in phosphate buffer (pH 7.0, 20mM) containing BSA (7.0 mg/mL) and the mixture was stirred at 37°C. 50µL of this mixture was taken at different time, diluted with water (85µL) and the enzyme was removed by centrifugal filtration through membrane (Molecular Weight cutoffs 10K, 10min, 12 000 rpm). The filtrate was the analyzed by HPLC.

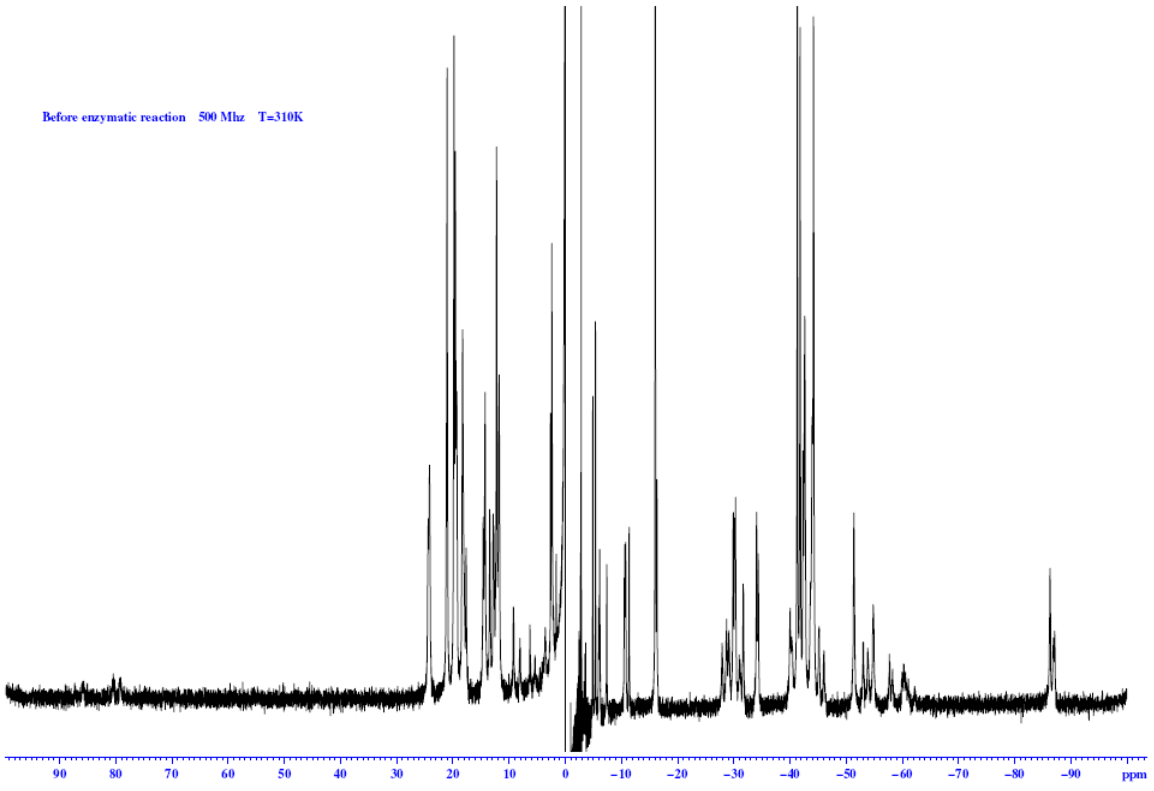


Fig. S1. 500 MHz ^1H NMR spectrum of Yb(DOTA-αbz-βGal)$^-$ **7** in H_2O.

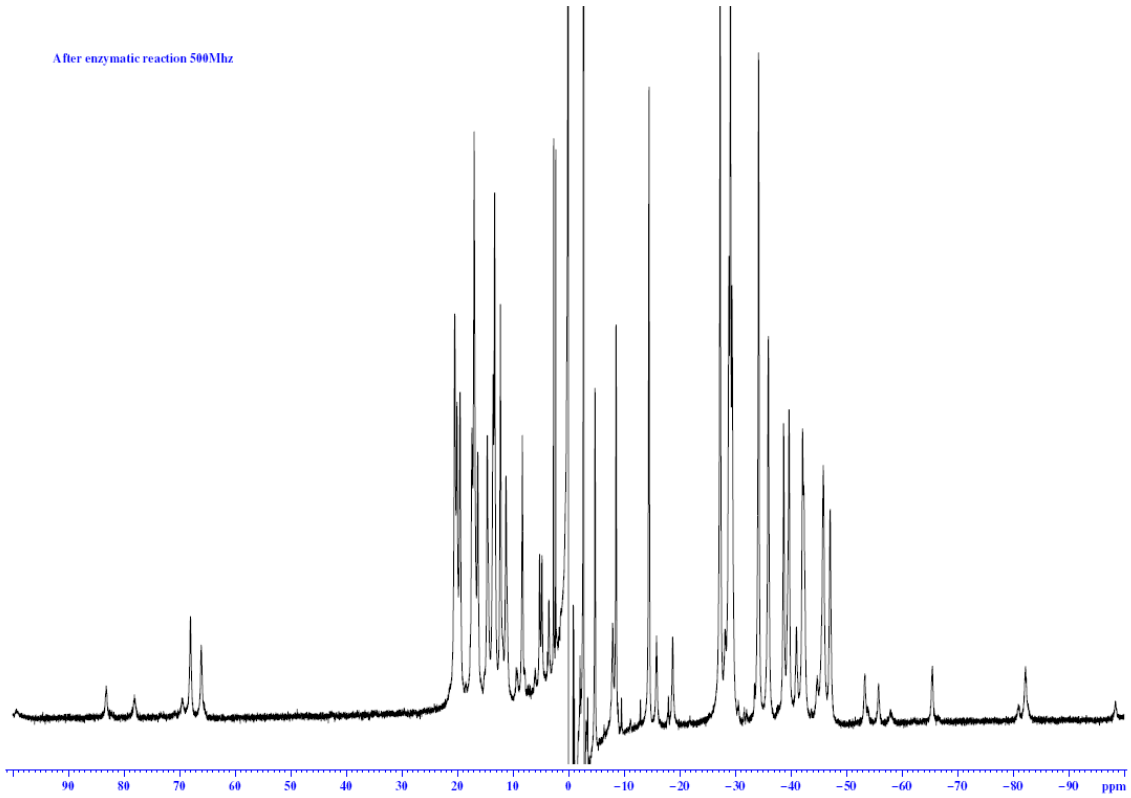


Fig. S2. 500 MHz ^{1}H NMR spectrum of the reaction mixture following addition of 31 U β-galactosidase to 0.5 mL of 20 mM Yb(DOTA-αbz-βGal)$^{-}$, pH 7.5, in H_2O.

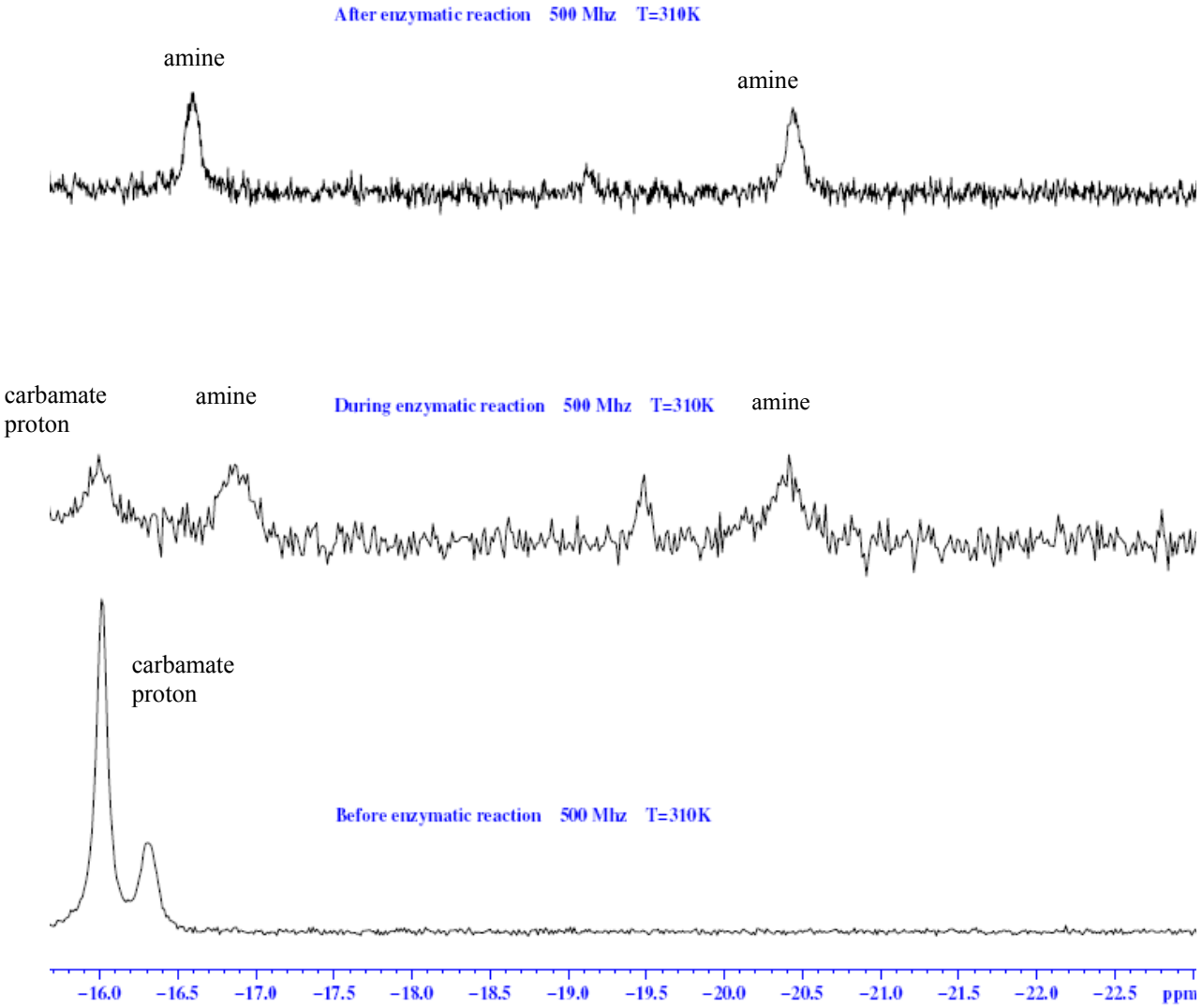


Fig. S3. The region of between -15 and – 23 ppm of the 500 MHz ^{1}H NMR spectrum of Yb(DOTA-αbz-βGal)$^{-}$ before enzymatic cleavage (bottom), during the enzymatic reaction (middle) and after completion of the enzymatic cleavage (top). pH 7.5, in H_2O.

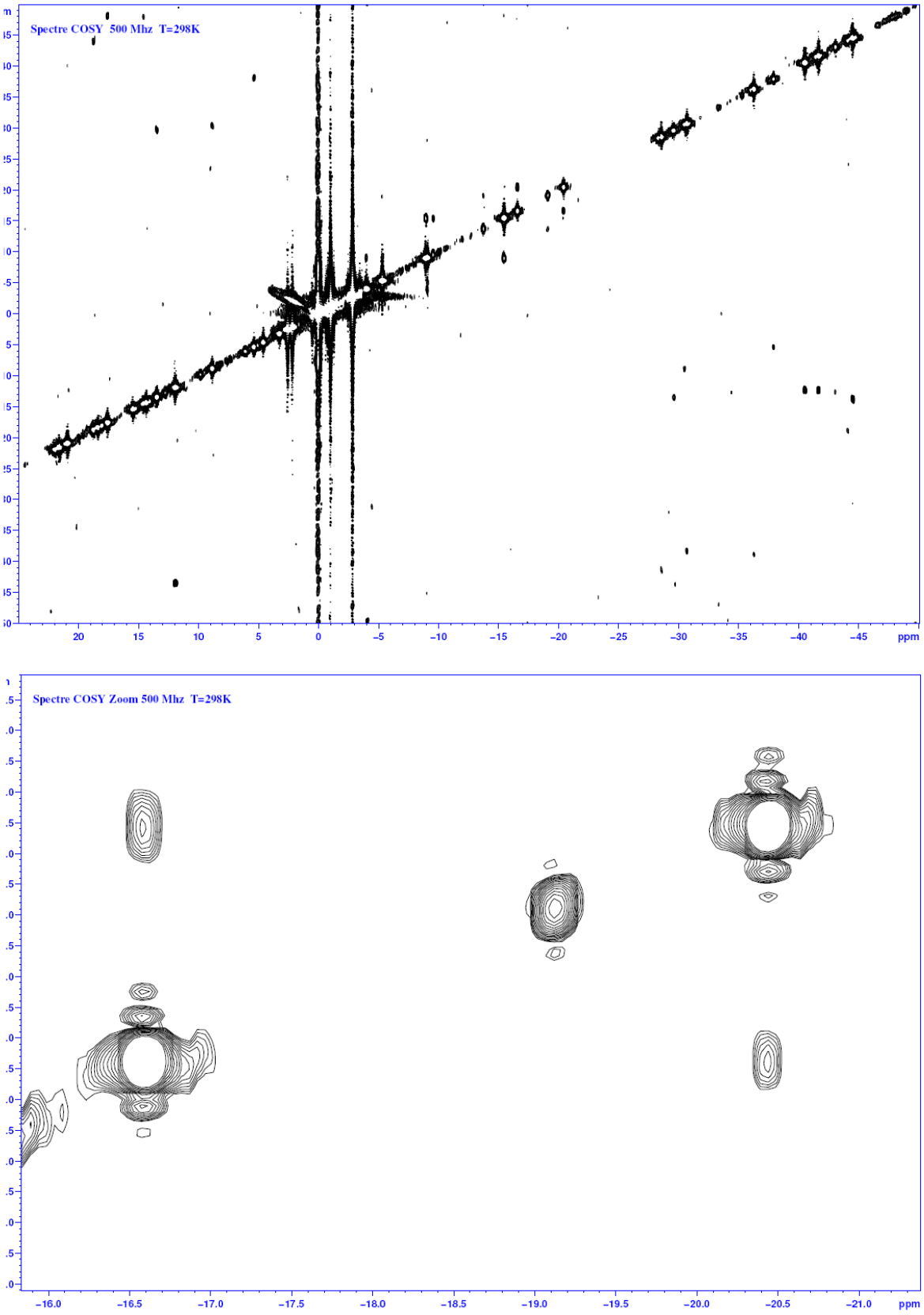


Fig. S4. COSY of the reaction mixture following addition of 31 U *β*-galactosidase to 0.5 mL of 20 mM Yb(DOTA-αbz-βGal)$^{-}$, pH 7.5.

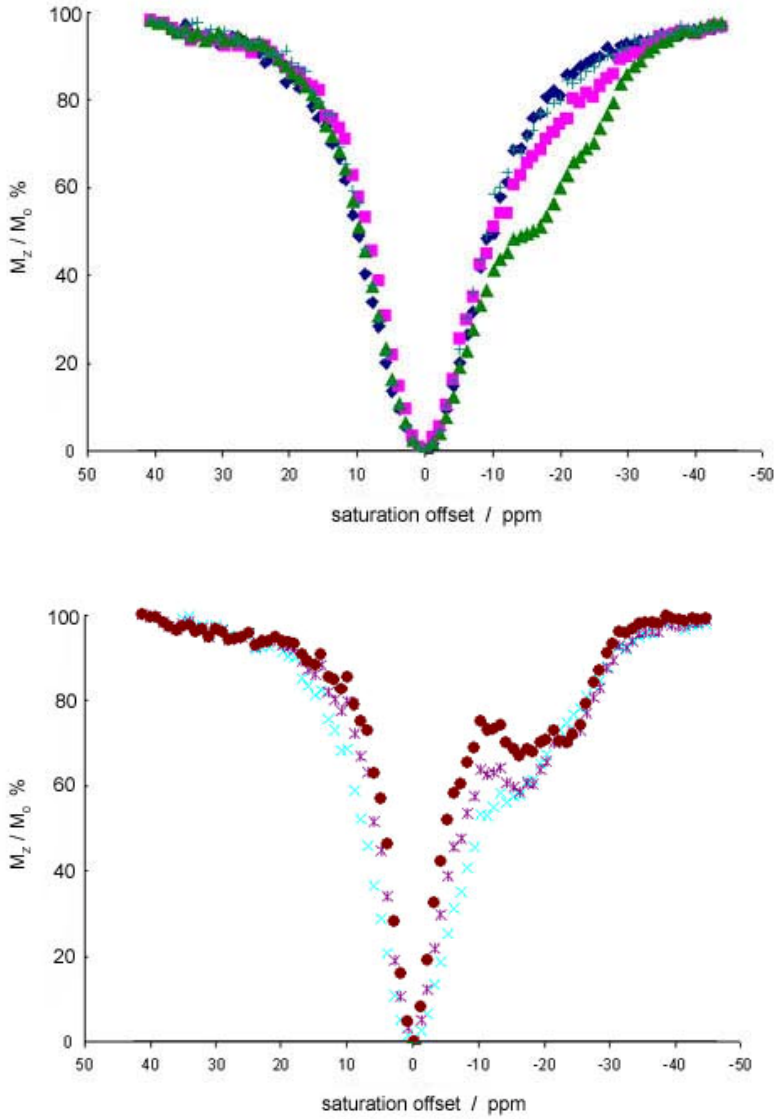


Figure S5. PARACEST spectra recorded after enzymatic cleavage of Yb(DOTA-α-bz-βGal)$^-$ by *β*-galactosidase at different pH values (+) 6.2 (♦) 6.9 (■) 7.2 (▲) 7.5 (x) 8.1 (*) 8.9 (●) 9.6. PARACEST spectra were collected with modified presaturation pulse sequence with a continuous wave saturation pulse, saturation pulse power of 31 μT, saturation delay of 3 sec and in 0.1ppm increments, 37°C.

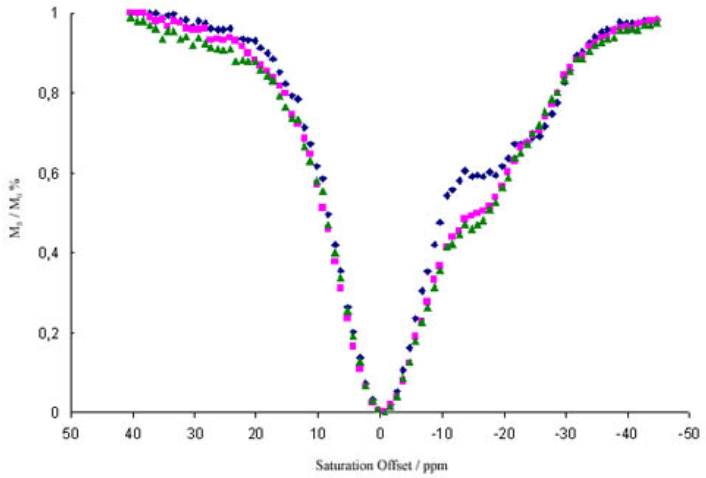


Figure S6. PARACEST spectra recorded after enzymatic cleavage of Yb(DOTA-α-bz-βGal)$^-$ by *β*-galactosidase at different temperatures (♦) 25°C (■) 37°C (▲) 45°C. PARACEST spectra were collected with modified presaturation pulse sequence with a continuous wave saturation pulse, saturation pulse power of 31 μT, saturation delay of 3 sec and in 0.1ppm increments.

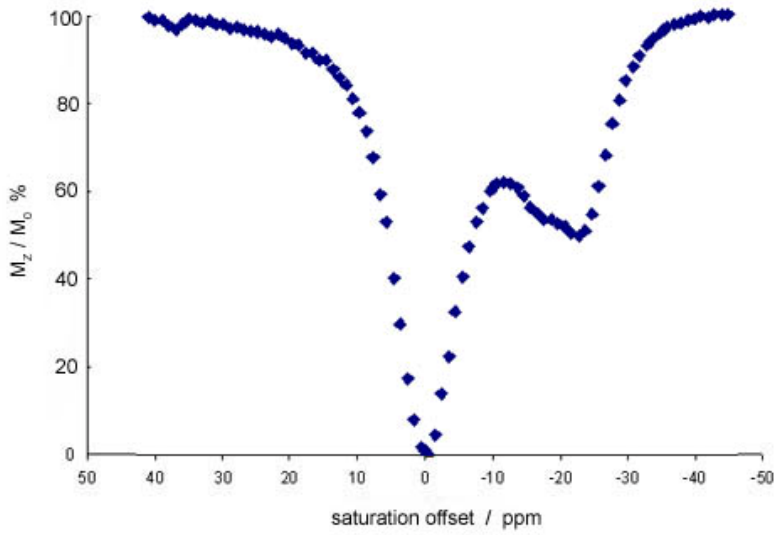
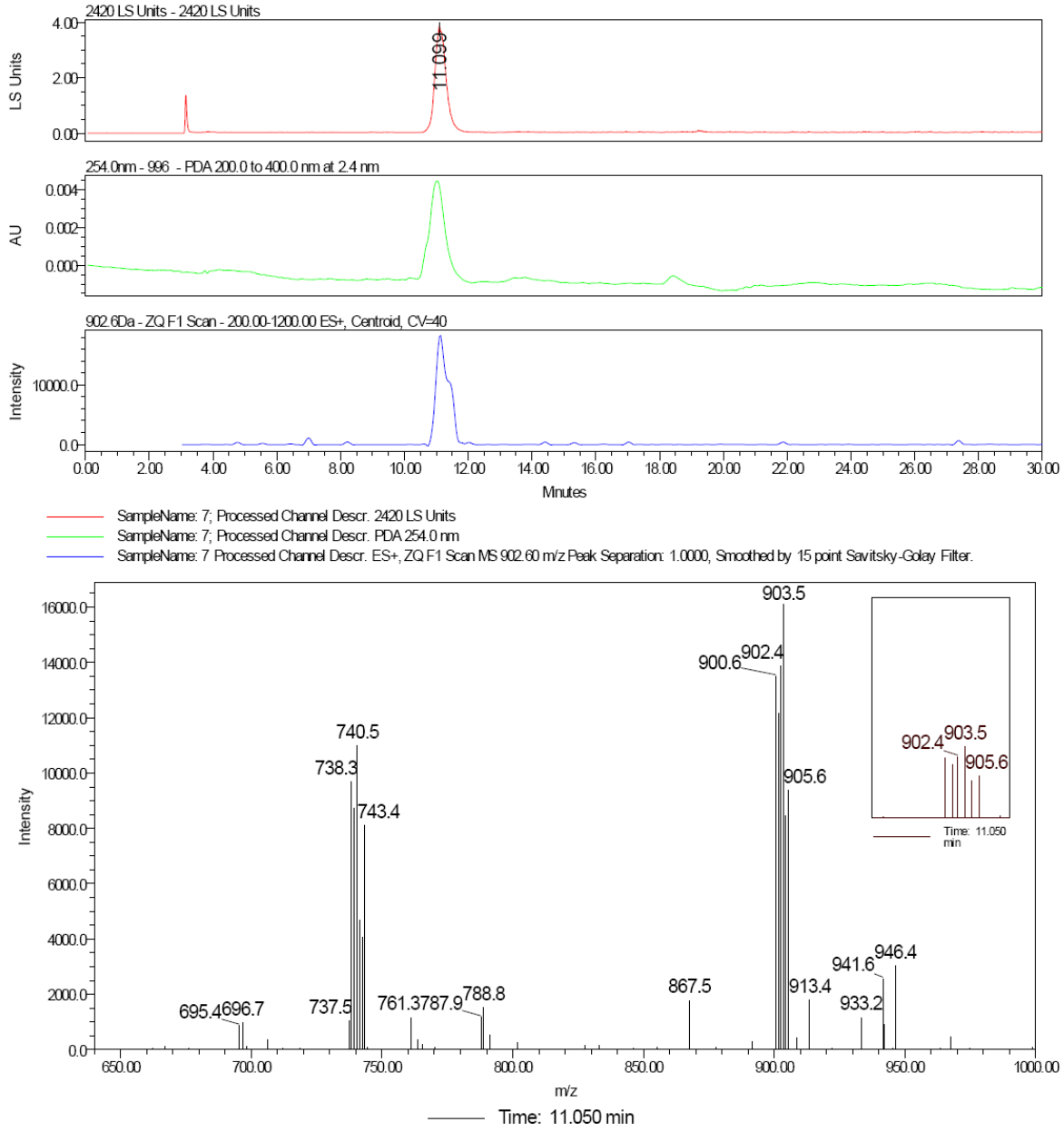


Figure S7. PARACEST spectrum of Yb(DOTA-NH_2) 37°C, pH = 8.3. PARACEST spectra were collected with modified presaturation pulse sequence with a continuous wave saturation pulse, saturation pulse power of 31 μT, saturation delay of 3 sec and in 0.1ppm increments.

HPLC Analysis of compound 7



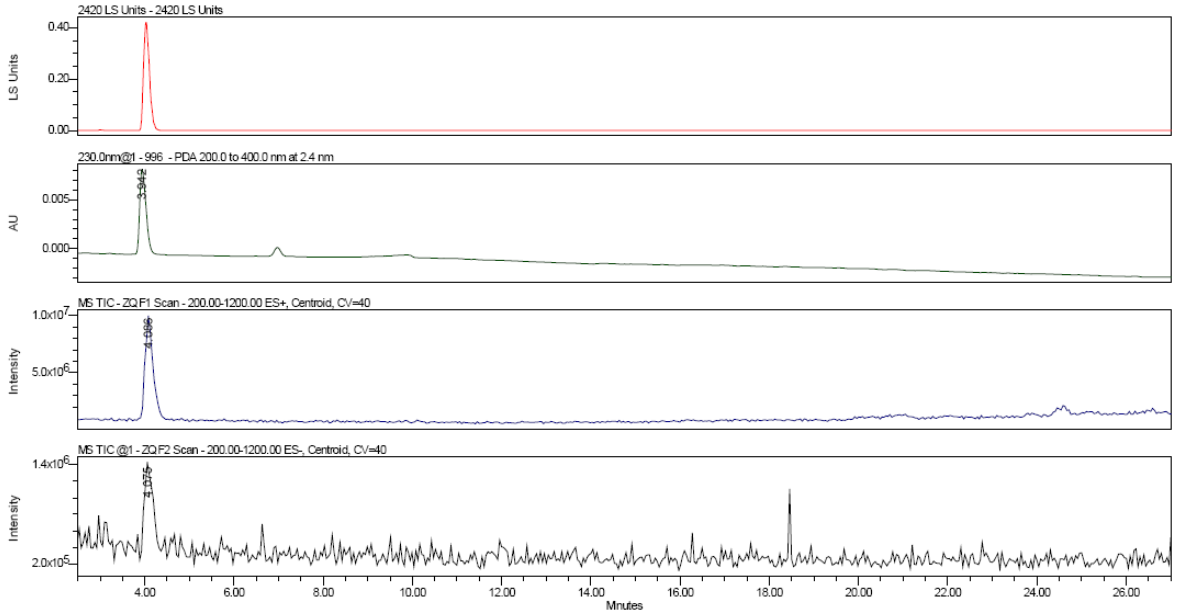
Gradient

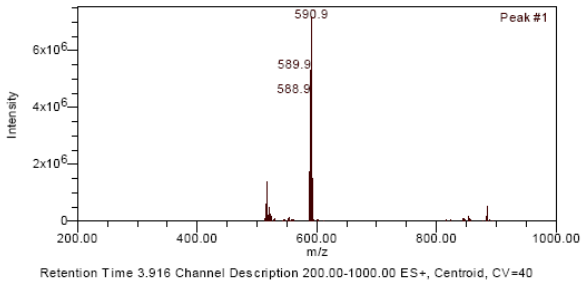
	Time	Flow	%A	%B
1	0.01	1.00	95.0	5.0
2	27.00	1.00	95.0	5.0
3	35.0	1.00	80.0	20.0

Column : Hypersil C18, 5µm, 250*4.6 mm
Solvent: A: H2O-AcONH4 25mM
B : CH3CN

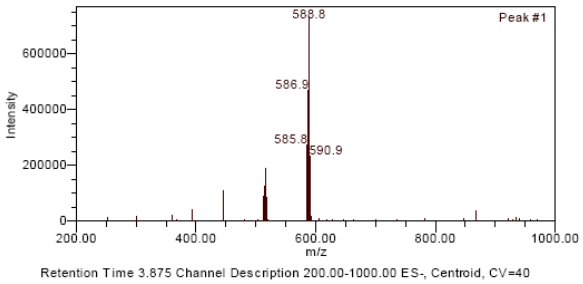
The HPLC chromatograms were respectively recorded using Light Scattering detection, 254 nm UV detection and Mass spectrometry detection with Electrospray ionisation in positive modes.

HPLC Analysis of compound 8





Retention Time 3.916 Channel Description 200.00-1000.00 ES+, Centroid, CV=40



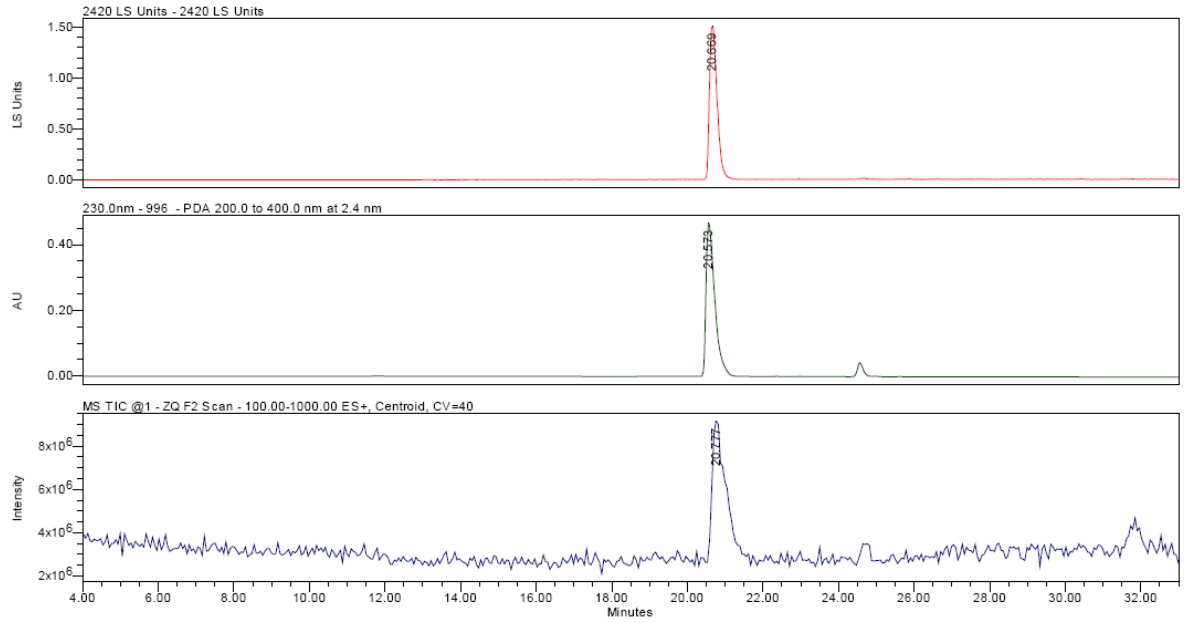
Retention Time 3.875 Channel Description 200.00-1000.00 ES-, Centroid, CV=40

Column : Hypersil C18, 5µm, 250*4.6 mm
Injection Volume : 10.00 µL
Solvents: A: H_20-$AcONH_4$(10mM) B:CH3CN
The HPLC chromatograms were respectively recorded using Light Scattering detection, 230nm UV and Mass spectrometry detection with Electrospray ionisation in positive and negativ modes.

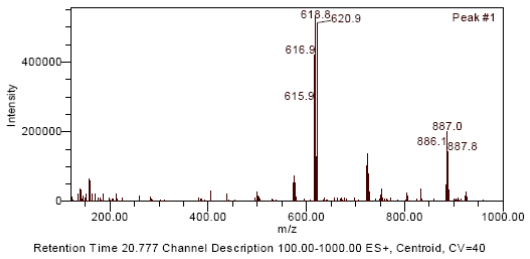
	Time	Flow	%A	%B	%C	%D	Curve
1		1.00	100.0	0.0	0.0	0.0	
2	5.00	1.00	100.0	0.0	0.0	0.0	6
3	25.00	1.00	90.0	10.0	0.0	0.0	6
4	30.00	1.00	90.0	10.0	0.0	0.0	6
5	32.00	1.00	50.0	50.0	0.0	0.0	6
6	35.00	1.00	50.0	50.0	0.0	0.0	6
7	36.00	1.00	100.0	0.0	0.0	0.0	6
8	50.00	1.00	100.0	0.0	0.0	0.0	6

HPLC Analysis of the Gd derivative (9) and its β galactosidase catalyzed hydrolysis

Column : Hypersil C18, 5µm, 250*4.6 mm
Injection Volume : 10.00 µL
Solvents: A: H_20-$AcONH_4$(10mM) B:CH3CN
The HPLC chromatograms were respectively recorded using Light Scattering detection, 230nm UV and Mass spectrometry detection with Electrospray ionisation in positive mode.
t=0:

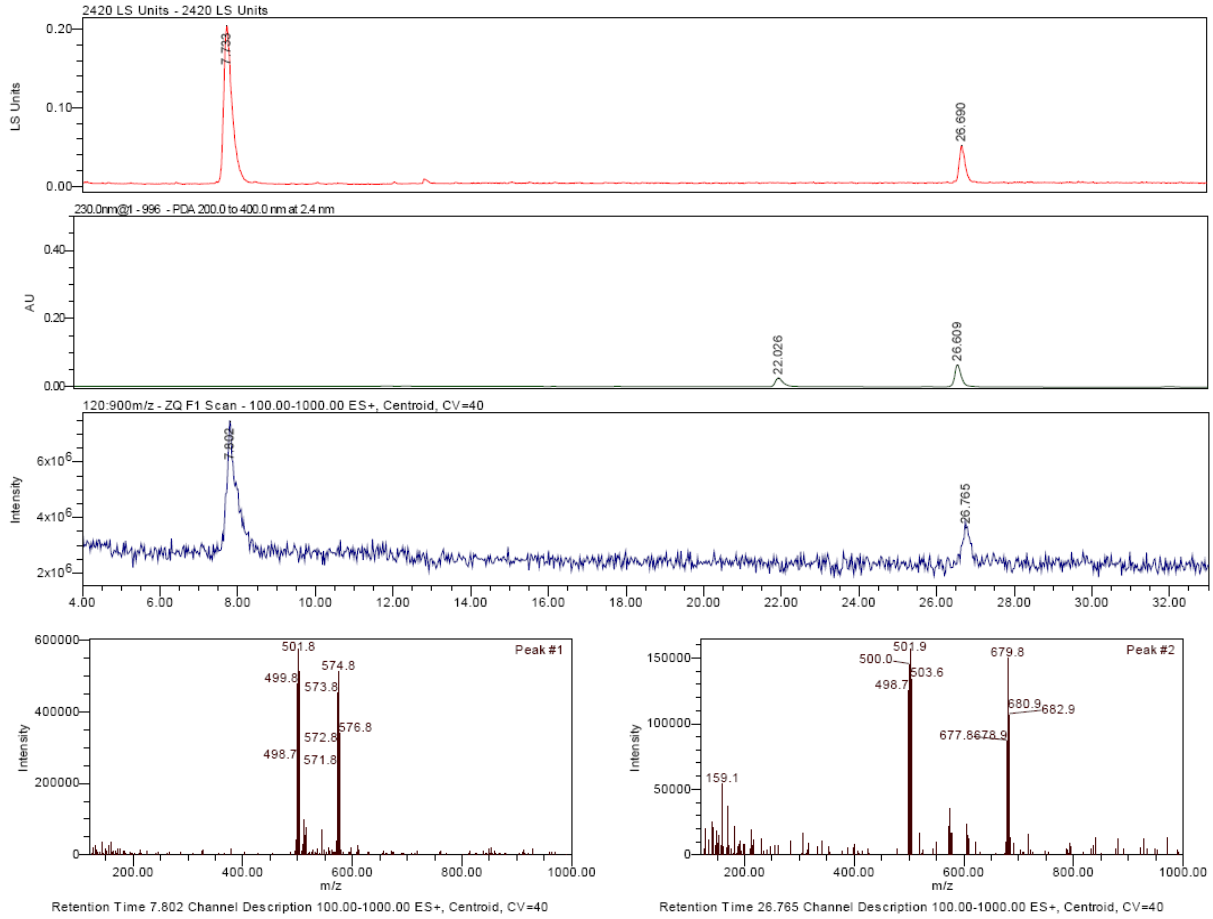


	Time	Flow	%A	%B	%C	%D	Curve
1		1.00	100.0	0.0	0.0	0.0	
2	5.00	1.00	100.0	0.0	0.0	0.0	6
3	25.00	1.00	90.0	10.0	0.0	0.0	6
4	30.00	1.00	90.0	10.0	0.0	0.0	6
5	32.00	1.00	50.0	50.0	0.0	0.0	6
6	38.00	1.00	50.0	50.0	0.0	0.0	6
7	39.00	1.00	100.0	0.0	0.0	0.0	6
8	52.00	0.10	100.0	0.0	0.0	0.0	6
9	59.00	1.00	100.0	0.0	0.0	0.0	6



HPLC Analysis of the β galactosidase catalyzed hydrolysis of the Gd derivative (9)

Column : Hypersil C18, 5µm, 250*4.6 mm
Injection Volume : 10.00 µL
Solvents: A: H_20-$AcONH_4$(10mM) B:CH3CN
Same elution conditions than for t=0
t=0:



Retention Time 7.802 Channel Description 100.00-1000.00 ES+, Centroid, CV=40

Retention Time 26.765 Channel Description 100.00-1000.00 ES+, Centroid, CV=40