A general method for affinity-based proteomic profiling of *exo*-α-glycosidases

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EXPERIMENTAL

General

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV600 (600 MHz for ^{1}H and 150 MHz for ^{13}C) (chemical shifts quoted relative to CD_3OD). Elemental analyses of all synthesized compounds used in enzyme assays were performed at the Australian National University Microanalytical Facility. Flash chromatography was performed on BDH silica gel with the specified solvents. Thin-layer chromatography (TLC) was effected on Merck silica gel 60 F_{254} aluminium-backed plates that were stained by heating (>200 °C) with 5% sulfuric acid in EtOH. Percentage yields for chemical reactions as described are quoted only for those compounds that were purified by column chromatography, and purity was assessed by TLC or ^{1}H NMR spectroscopy.

General procedure for preparation of affinity probes

To a solution of the iminosugar (0.1 mmol) in DMF (2 ml) was added potassium carbonate (7.0 mg, 0.055 mmol) and 1-azido-3-azidomethyl-5-(bromomethyl)benzene[1-3] (0.11 mmol) at 0 ºC and the solution stirred (2 h). The mixture was then filtered, concentrated and the resulting residue purified by flash chromatography (MeOH:$CHCl_3$, 1:9) to give the desired iminosugars.

N-(3-azido-5-[azidomethyl]benzyl)-1-deoxynojirimycin 3

Gave a colourless oil (81%). R_f 0.75 (MeOH/$CHCl_3$/NH_3 10:10:1); δ_H (600 MHz, CD_3OD): 7.06 (s, 1H, Ar), 6.96 (s, 1H, Ar), 6.95 (s, 1H, Ar), 4.30 (s, 2H, C**H**$_2$Ar), 4.04-4.00 (m, 2H, C**H**$_2$Ar, H6), 3.86 (dd, *J* = 2.5, 12.5, 1H, H6), 3.41-3.34 (m, 3H, H2, H4, C**H**$_2$Ar), 3.16 (dd, *J* = 9.3, 9.3, 1H, H3), 2.78 (dd, *J* = 5.0, 11.6, 1H, H1), 2.18-2.15 (m, 1H, H5), 1.93 (dd, *J* = 11.2, 11.2, 1H, H1). δ_C (150 MHz, $CDCl_3$): 141.4, 140.0, 138.6, 127.2, 121.0, 118.8 (Ar), 79.3 (C3), 71.0 (C4), 69.6 (C2), 67.4 (C5), 58.7, 56.8, 56.2, 54.6 (C1, C6, **C**H_2Ar, **C**H_2Ar). Anal. calcd for $C_{14}H_{19}N_7O_4$: C, 48.13; H, 5.48. Found: C, 48.22; H, 5.65%.

N-(3-azido-5-[azidomethyl]benzyl)-1-deoxymannojirimycin 6

Gave a colourless oil (83%). R_f 0.75 (MeOH/$CHCl_3$/NH_3 10:10:1); δ_H (600 MHz, CD_3OD): 7.21 (s, 1H, Ar), 7.13 (s, 1H, Ar), 7.06 (s, 1H, Ar), 4.43 (s, 2H, C**H**$_2$Ar), 4.20 (ABq, *J* = 13.7, 1H, C**H**$_2$Ar), 4.14 (dd, *J* = 2.1, 12.6, 1H, H6), 4.00 (dd, *J* = 1.5, 12.6, 1H, H6), 3.90-3.87 (m, 1H, H2), 3.77 (dd, *J* = 9.6, 9.6, 1H, H4), 3.49-3.45 (m, 2H, H3, C**H**$_2$Ar), 2.88 (dd, *J* = 1.1, 10.8, 1H, H1), 2.34-2.25 (m, 2H, H1, H5). δ_C (150 MHz, $CDCl_3$): 141.5, 140.5, 138.6, 127.0, 120.7, 118.7 (Ar), 75.3 (C3), 68.8, 68.6, 67.5 (C2, C4, C5), 58.9, 57.0, 55.5, 54.6 (C1, C6, **C**H_2Ar, **C**H_2Ar). Anal. calcd for $C_{14}H_{19}N_7O_4$: C, 48.13; H, 5.48. Found: C, 48.32; H, 5.41%.

***N*-(3-azido-5-[azidomethyl]benzyl)-1-deoxygalactonojirimycin 7**

Gave a colourless oil (87%). R_f 0.73 (MeOH/$CHCl_3$/NH_3 10:10:1); δ_H (600 MHz, CD_3OD): 7.20 (s, 1H, Ar), 7.10 (s, 1H, Ar), 7.08 (s, 1H, Ar), 4.43 (s, 2H, C**H**$_2$Ar), 4.10 (d, *J* = 3.0, 1H, H4), 4.05-4.01 (m, 2H, C**H**$_2$Ar, H6), 3.94 (dd, *J* = 6.2, 11.6, 1H, H6), 3.83 (ddd, *J* = 4.9, 11.2, 11.2, 1H, H2), 3.48 (ABq, *J* = 13.2, 1H, C**H**$_2$Ar), 3.39 (dd, *J* = 3.0, 11.2, 1H, H3), 2.88 (dd, *J* = 4.9, 11.2, 1H, H1), 2.56-2.53 (m, 1H, H5), 1.99 (dd, *J* = 11.2, 11.2, 1H, H1). δ_C (150 MHz, $CDCl_3$): 141.5, 140.5, 138.6, 127.2, 120.9, 118.9 (Ar), 76.0 (C3), 71.4 (C4), 67.7 (C2), 65.1 (C5), 61.8, 57.1, 56.6, 54.6 (C1, C6, **C**H_2Ar, **C**H_2Ar). Anal. calcd for $C_{14}H_{19}N_7O_4$: C, 48.13; H, 5.48. Found: C, 48.02; H, 5.53%.

ENZYMES USED IN THE STUDY

Yeast α-glucosidase, α-mannosidase from *C. ensiformis* and α-galactosidase from green coffee bean were obtainied from Sigma Aldrich. The α-glucosidase, YgjK from *E. coli* K-12 was cloned and expressed according to modified literature prodecdure.[4,5] *E. coli* K-12 genomic DNA used in the PCR reactions was obtained from the cultured bacterium. The PCR reactions were accomplished using the following primers (Sigma): 5′-GCGTAT**CCATAT**GAAAATAAAAACTATTTTAACGCC-3′ (*NdeI* cut site shown in bold) and 5′-GCGTAT**CTCGAG**TTATTGCTTACGGAAAAAATCG-3′ (*XhoI* cut site shown in bold). The conditions used to carry out the PCR were as follows: DNA was denatured at 95 °C for 30 s followed by annealing of primers for 30 s at 60 °C. The primers were then extended for 7 min at 72 °C with *Pfu* DNA polymerase and this process was repeated for 28 cycles. The reaction volume was 50 µl and contained 0.25 mM dNTPs, 4 µM of each primer, 50 ng of template DNA, and 1 µl of *Pfu* DNA polymerase. The PCR reaction mixture was then subjected to electrophoresis through a 0.8% agarose gel for 45 min at 90 V and the product was cut out and gel purified using a gel extraction kit (Qiagen). Double digest reaction mixtures with both sets of restriction endonucleases (*NdeI*/*XhoI*) were prepared to cut both PCR products and the empty pET28a vector. The reaction volumes were 100 µl and consisted of approximately 5 µg of DNA and 1.5 U of each restriction enzyme (Fermentas) and were allowed to proceed for 3-4 h at 37 °C. The reaction mixtures were then subjected to electrophoresis through a 0.8% agarose gel and the desired DNA was gel purified in the same matter as described above. 1 µl of the digested plasmid DNA was then combined with 3 µl of the appropriate digested PCR product and ligated together using T4 DNA Ligase (Fermentas) for one hour at 18 °C. 1 µl of each of the ligation mixtures were used to transform XL-10 gold ultracompetent *E. coli* cells according to the manufacturer's protocol (Stratagene) and plated in the presence of 50 µg/ml of kanamycin and incubated overnight at 37 °C. A colony from each plate was picked and used to inoculate 5 ml of Luria Broth (LB). After growing overnight at 37 °C the plasmid DNA was harvested from the bacteria using a mini-prep kit according to the manufacture's protocol (Qiagen). The correctly inserted gene sequences were confirmed by DNA sequencing. DNA encoding YgjK was used to transform Tuner(λDE3) cells (Novagen). A colony from both plates was selected and cultured to exponential phase in LB media (5 ml) containing 50 µg/ml of kanamycin. These cultures were used to inoculate 500 ml cultures that were grown to an $OD_{600} \approx 0.8$ at 37 °C. At this point protein expression was induced using 1 mM IPTG (Gold Biotechnology) for 6 h at 25 °C. Post-induction cells were

harvested by centrifugation for 15 min at 5000 rpm on a centrifuge (Beckman J2-HS) and resuspended in 20 ml of nickel-column binding buffer (50 mM Na_2PO_4, 500 mM NaCl, 5 mM imidazole; pH 7.4). The resuspended cells were incubated on ice for 30 min with 1 mg/ml of lysozyme (Sigma) and 1 mM PMSF followed by sonication (6 x 20 s at 60% power, Fisher Scientific, Model 500). The cell debris was then removed by centrifugation at 15000 rpm for 45 min and the supernatant was loaded *via* syringe onto a 5 ml FF HisTrap column (Amersham Biosciences). The column was washed with 100 ml of wash buffer (same as binding buffer but containing 60 mM imidazole) and eluted with 20 ml of elution buffer (same as binding buffer but containing 250 mM imidazole). The purified enzymes were subsequently dialyzed overnight against 1 l of PBS (pH 7.4) and the protein concentration of the resulting solutions was quantified with a Bradford assay using BSA as a standard.

The α-mannosidase from α-mannosidase, *At*MAN1b, from *A. thaliana* was cloned and expressed according to modified literature prodecdure.[6] cDNA encoding *At*MAN1b was obtained from the Arabidopsis Biological Resource Center. The PCR reactions were accomplished using the following primers (Sigma): 5′-GCGTAT**CCATAT**GCGAGAAGTAGATCGATTAGT-3′ (*NdeI* cut site shown in bold) and 5′-GCGTAT**GTCGAC**CTAAACGTTAATCTGATGACC-3′ (*SalI* cut site shown in bold). The PCR reaction and purication was carried out in the same way as for YgjK. Double digest reaction mixtures with both sets of restriction endonucleases (*NdeI*/*SalI*) were prepared to cut both PCR products and the empty pET28a vector and the digestion and ligation were carried out as for YgjK. The expression of *At*MAN1b was carried out as for YgjK above.

KINETIC ANALYSIS

All assays were carried out in triplicate at 37 °C for 60 min by using a stopped assay procedure in which the enzymatic reactions (50 μl) were quenched by the addition of a 4-fold excess (200 μl) of quenching buffer (200 mM glycine, pH 10.75). Assays were initiated by the careful addition, *via* pipette, of enzyme (5 μl), and in all cases the final pH of the resulting quenched solution was greater than 10. Time-dependent assay of the enzymes used revealed that the enzyme was stable in their respective buffers over the period of the assay. The progress of the reaction at the end of 60 min was determined by measuring the extent of 4-nitrophenol liberated as determined by visible measurements using a BMG Labtech Spectrophotometer 96-well plate system. The absorbance wavelength used was 400 nm and the appropriate 4-nitrophenyl α-D-glycopyranoside was used as substrate. Inhibitors were tested at six concentrations and K_I values were determined by linear regression of data from Dixon plots.

LABELLING EXPERIMENTS

Using Phosphine-FLAG

An aliquot of enzyme sample (20 μl) was treated with a solution (30 μl) of the appropriate affinity probe **3**,**6**, or **7** (0.5 mM, final concentration 300 μM) in PBS and incubated at room temperature for 10 min. The sample was then irradiated for 10 min using a portable UV-lamp (UVP, UVG-54, 254 nm, 6 W, 60 s × 2, from a distance of 1 cm, room temperature). The mixture was then taken, and 1 M pH 7.0 sodium

phosphate (20 μl), saturated urea (50 μl), and water (30 μl) were added. An aliquot of this mixture (25 μl) was then taken and added to an equal volume of a solution of phosphine-FLAG in water (250 μM final concentration), and the mixture was allowed to incubate at room temperature for 6 h. This sample was then taken and treated with SDS-PAGE loading buffer (3x) and without heating a portion of the sample (20 μl) was loaded onto precast 10% Tris-HCl polyacrylamide gels. After electrophoresis, the samples were electroblotted onto nitrocellulose membrane (0.45 μm, Biorad). The membrane was blocked using PBS containing 2% bovine serum albumin and 0.1% Tween-20 (blocking buffer) with rocking for 1 h at room temperature. The blocking solution was then decanted, and a solution of blocking buffer containing anti-FLAG-HRP mAb conjugate (1:3500, Sigma) was added. Membranes were incubated with rocking at room temperature for 1 h after which the blocking solution containing antibody was decanted and the membrane rinsed with PBS, containing 0.1% Tween-20 (wash buffer). Membranes were then washed for 2 x 5 min and 2 x 20 min with wash buffer. Detection of membrane-bound FLAG-HRP conjugates was accomplished by chemiluminescent detection using a SuperSignal West Pico Chemiluminescent Detection Kit (Pierce) and film (Pierce CL-XPosure).

Probing E. coli Cell Lysates

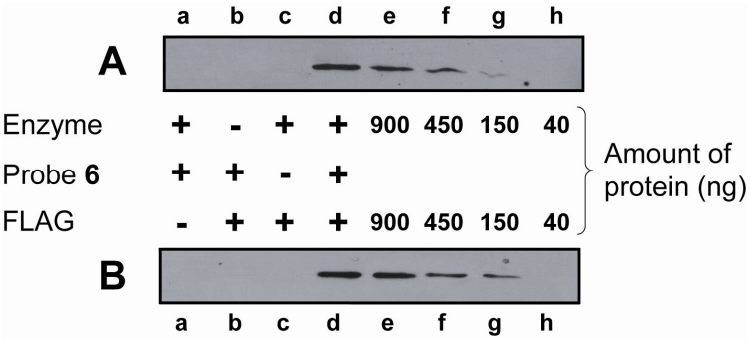
Cultures (20 ml) of *E. coli* K-12 were grown at 37 °C overnight to exponential phase, and the cells were harvested by centrifugation for 3 min at 13 000 rpm (3500 x g). The cells were washed with 250 μl of PBS buffer, pelleted, and then resuspended in fresh PBS buffer again. This suspension was then sonicated (4 x 15 s at 20% power, Fisher Scientific, model 500) after which the cell debris was removed by centrifugation at 15000 rpm for 60 min. A sample of the supernatant (20 μl) was treated with a solution (30 μl) of **3** (0.5 mM, final concentration 300 μM) in PBS and incubated at room temperature for 10 min. The mixture was then treated as described above in terms of labelling and attachment of phosphine-FLAG. The sample was then added to 10 μl of EZview Red ANTI-FLAG M2 Affinity Gel (Sigma) and allowed to stand for 4 h at 4 °C. The suspension was then centrifuged (Eppendorf 5417C) for 3 min at 5000 rpm. The supernatant was removed and a fresh 50 μl of PBS buffer was added, and after mixing, the suspension was kept for 1 h. The sample was washed in this way three more times, and then 20 μl of SDS-PAGE loading buffer (non-reducing) and after boiling the sample, (5 min) an aliquot (25 μl) was loaded onto precast 10% Tris-HCl polyacrylamide gels. After electrophoresis, the samples were stained with Coomassie blue to visualize the proteins of interest. Images of stained gels wee taken using a LAS 3000 (Fujifilm).

SUPPLEMENTARY TABLE

	K_I (μM)		
Compound	α-mannosidase from *C. ensiformis* (GH38)	α-mannosidase from *A. thaliana* (*At*MAN1B, GH47)	α-galactosidase from green coffee bean (GH27)
MNJ[a] **4**	68[7]	190	>1 mM
6	12	34	>1 mM
galacto-DNJ[b] **5**	>1 mM	>1 mM	0.016[8]
7	>1 mM	>1 mM	0.015

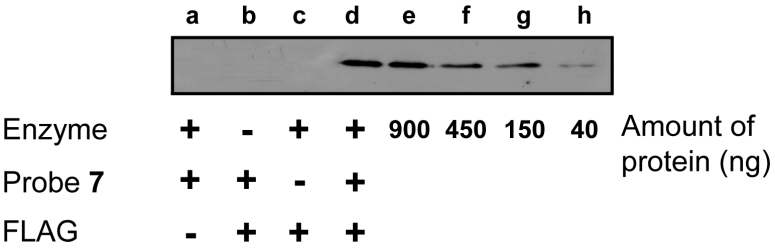
[a] 1-deoxymannojirimycin **4**
[b] 1-deoxygalactonojirimycin **5**

SUPPORTING FIGURE 1



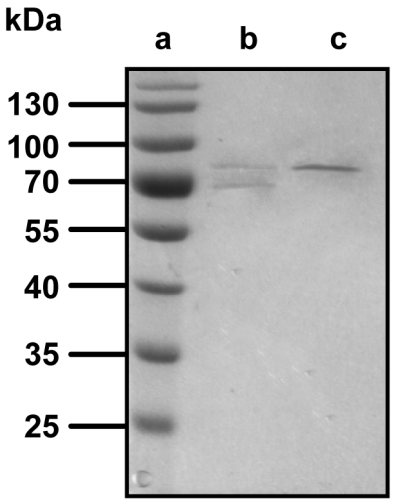
Visualization of **6**-labelled *At*MAN1B (A) and jack bean α-mannosidase (B) by denaturing SDS/PAGE Western blot. After labelling, the samples were labelled with phosphine-FLAG and analyzed by the Western blot technique using anti-FLAG-HRP (HRP, horseradish peroxidase). Lanes a-d demonstrate that all materials are required for successful labelling of the protein. Lanes e-h show the detection limit of **6**, used in conjunction with Staudinger ligation.

SUPPORTING FIGURE 2



Visualization of **7**-labelled green coffee been α-galactosidase by denaturing SDS/PAGE Western blot. After labelling, the samples were labelled with phosphine-FLAG and analyzed by the Western blot technique using anti-FLAG-HRP (HRP, horseradish peroxidase). Lanes a-d demonstrate that all materials are required for successful labelling of the protein. Lanes e-h show the detection limit of **7**, used in conjunction with Staudinger ligation.

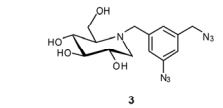
SUPPORTING FIGURE 3



10% SDS-PAGE analysis for the comparison of proteins eluted from anti-FLAG affinity beads compared to recombinantly expressed YgjK. Lane a) protein ladder b) proteins eluted from beads c) standard of purified recombinant YgjK.

REFERENCES

1. T. Hosoya, T. Hiramatsu, T. Ikemoto, H. Aoyama, T. Ohmae, M. Endo and M. Suzuki, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 1289-1294.
2. T. Hosoya, T. Hiramatsu, T. Ikemoto, M. Nakanishi, H. Aoyama, A. Hosoya, T. Iwata, K. Maruyama, M. Endo and M. Suzuki, *Org. Biomol. Chem.*, 2004, **2**, 637-641.
3. K. Yu, W. Sommer, J. M. Richardson, M. Weck and C. W. Jones, *Adv. Synth. Catal.*, 2005, **347**, 161-171.
4. Y. Kurakata, A. Uechi, H. Yoshida, S. Kamitori, Y. Sakano, A. Nishikawa and T. Tonozuka, *J. Mol. Biol.*, 2008, **381**, 116-128.
5. T. Tonozuka, A. Uechi, M. Mizuno, K. Ichikawa, A. Nishikawa and Y. Sakano, *Acta Crystallogr. Sect. D*, 2004, **60**, 1284–1285.
6. H. Kajiura, H. Koiwa, Y. Nakazawa, A. Okazawa, A. Kobayashi, T. Seki and K. Fujiyama, *Glycobiology*, 2010, **20**, 235-247.
7. G. Legler and E. Julich, *Carbohydr. Res.*, 1984, **128**, 61-72.
8. B. Ganem, *Acc. Chem. Res.*, 1996, **29**, 340-347.



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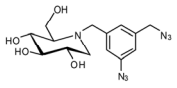

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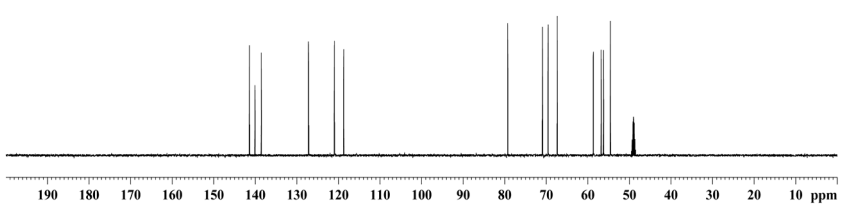

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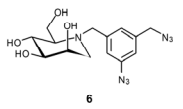

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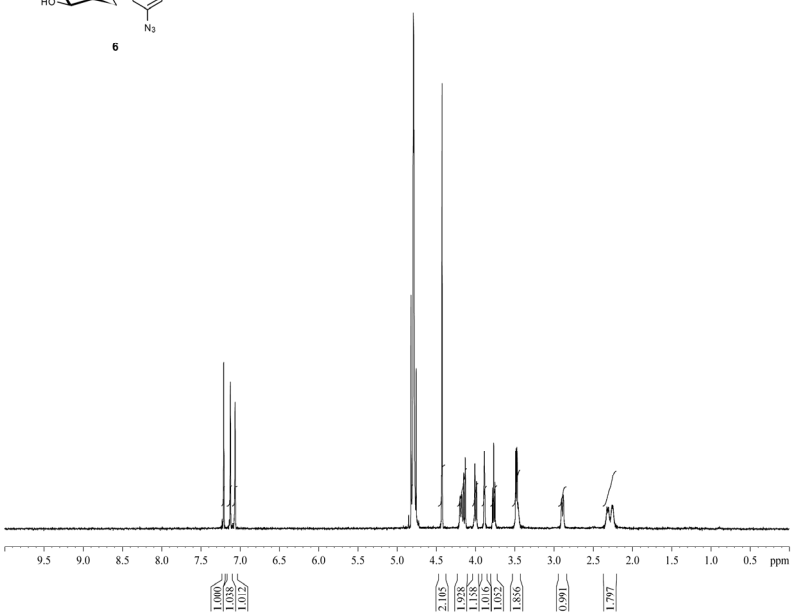


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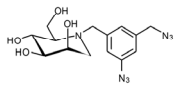

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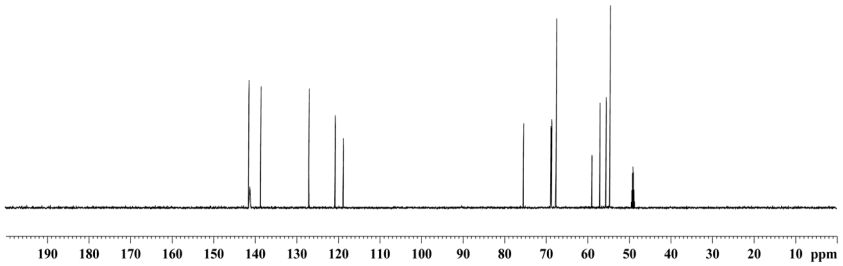


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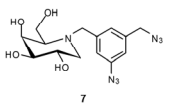

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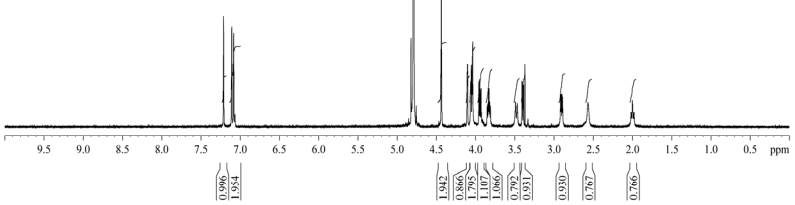


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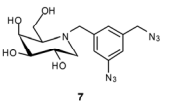
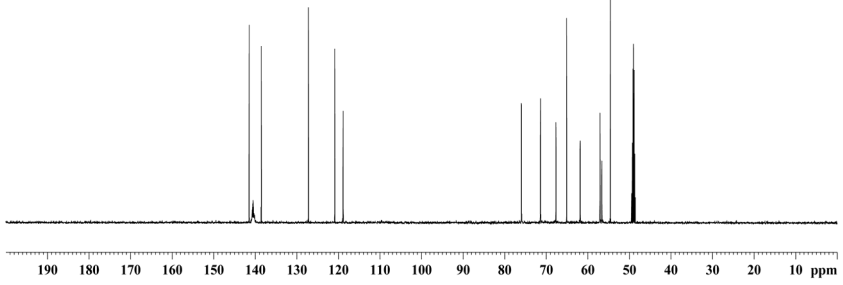
SSB                    0
LB                  0.00 Hz
GB                     0
PC                  0.50

1D NMR plot parameters
CX                 20.00 cm
CY                 50.00 cm
F1P               10.000 ppm
F1              6001.30 Hz
F2P                0.000 ppm
F2                 0.00 Hz
PPMCM           0.50000 ppm/cm
HZCM          300.06497 Hz/cm
```



7

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Current Data Parameters
NAME          KSGALDNJN3
EXPNO                  3
PROCNO                 1

F2 - Acquisition Parameters
Date_        20100830
Time              20.17
INSTRUM           spect
PROBHD  5 mm PHTBO BB-
PULPROG          zgpg
TD                65536
SOLVENT         Acetone
NS                13967
DS                    4
SWH           37593.984 Hz
FIDRES         0.573639 Hz
AQ             0.8716921 sec
RG                18390.4
DW                13.300 usec
DE                 7.00 usec
TE                298.0 K
D1          2.00000000 sec
d11         0.03000000 sec
DELTA       1.89999998 sec
MCREST      0.00000000 sec
MCWRK       0.01500000 sec

======== CHANNEL f1 ========
NUC1                 13C
P1                 3.00 usec
PL1               -3.00 dB
SFO1       150.9147813 MHz

======== CHANNEL f2 ========
CPDPRG2         waltz16
NUC2                  1H
PCPD2            100.00 usec
PL2               120.00 dB
PL12               12.70 dB
PL13               22.00 dB
SFO2       600.1324500 MHz

F2 - Processing parameters
SI                 65536
SF          150.9026637 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.00
```