

Supplementary Information

Highly efficient and selective biocatalytic production of glucosamine from chitin

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Supplementary Fig. S2. Amino acid sequence alignment of CmCBDA, EcCBDA and EfCBDA.

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Supplementary Fig. S6. ^1H NMR spectra of recycled CmCBDA beads.

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Supplementary Fig. S18. ^1H NMR spectra of CmCBDA-catalysed deacetylation reaction of GlcNAc to GlcN after 12 h (top panel) and acid hydrolysis of GlcNAc to GlcN hydrochloride (GlcN HCl) after 4 h (bottom panel). **B** Visual comparison of the enzymatic hydrolysis reaction with acid hydrolysis reaction and alkaline hydrolysis reaction.

Supplementary Fig. S19. ^1H NMR spectrum of the dialysate obtained after combined Zg β HexN2854 (1.25 U) and CmCBDA (5.5 U) treatment of chitinous mushroom extract.

1) Synthesis of N-acyl derivatives of glucosamine as CmCBDA substrates and experimental procedures for screening CmCBDA activity

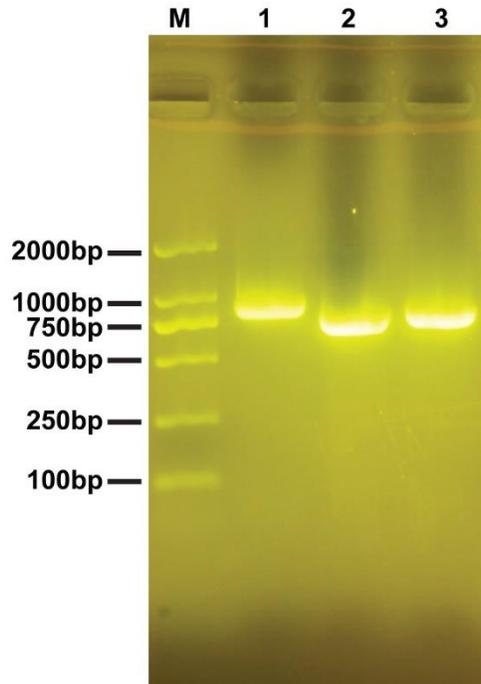
For the synthesis of N-acyl derivatives of glucosamine, a NHS-activated ester solution was prepared by stirring a solution containing the corresponding acid (0.186 mmol) (propanoic acid, butanoic acid and hexanoic acid) with N-hydroxysuccinimide (24 mg, 0.209 mmol) and N,N-dicyclohexylcarbodiimide (40 mg, 0.194 mmol) in AcOEt (200 μ L) overnight at room temperature. Then, a solution containing glucosamine (20 mg, 0.093 mmol) and Et₃N (14 μ L, 0.102 mmol) in MeOH (1 mL) was added to the NHS-activated ester solution. The resulting suspension was stirred for 6 h at room temperature. After evaporation of the solvent, N-propanoyl, N-butanoyl and N-hexanoylglucosamine were isolated by silica gel column using a mixture AcOEt/MeOH/AcOH 2:1:1 as the eluent with 70%, 98% and 91% yield, respectively (NMR spectra and the corresponding annotation are shown in the Supplementary Fig. S8-S10). The enzymatic reaction (25 μ L) containing 100 mM of N-acylglucosamine derivative, 200 mM of Na₂HPO₄ buffer (pH 8.0) and recombinant CmCBDA (0.013 U), were incubated for 6 h, and then quenched by adding 25 μ L of chloroform. After centrifugation, an aliquot of the upper-phase was used for the determination of the produced GlcN after o-phthalaldehyde (OPA) derivatisation using the colorimetric assay in the article section 'Activity and substrate specificity screening'. Screening Results are shown in the Supplementary Figure S7.

2) Alloc-, Boc-, Cbz- and Fmoc-protected glucosamines

Boc- and Cbz-protected glucosamine were purchased from commercial suppliers. Fmoc- and Alloc-protected glucosamine were synthesized. A solution of glucosamine hydrochloride (20 mg, 0.093 mmol), NaHCO₃ (14 mg, 0.186 mmol) and 9-fluorenylmethyl chloroformate or allyl chloroformate (0.140 mmol) in 1 mL H₂O/Acetone 1:1 was stirred for 24 h at room temperature. After evaporation of the reaction solvent, the residue was washed several times with diethyl ether. Then, the residue was subjected to purification by silica-gel chromatography using a mixture AcOEt/MeOH 4:1 as the eluent obtaining the desired carbamate (Fmoc-protected glucosamine: 79%, Alloc-protected glucosamine: 72% yield). (NMR spectra and the corresponding annotation are shown in the Supplementary Fig. S11-S12). Reaction mixtures (25 μ L) containing 100 mM of N-protected glucosamine, 200 mM of Na₂HPO₄ buffer (pH 8.0) and recombinant enzyme CmCBDA (0.013 U), were incubated for 6 h, and then quenched by adding 25 μ L of chloroform. After centrifugation, an aliquot of the upper-phase was used for the determination of the produced GlcN after o-phthalaldehyde (OPA) derivatisation using the colorimetric assay in the article section 'Activity and substrate specificity screening'. Screening Results are shown in the Supplementary Figure S7.

3) N-acetyl amino acids as CmCBDA substrates

N-acetyl amino acids of all proteinogenic amino acids were purchased from commercial suppliers. Reaction mixtures (25 μ L) containing 100 mM of N-acetyl amino acids, 200 mM of Na₂HPO₄ buffer (pH 8.0) and recombinant enzyme CmCBDA (0.013 U), were incubated for 6 h, and then quenched by adding 25 μ L of chloroform. After centrifugation, an aliquot of the upper-phase was used for the determination of the produced GlcN after o-phthalaldehyde (OPA) derivatisation using the colorimetric assay in the article section 'Activity and substrate specificity screening'. Screening Results are shown in the Supplementary Figure S7.



Supplementary Fig. S1. Agarose gel electrophoresis of the amplified DNA segments encoding CmCBDA (line-1), EcCBDA (line-2), EfCBDA (line-3); M – DNA marker

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CmCBDA : MNAAQKLGFTSETKLLI I HADDAG LAHAENRATI QSLOKGI VNSYSI M VPCPWFYEMAI FAK- N : 63
EcCBDA : -----MERLLI VNADDFGL SKGQNYGI I EACRNGI VTSTTAL VNGQAI DHAVQLSR- D : 52
EfcBDA : -----MSNKKLI I NADDFGYTPAVTOGI I EAHKRGVVTSTTALPTS PYFLEAMESARIS : 54
          : **.:*** * : . . . *.: :.*.* * : : : . : : :
          :

CmCBDA : NNQYDNGVHLTLTCEWENYRFGPVLPI SEVPSLVDENGYFFKRRDKLACNAKAEHVEKELTAQI : 127
EcCBDA : EPSLAI GMHFVLTVMGKP---- LTAMPGLTRDGVL--- GKWI WCL- AEEDALPLEEITQELVSCY : 108
EfcBDA : APTLAI GVHLTLTLNQA---- KPI LPREMVTSLVDEAGYFWHCS- I FEEKVNLEEVYNEWDAOI : 113
          *: :. : ** . : * . : : * : : : : * : : * : *
          :

CmCBDA : ERALKF- GI KPTHI DSHMYSV GAKPEFL NYRRI AKKYKLP LVLNQQLFEMVGLEMDLSDFKDE : 190
EcCBDA : LRFI ELFGRKPTHLDSH HVF- MFPOI FPI VARFAAEQGLALR- ADROYA----- FDLTVN : 162
EfcBDA : ISFMKS- GRRPDHI DSHHNVHGKNEKLL GVALALARKYQLPLRNASRSI ETK---- DYLELYQD : 172
          : : * : * * : *** : : : : * : : : * : : : : : : : : :
          :

CmCBDA : LLI DNVFMGEFKYFEKGE LANFYATA DKMEGG- LNLI LI HPAFDDDEMKGIT I NHPNFGSEWR : 253
EcCBDA : LRTTQGFSSAFYGEEL SESLFLQVL DDAGHRCDRSLEVMCHPAF DN----- TI R- QSAYCFPR : 220
EfcBDA : VRTPEMLYCFYDKAI STETI LQLLDVVCSECEVFEI NCHPAF DT----- I LQKOSGYCMPR : 231
          : : : * . : : . : : : : : : : : : : : : : : : : : : :
          :

CmCBDA : QI DFDFFTSEEAQSKLKEQN QLI TWDEI REKI YKD : 289
EcCBDA : LTEL DVLTSASLKGAI AQRGYRLGSYRDV----- : 249
EfcBDA : I REVEI LTSQEVKEAI EERGI LLANYESLAM----- : 262
          : : : : ** . : : : : * . : : :
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Supplementary Fig. S2. Amino acid sequence alignment of CmCBDA, EcCBDA and EfcBDA. Fully conserved amino acids are labelled with asterisk; conserved substitutions with colons, and semi-conserved substitutions with a dot.

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CmCBDA      : -----MNAACLKLGFTSTKLLI I HADDAGLAH : 27
EcCBDA      : -----MERLLI VNADDFGLSK : 16
EfCBDA      : -----MSNKKLI I NADDFGYTP : 17
C. albicans : MSFTRFTNCHLI DNGELYEFTDLVYVNNATKRI - CHPPANPELVSEVI DLKQKI LAPGFI DI QNNGI YGLNFSNLGEESTAEVVAEFK : 86
E. coli      : --MYALTQGRIF TGHEFLDDHAVVI ADGLI KSVCPVAELPPEI EQR- SLNGAI LSPGFI DVQLNGCGG- -- VQFNDTAEVSVETLE : 81
K. xylinus : -----MA : 2
L. monocytogenes : MANKVI TNATI YTGKGVLENAFVRFDKQI LEVGSMAADFQADKAEVI DAKGQKLVPGFI DVHSHG----- GYSFDAMDADPEALR : 80
P. horikoshii : -----NVVNFEDI DTFEEAFNK : 18
T. kodakaraensis : -----MVFEFNNFDEAFSA : 15

CmCBDA      : AENRATI QSLQKGI VNSYSI MVPCPWFYEMAI FAK- NNNQYDNG- -- VHLTLTCEWENY----- RFGPVLP I SEVPSLVDEN : 100
EcCBDA      : GQNYGI I EACRNGI VTSTTALVNGCAI DHAVQLSR- DEPSLAI G- --MHFVLT V GKP----- LTAMPGLTRDGVL- -- : 82
EfCBDA      : AVTQGI I EAHKRGVVTSTTALPTSPYFLEAMESARI SAPTLAI G- --VHLTLT V NQA----- KPI LPREMVTSLVDEA : 87
C. albicans : RFYRDAMAKYLSTGVTATCPTVTSFPEVYAKVL PMYKRSVLASQ- TDSLGAHVEGPF I NVQKKGCHPVET FVDAKEGESKLEVYG : 172
E. coli      : I MQKANEKSGCTNYLPTLI TTSDELKQGVVVMREYLAKHPNQALGLHLEGWVNLVKKG----- THNPFVVRKPDALVD : 157
K. xylinus : RIWKGPFI S- --PHRLGACP AHALPPV PARVS AVI RTGAVK----- : 40
L. monocytogenes : KQVNGMLNEGI TTYFPTTMTQSHENI EKALKVI NEVAQTEPVI GG- I HLEGPFVSKVFKG----- AQPEEYI QAPDLELFK : 155
P. horikoshii : LLREVLEFDLQNPFKDAKKVLCI EPHPDCCVI GGGTI KKLSDMG- VEVI YVCVTDG----- YMG- TDESLSGHELAA : 89
T. kodakaraensis : LLSK- LDFKI NEFPNDVKKVLCI EPHPDCAI GLGGTI KKL TDSG- I DVYLL VTDG----- SMGTTDGEVSGHELAL : 86

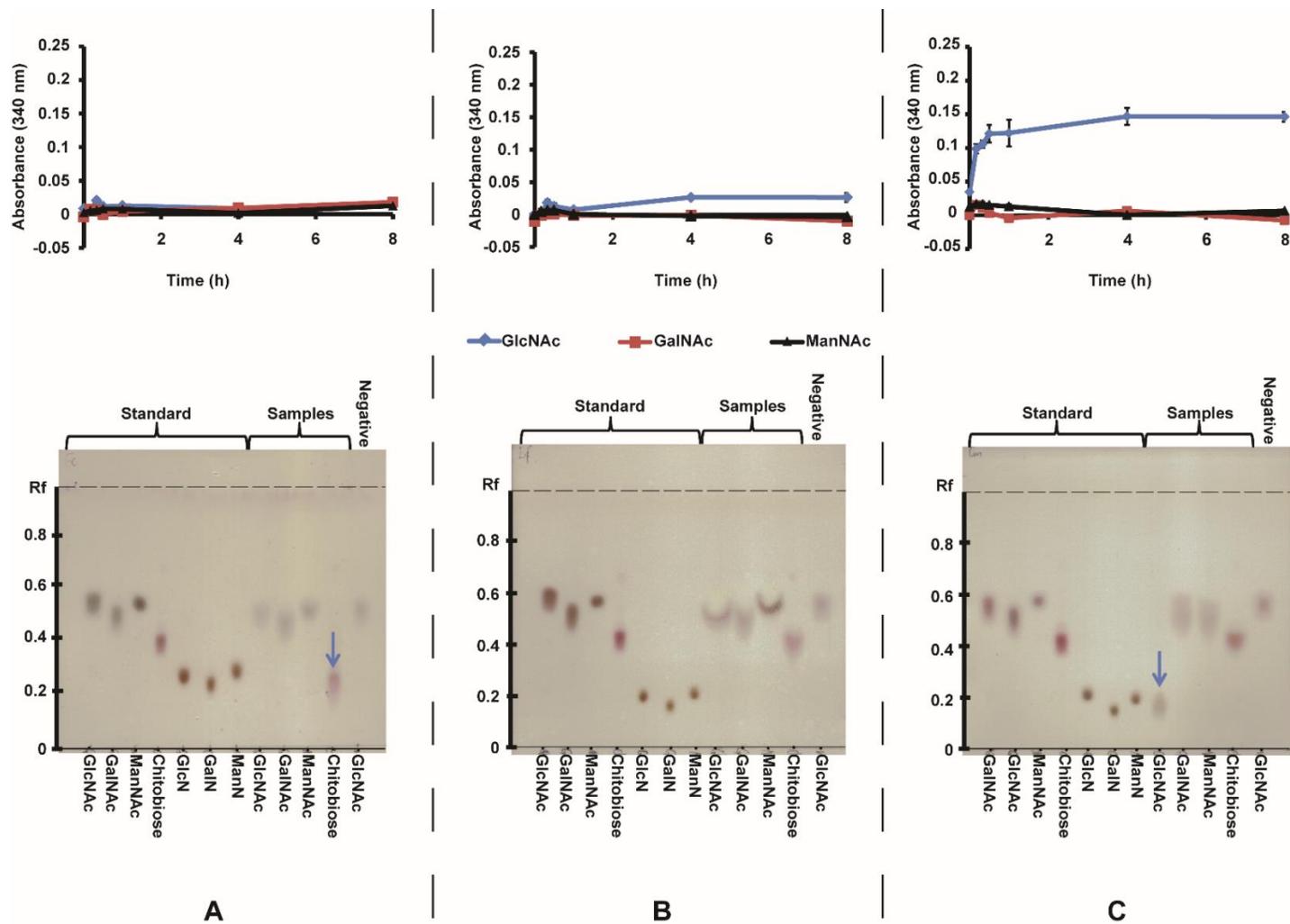
CmCBDA      : GYFFKK---- RDKLAQNAKAEHVEKELTAQI ERALKF- CI KPTHI DS----- HMYSV GAKPEFLNVYRRI AKIYKLPVLVNLQ : 173
EcCBDA      : GKWI WQ---- LAEEDALP EEEI TQ- ELVSQYLRFI ELFCRKPTHLDS----- HHVH- MFPQI FPI VARFAECGI ALR- ADR : 153
EfCBDA      : GYFWHQ---- SIFEEKVN EEVYN- EWDQAI I SFMKS- CRRPDHI DS----- HHNVHGKNEKLLGVALALARVYQLPLRNASR : 159
C. albicans : DLFDMV---- CI VTAAPE AGVLDLI PVVKS KNCVFSI CHTMSDYDTAV----- KAVEKGTAMI THLYNAMPQPHHINAGVGLI NS : 250
E. coli      : FLFCENAD- VI TKVTLAPEVYPAEVI SKLAN- AGI VVSACHSNATLKEAK----- AGFRAGI TFATHLYNAMPYI TGSEPLAGAI LD : 237
K. xylinus : -----LVTLAPEVEHADTAI QCFVNAGI RVSI CHTQADHEQTDRAI CRI CGGGVAGGT HMFNAMPVMAIAPGPATALMC : 116
L. monocytogenes : KWFDI SGLLI KLVTYAPEHDTSADEFNLCELFVGVPSI CHSNDVREHLK----- TSKATHATHLYNACHRMTHIEPVPVGHVLL : 234
P. horikoshii : IRRKEE---- EESARLLGVKKI YWLNYRDTLEPYSREVRKDLTKI LRKE----- QPDGVFAPDPWL PYESHDPDHRTG- LAI ESV : 164
T. kodakaraensis : RRLEEE---- KRSAEI LGVKKI HALDFGDTLEPYTREVRKEI VTVI RKE----- RPGI VLVNPDWLPYEGHPDHIHAGFLGI EAV : 162

CmCBDA      : LFEMVGLE---- MDLSDFKDELLI DNVF- MGEFKYFEKGE LANFYATALDKMEGGLNLI LI HP- AFDDIEMKGI TI NHPNFGEWRQ : 254
EcCBDA      : QMA----- FDLTVNLRRTQGF S- SAFYGEI SESLFLQVLDDAGHRGDRSLE MCHP- AFIDN----- TIR- QSAYCFPRI : 221
EfCBDA      : SIETK----- DYLELYQDVRTPEML- YQFYDKAI STETI LQLDMVVCSEGEVFEI NCHP- AFIDT----- I LQKQSGYCPRI : 232
C. albicans : PIVDTPYFGLI CDGVHVDPSMVNLAYRS- NPSKCVLVTDAHLI GLPDGHYKWDQVI VKTGDRLYLENTDTLAGAATLTPQCVRNL : 336
E. coli      : EADI YCG- I I ADGLHVDYANI RNAKRL- KGDKLCLVTDATAPAGANI EQFI FAGKTI YYRNLGCVDEY G- T LSGSSLTMI EGVNRL : 320
K. xylinus : SDDAY-- AEMI FDTHHVHPALFRLAHRV-- MGRLLFVTDA----- : 153
L. monocytogenes : ERGIN- AELI VDIG HVHPDMVKLAYQMKGPEHLCI I TDSMRAKMPGKSELGGQTV VVKDKQARLEFG- TLAGSVLTYDDGFRNM : 318
P. horikoshii : AFSQL----- PNFNTDLDI GLNPNY- SGSFI ALYYTHKPNYI VDI TDL MELKKA RVHRSQFPD----- IWEKWEFLRTI A : 238
T. kodakaraensis : SFAGL----- PNFNRSDLI AGLDPH- SI QAVGFYYTHKPNYFVDI SDVMEVKLRAVRTHESQFPE----- VWELWEPYLRTI A : 235

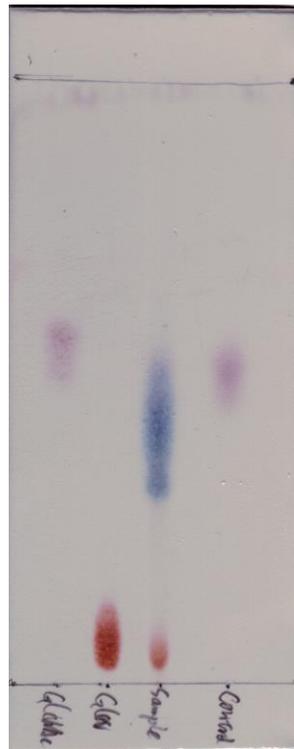
CmCBDA      : I DFDFFTSEE AQS K LKEQNI QLI TWDEI REKI YKD----- : 289
EcCBDA      : TELDVLTSLASKGAI ACRG-- YR LGSYRDV----- : 249
EfCBDA      : REVEI L TSQEVKEAI EERG-- I L LANYESLAM----- : 262
C. albicans : VKWSQI SLPAQAVMTVNNAARS I GVDHERGFLNVGCLADVFVLDKSGFVRKVKYKLGREVSQSDI PLDRATDKLTAVL : 413
E. coli      : VEHCGI ALDEVLRMATLYPARAI GVEKRLTLAAGKVANLAF TPDFKI KTI VNGNEVVQT----- : 382
K. xylinus : ----- : -
L. monocytogenes : I KFTGCSVEEAVLMSSGNQAREFNLT- QKGAIEAGKDADFNLLDEDLHI TATYSFGKKHS----- : 377
P. horikoshii : MFYGEKI GVRVYGEGRIMPLGFYH TPF TDL----- : 270
T. kodakaraensis : LYYGKMSGHRYAEGIRFVPGI FLHICPFAHI----- : 267

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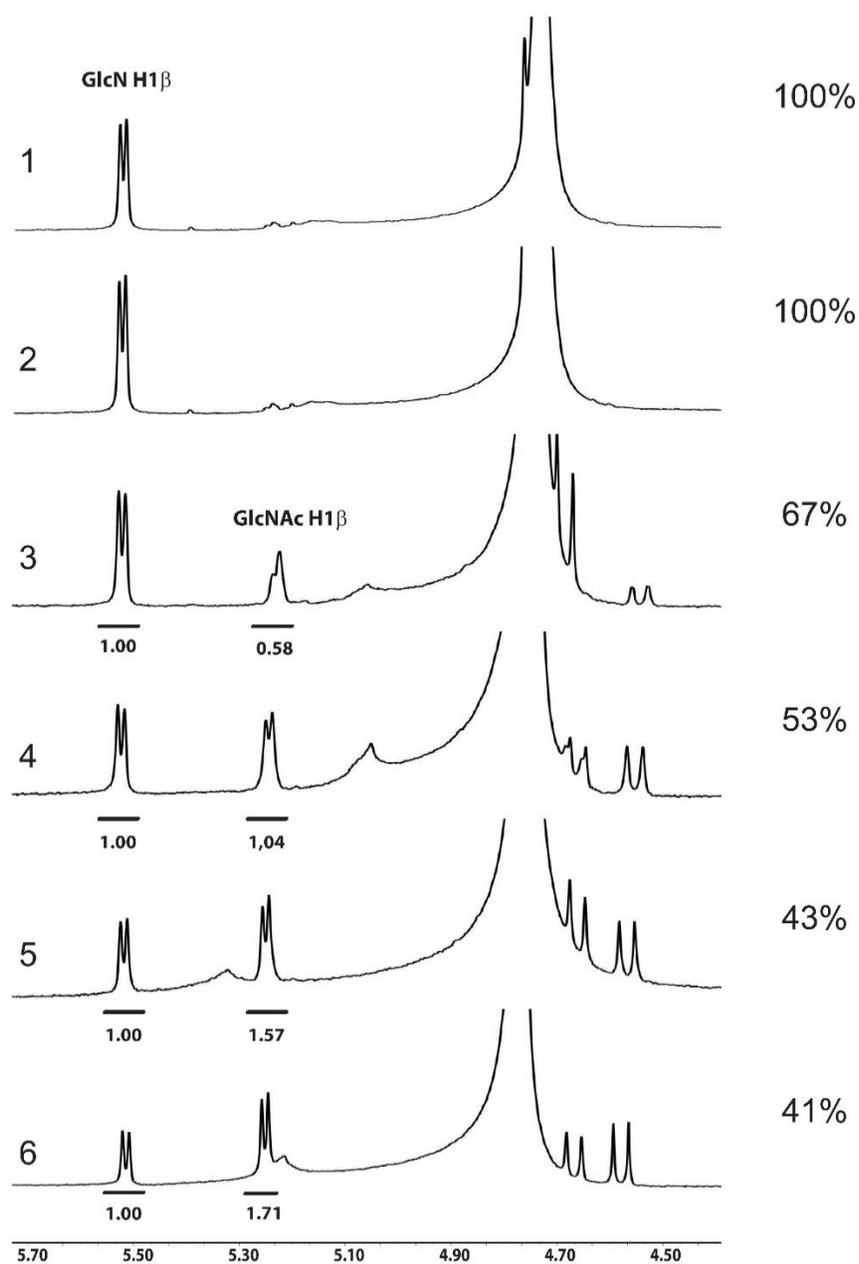
Supplementary Fig. S3. Amino acid sequence alignment of *N*-acetylglucosamine deacetylases (CmCBDA, EcCBDA, EfCBDA) and other reported relevant enzymes: *N*-acetylglucosamine 6-phosphate deacetylases from *Candida albicans*, *Escherichia coli*, *Komagataeibacter xylinus*, *Listeria monocytogenes*, *N,N'*-diacetylchitobiose deacetylases from *Pyrococcus horikoshii*, and *Thermococcus kodakaraensis*.



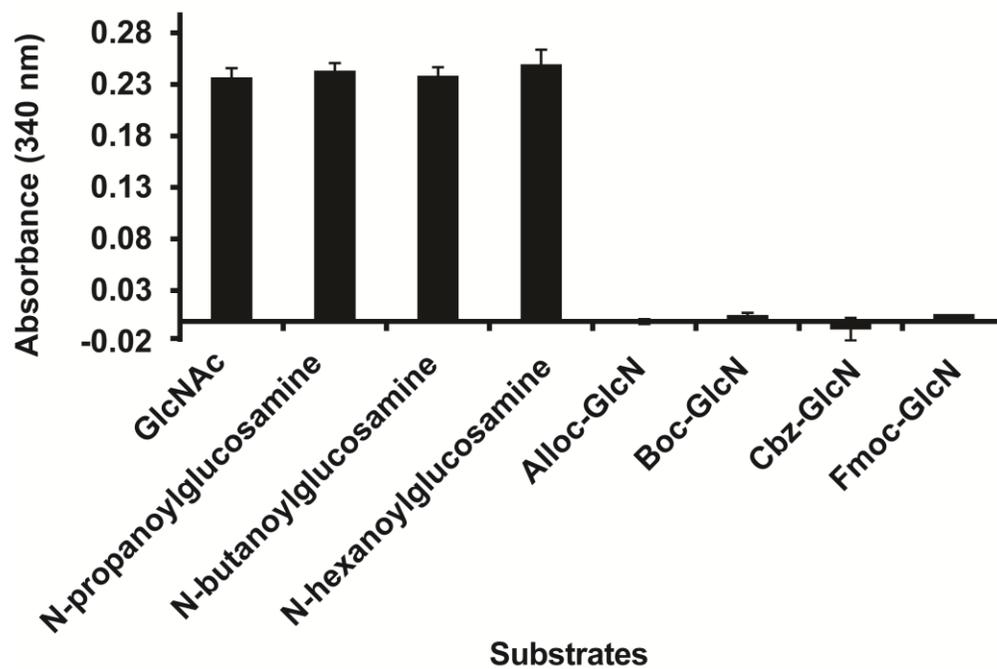
Supplementary Fig. S4. Colorimetric and TLC activity tests of **A** EcCDA, **B** EfCDA, or **C** CmCDA for the qualitative analysis of the conversion. Colorimetric tests were done using GlcNAc, GalNAc or ManNAc as substrates after 0 min, 10 min, 20 min, 30 min, 1 h, 4 h, 8 h, 16 h, and 24 h reaction time. TLC activity tests were done using GlcNAc, GalNAc, ManNAc or chitobiose as substrates after 24 h reaction time.



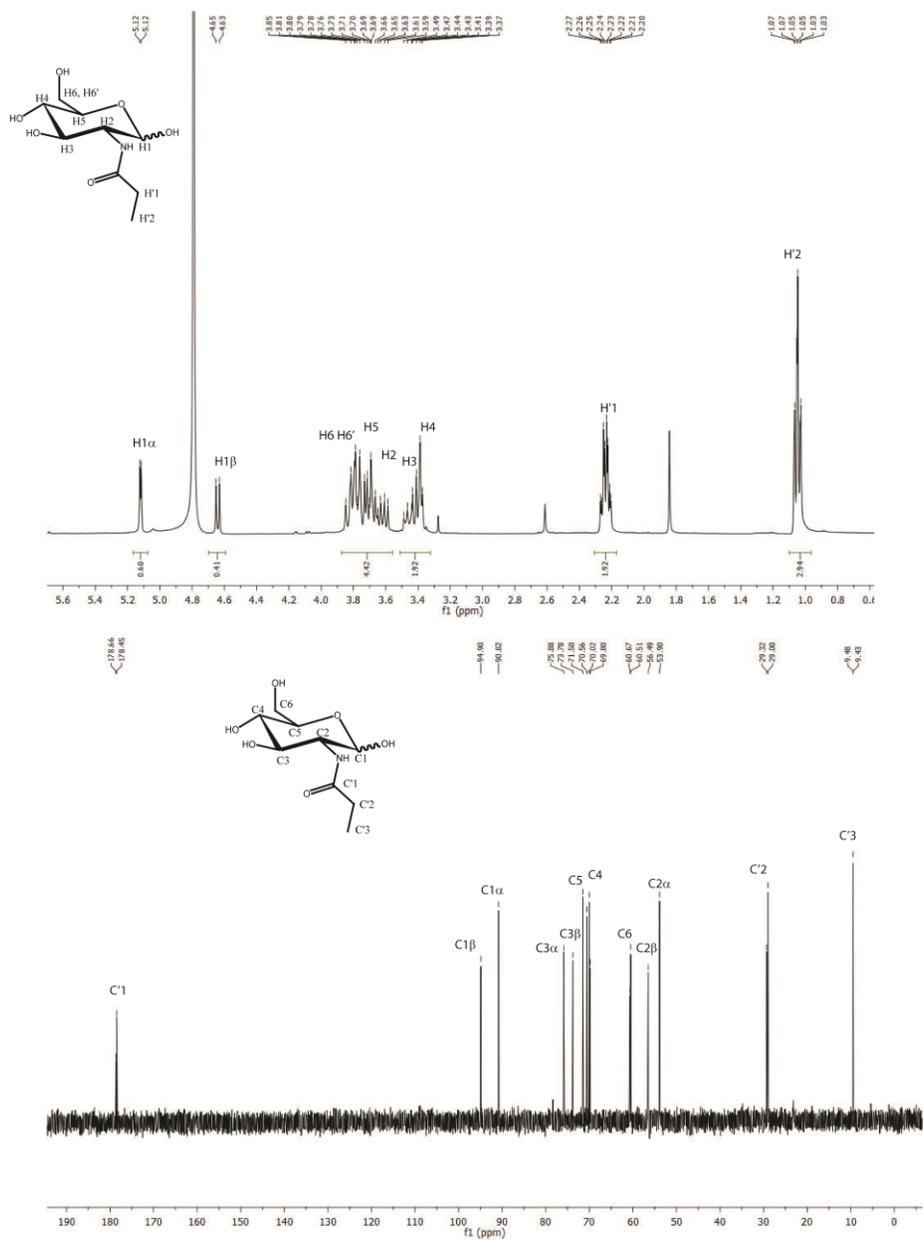
Supplementary Fig. S5. TLC result of CmCBDA-catalysed deacetylation of a 50 g/mL solution of GlcNAc. 0.05 U of CmCBDA were used in the reaction. The complete conversion of GlcNAc to GlcN could be observed.



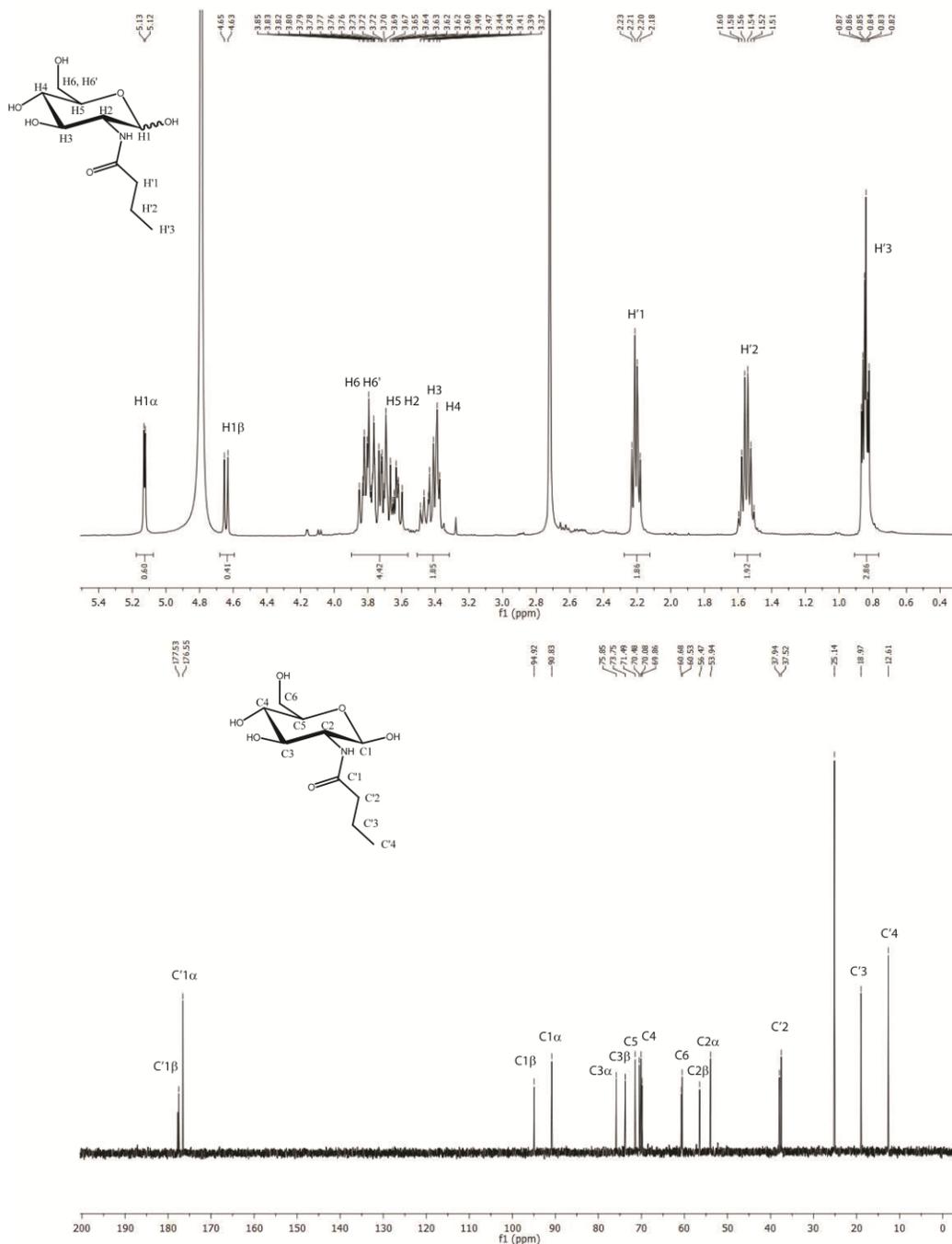
Supplementary Fig. S6. ^1H NMR spectra of recycled CmCBDA beads. The numbers 1 to 6 on the left side indicate the number of 24 h incubation cycles. Bead-immobilized CmCBDA were each time incubated with 100 μL of fresh GlcNAc solution (20 g/mL). The conversion rate (right side) was calculated by integration of the relative areas of the signals corresponding to the anomeric proton of the β anomer of glucosamine (GlcN H1 β) and of the proton of the β anomer of GlcNAc (GlcNAc H1 β) considering that the α/β ratios of glucosamine and GlcNAc are 0.53:0.47 and 0.61:0.39, respectively.



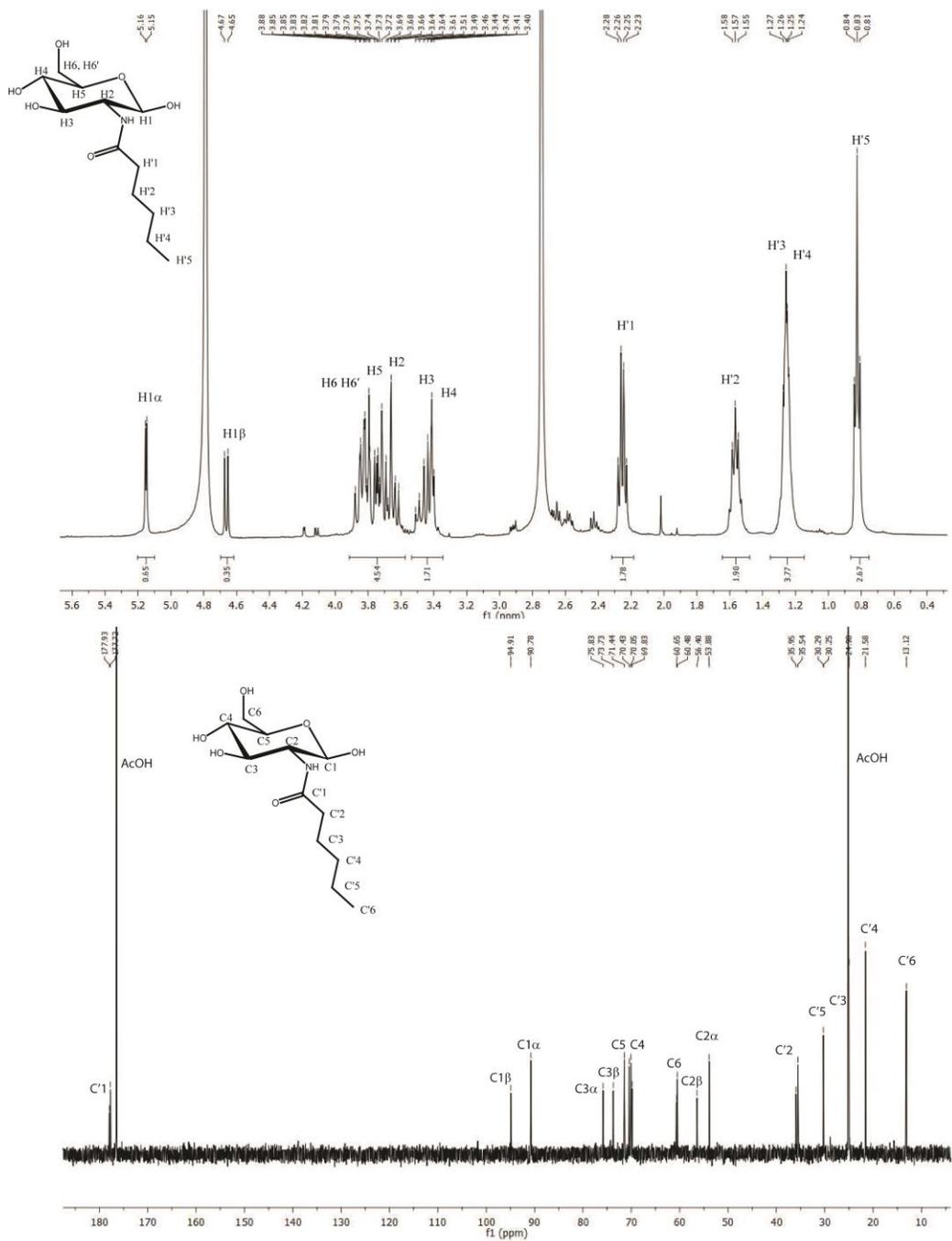
Supplementary Fig. S7. Colorimetric activity test of CmCBDA towards N-acylglucosamine derivatives and Alloc-, Boc-, Cbz- and Fmoc-protected glucosamine.



Supplementary Fig. S8. ¹H and ¹³C NMR spectra of N-propanoyl glucosamine. ¹H NMR (400 MHz, D₂O): δ (duplicated signals are observed for some protons; asterisks indicate those corresponding to the alpha anomer) 5.12* (d, 1H, *J* = 3.6 Hz), 4.64 (d, 1H, *J* = 8.4 Hz); 3.85-3.58 (m, 4H); 3.49-3.34 (m, 2H); 2.24 (q, 2H, *J* = 7.6 Hz), 2.24* (q, 2H, *J* = 7.6 Hz); 2.23 (q, 2H, *J* = 7.6 Hz); 1.05 (t, 3H, *J* = 7.6 Hz), 1.05* (t, 3H, *J* = 7.6 Hz). ¹³C NMR (100 MHz, D₂O): δ (duplicated signals are observed for some carbons; asterisks indicate those corresponding to the alpha anomer) 178.66, 178.45*; 94.90, 90.82*; 75.88*, 73.78; 71.50*, 70.56; 70.02*, 69.80; 60.67, 60.51*; 56.49, 53.90*; 29.32, 29.00*; 9.48*, 9.43.

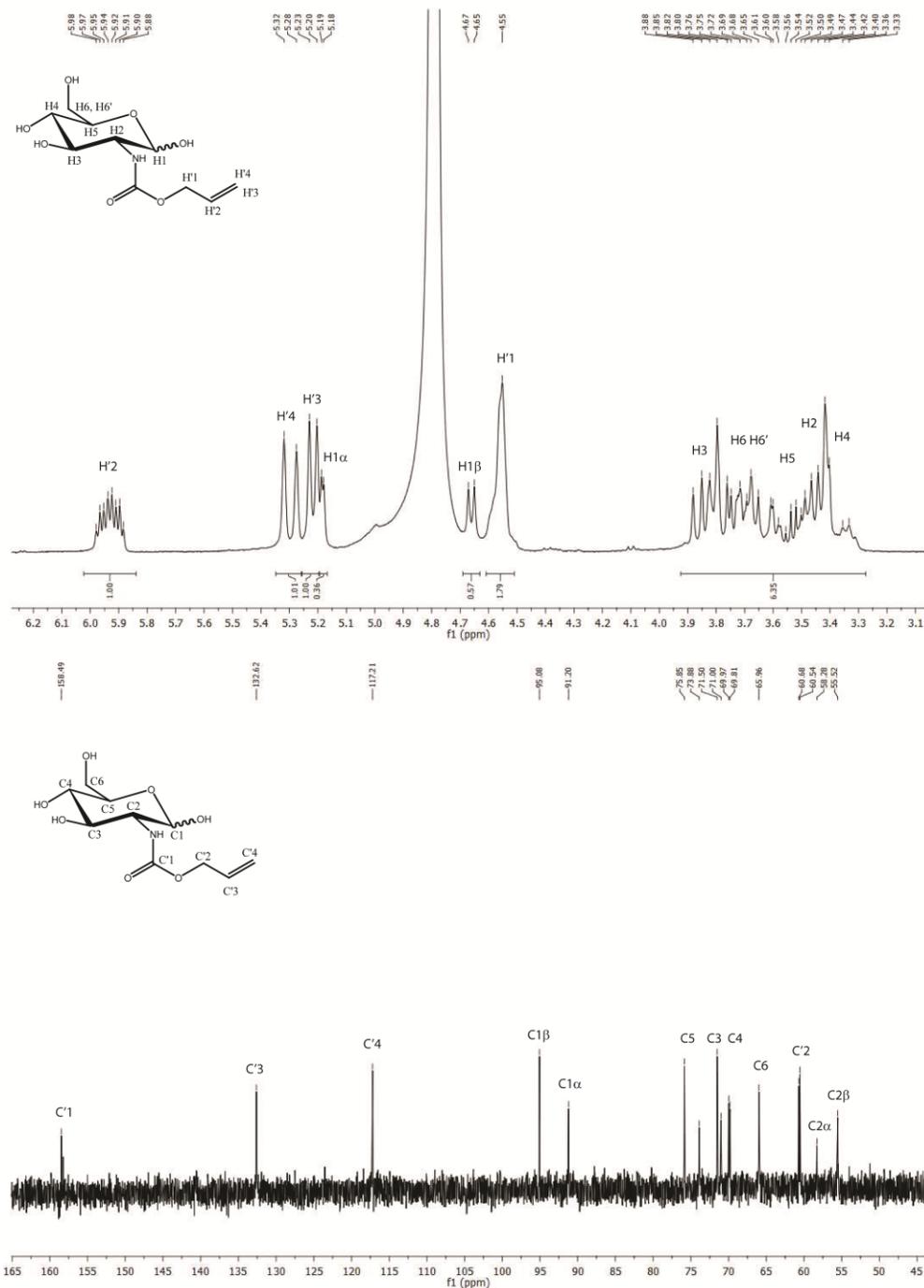


Supplementary Fig. S9. ^1H and ^{13}C NMR spectra of N-butanoyl glucosamine. ^1H NMR (400 MHz, D_2O): δ (duplicated signals are observed for some protons; asterisks indicate those corresponding to the alpha anomer) 5.12* (d, 1H, J = 3.6 Hz), 4.64 (d, 1H, J = 8.4 Hz); 3.86-3.59 (m, 4H); 3.49-3.37 (m, 2H); 2.21 (m, 2H); 1.55 (sext, 2H, J = 7.2 Hz); 0.85 (t, 3H, J = 7.6Hz), 0.84* (t, 3H, J = 7.6Hz). ^{13}C NMR (100 MHz, D_2O): δ (duplicated signals are observed for some carbons; asterisks indicate those corresponding to the alpha anomer) 177.53, 176.55*; 94.92, 90.83*; 75.85*, 73.75; 71.49, 70.48*; 70.08, 69.86*; 60.68, 60.53*; 56.47, 53.94*; 37.94, 37.52*; 18.97; 12.61.

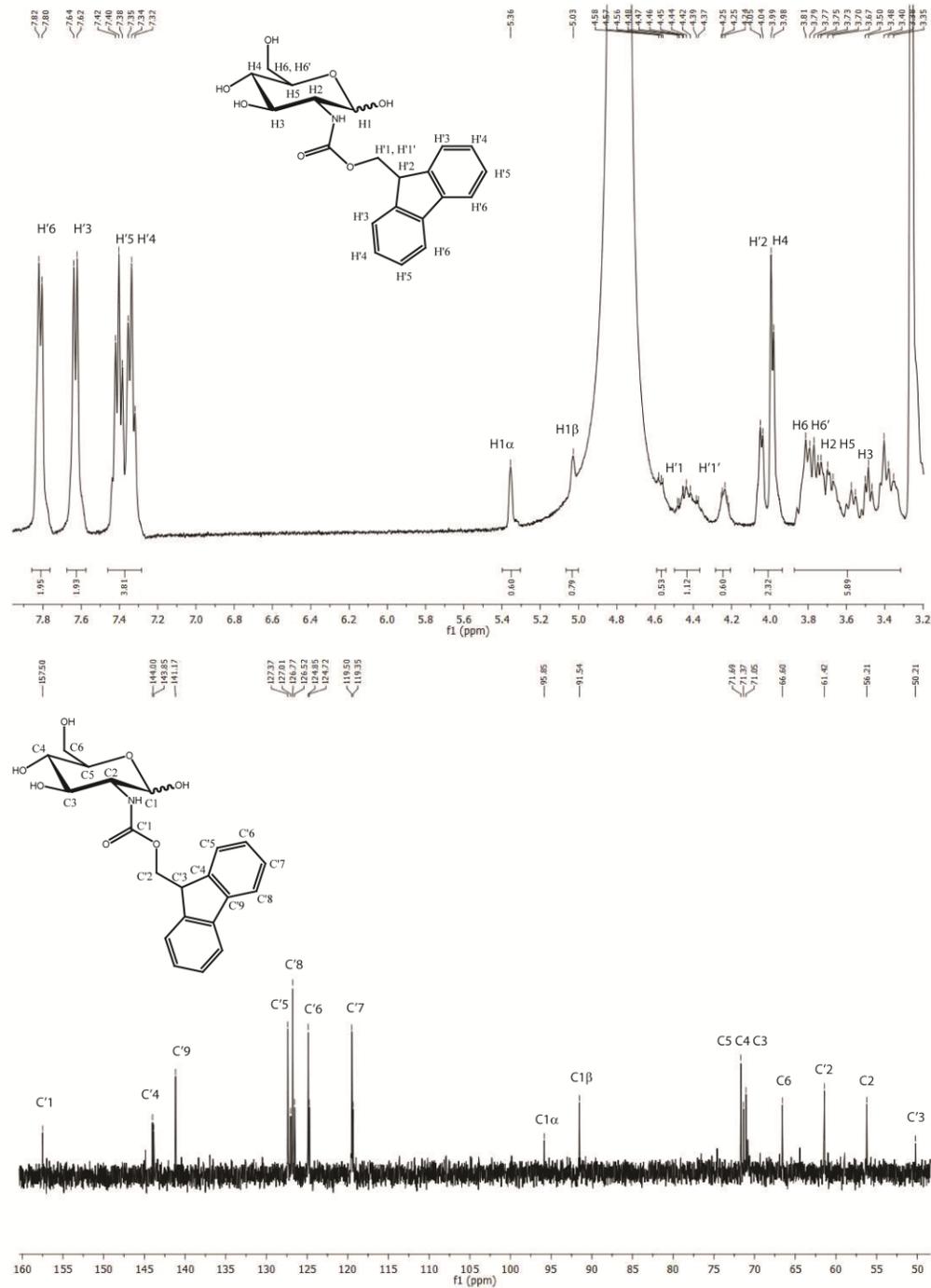


Supplementary Fig. S10. ^1H and ^{13}C NMR spectra of N-hexanoyl glucosamine. ^1H NMR (400 MHz, D_2O): δ (duplicated signals are observed for some protons; asterisks indicate those corresponding to the alpha anomer) 5.15* (d, 1H, $J = 3.2$ Hz), 4.66 (d, 1H, $J = 8.4$ Hz); 3.89-3.61 (m, 4H); 3.52-3.39 (m, 2H); 2.25 (m, 2H); 1.57 (qn, 2H, $J = 7.6$ Hz); 1.26 (m, 4H); 0.86 (t, 3H, $J = 6.4$ Hz). ^{13}C NMR (100 MHz, D_2O): δ (duplicated signals are observed for some carbons; asterisks indicate those corresponding to the alpha anomer) 177.93, 177.71*; 94.91, 90.78*; 75.83*,

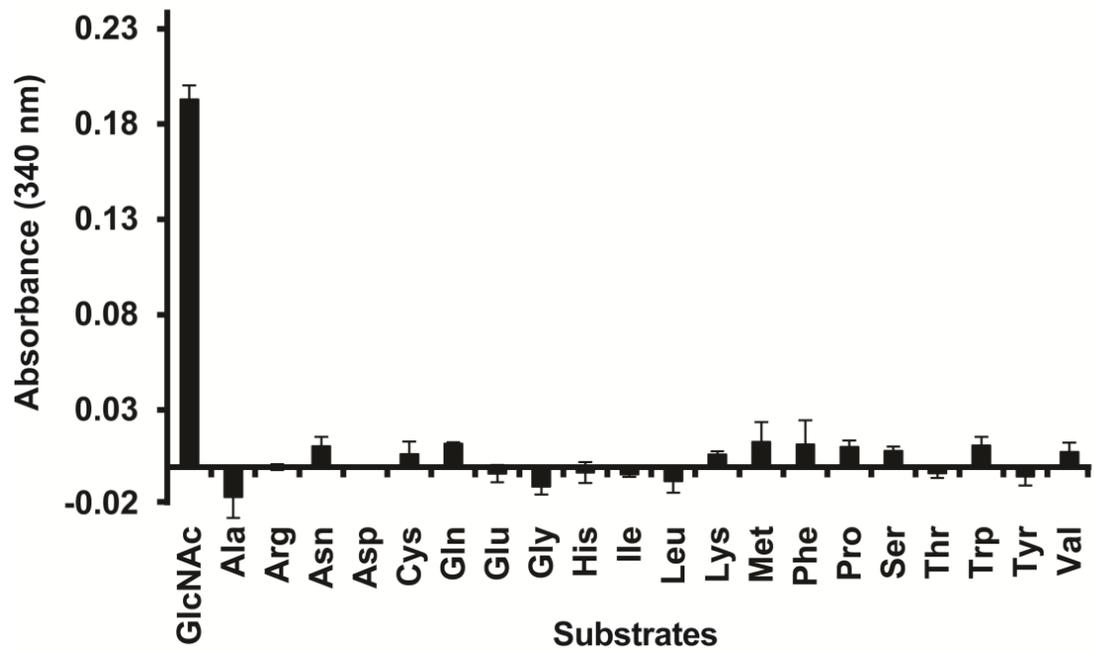
73.73; 71.44*, 70.43; 70.05*, 69.83; 60.65, 60.48*; 56.40, 53.88*; 35.95, 35.54*; 30.29*, 30.25; 24.98; 21.58;
13.12.



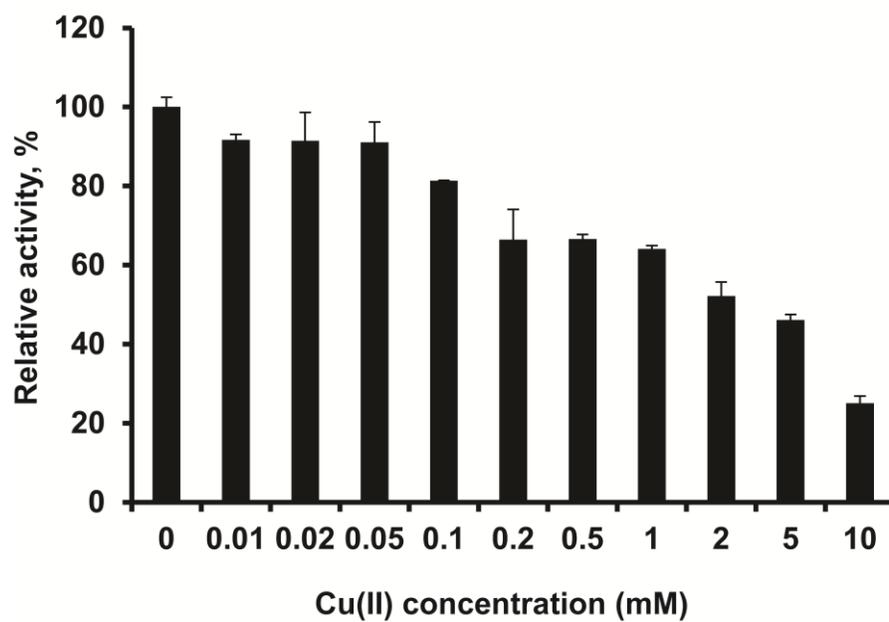
Supplementary Fig. S11. ¹H and ¹³C NMR spectra of Alloc-protected glucosamine. **¹H NMR (400 MHz, D₂O): δ** (duplicated signals are observed for some protons; asterisks indicate those corresponding to the alpha anomer) 5.93 (m, 1H); 5.30 (d, 1H, *J* = 17.6 Hz); 5.22 (d, 1H, *J* = 10.8 Hz); 5.18* (d, 1H, *J* = 3.2 Hz), 4.66 (d, 1H, *J* = 8.4 Hz); 4.61-4.53 (m, 2H); 3.89-3.29 (m, 6H). **¹³C NMR (100 MHz, D₂O): δ** (duplicated signals are observed for some carbons; asterisks indicate those corresponding to the alpha anomer) 198.49; 132.62; 117.21; 95.08, 91.20*; 75.85, 73.88*; 71.50, 71.00*; 69.97, 69.81*; 65.96; 60.68*, 60.54; 58.28*, 55.52.



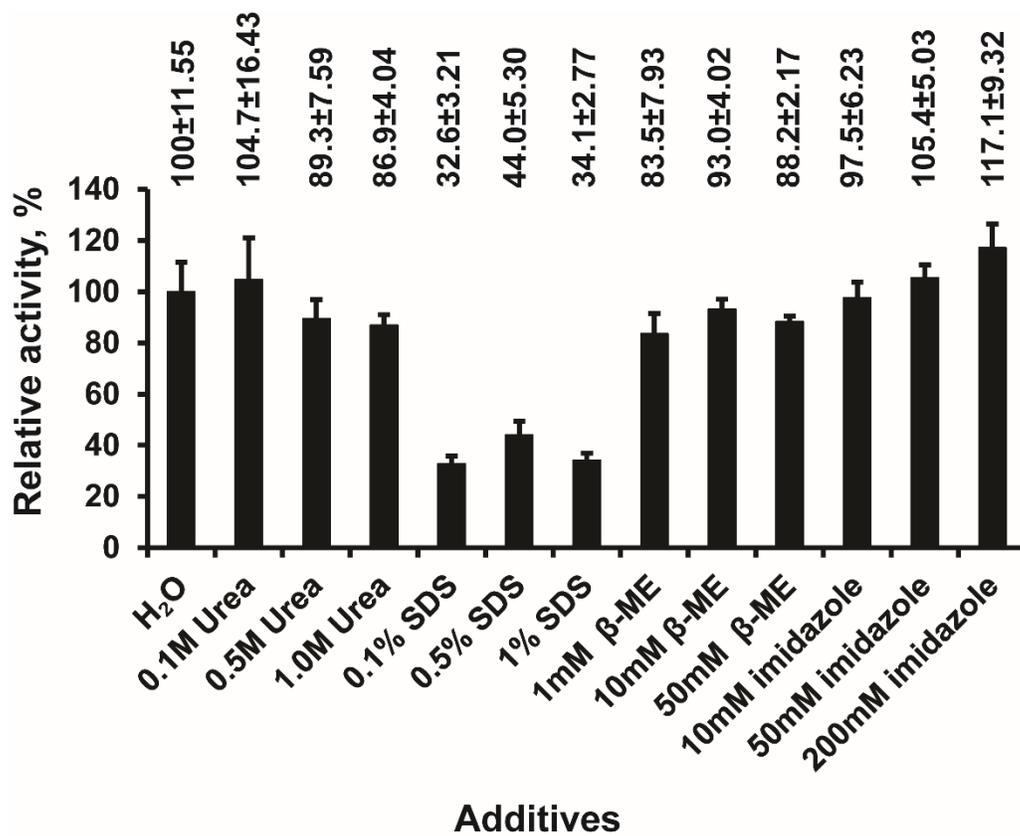
Supplementary Fig. S12. ^1H and ^{13}C NMR spectra of Fmoc-protected glucosamine. **^1H NMR (400 MHz, $\text{D}_2\text{O}/\text{MeOD}$ 1:1):** δ (duplicated signals are observed for some protons; asterisks indicate those corresponding to the alpha anomer) 7.85-7.79 (m, 2H); 7.66-7.61 (m, 2H); 7.45-7.31 (m, 2H); 5.36* (s, 1H), 5.03 (s, 1H); 4.59-4.35 (m, 2H); 4.08-3.96 (m, 2H); 3.87-3.32 (m, 5H). **^{13}C NMR (100 MHz, MeOD):** δ (duplicated signals are observed for some carbons; asterisks indicate those corresponding to the alpha anomer) 157.50; 144.00, 143.85*; 141.17; 127.37, 127.01*; 126.77, 126.52*; 124.85, 124.72*; 119.50, 119.35*; 95.85*, 91.54; 71.69; 71.37; 71.05; 66.60; 61.42; 56.21; 50.21.



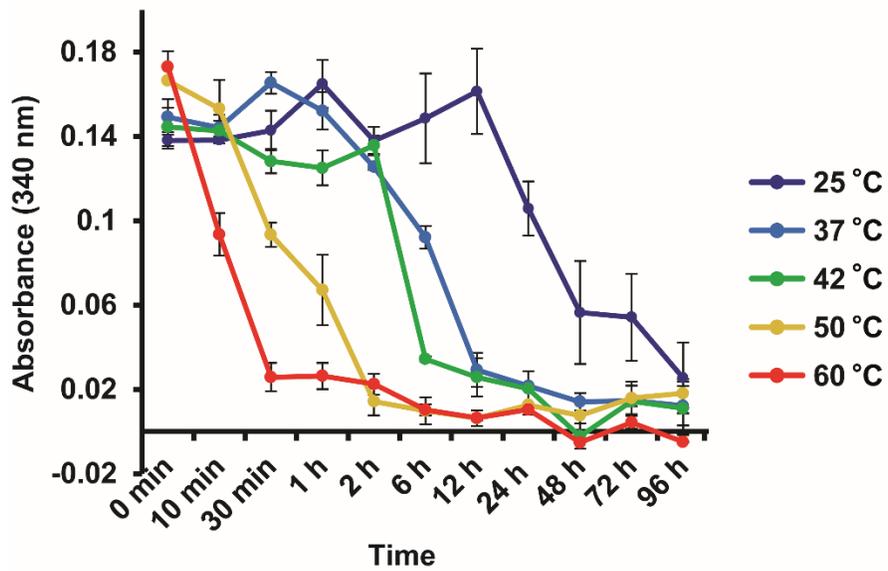
Supplementary Fig. S13. Activity of CmCBDA towards GlcNAc and various N-acetyl amino acids.



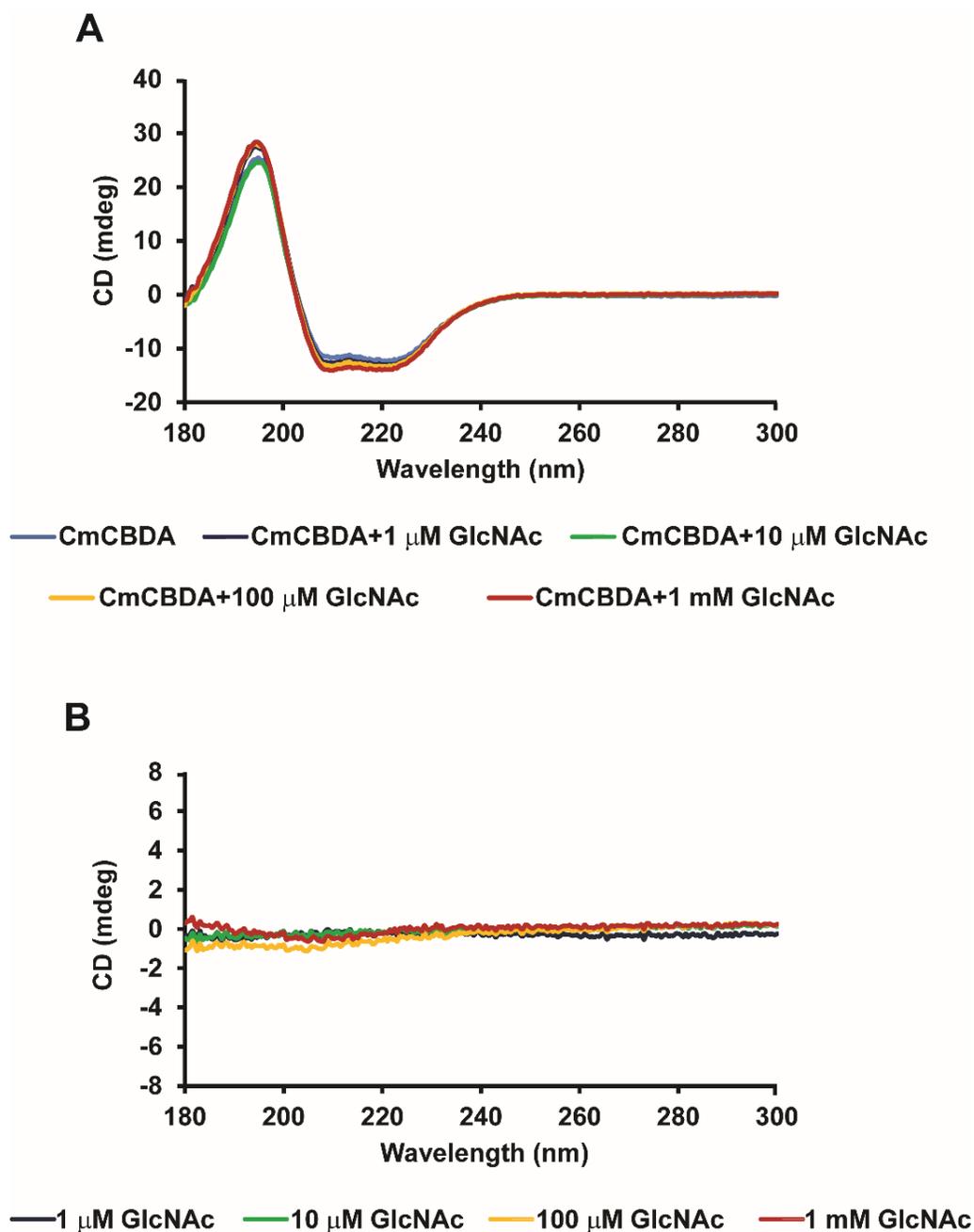
Supplementary Fig. S14. Influence of the Cu(II) ion concentration on CmCBDA activity.



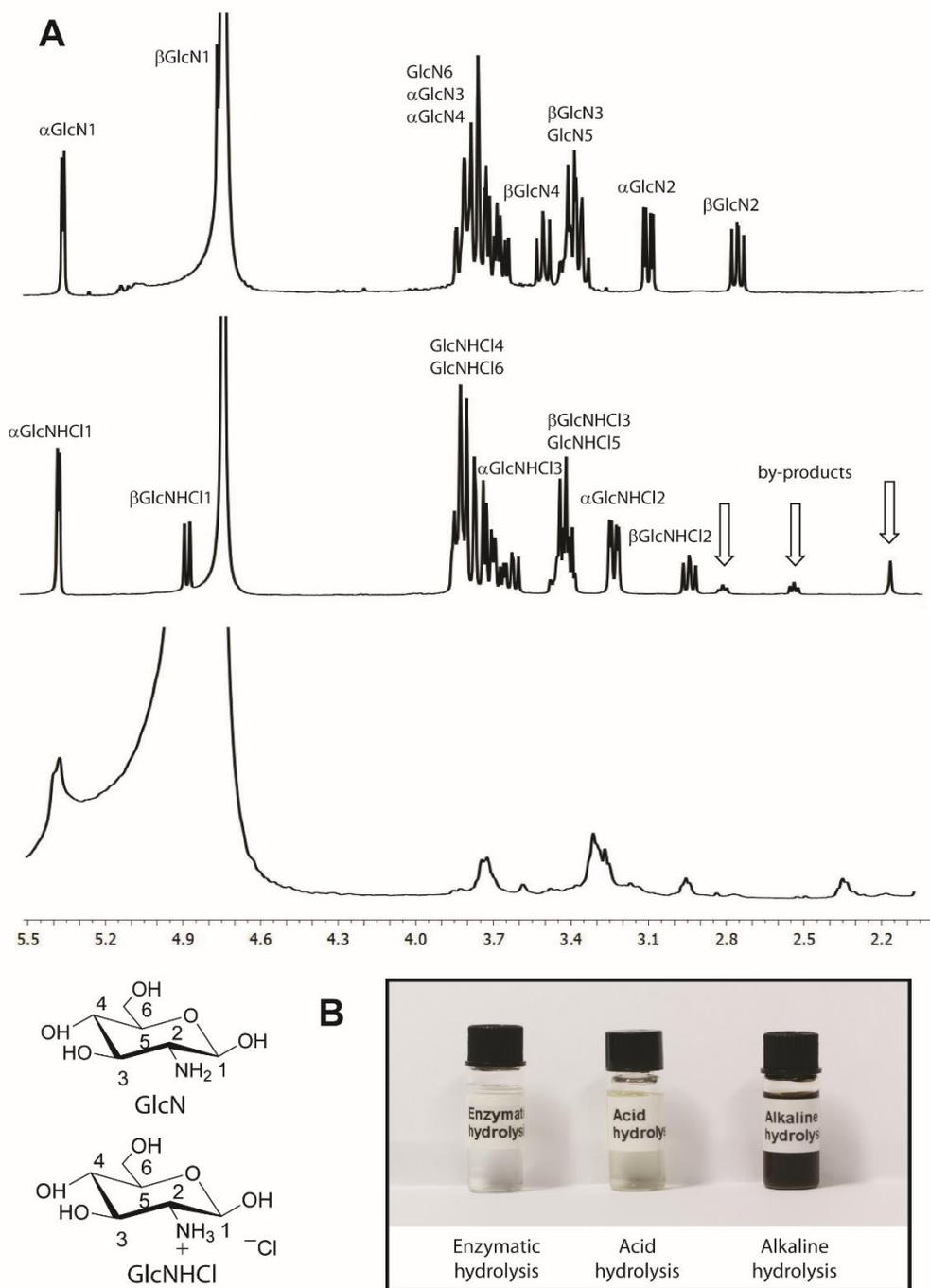
Supplementary Fig. S15. Relative activities of recombinant CmCBDA in presence of different concentrations of urea, 2-mercaptoethanol, imidazole and sodium dodecylsulphate (SDS).



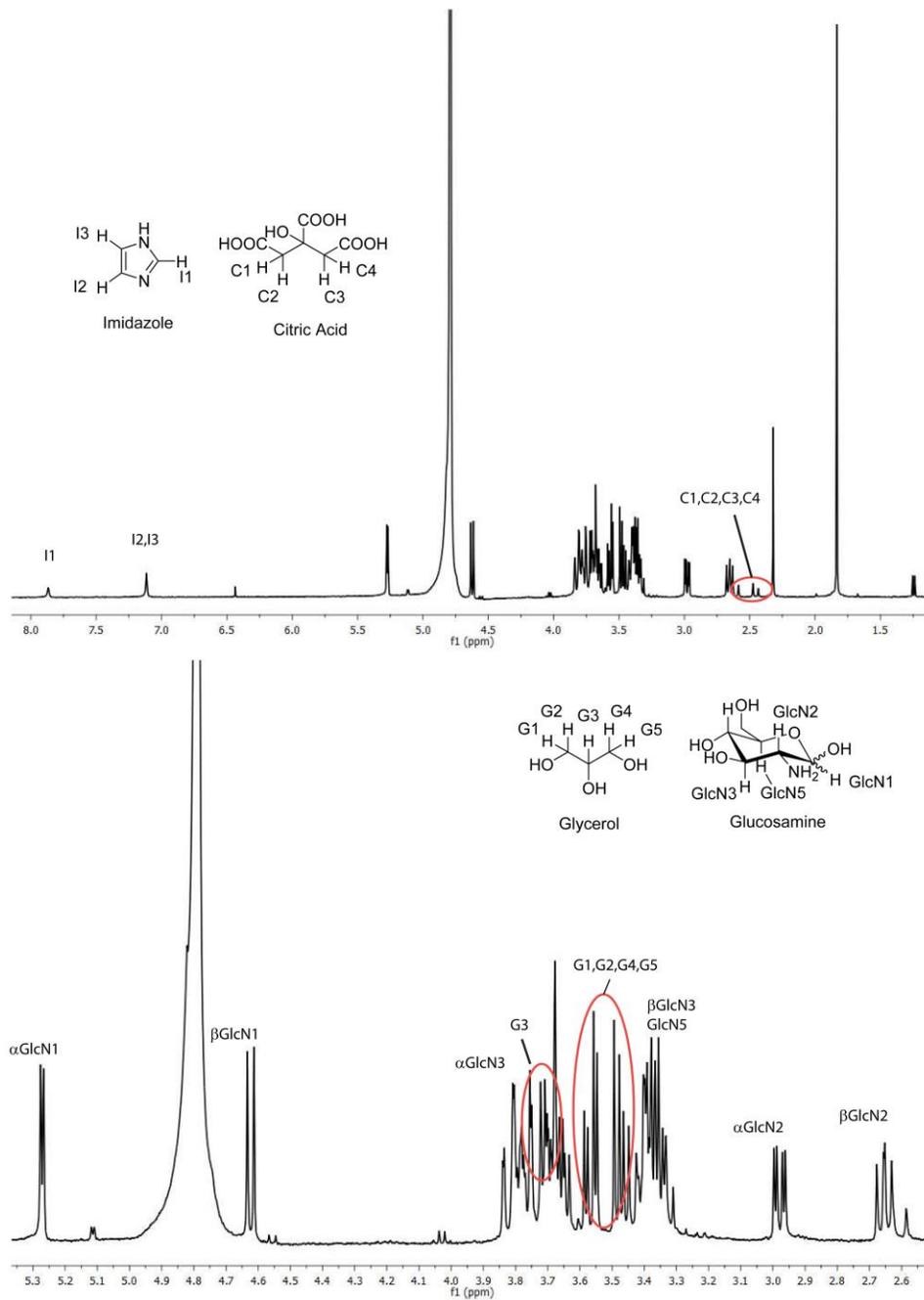
Supplementary Fig. S16. Temperature stability of CmCBDA.



Supplementary Fig. S17. Circular dichroism (CD) spectroscopy of CmCBDA incubated with various concentrations of GlcNAc (Panel A) and of an enzyme free control mixture (Panel B). Samples were analysed at a constant CmCBDA concentration of 1 mg/ml (in Panel A, Panel B had no CmCBDA added) in a 0.01 cm path length cuvette with different concentrations of GlcNAc ranging from 1 μ M to 10 mM. Each sample was recorded 5 times repeat. Sodium phosphate buffer(0.1 M, pH 8.0) was analysed under the same condition and subtracted, which were subsequently averaged to obtain the final result. The secondary structure of protein with different concentrations of GlcNAc was analysed by using CDNN software (version 2.0.3.188). No obvious changes in the secondary structure of CmCBDA were observed with increasing amounts of GlcNAc.



Supplementary Fig. S18. A ^1H NMR spectra of CmCBDA-catalysed deacetylation reaction of GlcNAc to GlcN after 12 h (top panel) and acid hydrolysis of GlcNAc to GlcN hydrochloride (GlcN HCl) after 4 h (bottom panel). **B** Visual comparison of the enzymatic hydrolysis reaction with acid hydrolysis reaction and alkaline hydrolysis reaction.



Supplementary Fig. S19. ¹H NMR spectrum of the dialysate obtained after combined ZgβHexN2854 (1.25 U) and CmCBDA (5.5 U) treatment of chitinous mushroom extract. Enzymes were added in a dialysis tubing containing 20 mL of the mushrooms extract. The reaction mixture was incubated in 100 mL of water over 72 h. Characteristic signals corresponding to GlcN are indicated. Signals corresponding to citrate, glycerol and imidazole could also be found. Signals corresponding with the hydrogens linked to C-4 and C-6 of GlcN overlap with the G3 signal of glycerol.