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Supporting Information

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for

The Engineering of Bacteria Bearing Azido-Pseudaminic Acid-Modified Flagella

Feng Liu, Annie J. Aubry, Ian C. Schoenhofen,
Susan M. Logan,* and Martin E. Tanner*

Materials and general methods: (+)-Biotin-$(PEO)_4$-amine (**6**) was purchased from Molecular Biosciences. Isopropyl-β-D-galactopyranoside (IPTG) was purchased from Invitrogen. All other chemicals were purchased from Sigma-Aldrich. ^{1}H NMR spectra were obtained on Bruker AV400 NMR spectrometers. Mass spectra were obtained on a Waters Micromass LCT mass spectrometer using electrospray ionization (ESI-MS).

Overexpression and Purification of His_6-tagged PseG, PseB, PseC and PseI: The recombinant *pseG* plasmid was transformed into *E. coli* BL21 (DE3) competent cells as described previously[1]. The cells were then incubated in Lucia-Bertani (LB) medium (10 mL) containing 50 mg/L kanamycin at 37 °C/225 rpm for 10 h. The overnight culture was then poured into LB medium (500 mL) containing 50 mg/L kanamycin and shaken at 37 ℃/225 rpm until an OD_{600} of 0.6 had been reached. The culture was allowed to continue to grow for 5 h after IPTG (70 mg/L) was added. Cells were harvested by centrifugation at 6000 *g* for 30 min, and then resuspended in sodium phosphate buffer (20 mM, 12 mL, pH 8.0) containing dithiothreitol (DTT, 2 mM), aprotinin (1 mg/L), and pepstatin A (1 mg/L). The cells were lysed by passage through a French Pressure cell at 20 000 psi. The lysate was centrifuged at 6000 *g* for 1 h and passed through a 0.22 μm filter.

A column containing Chelating Sepharose Fast Flow resin (10 mL, Pharmacia Biotech) was charged with $NiSO_4$ (100 mM, 20 mL), washed with distilled H_2O (20 mL) and sodium phosphate buffer (20 mM, 30 mL, pH 8.0, containing 0.5 M NaCl and 5 mM imidazole). The clarified lysate was loaded onto the column and eluted with same

buffer containing increasing amounts of imidazole in a step-wise fashion (5, 100 and 500 mM). Fractions that were eluted with 500 mM imidazole and showed absorbance at 280 nm were collected. These fractions were concentrated using Amicon Ultra Centricons (Millipore) before flash freezing with liquid N_2.

Plasmids used for the overexpression of PseB and PseC (pNRC 20.3 and pNRC 82.1, respectively) have been reported previously[2]. Plasmid used for the overexpression of PseI has also been reported previously[3]. The conditions used for overexpression of PseB, PseC and PseI were identical to those described above for PseG with the exception that the crude cell lysate was used directly without purification by Ni-affinity chromatography.

Chemoenzymatic Synthesis of UDP-2-acetamido-4-chloroacetamido-2,4,6-trideoxy-L-altrose (UDP-6-deoxy-AltNAc4NAcCl, **1)**: Samples of the crude lysates containing PseB and PseC (10 mL each) were added to a triethanolamine-HCl buffer (50 mM, 100 mL, pH 8.0) containing UDP-*N*-acetylglucosamine disodium salt (250 mg), monosodium glutamate (0.1 M) and pyridoxal 5'-phosphate (PLP, 0.25 mM). This solution was incubated for 4 h at 37 ℃ and the rea ction progress was monitored by ESI-MS(-). It was determined that > 90% of the UDP-GlcNAc (*m*/*z* 606 $[M\text{-H}]^+$) was converted to UDP-4-amino-2-acetamido-2,4,6-trideoxy-L-altrose (UDP-6-deoxy-AltNAc4N, *m*/*z* 589 $[M\text{-H}]^+$) during this time. Enzyme was then removed by centrifugation and centrifugal ultrafiltration. The resultant filtrate was loaded onto a 220 mL column of DEAE cellulose (DE 52, Whatman Inc.) and eluted with a linear gradient of 0.1 to 0.5 M triethylammonium bicarbonate buffer. The eluant was monitored at A_{254} and UV-active fractions were analyzed by ESI-MS(-). Those containing UDP-6-deoxy-AltNAc4N were lyophilized to dryness. The total amount was determined to be 250 mg (ditriethylammonium salt) by UV absorbance at 262 nm (ε_{262} = 9890 M^{-1}).

The lyophilized UDP-6-deoxy-AltNAc4N (240 mg, ditriethylammonium salt) was stirred with triethylamine (887 μL) and chloroacetic anhydride (1.08 g) in methanol (25 mL) at room temperature for 72 h. ESI-MS(-) showed that the starting material was completely converted to UDP-6-deoxy-AltNAc4NAcCl, **1**, (*m*/*z* 665 $[M\text{-H}]^+$) during this time. After filtration and evaporation of the solvent under reduced pressure, the product was loaded onto a DE-52 anion exchange column and eluted with a linear gradient of triethylammonium bicarbonate buffer as described above. Fractions containing the product were lyophilized to dryness and passed through 5 mL of DOWEX

50WX8-200 resin (sodium form). The yield of UDP-6-deoxy-AltNAc4NAcCl (**1**) was determined to be 160 mg (disodium salt) by UV absorbance as stated above. The product was lyophilized twice to obtain UDP-6-deoxy-AltNAc4NAcCl (**1**) as a white solid. ^{1}H NMR(D_2O, pD 6.4, 400MHz) δ 1.31 (d, 2H, $J_{5",6"}$ 6.2 Hz, H-6"), 2.10 (s, 3H, CH_3), 3.96 (dd, 1H, $J_{3",4"}$ 3.0 Hz, $J_{4",5"}$ 9.4 Hz, H-4"), 4.06 (dd, 1H, $J_{2",3"}$ 4.6 Hz, $J_{3",4"}$ 3.0 Hz, H-3"), 4.09 (dq, 1H, $J_{4",5"}$ 9.4 Hz, $J_{5",6"}$ 6.2 Hz, H-5"), 4.20 (s, 2H, Cl-CH_2), 4.21 (dd, 1H, $J_{1",2"}$ 2.2 Hz, $J_{2",3"}$ 4.6 Hz, H-2"), 4.27 (m, 2H, H-5'), 4.32 (m, 1H, H-4'), 4.39 (m, 1H, H-3'), 4.41 (m, 1H, H-2'), 5.69 (dd, 1H, $J_{1",2"}$ 2.2 Hz, $J_{H,P}$ 8.4 Hz, H-1"), 6.01 (m, 2H, H-1' and H-5), 8.02 (d, 1H, $J_{5,6}$ 8.1 Hz, H-6). ESI-MS(-) *m*/*z* 665 [*M*-H]$^+$.

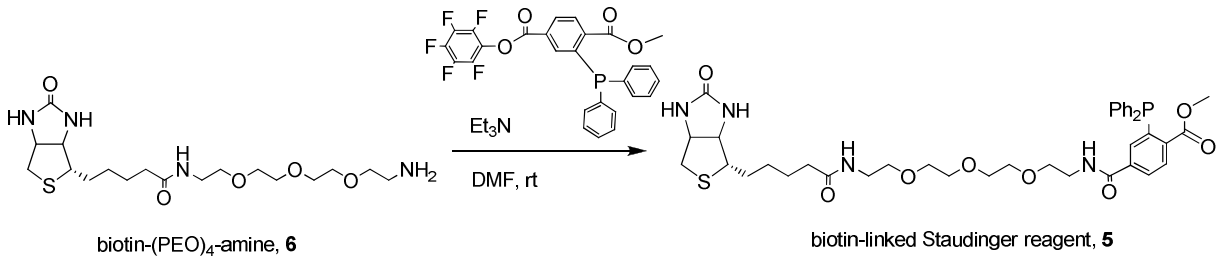
Enzymatic Synthesis of 2-acetamido-4-chloroacetamido-2,4,6-trideoxy-L-altrose (6-deoxy-AltNAc4NAcCl, 2): A solution of UDP-6-deoxy-AltNAc4NAcCl (**1**, disodium salt, 53 mg) in sodium phosphate buffer (1 mM, 5 mL, pH 7.5) containing PseG (2 mg) was incubated at 37 ℃ for 1 h. PseG was removed by centrifugal ultrafiltration. The resulting filtrate was then passed through BIO-RAD AG 1-X8 resin (10 mL) and DOWEX 50WX8-200 resin (5 mL) eluting with distilled water. The eluant (100 mL) was collected and 6-deoxy-AltNAc4NAcCl (**2,** 16mg) was obtained as a white solid after the removal of solvent in vacuo. ^{1}H (D_2O, pD=7.2, 400MHz), δ 1.24 (d, 3H, $J_{5\beta,6\beta}$ 6.1 Hz, H-6$_\beta$), 1.27 (d, 3H, $J_{5\alpha,6\alpha}$ 6.5 Hz, H-6$_\alpha$), 2.06 (s, 3H, α-CH_3), 2.11 (s, 3H, β-CH_3), 3.89 (dd, 1H, $J_{3,4}$ 2.8 Hz, $J_{4,5}$ 10.4 Hz, H-4), 3.98 (dd, 1H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 2.8 Hz, H-3) , 4.01 (dq, 1H, $J_{4,5}$ 10.4 Hz, $J_{5,6}$ 6.1 Hz, H-5), 4.08 (dd, 1H, $J_{1\alpha,2\alpha}$ 2.8 Hz, $J_{2\alpha,3\alpha}$ 4.3 Hz, H-2$_\alpha$), 4.13 (dd, 1H, $J_{1\beta,2\beta}$ 1.8 Hz, $J_{2\beta,3\beta}$ 3.2 Hz, H-2$_\beta$), 4.18 (s, 2H, β-Cl-CH_2), 4.20 (s, 2H, α-Cl-CH_2), 5.08 (d, 3H, $J_{1\alpha,2\alpha}$ 2.8 Hz, H-1$_\alpha$), 5.31 (d, 3H, $J_{1\beta,2\beta}$ 1.8 Hz, H-1$_\beta$). ESI-MS(+) *m*/*z* 303 [*M*+Na]$^+$.

Synthesis of 2-acetamido-4-azidoacetamido-2,4,6-trideoxy-L-altrose (6-deoxy-AltNAc4NAz, 3): 6-deoxy-AltNAc4NAcCl (**2**, 15 mg) was refluxed with sodium azide (17 mg) in acetone/water (15 mL/7 mL) for 24 h. After the removal of acetone at reduced pressure, the reaction mixture was passed through BIO-RAD AG 1-X8 and DOWEX 50WX8-200 resin as described above. This procedure yielded 14 mg of 6-deoxy-AltNAc4NAz (**3**). ^{1}H NMR (D_2O, pD 7.2, 400MHz), δ 1.24 (d, 3H, $J_{5\beta,6\beta}$ 6.0 Hz, H-6$_\beta$), 1.27 (d, 3H, $J_{5\alpha,6\alpha}$ 6.5 Hz, H-6$_\alpha$), 2.07 (s, 3H, α-CH_3), 2.11 (s, 3H, β-CH_3), 3.91 (dd, 1H, $J_{3,4}$ 3.1 Hz, $J_{4,5}$ 10.2 Hz, H-4), 3.94 (dd, 1H, $J_{2,3}$ 3.4 Hz, $J_{3,4}$ 3.1 Hz, H-3) , 3.98 (dq, 1H, $J_{4,5}$ 10.2 Hz, $J_{5\beta,6\beta}$ 6.0 Hz, H-5), 4.07 (dd, 1H, $J_{1\alpha,2\alpha}$ 2.5 Hz, $J_{2\alpha,3\alpha}$ 3.3 Hz, H-2$_\alpha$), 4.08 (s, 2H, β-Az-CH_2), 4.10 (s, 2H, α-Az-CH_2), 4.14 (dd, 1H, $J_{1\beta,2\beta}$ 1.9 Hz,

$J_{2\beta,3\beta}$ 3.4 Hz, H-2_β), 5.08 (d, 3H, $J_{1\alpha,2\alpha}$ 2.5 Hz, H-1_α), 5.31 (d, 3H, $J_{1\beta,2\beta}$ 1.9 Hz, H-1_β). ^{13}C NMR (D_2O, pD=7.2, 100MHz), δ 17.40, 22.13, 50.94, 51.94, 53.69, 68.22, 69.48, 90.75, 170.49, 175.01. HRMS calcd for $C_{10}H_{17}N_5O_5Na$ [*M*+Na]$^+$ 310.1127, found 310.1120.

Enzymatic synthesis of 5-acetamido-7-azidoacetamido-3,5,7,9-tetradeoxy-L-glycero-L-manno-nonulosonic acid (Az-Pse,4): 6-deoxy-AltNAc4NAz (**3**, 10 mg) was added to sodium phosphate buffer (15 mM, 6 mL, pH 8) containing the crude lysate of PseI from 150 mL of *E. coli* cell culture, phospho(enol)pyruvic acid monosodium salt hydrate (PEP, 16 mg), $MgCl_2$ (1.2 mg) and Na_2CO_3 (6.5 mg) and was incubated at 37 ℃ for 2.5 h. After removal of PseI by centrifug al ultrafiltration, the filtrate was loaded onto a column containing BIO-RAD AG 1-X8 resin (10 mL) and purified by stepwise elution with 0~1 M formic acid as described by Chou et al[3]. ^{1}H NMR (D_2O, pD 8.2, 400MHz), δ 1.11 (d, 3 H, $J_{8,9}$ 6.5 Hz, H-9), 1.78 (dd, 1 H, $J_{3ax,4}$ 12.9 Hz, $J_{3ax,3eq}$ 13.2 Hz, H-3ax), 1.94 (dd, 1 H, $J_{3eq,3ax}$ 13.2, $J_{3eq,4}$ 4.9 Hz, H-3eq), 2.01 (s, 3H, CH_3), 3.96 (d, 1 H, $J_{H,H}$ 16.4 Hz, Az-CH_2), 4.03 (d, 1 H, $J_{H,H}$ 16.4 Hz, Az-CH_2), 4.10 (dd, 1 H, $J_{6,7}$ 10.5 Hz, H-6), 4.15 (m, 1H, H-4), 4.16 (dq, 1H, $J_{7,8}$ 3.4 Hz, $J_{8,9}$ 6.5 Hz, H-8), 4.22 (dd, 1 H, $J_{6,7}$ 10.5 Hz, $J_{7,8}$ 3.4 Hz, H-7), 4.25 (dd, 1 H, H-5). ESI-MS(+) *m*/*z* 398 (M+Na$^+$), ESI-MS(-) *m*/*z* 374 [*M*+Na]$^+$.

Synthesis of biotin-linked Staudinger reagent (5): Compound **5** was synthesized using a slightly modified method from that described by Saxon and Bertozzi[4]. Commercially available 2-(diphenylphosphino) terephthalic acid 1-methyl 4-pentafluorophenyl diester (26 mg, 0.049 mmol) was stirred with (+)-biotin-$(PEO)_4$-amine (14.3 mg, 0.049 mmol) and triethylamine (43 μL, 0.245 mmol) in 2 mL of dimethylformamide (DMF) under argon at room temperature for 1 h. After the removal of DMF in vacuo, the product was purified by silica gel chromatography eluting with 9:1 dichloromethane (DCM): methanol (R_f = 0.2). ESI-MS(+) *m*/*z* 787 [*M*+Na]$^+$.



biotin-$(PEO)_4$-amine, **6**

biotin-linked Staudinger reagent, **5**

Scheme S1. Synthesis of Biotin-Linked Staudinger Reagent (**5**)

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[4] E. Saxon, C. R. Bertozzi, *Science* **2000**, *287*, 2007-2010.

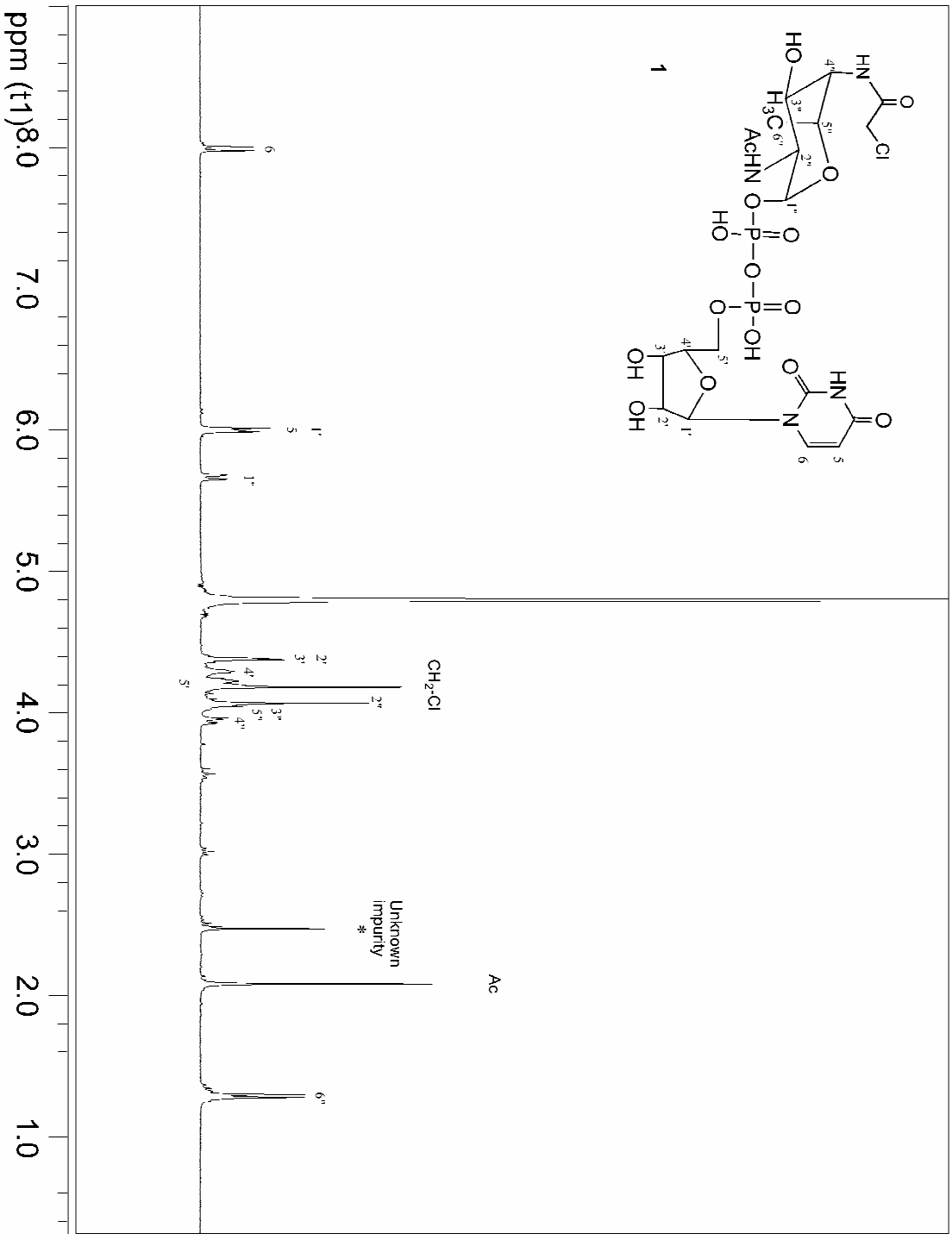

Figure S1. ^{1}H NMR of UDP-6-deoxy-AltNAc4NAcCl (**1**) (D_2O).

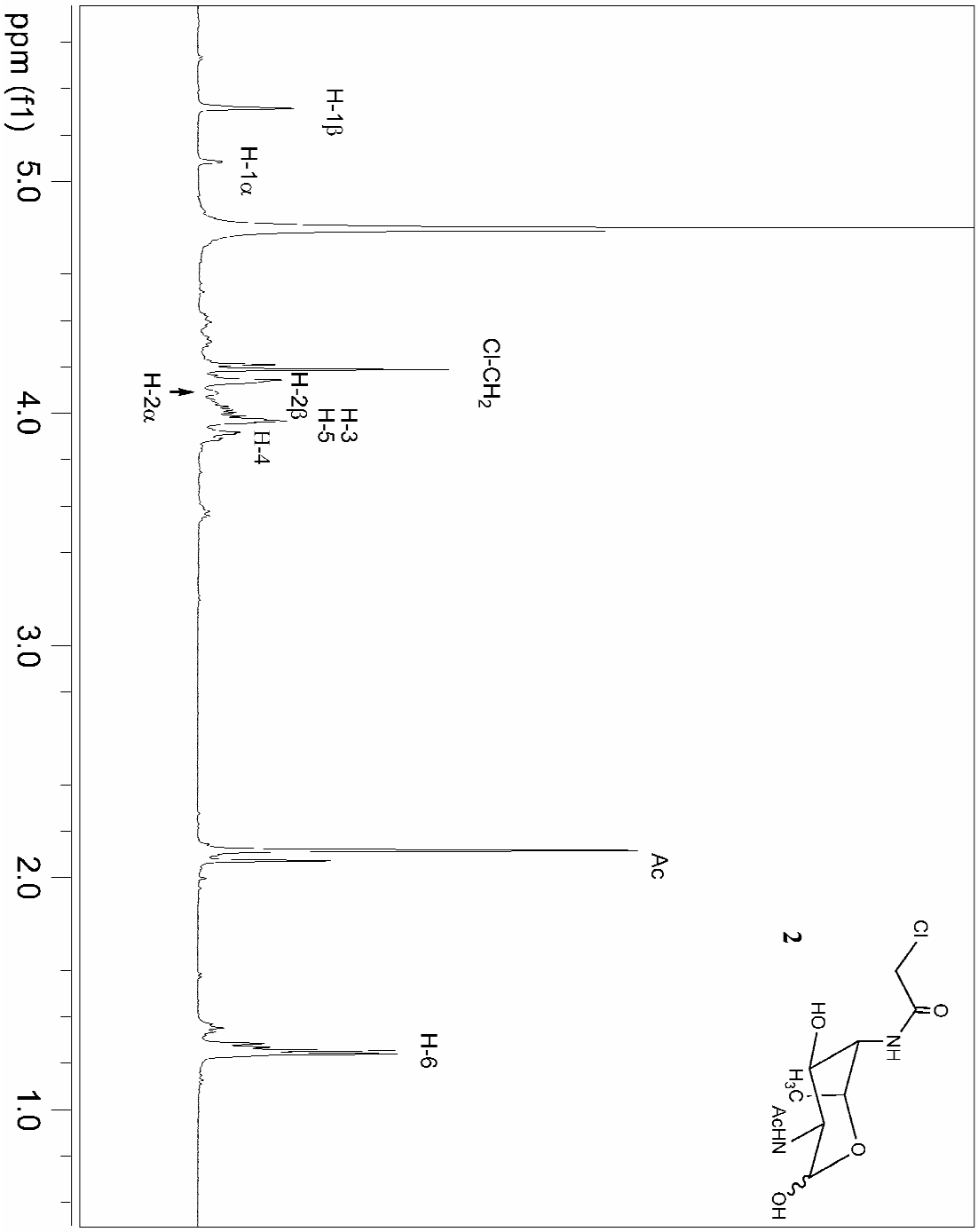

Figure S2. ^{1}H NMR of 6-deoxy-AltNAc4NAcCl (**2**) (D_2O).

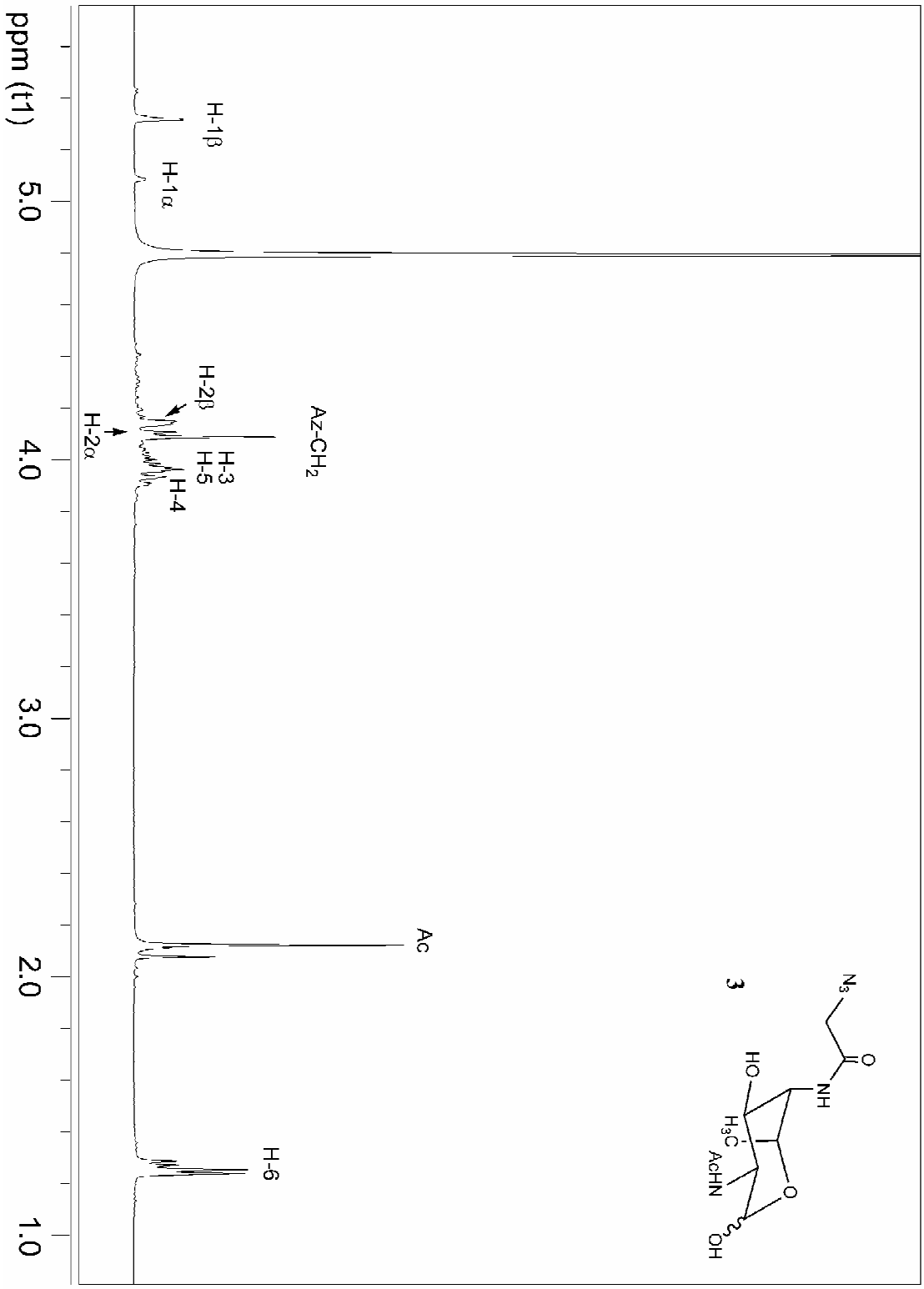

Figure S3. ^{1}H NMR of 6-deoxy-AltNAc4NAz (**3**) (D_2O).

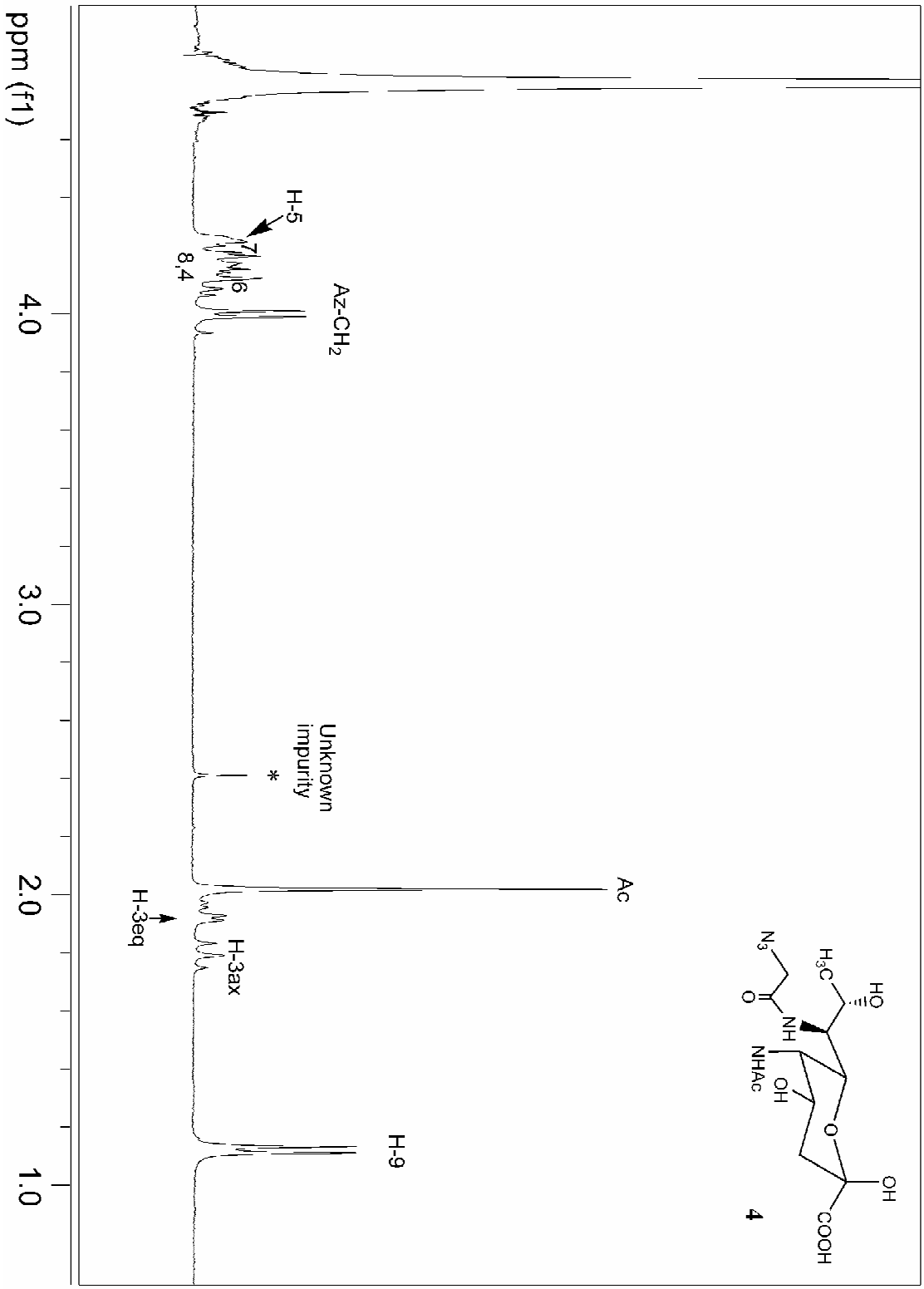

Figure S4. ^{1}H NMR of Az-Pse (**4**) (D_2O).

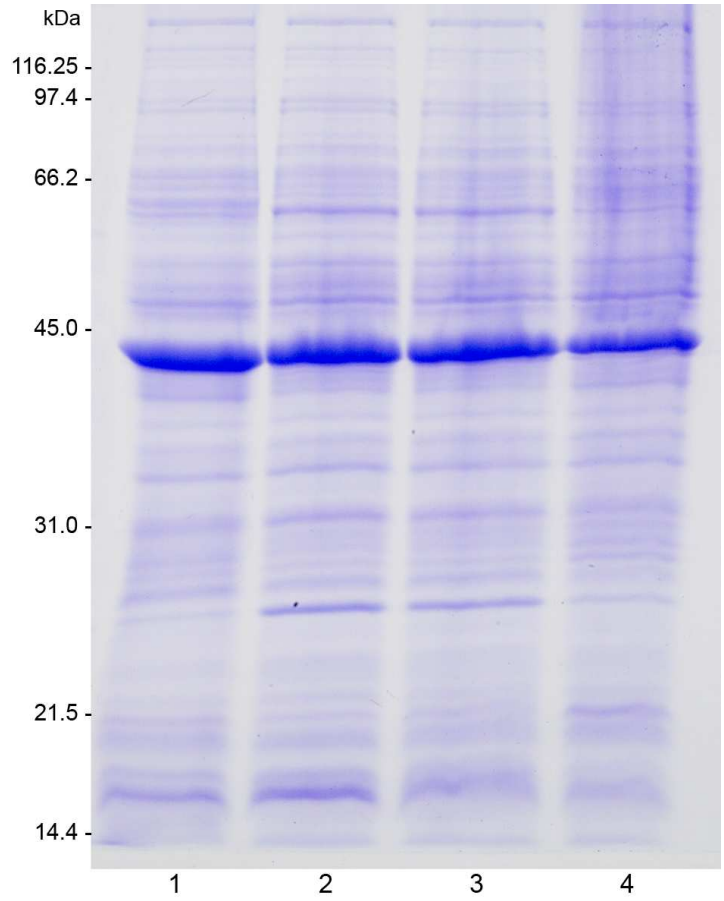

Figure S5. Coomassie blue-stained SDS-PAGE gel of samples used in Western blot. Lane 1. *C. jejuni* 81-176 grown on MH medium, Lane 2.*C. jejuni* 81-176 *pseG::cat* grown on MH medium, Lane 3. *C. jejuni* 81-176 *pseG::cat* grown on MH medium containing 6-deoxy AltNAc4NAz, Lane 4. *C. jejuni* 81-176 grown on MH medium containing 6-deoxy AltNAc4NAz. The major protein at 45 kDa is the major outer membrane protein (MOMP).