Electronic Supplementary Material (ESI) for RSC Advances.

SUPPORTING INFORMATION

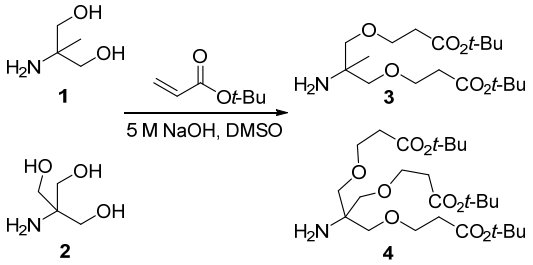
Analysis of Binding Properties of Pathogens and Toxins Using Multivalent Glycan Microarrays

Hyoung Sub Kim, Ji Young Hyun, Seonghyun Park, and Injae Shin*

Center for Biofunctional Biomolecules, Department of Chemistry, Yonsei University, Seoul 03722, Republic of Korea.

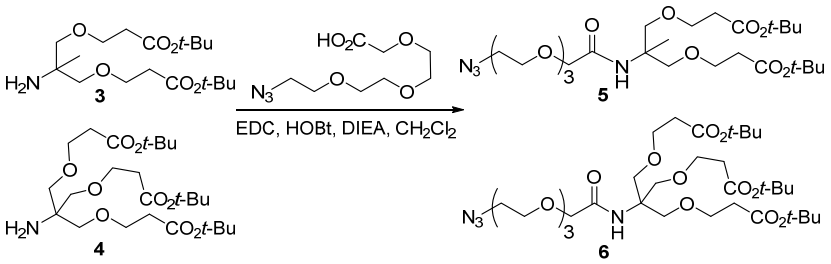
General. Analytical thin-layer chromatography (TLC) was conducted on silica gel 60 F254 glass plates. Compound spots were visualized by UV light (254 nm) and/or by staining with 10 wt% phosphomolybdic acid in ethanol. Flash column chromatography was performed using silica gel 60 (230–400 Mesh). NMR spectra were recorded on a Bruker Avance II 400 instrument. Mass spectra were obtained using a Waters 3100 LC/MS System.

Synthesis



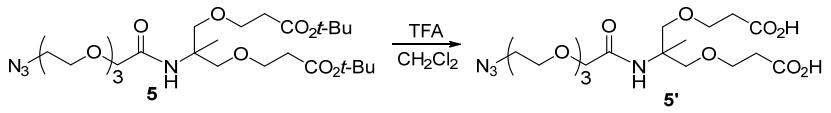
Compound 3. To a stirred solution of 2-amino-2-methyl-1,3-propanediol (4.3 g, 41.3 mmol) in DMSO (8.3 mL) was added 5.0 M sodium hydroxide (0.8 mL) at 15 °C and then *tert*-butyl acrylate (20.6 mL, 141 mmol) dropwise. The reaction mixture was warmed to room temperature. After stirring for 12 h, the reaction mixture was diluted with EtOAc, washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexane : EtOAc = 2:1 containing 0.05% NH_4OH) to afford a product as colorless oil in 45% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.67 (t, 4 H, J = 6.4 Hz), 3.24 (q, 4 H, J = 7.1 Hz), 2.47 (t, 4 H, J = 6.4 Hz), 1.45 (s, 18 H), 1.01 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 80.5, 76.9, 67.3, 53.0, 36.5, 28.2, 22.7; ESI-MS calcd for $C_{18}H_{35}NO_6$ $[M+H]^+$ 362.2, found 362.5.

Compound 4. To a stirred solution of tris(hydroxymethyl)aminomethane (5 g, 41.3 mmol) in DMSO (8.3 mL) was added 5.0 M sodium hydroxide (0.8 mL) at 15 °C and then *tert*-butyl acrylate (20.6 mL, 141 mmol) dropwise. The reaction mixture was warmed to room temperature. After stirring for 12 h, the reaction mixture was diluted with EtOAc, washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexane : EtOAc = 2:1 containing 0.05% NH_4OH) to afford a product as colorless oil in 50% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.65 (t, 6 H, J = 6.4 Hz), 3.32 (s, 6 H), 2.46 (t, 6 H, J = 6.4 Hz), 1.70 (s, 2 H), 1.45 (s, 27 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 80.5, 73.0, 67.3, 56.1, 36.5, 28.2; ESI-MS calcd for $C_{25}H_{47}NO_9$ $[M+H]^+$ 506.3, found 506.1.

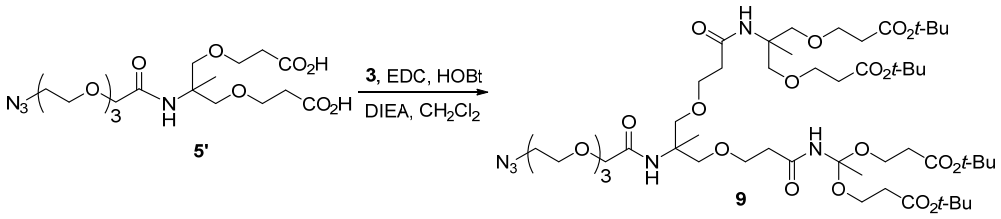


Compounds 5 and 6. To a stirred solution of **3** or **4** (2.78 mmol), HOBt (434.5 mg, 3.21 mmol), DIEA (0.56 mL, 3.21 mmol) and 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)acetic acid (0.5 g, 2.14 mmol) in CH_2Cl_2 (10 mL) was added EDC (616.4 mg, 3.21 mmol) at room temperature. After stirring for 12 h, the reaction mixture was diluted with CH_2Cl_2, washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (EtOAc : CH_2Cl_2 = 3:1) to afford **5** or **6** as colorless oil in 40-50% yield.

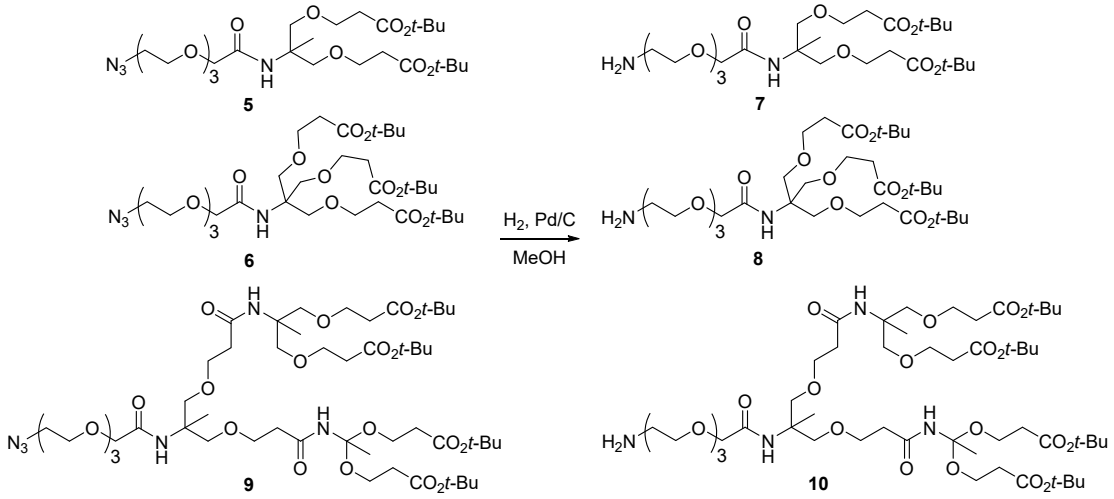
Compound 5: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.80 (s, 1 H), 3.90 (s, 2 H), 3.69–3.66 (m, 14 H), 3.63 (s, 2 H), 3.50 (d, 2 H, *J* = 8.9 Hz), 3.40 (t, 2 H, *J* = 5.2 Hz), 2.47 (t, 4 H, *J* = 6.4 Hz), 1.49 (s, 18 H), 1.36 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 169.5, 80.5, 72.7, 71.0, 70.9, 70.8, 70.7, 70.6, 70.2, 67.1, 56.5, 50.7, 36.3, 28.1, 19.2; ESI-MS calcd for $C_{26}H_{48}N_4O_{10}$ $[M+H]^+$ 577.3, found 577.8.
Compound 6: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.76 (s, 1 H), 3.90 (s, 2 H), 3.71–3.64 (m, 22 H), 3.40 (t, 2 H, *J* = 5.2 Hz), 2.44 (t, 6 H, *J* = 6.4 Hz), 1.45 (s, 27 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 169.6, 80.5, 71.2, 71.0, 70.9, 70.9, 70.7, 70.2, 69.3, 67.3, 59.7, 50.9, 36.4, 28.3; ESI-MS calcd for $C_{33}H_{60}N_4O_{13}$ $[M+H]^+$ 721.4, found 721.7.



Compound 5'. A solution of 50% TFA in CH_2Cl_2 (1.86 mL) was added to **5** (0.7 g, 1.50 mmol) in CH_2Cl_2 (3 mL) slowly. After stirring for 2 h, the volatile materials were removed under reduced pressure several times repeatedly. The compound **5'** as colorless oil was used for the next reaction without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.58 (br s, 2 H), 6.97 (s, 1 H), 3.91 (s, 2 H), 3.81–3.64 (m, 16 H), 3.53 (d, 2 H, *J* = 8.9 Hz), 3.39 (t, 2 H, *J* = 5.2 Hz), 2.58 (t, 4 H, *J* = 6.1 Hz), 1.38 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.2, 170.0, 72.9, 72.8, 72.8, 70.9, 70.6, 70.4, 70.1, 66.8, 56.8, 50.8, 35.0, 18.9; ESI-MS calcd for $C_{18}H_{32}N_4O_{10}$ $[M+H]^+$ 465.2, found 465.2.



Compound 9. To a stirred solution of **5′** (0.67 g, 1.44 mmol), **3** (1.19 g, 3.31mmol), HOBt (0.55 g, 3.60 mmol) and DIEA (0.63 mL, 3.60 mmol) in CH_2Cl_2 (10 mL) was added EDC (0.69 g, 3.60 mmol) at room temperature. After stirring for 12 h, the reaction mixture was diluted with CH_2Cl_2, washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (EtOAc : CH_2Cl_2 = 17:3) to afford a product as colorless oil in 45% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.76 (s, 1 H), 6.20 (s, 2 H), 3.89 (s, 2 H), 3.72–3.62 (m, 28 H), 3.50 (d, 2 H, *J* = 8.9 Hz), 3.45 (dd, 4 H, *J* = 8.9, 1.2 Hz), 3.38 (t, 2 H, *J* = 5.2 Hz), 2.45 (t, 8 H, *J* = 6.4 Hz), 2.39 (t, 4 H, *J* = 6.4 Hz), 1.45 (s, 36 H), 1.37 (s, 3 H) , 1.34 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 170.8, 169.5, 80.6, 73.0, 72.8, 71.1, 70.9, 70.8, 70.8, 70.6, 70.2, 67.7, 67.2, 56.8, 56.6, 50.8, 37.8, 36.3, 28.2, 19.3, 19.1; ESI-MS calcd for $C_{52}H_{94}N_6O_{20}$ $[M+H]^+$ 1123.6, found 1123.1.



Reduction of N_3 to NH_2. Compound **5**, **6** or **9** (1 g) dissolved in methanol (48 mL) was hydrogenated over 10% Pd/C (100 mg). After 4 h, the catalyst was filtered through Celite® pad and the solvent was removed under reduced pressure to afford **7**, **8** or **10** with quantitative yields.

Compound **7**: ^{1}H NMR (400 MHz, CD_3OD) δ 3.90 (s, 2 H), 3.69–3.53 (m, 18 H), 2.82–2.79 (m, 2 H), 2.47 (t, 4 H, *J* = 6.4 Hz), 1.46 (s, 18 H), 1.34 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 169.5, 80.6, 72.8, 71.1, 70.9, 70.7, 70.5, 67.2, 56.5, 49.4, 41.9, 36.4, 28.2, 19.3; ESI-MS calcd for $C_{26}H_{50}N_2O_{10}$ $[M+H]^+$ 551.3, found 551.0.

Compound **8**: ^{1}H NMR (400 MHz, CD_3OD) δ 3.91 (s, 2 H), 3.70–3.52 (m, 22 H), 2.81–2.68 (m, 2 H), 2.46 (t, 6 H, J = 6.4 Hz), 1.47 (s, 27 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 169.6, 80.5, 73.6, 71.0, 70.9, 70.7, 70.5, 70.4, 69.0, 67.1, 59.5, 41.9, 36.3, 28.2; ESI-MS calcd for $C_{33}H_{62}N_2O_{13}$ $[M+H]^+$ 695.4, found 695.1.
Compound **10**: ^{1}H NMR (400 MHz, CD_3OD) δ 3.96–3.91 (m, 2 H), 3.69–3.40 (m, 36 H), 2.48–2.41 (dt, 12 H, J = 16.7, 6.4 Hz), 1.46 (s, 36 H), 1.36 (s, 3 H) , 1.31 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 170.9, 169.5, 80.6, 73.5, 72.8, 72.6, 71.0, 70.8, 70.7, 70.5, 70.3, 67.6, 67.1, 56.7, 56.5, 41.8, 37.6, 36.2, 28.2, 19.2, 19.0; ESI-MS calcd for $C_{52}H_{96}N_4O_{20}$ $[M+H]^+$ 1097.6, found 1097.1.

Microarray studies

Preparation of hydrazide-functionalized glass slides. Monovalent hydrazide modified glass slides were prepared by using a known procedure.[1,2] For preparation of di-, tri- and tetravalent hydrazide modified glass slides, N-hydroxysuccinimide (NHS) ester functionalized glass slides, obtained from epoxide-coated glass slides,[1,2] were treated with a solution of respective **7**, **8** or **10** (1%) and 1% DIEA (w/v) in DMF for 5 h with gentle shaking. The glass slides were washed with DMF for 3 min three times and then with water. After purging with argon gas, the slides were treated with a solution of 2 M HCl in acetic acid for 2 h to remove t-Bu groups with gentle shaking. The glass slides were washed with water for 3 min three times. After purging with argon gas, the slides were treated with a solution of 3% DIC and 3% NHS in DMF for 3 h with gentle shaking. The slides were washed with DMF for 3 min three times. After purging with argon gas, the slides were treated with a solution of 3% hydrazine monohydrate in DMF for 3 h with gentle shaking. After washing with water several times, the slides were dried by purging with argon gas.

Construction of carbohydrate microarrays. Unmodified glycans, dissolved in 100 mM sodium phosphate buffer (pH 5.0) containing 30% glycerol, were placed into wells of a 384-well plate. Solutions of glycans (1 nL, 10 and 30 mM) were printed in duplicate in a predetermined place on a hydrazide coated glass slide with a distance of 250 μm between centers of adjacent spots by using an automatic pin-type microarrayer (SpotBot®, Arrayit). After printing carbohydrates on the slide surface, the slides were placed into a humidity chamber (55–60% humidity) at 50 °C for 10 h. The slide was then divided into several blocks by using a compartmentalized plastic film that was coated with adhesive on one side (thickness: 0.1–0.2 mm) to avoid cross-contamination. The slides were washed with PBS (pH 7.4) containing 0.1% Tween 20 for 5 min under gentle shaking. After drying the slide by purging with argon gas, a solution (15–20 μL) of PBS buffer (pH 7.4) containing 1% BSA was dropped onto the compartmented block and then left at room temperature for 1 h. The slide was washed three times with PBS buffer (pH 7.4) containing 0.1% Tween-20 for 5 min under gentle shaking. To obtain reproducible results, the prepared microarrays were used immediately.

Detection of glycan-protein interactions using microarrays. Carbohydrate microarrays were probed with Cy3-labeled RCA_{120} (50 μg/mL), ConA (50 μg/mL), AA (1 μg/mL) and cholera enterotoxin B (25 μg/mL) in PBS buffer (pH 7.4) containing 0.1% Tween 20 for 1 h at room temperature. For ConA binding, 1 mM $CaCl_2$ and 1 mM $MnCl_2$ were added. The unbound proteins were removed by washing with the same buffer and rinsed with water. After drying the treated microarray by purging with argon gas, the slide was scanned using GenePix® 4100A scanner (Applied Molecular Device). Fluorescence intensity was analyzed using GenePix Pro7 software (Molecular Device).

Cell culture. *H. pylori* J99 and 26695 strains (*H. pylori* Korean Type Culture Collection, HpKTCC, Gyeongsang National University) were grown on Brucella broth agar base plates (Neogen Corporation) supplemented with 2.5% (w/v) Bacto-Agar (Becton, Dickinson and Company) and 10% (v/v) heat-inactivated horse serum. *H. pylori* cells on the plates were cultured in a humidified incubator with 10% CO_2 for 1 day at 37 °C before use. *E. coli* ORN 178 and 208 strains were grown in Luria-Bertani *broth* media at 37 °C. *Staphylococcus aureus subspecies aureus* (KCTC 1916, Korean Collection for Type Culture, Daejeon, Korea) were grown in a tryptic soy broth (Difco Laboratories, Detroit, USA). *E. coli* and *Staphylococcus aureus subspecies aureus* were cultured in a shaking incubator for 12-18 h at 37°C before use.

Detection of interactions of carbohydrates with pathogenic cells using microarrays. *H. pylori* cells in Brucella broth media were treated with SYTO 83 (50 μM, 1% DMSO) to stain cells. After 5 min incubation on the shaker, cells were isolated by centrifugation and then washed twice with TBS buffer (50 mM Tris-Cl, 150 mM NaCl, pH 7.4). The labeled cells were suspended in TBS buffer containing 0.1% BSA and then applied onto carbohydrate microarrays. After 1 h at room temperature, unbound cells were removed by gently washing with PBS buffer containing 0.1% Tween 20 and water. After drying by purging with argon gas, the microarrays were scanned by using a GenePix® 4100A scanner (Applied Molecular Device). Fluorescence intensity was analyzed using GenePix Pro7 software (Molecular Device).

E. coli cells in PBS buffer (pH 7.4) containing $CaCl_2$ (1 mM) and $MnCl_2$ (1 mM) were treated with SYTO 83 (50 μM, 1% DMSO) to stain cells. After 1 h incubation on the shaker, cells were isolated by centrifugation and then washed twice with PBS buffer. The labeled cells were suspended in PBS buffer containing 0.1% BSA, 1 mM $CaCl_2$ and 1 mM $MnCl_2$ and then applied onto carbohydrate microarrays. After 1 h at room temperature, unbound cells were removed by gently washing with PBS buffer containing 0.1% Tween 20 and water. After drying by purging with argon gas, the slide was scanned using a scanner and fluorescence intensity was analyzed using GenePix Pro7 software.

S. aureus cells in TSB buffer (pH 7.4) were treated with SYTO 83 (50 μM, 1% DMSO) to stain cells. After 5 min incubation on the shaker, cells were isolated by centrifugation and then washed twice with TBS buffer. The labeled cells were suspended in TBS buffer

containing 0.1% BSA and 1 mM $CaCl_2$ and then applied onto the carbohydrate microarrays. After 1 h at room temperature, unbound cells were removed by gently washing with PBS buffer containing 0.1% Tween 20 and water. After drying by purging with argon gas, the slide was scanned using a scanner and fluorescence intensity was analyzed using GenePix Pro7 software.

Detection of *H. pylori* with Glyconanoparticles. Le^b- and H1-FMNPs were prepared and characterized according to known methods developed by us[3] *H. pylori* strains J99 and 26695 (10^6 cells) in PBS were treated with 1 μM Hoechst 33342 for 5 min. The cells were centrifuged and washed with PBS. The stained bacterial cells in PBS were incubated with 100 μg/mL of Le^b- or H1-FMNPs for 1 h at room temperature. After washing, images were obtained using confocal microscopy (LSM 700 META, Carl Zeiss, Germany).

Preparation of Cy3-BSA- Manα1,2Man conjugates. Cy3-NHS (0.08 mg, 0.14 μmol)[4] in DMSO (a final volume: 5% in buffer) was added to BSA (5 mg, 0.07 μmol) in 10 mM $NaHCO_3$ buffer (pH 8.0). The mixture was stirred for 1 h at room temperature. Low-molecular mass materials were removed by using an Amicon centrifugal filter device (cutoff: 30 KDa). Cy3-labeled BSA was lyophilized. The Cy3 conjugation ratio to BSA was determined by analyzing UV absorbance of a Cy3 dye.

Squarate-activated Manα1,2Man (0.6 mg, 1.13 μmol)[5] was added to Cy3-BSA (5 mg, 0.07 μmol) in 0.5 M borate buffer (pH 9.0). The mixture was gently stirred for 3 days. Low-molecular mass materials were removed by using an Amicon centrifugal filter device (cutoff: 30 KDa). The Manα1,2Man conjugation ratio to Cy3-BSA was determined by MS analysis.

Detection of *E. coli* with BSA-Manα1,2Man conjugate. *E. coli* strains ORN 178 ($FimH^+$) and ORN 208 ($FimH^-$) (10^6 cells) in PBS were treated with 1 μM Hoechst 33342 for 5 min. The cells were centrifuged and washed with PBS. The stained bacterial cells in PBS containing 1 mM $CaCl_2$ and 1 mM $MnCl_2$ were incubated with 200 μg/mL of Cy3-BSA-Manα1,2Man for 1 h at room temperature. After washing, images were obtained using confocal microscopy.

Supplementary References

1. M.-R. Lee and I. Shin, *Org. Lett*., 2005, **7**, 4269–4272; S. Park, M. R. Lee and I. Shin, *Bioconjugate Chem.*, 2009, **20**, 155-162

2. S. Park, M. R. Lee and I. Shin, *Nat. Protoc.*, 2007, **2**, 2747-2758

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5. J. Pai, J. Y. Hyun, J. Jeong, S. Loh, E. H. Cho, Y. S. Kang and I. Shin, *Chem. Sci.*, 2016, **7**, 2084-2093.

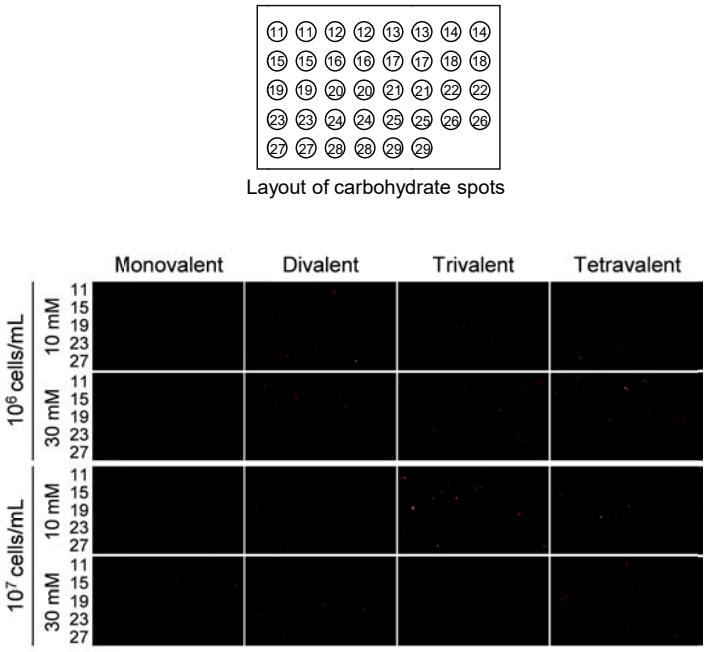


Figure S1. Fluorescence images of carbohydrate microarrays treated with SYTO 83-labeled *E. coli* ORN208 in 1 mM $CaCl_2$, 1 mM $MnCl_2$ PBS buffer (pH 7.4) containing 0.1% BSA. The distance between centers of adjacent spots is 250 μm.

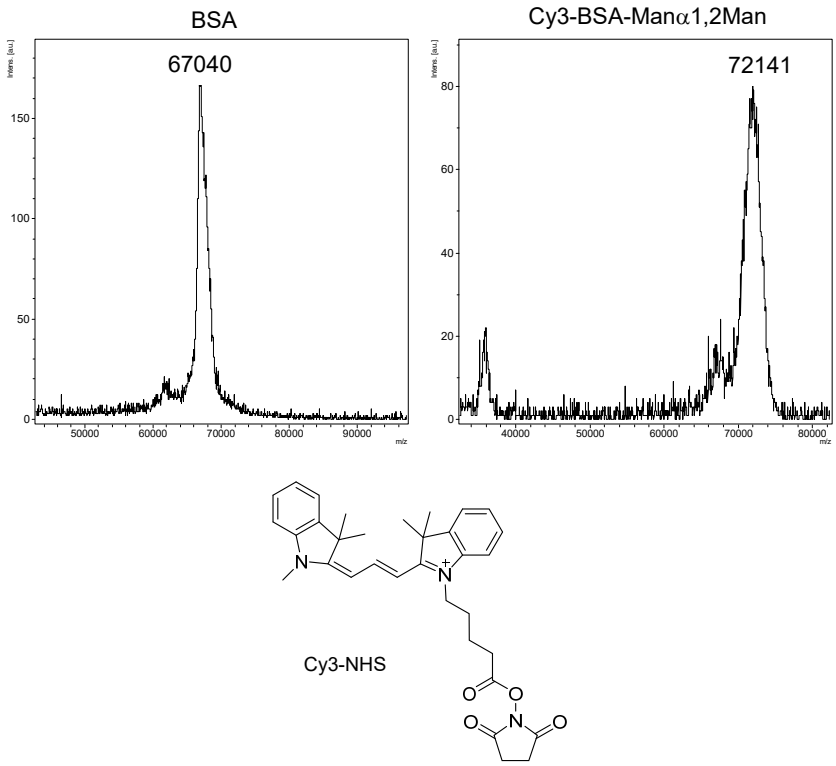


Figure S2. MS data for BSA and Cy3-BSA-Manα1,2Man conjugate. Shown is the chemical structure of Cy3-NHS.

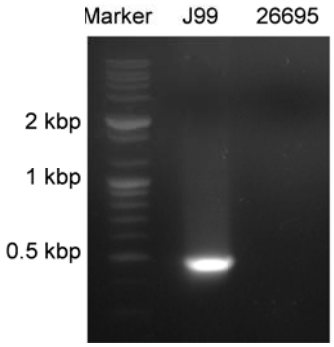


Figure S3. Expression of BabA in *H. pylori* strains 26695 and J99 was examined by RT-PCR.

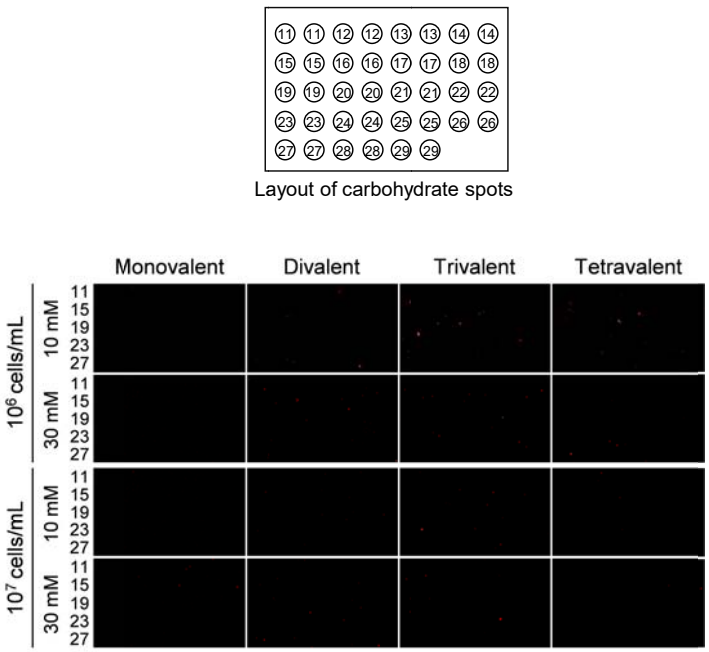
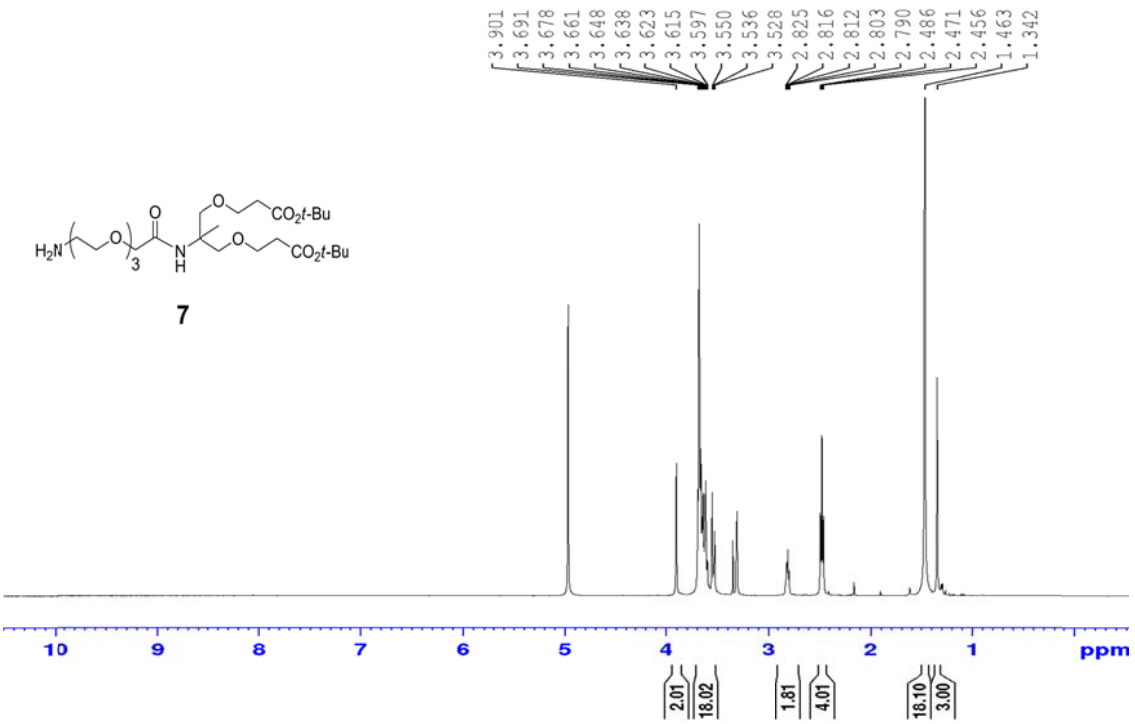
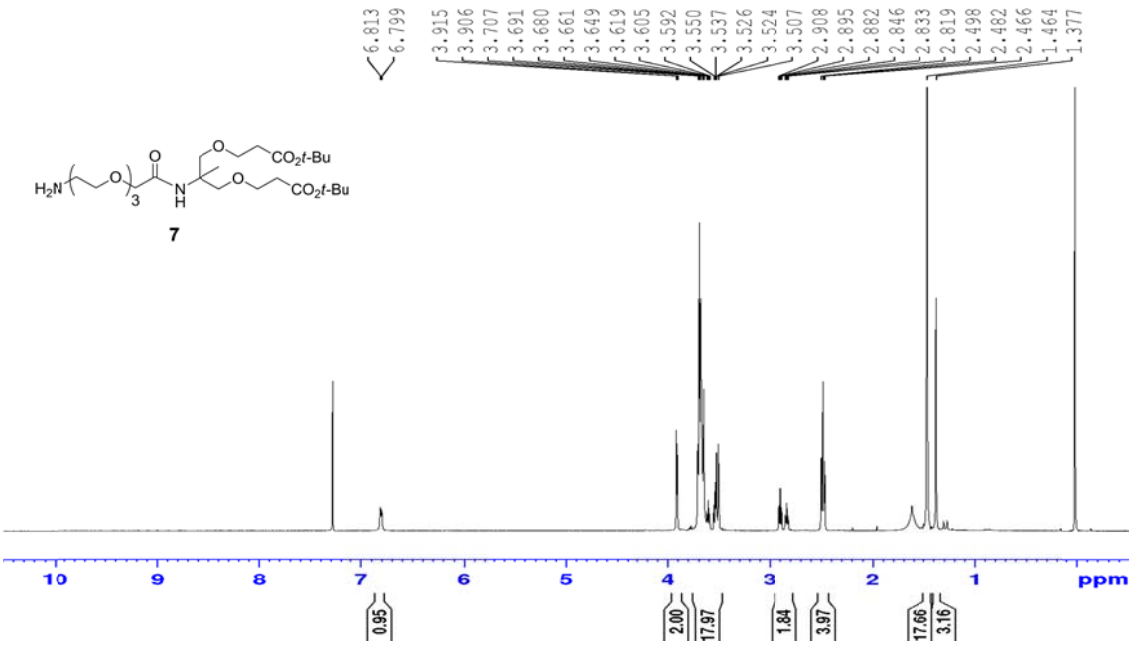


Figure S4. Fluorescence images of carbohydrate microarrays treated with SYTO 83-labeled *H. pylori* 26695 in TBS buffer (50 mM Tris-Cl, 150 mM NaCl, pH 7.4) containing 0.1% BSA for 1 h.. The distance between centers of adjacent spots is 250 μm.

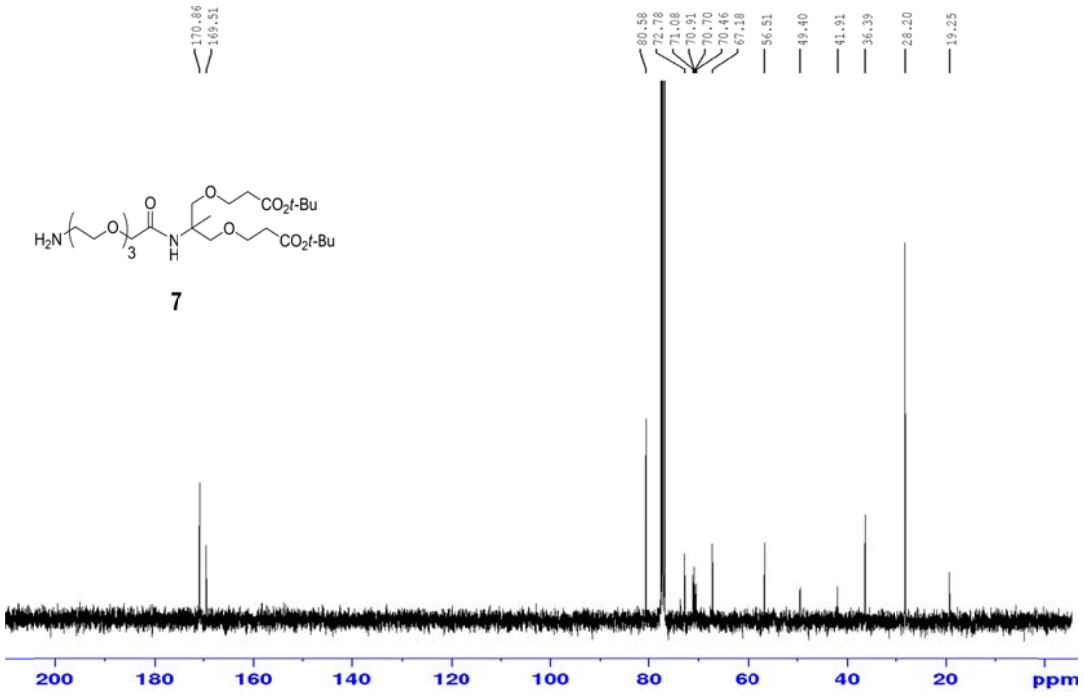
7 (CD_3OD, 400 MHz ^1H NMR)



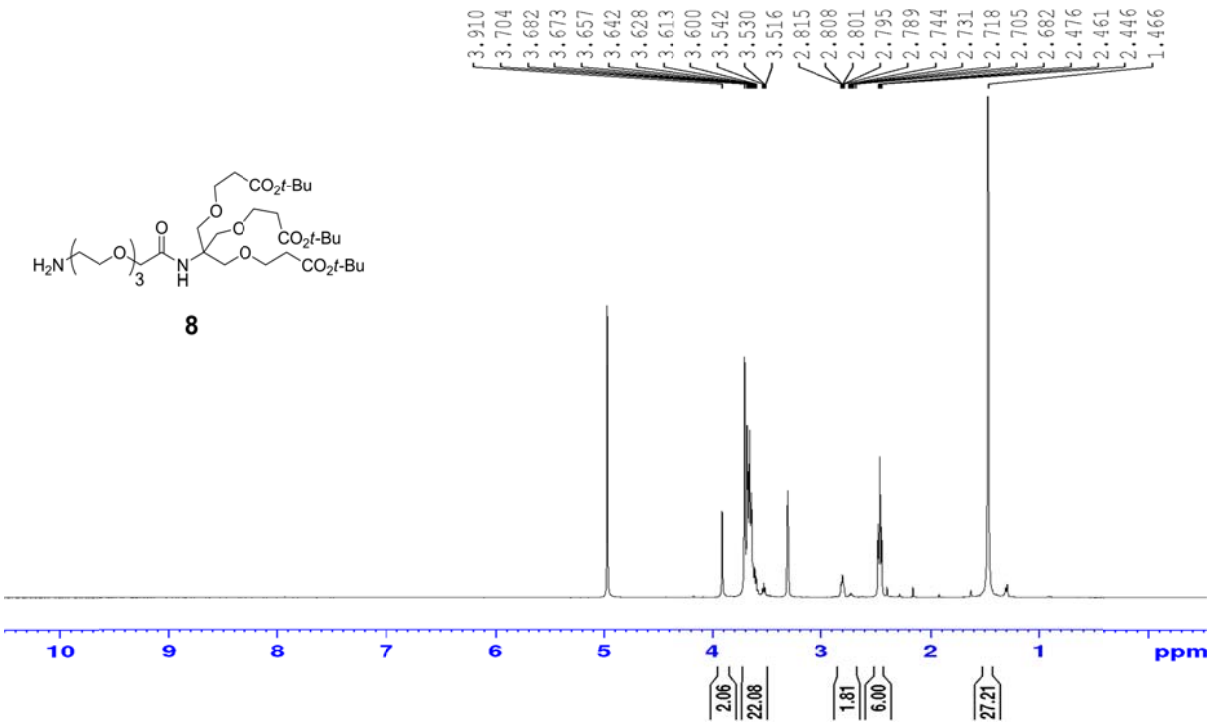
7 ($CDCl_3$, 400 MHz ^1H NMR)



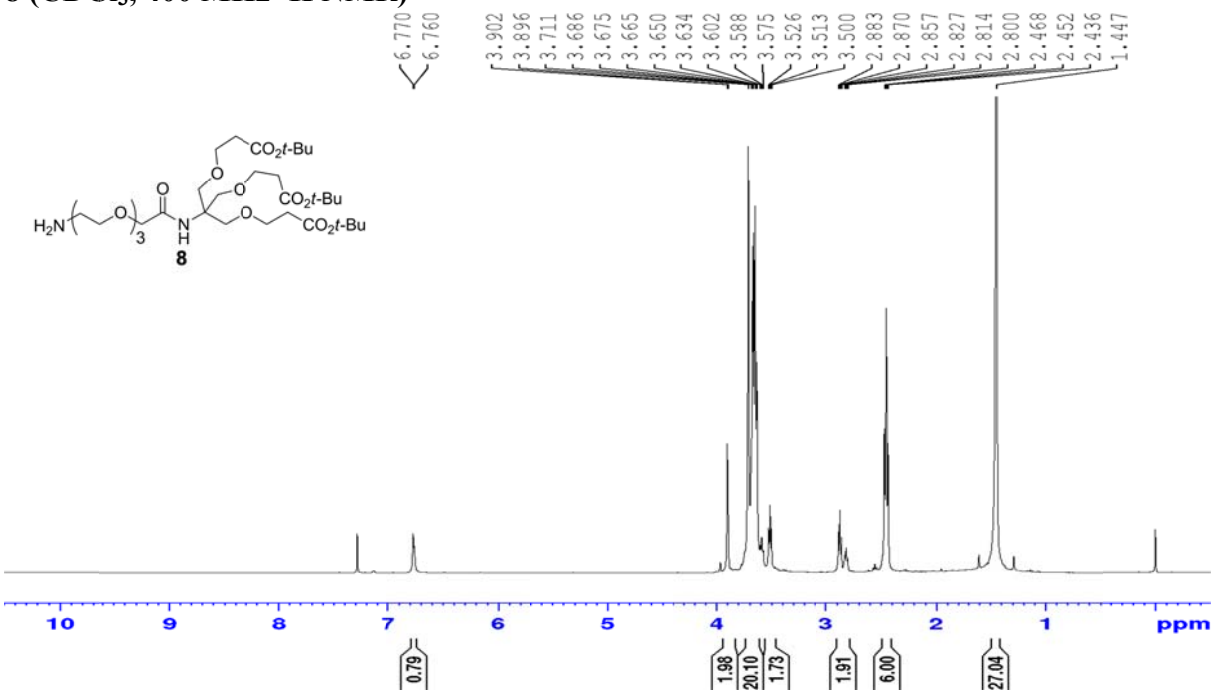
7 ($CDCl_3$, 100 MHz ^{13}C NMR)



8 (CD_3OD, 400 MHz ^1H NMR)



8 ($CDCl_3$, 400 MHz ^1H NMR)



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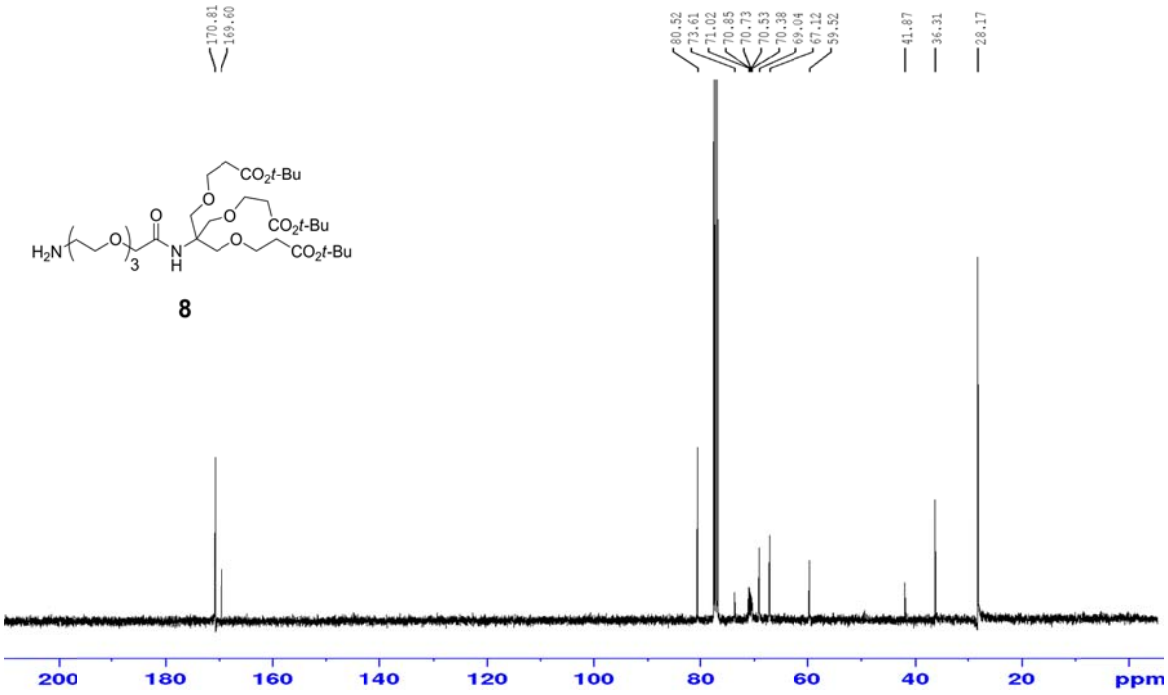
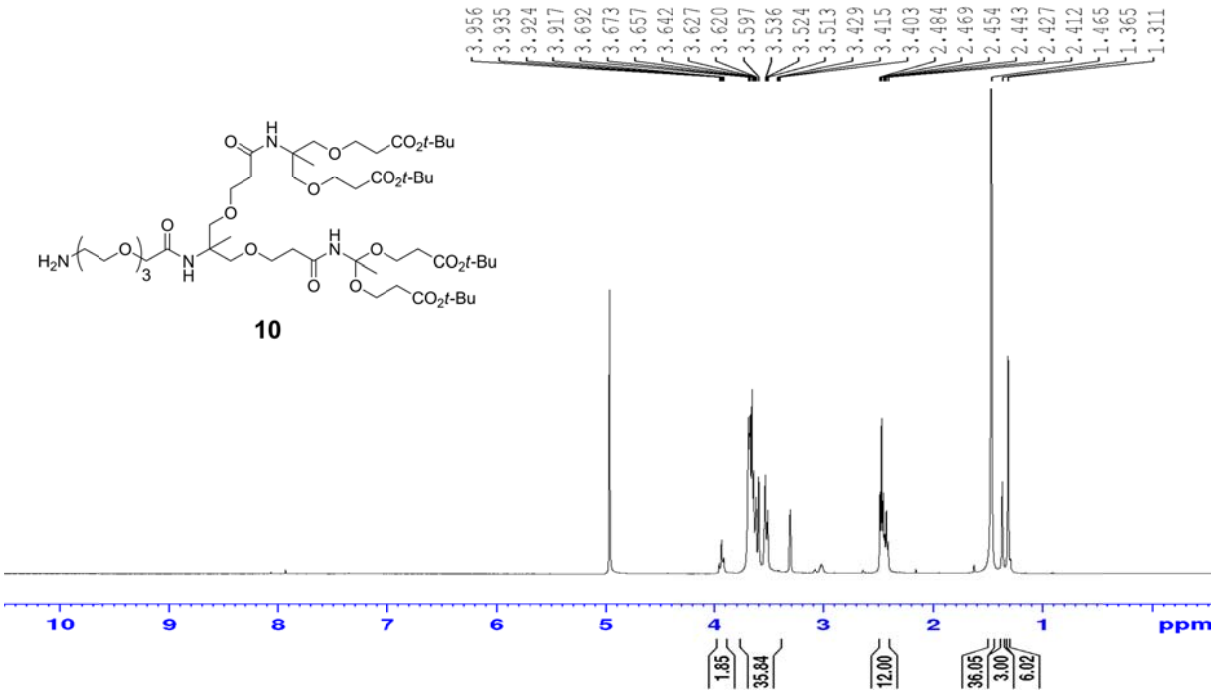
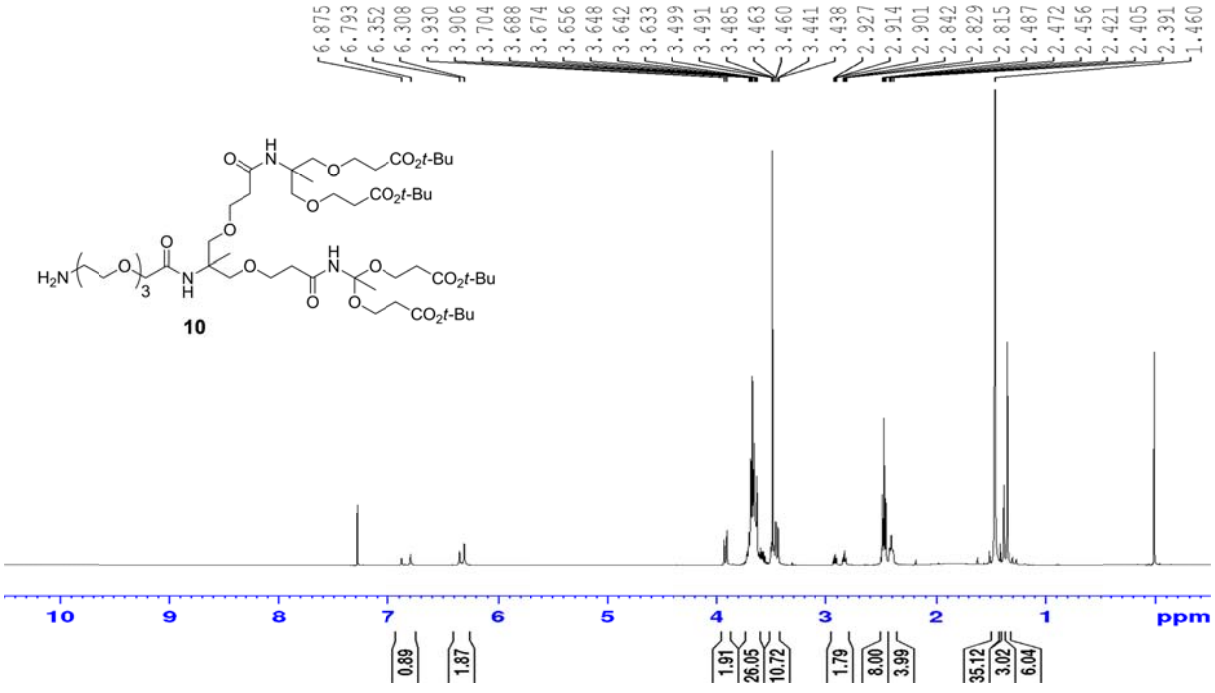


10 (CD_3OD, 400 MHz ^1H NMR)



10 ($CDCl_3$, 400 MHz ^1H NMR)



10 ($CDCl_3$, 100 MHz ^{13}C NMR)