

Photo-Click Immobilization of Carbohydrates on Polymeric Surfaces—A Quick Method to Functionalize Surfaces for Biomolecular Recognition Studies

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Methods to rapidly functionalize specific polymeric surfaces with alkynes, which can subsequently be linked to azide-containing carbohydrates, are presented. The methods comprise two main concepts: azide photoligation and Cu-catalyzed azide–alkyne cycloaddition. 2-Azidoethyl-functionalized α -D-mannopyranoside was synthesized and covalently attached to alkyne-functionalized polymeric surfaces using the techniques. The protein recognition properties of the carbohydrate-presenting surfaces were evaluated using quartz crystal microbalance biosensor instrumentation.

INTRODUCTION

Carbohydrates play highly diverse roles in biology. In polymeric form, they act as an energy source as well as a cell form stabilizer. In mono- and oligomeric forms, they build up DNA/RNA and function in the glycosylation process to increase protein diversity and stability. Carbohydrates also exist in high concentrations in the cell membrane where they have key functions as recognition markers, e.g., in cell–cell communication, cell adhesion and development, fertilization, immune response, signal transduction, and viral/bacterial infections (1–4). Considering the vast amount of data obtained from DNA and protein microarrays to date, it is desirable to apply the same methods to carbohydrates. Unfortunately, the analyses of these interactions are associated with several difficulties, which have made glycobiology a demanding area of research. The most important difference between carbohydrates and other biomolecules, like DNA and proteins, is that the biosynthesis of carbohydrates is not performed by replication of a template, but rather by a complex pathway of glycosyltransferases and glycosidases in several compartments of the cell, which severely complicates the *in vivo* amplification and purification of complex oligosaccharides. Further, the complex structure and reactivity aggravates the *in vitro* synthesis and quantification, creating a bottleneck that has delayed the development of high-throughput analysis methods, like microarrays, in glycobiology. Finally, since glycans in biology are presented in a specific direction, attached at the reducing end, the manufacturing of glycan arrays requires highly selective methods of immobilization (1, 5, 6). Despite, or perhaps due to, the difficulties in glycobiology, several promising examples of glycoarrays have appeared to date (7–11).

Herein, we present a 1-/2-step method to quickly functionalize specific polymeric surfaces with alkynes using a photoligation technique based on perfluorophenylazide (PFPA). The alkyne surfaces can subsequently be linked to azide-containing biomolecules like carbohydrates using Cu-catalyzed azide–alkyne

cycloaddition (CuAAC, click chemistry) (12, 13). Both azides and alkynes are rare in biological systems and have been shown to be highly inert under biological conditions (14). CuAAC has received a lot of attention since its discovery, especially within glycobiology where several examples of carbohydrate arrays utilizing CuAAC have been published (15–21).

EXPERIMENTAL PROCEDURES

General. All commercially available starting materials were of reagent grade and used as received. Lectins were purchased from Sigma-Aldrich and Vector Laboratories. QCM crystals were purchased from Attana AB. ¹H, ¹³C, and ¹⁹F NMR data were recorded on a Bruker Avance 400 instrument at 400 MHz (¹H) or a Bruker DMX 500 instrument at 500 MHz (¹H), 125 (¹³C), or 470 MHz (¹⁹F). Chemical shifts are reported as δ values (ppm) with CDCl₃ (¹H: δ = 7.26, ¹³C = 77.16), DMSO-*d*₆ (¹H: δ = 2.50, ¹³C = 39.52), or D₂O (¹H: δ = 4.79) as internal standard. *J* values are given in Hz. ¹H peak assignments were made by first-order analysis of the spectra supported by standard ¹H–¹H correlation spectroscopy (COSY). Thin layer chromatography (TLC) was performed on precoated Cromatofolios AL Silica gel 60 F₂₅₄ plates (Merck). Flash column chromatography was performed on silica gel 60, 0.040–0.063 mm (SDS). All experiments containing penta-/tetrafluorophenylazide groups were conducted in the absence of light with all reaction flasks covered with aluminum foil to prevent decomposition.

2-(2-(2-(Prop-2-ynyloxy)ethoxy)ethoxy)ethanol (2). Triethylene glycol (22.3 mL, 167 mmol) was added to a flask containing ground potassium hydroxide (3.7 g, 61.7 mmol). The mixture was stirred at 40 °C for 30 min after which propargyl bromide (6 mL, 55.7 mmol) was added. The mixture was then stirred at 60 °C for 3 h. The mixture was then diluted with water (50 mL) and acidified to pH 1 with 1 M hydrochloric acid (~80 mL). The resulting mixture was extracted with EtOAc (3 × 100 mL) and the extract was washed with brine (2 × 100 mL). The organic phase was dried (Na₂SO₄) and the solvent evaporated under reduced pressure giving a yellow oil (6.3 g). The crude product was purified by flash column chromatography using solvent system hexanes/EtOAc (1:1 v/v) giving **2** as a colorless oil (3.76 g, 36%).

¹H NMR (500 MHz, CDCl₃): δ 4.21 (d, 2 H, *J* = 2.2 Hz, CHCC₂H₂O), 3.74–3.66 (m, 11 H, OCH₂ and OH) 3.61 (t, 2 H,

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$J = 4.6$ Hz, CH_2OH), 2.43 (t, 1 H, $J = 2.4$ Hz, CHCCCH_2). ^{13}C NMR (125 MHz, CDCl_3): δ 79.68, 74.72, 72.60, 70.76, 70.51, 70.47, 69.20, 61.89, 58.54.

2-(2-(2-(Prop-2-ynyloxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (3). 2-(2-(2-(Prop-2-ynyloxy)ethoxy)ethoxy)ethanol (**2**) (1.639 g, 8.7 mmol) was dissolved in DCM (10 mL). Tosyl chloride (1.826 g, 9.6 mmol) was added, and the mixture was cooled to 0 °C on an ice bath. KOH (1.95 g, 34.8 mmol) was added slowly, and the mixture was stirred vigorously for 2 h. The mixture was then poured on ice water and extracted with DCM (3 $\times$ 50 mL). The combined organic phase was washed with brine, dried over MgSO_4 , and evaporated under reduced pressure to give **3** as white crystals in quantitative yield (2.977 g).

^1H NMR (500 MHz, CDCl_3): δ 7.80 (d, 2 H, $J = 8.2$ Hz, ArH), 7.34 (d, 2 H, $J = 8.2$ Hz, ArH), 4.19 (d, 2 H, 2.2 Hz, CHCCCH_2O), 4.16 (t, 2 H, $J = 4.7$ Hz, CH_2OTs), 3.70–3.67 (m, 4 H, OCH_2), 3.65–3.63 (m, 2 H, OCH_2), 3.59 (s, 4 H, OCH_2), 2.45 (s, 3 H, ArCH_3), 2.43 (t, 1 H, $J = 2.3$ Hz, CHCCCH_2). ^{13}C NMR (125 MHz, CDCl_3): δ 144.93, 133.18, 129.97, 128.14, 79.77, 74.68, 70.90, 70.72, 70.59, 69.37, 69.24, 68.85, 58.55, 21.79.

3-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)prop-1-yne (4). 2-(2-(2-(Prop-2-ynyloxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (**3**) (2.515 g, 7.34 mmol) was dissolved in DMF (20 mL). Then, NaN_3 (525 mg, 8.08 mmol) and tetrabutyl ammonium iodide (TBAI) (271 mg, 0.734 mmol) was added, and the mixture was stirred vigorously at 45 °C overnight. The resulting mixture was diluted with EtOAc and washed with saturated NaHCO_3 (aq). The water phase was extracted twice with EtOAc, and the combined organic phase was washed with water and brine and dried over MgSO_4 . Evaporation under reduced pressure yielded 1.705 g of crude product, which was purified by flash column chromatography using solvent system hexanes/EtOAc (1:1, v/v) yielding **4** (1.055 g, 67%).

^1H NMR (500 MHz, CDCl_3): δ 4.21 (d, 2 H, $J = 2.4$ Hz, CHCCCH_2O), 3.72–3.67 (m, 10 H, OCH_2), 3.39 (t, 2 H, $J = 5.1$ Hz, CH_2N_3), 2.43 (t, 1 H, $J = 2.4$ Hz, CHCCCH_2). ^{13}C NMR (125 MHz, CDCl_3): δ 79.65, 74.51, 70.71, 70.69, 70.52, 70.07, 69.13, 58.42, 50.71.

2-(2-(2-(Prop-2-ynyloxy)ethoxy)ethoxy)ethanamine (5). 3-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)prop-1-yne (**4**) (1.055 g, 4.95 mmol) was dissolved in dry THF (25 mL). PPh_3 (1.427 g, 5.44 mmol) was added, and the mixture was stirred at r.t. for 2.5 h. The temperature was then raised to 30 °C, and the mixture was stirred overnight. Water (0.2 mL) was added, and the mixture was stirred at 30 °C for 25 h, after which the solvent was evaporated under reduced pressure. The crude product was dissolved in EtOAc and extracted four times with 1 M HCl (aq). The combined water phase was then basified with ground NaOH to basic pH and extracted four times with EtOAc. The combined organic phase was dried over MgSO_4 and evaporated under reduced pressure to give the crude product, which was purified by flash column chromatography using solvent system hexanes/EtOAc (1:1, v/v) yielding **5** (648 mg, 70%).

^1H NMR (500 MHz, D_2O): δ 4.26 (s, 2 H, OCH_2CCH), 3.77–3.76 (m, 2 H, OCH_2), 3.73–3.68 (m, 6 H, OCH_2), 3.58 (t, 2 H, $J = 5.4$ Hz, CH_2NH_2). ^{13}C NMR (125 MHz, D_2O): δ 78.86 (t, $J = 7.4$ Hz, OCH_2CCH), 75.72 (t, $J = 38$ Hz, OCH_2CCH), 71.99, 69.60, 69.41, 69.31, 68.67, 57.87, 39.80.

Methyl 4-Azido-2,3,5,6-tetrafluorobenzoate (7). Methyl pentafluorobenzoate (2 mL, 13.55 mmol) was dissolved in a 2:1 (v/v) mixture of acetone and water (30 mL). Sodium azide (1.15 g, 17.6 mmol) was added to the flask and the mixture was refluxed at 90 °C for 2 h. The mixture was subsequently cooled to r.t., diluted with water (60 mL), and extracted with diethyl ether (3 $\times$ 60 mL). The extract was dried (Na_2SO_4) and

the solvent evaporated under reduced pressure, yielding **7** as white crystals (3.4 g, quant.).

^1H NMR (400 MHz, CDCl_3): δ 3.97 (s, 3 H, COCH_3). ^{19}F NMR (470 MHz, CDCl_3): δ -138.6 (m, 2 F), -150.9 (m, 2 F). ^{13}C NMR (125 MHz, CDCl_3): δ 159.98, 146.59–144.35 (m, $J_{\text{C-F}} = 260$ Hz), 141.69–139.50 (m, $J_{\text{C-F}} = 250$ Hz), 123.53, 107.77, 53.42.

4-Azido-2,3,5,6-tetrafluorobenzoic Acid (8). Methyl 4-azido-2,3,5,6-tetrafluorobenzoate (**7**) (3.86 g, 15.49 mmol) was dissolved in MeOH (14.6 mL), aqueous sodium hydroxide solution (20%, w/w; 1.5 mL) and water (3.1 mL). The mixture was then stirred at r.t. for 2.5 h, acidified with 1 M aqueous hydrochloric acid and extracted with DCM (3 $\times$ 60 mL). The solvent was evaporated under reduced pressure, yielding **8** (3.4 g, 84.4%).

^{13}C NMR (125 MHz, $\text{DMSO}-d_6$): δ 160.09 (s), 145.40–143.06 (m, $J_{\text{C-F}} = 250$ Hz), 141.32–139.02 (m, $J_{\text{C-F}} = 250$ Hz), 122.70 (m), 108.41 (m).

2,5-Dioxopyrrolidin-1-yl 4-azido-2,3,5,6-tetrafluorobenzoate (9). 4-Azido-2,3,5,6-tetrafluorobenzoic acid (**8**) (933 mg, 3.97 mmol), *N*-hydroxysuccinimide (502 mg, 4.36 mmol), and EDAC (836 mg, 4.36 mmol) were dissolved in DCM (15 mL) and stirred at 35 °C for 22 h. Additional EDAC (433 mg, 2.26 mmol) was then added, and the mixture was stirred at 35 °C for another 3 h. The resulting mixture was diluted with water and extracted twice with DCM. The combined organic phase was washed with water, dried over MgSO_4 , and evaporated under reduced pressure to give **9** as white crystals (1.244 g, 94%).

^1H NMR (500 MHz, CDCl_3): δ 2.91 (s, 4 H, COCH_2CH_2). ^{13}C NMR (125 MHz, CDCl_3): δ 168.47, 155.31, 147.72–145.30 (m, $J_{\text{C-F}} = 260$ Hz), 141.82–139.52 (m, $J_{\text{C-F}} = 250$ Hz), 126.49, 102.13, 25.75.

4-Azido-2,3,5,6-tetrafluoro-*N*-(2-(2-(2-(prop-2-ynyl-oxy)ethoxy)ethoxy)ethyl)benzamide (10). 2-(2-(2-(Prop-2-ynyl-oxy)ethoxy)ethoxy)ethanamine (**5**) (402 mg, 2.15 mmol) and 2,5-dioxopyrrolidin-1-yl 4-azido-2,3,5,6-tetrafluorobenzoate (**9**) (713 mg, 2.15 mmol) were dissolved in MeCN (6 mL) and stirred at r.t. overnight. A white precipitate was formed, and the mixture was filtered through a glass filter and washed with MeCN. The filtrate was evaporated under reduced pressure and the crude product purified by flash column chromatography using solvent system hexanes/EtOAc (1:1, v/v) yielding product **10** (605 mg, 70%).

^1H NMR (500 MHz, CDCl_3): δ 6.90 (s, 1 H, NH), 4.11 (d, 2 H, $J = 2.4$ Hz, CHCCCH_2O), 3.69–3.64 (m, 12 H, OCH_2CH_2), 2.41 (t, 1 H, $J = 2.4$ Hz, CHCCCH_2). ^{13}C NMR (125 MHz, CDCl_3): δ 157.92, 145.32–143.11 (m, $J_{\text{C-F}} = 250$ Hz), 141.67–139.48 (m, $J_{\text{C-F}} = 250$ Hz), 121.79, 111.98, 79.44, 74.74, 70.64, 70.40, 70.35, 69.54, 69.16, 58.45, 40.20. Anal. $\text{C}_{16}\text{H}_{16}\text{F}_4\text{N}_4\text{O}_4$ calcd. C 47.53, H 3.99, N 13.86; found C 47.60, H 4.10, N 13.79.

1-(2-Azidoethyl)-2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (12). A solution of 2-azidoethanol (424 mg, 4.88 mmol) in DCM (20 mL) was added to α -D-mannose pentaacetate (1.586 g, 4.06 mmol). The mixture was stirred at 0 °C and $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (2.04 mL, 16.25 mmol) was added slowly. After 1 h, the mixture was allowed to reach r.t., and after 18 h added to ice water (20 mL). The resulting mixture was extracted with DCM (2 $\times$ 20 mL), and the combined organic phase was washed with ice water, saturated NaHCO_3 (aq), and ice water. The organic phase was dried over Na_2SO_4 and the solvent evaporated under reduced pressure, yielding product **12** (1.411 g, 83%).

^1H NMR (500 MHz, CDCl_3): δ 5.34 (dd, 1 H, $J = 3.5$ and 9.8 Hz, H-3), 5.28 (d, 1 H, $J = 10.1$ Hz, H-4), 5.25 (dd, 1 H, $J = 1.9$ and 3.5 Hz, H-2), 4.85 (d, 1 H, $J = 1.9$ Hz, H-1), 4.26 (dd, 1 H, $J = 5.4$ and 12.3 Hz, H-6), 4.10 (dd, 1 H, $J = 2.2$ and

12.3 Hz, H-6'), 4.02 (ddd, 1 H, $J = 2.5, 5.4$, and 9.8 Hz, H-5), 3.85 (ddd, 1 H, $J = 3.8, 6.9$, and 10.7 Hz, $\text{CH}_2\text{CH}_2\text{N}_3$), 3.65 (ddd, 1 H, $J = 3.9, 6.2$, and 10.3 Hz, $\text{CH}_2\text{CH}_2\text{N}_3$), 3.47 (ddd, 1 H, $J = 3.5, 6.9$, and 13.2 Hz, CH_2N_3), 3.42 (ddd, 1 H, $J = 3.8, 6.0$, and 13.2 Hz, CH_2N_3), 2.14 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.03 (s, 3 H, Ac), 1.97 (s, 3 H, Ac). ^{13}C NMR (125 MHz, CDCl_3): δ 170.74, 170.13, 169.93, 169.88, 97.88, 69.53, 69.00, 68.98, 67.18, 66.14, 62.59, 50.49, 21.00, 20.87, 20.83, 20.78.

Compound 13. 1-(2-Azidoethyl)-2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (**12**) (203 mg, 0.486 mmol), 2-(2-(2-(prop-2-ynyloxy)ethoxy)ethoxy)ethyl 4-methyl-benzenesulfonate (**3**) (200 mg, 0.5837 mmol), CuI (4.6 mg, 0.024 mmol), and DIPEA (8.5 μL , 0.049 mmol) were dissolved in MeCN (15 mL) and stirred at r.t. for 28 h. Additional CuI (5 mg) was subsequently added, and the mixture was stirred at r.t. for 20 h. The resulting mixture was evaporated under reduced pressure, and the crude product was purified by flash column chromatography using solvent system DCM/MeCN (1:1, v/v) yielding product **13** (279 mg, 76%).

^1H NMR (500 MHz, $\text{DMSO}-d_6$): δ 8.09 (s, 1 H, triazol-H), 7.78 (d, 2 H, $J = 8.3$ Hz, Ar-H), 7.47 (d, 2 H, $J = 8.3$ Hz, Ar-H), 5.08–5.04 (m, 2 H, H-2 and H-4), 5.01 (dd, 1 H, $J = 3.4$ and 10.2, H-3), 4.90 (s, 1 H, H-1), 4.67–4.58 (m, 2 H, $\text{OCH}_2\text{CH}_2\text{N}$ -triazol), 4.52 (s, 2 H, OCH_2C -triazol), 4.11 (t, 2 H, $J = 4.4$ Hz, OCH_2), 4.07 (dd, 1 H, $J = 5.0$ and 12.3 Hz, H-6), 4.02–3.89 (m, 3 H, H-7 and $\text{OCH}_2\text{CH}_2\text{N}$ -triazol), 3.57 (t, 2 H, $J = 4.4$ Hz, OCH_2), 3.54–3.51 (m, 3 H, H-5 and OCH_2), 3.50–3.48 (m, 2 H, OCH_2), 3.44 (s, 4 H, OCH_2), 2.41 (s, 3 H, Ar- CH_3), 2.09 (s, 3 H, Ac), 2.01 (s, 3 H, Ac), 2.00 (s, 3 H, Ac), 1.92 (s, 3 H, Ac). ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$): δ 169.99, 169.55, 169.54, 169.40, 144.89, 143.98, 132.41, 130.12, 127.61, 124.29, 96.33, 69.97, 69.68, 69.67, 69.65, 68.87, 68.57, 68.48, 67.92, 67.88, 65.71, 65.09, 63.46, 61.65, 48.94, 21.07, 20.55, 20.48, 20.39, 20.37. $[\alpha]_D^{20} + 27.0$ (c 0.673).

Compound 14. Compound **13** (279 mg, 0.368 mmol) and NaN_3 (29 mg, 0.441 mmol) were dissolved in DMF (25 mL) and stirred at 65 °C for 20 h. The solvent was then evaporated under reduced pressure yielding a crude product, which was dissolved in a small amount of DCM/MeCN (1:1, v/v) and passed through a short silica column yielding product **14** (221 mg, 95%).

^1H NMR (500 MHz, $\text{DMSO}-d_6$): δ 8.09 (s, 1 H, triazol-H), 5.08–5.04 (m, 2 H, H-2 and H-4), 5.00 (dd, 1 H, $J = 3.3$ and 10.2 Hz, H-3), 4.90 (d, 1 H, $J = 1.3$ Hz, H-1), 4.67–4.58 (m, 2 H, $\text{OCH}_2\text{CH}_2\text{N}$ -triazol), 4.53 (s, 2 H, OCH_2C -triazol), 4.06 (dd, 1 H, $J = 5.0$ and 12.3 Hz, H-6), 4.01–3.89 (m, 3 H, H-7 and $\text{OCH}_2\text{CH}_2\text{N}$ -triazol), 3.59 (t, 2 H, $J = 4.9$ Hz, OCH_2), 3.56–3.51 (m, 9 H, H-5 and OCH_2), 3.38 (t, 2 H, $J = 4.9$ Hz, OCH_2), 2.09 (s, 3 H, Ac), 2.02 (s, 3 H, Ac), 2.00 (s, 3 H, Ac), 1.92 (s, 3 H, Ac). ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$): δ 170.00, 169.56, 169.53, 169.41, 144.00, 124.28, 96.33, 69.80, 69.73, 69.67, 69.23, 68.91, 68.57, 68.48, 67.91, 65.72, 65.09, 63.47, 61.64, 49.98, 48.94, 20.55, 20.49, 20.39, 20.38. MS (ESI $^+$): $[\text{M}+\text{Na}]^+$ m/z calcd for $\text{C}_{25}\text{H}_{38}\text{N}_6\text{O}_{13}$, 653.24; found 653.27. $[\alpha]_D^{20} + 30.6$ (c 0.727).

Compound 15. Compound **14** (399 mg, 0.632 mmol) was dissolved in MeOH (15 mL) under nitrogen atmosphere. NaOMe (17 mg, 0.316 mmol) in MeOH (1 mL) was added, and the mixture was stirred at r.t. for 2 h. The resulting mixture was neutralized with Amberlyst 15 and filtered. Pd/C (10%, 67 mg, 0.0632 mmol) was added, and the reaction vessel was subjected to H_2 -atmosphere (1 atm) for 5 h at r.t. The mixture was subsequently filtered through Celite, and the filtrate evaporated under reduced pressure to yield compound **15** (204 mg, 74%).

^1H NMR (500 MHz, D_2O): δ 8.13 (s, 1 H, triazol-H), 4.82 (d, 1 H, $J = 1.4$ Hz, H-1), 4.73–4.70 (m, 4 H, CH_2N -triazol + CH_2C -triazol), 4.14 (ddd, 1 H, $J = 11.0, 7.0, 4.1$ Hz, $\text{CH}_2\text{CH}_2\text{N}$ -

triazol), 3.96 (dt, 1 H, $J = 9.1, 4.3$ Hz, $\text{CH}_2\text{CH}_2\text{NNC}$), 3.88 (dd, 1 H, $J = 3.2, 1.7$ Hz, H-2), 3.77–3.59 (m, 15 H), 3.07 (ddd, 1 H, $J = 9.4, 5.8, 2.2$ Hz), 2.94 (t, 1 H, $J = 5.3$ Hz, CH_2NH_2). ^{13}C NMR (125 MHz, D_2O) δ 143.91, 125.59, 99.56, 72.79, 70.39, 70.22, 69.85, 69.58, 69.48, 69.35, 68.89, 66.35, 65.48, 62.97, 60.64, 50.08, 39.57. MS (ESI $^+$): $[\text{M}+\text{H}]^+$ m/z calcd for $\text{C}_{17}\text{H}_{32}\text{N}_4\text{O}_9$, 437.23; found 437.27.

Compound 16. 2,5-Dioxypyrrolidin-1-yl 4-azido-2,3,5,6-tetra-fluorobenzoate (**9**) (178 mg, 0.537 mmol) and compound **15** (195 mg, 0.448 mmol) were dissolved in 10 mL of DMF and stirred at r.t. for 22 h. The solvent was then evaporated under reduced pressure to give 289 mg of crude product which was purified by flash column chromatography with solvent system DCM/MeOH (6:1, v/v) yielding product **16** (201 mg, 69%).

^1H NMR (500 MHz, D_2O): δ 8.10 (s, 1 H, triazol-H), 4.80 (d, 1 H, $J = 1.6$ Hz, H-1), 4.71–4.67 (m, 4 H, $\text{CH}_2\text{NNC} + \text{CH}_2\text{CNC}$), 4.12 (ddd, 1 H, $J = 11.0, 7.0$, and 4.0 Hz, $\text{CH}_2\text{CH}_2\text{NNC}$), 3.97–3.91 (m, 1H, $\text{CH}_2\text{CH}_2\text{NNC}$), 3.87 (dd, 1 H, $J = 3.2$ and 1.7 Hz, H-2), 3.76–3.57 (m, 17 H), 3.07 (ddd, 1 H, $J = 9.5, 5.7$, and 2.2 Hz). ^{13}C NMR (125 MHz, D_2O): δ 160.66, 144.60–142.39 (dm, $J = 250$ Hz), 143.97, 141.44–139.30 (dm, $J = 252$ Hz), 125.46, 122.51–122.26 (m), 110.12 (t, $J = 19.1$ Hz), 99.57, 72.79, 70.40, 69.86, 69.65, 69.55, 69.49, 68.96, 68.46, 66.36, 65.47, 63.01, 60.64, 50.06, 39.72. MS (ESI $^+$): $[\text{M}+\text{Na}]^+$ m/z calcd for $\text{C}_{24}\text{H}_{31}\text{F}_4\text{N}_7\text{O}_{10}$, 653.21; found 653.27. Anal. $\text{C}_{24}\text{H}_{31}\text{F}_4\text{N}_7\text{O}_{10}$ calcd. C 44.11, H 4.78, N 15.00; found C 44.07, H 4.77, N 14.92. $[\alpha]_D^{20} + 21.4$ (c 0.58).

1-(2-Azidoethyl)- α -D-mannopyranoside (17). 1-(2-Azidoethyl)-2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (**12**) (66 mg, 0.158 mmol) was dissolved in 30 mL of MeOH. Then sodium methoxide (25.5 mg, 0.474 mmol) was added and the reaction mixture was stirred at r.t. until TLC indicated full conversion. Then, Amberlyst 15 was added until pH reached ~ 7 . The Amberlyst was then filtered off and the solvent was evaporated under reduced pressure, giving the pure product **17** (37 mg, 95%).

^1H NMR (500 MHz, D_2O): δ 4.93 (d, 1 H, $J = 1.6$ Hz, H-1), 3.99 (dd, 1 H, $J = 1.6$ and 3.5 Hz, H-2), 3.93 (ddd, 1 H, $J = 3.4, 7.0$, and 10.8 Hz, $\text{CH}_2\text{CH}_2\text{N}_3$), 3.91 (dd, 1 H, $J = 1.3$ and 12.3 Hz, H-6), 3.85 (dd, 1 H, $J = 3.5$ and 9.1 Hz, H-3), 3.77 (dd, 1 H, $J = 5.7$ and 12.0 Hz, H-6'), 3.73 (ddd, 1 H, $J = 3.2, 6.3$, and 11.0 Hz, $\text{CH}_2\text{CH}_2\text{N}_3$), 3.70–3.65 (m, 2 H, H-4 and H-5), 3.56 (ddd, 1 H, $J = 3.1, 6.9$, and 13.6, CH_2N_3), 3.50 (ddd, 1 H, $J = 3.2, 6.4$ and 13.6, CH_2N_3). ^{13}C NMR (125 MHz, D_2O): δ 99.80, 72.88, 70.37, 69.92, 66.67, 66.29, 60.90, 50.19.

General Surface Modification (cf. Figure 1). All QCM experiments were performed on gold-plated 10 MHz quartz crystals (Attana), either coated with poly(ethylene glycol) by a previously reported method (22) or purchased precoated with polystyrene. The photoreaction step was performed at 240–400 nm at a measured intensity of 13.3–13.5 mW/cm 2 with a LC8 equipped Hg–Xe UV-lamp from Hamamatsu Photonics. The fabricated crystals were mounted in a flowthrough QCM system (Attana 100).

Method A - 1-Step Functionalization. The precoated QCM crystal was immersed in a methanol solution of **16** (2.0 mM) for 5 min, after which it was dried under a stream of N_2 for 5 min, and irradiated for 5 min with UV light. The crystal was then rinsed with methanol in a supersonic bath for 5 min, dried under N_2 , and placed in the QCM flowthrough system.

Method B - 2-Step Functionalization. The precoated QCM crystal was immersed in a methanol solution of **10** (2.0 mM) for 5 min, after which it was dried under a stream of N_2 for 5 min, and irradiated for 5 min with UV light. The crystal was then rinsed with methanol in a supersonic bath for 5 min and dried under N_2 . The crystal was then immersed in a solution of

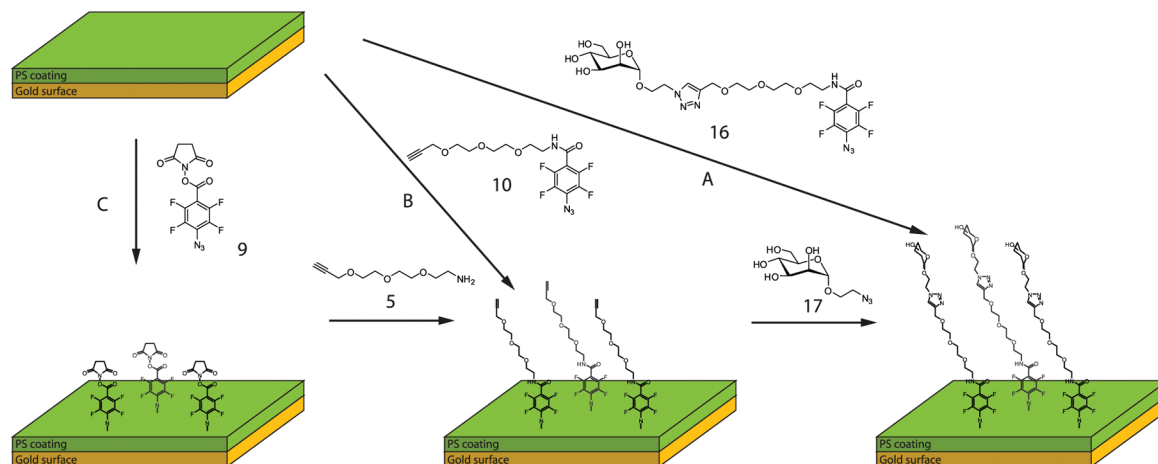


Figure 1. Developed methods for carbohydrate functionalization on polystyrene (PS) surfaces. Method A consists of one step in which a carbohydrate-functionalized PFP ester is covalently bound to the polystyrene surface using UV-irradiation. In method B, the surface is primarily functionalized with alkynes and subsequently connected to carbohydrate-azides via Click chemistry. Method C consists of three steps in which a NHS-activated PFP ester is immobilized using UV irradiation. Then, compound **5** is attached to the surface, by the formation of a stable amide bond, creating an alkyne-functionalized surface (equivalent to method B), which is subsequently bound to carbohydrate-azides using Click chemistry.

water:acetonitrile (1:1, v/v) containing **17** (19.2 mM), CuI (0.96 mM), and DIPEA (9.7 mM) for 10 h. The crystal was then rinsed, first with acetonitrile/methanol, and then with acetonitrile/water (1:1, v/v) in a supersonic bath for 5 min. The crystal was then dried under N₂ and placed in the QCM flowthrough system.

Method C - 3-Step Functionalization. The precoated QCM crystal was immersed in an ethanol solution of **9** (5.0 mM) for 5 min after which it was dried under a stream of N₂ for 5 min and irradiated for 5 min with UV light. The crystal was then immersed in an acetonitrile solution of **5** (50 mM) for 8 h after which it was rinsed with methanol in a supersonic bath for 5 min. The crystal was then dried under N₂ for 5 min and subsequently immersed in a solution of water/acetonitrile (1:1, v/v) containing **17** (19.2 mM), CuI (0.96 mM), and DIPEA (9.7 mM) for 10 h. The crystal was then rinsed, first with acetonitrile/methanol, and then with acetonitrile/water (1:1, v/v) in a supersonic bath for 5 min. The crystal was then dried under N₂ and placed in the QCM flowthrough system.

General Surface Analysis. A continuous flow of running buffer (PBS 10 mM, pH 7.4, 25–50 μ L/min) was used throughout the experiments, and samples of Concanavalin A (Con A) and peanut agglutinin (PNA) were prepared in the same buffer (sample injection volume: 50 μ L). The crystals were washed/equilibrated with buffer solution prior to manipulations/measurements. After the crystal was equilibrated in the flowthrough system, it was subjected to several injections of BSA (1 mg/mL), low pH buffer (pH 1.5), and BSA (1 mg/mL) to fully block any nonfunctionalized surface. Binding to the surface was monitored by frequency logging with Attester 1.1 (Attana) and adsorption/desorption to the surface recorded as the resulting frequency shifts. Solutions (0.2 mg/mL) of Con A (1.92 μ M) or PNA (1.82 μ M) were then injected on the system, where the resulting shift in frequency corresponds to the binding to the surface. Bound lectin was released from the surface between measurements by several successive injections of low pH buffer (PBS 10 mM, pH 1.5). The procedure was then repeated several times for each lectin to determine the surface stability over time.

RESULTS AND DISCUSSION

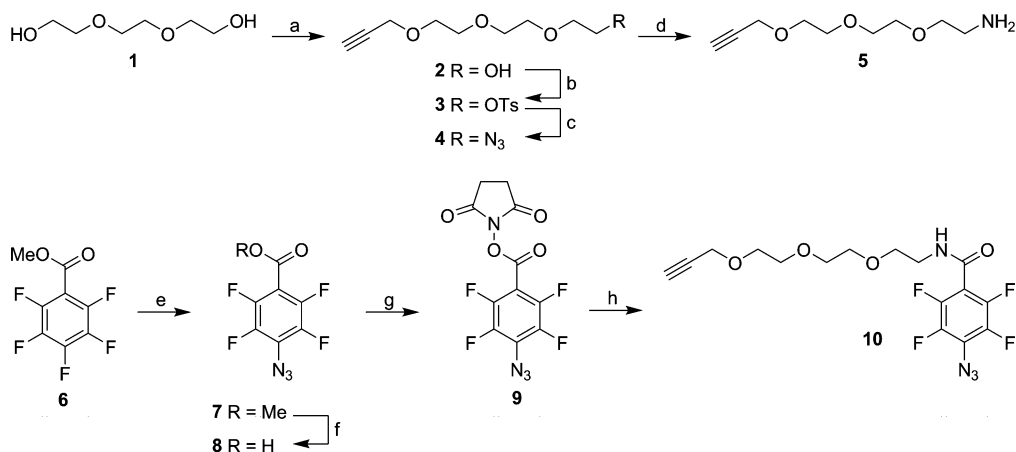
Perfluorophenylazides (PFPAs) have been shown to be efficient reagents in photolabeling using UV light. Unlike other aryl azides, which quickly decompose upon radiation, PFPFA forms a less reactive nitrene intermediate, which inserts into

C–H bonds with moderate to high efficiency (23–25). We have previously reported the use of PFPFA in the immobilization of carbohydrates to poly(ethylene glycol) surfaces for quartz crystal microbalance (QCM) and microarray studies (22, 26). In the present approach, the versatility of the system is further improved by introducing the Cu-catalyzed azide–alkyne cycloaddition (click chemistry) step. This enables the use of more readily available carbohydrate-azides in the manufacturing of the surfaces.

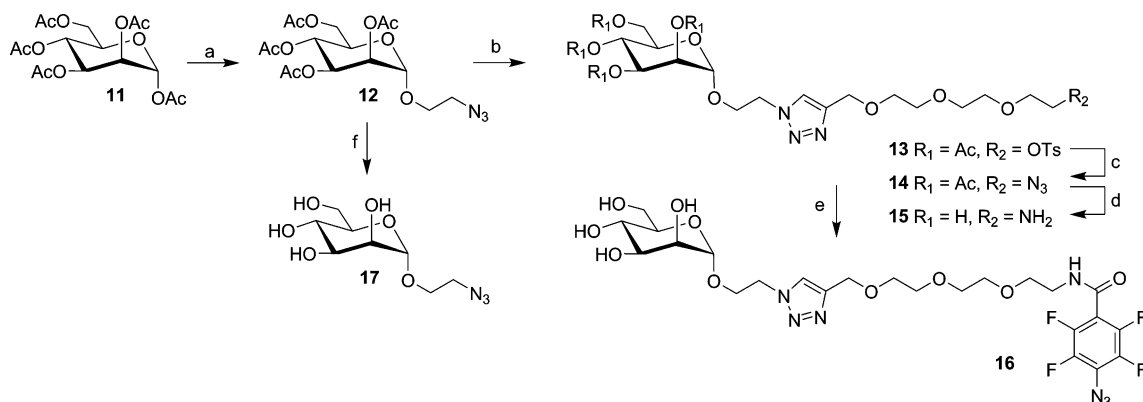
To demonstrate the concept of the method, three experimental procedures of immobilizing the carbohydrates were designed, all of which resulted in very similar final surfaces (Figure 1). This experimental design also enabled the evaluation and analysis of the efficiency of the different steps, i.e., the photoligation and the CuAAC reaction. The simplest method (A) consists of a single photoligation step in which a carbohydrate-derivatized photoprobe (α -D-mannopyranose derivative **16** in the example) is immobilized on the surface by UV irradiation. In method B, an alkyne-functionalized PFPFA (**10**) is primarily coupled to the surface by UV irradiation. The alkyne surface is then coupled to a 2-azidoethyl functionalized carbohydrate species (**17**) using CuAAC. In method C, the alkyne surface is produced in two steps. First, the polymeric surface is functionalized with an NHS-activated PFPFA-ester (**9**) (24, 25), and the resulting surface is subsequently submerged in an acetonitrile solution of an alkyne-functionalized amine linker (**5**), forming a stable amide bond with the surface. The alkyne surface is then subjected to the same procedure as the last step in method B.

All three methods result in carbohydrate-functionalized surfaces of the same type. Methods B and C, however, generate stable alkyne-functionalized intermediate surfaces, which in a one-step highly chemoselective reaction can be linked to azide-containing molecules, in this case, carbohydrates. The photoligation step, in the presence of surface C–H bonds, results in stable amine bonds, which makes hydrocarbon-based polymers a suitable substrate. This expands the end use of this method to a large extent, considering the extensive use of polymeric analysis equipment in life sciences. In this study, we have evaluated the methods on poly(ethylene glycol) (PEG) and polystyrene (PS) surfaces with successful results in both cases.

All compounds used were synthesized according to Schemes 1 and 2. The NHS-activation of compound **8** was essential for the synthesis of compounds **10** and **16**. The reaction between compound **10** and compounds **12** and **17**, to form structure **16**,

Scheme 1^a

^a (a) KOH, propargyl bromide, 60 °C, 3 h (36%); (b) TsCl, KOH, 0 °C, 2 h (quant.); (c) NaN₃, TBAI, DMF, 45 °C, overnight (67%); (d) (i) PPh₃, THF, 30 °C, 19 h, (ii) H₂O, 30 °C, 25 h (70%); (e) NaN₃, Acetone/H₂O (2:1), 90 °C, 2 h (quant.); (f) NaOH aq (20% w/w):MeOH (1:18), rt, 5 h (97%); (g) NHS, EDAC, 35 °C, 25 h (94%); (h) 5, MeCN, rt, overnight (70%).

Scheme 2^a

^a (a) 2-azidoethanol, BF₃·(Et₂O)₂, DCM, 0 °C, 18 h (83%); (b) 3, CuI, DIPEA, MeCN, rt, 48 h (76%); (c) NaN₃, DMF, 65 °C, 20 h (95%); (d) (i) NaOMe, MeOH, rt, 5 h, (ii) 10 mol % Pd(C) under H₂, 6 h (74%); (e) 9, MeCN, rt, overnight (69%); (f) NaOMe, MeOH, rt, 2 h (quant.).

resulted in complex mixtures due to the higher reactivity of the aryl azide group compared to the carbohydrate azide moiety. This reactivity difference necessitated the chosen route to compound **16**. Compound **10** was also found to undergo self-cycloaddition in concentrated form, but proved stable in dilute solution at low temperature.

During initial studies, different catalytic systems for the cycloaddition reaction were examined, including CuSO₄·5H₂O + sodium ascorbate in water and water/*t*-butanol (1:1, v/v), Cu(OAc)₂ in water, and CuI + DIPEA in water/acetonitrile (1:1, v/v). The CuI system was, however, found to be the most appropriate for the surfaces used. Experiments were also performed for method B without the copper catalyst, in which the concentration of compound **17** was increased from 19.2 mM to 300 mM. Preliminary results indicated similar efficiencies of the thermal cycloaddition reaction but required longer reaction times (>19 h) and heating to 40 °C. This could, however, be a useful approach if copper needs to be avoided during immobilization.

The biorecognition properties of the surfaces were subsequently analyzed by injections of the lectins Con A (primarily specific for α-D-mannopyranose units) and PNA (primarily specific for β-D-galactopyranose units) in the flowthrough QCM system. The QCM technique allows for real-time monitoring of the association and dissociation of the lectins and provides an easy way to assess the stability of the immobilization over several injections.

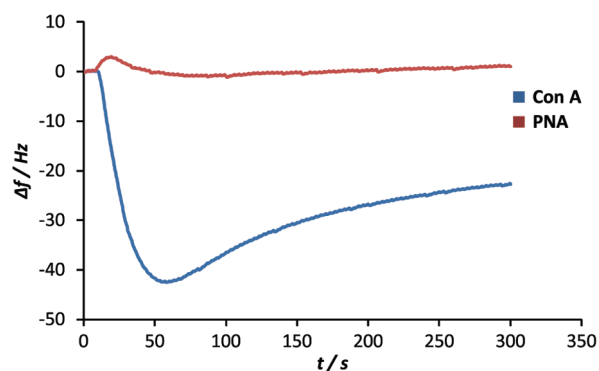


Figure 2. General binding curve of Con A and PNA to mannose-functionalized surface.

The binding study was performed by measuring the decrease in frequency upon lectin injection. Typical binding curves for the two lectins are presented in Figure 2. As can be seen for the α-D-mannopyranose-specific lectin Con A, the frequency drops immediately upon injection as a consequence of the association of the lectin to the surface. As the injection plug comes to an end, dissociation takes place where the lectin is washed away from the surface. In comparison, PNA shows no binding in agreement with the specificities for this protein.

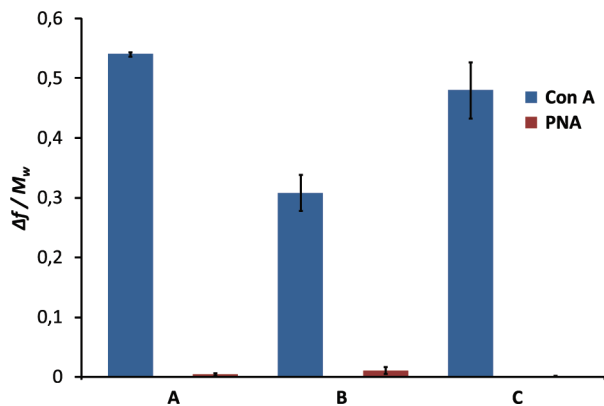


Figure 3. Binding results for the three different methods (normalized for the molecular weight of the lectins).

The resulting binding analyses for all three methods are presented in Figure 3, where the maximum decrease in frequency corresponds to the binding of the lectins at the conditions used. As can be seen, method A produces the highest and most consistent binding of Con A, with negligible nonspecific binding of PNA to the surfaces. This observation is reasonable, since method A is the simplest approach with only one photoligation step and is also most likely to produce the most carbohydrate-dense surface. On the other hand, both methods B and C show high specific binding of Con A with good consistency, although the capacity differs slightly between the methods. These results confirm the generality of the different methods to produce surfaces of similar quality. Method C requires roughly 8 h longer preparation time than method B, but in return produces surfaces which stabilize faster in the QCM system than surfaces produced by method B (cf. Figure 4). This indicates that surfaces produced by method C have lower thickness and are more uniform, which is most likely due to the increased potential cross-linking of compound **10** compared to compound **9** in the photoligation step. This also explains the slightly lower capacity of method B, where more alkyne moieties are likely buried, resulting in lower surface density of alkynes in method B than in method C. Method B further deviates from method A and C in the regeneration step, where it results in considerably larger responses to the change in pH, similar to the 3D pH-responsive dynamic polymer surfaces previously reported (27). The response times for all methods are, however, generally good, although surfaces produced by method A or C respond much faster than surfaces produced by method B, a desirable feature in real-time monitoring instrumentations. This supports the theory that methods A and C produce more ordered surfaces than method B.

CONCLUSIONS

Fast and straightforward methods to regio- and chemoselective functionalization of polymeric surfaces with carbohydrate structures have been developed and evaluated. The methods make use of the highly efficient PFPA-photoligation technique, combined with the CuAAC chemistry. The surfaces were evaluated using a QCM instrumentation, allowing for real-time analysis of the association/dissociation effects of unlabeled proteins. High specificities and capacities were recorded for all surfaces. All methods can be used for a variety of polymeric substrates, and methods B and C can furthermore be easily expanded with more azide-functionalized carbohydrates, as well as other structures. These

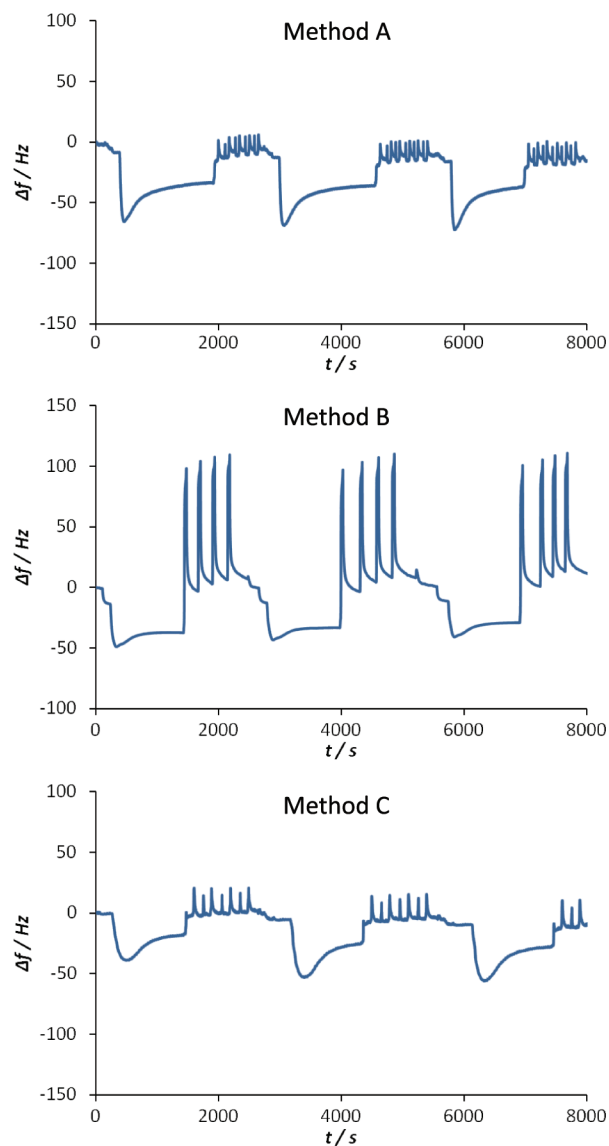


Figure 4. General frequency response curves for the three different methods. Con A was injected at 400 s, 3000 s, and 5900 s. The positive frequency peaks between lectin injections correspond to regeneration by release buffer solution. The average Con A binding values are presented in Figure 3.

features make the developed technology highly versatile, and show the potential for other applications.

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