Preparation of Carbohydrate Derivatives with PCDA Tails: Applicationfor Cell Surface Recognition

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Modifications of carbohydrates are found in nature as constituents of many structures by biological activity and recognition. Sugar rings have been systematically varied to probe binding sites of carbohydrate-specific proteins, such as enzymes or lectins, which are important for its recognition.[1]

The functionalization at the anomeric center of a carbohydrates can serve as sensor in biological sytem.

Polyacetylene based sensor systems are unique because of blue to red color transitions due to their polymerized diacetylene unit.[2-11] It is well known that the spacially aligned monomeric diacetylene moieties undergo photo polymerization process via a 1,4-addition mechanism and form conjugated chains that give the molecule a significant color change.[12] Due to this unique color change, efforts have been devoted to develop efficient sensor systems based on this polyacetylenes.[12-14] Our plan is devising PCDA (10,12-pentacoasdiynoic acid) biosensor, which is tagging carbohydrates with PCDA dye (Figure 1).

Commercially available β-D-glucose pentaacetate (**1**) was transformed into corresponding β-azide **2** by the known method.[15] β-D-glucose pentaacetate azide **2** is reduced with *p*-toluen sulfonic acid (1.2 equiv.) in CH_3OH/CH_2Cl_2 as cosolvent system to get carbohydrate *p*-TSA ammonium salt **3** in 90% yield. The crude 10,12-pentacosadiynoyl chloride which was prepared from the PCDA and oxalyl chloride was react with carbohydrate *p*-TSA ammonium salt **3** in situ to get glucose derivative **4** with PCDA tailed in 60% yield (Scheme 1).[16]

Similarly, galactose-PCDA amide **8** was prepared from commercially available β-D-galactose pentaacetate (**5**). β-D-glucose pentaacetate azide **6** is reduced with *p*-toluen sulfonic acid (1.2 equiv.) in CH_3OH/CH_2Cl_2 as co-solvent system to get carbohydrate *p*-TSA ammonium salt **7** in 85% yield. Treatment of *p*-TSA ammonium salt **7** with the crude 12-pentacosadiynoyl chloride which is prepared from PCDA and oxalyl chloride give the PCDA connecting galactose derivative **8** in 55% yield (Scheme 2).[16]

In summary, we have prepared precursors of dye labeled carbohydrate ligands which will be tested as a new biosensor. Further studies of biosensing living cell systems such as Concanavalin A[17] or tumor cells are now on going and will be discussed soon.

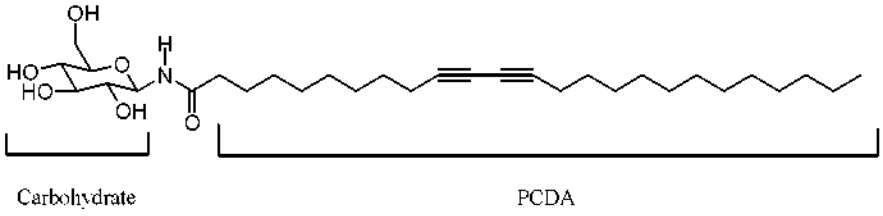
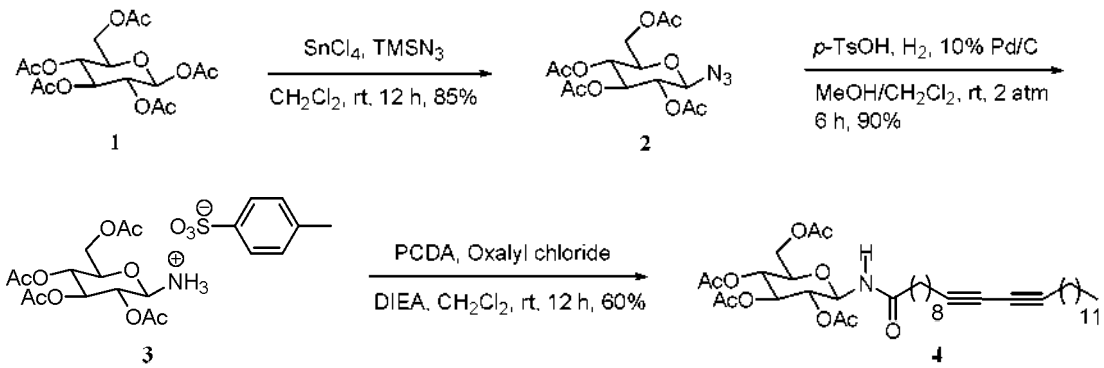
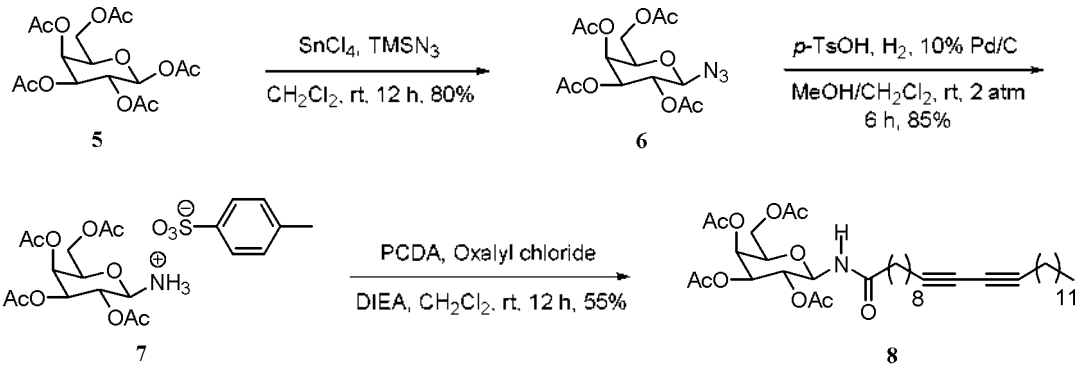


Figure 1



Scheme 1



Scheme 2

Experimental Section

General procedure for preparation of β-D-glucose pentaacetate-PCDA amide 4 and β-D-galactose pentaacetate-PCDA amide 8. To the solution of PCDA(10,12-pentacosadiynoic acid) (10.0 mmol) in 70 mL of dried CH_2Cl_2, oxalyl chloride (25.2 mmol) was slowly added at room temperature in nitrogen atmosphere. The resulting solution was stirred at room temperature for 1 h. To the solution was added catalytic amount of DMF and stirred for additional 1 h. After concentrating *in vacuo*, the residue was redissolved in 60 mL of CH_2Cl_2. The resulting solution was added dropwise to the each solutions containing ammonium p-TSA salts **3** and **4** (12.0 mmole) and *N,N*-diisopropylethylamine (83.9 mmole) in 100 mL of anhydrous THF. Each resulting mixture was stirred for 12 h at room temperature. After checking TLC, each reaction mixture was poured into 500 mL separatory funnel with washing 200 mL CH_2Cl_2. The organic phase was successively washed by 5% HCl, saturated $NaHCO_3$, brine and the combined organic fractions were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. *Silica gel* flash column chromatography (Hexane/Ethyl Acetate 1/1) gave glucose-PCDA amide **4** and galactose-PCDA amide **8** as well.

β-D-Glucose pentaacetate-PCDA amide 4: purple solid (yield 60%); mp 49-52 °C (dec.) FT-IR (ZnS window); 2294.1, 2853.5, 1747.0, 1675.0, 1531.7, 1223.2, 1040.7 cm^{-1}. $[\alpha]_D^{15}$ = +18.40 (*c* 0.01, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$) δ 6.35 (d, *J* = 9.4 Hz, 1H), 5.30 (m, 2H), 5.07 (dd, *J* = 10.3, 9.8 Hz, 1H), 4.93 (t, *J* = 9.6 Hz, 1H), 4.33 (dd, *J* = 12.7, 4.2 Hz, 1H), 4.07 (dd, *J* = 12.7, 1.7 Hz, 1H), 3.85 (m, 1H), 2.24 (t, *J* = 7.0 Hz, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H) 1.4-1.1 (m, 36H), 0.87 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.37, 170.92, 170.58, 169.81, 169.53, 77.95, 77.52, 77.26, 735.03, 61.49, 36.49, 31.82, 29.55, 29.53, 29.51, 29.39, 29.26, 29.02, 29.00, 28.94, 28.80, 28.75, 28.65, 28.22, 28.16, 25.00, 22.60, 20.68, 20.60, 20.52, 19.08, 19.05, 14.07.

β-D-Galactose pentaacetate-PCDA amide 8: colorless oil (yield 55%); IR (ZnS window); 2925.6, 2835.8, 1750.6, 1700.4, 1533.9, 1277.8 cm^{-1}. $[\alpha]_D^{18}$ = +21.15 (*c* 0.0193, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$) δ 6.37 (d, *J* = 9.5 Hz, 1H), 5.45 (d, *J* = 2.4 Hz, 1H), 5.28 (t, *J* = 9.2 Hz, 1H), 5.13 (m, 2H), 4.08 (m, 3H), 2.22 (t, *J* = 7.0 Hz, 3H), 2.16 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H), 1.7-1.2 (m, 36H), 0.89 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.03, 170.92, 170.09, 169.73, 169.48, 77.98, 77.25, 76.51, 71.88, 70.46, 67.90, 66.79, 64.81, 60.77, 36.26, 31.58, 29.31, 29.29, 29.27, 29.15, 29.02, 28.80, 28.77, 28.71, 28.57, 28.51, 28.41, 27.99, 27.82, 24.80, 22.37, 20.46, 20.39, 20.31, 20.25, 18.84, 18.81, 13.83.

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17. Concanavalin A is the first commercialized lectin protein. It reacts with specific terminal sugar residues and has been used as a useful tool in studying carbohydrates of cell surfaces.