Bioconjugate Chemistry

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Aniline-Catalyzed Reductive Amination as a Powerful Method for the Preparation of Reducing End-"Clickable" Chitooligosaccharides

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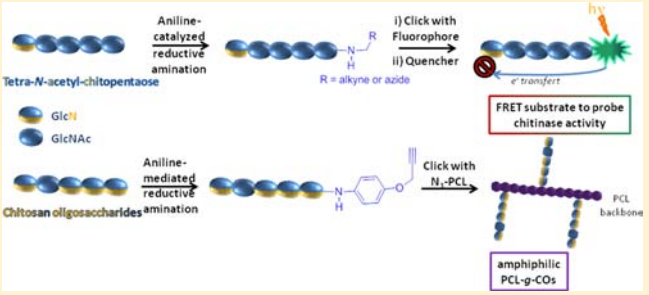
Supporting Information

ABSTRACT: Functionalized oligosaccharides are useful intermediates to prepare products for biological research or for the development of advanced functional materials. Here, we report the unprecedented use of aniline as an efficient organocatalyst reaction with "clickable" (azide or alkyne) amine for the transimination-mediated reductive amination of a chitooligosaccharide. Moreover, we demonstrate that alkyne-bearing aniline constitutes an excellent tool for the easy derivatization of chitosan oligosaccharides. Evidence for such improvement has been illustrated by the straightforward design of a FRET substrate to probe chitinase activity and of amphiphilic polycaprolactone-*grafted*-chitosan. This efficient methodology paves the way to the preparation of novel chitosan oligosaccharide-based advanced materials.



INTRODUCTION

Chitin is a natural linear polysaccharide essentially constituted of β(1,4)-linked *N*-acetylglucosaminyl (GlcNAc) units. Chitin, which is the second most abundant natural polysaccharide on earth, can be found in insect or crustacean exoskeleton as well as in cell walls of bacteria and fungi. Chitosan is obtained by partial *N*-deacetylation of chitin. This copolymer composed of randomly distributed *N*-acetyl-D-glucosamine and D-glucosamine motifs exhibits a degree of acetylation lower than 50% and can be dissolved in its protonated state in acidic aqueous solutions. Both polysaccharides are nontoxic, biorenewable, and biodegradable and display highly desirable biological properties, namely, anti-inflamatory, mucoadhesive, or antimicrobial activities, with regard to biomedical and pharmaceutical applications.[1]

Significant attention has naturally been devoted to the development of advanced functional chitin/chitosan-based hybrid materials. A convenient route to chemically modified chitosan relies on the presence of ubiquitous hydroxyl and amino reactive groups along the polysaccharide backbones.[2,3] However, as numerous biological properties of chitosan actually stem from the amino groups, this simple strategy is often detrimental for biomedical applications.

An approach to confer new chemical or physical properties to polysaccharides while retaining their inherent structures and functionalities consists of exploiting the presence of a "masked" aldehyde at the reducing end residue to anchor amino-functional derivatives through imine chemistry.[4] The synthesis of imines relies on the nucleophilic attack of a free amine (primary amine, oxime, hydrazone) on a protonated carbonyl group (ketone or aldehyde) generated under acidic conditions and a subsequent dehydration. As the pK_a of protonated carbonyl groups ranges from −4 to −10, the concentration of such species under typical conditions of imine formation (pH 5) is extremely low, limiting the rate of imine-based reactions.

In recent contributions, Dawson and co-workers showed that aniline acts as a very efficient nucleophilic catalyst in hydrazone or oxime formation under acidic aqueous conditions.[5−7] The rate enhancement of the reaction was attributed to the formation of significant concentrations of protonated aniline Schiff base owing to the pK_a of aniline (4.62) and rapid subsequent transimination with nucleophilic oxyamine or hydrazine derivatives.

Surprisingly, to our knowledge, the application of aniline as catalyst has not been extended so far to transimination reactions with primary amines despite its obvious importance in the conjugation of biomolecules[8,9] or surface immobilization.[10]

In this context, we report herein a study on aniline catalysis of primary amine ligation through reductive amination. In the framework of our investigations on the preparation of hardly accessible chitin and chitosan oligosaccharide (CO) conjugates, we are focusing on the incorporation of functional groups through reductive amination, i.e., propargylamine, allowing subsequent derivatization using useful copper(I)-catalyzed

Received: July 6, 2012
Revised: February 28, 2013
Published: March 4, 2013

   dx.doi.org/10.1021/bc3003716 | *Bioconjugate Chem.* 2013, 24, 544−549

azide−alkyne cycloaddition (CuAAC), so-called "click" reaction.[11−13]

EXPERIMENTAL PROCEDURES

Materials. Tetra-*N*,N^{II},N^{III},N^{IV}-acetyl-chitopentaose **1** was obtained from metabolically engineered *Escherichia coli* as described.[14] FACOS **4** was kindly provided by Kitto Life Co., Ltd. and characterized by ^{1}H NMR and MALDI-TOF MS. FACOS is a low molecular weight chitosan inferior to 2 kDa and with an acetylation degree of 10%. Poly(α-azido-ε-caprolactone-*co*-ε-caprolactone) **9** (poly(α-N_3-ε-CL-*co*-ε-CL)) was prepared by nucleophilic substitution with sodium azide on poly(α-chloro-ε-caprolactone-*co*-ε-caprolactone) according to methods reported in the literature.[15,16] The catalyst CuI·P(OEt)$_3$ was synthesized as reported in the literature.[17] All other chemicals were obtained from commercial suppliers and used without further purification. Deionized water was used in all experiments. Reactions were magnetically stirred and monitored by thin-layer chromatography (TLC) on silica gel. TLC was carried out using Merck Kieselgel 60 F254 (230−400 mesh) fluorescent treated silica and were visualized under UV light (254 nm) and by staining with a solution of ethanol−sulfuric acid (9:1, v/v) or aqueous potassium permanganate solutions followed by heating.

Instruments. Flash chromatography was performed on Reveleris Flash System with C18 Flash Cartridges or on silica gel column chromatography with Merck Silica gel 40−63 μm. NMR spectra were recorded on dilute solutions with D_2O, CD_3OD, or $CDCl_3$ as solvent on a Bruker DPX400 spectrometer with 5 mm QNP probe. The solvent residual peaks of D_2O, CD_3OD, and $CDCl_3$ were used as internal standards, at 4.79 ppm (298 K) or 4.20 ppm (353 K), 3.31 ppm (298K), and 7.26 ppm (298 K), respectively. General experiences for NMR characterization are ^{1}H NMR, ^{13}C NMR, COSY-NMR, HMQC-NMR, and DOSY-NMR. Chemical shifts are given in δ values and coupling constants *J* are given in Hz. The splitting patterns are reported as s (singlet), d (doublet), t (triplet), m (multiplet), and dd (double of doublets). MALDI-TOF measurements were performed using a Bruker Daltonics Autoflex apparatus. Fluorescence spectra were recorded on a LS-50B Perkin-Elmer spectrofluorimeter, equipped with a thermostated cell holder. All measurements were made at 40 °C. Samples were irradiated at 340 nm and emission spectra were recorded at 490 nm. Size exclusion chromatography (SEC) was performed at 40 °C using an Agilent 390-MDS system (290-LC pump injector, ProStar 510 column oven, 390-MDS refractive index detector) equipped with Knauer Smartline UV detector 2500 and two Agilent PolyPore PL1113−6500 columns (linear, 7.5 mm × 300 mm; particle size, 5 μm; exclusion limit, 200−2 000 000) in DMF containing lithium chloride (0.01 M) at a flow rate of 1.0 mL min^{-1}. Infrared (FT-IR) spectra were recorded using a Perkin-Elmer Spectrum RXI FT-IR Spectrometer.

Synthesis of Chitooligosaccharide-Based Derivatives. *(Tri-N,N^{II},N^{III}-acetyl-chitotetraose)-(1→4)-N-acetyl-1-deoxy-1-N-propyne-D-glucosaminitol (**2**).* Tetra-*N*,N^{II},N^{III},N^{IV}-acetyl-chitopentaose **1** (500 mg, 0.5 mmol), propargylamine (161 μL, 2.5 mmol) and aniline (40 μL, 0.25 mmol) were dissolved in aqueous ammonium acetate buffer (17 mL, 50 mM, pH 5.0). The mixture was stirred for 5 min at room temperature, and sodium cyanoborohydride (160 mg, 2.5 mmol) was added. The solution was stirred for 4 days at 40 °C. Subsequently, the mixture was evaporated under reduced pressure and coevaporated five times with 10 mL of water. The crude product was purified by reversed phase flash column chromatography (water to 8:2 acetonitrile−water). The solid product **2** was obtained after lyophilization (70%). ^{1}H NMR: (D_2O, 400 MHz): δ (ppm) = 4.62−4.57 (m, 3H, H-1^2, H-1^3, H-1^4), 4.49 (d, 1H, *J* = 8.0 Hz, H-1^5), 3.97−3.44 (m, 28H, H-2^{1-4}, H-3^{1-4}, H-4^{1-5}, H-5^{1-5}, H-6a$^{1-5}$, H-6b$^{1-5}$), 3.41−3.38 (m, 4H, H-3^5, CH_2−C≡, H-≡), 2.94 (dd, 1H, *J* = 2.64 and 12.23 Hz, H-1^{1a}), 2.76 (dd, 1H, *J* = 9.1 and 12.1 Hz, H-1^{1b}), 2.69 (t, 1H, *J* = 8.7 Hz, H-2^5), 2.06 (s, 12H, COCH_3); ^{13}C NMR: (D_2O): δ (ppm) = 175.2 (CO), 101.6, 101.2 (C-1^2, C-1^3, C-1^4, C-1^5), 79.5, 79.3, 79.0, 77.1, 76.7, 74.9, 74.8, 72.9, 72.5, 72.4, 71.9, 71.5, 69.9, 69.8, 62.1, 61.4, 60.7, 60.6, 60.4, 60.3, 56.3, 55.8, 55.5, 55.4 (C-2^{1-5}, C-3^{1-5}, C-4^{1-5}, C-5^{1-5}, C-6^{1-5}, HC≡), 49.4, 49.1 (C-1^1), 36.8 (CH_2-≡), 22.6, 22.5 (CH_3); MALDI-TOF-MS: *m*/*z* 1031.48 [M+H]$^+$, 1053.59 [M+Na]$^+$, 1069.37 [M+K]$^+$.

*(Tri-N,N^{II},N^{III}-acetyl-chitotetraose)-(1→4)-N-acetyl-1-deoxy-1-N-azidoethyl-D-glucosaminitol (**3**).* Tetra-*N*,N^{II},N^{III},N^{IV}-acetyl-chitopentaose **1** (50 mg, 0.05 mmol), 2-azidoethylamine (21.5 μL, 0.25 mmol), and aniline (4 μL, 0.025 mmol) were dissolved into aqueous ammonium acetate buffer (3 mL, 50 mM, pH 5.0). The mixture was stirred for 5 min at room temperature, and sodium cyanoborohydride (16 mg, 025 mmol) was added. The solution was stirred for 4 days at 40 °C. Subsequently, the mixture was evaporated under reduced pressure and coevaporated five times with 5 mL of water. The crude product was purified by reversed phase flash chromatography (water to 8:2 acetonitrile−water). The solid product **3** was obtained after lyophilization (74%). ^{1}H NMR: (D_2O, 400 MHz): δ (ppm) = 4.65−4.60 (m, 3H, H-1^2, H-1^3, H-1^4), 4.51 (d, 1H, *J* = 8.0 Hz, H-1^5), 4.00−3.35 (m, 30H, H-2^{1-4}, H-3^{1-5}, H-4^{1-5}, H-5^{1-5}, H-6a$^{1-5}$, H-6b$^{1-5}$, CH_2), 2.97−2.69 (m, 5H, CH_2, H-1^{1a}, H-1^{1b}, H-2^5), 2.09 (s, 12H, COCH_3); ESI-MS: *m*/*z* 542.5 [M+H+Na]$^{2+}$, 553.5 [M+2Na]$^{2+}$, 1062.5 [M+H]$^+$, 1084.5 [M+Na]$^+$.

*4-Propargyloxyaniline (**5**).* 4-Propargyloxyaniline **5** was easily prepared from commercial 4-nitrophenol, as reported elsewhere.[18] Characterization was in agreement with literature data. ^{1}H NMR: ($CDCl_3$, 400 MHz): δ (ppm) = 6.83 (d, 2H, *J* = 9.0 Hz, H−Ar^2, H−Ar^6), 6.64 (d, 2H, *J* = 9.0 Hz, H−Ar^3, H−Ar^5), 4.61 (s, 2H, CH_2-≡), 3.48 (br. s, 2H, NH_2), 2.51 (s, 1H, HC≡); ^{13}C NMR: ($CDCl_3$): δ (ppm) = 116.84, 116.81, 116.66, 116.62 (C−Ar), 79.58 (HC≡), 75.60 (-C≡), 57.15 (CH_2−C≡); ESI MS: *m*/*z* 147.9 [M+H]$^+$.

*(Chitosan)-(1→4)-1-deoxy-1-N-(4-propargyloxyaniline)-D-glucosaminitol (**6**).* Commercial chitosan oligosaccharides (COs) **4** (FACOS, 680 mg, 0.68 mmol) and 4-propargyloxyaniline **5** (500 mg, 3.4 mmol) were dissolved into aqueous ammonium acetate buffer (20 mL, 50 mM, pH 5.0). The mixture was stirred for 5 min at room temperature, and sodium cyanoborohydride (210 mg, 3.4 mmol) was added. The solution was stirred for 4 days at 40 °C. Subsequently, the mixture was poured into 50 mL of distilled water, and extracted twice with methylene chloride (50 mL). The aqueous phase was evaporated under reduced pressure and coevaporated five times with 10 mL of water. The crude product was purified by reversed phase flash chromatography (water to 8:2 acetonitrile−water). The solid product **6** was obtained after lyophilization (71%). ^{1}H NMR: (D_2O, 400 MHz): δ (ppm) = 6.93−6.72 (m, 4H, H−Ar), 4.65 (s, 2H, CH_2-≡), 4.60−4.47 (m, 4H, H-1^n), 3.95−3.64 (m, 18H, H-5^n, H-6^n), 3.61−3.27 (m, 14H, H-3^n, H-4^n, H-1^{1a}, H-1^{1b}), 2.83−2.73 (m, 4H, H-2^n), 1.98 (s, 1H, COCH_3); ^{13}C NMR: (D_2O): δ (ppm) = 175.0

dx.doi.org/10.1021/bc3003716 | *Bioconjugate Chem.* 2013, 24, 544−549

(CO), 118.2, 117.4, 117.1, 115.6 (C−Ar), 101.7, 101.3, 101.2 (C-1^n), 79.1 (HC≡), 77.9, 77.7, 76.6, 75.2, 75.1, 74.9, 74.6, 74.4, 74.4, 73.3, 73.1, 72.1, 71.0, 70.0, 67.0, 62.4, 60.9, 60.5, 53.2 (C-3^n, C-4^n, C-5^n, C-6^n), 57.4 (CH_2−C≡), 57.3, 56.7, 55.8 (C-2), 22.5 (CH_3). Chitosan-(1→4)-1-deoxy-1-*N*-(4-propargyloxyaniline)-D-glucosaminitol **6** was *N*-acetylated to obtain better desorption of the ionized molecule from the DHB matrix for MALDI-TOF MS measurement. The COs **6** (100 mg, 0.089 mmol) was dissolved in methanol−water solution (25 mL, 1:1). Acetic anhydride (7 mL) was added slowly to the solution. The mixture was stirred overnight at room temperature, then evaporated under reduced pressure and coevaporated five times with water (10 mL). The resulting solution was freeze-dried to give the per-*N*-acetylated **6′**. MALDI-TOF MS: *m*/*z* 620.25 $[M1+Na]^+$; 823.31 $[M2+Na]^+$; 1026.40 $[M3+Na]^+$; 1229.48 $[M4+Na]^+$; 1432.56 $[M5+Na]^+$.

*Sodium N-[[(Azidoacetyl)amino]ethyl]-5-napthylamine-1-sulfonate (**7**).* Sodium azide (32 mg, 0.5 mmol) was added to a solution *N*-[[(iodoacetyl)amino]ethyl]-5-napthylamine-1-sulfonic acid (I-EDANS) (43 mg, 0.1 mmol) in 1 mL DMF and stirred for 48 h. The reaction was followed by TLC (1:1 Toluene-MeOH). After completion of the reaction, the solvent was removed under reduced pressure, and then the solid was redissolved in toluene and subjected to flash column chromatography on silica gel (step gradient: 0−50% MeOH in toluene) to give 34 mg of *N*-[[(azidoacetyl)amino]ethyl]-5-napthylamine-1-sulfonic acid. The sodium salt was generated by adding 2 equiv of MeONa 1 M, then neutralization with weakly acidic Amberlite resin CG50 in MeOH. After evaporation to dryness, then dissolution in water and freeze-drying, 36 mg of sodium *N*-[[(azidoacetyl)amino]ethyl]-5-napthylamine-1-sulfonate (N_3EDANS) **7** was obtained with quantitative yield. ^{1}H NMR: (D_2O/CD_3OD, 300 MHz): δ (ppm) = 8.12 (m, 3H, H−Ar^{2,3,4}), 7.32 (m, 2H, H−Ar^{7,8}), 6.60 (d, 1H, *J* = 9 Hz, H−Ar^6), 3.87 (s, 2H, CH_2N_3), 3.52 and 3.31 (2 × m, 2 × 2H, $_\alpha NCH_2$ or $_\beta CH_2N$); ^{13}C NMR: (D_2O/CD_3OD): δ (ppm) = 170.9 (C═O), 145.4, 141.5, 131.4, 128.7, 126.8, 125.7, 123.6, 116.2, 105.1, 102.6, 95.6 (C−Ar), 53.1 (CH_2N_3), 44.7 and 39.7 ($_\alpha NCH_2$ or $_\beta CH_2N$); ESI MS: *m*/*z* 393.9 $[M+Na]^+$; FT-IR cm^{-1}: 3388 (N−H), 2114 (N_3), 1653, 1578, and 1531 (C═C).

*4-[N^{IV}-Dimethylaminophenylazophenyl-thioureido-(tri-N,N^{II},N^{III}-acetyl-chitotetraose)-(1→4)-(N-acetyl-1-desoxy-1-N-methyl-D-glucosaminitol)]-1-[(sodium N-(acetylamino)-ethyl)-5-napthylamine-1-sulfonate]-1H-[1,2,3]-triazole (**8b**).* To a stirred solution of alkyne-chitopentaose **2** (20.6 mg, 0.025 mmol) and N_3-EDANS **7** (11 mg, 0.03 mmol) in 1 mL of water at 50 °C was added a solution of sulfate copper pentahydrate 1 M (50 μL, 0.05 mmol) and sodium ascorbate (20 mg, 0.1 mmol). The reaction mixture was stirred for 3 days at 50 °C. After concentration under reduced pressure, the residue was dissolved in water, and subjected to purification over a C-18 Sep-Pack plus cartridge, by stepwise elution with increasing concentration of MeOH in H_2O, giving the 1,2,3-triazole derivative **8a** (15.5 mg, 55% yield). Analysis by MALDI-TOF MS revealed the presence of the expected product which (6 mg, 4.3 μmol) was allowed to react at 37 °C with 4-dimethylaminoazobenzene-4′-isothiocyanate (DABITC) (2 mg, 7 μmol) in *N*,*N*-dimethylformamide (0.5 mL) and 0.2 mL of aqueous sodium hydrogenocarbonate (30 mg.mL^{-1}) for 24 h. The reaction mixture was evaporated in the presence of silica and the crude solid deposited on the top of a column and subjected to flash column chromatography (step gradient: 0−20% water in acetonitrile) to yield the FRET probe **8b** (6.3 mg, 87%) as an orange solid. ^{1}H NMR: (D_2O, 400 MHz): δ (ppm) = 8.58 (H^5-triazole), 8.20−6.00 (m, H-aryl), 5.20−4.30 [m, H-1^{II}, H-1^{III}, H-1^{IV} and H-1^V], 4.00−3.00 (m, H-sugars, 2 × NCH_3, 5 × CH_2N), 1.9−1.7 (m, $NCOCH_3$).

*Chitosan-Grafted-Polycaprolactone (**9**).* Poly(α-N_3-ε-CL-*co*-ε-CL) **9** (M_n = 10100 g·mol^{-1}; PDI = 1.50; 20% molar ratio of azido monomers, see Figure S14 in Supporting Information) (50 mg, 4.1 μmol, 0.082 mmol in azide functional group), and alkyne-functionalized COs **6** (123 mg, 0.123 mmol) were dissolved into a mixture of 5:1 DMF/water (6 mL). The solution was stirred for 5 min at room temperature, and CuI·P(OEt)$_3$ (58 mg, 0,164 mmol) was added. The reaction mixture was heated to 40 °C and stirred for 36 h. The solution was diluted in water (80 mL) and acetic acid (200 μL), and centrifuged at 8000 rpm at 4 °C for 20 min. The supernatant was filtered on 10 000 Da cellulose acetate membrane against 2 L of water. The solid product **10** was obtained after lyophilization (32 mg, 80%). ^{1}H NMR: (D_2O with 5% CD_3COOD, 400 MHz): δ (ppm) = 7.9 (m, 1H, H−F), 6.97−6.77 (m, 4H, H−Ar), 4.85 (m, 12H, H-E), 4.60 (m, 11H, H-1^n), 4.01−3.4 (m, 128H, H-3^n, H-4^n, H-5^n, H-6^n), 3.17 (m, 11H, H-2^n), 2.24 (m, 12, H-D), 2.05 (s, $COCH_3$), 1.58−1.25 (m, 36H, H-J).

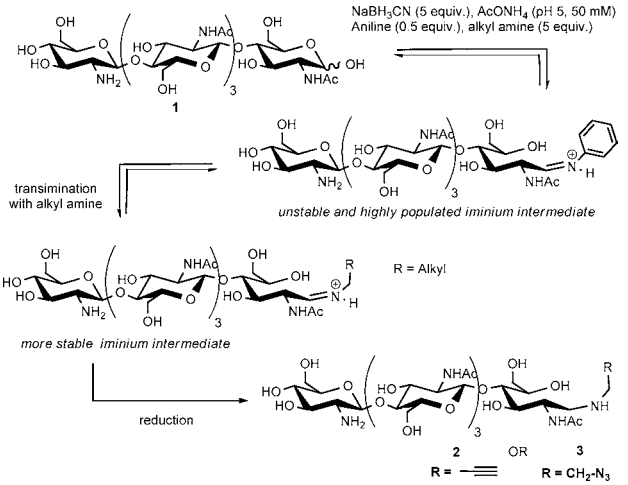
RESULTS AND DISCUSSION

Commercially available COs are generally constituted of oligomers with different degree of polymerization, significantly complicating the follow-up of the reaction, the purification, and the characterization of the products. For these reasons, we decided to begin our investigations with a well-defined chitooligosaccharide, the tetra-*N*-acetyl-chitopentaose **1**,[14] which was produced in our laboratory by genetic engineering of *Escherichia coli*.[14,19,20] The first attempts to incorporate an alkyne group at the CO reducing end were carried out according to classical methods of carbohydrate reductive amination. Typically, tetra-*N*-acetyl-chitopentaose **1** was dissolved in ammonium acetate buffer at pH 5 with subsequent addition of propargylamine (5 equiv) followed by $NaBH_3CN$ (5 equiv) at 40 °C for 4 days. MALDI-MS analysis of the major purified fraction highlighted the presence of a complex mixture, compound **2** being detected as a minor population together with several other unidentified products suggesting a very low yield of functionalization (p. S2 (Top), ESI†). The causes of this poor result are probably numerous. A first issue stems from the substantial protonation of the propargylamine under the required acidic conditions (pK_a = 8.15).[21] Another matter has to do with the very weak reactivity of the masked aldehyde group. It has indeed been previously established that the anomeric centers of sugars with amino group at C-2 are far less reactive than hydroxy counterparts.[22−24]

In view of the aniline iminium pK_a, around 2 units below the pK_a of aniline (4.62) (vs 6−8.5 for aliphatic amine iminium[25]) that ensures an almost complete protonation under ligation conditions[26] and the small equilibrium constant (K_{eq}) associated to its formation,[27,28] we anticipated that the addition of aniline and the *in situ* generation of the aniline iminium could significantly enhance the formation of the propargylamine iminium through transimination (Scheme 1). The coupling reaction was thus subsequently performed in the presence of aniline ([propargylamine]/[**1**]/[aniline] = 5.0/1.0/0.5). With this modified procedure, the targeted product **2** was obtained in good isolated yield (70%) confirming that aniline-mediated reductive amination promotes the formation of

dx.doi.org/10.1021/bc3003716 | *Bioconjugate Chem.* 2013, 24, 544−549

Scheme 1. Synthetic Pathway of Chitopentaose−Alkyne Conjugate 2 or 3 by Aniline-Mediated Reductive Amination of Tetra-*N*-acetyl-chitopentaose 1

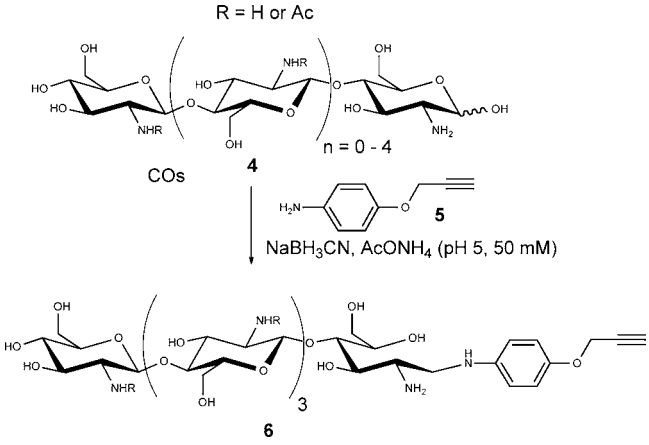


propargylamine iminium and its subsequent reduction. It is worth noting that the reduced tetra-*N*-acetyl-chitopentaose derivative of aniline was also detected by mass spectrometry in the crude mixture. A 10/1 [propargylamine]/[aniline] ratio was shown to minimize the formation of such species.

To demonstrate the versatility of our synthetic approach, we further explored the synthesis of another clickable chitooligosaccharide **3** using 2-azidoethylamine. Again, the expected azide-functionalized chitooligosaccharide **3** was obtained with good yield (~70%) thanks to aniline-catalyzed reductive amination. The presence of the terminal azido group was confirmed by FT-IR analysis with the stretching frequency around 2100 cm^{-1} and the mass spectrometry confirmed that the chitopentaose **1** carries the azidoethylamine modification (p S5−6, Supporting Infomation).

When applying this methodology to a mixture of commercially available chitosan oligomers (COs) **4** displaying a *N*-deacetylation degree above 90%, the expected *N*-propargyl conjugate could not be observed regardless of various conditions of reaction. Waldron et al.[29] have already reported that chitosan oligomer reductive amination was even more problematic than for chitin oligomers, and this difference of reactivity notably increased with the degree of polymerization. Therefore, the aniline-catalyzed reductive amination through a transimination reaction showed some limitations with weakly reactive anomeric centers as chitosan oligosaccharides. However, in the course of our experimentations, it was shown that, upon addition of aniline in excess over propargylamine, the reduced form of aniline end-functionalized COs was preferentially generated. This result is consistent with previous works of Koshida et al. that underlined a preferential incorporation of aniline derivatives (vs ethanolamine) on sulfated disaccharides by reductive amination.[30] Aniline and others aromatic amines[31] were already reported as useful reagents for glycan derivatization affording better detection and analysis of biological sugars by liquid chromatography or mass spectrometry, but this approach has never been used to incorporate new functionalities on COs. These observations prompted us to examine the straightforward introduction of "clickable" groups onto COs using 4-(oxypropargyl)aniline **5**, an alkyne-functionalized aniline derivative generated from 4-nitrophenol[32,18] (Scheme 2). The coupling reaction involving

Scheme 2. Reductive Amination of Chitosan Oligosaccharides (COs) 4 with Alkyne-Modified Aniline 5



COs, 4-(oxypropargyl)aniline, and $NaBH_3CN$ was performed in ammonium acetate buffer (50 mM, pH 5) for 4 days at 40 °C affording the corresponding alkyne-functionalized COs conjugate **6** in good yield (71%). Importantly, the presence of an aromatic ring considerably improved the purification procedure using reversed phase chromatography with UV-detection.

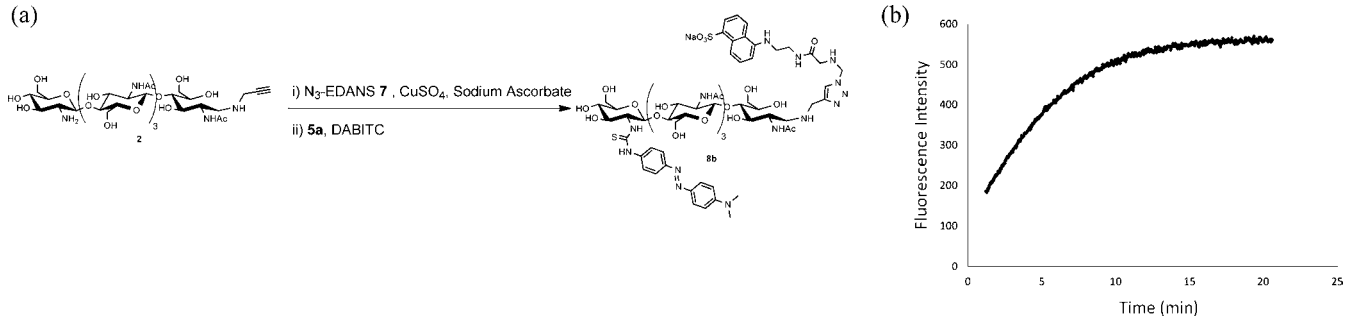
To illustrate the potential of alkyne end-functionalized oligosaccharidic materials in bioapplications, we further investigated the preparation of a fluorescence resonance energy transfer (FRET) probe from chitopentaose **2** to specifically assay chitinase activity (Scheme 3). Chitopentaose derivative harboring a fluorophore 5-(2- aminoethyl)amino-1-naphthalene-sulfonic acid (EDANS) and a quenching group, dimethylaminophenylazophenyl (DAB), at both chain ends has been proven to be extremely useful for the specific assay of chitinases.[33] Unfortunately, the availability of such fluorogenic probes is limited because of the low yield of introduction of EDANS group at the reducing end chain of the chitin oligomer. As an alternative route, we explored the introduction of EDANS through our new methodology, then CuAAC ligation ($CuSO_4$/sodium ascorbate/DMF) between the chitopentaose alkyne conjugate **2** and azido-EDANS **7** (prepared from commercially available I-EDANS).

In a following step, the DAB quenching group was then anchored to the fluorescent-tagged chitopentaosyl **8a** by reacting 4-dimethylaminoazobenzene-4′-isothiocyanate (DABITC) in the presence of sodium carbonate to give the bifunctionalized chitopentaose derivative **8b** with an overall yield of 48%. Fluorescence-quenched substrate **8b** was finally evaluated as substrate for Chitinase A1 from *Bacillus circulans*. As shown in Scheme 3, time-dependent increase of fluorescence was detected at 490 nm upon addition of the enzyme confirming the potential use of **8b** as FRET substrate to probe the chitinase activity.

COs conjugate **6** constitutes another valuable intermediate for the preparation of a CO-based copolymer, in view of reaching nanostructured advanced materials in solution or in bulk. The amphiphilic block copolymers displaying biosourced polysaccharides at the surface are promising systems for the vectorization of drugs through self-assembled micelles.[34] In this context, poly(caprolactone)-*graft*-COs graft copolymer **10** were first generated through CuAAC ligation with a efficiency of 75% by coupling terminal alkyne-functionalized COs conjugate **6**

dx.doi.org/10.1021/bc3003716 | *Bioconjugate Chem.* 2013, 24, 544−549

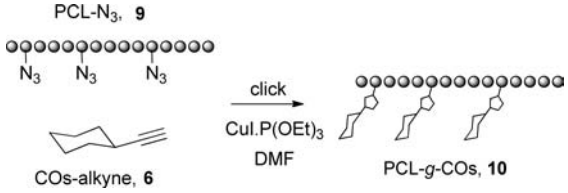
Scheme 3[a]



[a](a) Synthesis of chitopentaose-based FRET probe **8b** and (b) the enzymatic hydrolysis kinetic with *B. circulans* Chitinase A1. Excitation at 340 nm and hydrolysis monitored at 490 nm.

with poly(caprolactone) **9** having pendant azide groups[16] (11 400 g·mol^{-1}, 20% molar azide, PDI = 1.5) using CuI·P(OEt)$_3$ as catalytic system[35,36] (Scheme 4).

Scheme 4. Schematic representation of the Preparation of Poly(Caprolactone)-*graft*-COs Copolymer 10 by Click Conjugation



The coupling of blocks was confirmed by ^{1}H NMR which indicated the expected resonances that are distinctive for each block and showed a characteristic peak of proton on the triazole ring around 8 ppm (p. S15, Supporting Infomation). The monomodal peak merged on SEC (p. S15, Supporting Information) and the unique diffusion coefficient by diffusion-ordered NMR spectroscopy (DOSY) experiments (p. S16, Supporting Information) confirmed the formation of the PCL-*g*-COs **10**.

CONCLUSIONS

In conclusion, we have developed a novel and straightforward approach for derivatization of biologically important COs by aniline-catalyzed or -mediated reductive amination. Unlike previous modifications of chitin/chitosan, this approach confers a new opportunity toward attractive conjugatable COs without altering the chemical nature of CO backbone and their inherent biological activity. The efficiency of this strategy to incorporate new functional groups onto COs through aniline-mediated transimination or reductive amination in the presence of aniline derivatives paves the way to the preparation of advanced functional chitin/chitosan-based hybrid materials. This methodology could also be extended to glycosaminoglycan oligosaccharides that represent one of the most important classes of biologically active carbohydrates.

ASSOCIATED CONTENT

Supporting Information

Spectroscopic (NMR and mass spectrometry) and chromatography (SEC) data of synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the Région Rhône-Alpes (Cluster de Recherche Chimie). The authors thank Kitto Life Co., Ltd, (Seoul, Korea) for the kind gift of COs referred as FACOS, ICMG mass spectrometry platform for analyses, and I. Jeacomine for the technical assistance in NMR spectrometry.

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