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Regioselective Benzylation of 2-Deoxy-2-aminosugars using Crown Ethers: Application to a Shortened Synthesis of Hyaluronic Acid Oligomers

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Abstract: The combination of benzyl bromide, sodium hydroxide and 15-crown-5 in tetrahydrofuran is shown to be an efficient method for installing benzyl groups at both the 4- and 6-positions regioselectively directly from peracetylated *N*-trichloroacetyl-protected glucosamine and galactosamine. Application of this benzylation strategy proved to significantly shorten the synthetic route to hyaluronic acid tetra- and hexamers.

Keywords: benzylation; carbohydrates; phase-transfer catalysis; protecting groups; trichloroacetyl group

Introduction

In the synthesis of complex carbohydrates, the main focus lies logically on the stereoselective formation of glycosidic bonds; however, a significant portion of effort in the development of a route for the chemical synthesis of oligosaccharides is unavoidably devoted to protecting group manipulations.[1] The chosen protecting groups are much more than inert groups in carbohydrates synthesis, since they can also have a profound impact on the reactivity in glycosylation of the substrate.[2] For example, we recently demonstrated that the protecting groups greatly influence the reactivity of intermediates in the synthesis a carba-sugar.[3] The protecting groups on the donor and acceptors also play key roles in determining outcomes in glycosylation reactions.[4] For example, we recently noted that a fluorous phosphate protecting group could change the stereochemical outcome of a glycosylation unexpectedly.[5] In addition, efforts to install a particular protecting group pattern can be hampered by incompatibilities in conditions required to install different protecting groups sequentially. For instance, the installation of multiple ether protecting groups, such as the often-used "permanent" benzyl (Bn) ether,[6] can be challenging in the presence of base-sensitive ester or amide bonds. This incompatibility can then lead to lengthy building block syntheses or even to completely changed synthetic plans. More effective protecting group manipulations, avoiding lengthy multiple transformations and tedious separations, are essential to the practicability of a certain synthetic route and thus are highly attractive. In the past few years, increasing attention has been given to streamlining such protecting group manipulations. The groups of both Hung[7] and Beau[8] have reported highly selective 4,6-benzylidene acetal formation and benzyl ether installations at the 3-position of monosaccharides using per-silylated substrates under Lewis acid conditions to significantly shorten the steps needed to obtain certain building blocks. However, due to the great diversity of the building blocks needed for oligosaccharide synthesis, much creative effort is still needed to enhance protecting group manipulations. Efforts have also been done to optimize the protecting group manipulations on glucosamine. Hung[9] and Beau[10] with co-workers have reported one-pot syntheses of differently protected glucosamine building blocks. We have recently reported three new fluorous amine protecting groups appropriate for glucosamine building blocks.[11]

As part of our ongoing work on glycosaminoglycans, we specifically needed glucosamine and galactosamine acceptors having the 3-OH group unprotected. A hindered group at the 2-N of an amino sugar is known to exert hindrance on the incoming electrophile approaching to the 3-OH group; in the presence of a bulkier group, for example, a 2-*N*-phthaloyl[12] or 2-N-TCP (*N*-tetrachlorophthaloyl) group,[13] glycosylation preferentially occurs at the 4-OH if both the 3-

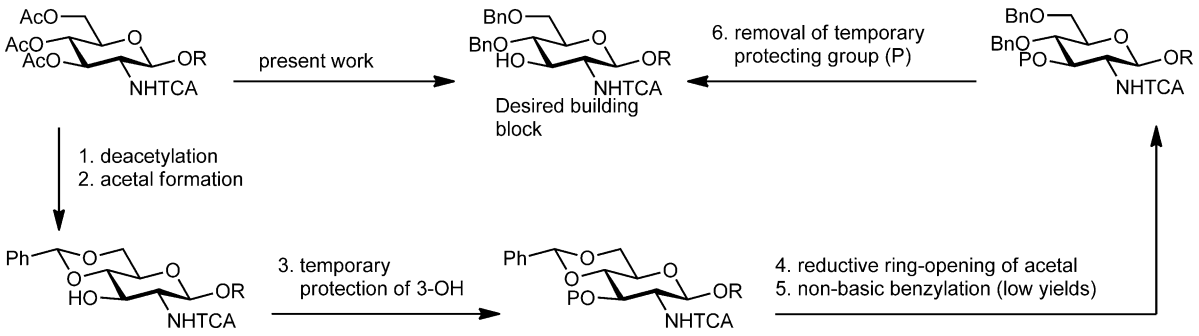


Figure 1. Comparison between the present work and a previously reported strategy[20] for the synthesis of 4,6-di-*O*-benzyl-2-NHTCA-protected amino sugars using existing methods.

OH and 4-OH groups are free whereas similar reactions with smaller protecting groups – for example, N_3[14] or NHAc (acetyl)[15] or Troc (2,2,2-trichloroethyl carbonate)[16] – provide regioisomers. However, our application required a protecting group at the 2-N that would exhibit both bulkiness and the flexibility to control reactions on the 3-OH site under certain conditions. To attempt to keep the 3-OH group free for chain extension reactions, we decided to probe the possibility of a regioselective dibenzylation reaction – preferentially at the 4-OH and 6-OH – by utilizing this known hindrance factor.

Recently, the trichloroacetyl group (TCA) has become a popular protecting group for amino sugars; it has been used in the synthesis of hyaluronic acid, chondroitin and other amino sugar-containing structures as a participating group due to the fact that TCA could be either removed at the end of the synthesis under mild basic conditions[17] or transformed to NHAc group under radical conditions.[18] We envisioned that a 2-NHTCA group might be a good choice as it is neither too small nor too big in size and could provide the optimum masking group for preventing the neighboring 3-OH from undergoing a subsequent benzylation reaction. In our efforts towards the synthesis of various glycosaminoglycans, use of the common benzyl ether as the permanent protecting group at the 4- and 6- position of the *N*-trichloroacetyl-protected glucosamine or galactosamine made most sense. However, even though benzyl ether groups have been so widely used, very much to our surprise, there have only been a few reports using this building block pattern. Most of the existing studies involving NHTCA-GalN (galactosamine) or GlcN (glucosamine) used either a 4,6-*O*-benzylidene or 4,6-*O*-di-*tert*-butylsilyl group to block these two hydroxy groups. We envision the lack of usage of benzyl ether groups at the 4,6 positions in the presence of NHTCA is due to the incompatibility between the basic conditions used to install benzyl ethers and the trichloroacetamide groups. *N*-Benzylation or amide bond cleavage could be possible side reactions under commonly utilized basic conditions like NaH or NaOH.[19]

In the few cases of benzylated NHTCA substrates, the benzyl group was installed on 2-azido sugars,[21] and TCA was installed after azido reduction. This strategy utilizes the azido group to circumvent the incompatibility problem, but adds additional steps to the route. In other cases, a lengthy synthesis was used to install the benzyl ether groups on the 4- and 6- positions.[20] (Figure 1) This route involves the benzylidene acetal formation at the 4,6 positions, installation of a temporary protecting group at the 3-OH, selective benzylidene opening to give 4-OBn and a free primary hydroxy at C-6, followed by benzylation of the 6-OH under non-basic conditions like silver oxide or Dudley reagent.[22] In this route, it takes 6 steps to obtain the 4, 6-di-*O*-benzyl NHTCA substrates from a 3,4,6-tri-*O*-acetyl-2-NHTCA substrate with a chosen anomeric protecting group. Sometimes, due to incompatibilities amongst certain protecting groups, the 3-*O*-protecting group needs to be swapped, thereby leading to an even longer route. For example, in the recently reported synthesis of an *E. coli* O111 *O*-specific polysaccharide repeating unit, a 4,6-di-*O*-benzyl NHTCA substrate with SEt as the anomeric protecting group and Fmoc (fluorenylmethyloxycarbonyl) as the 3-*O* protecting group was synthesized in 7 steps from per-acetylated NHTCA substrate.[23] The steps for the currently employed methods are long, and in our hands, we obtained highly variable results using the reported non-basic benzylation methods on carbohydrate substrates.

The lack of good synthetic methods to generate 4,6-di-*O*-benzyl-protected NHTCA substrates seriously hampered our syntheses of glycosaminoglycans using TCA groups. We decided to study if any improvement could be made to solve the problem. In a larger effort to significantly simplify the synthesis of carbohydrate building blocks for our automated oligosaccharide synthesis platform,[24] we report herein the discovery of efficient conditions to 4,6-*O*-dibenzylated glucosamine and galactosamine building blocks and the ap-

plication of this method to the synthesis of a hyaluronan hexasaccharide.

Results and Discussion

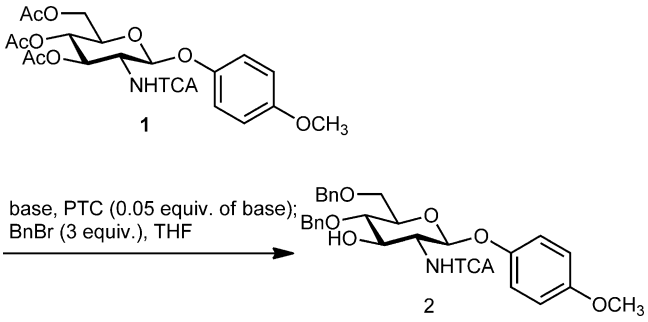
The most widely used method to install benzyl ethers is to use NaH/BnBr in a polar aprotic solvent.[6] Under certain circumstances, a low temperature benzylation could be performed at the 3-OH of a benzylidene-protected NHTCA-bearing carbohydrate substrate; however, this method could not solve our need, as the free 3-OH group would be difficult to retain under stronger basic conditions, even at low temperature. During the course of this work, new procedures to perform benzylation under acidic conditions have been reported;[25] however, these reagents also do not provide the regioselectivity needed. In our efforts towards effective benzylation in the presence of the TCA group, we realized that the key to successful benzylation on such substrates might be the chemical stability difference between acyl groups and amide groups. By carefully choosing the reaction conditions, we may have a chance of performing benzylation on hydroxy moieties or acyl-protected hydroxy groups without affecting the TCA group.

Benzylation reactions can be performed directly from acyl-protected sugar substrates using a nucleophilic base.[26] In that case a one-pot, two-step reaction can be performed first by *in situ* deacylation, followed by benzylation. We decided to use compound **1**, a 3,4,6-tri-*O*-acetylated 2-NHTCA-protected glucosamine substrate with a methoxyphenol group at the anomeric position, as the model compound to study benzylation. Tetrahydrofuran (THF) was chosen as the solvent, for its lesser polarity might improve selectivity.[27] Phase-transfer conditions have been used in benzylation reactions for a long time,[28] and allow the usage of milder bases in organic solvents. In many cases, phase-transfer conditions lead to selective benzylation on carbohydrate substrates.[29] However, the possibility of using such phase-transfer conditions in benzylation reactions in the presence of an amide bond has not been thoroughly explored. In our study, different bases with different phase-transfer reagents in the presence of 3 equiv. of benzyl bromide were screened (Table 1).

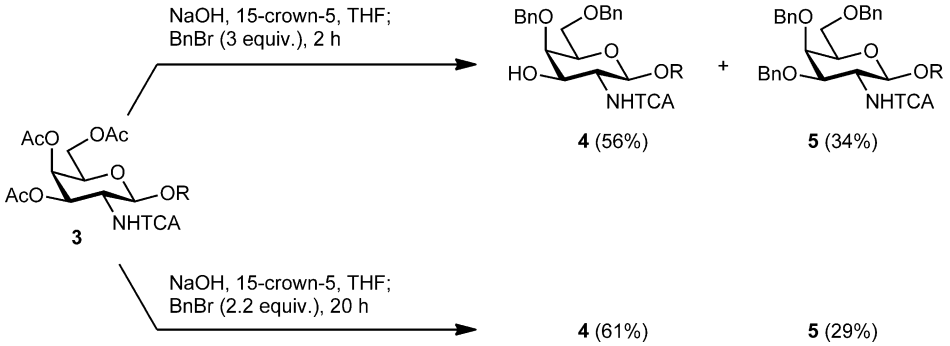
Potassium hydroxide has been used earlier in benzylation reactions directly on acylated sugars,[30] and therefore served as the starting point for our study. The preliminary benzylation reaction of substrate **1** was performed using a combination of BnBr/KOH in the presence of 18-crown-6, a phase-transfer catalyst. Before addition of benzyl bromide, substrate **1** was first treated with 6 equiv. of powdered KOH and 18-crown-6 (0.05 equiv. of base) in anhydrous THF for 1 hour. During this time all the acetyl groups were cleaved and then benzyl bromide (3 equiv) was added slowly to the resulted slurry under an inert atmosphere. A non-polar spot appeared, based on TLC (thin layer chromatography) analysis, in a few hours but no satisfactory progress of the reaction was seen even after 20 h, as a large amount of unreacted polar compound could be seen on TLC. Chromatographic separation yielded only 21% of that non-polar compound (entry 1, Table 1). A careful nuclear magnetic resonance (NMR) study of the compound revealed the formation of our expected 4,6-di-*O*-benzylated compound **2**. The presence of four benzylic protons [at $\delta = 4.67$, 4.58 (2 d, $J = 11.2$ Hz each), 4.55, 4.47 (2 d, $J = 12.0$ Hz each)] and a doublet for the NHTCA proton at $\delta = 7.04$ ($J = 7.4$ Hz) in the ^{1}H NMR spectrum confirmed the incorporation of two benzyl groups without any base-catalyzed hydrolysis of the amide bond or *N*-benzylation. Absence of any acetyl group in the NMR spectra confirmed that a complete deacetylation reaction occurred prior to the benzylation reaction. The exact structure of the compound was established through a cross-peak correlation between H-3 and 3-OH protons in a ^{1}H-^{1}H COSY spectrum (please see the Supporting Information).

Encouraged by this result, efforts were directed towards other available bases for regioselective benzylation. A combination of CsOH/TBAI (tetrabutylammonium iodide) only gave an 18% yield of the dibenzylated product under similar conditions, along with triols from the starting materials in which the acetyl groups were cleaved by the treatment of base (entry 2, Table 1). $BaO/Ba(OH)_2$ has been reported to perform ether formation in the presence of an amide bond.[31] Using $BaO/Ba(OH)_2$ only gave trace amounts of the 4,6-di-*O*-benzyl product **2** (entry 3,

Table 1. Initial screening of benzylation conditions.



Entry	Base (equiv.)	PTC	Time	Yield
1	KOH (6)	18-crown-6	20 h	21%
2	CsOH (6)	TBAI	20 h	18%
3	$BaO/Ba(OH)_2$ (6)	–	20 h	<5%
4	50 wt% NaOH-Al_2O_3 (12)	15-crown-5	20 h	16%
5	NaOH (6)	15-crown-5	20 h	68%
6	NaOH (9)	15-crown-5	6 h	69%
7	**NaOH (12)**	**15-crown-5**	**6 h**	**80%**



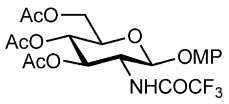
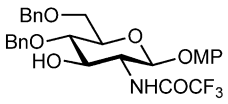
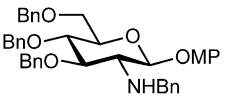
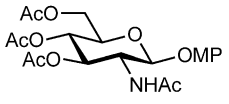
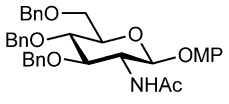
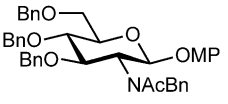
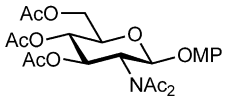
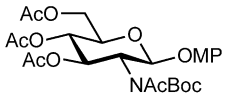
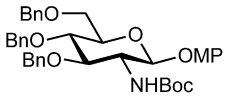
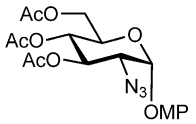
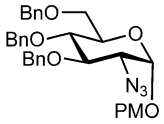
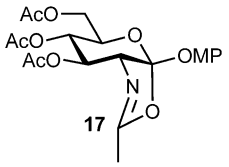
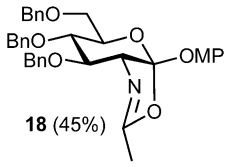
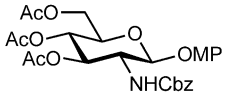
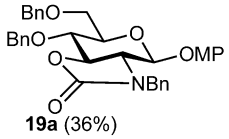
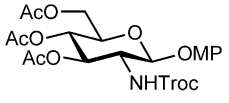
Scheme 1. Selective benzylation on NHTCA-protected GlaN; R = 4-methoxyphenyl.

Table 1). No tribenzylated product was isolated. When a mixed base of NaOH and Al_2O_3[32] was used (entry 4, Table 1), in the presence of 15-crown-5, the yield of **2** was only 16%. After getting unsatisfactory results with these bases, we turned our attention to the inexpensive base sodium hydroxide. A BnBr/NaOH/TBAI combination had been reported by Misra and co-workers for the benzylation of acylated non-amino sugar substrates.[26] In our case, a combination of NaOH (6 equiv.), 15-crown-5 (0.05 equiv. of base) and benzyl bromide (3 equiv.) furnished **2** in 68% yield in 20 h (entry 5, Table 1). Delighted with this result, we decided to proceed with NaOH/15-crown-5 to optimize the yield of the desired dibenzylated product. When the amount of NaOH was increased to 9 equiv., the reaction gave a similar yield (69%) in only 6 h (entry 6, Table 1). This prompted us to use more NaOH in the reaction. With 12 equiv. of NaOH, the reaction gave **2** in 80% yield in 6 h (entry 7, Table 1). By using these optimized conditions, we could synthesize **2** easily on a gram scale from **1** in only one step, thereby eliminating the previously reported[20] six-step method (Figure 1).

Next, we applied these same conditions to a galactosamine substrate. Interestingly, unlike the 4-position epimer, glucosamine **1**, the tribenzylated compound was also formed in moderate yield when the same conditions were applied to galactosamine **3**. The dibenzylated product **4** was isolated in a 56% yield, along with 34% of the tribenzylated product **5**. This result reflects its more reactive character[33] as compared to the analogous glucosamine derivative (Scheme 1). When 2.2 equiv. of benzyl bromide were used, a slightly better yield of dibenzylated derivative was found, though the reaction time was comparatively much longer. As in the case of compound **2**, the formation of dibenzylated **4** was confirmed through the appearance of a correlation peak between the H-3 and 3-OH in the ^{1}H-^{1}H COSY spectrum.

To study the role of the *N*-trichloroacetyl protecting group in the observed selectivity, various other amino protecting groups were tested (Table 2). Both smaller than trichloroacetyl and slightly hindered groups were considered for this purpose. From an *N*-trifluoroacetyl (TFA) – which is smaller but more electronegative in comparison to the TCA group – protected glucosamine substrate **6**,[34] the dibenzylated product **7** was isolated in 53% yield, along with 20% of the tetrabenzylated product **8**. Compound **8** was likely formed first by *N*-benzylation followed by amide hydrolysis. The presence of four doublets from benzylic protons at $\delta = 4.64$, 4.57, 4.54 and 4.46, respectively and a doublet of NHTFA proton at $\delta = 6.63$ ($J = 7.6$ Hz) in ^{1}H NMR and two quartets at $\delta = 157.8$ ($^2J_{C,F} = 38.0$ Hz, *C*OCF$_3$) and $\delta = 115.8$ ($^1J_{C,F} = 287$ Hz, *C*F$_3$), respectively, in ^{13}C NMR supported the formation of the dibenzylated product. The exact position of the free hydroxy group was confirmed from a ^{1}H-^{13}C HMBC spectrum, as no correlation peaks between 3-OH and H-3 protons were observed in the ^{1}H-^{1}H COSY spectrum due to the appearance of a broad singlet for the 3-OH proton. The presence of correlation peaks between the C-4 and C-6 carbons with their adjacent benzylic protons and the absence of any correlation peaks of benzylic protons with C-3 in ^{1}H-^{13}C HMBC confirmed the formation of compound **7** (please see the Supporting Information). The formation of tetrabenzylated product **8** was easily confirmed through the presence of four benzyl groups and the absence of the NHTFA proton in ^{1}H NMR as well as absence of two quartets of the COCF$_3$ group in ^{13}C NMR. As a TFA group is smaller than a TCA moiety, comparatively less hindrance from the former group is expected and therefore there is a greater possibility of forming the tribenzylated compound under these conditions. Interestingly, no 3,4,6-tri-*O*-benzylated product of Glc-NHTFA was formed; this may be due to an electronic factor.[33] When compound **9**[35] with an acetamido group was used, the tribenzylated and tetrabenzylated compounds **10** and **11**, respectively, were the major products, which were isolated in 35% and 33% yields, respectively. To make the region more hindered, NHAc was replaced by an *N*-diacetyl group. Treatment of compound **9** with isopropenyl acetate in the presence of acid catalyst at elevated temperature furnished compound **12** in 97% yield. A

Table 2. Scope for the phase-transfer benzylation method: different amino-protecting groups.[a]

Substrate	Dibenzylated product	Tribenzylated product	Tetrabenzylated product
 **6**	 **7** (53%)		 **8** (20%)
 **9**		 **10** (35%)	 **11** (33%)
 **12**		**10** (16%)	**11** (49%)
 **13**		 **14** (66%)	
 **15**[b]		 **16** (92%)	
 **17**		 **18** (45%)	
 **19**		 **19a** (36%)	
 **20**		**19a** (60%)	

[a] Reaction time was 20 h for each example except compound **15**.
[b] Reaction time was 2 h.

similar benzylation reaction on **12** furnished tribenzylated product **10** in 16% and tetrabenzylated product **11** in 49% yields as major products. Presumably, the diacetyl group did not survive the initial basic conditions and thus directly transformed into NHAc to behave similarly to compound **9**.

We also discovered that an azide group cannot affect the selective benzylation. When a similar benzylation reaction was carried out on compound **15**, tribenzylated product **16** was obtained almost exclusively. When only 2.2 equiv. of BnBr were used, **16** was still isolated as the major product. An oxazoline group in compound **17** also did not allow a selective benzylation reaction. Tribenzylated compound **18** was found as the major product even when 2.2 equiv. of BnBr were used. Carbamate derivatives (**19**, **20**) were transformed into *N*-benzyl-2,3-*trans*-oxazolidinone **19a** under basic conditions.[36] Clearly, the TCA-protected nitrogen is key to the selectivity under these conditions.

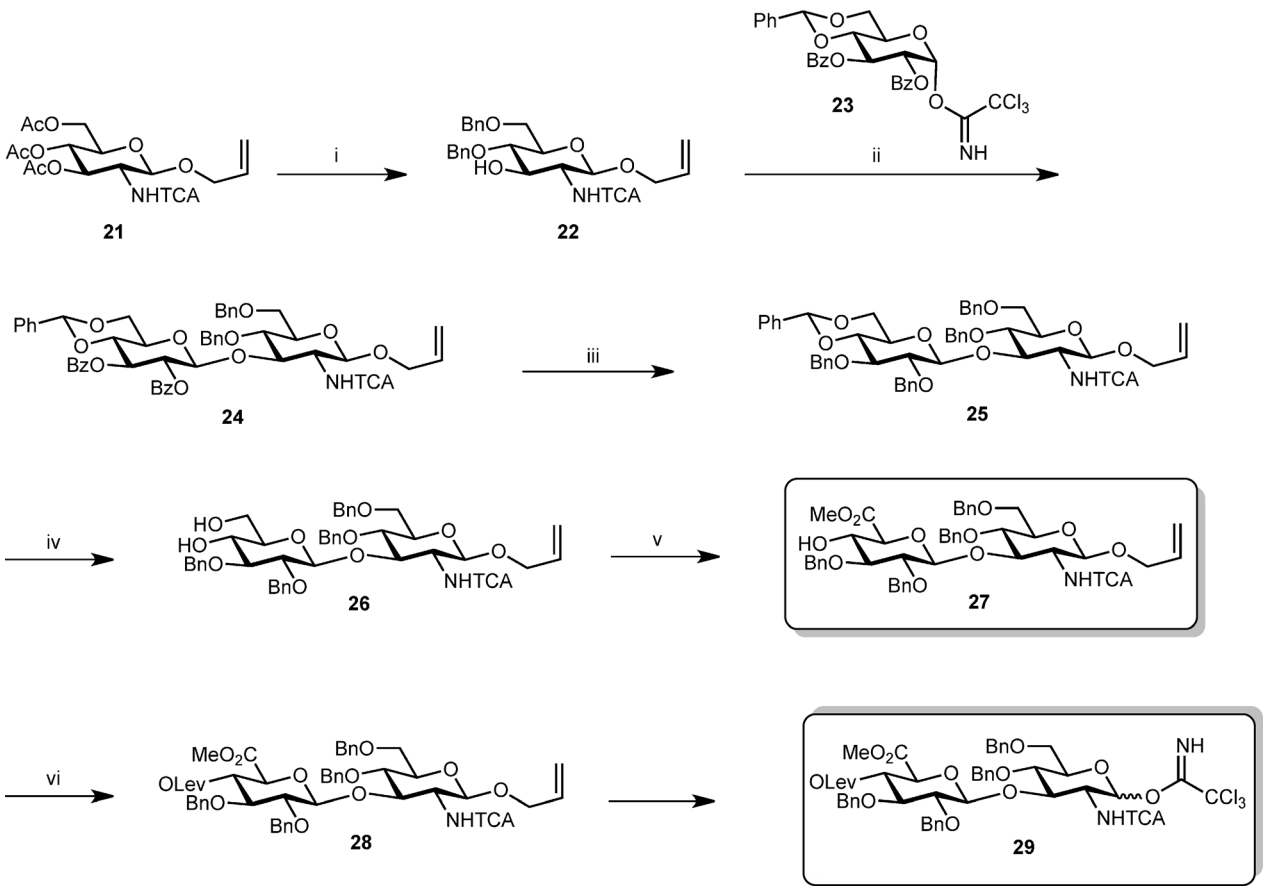
Based on the above studies, BnBr/NaOH/15-crown-5 in THF provides an efficient method to efficiently create the desired protecting group pattern for a building block needed to make hyaluronic acid and related glycosaminoglycans. Hyaluronic acid (HA) is a linear, unbranched repeating polymer of *N*-acetylglucosamine and D-glucuronic acid. Hyaluronic acid is distributed widely throughout connective, epithelial, and neural tissues and is involved with multiple functions including cellular proliferation, cell-cell recognition, and cell migration.[37] As the main function of this glycosaminoglycan family member was believed to be as a polymer required for the lubrication of joints, HA received relatively little attention for years. However, more recently biological functions of shorter hyaluronic acid fragments have been uncovered.[38] The degradation products of hyaluronic acid, small oligosaccharides with varying lengths, exhibit pro-angiogenic properties.[39] Short fragments of hyaluronic acid can induce inflammatory responses in macrophages and dendritic cells in cases of tissue injury or skin transplant rejection.[40] Hyaluronic acid is also the main component of the capsular polysaccharide of group A Streptococci (*Streptococcus pyogenes* or GAS).[41] Abundant production of hyaluronic acid in GAS is a key virulence determinant and is correlated with serious GAS infections.[42] As a result, low molecular weight hyaluronic acid is now being studied as a treatment and prevention of infection and disease caused by group A and group C Streptococci.[43]

Given the increasing biological interest in HA, there have been many reports on the synthesis of hyaluronic acid fragment;[44] those syntheses mainly differ in the choice of protecting groups (especially on the amino group), the choice of anomeric activating group and the strategy of the oxidation.[45] In recent years, great advancements were made in this area. For example, van der Marel and co-workers have reported the synthesis of the tri-, penta-, and heptamer of hyaluronic acid.[46] Huang and co-workers have reported the synthesis of a dodecamer of hyaluronic acid.[44,47] Sleeman and Bräse reported the multi-gram synthesis of a hyaluronic acid building block and a fully protected oligomer.[48] Recently, van der Marel and Codée reported the automated solid-phase synthesis of hyaluronan oligosaccharides, obtaining a pentadecamer of hyaluronic acid.[49] Despite these efforts, it is still a daunting task to perform the synthesis to obtain different lengths of hyaluronic acid fragments, partially due to the difficulties in synthesizing the required building blocks on larger scales.

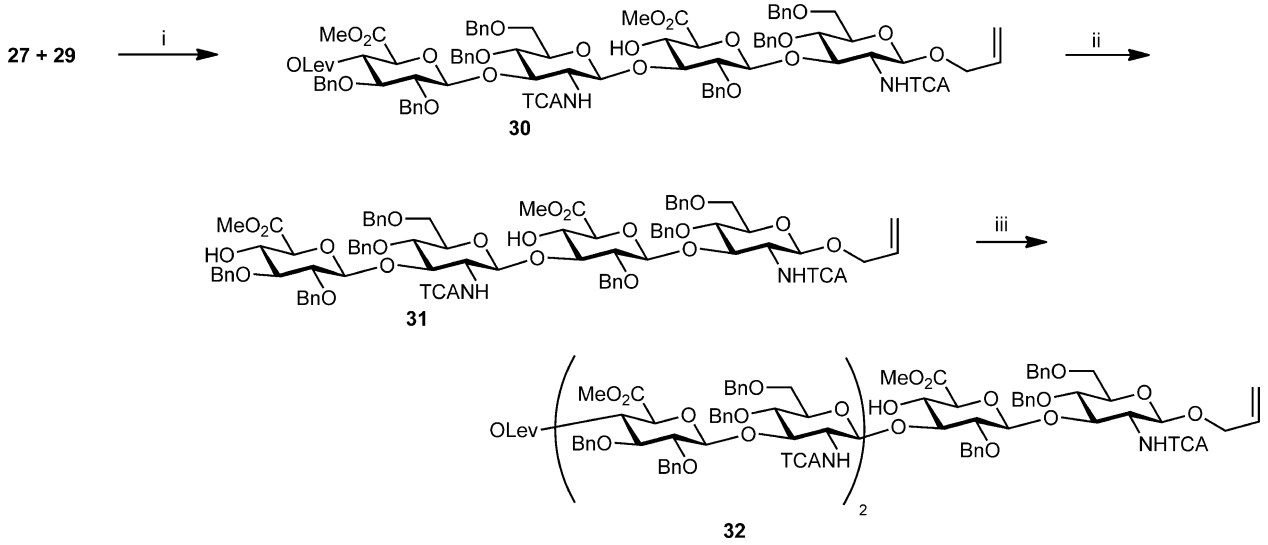
We envision that application of this phase-transfer benzylation reaction could afford a concise method for the production of building blocks of hyaluronic acid. When the donor and acceptor become larger, their reactivity decreases for steric and electronic reasons. Therefore, increasing the reactivity, especially for the uronic acid disaccharide acceptor, has become an important problem. To increase the reactivity of the acceptor, more electron-donating groups rather than electron-withdrawing groups should be installed on the glucuronic building block. In the former synthesis of hyaluronic acids, 2-Bz,3-Bn-protected uronic acid building blocks had been used several times to improve the reactivity of the block.[47b,50] We reasoned that if we could synthesize a disaccharide building block with a uronic acid residue bearing 2,3-dibenzyl protecting groups, the reactivity of the building block might be further improved. 2-Bz,3-Bn-protected uronic acid building blocks could be synthesized fairly easily; however, synthesizing a disaccharide building block with a 2,3-dibenzyluronic acid residue was much more difficult and has not yet been reported. The benzyl group cannot be installed on the 2-position of the glucuronic building block prior to glycosylation since neighboring group participation from an acyl group helps form the required beta-glycosidic linkage. Therefore, a method to affect benzylation on the disaccharide had to be developed to synthesize the disaccharide building block with a 2,3-dibenzylglucuronic acid residue. Our new benzylation method provides a unique strategy to install benzyl groups on the 2 and 3-positions of the GlcA building block.

From the easily obtained allyl-protected per-acetylated NHTCA substrate **21**,[48] in one pot using the new benzylation reaction described above, the GlcNHTCA building block **22** was obtained in good yield (Scheme 2). The glycosylation between **21** and **23**,[51] the precursor of the GlcA unit, went well to provide disaccharide **24** in high yield. The new benzylation reaction was performed on disaccharide **24** to readily provide the desired tetrabenzylated disaccharide **25** in 72% yield. The benzylidene group was then removed, and the primary hydroxy group was oxidized and protected as the methyl ester. The 4-OH on the GlcA unit was protected using a levulinate (Lev) group to give the fully assembled hyaluronic acid disaccharide building block **28**.

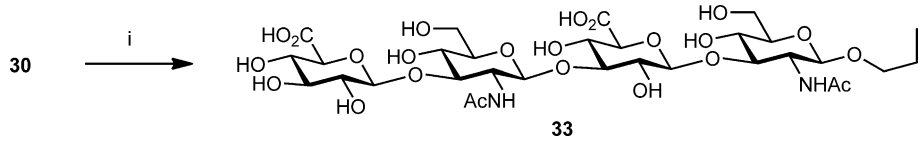
Trichloroacetamide donor **29** was obtained following routine steps. The corresponding tetrasaccharide



Scheme 2. Synthesis of the HA disaccharide building blocks using phase transfer benzylation reactions. *Reaction conditions:* i) BnBr, NaOH, 15-crown-6, THF, room temperature, 8 h, 77%; ii) TMSOTf, CH_2Cl_2, 0 °C, 1 h, 98%; iii) BnBr, NaOH, 15-crown-5, THF, room temperature, 36 h, 72%; iv) TFA, CH_2Cl_2-H_2O, 0 °C to room temperature, 30 min, 92%; v) a) TEMPO, BAIB, CH_2Cl_2-H_2O (2:1), room temperature, 1 h, b) CH_3I, K_2CO_3, DMF, room temperature, 2 h, 74% in two steps; vi) levulinic acid, DCC, DMAP, CH_2Cl_2, room temperature, 3 h, 96%; vii) a) $[Ir(COD)(PMePh_2)_2]PF_6$, THF then H_2 (10–20 sec), room temperature, 3 h, b) HgO, $HgCl_2$, acetone-H_2O (5:1, v/v), room temperature, 20 h, c) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 2 h, 67% in three steps.



Scheme 3. Synthesis of hyaluronic acid hexamer as its allyl glycoside. *Reaction conditions:* i) TMSOTf, CH_2Cl_2, 0 °C to room temperature, 3 h, 70%; i) NH_2NH_2·H_2O, allyl alcohol, pyridine-AcOH (4:1; v/v), 0 °C to room temperature, 2 h, 87%; iii) **29**, TMSOTf, CH_2Cl_2, 0 °C to room temperature, 1 h, 64%.



Scheme 4. Synthesis of hyaluronic acid tetramer as its *n*-propyl glycoside. *Reaction conditions:* i) a) Bu_3SnH, AIBN, benzene-dimethylacetamide (4:1; v/v), 80 °C, 2 h, b) 1 M aqueous LiOH, 30% H_2O_2, THF, H_2O, room temperature, 16 h; then 4 M NaOH, MeOH, room temprature, 22 h; c) 20% $Pd(OH)_2$-C, H_2, MeOH-H_2O (3:1, v/v), AcOH, room temperature, 22 h, 36% in three steps.

30 and hexasaccharide **32** were obtained in good yield after 2+2 and 2+4 glycosylations (Scheme 3). There are several reports on the chemical synthesis of hyaluronic acid oligomers where amino functionalities have been temporarily protected by trichloroacetyl groups and the conversion of multiple NHTCA→NHAc groups was performed successfully, firstly by deprotection of the TCA group in basic conditions, followed by *N*-acetylation.[17b,c,49] Direct conversion of NHTCA→NHAc was also reported by Bräse et al.[48] They used Zn/AcOH conditions for this purpose; however, yields were not satisfactory (only 15%) as they observed more of decomposition products. They also tried the conversion with a tributyltin hydride/AIBN-mediated radical reaction at elevated temperature. Although the method was successful with a disaccharide unit (one NHTCA group), only decomposition products were obtained when the approach was applied to an HA tetrasaccharide. They reasoned that the β-1,4 glycosyl bond of the tetrasaccharide was cleaved during the elevated temperature reaction. Earlier, Hsieh-Wilson and co-workers performed the conversion of NHTCA to NHAc successfully on a precursor of a chondroitin sulfate tetramer using Bu_3SnH/AIBN.[17d] They used dimethylacetamide as a co-solvent with benzene and performed the reaction at elevated temperature. We envisioned that a similar strategy could also be applied to hyaluronic acid oligomers and thereby provide an additional method to evaluate in the design of the global deprotection of fully protected hyaluronic acid oligomers. Tetramer **30** was chosen as an experimental target to see whether these modified conditions would also be successful with hyaluronic acid; gratifyingly, the NHTCA→NHAc conversion was completed within two hours (Scheme 4). This reaction, followed by saponification and hydrogenolysis, respectively, nicely provided the fully deprotected tetrasaccharide **33** in reasonable overall yield.

Conclusions

To summarize, a combination of BnBr/NaOH/15-crown-5 in THF is an efficient method for installing benzyl groups at the 4- and 6-positions on acetylated *N*-trichloroacetyl-protected 2-deoxy-2-amino sugars while leaving a free 3-position hydroxy for chain extension or further elaboration. The *N*-trichloroacetyl group was shown to be the key to obtaining the dibenzylated product. This new method then allowed a tetrabenzylated hyaluronic acid disaccharide building block to be readily synthesized. Based on this tetrabenzylated building block strategy, the tetramer and hexamer of hyaluronic acid were also easily made. We conclude that the greatly simplified method for selective benzylation on trichloroacetyl-protected 2-deoxy-2-amino sugars provides a convenient route to a previously under-utilized protecting group pattern not readily available by existing synthetic methods, and should provide a nice alternative strategy in the syntheses of a variety of glycosaminoglycans.

Experimental Section

Detailed experimental procedures and characterization data of synthetic compounds **2**--**33** are given in Supporting Information.

General Procedure for the Benzylation Reaction

To a solution of the acetylated derivative (1 equiv.) in anhydrous THF (10 mL mmol^{-1}) was added powdered base (12 equiv.) and 15-crown-5 (0.05 equiv. of base), respectively, and the reaction mixture was stirred at ambient temperature under an argon atmosphere for 1 h. BnBr (3 equiv.) was added to the slurry dropwise and the reaction mixture was stirred for 2–20 h (required time for each reaction is given on the Table 1 and Table 2). The reaction mixture was then filtered through Celite and washed with dichloromethane; the filtrate was concentrated under reduced pressure. The crude mixture was diluted with dichloromethane and washed with water and then brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Alternatively, an easier and quicker work-up procedure was performed as follows. The reaction mixture was neutralized with Dowex® 50WX8 (H^+), filtered and washed with ethyl acetate. The combined filtrate was concentrated under reduced pressure. The crude mixture was then purified through normal phase silica gel flash column chromatography using a hexane and ethyl acetate solvent system as the eluent to afford pure product. With this procedure, compounds **2**, **4**, **5**, **7**, **8**, **10**, **11**, **14**, **16**, **18**, **19a** and **22** were obtained.

Acknowledgements

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