Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 16 (2005) 411–424

α:β Selectivity in the synthesis of 3-substituted, 4-methyl umbelliferone glycosides of *N*-acetyl glucosamine and chitobiose

Anjali R. S. Ganguli and James K. Coward*

Departments of Medicinal Chemistry and Chemistry, The University of Michigan, Ann Arbor, MI 48109-1055, USA

Received 8 November 2004; accepted 19 November 2004
Available online 11 January 2005

Abstract—The influence of phenolic acceptor nucleophilicity; for example, 3-substituted, 4-methylumbelliferones, and glycosyl donor electrophilicity; for example, 3- and 4-substituted *N*-acetylglucosamines, on glycosylation stereochemistry has been evaluated. In a systematic comparison, the stereochemical outcome as well as the reaction yield appeared to be influenced by the 3- and 4-substituents of the donor as well as the 3-substituent of the aryl acceptor. In the context of synthesizing a fluorogenic substrate for oligosaccharyltransferase, an α-glycoside was desired. Although most acceptor–donor pairs led to predominantly or exclusively the β-glycoside, reaction of the most activated (3,4-di-*O*-benzyl) donor and the least nucleophilic acceptor (3-Br), resulted in a 1:1 ratio of α,β arylglycosides.

1. Introduction

One of the most sensitive methods of monitoring the progress of enzymatic reactions is by the use of a continuous fluorescent assay. In an effort to develop such an assay for use in studies of oligosaccharyltransferase (OST, EC 2.4.2.119) activity, we explored the insertion of a fluorescent leaving group to replace the naturally occurring pyrophosphate leaving group of the donor substrate, **1** (Fig. 1). Currently, glycosides of 4-methylumbelliferone (4-MU, 7-hydroxy-4-methyl coumarin) are commonly used as fluorogenic substrates to study a wide variety of glycosidases including chitinase.[1–5] Molecular modeling (INSIGHT II) indicated that derivatives of 4-MU, such as **2**, containing a 3-polyprenyl substituent, for example, dolichyl (C_{95}),[6] or undecaprenyl (C_{55}),[7] could serve as a neutral, isosteric leaving group and allow for development of a continuous fluorescence assay of OST activity.

Chitin hydrolyzing enzymes act on β-glycosides, and consequently the fluorogenic mono-, di-, and even trisaccharides have been obtained through an S_N2 displacement by the fluorogenic moiety of a readily accessible α-halo glycoside, derived from hydrolysis of chitin polymers.[1,8–10] Though this route produces the desired product efficiently, it is limited to the synthesis of β-glycosides. The donor used in the OST-catalyzed reaction contains an α-linked leaving group, requiring the corresponding fluorescent leaving group also to have the same α-stereochemistry. Very little precedent exists for the synthesis of 1,2-*cis*-phenolic 2-deoxy-2-acetamido glycosides, and the methods currently available result in very low yields.[11–15] This communication presents the results of studies on the synthesis of 4-MU glycosides of *N*-acetyl glucosamine (GlcNAc) as well as the β-1→3 and β-1→4 GlcNAc disaccharides.

2. Results

Three key transformations are required for the successful synthesis of chitobiose 4-MU-lipid, **2**: (1) formation of the disaccharide linkage, (2) formation of the α-(4-MU)-glycoside, and (3) attachment of the lipid to the umbelliferone ring (Fig. 1). It was initially unclear as to the order that these bonds should be constructed for optimal yield and stereoselectivity. Consequently, we studied the chemistry and reactivity of model compounds in order to design the appropriate synthetic route. These investigations would indicate whether a mono- or disaccharide should be used in the glycosylation reaction and when the lipid substituent at C-3 should be installed on the 4-MU core.

* Corresponding author. Tel.: +1 734 936 2843; fax: +1 734 647 4865; e-mail: jkcoward@umich.edu

0957-4166/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2004.11.053

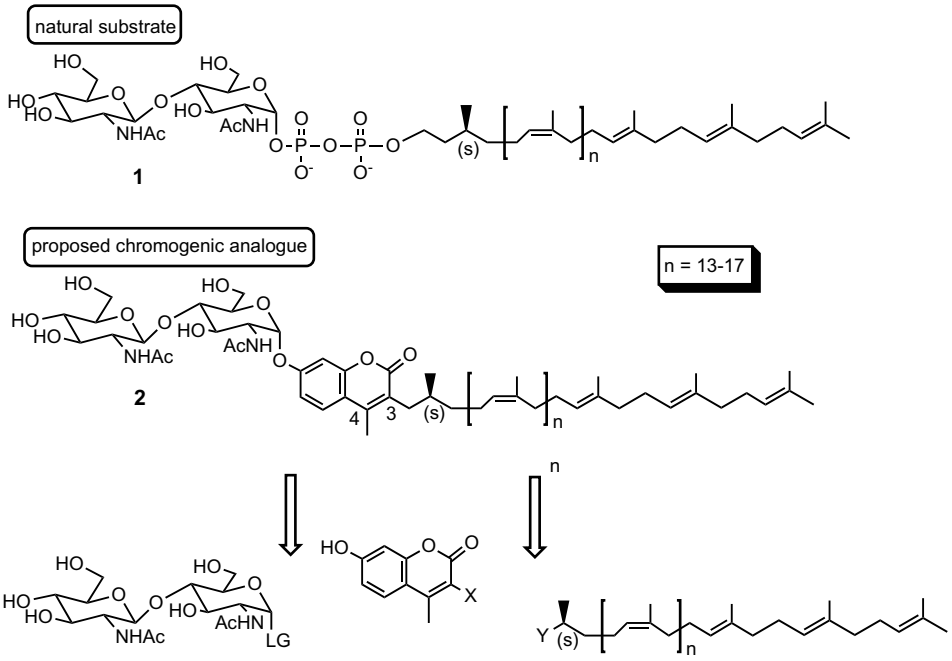
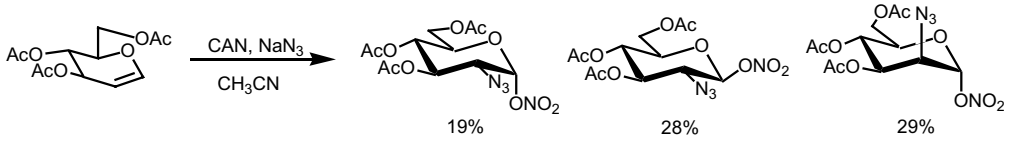


Figure 1.

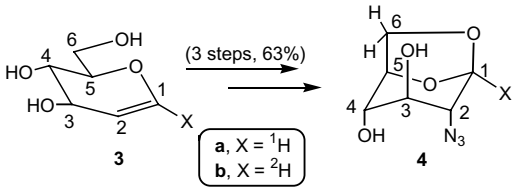
Traditionally, the synthesis of any 1,2-*cis*-glycoside begins by disguising the C-2 participatory group (such as an amide, ester, carbamate, etc.) as a small, nonparticipating moiety. For amides, the surrogate group is normally a C-2 azide since this substitution removes steric hindrance on the α-face, increasing the possibility of forming the 1,2-*cis* linkage, and is readily transformed back into the amide in one step.[16] There are two well-known methods for installing an azide at C-2 on the pyranose ring; either through azido-nitration of glycals[17,18] or via the anhydro sugar[19,20] methodology. The azido-nitration strategy allows for installation of the azide in one step, however it does not incorporate a means to discriminate between the C-3 and C-4 positions and requires extensive protecting group manipulations subsequent to azide installation. In addition, this method produces only low yields of the desired *gluco* product, which is difficult to purify from a mixture of *gluco* and *manno* isomers (Scheme 1).[17,18]

Incorporation of an azide through the anhydro route (Scheme 2) is more complicated but orients the pyranose in a conformation that inherently distinguishes between the C-3 and C-4 positions; the steric constraints are much greater on the C-3 hydroxyl due to the acetal and anhydro rings, as well as the C-6 CH_2 group.[21–23] The ability to distinguish between these positions reduces the number of protecting group manipulations required for synthesizing a variety of mono- and disaccharides. There are many additional benefits to working with anhydro sugars. In particular, these sugars are known to exhibit enhanced reactivity of the C-4 hydroxyl group over their unconstrained counterparts.[24] Since the C-4 hydroxyl will serve as the acceptor in formation of the β-1→4 disaccharide linkage of **2**, any improvement in reactivity is highly desirable. Although the β-1→4 disaccharide, chitobiose, is readily available via acid hydrolysis of chitin,[1,8,9,25,26] use of 1,4-anhydro sugars derived from isotopically enriched glucal **3b**[27,28] could permit installation of deuterium at C-1 of the anhydro sugar, **4b**, and lead ultimately to a C-1 deuterated isotopomer of **2**. This substitution would enable the study of α-secondary kinetic isotope effects on glycosyl transfer reactions (e.g., OST) using isotopically enriched donor substrates.[29]

Therefore, we chose the anhydro method over the azido-nitration method to access the azido sugar donors. The synthetic route began with a one-step oxidative cyclization of glucal **3**, into 1,6-anhydro-2-deoxy-2-iodo-β-D-glucopyranose **5**, using the method of Leteux and Veyrières.[19] The 2-iodo substituent is traditionally transformed into the 2-azido sugar **4**, through a one-pot displacement reaction.[20] However, purification of **4** from the reaction mixture involves a lengthy continuous extraction. Instead, we adapted a sequential method to

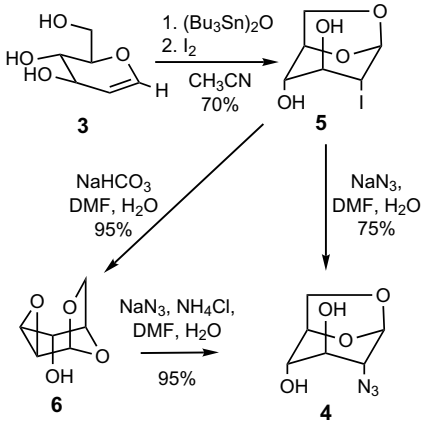


Scheme 1.



Scheme 2.

4 using known methods to first generate a 1,6:2,3-dianhydroglycopyranose, **6**,[30] and subsequently open the epoxide with ammonium chloride and sodium azide to yield **4**[31] (Scheme 3). Though it involved an extra purification step, the sequential method was preferable in our hands because it produced a 2-azido product in higher yields and purity.

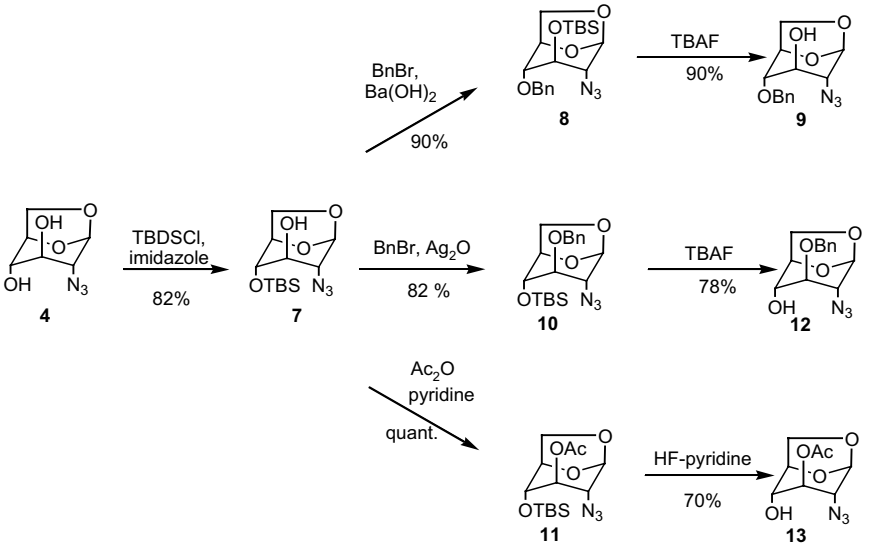


Scheme 3.

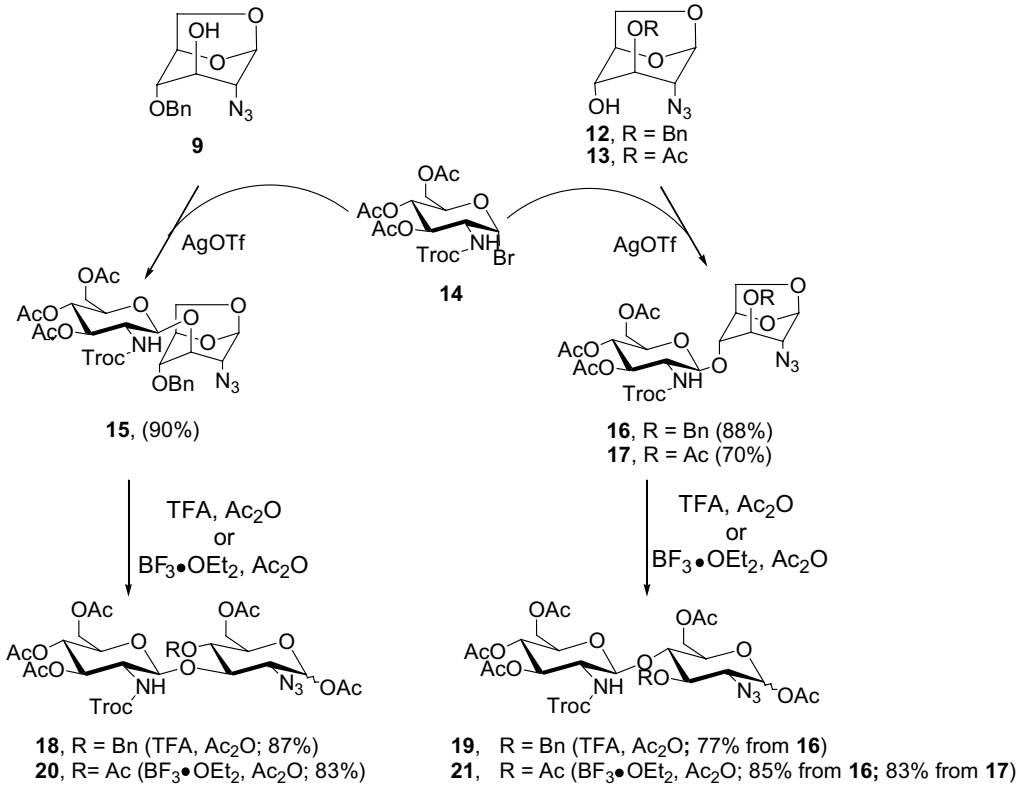
As mentioned, one can readily distinguish between the hydroxyl groups of **4** (C-3 vs C-4) and selectively form a TBS ether **7**, of the more accessible C-4 OH (Scheme 4).[21–23] Treatment of **7** with $Ba(OH)_2$ and BnBr, after the method first described by van Boeckel et al.,[21] effects stepwise migration of the TBS group from the hydroxyl at C-4 to that at C-3, followed by benzylation of the C-4 hydroxyl (**7**→**8**). Desilylation (TBAF) of **8** provides **9**, containing a free hydroxyl group at C-3. Alternatively, one can access the 4-hydroxyl regioisomers, **12** or **13**, from the same 4-OTBS molecule using less basic conditions (BnBr, Ag_2O (**7**→**10**) or Ac_2O, pyridine (**7**→**11**[32]) to prevent migration of the TBS group while protecting the 3-hydroxyl. Subsequent desilylation with TBAF or HF–pyridine then generates **12** and **13**, respectively, with a free hydroxyl group at C-4.

This methodology provides stable, readily available, and highly reactive glycosyl acceptor precursors to either a 1→3- or 1→4-linked disaccharide (vide infra). Monosaccharides **9**, **12**, and **13** were then either transformed directly into the monosaccharide donors or elaborated into disaccharide donor molecules, both of which can be used to explore the stereochemistry of aryl glycoside formation.

To synthesize the disaccharides, 2-deoxy-2-(2,2,2-trichloroethanoxycarbonylamino)glucopyranosyl bromide, **14**, was chosen as the donor. The 2,2,2-trichloroethanoxycarbonyl (Troc) group has recently gained popularity in glycosylation reactions because, unlike the 2-acetamido moiety, it can regioselectively direct reactions without the formation of a formal dioxocarbenium species.[33] This property gives rise to the stereoselective formation of β-glycosides in high yields.[34] The Troc group is also favored because it can be easily and selectively removed under very mild conditions (Zn–HOAc) that do not affect most other protecting groups used in carbohydrate chemistry.[35] Coupling of the acceptors with the *N*-Troc glycosyl bromide **14**, proceeded efficiently using AgOTf as the promoter. Use of 4-*O*-benzyl **9** or 3-*O*-benzyl **12** acceptors, produced 1→3-**15** and 1→4-**16** disaccharides, respectively, in high



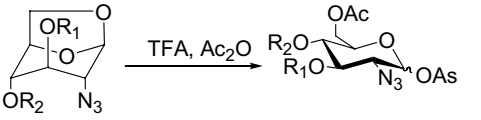
Scheme 4.



Scheme 5.

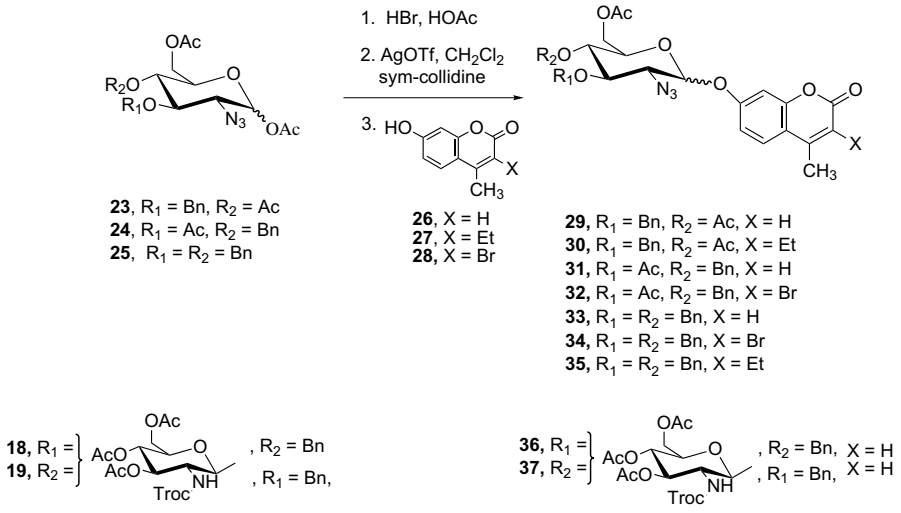
yields (Scheme 5). Use of the 3-*O*-acetyl acceptor, **13**, resulted in somewhat lower yields of the disaccharide product, **17**, presumably due to the stronger electron-withdrawing properties of the ester (*O*-acetyl) versus ether (*O*-benzyl) protecting group at C-3; this effect has considerable precedent in glycosylation reactivity studies.[36–41] After forming the disaccharide bond, the anhydro ring could be opened by one of two methods. TFA/Ac_2O-mediated reaction (**15**→**18** or **16**→**19**) furnished the disaccharide in high purity and in good yield, but was quite slow and required 3 days for completion. In contrast, the alternate method ($BF_3{\cdot}OEt_2$, Ac_2O) proceeded much faster, leading to anhydro sugar ring opening in 20 min at 0 °C[42] (**15**→**20**, **16**, or **17**→**21**); however, partial conversion of the benzyl ether (**15** or **16**) to the acetate ester (**20** or **21**) was observed. Complete removal of the benzyl protecting group (**15**→**20** or **16**→**21**) required 15 h at 4 °C then 2 h at rt, but the prolonged reaction time also caused 5–10% cleavage of the disaccharide linkage.

The anhydro monosaccharides **9**, **12**, and **22** were similarly transformed into the glycosyl acetates (Scheme 6). Starting from 4-*O*-benzyl-3-hydroxy-2-azido anhydro sugar **9**, or 3-*O*-benzyl-4-hydroxy-2-azido anhydro sugar **12**, TFA/Ac_2O-mediated ring opening gave either the 1,3,6-tri-*O*-acetyl-4-*O*-benzyl-2-azido-2-deoxy-α,β-D-glucopyranose **23**[50] or 1,4,6-tri-*O*-acetyl-3-*O*-benzyl-2-azido-2-deoxy-α,β-D-glucopyranose **24**,[20] respectively. Unlike the slow reaction of the disaccharides, ring opening of the monosaccharides proceeded overnight at room temperature. One can also access the 1,6-di-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-α,β-D-glucopyranose, **25**,[20] from the diol precursor, 1,6-anhydro-2-deoxy-2-azido-glucose **4**, via the 3,4-dibenzyl anhydro sugar **22**[43] followed by TFA/Ac_2O ring opening. Protection of the sugar hydroxyl with electron-donating benzyl ethers was desired because of the known rate-enhancing effect of benzyl ethers on the reactivity of glycosyl donors.[37,38,40,44] These monosaccharides were investigated for their ability to form 3-substituted, 4-MU glycosides as potential precursors of **2**.



Scheme 6.

In order to determine the optimal glycosylation partners for the synthesis of various 3-substituted, 4-MU glycosides of GlcNAc-containing disaccharides, three glycosyl acceptors, 4-MU **26**, as well as the 3-ethyl, **27**, and 3-bromo, **28**, derivatives, were studied. Since the 4-MU acceptor is a weak nucleophile ($pK_a = 7.8$),[45] it was anticipated that enhanced reactivity of the glycosyl donor would be necessary to achieve acceptable yields of the 4-MU glycoside products. Conversely, a change in



Scheme 7.

nucleophilicity of the acceptor, due to the effect of C-3 substituents on the phenolic pK_a,[45] could affect the yield and/or α:β selectivity of the glycosylation reaction. In fact, this trend was observed within the monosaccharide series used in the research reported herein. The ethyl coumarin **27**, was synthesized as described by Chakravarti,[46] and used as a model system to explore the effects of an alkyl substituent at C-3 on glycosylation stereoselectivity in case attachment of the lipid at C-3 prior to glycosylation was required. Bromination at C-3 of the umbelliferone ring system to provide **28** was effected using NBS.[47] The bromo substituent at C-3 on the coumarin ring system should allow for lipid attachment using transmetalation reactions (e.g., Suzuki cross-coupling) subsequent to glycosylation.

The ring-open disaccharides, **18**–**21**, and monosaccharides, **23**–**25** were transformed into the corresponding 1-bromo glycopyranosyl donors in quantitative yield using HBr/HOAc. Subsequent glycosylation reactions followed the approach developed by Crich et al. in their efforts to prepare 1,2-*cis*-glycosides in the *manno* series,[48] namely in situ isomerization of the α-bromide to the β-triflate, followed by glycosylation with the incoming acceptor (Scheme 7). Unfortunately, this method did not provide a satisfactory means to the desired 1,2-*cis*-glycoside with our system. However, a profound influence of the protecting groups on the stereochemical outcome of the glycosylation reaction was observed (Table 1). In the series using 4-MU, **26**, as the glycosyl acceptor, only donor **25**, containing two electron-donating (benzyl) hydroxyl-protecting groups, produced any of the desired 1,2-*cis* linkage. In the cases where even one of these benzyl groups was replaced with an electron-withdrawing (acetyl) group (**23**, **24**), no 1,2-*cis* (α) linkage was formed. The alkyl-substituted, 3-ethyl-4-MU acceptor **27**, showed slightly better selectivity over the unsubstituted 4-MU acceptor with the di-*O*-benzyl donor **25**, although the undesired 1,2-*trans* product still predominated. And not surprisingly, acceptor **27** showed the same selectivity as **26** with the mono-*O*-benzylated donor **23**, to give only the 1,2-*trans* product. In contrast, when 3-bromo-4-MU **28**, was the acceptor, increased amounts of the 1,2-*cis* linkage was observed with either the mono- or di-*O*-benzylated donors, **24** and **25**, though neither gave stereoselective formation of the 1,2-*cis* product.

Table 1. Effect of donor and acceptor structures on α:β selectivity

Saccharide donor	Phenolic acceptor (X)	Glycoside product	α (%)	β (%)	Yield (%)
Monosaccharides					
23	**26** (H)	**29**	0	100	64
24	**26** (H)	**31**	0	100	54
25	**26** (H)	**33**	17	83	77
23	**27** (Et)	**30**	0	100	43
25	**27** (Et)	**35**	9	91	58
24	**28** (Br)	**32**	44	56	64
25	**28** (Br)	**34**	59	41	50
Disaccharides					
18	**26** (H)	**36**	0	100	33
19	**26** (H)	**37**	0	100	34

These results suggest that decreasing the nucleophilicity of the phenol acceptor through the incorporation of electron-withdrawing substituents tends to favor formation of the desired α-linked product. Overall, substitutions on both the glycosyl donors and acceptors exert important influences over reaction reactivity and stereoselectivity.[41,44] These results led to selection of glycosyl bromide donors derived from the benzylated disaccharides **18** and **19** rather than the less reactive acetylated versions **20** and **21**, to extend the investigation from monosaccharides to disaccharides. However, as seen previously with the monosaccharide donors, the presence of only a single electron-donating group (3- or 4-OBn) was not sufficient to produce any of the 1,2-*cis*-glycosylation product. Since the yields and α-selectivity of the glycosylation reaction were very poor with the unsubstituted 4-MU acceptor **26**, attempts to couple the disaccharide to 3-ethyl-4-MU **27**, or 3-bromo-4-MU **28**, were not pursued.

3. Discussion

To develop an efficient method for synthesizing an aryl glycoside *cis* to an amide, a nonparticipating group was desired at C-2 as a surrogate for the NHAc group found in 2-deoxy-2-acetamido sugars. An azide is a well known nonparticipating amide surrogate[16] because it can be easily transformed to an acetamido group. Therefore, synthesis of the fluorogenic compound **2**, began with formation of 1,6-anhydro-2-deoxy-2-azido-β-D-glucopyranose **4**, from glucal **3**, by adapting established literature procedures. Further elaboration of the anhydro skeleton provided access to a variety of monosaccharide synthons from which both 1→3 and 1→4 disaccharides were obtained.

Masking the C-2-amide was, however, insufficient for effecting a stereoselective synthesis of the desired 1,2-*cis* linkage between the sugar donor and the fluorescent 4-MU acceptor. The systematic comparison of glycosylation efficiency performed for a variety of donors and acceptors in this study has shown that effects of the substituents on the pyranose donor as well as the electronic properties of the phenolic acceptor play influential roles in governing the stereochemical outcome of the glycosylation reactions (Table 1). These effects have been discussed previously when comparing efficiencies of carbohydrate donors and acceptors,[36–41,44] and further emphasize the importance of protecting group strategy in the design and synthesis of complex carbohydrates. The highest yield of an α-aryl glycoside was obtained using the Crich activation method to link the 3-bromo acceptor **28**, with the 3,4-di-*O*-benzyl donor **25**. In this case, ca. 1:1 α:β mixture of $GlcN_3$-*O*-(3-Br-4-MU) **34**, was obtained in 50% yield. This route could be useful for isolating sufficient amounts of **34** to complete the synthesis of the substrate analogue **2**. The C-3 bromide should provide a useful handle to direct alkylation of the polyisoprene chain onto the umbelliferone ring to complete the synthesis. Dasgupta and Masuda[49] recently reported the isolation of a 3:2 α:β mixture of glycosides when coupling 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy glucopyranosyl fluoride with 7-*O*-trimethylsilyl-4-methylcoumarin (4-MU-OTMS). Taking their data together with the data presented in this paper, it is evident that distal substituents as well as the nature of the leaving group leading to the transient oxocarbenium ion, at C-1 of the donor, strongly influence the stereochemical outcome of the glycosylation reaction.

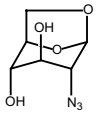
In conclusion, a systematic study of aryl glycoside formation within a series of 3-substituted, 4-methyl-umbelliferone (4-MU) acceptors, and mono- and disaccharide donors has led to conditions whereby one can synthesize aryl glycosides of GlcNAc and chitobiose. The β-glycoside of both GlcNAc and chitobiose is favored in the case of 4-MU acceptors with either H or an alkyl group at the 3-position. In contrast, a 1:1 mixture of α,β glycosides is obtained with a 3-Br-4-MU acceptor. Due to the differential protection of the carbohydrate moieties of the donors, the approach described herein allows for the synthesis of complex GlcNAc-containing oligosaccharides possessing a fluorescent leaving group that could be of great use in the development of continuous fluorescence assays of previously uninvestigated glycosidases and glycosyltransferases.

4. Experimental

4.1. General methods

All air- and moisture-sensitive reactions were carried out under nitrogen or argon using oven-dried glassware, and distilled solvents (MeOH, Et_3N, $^iPr_2NH$, and CH_2Cl_2 distilled from CaH_2, THF distilled from Na–benzophenone). All flash column chromatography was carried out using silica gel (40–60 μm, 230–400 mesh) as the stationary phase, and thin layer chromatography preformed on Whatman 250 μm silica gel plates. All NMR spectra were recorded on Bruker AVANCE DRX300 or DRX500 spectrometers. ^1H NMR spectra were recorded at 300 or 500 MHz and are reported as follows: chemical shifts in ppm downfield from internal tetramethylsilate (multiplicity, integrated intensity, coupling constant in Hz). ^{13}C NMR spectra were obtained at 75 or 126 MHz and referenced to tetramethylsilane. ^{31}P NMR spectra were recorded at 121 MHz with 1% aqueous phosphoric acid as an external reference. All ^{13}C and ^{31}P NMR spectra are proton decoupled. MS data for chlorinated and brominated compounds contain multiple peaks correlated to the natural abundance ratios of the halogen isotopes. Common reagent chemicals were purchased from commercial sources. The following carbohydrates were synthesized as described in the literature; 1,6-anhydro-2-deoxy-2-iodo-β-D-glucopyranose **5**,[19] 1,6-anhydro-2-azido-2-deoxy-4-*O*-(*tert*-butyl-dimethylsilyl)-glucopyranose **7**,[21–23] 1,6-anhydro-2-azido-2-deoxy-4-*O*-benzyl-3-*O*-(*tert*-butyl-dimethylsilyl)-glucopyranose **8**,[21] 1,6-anhydro-2-azido-2-deoxy-4-*O*-benzyl-glucopyranose **9**,[21] 1,6-anhydro-3-*O*-acetyl-2-azido-2-deoxy-4-*O*-(*tert*-butyl-dimethylsilyl)-glucopyranose **11**,[32] 1,6-anhydro-3-*O*-acetyl-2-azido-2-deoxy-glucopyranose **13**,[32] 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-glycopyranosyl bromide **14**[34] 1,6-anhydro-2-azido-2-deoxy-3,4-di-*O*-benzyl-β-D-glucopyranose **22**,[20,43] 1,3,6-tri-*O*-acetyl-2-azido-2-deoxy-4-*O*-benzyl-β-D-glucopyranose **23**,[50] 1,4,6-tri-*O*-acetyl-2-azido-2-deoxy-3-*O*-benzyl-β-D-glucopyranose **24**,[20] 1,6-di-*O*-acetyl-2-azido-2-deoxy-3,4-di-*O*-benzyl-β-D-glucopyranose **25**,[20] 4-methylumbelliferone **26**,[46] and 3-ethyl-4-methylumbelliferone **27**.[46]

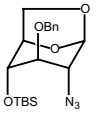
4.2. 1,6-Anhydro-2-azido-2-deoxy-β-D-glucopyranose 4



A heterogeneous solution of 1,6-anhydro-2-deoxy-2-iodo-β-D-glucopyranose **5** (3.47 g, 12.8 mmol) and $NaHCO_3$ (2.52 g, 30 mmol) in DMF–H_2O (10:1) was

heated to 120 °C. After 4 h, the reaction mixture was cooled, concentrated (in vacuo) and subjected to chromatography (SiO_2, EtOAc containing 0–10% MeOH) to yield 1,6:2,3-di-anhydro-β-D-glucopyranose **6**, in quantitative yield (1.84 g, 12.8 mmol). ^{1}H NMR ($CDCl_3$): δ 3.13 (d, 1H, H-2, $J_{2,1}$ = 3.3 Hz), 3.47 (dd, 1H, H-4, $J_{4,3}$ = 2.7 Hz, $J_{4,5}$ = 2.7 Hz), 3.68 (m, 2H, H-3, H-6), 3.93 (d, 1H, H-6, $J_{6,6}$ = 5.7 Hz), 4.44 (m, 1H, H-5), 5.3 (d, 1H, OH, J = 6.3 Hz), 5.7 (d, 1H, H-1β, $J_{1\beta,2}$ = 2.7 Hz,). ^{13}C NMR ($CDCl_3$): δ 49.5, 54.1, 65.6, 66.8, 75.9, 100.5. The di-anhydro sugar **6** (1.84 g, 12.8 mmol) was heated to reflux temperature in a 10:1 MeOH–H_2O solution containing NaN_3 (8.33 g, 128 mmol), and NH_4Cl (5.14 g, 96 mmol). After ^{1}H NMR showed complete conversion to the azide **4** (4.5 days), the solution was cooled and concentrated (in vacuo). Column chromatography yielded pure product **4**, as a reddish semi-solid (2.2 g, 92%). IR (cm^{-1}): 3428.1, 2113, 1263, 1050. ^{1}H NMR ($CDCl_3$): δ 3.02 (s, 1H, H-2), 3.41 (s, 1H, H-4), 3.55 (m, 2H, H-3, H-6), 3.93 (d, 1H, H-6, $J_{6,5}$ = 6.9 Hz), 4.43 (m, 1H, H-5), 5.28 (d, 1H, OH, J = 3.6 Hz), 5.38 (s, 1H, H-1β), 5.41 (d, 1H, OH, J = 4.2 Hz). ^{13}C NMR (DMSO): δ 100.7, 77.2, 72.4, 71.5, 65.8, 62.9.

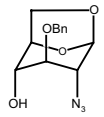
4.3. 1,6-Anhydro-2-azido-2-deoxy-3-*O*-benzyl-4-*O*-(*tert*-butyl-dimethylsilyl)-β-D-glucopyranose 10



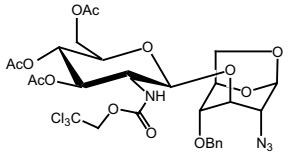
Benzyl bromide (32 μL, 0.30 mmol) was added to a solution of **7**, (75 mg, 0.25 mmol) and Ag_2O (92 mg, 0.4 mmol) in DMF (2 mL) and stirred at room temperature. After 15 h, the reaction mixture was filtered through Celite (1:1 hexanes–EtOAc) diluted with 20 mL of 1:1 hexanes–EtOAc and 20 mL water and stirred vigorously for 45 min. The aqueous layer was re-extracted with hexanes–EtOAc; and the pooled organic layers were washed with water, satd aq $NaHCO_3$, and brine, then dried (Na_2SO_4), filtered, and concentrated (in vacuo). Column chromatography (SiO_2, 20:1 hexanes–EtOAc) afforded pure **10** (80 mg, 82%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 0.16 (s, 6H, 2CH_3), 0.93 (s, 9H, *t*-Bu–CH_3), 3.13 (s, 1H, H-2), 3.67–3.77 (m, 3H, H-3, H-6, H-4), 4.06 (d, 1H, H-6, $J_{6,6}$ = 7.5 Hz), 4.47 (m, 1H, H-5), 4.7 (s, 2H, CH_2Ph), 5.51 (s, 1H, H-1β) 7.30 (m, 5H, Ph–*H*). ^{13}C NMR ($CDCl_3$): δ −4.5, −4.4, 26.1, 59.2, 65.5, 70.9, 72.7, 77.6, 79.6, 101.1, 128.2, 128.5, 128.9, 137.7. MS (ESI) *m*/*z* (%) 414.2 (100.0, $(M+Na)^+$). HRMS (ESI) calcd for $C_{19}O_4N_3H_{30}SiNa$ $(M+Na)^+$ 414.1825. Found 414.1831.

4.4. 1,6-Anhydro-2-azido-2-deoxy-3-*O*-benzyl-β-D-glucopyranose 12

TBAF (1 M in THF, 0.23 mL, 0.23 mmol) was added dropwise to a 0 °C solution of **10** (76 mg, 0.195 mmol) in THF (2 mL), and slowly warmed to room temperature. After 1.5 h, the reaction was concentrated (in vacuo). Column chromatography (SiO_2, 1.5:1 hexanes–EtOAc–2% MeOH) yielded pure product **12** (40 mg, 78%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 3.52 (s, 1H, H-2), 3.67 (s, 1H, H-3), 3.81 (dd, 1H, H-6, $J_{6,5}$ = 7.6 Hz, $J_{6,6}$ = 5.4 Hz), 3.90 (m, 2H, H-4, OH), 4.25 (dd, 1H, H-6, $J_{6,5}$ = 1.4 Hz, $J_{6,6}$ = 5.8 Hz), 4.64–4.5 (m, 3H, H-5, CH_2Ph), 5.51 (s, 1H, H-1β), 7.35 (m, 5H, Ph–*H*). ^{13}C NMR ($CDCl_3$): δ 59.9, 65.5, 69.2, 72.9, 76.7, 78.4, 100.5, 128.1, 128.5, 129.0, 137.6. MS (CI) *m*/*z* (%) 295.0 (100.0, $(M+NH_4)^+$). HRMS (CI) calcd for $C_{13}O_4N_4H_{14}$ $(M+NH_4)^+$ 295.1406. Found 295.1400.

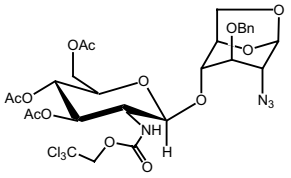


4.5. 1,6-Anhydro-2-azido-4-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranose 15



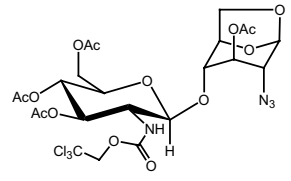
A solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl bromide **14** (0.35 g, 0.63 mmol), 1,6-anhydro-2-azido-2-deoxy-4-*O*-benzyl-β-D-glucopyranose, **9** (0.125 g, 0.45 mmol) and 3 Å molecular sieves (0.05 g) in freshly distilled CH_2Cl_2 (8 mL) was stirred at room temperature for 15 min, then cooled to 0 °C. After 30 min, AgOTf (0.231 g, 0.90 mmol) was added and the solution warmed slowly to room temperature. After 15 h, the reaction was filtered through Celite (CH_2Cl_2), washed with water, satd aq $NaHCO_3$, and brine, then dried (Na_2SO_4), filtered, and concentrated (in vacuo) to obtain the crude product. Column chromatography (SiO_2, 2:1 hexanes–EtOAc) afforded pure product, **15** (0.320 g, 90%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 2.01–2.04 (s, 9H, 3CH_3), 3.10 (s, 1H, H-2), 3.56 (s, 1H, H-3), 3.62–3.71 (m, 3H, H-4, H-5′, H-2′), 4.0 (m, 2H, H-6, H-5), 4.12 (dd, 1H, H-6′, $J_{6',6'}$ = 12.3 Hz, $J_{6',5'}$ = 1.9 Hz), 4.23 (dd, 1H, H-6′, $J_{6',6'}$ = 12.4 Hz, $J_{6',5'}$ = 5 Hz), 4.62–4.83 (m, 6H, CH_2Ph, TrocCH_2, H-1′β, H-6), 5.07 (dd, 1H, H-4′, $J_{4',3'}$ = 9.7 Hz, $J_{4',5'}$ = 9.7 Hz), 5.20 (m, 2H, H-3′, NH), 5.51 (s, 1H, H-1β), 7.33–7.42 (m, 5H, Ph–*H*). ^{13}C NMR ($CDCl_3$): δ 21.0, 21.1, 21.5, 53.9, 56.6, 60.9, 62.3, 65.4, 68.7, 71.7, 72.5, 74.7, 75.0, 77.6, 95.7, 100.5, 128.3, 128.5, 128.9, 137.8, 157.9, 169.8, 171.1, 170.9, 171.1. MS (ESI) *m*/*z* (%) 761.1 (94.43, $(M+Na)^+$), 763.1 (100.0), 765.1 (33.92), 767.1 (6.60). HRMS (ESI) calcd for $C_{28}H_{33}Cl_3N_4O_{13}Na$ $(M+Na)^+$ 761.1007. Found 761.1011.

4.6. 1,6-Anhydro-2-deoxy-2-azido-3-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonyl-amino)-β-D-glucopyranosyl)-β-D-glucopyranose 16



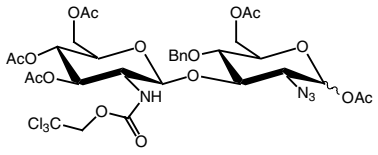
A solution of the 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl bromide **14** (106 mg, 0.191 mmol), 1,6-anhydro-2-de-oxy-2-azido-3-*O*-benzyl-β-D-glucopyranose, **12** (35 mg, 0.127 mmol), and 3 Å molecular sieves (35 mg) in freshly distilled CH_2Cl_2 (3 mL) was stirred at room temperature for 15 min, then cooled to 0 °C. After 30 min, AgOTf (75 mg, 0.29 mmol) was added and the solution warmed slowly to room temperature. After 15 h, the reaction was filtered through Celite (CH_2Cl_2), washed with water, satd aq $NaHCO_3$, and brine, then dried (Na_2SO_4), filtered, and concentrated (in vacuo). Column chromatography (SiO_2, 1:1 hexanes–EtOAc) yielded pure **16** (0.410 g, 88%) as a clear colorless oil. ^{1}H NMR ($CDCl_3$): δ 2.02 (m, 9H, 3CH_3), 3.17 (s, 1H, H-2), 3.39 (m, 1H, H-2′), 3.71-3.83 (m, 4H, H-3, H-4, H-5′, H-6), 4.09 (m, 2H, H-6, H-6′), 4.19 (m, 1H, H-6′, $J_{6',5'}$ = 4.4 Hz, $J_{6',6'}$ = 12.8 Hz), 4.56–4.79 (m, 5H, H-5, TrocCH_2, CH_2Ph), 5.04 (dd, 1H, H-4′, $J_{4',3'}$ = 9.6 Hz, $J_{4',3'}$ = 9.7 Hz), 5.18 (d, 1H, H-1′β, $J_{1',2'}$ = 8.0 Hz), 5.48 (s, 1H, H-1), 5.55 (m, 2H, H-3′, NH) 7.33–7.42 (m, 5H, Ph–*H*). ^{13}C NMR ($CDCl_3$): δ 21.1, 56.9, 59.4, 62.3, 65.3, 69.2, 71.4, 72.3, 72.7, 74.1, 74.8, 75.6, 77.3, 95.8, 98.9, 101.0, 128.0, 128.5, 129.0, 137.7, 154.4, 169.9, 170.78, 171.0. MS (ESI) *m/z* (%) 761.1 (94.87, $(M+Na)^+$), 763.1 (100.0), 765.1 (35.90), 767.1 (5.22). HRMS (ESI) calcd for $C_{28}O_{13}N_4H_{33}Cl_3Na$ $(M+Na)^+$ 761.1007. Found 761.1006.

4.7. 1,6-Anhydro-3-*O*-acetyl-2-azido-2-deoxy-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonyl-amino)-β-D-glucopyranosyl)-β-D-glucopyranose 17



A solution of the 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl bromide **14** (0.65 g, 1.19 mmol), 1,6-anhydro-2-deoxy-2-azido-3-*O*-acetyl-β-D-glucopyranose, **13** (0.195 g, 0.78 mmol) and 3 Å molecular sieves (0.05 g) in freshly distilled CH_2Cl_2 (25 mL) was stirred at room temperature for 15 min, then cooled to 0 °C. After 30 min, AgOTf (0.442 g, 1.72 mmol) was added and the solution warmed slowly to room temperature. After 15 h, the reaction was filtered through Celite (CH_2Cl_2), washed with water, satd aq $NaHCO_3$, and brine, then dried (Na_2SO_4), filtered, and concentrated (in vacuo). Column chromatography (SiO_2, 1:1 hexanes–EtOAc) yielded pure **17** (0.410 g, 70%) as a clear colorless oil. ^{1}H NMR ($CDCl_3$): δ 2.04–2.1 (m, 12H, 4CH_3), 3.19 (s, 1H, H-2), 3.49 (m, 1H, H-2′), 3.67 (s, 1H, H-4), 3.79–3.87 (m, 2H, H-5′, H-6), 4.03 (d, 1H, H-6, $J_{6,6}$ = 7.6 Hz), 4.25 (m, 2H, H-6′, H-6′), 4.65 (m, 2H, 1/2 TrocCH_2, H-5), 4.87 (d, 1H, 1/2 TrocCH_2, J = 12.0 Hz), 5.08 (dd, 1H, H-4′, $J_{4',3'}$ = 9.4 Hz, $J_{4',5'}$ = 9.0 Hz) 5.36 (d, 1H, NH, $J_{NH,H2}$ = 8.1 Hz), 5.48 (s, 1H, H-1), 5.58 (dd, 1H, H-3′, $J_{3',4'}$ = 9.8, $J_{3',2'}$ = 9.2 Hz). ^{13}C NMR ($CDCl_3$): δ 21.1, 21.1, 21.4, 23.1, 56.9, 59.0, 62.3, 65.3, 69.2, 70.7, 71.5, 72.5, 74.4, 74.8, 75.7, 95.8, 99.67, 100.6, 154.3, 169.8, 169.9, 170.7, 171.1. MS (ESI) *m/z* (%) 713.0 (99.30, $(M+Na)^+$), 715.0 (99.90), 717.0 (35.61), 719.0 (5.38). HRMS (ESI) calcd for $C_{23}O_{14}N_4H_{29}Cl_3Na$ $(M+Na)^+$ 713.0644. Found 713.0645.

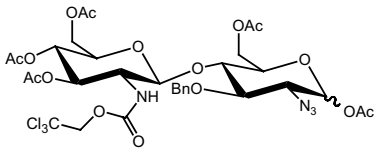
4.8. 1,6-Di-*O*-acetyl-2-azido-4-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-2-deoxy-α,β-D-glucopyranose 18



Trifluoroacetic acid (63 mg, 0.55 mmol) was added to a solution of disaccharide **15** (0.10 g, 0.14 mmol) in acetic anhydride (0.429 g, 4.2 mmol), and stirred at room temperature. After 3 days, the reaction was concentrated (in vacuo), and the residual acid removed by multiple triturations with toluene. Column chromatography (SiO_2, 2:1 hexanes–EtOAc) afforded pure product, **18** (0.103 g, 87%) as a light yellow semi-solid. ^{1}H NMR ($CDCl_3$): δ 2.10–2.21 (m, 15H, 5 CH_3), 3.56 (m, 1H, H-2′), 3.75 (m, 1H, H-5′), 3.89 (m, 2H, H-2, H-5), 4.07–4.34 (m, 5H, H-6, H-6, H-6′, H-4, H-3), 4.55 (dd, 1H, H-6′, $J_{6',6'}$ = 12.4 Hz, $J_{6',5'}$ = 4.0 Hz), 4.80 (m, 3H, 1/2 CH_2Ph, TrocCH_2), 4.92 (d, 1H, H-1′β, $J_{1',2'}$ = 8.3 Hz), 5.05 (d, 1H, 1/2 CH_2Ph, J = 10.5 Hz), 5.16 (dd, 1H, H-4′, $J_{4',3'}$ = 9.74 Hz, $J_{4',5'}$ = 9.7 Hz), 5.27 (dd, 1H, H-3′, $J_{3',4'}$ = 10.02 Hz, $J_{3',2'}$ = 9.2 Hz) 5.48 (d, 1H, NH, $J_{NH,H2}$ = 9.2 Hz), 5.75 (d, 1H, H-1β, $J_{1,2}$ = 8.7 Hz), 6.27 (d, 1H, H-1α, $J_{1,2}$ = 3.5 Hz), 7.3 (m, 5H, Ph–*H*). ^{13}C NMR ($CDCl_3$): δ 21.0, 21.2, 21.4, 21.4, 21.5, 23.0, 62.2, 62.2, 63.2, 65.2, 68.7, 71.2, 72.3, 72.5, 74.9, 75.5, 78.9, 90.5, 95.7, 100.5, 128.6, 128.7, 128.8, 137.8, 154.9, 169.1, 170.9, 171.0, 171.2. MS (ESI) *m/z* (%) 863.2 (98.27, $(M+Na)^+$), 865.2 (100.0), 867.2 (39.11), 869.2 (6.63). HRMS (ESI) calcd for $C_{32}O_{16}N_4H_{39}Cl_3Na$ $(M+Na)^+$ 831.1324. Found 831.1327.

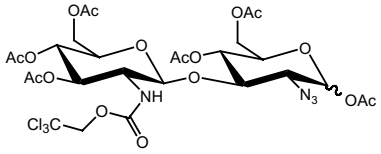
4.9. 1,6-Di-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-2-deoxy-α,β-D-glucopyranose 19

Trifluoroacetic acid (20 μL, 0.264 mmol) was added to a solution of disaccharide **16** (48 mg, 0.07 mmol) in acetic



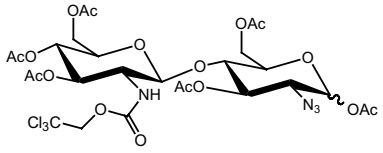
anhydride (0.373 mL, 3.95 mmol), and stirred at room temperature. After 3 days, the reaction was concentrated (in vacuo), and the residual acid removed by multiple triturations with toluene. Column chromatography (SiO_2, 2:3 hexanes–EtOAc) afforded pure product, **19** (42 mg, 77%) as a light yellow semi-solid. ^{1}H NMR ($CDCl_3$): δ 1.95–2.17 (m, 15H, 5CH_3), 3.54 (m, 3H, H-2′, H-5′, H-3), 3.87 (m, 4H, H-4, H-5, H-2, H-6′), 4.19 (m, 2H, H-6, H-6′), 4.47 (dd, 1H, H-6, $J_{6,6}$ = 12.0 Hz), 4.60–4.86 (m, 4H, NH, TrocCH_2, H-1′β), 4.98–5.12 (m, 4H, CH_2Ph, H-4′, H-3′) 6.21 (d, 1H, H-1β, $J_{1,2}$ = 8.1 Hz), 6.21 (d, 1H, H-1α, $J_{1,2}$ = 3.5 Hz), 7.27–7.34 (m, 5H, Ph–*H*). ^{13}C NMR ($CDCl_3$): δ 20.9, 21.29, 57.2, 60.7, 62.2, 62.4, 62.8, 71.4, 72.4, 74.9, 75.5, 78.9, 90.5, 95.9, 101.6, 127.6, 127.7, 128.1, 128.7, 178.8, 138.5, 154.7, 169.0, 169.7, 170.8, 170.9, 171.4. MS (ESI) *m*/*z* (%) 863.1 (96.77, $(M + Na)^+$), 865.1 (100.0), 867.1 (37.20), 869.1 (5.73). HRMS (ESI) calcd for $C_{32}O_{16}N_4H_{39}Cl_3Na$ $(M+Na)^+$ 863.1324. Found 863.1339.

4.10. 1,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-3-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-α,β-D-glucopyranose 20



$BF_3{\cdot}OEt_2$ (0.345 mL, 2.99 mmol) was added to a 0 °C of 1,6-anhydro-2-azido-4-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranose **15** (440 mg, 0.597 mmol) in Ac_2O (0.5 mL), and stirred at 0 °C for 14.5 h. The solution was warmed to room temperature, stirred for an additional 2 h, then quenched with Et_3N (0.4 mL), diluted with toluene (3 mL), and concentrated (in vacuo). Column chromatography (SiO_2, 1:1 hexanes–EtOAc) yielded pure product **20** (390 mg, 83%) as a yellowish oil. ^{1}H NMR ($CDCl_3$): δ 2.04–2.1 (m, 15H, 5CH_3), 2.2 (s, 3H, CH_3), 3.54 (m, 1H, H-2′), 3.67 (m, 1H, H-2), 3.76 (m, 1H, H-5′), 3.97 (dd, 1H, H-3, $J_{3,4}$ = $J_{3,2}$ = 8.2 Hz), 4.10–4.02 (m, 4H, H-4, H-6, H-5, H-6′), 4.25 (dd, 1H, H-6, $J_{6,6}$ = 12.3 Hz, $J_{6,5}$ = 3.6 Hz), 4.44 (dd, 1H, H-6′, $J_{6',6'}$ = 12.5 Hz, $J_{6',5'}$ = 3.7 Hz), 4.73 (m, 2H, TrocCH_2), 4.92 (d, 1H, H-1′β, $J_{1',2'}$ = 8.4 Hz), 5.1 (m, 3H, H-4′, H-1β), 5.28 (d, 1H, NH, $J_{NH,H2}$ = 8.2 Hz), 5.42 (dd, 1H, H-3′, $J_{3',4'}$ = $J_{3',2'}$ = 9.6 Hz), 6.34 (d, 1H, H-1α, $J_{1,2}$ = 3.5 Hz). ^{13}C NMR ($CDCl_3$): δ 20.9, 21.0, 21.1, 21.3, 57.1, 62.7, 62.1, 63.1, 67.7, 68.6, 70.3, 71.9, 72.1, 74.9, 90.3, 95.7, 96.5, 100.7, 154.3, 168.9, 169.7, 169.8, 170.7, 170.8, 171.0. MS (ESI) *m*/*z* (%) 815.1 (97.12, $(M+Na)^+$), 817.1 (100.0), 819.1 (35.81), 821.1 (5.66%). HRMS (ESI) calcd for $C_{27}H_{35}Cl_3N_4O_{17}Na$ $(M+Na)^+$ 815.0960. Found 815.0975.

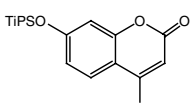
4.11. 1,3,6-Tri-*O*-acetyl-2-azido-2-deoxy-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-α,β-D-glucopyranose 21



From **16**: $BF_3{\cdot}OEt_2$ (22 μL, 0.18 mmol) was added to a 0 °C solution of 1,6-anhydro-2-azido-2-deoxy-3-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxy-carbonylamino)-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranose **16** (26 mg, 0.04 mmol) in Ac_2O (0.4 mL). After 14.5 h, the solution was warmed to room temperature. After 2 h, the reaction was quenched with Et_3N (0.4 mL), diluted with toluene (5 mL), and concentrated (in vacuo). Chromatography (preparative TLC, 3:2 hexanes–EtOAc) yielded pure **21** (25 mg, 85%) as a yellowish oil. Spectral data were identical to those given below for **21** derived from **17**.

From **17**: $BF_3{\cdot}OEt_2$ (84 μL, 0.724 mmol) was added to a 0 °C solution of 1,6-anhydro-2-azido-2-deoxy-3-*O*-acetyl-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranose **17** (98 mg, 0.145 mmol) in Ac_2O (1.3 mL). After 30 min, the reaction was quenched with Et_3N (0.8 mL), diluted with toluene (5 mL), and concentrated (in vacuo). Chromatography (SiO_2, 1:1 hexanes–EtOAc) yielded pure **21** (62 mg, 83%) as a yellowish oil. ^{1}H NMR ($CDCl_3$): δ 1.98–2.17 (m, 18 H, 6 CH_3), 3.53 (m, 2H, H-2′, H-2), 3.67 (m, 1H, H-5′), 3.81 (dd, 1H, H-4, $J_{4,3}$ = 9.5 Hz, $J_{4,5}$ = 9.6 Hz), 4.03 (dd, 1H, H-6′, $J_{6',6'}$ = 12.3 Hz), 4.26 (s, 2H, H-6, H-6), 4.40 (dd, 1H, H-6′, $J_{6',6'}$ = 12.2 Hz, $J_{6',5'}$ = 3.6 Hz), 4.66–4.74 (m, 3H, TrocCH_2, H-1′β), 5.03 (dd, 1H, H-4′, $J_{4',3'}$ = 9.7 Hz, $J_{4',5'}$ = 9.5 Hz), 5.30 (dd, 1H, H-3′, $J_{3',4'}$ = 9.6 Hz, $J_{3',2'}$ = 8.9 Hz), 5.42 (dd, 1H, H-3, $J_{3,4}$ = 9.6 Hz, $J_{3,2}$ = 9.5 Hz), 5.55 (d, 1H, H-1β, $J_{1,2}$ = 8.5 Hz), 5.72 (d, 1H, NH, $J_{NH,H2}$ = 8.4 Hz), 6.22 (d, 1H, H-1α, $J_{1,2}$ = 3.4 Hz). ^{13}C NMR ($CDCl_3$): δ 20.9, 21.1, 21.1, 21.3, 21.4, 56.9, 60.9, 62.2, 62.2, 68.7, 70.5, 70.9, 71.9, 72.3, 74.9, 75.6, 90.3, 95.8, 100.7, 154.4, 169.8, 170.3, 170.5, 170.8, 171.9, 171.3. MS (ESI) *m*/*z* (%) 815.1 (95.53, $(M+Na)^+$), 817.1 (99.91), 819.1 (34.48), 821.1 (5.34). HRMS (ESI) calcd for $C_{27}O_{17}N_4H_{35}Cl_3Na$ $(M+Na)^+$ 815.0960. Found 815.0975.

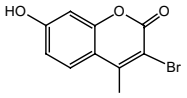
4.12. 7-Triisopropylsilyloxy-4-methylcoumarin



7-Hydroxy-4-methylcoumarin **26** (2 g, 11.4 mmol) in THF (80 mL) was added dropwise to a 0 °C solution of sodium hydride (0.38 g, 12.4 mmol) in THF (5 mL).

After addition was complete, stirring continued at 0 °C for 5 min, then the solution was warmed to room temperature, and TiPS–chloride (0.132 mL, 0.688 mmol) was added. After 15 h, the reaction mixture was condensed (in vacuo) diluted up $CHCl_3$, washed with H_2O, brine, dried (Na_2SO_4), and concentrated (in vacuo). The crude mixture was then passed through a plug of silica using 5:1 hexanes–EtOAc as the eluant and concentrated to obtain pure product (2.96 g, 82%) as a white solid. Mp 63-65 °C. ^{1}H NMR ($CDCl_3$): δ 1.11 (m, 18H, TiPS–CH_3), 1.28 (m, 3H, TiPS–CH), 2.41 (s, 3H, CH_3), 6.16 (s, 1H, H-3), 6.85 (m, 2H, H-6, H-8), 7.46 (d, 1H, H-5 $J_{5,6}$ = 8.8 Hz). ^{13}C NMR ($CDCl_3$): δ 12.9, 12.9, 18.5, 18.7, 18.9, 107.5, 112.4, 114.8, 117.5, 126.3, 127.6, 154.1, 155.3, 159.6, 160.9. MS (ESI) *m/z* (%) 333.2 (100.0, $(M+H)^+$). HRMS (ESI) calcd for $C_{19}O_3H_{29}Si$ $(M+H)^+$ 333.1886. Found 333.1877.

4.13. 3-Bromo-7-hydroxy-4-methylcoumarin 28



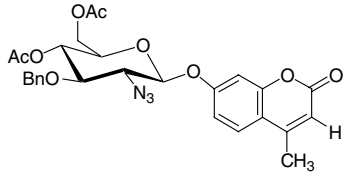
A solution of NBS (0.89 g, 5 mmol) in 10 mL acetonitrile was added to a solution of 4-methyl-7-triisopropylsilanoxycoumarin (1.67 g, 5 mmol) in acetonitrile (20 mL) at room temperature. After 26 h, the solvent was removed (in vacuo) the residue was dissolved up in CH_2Cl_2 and extracted with water, satd aq $NaHCO_3$, and brine, dried (Na_2SO_4), filtered, and concentrated (in vacuo). Column chromatography (silica gel, 20:1 hexanes–EtOAc) yielded pure 3-bromo-7-triisopropylsilanoxy-4-methylcoumarin as a white solid (2.04 g, 99% yield). Mp 104–105 °C. ^{1}H NMR ($CDCl_3$): δ 1.11 (d, 18H, TiPS–CH_3), 1.28 (m, 3H, TiPS–CH), 2.40 (s, 3H, CH_3), 6.86 (m, 2H, H-6, H-8), 7.53 (d, 1H, H-5, $J_{5,6}$ = 8.7 Hz). ^{13}C NMR ($CDCl_3$): δ 13.0, 18.2, 19.9, 107.8, 110.2, 114.3, 118.2, 126.4, 151.6, 157.8, 157.9, 160.2. MS (ESI) *m/z* (%) 411.1 (92.51, $(M+H)^+$), 413.1 (100.0). HRMS (ESI) calcd for $C_{19}O_3H_{28}SiBr$ $(M+H)^+$ 411.0991. Found 411.1014.

To a solution of 3-bromo-7-triisopropylsilanoxy-4-methylcoumarin (380 mg, 0.92 mmol) in EtOH (15 mL) was added 2 M HCl (3 mL), and the resulting solution was then heated at reflux temperature. After 4 h, the solution was poured onto ice and the precipitated product was isolated by filtration and washed with cold water to give **28** (225 mg, 96%) as a white powder. Mp 205–209 °C. ^{1}H NMR (DMSO): δ 2.49 (s, 3H, CH_3), 6.75 (d, 1H, H-8, $J_{8,6}$ = 2.4 Hz), 6.84 (dd, H, H-6, $J_{6,5}$ = 8.9 Hz, $J_{6,8}$ = 2.5 Hz), 7.72 (d, 1H, H-5, $J_{5,6}$ = 8.8 Hz) 10.71 (br s, 1H, OH). ^{13}C NMR ($CDCl_3$): δ 20.2, 102.9, 109.2, 112.7, 114.4, 128.3, 152.9, 154.0, 157.9, 162.3. MS (EI) *m/z* (%) 253.8 (M^+, 100%), 255.8 (M^+, 96.7%). HRMS (EI) calcd for $C_{10}O_3H_7Br$ (M^+) 253.9579. Found 253.9587.

4.14. General procedure for 4-methylumbelliferyl glycosylation

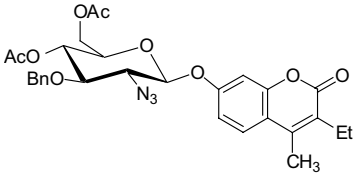
A 30% solution of HBr in AcOH (47 μL, 0.35 mmol) was added to a solution of the saccharide (0.11 mmol) in AcOH (0.5 mL) at room temperature. After 3.5 h, the reaction mixture was diluted with CH_2Cl_2, washed with satd aq $NaHCO_3$, and brine, dried (Na_2SO_4), filtered, and concentrated (in vacuo) to obtain the α-bromide. Silver triflate (0.14 mmol) was added to a solution (−78 °C) of the α-bromide (70 μmol) in CH_2Cl_2 (1.5 mL) containing 3 Å molecular sieves, and sym-collidine (13 μL, 0.1 mmol) under Ar, in the dark. After stirring 5 min at −78 °C, the umbelliferone acceptor (0.14 mmol) was added, and the mixture warmed to room temperature. After 16 h, the mixture was filtered through Celite, washed with satd aq $NaHCO_3$, and brine, dried (Na_2SO_4), filtered, and concentrated in vacuo.

4.15. 4-Methylumbelliferyl 2-azido-4,6-di-*O*-acetyl-3-*O*-benzyl-2-deoxy-β-D-glucopyranose 29



1,4,6-Tri-*O*-acetyl-2-deoxy-2-azido-3-*O*-benzyl-α-D-glucopyranose **23** (30 mg, 70 μmol) was coupled with 4-methylumbelliferone **26** (0.14 mmol) following the general procedure outlined above. Column chromatography (SiO_2, 1.5:1 hexanes–EtOAc) yielded pure **29**, (23 mg, 64%, 100% β) as light yellow semi-solid. ^{1}H NMR ($CDCl_3$): δ 1.77 (s, 3H, CH_3), 2.14 (s, 3H, CH_3), 2.43 (s, 3H, CH_3) 3.53 (dd, 1H, H-3, $J_{3,4}$ = 9.53 Hz, $J_{3,2}$ = 10.41 Hz), 3.75 (m, 1H, H-5), 3.82 (dd, 1H, H-2, $J_{2,1}$ = 8.4 Hz, $J_{2,3}$ = 9.6 Hz), 4.13 (dd, 1H, H-6, $J_{6,6}$ = 11.9 Hz, $J_{6,5}$ = 2.0 Hz), 4.27 (dd, 1H, H-6, $J_{6,6}$ = 12.4 Hz, $J_{6,5}$ = 6.2 Hz), 4.72 (d, 1H, 1/2 CH_2Ph, J = 11.4), 4.88 (d, 1H, 1/2 CH_2Ph, J = 11.4), 4.95 (d, 1H, H-1β, $J_{1,2}$ = 8.1 Hz), 5.12 (dd, 1H, H-4, $J_{4,3}$ = 9.8 Hz, $J_{4,5}$ = 9.5 Hz), 6.21 (s, 1H, H-3c), 7.03 (m, 2H, H-8c, H-6c), 7.31–7.36 (m, 5H, Ph–*H*), 7.56 (d, 1H, H-5c, $J_{5c,6c}$ = 8.4 Hz). ^{13}C NMR ($CDCl_3$): δ 19.1, 21.2, 62.4, 65.9, 69.8, 73.1, 75.7, 80.4, 100.2, 104.2, 113.6, 114.6, 115.9, 126.2, 128.5, 128.6, 128.9, 137.7, 152.7, 155.3, 159.6, 161.2, 166.8, 169.9, 171.1. MS (ESI) *m/z* (%) 560.2 (100.0, $(M+Na)^+$). HRMS (ESI) calcd for $C_{27}O_9N_3H_{27}Na$ $(M+Na)^+$ 560.1645. Found 560.1647.

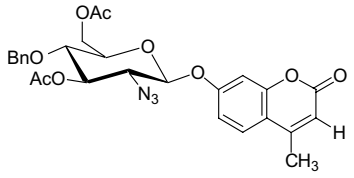
4.16. 3-Ethyl-4-methylumbelliferyl 2-azido-4,6-di-*O*-acetyl-3-*O*-benzyl-2-deoxy-β-D-glucopyranose 30



1,4,6-Tri-*O*-acetyl-2-deoxy-2-azido-3-*O*-benzyl-α-D-glucopyranose **23** (30 mg, 70 μmol) was coupled with 3-ethyl-4-methylumbelliferone **27**, following the general

procedure outlined above. Column chromatography (SiO_2, 8:1 $CHCl_3$–EtOAc) yielded pure **30** (9 mg, 43%, 100% β) as a faint yellow semi-solid. ^1H NMR ($CDCl_3$): δ 1.15 (t, 3H, CH_3, J = 7.5 Hz), 1.97 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.67 (q, 2H, CH_2, J = 7.5 Hz), 3.54 (dd, 1H, H-3, $J_{3,2}$ = 9.6 Hz, $J_{3,4}$ = 9.5), 3.73 (m, 1H, H-5), 3.81 (dd, 1H, H-2, $J_{2,3}$ = 9.7 Hz, $J_{2,1}$ = 8.2 Hz), 4.11 (dd, 1H, H-6, $J_{6,5}$ = 2.3 Hz, $J_{6,6}$ = 12.2 Hz), 4.25 (dd, 1H, H-6, $J_{6,5}$ = 6.2 Hz, $J_{6,6}$ = 12.4 Hz), 4.67 (d, 1H, 1/2 CH_2Ph, J = 11.4 Hz), 4.86 (d, 1H, 1/2 CH_2Ph, J = 11.4 Hz), 4.86 (d, 1H, H-1β, $J_{1,2}$ = 8.2 Hz), 5.10 (dd, 1H, H-4, $J_{4,5}$ = 9.9 Hz, $J_{4,3}$ = 9.4 Hz), 6.97–6.99 (m, 2H, H-8c, H-6c), 7.30–7.38 (m, 5H, Ph–*H*), 7.53 (d, 1H, H-5c, $J_{5c,6c}$ = 8.6 Hz). ^{13}C NMR ($CDCl_3$): δ 13.5, 15.0, 21.1, 21.2, 21.3, 62.4, 65.9, 73.1, 75.7, 80.4, 100.4, 104.0, 114.4, 116.8, 125.9, 126.7, 128.4, 128.5, 137.8, 153.7, 158.6, 161.9, 169.8, 171.1. MS (ESI) *m/z* (%) 588.2 (100.0, $(M+Na)^+$). HRMS (ESI) calcd for $C_{29}O_9N_3H_{31}Na$ $(M+Na)^+$ 588.1958. Found 588.1961.

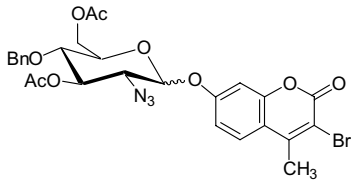
4.17. 4-Methylumbelliferyl 2-azido-3,6-di-*O*-acetyl-4-*O*-benzyl-2-deoxy-β-D-glucopyranose 31



1,3,6-Tri-*O*-acetyl-2-deoxy-2-azido-4-*O*-benzyl-α-D-glucopyranose, **24** (12 mg, 30 μmol) was coupled with 4-methylumbelliferone **26** (12 mg, 0.07 mmol) following the general procedure outlined above. Column chromatography (SiO_2, 1:2 hexanes–EtOAc) yielded pure **31**, (23 mg, 54%, 100% β) as an off-white semi-solid. ^1H NMR: δ 2.12 (s, 6H, 2CH_3), 2.43 (s, 3H, CH_3) 3.63 (dd, 1H, H-4, $J_{4,3}$ = 9.48 Hz, $J_{4,5}$ = 9.33 Hz), 3.72–3.81 (m, 2H, H-2, H-5), 4.27 (dd, 1H, H-6, $J_{6,6}$ = 11.1 Hz, $J_{6,5}$ = 5.6 Hz), 4.40 (m, 1H, H-6, $J_{6,6}$ = 10.4 Hz, $J_{6,5}$ = 1.2 Hz), 4.60 (m, 2H, CH_2Ph), 5.02 (d, 1H, H-1β, $J_{1,2}$ = 8.0 Hz), 5.20 (dd, 1H, H-3, $J_{3,4}$ = 9.8 Hz, $J_{3,2}$ = 9.6 Hz), 6.22 (s, 1H, H-3c), 6.98–7.03 (m, 2H, H-8c, H-6c), 7.28–7.38 (m, 5H, Ph–*H*), 7.55 (d, 1H, H-5c, $J_{5c,6c}$ = 8.5 Hz). ^{13}C NMR ($CDCl_3$): δ 19.1, 21.2, 62.4, 65.9, 69.8, 73.0, 75.7, 80.4, 100.2, 104.2, 113.6, 114.6, 115.9, 126.2, 128.5, 128.6, 128.9, 137.7, 152.7, 155.23, 159.6, 161.2, 166.8, 169.9, 171.1. MS (ESI) *m/z* (%) 560.4 (100.0, $(M+Na)^+$). HRMS (ESI) calcd for $C_{27}O_9N_3H_{27}Na$ $(M+Na)^+$ 560.1645. Found 560.1648.

4.18. 3-Bromo-4-methylumbelliferyl 2-azido-3,6-di-*O*-acetyl-4-*O*-benzyl-2-deoxy-α,β-D-glucopyranose 32

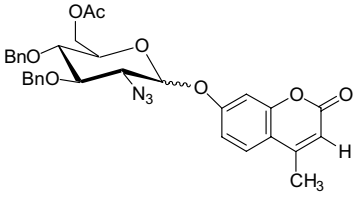
1,3,6-Tri-*O*-acetyl-2-deoxy-2-azido-4-*O*-benzyl-α-D-glucopyranose **24** (21 mg, 50 μmol) was coupled to 3-bromo-4-methylumbelliferone **28** (0.1 mmol) following the general procedure outlined above. Column chromatography (SiO_2, 8:1 $CHCl_3$–EtOAc) separated the α- and β-anomers and yielded **32** (19 mg, 64%, α:β = 44:56) as a yellowish semi-solid.



β-Anomer: ^1H NMR ($CDCl_3$): δ 1.9–1.8 (m, 6H, 2 CH_3), 3.75–3.5 (m, 3H, H-4, H-5, H-2), 4.3–4.2 (m, 2H, H-6, H-6), 4.6 (m, 2H, CH_2Ph), 5.01 (d, 1H, H-1β, J = 8.6 Hz), 5.24 (dd, 1H, H-3, $J_{3,4\,=\,3,2}$ = 7.6 Hz), 7.0 (m, 2H, H-8c, H-6c), 7.2 (m, 5H, Ph–*H*), 7.5 (d, 1H, H-5c, $J_{5c,6c}$ = 8.9 Hz).

α-Anomer: ^1H NMR ($CDCl_3$): δ 2.10 (s, 3H, 1CH_3), 2.61 (s, 3H, CH_3), 3.39 (dd, 1H, H-2, $J_{2,1}$ = 3.4 Hz, $J_{2,3}$ = 10.7 Hz), 3.72 (dd, 1H, H-4, $J_{4,3}$ = 9.3 Hz, $J_{4,5}$ = 9.9 Hz), 4.00 (m, 1H, H-5), 4.27 (m, 2H, H-6, H-6), 4.62 (q, 2H, CH_2Ph, J = 11.2 Hz), 5.67 (dd, 1H, H-1α, $J_{1,2}$ = 3.4 Hz,), 5.78 (dd, 1H, H-3, $J_{3,4}$ = 9.0 Hz, $J_{3,2}$ = 10.7 Hz), 7.10 (dd, 1H, H-6c, $J_{6c,5c}$ = 8.8 Hz, $J_{6c,8c}$ = 2.4 Hz) 7.17 (d, 1H, H-8c, $J_{8c,6c}$ = 2.4 Hz), 7.26–7.39 (m, 10H, Ph–*H*), 7.63 (d, 1H, H-5c, $J_{5c,6c}$ = 8.9 Hz). ^{13}C NMR ($CDCl_3$): δ 19.9, 21.2, 63.4, 62.6, 70.5, 72.3, 75.3, 76.1, 80.6, 97.4, 104.5, 114.5, 115.8, 126.7, 128.4, 128.7, 129.01, 137.3, 151.1, 153.6, 157.3, 158.9, 170.2, 170.8. MS (ESI) *m/z* (%) 638.0 (87.56, $(M+Na)^+$), 640.0 (100.0). HRMS (ESI) calcd for $C_{27}O_9N_3H_{26}BrNa$ $(M+Na)^+$ 638.0750. Found 638.0764.

4.19. 4-Methylumbelliferyl 2-azido-6-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-α,β-D-glucopyranose 33



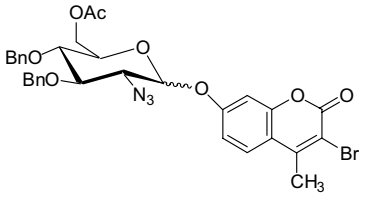
1,6-Di-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranose **25** (38 mg, 80 μmol) was coupled to 4-methylumbelliferone **26** (0.16 mmol), following the general procedure outlined above. Column chromatography (SiO_2, 1:1 hexanes–EtOAc) separated the α- and β-anomers and afforded pure **33** (35 mg, 77%, α:β = 17:83) as an off-white semi-solid.

β-Anomer: ^1H NMR ($CDCl_3$): δ 2.08 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 3.18 (m, 1H, H-5) 3.24–3.33 (m, 2H, H-3, H-4), 3.55 (dd, 1H, H-2, $J_{2,1}$ = 8.7 Hz, $J_{2,3}$ = 9.3 Hz), 4.10 (dd, 1H, H-6, $J_{6,5}$ = 6.4 Hz, $J_{6,6}$ = 11.9 Hz), 4.31–4.35 (m, 2H, H-6, 1/2 CH_2), 4.52 (d, 1H, H-1β, $J_{1,2}$ = 8.1 Hz), 4.65–4.78 (m, 2H, CH_2Ph), 4.85 (d, 1H, 1/2 CH_2, J = 11.1 Hz), 6.18 (s, 1H, H_{3c}), 7.01 (m, 2H,

H_{6c}, H_{8c}), 7.29–7.41 (m, 10H, Ph–*H*), 7.53 (d, 1H, H-5c, $J_{5c,6c}$ = 8.8 Hz).

α-Anomer: ^{1}H NMR ($CDCl_3$): δ 2.10 (s, 3H, C*H*$_3$), 2.42 (s, 3H, C*H*$_3$), 3.05 (dd, 1H, H-2, $J_{2,1}$ = 3.4 Hz, $J_{2,3}$ = 10.4 Hz), 3.27 (m, 1H, H-4), 3.40 (dd, 1H, H-3, $J_{3,4}$ = 9.2 Hz, $J_{3,2}$ = 9.7 Hz), 3.92 (m, 1H, H-5), 4.20–4.28 (m, 2H, H-6, H-6), 4.45 (d, 2H, C*H*$_2$Ph, *J* = 11.0 Hz), 4.65–4.78 (m, 2H, C*H*$_2$Ph), 5.61 (d, 1H, H-1α, $J_{1,2}$ = 3.4 Hz), 6.18 (s, 1H, H-3c), 7.01 (m, 2H, H-6c, H-8c), 7.29–7.41 (m, 10H, Ph–*H*), 7.53 (d, 1H, H-5c, $J_{5c,6c}$ = 8.8 Hz). ^{13}C NMR ($CDCl_3$): δ 18.0, 20.7, 62.9, 66.4, 73.9, 75.2, 75.78, 77.8, 83.1, 99.9, 104.3, 113.8, 114.12, 115.6, 125.9, 128.0, 128.4, 128.9, 138.3, 138.6, 151.3, 155.7, 159.7, 159.9, 170.1, 171.2. MS (ESI) *m*/*z* (%) 608.2 (100.0, $(M+Na)^+$). HRMS (ESI) calcd for $C_{32}O_8N_3H_{31}Na$ $(M+Na)^+$ 608.2009. Found 608.2014.

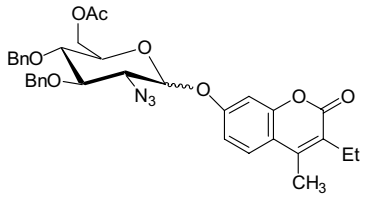
4.20. 3-Bromo-4-methylumbelliferyl 2-azido-6-*O*-acetyl-3,4-*O*-benzyl-2-deoxy-αβ-D-glucopyranose 34



1,6-Di-*O*-acetyl-2-deoxy-2-azido-3,4-di-*O*-benzyl-α-D-glucopyranose **25** (5 mg, 10 μmol) was coupled to 3-bromo-4-methylumbelliferone **28** (20 μmol) following the general procedure outlined above. Column chromatography (SiO_2, 2:1 hexanes–EtOAc) yielded pure **34**, an inseparable mixture of α- and β-anomers (4 mg, 50%, α:β = 59:41), as a yellowish, semi-solid. ^{1}H NMR ($CDCl_3$): δ 2.08 (s, 3H, C*H*$_3$), 2.67 (s, 3H, C*H*$_3$), 3.56–3.81 (m, 6H, H-4β, H-3β, H-2β, H-4α, H-3α, H-2α), 3.98 (m, 1H, H-5α), 4.29 (m, 4H, H-5β, H-6β, H-6α, H-6α), 4.67 (d, 2H, C*H*$_2$Ph, *J* = 10.5 Hz), 5.07–4.95 (m, 7H, 3C*H*$_2$Ph, H-1β), 5.68 (d, 1H, H-1α, $J_{1\alpha,2}$ = 3.3), 7.07–7.19 (m, 4H, H-6c, H-8c), 7.32–7.48 (m, 20H, Ph–*H*), 7.66 (d, 2H, H-5c, $J_{5c,6c}$ = 8.8 Hz). ^{13}C NMR ($CDCl_3$): δ 19.9, 21.2, 62.7, 63.4, 70.8, 75.7, 76.12, 80.6, 97.1, 104.8, 111.5, 114.6, 115.6, 126.7, 128.4, 128.5, 129.0, 137.6, 137.8, 151.1, 153.6, 157.4, 159.1, 170.9. MS (ESI) *m*/*z* (%) 686.1 (100.0, $(M+Na)^+$), 688.1 (99.78). HRMS (ESI) calcd for $C_{32}O_8N_3H_{30}BrNa$ $(M+Na)^+$ 686.1114. Found 686.1117.

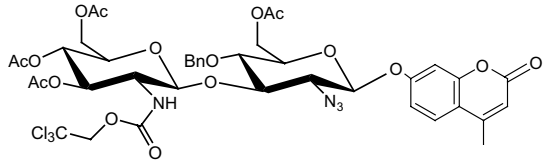
4.21. 3-Ethyl-4-methylumbelliferyl 2-azido-6-*O*-acetyl-3,4-*O*-benzyl-2-deoxy-α,β-D-glucopyranose 35

1,6-Di-*O*-acetyl-2-deoxy-2-azido-3,4-di-*O*-benzyl-α-D-glucopyranose **25** (23 mg, 50 μmol) was coupled to 3-ethyl-4-methylumbelliferone **27** (0.09 mmol) following the general procedure outlined above. Column chromatography (SiO_2, 2:1 hexanes–EtOAc) yielded pure **35**, an inseparable mixture of α- and β-anomers (18 mg, 58%,



α:β = 1:10), as an off-white semi-solid. ^{1}H NMR ($CDCl_3$): δ 1.17 (t, 3H, C*H*$_3$, *J* = 7.4 Hz), 2.08 (s, 3H, C*H*$_3$), 2.39 (s, 3H, C*H*$_3$), 2.67 (q, 2H, C*H*$_2$, *J* = 7.5 Hz), 3.59 (m, 2H, H-3, H-4), 3.67 (m, 1H, H-5), 3.74 (dd, 1H, H-2, $J_{2,3}$ = 9.2 Hz, $J_{2,1}$ = 8.4 Hz), 4.23 (dd, 1H, H-6, $J_{6,5}$ = 5.8 Hz, $J_{6,6}$ = 12.0 Hz), 4.35 (dd, 1H, H-6, $J_{6,5}$ = 2.1 Hz, $J_{6,6}$ = 12.0 Hz), 4.61 (d, 1H, 1/2 C*H*$_2$, *J* = 10.7 Hz) 4.84–4.90 (m, 3H, C*H*$_2$, $H_{1\beta}$), 5.59 (d, 1H, $H_{1\alpha}$), 6.97 (m, 2H, H-8c, H-6c), 7.28–7.42 (m, 10H, Ph–*H*), 7.52 (d, 1H, H-5c, $J_{5c,6c}$ = 9.5 Hz). ^{13}C NMR ($CDCl_3$): δ 13.5, 14.9, 21.2, 21.3, 63.1, 66.3, 74.0, 75.6, 76.2, 83.3, 100.4, 104.2, 114.9, 116.7, 125.9, 126.6, 128.4, 128.5, 128.5, 128.6, 128.6, 128.9, 129.0, 137.6, 137.9, 145.8, 153.7, 158.7, 161.9, 171.1. MS (ESI) *m*/*z* (%) 636.3 (99.84, $(M+Na)^+$). HRMS (ESI) calcd for $C_{34}O_8N_3H_{35}Na$ $(M+Na)^+$ 636.2322. Found 636.2329.

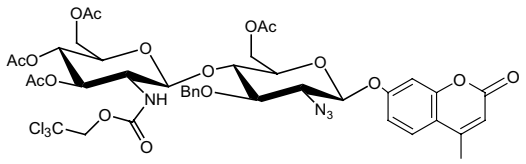
4.22. 4-Methylumbelliferyl 6-*O*-acetyl-2-azido-4-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranose 36



1,6-Di-*O*-acetyl-2-azido-4-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-2-deoxy-α-D-glucopyranose **18** (64 mg, 76 μmol) was coupled to 4-methylumbelliferone **26** (0.14 mmol) following the general procedure outlined above. Column chromatography (SiO_2, 8:1 $CHCl_3$–EtOAc) yielded pure product **36** (25 mg, 33%, 100% β) as an off-white semi-solid. ^{1}H NMR ($CDCl_3$): δ 1.8–1.9 (m, 12H, 4C*H*$_3$), 2.10 (s, 3H, C*H*$_3$), 3.4–3.5 (m, 3H, H-5, H-5′, H-2), 3.75–3.80 (m, 2H, H-3, H-2), 3.94 (m, 1H, H-2′), 4.14 (d, 1H, H-6′, *J* = 12.5 Hz), 4.4–4.3 (m, 3H, H-6, H-6, H-6′), 4.57 (d, 1H, 1/2 C*H*$_2$Ph, *J* = 11.0 Hz), 4.71 (d, 1H, H-1β, *J* = 7.5 Hz), 4.83 (dd, 2H, TrocC*H*$_2$, *J* = 12.1 Hz), 5.07 (d, 1H, H-1′β, *J* = 8.3 Hz), 5.17 (d, 1H, 1/2 C*H*$_2$Ph, *J* = 10.5 Hz), 5.27 (d, 1H, NH, *J* = 8.8 Hz), 5.34 (dd, 1H, H-4′, $J_{4',3'}$ = 9.3 Hz, $J_{4',5'}$ = 9.0 Hz), 5.56 (dd, H-3′, $J_{3',4'}$ = 9.8 Hz, $J_{3',2'}$ = 10.1 Hz), 5.9 (s, 1H, H-3c), 6.9 (s, 1H, H-8c), 7.0 (s, 1H, H-6c), 7.2 (m, 5h, Ph–*H*), 7.5 (d, 1H, H-5c). ^{13}C NMR ($CDCl_3$): δ 18.0, 20.34, 20.5, 20.6, 30.4, 62.2, 63.0, 63.3, 66.2, 66.2, 69.2, 72.4, 72.7, 73.9, 74.9, 75.8, 83.9, 91.4, 96.8, 102.5, 104.8, 112.8,

114.0, 115.7, 125.9, 128.0, 128.2, 128.7, 130.6, 138.67, 151.4, 154.6, 155.7, 159.4, 160.0, 169.4, 170.2, 170.5. MS (ESI) *m/z* (%) 979.2 (100.0, $(M+Na)^+$), 981.2 (97.97), 983.2 (42.18), 985.2 (8.10). HRMS (ESI) calcd for $C_{40}O_{17}N_4H_{43}Cl_3Na$ $(M+Na)^+$ 979.1586. Found 979.1595.

4.23. 4-Methylumbelliferyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranose 37



1,6-Di-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-2-deoxy-α-D-glucopyranose **19** (17 mg, 20 μmol) was coupled to 4-methylumbelliferone **26** (40 μmol), following the general procedure outlined above. Column chromatography (SiO_2, 2:1 hexanes–EtOAc) yielded pure product **37** (12 mg, 34%, 100% β) as an off-white semi-solid. ^1H NMR ($CDCl_3$): δ 1.63–1.84 (m, 12H, $4CH_3$), 2.11 (s, 3H, CH_3), 3.39 (m, 2H, H-5, H-5′), 3.70–3.85 (m, 3H, H-2′, H-2, H-4), 4.08 (dd, 1H, H-6′, $J_{6',6'}$ = 12.0 Hz), 4.29 (m, 2H, H-6, H-6′), 4.56 (dd, 1H, H-6, $J_{6,6}$ = 11.4 Hz), 4.61 (d, 1H, H-1β, $J_{1,2}$ = 8.2 Hz), 4.68 (d, 1H, H-1′β, $J_{1,2}$ = 8.1 Hz), 4.82 (dd, 1H, 1/2 TrocCH_2, J = 12.1 Hz), 4.95 (d, 1H, NH, $J_{NH,H2}$ = 9.2 Hz), 5.09 (dd, 2H, 1/2 CH_2Ph, 1/2 TrocCH_2 1/2 J = 11.7) 5.17 (dd, 1H, 1/2 CH_2Ph, J = 11.7 Hz), 5.34 (d, 1H, H-4′, $J_{4',3'}$ = 9.6 Hz, $J_{4',5'}$ = 9.3 Hz), 5.41 (dd, 1H, H-3′, $J_{3',2'}$ = 9.9 Hz, $J_{3',4'}$ = 9.8 Hz), 5.95 (s, 1H, H-3c), 6.90–7.11 (m, 2H, H-6c, H-8c), 7.27–7.43 (m, 5H, Ph–*H*), 7.62 (d, 1H, H-5c, $J_{5c,6c}$ = 7.4 Hz). ^{13}C NMR ($CDCl_3$): δ 18.0, 20.4, 20.5, 20.8, 30.4, 57.5, 61.7, 63.1, 66.5, 68.9, 72.4, 72.5, 73.5, 75.0, 75.3, 78.0, 81.5, 96.4, 99.9, 102.8, 104.3, 113.8, 114.2, 115.6, 125.5, 127.5, 128.0, 128.2, 130.7, 139.2, 151.3, 154.6, 155.7, 159.6, 159.9, 169.3, 170.2, 170.3, 170.7. MS (ESI) *m/z* (%) 979.1 (99.73, $(M+Na)^+$), 981.1 (100.0), 983.1 (41.95), 985.2 (5.85). HRMS (ESI) calcd for $C_{40}O_{17}N_4H_{43}Cl_3Na$ $(M+Na)^+$ 979.1586. Found 979.1590.

Acknowledgements

This research was supported in part by the Hans Vahlteich and Ella McCollum Research Endowment Fund administered by the College of Pharmacy, University of Michigan. A.G. is the recipient of a Fred W. Lyons Fellowship from the College of Pharmacy, University of Michigan, and a fellowship from the American Foundation for Pharmaceutical Education. A.G. is a trainee in the Pharmacological Sciences Training Program supported by a grant from the National Institutes of General Medical Sciences (GM 07767).

References

1. Malet, C.; Viladot, J. L.; Ochoa, A.; Gallego, B.; Brosa, C.; Planas, A. *Carbohydr. Res.* **1995**, *274*, 285–301.
2. Howard, M. B.; Ekborg, N. A.; Taylor, L. E., II; Weiner, R. M.; Hutcheson, S. W. *J. Bacteriol.* **2004**, *186*, 1297–1303.
3. Omero, C.; Horwitz, B. A.; Chet, I. *J. Microbiol. Methods* **2001**, *43*, 165–169.
4. Pritsch, K.; Raidl, S.; Marksteiner, E.; Blaschke, H.; Agerer, R.; Schloter, M.; Hartmann, A. *J. Microbiol. Methods* **2004**, *58*, 233–241.
5. Vinetz, J. M.; Valenzuela, J. G.; Specht, C. A.; Aravind, L.; Langer, R. C.; Ribeiro, J. M. C.; Kaslow, D. C. *J. Biol. Chem.* **2000**, *275*, 10331–10341.
6. Krag, S. S. *Biochem. Biophys. Res. Commun.* **1998**, *243*, 1–5.
7. Wacker, M.; Linton, D.; Hitchen, P. G.; Nita-Lazar, M.; Haslam, S. M.; North, S. J.; Panico, M.; Morris, H. R.; Dell, A.; Wren, B. W.; Aebi, M. *Science* **2002**, *298*, 1790–1793.
8. Delmotte, F. M.; Privat, J.-P. D. J.; Monsigny, M. L. P. *Carbohydr. Res.* **1975**, *40*, 353–364.
9. Courtin-Duchateau, M.-C. *Veyrieres Carbohydr. Res.* **1978**, *65*, 23–33.
10. Honda, Y.; Tanimori, S.; Kirihata, M.; Kaneko, S.; Tokuyasu, K.; Hashimoto, M.; Watanabe, T. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 827–829.
11. Szweda, R.; Spohr, U.; Lemieux, R. U. *Can. J. Chem.* **1989**, *67*, 1388–1391.
12. Chow, P.; Weissmann, B. *Carbohydr. Res.* **1981**, *96*, 87–93.
13. Roy, R. T. F. *Synth. Commun.* **1990**, *20*, 2097–2102.
14. Ponticelli, F.; Trendafilova, A.; Valoti, M.; Saponara, S.; Sgaragli, G. *Carbohydr. Res.* **2001**, *330*, 459–468.
15. Vozniyi, Y. V.; Afanasyeva, S. V.; Kalicheva, I. S.; Galoyan, A. A. *Bioorg. Khim.* **1991**, *17*, 510–516.
16. Paulsen, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 155–173.
17. Lemieux, R. U.; Ratcliffe, R. M. *Can. J. Chem.* **1979**, *57*, 1244–1251.
18. Briner, K.; Vasella, A. *Helv. Chim. Acta* **1987**, *70*, 1341–1356.
19. Leteux, C.; Veyrières, A.; Robert, F. *Carbohydr. Res.* **1993**, *242*, 119–130.
20. Tailler, D.; Jacquinet, J.-C.; Noirot, A.-M.; Beau, J.-M. *J. Chem. Soc., Perkin. Trans 1* **1992**, 3163–3164.
21. van Boeckel, C. A. A.; van Aelst, S. F.; Beetz, T. *Recl. Trav. Chim. Pays-Bas* **1983**, *102*, 415–416.
22. Izumi, M.; Tsuruta, O.; Hashimoto, H.; Yazawa, S. *Tetrahedron Lett.* **1996**, *37*, 1809–1812.
23. Roush, W. R.; Bennett, C. E. *J. Am. Chem. Soc.* **1999**, *121*, 3541–3542.
24. Shapiro, D.; Rabinsohn, Y.; Acher, A. J.; Diver-Haber, A. *J. Org. Chem.* **1970**, *35*, 1464–1467.
25. Lee, J.; Coward, J. K. *J. Org. Chem.* **1992**, *57*, 4126–4135.
26. Shaban, M.; Jeanloz, R. W. *Carbohydr. Res.* **1971**, *19*, 311–318.
27. Friesen, R. W.; Sturino, C. F.; Daljeet, A. K.; Kolaczewska, A. *J. Org. Chem.* **1991**, *56*, 1944–1947.
28. Friesen, R. W.; Trimble, L. A. *J. Org. Chem.* **1996**, *61*, 1165–1168.
29. Srinivasan, A. Ph.D. Thesis, University of Michigan, 2004.
30. Xue, J.; Guo, Z. *Tetrahedron Lett.* **2001**, *42*, 6487–6489.
31. Hernández, N.; Martín, V. S. *J. Org. Chem.* **2001**, *66*, 4934–4938.
32. Izumi, M.; Tsuruta, O.; Harayama, S.; Hashimoto, H. *J. Org. Chem.* **1997**, *62*, 992–998.

33. Dullenkopf, W.; Castro-Palomino, J. C.; Manzoni, L.; Schmidt, R. R. *Carbohydr. Res.* **1996**, *296*, 135–147.
34. Ellervik, U.; Magnusson, G. *Carbohydr. Res.* **1996**, *280*, 251–260.
35. Ellervik, U.; Magnusson, G. *J. Org. Chem.* **1998**, *63*, 9314–9322.
36. Friesen, R. W.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1989**, *111*, 6656–6660.
37. Veeneman, G. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 275–278.
38. Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331–1334.
39. Frasier-Reid, B.; Wu, Z.; Andrews, C. W.; Skowronski, E. *J. Am. Chem. Soc.* **1991**, *113*, 1434–1435.
40. Chenault, H. K.; Castro, A. *Tetrahedron Lett.* **1994**, *35*, 9145–9148.
41. Mong, T. K.-K.; Huang, C.-Y.; Wong, C.-H. *J. Org. Chem.* **2003**, *68*, 2135–2142.
42. Umino, A.; Hinou, H.; Matsuoka, K.; Terunuma, D.; Takahashi, S.; Kuzuhara, H. *J. Carbohydr. Chem.* **1998**, *17*, 231–239.
43. Hori, H.; Nishida, Y.; Ohrui, H.; Meguro, H. *J. Org. Chem.* **1989**, *54*, 1346–1353.
44. Zhang, Z.; Ollmann, I. R.; Ye, X.-S.; Wischnat, R.; Baasov, T.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 734–753.
45. Sun, W.-C.; Gee, K. R.; Haugland, R. P. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3107–3110.
46. Chakravarti, D. *J. Indian Chem. Soc.* **1931**, *8*, 129–136.
47. Gordeeva, N. A.; Kirpichenok, M. A.; Patalakha, N. S.; Grandberg, A. *Chem. Heterocycl. Compd.* **1990**, *26*, 1329–1337.
48. Crich, D.; Sun, S. *Tetrahedron* **1998**, *54*, 8321–8348.
49. Dasgupta, F.; Masada, R. I. *Carbohydr. Res.* **2002**, *337*, 1055–1058.
50. Paulsen, H.; Richter, A.; Sinnwell, V.; Stenzel, W. *Carbohydr. Res.* **1978**, *64*, 339–362.