DOI: 10.1002/asia.200900765

Rate-Dependent Inverse-Addition β-Selective Mannosylation and Contiguous Sequential Glycosylation Involving β-Mannosidic Bond Formation

Shih-Sheng Chang, Che-Hao Shih, Kwun-Cheng Lai, and Kwok-Kong Tony Mong*[a]

Dedicated to Professor Tse-Lok Chan at CUHK on the occasion of his 70th birthday

Abstract: The β-selectivity of mannosylation has been found to be dependent on the addition rate of the mannosyl trichloroacetimidate donor in an inverse-addition (I-A) procedure. This rate dependent I-A procedure can improve the selectivity of direct β-mannosylation and is applicable to orthogonal glycosylations of thioglycoside acceptors. Further elaboration of this novel procedure enables the development of the contiguous sequential glycosylation strategy, which streamlines the preparation of oligosaccharides invoking β-mannosidic bond formation. The synthetic utility of the contiguous glycosylation strategy was demonstrated by the preparation of the trisaccharide core of human N-linked glycoproteins and the trisaccharide repeating unit of the O-specific polysaccharide found in the cellular capsule of Salmonelle bacteria.

Keywords: glycosylation · inverse addition · mannosylation · oligosaccharides · β-selectivity

Introduction

The chemical synthesis of oligosaccharides is labor intensive because it demands the preparation of monosaccharide building blocks and the coupling of these building blocks in a stereospecific manner.[1] Although various technologies have been developed to expedite these processes,[2–4] there remain no universal methods applicable to all oligosaccharide structures. This is best illustrated by the synthesis of oligosaccharides involving β-mannosidic bond formation. In past decades, a few reports in the literature have investigated the construction of the β-mannosidic bond on solid support or the use of solid-phase synthesizers for the preparation of these oligosaccharide targets.[5] However, in the solid-phase synthesis, a large excess of glycosyl substrate is required to achieve quantitative conversion. Since glycosyl substrates are generally prepared by multiple-step synthesis, such a solid-phase process would be prohibitively expensive. Moreover, particular solid-phase β-selective mannosylations suffer from drawbacks stemming from their innate chemistry. For example, the acetal linkage connecting the mannosyl donor and the support is cleaved in the intramolecular aglycon delivery (IAD) mannosylations.[5a] On some occasions, the β-selectivities of solid-supported mannosylation decreased to some extent.[5b]

Regarding the frontiers in solution-phase synthesis, the incorporation of β-selective mannosylation to sequential glycoysylation is well-recognized as a challenging task despite the recent advances in glycosylation technologies.[3] Reports concerning the use of contiguous sequential glycosylation that invoke β-mannosidic bond formation remain scarce. In most scenarios, a disaccharide bearing a β-mannosidic bond is prepared first, which upon chromatographic purification and modification of the anomeric function is engaged in subsequent glycosylation.[6–10] This stepwise process is lengthy and inefficient. Given the frequent occurrence of oligosaccharides with a β-mannosidic linkage and the demand of homogeneous samples for interdisciplinary studies, developing a straightforward synthetic strategy for such oligosaccharide targets is an urgent and necessary task.[11]

In the light of the discussion above, this paper reports for the first time a rate-dependent inverse-addition β-mannosylation with the use of an easily available mannosyl tri-

[a] S.-S. Chang, C.-H. Shih, K.-C. Lai, K.-K. T. Mong
Department of Applied Chemistry
National Chiao Tung University
Taiwan, ROC
1001, Ta Hsueh Road, Hsinchu, Taiwan, 300 (Taiwan)
Fax: (+886) 3572-3764
E-mail: tmong@mail.nctu.edu.tw

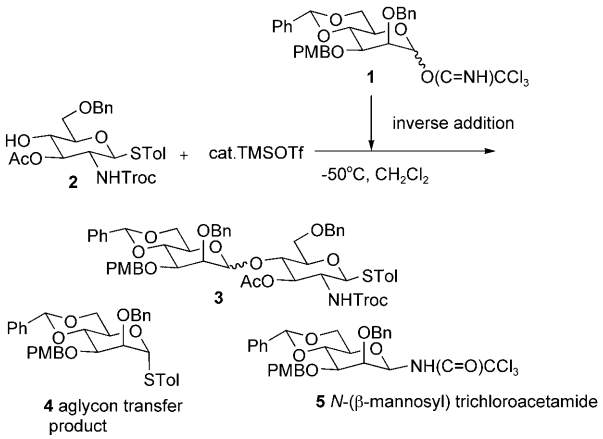
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/asia.200900765.

chloroacetimidate donor and its elaboration to orthogonal glycosylations. This orthogonal glycosylation method is further exploited in the development of the contiguous sequential glycosylation strategy, which has been found to be useful for the preparation of the trisaccharide core of human N-linked glycoproteins[12] and the repeating unit of the O-specific polysaccharide found in *Salmonella anatum*.[13]

Results and Discussion

Rate-dependent inverse-addition β-selective mannosylation: In a project relating to the synthesis of oligosaccharides of human N-linked glycoproteins, the synthesis of a trisaccharide, namely, Man-β(1→4)-GlcNAc-β(1→4)-GlcNAc was required.[12] This trisaccharide is composed of three monosaccharides that are joined by Man-β(1→4)-GlcNAc and GlcNAc-β(1→4)-GlcNAc glycosidic bonds. Owing to the hindered positions, the chemical constructions of these glycosidic linkages are difficult. Though numerous attempts that have targeted the preparation of this structure have been reported, there is still the lack of a straightforward glycosylation strategy.[14,15] To tackle this challenge, an efficient glycosylation method for the formation of the Man-β(1→4)-GlcNAc glycosidic bond is required. In this regard, we sought to use the direct β-selective mannosylation approach rather than the indirect one because the former approach does not involve additional synthetic steps.[16–18] Among different mannosyl donors that have been explored in β-mannosylations, the mannosyl trichloroacetimidate donor is preferred because the imidate donor can be activated by a catalytic amount of Lewis acid, such as trimethylsilyl trifluoromethanesulfonate (TMSOTf).[19] This catalytic reaction is beneficial to the development of a sequential glycosylation strategy as it presumably results in a less complex reaction mixture.[20] Thus, 4,6-*O*-benzylidene mannosyl trichloroacetimidate **1** was prepared (Scheme 1).[17b,d,e,21] For the GlcNAc acceptor, 2-deoxy-2-trichloroethoxycarbonyl thioglucopyranoside **2** was selected for its simpler preparation. The C-2 carbamate protecting function in **2** would guide the formation of the GlcNAc-β(1→4)-GlcNAc glycosidic bond by neighboring-group participation.[22,23,24] Furthermore, the anomeric thioacetal function of **2** is stable to the activation conditions for the mannosyl donor; if needed, this thioacetal function can be readily activated by a suitable thiophilic reagent.[22]

Abstract in Chinese:
本論文敘述在運用"醣給體逆向加入法(inverse-addition)"進行合成 1,2-順式□-甘露醣質化反應時，只要控制甘露醣給體加入的速度，便能有效壓低甘露醣衍生陽離子(mannosyl oxocarbenium ion)的濃度，減少 S_N1-like 親核性取代反應，因此便提高合成 1,2-順式□-甘露醣質化反應的立體選擇性。利用上述成果，本實驗室首次研發連實式醣質化策略(contiguous sequential glycosylation strategy)，合成氮-共軛醣蛋白質的核心三醣體和沙門氏細菌細胞囊膜上的寡醣單元。



Scheme 1. The inverse-addition (I-A) protocol for the glycosylation of GlcNAc acceptor **2** with mannopyranosyl trichloroacetimidate **1**. PMB = *p*-methoxy benzyl; Troc = trichloroethoxycarbonyl.

Concerning the glycosylation procedure in Man-β(1→4)-GlcNAc glycosidic bond formation, both inverse addition[16b,17d] and conventional procedures have been exploited.[16e,17h,i,l] In the conventional procedure, TMSOTf promoter is added to a mixture of mannosyl donor and GlcNAc acceptor. In the I-A procedure, mannosyl donor is added to a mixture of TMSOTf promoter and GlcNAc acceptor. A search in the literature reveals that the I-A procedure is primarily designed for suppression of undesired side reactions in glycosylations when highly reactive glycosyl trichoroacetimidate donor is employed.[25] We speculated that the manner of donor addition might have some bearing on the β-selectivity of mannosylation. To attest this speculation, both the conventional and I-A procedures were used for glycosylations of GlcNAc acceptor **2** with mannsoyl trichoroacetimidate **1** (Scheme 1, Table 1). For the donor addition rate used in the I-A procedure, 0.25 M of mannosyl donor **1** was added to 50 mM of glycosyl acceptor **2** in CH_2Cl_2 over a period of 5, 15, or 30 min, which was corresponded to the donor addition rates of 0.2, 0.07, or 0.035 mL min^{-1}.[26]

The conventional glycosylation of GlcNAc acceptor **2** with **1** produced the expected disaccharide **3** in 48% yield with a 1:2 α/β-anomeric ratio along with approximately 20% aglycon transfer product **4** (Table 1, entry 1).[27] Grate-

Table 1. Glycosylations of GlcNAc acceptor **2** with mannosyl trichloroacetimidate **1** by the conventional and inverse-addition procedures.

Entry	Addition rate of 0.25 M of **1** [mL min^{-1}]	Yield **3** [%]	α/β ratio[a] of **3**	Yield **4** [%]	Yield **5** [%]
1	conventional	48	1:2	20	0
2	0.20[b]	65	1:9.5	5	5
3	0.07	47	1:5.2	5	10
4	0.035	40	1:4.5	10	10

[a] α/β-Anomeric ratios were determined by HPLC analysis (Hitachi HPLC system: L-2300 pump, L-2400 UV-detector; Mightysil normal phase Si 4.6-250 column; mobile phase: CH_2Cl_2/hexane/EtOAc gradient from 10:75:15 to 10:70:20 at a flow rate of 0.8 mL min^{-1}). [b] This particular reaction was repeated twice for validation.

fully, this aglycon transfer product was reduced when the I-A mannosylation procedure was applied (column 4). More significantly, a higher β-selectivity was observed in the I-A mannosylations, and such selectivity was found to be dependent on the addition rate of mannosyl donor **1**. For instance, at the donor addition rate of 0.2 mL min^{-1}, the disaccharide **3** was produced in 65% yield with a 1:9.5 α/β-anomeric ratio (entry 2), but this selectivity declined to some degree at slower donor additions (0.07 and 0.035 mL min^{-1}; Table 1, entries 3 and 4). A minor drawback of the present I-A mannosylations was the formation of approximately 5–10% *N*-(β-mannosyl) trichloroacetamide **5** (column 5).[28] One possible solution to eliminate amide product formation is to use the mannosyl (*N*-phenyl) trifluoroacetimidate donor.[17h,i,l] However, the synthesis of such a mannosyl donor requires the use of unstable *N*-phenyl trifluoroacetimidoyl chloride, which is not commercially available.[29] Nevertheless, the amide byproduct **5** does not interfere with the development of a sequential glycosylation strategy. Thus, this study selected the easily accessible mannosyl trichloroacetimidate as the donor for subsequent glycosylations after the trade-off between the preparatory convenience and optimization of the glycosylation yield.

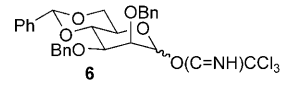
Guided by the results of the foregoing glycosylations, we extended the scope of this study to mannosylations of glycosyl acceptors **7**, **8**, and **9** with mannosyl trichloroacetimidate donor **6** (Table 2).[30] It should be noted that the mannosyl donor **6** was employed instead of **1** for the reason of its simpler preparation. For the glycosylation of acceptor **7** with mannosyl donor **6**, the I-A procedure furnished a higher β-selectivity than that given by the conventional procedure (Table 2, entries 1–3). However, the improvement of β-selectivity was less pronounced in the glycosylations of diisopropylidene acetal protected galactopyranosyl acceptor **8** (entries 4–6). A search in literature revealed that the β-mannosylations of the galactopyranosyl acceptor **8** often result in modest β-selectivities to other glycosyl acceptors.[17k,31] This peculiar result may be due to a mismatched interaction between the mannosyl donor **6** and galactopyranosyl acceptor **8** in the transition state leading to β-anomer formation.[32] Another possible explanation is the erosion in selectivity of β-mannosylation when a highly reactive acceptor is employed.[17d,j,k] To clarify the exact cause, methyl 2,3,4-tri-*O* benzyl glucopyranoside **9**, which bears a C-6 hydroxyl function, was prepared and used as a reactive acceptor for glycosylation with mannosyl donor **6**. Remarkably, the glycosylation produced the desired disaccharide **12** with excellent β-selectivity (entry 7). The experimental result is in line with the explanation of mismatching interactions.

Table 2. Conventional and I-A mannosylations of glycosyl acceptors **7**, **8**, and **9** with mannosyl trichloroacetimidate **6**.

Entry	Donor addition rate of 0.25 M of **6** [mL min^{-1}]	Acceptor	Product (yield [%])	α/β ratio[a]	Reported α/β ratios in the literature
1	conventional	**7**	**10** (88)	1:2	for **10**, 1:9 to 1:16[17c,g,k]
2	0.26	**7**	**10** (90)	1:10	
3	0.07	**7**	**10** (85)	1:8	
4	conventional	**8**	**11** (85)	1:2	for **11**, 1:2 to 1:5.0[17a,d,j,k]
5	0.46	**8**	**11** (92)	1:2.3	
6	0.14	**8**	**11** (87)	1:2.3	
7	0.26	**9**	**12** (87)	1:>15[b]	for **12**, 1:10[17k]
8	conventional	**8**	**11** (50)	2:1[c]	

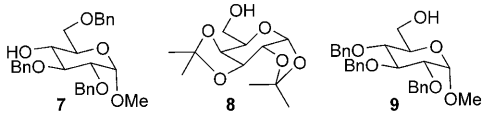
[a] α/β-Anomeric ratios were determined by HPLC analysis. [b] In practice, no α-anomer was detected on the base of NMR spectroscopy, and the ratio of 1:>15 was a conservative minimum. [c] BF_3•Et_2O (1 mol equiv with respect to the donor) was used for activation of **6**.

Mannosyl trichloroacetimidate donor



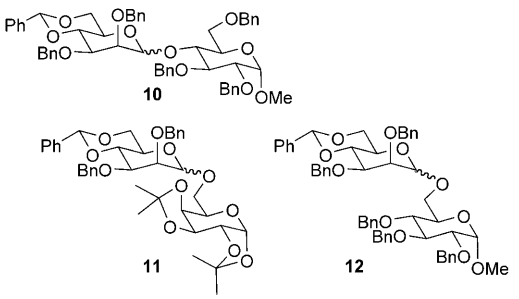
6

Glycosyl acceptors



7 **8** **9**

Glycosylation products

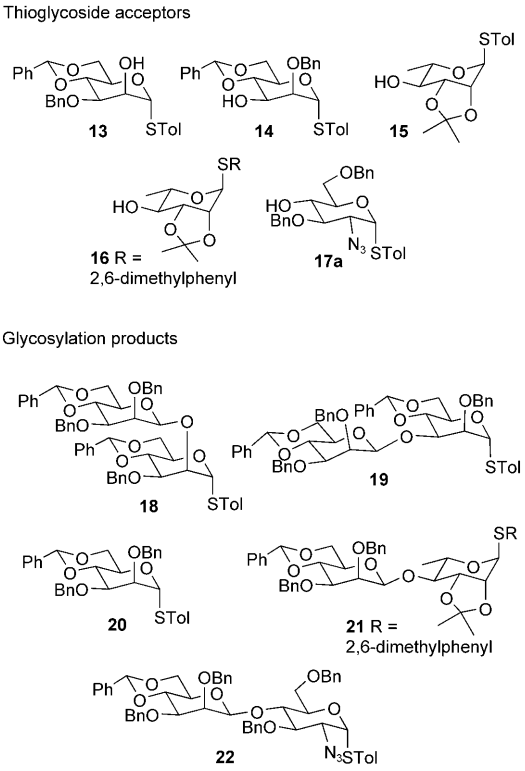


10 **11** **12**

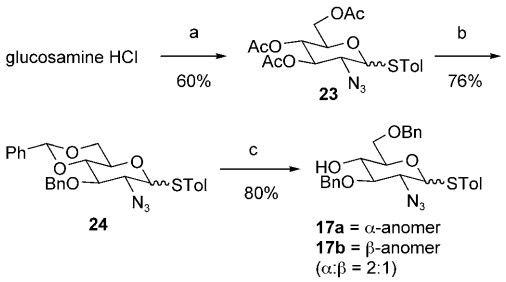
Since TMSOTf and BF_3•Et_2O are widely used as promoters for the activation of trichloroacetimidate donors, it is reasonable to question if BF_3•Et_2O could also be used in the present mannosylation context.[33] To answer this quest, the glycosylation of acceptor **8** with donor **6** was repeated with the conventional procedure by using BF_3•Et_2O as the promoter. In sharp contrast, this change of promoter reversed the selectivity of mannosylation and a 2:1 α/β-anomeric mixture of **11** was obtained (Table 2, entry 8). It should be noted that the loss of β-selectivity in the absence of triflate-based promoters has also been reported by other studies.[16e,17k,34] A possible explanation is that when TMSOTf was used, the counter anion of the mannosyl oxocarbenium ion was the triflate anion and thus the two ions quickly coupled to generate the covalent α-mannosyl triflate. This α-mannosyl triflate would follow an S_N2-like pathway and produce the β-mannoside. On the other hand, when BF_3•OEt_2 was used, the mannosyl oxocarbenium ion would directly react with the acceptor to give the α-mannoside as the major product.[35]

CHEMISTRY
AN ASIAN JOURNAL

Orthogonal β-mannosylation: After establishing the rate-dependent I-A mannosylation procedure, we proceeded to apply the procedure to orthogonal glycosylations. Thus, thioglycosides **13**–**17a** were synthesized and used as glycosyl acceptors for glycosylation with mannosyl donor **6** (Table 3).[36] The synthesis of **17a** employed a modified procedure, which, therefore, deserved some elaboration (Scheme 2).



Glucosamine hydrogen chloride was first converted to the glucosamine azido derivative by a recently reported diazo transfer reaction.[37] Acetylation and thioglycosidation of the crude azido derivative produced the peracetyl GlcNAc thioglycoside **23**. Followed by standard protecting-group manipulations, the GlcNAc thioglycoside **23** was converted to α- and β-thioglycosides **17a** and **17b** via intermediate **24**. The



Scheme 2. Reagents and conditions: a) i) azido-sulfonyl imidazolium chloride, Et_3N, cat. $CuSO_4$, MeOH, RT; ii) Ac_2O, py. CH_2Cl_2, RT; iii) thiocresol, $BF_3{\cdot}Et_2O$, CH_2Cl_2, RT, 2 days, 60% 3 steps; b) i) Na (s), MeOH/CH_2Cl_2, RT, 90%; ii) $C_6H_5CH(OMe)_2$, cat. TsOH, CH_3CN; iii) benzyl bromide, NaH, DMF, 0 °C–RT; 84% over 2 steps; c) Et_3SiH, TFA, CH_2Cl_2, −15 °C, 80%. py = pyridine.

Table 3. Results of orthogonal β-mannosylations with the rate dependent I-A procedure.

Entry	Thioglycoside acceptor	Glycosylation product (yield [%])	Yield **20** [%]	α/β ratio[a]
1	**13**	**18** (75)	<5	1:>15
2	**14**	**19** (70)	<5	1:>15
3	**15**	–	80	–[b]
4	**16**	**21** (90)	–	1:>15
5	**17a**	**22** (60)	<5	1:>15[c]

[a] No α-anomers were detected based on NMR spectroscopy of the crude chromatography products and ratios of 1:>15 were based on conservative estimate. [b] Predominant formation of α-thiomannopyranoside was observed. [c] This particular glycosylation has been repeated twice for validation.

thioglycosides **17a** and **17b** could be separated by standard chromatography.

With thioglycoside acceptors **13**–**17a** and mannosyl donor **6** in hand, the stage was set for studying the orthogonal glycosylations. With the exception of thioglycoside **15**, all the examined glycosylations furnished the expected disaccharide thioglycosides **18**, **19**, **21**, and **22** in 60–90% yields with excellent β-selectivities (1:>15 α/β). In practice, no α-anomers were isolated by chromatographic purification (Table 3, entries 1, 2, 4, and 5). To our delight, the aglycon transfer side reaction was not significant, which may probably be attributed to the relatively mild promoting conditions used. However for the glycosylation of deoxy thioglycoside **15**, aglycon transfer-product **20** was obtained in the majority. This peculiar phenomenon can be explained by the higher reactivity of the deoxy glycosyl substrate, which accelerates the aglycon transfer reaction (Table 3, entry 3).[20d,27] Nevertheless, the adverse reaction was rectified by using a bulky thioacetal function in the thioglycoside acceptor, which was illustrated by the glycosylation of thioglycoside **16** (Table 3, entry 4).

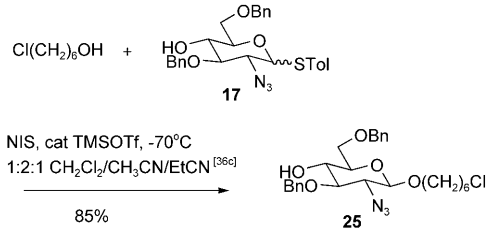
Since disaccharides **3**, **18**,[9c] **19**,[9a] **21**, and **22** are potential glycosyl donors, it is necessary to confirm their structures for future applications. Thus, all these disaccharides were rigorously characterized by NMR spectroscopy and selected characteristic data are depicted in Table 4. The β-configurations of the mannosidic linkages in disaccharides **3**, **18**, **19**, **21**, and **22** are evidenced by 1) the ^{13}C chemical shifts of C-1′, which lie between $\delta = 98.1$ and 102.4 ppm (Table 4, column 3), 2) the ^{1}H chemical shifts of H-1′, which lie between $\delta = 4.08$ and 5.06 ppm (column 5), and 3) the $^{1}J_{CH}$ coupling constants, which span from 153 to 159 Hz (column 3).[38]

Development of contiguous sequential glycosylation: Encouraged by the results of the orthogonal glycosylations, this study resumed the synthesis of the Man-β(1→4)-GlcNAc-β-(1→4)-GlcNAc trisaccharide (Schemes 3 and 4). As a model study, a stepwise glycosylation approach was examined to test the suitability of the GlcNAc glycoside **25** as an acceptor. The GlcNAc glycoside **25** was prepared from glycosyla-

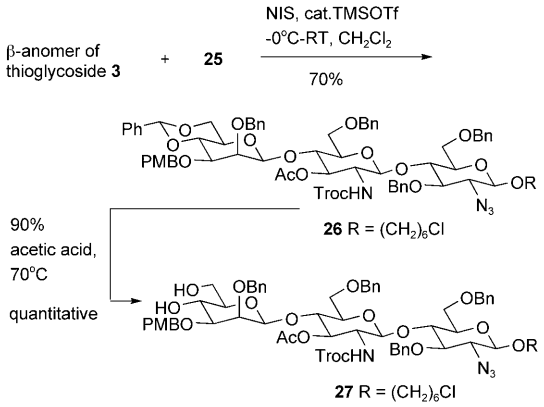
Table 4. Selected NMR spectroscopic chemical shifts and $^1J_{CH}$ coupling constants of the disaccharide thioglycosides **3**, **18**, **19**, **21**, and **22**.

Entry	Disaccharide thioglycoside	δ [ppm] and $^1J_{CH}$ [Hz] of C-1	δ [ppm] and $^1J_{CH}$ [Hz] of C-1′	δ [ppm] of H-1	δ [ppm] of H-1′
1	α-anomer **3**	86.5, 158.4	100.7, 167	4.58	5.01
2	β-anomer **3**	87.3, 158.4	102.4, 159	–[a]	4.52
3	**18**	86.6, 164	100.8, 153	5.47	4.65
4	**19**	86.1, 167	98.1, 158	5.56	4.08[b]
5	**21**	84.9, 165	100.4, 158	5.47	5.06
6	**22**	87.5, 167	101.1, 157	5.49	4.36

[a] H-1 anomeric proton of the β-anomer of **3** was obscured by the benzylic protons, but its presence was clearly implicated by noting the cross-peak in the ^{1}H–^{13}C HMQC spectrum. [b] This upfield anomeric ^{1}H signal was confirmed by the ^{1}H–^{13}C HMQC spectrum.



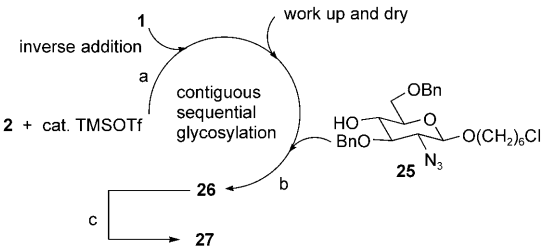
Scheme 3. Synthesis of GlcNAc glycoside **25** by the low substrate concentration β-selective glycosylation.



Scheme 4. Stepwise synthesis of the protected trisaccharide core of N-linked glycoproteins **26** and **27**.

tion of 3-chloropropanol with GlcNAc thioglycoside **17** by the low substrate concentration β-selective glycosylation (Scheme 3).[36c] Subsequent glycosylation of the GlcNAc glycoside **25** with the β-anomer of disaccharide thioglycoside **3** produced the expected trisaccharide **26** (Scheme 4). To simplify the purification process, the benzylidene acetal function in trisaccharide **26** was removed to give trisaccharide diol **27**. The β-configurations of the anomeric centers in **27** are evidenced by 1) the ^{13}C chemical shifts at $\delta = 102.5$, 101.2, and 101.1 ppm and 2) the corresponding $^1J_{CH}$ coupling constants of 158.2, 158.6, and 160.8 Hz.[38]

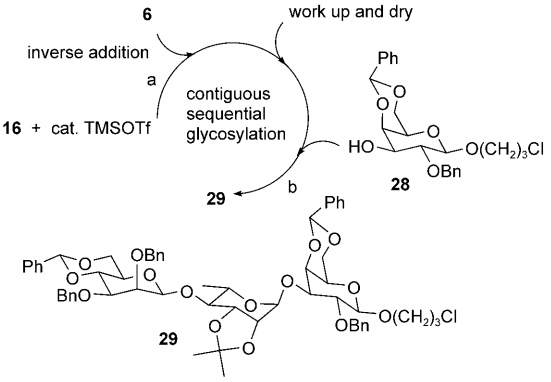
After proving the utility of GlcNAc glycoside **25** as an acceptor, the stage was mature for developing the contiguous sequential glycosylation strategy, which skipped the need for isolating the disaccharide intermediate **3** (Scheme 5). Thus, GlcNAc acceptor **2** was glycosylated with mannosyl donor **1** by the rate-dependent I-A procedure to furnish Man-β(1→4)-GlcNAc disaccharide **3**. The crude thioglycoside **3** obtained upon standard workup was used directly as a donor for glycosylation of GlcNAc glycoside **25** to produce trisaccharide **26**. Subsequent removal of the benzylidene acetal function of **26**



Scheme 5. Reagents and conditions: a) cat. TMSOTf, CH_2Cl_2, 4 Å MS, −50 °C, addition of 0.25 M of **1** at 0.2 mL min^{-1}; b) cat. TMSOTf, NIS, CH_2Cl_2, 4 Å MS, 0 °C–RT, 16 h; c) 90% AcOH/H_2O, 70 °C, 1 h, 40% over a to c.

produced the target trisaccharide **27** in 40% yield as the sole isolable isomer.

The synthetic utility of the foregoing contiguous sequential glycosylation was further exploited in the preparation of Man-β(1→4)-Rha-α(1→3)-Gal trisaccharide. Previous synthesis of this trisaccharide target employed a stepwise glycosylation approach.[7,39] We envisaged that the application of the contiguous glycosylation strategy should simplify the synthetic endeavor (Scheme 6). Thus, thiorhamopyranoside **16** was glycosylated with mannosyl trichloroacetimidate **6** to produce disaccharide thioglycoside **19**. The crude disaccharide **19** obtained from standard workup was directly coupled with the galactopyranoside acceptor **28** to furnish the de-

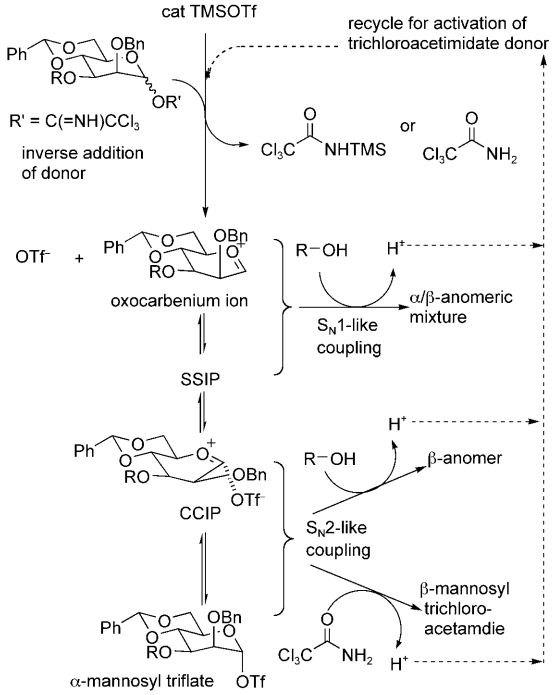


Scheme 6. Reagents and conditions: a) cat. TMSOTf, CH_2Cl_2, 4 Å MS, −50 °C, 10 min, addition of 0.25 M of **6** at 0.4 mL min^{-1}; b) cat. TMSOTf, NIS, CH_2Cl_2, 4 Å MS, −20 to 0 °C, 30 min; 70% over a and b.

CHEMISTRY
AN ASIAN JOURNAL

sired trisaccharide **29** as the single isolable isomer in a high 70% yield.[40] The configurations of the anomeric centers in trisaccharide **29** are evidenced by 1) the ^{13}C chemical shifts at $\delta = 104.0$, 100.6, and 100.5 ppm and 2) the corresponding $^{1}J_{CH}$ coupling constants of 156.5, 157.5, and 167.9 Hz.[38]

Mechanistic discussion: Based on experimental observations and literature review, we propose a mechanistic model to account for the observations in this study (Scheme 7).[16b,17,41,42] At first, the mannosyl trichloroacetimidate is activated by TMSOTf to produce free oxocarbenium and triflate ions.



Scheme 7. Proposed mechanism for the rate-dependent I-A β-selective mannosylation.

These ions are coupled to each other and generate a series of glycosyl intermediates, namely, closed-contact ion pairs (CCIPs), solvent-separated ion pairs (SSIPs), and α-mannosyl triflate, which are engaged in a complex equilibrium network. Each of these intermediates can react with the nucleophilic acceptor to furnish the glycosylation product through either the S_N1- or S_N2-like pathway. The former reaction pathway leads to the formation of α- and β-anomers, whereas the latter pathway furnishes the β-anomeric product. When these coupling reactions are complete, a proton is released and recycled to activate other trichloroacetimidate donors. It is reasoned that the optimized rate-dependent I-A protocol results in a low concentration of free oxocarbenium ions. Under these conditions, the S_N1-like reaction pathway is suppressed, thus reducing the formation of the undesired α-anomer.

In hindsight, in earlier 4,6-*O*-benzylidene-directed β-mannosylations, the β-selectivity was enhanced when the acceptor was added to the reaction mixture several minutes after the donor was completely activated by the promoter.[17a,17e] It is believed that such a preactivation approach allows the quantitative conversion of the oxocarbenium ion to the α-mannosyl triflate before charging the acceptor to the reaction mixture. Thus, both the present rate-dependent I-A approach and previous preactivation approach enhance the β-selectivity by avoiding the accumulation of the mannosyl oxocarbenium ions. Regarding the decline of β-selectivity in slower donor additions, we are unable to give an explanation at the present stage and further investigations along this line are being undertaken.

Conclusions

This study describes for the first time the rate-dependent inverse-addition procedure. This procedure improves the selectivity of direct β-mannosylations with the use of an easily accessible mannosyl trichloroacetimidate donor. Further elaboration of this procedure leads to the incorporation of β-mannosidic bond formation to a contiguous sequential glycosylation strategy, which streamlines the preparation of oligosaccharides bearing β-mannosidic linkages. The synthetic utility of this glycosylation strategy was demonstrated by the synthesis of the trisaccharide core of human N-linked glycoproteins and the trisaccharide repeating units of O-specific polysaccharide in the cellular capsule of Salmonella bacteria. Owing to the frequent occurrence of natural oligosaccharides with β-mannosidic linkages and their biological relevance, the proposed method should prove useful in their preparation.

Experimental Section

General: Chemicals employed in this study were purchased as ACS reagent grande from loval commercial vendors and used without further purification. CH_2Cl_2 (Mallinckrodt), MeOH, and CH_3CN (J. T. Baker) were distilled over calcium hydride before use. Addition rates of mannosyl donor were controlled by KSD 101 syringe pump. Chemical reactions were monitored by thin layer chromatography (TLC) on a silca gel F-254 plate (Merck). Compounds on the TLC plate were visualized with UV illumination (254 nm) and/or by staining with *p*-anisaldehyde staining reagent. Optical rotations of new compounds were acquired using a JASCO DIP-1000 polarimeter at RT. Chromatographic purification was performed on either 70–230 or 230–400 mesh size silca gel (Merck). Elution of 230–499 mesh size silica gel was achieved by medium pressure liquid chromatography (MPLC) (Buchi 688 pump). ^{1}H NMR spectra were recorded by 300 or 500 MHz spectrometers (^{13}C NMR spectra by 75 or 125 MHz spectrometers), which are either configured in the Bruker or Varian console as specified. Chemical shifts are calibrated against the residual ^{1}H resonance signal and ^{13}C resonance signal of the deuterated solvent used. Coupling constants measured in hertz (Hz) were derived from the difference of chemical shifts in the ^{1}H NMR spectra. α/β-Anomeric ratiosAnomeric ratios of glycosylation products were determined either by HPLC or NMR analysis of the isolated products. HPLC analysis was performed by 1) Hitachi L-2130 gradient pump and L-2400 UV/Vis detector; and 2) Mightysil Si 260-4.6 normal phase column.

Rate-dependent inverse-addition procedure for disaccharide 3 and its side products 4 and 5: A mixture of GlcNAc thioglycoside **2**[23] (100 mg, 0.17 mmol) and activated 4 Å molecular sieves (MS; AW300) (500 mg) in

CH_2Cl_2 (3.5 mL) was stirred at −50 °C for 10 min under N_2, followed by the addition of TMSOTf (5.0 μL, 0.026 mmol). A solution of mannosyl trichloroacetimidate **1** (158 mg, 0.25 mmol) in CH_2Cl_2 (1 mL, 0.25 M) was added at 0.20, 0.07, or 0.035 mL min^{-1} (by KDS100 syringe pump) to the reaction mixture. Upon completion of the reaction as judged by TLC, (R_f of **3** = 0.22; EtOAc/hexane 1:4), a few drops of Et_3N were added to quench the reaction, which was followed by the removal of the MS by filtration over Celite. The resulting filtrate was concentrated for purification by MPLC over 230–400 mesh silica gel (EtOAc/hexane 1:4) to furnish tolyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-*p*-methoxy-benzyl-β-D-mannopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroethoxycarbonyl-1-thio-β- D-glucopyranoside (**3**) (118 mg, 65%, α/β 1:9.5), tolyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside (**4**) as a colorless syrup (6 mg, 5%), and *N*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-*p*-methoxybenzyl-1-β-D-mannopyranosyl) trichloroacetamide (**5**) as a colorless syrup (10 mg, 10%). The α/β-anomeric ratio of **3** was determined by HPLC analysis (Hitachi HPLC system: L-2300 pump, L-2400 UV-detector and Mightysil Si 60 250–4.6 normal phase column; mobile phase: CH_2Cl_2/hexane/EtOAc, elution gradient from 10:75:15 to 10:70:20 at a flow rate of 0.8 mL min^{-1}).

For the β-anomer of ***3***: R_f = 0.22 (EtOAc/hexane 1:4); $[\alpha]_D^{27} = -30.7$ (c = 0.63 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.50 (d, J = 7.2 Hz, 2H; Ar*H*), 7.44 (d, J = 7.8 Hz, 2H; Ar*H*), 7.42–7.22 (m, 15H; Ar*H*), 7.09 (d, J = 7.9 Hz, 2H; Ar*H*), 6.86 (d, J = 8.3 Hz, 2H; Ar*H*), 5.58 (s, 1H; benzylidene-C*H*), 5.35 (d, J = 9.3 Hz, 1H), 5.17 (t, J = 9.5 Hz, 1H), 4.84 (d, J = 12.0 Hz, 1H), 4.79–4.62 (m, 5H), 4.56 (dd, J = 22.9, 11.9 Hz, 2H), 4.42 (d, J = 12.0 Hz, 2H; PhCH_2, H-1′), 4.28 (dd, J = 10.1, 4.5 Hz, 1H), 4.09 (t, J = 9.5 Hz, 1H), 3.89 (t, J = 9.2 Hz, 1H), 3.84–3.77 (m, 4H), 3.72 (d, J = 9.8 Hz, 1H), 3.67 (d, J = 8.4 Hz, 2H), 3.54 (d, J = 10.9 Hz, 1H), 3.44 (d, J = 7.1 Hz, 1H), 3.18 (d, J = 4.2 Hz, 1), 2.33 (s, 3H; ArCH_3), 2.01 ppm (s, 3H; CH_3C=O); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.3 (*C*=O), 159.5-(*Ar*OCH$_3$), 154.6 (carbamate-*C*=O), 138.8, 138.7, 138.0, 137.8, 134.0, 130.8, 130.1, 129.5, 129.3, 128.9, 128.9, 128.6, 128.5, 128.4, 128.2, 128.0, 126.4, 114.1, 102.4 ($^1J_{CH}$ = 158.6 Hz; C-1′), 101.8 (benzylidene-*C*H), 95.9 (*C*Cl$_3$), 87.3 ($^1J_{CH}$ = 158.4 Hz; C-1), 79.1, 78.9, 78.0, 77.7, 76.9, 76.3, 75.1, 74.9, 74.4, 73.9, 72.6, 68.9, 68.8, 67.8, 55.6, 55.3, 21.6 (*C*H$_3$), 21.4 ppm (*C*H$_3$); HRMS-ESI: m/z: calcd for $C_{53}H_{56}Cl_3NO_{13}SNa$: 1074.2436; found: 1074.2430 $[M+Na]^+$. The crude NMR (including ^{1}H-, ^{13}C-, HMQC-, and gated decoupling ^{13}C NMR) spectra of α-anomer of **3** are provided in the Supporting Information.

For side-product ***4***: R_f = 0.75 (EtOAc/hexane 1:4); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.63 (dd, J = 7.6, 1.8 Hz, 2H; Ar*H*), 7.54–7.37 (m, 12H; Ar*H*), 7.19 (d, J = 8.1 Hz, 2H; Ar*H*), 7.01–6.93 (m, 2H; Ar*H*), 5.73 (s, 1H; benzylidene-C*H*), 5.54 (d, J = 0.9 Hz, 1H; H-1), 4.88–4.77 (m, 3H), 4.68 (d, J = 11.8 Hz, 1H), 4.45–4.27 (m, 3H), 4.14–4.04 (m, 2H), 4.02–3.91 (m, 1H), 3.87 (s, 3H; OCH_3), 2.41 ppm (s, 3H; ArCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 159.7 (*Ar*OCH$_3$), 138.4, 138.3, 138.1, 132.8, 130.9, 130.4, 130.3, 129.8, 129.3, 128.9, 128.6, 128.5, 128.3, 126.6, 114.2, 101.9 (benzylidene-C*H*), 87.9 (C-1), 79.5, 78.4, 76.2, 73.4, 73.1, 69.0, 65.9, 55.7 (ArOCH$_3$), 21.6 ppm (*C*H$_3$); HRMS-ESI: m/z: calcd for $C_{35}H_{37}O_6SNa$: 608.2209; found: 608.2203 $[M+Na]^+$.

For side-product ***5***: R_f = 0.65 (EtOAc/hexane 1:4); $[\alpha]_D^{27} = +17.3$ (c = 0.75 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.60–7.48 (m, 3H; Ar*H*), 7.48–7.30 (m, 10H; Ar*H*), 6.98–6.89 (m, 2H; Ar*H*), 5.67 (s, 1H; benzylidene-C*H*), 5.22 (dd, J = 9.2, 1.35 Hz, 1H; H-1), 5.15 (d, J = 11.5 Hz, 1H), 4.93 (d, J = 11.7 Hz, 1H), 4.75 (d, J = 11.4 Hz, 2H), 4.36 (dd, J = 10.4, 4.9 Hz, 1H), 4.22 (t, J = 9.6 Hz, 1H), 3.97–3.90 (m, 1H), 3.89–3.79 (m, 5H), 3.52 ppm (td, J = 9.8, 4.9 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.7 (*C*=O), 159.8 (*Ar*OCH$_3$), 137.7, 137.6, 130.4, 129.9, 129.4, 129.3, 128.9, 128.6, 126.4, 114.3, 101.9 (benzylidene-*C*H), 92.3 (*C*Cl$_3$), 79.4 ($^1J_{CH}$ = 160.7 Hz), 78.98, 77.9, 77.4, 77.0, 76.7, 75.9, 73.9, 69.4, 68.7, 55.7 ppm (ArO*C*H$_3$); HRMS-FAB: m/z: calcd for $C_{30}H_{30}NO_7Cl_3$: 621.1088; found: 621.1085 $[M]^+$.

Rate-dependent inverse-addition (I-A) procedure for the synthesis of 10–12, 18, 19, 21, and 22: A suspension of *O*-glycoside acceptor (**7**, **8**, or **9**) (1 equiv, 100 mg) or thioglycoside acceptor (**13**, **14**, **16**, or **17a**) (1 equiv, 100 mg) and activated 4 Å MS (AW300) in CH_2Cl_2 was stirred at −50 °C for 10 min under N_2, which was followed by the addition of TMSOTf (0.15 equiv to acceptor). A solution of mannosyl trichloroacetimidate **6** in CH_2Cl_2 (1.5 equiv, 0.25 M) was added at the designated addition rate (0.26, 0.07 for **7**; 0.46, 0.14 for **8**; 0.26 for **9**, **13**, and **14**; 0.40 for **16**; and 0.24 mL mim^{-1} for **17a**). The progress of the reaction was monitored by TLC with EtOAc/hexane or EtOAc/CH_2Cl_2/hexane mixture as the developing solvent. Upon complete consumption of the glycosyl acceptor, a few drops of Et_3N were added to quench the reaction, which was then followed by filtration over Celite. The resulting filtrate was concentrated for standard chromatography or MPLC purification to furnish disaccharide **10** (from glycosylation of **7**), **11** (from glycosylation of **8**), **12** (from glycosylation of **9**), **18** (from glycosylation of **13**), **19** (from glycosylation of **14**), **21** (from glycosylation of **16**), or **22** (from glycosylation of **17a**). α/β-Anomeric ratios of the glycosylation products were determined by either HPLC analysis (HPLC system: Hitachi L-2300 pump, L-2400 UV-detector, and Mightysil Si 60 250–4.6 normal phase column; mobile phase for elution: hexane/CH_2Cl_2/EtOAc mixture gradient from 75:10:15 to 70:10:20 at a flow rate of 0.8 mL min^{-1}) (for disaccharides **10**–**12**) or NMR spectroscopy of the isolated products (for disaccharides **18**, **19**, **21**, and **22**).

*Methyl 2,3-di-O-benzyl-4,6-O-benzylidene-β-*D*-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-1-α-*D*-glucopyranoside (**10**)*: Disaccharide **10** was prepared from glucopyranoside acceptor **7** (100 mg, 0.216 mmol) and mannosyl trichloroacetimidate **6** (192 mg, 0.324 mmol) according to the general I-A procedure at a donor addition rate of 0.26 mL min^{-1}. The crude disaccharide **10** was purified by MPLC over silica gel (230–400 mesh) (EtOAc/hexane 1:3) to furnish the β-anomer of disaccharide **10** as an amber-colored syrup (170 mg, 90%, α/β 1:10 based on HPLC analysis). For the β-anomer of disaccharide **10**: R_f = 0.30 (EtOAc/hexane 1:3); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.50 (dt, J = 8.0, 4.3 Hz, 4H; Ar*H*), 7.46–7.23 (m, 26H; Ar*H*), 5.55 (s, 1H; benzylidene-C*H*), 5.08 (d, J = 10.7 Hz, 1H; PhCH_2), 4.87–4.73 (m, 5H), 4.72–4.57 (m, 5H), 4.39 (s, 1H), 4.31 (d, J = 12.1 Hz, 1H), 4.15–4.03 (m, 2H), 3.96–3.84 (m, 2H), 3.67 (d, J = 2.9 Hz, 1H), 3.62 (dd, J = 8.0, 4.8 Hz, 1H), 3.58–3.52 (m, 1H), 3.49 (dd, J = 11.1, 2.7 Hz, 1H), 3.43 (s, 3H; OCH_3), 3.36 (dd, J = 10.0, 2.9 Hz, 2H), 3.08–3.01 ppm (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 139.8, 139.0, 138.9, 138.7, 138.0, 137.9, 129.2, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.2, 128.1, 127.96, 127.91, 127.7, 127.6, 126.5, 101.9 (benzylidene-*C*H), 101.7 (C-1′), 98.8 (C-1), 80.6, 79.3, 79.1, 78.6, 78.0, 77.6, 75.7, 75.4, 74.0, 73.9, 72.9, 70.0, 68.9, 68.7, 67.6, 55.7 ppm (O*C*H$_3$).[17c,g,k]

*2,3-Di-O-benzyl-4,6-O-benzylidene-β-*D*-mannopyranosyl-(1→6)-1,2:3,4-di-O-isopropylidene-α-*D*-galactopyranose (**11**)*: Disaccharide **11** was prepared from galactopyranosyl acceptor **8** (100 mg, 0.385 mmol) and mannosyl trichloroacetimidate **6** (342 mg, 0.578 mmol) according to the general I-A procedure at a donor addition rate of 0.46 mL min^{-1}. The crude reaction product was purified by MPLC over 230–400 mesh silica gel (EtOAc/hexane 1:4). The disaccharide **11** was obtained as an amber-colored syrup (250 mg, 90%, α/β 1:2.3 based on HPLC analysis). For the β-anomer of disaccharide **11**, R_f = 0.35 (EtOAc/hexane 1:4); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.56–7.47 (m, 5H; Ar*H*), 7.45–7.28 (m, 10H; Ar*H*), 5.63 (s, 1H; benzylidene-C*H*), 5.61 (d, J = 5.0 Hz, 1H; H-1), 5.02 (d, J = 12.1 Hz, 1H; PhCH_2), 4.91 (d, J = 12.1 Hz, 1H; PhCH_2), 4.68–4.53 (m, 4H), 4.38–4.32 (m, 2H), 4.29–4.15 (m, 3H), 4.12–4.08 (m, 1H), 4.07 (d, J = 3.1 Hz, 1H), 3.94 (t, J = 10.3 Hz, 1H), 3.67 (dd, J = 10.7, 8.2 Hz, 1H), 3.59 (dd, J = 9.9, 3.1 Hz, 1H), 3.39–3.31 (dt, J = 9, 3 Hz, 1H), 1.53 (s, 3H; CH_3), 1.48 (s, 3H; CH_3), 1.36 ppm (d, J = 3.6 Hz, 6H; 2×CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.6, 138.5, 137.9, 129.3, 129.2, 128.7, 128.66, 128.61, 128.0, 127.9, 126.4, 109.9 (quaternary-*C*), 109.2 (quaternary-*C*), 103.2 (C-1′), 101.8 (benzylidene-*C*H), 96.7 (C-1), 78.8, 77.6, 75.5, 75.1, 72.5, 71.9, 71.1, 70.8, 70.4, 68.9, 68.3, 67.9, 26.4 (*C*H$_3$), 26.3 (*C*H$_3$), 25.4 (*C*H$_3$), 24.7 ppm (*C*H$_3$).[17a,d,j,k]

*Methyl 2,3-di-O-benzyl-4,6-benzylidene-β-*D*-mannopyranosyl-(1→6)-2,3,4-tri -O-benzyl-α-*D*-glucopyranoside (**12**)*: Disaccharide **12** was prepared from galactopyranosyl acceptor **9** (100 mg, 0.215 mmol) and mannosyl trichloroacetimidate **6** (191 mg, 0.324 mmol) according to the general I-A procedure at a donor addition rate of 0.26 mL min^{-1}. Purification of **12** was achieved by standard column chromatography (hexane/CH_2Cl_2/EtOAc 3:1:0.5) and **12** was obtained as white glassy substance (135 mg, 68%, α/β 1:>15 based on NMR spectroscopy). For the β-anomer of di-

saccharide **12**: R_f=0.4 (EtOAc/hexane/CH_2Cl_2 1:3:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.52–7.49 (m, 2H; Ar*H*), 7.32–7.14 (m, 30H; Ar*H*), 5.59 (s, 1H; benzylidene-C*H*), 5.03 (d, J=12 Hz, 1H), 4.91 (d, J=12 Hz, 1H), 4.86–4.71 (m, 4H), 4.67 (d, J=12 Hz, 1H), 4.62–4.59 (m, 2H), 4.54 (d, J=12 Hz, 1H), 4.28–4.17 (m, 2H), 4.14–3.98 (m, 3H), 3.90 (t, J=12 Hz, 1H), 3.77–3.73 (m, 2H), 3.53–3.42 (m, 4H), 3.34 (s, 1H; OCH_3), 3.28–3.18 ppm (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=139.0, 138.5, 138.2, 137.7, 128.6, 128.4, 128.2, 127.9, 126.2, 102.1 (C-1′), 101.6 (benzylidene-*C*H), 98.0 (C-1), 77.2, 75.8, 74.9, 74.8, 73.5, 72.8, 69.8, 68.8, 68.3, 67.7, 55.4 ppm.[17k]

*Tolyl 2,3-di-O-benzyl-4,6-O-benzylidene-β-*D*-mannopyranosyl-(1→2)-3-O-benzyl-4,6-O-benzylidene-1-thio-α-*D*-mannopyranoside (**18**)*: Disaccharide **18** was prepared from thio-α-D-mannopyranosyl acceptor **13** (100 mg, 0.216 mmol) and mannosyl trichloroacetimidate **6** (192 mg, 0.325 mmol) according to the general I-A procedure at a donor addition rate of 0.26 mL min^{-1}. Disaccharide **18** was purified by MPLC over 230–400 mesh silica gel (EtOAc/hexane 1:4). The β-anomer of disaccharide **18** was obtained as an amber-colored syrup (145 mg, 75%, α/β 1:>15, based on NMR spectroscopy). For disaccharide **18**: R_f=0.29 (EtOAc/hexane/CH_2Cl_2 1:4); ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.58–7.33 (m, 27H; Ar*H*), 7.18 (d, J=8 Hz, 2H; Ar*H*), 5.64 (s, 1H; benzylidene-C*H*), 5.55 (s, 1H; benzylidene-C*H*), 5.47 (d, J=0.5 Hz, 1H; H-1), 5.08 (d, J=12.5 Hz, 1H; PhCH_2), 4.99 (d, J=12.5 Hz, 1H; PhCH_2), 4.85 (d, J=12 Hz, 1H), 4.80 (d, J=12 Hz, 1H), 4.73 (d, J=12 Hz, 1H), 4.651 (d, J=12 Hz, 1H), 4.649 (s, 1H; H-1′), 4.56–4.54 (m, 1H), 4.39 (dt, J=5, 1.5 Hz, 1H), 4.31–4.27 (m, 3H), 4.21 (t, J=10 Hz, 1H), 4.01 (dd, J=3.5, 11 Hz; 1H), 3.99 (d, J=3 Hz, 1H), 3.91 (t, J=10.5 Hz, 1H), 3.82 (t, J=10.2 Hz, 1H), 3.62 (dd, J=13.5, 3.5 Hz, 1H), 3.31 (m, 1H), 2.33 ppm (s, 1H; ArCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ=138.4, 138.3, 137.4, 132.4, 129.9, 129.6, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.7, 127.6, 127.5, 127.4, 127.3, 126.1, 101.6 (benzylidene-*C*H), 101.3 (benzylidene-*C*H), 100.8 ($^1J_{CH}$=153 Hz; C-1′), 86.6 ($^1J_{CH}$=164 Hz; C-1), 78.6, 78.5, 77.9, 77.4, 77.2, 77.0, 76.7, 76.0, 74.7, 74.6, 74.2, 72.3, 71.4, 71.03, 68.5, 68.4, 67.7, 76.5, 65.3, 21.1 ppm (Ar*C*H$_3$).[9c]

*Tolyl 2,3-di-O-benzyl-4,6-O-benzylidene-β-*D*-mannopyranosyl-(1→3)-2-O-benzyl-4,6-O-benzylidene-1-thio-α-*D*-mannopyranoside (**19**)*: Disaccharide **19** was prepared from thio-α-D-mannopyranosyl acceptor **14** (100 mg, 0.216 mmol) and mannosyl trichloroacetimidate **6** (192 mg, 0.325 mmol) according to the general I-A procedure at a donor addition rate of 0.26 mL min^{-1}. Disaccharide **19** was purified by MPLC over 230–400 mesh silica gel (EtOAc/hexane 4:1). The β-anomer of disaccharide **19** was furnished as a colorless syrup (135 mg, 70%, α/β 1:>15). For disaccharide **19**: R_f=0.28 (EtOAc/CH_2Cl_2/hexane 0.5:1:4); ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.50–7.14 (m, 24H; Ar*H*), 5.64 (s, 1H; benzylidene-C*H*), 5.56 (d, J=1.0 Hz, 1H; H-1), 5.53 (s, 1H; benzylidene-C*H*), 4.96 (d, J=12.0 Hz, 1H; PhCH_2), 4.78 (s, 1H; PhCH_2), 4.701 (s, 1H; PhCH_2), 4.695 (s, 1H), 4.62 (d, J=12.0 Hz, 1H; PhCH_2), 4.46–4.35 (m, 2H), 4.34–4.18 (m, 4H), 4.12 (t, J=9 Hz, 1H), 4.08 (s, 1H; H-1′), 4.05–4.03 (m, 1H), 3.88 (dt, J=9.0, 5.4 Hz, 2H), 3.75 (d, J=3.3 Hz, 1H), 3.32 (dd, J=9.7, 3.3 Hz, 1H), 2.97 (dt, J=4.8, 9.0 Hz, 1H), 2.36 ppm (s, 3H; ArCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ=138.7, 138.6, 138.2, 137.7, 137.4, 137.1, 132.4, 130.0, 129.8, 128.9, 128.8, 128.5, 128.33, 128.30, 128.2, 128.1, 128.03, 128.99, 127.54, 127.48, 127.42, 127.3, 126.2, 126.1, 101.8 (benzylidene-*C*H), 101.3 (benzylidene-*C*H), 98.1 ($^1J_{CH}$=158 Hz; C-1′), 86.1 ($^1J_{CH}$=167 Hz; C-1), 78.5, 77.4, 77.3, 77.2, 76.1, 75.2, 74.7, 72.3. 72.0, 71.8, 68.5, 67.7, 65.4, 21.1 ppm (Ar*C*H$_3$); HRMS-ESI: m/z: calcd for $C_{54}H_{54}O_{10}$SNa: 917.3330; found: 917.3327 [M+Na]$^+$.[9a]

*2,6-Dimethylphenyl 2,3-di-O-benzyl-4,6-O-benzylidene-1-β-*D*-mannopyranosyl-(1→4)-2,3-di-O-isopropylidene-1-thio-α-*L*-rhamnopyranoside (**21**)*: Disaccharide **21** was prepared from thio-α-L-rhamnopyranosyl acceptor **16** (100 mg, 0.31 mmol) and mannosyl trichloroacetimidate **6** (274 mg, 0.46 mmol) according to the general I-A procedure at a donor addition rate of 0.26 mL min^{-1}. Disaccharide **21** was purified by MPLC over 230–400 mesh silica gel (EtOAc/hexane 1:8). The β-anomer of disaccharide **21** was furnished as a colorless syrup (210 mg, 90%, α/β 1:>15). For disaccharide **21**: R_f=0.35 (EtOAc/hexane 1:8); $[\alpha]_D^{27}$=−140.4 (c=0.77 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.54 (m, 4H; Ar*H*), 7.47–7.29 (m, 12H; Ar*H*), 7.24–7.11 (m, 2H; Ar*H*), 5.67 (s, 1H; benzylidene-C*H*), 5.47 (s, 1H; H-1), 5.06 (s, 1H; H-1′), 4.95 (q, J=12.2 Hz, 2H; PhCH_2), 4.72 (q, J=12.6 Hz, 2H; PhCH_2), 4.44 (d, J=5.3 Hz, 1H), 4.27 (ddd, J=15.4, 11.8, 5.3 Hz, 3H), 4.10–3.94 (m, 3H), 3.73 (ddd, J=12.9, 9.9, 5.4 Hz, 2H), 3.37 (dt, J=9.8, 4.9 Hz, 1H), 2.60 (s, 6H; ArCH_3), 1.53 (s, 3H; CH_3), 1.40 (s, 3H; CH_3), 1.30 ppm (d, J=6.2 Hz, 3H; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ=143.4, 139.0, 138.8, 138.0, 131.9, 129.3, 129.2, 128.9, 128.8, 128.7, 128.6, 128.5, 128.3, 128.2, 128.1, 128.04, 128.00, 127.9, 126.4, 109.9 (quaternary-*C*), 101.8 (benzylidene-*C*H), 100.4 ($^1J_{CH}$=158.2 Hz; C-1′), 84.9 ($^1J_{CH}$=164.6 Hz; C-1), 79.1, 78.5, 78.4, 78.2, 78.1, 76.8, 75.3, 72.6, 69.0, 68.1, 67.4, 28.2, 27.0, 22.6, 18.0 ppm; HRMS-ESI: m/z: calcd for $C_{44}H_{50}O_9$SNa: 777.3073; found: 777.3068 [M+Na]$^+$.

*Tolyl 2,3-di-O-benzyl-4,6-O-benzylidene-1-β-*D*-mannopyranosyl-(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-1-thio-α-*D*-glucopyranoside (**22**)*: Disaccharide **22** was prepared from 2-azido-2-deoxy thio-α-D-glucopyranoside **17a** (100 mg, 0.20 mmol) and mannosyl trichloroacetimidate **6** (180 mg, 0.30 mmol) according to the general I-A procedure at a donor addition rate of 0.24 mL min^{-1}. Disaccharide **22** was purified by MPLC over 230–400 mesh silica gel (EtOAc/hexane 1:8). The β-anomer of disaccharide **22** was furnished as a colorless syrup (120 mg, 65%, α/β 1:>15). For disaccharide thioglycoside **22**: R_f=0.25 (EtOAc/CH_2Cl_2/hexane 0.5:1:4); $[\alpha]_D^{27}$=+20.84 (c=0.5 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.50–7.18 (m, 32H; Ar*H*), 7.11 (d, J=7.5 Hz, 2H; Ar*H*), 5.51 (s, 1H; benzylidene-C*H*), 5.49 (s, 1H; H-1), 5.16 (d, J=10 Hz, 1H; PhCH_2), 4.90 (d, J=11.5 Hz, 1H; PhCH_2), 4.81(d, J=12 Hz, 1H), 4.76 (d, J=12.5 Hz, 1H), 4.62 (d, J=6.5 Hz, 1H), 4.58 (d, J=12 Hz, 1H), 4.36 (s, 1H; H-1′), 4.26 (d, J=12 Hz, 1H), 4.21 (d, J=10 Hz, 1H), 4.07 (t, J=10 Hz, 1H), 4.03–3.99 (m, 2H), 3.90–3.88 (m, 1H), 3.70 (d, J=2.5 Hz, 1H), 3.67 (t, J=5.5 Hz, 1H), 3.54–3.49 (m, 2H), 3.39 (t, J=5.5 Hz, 1H), 3.33 (dd, J=6.5, 10 Hz, 1H), 3.03 (dt, J=5, 9.5 Hz, 1H), 2.312 ppm (s, 3H; ArCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ=138.5, 138.4, 137.8, 137.6, 137.3, 132.3, 129.8, 129.7, 128.9, 128.8, 128.5, 128.3, 128.2, 128.1, 128.0, 127.7, 127.6, 127.5, 127.4, 127.3, 126.1, 101.3 (benzylidene-*C*H), 101.1 ($^1J_{CH}$=157 Hz; C-1′), 87.5 ($^1J_{CH}$=167 Hz; C-1), 79.8, 78.6, 78.2, 77.2, 77.0, 76.7, 75.2, 75.0, 73.5, 72.6, 71.3, 68.3, 68.1, 67.2, 63.2, 21.1 ppm (Ar*C*H$_3$); HRMS-ESI: m/z: calcd for $C_{54}H_{55}N_3O_9$SNa: 944.3551; found: 944.3546 [M+Na]$^+$.

Synthesis of tolyl 2-azido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-D-glucopyranoside (23): $CuSO_4$ (17 mg, 0.07 mmol) was added to a mixture of glucosamine hydrogen chloride salt (1.5 g, 6.9 mmol), Et_3N (2.8 mL, 21 mmol), and azido sulfonylimidazolium chloride (1.8 g, 9 mmol) in MeOH (35 mL) that was stirred at RT.[37] The progress of reaction was monitored by TLC (R_f=0.45, CH_2Cl_2/MeOH 3.5:1). After stirring for 1–2 h, the reaction solvent was removed by a rotary evaporator. The crude residue was redissolved in pyridine (6.5 mL) and Ac_2O (5.4 mL, 57 mmol) was then added. The resulting mixture was stirred for 10 h at RT and then excessive pyridine was removed in vacuo to give the crude peracetyl glucoamine derivative. This derivative was diluted with EtOAc, washed with 0.1 M HCl (25 mL×3), H_2O (25 mL×1), and saturated NaCl (25 mL×1), dried ($MgSO_4$), filtered, and concentrated for thioglycosidation. The crude peracetyl glucosamine derivative (2.1 g, 5.8 mmol) obtained from the preceding step was dissolved in CH_2Cl_2 and then thiocresol (1.5 g, 12 mmol) and BF_3·Et_2O (1.6 g, 13.2 mmol) were added. The resulting mixture was stirred at RT under N_2 for 2 days and the progress of the reaction was monitored by TLC (R_f=0.4, hexane/EtOAc 2:1). The reaction mixture was diluted with CH_2Cl_2, washed with 1.0 M NaOH (25 mL×3), HCl (25 mL×1), and saturated NaCl (25 mL×1), and then dried ($MgSO_4$), filtered, and concentrated for standard chromatographic purification (hexane/EtOAc 3:1) to furnish 2-azido-2-deoxy-D-thioglucopyranoside **23** as a colorless oily syrup (1.8 g, 60% over three steps, α/β 2:1).[36c]

Synthesis of tolyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-1-thio-D-glucopyranoside (24): 2-Azido-2-deoxy thioglucopyranoside **23** (2.3 g, 5.2 mmol) was dissolved in CH_2Cl_2/MeOH 1:2 (ca. 9 mL), followed by the addition of slumps of freshly cut Na metal (~10 mg). The reaction mixture was stirred at RT for 3 h and then diluted with MeOH (3 mL) before being neutralized with IR-120 (H^+). After the removal of the resin by filtration, the filtrate was concentrated and the residue was dried in vacuo for few hours to give the crude deacetylated 2-azido-2-deoxy thioglucopyranoside as a white glassy solid (1.45 g, 90%). The deacetylat-

ed 2-azido-2-deoxy thioglucopyranoside from the preceding step (1 g, 3.2 mmol) was suspended in dried CH_3CN (10 mL), followed by the addition of *p*-toluenylsulfonic acid monohydrate (61 mg, 0.32 mmol) and benzaldehyde dimethyl acetal (0.72 mL, 4.8 mmol). The reaction mixture was stirred at RT for 4 h and the progress of reaction was monitored by TLC (hexane/CH_2Cl_2/EtOAc 3:1:1; the R_f values of the α- and β-anomers = 0.4 and 0.45). After completion of the reaction, a few drops of Et_3N were added and the solvent was reduced by a rotary evaporator. The crude product from the preceding procedure was purified by standard chromatographic purification (hexane/CH_2Cl_2/EtOAc 3:1:1) and the resulting product was used as a substrate for benzylation. The anomeric mixture of the benzylidene derivative from the preceding step (0.9 g, 2.3 mmol) was dissolved in dried DMF (5 mL) and stirred at 0 °C under N_2, to which 60% NaH (0.3 g, 4.4 mmol) and benzyl bromide (BnBr) (0.5 mL, 4.4 mmol) were added. The mixture was stirred from 0 °C to RT for 2 h and the reaction was then quenched by the addition of MeOH (2 mL). The resulting mixture was diluted with H_2O (20 mL) and the product was extracted with Et_2O (15 mL × 3). The pooled ether solution was washed with 0.1 M HCl (50 mL × 2) and saturated NaCl (50 mL × 1), dried ($MgSO_4$), and then concentrated for standard chromatographic purification (hexane/CH_2Cl_2/EtOAc 3:1:0.3) to give **24** as a white glassy solid (0.95 g, 84% over two steps, α/β 2:1). For the α-anomer of **24**: R_f = 0.31 (EtOAc/hexane/CH_2Cl_2 1:4:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.52–7.31 (m, 12H; Ar*H*), 7.13 (d, J = 7.8 Hz, 2H; Ar*H*), 5.60 (s, 1H; benzylidene-C*H*), 5.49 (d, J = 2.4 Hz, 1H; H-1), 4.89 (dd, J = 11, 45 Hz, 2H; PhCH_2), 4.44 (dt, J = 5, 11 Hz, 1H), 4.23 (dd, J = 5, 10 Hz, 1H), 4.01–3.92 (m, 2H), 3.80–3.73 (m, 2H), 2.34 ppm (s, 3H; ArCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.0, 133.5, 130.4, 129.5, 129.3, 128.8, 128.7, 128.6, 128.4, 126.4, 101.8 (benzylidene-*C*H), 88.5 (C-1), 83.1, 78.2, 77.8, 77.4, 77.0, 69.0, 64.0, 63.9, 21.6 ppm (Ar*C*H$_3$).

Synthesis of tolyl 2-azido-3,6-di-*O*-benzyl-6-*O*-benzyl-2-deoxy-D-thioglucopyranoside (17) (α-anomer: 17a β-anomer: 17b): A suspension of an anomeric mixture of **24** (0.8 g, 1.64 mmol), Et_3SiH (2.8 mL, 16.4 mml), and activated 4 Å MS (AW300) (1 g) in dried CH_2Cl_2 (6 mL) was stirred under N_2 at RT and then at −15 °C for 20 min, which was followed by the addition of trifluoroacetic acid (TFA) (1.1 mL, 16 mmol). After stirring at −15 °C for 2 h, the reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with 0.1 M NaOH (30 mL × 2), 0.1 M HCl (30 mL × 1), and saturated NaCl (30 mL × 1), dried ($MgSO_4$), filtered, and concentrated for standard chromatographic purification (hexane/CH_2Cl_2/EtOAc 4:1:1) to produce **17** as a white amorphous solid (0.63 g, 78%, α/β 2:1).

For α-anomer: ***17a***: R_f = 0.25 (EtOAc/hexane/CH_2Cl_2 1:4:1); $[\alpha]_D^{27}$ = +144.2 (c = 0.6 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.42–7.34 (m, 6H; Ar*H*), 7.32–7.28 (m, 6H; Ar*H*), 7.04 (d, J = 8.1 Hz, 2H; Ar*H*), 5.47 (d, J = 5.4 Hz, 1H; H-1), 4.87 (dd, J = 11, 31 Hz, 2H; PhCH_2), 4.51 (dd, J = 11.1, 27 Hz, 2H; PhCH_2), 4.34 (dt, J = 4, 9 Hz, 1H), 3.84 (dd, J = 5, 10 Hz, 1H; H-6), 3.75–3.61 (m, 4H), 2.63 (d, J = 2.6 Hz, 1H; O*H*), 2.30 ppm (s, 3H; ArCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 137.9, 137.6, 133.8, 129.8, 128.5,128.3, 128.0, 87.4 (C-1), 81.2, 75.3, 74.1, 73.4, 72.1, 70.1, 63.4, 21.0 ppm (Ar*C*H$_3$).

For β-anomer ***17b***: R_f = 0.31 (EtOAc/CH_2Cl_2/hexane 1:1:4); $[\alpha]_D^{27}$ = −95.8 (c = 1.0 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.45 (d, J = 8.4 Hz, 2H; Ar*H*), 7.35–7.27 (m, 10H; Ar*H*), 7.06 (d, J = 8.4 Hz, 2H; Ar*H*), 4.83 (d, J = 11, 35 Hz, 2H; PhCH_2), 4.55 (d, J = 12, 25 Hz, 2H; PhCH_2), 4.35 (d, J = 9.6 Hz, 1H; H-1), 3.79–3.70 (m, 2H), 3.58 (t, J = 9 Hz, 1H), 3.43–3.23 (m, 3H), 2.78 (s, 1H; O*H*), 2.31 ppm (s, 3H; ArCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.6, 137.6, 134.1, 129.7, 128.5, 128.2, 127.6, 86.1 (C-1), 84.5, 78.0, 75.4, 73.7, 71.7, 70.1, 64.3, 21.1 ppm (Ar*C*H$_3$); HRMS-ESI: m/z: calcd for $C_{27}H_{29}N_3O_4SNa$: 514.1771; found: 514.1777 $[M+Na]^+$.

Preparation of 6-chlorohexyl 2-*O*-benzyl-3-*O*-*p*-methoxybenzyl-β-D-mannopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroethoxycarbonyl-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-azido-2-deoxy-β-D-glucopyranoside (27)

By a stepwise approach: A mixture of the β-anomer of disaccharide **3** (310 mg, 0.30 mmol), GlcNAc acceptor **25** (100 mg, 0.20 mmol), and 4 Å MS (AW300) (500 mg) in CH_2Cl_2 (6.0 mL) was stirred at −20 °C for 10 min under N_2, followed by the addition of *N*-iodosuccinimide (NIS) (67 mg, 0.30 mmol) and TMSOTf (5 μL, 0.03 mmol). Then, the reaction mixture was stirred from 0 °C to RT for 16 h. Upon completion of the glycosylation, the reaction was quenched by the addition of a few drops of saturated $NaHCO_3$ and small pieces of solid $Na_2S_2O_3$, and was then stirred at RT for ca. 30 min. The resulting solution was filtered over Celite to remove the MS, and the filtrate was concentrated to furnish crude trisaccharide **26** (the crude ^{1}H NMR spectrum of **26** is given in the Supporting Information for reference). Crude trisaccharide **26** was dissolved in 90% aqueous acetic acid (1.0 mL) and the resulting mixture was stirred at 70 °C for 1 h. Upon complete removal of the acetal function as implicated by TLC examination, the reaction mixture was concentrated for MPLC purification over 230–400 mesh silica gel (EtOAc/hexane 1:1+1% MeOH) to furnish trisaccharide diol **27** as a colorless syrup (188 mg, 70% over two steps).

By the contiguous sequential glycosylation approach: A mixture of GlcNAc thioglycoside **2** (100 mg, 0.17 mmol) and 4 Å MS (AW300) (300 mg) in CH_2Cl_2 (3.5 mL, 50 mM) was stirred at −50 °C for 10 min under N_2, followed by the addition of TMSOTf (5.0 μL, 0.025 mmol). In the meantime, a solution of 0.25 M of mannosyl trichloroacetimidate **1** (160 mg, 0.25 mmol) in CH_2Cl_2 (1.0 mL) was prepared and added to the reaction mixture of **2** and TMSOTf at 0.2 mL min^{-1} by a syringe pump (KSD 100). Upon completion of the glycosylation, the reaction was quenched with few drops of Et_3N, and the mixture was filtered over Celite. The resulting filtrate was washed with water (20 mL × 1) and brine (20 mL × 1), dried ($MgSO_4$), filtered, and concentrated to give crude disaccharide **3**. After drying in vacuo for couple of hours, disaccharide **3** was dissolved in CH_2Cl_2 (4.0 mL) and GlcNAc thioglycoside **25** (100 mg, 0.20 mmol) and 4 Å molecular sieves (AW300) (500 mg) were added and the mixture was stirred under N_2 at −20 °C for 10 min. Then, the resulting mixture was treated with NIS (45 mg, 0.20 mmol) and TMSOTf (3.0 μL, 0.017 mmol). The resulting mixture was stirred from 0 °C to RT for 16 h under N_2. Upon completion of the glycosylation, the reaction was quenched and worked up as described previously to give crude trisaccharide **26**. The crude trisaccharide **26** was dissolved in 90% aqueous acetic acid (1.0 mL) and stirred at 70 °C for 1 h. After completion of acetal deprotection, the reaction mixture was concentrated for purification by MPLC over 230–400 mesh silica gel (EtOAc/hexane 1:1+1% MeOH) to furnish target trisaccharide diol **27** (90 mg, 40% over three steps). For trisaccharide diol **27**: R_f = 0.30 (EtOAc/hexane 1:1+1% MeOH); $[\alpha]_D^{27}$ = −45.9 (c = 0.20 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.52–7.22 (m, 17H; Ar*H*), 6.94–6.82 (m, 2H; Ar*H*), 5.02 (d, J = 11.6 Hz, 1H; PhCH_2), 4.90–4.54 (m, 6H), 4.46–4.11 (m, 8H), 3.90 (ddd, J = 19.8, 11.3, 6.9 Hz, 4H), 3.81 (s, 3H; OCH_3), 3.78–3.59 (m, 4H), 3.56 (t, J = 6.7 Hz, 3H), 3.43–3.23 (m, 4H), 3.19 (dd, J = 6.3, 3.0 Hz, 1H), 3.07 (dd, J = 9.4, 2.8 Hz, 1H), 2.04 (s, 3H; CH_3C=O), 1.86–1.74 (m, 4H; CH_2), 1.72–1.59 (m, 2H; CH_2), 1.55–1.38 ppm (m, 4H; CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.0 (*C*=O), 159.8 (*Ar*OCH$_3$), 154.5 (carbamate-*C*=O), 139.1, 138.9, 138.0, 137.6, 129.9, 129.7, 129.6, 129.4, 129.3, 128.9, 128.7, 128.6, 128.55, 128.52, 128.3, 128.2, 128.1, 128.05, 128.0, 127.7, 114.3, 102.5 ($^1J_{CH}$ = 158.2 Hz), 101.2 ($^1J_{CH}$ = 158.6 Hz), 101.1 ($^1J_{CH}$ = 160.8 Hz), 96.0 (*C*Cl$_3$), 81.6, 81.4, 77.6, 76.4, 75.7, 75.5, 74.9, 74.8, 74.6, 74.5, 74.1, 74.0, 73.8, 73.0, 71.1, 70.4, 69.3, 67.6, 67.5, 66.5, 63.4, 56.7, 55.7, 45.4 (*C*H$_2$Cl), 32.9, 29.7, 26.9, 25.6, 21.2 ppm; HRMS-ESI: m/z: calcd for $C_{65}H_{79}Cl_4N_4O_{18}Na$: 1366.4041; found: 1366.4036 $[M+Na]^+$.

Preparation of 3-chloropropyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (29): A mixture of thio-L-rhamnopyranoside **16** (100 mg, 0.31 mmol) and 4 Å MS-AW (300 mg) in CH_2Cl_2 (6.0 mL, 50 mM) was stirred under N_2 at −50 °C for 10 min, followed by the addition of TMSOTf (8.0 μL, 0.046 mmol). Meanwhile, 0.25 M of mannosyl trichloroacetimidate **6** (274 mg, 0.46 mmol) in CH_2Cl_2 (2.0 mL) was prepared and added to the mixture of **16** and TMSOTf at 0.40 mL min^{-1} by a syringe pump (KSD 100). Upon completion of the glycosylation, the reaction was quenched by the addition of a few drops of Et_3N, and the resulting mixture was filtered over Celite to remove the MS. The resulting filtrate was then washed with water (20 mL × 1) and brine (20 mL × 1), dried ($MgSO_4$), filtered, and concentrated to yield crude disaccharide **21**. Upon drying in vacuo for couple of hours, the crude disaccharide **21** was dissolved in CH_2Cl_2 (6.0 mL), to which β-galactopyranoside **28** (162 mg, 0.37 mmol) and 4 Å

MS (AW300) (500 mg) were added, and the mixture was stirred under N_2 for 10 min at −20 °C. Then, NIS (84 mg, 0.37 mmol) and TMSOTf (6.0 μL, 0.031 mmol) were added, and the resulting mixture was stirred from −20 to 0 °C for ca. 30 min. The reaction was then quenched by the addition of a few drops of saturated $NaHCO_3$ and small pieces of solid $Na_2S_2O_3$, followed by vigorous stirring at RT for 15 min. The resulting mixture was filtered over Celite to remove the MS and the filtrate was concentrated for MPLC purification over 230–400 mesh silica gel (EtOAc/CH_2Cl_2/hexane 1:1:4) to furnish target trisaccharide **29** as a colorless syrup (272 mg, 70%). For trisaccharide **29**: R_f=0.20 (EtOAc/CH_2Cl_2/hexane 1:1:4); $[\alpha]_D^{27}=-3.33$ (c=0.30 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.54–7.19 (m, 25H; Ar*H*), 5.71–5.48 (m, 2H), 5.25 (s, 1H; H-1‴), 5.01 (s, 1H; H-1″), 4.95–4.81 (m, 3H), 4.75–4.67 (m, 2H), 4.63 (d, J=9.1 Hz, 1H), 4.47 (d, J=7.2 Hz, 1H; H-1′), 4.35 (t, J=8.3 Hz, 2H), 4.32–4.19 (m, 3H), 4.18–4.06 (m, 3H), 4.02–3.91 (m, 3H), 3.85–3.59 (m, 7H), 3.46 (d, J=8.1 Hz, 1H), 3.33 (td, J=9.8, 4.9 Hz, 1H), 2.28–2.01 (m, 2H; CH_2), 1.52 (s, 3H; CH_3), 1.34 ppm (s, 6H; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ=138.9, 138.8, 138.7, 138.1, 137.9, 129.5, 129.2, 128.88, 128.81, 128.7, 128.65, 128.60, 128.5, 128.4, 128.3, 128.28, 128.23, 128.1, 127.9, 127.8, 126.69, 126.65, 126.5, 126.4, 109.6 (quaternary-*C*), 104.0 ($^1J_{CH}$=156.5 Hz; C-1′), 101.8 (benzylidene-*C*H), 101.7 (benzylidene-*C*H), 100.6 ($^1J_{CH}$=157.5 Hz; C-1‴), 100.5 ($^1J_{CH}$=167.9 Hz; C-1″), 81.6, 79.0, 78.5, 78.3, 78.2, 77.67, 76.65, 76.5, 76.4, 75.7, 75.2, 72.4, 69.6, 69.0, 68.0, 66.7, 66.6, 65.2, 42.2 (*C*H_2Cl), 33.1, 28.2, 26.8, 18.2 ppm; HRMS-ESI: m/z: calcd for $C_{59}H_{67}ClO_{15}Na$: 1073.4066; found: 1073.4061 $[M+Na]^+$.[7,38]

Acknowledgements

We thank the National Science Council of Taiwan for financial support (grant no. NSC 97-2113M-009-007), Ms. C.-C. Chang of NCTU for 500 MHz NMR spectral analysis, and Mr. T.-Y. Chen for MS analysis.

[1] a) Z. Guo, Chemical synthesis of complex carbohydrates in *Carbohydrate Chemistry, Biology and Medical Applications* (Ed.: H. G. Garg, M. K. Cowman, C. A. Hales), Elsevier Ltd., Oxford, UK, **2008**, pp. 59–83; b) A. T. Carmona, A. J. Moreno-Vargas, I. Robina, *Curr. Org. Synth.* **2008**, *5*, 81–116; c) X. Zhu, R. R. Schmidt, *Angew. Chem.* **2009**, *121*, 1932–1967; *Angew. Chem. Int. Ed.* **2009**, *48*, 1900–1934.

[2] P. H. Seeberger, *Chem. Soc. Rev.* **2008**, *37*, 19–28 and references therein.

[3] a) J.-C. Lee, W.-A. Greenberg, C.-H. Wong, *Nat. Protoc.* **2007**, *1*, 3143–3152; b) Y. Wang, X.-S. Ye, L.-H. Zhang, *Org. Biomol. Chem.* **2007**, *5*, 2189–2200; c) S. Valerio, A. Pastore, M. Adinolfi, A. Iadonisi, *J. Org. Chem.* **2008**, *73*, 4496–4503.

[4] a) S. Hanashima, S. Manabe, Y. Ito, *Angew. Chem.* **2005**, *117*, 4290–4296; *Angew. Chem. Int. Ed.* **2005**, *44*, 4218–4224; b) E. Kantchev, B. Assen, S. J. Bader, J. R. Parquette, *Tetrahedron* **2005**, *61*, 8329–8338; c) M. Dhanawat, S. K. Shrivastava, *Mini-Rev. Med. Chem.* **2009**, *9*, 169–185.

[5] a) Y. Ito, T. Ogawa, *J. Am. Chem. Soc.* **1997**, *119*, 5562–5566; b) D. Crich, M. Smith, *J. Am. Chem. Soc.* **2002**, *124*, 8867–8869; c) J. D. C. Codée, L. Kröck, B. Castagner, P. H. Seeberger, *Chem. Eur. J.* **2008**, *14*, 3987–3994.

[6] V. Y. Dudkin, D. Crich, *Tetrahedron Lett.* **2003**, *44*, 1787–1789.

[7] D. Crich, H. Li, *J. Org. Chem.* **2002**, *67*, 4640–4646.

[8] J. J. Gridley, H. M. I. Osborn, *J. Chem. Soc. Perkin Trans. 1* **2000**, 1471–1491.

[9] a) D. Crich, B. Wu, P. Jayalath, *J. Org. Chem.* **2007**, *72*, 6806–6815; b) D. Crich, W. Li, H. Li, *J. Am. Chem. Soc.* **2004**, *126*, 15081–15086; c) M. Poláková, M. U. Roslund, F. S. Ekholm, T. Saloranta, R. Leino, *Eur. J. Org. Chem.* **2009**, 870–888.

[10] B. Sun, B. Srinivasan, X. Huang, *Chem. Eur. J.* **2008**, *14*, 7072–7081.

[11] a) B. G. Davis, *Chem. Rev.* **2002**, *102*, 579–602; b) S. R. Shenoy, L. G. Barrientos, D. M. Ratner, B. R. O'Keefe, P. H. Seeberger, A. M. Gronenborn, M. R. Boyd, *Chem. Biol.* **2002**, *9*, 1109–1118; c) H. Li, L. Wang, *Org. Biomol. Chem.* **2004**, *2*, 483–488; d) D. E. Szymkowshi, *Curr. Opin. Drug Discovery Dev.* **2005**, *8*, 590–660.

[12] Processing of Protein-Bound *N*-Glycans: H. Schachter in *Comprehensive Glycoscience from Chemistry to System Biology*, *Vol. 2* (Editor-in-Chief: J. P. Kamerling, Eds.: G.-J. Boons, Y. C. Lee, A. Suzuki, N. Taniguchi, A. G. J. Voragen), Elsevier, Spain, **2007**, pp. 11–32.

[13] a) O. Lüderitz, *Angew. Chem.* **1970**, *82*, 708–722; *Angew. Chem. Int. Ed. Engl.* **1970**, *9*, 649–663; b) O. Lüderitz, O. Westphal, A. M. Staub in *Microbial Toxins*, *Vol. 4* (Eds.: G. Weinbaum, S. Kadis, S. J. Ajl), Academic Press, New York, **1971**, pp. 145–233.

[14] M. A. E. Shaban, R. W. Jeanloz, *Carbohydr. Res.* **1976**, *52*, 115–127.

[15] Selected reports for the synthesis of the trisaccharide core of N-linked glycoproteins: a) V. Y. Dudkin, D. Crich, *Tetrahedron Lett.* **2003**, *44*, 1787–1789; b) C. Unverzagt, *Chem. Eur. J.* **2003**, *9*, 1369–1376; c) E. Attolino, T. W. D. F. Rising, C. D. Heidecke, A. J. Fairbanks, *Tetrahedron: Asymmetry* **2007**, *18*, 1721–1734; d) G. Wang, W. Zhang, Z.-C. Lu, P. Wang, X.-L. Zhang, Y. Li, *J. Org. Chem.* **2009**, *74*, 2508–2515.

[16] Direct β-selective mannosylation without using a 4,6-*O*-benzylidene acetal function: a) V. K. Srivastava, C. Schuerch, *J. Org. Chem.* **1981**, *46*, 1121–1126; b) A. A.-H. Abdel-Rahman, S. Jonke, E. S. H. E. Ashry, R. R. Schmidt, *Angew. Chem.* **2002** *116*, 3100–3103; *Angew. Chem. Int. Ed.* **2002**, *41*, 2972–2974; c) H. Mandi, T. Mukaiyama, *Chem. Lett.* **2005**, *34*, 702–703; d) K. J. Doores, B. G. Davis, *Org. Biomol. Chem.* **2008**, *6*, 2692–2696; e) J.-Y. Baek, B.-Y. Lee, M.-G. Jo, S. K. Kim, *J. Am. Chem. Soc.* **2009**, *131*, 17705–17713.

[17] Direct β-selective mannosylation invoking the use of a 4,6-*O*-benzylidene acetal function: a) D. Crich, S. Sun, *J. Org. Chem.* **1996**, *21*, 4506–4507; b) D. Crich, S. Sun, *J. Am. Chem. Soc.* **1998**, *120*, 435–436; c) D. Crich, P. Jayalath, T. K. Hutton, *J. Org. Chem.* **2006**, *71*, 3064–3070; d) R. Weingart, R. R. Schmidt, *Tetrahedron Lett.* **2000**, *41*, 8753–8758; e) K.-S. Kim, J. H. Kim, Y.-Jon Lee, Y.-Jun Lee, J. Park, *J. Am. Chem. Soc.* **2001**, *123*, 8477–8481; f) J.-Y. Baek, T. J. Choi, H.-B. Jeon, K.-S. Kim, *Angew. Chem.* **2006**, *118*, 7596–7600; *Angew. Chem. Int. Ed.* **2006**, *45*, 7436–7440; g) K. S. Kim, D. B. Fulse, J.-Y. Baek, B.-Y. Lee, H.-B. Jeon, *J. Am. Chem. Soc.* **2008**, *130*, 8537–8547; h) S. Tanaka, M. Takashina, H. Tokimoto, Y. Fujimoto, K. Tanaka, K. Fukase, *Synlett* **2005**, 2325–2328; i) K. Tanaka, Y. Mori, K. Fukase, *J. Carbohydr. Chem.* **2009**, *28*, 1–11; j) J. D. C. Codée, L. H. Hossain, P. H. Seeberger, *Org. Lett.* **2005**, *7*, 3251–3254; k) T. Tsuda, R. Arihara, S. Sato, M. Koshiba, S. Nakamura, S. Hashimoto, *Tetrahedron* **2005**, *61*, 10719–10733; l) K. Tanaka, Y. Fuji, h. Tokimoto, Y. Mori, S. Tanaka, G.-M. Bao, E. R. O. Siwu, A. Nakayabu, K. Fukase, *Chem. Asian J.* **2009**, *4*, 574–580.

[18] Indirect β-selective mannosylation: a) J. Alais, S. David, *Carbohydr. Res.* **1990**, *201*, 69–77; b) M. A. Shaban, R. W. Jeanloz, *Carbohydr. Res.* **1976**, *52*, 103–114; c) W. Günther, H. Kunz, *Carbohydr. Res.* **1992**, *228*, 217–241; d) F. Barresi, O. Hindsgaul, *Can. J. Chem.* **1994**, *72*, 1447–1465; e) I. Cumpstey, A. J. Fairbanks, A. J. Redgrave, *Org. Lett.* **2001**, *3*, 2371–2374; f) A. Ishiwata, Y. Munemura, Y. Ito, *Eur. J. Org. Chem.* **2008**, 4250–4263.

[19] Reviews for glycosyl trichloroacetimidates: a) X. Zhu, R. R. Schmidt in *Handbook of Chemical Glycosylation: Advances in Stereoselectivity and Therapeutic Relevance* (Ed.: A. V. Demchenko), Wiley-VCH, Weinheim, **2008**, pp. 143–185; b) R. R. Schmidt, X. Zhu, In *Glycoscience: Chemistry and Chemical Biology*, *2nd ed.* (Eds.: B. O. Fraser-Reid, K. Tatsuta, J. Thiem), Springer-Verlag, Berlin, **2008**, pp. 452–524.

[20] For the first report of orthogonal glycosylation: a) O. Kanie, Y. Ito, T. Ogawa, *J. Am. Chem. Soc.* **1994**, *116*, 12073–12074; for orthogonal glycosylations of thioglycosides with glycosyl trichloroacetimidates: b) H. Yamada, T. Harada, T. Takahashi, *J. Am. Chem. Soc.* **1994**, *116*, 7919–7920; c) H. Yamada, K. Tetsuya, T. Takahashi, *Tet-*

rahedron Lett. **1999**, *40*, 4581–4584; d) H. Yu, Y. Biao, X. Wu, Y. Hui, X. Han, *J. Chem. Soc. Perkin Trans. 1* **2000**, 1445–1453.
[21] Synthesis of the mannosyl trichloroacetimidate **1** was described in the Supporting Information.
[22] For a recent review of thioglycosides: J. D. C. Codée, R. E. J. N. Litjens, L. J. van den Bos, H. S. Overkleeft, G. A. van der Marel, *Chem. Soc. Rev.* **2005**, *34*, 769–782.
[23] Synthesis of **2**: K.-K. T. Mong, C.-Y. Huang, C.-H. Wong, *J. Org. Chem.* **2003**, *68*, 2135–2142.
[24] a) G. Wulff, G. Röhle, *Angew. Chem.* **1974**, *86*, 173–187; *Angew. Chem. Int. Ed. Engl.* **1974**, *13*, 157–160; b) B. Capon, S. P. McManus, *Neighboring-Group Participation*, Plenum, New York, **1976**.
[25] R. R. Schmidt, A. Toepfer, *Tetrahedron Lett.* **1991**, *32*, 3353–3356.
[26] As the information concerning the relationship of the β-selectivity of mannosylation and the addition rate of the mannosyl donor (see references [17d] and [25]) is not available, the donor addition rate reported in the literature[17d] (0.25 M of mannosyl donor was added over a period of 25 min) was used as a reference in this study.
[27] For aglycon transfer reaction, a) F. Belot, J. C. Jacquinet, *Carbohydr. Res.* **1996**, *290*, 79–86; b) Z. Li, J. C. Gildersleeve, *J. Am. Chem. Soc.* **2006**, *128*, 11612–11619; c) Z. Li, J. C. Gildersleeve, *Tetrahedron Lett.* **2007**, *48*, 559–562.
[28] *N*-(β-Mannopyranosyl) trichloroacetamide **5** was isolated and the anomeric configuration was evidenced by the ^{13}C NMR spectroscopic chemical shift at $\delta = 79.4$ ppm and the $^{1}J_{CH}$ coupling constant of 161 Hz (see the Experimental Section).
[29] During the revision of this manuscript, one of the referees recommended this study to use the mannosyl *N*-phenyltrifluoroacetimidate donor for the elimination the formation of the amide byproduct (**5**) (see references [17h], [17i], and [17l]). However, the preparation of this imidate donor requires the use of unstable *N*-phenyl trifluoroacetimidoyl chloride, which has not been realized in our hands. For the preparation of acetimidoyl chlorides: a) Y.-H. Yang, M. Shi, *Tetrahedron* **2006**, *62*, 2420–2427; b) K. Tamura, H. K Mizukami, K. Maeda, H. Watanabe, K. Uneyama, *J. Org. Chem.* **1993**, *58*, 32–35.
[30] a) Synthesis of mannosyl trichloroacetimidate **6**: S. Roy, N. Roy, *J. Carbohydr. Chem.* **2003**, *22*, 521–535; b) synthesis of methyl 2,3,6-tri-*O*-benzyl β-D-glucopyranoside **7**: M. P. DeNinno, J. B. Etienne, K. C. Duplantier, *Tetrahedron Lett.* **1995**, *36*, 669–672; c) diisopropylidene galactopyranose **8** is commercially available; d) synthesis of methyl 2,3,4-tri-*O*-benzyl β-D-glucopyranoside **9**: C.-R. Shie, Z.-H. Tzeng, S. S. Kulkarni, B.-J. Uang, C.-Y. Hsu, S.-C. Hung, *Angew. Chem.* **2005**, *117*, 1693–1696; *Angew. Chem. Int. Ed.* **2005**, *44*, 1665–1668.
[31] a) D. Crich, S. Sun, *J. Org. Chem.* **1997**, *62*, 1198–1199; b) D. Crich, S. Sun, *Tetrahedron* **1998**, *54*, 8321–8348.
[32] N. M. Spijker, C. A. Boeckel, *Angew. Chem.* **1991**, *103*, 179–182; *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 180–183.
[33] a) G. Grundler, R. R. Schmidt, *Liebigs Ann. Chem.* **1984**, 1826–1847; b) R. R. Schmidt, J. Michel, *J. Carbohydr. Chem.* **1985**, *4*, 141–169; c) R. Schaubach, J. Hemberger, W. Kinzy, *Liebigs Ann. Chem.* **1991**, 607–614.
[34] E. A. Mensah, J. M. Azzarelli, H. M. Nguyen, *J. Org. Chem.* **2009**, *74*, 1650–1657.
[35] This explanantion was suggested by one of the referees in the revision of this manuscript, whereas in our perspective, we do not exclude other possible causes unless further experimental evidence is obtained.
[36] Syntheses of **13**, **14**, **16**, **17**, **23**, **24**, and **25** were detailed in the Supporting Information. Since synthesis of **15** and **16** employed the same route, only the synthesis of **16** was described in the Supporting Information. For the synthesis of **16**: a) J. Gildersleeve, R. A. Pascal, D. Kahn, *J. Am. Chem. Soc.* **1998**, *120*, 5961–5969; b) A. Irina, A. J. Ivanova, M. A. J. F. Ross, V. N. Andrei, *J. Chem. Soc. Perkin Trans. 1* **1999**, 1743–1753; c) for the synthesis of **23**: C.-S. Chao, C.-W. Li, M.-C. Chen, S.-S. Chang, K.-K. T. Mong, *Chem. Eur. J.* **2009**, *15*, 10972–10982.
[37] E.-D. Goddard-Borger, R. V. Stick, *Org. Lett.* **2007**, *9*, 3797–3800.
[38] K. Bock, C. Pedersen, *J. Chem. Soc. Perkin Trans. 2* **1974**, 293–297.
[39] a) N. K. Kochetkov, B. A. Dmitriev, O. S. Chizhov, E. M. Klimov, N. N. Malysheva, A. Y. Chernyak, N. E. Bayramova, V. I. Torgov, *Carbohydr. Res.* **1974**, *33*, C5–C7; b) N. K. Kochetkov, B. A. Dmitriev, O. S. N. N. Malysheva, A. Y. Chernyak, E. M. Klimov, N. E. Bayramova, V. I. Torgov, *Carbohydr. Res.* **1975**, *45*, 283–290.
[40] Synthesis of **28** was described in the Supporting Information.
[41] a) D. Crich, S. Sun, *J. Am. Chem. Soc.* **1997**, *119*, 11217–11223; b) D. Crich, N. S. Chandrasekera, *Angew. Chem.* **2004**, *116*, 5500–5503; *Angew. Chem. Int. Ed.* **2004**, *43*, 5386–5389.
[42] L. K. Mydock, A. V. Demchenko, *Org. Bioorg. Chem.* **2010**, *8*, 497–510.

Received: December 31, 2009
Published online: March 23, 2010

