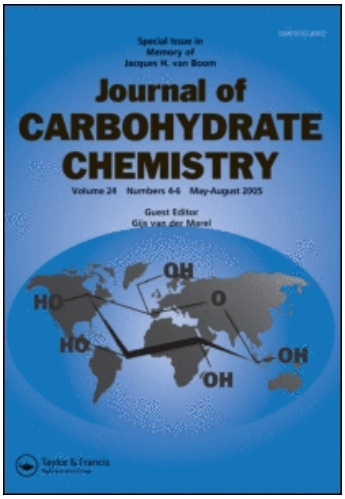

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Glycosyl β-Bromo-α-ketonitriles: Useful Intermediates for a Rapid Conversion of Dialdoses into Glycoamidoesters and Glycothioesters

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Glycosyl β-Bromo-α-ketonitriles: Useful Intermediates for a Rapid Conversion of Dialdoses into Glycoamidoesters and Glycothioesters

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Glycosyl β-bromo-α-ketonitriles can be conveniently accessed through a versatile reaction of easily available dialdoses with potassium dibromoacetonitrile anion in isopropanol. They proved to be very useful intermediates in the direct preparation of new glycoamidoesters and glycothioesters. The synthesis of the title compounds involves coupling of glycosyl β-bromo-α-ketonitrile intermediates with suitably protected amino acid esters and 1-propanethiol, respectively. The coupling reaction proceeded smoothly at rt affording the targeted products in good yields and with moderate diastereoselectivities. The route presented here constitutes an important synthetic potential for the rapid preparation of new glycoamidoesters and glycothioesters that can find application as useful building blocks in the construction of more complex glycopeptides.

Keywords Glycoamidoesters; Dialdoses; β-Bromo-α-ketonitriles; Thioesters; Activated uronic acids

INTRODUCTION

Glycoproteins play an important role in biological processes and have found many biological and biotechnological applications. Besides the influence of saccharide residues on the conformational and physicochemical properties of the proteins, important biological recognition processes depend on the interplay

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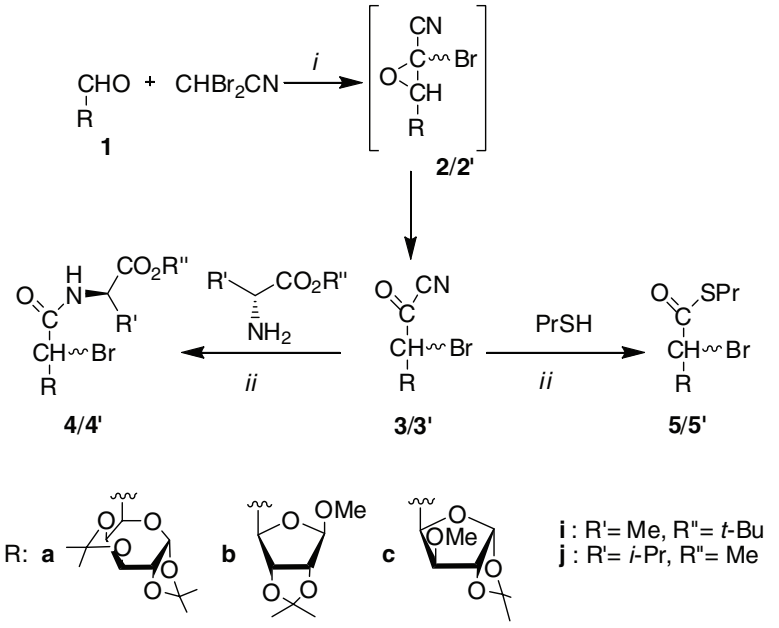
between peptidic and saccharidic elements[1–3]; the carbohydrate moieties of glycopeptides play different decisive roles, especially in recognition phenomena.[4–6] Additionally, the conformation and solubility of glycoproteins are influenced by the oligosaccharide chain that can also prohibit the proteolytic cleavage.[7,8]

Eucaryotic cells synthesize glycoproteins by modifying ribosomal proteins enzymatically, and a purely enzymatic approach can enable elegant access to natural molecules.[2,9–11] However, nonnatural glycopeptides and derivatives of pharmacological interest, as a rule, require the application of synthetic methods. Glycoproteins of defined chemical structures can be prepared by total or semisynthesis based on modern techniques, especially chemical ligation methods,[12,13] and chemistry can then be used to control both the site and structure of glycosylation. The synthesis of glycopeptides is demanding because of the polyfunctionality of the target molecules. Moreover, the complexity rises with the occurrence of multiple functional groups on a single molecule. Furthermore, another important issue in the synthesis of glycoprotein conjugates is the compatibility of the protecting groups required for peptide and carbohydrate assemblies. The accomplishment of this goal is not a trivial matter and a complex glycan is often unstable under the highly acidic conditions that are required to remove peptide protecting groups. Correspondingly, peptides may be unstable under the basic conditions that are required for the deprotection and retrieval of the oligosaccharide ensembles. As a consequence, the synthetic methods to be employed in the preparation of glycopeptides must be compatible with both the carbohydrate and peptide domains.[3,14,15]

As a part of a program aimed at developing new versatile procedures for glycopeptide synthesis, herein we wish to report on the synthetic utility of β-bromo-α-ketonitriles as reactive intermediates for the efficient and straightforward preparation of new glycoamidoesters and glycothioesters. These compounds can possess interesting biological activity as it is known that glycosyl-α-aminoacids and their derivatives are good enzyme inhibitors[16] and antifungal agents.[17] In addition to their potential biological application, glycoamidoesters and glycothioesters can be considered as useful building blocks for glycopeptide synthesis.

RESULTS AND DISCUSSION

We have previously reported on the application of β-bromo-α-ketonitriles, and we have developed a protocol for an efficient synthesis of α-bromo esters from carbonyl compounds using dibromoacetonitrile.[18–20] More recently, we have demonstrated the versatility of this methodology in the field of glycochemistry by the preparation of 6-amino-6-deoxy pyranuronic and 5-amino-5-deoxy



Scheme 1: Preparation of glycoamidoesters **4**/**4**′ and glycothioesters **5**/**5**′ *via* β-bromo-α-ketonitriles **3**/**3**′. *Reagents and conditions:* (*i*) *i*-PrOK, *i*-PrOH, Et_2O, –5°C, 2 h 30 min, then HCl/Et_2O and centrifugation in acetone; (*ii*) CH_2Cl_2, Et_3N, 2 h, 20°C.

furanuronic acid derivatives, which are potentially interesting precursors of nikkomycins and polyoxins.[21] In continuation of those studies, here we describe the utility of β-bromo-α-ketonitriles **3/3′** for the synthesis of glycoamidoesters and glycothioesters, useful building blocks for construction of complex glycopeptides.

As depicted in Scheme 1, β-bromo-α-ketonitriles **3/3′** can be viewed as pivotal intermediates for the preparation of glycoamidoesters **4/4′** and glycothioesters **5/5′**. Due to the high reactivity of the nitrile group in β-bromo-α-ketonitriles **3/3′**, we postulated that these compounds could behave like acid halides in coupling reactions.

The synthesis of **3/3′** is direct and straightforward. Reaction of readily available dialdoses **1a–c**, dibromoacetonitrile in diethyl ether with potassium isopropylate in isopropanol yield α-bromo epoxynitrile intermediates **2/2′**, which spontaneously isomerize into desired β-bromo-α-ketonitriles **3/3′** (Sch. 1).

In particular, we found that the temperature and reaction times were crucial for the success of the protocol. Low temperature (–5°C) and the reaction time of 2 h 30 min avoided the undesired self-condensation of $CHBr_2CN$, as could be deduced by following the progress of the reaction using IR spectroscopy [$CHBr_2CN$: ν (C≡N) 2240 cm^{-1}; $CHBr_2C{=}NH(CBr_2CN)$: ν (C≡N) 2195 cm^{-1}]. In turn, prolonged reaction times led to the formation of the α-bromoester [ν (C=O) 1745 cm^{-1}], which resulted from the addition of isopropanol to the β-bromo-α-keto unit of **3/3′**.

The advantage of using β-bromo-α-ketonitriles **3/3′** as building blocks is that they can be easily stored and used without purification; however, a special workup is required. The usual reactivity of the nitrile group toward acidic or basic medium requires a careful control of pH before storage. These problems were simply overcome by the neutralization of the reaction mixture with diethyl ether saturated with HCl, which prevents the formation of side products such as iminoether [RCHBrC(O)C=NH(O-*i*-Pr): ν (C=O) 1630 cm^{-1}]. Next, after the removal of the reaction solvent, the crude β-bromo-α-ketonitriles **3/3′** were submitted for centrifugation with acetone. The role of acetone is important because following successive centrifugation cycles, it fully separates β-bromo-α-ketonitriles **3/3′** from KBr salts, formed during the course of reaction. Acetone has good extracting properties, and other solvents such as diethyl ether or THF were found to give less favorable results. It is noteworthy that the glycosyl β-bromo-α-ketonitriles **3/3′** could be stored at 5°C for up to 4 weeks with rigorous exclusion of moisture.

After optimizing the reaction conditions for the preparation of β-bromo-α-ketonitriles **3/3′** and finding the best protocol for their safe storage, we began testing their reactivity toward functionalized nucleophiles. D-Alanine *t*-butylester, D-valine methyl ester, and 1-propanethiol were used to evaluate the application of **3/3′** in the synthesis of glycoamidoesters and glycothioesters

Table 1: Glycoamidoesters **4**/**4′** and glycothioesters **5**/**5′** produced via Scheme 1

Entry	β-Bromo-α-ketonitrile	Nucleophile	Product	Yield (%)[a]/ epimeric ratio[b]
1.	**3a**/**3′a**	$(CH_3)_2CHCH(NH_2)CO_2CH_3$	**4aj**/**4′aj**	53/(66/34)
2.	**3a**/**3′a**	$CH_3CH(NH_2)CO_2C(CH_3)_3$	**4ai**/**4′ai**	66/(75/25)
3.	**3b**/**3′b**	$(CH_3)_2CHCH(NH_2)CO_2CH_3$	**4bj**/**4′bj**	58/(66/34)
4.	**3b**/**3′b**	$CH_3CH(NH_2)CO_2C(CH_3)_3$	**4bi**/**4′bi**	56/(54/46)
5.	**3c**/**3′c**	$CH_3CH(NH_2)CO_2C(CH_3)_3$	**4ci**/**4′ci**	66/(85/15)
6.	**3a**/**3′a**	PrSH	**5a**/**5′a**	57/(77/23)
7.	**3b**/**3′b**	PrSH	**5b**/**5′b**	52/(54/46)

[a] Isolated yield after purification.
[b] Epimeric ratio determined on the crude product by ^{1}H NMR.

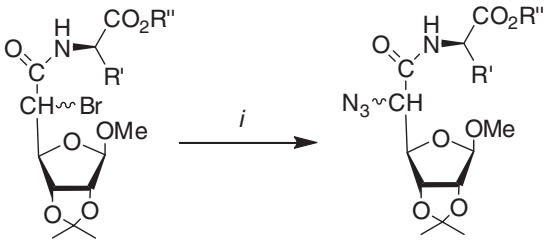
(Sch. 1). To our delight, the coupling reaction proceeded under very mild conditions, that is, rt and the use of 1.1 equiv. of Et_3N as a base. β-Bromo-α-ketonitriles **3**/**3′** reacted smoothly, and the desired glycoamidoesters **4**/**4′** and glycothioesters **5**/**5′** were obtained in good overall yields (Table 1). Reaction progress was monitored by IR spectroscopy, and the formation of the expected compounds could be clearly observed [**3**/**3′** ν (C≡N) 2224 cm^{-1}, ν (C=O) 1727 cm^{-1}; **4**/**4′**: ν (C=O) 1733–1745 cm^{-1}; **5**/**5′**: ν (C=O) 1689–1692 cm^{-1}].

A palette of sugar substrates such as dialdogalactose **1a**, dialdoribose **1b,** and dialdoxylose **1c** were tolerated under the reaction conditions and the selection of amino-partners showed that the steric hindrance present in R' and R" did not affect the coupling. The reaction was found to be free from side products. Additionally, the chemical manipulation of the R" group (methyl and *t*-butyl) in glycoamidoesters **4**/**4′** and glycothioesters **5**/**5′** offers the possibility for further functionalization.

Glycoamidoesters **4**/**4′** were formed as a mixture of diastereomers and were characterized by 1D and 2D NMR spectroscopy (^{1}H, ^{13}C, COSY, HMBC). The epimeric ratios were evaluated based on the integration of H-1 signals in the ^{1}H NMR (250 MHz) spectra of the crude products. The values obtained indicated a satisfactory overall diastereoselectivity in the case of the D-galacto and D-xylo derivatives (Table 1).

The presence of bromine in compounds **4**/**4′** has an additional advantage, namely the possibility of introducing a nitrogen nucleophile, which could be subsequently transformed into the amine functionality. Treatment of **4b**/**4′b** with NaN_3 indicates the true utility and versatility of these compounds. The use of an excess of NaN_3 (5 equiv.) at 100°C in DMF for 5 h led to the α-azidoesters **6b**/**6′b** with a satisfactory yield (65%–68%) (Sch. 2).

The glycothioesters **5**/**5′** were also obtained as a mixture of diastereomers, although they could only be characterized by ^{1}H NMR spectroscopy, because of



Scheme 2: Preparation of azidoglycoamidoesters **6b**/**6′b**. *Reagents and conditions:* (*i*) NaN_3 (5 equiv.), DMF, 100°C, 5 h.

their fragility. Purification by standard chromatographic techniques was not possible due to the high reactivity of those compounds, and hence the analytically pure samples were obtained after fast filtration of the crude reaction mixtures through a pad on silica gel. It is noteworthy that the formation of glycothioesters **5/5′** is an important achievement because it is known that aminolysis of alkyl thioesters allows the preparation of amides from blocked amino acids or peptides. Additionally, peptide and glycopeptide thioesters have recently attracted considerable attention generated after the emergence of native chemical ligation methodology used widely for protein and glycoprotein synthesis.[12,13,22–24]

CONCLUSION

We have reported here on a facile two-step synthetic route to new glycoamidoesters and glycothioesters via the coupling of glycosyl β-bromo-α-ketonitriles with suitably protected amino acids esters and 1-propanethiol, respectively. The glycosyl β-bromo-α-ketonitriles proved to be useful reactive intermediates that are easily accessible through a reaction of dialdoses with potassium dibromoacetonitrile anion in isopropanol. The nitrile group of the glycosyl β-bromo-α-ketonitriles behaves like an activated carboxylic acid and reacted easily with the examined nucleophiles affording the desired glycoamidoesters and glycothioesters, with good yields and moderate diastereoselectivity. The presented approach is practical, simple, and fairly inexpensive and represents an interesting alternative to the known, classical coupling methods, especially in the field of glycochemistry.[25–28] Furthermore, the route presented here constitutes an important synthetic potential for the construction of new functionalized glycoamidoesters and glycothioesters that, without a doubt, can find application in the synthesis of more complex glycopeptides. The usefulness of the glycoamidoesters and glycothioesters for the preparation of glycopeptides is currently

a subject of intensive investigation in our group. Additionally, we are studying the use of glycosyl β-bromo-α-ketonitriles as acyl donors in the reactions with small peptides as N-nucleophiles, and the results of these studies will be reported in due course.

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EXPERIMENTAL

IR spectra were recorded using a Nicolet 205 spectrometer, and only the most representative frequencies (cm^{-1}) are given. ^1H NMR spectra were recorded using a Bruker AM 400 or AC250 and ^{13}C NMR spectra were recorded using a Bruker AC250. Data for ^1H NMR spectra are reported in δ units downfield from internal Me_4Si (TMS) or from the $CHCl_3$ solvent at 7.25 ppm relative to TMS. ^{13}C NMR spectra were referenced to the $CDCl_3$ peak at 77.2 ppm relative to TMS. Coupling constants (J) are quoted in hertz. Multiplicities are quoted as s (singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). Mass spectra were obtained on an Autospec Fited Cesium Gun (Micro Mass Manchester). Preparative chromatographic separations were carried out on Merck silica gel 60 (230–400 mesh). Products were revealed by spraying sulfuric acid followed by calcinations or iodine, or with UV light. Reactions were conduced in oven-dried glassware under inert atmosphere of nitrogen. All solvents were purified according to standard procedures and distilled prior to use.

Procedure for the Preparation of Glycosyl β-bromo-α-ketonitriles 3/3′

The reaction conditions are crucial and required a precise temperature control. In a typical experiment, potassium isopropylate (3.52 mmol, 15 mL isopropanol) was added dropwise at –5°C, with stirring, to the mixture of dialdogalactose **1a** (3.87 mmol, 1 g) and dibromoacetonitrile (3.52 mmol) in 15 mL of diethyl ether. After stirring at –5°C for 2 h 30 min, the reaction mixture was neutralized with saturated HCl diethyl ether solution, and the solvent was removed. For product isolation, the crude residue was treated with 15 mL of acetone and centrifuged. After removal of the solvent, the crude galactosyl β-bromo-α-ketonitrile **3a/3a′** was obtained as a pale yellow pasty solid and was

used without purification. Storage is possible for up to 4 weeks under an atmosphere of dry N_2.[21]

General Procedure for the Coupling of Glycosyl β-Bromo-α-ketonitriles 3/3′ with Nucleophiles

A 50-mL round-bottom flask was charged with a solution of commercially available 1-propanethiol or appropriate amino ester hydrochloride (3.0 mmol) in CH_2Cl_2 (15 mL). Subsequently, neat Et_3N (3.3 mmol) was added. After stirring for 5 min at rt, a solution of the appropriate glycosyl β-bromo-α-ketonitrile **3/3′** (3.0 mmol) in CH_2Cl_2 (15 mL) was injected, and the progress of the reaction was followed by TLC. When the reaction was completed, aqueous 1N HCl was added to the reaction vessel until pH = 4. The layers were separated, and the organic layer was washed with H_2O (2 × 10 mL), dried over anhydrous $MgSO_4$, and concentrated under vacuum to afford the crude products. Crude thioesters **5/5′**, due to their fragility, were dissolved in CH_2Cl_2 and rapidly filtrated through a pad of silica gel to afford analytically pure samples. Crude glycoamidoesters **4/4′** were purified by flash chromatography on silica gel.

Methyl N-(6-bromo-6-deoxy-1,2:3,4-di-*O*-isopropylidene-D-*glycero*-α-D-*galacto*heptopyranuronoyl)-L-valinate/Methyl N-(6-bromo-6-deoxy-1,2:3,4-di-O-isopropylidene-L-*glycero*-α-D-*galacto*heptopyranuronoyl)-L-valinate 4aj/4′aj

Colorless oil; Yield: 53%; R_f 0.30 (EtOAc/Hexane 1:6).

^{1}H NMR ($CDCl_3$) δ_H (ppm): *major diastereomer:* 6.48 (d, 1H, H_8, $^3J_{H8\text{-}H9}$ = 8 Hz), 5.54 (d, 1H, H_1, $^3J_{H1\text{-}H2}$ = 4.5 Hz), 4.75–4.25 (m, 6H, H_2, H_3, H_4, H_5, H_6, H_9), 3.77 (s, 3H, OCH_3), 2.30–2.10 (m, 1H, H_{12}), 1.60 (s, 3H), 1.49–1.39 (s, s, s, 3H, 3H, 3H), 0.99 (d, 3H, H_{13}, $^3J_{H13\text{-}H12}$ = 10.0 Hz), 0.96 (d, 3H, $H_{13'}$, $^3J_{H13'\text{-}H12}$ = 10.5 Hz); *minor diastereomer:* 6.51 (d, 1H, H_8, $^3J_{H8\text{-}H9}$ = 9 Hz), 5.60 (d, 1H, H_1, $^3J_{H1\text{-}H2}$ = 5.0 Hz), 4.75–4.25 (m, 6H, H_2, H_3, H_4, H_5, H_6, H_9), 3.79 (s, 3H, OCH_3), 2.30–2.10 (m, 1H, H_{12}), 1.61 (s, 3H), 1.51–1.28 (s, s, s, 3H, 3H, 3H), 0.99 (d, 3H, H_{13}, $^3J_{H13\text{-}H12}$ = 10.0 Hz), 0.96 (d, 3H, $H_{13'}$, $^3J_{H13'\text{-}H12}$ = 10.5 Hz); ^{13}C NMR ($CDCl_3$) δ_C (ppm): *major diastereomer:* 171.9 (C_7), 166.9 (C_{10}), 109.3 (C_{14}), 109.0 (C_{15}), 96.2 (C_1), 71.0–68.0 (m, C_2, C_3, C_4, C_5), 57.0 (C_9), 52.0 (C_{11}), 43.9 (C_6), 31.7 (C_{12}), 25.8 (CH_3), 25.7 (CH_3), 24.7 (CH_3), 24.1 (CH_3), 18.5 ($C_{13'}$), 17.5 (C_{13}); *minor diastereomer:* 171.9 (C_7), 166.8 (C_{10}), 109.3 (C_{14}), 109.0 (C_{15}), 95.8 (C_1), 71.0–66.0 (m, C_2, C_3, C_4, C_5), 54.8 (C_9), 50.4 (C_{11}), 43.9 (C_6), 31.5 (C_{12}), 25.8 (CH_3), 25.7 (CH_3), 24.7 (CH_3), 24.1 (CH_3), 18.5 ($C_{13'}$), 17.5 (C_{13}). IR, ν_{max} (film) cm^{-1}: 1740 (C=O), 1692 (C=O). MS (FAB): m/z calcd for $C_{19}H_{30}BrNO_8$ 479: 479 (100%), 481 (97%), 480 (21%), 482 (21%).

t-Butyl N-(6-bromo-6-deoxy-1,2:3,4-di-*O*-isopropylidene-D-*glycero*-α-D-*galacto*hepto- pyranuronoyl)-L-alaninate/*t*-butyl N-(6-bromo-6-deoxy-1,2:3,4-di-*O*-isopropylidene-L-*glycero*-α-D-*galacto*heptopyranuronoyl)-L-alaninate 4ai/4′ai

Colorless oil; Yield: 66%; R_f 0.27 (EtOAc/Hexane 1:6).

^{1}H NMR ($CDCl_3$) δ_H (ppm): *major diastereomer:* 6.52 (d, 1H, H_8, $^3J_{H8\text{-}H9}$ = 7 Hz), 5.48 (d, 1H, H_1, $^3J_{H1\text{-}H2}$ = 4.5 Hz), 4.68 (dd, 1H, H_3, $^3J_{H3\text{-}H2}$ = 2 Hz, $^3J_{H3\text{-}H4}$ = 8 Hz), 4.59 (dd, 1H, H_4, $^3J_{H3\text{-}H4}$ = 8 Hz, $^3J_{H4\text{-}H5}$ = 1 Hz), 4.59 (dq, 1H, H_9, $^3J_{H8\text{-}H9}$ = 7 Hz, $^3J_{H9\text{-}H15}$ = 6.5 Hz), 4.31 (dd, 1H, H_2, $^3J_{H2\text{-}H3}$ = 2 Hz, $^3J_{H2\text{-}H1}$ = 4.5 Hz), 4.24–4.21 (m, 2H, H_5, H_6), 1.65–1.30 (m, 15H, CH_3, H_{15}), 1.48 (s, H_{12}, 9H); *minor diastereomer:* 6.61 (d, 1H, H_8, $^3J_{H8\text{-}H9}$ = 7.5 Hz), 5.58 (d, 1H, H_1, $^3J_{H1\text{-}H2}$ = 5.0 Hz), 4.68 (dd, 1H, H_3, $^3J_{H3\text{-}H2}$ = 2 Hz, $^3J_{H3\text{-}H4}$ = 8 Hz), 4.59 (dq, 1H, H_9, $^3J_{H8\text{-}H9}$ = 7 Hz, $^3J_{H9\text{-}H15}$ = 6.5 Hz), 4.45 (dd, 1H, H_4, $^3J_{H3\text{-}H4}$ = 8 Hz, $^3J_{H4\text{-}H5}$ = 1.5 Hz), 4.37 (dd, 1H, H_2, $^3J_{H2\text{-}H3}$ = 5 Hz, $^3J_{H2\text{-}H1}$ = 2.5 Hz), 4.24–4.21 (m, 2H, H_5, H_6), 1.65–1.30 (m, 15H, CH_3, H_{15}), 1.48 (s, H_{12}, 9H); ^{13}C NMR ($CDCl_3$) δ_C (ppm): *major diastereomer:* 171.8 (C_7), 166.4 (C_{10}), 109.5 (C_{13}), 109.2 (C_{14}), 96.6 (C_1), 82.2 (C_{11}), 70.9 (C_2), 70.6 (C_3), 70.5 (C_4), 68.5 (C_5), 49.0 (C_9), 44.2 (C_6), 27.9 (C_{12}), 26.0 (CH_3), 25.9 (CH_3), 24.9 (CH_3), 24.3 (CH_3), 18.2 (C_{15}); *minor diastereomer:* 172.8 (C_7), 166.3 (C_{10}), 110.1 (C_{13}), 108.9 (C_{14}), 96.3 (C_1), 81.6 (C_{11}), 70.9 (C_2), 70.6 (C_3), 70.5 (C_4), 68.5 (C_5), 48.7 (C_9), 44.2 (C_6), 25.8 (C_{12}), 26.0 (CH_3), 25.9 (CH_3), 24.9 (CH_3), 24.3 (CH_3), 18.0 (C_{15}). IR, ν_{max} (film) cm^{-1}: 1733 (C=O), 1694 (C=O). MS (FAB) m/z calcd for $C_{20}H_{32}BrNO_8$ 493: 493 (100%), 495 (97%), 494 (22%), 496 (21%).

Methyl N-(methyl 5-bromo-5-deoxy-2,3-di-*O*-isopropylidene-β-D-*allo*hexofuranuronoyl)-L-valinate/Methyl N-(methyl 5-bromo-5-deoxy-2,3-di-*O*-isopropylidene-β-L-t*alo*hexofuranuronoyl)-L-valinate 4bj/4′bj

Colorless oil; Yield: 58%; R_f 0.37 (EtOAc/Hexane 1:5).

^{1}H NMR ($CDCl_3$) δ_H (ppm): *major diastereomer:* 6.78 (d, 1H, H_7, $^3J_{H7\text{-}H8}$ = 8 Hz), 5.05 (s, 1H, H_1), 5.20–4.20 (m, 5H, H_2, H_3, H_4, H_5, H_8), 3.77 (s, 3H, H_{10}), 3.48 (s, 3H, OCH_3), 2.30–2.15 (m, 1H, H_{13}), 1.49 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 1.0–0.9 (m, 2H, H_{14}, $H_{14'}$); *minor diastereomer:* 6.53 (d, 1H, H_7, $^3J_{H7\text{-}H8}$ = 9 Hz), 5.06 (s, 1H, H_1), 5.20–4.20 (m, 5H, H_2, H_3, H_4, H_5, H_8), 3.76 (s, 3H, H_{10}), 3.39 (s, 3H, OCH_3), 2.30–2.15 (m, 1H, H_{13}), 1.49 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.0–0.9 (m, 2H, H_{14}, $H_{14'}$). ^{13}C NMR ($CDCl_3$) δ_C (ppm): *major diastereomer:* 171.6 (C_6), 166.8 (C_9), 112.9 (C_{12}), 109.9 (C_1), 87.2 (C_2), 85.0 (C_3), 82.4 (C_4), 57.6 (C_8), 56.1 (OCH_3), 51.2 (C_{10}), 42.7 (C_5), 31.3 (C_{13}), 26.5 (CH_3), 25.0 (CH_3), 18.9 (C_{14}), 17.8 ($C_{14'}$); *minor diastereomer:* 172.6 (C_6), 166.9 (C_9), 112.8 (C_{12}), 109.6 (C_1), 86.8 (C_2), 84.5 (C_3), 82.4 (C_4), 57.5 (C_8), 54.7 (OCH_3), 52.3 (C_{10}), 42.1 (C_5), 31.7 (C_{13}), 26.3 (CH_3), 24.8 (CH_3), 18.7 (C_{14}), 17.5 ($C_{14'}$). IR, ν_{max} (film) cm^{-1}: 1743 (C=O),

1683 (C=O). MS (FAB) m/z calcd for $C_{16}H_{26}BrNO_7$ 423: 421 (100%), 423 (99%), 422 (19%), 424 (18%).

t-Butyl N-(methyl 5-bromo-5-deoxy-2,3-di-*O*-isopropylidene-*β*-D-*allo*hexofuranuronoyl)-L-alaninate/*t*-butyl N-(methyl 5-bromo-5-deoxy-2,3-di-*O*-isopropylidene-*β*-L-t*alo*hexofuranuronoyl)-L-alaninate 4bi/4′bi

Colorless oil; Yield: 56%; R_f 0.25 (EtOAc/Hexane 1:6).

^{1}H NMR ($CDCl_3$) δ_H (ppm): *major diastereomer:* 6.81 (d, 1H, H_7, $^3J_{H7\text{-}H8}$ = 8 Hz), 5.05 (s, 1H, H_1), 5.10–4.00 (m, 5H, H_2, H_3, H_4, H_5, H_8), 3.42 (s, 3H, OCH_3), 1.70–1.20 (m, 9H, CH_3, H_{14}), 1.50 (m, 9H, H_{11}); *minor diastereomer:* 6.50 (d, 1H, H_7, $^3J_{H7\text{-}H8}$ = 9 Hz), 5.03 (s, 1H, H_1), 5.10–4.00 (m, 5H, H_2, H_3, H_4, H_5, H_8), 3.38 (s, 3H, OCH_3), 1.70–1.20 (m, 9H, CH_3, H_{14}), 1.49 (m, 9H, H_{11}). ^{13}C NMR ($CDCl_3$) δ_C (ppm): *major diastereomer:* 171.5 (C_6), 166.8 (C_9), 112.9 (C_{12}), 109.2 (C_1), 87.2 (C_2), 85.1 (C_3), 82.4 (C_4), 81.2 (C_{10}), 56.5 (C_8), 56.1 (OCH_3), 42.7 (C_5), 26.5 (CH_3), 25.0 (CH_3), 18.2 (C_{11}), 17.8 (C_{13}); *minor diastereomer:* 172.4 (C_6), 166.9 (C_9), 112.8 (C_{12}), 109.0 (C_1), 86.8 (C_2), 84.6 (C_3), 82.4 (C_4), 80.3 (C_{10}), 56.4 (C_8), 54.7 (OCH_3), 42.1 (C_5), 26.3 (CH_3), 24.8 (CH_3), 18.1 (C_{11}), 17.5 (C_{13}). IR, ν_{max} (film) cm^{-1}: 1745 (C=O), 1685 (C=O). MS (FAB) m/z calcd for $C_{17}H_{28}BrNO_7$ 437: 435 (100%), 437 (97%), 436 (20%), 438 (20%).

t-Butyl N-[methyl 5-bromo-5-deoxy-3-*O*-methyl-1,2-di-*O*-isopropylidene-*α*-D-*gluco*hexofuranuronoyl]-L-alaninate/t-butyl N-[methyl 5-bromo-5-deoxy-3-*O*-methyl-1,2-di-*O*-isopropylidene-*α*-L-*ido*hexofuranuronoyl]-L-alaninate 4ci/4′ci

Colorless oil; Yield: 66%; R_f 0.28 (EtOAc/Hexane 1:5).

^{1}H NMR ($CDCl_3$) δ_H (ppm): *major diastereomer:* 6.63 (d, 1H, H_7, $^3J_{H7\text{-}H8}$ = 7 Hz), 5.93 (d, 1H, H_1, $^3J_{H1\text{-}H2}$ = 3.5 Hz), 4.68 (dd, 1H, H_4, $^3J_{H4\text{-}H3}$ = 10.0 Hz, $^3J_{H4\text{-}H5}$ = 3.0 Hz), 4.58 (d, 1H, H_2, $^3J_{H2\text{-}H1}$ = 3.5 Hz), 5.56–4.42 (m, 1H, H_8), 4.28 (d, 1H, H_3, $^3J_{H3\text{-}H4}$ = 10.0 Hz), 3.91 (d, 1H, H_5, $^3J_{H5\text{-}H4}$ = 3.0 Hz), 3.48 (s, 3H, OCH_3), 1.55–1.30 (m, 9H, 2 × CH_3, H_9), 1.48 (s, 9H, H_{11}); *minor diastereomer:* 6.54 (d, 1H, H_7, $^3J_{H7\text{-}H8}$ = 7 Hz), 6.01 (d, 1H, H_1, $^3J_{H1\text{-}H2}$ = 4.0 Hz), 4.68 (dd, 1H, H_4, $^3J_{H4\text{-}H3}$ = 10.0 Hz, $^3J_{H4\text{-}H5}$ = 3.0 Hz), 4.58 (d, 1H, H_2, $^3J_{H2\text{-}H1}$ = 3.5 Hz), 5.56–4.42 (m, 1H, H_8), 4.28 (d, 1H, H_3, $^3J_{H3\text{-}H4}$ = 10.0 Hz), 3.94 (d, 1H, H_5, $^3J_{H5\text{-}H4}$ = 3.0 Hz), 3.35 (s, 3H, OCH_3), 1.55–1.30 (m, 9H, 2 × CH_3, H_9), 1.50 (s, 9H, H_{11}). ^{13}C NMR ($CDCl_3$) δ_C (ppm): *major diastereomer:* 171.6 (C_6), 166.3 (C_9), 112.3 (C_{12}), 105.9 (C_1), 83.5 (C_2), 80.8 (C_3), 80.5 (C_4), 82.0 (C_{10}), 58.2 (OCH_3), 49.1 (C_8), 42.9 (C_5), 27.8 (C_{14}), 26.8 (CH_3), 26.3 (CH_3), 18.1 (C_{11}); *minor diastereomer:* 172.9 (C_6), 166.5 (C_9), 112.1 (C_{12}), 105.9 (C_1), 83.8 (C_2), 81.8

(C_3), 80.1 (C_4), 81.7 (C_{10}), 57.9 (OCH_3), 48.4 (C_8), 42.6 (C_5), 27.8 (C_{14}), 26.8 (CH_3), 26.3 (CH_3), 18.2 (C_{11}). IR, ν_{max} (film) cm^{-1}: 1745 (C=O), 1685 (C=O). MS (FAB) m/z calcd for $C_{17}H_{28}BrNO_7$ 437: 435 (100%), 437 (97%), 436 (20%), 438 (20%).

S-Propyl (6-bromo-6-deoxy-1,2:3,4-di-O-isopropylidene-D-*glycero*-α-D-*galacto*heptopyranosid)thiouronate/S-propyl (6-bromo-6-deoxy-1,2:3,4-di-O-isopropylidene-L-*glycero*-α-D-*galacto*heptopyranosid)thiouronate 5a/5′a

Colorless oil; Yield: 57%.

^1H NMR ($CDCl_3$) δ_H (ppm): *major diastereomer:* 5.44 (d, 1H, H_1, $^3J_{H1\text{-}H2}$ = 5 Hz), 4.72 (dd, 1H, H_3, $^3J_{H3\text{-}H2}$ = 2.5 Hz, $^3J_{H3\text{-}H4}$ = 7.5 Hz), 4.70–4.10 (m, 4H, H_2, H_4, H_5, H_6), 2.68 (t, 2H, H_8, $^3J_{H8\text{-}H9}$ = 7.5 Hz), 1.80–1.50 (m, 2H, H_9), 1.60 (s, 3H), 1.47–1.34 (s, s, s, 3H, 3H, 3H), 1.01 (t, 3H, H_{10}, $^3J_{H10\text{-}H9}$ = 9.5 Hz); *minor diastereomer:* 5.60 (d, 1H, H_1, $^3J_{H1\text{-}H2}$ = 4.5 Hz), 4.72 (dd, 1H, H_3, $^3J_{H3\text{-}H2}$ = 2.5 Hz, $^3J_{H3\text{-}H4}$ = 7.5 Hz), 4.70–4.10 (m, 4H, H_2, H_4, H_5, H_6), 2.68 (t, 2H, H_8, $^3J_{H8\text{-}H9}$ = 7.5 Hz), 1.80–1.50 (m, 2H, H_9), 1.54 (s, 3H), 1.46–1.35 (s, s, s, 3H, 3H, 3H), 1.02 (t, 3H, H_{10}, $^3J_{H10\text{-}H9}$ = 9.0 Hz). IR, ν_{max} (film) cm^{-1}: 1689 (C=O).

S-Propyl (methyl 5-bromo-5-deoxy-2,3-di-O-isopropylidene -β-D-*allo*hexofuranosid)thiouronate/S-propyl (methyl 5-bromo-5-deoxy-2,3-di-O-isopropylidene-β -L-t*alo*hexofuranosid)thiouronate 5b/5′b

Colorless oil; Yield: 52%.

^1H NMR ($CDCl_3$) δ_H (ppm): *major diastereomer:* 5.20–4.20 (m, 5H, H_1, H_2, H_3, H_4, H_5), 3.40 (s, 3H, OCH_3), 2.90–2.50 (m, 2H, H_7, H_8), 1.45–1.35 (m, 2 × CH_3, H_9). IR, ν_{max} (film) cm^{-1}: 1692 (C=O).

Procedure for the Preparation of Glycosyl α-Azidoamidoesters 6b/6′b

A 50-mL round-bottom flask was charged with a solution of glycosylamidoesters **4b/4′b** (1 mmol) in DMF (5 mL). Subsequently, NaN_3 (5.0 mmol) was added. The reaction mixture was heated at 100°C for 5 h and then cooled down to rt and CH_2Cl_2 (15 mL) was added. The organic layer was washed with H_2O (3 × 10 mL), dried over anhydrous $MgSO_4$, and concentrated under vacuum to afford the crude products. Crude glycosyl α-azidoamidoesters **6b/6′b** were purified by flash chromatography on silica gel.

Methyl N-(methyl 5-azido-5-deoxy-2,3-di-*O*-isopropylidene-β-D-*allo*hexofuranuronoyl)-L-valinate/Methyl N-(methyl 5-azido-5-deoxy-2,3-di-*O*-isopropylidene-β-L-*talo*hexofuranuronoyl)-L-valinate 6bj/6′bj

Colorless oil; Yield: 65%; R_f 0.34 (EtOAc/Hexane 1:5).

^{1}H NMR ($CDCl_3$) δ_H (ppm): *major diastereomer:* 6.55 (d, 1H, H_7, $^3J_{H7\text{-}H8}$ = 9 Hz), 5.00 (s, 1H, H_1), 5.20–4.20 (m, 4H, H_2, H_3, H_4, H_8), 3.81 (s, 1H, H_5), 3.76 (s, 3H, H_{10}), 3.39 (s, 3H, OCH_3), 2.30–2.15 (m, 1H, H_{13}), 1.49 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.0–0.9 (m, H_{14}, $H_{14'}$); *minor diastereomer:* 6.78 (d, 1H, H_7, $^3J_{H7\text{-}H8}$ = 8 Hz), 5.05 (s, 1H, H_1), 5.20–4.20 (m, 4H, H_2, H_3, H_4, H_8), 3.77 (s, 3H, H_{10}), 3.71 (s, 1H, H_5), 3.48 (s, 3H, OCH_3), 2.30–2.15 (m, 1H, H_{13}), 1.49 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 1.0–0.9 (m, H_{14}, $H_{14'}$); ^{13}C NMR ($CDCl_3$) δ_C (ppm): *major diastereomer:* 172.6 (C_6), 166.9 (C_9), 112.8 (C_{12}), 109.6 (C_1), 86.8 (C_2), 84.5 (C_3), 82.4 (C_4), 62.1 (C_5), 57.5 (C_8), 54.7 (OCH_3), 52.3 (C_{10}), 31.7 (C_{13}), 26.3 (CH_3), 24.8 (CH_3), 18.7 (C_{14}), 17.5 ($C_{14'}$); *minor diastereomer:* 171.6 (C_6), 166.8 (C_9), 112.9 (C_{12}), 109.9 (C_1), 87.2 (C_2), 85.0 (C_3), 82.4 (C_4), 63.1 (C_5), 57.6 (C_8), 56.1 (OCH_3), 51.2 (C_{10}), 31.3 (C_{13}), 26.5 (CH_3), 25.0 (CH_3), 18.9 (C_{14}), 17.8 ($C_{14'}$); IR, ν_{max} (film) cm^{-1}: 2110 (N_3), 1743 (C=O), 1683 (C=O). MS (FAB) m/z calcd for $C_{16}H_{26}N_4O_7$ 386: 386 (100%), 387 (19%).

t-Butyl N-(methyl 5-azido-5-deoxy-2,3-di-*O*-isopropylidene-β-D-*allo*hexofuranuronoyl)-L-alaninate/*t*-butyl N-(methyl 5-azido-5-deoxy-2,3-di-*O*-isopropylidene-β-L-*talo*hexofuranuronoyl)-L-alaninate 6bi/6′bi

Colorless oil; Yield: 68%; R_f 0.21 (EtOAc/Hexane 1:5).

^{1}H NMR ($CDCl_3$) δ_H (ppm): *major diastereomer:* 6.50 (d, 1H, H_7, $^3J_{H7\text{-}H8}$ = 9 Hz), 5.03 (s, 1H, H_1), 5.10–4.00 (m, 4H, H_2, H_3, H_4, H_8), 3.81 (s, 1H, H_5), 3.38 (s, 3H, OCH_3), 1.70–1.20 (m, 9H, CH_3, H_{14}), 1.49 (m, 9H, H_{11}); *minor diastereomer:* 6.81 (d, 1H, H_7, $^3J_{H7\text{-}H8}$ = 8 Hz), 5.05 (s, 1H, H_1), 5.10–4.00 (m, 4H, H_2, H_3, H_4, H_8), 3.73 (s, 1H, H_5), 3.42 (s, 3H, OCH_3), 1.70–1.20 (m, 9H, CH_3, H_{14}), 1.50 (m, 9H, H_{11}). ^{13}C NMR ($CDCl_3$) δ_C (ppm): *major diastereomer:* 172.4 (C_6), 166.9 (C_9), 112.8 (C_{12}), 109.0 (C_1), 86.8 (C_2), 84.6 (C_3), 82.4 (C_4), 80.3 (C_{10}), 62.3 (C_5), 56.4 (C_8), 54.7 (OCH_3), 26.3 (CH_3), 24.8 (CH_3), 18.1 (C_{11}), 17.5 (C_{13}); *minor diastereomer:* 171.5 (C_6), 166.8 (C_9), 112.9 (C_{12}), 109.2 (C_1), 87.2 (C_2), 85.1 (C_3), 82.4 (C_4), 81.2 (C_{10}), 62.6 (C_5), 56.5 (C_8), 56.1 (OCH_3), 26.5 (CH_3), 25.0 (CH_3), 18.2 (C_{11}), 17.8 (C_{13}). IR, ν_{max} (film) cm^{-1}: 2110 (N_3), 1745 (C=O), 1685 (C=O). MS (FAB) m/z calcd for $C_{17}H_{28}N_4O7$ 400: 400 (100%), 401 (19%), 402 (3.5%).

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