A Facile and General Entry to *C*-Glycosyl (*R*)- and (*S*)-β-Amino Acid Pairs from Glycosyl Cyanides through Enamino Ester Intermediates

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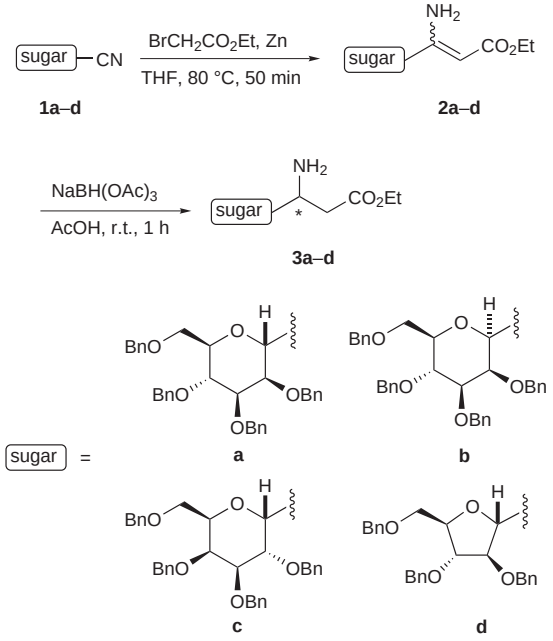
Abstract: Four O-perbenzylated glycosyl cyanides (α- and β-D-mannopyranosyl, α-D-galactopyranosyl, and α-D-arabinofuranosyl) were converted by treatment with $BrCH_2CO_2Et$/Zn in THF at reflux (Blaise–Kishi reaction) into the corresponding *C*-glycosyl β-enamino esters which in turn were reduced by $NaBH(OAc)_3$ to give four pairs of *C*-glycosyl (*R*)- and (*S*)-β-amino esters.

Key words: amino acids, glycopeptides, glycosides, reductions, nitriles

It is abundantly demonstrated that oligosaccharide fragments attached through *O*- and *N*-glycosidic linkages to the polyamide backbone derived from α-amino acid residues, i.e. in native glycoproteins, exert crucial roles on the various properties (structure, protease resistance, stability, and solubility) and biological activities of these biomolecules.[1] Protein glycosylation has been implicated in a variety of processes such as the immune response, intracellular targeting, intercellular recognition, infection by viruses and bacteria adhesion. The introduction of one or more carbon-linked glycosyl β-amino acid residues in a natural glycopeptide induces two elements of diversity at the same time, namely the nature of the linkage holding the sugar moieties and the primary structure of the peptide backbone. Both changes may have dramatic effects on the structure and function of the original α-peptide and therefore these artificial products can be used as valuable probes in mechanistic studies of biological processes at the molecular level and may serve to modulate one or more of those processes. In line with these concepts we began to tackle a project to synthesize *C*-glycosyl β-amino acids[2] which, in contrast with *C*-glycosyl α-amino acids,[3] are difficult to prepare. Accordingly, we reported very recently on the three-component Mannich- and Reformatsky-type syntheses of *C*-glycosyl β-amino acids and in this way paved the route for the preparation of libraries of these compounds.[4] Both methods involved, as the initial step, the coupling of a *C*-glycosyl formaldehyde with *p*-methoxybenzylamine to furnish an intermediate imine which in turn was captured by a ketene silyl acetal in the Mannich route and a bromozinc enolate in the Reformatsky route. Both processes offered a high degree of chemical efficiency and an astonishing stereoselectivity as demonstrated by the formation of a single β-amino acid diastereoisomer in very good yield from each reaction. However, the execution of these reactions with such a degree of efficiency appeared to be conditioned by the availability and stability of the starting sugar aldehydes. Hence the intrinsic configurational instability of α-linked formyl *C*-glycosides[5] precluded the synthesis of the corresponding α-linked *C*-glycosyl β-amino acids. To overcome this limitation we have developed a new reaction sequence that avoids the use of sugar aldehydes and exploits instead glycosyl cyanides as starting material. These *C*-glycosides are readily accessible as both α- or β-anomers via Lewis acid catalyzed coupling of activated glycosyl donors with trimethylsilyl or tetrabutylammonium cyanide[6] and are configurationally stable. Hence, following in part a route developed in our laboratory and leading to C(2)-glycosylated Hantzsch dihydropyridines,[7] we have transformed four known glycosyl cyanides **1a–d** (**a**, tetra-*O*-benzyl-α-D-mannopyranosyl;[6a] **b**, tetra-*O*-benzyl-β-D-mannopyranosyl;[6a] **c**, tetra-*O*-benzyl-α-D-galactopyranosyl;[6a] **d**, tri-*O*-benzyl-α-D-arabinofuranosyl[8]) into the corresponding glycosylated β-amino acrylates **2a–d** (enamino esters) and these were submitted to hydride reduction to give pairs of (*R*)- and (*S*)-β-amino esters **3a–d** (Scheme 1). The results of this study are presented below.



Scheme 1

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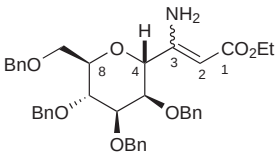
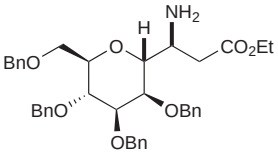
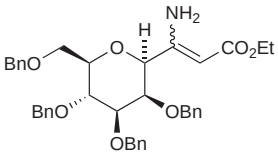
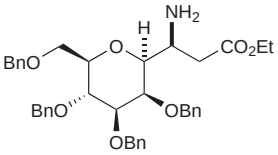
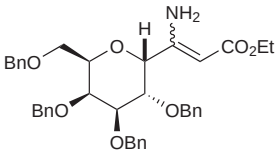
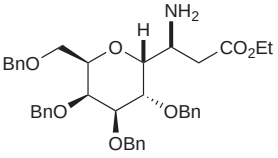
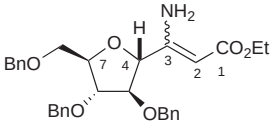
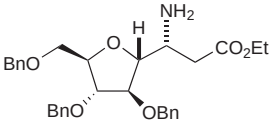
Treatment of the glycosyl cyanides **1a**–**d** with a four molar excess of ethyl bromoacetate in the presence of zinc dust in THF at reflux according to the Kishi improved conditions of the Blaise reaction[9] gave the corresponding *C*-glycosyl enamino esters **2a**–**d** in yields ranging from 86% up to 98% (Table 1), each product being present as a single (unidentified) *E*/*Z* stereoisomer.[10] On the other hand, the retention of the original anomeric configuration of starting glycosyl cyanides was confirmed for enamino esters **2a**–**d** by estimating the $J_{4,5}$ values in the corresponding ^{1}H NMR spectra or by the aid of NOE measurements, as appropriate. In fact, the α-galactopyranosyl derivative **2c** showed a $J_{4,5}$ value around 6.0 Hz. The α- and β-mannopyranosyl derivatives **2a**,**b** instead displayed probing NOE values between H-4 and H-7 or H-4 and H-8, respectively, while the α-arabinofuranosyl **2d** showed NOE interactions between H-4 and H-6. Next we considered the key transformation of the enamino esters **2** into the target β-amino esters **3**. This kind of process has been earlier reported by Palmieri and co-workers[11] to occur readily and chemoselectively in achiral systems by the use of sodium triacetoxyborohydride [$NaBH(OAc)_3$] in acetic acid at room temperature. Hence we were delighted to observe that this hydride releasing agent under the same conditions worked efficiently on our *C*-glycosyl enamino esters **2a**–**d** to give the corresponding β-amino esters **3a**–**d**, in each case as a mixture of diastereoisomers, in good isolated yields (Table 1).[12] The chiral sugar fragment adjacent to the enamino group exerted a very light asymmetric induction on the formation of the new stereocenter as shown by the small to modest diastereomeric excess (de) values of the major products reported in Table 1. Attempts to improve the diastereoselectivity of this reaction by the use of other hydride donor reagents [$NaBH_4$, ZnI_2–$NaBH_4$, $NaBH_3CN$, and $(i\text{-Bu})_2AlH$] have been unsuccessful so far. Hence we were forcedly led to consider the lack of stereoselectivity in a positive sense as a way to obtain pairs of β-amino esters (*R*)- **3** and (*S*)-**3** in almost equal amounts by the reduction of each individual enamino ester **2**. This approach may allow the construction of a collection of these artificial sugar amino acids having the configuration of the β-amino acid moiety as one of the elements of diversity.

In order to transform the β-amino esters **3** to N-protected derivatives suitable for peptide synthesis, the inseparable pair of α-D-mannosyl (*S*)-**3a** and (*R*)-**3a** and each individual compound β-D-mannosyl (*S*)-**3b** and (*R*)-**3b**, α-D-galactosyl (*S*)-**3c** and (*R*)-**3c**, and α-D-arabinosyl (*S*)-**3d** and (*R*)-**3d** were transformed by a standard method into the corresponding *N*-Boc derivatives **4a**–**d** in very high yields

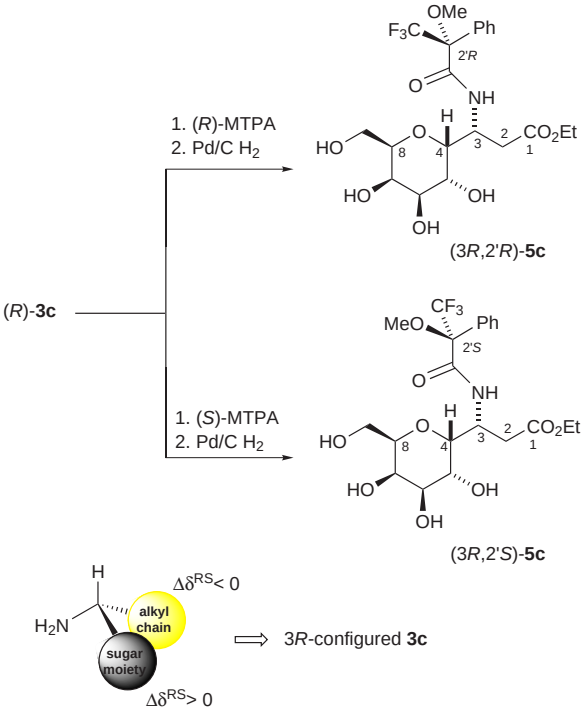
Table 1 *C*-Glycosyl Enamino Esters **2** and *C*-Glycosyl β-Amino Esters **3** Prepared

Sugar nitrile	Enamino ester	Yield (%)[a]	β-Amino ester[b]	Yield,[c] de[d] (%)
1a		87		70, 15
	2a		(*S*)-**3a**	
1b		97		70, 25
	2b		(*S*)-**3b**	
1c		86		65, 5
	2c		(*S*)-**3c**	
1d		98		75, 35
	2d		(*R*)-**3d**	

[a] Isolated yield.
[b] Only the major diastereoisomer is shown.
[c] Overall yield of the mixture of diastereoisomers.
[d] Determined by ^{1}H NMR analysis of the crude reaction mixture.

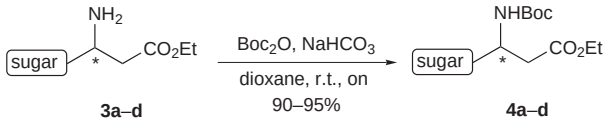
(Table 2). Fortunately, the α-D-mannosyl (*S*)-**4a** and (*R*)-**4a** were separable by column chromatography and therefore the eight compounds reported in Table 2 constitute a collection of pure *C*-glycosyl *N*-Boc β-amino esters.[13]

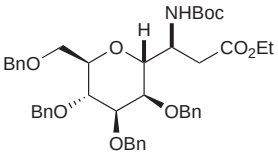
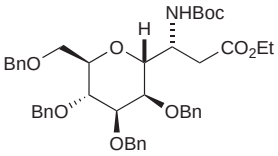
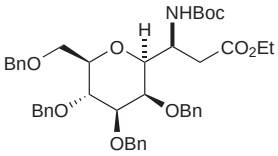
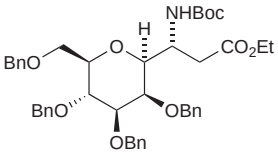
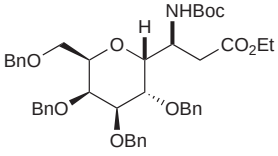
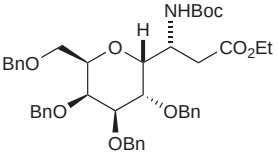
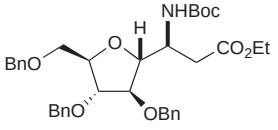
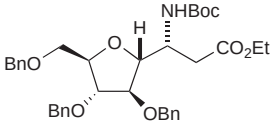
The anomeric configuration of the sugar fragment of β-amino esters **3** and **4** was taken as that established in the corresponding β-enamino esters **2** (see above), while the absolute configuration of the carbon atom bearing the amino group in the side chain of **3** was assigned by the same NMR-based procedure illustrated in our recent report.[4] This procedure follows the protocol developed by Riguera and co-workers[14] for the assignment of the absolute configuration of chiral primary amines. Succinctly, each pure β-amino ester **3** was transformed into the corresponding hydroxy-free Mosher's amides (2′*R*)-**5** and (2′*S*)-**5** by treatment with (*R*)- and (*S*)-methoxytrifluoromethylphenylacetic acid (MTPA) in the presence of dicyclohexylcarbodiimide (DCC) followed by benzyl group hydrogenolysis. Next, the ^{1}H NMR spectra were compared to give chemical shift difference values $\Delta\delta^{RS}$ of proton signals belonging to the two groups linked to the asymmetric carbon, i.e. the sugar moiety and the alkyl chain.[15] The positive or negative $\Delta\delta^{RS}$ values of each set of signals allowed the spatial disposition of the relevant groups attached to the stereogenic carbon to be established and this allowed the assignment of the absolute configuration. The example reported in Scheme 2 illustrates the various steps of this protocol leading to the assignment of the 3*R* configuration of the β-amino ester (*R*)-**3c**.



Scheme 2

Table 2 Transformation of *C*-Glycosyl β-Amino Esters **3** into their Corresponding *N*-Boc Derivatives **4**



(*S*)-Epimer	(*R*)-Epimer
	
(*S*)-**4a**	(*R*)-**4a**
	
(*S*)-**4b**	(*R*)-**4b**
	
(*S*)-**4c**	(*R*)-**4c**
	
(*S*)-**4d**	(*R*)-**4d**

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(9) Hannick, S. M.; Kishi, Y. *J. Org. Chem.* **1983**, *48*, 3833.
(10) **Typical Procedure for Entry to β-Enamino Esters (2).** A suspension of zinc dust (588 mg, 9.00 mmol) in anhyd THF (8 mL) was heated under reflux then a few drops of ethyl bromoacetate were added. After a green color had appeared (ca. 15 min), a solution of glycosyl cyanide **1** (1.50 mmol) in anhyd THF (2 mL) was added in one portion. The remaining bromoacetate was added dropwise over 50 min (total amount of bromoacetate: 0.66 mL, 6.0 mmol). The reaction mixture was cooled to r.t., treated with sat. aq $NaHCO_3$ solution (10 mL) and filtered through a pad of Celite®. The filtrate was extracted with Et_2O (3 × 75 mL) and the combined organic layers were dried (Na_2SO_4) and concentrated in vacuo. The residue was eluted from a column of silica gel using a suitable elution system to afford the corresponding β-enamino ester **2** as a single stereoisomer.
Analytical data for compound **2b**: $[\alpha]_D$ 12.7 (*c* 1.4, $CHCl_3$). ^1H NMR ($CDCl_3$ + D_2O): δ = 7.45–7.10 (m, 20 H, Ph), 4.92 and 4.68 (2 d, 2 H, *J* = 11.2 Hz, PhCH_2), 4.84 and 4.59 (2 d, 2 H, *J* = 11.5 Hz, PhCH_2), 4.73 and 4.66 (2 d, 2 H, *J* = 11.0 Hz, PhCH_2), 4.63 and 4.56 (2 d, 2 H, *J* = 12.0 Hz, PhCH_2), 4.47 (s, 1 H, H-2), 4.15 (q, 2 H, *J* = 7.0 Hz, OCH_2CH$_3$), 4.00 (dd, 1 H, $J_{4,5}$ = ca. 0.5 Hz, $J_{5,6}$ = 3.0 Hz, H-5), 3.93 (dd, 1 H, $J_{6,7}$ = 9.1 Hz, $J_{7,8}$ = 9.0 Hz, H-7), 3.91 (d, 1 H, H-4), 3.77 (dd, 1 H, $J_{8,9a}$ = 2.5 Hz, $J_{9a,9b}$ = 10.8 Hz, H-9a), 3.73 (dd, 1 H, $J_{8,9b}$ = 4.8 Hz, H-9b), 3.66 (dd, 1 H, H-6), 3.51 (dd, 1 H, H-8), 1.30 (t, 3 H, OCH$_2$CH_3). Anal. Calcd for $C_{39}H_{43}NO_7$: C, 73.45; H, 6.80; N, 2.20. Found: C, 73.48; H, 6.81; N, 2.24. MALDI-TOF MS: 638.5 [M^+ + H], 660.9 [M^+ + Na].
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(12) **Typical Procedure for Entry to β-Amino Esters (3).** A solution of $NaBH(OAc)_3$ was prepared by adding $NaBH_4$ (57 mg, 1.50 mmol) to glacial AcOH (1.5 mL) while the temperature was kept at 10 °C. After the H_2 evolution ceased (30 min), a solution of β-enamino ester **2** (0.50 mmol) in glacial AcOH (0.5 mL) was added slowly. The solution was stirred at r.t. for an additional 1 h, and then concentrated in vacuo. The residue was suspended in EtOAc (80 mL) and washed with sat. aq $NaHCO_3$ solution (2 × 10 mL). The organic phase was dried (Na_2SO_4), concentrated, and purified by column chromatography on silica gel using a suitable elution system to give the corresponding (*R*)- and (*S*)-β-amino esters **3** in a variable diastereomeric ratio (see Table 1).
Analytical data for compound (*S*)-**3b**: ^1H NMR ($CDCl_3$): δ = 7.50–7.10 (m, 20 H, Ph), 5.09 and 4.78 (2 d, 2 H, *J* = 11.8 Hz, PhCH_2), 4.92 and 4.60 (2 d, 2 H, *J* = 10.8 Hz, PhCH_2), 4.86 and 4.77 (2 d, 2 H, *J* = 11.5 Hz, PhCH_2), 4.64 and 4.54 (2 d, 2 H, *J* = 12.0 Hz, PhCH_2), 4.21 (dd, 1 H, $J_{4,5}$ = ca. 0.5 Hz, $J_{5,6}$ = 2.5 Hz, H-5), 4.20–4.08 (m, 2 H, OCH_2CH$_3$), 3.96 (dd, 1 H, $J_{6,7}$ = 9.0 Hz, $J_{7,8}$ = 9.2 Hz, H-7), 3.80–3.70 (m, 2 H, 2 H-9), 3.67 (dd, 1 H, H-6), 3.44 (ddd, 1 H, $J_{2a,3}$ = 3.2 Hz, $J_{2b,3}$ = 8.8 Hz, $J_{3,4}$ = 8.5 Hz, H-3), 3.42 (m, 1 H, H-8), 3.12 (dd, 1 H, H-4), 2.88 (dd, 1 H, $J_{2a,2b}$ = 16.2 Hz, H-2a), 2.37 (dd, 1 H, H-2b), 2.00 (bs, 2 H, NH_2), 1.26 (t, 3 H, *J* = 7.0 Hz, OCH$_2$CH_3). Anal. Calcd for $C_{39}H_{45}NO_7$: C, 73.22; H, 7.09; N, 2.19. Found: C, 73.20; H, 7.05; N, 2.15. MALDI-TOF MS: 640.8 [M^+ + H], 662.8 [M^+ + Na].
(13) **Typical Procedure for Entry to *N*-Boc Derivatives (4).** To a stirred mixture of β-amino ester **3** (0.50 mmol), dioxane (8 mL), and Boc_2O (546 mg, 2.50 mmol) a few drops of sat. aq $NaHCO_3$ solution (until basic pH) were added. The solution was stirred at r.t. for an additional 12 h then diluted with Et_2O (100 mL) and washed with a 10% aq solution of citric acid (2 × 10 mL). The organic phase was separated, washed with brine (2 × 10 mL), dried (Na_2SO_4) and concentrated in vacuo. The residue was then purified by column chromatography on silica gel with the suitable elution system to give the corresponding *N*-Boc derivative **4** in almost quantitative yield.
Analytical data for compounds **4**: (*S*)-**4a**: $[\alpha]_D$ 1.7 (*c* 1.3, $CHCl_3$). (*R*)-**4a**: $[\alpha]_D$ –5.8 (*c* 1.0, $CHCl_3$). (*S*)-**4b**: $[\alpha]_D$ –12.0 (*c* 1.5, $CHCl_3$). (*R*)-**4b**: $[\alpha]_D$ –11.5 (*c* 1.2, $CHCl_3$). (*S*)-**4c**: $[\alpha]_D$ 22.1 (*c* 0.9, $CHCl_3$). (*R*)-**4c**: $[\alpha]_D$ 37.0 (*c* 0.8, $CHCl_3$). (*S*)-**4d**: slightly contaminated by uncharacterized by-products. (*R*)-**4d**: $[\alpha]_D$ 8.2 (*c* 0.7, $CHCl_3$). ^1H NMR ($CDCl_3$) for (*S*)-**4b**: δ = 7.50–7.10 (m, 20 H, Ph), 5.17 (br d, 1 H, $J_{3,NH}$ = 8.5 Hz, NH), 5.02 and 4.70 (2 d, 2 H, *J* = 10.5 Hz, PhCH_2), 4.90 and 4.75 (2 d, 2 H, *J* = 11.0 Hz, PhCH_2), 4.82 and 4.60 (2 d, 2 H, *J* = 11.5 Hz, PhCH_2), 4.62 and 4.51 (2 d, 2 H, *J* = 12.0 Hz, PhCH_2), 4.30–4.20 (m, 1 H, H-3), 4.11 (q, 2 H, *J* = 7.0 Hz, OCH_2CH$_3$), 4.02 (dd, 1 H, $J_{4,5}$ = ca. 0.5 Hz, $J_{5,6}$ = 2.5 Hz, H-5), 3.88 (dd, 1 H, $J_{6,7}$ = 9.2 Hz, $J_{7,8}$ = 9.5 Hz, H-7), 3.76 (dd, 1 H, $J_{8,9a}$ = 3.5 Hz, $J_{9a,9b}$ = 12.0 Hz, H-9a), 3.70 (dd, 1 H, $J_{8,9b}$ = 3.8 Hz, H-9b), 3.65 (dd, 1 H, H-6), 3.53 (dd, 1 H, $J_{3,4}$ = 8.5 Hz, H-4), 3.42 (ddd, 1 H, H-8), 2.83 (dd, 1 H, $J_{2a,2b}$ = 16.5 Hz, $J_{2a,3}$ = 6.0 Hz, H-2a), 2.66 (dd, 1 H, $J_{2b,3}$ = 4.0 Hz, H-2b), 1.42 (s, 9 H, *t*-Bu), 1.22 (t, 3 H, OCH$_2$CH_3). Anal. Calcd for $C_{44}H_{43}NO_9$: C, 71.43; H, 7.22; N, 1.89. Found: C, 71.40; H, 7.20; N, 1.80. MALDI-TOF MS: 762.5 [M^+ + Na], 778.5 [M^+ + K].
(14) Seco, J. M.; Quinoa, E.; Riguera, R. *Tetrahedron: Asymmetry* **2001**, *12*, 2915.
(15) The removal of benzyl groups was essential to guarantee $\Delta\delta^{RS}$ values with a regular sign distribution for all protons of Mosher's amides **5**.