[11] Note added in proof (May 24, 1992): The thermolysis of tetranitromethane has also been performed with largely comparable results by L. K. Koo, W. S. Chin, C. Y. Mok, H. H. Huang (personal communication, C. Y. Mok; cf. also *Bull Singapore Nat. Inst. Chem.* **1990**, *18*, 21). For a structurally characterized intermediate of the nitration of naphthalene by tetranitromethane, see L. Eberson, M. P. Hartshorn, F. Radner, W. T. Robinson, *J. Chem. Soc. Chem. Commun.* **1992**, 566.

(*S*)-10 – (*S*)-20 (*R*)-10 – (*R*)-20

Scheme 1. Synthesis of *C*-glycosides **10**–**20** by free radical addition of glycosyl bromides to dehydroalanine derivatives. (*S*) and (*R*) refer to the configuration at C2. **1**: R^1 = OAc, R^2 = H; **2**: R^1 = H, R^2 = OAc; **3**: R^1 = H, R^2 = *O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl); **4**; R^3 = Fmoc, R^4 = OBzl; **5**: R^3 = Z, R^4 = OBzl; **6**: R^3 = Boc, R^4 = OBzl; **7**: R^3 = Fmoc, R^4 = Phe-OBzl; **8**: R^3 = Fmoc, R^4 = D-Phe-OBzl; **9**: R^3 = Fmoc-Pro, R^4 = Ala-OBzl. (a) Bu_3SnH, AIBN, dry toluene, 50–65 °C (see experimental procedure). For abbreviations, see Table 1.

Synthesis of *C*-Glycopeptides via Free Radical Addition of Glycosyl Bromides to Dehydroalanine Derivatives**

By *Horst Kessler**, *Valentin Wittmann*, *Matthias Köck*, and *Matthias Kottenhahn*

Dedicated to Professor Ernst Bayer on the occasion of his 65th birthday

Glycoproteins are widely distributed in living organisms and their carbohydrate part plays a fundamental role in many processes of molecular recognition.[1, 2] The oligosaccharide side chain influences the conformation and solubility of proteins and can prohibit proteolytic cleavage.[3] In most cases the sugar residues form an *N*-glycosidic bond to asparagine or an *O*-glycosidic bond to hydroxy-containing amino acids. Unusual and more stable linkages between carbohydrates and peptides are important for an understanding of the mutual interactions between both moieties.

We now report the facile synthesis of *C*-glycopeptides via free radicals according to the tin-hydride procedure of Giese et al.[4] This new class of compounds[5] is formally derived

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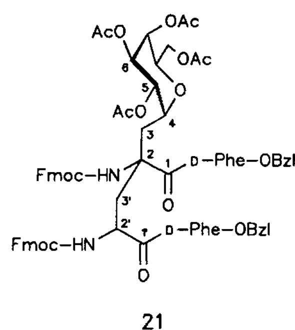
reagents were mixed synchronously after they had been warmed to the reaction temperature. The sugars dehalogenated at C1 could be isolated as side products. Slow addition of a solution of Bu_3SnH and AIBN in toluene to the reaction mixture resulted in reduced yields. As expected,[9] the products are exclusively (>97%) *α*-configurated at the "anomeric center" in the case of the monosaccharides **1** and **2**. Only small amounts of *β*-*C*-glycosides were formed when lactosyl bromide (**3**) was used (12% when reacting with **4**, 4% with **5**). In contrast to the high diastereoselectivity at the sugar, the hydrogen atom transfer to C2 of the amino acid yields mixtures of epimers. The diastereomeric ratios vary depending on the protecting group at the *N*-terminus of the amino acid. The best selectivity (3.8:1 in favor of the (2*S*)-epimer) is obtained with Boc-ΔAla-OBzl (**6**). The epimeric mixtures can be separated by HPLC. After the removal of the urethane group, gram-scale separation is possible by chromatography on a silica gel column.

To examine the possiblity of higher distereoselectivity through chirality in the dehydroamino acid building block, the enantiomeric dipeptides **7** and **8**, as well as the tripeptide **9**, were used. In all cases, even lower selectivity was observed (Table 1). In addition, the use of *α*,*β*-unsaturated carb*amides* results in lower chemical yields. For electronic reasons,[10] addition to a second dehydroalanine unit occurs (e.g., with Fmoc-ΔAla-D-Phe-OBzl (**8**) a single diastereomer of **21**[11] is

Table 1. Results of free-radical addition of glycosyl bromides **1**–**3** to dehydroalanine derivatives **4**–**9** according to Scheme 1. Abbreviations: Fmoc = 9-fluorenylmethoxycarbonyl, Z = benzyloxycarbonyl, Boc = *tert*-butyloxycarbonyl, ΔAla = dehydroalanine, TA-gal = *C*-(2,3,4,6-tetra-*O*-acetyl-D-galactopyranosyl)-α(1 → 3), TA-glc = *C*-(2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl)-α(1 → 3), HA-lac = *C*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-D-lactosyl)-α(1 → 3).

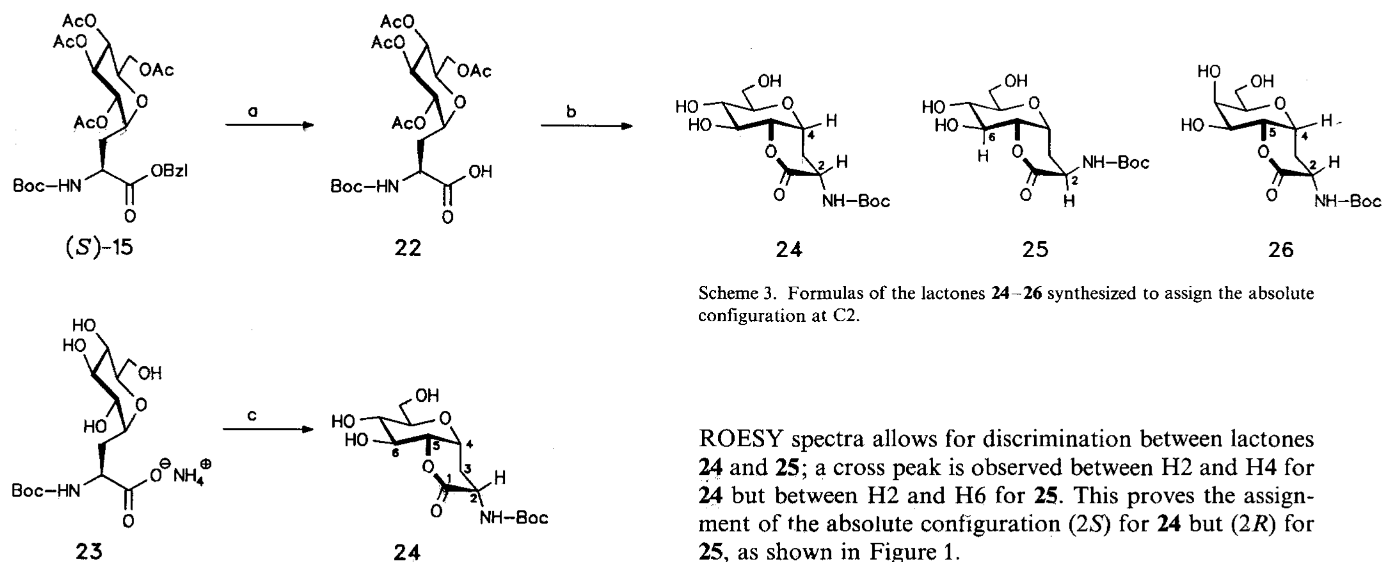
Bromide	Dehydroalanine Derivative		Product [a]		Yield [b]	*S*:*R* [c]
1	Fmoc-ΔAla-OBzl	**4**	Fmoc-Ala(TA-gal)-OBzl	(*S*)-**10**/(*R*)-**10**	65%	2.5:1
1	Z-ΔAla-OBzl	**5**	Z-Ala(TA-gal)-OBzl	(*S*)-**11**/(*R*)-**11**	60%	2.4:1
1	Boc-ΔAla-OBzl	**6**	Boc-Ala(TA-gal)-OBzl	(*S*)-**12**/(*R*)-**12**	61%	3.8:1
2	Fmoc-ΔAla-OBzl	**4**	Fmoc-Ala(TA-glc)-OBzl	(*S*)-**13**/(*R*)-**13**	73%	2.6:1
2	Z-ΔAla-OBzl	**5**	Z-Ala(TA-glc)-OBzl	(*S*)-**14**/(*R*)-**14**	55%	2.3:1
2	Boc-ΔAla-OBzl	**6**	Boc-Ala(TA-glc)-OBzl	(*S*)-**15**/(*R*)-**15**	65%	3.8:1
3	Fmoc-ΔAla-OBzl	**4**	Fmoc-Ala(HA-lac)-OBzl	(*S*)-**16**/(*R*)-**16**	55%	(1.9:1)
3	Z-ΔAla-OBzl	**5**	Z-Ala(HA-lac)-OBzl	(*S*)-**17**/(*R*)-**17**	13%	(3.2:1)
1	Fmoc-ΔAla-Phe-OBzl	**7**	Fmoc-Ala(Ta-gal)-Phe-OBzl	(*S*)-**18**/(*R*)-**18**	45%	1.6:1
1	Fmoc-ΔAla-D-Phe-OBzl	**8**	Fmoc-Ala(TA-gal)-D-Phe-OBzl	(*S*)-**19**/(*R*)-**19**	23%	≥ 2.0:1
1	Z-Pro-ΔAla-Ala-OBzl	**9**	Z-Pro-Ala(TA-gal)-Ala-OBzl	(*S*)-**20**/(*R*)-**20**	40%	1.0:1

[a] (*S*) and (*R*) correspond to the configuration at C2 of the glycosidized amino acid. The products were characterized by ^{1}H and ^{13}C NMR spectroscopy and FAB-MS. [b] Overall yields of the α anomers after chromatography on silica gel. With the exception of two cases (12% for **16**, 4% for **17**), no β anomer could be detected (limit of detection <3%). [c] Isomeric ratios were determined by integration of corresponding signals in the ^{1}H NMR spectra or the HPLC chromatograms. The configuration at C2 was not assigned for compounds **16** and **17** (see text).



obtained in 53% yield). The higher stereoselectivity can be understood by the principle of double stereodifferentiation.[12]

The determination of the absolute configuration at C2 of the amino acids was carried out by NMR spectroscopy of the conformationally restricted lactones **24**, **25**, and **26** (see Scheme 3) and subsequent refinement by molecular dynamics calculations. The lactones were prepared in three steps starting from the corresponding *C*-glycosyl alanine derivatives (*S*)-**15**, (*R*)-**15**, and (*S*)-**12** (Scheme 2). The assignment of proton and carbon atoms was performed by COSY and HMQC[13] spectra. A mixture of [D$_6$]DMSO/CDCl$_3$ was used in the ratio 1:2 for the lactones **24** and **26** but 1:1.4 for lactone **25**. Pure [D$_6$]DMSO could not be used because of overlap in the ^{1}H NMR spectrum. An HMBCS-270 experiment[14] confirmed the cyclization to the δ-lactone ($^3J_{C1,H5}$). ^{1}H,^{1}H coupling constants were extracted from 1D spectra after apodization. The interproton distances were obtained from 500 MHz ROESY spectra[15] with a mixing time of 150 ms. Using these data we could assign both diastereotopic protons at C3. A qualitative inspection of the ROESY spectra allows for discrimination between lactones **24** and **25**; a cross peak is observed between H2 and H4 for **24** but between H2 and H6 for **25**. This proves the assignment of the absolute configuration (2*S*) for **24** but (2*R*) for **25**, as shown in Figure 1.



Scheme 2. Typical reaction sequence illustrated for the synthesis of compound **24**. The lactones **25** and **26** were obtained analogously from coumpounds (*R*)-**15** and (*S*)-**12**, respectively. a) H_2, 10% Pd-C, MeOH, room temperature, 1 h; b) NH_3, MeOH, room temperature, 14 h; c) *N*-ethyl-*N′*-(*N″*,*N″*-dimethylaminopropyl)carbodiimide (EDCI), 1-hydroxybenzotriazole (HOBT), dimethylacetamide (DMA)/THF (1:1), room temperature, 14 h. Quantitative yields were obtained for all three reaction steps.

Scheme 3. Formulas of the lactones **24**–**26** synthesized to assign the absolute configuration at C2.

To refine the structures, restrained MD simulations (Discover[16]) were performed by using the interproton distances (25 distances for compound **24** and 16 distances for **25**).[17] The calculated structures (Fig. 1) are in agreement with all measured distances and coupling constants (Table 2). The simulation was then continued for 300 ps without restraints

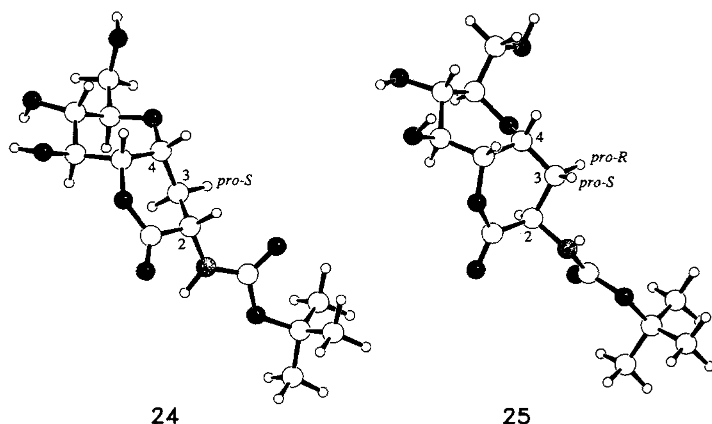


Fig. 1. MD structures of the lactones **24** and **25**. Oxygen atoms are filled, and nitrogen atoms are stippled.

and exhibited the stability of the obtained structures. To prove the configuration at C2, a calculation under identical conditions but with exchanged starting configurations was performed. As expected, considerable deviations from the interproton distances obtained from NMR spectroscopy were observed. In lactone **26**, which stems from the galactose series, strong ROEs from H2 to H4 and H5 are observed, proving the (2*S*) configuration as in **24**. Again, additional evidence is provided by the $^1H,^1H$ coupling constants (Table 2). The absolute configurations of the lactosyl compounds **16** and **17** were not determined but assumed to be the (2*S*) epimers from analogy with the main products. Transformation of the *C*-glycosides (*S*)-**12** and (*S*)-**15** into (*S*)-**10**, (*S*)-**11**, (*S*)-**18**, (*S*)-**19**, and (*S*)-**13**, (*S*)-**14**, respectively, indicates the natural (2*S*) configuration at the amino acids for all other major products.

Table 2. Selected $^1H,^1H$ coupling constants for compounds **24**–**26**.

^3J(H,H)	2,3(*pro-R*)	2,3(*pro-S*)	4,3(*pro-R*)	4,3(*pro-S*)
24	13.1	6.5	9.4	6.6
25	6.6	12.1	4.3	3.7
26	12.7	7.6	5.7	8.1

The procedure described here yields nonnatural *C*-glycosyl amino acids and *C*-glycosyl peptides in one step with high diastereoselectivity at the anomeric center, from readily available starting materials. The products exhibit high chemical stability, are suitable as potential glycosidase inhibitors, and provide a method to improve pharmaco kinetic properties of peptides.

General procedure

The protected dehydroamino acid is dissolved in dry toluene (about 0.1 M) together with 1.2–2 equivalents of glycosyl bromide and warmed to approximately 60 °C. Bu_3SnH (1.2–2 equiv) and catalytic amounts of AIBN are added under nitrogen or argon.

After completion of the reaction (monitored by TLC), the solvent is evaporated, the residue dissolved in acetonitrile and extracted three to five times with hexane to remove the tin compounds. The acetonitrile is evaporated, and the remaining oil purified by silica gel chromatography. The products are distereomeric mixtures of L- and D-amino acids.

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[8] For example (*S*)-**15**: $[\alpha]_D^{22} = 50.6$ ($c = 1$, $CDCl_3$); FAB-MS: *m/z* 610 ($M + H^{\oplus}$); ^1H NMR (250 MHz, $CDCl_3$, 300 K, TMS): $\delta = 7.42$–7.32 (m, 5H, C_6H_5), 5.35 (d, 1H, NH), 5.20 (dd, 1H, H-6), 5.30–5.14 (m, 2H, CH_2-Ph), 5.02 (dd, 1H, H-5), 4.96 (dd, 1H, H-7), 4.46 (m, 1H, H-2), 4.35 (ddd, 1H, H-4), 4.21 (dd, 1H, H-9b), 3.95 (dd, 1H, H-9a), 3.85 (ddd, 1H, H-8), 2.24 (ddd, 1H, H-3b), 2.08 (ddd, 1H, H-3a), 2.10–2.00 (4 s, 12H, OAc), 1.42 (s, 9H, Boc); J(NH,2) = 7.2, J(4,5) = 5.3, J(5,6) = 8.7, J(6,7) = 7.4, J(7,8) = 8.6, J(8,9a) = 2.7, J(8,9b) = 4.8, J(9a,9b) = 12.2 Hz; ^{13}C NMR (62.5 mHz, $CDCl_3$, 300 K): $\delta = 171.4$, 170.6, 169.8, 169.4, 169.3, 154.9 (C=O), 135.0, 128.6, 128.5, 128.3 (C_6H_5), 80.1 (CMe_3), 69.9 (C-8), 69.8 (C-6), 69.5 (C-5), 68.7 (C-4), 67.9 (C-7), 67.3 (CH_2-Ph), 61.5 (C-9), 50.5 (C-2), 28.2 (C-3 and $C(CH_3)_3$), 20.6 (4(CO)CH_3).
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[11] **21**: FAB-MS: *m/z* 1425.5 ($M + H^{\oplus}$); ^1H NMR (500 MHz, $CDCl_3$, 300 K, TMS): $\delta = 8.12$ (d, 1H, Phe-NH), 7.70–7.10 (m, 36H, arenes), 7.05 (d, 1H, Phe-NH), 6.97 (s, 1H, NH), 5.73 (d, 1H, NH), 5.23–5.13 (m, 4H, 2 CH_2-Ph), 5.09 (dd, 1H, H-7), 5.04 (dd, 1H, H-5), 4.93 (dd, 1H, H-6), 4.90, 4.64 (each ddd, 2H, each Phe-H-α), 4.33, 3.85 (each m, 2H, Fmoc-CH_2), 4,27, 3.92 (each m, 2H, Fmoc-CH_2), 4.25 (ddd, 1H, H-4), 4.12 (dd, 1H, H-9b), 4.06 (ddd, 1H, H-2'), 4.05, 3.94 (each m, 2H, each Fmoc-H-9), 3.74 (dd, 1H, H-9a), 3.30 (ddd, 1H, H-8), 3.10, 2.91 (each dd, 2H, Phe-H-β), 3.08, 3.00 (each dd, 2H, Phe-H-β), 2.62 (dd, 1H, H-3'b), 2.42 (dd, 1H, H-3b), 2.30 (dd, 1H, H-3'a), 1.94 (dd, 1H, H-3a), 2.07–1.94 (4 s, 12H, OAc); ^{13}C NMR (125 MHz, $CDCl_3$, 300 K): $\delta = 172.5$, 172.3, 172.2, 170.7, 169.9, 169.7, 169.6, 169.5, 155.9, 155.4 (C=O), 143.8–119.8 (arenes), 68.5 (C-8), 67.8 (C-4, C-5, CH_2-Ph), 67.3 (C-6, CH_2-Ph), 67.2 (C-7), 67.1 (2 Fmoc-CH_2), 61.2 (C-9), 60.8 (C-2), 54.9, 53.9 (each Phe-C-α), 51.2 (C-2'), 47.0, 46.8 (each Fmoc-C-9), 40.8 (C-3'), 37.4, 36.7 (each Phe-C-β), 32.5 (C-3), 20.9–20.6 (4 (CO)CH_3).
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[17] Restrained MD simulations in vacuum were performed over 150 ps. The time step for the integration of Newton's equations of motion was 1.0 fs. The starting structures were built interactively with INSIGHT. To take into account errors in the ROESY cross-peak intensities and for the interconversion into distanes, 5% were subtracted from the lower and added to the upper bounds. The simulations were performed for 5 ps at 1000 K, then for 10 ps at 500 K, and finally for an additional 135 ps at 300 K. Only the last 100 ps were used for analysis.