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A New Approach to Glycopeptides**

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The importance of carbohydrates for a living organism goes far beyond the function of serving as an energy reservoir.[1] Carbohydrates are elementary building blocks of ribonucleic acids and glycoconjugates such as glycoproteins, -lipids, and -phospholipids,[2] and in most cases the carbohydrate portion of a given molecule is the carrier of the biological information.[3] As a component of the cell membrane, glycoconjugates fulfil important functions[2–4] during cell–cell recognition, cell–matrix interaction, and cell growth regulation, and thus also in the development of tumors.[5] Furthermore, they play a significant role in the interaction with biologically active factors such as enzymes, hormones, bacteriotoxins, and viruses.[1, 4]

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The substructure of a carbohydrate, which undergoes permanent change[6] during the life cycle of a cell, influences the biological properties of peptides and proteins.[1] In general, it increases their proteolytic stability,[7] improves their solubility and properties for transmembrane transport, and decreases their excretion rate[8] thus enhancing their bioavailability. Since glycosylations can cause restrictions of the conformational flexibility of peptides, they play a significant role in peptide folding processes.[9]

In many cases, the glycosylation of naturally occurring and non-natural peptides leads to a change in their activity profile. For instance, the analgesic activity of enkephalines[10] could be increased by glycosylation which is attributed, among others, to an improved passage of the glycosylated form across the blood/brain barrier.[11]

The convergent synthesis of *O*-glycosides by coupling reactions at the carbohydrate/peptide link is problematic because of the generally poor solubility of peptides under the glycosylation conditions and also because of regio- and stereochemical aspects.[9a, 12] Therefore, the method of choice[12] is an alternative synthetic strategy that involves the stepwise construction starting from O-glycosylated amino acid derivatives as well as small O-glycosylated peptide fragments, especially since further coupling reactions can be carried out enzymatically and in solid phase reactions.[14]

The conventional synthesis of glycosyl amino acids and peptides requires the orthogonal protection of the α-amino and the α-carboxylic functionalities. After glycosylation of the free hydroxy group, the carboxylic acid group is deprotected, activated, and the C-terminus is coupled with an amino ester.

The new protecting group/activation strategy described herein offers significant advantages: The introduction and cleavage of the protecting group occurs under mild conditions. This resolves the issue of the acid lability of the *O*-glycoside bond as well as the tendency of the *O*-glycosylated serine and threonine derivatives to undergo β-elimination in the presence of strong bases. As a result, the new strategy offers the possibility of a shorter reaction sequence. Moreover, the reaction can be monitored easily and quickly by ^{19}F NMR spectroscopy without any material loss.

Multifunctional α-amino acids such as serine, threonine, 4-hydroxyproline, and tyrosine react with hexafluoroacetone in very good yields to form 2,2-bis(trifluoromethyl)-1,3-oxazolidin-5-ones. The regioselective heterocyclization process in the case of serine and threonine not only allows the protection of the α-amino and the α-carboxylic groups in one step, but at the same time an additional activation of the carboxylic group. Since an excess of hexafluoroacetone is normally used, the hydroxy groups of serine, threonine, and 4-hydroxyproline in the crude product are partially present as hemiketals. The hexafluoroacetone can be cleaved by stirring a solution of the respective hemiketal in dichloromethane in the presence of silica gel at room temperature; the progress of the cleavage reaction can be monitored easily by ^{19}F NMR spectroscopy. The 2,2-bis(trifluoromethyl)-1,3-oxazolidin-5-ones obtained in this manner can be stored for several months when kept refrigerated in a moisture-free environment.[15]

The HFA-protected amino acids **5**–**8** (HFA = hexafluoroacetone) were allowed to react with 2,3,4,6-tetra-*O*-acetyl-α-

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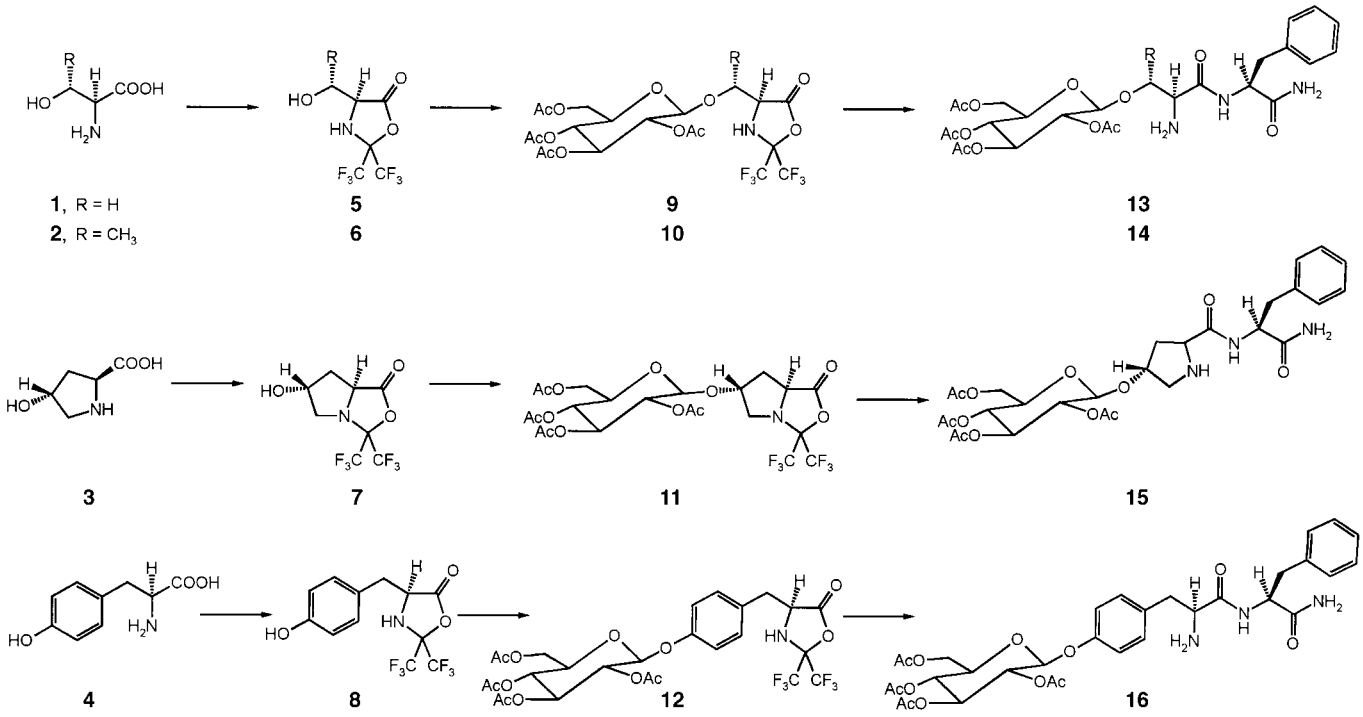


D-glucopyranosyl bromide/$Hg(CN)_2$ according to Helferich,[16] with penta-*O*-acetyl-β-D-glucopyranose/BF_3 according to Paulsen,[17] and with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl trichloroacetimidate according to Schmidt.[18] The good yields of β-D-Glc-(HFA)-Xaa (Table 1) indicate that the new protecting group strategy can be used successfully for all three glycosylation procedures. Compared to the glycosylation of HFA-Ser **5** and HFA-Thr **6**, the yield of HFA-Hyp (**7** → **11**) was surprisingly low (27%) even with the imidate method. The main product in this case (55%) was 4-Ac-(HFA)-Hyp which indicates that an orthoester is formed as an intermediate.[12]

Table 1. Glycosylation of HFA-protected amino acids according to various methods.

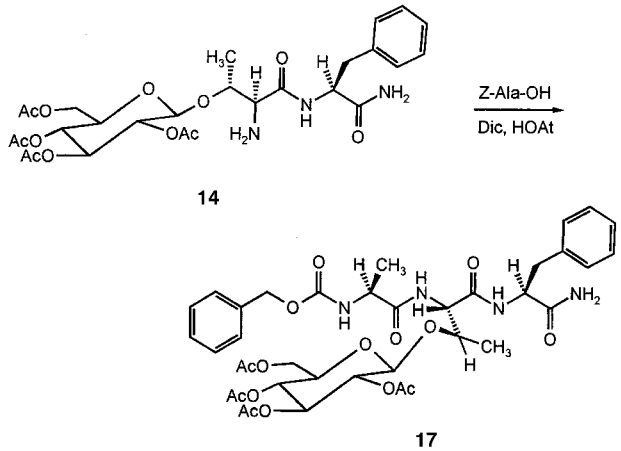
Compound	Method[a]/Yield [%]			m.p. [°C]
	A	B	C	
HFA-Ser(Glc)	57	61	78	173–174
HFA-Thr(Glc)	86	70	82	111–112
HFA-Hyp(Glc)	21	40[b]	27	106–107
HFA-Tyr(Glc)	–	83	93	105–106

[a] Method A: Helferich variant of the Koenigs-Knorr reaction; method B: Procedure according to Paulsen; Method C: Procedure according to Schmidt. [b] Yield determined by ^{19}F NMR spectroscopy.

To date, only a small number of glycopeptides containing glycosyl-Tyr groups have been isolated from natural sources.[12] β-D-Glycosyl-*O*-tyrosine itself was found as a tyrosine metabolite in *Lepidoptera*.[19] The moderate yields (ca. 40%) in the glycosylation of 9-Fmoc-Tyr-OPfp (9-fluorenylmethoxycarbonyl-tyrosine-*O*-pentafluorophenyl) have been attributed the low nucleophilic character of the phenolic hydroxy group.[20] The glycosylation of HFA-Tyr (**8** → **12**) by the imidate method gives a yield of 93%.

The O-glycosylated oxazolidin-5-ones **9**–**12** can be used directly as activated esters in peptide syntheses. When the lactone ring is opened with amino acid amides, glycosylated dipeptides are formed as solids in very good yields. With the new strategy, Tyr(β-D-Glc)-Phe-NH_2 was obtained from Tyr in only three steps with an overall yield of 70%. The deprotection of the amino group is coupled to the formation of the amide bond so that an extension of the peptide chain at the N-terminus can occur without any additional steps.

The synthesis of the glycosylated tripeptide Z-Ala-Thr(TAcGlc)-Phe-NH_2 **17** (Z = benzyloxycarbonyl) starting from **14** can be achieved with diisopropylcarbodiimide(Dic)/7-aza-1-hydroxy-1*H*-benzotriazole (HOAt) in 87% yield.



The protecting group/activation concept presented herein allows the construction of glycosylated dipeptide and tripeptide fragments starting from hydroxy amino acids in only three or four synthetic steps, and can therefore be considered superior to the pentafluorophenyl estermethod.[21]

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Experimental Section

The experimental data of the threonine derivates **10**, **14**, and **17** only are presented as examples. The NMR data have been assigned from ^{1}H, ^{13}C, APT, H,H-COSY, and HMQC spectra.

10: HFA-Thr **6**,[15] (2 mmol, 534 mg), 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl trichloroacetimidate (2 mmol, 984 mg) and $BF_3 \cdot Et_2O$ (1 mmol, 142 mg, 125 μL) were stirred at room temperature in anhydrous CH_2Cl_2 (20 mL) for 1 h. H_2O (10 mL) and CH_2Cl_2 (20 mL) were added, and the resulting mixture was stirred vigorously for an additional 5 min. The organic layer was separated, dried ($MgSO_4$), and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel (toluene/ethyl acetate, 5/1). Yield: 1 g (68 %), amorphous solid; m.p. 112 – 113 °C; IR (KBr): $\tilde{\nu} = 1830$, 1750 cm^{-1}; MS (70 eV): m/z: 597.42 [M^+]; ^{1}H NMR ($CDCl_3$): $\delta = 1.38$ (d, $J = 6.5$ Hz, 3H), 2.01 (s, 3H), 2.02 (s, 3H), 2.06 (s, 3H), 2.07 (s, 3H), 3.66 – 3.76 (m, 3H), 4.06 – 4.13 (m, 2H), 4.22 (dd, $J = 12.5$ Hz, 4.2 Hz, 1H), 4.57 (d, $J = 8.0$ Hz, 1H), 4.95 (dd, $J = 9.6$ Hz, 8.0 Hz, 1H), 5.09 (dd, $J = 9.7$, 9.7 Hz, 1H), 5.20 (dd, $J = 9.7$, 9.6 Hz, 1H); ^{13}C NMR ($CDCl_3$): $\delta = 18.1$, 20.4, 20.5, 20.8, 59.9, 61.3, 68.0, 71.2, 72.2, 72.6, 75.7, 89.3 (qq, $J = 34$, 34 Hz), 100.2, 120.2 (q, $J = 286$ Hz), 121.1 (q, $J = 287$ Hz), 169.2, 169.3, 169.4, 170.2, 170.7; ^{19}F NMR ($CDCl_3$): $\delta = -0.94$ (q, $J = 8.6$ Hz, 3F), -2.89 (q, $J = 8.6$ Hz, 3F).

14: HFA-Thr(TAcGlc) **10** (1 mmol, 597 mg) and L-phenylalanine amide (1 mmol, 164 mg) were stirred at room temperature in anhydrous ethyl acetate (20 mL) for 3 d (reaction control by ^{19}F NMR spectroscopy). The solvent was removed in vacuo and the residue was purified by flash chromatography on silica gel ($CHCl_3$/MeOH, 8/1). Yield: 403 mg (68 %), amorphous solid; m.p. 75 – 78 °C; $M_{calcd} = 595.56$, MS (MALDI-TOF): m/z: 618.19 [M^++Na], 635.25 [M^++K]; ^{1}H NMR (CD_3OD): $\delta = 1.12$ (d, $J = 6.5$ Hz, 3H), 1.96 (s, 3H), 1.97 (s, 3H), 1.99 (s, 3H), 2.02 (s, 3H), 3.00 (dd_{ABX}, $J = 14$, 7.6 Hz, 1H), 3.13 (dd_{ABX}, $J = 14$, 5.8 Hz, 1H), 3.28 (d, $J = 6.5$ Hz, 1H), 3.86 (m, 1H), 3.99 (dq, $J = 5.0$, 6.5 Hz, 1H), 4.09 (dd_{ABX}, $J = 12.3$, 4.8 Hz, 1H), 4.28 (dd_{ABX}, $J = 12.3$, 2.2 Hz, 1H), 4.61 (dd, $J = 6.0$, 8.0 Hz, 1H), 4.73 (d, $J = 8.0$ Hz, 1H), 4.84 (dd, $J = 8.0$, 9.6 Hz, 1H), 4.98 (dd, $J = 9.6$, 9.9 Hz, 1H), 5.26 (dd, $J = 9.6$, 9.6 Hz, 1H), 7.21 – 7.33 (m, 5H); ^{13}C NMR (CD_3OD): $\delta = 16.81$, 20.52, 20.53, 20.63, 20.65, 38.93, 55.47, 60.25, 63.01, 69.80, 72.83, 72.95, 74.11, 78.91, 100.57, 127.94, 129.54, 130.55, 138.20, 171.17, 171.25, 171.59, 172.37, 174.19, 175.72.

17: HOAt (0.34 mmol, 46.8 mg) and DIC (0.34 mmol, 53.3 μL) were added subsequently to a solution of Z-Ala-OH (0.34 mmol, 76.7 mg) in CH_2Cl_2 while stirring at room temperature. A solution of *O*-(2,3,4,5-tetra-*O*-acetyl-β-D-glucopyranosyl)-L-threonyl-L-phenylalanine amide **14** (0.17 mmol, 100 mg) in DMF (1 mL) was then added to the reaction mixture. After 2 h, the reaction mixture was concentrated, the residue was dissolved in ethyl acetate and then washed three times each with citric acid, saturated $NaHCO_3$ solution, and water. The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by flash chromatography on silica gel (eluent: $CHCl_3$/CH_3OH, 10/1). Yield: 101 mg (73 %), crystalline solid; m.p. 190 °C, $M_{calcd} = 800.81$, MS (MALDI-TOF): m/z: 823.23 [M^++Na], 839.12 [M^++K]; ^{1}H NMR (CD_3OD): $\delta = 1.12$ (d, $J = 6.2$ Hz, 3H), 1.30 (d, $J = 7.2$ Hz, 3H), 1.93 (s, 3H), 1.98 (s, 3H), 2.00 (s, 3H), 2.03 (s, 3H), 3.04 (dd_{ABX}, $J = 14$, 7.7 Hz 1H), 3.17 (dd_{ABX}, $J = 14$, 5.5 Hz, 1H), 3.90 (m, 1H), 4.07 (dd_{ABX}, $J = 12.3$, 5.3 Hz, 1H), 4.14 (m, 1H), 4.16 (m, 1H), 4.28 (dd_{ABX}, $J = 12.3$, 5.3 Hz, 1H), 4.45 (d, $J = 6.2$ Hz, 1H), 4.60 (m, 1H), 4.77 (d, $J = 8.0$ Hz, 1H), 4.85 (dd, $J = 9.6$, 9.6 Hz, 1H), 5.01 (dd, $J = 9.6$, 9.6 Hz, 1H), 5.05/5.10 (dd, $J = 12.5$, 12.5 Hz, 2H), 5.27 (dd, $J = 9.6$, 9.6 Hz, 1H), 7.15 – 7.35 (m, 10H); ^{13}C NMR (CD_3OD): $\delta = 16.47$, 17.82, 20.53, 20.55, 20.65, 20.70, 38.62, 52.28, 55.75, 58.58, 63.08, 67.81, 69.80, 72.83, 73.17, 74.06, 76.44, 100.84, 127.98, 128.89, 129.03, 129.50, 129.60, 130.49, 138.25, 138.27, 158.50, 171.26, 171.40, 171.58, 172.42, 175.49, 175.87, 176.86.

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