SYNTHESIS
COMMUNICATIONS

A New Utility of *O*-Glycosylpseudoureas for Synthesis of Glucopeptides and (1 → 6)-Disaccharides

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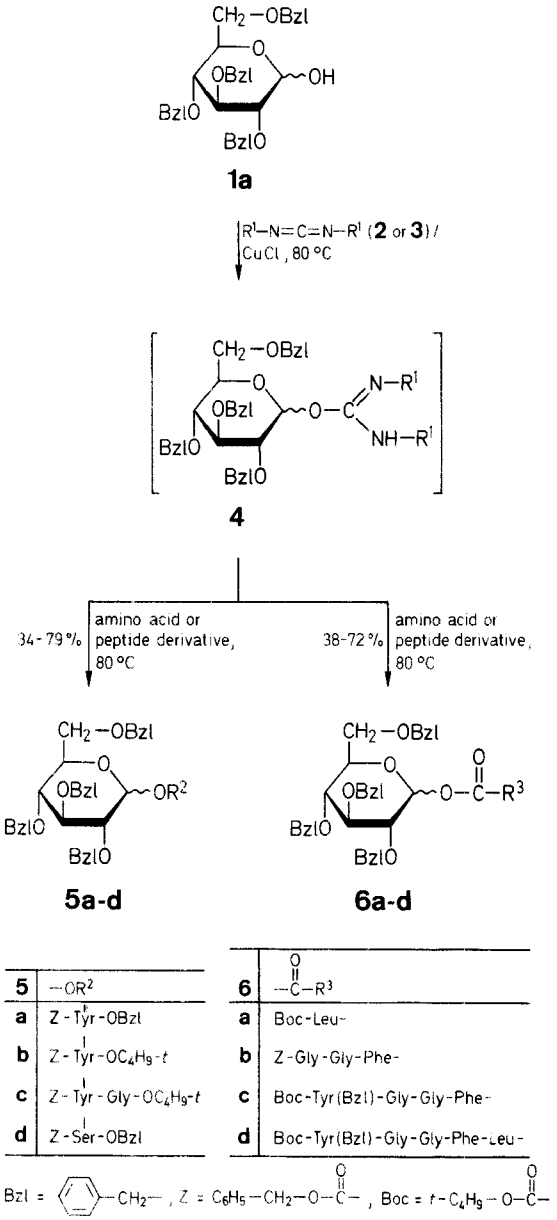
The synthetic utility of *O*-glycosylpseudourea derivatives for the preparation of glucosyl ethers and esters of amino acids and peptides (**5, 6**), and (1 → 6)-disaccharides (**8**) is reported. Simple two-step transformation of 1-*O*-unprotected sugars **1** is accomplished by a one-pot procedure giving products with high stereoselectivity in some cases.

A research program in our laboratory has been concerned with the synthesis of glycopeptides, due to their uses as model substrates in chemical, physicochemical, and biological studies[1]. Recently, our attention has been focused on the synthesis of novel carbohydrate derivatives of Leu-enkephaline, a naturally occurring pentapeptide with morphine-like action[2].

Various methods for the synthesis of glycosidically linked amino acids and peptides have been developed[3–6], although they are not applicable to the synthesis of carbohydrate conjugates of larger peptides such as, for example, enkephalin. Recently, a convenient method for 1-*O*-acylation of carbohydrate molecules via the corresponding *O*-glycosylpseudoureas was described[7].

We now report an efficient, operationally simple synthetic approach to the compounds **5, 6** and **8** via *O*-glycosylpseudourea derivatives. As shown in Scheme **A**, tetra-*O*-benzyl-D-glucopyranose (**1a**); (ratio of α- and β-anomers 4:1), *N,N'*-dicyclohexylcarbodiimide (**2**; Method A) or *N,N'*-di-*p*-tolylcarbodiimide (**3**; Methods B and C), and a catalytic amount of copper(I) chloride were cofused at 80 °C generating the activated *O*-glucosylpseudourea intermediate **4** which was not isolated. Fusion of **4** with amino acid or peptide derivatives at 80 °C afforded the corresponding D-glucopyranosyl ethers **5a–d** and esters **6a–d** as anomeric mixtures in different proportions (Table 1.). Products obtained by Methods A or B contained very high proportions of the corresponding β-D-glucopyranoside anomer which is consistent with previous conclusions[7] that the reaction proceeds via an S_N2-like mechanism.



5	–OR²
a	Z-Tyr-OBzl
b	Z-Tyr-OC₄H₉-t
c	Z-Tyr-Gly-OC₄H₉-t
d	Z-Ser-OBzl

6	–C(=O)–R³
a	Boc-Leu-
b	Z-Gly-Gly-Phe-
c	Boc-Tyr(Bzl)-Gly-Gly-Phe-
d	Boc-Tyr(Bzl)-Gly-Gly-Phe-Leu-

Bzl = C₆H₅–CH₂–, Z = C₆H₅–CH₂–O–C(=O)–, Boc = t-C₄H₉–O–C(=O)–

Scheme A

Comparison of the data in Table 1 reveals that the glucosyl ether synthesis (compounds **5a–c**) by the Method A is superior in stereoselectivity to the Method B. In the glucosyl ester formation (compounds **6a–d**) moderate to fairly good stereoselectivity was achieved by both Methods. The best stereoselectivity was obtained in the preparations of D-glucopyranosyl esters of leucine (**6a**) and Leu-enkephaline (**6d**) having the hindered amino acid involved in the bond formation.

Both Methods A and B were satisfactory for the fusion of **4** with amino acids and peptides having phenolic hydroxy groups (tyrosine derivatives) or free carboxy groups available for coupling, however, they could not be applied for the synthesis of serine glucosyl ether **5d**. This problem has been overcome by the use of acid catalyst (*p*-toluenesulfonic acid) in the second-stage of the reaction (Method C) giving compound **5d** in excellent yield. However, significant loss of stereoselectivity was observed due to anomerization of **4** in the presence of the acid catalyst.

Furthermore, in continuation of these investigations, we have found that the Method C is applicable to the preparation of (1 → 6)-disaccharides **8**. As illustrated in Scheme **B**, three protected disaccharides, having *O*-α-D-glucopyranosyl-(1 → 6)-*O*-2-acetamido-2-deoxy-α-D-glucopyranose (**8a**), *O*-β-L-arabinopyranosyl-(1 → 6)-*O*-2-acetamido-2-deoxy-α-D-glucopyranose (**8b**), and *O*-α,β-L-arabinofuranosyl-(1 → 6)-*O*-2-acetamido-2-deoxy-α-D-

Table 1. Formation of D-Glucopyranosyl Ethers **5a–d** and Esters **6a–d** by Fusion of *O*-Glucosylpseudoureas **4** with Amino Acid or Peptide Derivatives

Amino Acid or Peptide Derivative	Product	Procedure	Yield[a] [%]	Ratio[b] $\alpha:\beta$	^{1}H-N.M.R. (CDCl$_3$/TMS)[c] δ_{H-1} [ppm]	$J_{1,2}$ [Hz]	^{13}C-N.M.R.[d] δ_{C-1} [ppm]
Z-Tyr-OBzl	**5a**	A	68	1:9			95.7
		B	59	1:4			101.8
Z-Tyr-OC$_4$H$_9$-*t*	**5b**	A	58	β only			95.7
		B	40	1:4			101.9
Z-Tyr-Gly-OC$_4$H$_9$-*t*	**5c**	A	34	β only			95.7
		B	21	1:4			101.9
		C	18	1:1			
Z-Ser-OBzl	**5d**	A or B	0				98.6
		C	79	1:1			103.8
		C[e]	58	1.8:1			
Boc-Leu-OH	**6a**	A	70	1:3.6	6.41	2.7	90.9
		B	72	1:9	5.65	7.1	94.8
Z-Gly-Gly-Phe-OH	**6b**	B	58	1:4.5	6.35	3.6	91.5
					5.65	7.2	94.8
Boc-Tyr(Bzl)-Gly-Gly-Phe-OH	**6c**	A	60	1:5.2	6.38	3.6	91.5
		B	43	1:3.3	5.69	7.0	94.9
Boc-Tyr(Bzl)-Gly-Gly-Phe-Leu-OH	**6d**	A	37	1:9	6.43	3.4	89.2
		B	38	1:9	5.63	7.3	96.2

[a] Yields refer to the isolated products and are based on the amount of amino acid or peptide used. Eluents for column chromatography were: chloroform/ether, 96/4 (**5a,b,d**, **6a**); ethyl acetate/hexane, 5/7 (**5c**); dichloromethane/ethyl acetate, 1/1 (**6b–d**).
[b] Ratio of the anomers determined on the basis of the area-ratios of the C-1 signals (compounds **5a–d**) or H-1 signals (compounds **6a–d**) in the N.M.R. spectrum.
[c] α-Anomers having higher δ_{H-1} values.
[d] α-Anomers having lower δ_{C-1} values.
[e] *p*-Toluenesulfonic acid added in the first stage of the reaction.

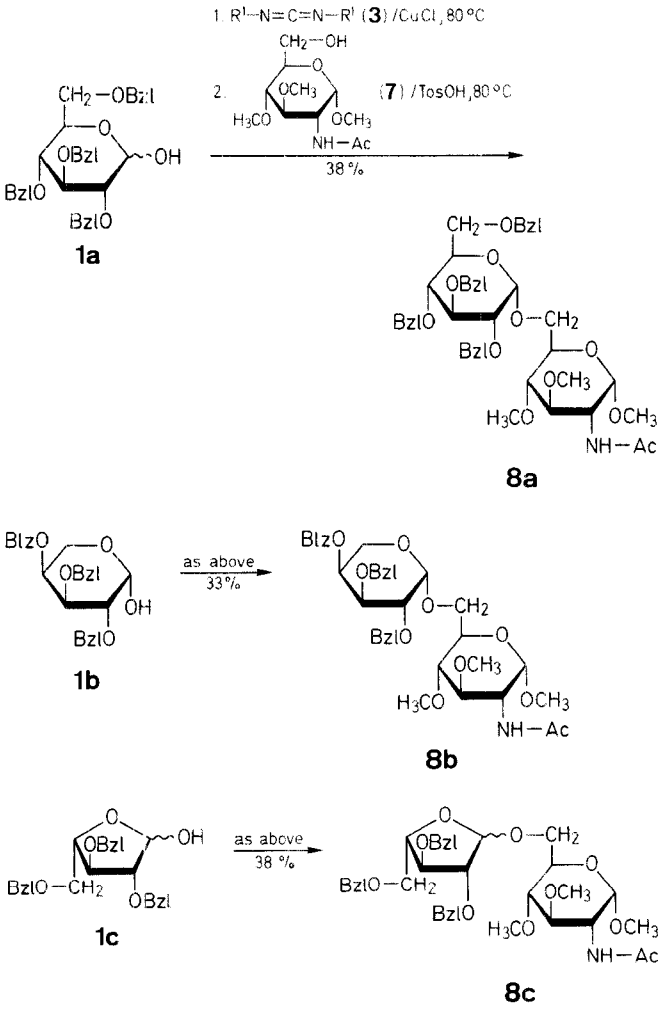
Table 2. Synthesis of Disaccharides **8a–c** from **1a–c** and **7**

Substrate[a]	Product	Procedure	Yield[b] [%]	^{13}C-N.M.R. (CDCl$_3$/TMS)[c] δ_{C-1} [ppm]	$\delta_{C-1'}$ [ppm]
1a	**8a**	A or B	0		
		C	38	97.2	98.3
1b	**8b**	A or B	0		
		C	33	98.6	98.3
1c	**8c**	A or B	0		
		C	38	100.7, 106.4[d]	98.3, 98.1

[a] The α/β ratio being 4:1 for compound **1a**, and 1:4 for compound **1c**.
[b] Yields refer to the isolated products. Eluent for column chromatography: benzene/ethyl acetate, 1/1.
[c] Primed carbon atom refers to 2-acetamido-2-deoxy-D-glucopyranose.
[d] α-L-Anomer having higher δ_{C-1} value.

glucopyranose (**8c**) structures, were prepared in moderate yields from the parent sugars **1** and the fully methylated 6-*O*-unsubstituted *N*-acetyl-α-D-glucosamine **7**, under the conditions of Method C. The synthesis of **8a** and **8b** proceeded with high stereoselectivity, whereas **8c** was obtained as an anomeric mixture (ratio of α- and β-anomers 1:1) not separable by column chromatography and crystallization. As has been demonstrated[8] glycosylation with tri-*O*-benzylarabinofuranose often results in low stereoselectivity.

Evidence for the anomeric configuration of (1→6)-disaccharides **8** was obtained from their ^{13}C-N.M.R. spectra. Assignments of anomeric carbon atoms (Table 2) were based on reported chemical shifts of model compounds[3,9,10], and off-resonance proton decoupling.



Scheme **B**

Table 3. Characterization of Glycopyranoses **5**, **6**, and **8**

Product	Anomer	m.p. [°C] (solvent)	$[\alpha]_D^{22}$ (*c* 1, $CHCl_3$)	Molecular Formula[a] or Lit. Data
5a	β	66–68° (methanol)	+ 3°	$C_{58}H_{57}NO_{10}$ (928.1)
5b	β	76–78° (isopropanol)	+15°	$C_{55}H_{59}NO_{10}$ (894.1)
5c	β	130–132° (isopropanol)	+ 4°	$C_{57}H_{62}N_2O_{11}$ (951.1)
5d	α	oil	+36°	oil; $[\alpha]_D^{22}$: +33° ($CHCl_3$)[3]
5d	β	79–81° (ether/petroleum ether)	+15°	m.p. 80–81°C; $[\alpha]_D^{22}$: +16.3° ($CHCl_3$)[3]
6a	β	oil	+ 6	$C_{45}H_{55}NO_9$ (753.9)
6b	β	137–139° (ethanol)	+12	m.p. 137–139°C; $[\alpha]_D^{22}$: +12° ($CHCl_3$)[4]
6c	β	149–150° (isopropanol)	+17°	$C_{68}H_{74}N_4O_{13}$ (1155.3)
6d	β	83–90° (isopropanol)	+11	$C_{74}H_{85}N_5O_{14}$ (1268.5)
8a		187–189° (ethanol)	+87°	$C_{45}H_{55}NO_{11}$ (785.9)
8b		177–179° (DMF/water)	+58°[b]	$C_{37}H_{47}NO_{10}$ (665.8)
8c	α, β	138–140° (DMF/water)	+50°[b]	$C_{37}H_{47}NO_{10}$ (665.8)

[a] Satisfactory microanalyses obtained: C ±0.23, H ±0.29, N ±0.29.
[b] Determined in dimethylformamide.

The procedures reported in this paper provide a convenient route for the synthesis of such biologically active substances.

The purity and structures of the new compounds **5**, **6**, and **8** were established by T.L.C. on Merck GF 254 silica gel plates, by microanalysis (C,H,N) as well as by ^1H- and ^{13}C-N.M.R. spectra recorded with JEOL FX 90Q Fourier transform spectrometer. The melting points were determined on Tottoli (Büchi) melting point apparatus and are uncorrected. Optical rotations were measured with Carl-Zeiss polarimeter.

2,3,4,6-Tetra-*O*-benzyl-D-glucopyranose (**1a**), 2,3,4-tri-*O*-benzyl-β-L-arabinopyranose (**1b**), 2,3,5-tri-*O*-benzyl-L-arabinofuranose (**1c**), and methyl 2-acetamido-2-deoxy-3,4-di-*O*-methyl-α-D-glucopyranoside (**7**) were prepared according to literature procedures [11,12,13]. The amino acid and peptide derivatives Z-Tyr-OBzl, Z-Tyr-OC_4H_9-*t*, Z-Ser-OBzl, Boc-Leu-OH, Z-Gly-Gly-Phe-OH, Boc-Tyr(Bzl)-Gly-Gly-Phe-OH and Boc-Tyr(Bzl)-Gly-Gly-Phe-Leu-OH were synthesized as previously reported[14–19].

Z-Tyr-Gly-OC_4H_9-*t* was prepared by condensing Z-Tyr-OH with H-Gly-OC_4H_9-*t* in the presence of *N,N'*-dicyclohexylcarbodiimide (**2**) and 1-hydroxybenztriazole; yield: 56%; m.p. 110–112°C (isopropanol); $[\alpha]_D^{22}$: + 3.7° (*c* 1, $CHCl_3$).

Glycosyl Ethers 5, Esters 6, and Disaccharides 8; General Procedures:

Method A: A mixture of **1** (2.0 mmol), *N,N'*-dicyclohexylcarbodiimide (**2**; 212 mg, 2.0 mmol; Fluka), and copper(I) chloride (2 mg, 0.02 mmol) is fused for 0.5 h at 80°C. To the resulting melt the amino acid or peptide derivative or **7** (1.0 mmol) is added and the mixture is heated for 1 h at 80°C. The melt is dissolved in dichloromethane (40 ml), the *N,N'*-dicyclohexylurea is filtered off, the filtrate evaporated, and the residue is purified by column chromatography on silica gel. Crystallization of the chromatographically homogeneous anomeric mixtures affords pure β-anomers **5a–c** and **6b–d**. Anomeric separation of compound **6a** is effected on silica gel column; the α-anomer migrates slightly faster than the corresponding β-anomer. Yields and analytical data are summarized in the Tables.

Method B: A mixture composed of **1** (1.0 mmol), *N,N'*-di-*p*-tolylcarbodiimide (**3**; 222 mg, 1.0 mmol; Fluka), and copper(I) chloride (10 mg, 0.1 mmol) is fused for 5 minutes at 80°C; amino acid or peptide derivative or **7** (1.0 mmol) is then added, and the mixture is heated for 30 min at 80°C. The resulting melt is treated as described in Method A. The yields and properties of the products are given in the Tables.

Method C: This is analogous to Method B, except that, after the formation of the *O*-D-glycosylpseudourea derivatives from **1**, **3**, and copper(I) chloride, *p*-toluenesulfonic acid (0.1 mmol) is added to the reaction mixture together with the amino acid or peptide derivative or **7**. The fusion is continued for 30 min at 80°C, the resulting mixture is taken up in dichloromethane (40 ml), and the precipitated urea derivative filtered off. The filtrate is washed with aqueous sodium hydrogen carbonate solution, then with water, and dried with sodium sulfate. After evaporation of the solvent, the residue is purified by the column chromatography on silica gel to give pure products **5c, d** and **8a–c**. Anomeric separation of compound **5c** is achieved by crystallization and of **5d** in the manner analogous to that described for **6a** (Tables 1–3).

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