Glycosylation of Phenols: Preparation of 1,2-*cis* and 1,2-*trans* Glycosylated Tyrosine Derivatives to be used in Solid-phase Glycopeptide Synthesis

Knud J. Jensen, Morten Meldal and Klaus Bock*
Department of Chemistry, Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Valby, Copenhagen, Denmark

The synthesis of four building blocks, N^{α}-Fmoc-Tyr(Ac_4-β-D-Glc)-OPfp **6**, N^{α}-Fmoc-Tyr(Bz_4-α-D-Glc)-OPfp **16**, N^{α}-Fmoc-Tyr[Ac_4-α-D-Glc-(1→4)-Ac_3-β-D-Glc]-OPfp **9** and N^{α}-Fmoc-Tyr[Bz_4-α-D-Glc-(1→4)-Bz_3-α-D-Glc]-OPfp **19**, suitable for solid-phase glycopeptide synthesis is described.† Several different glycosylation procedures were evaluated for this purpose. A remarkable solvent effect on the α:β ratio was observed on going from dichloromethane to the nucleophilic solvent acetonitrile for the glycosylation reactions promoted by silver triflate with participating protecting groups in the 2-position of the glycosyl donor.

In recent years there has been an increasing interest in glycoproteins and it is now well established that a majority of proteins found in nature are glycosylated.[1,2] The glycan moiety of a glycoprotein can be either *N*-linked to asparagine[1,3–5] or *O*-linked to serine, threonine, 5-hydroxylysine, 4-hydroxyproline or tyrosine.[1,6–12] Glycosylated tyrosine and glycopeptides with the glycan part linked to the phenolic side-chain hydroxy group on tyrosine are found at various places in nature. For example, β-D-glucosyl-*O*-tyrosine has been shown to be a transient metabolite of some insects and it appears to be the major tyrosine-storage metabolite for the production of tanning diphenol substrates in lepidoptera.[10,13,14] Furthermore, in a number of polycyclic glycopeptide antibiotics of the vancomycin and ristocetin group β-D-glucose is attached to the peptide *via* a phenolic side-chain hydroxy group and, subsequently, unusual amino sugars are attached to this glucose unit.[12] In another cyclic glycopeptide antibiotic, ramoplanose, α-D-Man-(1→3)-[α-D-Man-(1→2)]-D-Man is found α-linked to a 4-hydroxyphenylglycine residue.[15] In the S-layer glycoproteins a tyrosine is glycosylated with a linear hexasaccharide where glucose is β-linked to tyrosine.[16] Finally, in glycogenin, the 38 kDa primer protein for the biosynthesis of glycogen, the aromatic hydroxy side-chain of Tyr-194 is gluco- or maltosylated.[11,17] The anomeric configuration of the Tyr–Glc linkages is unproved, but has been proposed to be α.[18]

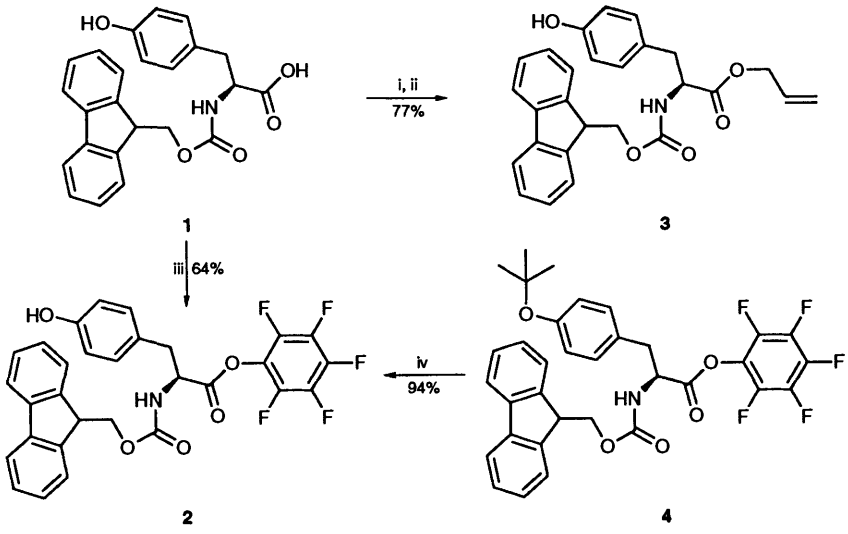
Recently, we published a new strategy for the solid-phase peptide synthesis of *O*-linked glycopeptides.[19,20] It involves the direct glycosylation of the active ester derivatives N^{α}-Fmoc-Ser-OPfp[19,21] and N^{α}-Fmoc-Thr-OPfp.[22,23] This strategy circumvents the need for multistep procedures that hitherto have been used, involving activation of the carboxylic group after selective removal of the protecting group and in some cases exchange of the N^{α}-protecting group.[24,25] For the protection of the glycan moiety the easily removable ester-type protecting groups, acetyl and benzoyl, are preferred.

Compared with the chemical synthesis of aliphatic glycosides, the synthesis of aromatic glycosides in general is more difficult due to the lower nucleophilicity of phenolic hydroxy groups. The phenolic hydroxy group can, however, easily be deprotonated, generating a better nucleophile. It may be safely assumed that a high 1,2-*trans* selectivity in the formation of aryl glycosides bearing a participating neighbouring group in the 2-position is most likely due to the intermediate formation of a 1,2-dioxocarbenium glycosyl donor.[26] However, a few reports have been published where 1,2-*cis*-linked phenyl glycosides are formed, having a participating protecting group at the C-2 position of the glycosyl donor.[27–36] The formation of 1,2-*cis* glycosides with participating neighbouring groups at the C-2 position, on the other hand, proceeds either *via* an S_N2-type concerted displacement reaction of a 1,2-*trans* oriented leaving group at the anomeric carbon or *via* an S_N1-type glycosylation reaction involving an oxocarbenium ion.[37]

Only a few reports on the successful glycosylation of tyrosine have been published. Lu *et al.* obtained N^{α}-Z-Tyr(Ac_4-β-D-Glc)-OMe in 47% yield by reaction of peracetylated sugars in the presence of Lewis acids (Helferich conditions) at elevated temperatures.[14] Horvat *et al.* coupled 2,3,4,6-tetra-*O*-benzyl-glycopyranose with N^{α}-Z-Tyr-OBu^t and N^{α}-Z-Tyr-OBn at 80 °C by *in situ* activation with a carbodiimide and CuCl, and obtained in most cases an α:β mixture in 40–68% yields.[38,39] The β-glycoside obtained was deblocked at the carboxylic acid and used in the last step of a solution-phase synthesis of a glycopeptide. These authors also glycosylated a tetra- and a penta-peptide at the tyrosine hydroxy group but in very low yield. Smiatacz *et al.* treated N^{α}-Z-Tyr-OMe and N^{α}-Boc-Tyr-OMe with dimeric 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride in DMF at ambient temperature for 24 h.[40,41] The α-D-glycosides were obtained in 27 and 25% yield, respectively. A major disadvantage of this approach is that the 2-hydroxyimino group in the product has to be transformed into a suitably protected hydroxy group after the glycosylation. Finally, Wiesner and Leupold have glucosylated N^{α}-Ac-Tyr-OMe at −10 °C with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl fluoride and BF_3·OEt_2 in acetonitrile and obtained the β-glycoside in 71% yield.[42]

Since the configuration at the anomeric centre in the glucosylated tyrosine-194 in glycogenin has not been determined with certainty, both the 1,2-*trans* and 1,2-*cis* aryl glycosides with ester-type protecting group are targets in syntheses of glycogenin-related glycopeptides. The purpose of the present investigation is therefore to develop an efficient methodology for the synthesis of α- or β-linked glycosides of N^{α}-Fmoc-Tyr-OPfp suitable for solid-phase glycopeptide synthesis, of *e.g.* glycogenin-related glycopeptides, which will be reported in a forthcoming publication.

† *Abbreviations used:* Ac = acetyl, All = allyl, Bn = benzyl, Bz = benzoyl, DCC = dicyclohexylcarbodiimide, DIPEA = diisopropylethylamine, DMF = *N*,*N*-dimethylformamide, Fmoc = fluoren-9-ylmethoxycarbonyl, HPLC = high-performance liquid chromatography, MPLC = medium performance liquid chromatography, Pfp = pentafluorophenyl, Su = succinimidyl, TFA = trifluoroacetic acid, Tfl = trifluoromethanesulfonyl, TMS = trimethylsilyl, VLC = vacuum liquid chromatography, Z = benzyloxycarbonyl.

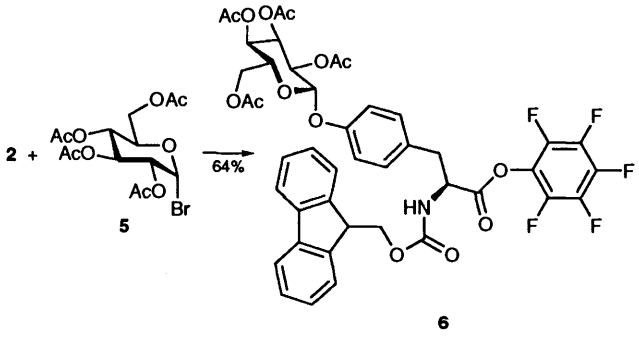


Scheme 1 *Reagents:* i, Cs_2CO_3; ii, $CH_2{=}CHCH_2Br$; iii, DCC, PfpOH; iv, TFA

Results

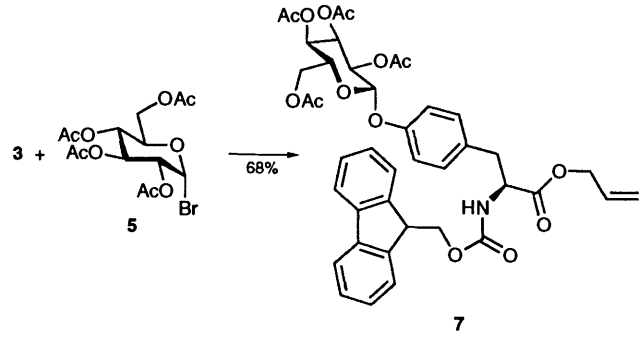
N^{α}-Fmoc-Tyr-OH,[43] **1**, was converted into N^{α}-Fmoc-Tyr-OPfp,[32] **2**, in 64% yield *via* a chemoselective dicyclohexylcarbodiimide (DCC)-mediated coupling with Pfp-OH. Compound **2** was also prepared in 94% yield by treatment of commercially available N^{α}-Fmoc-Tyr(Bu^t)-OPfp, **4**, with neat TFA. As a more stable analogue of compound **2**, N^{α}-Fmoc-Tyr-OAll, **3**, was prepared in 77% yield (Scheme 1). The reactivities and stabilities of compounds **2**, **3** and **4** towards different glycosylation mixtures were studied. In some cases phenol, **10**, was used as glycosyl acceptor to evaluate the glycosylation conditions, since the anomeric mixture of phenyl glycosides is easily separated and the ^{1}H NMR spectra of these compounds are simple, the latter being important when the reactions are followed by ^{1}H NMR spectroscopy.

First, glycosyl bromides were studied as glycosyl donors and 1,2-*trans* glycosides were prepared by silver triflate-promoted reactions where 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **5** and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl bromide **8** were treated with compound **2** or **3**, at −10 °C in dichloromethane in the presence of AgOTfl (Scheme 2). The reactions proceeded



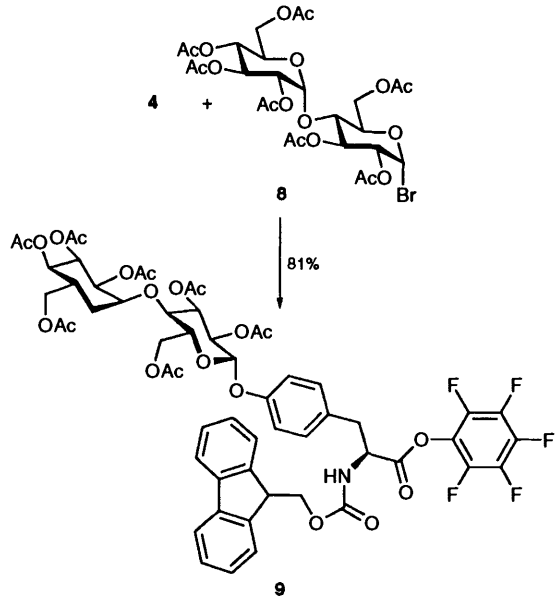
Scheme 2 *Reagents:* AgOTfl, CH_2Cl_2

smoothly under these mild conditions and gave high yields. According to HPLC analysis, the Pfp-ester moiety proved to be completely stable towards the glycosylation conditions. The formation of N^{α}-Fmoc-Tyr(Ac_4-β-D-Glc)-OPfp, **6**, was complete within 90 min and was isolated in 64% yield. Under the same conditions N^{α}-Fmoc-Tyr(Ac_4-β-D-Glc)-OAll, **7**, was prepared in 68% yield, confirming that no significant decomposition of the Pfp-ester had taken place (Scheme 3). The formation of N^{α}-Fmoc-Tyr[Ac_4-α-D-Glc-(1→4)-Ac_3-β-D-Glc]-OPfp, **9**, was complete within 120 min and it was isolated in 42%



Scheme 3 *Reagents:* AgOTfl, CH_2Cl_2

yield. Compound **9** was also prepared in 81% yield by the direct glycosylation of the *tert*-butyl ether **4** with bromide **8** (Scheme 4). The reaction was complete in less than 30 min. Although the markedly better yield may partly be the result of a more efficient purification procedure, the acceleration of the reaction and (in part) the improved yield is most likely due to the enhanced nucleophilicity of the oxygen in the *tert*-butyl ether (Table 1). This is to our knowledge the first example of a glycosylation of a protected aromatic hydroxy group in an amino acid derivative



Scheme 4 *Reagents:* AgOTfl, CH_2Cl_2

Table 1 Silver triflate-promoted glycosylation of tyrosine derivatives in CH_2Cl_2 at −10 °C

Donor	Promoter	Acceptor	Time (*t*/h)	HPLC α/β	HPLC yield (%)	Isolated yield (%)
5[a]	AgOTfl[d]	**3**	1.5	< 1/99	80	β: 68
5[a]	AgOTfl[d]	**2**	1.5	1/99	> 99	α: < 2; β: 64
8[b]	AgOTfl[d]	**2**	2.0	< 1/99	92	β: 42
8[b]	AgOTfl[d]	**4**	1.0	2/98	> 99	β: 81
15[a]	AgOTfl[d]	**2**	1.5	2/98	> 99	n.d.
15[a]	AgOTfl[d]	**3**	1.5	5/95	> 99	β: 47
15[c]	AgOTfl[d], BF_3·OEt_2[e]	**2**	1.5	< 1/99	83	n.d.

[a] 1.5 mol equiv. [b] 2.0 mol equiv. [c] 3.0 mol equiv. [d] Equimolar with the donor. [e] 64 mol equiv. n.d., not determined.

Table 2 Glycosylation of phenol and tyrosine derivatives with glycosyl fluorides at 22 °C.

Donor	Promoter	Acceptor	Conditions: Solvent	Time (*t*/h)	α:β Ratio [yield (%)][j]
11[a]	BF_3·OEt_2[b]	**2**	CH_2Cl_2	20	40:60[g] (> 95)
11[b]	TMSOTfl[e]	**2**	CH_2Cl_2	24	35:65[g] (75)
11[c]	BF_3·OEt_2[f]	**10**	$CDCl_3$	1.1	59:41[h]
11[c]	TMSOTfl[f]	**10**	$CDCl_3$	20	46:54[h]
14[c]	BF_3·OEt_2[f]	**10**	$CDCl_3$	2.6	37:63[h]
11[a]	BF_3·OEt_2[b]	**10**	CH_2Cl_2	5	α[i] (45)
13[c]	BF_3·OEt_2[f]	**10**	$CDCl_3$	1.3	22:78[h]
13[c,d]	BF_3·OEt_2[f]	**10**	$CDCl_3$	4	46:54[h]
13[a,d]	BF_3·OEt_2[b]	**2**	CH_2Cl_2	1.5	2:98[j] (63)

[a] 1.5 mol equiv. [b] 3.0 mol equiv. [c] 1.1 mol equiv. [d] BF_3·OEt_2 added 8 min prior to the addition of the acceptor. [e] 2.2 mol equiv. [f] 1.3 mol equiv. [g] Anomers not separated in HPLC. [h] Determined by NMR. [i] Isolated yield. [j] Determined by HPLC.

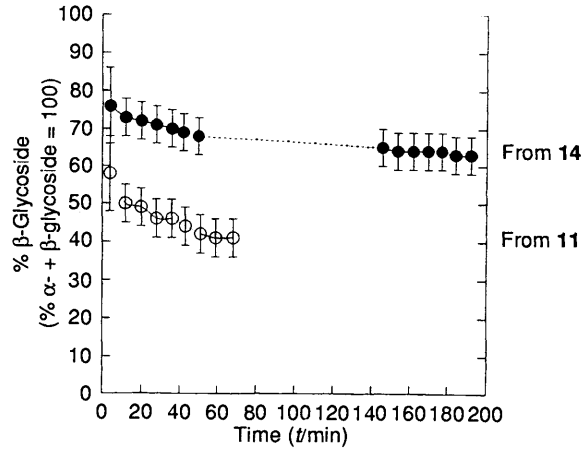
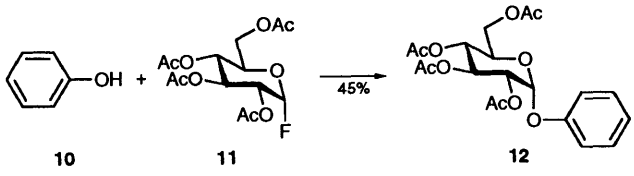


Fig. 1

suitable for solid-phase peptide synthesis. However, glycosides have previously been reported to be formed by reaction of a glycosyl bromide donor and a *tert*-butyl-protected aliphatic alcohol of the aglycone.[44] The glycosylated Pfp-esters could be purified by silica gel chromatography (flash, MPLC and VLC) using predried silica gel and dried solvents as well as by reversed-phase HPLC using acetonitrile and water with or without the addition of TFA as eluent. Thus, the Pfp-esters are relatively stable towards oxygen nucleophiles under neutral or acidic conditions.

Subsequently, three glucosyl fluorides were studied as glycosyl donors promoted by BF_3·OEt_2 and TMSOTfl as promoters. As above, the reactions were carried out in dichloromethane but were found to be slower than the silver triflate-promoted reactions and were therefore carried out at ambient temperature. By using 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl fluoride,[45] **11**, as donor both α- and β-glucosides were obtained, but the glycosides proved difficult to separate when compound **2** was used as glycosyl acceptor. However, their identity and the ratio between them was determined by ^{13}C and ^1H NMR spectroscopy (Table 2).

In the BF_3·OEt_2-promoted glycosylation of phenol, **10**, with fluoride **11** the α-glycoside **12** was obtained in 45% yield after silica gel chromatography (Scheme 5). This glycosylation



10 11 12

Scheme 5 *Reagents:* BF_3·OEt_2, CH_2Cl_2

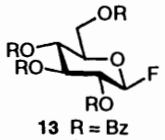
reaction was also carried out in $CDCl_3$ and followed by NMR spectroscopy. As shown in Fig. 1, the α:β ratio increased only slightly during the reaction. This indicates that the formation of α-glycosides (1,2-*cis* glycosides) in these reactions is not due to acid-catalysed rearrangement of initially formed β-glycoside as previously suggested.*† Use of TMSOTfl as catalyst also yielded both the β- and α-glycoside as seen from an experiment where the course of reaction was followed by ^1H NMR spectroscopy. After 20 h an α:β ratio of 46:54 was measured with TMSOTfl as catalyst.

Another experiment carried out in an NMR tube showed that 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl fluoride **13** and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl fluoride[45] **14** rearrange to the corresponding α-fluorides in less than 4 min in $CDCl_3$ with BF_3·OEt_2 as catalyst. This, however, does not mean that the configuration at the anomeric centre of the starting glycosyl donor is without significance for the stereochemical outcome of the glycosylation reaction. When phenol was treated in an NMR tube with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl fluoride, **14**, and BF_3·OEt_2 a final α:β ratio of 37:63 was obtained. As shown in Fig. 1 the α:β ratio gradually increased after the initial phase before reaching the constant final level. The final α:β ratio was significantly lower

* The formation of phenyl α- and β-glycosides from 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl fluoride promoted by BF_3·OEt_2 has been explained by proposing that the initially formed phenyl β-glycoside anomerizes under the acidic conditions used to the thermodynamically more stable α-anomer (Ref.29).

† It has been demonstrated that Lewis acids such as $TiCl_4$, $SnCl_4$ and $ZnCl_2$ do not cause anomerization of β-glycosides to the more stable α-glycosides (refs. 28, 46). The formation of α- (1,2-*cis*) glycosides under Helferich conditions, where an excess of the phenol is used, is presumably due to a transglycosylation of the initially formed β-(1,2-*trans*) glycoside by excess of the phenol (refs. 36, 37).

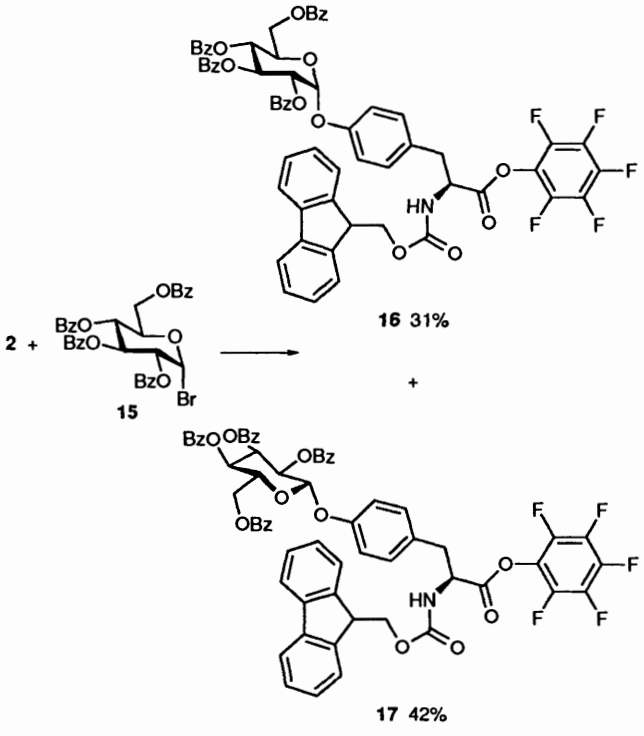
when starting from the β-fluoride than when starting from the corresponding α-derivative. The benzoyl-protected β-fluoride **13** gave, under the same conditions, a final α:β ratio of 22:78. To



13 R = Bz
14 R = Ac

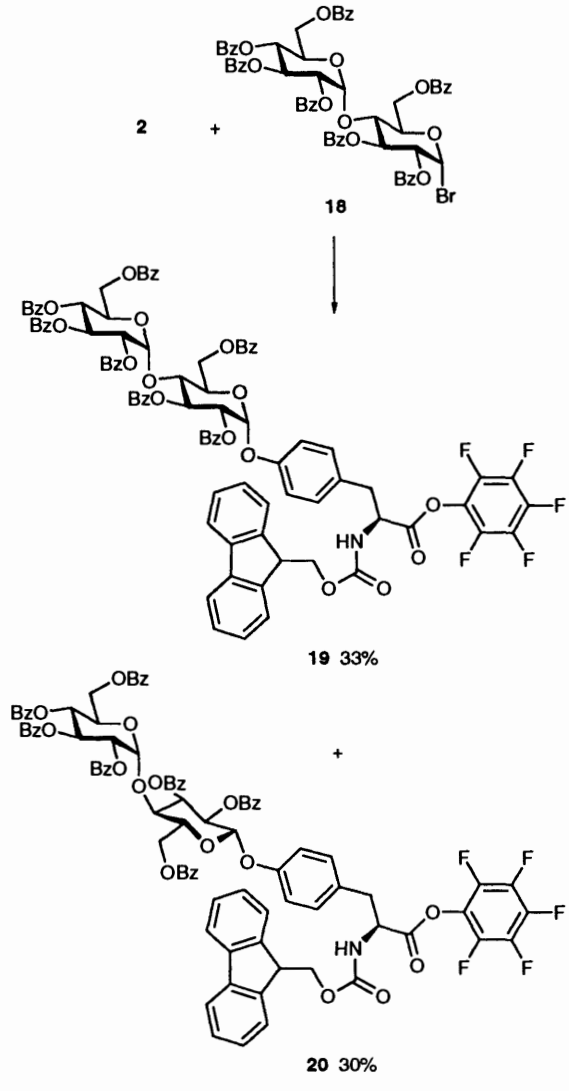
test whether a higher α:β ratio of phenyl glycosides could be obtained from the corresponding α-fluoride, the β-fluoride rearranged to the α-derivative prior to addition of the glycosyl-acceptor phenol. This yielded a final α:β ratio of 46:54. However, glycosylation of compound **2** with the benzoyl-protected β-fluoride gave an α:β ratio of 2:98 (Table 2).

A remarkable solvent effect was found upon changing from dichloromethane to acetonitrile in silver triflate-promoted reactions with glycosyl bromides. While the silver triflate-promoted reactions in dichloromethane led to almost exclusive formation of β-glycosides, the reaction of α-bromide **15** and AgOTfl in acetonitrile with the Pfp-ester **2** gave the α-glycoside **16** (31%) and the β-glycoside **17** (42%) (α:β ratio 42:58). While the reactions carried out in dichloromethane were complete at −10 °C within 90 or 120 min the reactions in acetonitrile were much slower and had to be carried out at ambient temperature for at least 20 h (Scheme 6).



Scheme 6 *Reagents:* AgOTfl, MeCN

For reactions in acetonitrile the choice of protecting groups was also shown to have an influence on the α:β ratio. Thus when the glucosyl donor was benzoylated rather than acetylated the α:β ratio decreased from 63:37 to 43:57 for the glycosylation of N^{α}-Fmoc-Tyr-OAll **3**. However, the protecting groups also influence the reaction in another way. While the use of acetyl-protected glycosyl donors in silver triflate-promoted reactions in acetonitrile led to decomposition of N^{α}-Fmoc-Tyr-OPfp **2** (presumably by cleavage of the Pfp-ester group) it could be treated with benzoyl-protected glycosyl donors with the formation of only minor amounts of by-products. It proved difficult to separate the acetyl-protected anomers by both reversed-phase HPLC and silica gel chromatography, but the benzoylated glycosyl amino acids could easily be separated by reversed-phase HPLC. Separation of the reaction mixture immediately after neutralization was essential, since prolonged storage reduced the yield considerably. Through the use of the glycosyl donor 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranosyl bromide **18**, this approach to the synthesis of benzoyl-protected 1,2-*cis*-linked glycosides was extended to the synthesis of the corresponding maltosyl derivative, N^{α}-Fmoc-Tyr[Bz_4-α-D-Glc(1→4)-Bz_3-α-D-Glc]-OPfp **19**. A 33% yield of the α-glycoside **19** and a 30% yield of N^{α}-Fmoc-Tyr[Bz_4-α-D-Glc-(1→4)-Bz_3-β-D-Glc]-OPfp **20** (α:β ratio 52:48) was obtained (Scheme 7). In diethyl



Scheme 7 *Reagents:* AgOTfl, MeCN

ether, another nucleophilic solvent, the rate of reaction between substrates **2** and **15** increased while the α:β ratio decreased. Dilution of the acetonitrile with toluene as well as glycosylation in pure benzonitrile also gave a lower α:β ratio. The results are summarized in Table 3.

In an alternative approach to the synthesis of α-linked aryl glycosides the use of a glycosyl donor with a non-participating neighbouring group at the 2-position was evaluated. The trichloroacetimidate of 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (α:β ratio 1:8), compound **21**, was used for this purpose due to

Table 3 Silver triflate[a] promoted glycosylation of tyrosine derivatives in nucleophilic solvents at 22 °C

Donor	Acceptor	Conditions: Solvent	Conditions: Time (*t*/h)	HPLC α/β ratio [yield (%)]	Isolated yield (%)
5[b]	**2**	Et_2O	1.5	10/90 (61)	n.d.
5[c]	**3**	Toluene–MeCN (4:1)	1.5	19/81 (85)	n.d.
15[d]	**3**	PhCN	21	34/66 (70)	n.d.
5[d]	**3**	MeCN	4.0	63/37 (80)	n.d.
5[d]	**2**	MeCN	4.0	Decomposition	n.d.
15[d]	**3**	MeCN	44	43/57 (93)	n.d.
15[d]	**2**	MeCN	24	< 47/53 (> 95)	α: 31; β: 42
18[d]	**2**	MeCN	21	51/49 (74)	α: 33; β: 30

[a] Equimolar with the donor. [b] 3.0 mol equiv. [c] 1.5 mol equiv. [d] 2.0 mol equiv. n.d., not determined.

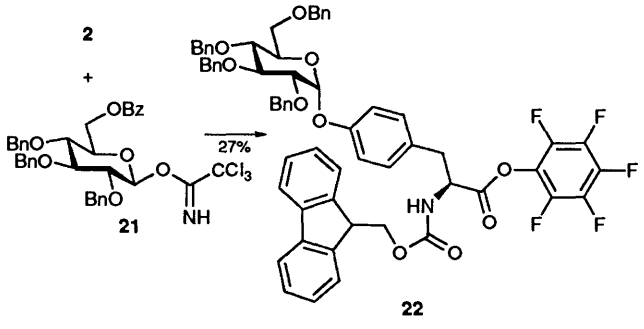
Table 4 Glycosylation of Fmoc-Tyr-OPfp **2** with trichloroacetimidate **21**

Donor	Promoter	Acceptor	Conditions: Solvent	Conditions: *T*/°C	Conditions: Time (*t*/h)	HPLC α/β ratio (yield %)	Isolated yield (%)
21[a]	BF_3·OEt_2[d]	**2**	CH_2Cl_2	−10	1.5	33/67 (80)	n.d.
21[b]	BF_3·OEt_2[b]	**2**	Toluene	22	1.5	38/62 (76)	n.d.
21[c]	TMSOTfl[c]	**2**	Et_2O	−30	2.5	69/31 (83)	α: 27
21[b]	TMSOTfl[b]	**2**	MeCN	22	3.0	74/26 (54)	n.d.

[a] 1.5 mol equiv. [b] 3.0 mol equiv. [c] 4.0 mol equiv. [d] 2.0 mol equiv., n.d., not determined.

its easy accessibility and its reactivity.[47,48] The reactions were in most cases carried out by using 2.0–4.0 mol equiv. of a Lewis acid catalyst (BF_3·OEt_2 or TMSOTfl). The highest α:β ratios were obtained by using diethyl ether (69:31) and acetonitrile (74:26) as solvents. The enhanced formation of α-glycosides in diethyl ether has been ascribed to the intermediate formation of a reactive β-diethyloxonium complex with the activated glycosylating agent leading to preferred attack from the α-face.[49,50] The high α:β ratio for the glycosylation in acetonitrile was surprising, since acetonitrile in aliphatic glycosylations with non-participating protecting groups at C-2 of the glycosyl donors tend to lower the α:β ratio.[51]

Owing to the low reactivity of the phenolic hydroxy groups and the consequently extended reaction times, rearrangement or decomposition of the trichloroacetimidate was pronounced and additional portions of the trichloroacetimidate had to be added during the reaction. The separation of the anomeric mixture of glycosides proved to be difficult even with reversed-phase HPLC. Pure N^{α}-Fmoc-Tyr(Bn_4-α-D-Glc)-OPfp **22** could therefore only be isolated in 27% yield (Scheme 8). The results are summarized in Table 4.



Scheme 8 *Reagents:* TMSOTfl, Et_2O

Discussion

The goal of the present work to develop a methodology for the synthesis of β- and α-linked tyrosine derivatives suitable for solid-phase glycopeptide synthesis has been realized using silver triflate-promoted reactions in dichloromethane and acetonitrile. In dichloromethane these conditions gave rise to stereoselective (and in some cases stereospecific) formation of β-(1,2-*trans*) glycosides. The reactions were carried out at moderately low temperatures with acetyl- or benzoyl-protected glycosyl bromides. A mixture of glycosides was obtained when the silver triflate-promoted reactions were carried out in acetonitrile. With benzoyl-protected glycosyl donors the anomers were easily separated and good yields were obtained. The fact that the *tert*-butyl ether **4** could be glycosylated directly constitutes a simplification of the general strategy for the synthesis of glycosylated amino acid derivatives suitable for solid-phase glycopeptide synthesis, since the *tert*-butyl ether protecting group is very common in peptide chemistry. The rate enhancement and improved yields are other beneficial aspects of this approach. This result demonstrates, however, that there may be inherent difficulties in direct glycosylation of peptides if the *tert*-butyl group is used for the blocking of those side-chain hydroxy groups that should not be glycosylated. The acetyl-protected glucosyl fluoride **11** also yielded both α- and β-glycosides, but the products proved difficult to separate. Benzoyl-protected glucosyl fluorides yielded almost exclusively the β-glucoside. The trichloroacetimidate **21** proved not to be efficient in aromatic glycosylation reactions.

Although our goal has been realized, the results raise some questions about the mechanism of the reactions and the nature of the intermediate species. The fact that the glucosyl fluoride **11** but not the glycosyl bromides yielded significant amounts of α-glycoside together with the β-glycoside in dichloromethane must be explained by either assuming that the reaction in the former case at least partially proceeds *via* a displacement of a 1,2-*trans* oriented leaving group or by assuming an oxocarbenium mechanism without participation of the acetyl group at the 2-position. In the latter case, the counterion should confer some unique stabilization to the oxocarbenium intermediate or steric hindrance for nucleophilic attack from the β-face. However, since the use of both BF_3·OEt_2 and TMSOTfl as promoters gave a mixture of α- and β-glycosides it seems unlikely that the lack of stereoselectivity can be attributed to steric influences only from the counterion. Although we are not able to propose a definite rationale for the lack of stereoselectivity, it should be pointed out that the anomerization of β-fluorides to the corresponding α-fluorides implies

that, in a glycosylation reaction with the α-fluoride activated with BF_3·OEt_2, a small amount of β-fluoride is present during the reaction. It must be assumed that the β-fluoride is more reactive than the α-fluoride due to lack of stabilization *via* the anomeric effect. An explanation for the formation of both α- and β-glycosides could be that the reaction at least partly proceeds *via* the β-fluoride in an *in situ* anomerization mechanism conceptually similar to the *in situ* anomerization by an auxiliary nucleophile described by Lemieux *et al.*[52] β-Fluorides will not react with neutral oxygen nucleophiles without activation by an electrophile. The formation of α-glycosides from glycosyl fluorides carrying participating neighbouring groups at C-2 can be explained by assuming that the β-configured fluoride activated by complexation to BF_3 is displaced in a concerted reaction, thereby allowing attack from only the α-face. While the formation of α-glycosides (together with the corresponding β-derivatives) starting from the α-fluorides according to this is due to the establishment of a **thermodynamic** equilibrium between the α- and β-fluorides (as well as other reactive species), the lower α:β product ratio obtained when starting from the β-fluoride (followed by an increase in the α:β ratio before reaching a constant level) should be attributed to a **kinetic** distribution of reactive intermediates in the initial phase of the glycosylation reaction. Paulsen *et al.*[53] have demonstrated that the 1,2-dioxocarbenium ion is formed upon treatment of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl fluoride with BF_3·OEt_2 and therefore the 1,2-dioxocarbenium ion is likely to be present when establishing the equilibrium between the β-fluoride and the corresponding α-fluoride. The significantly lower α:β ratio of phenyl glycosides formed in the glycosylation reaction with the β-fluoride as compared with the glycosylation with the α-fluoride under the same conditions, is most likely due to a higher concentration of the 1,2-dioxcarbenium ion in the former case. We have no other explanation for this observation. The increased α:β ratio in both cases after the initial phase before reaching the final constant level should consequently be ascribed to product formation from a **thermodynamic** distribution of intermediates; that is, with a significantly lower concentration of the 1,2-dioxocarbenium ion.

Schmidt *et al.*,[51,54] Pougny and Sinay,[55] Ratcliffe and Fraser-Reid,[56] as well as Lemieux and Ratcliffe[57] have shown that glycosyl donors protected by non-participating groups under S_N1 conditions in acetonitrile can form nitrilium–nitrile intermediates. Schmidt *et al.* have shown that for aliphatic glycosyl acceptors the formation of β-glycosides is preferred under these conditions.[51,54] It has been argued that although the β-nitrilium–nitrile complex is thermodynamically preferred due to the reverse anomeric effect, the reaction proceeds mainly *via* an S_N2-type displacement of the more reactive α-nitrilium–nitrile, which is initially formed faster than the corresponding β-complex.[51,54]

The formation of nitrilium–nitrile complexes when the glycosyl donor is protected with participating neighbouring groups has not previously been reported. The above mentioned results for the glycosylation of tyrosine derivatives in silver triflate-promoted reactions in acetonitrile indicate that the reactions at least partly proceed *via* reactive nitrilium–nitrile complexes as two of the predominant glycosyl donors in the reaction mixture. This assumption is based on two observations. First, the glycosylation reactions in acetonitrile are considerably slower than in the non-nucleophilic solvent dichloromethane. Second, the reactions performed in acetonitrile lead to the formation of both α- and β-glycosides. The formation of α-glycosides may be accounted for by a concerted nucleophilic displacement of a 1,2-*trans* oriented nitrilium–nitrile leaving group at the anomeric centre. Since the tyrosine hydroxy group is less nucleophilic than aliphatic hydroxy groups, the glycosylation reactions reported here proceed at a lower rate. The formation of both α- and β-glycosides implies that a part of the glycosylation proceeds *via* the thermodynamically more favourable β-nitrilium–nitrile complex. This also seems likely on the basis of the low rate of reaction of the phenolic hydroxy groups. The interconversion between the anomeric nitrilium–nitrile intermediates is a solvolytic *in situ* anomerization conceptually similar to the *in situ* anomerization by an auxiliary nucleophile described by Lemieux *et al.*[52]

Experimental

General Procedures.—HPLC grade solvents were purchased from Labscan Ltd. (Dublin, Ireland). Dichloromethane was distilled from $CaCl_2$, while acetonitrile and benzonitrile were distilled from P_4O_{10}. These solvents were stored over molecular sieves 3 Å under argon in sealed vessels. DMF was distilled by fractional distillation under reduced pressure at 45 °C through a column of Raschig rings prior to use. Ethyl acetate (EtOAc) for dry silica gel chromatography was distilled from $CaCl_2$ and stored over molecular sieves 3 Å. Light petroleum was the 60–80 °C fraction. Other chemicals were purchased as follows and used without further purification. Pfp-OH and DCC were purchased from Fluka (Buchs, Schwitzerland), while Fmoc-OSu and the Fmoc amino acids were purchased from Novabiochem (Läufelfingen, CH) and Milligen (Bedford, USA). Silica-coated aluminium TLC plates [Merck (Darmstadt, FRG), Kieselgel 60 F_{254}] were eluted with mixtures of ethyl acetate and light petroleum and visualized with UV light and by charring with 10% aq. sulfuric acid spray. The silica gel Merck 60 H for VLC (forcing the eluent through a packed column by applying a vacuum to the outlet); Merck 60 (Korngrösse 0.015–0.040) for MPLC (Büchi) and Merck 60 (Korngrösse 0.040–0.063) for 'flash' chromatography (forcing the eluent through a packed column with an air pressure applied to the column inlet) for the chromatography of Pfp-esters was dried at 120 °C for at least 24 h. Round-bottomed flasks for glycosylation reactions were either flame dried or stored at 120 °C for 24 h prior to use. All reactions with silver salts were carried out in the dark. Unless otherwise indicated all concentrations were carried out at reduced pressure at a temperature < 40 °C.

M.p.s were measured on a Büchi melting point apparatus and are uncorrected. Microanalysis was generously carried out at Leo pharmaceutical products (Ballerup, Denmark). Optical rotations were recorded on a Perkin-Elmer 241 instrument and are given in units of 10^{-1} deg cm^2 g^{-1}. HPLC was carried out on a Waters Delta Prep 3000 equipped with a Waters 991 Photodiode Array detector. Analytical chromatograms were obtained using a Waters RCM 8 × 10 module with a Waters 8 NV C_{18} (4μ) column, while semi-preparative purifications were carried out on a Waters RCM 25 × 10 with a 25 NV C_{18} (6μ) column. Preparative purifications were carried out on a Waters 1000 Prep Pak module. Buffer A was 0.1% aq. TFA, while B buffer was 90% acetonitrile, 9.9% water and 0.1% TFA. All the active ester derivatives were analysed by using a linear gradient of 50–100% B in 24 min followed by isocratic elution with 100% B at a flow rate of 1.3 cm^3 min^{-1}. When establishing the ratio between anomers by analytical reversed-phase HPLC the level of sensitivity was estimated to be 1:99. After preparative reversed-phase HPLC of Pfp-esters the relevant fractions were combined in a separation funnel and extracted twice with dichloromethane. The combined organic phases were dried over $MgSO_4$, filtered and concentrated to dryness.

^1H NMR spectroscopy was performed on a Bruker AM 500 operating at 500.14 MHz. ^{13}C NMR spectroscopy was performed on a Bruker AM 500 operating at 125.76 MHz. Unless otherwise indicated the NMR experiments were carried out at 300 K. For all compounds the assignment of the ^1H NMR

Table 5 ^{1}H NMR data (500 MHz) of tyrosine and Fmoc protons in the tyrosine derivatives, δ-values in ppm (*J*-values in Hz)

	Tyrosine							Fmoc		
Compound	NH	H_α	H_β	$H_{\beta'}$	H_δ	H_ε	OH	H_α	H_β	H_β
1[a]	7.64	4.09	2.96	2.76	7.05	6.66	9.18		4.15–4.22	
	(8.4)		(14.0, 4.4)	(10.5)	(8.4)					
2[b]	5.33	4.70	3.12	3.06	6.99	6.77	n.a.	4.22	4.36	4.30
	(8)		(14.0, 5.6)	(5.6)	(8.1)				(10.0, 6.9)	(6.9)
2[c]	5.22	5.01	3.29	3.27	7.11	6.83	5.01	4.26	4.52	4.45
	(8.2)		(14.1, 6.0)	(6.2)	(8)				(10.7, 6.9)	(6.9)
3[d]	8.21	4.61	3.13	3.02	7.14	6.72	9.14	4.25	4.47	4.40
	(7.8)		(14.0, 5.2)	(10.0)	(8.3)				(10.5, 7.3)	(6.9)
6[d]	5.25	5.04	3.34	3.25	7.18	7.01		4.23	4.52	4.43
	(8.5)		(14.3, 5.9)	(6.3)	(8)				(10.7, 7.0)	(6.9)
7[e]	5.3	4.72	3.18	3.10	7.08	6.94		4.23	4.48	4.36
	(8)		(14.0, 5.6)	(6.0)	(8)				(10.5, 7.1)	(7.1)
9[c]	5.25	5.02	3.35	3.23	7.18	6.98		4.25	4.53	4.40
	(8.5)		(14.1, 5.6)	(6.4)	(8)				(10.5, 6.8)	(6.9)
16[c]	5.19	4.97	3.26	3.18	7.16	7.07		4.23	4.51	4.45
	(8.1)		(14.0, 5.7)	(6.1)	(8)					(10.6, 6.7)
17[c]	5.22	5.03	3.31	3.20	7.08	7.04		4.25	4.55	4.44
	(8.4)		(14.2, 6.0)	(6.4)	(8.6)				(11.0, 7.0)	(7.0)
19[e]	5.15	5.07	3.26	3.16	7.12	7.05		4.20	4.52	4.40
	(8.5)		(14.0, 6.0)	(6.5)	(8.4)					(10.5, 6.8)
20[c]	5.26	5.02	3.30	3.16	7.02	6.94		4.20	4.55	4.40
			(13.6, 5.7)	(6.4)	(8)					(7.4)
22[c]	5.29	5.04	3.31	3.24	n.a.	n.a.		4.26	4.51	4.46
	(8.5)		(14.2, 5.7)	(6.2)				(6.5)	(10.7)	(6.7)

[a] [$^2H_6$] DMSO, 310 K. [b] [$^2H_6$] DMSO, 300 K. [c] $CDCl_3$, 310 K. [d] $CDCl_3$, 298 K. [e] $CDCl_3$, 300 K. n.a., not assigned. The *J*-values at a certain proton refer to the coupling between this and a proton following in the Table. When the coupling constant could only be measured at one proton it is presented last at the chemical shift of this proton.

Table 6 ^{1}H NMR data (500 MHz) of glucose moieties, δ-values in ppm (*J*-values in Hz)

Compound	1-H	2-H	3-H	4-H	5-H	6-H	6'-H
6[a]	5.03	5.31	5.30	5.19	3.80	4.30	4.16
	(7.3)	(10.4)	(9.3)	(9.6)		(12.3, 5.3)	
7[b]	5.00	5.29	5.31	5.19	3.78	4.29	4.15
	(7.4)			(9.6, 9.3)		(11.6, 5.2)	
16[c]	6.00	5.53	6.44	5.81	4.6	4.6	4.51
	(3.7)	(10.2)	(9.7)	(9.5)			
17[c]	5.35	5.86	6.04	5.79	4.29	4.70	4.58
	(7.7)	(9.6)	(9.5)	(9.5)		(12.0, 2.8)	(6.0)
22[c]	5.46	3.77	4.24	3.81	3.85	n.a.	n.a.
	(3.4)	(9.6)	(9.4)	(9.4)			

[a] $CDCl_3$, 298 K. [b] $CDCl_3$, 300 K. [c] $CDCl_3$, 310 K. n.a.; not assigned. The *J*-values at a certain proton refer to the coupling between this and a proton following in the Table. When the coupling constant could only be measured at one proton it is presented last at the chemical shift of this proton.

spectra was based on 2D proton–proton shift-correlation spectra. The assignment of ^{13}C NMR spectra was based on carbon–proton shift-correlation spectra of representative compounds. The assigned ^{1}H NMR data are given in Tables 5–7 and the corresponding ^{13}C NMR data in Tables 8–10, respectively. The completely assigned NMR spectra of β-glycosides **6** and **7** and the corresponding α-anomers (data not presented) isolated in low yield in separate glycosylation experiments were used as standards for integration of anomeric ratios on crude glycosylation reaction mixtures.

General Description of Analytical Glycosylations monitored by Reversed-Phase HPLC and NMR Spectroscopy.—Silver triflate-promoted glycosylations in dichloromethane. The glycosyl acceptor (0.1 or 0.05 mmol), AgOTfl (equimolar with the donor), molecular sieves 3 Å and a magnet were placed in a predried flask (5 or 10 cm^3). After evacuation through a three-way valve on an oil-pump, the flask was filled with argon and dry dichloromethane (2 cm^3) was injected through the valve. The suspension was cooled to −10 °C and a solution of the glycosyl donor in dry dichloromethane (0.5–1.0 cm^3) was injected.

Glycosylations with glycosyl fluorides. The glycosyl acceptor (0.1 or 0.05 mmol), glycosyl fluoride, molecular sieves 3 Å and a magnet were placed in a predried flask (5 or 10 cm^3). After evacuation through a three-way valve on an oil-pump, the flask was filled with argon and dry dichloromethane (2 cm^3) was injected through the valve. If indicated, the suspension was cooled to −10 °C. The promoter was injected through the valve.

Silver triflate-promoted glycosylations in nucleophilic solvents. The glycosyl acceptor (0.1 or 0.05 mmol), the glycosyl donor, AgOTfl (equimolar with the donor), molecular sieves 3 Å and a magnet were placed in a predried flask (5 or 10 cm^3). After evacuation through a three-way valve on an oil-pump, the flask was filled with argon and dry solvent (2–3 cm^3) was injected through the valve.

Glycosylations with trichloroacetimidate **21**. The glycosyl acceptor **21** (0.1 mmol), molecular sieves 3 Å, and a magnet were placed in a predried flask (5 or 10 cm^3). After evacuation through a three-way valve on an oil-pump, the flask was filled with argon and dry solvent was injected (2–3 cm^3). The suspension was cooled as indicated and the promoter was injected through the valve.

Reversed-phase HPLC analysis of analytical glycosylations. Samples (0.1 cm^3) were taken out through the valve with a syringe, diluted with acetonitrile (0.5 cm^3), and neutralized with DIPEA or triethylamine.

NMR analysis of analytical glycosylations. The reaction mixture was neutralized with DIPEA (equimolar with the acceptor), diluted with acetonitrile and centrifuged. The supernatant was concentrated to dryness, redissolved in $CDCl_3$, concentrated to dryness, and extracted with $CDCl_3$ (700 mm^3).

Table 7 ^{1}H NMR data (500 MHz) of the maltose moieties on the tyrosine derivatives, δ-values are in ppm (*J*-values are in Hz)

Compound	1-H	2-H	3-H	4-H	5-H	6-H[a]	6-H[b]	1'-H	2'-H	3'-H	4'-H	5'-H	6'-H[a]	6'-H[b]
9[a]	5.01 (7.6)	5.11 (9)	5.33 (8.9)	4.10	3.74	4.29 (12.5, 4.1)	4.09	5.47 (3.9)	4.92 (10.5)	5.43 (10.0)	5.10 (9.7)	3.99	4.44 (11.5, 2)	4.24 (5.5)
19[b]	5.87 (3.8)	5.25 (10.1)	6.30 (8.5)	4.51	4.49	4.82[c] (12.0)	4.70[c] (4.2)	5.81 (4.0)	5.29 (10.3)	6.11 (9.9)	5.69	4.49	4.51	4.33[c]
20[a]	5.26	5.59 (8, 8)	5.82 (9.3)	4.59	4.17	4.93	4.71 (12.0, 5.6)	5.81 (3.9)	5.32 (10.2)	6.16 (10.0)	5.72	4.51	4.55	4.38 (12.0, 4.0)

[a] $CDCl_3$, 310 K. [b] $CDCl_3$, 300 K. [c] Assignments may have to be reversed. The *J*-values at a certain proton refer to the coupling between this and a proton following in the Table. When the coupling constant could only be measured at one proton it is presented last at the chemical shift of this proton.

Table 8 ^{13}C NMR data (125.8 MHz) of tyrosine and Fmoc carbons in tyrosine derivatives; δ-values are in ppm

	Tyrosine							Fmoc			
Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-7[c]	CH	CH_2	Aromatic	Carbamate[c]
1[a]	173.4	55.8	35.7	128.0	130.0	115.0	155.9	46.6	65.6	120.1, 125.2, 127.0, 127.6, 140.7, 143.7	155.8
2[a]	168.6	55.7	35.2	126.5	130.2	115.1	156.2	46.5	65.8	120.1, 125.1, 127.0, 127.6, 140.7, 143.6	156.0
3[b]	171.4	54.9	37.4	n.a.	130.5	115.5	155.7	47.1	67.0	120.0, 125.0, 127.0, 127.7, 141.3, 143.7	155.0
6[b]	168.0	54.5	37.1	129.5	130.5	117.5	156.2	47.1	67.2	120.1, 125.0, 127.1, 127.8, 141.3, 143.6	155.4
7[b]	171.1	54.7	37.5	130.5	130.5	119.2	156.0	47.1	67.0	120.0, 125.0, 127.1, 127.8, 141.3, 143.7	155.4
9[b]	170.5	54.5	37.2	129.6	130.5	117.6	156.2	47.1	67.2	120.1, 125.0, 127.1, 127.8, 141.3, 143.7	155.5
16[a]	168.5	55.3	35.0	n.a.	n.a.	117.2	155.8	46.5	68.0	120.0, 125.1, 126.9, 127.5, 140.6, 143.6	154.8
17[b]	168.0	54.5	37.1	n.a.	n.a.	118.0	156.4	47.0	67.2	120.1, 125.0, 127.1, 127.8, 141.3, 143.7	155.3
19[b]	167.9	54.9	36.9	n.a.	130.5	117.4	156.0	47.0	67.5	120.1, 125.1, 127.1, 127.8, 141.4, 143.6	156.0
20[b]	167.6	54.4	37.1	n.a.	130.4	118.0	156.3	47.0	67.5	120.1, 125.0, 127.1, 127.9, 141.3, n.a.	n.a.
22[b]	n.a.	54.6	37.1	n.a.	na.	117.1	n.a.	47.0	67.5	120.1, 124.9, 127.1, n.a., n.a., n.a.	n.a.

[a] [$^2H_6$] DMSO, 300 K. [b] $CDCl_3$, 300 K. [c] Assignments may have to be reversed. n.a., not assigned.

Table 9 ^{13}C NMR data (125.8 MHz) of the glucose moieties, δ-values are in ppm.

Compound	C-1	C-2	C-3	C-4	C-5	C-6
6[a]	99.1	71.1	72.7	68.2	72.0	61.9
7[a]	99.2	71.1	72.7	68.2	72.0	61.9
16[b]	94.8	70.7	68.8	68.0	65.7	62.6
17[a]	99.8	71.7	72.7[c]	69.6	72.6[c]	63.1
22[a]	95.4	79.5	81.8	77.0	70.8	68.0

[a] $CDCl_3$, 300 K. [b] [$^2H_6$]DMSO, 300 K. [c] Assignments may have to be reversed.

Glycosylations in an NMR tube. Phenol (0.03 mmol) and the glycosyl fluoride (0.033 mmol) were placed in a predried NMR tube, the tube was flushed with argon, and dry $CDCl_3$ (700 mm^3, dried over molecular sieves 3 Å for at least 24 h) was added. The reaction was started by addition of the promoter (0.04 mmol). The first spectrum was obtained 4 min after initiation.

Synthesis.—*N$^{\alpha}$-Fmoc-Tyr-OH* **1**. Tyrosine (3.62 g, 20.0 mmol) was suspended in a mixture of 10% aq. Na_2CO_3 (50 cm^3) and 1,4-dioxane (20 cm^3). The suspension was cooled on an ice-bath. Fmoc-OSu (7.08 g, 21.0 mmol) was dissolved in 1,4-dioxane (25 cm^3) by gentle heating and the solution was added through a dropping funnel over a period of 30 min with efficient magnetic stirring. The reaction mixture was allowed to warm up to 20 °C overnight. 1,4-Dioxane was removed by evaporation at 40 °C and the suspension was diluted with water (200 cm^3). The suspension was transferred to a separation funnel and washed twice with diethyl ether (350 cm^3 in total). At this step both the aqueous and the ether phase were clear. The aqueous phase was acidified (pH 3.5) (pH paper) with citric acid (14 g). The precipitate was extracted with EtOAc. The organic phase was dried over $MgSO_4$, filtered, and concentrated at 35 °C to afford a foam, which was dissolved in dichloromethane and crystallized after the addition of light petroleum to yield *title compound* **1** (5.21 g, 65%), m.p. 120.5–124 °C (lit.,[43] 98–107 °C); $[\alpha]_D^{25}$ −18.8 (*c* 1.1, DMF) [lit.,[43] −19.9 (*c* 1, DMF)].

N$^{\alpha}$-Fmoc-Tyr-OPfp **2**. *Method A*. Pentafluorophenol (1.38 g, 7.5 mmol) was dissolved in THF (50 cm^3). The solution was cooled to −20 °C on a solid CO_2-bath and DCC (1.14 g, 5.5 mmol) was added to the magnetically stirred solution. After 30 min compound **1** was added and the mixture was stirred overnight at −20 °C. The precipitate was removed by filtration and the solution was concentrated to give an oil. Crystals formed by crystallization from acetonitrile contained an impurity (presumably dicyclohexylurea). Therefore they were redissolved in THF, diluted with diethyl ether, evaporated to a small volume, diluted with diethyl ether, and then applied to a dry VLC column and eluted with EtOAc–light petroleum (1 : 5) to yield compound **2** (1.24 g, 64%), m.p. 170.5–172.5 °C (lit.,[43] 152–156 °C) (Found: C, 63.35; H, 3.7; N, 2.45. Calc. for

Table 10 ^{13}C NMR data (125.8 MHz) of the maltose moieties, δ-values are in ppm

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
9[a]	98.6	71.9	75.2	72.6	72.2	61.5	95.6	70.0	69.3	68.0	68.6	62.7
19[a]	94.5	72.0	72.3	73.7	69.1[b]	63.2[c]	96.9	71.0	69.9	69.3	69.1[b]	62.6[c]
20[a]	99.0	72.2	75.0	73.3	73.0	62.7[b]	96.6	71.0	70.0	69.1[c]	69.2[c]	63.5[b]

[a] $CDCl_3$, 300 K. [b,c] Assignments may have to be reversed.

$C_{30}H_{25}F_5NO_5$: C, 63.25; H, 3.55; N, 2.45%); $[\alpha]_D^{25}$ −17.8 (*c* 0.4, $CHCl_3$) [lit.,[43] −15.7 (*c* 1, $CHCl_3$)].

N$^{\alpha}$-*Fmoc-Tyr-OPfp* **2**. *Method B*. Compound **4** (1.25 g, 2.00 mmol) was treated with TFA (35 cm^3) in a 100 cm^3 flask with efficient stirring. After 1 h the mixture was concentrated to dryness, toluene was added, and the mixture was concentrated twice (oil-pump) to yield compound **2** (1.07 g, 94%).

N$^{\alpha}$-*Fmoc-Tyr-OAll* **3**. In a 250 cm^3 flask compound **1** (3.00 g, 7.44 mmol) was dissolved in 1,4-dioxane (40 cm^3) and diluted with water (30 cm^3). The turbid solution was cooled on an ice-bath. Cs_2CO_3 (1.22 g, 3.72 mmol) was dissolved in the minimum amount of water and added to the magnetically stirred solution over a period of 5 min. After 10 min the solution was concentrated to dryness at 45 °C and lyophilized overnight. It was suspended in dry DMF (25 cm^3, distilled and dried over molecular sieves 3 Å), 3-bromopropene (0.71 cm^3, 8.18 mmol) was added and the stirred reaction mixture was left overnight. The suspension was concentrated at ~0.1 mmHg and lyophilized overnight. The solid residue was extracted twice with dichloromethane (2–30 cm^3 in total) which was applied directly to a VLC column and the extract was eluted with ethyl acetate–light petroleum (1 : 2) to yield, after crystallization from dichloromethane–light petroleum, *compound* **3** (2.55 g, 77%), m.p. 98–99 °C (Found: C, 73.2; H, 5.85; N, 3.0. $C_{27}H_{25}NO_5$ requires C, 73.1; H, 5.6; N, 3.15%); $[\alpha]_D^{25}$ +15.1 (*c* 0.4, $CHCl_3$).

N$^{\alpha}$-*Fmoc-Tyr*(*Ac*$_4$-β-D-*Glc*)-*OPfp* **6**. Compound **2** (285 mg, 0.50 mmol), AgOTfl (193 mg, 0.75 mmol), molecular sieves 3 Å, and a magnet were placed in a 50 cm^3 predried flask. After evacuation through a three-way valve the flask was filled with argon, and dry dichloromethane was injected through the valve. The suspension was cooled to −10 °C and a solution of the glycosyl bromide **5** (305 mg, 0.75 mmol) in dry dichloromethane (2 cm^3) was injected. After 90 min at −10 °C the suspension was neutralized with 2,4,6-trimethylpyridine (0.13 cm^3, 1.00 mmol) and centrifuged. The supernatant was concentrated to dryness, redissolved in dry dichloromethane, and applied onto a dry VLC column and eluted with ethyl acetate–light petroleum (1 : 2). The β-*glycoside* **6** was obtained pure and was crystallized from dichloromethane–light petroleum (286 mg, 64%) (Found: C, 58.5; H, 4.25; N, 1.6. $C_{44}H_{38}F_5NO_{14}$ requires C, 58.75; H, 4.25; N, 1.55%); $[\alpha]_D^{25}$ −17.7 (*c* 0.3, $CHCl_3$).

N$^{\alpha}$-*Fmoc-Tyr*(*Ac*$_4$-β-D-*Glc*)-*OAll* **7**. Compound **3** (222 mg, 0.5 mmol), AgOTfl (193 mg, 0.75 mmol), molecular sieves 3 Å, and a magnet were placed in a 25 cm^3 predried flask. After evacuation through a three-way valve the flask was filled with argon and dry dichloromethane (5 cm^3) was injected. The solution was cooled to −10 °C and a solution of glycosyl bromide **5** (305 mg, 0.75 mmol) in dry dichloromethane (2 cm^3) was injected. After 90 min at −10 °C the suspension was neutralized with 2,4,6-trimethylpyridine (0.132 cm^3, 1.2 mmol) and centrifuged. The supernatant was concentrated to dryness, redissolved in dichloromethane, and applied onto a VLC (silica gel) column and eluted with ethyl acetate–light petroleum (2 : 3). After crystallization from dichloromethane–light petroleum this yielded the pure β-*glycoside* **7** (254 mg, 68%) (Found: C, 63.4; H, 5.55; N, 1.8. $C_{41}H_{43}NO_{14}$ requires C, 63.65; H, 5.6: N, 1.8%); $[\alpha]_D^{25}$ −3.5 (*c* 0.5, $CHCl_3$).

N$^{\alpha}$-*Fmoc-Tyr*[*Ac*$_4$-α-D-*Glc*-(1→4)-*Ac*$_3$-β-D-*Glc*]-*OPfp* **9**. *Method A*. Compound **2** (557 mg, 1.0 mmol), AgOTfl (514 mg, 2.0 mmol), molecular sieves 3 Å, and a magnet were placed in a predried 50 cm^3 flask. After evacuation with an oil-pump through a three-way valve the flask was filled with argon, and dry dichloromethane (20 cm^3) was injected through the valve. The suspension was cooled to −10 °C and a solution of the glycosyl bromide **8** (1.399 g, 2.0 mmol) in dry dichloromethane (5 cm^3) was injected. After 2 h the suspension was neutralized with DIPEA (0.34 cm^3, 2.0 mmol) and centrifuged. The supernatant was concentrated, and the remaining solid was redissolved in dichloromethane and applied onto a dry VLC column. After elution with ethyl acetate–light petroleum (2 : 3) the fractions containing the main product were combined, concentrated, and crystallized from dichloromethane–light petroleum to yield *compound* **9** (489 mg, 42%) (Found: C, 56.2; H, 4.65; N, 1.2. $C_{50}H_{53}F_5NO_{14}$ requires C, 56.65; H, 4.5; N, 1.2%); $[\alpha]_D^{25}$ +16.8 (*c* 1.1, $CHCl_3$).

N$^{\alpha}$-*Fmoc-Tyr*[*Ac*$_4$-α-D-*Glc*(1→4)-*Ac*$_3$-β-D-*Glc*]-*OPfp* **9**. *Method B*. Compound **4** (313 mg, 0.5 mmol), AgOTfl (257 mg, 1.0 mmol), molecular sieves 3 Å, and a magnet were placed in a predried 50 cm^3 flask. After evacuation with an oil-pump through a three-way valve the flask was filled with argon, and dry dichloromethane (10 cm^3) was injected through the valve. The suspension was cooled to −10 °C and a solution of the glycosyl bromide **8** (689 g, 1.0 mmol) in dry dichloromethane (5 cm^3) was injected. According to HPLC the reaction was finished after 30 min. After 1 h the suspension was neutralized with DIPEA (0.17 cm^3, 1.0 mmol) and centrifuged. The supernatant was concentrated, redissolved in acetonitrile (10 cm^3), and purified by preparative HPLC. Compound **9** was obtained as a foam (520 mg), which was crystallized from THF–diethyl ether–light petroleum (454 mg, 81%). Analytical data were in accord with the results presented above.

Phenyl 2,3,4,6-*tetra*-O-*acetyl*-α-D-*glucopyranoside* **12**. Phenol (94 mg, 1.0 mmol), the glycosyl fluoride **11**[45] (525 mg, 1.5 mmol), molecular sieves 3 Å, and a magnet were placed in a predried 10 cm^3 flask. After evacuation with an oil-pump through a three-way valve the flask was filled wih argon, and dry dichloromethane (5 cm^3) and $BF_3{\cdot}OEt_2$ (0.38 cm^3, 3.0 mmol) were injected. After 5 h the mixture was diluted with dichloromethane and washed with saturated aq. $NaHCO_3$ in a separation funnel. The organic phase was dried over $MgSO_4$, filtered and concentrated to a small volume (~10 cm^3). The solution was applied to a VLC column and eluted with ethyl acetate–light petroleum (1 : 3 to 1 : 2) to give, after crystallization from dichloromethane–light petroleum, pure α-glycoside **12** (193 mg, 45%), m.p. 113–114 °C (lit.,[58] 114–115 °C) (Found: C, 56.45; H, 5.7. Calc. for $C_{20}H_{24}O_{10}$: C, 56.6; H, 5.7%); $[\alpha]_D^{25}$ +159 (*c* 1.0, $CHCl_3$) [lit.,[58] +168 ($CHCl_3$)].

2,3,4,6-*Tetra*-O-*benzoyl*-β-D-*glucopyranosyl fluoride* **13**. The glycosyl bromide **15**[59] (654 mg, 1.0 mmol), AgF (381 mg, 3.0 mmol), molecular sieves 3 Å, and a magnet were placed in a predried 10 cm^3 flask. After evacuation on an oil-pump through a three-way valve the flask was filled with argon and dry acetonitrile (2 cm^3) was injected through the valve. After the mixture had been efficiently stirred for 20 h, acetonitrile (10 cm^3) was added, the suspension was centrifuged, and the supernatant was concentrated to dryness. The remaining oil was dissolved in diethyl ether (40 cm^3) and decolourized with charcoal. After filtration the solution was concentrated to a small volume and

light petroleum was added to yield *crystalline fluoride* **13** (435 mg, 73%), m.p. 139–142 °C (lit.,[60] 150–151 °C) (Found: C, 68.3; H, 4.55. $C_{34}H_{27}FO_9$ requires C, 68.2; H, 4.55%); $[\alpha]_D^{25}$ +44.4 (*c* 1.0, $CHCl_3$) {lit.,[60] $[\alpha]_D^{22}$ +53.8 (*c* 0.9, $CHCl_3$)}.

N^{α}-*Fmoc-Tyr*(*Bz*$_4$-α-D-*Glc*)-*OPfp* **16**. Compound **2** (427 mg, 0.75 mmol), the glycosyl bromide **15** (989 mg, 1.5 mmol), AgOTfl (206 mg, 1.5 mmol), molecular sieves 3 Å, and a magnet were placed in a predried 50 cm^3 flask. After evacuation with an oil-pump through a three-way valve the flask was filled with argon, and dry acetonitrile (20 cm^3) was injected and after 24 h the suspension was neutralized with DIPEA (257 mm^3, 1.5 mmol). After filtration the solution was concentrated to 10 cm^3 and immediately purified by preparative reversed-phase HPLC. This yielded pure α-*glycoside* **16** (267 mg, 31%) (Found: C, 66.55; H, 4.15; N, 1.3. $C_{64}H_{46}F_5NO_{14}$ requires C, 66.95; H, 4.05; N, 1.2%); $[\alpha]_D^{25}$ +38.9 (*c* 0.6, $CHCl_3$), as well as the pure β-*glycoside* **17** (362 mg, 42%) (Found: C, 67.15; H, 4.29; N, 0.95%); $[\alpha]_D^{25}$ +27.3 (*c* 0.6, $CHCl_3$).

2,3,4,6-*Tetra*-O-*benzoyl*-α-D-*glucopyranosyl*-(1→4)-2,3,6-*tri*-O-*benzoyl*-α-D-*glucopyranosyl bromide* **18**. 2,3,4,6-Tetra-*O*-benzoyl-α-D-glucopyranosyl-(1→4)-1,2,3,6-tetra-*O*-benzoyl-D-glucopyranose (35.3 g, 30.0 mmol) were placed in a 500 cm^3 flask and dissolved in dichloromethane (200 cm^3). 33% HBr in acetic acid (53 cm^3) was added, and after 12 h the reaction mixture was diluted with dichloromethane (400 cm^3). The solution was washed twice with ice-cold water and twice with ice-cold, 10% aq. sodium hydrogen carbonate. The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness. The remaining foam was precipitated from diethyl ether–light petroleum. A part (8.2 g) was purified by VLC with ethyl acetate–light petroleum (1 : 5) as eluent. The relevant fractions were concentrated and the resulting foam was crystallized from diethyl ether to yield *compound* **18** (6.18 g, 76%), m.p. 167–169.5 °C (lit.,[61] 161–163 °C) (Found: C, 64.6; H, 4.4; Br, 7.0. $C_{61}H_{49}BrO_{17}$ requires C, 64.6; H, 4.4; Br, 7.1%); $[\alpha]_D^{25}$ +108 (*c* 1.3, $CHCl_3$) {lit.,[61] $[\alpha]_D^{20}$ +107.4 (*c* 1, $CHCl_3$)}.

N^{α}-*Fmoc-Tyr*[*Bz*$_4$-α-D-*Glc*(*1*→*4*)-*Bz*$_3$-α-D-*Glc*]-*OPfp* **19**. Compound **2** (228 mg, 0.4 mmol), the glycosyl bromide **18** (908 mg, 0.8 mmol), AgOTfl (206 mg, 0.8 mmol), molecular sieves 3 Å, and a magnet were placed in a predried 50 cm^3 flask. After evacuation with an oil-pump through a three-way valve the flask was filled with argon and dry acetonitrile (20 cm^3) was injected. The suspension was neutralized with DIPEA (68 mm^3, 0.4 mmol) after 21 h, followed by centrifugation, and the supernatant was concentrated to 10 cm^3 and was immediately purified by preparative reversed-phase HPLC. This yielded pure α-*glycoside* **19** (217 mg, 33%) (Found: C, 66.75; H, 4.3. $C_{91}H_{68}F_5NO_{14}$ requires C, 67.35; H, 4.2%); $[\alpha]_D^{25}$ +51.2 (*c* 0.4, $CHCl_3$) as well as the pure β-*glycoside* **20** (197 mg, 30%); $[\alpha]_D^{25}$ +29.9 (*c* 0.6, $CHCl_3$).

N^{α}-*Fmoc-Tyr*(*Bn*$_4$-α-D-*Glc*)-*OPfp* **22**. Compound **2** (111 mg, 0.2 mmol), molecular sieves 3 Å and a magnet were placed in a predried 25 cm^3 flask. After evacuation through a three-way valve with an oil-pump the flask was filled wih argon, and dry diethyl ether (10 cm^3) was injected through the valve. The solution was cooled to −30 °C on a solid CO_2-bath and solutions of the trichloroacetimidate **21**[47,48] (137 mg, 0.2 mmol) in dry diethyl ether (1 cm^3) and TMSOTfl (0.04 cm^3, 0.2 mmol) in diethyl ether (0.5 cm^3) were injected separately four times at intervals of 30 min. 30 Min after the last addition the solution was neutralized and centrifuged. The supernatant was concentrated and the residue was redissolved in dichloromethane and purified by VLC with ethyl acetate–light petroleum (1 : 6) as eluent and preparatative HPLC to yield pure *compound* **22** (58 mg, 27%), which was crystallized from diethyl ether–light petroleum (Found: C, 70.25; H, 5.15; N, 1.15. $C_{64}H_{54}F_5NO_{10}$ requires C, 70.4; H, 5.0; N, 1.3%); $[\alpha]_D^{25}$ +33.1 (*c* 1.3, $CHCl_3$). A fraction containing a mixture of the α- and β-glycosides was also obtained (34 mg, 16%).

Acknowledgements

We thank Mr. Bent O. Petersen for recording the NMR spectra and Leo pharmaceutical products for microanalyses. A gift of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl fluoride **11** from Prof. Christian Pedersen is gratefully acknowledged. This work was supported by a grant from the Danish Technical Science Research Council (K. J. J.).

References

1 J. Montreuil, *Adv. Carbohydr. Chem. Biochem.*, 1982, **37**, 157.
2 N. Sharon, *Trends Biochem. Sci.*, 1984, **9**, 198.
3 F. Wieland, *Biochemie*, 1988, **70**, 1493.
4 F. Wieland, R. Heitzer and W. Schaefer, *Proc. Natl. Acad. Sci. USA*, 1983, **80**, 5470.
5 S. Shibata, T. Takeda and Y. Natori, *J. Biol. Chem.*, 1988, **263**, 12483.
6 G. W. Hart, G. D. Holt and R. S. Haltiwanger, *Trends Biochem. Sci.*, 1988, **13**, 380.
7 S. Hase, H. Nishimura, S. Kawabata, S. Iwanga and T. Ikenaka, *J. Biol. Chem.*, 1990, **265**, 1858.
8 W. Tanner and L. Lehle, *Biochim. Biophys. Acta*, 1987, **906**, 81.
9 P. Messner and U. B. Sleytr, *FEBS Lett.*, 1988, **228**, 317.
10 P. S. Chen, H. K. Mitchell and M. Neuweg, *Insect Biochem.*, 1978, **8**, 279.
11 I. R. Rodriguez and W. J. Whelan, *Biochem. Biophys. Res. Commun.*, 1985, **132**, 829.
12 D. H. Williams, V. Rajananda, M. P. Williamson and G. Bojesen, *Topics in Antibiotic Chemistry*, ed. P. G. Sammes, Wiley, New York, 1980, vol. 5, p. 119.
13 K. J. Kramer, T. L. Hopkins, R. F. Ahmed, D. Mueller and G. Lookhart, *Arch. Biochem. Biophys.*, 1980, **205**, 146.
14 P. W. Lu, K. J. Kramer, P. A. Seib, D. D. Mueller, R. Ahmed and T. L. Hopkins, *Insect Biochem.*, 1982, **12**, 377.
15 N. J. Skelton, M. M. Harding, R. J. Mortishire-Smith, S. K. Rahman, D. H. Williams, M. J. Rance and J. C. Ruddock, *J. Am. Chem. Soc.*, 1991, **113**, 7522.
16 P. Messner, R. Christian, J. Kolbe, G. Schulz and U. B. Sleytr, *J. Bacteriol.*, 1992, **174**, 2236.
17 D. G. Campbell and P. Cohen, *Eur. J. Biochem.*, 1989, **185**, 119.
18 J. Lomako, W. M. Lomako and W. J. Whelan, *Carbohydr. Res.*, 1992, **227**, 331.
19 M. Meldal and K. J. Jensen, *J. Chem. Soc., Chem. Commun.*, 1990, 483.
20 M. Meldal, K. J. Jensen, A. M. Jansson and K. Bock, *Innovations and Perspectives in Solid Phase Synthesis: Peptides, Polypeptides and Oligonucleotides*, ed. R. Epton, SPCC, Birmingham, 1992, p. 587.
21 M. Meldal, S. Mouritsen and K. Bock, *A. C. S. Symp. Ser.*, 1993, **519**, 19.
22 A. M. Jansson, M. Meldal and K. Bock, *Tetrahedron Lett.*, 1990, **31**, 6991.
23 A. M. Jansson, M. Meldal and K. Bock, *J. Chem. Soc., Perkin Trans. 1*, 1992, 1.
24 H. Paulsen, *Angew. Chem.*, 1990, **102**, 851.
25 H. Kunz, *Angew. Chem.*, 1987, **99**, 297.
26 H. Paulsen, *Angew. Chem., Int. Ed. Engl.*, 1982, **21**, 155.
27 B. Helferich and E. Schmitz-Hillebrecht, *Ber. Dtsch. Chem. Ges.*, 1933, **66**, 378.
28 E. M. Montgomery, N. K. Richtmeyer and C. S. Hudson, Jr., *J. Am. Chem. Soc.*, 1942, **64**, 690.
29 M. Yamaguchi, A. Horiguchi, A. Fukuda and T. Minami, *J. Chem. Soc., Perkin Trans. 1*, 1990, 1079.
30 Y. V. Voznyi, I. S. Kalicheva and A. A. Galoyan, *Bioorg. Khim.*, 1984, **10**, 1256; 1986, **12**, 521.
31 N. M. Spijker and C. A. A. van Boeckel, *Angew. Chem.*, 1991, **103**, 179.
32 S. Sato, Y. Ito, T. Nukuda, Y. Nakahara and T. Ogawa, *Carbohydr. Res.*, 1987, **167**, 197.
33 M. E. Chacón-Fuertes and M. Martín-Lomas, *Carbohydr. Res.*, 1975, **43**, 51.
34 N. E. Nifant'ev, A. S. Shaskov and N. K. Kochetkov, *Carbohydr. Res.*, 1992, **226**, 331.
35 N. K. Kochetkov, E. M. Klimov and N. M. Malysheva, *Tetrahedron Lett.*, 1989, **30**, 5459.
36 B. Lindberg, *Acta Chem. Scand.*, 1950, **4**, 49.

37 K. J. Jensen, M. Meldal and K. Bock, *J. Carbohydr. Chem.*, review in preparation.
38 S. Horvat, L. Varga and J. Horvat, *Synthesis*, 1986, 209.
39 L. Varga, S. Horvat, C. Lemieux and P. W. Schiller, *Int. J. Pept. Protein Res.*, 1987, **30**, 371.
40 Z. Smiatacz and E. Paskiewicz, *Bull. Pol. Acad. Sci., Chem.*, 1986, **34**, 381.
41 Z. Smiatacz and H. Myszka, *Carbohydr. Res.*, 1990, **196**, 167.
42 M. Wiesner and I. Leupold, personal communication.
43 I. Schön and L. Kisfaludy, *Synthesis*, 1986, 303.
44 N. K. Kochetkov, V. A. Derevitskaya and E. M. Klimov, *Tetrahedron Lett.*, 1969, 4769; N. K. Kochetkov, E. M. Klimov and V. I. Torgov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1975, **11**, 2587.
45 F. Micheel and A. Klemer, *Adv. Carbohydr. Chem.*, 1961, **16**, 85.
46 M.-C. Courtin-Duchateau and A. Veyrières, *Carbohydr. Res.*, 1978, **65**, 23; K. Horma, K. Nakazimi, T. Vematsu and A. Hamada, *Chem. Pharm. Bull.*, 1976, **24**, 394.
47 R. R. Schmidt, J. Michel and M. Roos, *Liebigs Ann. Chem.*, 1984, 1343.
48 R. R. Schmidt and J. Michel, *Angew. Chem.*, 1980, **92**, 763.
49 G. Wulff and G. Röhle, *Angew. Chem.*, 1974, **86**, 173.
50 R. R. Schmidt, *Angew. Chem.*, 1986, **98**, 213.
51 R. R. Schmidt, M. Behrendt and A. Toepfer, Synlett, 1990, 694.
52 R. U. Lemieux, K. B. Hendriks, R. V. Stick and K. James, *J. Am. Chem. Soc.*, 1975, **97**, 4056.
53 H. Paulsen, W.-P. Trautwein, F. G. Espinosa and K. Heyns, *Chem. Ber.*, 1967, **100**, 2822.
54 Y. D. Vankar, P. S. Vankar, M. Behrendt and R. R. Schmidt, *Tetrahedron*, 1991, **47**, 9985.
55 J. R. Pougny and P. Sinay, *Tetrahedron Lett.*, 1976, 4073.
56 A. J. Ratcliffe and B. Fraser-Reid, *J. Chem. Soc., Perkin Trans. 1*, 1990, 747.
57 R. U. Lemieux and R. M. Ratcliffe, *Can. J. Chem.*, 1979, **57**, 1244.
58 J. Conchie, G. A. Levvy and C. A. Marsh, *Adv. Carbohydr. Chem.*, 1957, **12**, 157.
59 R. K. Ness, H. G. Fletcher and C. S. Hudson, Jr., *J. Am. Chem. Soc.*, 1950, **72**, 2200.
60 I. Lundt and C. Pedersen, *Microchim. Acta*, 1966, 126.
61 F. W. Lichtenthaler, E. Kaji and S. Weprek, *J. Org. Chem.*, 1985, **50**, 3505.

Paper 3/00125C
Received 8*th January* 1993
Accepted 13*th May* 1993