p-Methoxybenzylidene-tethered β-Mannosylation for Stereoselective Synthesis of Asparagine-Linked Glycan Chains

Akihito Dan, Matthias Lergenmüller, Masayuki Amano, Yoshiaki Nakahara, Tomoya Ogawa, and Yukishige Ito*

Abstract: One of the main obstacles in the chemical synthesis of Asn-linked glycoprotein oligosaccharides has been the formation of β-manno glycoside linked to the 4 position of *N*-acetylglucosamine. Addressing this fundamental problem, we previously developed the use of a 2-*O*-*p*-methoxybenzyl-protected mannosyl donor as a new variant of intramolecular aglycon delivery (IAD). Now, the flexibility of this approach is demonstrated in the synthesis of fucose-containing hexasaccharide, which constitutes the core structure of biomedically significant glycoproteins, in its reducing end-protected (**1a**) and Asn-linked (**1b**) forms. The key transformation is the stereoselective β-mannosylation of the disaccharide donor **5b**, which was treated with **10** to form trisaccharide **12**. Further conversion into trichloroacetimidate **15**, coupling with disaccharide segment **17**, and introduction of α-linked mannose residue afforded hexasaccharides **26a** and **26b**, which were transformed into **1a** and **1b**.

Keywords: glycosides · intramolecular aglycon delivery · oligosaccharides · saccharide chemistry · stereoselectivity

Introduction

Asparagine-linked glycoprotein oligosaccharides (Figure 1) have been revealed to play various important roles in numerous biological events, including cell differentiation, malignant transformation, cell adhesion, and intra- and intercellular protein transport. They are also functional constituents of proteins, defining three dimensional structures, stabilizing proteins under physiological conditions, tuning enzymatic activities, and so on.[1] They are usually divided into three major subgroups, high-mannose type, hybrid type, and complex type, and each of them consists of diverse structures, which result from the presence of a variable degree of branching as well as additional terminal and nonterminal modifications such as sialylation, fucosylation, polylactosaminylation, sulfation, and phosphorylation.[2] In spite of such diversity, all members of this family share a common pentasaccharide unit, in which the trimannosyl unit is linked to chitobiose (GlcNAcβ1 →4GlcNAc), which in turn is connected to the protein backbone through an *N*-glycosidic linkage to an Asn residue.

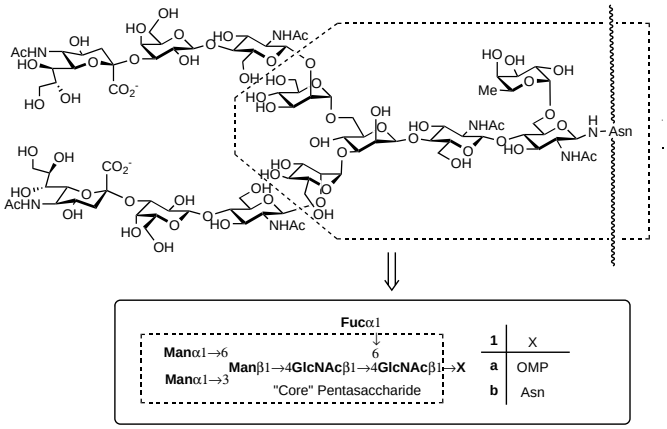


Figure 1. Typical structure of Asn-linked glycan chain.

With the aim of the chemical synthesis of this biologically important class of molecules, the main obstacle has been the formation of β-manno (Man) glycoside[3] linked to the 4 position of *N*-acetylglucosamine (GlcNAc). β-Manno glycoside has been considered to be one of the most difficult types of *O*-glycoside to synthesize selectively. This derives from its unique structural feature (1,2-*cis* equatorial glycoside) that makes neither stereoelectronic control (i.e., by an anomeric effect) nor neighboring group participation available. Addressing these problems, a number of intriguing approaches

[*] Dr. Y. Ito, Dr. A. Dan, M. Lergenmüller,[+] Dr. T. Ogawa
The Institute of Physical and Chemical Research
(RIKEN) and CREST
Japan Science and Technology Corporation (JST)
2-1 Hirosawa, Wako-shi, Saitama 351-0198 (Japan)
Fax: (+81) 48-462-4680
E-mail: yukito@postman.riken.go.jp
M. Amano, Dr. Y. Nakahara
Department of Industrial Chemistry, Tokai University
1117 Kitakaname, Hiratsuka-shi, Kanagawa 259-1292 (Japan)

[+] Recipient of STA fellowship award (1995 – 1997).

0947-6539/98/0411-2182 $ 17.50+.50/0 *Chem. Eur. J.* **1998**, *4*, No. 11

have been investigated. However, it would be most fair to mention that the classical insoluble silver salt approach[4] is still competitive with more recently developed methods in terms of its practicality. Although this method has been successfully applied to the synthesis of glycoprotein-related glycans and glycolipids, the stereoselectivity is critically dependent upon the reactivity of the acceptor and the nature of protecting groups of the mannosyl donor.[5] As a result, it is often observed that β-selectivity quickly diminishes once applied to a sterically demanding acceptor. In addition, the use of unstable bromide required in this approach eliminates the possibility to accommodate modern glycosylation technologies[6] (glycosyl fluoride, trichloroacetimidate, thioglycoside etc.), which have been demonstrated to be well-adapted for block condensation of oligosaccharide fragments.

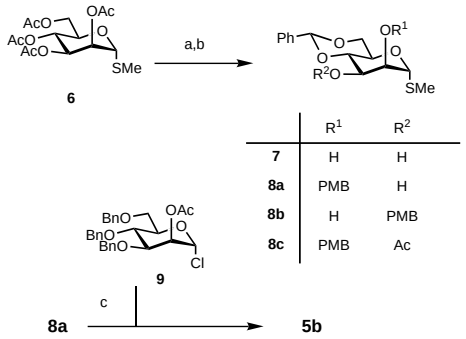
The intramolecular aglycon delivery (IAD) approach, which was introduced by Baressi and Hindsgaul,[7] and later by Stork and co-workers,[8] is endowed with an evident advantage over others, because it guarantees the exclusive formation of the correct anomer as a result of the geometrical constraint. As a newer variant of this approach, we have reported the use of the 2-*O*-*p*-methoxybenzyl (PMB) protected mannose donor **2**.[9] Starting from **2**, treatment with DDQ in the presence of aglycon affords mixed acetal **3**, presumably via quinonemethide like species, which serves as a tethered intermediate in the forthcoming IAD process (Scheme 1). Subsequent activation of the anomeric position then triggers IAD to afford β-manno glycoside **4**. In order to apply this methodology into the synthesis of biologically relevant oligosaccharides, especially asparagine-linked complex-type glycans, appropriately protected mannosyl donors (**5a**,**b**) have been examined.[9b] Most significantly, exclusive formation of β-manno glycoside at the oligosaccharide block-condensation stage was realized for the first time.[9b,c]

Results

As a demonstration of the versatility of our PMB-assisted approach, we disclose here the synthesis of the fucose-containing core structure (Fuc) of Asn-linked glycans in reducing end-protected (**1a**) and Asn-linked (**1b**) forms. Addition of a Fuc residue onto C-6 of the innermost GlcNAc is a frequently observed structural modification in complex- and hybrid-type glycans.[10] This also has a biomedical significance in connection with intracellular trafficking of certain types of glycoproteins and malignant transformations.[11]

Glycosyl donor **5b** was strategically designed, so that various types of Asn-linked glycans can be synthesized based on the PMB-assisted β-mannosylation protocol. To be more precise, the expected β-mannoside product has an available hydroxy group at C-2 and an acetal protected diol at C-4 and C-6. Therefore, all hydroxy groups on this particular mannose residue can be differentiated from others by selective manipulation of protective groups in combination with the well established reactivity order[12] that favors the reaction at C-6 position (vide infra).

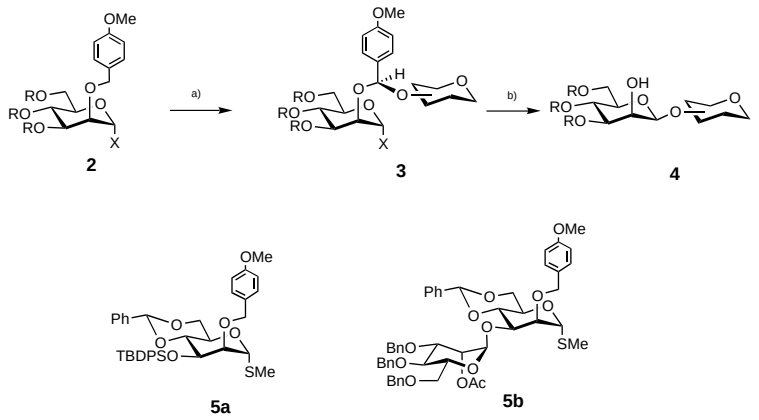
Preparation of **5b** was performed as depicted in Scheme 2. Thus, starting from the known methyl thiomannoside **6**,[13] deacetylation–benzylidenation afforded 4,6-*O*-protected **7**.



Scheme 2. Preparation of dimannosyl donor. a) $PhCH(OMe)_2$, CSA/DMF, 77%; b) *p* -$MeOC_6H_4CH_2Cl$, Bu_4NHSO_4, aq NaOH/CH_2Cl_2, 50%; c) AgOTf, 2,6-di-*tert*-butyl-4-methylpyridine, MS 4 A/CH_2Cl_2, 81%.

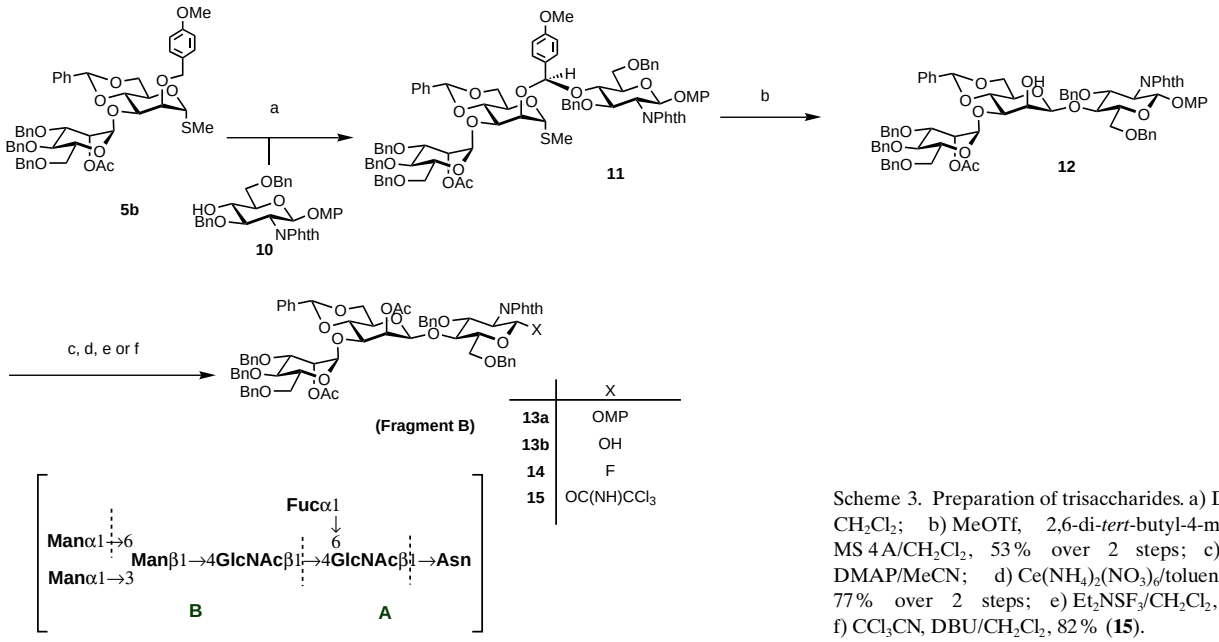
Regioselective alkylation was performed under phase-transfer conditions[14] to afford **8a** as a major product, together with the corresponding regioisomer **8b**. The introduction of the PMB group onto the C-2 position of **8a** was confirmed by NMR analysis of acetylated **8c**. Stereoselective incorporation of an α-linked mannose residue onto **8a** was performed by use of chloride **9**[15] as a glycosyl donor to afford disaccharide **5b**.

Having the designed dimannoside donor in hand, we performed the β-mannosylation by using the latent GlcNAc component **10**[16] as an acceptor (Scheme 3). Initial transformation into mixed acetal **11** was effected by DDQ[17] according to our standard protocol. This material was purified by size exclusion chromatography into a spectroscopically homogeneous form (86% yield), although IAD proceeded in a nearly equal efficiency via nonpurified **11**.[18] ^{1}H NMR analysis of **11** revealed that it consists of a single isomer, within the detection limit of a 270 MHz NMR spectrometer, indicating that the mixed acetal formation proceeds with a high degree of stereoselectivity. Characteristic low-field shifts of H-1_{Man} (δ_H = 5.71) and one of the benzylic protons (δ_H = 5.40) allowed us to assign the configuration of acetalic carbon as *S*.[19]



Scheme 1. Intramolecular aglycon delivery via *p*-methoxybenzylidene acetal. a) Aglycon, DDQ. b) IAD.

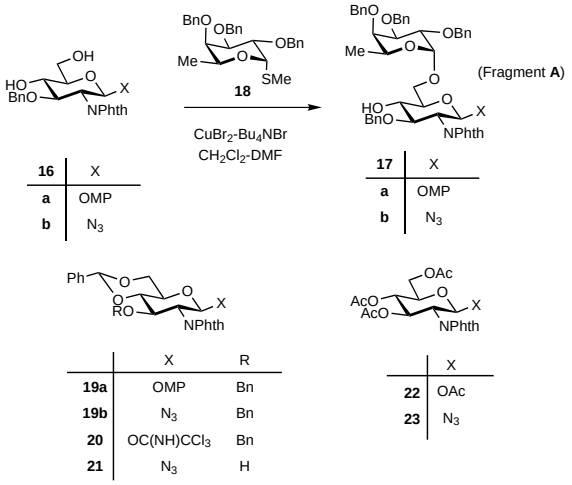
  0947-6539/98/0411-2183 $ 17.50+.25/0



Scheme 3. Preparation of trisaccharides. a) DDQ, MS 4 A/CH_2Cl_2; b) MeOTf, 2,6-di-*tert*-butyl-4-methylpyridine, MS 4 A/CH_2Cl_2, 53% over 2 steps; c) Ac_2O, Et_3N, DMAP/MeCN; d) $Ce(NH_4)_2(NO_3)_6$/toluene/MeCN/H_2O, 77% over 2 steps; e) Et_2NSF_3/CH_2Cl_2, 76% (**14**); f) CCl_3CN, DBU/CH_2Cl_2, 82% (**15**).

IAD was effected by exposure of **11** to $MeOSO_2CF_3$ (MeOTf[20]) and 2,6-di-*tert*-butyl-4-methylpyridine (DBMP) in 1,2-dichloroethane to afford the β-manno glycoside product **12** (53% yield), which corresponds to the backbone trisaccharide structure (αMan1 →3βMan1 →4GlcNAc; fragment **B**) of Asn-linked glycan chains . Further transformations into fluoride **14** and trichloroacetimidate **15** were performed via **13a** and **13b** in a standard manner.

The designed fucosyl glucosamine component (**17a/b**; fragment **A**) was synthesized by regioselective glycosylation of the 4,6-diol **16a/b** with fucose donor **18** (Scheme 4).[21]



Scheme 4. Synthesis of fucosyl glucosamine.

Requisite **16a/b** were prepared from 4,6-benzylidene protected **19a**, which was reported previously. Thus, transformation into trichloroacetimidated **20** [a): CAN[22]; b): CCl_3CN, DBU] followed by introduction of an azide group[23] afforded **19b**. Subsequent acidic removal of benzylidene groups from **19a/b** afforded **16a/b**. Alternatively, azide **19b** was prepared from tetraacetate **22**, in a similar manner as described by Kunz and co-workers.[24] Namely, **22** was first converted into glycosyl azide **23** ($TMSN_3$, TMSOTf/MeCN), which was then subjected to a three step sequence [a): NaOMe/MeOH; b): $PhCH(OMe)_2$, CSA/MeCN; c): $PhCH_2Br$, NaH/DMF] to afford **19b** via **21**. Fucosylation was performed by use of the thioglycoside **18** under in situ anomerization-like[25] conditions ($CuBr_2$, Bu_4NBr/CH_2Cl_2/DMF[26]) to afford **17a** (78%) and **17b** (73%).

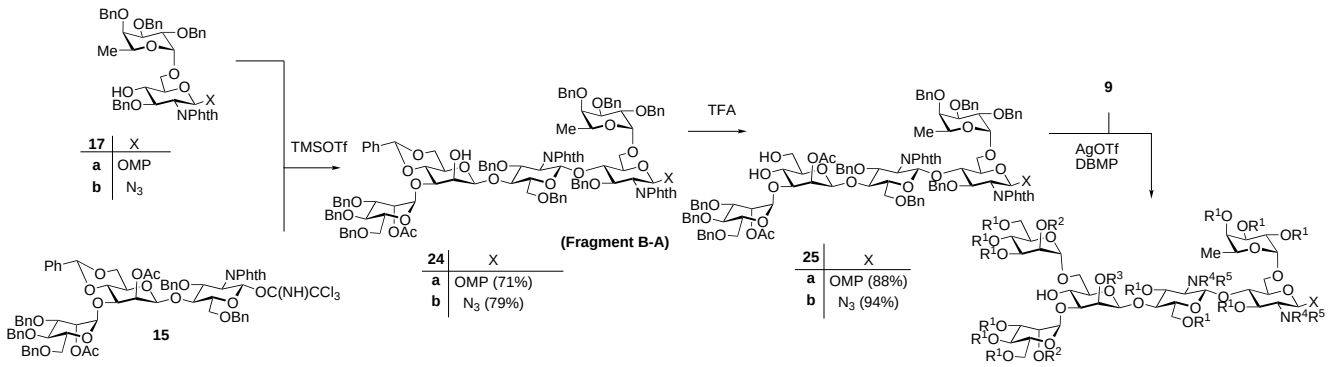
Critical fragment coupling of **17a/b** with **15** was successfully performed by the action of $Me_3SiOSO_2CF_3$ (TMSOTf) and pentasaccharides **24a** and **24b** were obtained in 71% and 79% yield, respectively (Scheme 5). Fluoride **14** proved less effective for this purpose even under the conditions optimized for the strongest activation (AgOTf, Cp_2HfCl_2[27]/CH_2Cl_2, 39% **24a**).

Since the benzylated fucose residue is known to be acid labile,[28] subsequent debenzylidenation of **24a/b** required carefully controlled conditions. This transformation was best achieved by treatment with dilute trifluoroacetic acid in CH_2Cl_2 to afford **25a** (88%) and **25b** (94%). Regioselective introduction of an α-mannose residue was achieved by use of the chloride **9** (AgOTf/CH_2Cl_2) to afford **26a** (77%) and **26b** (74%).

Deprotection of **26a** was performed as follows. Removal of phthalimide groups (ethylenediamine[29]/EtOH) followed by *N*-acetylation afforded **27a** (Scheme 5). Somewhat surprisingly, the acetyl group of the β-linked mannose residue mostly remained intact, while those of α-linked mannose residues were cleaved. Compound **27a** was further deprotected [a): H_2, Pd-C; b): aq NaOH] into **1a** via **28**.

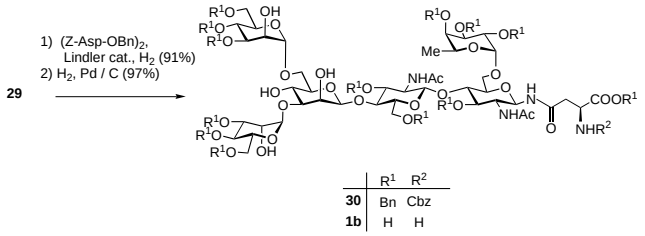
On the other hand, azide-carrying **26b** was converted into glycosyl asparagine **1b** as follows. Dephthaloylation – acetylation afforded **27b**, which was de-*O*-acetylated to give **29** (Scheme 6). Compound **29** was then subjected to the conditions for chemoselective reduction of an azide group with a Lindlar catalyst to produce the corresponding glycosylamine,

0947-6539/98/0411-2184 $ 17.50+.50/0 *Chem. Eur. J.* **1998**, *4*, No. 11



Scheme 5. Coupling of fragments **A** and **B**.

	X	R^1	R^2	R^3	R^4R^5
26a	OMP	Bn	Ac	Ac	Phth
b	N_3	Bn	Ac	Ac	Phth
27a	OMP	Bn	H	Ac	H,Ac
b	N_3	Bn	H	Ac	H,Ac
28	OMP	H	H	Ac	H,Ac
29	N_3	Bn	H	H	H,Ac
1a	OMP	H	H	H	H,Ac

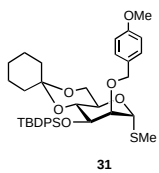


Scheme 6. Synthesis of hexasaccharide.

which was trapped in situ with a Cbz-Asp-OBn derived acid anhydride[30] to give **30** in 91% yield. Deprotection of **30** was performed in a standard manner to afford hexaglycosyl asparagine **1b**.

Discussion

The utility of PMB-tethered β-mannosylation in Asn-linked glycans is clearly demonstrated in the synthesis of **1a/b**. Now, the fully stereocontrolled construction of β-manno glycoside is possible in a practical manner. The following features make our PMB-based strategy clearly distinct from others; a) β-manno glycoside can be formed with complete stereochemical control, b) it is applicable to oligosaccharide condensation, and c) a variety of protecting group patterns can be accommodated (i.e., acetyl, benzyl, benzylidene, silyl, *p*-methoxyphenyl, phthalimide). From these results, it can be concluded that the IAD approach has gained practical utility in synthetic studies on Asn-linked glycans.



31

Recently, the efficiency of PMB-assisted β-mannosylation was further improved by the use of 4,6-cyclohexylidene-protected mannosyl donor **31**, which now realizes the formation of the β-Man1 $\rightarrow$4βGlcNAc equivalent in as high as >80% yield.[31] Furthermore, the question with regard to the stereochemistry of the mixed acetal **3** was recently solved.[19] Namely, it is now clear that mixed acetal formation proceeds with a high degree of stereoselectivity to afford **3** with *S*- configuration at the acetalic carbon (see Scheme 1).

An elegant synthetic approach to asparagine linked glycans has been reported by Unverzagt.[32] In this case, the β-mannosylation protocol developed by Kunz was used as the key transformation, in which the β-gluco-configurated glycosylation product was transformed into β-manno glycoside by intramolecular S_N2 type replacement.[33] Our IAD based methodology would be able to provide more flexible synthetic route, with respect to the substitution pattern at the C-3 position. For instance, in our case the mannose donor that has an additional sugar residue at C-3 (i.e., **5b**) can be used to provide building blocks for a more convergent synthetic approach to Asn-linked glycans.

On the other hand, efficient methods for the stereoselective introduction of sialic acid (NeuAc) residue, which was considered as another challenge in synthetic carbohydrate chemistry, have been developed by various approaches utilizing either chemical[34] or enzymatic means.[35] Taking all of these aspects together, it is now possible to conceive highly stereocontrolled synthetic routes to a wide range of Asn-linked glycans.

Experimental Section

General Procedures: Melting points were determined with a Yanagimoto micro melting-point apparatus and are not corrected. Optical rotations were measured with a JASCO DIP 370 polarimeter at ambient temperature ($20\pm3\,^{\circ}C$). NMR spectra were recorded with a JEOL EX-270 or GX-400 spectrometers. Me_4Si ($\delta=0.00$) and *t*BuOH ($\delta=1.23$ ppm) were used as internal standards for ^1H NMR in $CDCl_3$ and D_2O, respectively. ^{13}C NMR spectra in $CDCl_3$ were measured with the solvent peaks as an internal standard adjusted to $\delta=77.0$. 1,4-Dioxane ($\delta=67.2$) was used as an internal standard for ^{13}C NMR spectra in D_2O. TLC on silica gel 60 F_{254} (Merck, Darmstadt) was used to monitor the reactions and to ascertain the purity of

the products. Silica-gel column chromatography was performed with Merck silica gel 60 (63–200 μm) or Cica silica gel 60N (spherical, 40–100 or 100–210 μm). Molecular sieves were activated by heating to 180 °C in vacuo for 24 h prior to use. All reactions require anhydrous conditions were performed under atmosphere of N_2 or Ar.

Methyl 4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (7): A mixture of compound **6** (10.0 g, 47.6 mmol), benzaldehyde dimethylacetal (7.85 mL, 52.3 mmol), and DL-camphorsulfonic acid (330 mg, 1.4 mmol) in DMF (150 mL) was stirred at room temperature under vacuum (≈15 mmHg) for 4 h. An additional amount of benzaldehyde dimethylaectal (1.4 mL, 9.3 mmol) was added and stirring was continued for 3 h. The reaction was quenched with triethylamine (3 mL) and diluted with ether/dichloromethane (1:1). The solution was washed three times with water, and the combined aqueous layers were extracted with AcOEt. The combined organic layers were washed with brine and dried ($MgSO_4$), and the solvent was evaporated in vacuo. The residue was crystallized from AcOEt/EtOH to afford 6.09 g of **7**. The mother liquor was evaporated in vacuo and purified by silica-gel column chromatography (hexane/AcOEt 5:1–1:3) to afford an additional 4.85 g of **7**. Total yield 10.94 g (77%). M.p. 173–175 °C; $[\alpha]_D = +171.5$ ($c = 1.1$ in chloroform); ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 5.57$ (s, 1H; benzylidene CH), 5.25 (s, 1H; H-1), 2.16 (s, 3H; SMe); ^{13}C NMR (67.5 MHz, $CDCl_3$, 25 °C): $\delta = 102.1$ (benzylidene CH), 83.9 (C-1), 79.7, 79.5, 72.7, 68.9, 68.6, 63.9, 55.3; $C_{14}H_{18}O_5S$ (298.4): calcd C 56.36, H 6.08, S 10.75; found C 56.62, H 6.03, S 10.35.

Methyl 4,6-*O*-benzylidene-2-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside (8a): *p*-Methoxybenzyl chloride (1.0 mL, 7.4 mmol), Bu_4NHSO_4 (240 mg, 0.71 mmol), and 5% aq NaOH (5 mL) were added to a solution of compound **7** (1.08 g, 3.62 mmol) in CH_2Cl_2 (60 mL), and the whole was stirred vigorously and heated under reflux for 2 days. The mixture was washed with water and brine successively, and dried over $MgSO_4$; the solvent was then evaporated in vacuo. The residue was separated by silica-gel column chromatography (toluene/AcOEt 4:1) to afford 758 mg (50%) of compound **8a** as well as the corresponding regioisomer **8b** (222 mg, 15%). Compound **8a**: ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.55–6.84$ (m, 9H; aromatic), 5.56 (s, 1H; benzylidene CH), 5.26 (s, 1H; H-1), 3.81 (s, 3H; OMe), 2.13 (s, 3H; SMe); ^{13}C NMR (67.5 MHz, $CDCl_3$, 25 °C): $\delta = 102.1$ (benzylidene CH), 83.9 (C-1), 79.7, 79.5, 72.7, 68.9, 68.6, 63.9, 55.3; $C_{22}H_{26}O_6S$ (418.5): calcd C 63.14, H 6.26; found C 63.01, H 6.29.

The regiochemistry of **8a** was confirmed by converting into corresponding acetate **8c**: $[\alpha]_D = +59.6$ ($c = 1.0$ in chloroform); ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.32–7.48$ (m, 5H; C_6H_5CH), 6.89 and 7.28 (2 d, each 2H; $CH_3OC_6H_4CH_2$), 5.56 (s, 1H; C_6H_5CH), 5.21 (dd, $J = 3.6$, 9.6 Hz, 1H; 3-H), 5.20 (d, $J = 1.3$ Hz, 1H; H-1), 4.48 and 4.63 (ABq, $J = 11.9$ Hz, each 1H; $MeOC_6H_4CH_2$), 4.21–4.31 (m, 2H; H-5, H-6_b), 4.18 (dd, $J = 9.7$, 9.6 Hz, 1H; H-4), 4.05 (dd, $J = 1.3$, 3.6 Hz, 1H; H-2), 3.89 (m, 1H; H-6_a), 3.81 (s, 3H; $CH_3OC_6H_4CH_2$), 2.13 and 2.01 (2s, each 3H; MeS, Ac); $C_{24}H_{28}O_7S$ (460.5): calcd C 62.59, H 6.13; found C 62.73, H 6.13.

Regioisomer **8b**: ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 5.61$ (s, 1H; benzylidene CH), 5.22 (s, 1H; H-1), 2.14 (s, 3H; SMe). Corresponding acetate: ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.68–7.32$ (m, 5H; Ar), 7.26 (m, 2H; Ar), 6.84 (m, 2H; Ar), 5.62 (s, 1H; benzylidene CH), 5.45 (dd, $J = 3.4$, 1.3 Hz, 1H; H-2), 5.14 (d, $J = 1.3$ Hz, 1H; H-1), 4.63 and 4.56 (each ABq, $J = 11.6$ Hz, 1H; $MeOC_6H_4CH_2$), 4.25 (dd, $J = 9.7$, 4.9 Hz, 1H; H-6), 4.19 (ddd, $J = 9.5$, 4.9, 4.9 Hz, 1H; H-5), 4.08 (dd, $J = 9.6$, 9.5 Hz, 1H; H-4), 3.95 (dd, $J = 9.6$, 3.4 Hz, 1H; H-3), 3.87 (m, 1H; H-6′), 3.79 (s, 3H; OMe), 2.14 and 2.17 (2s, each 3H; SMe, Ac).

Methyl *O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-4,6-*O*-benzylidene-2-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside (5b): Methyl thiomannopyranoside **8a** (1.15 g, 2.75 mmol) and 2,6-di-*t*-butyl-4-methylpyridine (DBMP, 1.02 g, 4.95 mmol) were dissolved in anhydrous CH_2Cl_2 (25 mL) and stirred for 30 min under Ar and exclusion of light with AgOTf (1.27 g, 4.95 mmol) over freshly activated molecular sieves 4A (5.0 g). After cooling to −15 °C, **9** (1.97 g, 3.85 mmol) was added as a solution in CH_2Cl_2 (15 mL). The mixture was gradually warmed up to room temperature, stirred for 90 min, and diluted with CH_2Cl_2 (100 mL). The suspension was filtered through Celite and the filtrate was washed with satd. $NaHCO_3$ solution (30 mL) and 10% $Na_2S_2O_3$ solution (30 mL), and dried (Na_2SO_4). Removal of the solvent in vacuo gave a colorless foam (3.79 g), which was purified by elution from silica gel with toluene/AcOEt 10:1 to afford 2.10 g (86%) of **5** as a colorless foam. $[\alpha]_D = +57.0$ ($c = 1.1$, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.64–6.70$ (m, 24H; aromatic), 5.61 (s, 1H; benzylidene CH), 5.60 (dd, $J = 3.3$, 2.0 Hz, 1H; H-2^2), 5.30 (d, $J = 2.0$ Hz, 1H; H-1^2), 5.15 (d, $J = 1.0$ Hz, 1H; H-1^1), 4.89–4.55 (m, 4H; benzylic CH_2), 3.96 (dd, $J = 8.6$, 3.3 Hz, 1H; H-3^2), 3.67 (m, 1H; H-4^2), 3.63 (s, 3H; OMe), 2.09 and 2.07 (2s, each 3H; SMe, Ac); ^{13}C NMR (67.5 MHz, $CDCl_3$, 25 °C): $\delta = 101.6$ (benzylidene CH), 99.2 (C-1^2), 85.2 (C-1^1), 79.5, 78.7, 78.3, 75.4, 74.7, 73.7, 73.6, 72.9, 72.5, 71.9, 69.2, 68.9, 68.5, 64.8, 55.4; $C_{51}H_{56}O_{12}S$ (893.07): calcd C 68.59, H 6.32; found C 68.34, H 6.34.

***p*-Methoxyphenyl *O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-*O*-(4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (12) via mixed acetal 11**: Compounds **5b** (43 mg, 0.048 mmol) and **10** (20.8 mg, 0.035 mmol) as a solution in CH_2Cl_2 (1.3 mL) were added to an ice-cold mixture of DDQ (14 mg, 0.062 mmol), molecular sieves 4A (0.2 g) in CH_2Cl_2 (0.5 mL). The mixture was stirred at room temperature for 3 h and quenched with an aqueous solution of ascorbic acid (0.7%)/citric acid (1.3%)/NaOH (0.9%) (2 mL). The resulting solution was stirred for 10 min to form a lemon-yellow suspension, that was subsequently diluted with AcOEt and filtered through Celite. The filtrate was washed with water, aq $NaHCO_3$, and brine successively, and dried over $MgSO_4$. The solvent was then evaporated in vacuo, and the residue was subjected to size-exclusion column chromatography with Bio-Beads S-X4 (toluene). Fractions containing mixed acetal **11** were collected, concentrated, and, after brief exposure to high vacuum, added as a solution in 1,2-dichloroethane (5 mL) to a flask containing molecular sieves 4A (0.2 g) and DBMP (39 mg, 0.19 mmol). Methyl trifluoromethanesulfonate (MeOTf; 20 μl, 0.18 mmol) was added, and the mixture was stirred at 40 °C for 14 h. The reaction was quenched with Et_3N (0.2 mL; RT, 10 min), diluted with AcOEt/water, and filtered through Celite. The filtrate was washed with water and brine successively, and dried over $MgSO_4$; the solvent was then evaporated in vacuo. The residue was purified by chromatography with Bio-Beads S-X4 (toluene) and then with silica gel (hexane/AcOEt 1:3) to afford 24.3 mg (53%) of compound **12**. $[\alpha]_D = +24.1$ ($c = 0.8$ in chloroform); ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.61–6.61$ (m, 38H; aromatic), 5.52 (d, $J = 8.3$ Hz, 1H; H-1^1), 5.40 (s, 1H; benzylidene CH), 5.04 (d, $J = 1.3$ Hz, 1H; H-1^3), 4.59 (s, 1H; H-1^2), 4.31 (dd, $J = 8.3$, 3.7 Hz, 1H), 3.97 (dd, $J = 9.2$, 3.3 Hz, 1H; H-3^3), 3.89 (t, $J = 9.6$ Hz, 1H; H-4^2), 3.64 (s, 3H; OMe), 3.07 (ddd, $J = 9.6$, 4.6, 4.6 Hz, 1H; H-5^2), 2.80 (d, $J = 3$ Hz, 1H; OH), 2.05 (s, 3H; Ac); ^{13}C NMR (67.5 MHz, $CDCl_3$, 25 °C): $\delta = 101.4$ (benzylidene CH), 100.6 (C-1^2, $^1J_{CH} = 161$ Hz), 98.8 (C-1^3), 97.7 (C-1^1), 78.7, 77.9, 77.2, 77.1, 75.6, 74.7, 74.4, 73.6, 73.5, 71.9, 71.8, 70.6, 69.4, 68.5, 68.4, 68.3, 66.8, 55.6, 55.5; $C_{77}H_{77}NO_{19}$ (1320.47): calcd C 68.59, H 6.32, N 1.06; found C 69.90, H 5.90, N 0.95.

In a separate experiment, mixed acetal **11** was prepared with a slight excess amount of **10** and was more rigorously purified into a spectroscopically pure form. To be brief, compounds **5b** (44.1 mg, 0.0494 mmol) and **10** (38.3 mg, 0.0643 mmol) were treated with DDQ (13.5 mg, 0.0593 mmol) in CH_2Cl_2 (0.4 mL) in the presence of molecular sieves 4A (0.1 g) at room temperature for 3 h. The mixture was processed as described above and purified by a column of Bio-Beads S-X4 (toluene) to afford 63.2 mg (86%) of **11**. ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 5.71$ (s, 1H; H-1^2), 5.66 (d, $J = 8.3$ Hz, 1H; H-1^1), 5.63 (br s, 2H; mixed acetal CH and H-2^3), 5.40 (d, $J = 11.2$ Hz, 1H; benzylic CH_2), 5.26 (d, $J < 1$ Hz, 1H; H-1^3), 4.85 (s, 1H; benzylidene CH), 3.78 (s, 3H; OMe), 3.25 (s, 3H; OMe), 2.08 and 1.99 (2s, each 3H; SMe, Ac); ^{13}C NMR (67.5 MHz, $CDCl_3$, 25 °C): $\delta = 100.5$, 99.4, 98.1, 97.5 (C-1^1, C-1^3, benzylidene CH, mixed acetal CH), 82.1, 78.6, 78.4, 77.8, 77.2, 76.1, 76.0, 75.3, 75.2, 74.4, 74.0, 73.5, 73.2, 71.8, 71.2, 68.5, 68.1, 64.9, 55.9, 55.6, 54.8, 21.0 (Ac), 13.5 (SMe).

***O*-(2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranose (13b)**: Compound **12** (65.6 mg, 0.0497 mmol) was dissolved in acetonitrile (1.5 mL), and triethylamine (21 μl, 0.15 mmol), *N*,*N*-dimethylaminopyridine (DMAP; 0.3 mg, 0.003 mmol), and acetic anhydride (7 μl, 0.07 mmol) were added successively. The mixture was stirred at room temperature overnight and quenched with MeOH. Volatiles were removed in vacuo, and the residue was co-evaporated successively with ethanol and toluene to afford the acetylated product **13a**, which was then diluted with toluene/acetonitrile/water (3:4:3; 4 mL). Ceric ammnonium nitrate (CAN; 82 mg, 0.15 mmol) was added, and the mixture was stirred at room temperature for 2 h. An additional amount of CAN (40 mg, 0.073 mmol) was added and stirring was continued for 2 h. The resulting mixture was diluted with AcOEt, washed

0947-6539/98/0411-2186 $ 17.50+.50/0 *Chem. Eur. J.* **1998**, *4*, No. 11

with water, 5% aq NaOH, and brine successively, and dried over $MgSO_4$; the solvent was then evaporated in vacuo. The residue was purified by silica-gel column chromatography (hexane/AcOEt 10:1–5:1) to afford 48.2 mg (77%) of compound **13b**.

Compound **13a**: $[\alpha]_D = +26.4$ ($c = 0.9$ in chloroform); ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.68-6.67$ (m, 38H; aromatic), 5.58 (d, $J = 8.3$ Hz, 1H; H-1^1), 5.50 (s, 1H; benzylidene CH), 5.46 (dd, $J = 3.3$, 1.7 Hz, 1H; H-2^3), 5.37 (br s, 1H; H-2^2), 5.22 (d, $J = 1.7$ Hz, 1H; H-1^3), 4.67 (d, $J < 1$ Hz, 1H; H-1^2), 3.71 (s, 3H; OMe); ^{13}C NMR (67.5 MHz, $CDCl_3$, 25 °C): $\delta =$ 101.2 (benzylidene CH), 99.2, 98.6 (C-1^2, C-1^3), 97.6 (C-1^1), 78.7, 78.6, 76.5, 74.7, 74.7, 74.6, 74.1, 73.4, 73.4, 72.7, 72.1, 71.7, 70.7, 68.9, 68.5, 68.3, 68.0, 66.4, 55.6; $C_{79}H_{79}NO_{20}$ (1362.51): calcd C 69.94, H 5.84, N 1.03; foumd C 69.46, H 5.85, N 1.16.

Compound **13b**: ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.69-6.93$ (m, 34H; aromatic), 5.49 (s, 1H; benzylidene CH), 5.46 (dd, $J = 2.6$, 1.6 Hz, 1H; H-2^3), 5.33 (br, 1H; H-2^2), 5.27 (d, $J = 8.6$ Hz, 1H; H-1^1), 5.21 (d, $J =$ 1.6 Hz, 1H; H-1^3), 2.88 (d, $J = 8.6$ Hz, 1H; OH), 2.09 and 2.04 (2s, each 3H; Ac);^{13}C NMR (67.5 MHz, $CDCl_3$, 25 °C): $\delta = 99.0$, 98.6 (C-1^2, C-1^3), 93.0 (C-1^1), 78.6, 78.4, 74.5, 74.4, 74.1, 73.5, 73.4, 72.7, 72.1, 71.7, 70.6, 68.9, 68.5, 68.3, 68.1, 66.4, 57.5.

***O*-(2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-*α*-D-mannopyranosyl)-(1→3)-*O*-(4,6-*O*-benzylidene-*β*-D-mannopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl fluoride (14)**: Dimethylaminosulfur trifluoride (DAST; 2.4 µl, 0.018 mmol) was added to a precooled (−20 °C) solution of compound **13b** (16.5 mg, 0.0131 mmol) in CH_2Cl_2 (1 mL). The mixture was stirred at this temperature for 30 min and quenched with ice-cold aq $NaHCO_3$. After being diluted with AcOEt, the layers were separated. The organic layer was washed with aq $NaHCO_3$ and brine successively, and dried over $MgSO_4$; the solvent was then evaporated in vacuo. The residue was purified by silica-gel column chromatography (toluene/AcOEt 20:1–10:1) to afford 12.5 mg (76%) of compound **14**. ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.74-6.90$ (m, 34H; aromatic), 5.81 (dd, ^1J(H,F) = 53.8 Hz, $J = 7.7$ Hz, 1H; H-1^1), 5.49 (s, 1H; benzylidene CH), 5.46 (dd, $J = 2.2$, 1.7 Hz, 1H; H-2^3), 5.34 (br s, 1H; H-2^2), 5.22 (d, $J =$ 1.7 Hz, 1H; H-1^3), 2.09 and 2.04 (2s, each 3H; Ac).

***O*-(2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-*α*-D-mannopyranosyl)-(1→3)-*O*-(4,6-*O*-benzylidene-*β*-D-mannopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl trichloroacetimidate (15)**: Trichloroacetonitrile (15 µl, 0.15 mmol) was added to a solution of compound **13b** (18.4 mg, 0.0147 mmol) in 1,2-dichloroethane (0.5 mL), and the solution was cooled down to 0 °C. DBU (10% in 1,2-dichloroethane; 2 µl, 0.001 mmol) was added and the mixture was stirred for 2 h. The mixture was applied to a column of silica gel (10–30% AcOEt in hexane) to afford 16.8 mg (82%) of compound **15**. ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 8.54$ (s, 1H; =NH), 7.67–6.91 (m, 34H; aromatic), 6.36 (d, $J = 8.3$ Hz, 1H; H-1^1), 5.50 (s, 1H; benzylidene CH), 5.46 (dd, $J = 3.6$, 1.6 Hz, 1H; H-2^3), 5.36 (br s, 1H; H-2^2), 5.22 (d, $J = 1.6$ Hz, 1H; H-1^3), 2.09 and 2.05 (2s, each 3H; Ac).

***p*-Methoxyphenyl *O*-(2,3,4-tri-*O*-benzyl-*α*-D-fucopyranosyl)-(1→6)-3-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (17a)**: A mixture of $CuBr_2$ (69.2 mg, 0.31 mmol), Bu_4NBr (100 mg, 0.31 mmol), and molecular sieves 4 A (0.13 g) in CH_2Cl_2/DMF (2:1, 3 mL) was stirred and cooled over an ice–water bath. A solution of comounds **16a** (73.0 mg, 0.145 mmol) and **18** (72.0 mg, 0.155 mmol) in CH_2Cl_2 (4 mL in total) was added dropwise and the mixture stirred at 0 °C for 4.5 h. An additional amount of compound **18** (12.0 mg, 0.026 mmol) was added as a solution in CH_2Cl_2 (0.5 mL) and stirring was continued overnight at room temperature. The resulting mixture was quenched with aq $NaHCO_3$ (RT, 10 min), diluted with AcOEt, and filtered through Celite. The filtrate was washed successively with aq $NaHCO_3$ and brine, and dried over Na_2SO_4; the solvent was then evaporated in vacuo. The residue was purified by silica-gel column chromatography (4–8% AcOEt in toluene) to afford 104.4 mg (78%) of compound **17a**, together with 10.5 mg (8%) of corresponding β-isomer. Compound **17a**: $[\alpha]_D = -5.1$ ($c = 0.8$ in chloroform); ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.62-6.58$ (m, 28H; aromatic), 5.53 (d, $J = 8.3$ Hz, 1H; H-1^1), 4.31–4.13 (m, 2H; H-2^1, H-3^1), 3.98 (m, 1H; H-5^F), 3.60 (s, 3H; OMe), 1.00 (d, $J = 6.3$ Hz, 3H; H-6^F); ^{13}C NMR (67.5 MHz, $CDCl_3$): $\delta =$ 98.5 and 97.8 (anomeric carbons), 79.3, 77.7, 77.2, 76.3, 74.9, 74.3, 74.2, 73.5, 73.3, 72.9, 68.3, 66.9, 55.5, 55.5.

3-*O*-Benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-*β*-D-glucopyranose (19c): Ceric ammonium nitrate (CAN; 961 mg, 1.75 mmol) was added to a solution of **19a** (520 mg, 0.88 mmol) in toluene/MeCN/H_2O (3:4:3; 10 mL), and the mixture stirred at room temperature. After 3 h, additional amounts of toluene/MeCN/H_2O (3:4:3; 10 mL) and CAN (961 mg, 1.75 mmol) were added. After being stirred for additional 30 min, the mixture was diluted with AcOEt, washed successively with water, 5% aq NaOH, and brine, and dried over $MgSO_4$; the solvent was then evaporated in vacuo. The residue was triturated with hexane/AcOEt and a solid material was collected by filtration to afford 343 mg of compound **19c**. The mother liquor was concentrated to dryness and purified by silica-gel column chromatography (10–30% AcOEt in hexane) to afford additional 67 mg of **19c**. Total yield 410 mg (96%). ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.71-6.79$ (m, 14H; aromatic), 5.63 (s, 1H; benzylidene CH), 5.42 (br t, $J = 8$ Hz, 1H; H-1), 4.81 and 4.53 (ABq, $J = 12.2$ Hz, each 1H; benzyl CH_2), 4.52 (dd, $J =$ 10.2, 8.9 Hz, 1H; H-3), 4.14 (dd, $J = 10.2$, 8.6 Hz, 1H; H-2), 3.84 (m, 2H; H-6), 3.70 (m, 1H; H-5), 3.21 (d, $J = 7.6$ Hz, 1H; OH).

3-*O*-Benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl trichloroacetimidate (20): DBU (5 µl, 0.03 mmol) was added to an ice-cold solution of compound **19c** (195 mg, 0.40 mmol) and trichloroacetonitrile (0.4 mL, 4.0 mmol) in 1,2-dichloroethane (4 mL), and the mixture was stirred for 1.5 h. The mixture was subjected to a column of silica gel, which was eluted with 10–30% AcOEt in toluene to afford 228 mg (91%) of compound **20**. ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 8.57$ (s, 1H; C=NH), 7.70–6.85 (m, 14H; aromatic), 6.49 (d, 1H, $J = 8.6$ Hz; H-1), 5.65 (s, 1H; benzylidene CH), 4.82 and 4.53 (ABq, $J = 12.2$ Hz, each 1H; benzyl CH_2), 4.59-4.47 (m, 3H; H-2, H-3, H-4), 3.89 (m, 3H; H-5, H-6).

3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl azide (23): Trimethylsilyl triflate (38 µl, 0.21 mmol) was added to a solution of compound **22** (1.00 g, 2.1 mmol) and trimethylsilyl azide (420 µmL, 3.16 mmol) in acetonitrile (20 mL), and the solution was stirred at room temperature for 4 h. The resulting mixture was diluted with AcOEt and washed with ice-cold water, aq $NaHCO_3$, and brine, successively. The organic layer was dried over $MgSO_4$ and the solvent was evaporated in vacuo. The residue was purified by silica-gel column chromatography (toluene/AcOEt 2:1) to afford 0.817 g (84%) of compound **23**. M.p. 135–138°; ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.90-7.75$ (m, 4H; Phth), 5.74 (dd, $J = 10.6$, 9.2 Hz, 1H; H-3), 5.66 (d, $J = 9.2$ Hz, 1H; H-1), 5.20 (t, $J = 9.6$ Hz, 1H; H-4), 4.36 (dd, $J = 12.5$, 4.6 Hz, 1H; H-6), 4.25 (dd, $J = 12.5$, 2.3 Hz, 1H; H-6′), 4.24 (dd, $J = 10.6$, 9.2 Hz, 1H; H-2), 3.98 (m, 1H; H-5), 2.14, 2.05 and 1.87 (s, 3H; Ac); $C_{20}H_{20}N_4O_9$ (460.40): calcd C 52.18, H 4.38, N 12.17; found C 52.18, H 4.33, N 12.01.

4,6-*O*-Benzylidene-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl azide (21): A solution of **23** (1.57 g, 4.69 mmol), benzaldehyde dimethylacetal (1.05 mL, 7.0 mmol), and DL-camphorsulfomic acid (0.11 g, 0.47 mmol) in acetonitrile (40 mL) was stirred at room temperature for 4 d. The mixture was made slightly basic with NaOMe (0.5 M; phenolphthalein indicator) and immediately neutralized with a drop of acetic acid. After the solvent was evaporated in vacuo, purification by silica-gel column chromatography (toluene/EtOH 19:1) afforded 1.83 g (94%) of compound **21**. M.p. 187–191°; ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.90-7.72$ (m, 4H; Phth), 7.57–7.32 (m, 5H; Ph), 5.59 (s, 1H; benzylidene CH), 5.48 (d, $J =$ 9.2 Hz, 1H; H-1), 4.69 (ddd, $J = 10.4$, 8.9, 3.6 Hz, 1H; H-3), 4.44 (dd, $J =$ 9.9, 4.3 Hz, 1H; H-6), 4.18 (dd, $J = 10.4$, 9.4 Hz, 1H; H-2), 3.86 (t, $J =$ 10 Hz, 1H; H-6′), 3.76 (m, 1H; H-5), 3.64 (t, $J = 8.9$ Hz, 1H; H-4).

3-*O*-Benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl azide (19b)

*From **20***: Compound **20** (112 mg, 0.178 mmol) and trimethylsilyl azide (0.22 mL, 1.7 mmol) were dissolved in CH_2Cl_2 (1 mL) containing molecular sieves 4 A (0.16 g), and the mixture was cooled down to −78 °C. A solution of TMSOTf in 1,2-dichloroethane (0.2 M; 50 µl, 0.01 mmol) was added, and the whole was stirred for 2 h, while being warmed up to −20 °C. The reaction was quenched with Et_3N (0.2 mL), diluted with AcOEt, and filtered through Celite. The filtrate was washed successively with water and brine, and dried over Na_2SO_4; the solvent was evaporated in vacuo. The residue was purified by silica-gel column chromatography (10–20% AcOEt in hexane) to afford 63.7 mg (70%) of **19b**. M.p. 129–131°; $[\alpha]_D = 37.1$ ($c = 1.1$ in chloroform); ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.83-7.72$ (m, 4H; Phth), 7.55–7.37 (m, 5H; Ph), 7.00–6.85 (m, 5H; Ph), 5.64 (s, 1H; benzylidene CH), 5.43 (d, $J = 9.6$ Hz, 1H; H-1), 4.80 and 4.45 (ABq, $J = 12.2$ Hz, each 1H; benzyl CH_2), 4.49–4.42 (m, 2H; H-3, H-6), 4.14 (dd, $J = 10.2$, 9.2 Hz, 1H; H-2), 3.91–3.70 (m, 3H; H-4, H-5,

H-6′); $C_{28}H_{24}N_4O_6$ (512.52): calcd C 65.62, H 4.72, N 10.53; found C 65.80, H 4.73, N 10.53.

From ***21***: NaH (60%, 792 mg, 20 mmol) was added to an ice-cold solution of compound **21** (4.60 g, 9.9 mmol) and benzyl bromide (2.35 mL, 19.8 mmol) in DMF, and the mixture was stirred for 19 h, while being gradually warmed up to room temperature. The solution was quenched with Et_3N, diluted with AcOEt, washed successively with aq NH_4Cl and brine, and dried over Na_2SO_4; the the solvent was evaporated in vacuo. The residue was purified by silica-gel column chromatography (toluene/EtOH 19:1) to afford 3.9 g (72%) of compound **19b**.

3-*O*-Benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl azide (16b): A solution of **19b** (500 mg, 0.98 mmol) and DL-camphorsulfonic acid (21 mg, 0.09 mmol) in MeOH (10 mL) was stirred under heating with oil bath (50 °C). After 30 min, CH_2Cl_2 (2 mL) was added and stirring was continued at the same temperature for additional 1.5 h. The mixture was processed as described for **21** and purified by silica-gel column chromatography (AcOEt/toluene 1:1) to afford 317 mg (83%) of compound **16b**. M.p. 147–149 °C; $[\alpha]_D = +26.9$ ($c = 1.1$ in chloroform); ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.81-6.92$ (m, 9H; aromatic), 5.42 (d, $J = 9.2$ Hz, 1H; H-1), 5.42 and 4.73 (ABq, $J = 12.2$ Hz, each 1H; benzyl CH_2), 4.31 (dd, $J = 10.6$, 8.6 Hz, 1H; H-3), 4.08 (dd, $J = 10.6$, 9.2 Hz, 1H; H-2), 3.98 (m, 2H; H-6), 3.86 (ddd, $J = 9.6$, 8.6, 3.6 Hz, 1H; H-4), 3.65 (ddd, $J = 9.6$, 3.6, 3.6 Hz, 1H; H-5), 3.01 (br d, $J = 3.6$ Hz, 1H; 4-OH), 2.35 (br s, 1H; 6-OH); ^{13}C NMR (67.5 MHz, $CDCl_3$, 25 °C): $\delta = 85.8$ (C-1), 78.9, 77.2, 74.2, 71.7, 62.1, 55.0; $C_{21}H_{20}N_4O_6$ (424.42): calcd C 59.43, H 4.75, N 13.20; found C 59.18, H 4.64, N 13.19.

***O*-(2,3,4-Tri-*O*-benzyl-*α*-D-fucopyranosyl)-(1→6)-3-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl azide (17b)**: Compounds **16b** (55 mg, 0.13 mmol) and **18** (67 mg, 0.14 mmol) were treated as described for the preparation of **17a**, with the use of $CuBr_2$ (64 mg, 0.29 mmol), Bu_4NBr (93 mg, 0.29 mmol), and molecular sieves 4A (0.2 g) in CH_2Cl_2/DMF (5:1, −10 °C–RT., 17 h) to afford 80.2 mg (73%) of **17b**, together with corresponding β-isomer (7.2 mg, 7%). $[\alpha]_D = -34.1$ ($c = 1.0$ in chloroform); ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.83-6.84$ (m, 24H; aromatic), 5.32 (d, $J = 9.6$ Hz, 1H; H-1^1), 3.71 (m, 1H; H-4^F), 3.67 (m, 1H; H-5^1), 1.14 (d, $J = 6.4$ Hz, 3H; H-6^F); ^{13}C NMR (67.5 MHz, $CDCl_3$): $\delta = 98.7$ (C-1^F), 85.7 (C-1^1), 79.2 77.2, 76.8, 76.4, 75.6, 74.9, 74.4, 73.0, 72.8, 67.9, 66.9, 55.0; $C_{48}H_{48}N_4O_{10}$ (840.94): calcd C 68.56, H 5.75, N 6.66; found C 68.21, H 5.67, N 6.49.

β-Isomer: ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 5.34$ (d, $J = 9.6$ Hz, 1H; H-1^1), 4.41 (d, $J = 7.6$ Hz, 1H; H-1^F), 1.19 (d, $J = 6.3$ Hz, 3H; H-6^F); ^{13}CNMR (67.5 MHz, $CDCl_3$): $\delta = 103.8$ (C-1^F), 85.8 (C-1^1), 82.4, 79.0, 77.2, 76.9, 76.5, 76.2, 75.3, 74.6, 74.4, 73.2, 72.1, 70.7, 68.3, 55.1.

***p*-Methoxyphenyl *O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-*α*-D-mannopyranosyl)-(1→3)-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-*β*-D-mannopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-*α*-L-fucopyranosyl)-(1→6)]-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (24a)**

Method A (with trichloroacetimidate ***15****)*: A solution of TMSOTf (0.2 M in 1,2-dichloroethane; 1.5 µl, 0.3 µmol) was added to a precooled (−78 °C) mixture of compounds **15** (16.5 mg, 0.0118 mmol) and **17a** (27.1 mg, 0.0294 mmol) in CH_2Cl_2 (0.3 mL) containing molecular sieves 4A (0.04 g), and the mixture was stirred at this temperature. After 1 h, an additional amount of TMSTOf (0.3 µmol) was added and the mixture was gradually warmed up to −45 °C (≈1 h) and kept at that temperature for 0.5 h. The reaction mixture was quenched with Et_3N, diluted with AcOEt, and filtered through Celite. The filtrate was washed successively with aq $NaHCO_3$ and brine, and dried over Na_2SO_4; the solvent was evaporated in vacuo. The residue was subjected to a column of Bio-Beads S-X1 (toluene/AcOEt 1:1) and pentasaccharide-containing fractions were further purified by silica-gel column chromatography (hexane/AcOEt 1:1) to afford 18.0 mg (71%) of compound **24a**. $[\alpha]_D = -0.5$(c = 0.9 in chloroform); ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.86-6.55$ (m, 62H; aromatic), 5.47 (d, $J = 8.3$ Hz, 1H; H-1^1), 5.45 (br s, 2H; H-2^4, benzylidene CH), 5.39 (d, $J = 8.3$ Hz, 1H; H-1^2), 5.38 (br s, 1H; H-2^3), 5.25 (d, $J = 1.5$ Hz, 1H; H-1^4), 3.61 (s, 3H; OMe), 2.08 and 1.74 (2s, each 3H; Ac), 0.86 (d, $J = 6.3$ Hz, 3H; H-6^F); ^{13}C NMR (67.5 MHz, $CDCl_3$, 25 °C): $\delta = 101.1$ (benzylidene CH), 99.3, 98.6, 98.0, 96.94, 96.93 (anomeric carbons), 79.9, 79.2, 78.8, 77.6, 76.4, 75.4, 75.2, 75.0, 74.8, 74.7, 74.6, 74.1, 73.8, 73.4, 72.7, 72.1, 71.7, 70.5, 68.7, 66.3, 66.0, 63.7, 56.5, 55.8, 55.5; $C_{122}H_{120}N_2O_{30}$ (2094.31): calcd C 70.60, H 5.88, N 1.30; found C 70.13, H 5.82, N 1.19.

Method B (with fluoride ***14****)*: A mixture of Cp_2HfCl_2 (5.1 mg, 0.013 mmol), AgOTf (6.9 mg, 0.027 mmol), and molecular sieves 4A (0.04 g) in CH_2Cl_2 (0.2 mL) was stirred at 0 °C for 10 min and then cooled down to −78 °C. Compounds **14** (12.0 mg, 0.0095 mmol) and **17a** (26.4 mg, 0.029 mmol) were added as a solution in CH_2Cl_2 (0.6 mL), and the whole was stirred for 3 h, while being gradually warmed up to −10 °C. After being stirred for additional 2 h at −10 °C, additional amounts of Cp_2HfCl_2 (2.0 mg, 0.005 mmol) and AgOTf (2.8 mg, 0.011 mmol) were added and stirring was continued at room temperature overnight. The resulting mixture was quenched with aq $NaHCO_3$ (RT, 10 min), diluted with AcOEt, and filtered through Celite. The filtrate was washed successively with aq $NaHCO_3$ and brine, and dried over Na_2SO_4; the solvent was evaporated in vacuo. The residue was subjected to a column of Bio-Beads S-X1 (toluene). Fractions containing pentasaccharide were collected and further purified by preparaetive TLC (hexane/AcOEt 1:1) to afford 8.1 mg (39%) of compound **24a**.

***O*-(2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-*α*-D-mannopyranosyl)-(1→3)-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-*β*-D-mannopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-*α*-L-fucopyranosyl)-(1→6)]-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl azide (24b)**: Compounds **15** (34.0 mg, 0.0486 mmol) and **17b** (40.9 mg, 0.0486 mmol) were treated with TMSOTf (1 µmol) and molecular sieves 4A (0.04 g) in CH_2Cl_2 (0.6 mL; −78– −40 °C, 1 h) as desribed for **24a**, method A. The mixture was processed as described above and purified by Bio-Beads S-X1 (toluene/AcOEt 1:1) and by silice-gel column chromatography (10–30% AcOEt in hexane) to afford 40.1 mg (79%) of compound **24b**. $[\alpha]_D = -10.8$ ($c = 1.0$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.87-6.72$ (m, 58H; aromatic), 5.48 (d, $J = 8.3$ Hz, 1H; H-1^1), 5.44 (m, 2H; H-2^4, benzylidene CH), 5.37 (dd, $J = 2.6$, < 1 Hz, 1H; H-2^3), 5.22 (d, $J = 1.5$ Hz, 1H; H-1^4), 5.11 (d, $J = 9.3$ Hz, 1H; H-1^2), 2.07 and 1.74 (2s, each 3H; Ac), 1.02 (d, $J = 6.3$ Hz, 3H; H-6^F); ^{13}C (67.5 MHz, $CDCl_3$): $\delta = 101.1$ (benzylidene CH), 99.3, 98.6, 96.9, 96.8 (C-1^{2,3,4,F}), 84.9 (C-1^1), 79.4, 79.1, 78.8, 77.8, 77.6, 77.2, 76.9, 76.0, 76.0, 75.1, 75.0, 74.8, 74.7, 74.6, 74.0, 73.4, 73.3, 73.3, 73.2, 72.7, 72.1, 71.7, 70.4, 68.7, 68.5, 68.3, 67.9, 66.3, 66.1, 63.2, 56.4, 55.4; $C_{120}H_{119}N_5O_{28}$ (2079.30): calcd C 69.32, H 5.77, N 3.37; found C 69.00, H 5.75, N 3.27.

***p*-Methoxyphenyl *O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-*α*-D-mannopyranosyl)-(1→3)-*O*-(2-*O*-acetyl-*β*-D-mannopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-*α*-L-fucopyranosyl)-(1→6)]-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (25a)**: Compound **24a** (12.8 mg, 5.9 µmol) was dissolved in a precooled (0 °C) solution of 5% trifluoroacetic acid in CH_2Cl_2 (1 mL). After 10 min, the reaction was quenched with aq $NaHCO_3$ and extracted three times with $CHCl_3$. The combined organic layers were dried over Na_2SO_4, and the solvent was evaporated in vacuo. The residue was purified by preparative TLC (toluene/AcOEt 1:1) to afford 10.8 mg (88%) of compound **25a**. $[\alpha]_D = +2.4$ ($c = 0.7$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.87-6.55$ (m, 57H; aromatic), 5.46 (d, $J = 7.8$ Hz, 1H; H-1^1), 5.39 (d, $J = 8.3$ Hz, 1H; H-1^2), 5.32 (dd, $J = 3.4$, < 1 Hz, 1H; H-2^3), 5.23 (br s, 1H; H-1^4), 5.21 (dd, $J = 2.9$, 1.5 Hz, 1H; H-2^3), 3.61 (s, 3H, OMe), 2.12 and 1.72 (2s, each 3H; Ac); ^{13}C NMR (67.5 MHz, $CDCl_3$): $\delta =$ 98.5, 98.0, 97.6, 97.0 and 96.9 (anomeric carbons), 79.6, 78.7, 77.7, 77.3, 76.9, 75.5, 75.3, 75.2, 74.8, 74.7, 74.4, 74.3, 74.0, 73.8, 73.4, 73.4, 73.2, 72.7, 71.8, 71.7, 70.9, 69.3, 69.0, 66.9, 65.9, 63.6, 62.3, 56.4, 55.8, 55.5; $C_{120}H_{120}N_2O_{30}$ (2070.29): calcd C 69.55, H 5.93, N 1.35; found C 69.18, H 5.88, N 1.46.

***O*-(2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-*α*-D-mannopyranosyl)-(1→3)-*O*-(2-*O*-acetyl-*β*-D-mannopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-*α*-L-fucopyranosyl)-(1→6)]-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl azide (25b)**: Compound **24b** (27.8 mg, 0.0134 mmol) was treated with 5% trifluoroacetic acid in CH_2Cl_2 (0 °C, 30 min), and the proceedure described for **25a** was followed. Purification by silica-gel column chromatography (toluene/AcOEt 5:1–3:1) afforded 25.0 mg (94%) of compound **25b**. $[\alpha]_D = +10.7$ ($c = 1.1$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.88-6.72$ (m, 53H; aromatic), 5.46 (d, $J = 7.8$ Hz, 1H; H-1^1), 5.32 (br d, $J = 3.2$ Hz, 1H; H-2^4), 5.23 (d, $J = 1.5$ Hz, 1H; H-1^4), 5.20 (dd, $J = 3.4$, 2.0 Hz, 1H; H-2^3), 5.11 (d, $J = 9.3$ Hz, 1H; H-1^2), 2.11 and 1.74 (2s, each 3H; Ac), 1.02 (d, $J = 6.8$ Hz, 3H; H-6^F); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 98.45$, 97.62, 96.94 and 96.19 (C-1^{2,3,4,F}), 84.80 (C-1^1), 79.5, 78.7, 78.6, 77.8, 77.4, 77.4, 76.9, 76.1, 76.0, 75.3, 75.2, 75.2, 74.8, 74.8, 74.8, 74.5, 74.3, 74.0, 73.5, 73.3, 73.2, 72.8, 71.8, 71.7, 70.9, 69.3, 69.0, 67.7, 66.9, 66.1, 63.2, 62.2,

0947-6539/98/0411-2188 $ 17.50+.50/0 *Chem. Eur. J.* **1998**, *4*, No. 11

56.4, 55.3; $C_{113}H_{115}N_5O_{28}$ (1991.19): calcd C 68.16, H 5.82, N 3.52; found C 67.71, H 5.85, N 3.43.

***p*-Methoxyphenyl *O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-*O*-[(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)]-(2-*O*-acetyl-β-D-mannopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→6)]-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside** (**26a**): A mixture of compound **25a** (20.0 mg, 9.7 μmol), silver trifluoromethenesulfonate (AgOTf; 4.0 mg, 16 μmol), and molecular sieves 4 A (0.03 g) in CH_2Cl_2 (0.8 mL) was stirred at 0 °C. Compound **9** (5.4 mg, 11 μmol) was added dropwise as a solution in CH_2Cl_2 (0.2 ml), and the mixture was warmed up to room temperature and stirred for 4 h. The reaction was quenched by aq $NaHCO_3$ (RT, 10 min), diluted with $CHCl_3$, and filtered through Celite. The filtrate was washed with $CHCl_3$ and the aqueous layer was back-extracted twice with $CHCl_3$. The combined organic layers were dried over Na_2SO_4, and the solvent was evaporated in vacuo. The residue was purified by preparative TLC (toluene/AcOEt 2:1) to afford 18.8 mg (77 %) of compound **26a** together with recovered **25a** (3.2 mg). $[\alpha]_D = +9.5$ ($c = 1.3$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.99-6.54$ (m, 72H; aromatic), 5.43 (d, $J = 8.3$ Hz, 1H; H-1^1), 5.37 (d, $J = 8.8$ Hz, 1H; H-1^2), 5.39 and 5.35 (each 1H; H-2^4, H-2^4'), 5.24 (br s, 1H; H-2^3), 3.60 (s, 3H; OMe), 2.10, 1.95 and 1.77 (3s, each 3H; Ac); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 99.3$, 99.2, 97.93, 97.75, 96.93 and 96.85 (anomeric carbons), 79.5, 79.4, 78.2, 78.0, 77.7, 77.2, 76.4, 75.4, 75.3, 75.2, 74.7, 74.5, 74.4, 74.2, 74.1, 73.8, 73.4, 73.0, 72.6, 71.9, 71.7, 71.4, 70.8, 68.9, 68.8, 68.6, 68.4, 67.8, 67.0, 66.1, 65.9, 63.8, 56.6, 55.7, 55.4; $C_{149}H_{152}N_2O_{36} \cdot H_2O$ (2564.88): calcd C 69.77, H 6.05, N 1.09; found C 69.61, H 6.01, N 1.05.

***O*-(2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-*O*-[(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)]-(2-*O*-acetyl-β-D-mannopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→6)]-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl azide** (**26b**): Compound **25b** was transformed into **26b** with the procedure described for the preparation of **26a**. Compound **25b** (39.6 mg, 0.0199 mmol) was treated with **9** (11.8 mg, 0.023 mmol) in the presence of AgOTf (10.3 mg, 0.040 mmol), and molecular sieves 4 A (0.1 g) (0 °C – RT, 4 h). Purification by silica-gel column chromatography (10–30 % AcOEt in toluene) afforded 36.1 mg (74 %) of compound **26b** as well as recovered **25b** (6.0 mg). $[\alpha]_D = +4.4$ ($c = 0.6$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.80-6.71$ (m, 68H; aromatic), 5.43 (d, $J = 8.3$ Hz, 1H; H-1^1), 5.38 and 5.35 (each dd, $J = 3$, 1 Hz, 1H; H-2$^{4,4'}$), 5.24 (br s, 1H; H-2^3), 5.21 (d, $J = 1.5$ Hz, 1H; H-1^4), 2.10, 1.95 and 1.76 (3s, each 3H; Ac); ^{13}C NMR (67.5 MHz, $CDCl_3$): $\delta = 99.3$, 99.2, 97.9, 96.9 and 96.8 (C-1$^{2,3,4,4',F}$), 84.9 (C-1^1), 79.4, 78.2, 78.0, 77.9, 77.7, 77.2, 76.7, 76.1, 76.0, 75.3, 75.2, 75.0, 74.7, 74.7, 74.6, 74.4, 74.3, 74.1, 73.4, 73.1, 72.7, 71.9, 71.8, 71.4, 70.8, 69.0, 68.8, 68.6, 68.4, 67.9, 67.0, 66.1, 63.3; $C_{142}H_{145}N_5O_{34} \cdot H_2O$ (2483.77): C 68.67, H 5.97, N 2.82; found C 68.73, H 6.00, N 2.60.

***p*-Methoxyphenyl *O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-*O*-[(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)]-(2-*O*-acetyl-β-D-mannopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl]-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→6)]-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside** (**27a**): Compound **26a** (14.6 mg, 5.7 μmol) was dissolved in EtOH (2 mL), which contained ethylenediamine (0.3 mL), and the solution was heated under reflux for 7 h. Volatiles were removed in vacuo, and the residue was co-evaporated with toluene. The residue was dissolved in MeOH (3 mL) and treated at 0 °C with acetic anhydride (0.5 mL) for 30 min. The mixture was evaporated in vacuo and purified by preparative TLC (toluene/AcOEt 7:1) to afford 10.9 mg (85 %) of compound **27a**. $[\alpha]_D = -25.3$ ($c = 0.7$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.37-6.71$ (m, 64H; aromatic), 6.51 (br d, $J = 9.2$ Hz, 1H; NHCO), 5.31 (br d, $J = 3.0$ Hz, 1H; H-2^3), 5.27 (d, $J = 1.5$ Hz, 1H; H-1^4), 5.10 (br d, $J = 8.6$ Hz, 1H; NHCO), 5.04 (d, $J = 5.0$ Hz, 1H; H-1^1), 3.71 (s, 3H; OMe), 1.98, 1.94 and 1.64 (3s, each 3H; Ac), 0.88 (d, $J = 6.6$ Hz, 3H; H-6^F); ^{13}C NMR (67.5 MHz, $CDCl_3$): $\delta = 100.6$, 100.4, 99.4, 99.3, 98.6 and 98.0 (anomeric carbons), 80.2, 79.7, 79.5, 78.9, 77.9, 77.2, 76.0, 75.4, 74.8, 74.7, 74.4, 74.3, 74.1, 73.5, 73.4, 73.3, 73.0, 72.8, 72.2, 72.0, 71.7, 71.5, 71.2, 68.9, 68.8, 68.5, 68.3, 68.2, 67.6, 66.7, 66.4, 66.3, 55.6, 55.0, 50.0, 23.4 and 23.2 (NHCO*C*H$_3$), 21.0 (OCO*C*H$_3$).

***p*-Methoxyphenyl *O*-(α-D-mannopyranosyl)-(1→6)-*O*-[(α-D-mannopyranosyl)-(1→3)]-(2-*O*-acetyl-β-D-mannopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl]-(1→4)-*O*-[(α-L-fucopyranosyl)-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranoside** (**1a**): Compound **27a** (10.9 mg, 4.9 μmol) was hydrogenated over 10 % Pd-C (6 mg) in MeOH/AcOH/H_2O (7:2:1) at room temperature for 24 h. The mixture was filtered through a membrane filter and the filtrate was subjected to a column of Bio-Gel P-2 (H_2O) to afford 5.3 mg (91 %) of **28**. ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.01$ (m, 4H, aromatic), 5.51 (br d, $J = 4$ Hz, 1H; H-2^3), 5.12 (d, $J = 1.0$ Hz, 1H; H-1^4), 4.97 (d, $J = 8.3$ Hz, 1H; H-1^1), 4.91 (d, $J < 1$ Hz, 1H;, H-1^4), 4.83 (d, $J = 3.3$ Hz, 1H; H-1^F), 4.63 (d, $J = 7.9$ Hz, 1H; H-1^2), 3.80 (s, 3H; OMe), 2.18, 2.07 and 2.04 (3s, each 3H; Ac), 1.01 (d, 3H, $J = 6$ Hz; H-6^F).

This material was dissolved in 50 mM NaOH in D_2O and the progress of the reaction was monitored by ^{1}H NMR. After standing for 2 h, the solution was neutralized with acetic acid, and the solvent was evaporated in vacuo. The residue was subjected to a column of Bio-Beads P-2 (H_2O) to afford, after lyophilization, 5.0 mg (88 %) of **1a**. ^{1}H NMR (400 MHz, D_2O, 60 °C, *t*BuOH adjusted to $\delta = 1.23$): $\delta = 7.01$ (m, 4H; aromatic), 5.10 (d, $J = 1.0$ Hz, 1H; H-1^4), 4.98 (d, $J = 8.3$ Hz, 1H; H-1^1), 4.90 (d, $J = 1.5$ Hz, 1H; H-1^4), 4.84 (d, $J = 3.9$ Hz, 1H; H-1^F), 4.76 (s, 1H; H-1^3), 4.66 (d, $J = 7.8$ Hz, 1H; H-1^2), 4.22 (br s, 1H; H-2), 4.06 (dd, $J = 3.4$, 1.5 Hz, 1H; H-2^4), 3.80 (s, 3H; OMe), 2.07 and 2.04 (2s, each 3H; Ac), 1.01 (d, $J = 6.8$ Hz, 3H; H-6^F); ^{13}C NMR (100 MHz, D_2O): $\delta = 104.3$, 102.8, 102.1 and 101.3 (anomeric carbons), 82.2, 82.5, 80.6, 76.1, 75.9, 75.3, 75.2, 74.4, 73.9, 73.7, 72.1, 72.0, 71.9, 71.7, 71.6, 71.5, 71.2, 69.8, 68.7, 68.6, 68.5, 68.4, 67.5, 62.9, 62.7, 62.2, 61.7, 57.5, 56.8, 56.6.

***O*-(3,4,6-Tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-*O*-[(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)]-(2-*O*-acetyl-β-D-mannopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl]-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→6)]-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside** (**29**): Compound **26b** (20.2 mg, 8.2 μmol) was treated with ethylenediamine (0.3 mL) in EtOH (1 mL) under reflux for 22 h. The resulting mixture was evaporated in vacuo and co-evaporated with EtOH and then with toluene, and was dissolved in MeOH (1.5 mL). The solution was treated at 0 °C with acetic anhydride (0.5 mL) for 1 h and evaporated in vacuo to afford crude **27b**. This material was dissolved in 0.2 M methanolic NaOMe (1 mL) and stirred at room temperature for 22 h. The mixture was quenched with acetic acid, evaporated in vacuo and purified through a column of Sephadex LH-20 (H_2O) to afford compound **29**. $[\alpha]_D = -17.6$ ($c = 1.1$ in chloroform); ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.65-7.09$ (m, 60H, aromatic), 5.80 (br d, $J = 8.9$ Hz, 1H; NHCO), 5.65 (br d, $J = 8.6$ Hz, 1H; NHCO), 5.12 (br s, 1H; H-1^4), 2.98 and 2.84 (2br s, each 1H; OH), 2.52 (br s, 2H; 2OH), 1.84 and 1.72 (2s, each 3H; Ac), 1.06 (d, $J = 6.6$ Hz, 3H; H-6^F); ^{13}C NMR (67.5 MHz, $CDCl_3$): $\delta = 100.8$, 100.6, 99.6, 99.4 and 98.0 (C-1$^{2,3,4,4',F}$), 88.3 (C-1^1), 82.4, 80.1, 80.8, 79.7, 79.3, 78.2, 76.3, 76.2, 75.9, 75.2, 74.9, 74.4, 73.8, 73.4, 73.0, 72.8, 72.2, 71.7, 71.5, 71.3, 70.3, 69.1, 68.9, 68.1, 66.6, 66.5, 65.3.

***N*^{2}-Benzyloxycarbonyl-*N*^{4}-{*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-*O*-[(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)]-(β-D-mannopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-D-fucopyranosyl)-(1→6)]-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl}-L-asparagine benzyl ester** (**30**): DCC (4.8 mg, 0.023 mmol) was added to a solution of Z-Asp-OBn (16.0 mg) in CH_2Cl_2 (1 mL), and the mixture was stirred at 0 °C for 30 min. The precipitate was filtered off, and the filtrate was evaporated in vacuo to afford the crude anhydride, which was dissolved in AcOEt (1 mL). A solution of compound **29** (8.0 mg, 3.7 μmol) in MeOH was added followed by Lindlar catalyst (5 mg). The mixture was stirred under an H_2 atmosphere at room temperature for 2 h and then filtered through Celite. The filtrate was evaporated in vacuo and purified through a column of Sephadex LH-20 (MeOH) to afford 8.3 mg (91 %) of compound **30**. $[\alpha]_D = -2.5$ ($c = 0.5$ in chloroform); ^{1}H NMR (270 MHz, $CDCl_3$-D_2O, 25 °C, TMS): $\delta = 7.35-7.09$ (m, 60H; aromatic), 5.11 (m, 2H; H-1^4, CO_2CH_2Ph), 5.07 and 5.02 (ABq, $J = 12.2$ Hz, each 1H; CO_2CH_2Ph), 2.83 (dd, $J = 16.1$, 4.9 Hz, 1H; CH_{2Asn}), 2.58 (dd, $J = 16.1$, 3.9 Hz, 1H; CH_{2Asn}), 1.73 and 1.67 (2s, each 3H; Ac), 1.03 (d, $J = 6.3$ Hz, 3H, H-6^{Fuc}); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 100.8$, 100.6, 99.7, 99.4 and 97.9 (C-1$^{2,3,4,4',F}$), 79.8 (C-1^1), 82.4, 80.1, 80.0, 80.0, 78.7, 77.2, 76.1, 75.9, 75.1, 74.9, 74.8, 74.4, 73.7, 73.4, 73.1, 72.7, 72.2, 71.7, 71.5, 71.2, 70.3, 69.1, 68.8, 68.6, 68.1, 67.1, 67.1, 66.9, 66.5, 66.2, 65.3; $C_{143}H_{158}N_4O_{35}$ (2492.86): calcd C 68.90, H 6.39, N 2.25; found C 68.41, H 6.53, N 2.28.

***N*^{4}-[*O*-(α-D-mannopyranosyl)-(1→3)-*O*-[(-α-D-mannopyranosyl)-(1→6)]-(β-D-mannopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-**

(1→4)-*O*-[(α-D-fucopyranosyl)-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl}-L-asparagine (1b): Compound **30** (9.0 mg, 3.6 μmol) was hydrogenated over 10% Pd-C in MeOH/AcOH/H_2O (7:2:1; 1.5 mL) at room temperature for 44 h. The resulting mixture was filtered through membrane filter and the filtrate was evaporated and subjected to a column of Sephadex G-50 (H_2O) to afford, after lyophilization, 4.1 mg (97%) of compound **1b**. ^{1}H NMR (400 MHz, D_2O, 60 °C, *t*BuOH adjusted to $\delta = 1.23$): $\delta = 5.10$ (d, $J = 1.5$ Hz, 1H; H-1^4), 5.06 (d, $J = 9.8$ Hz, 1H; H-1^1), 4.90 (d, $J = 1.5$ Hz, 1H; H-1$^{4'}$), 4.86 (d, $J = 3.9$ Hz, 1H; H-1^F), 4.76 (s, 1H; H-1^3), 4.67 (d, $J = 7.8$ Hz, 1H; H-1^2), 4.22 (br s, 1H; H-2^3), 4.10 (q, $J = 6.8$ Hz, 1H; H-5^F), 4.06 (dd, $J = 3.4$, 1.5 Hz, 1H; H-2^4), 3.96 (dd, $J = 3.4$, 1.5 Hz, 1H; H-2$^{4'}$), 2.88 (dd, $J = 16.8$, 4.2 Hz, 1H; CH_{2Asn}), 2.74 (dd, $J = 16.8$, 7.6 Hz, 1H; CH_{2Asn}), 2.07 and 2.00 (2s, each 3H; Ac), 1.19 (d, 3H, $J = 6.6$ Hz; H-6^F); ^{13}C NMR (100 MHz, D_2O): $\delta = 104.3$, 102.7, 102.1, 101.3 and 101.0 (C-1$^{2,3,4,4',F}$), 79.9 (C-1^1), 82.2, 81.5, 79.8, 76.9, 76.1, 75.9, 75.2, 74.5, 74.4, 73.7, 73.5, 72.1, 72.0, 71.9, 71.7, 71.6, 71.5, 71.2, 69.9, 68.5, 68.5, 68.2, 67.5, 62.9, 62.7, 61.7, 56.6, 55.3, 53.0.

Received: April 20, 1998 [F1105]

[1] T. W. Rademacher, R. B. Parekh, R. A. Dwek, *Ann. Rev. Biochem.* **1988**, *57*, 785; A. Varki, *Glycobiology* **1993**, *3*, 97.

[2] A. Kobata, *Acc. Chem. Res.* **1993**, *26* , 319

[3] F. Barresi, O. Hindsgaul, in *Modern Methods in Carbohydrate Synthesis*, (Eds.: S. H. Khan, R. O. O'Neill), Harwood, Amsterdam, **1996**, p. 251. For more recent publications, see; T. Iimori, H. Ohtake, S. Ikegami, *Tetrahedron Lett*. **1997**, *38*, 3415; G. Hodosi, P. Kovàc, *J. Am. Chem. Soc*. **1997**, *119*, 2335; D. Crich, S. Sun, *J. Org. Chem*. **1997**, *62*, 1198, and references therein. For enzymatic approaches, see: G. Watt, L. Revers, M. C. Webberley, I. B. H. Wilson, S. Flitsch, *Angew. Chem.* **1997**, *109*, 2445; *Angew. Chem. Int. Ed. Engl*. **1997**, *36*, 2354; J. Taubken, B. Sauerbrei, J. Thiem, *J. Carbohydr. Chem*. **1993**, *12*, 651; S. Singh, M. Scigelova, D. H. G. Crout, *Chem. Commun*. **1996**, 993.

[4] a) H. Paulsen, O. Lockhoff, *Chem. Ber.* **1981**, *114*, 3101; b) P. J. Garegg, P. Ossowski, *Acta Chem. Scand. Ser. B* **1983**, *37*, 249; c) C. A. A. van Boechkel, T. Beetz, S. F. van Aelst, *Tetrahedron* **1984**, *40*, 4097.

[5] C. A. A. van Boeckel, T. Beetz, *Recl. Trav. Chim. Pays-Bas* **1985**, *104*, 171.

[6] K. Toshima, K. Tatsuta, *Chem. Rev.* **1993**, 1503.

[7] F. Barresi, O. Hindsgaul, *J. Am. Chem. Soc.* **1991**, *113*, 9376; F. Barresi, O. Hindsgaul, *Synlett* **1992**, 759.

[8] G. Stork, G. Kim, *J. Am. Chem. Soc.* **1992**, *114*, 1087; G. Stork, J. J. La Clair, *J. Am. Chem. Soc.* **1996**, *118*, 247.

[9] a) Y. Ito, T. Ogawa, *Angew. Chem.* **1994**, *106*, 1843; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1765; b) A. Dan, Y. Ito, T. Ogawa, *J. Org. Chem.* **1995**, *60*, 4690; c) A. Dan, Y. Ito, T. Ogawa, *Tetrahedron Lett.* **1995**, *36*, 7487; d) A. Dan, Y. Ito, T. Ogawa, *Carbohydr. Lett.* **1996**, *1*, 469; e) Y. Ito, T. Ogawa, *J. Am. Chem. Soc.* **1997**, *119*, 5562.

[10] M. Fukuda, in *Molecular Glycobiology* (Eds.: M. Fukuda, O. Hindsgaul) IRL, Oxford, **1994**, p. 1.

[11] For example: K. Yamashita, K. Totani, Y. Iwaki, I. Takamizawa, N. Tateishi, T. Higasi, Y. Sakamoto, A. Kobata, *J. Biochem. (Tokyo)* **1989**, *105*, 728; K. Yamashita, K. Taketa, S. Nishi, K. Fukushima, *Cancer Res.* **1993**, *53*, 2970.

[12] For general discussion, see: H. Paulsen, *Angew. Chem.* **1982**, *94*, 184; *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 155.

[13] T. Ogawa, M. Matsui, *Carbohydr. Res.* **1977**, *54*, C17.

[14] P. J. Garegg, I. Kvarnström, A. Niklasson, G. Niklasson, S. C. T. Svensson, *J. Carbohydr. Chem.* **1993**, *12*, 933.

[15] T. Ogawa, K. Katano, M. Matsui, *Carbohydr. Res.* **1978**, *64*, C3; F. Yamazaki, S. Sato, T. Nukada, Y. Ito, T. Ogawa, *Carbohydr. Res.* **1990**, *201*, 31.

[16] T. Nakano, Y. Ito, T. Ogawa, *Carbohydr. Res.* **1993**, *243*, 43.

[17] The PMB group has been extensively used as a valuable hydroxy-protecting group: Y. Oikawa, T. Yoshioka, O. Yonemitsu, *Tetrahedron Lett*. **1982**, *23*, 885; T. W. Greene, P. G. Wuts, *Protective Groups in Organic Synthesis*, 2nd ed., Wiley, New York, **1991**, p. 53. For cyclic *p*-methoxybenzylidene acetal formation under oxidative conditions, see: Y. Oikawa, T. Yoshioka, O. Yonemitsu, *Tetrahedron Lett*. **1982**, *23*, 889.

[18] When the purification by size-exclusion column chromatography was omitted at the stage of the mixed acetal **12**, disaccharide **11** with identical quality was obtained in 51% overall yield based upon **10**.

[19] *S* configuration does not seem to be a critical factor for successful intramolecular aglycon delivery. Detailed discussion will be reported in due course. (M. Lergenmüller, T. Nukada, K. Kuramochi, A. Dan, T. Ogawa, Y. Ito, unpublished results)

[20] H. Lönn, *J. Carbohydr. Chem.* **1987**, *6*, 301.

[21] S. Sato, Y. Ito, T. Nukada, T. Ogawa, *Carbohydr. Res.* **1987**, *167*, 197.

[22] T. Fukuyama, A. A. Laird, L. M. Hotchkiss, *Tetrahedron Lett.* **1985**, *26*, 6291.

[23] For a review on preparations and reactions of glycosyl azides, see: Z. Györgydeák, L. Szilágy, H. Paulsen, *J. Carbohydr. Chem.* **1993**, *12* , 139.

[24] a) C. Unversagt, H. Kunz, *J. Prakt. Chem*. **1992**, *334*, 570; b) W. Bröder, H. Kunz, *Carbohydr. Res.* **1993**, *249*, 221.

[25] R. U. Lemieux, K. B. Hendricks, R. V. Stick, K. James, *J. Am. Chem. Soc.* **1975**, *97*, 4056.

[26] S. Sato, M. Mori, Y. Ito, T. Ogawa, *Carbohydr. Res.* **1986**, *155*, C6.

[27] K. Suzuki, T. Maeta, T. Suzuki, T. Matsumoto, *Tetrahedron Lett*. **1989**, *30*, 6879.

[28] For instance: C. Unverzagt, H. Kunz, *Bioorg. Med. Chem.* **1994**, *2*, 1189.

[29] O. Kanie, S. C. Crawley, M. M. Palcic, O. Hindsgaul, *Carbohydr. Res.* **1993**, *243*, 139.

[30] I. Matsuo, Yu. Nakahara, Y. Ito, T. Nukada, Y. Nakahara, T. Ogawa, *Bioorg. Med. Chem.* **1995**, *3*, 1455.

[31] Y. Ito, Y. Ohnishi, T. Ogawa, Y. Nakahara, *Synlett*, in press.

[32] C. Unverzagt, *Angew. Chem.* **1994**, *106*, 1170; *Angew. Chem. Int. Ed. Engl*. **1994**, 33, 1102; J. Seifert, C. Unverzagt, *Tetrahedron Lett.* **1996**, 37, 6527; C. Unverzagt, *Angew. Chem.* **1996**, *108*, 2507; *Angew. Chem. Int. Ed. Engl.* **1996**, 35, 2350.

[33] W. Gunther, H. Kunz, *Angew. Chem.* **1990**, *102*, 1068; *Angew. Chem. Int. Ed. Engl.* **1990**, 29, 1050.

[34] Y. Ito, T. Ogawa, M. Numata, M. Sugimoto, *J. Am. Chem. Soc.* **1988**; A. Hasegawa, T. Nagahama, H. Ohki, K. Hotta, H. Ishida, M. Kiso, *J. Carbohydr. Chem.* **1991**, 10, 493; A. Hasegawa, H. Ohki, T. Nagahama, H. Ishida, M. Kiso, *Carbohydr. Res.* **1991**, 212, 277; T. J. Martin, R. R. Schmidt, *Tetrahedron Lett.* **1992**, 33, 6123; A. V. Demchenko, G.-J. Boons, *Tetrahedron Lett*. **1998**, 39, 3065; J. C. Castro-Palomino, Y. E. Tsvetkov, R. R. Schmidt, *J. Am Chem. Soc.* **1998**, *120*, 5434.

[35] Y. Ichikawa, in *Glycopeptides and Related Compounds* (Eds.: D. G. Large, C. D. Warren), Marcel Dekker, New York, **1997**, p. 79; C.-H. Wong, R. L. Halcomb, Y. Ichikawa, T. Kajimoto, *Angew. Chem.* **1995**, *107*, 453, 569; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 412, 521.

  0947-6539/98/0411-2190 $ 17.50+.50/0