# Synthesis of aliphatic *O*-dimannosyl amino acid building blocks for solid-phase assembly of glycopeptide libraries

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The preparation of α(1,2)-, α(1,3)- and α(1,6)-linked mannose disaccharides is described. The protected α-D-Man(1→3)-D-Man disaccharide was synthesized using the trichloroacetimidate method, while the Königs–Knorr procedure was employed in the preparation of the 1→2- and 1→6-linked disaccharides. Glycosylation of $N^{\alpha}$-Fmoc-Ser-OPfp, $N^{\alpha}$-Fmoc-Thr-OPfp and $N^{\alpha}$-Fmoc-Hyp-OPfp with the dimannosyl bromides afforded the activated building blocks, in moderate to high yield, for direct use in solid-phase synthesis of glycopeptide libraries.

## Introduction

During the last decade, animal lectins have been recognized as important sugar-binding proteins recognizing endogenous carbohydrate ligands as well as sugar chains on the surface of bacteria, viruses and parasites. Three major classes of lectins have been outlined:[1] the C-type, the P-type (*e.g.*, the mannose 6-phosphate receptor) and the galectins (preferential binding of β-galactoside containing glycoconjugates). All C-type lectins require $Ca^{2+}$ for binding of carbohydrates and three subgroups can be distinguished: (*i*) endocytic receptors with both type I (*e.g.*, the mannose receptor) and type II (*e.g.*, the asialoglycoprotein receptor) transmembrane orientation, (*ii*) cell-adhesion molecules (*e.g.*, the selectins) and (*iii*) the collectins. The latter subgroup comprises a number of soluble, circulating proteins, of which the mannose-binding proteins (MBPs), conglutinin, collectin-43 (CL-43) and the lung surfactant proteins (*e.g.*, SP-A and SP-D) are the main members.[1–3] The collectins form oligomers (bouquets or cruciform structures, except for the monomeric CL-43) of trimeric subunits each composed of a collagenous triple-helical 'stalk' formed by three identical polypeptide chains ending in a C-terminal globular region.[2,3] Each globular head thus contains three carbohydrate-recognizing domains (CRDs). A wide range of sugar ligands having equatorial 3- and 4-OH groups bind to the CRDs of collectins; mannose, glucose, ManNAc, GlcNAc, and L-fucose (probably *via* 2- and 3-OH groups) being the most important.[2]

Recently, a high-resolution (1.8 Å) crystal structure[4] of the head-and-neck part of a MBP (rat serum mannose-binding protein A) showed that the individual carbohydrate-binding sites of the trimeric subunit are separated from each other by 53 Å. This distance is too long to allow multivalent binding of a single trimer to a typical high-mannose oligosaccharide. Also, the CRDs of different trimers in the bouquet-like oligomers seem too far apart to enable binding of the same ligand. However, simultaneous ligand-binding to two different oligomeric bouquets may be possible due to the proximity of the sugar-binding site to the edge of the trimer.[4] These facts could explain why the natural oligosaccharides and synthetic mannose neoglyconjugate ligands and the mannose monosaccharide bind to MBP with essentially equal affinity. Given this detailed knowledge about the structure of the MBPs, we have been challenged to synthesize building blocks for the assembly of triantennary glycopeptide ligand libraries to study their binding to the MBP. Access to such a large variety of glycopeptide mimics may be achieved by multi-column peptide synthesis[5,6] or in library methods.[7] The methods of Furka[8] and Lam[9] (the one bead–one peptide procedure) have been further refined in our laboratory to a portion-mixing procedure using the biocompatible poly(ethylene glycol)–poly-(*N*,*N*-dimethylacrylamide) copolymer (PEGA) resin.[10,11]

At present the most efficient and versatile method for the preparation of O-glycopeptides employs protected glycosylated amino acids as building blocks in a sequential assembly of peptides.[12–14] In general, synthesis of glycosylated amino acid building blocks involves a multistep sequence of glycosylation, selective removal of the α-carboxylic protecting group, activation of the α-carboxylic acid, and in some cases even exchange of the $N^{\alpha}$-protecting group.[15,16] In recent years we have described[17] and further developed[18–24] an alternative, simplified strategy for the preparation of O-glycosylated amino acids. This protocol involves a direct glycosylation of a fluoren-9-ylmethoxycarbonyl-(Fmoc) protected amino acid with a preactivated glycosyl donor.

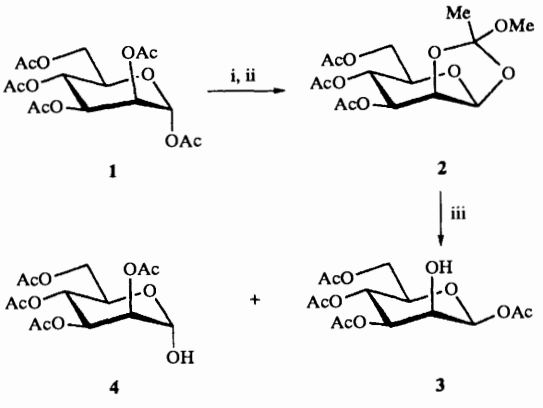
The sugar hydroxy groups are preferably protected as esters, *e.g.* acetates and benzoates, which can be easily removed under mild homogeneous conditions.[25] The Fmoc group is excellent as an $N^{\alpha}$-protecting group in glycopeptide synthesis since only mild bases (morpholine[26] or piperidine[27]) are required for its cleavage. Furthermore, the Fmoc group is ideally stable under the acidic conditions normally used in O-glycosylations.[12] Application[17] of pentafluorophenyl (Pfp) esters as protection for the α-carboxylic function has proved advantageous[18–24] as they are stable to both the acidic glycosylation conditions and purification by silica gel (flash,[17–19] vacuum liquid[20,23]) chromatography or reversed-phase HPLC.[22] Moreover, such protected building blocks can subsequently be used directly in the solid-phase synthesis of O-linked glycopeptides. Here, we report on the synthesis of $N^{\alpha}$-Fmoc-Ser-OPfp, $N^{\alpha}$-Fmoc-Thr-OPfp and $N^{\alpha}$-Fmoc-Hyp-OPfp glycosylated with α(1,2)-, α(1,3)- and α(1,6)-linked mannose disaccharides. While the synthesis of suitably protected glycosylated serine and threonine building blocks is well established,[18,21,23] most reports[28–34] on the glycosylation of *trans*-L-hydroxyproline (Hyp) have involved benzyloxycarbonyl (Cbz)-protected methyl and benzyl esters of Hyp, which do not allow immediate use of the resulting building blocks in solid-phase glycopeptide synthesis. However, in a single report[35] Fmoc-Hyp-OMe was employed successfully.

Previously, 1,3,4,6-tetra-*O*-acetyl-2-*O*-(tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-mannopyranose has been prepared for the synthesis of α(1,2)-dimannosyl-Ser and -Thr building blocks;[18,19] however, owing to difficulties with the reproducibil-

ity in this synthesis, we decided to use benzoyl protecting groups in the non-reducing ring in the present work. Likewise, the successful synthesis of a glycosylated Fmoc-Thr-OPfp building block having a phosphorylated α(1,6)-mannobiosyl moiety has been reported.[20] A similar approach was adapted in the present work for the synthesis of $N^{\alpha}$-Fmoc-Thr[α-D-Man(1→6)-D-Man]-OPfp and the corresponding Ser and Hyp building blocks. In order to reduce the synthetic work on selectively protected monosaccharide glycosyl acceptors, the desired acceptor having a free hydroxy group in the 3-position should preferably be obtained *via* either the orthoacetate **2** or the phenyl thioglycoside **15**. Although the synthesis[36] of 1,2,4,6-tetra-*O*-acetyl-α-D-mannopyranose starting from orthoacetate **2** has been improved considerably[37] this procedure was rejected owing to a lack of experimental details for the modified procedure. Instead, the thioglycoside approach was considered. Liptak and co-workers[38] prepared phenyl 2-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside in two steps from phenyl 1-thio-α-D-mannopyranoside; however, the overall yield was only 21%. Therefore, another procedure involving the selective 4,6-*O*-monobenzylidenation[39] of phenyl 1-thio-α-D-mannopyranoside and a subsequent phase-transfer-catalysed (PTC) selective benzoylation, recently reported for the corresponding ethyl thioglycoside by Garegg *et al.*,[40] was chosen for the preparation of the acceptor **17**.

## Results and discussion

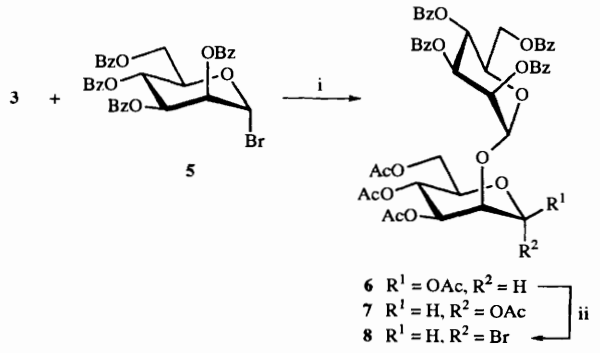
First penta-*O*-acetyl-α-D-mannopyranose **1** was converted into the corresponding bromide, which was treated directly with methanol and 2,6-dimethylpyridine (2,6-lutidine) in chloroform to give 3,4,6-tri-*O*-acetyl-1,2-*O*-methoxyethylidene-β-D-mannopyranose **2** in 72.5% overall yield. Subsequent hydrolysis of the orthoester **2** in 10% aq. trifluoroacetic acid (TFA)–acetonitrile at 0 °C afforded crude 1,3,4,6-tetra-*O*-acetyl-β-D-mannopyranose **3** (29%) by direct crystallization of the product mixture (Scheme 1); recrystallization yielded pure acceptor **3**




**Scheme 1** *Reagents and conditions:* i, HBr–HOAc, $CH_2Cl_2$, 4 Å MS; ii, MeOH, 2,6-lutidine, $CHCl_3$, room temp. 72.5% overall; iii, 10% TFA (aq.)–MeCN (1:10), 0 °C, 27% **3** cryst. ($Et_2O$–light petroleum); 25% **4** (containing 2% β-anomer and 2% of an impurity cryst. (hexane–ethyl acetate)

(27% overall). From the mother-liquor a substantial amount (25%) of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranose **4** was obtained by successive vacuum liquid chromatography (VLC) on silica gel and crystallization; only a trace (~2%) of the β-anomer could be detected by NMR spectroscopy.

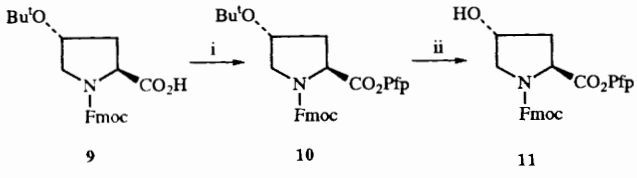
Initially, the glycosylation of acceptor **3** with 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide[41] **5** (Scheme 2) was performed under the Königs–Knorr conditions (in dichloromethane at −40 °C) reported[18,19] for the reaction involving




**Scheme 2** *Reagents and conditions:* i, AgOTf, 2,6-di-Bu$^t$-4-Me-pyridine, −55 to −40 °C, 80%; ii, HBr–HOAc, $CH_2Cl_2$, 4 Å MS, 86–92%

the acetylated donor. However, owing to the low yields (~50%) of a mixture of anomers (**6** and **7**) obtained, a careful optimization was performed. Addition of the non-nucleophilic base 2,6-di-*tert*-butyl-4-methylpyridine (0.3–0.8 mol equiv.), as well as the reaction temperature, were found to be important. Optimal yields (~80%) of pure compound **6** were obtained when 0.8 mol equiv. of base was employed at −55 to −40 °C. A similar result was recently noted by Nikolaev *et al.*[42]

Condensation of $N^{\alpha}$-Fmoc-Hyp(Bu$^t$)-OH **9** and pentafluorophenol mediated by dicyclohexylcarbodiimide (DCCI) gave $N^{\alpha}$-Fmoc-Hyp(Bu$^t$)-OPfp **10** in moderate yield (65%) after purification by VLC on silica gel. Treatment of compound **10** with neat TFA[23] gave a slightly impure product which failed to crystallize from diethyl ether, ethyl acetate–hexane and dichloromethane–light petroleum (boiling range 60–80 °C). Purification by VLC yielded 80% of the partially protected compound **11** (Scheme 3). All the hydroxyproline derivatives




**Scheme 3** *Reagents and conditions:* i, DCCI, Pfp-OH, THF, −20 °C, 65%; ii, TFA, room temp. 80%

**9–11** appeared as mixtures of *cis–trans* isomers[29,31,43–45] in NMR spectroscopy (Tables 1 and 2) due to the rotational barrier of the carbimide bond between the hydroxyproline and the Fmoc group; however, TLC showed no distinguishable separation into two bands. Apparently, the major isomer for all three compounds has the same structure (either *cis* or *trans*) since the α-protons consistently are seen at a slightly lower field. Also, in the $^{13}C$ NMR spectra several general features of the major isomer were observed. Thus, the chemical shifts of $C^{\alpha}$, $C^{\gamma}$ and the amide carbonyl were at slightly lower field than those of the minor conformers while the opposite trend was seen for $C^{\beta}$ and the acid/ester carbonyl group. These observations are in accord with the $^{13}C$ NMR data of other simple derivatives of Hyp.[43–46] When compared to simple dipeptides containing Hyp[45] the most abundant conformer of compounds **9–11** is most likely the *trans* isomer.

Silver triflate-promoted glycosylation of $N^{\alpha}$-Fmoc-Ser-OPfp,[17] $N^{\alpha}$-Fmoc-Thr-OPfp[17] and $N^{\alpha}$-Fmoc-Hyp-OPfp **11** with the α-bromide **8** in dry dichloromethane afforded the corresponding protected dimannosylated building blocks **12** (69%), **13** (76%) and **14** (56%), respectively (Scheme 4). A suitable reaction temperature which limited the reaction time to 4–5.5 h was determined from small-scale experiments starting at −60 °C. The temperature was then gradually raised

**Table 1** $^1$H NMR data (500 MHz; $CDCl_3$; ~ 5 mg/cm$^3$) for hydroxyproline derivatives **9**–**11**. $\delta$-Values in ppm and coupling constants in Hz

| | **9** major:minor isomer 2:1 | | **10** major:minor isomer 5:4 | | **11** major:minor isomer 5:4 | |
|---|---|---|---|---|---|---|
| $H^\alpha$ | 4.54 (8.8, 4.4) | 4.41 | 4.85 (8.5, 5.2) | 4.81 (8.6, 5.2) | 4.89 (8.0) | 4.86 (8.0) |
| $H^\beta$ | 2.34 (12.9, 5.2) | 2.29 | 2.53–2.33 | 2.53–2.33 | 2.55 (13.5, 8.0) | 2.64 (13.0, 8.0, 2.2) |
| | 2.22 (12.9, 8.5, 6.5) | 2.22 | | | 2.35 (13.5, 8.0, 4.8) | 2.39 (13.0, 8.0, 4.9) |
| $H^\gamma$ | 4.35 (6.0) | 4.35 | 4.43 | 4.43 | 4.67 | 4.67 |
| $H^\delta$ | 3.74 (10.4, 6.2) | 3.78 (10.9, 6.1) | 3.84 (10.6, 6.2) | 3.86 (10.9, 6.4) | 3.81–3.77 | 3.81–3.77 |
| | 3.39 (10.4, 5.2) | 3.41 (10.9, 4.8) | 3.46 (10.6, 4.8) | 3.50 (10.9, 4.7) | | 3.61 (11.5) |
| Fmoc-CH | 4.32 (7.1) | 4.20 (6.6) | 4.34 (7.2) | 4.28 (7.1) | 4.32 (7.0) | 4.28 (7.0) |
| Fmoc-$CH_2$ | 4.49–4.42 | 4.49–4.42 | 4.51–4.41 | 4.51–4.41 | 4.53–4.46 | 4.53–4.46 |
| Fmoc-ArH | 7.80 (7.5) | 7.74 (7.6) | 7.81 (7.0) | 7.80 | 7.81 (7.3) | 7.80 |
| | 7.62 (8.0) | 7.58 (7.3) | 7.64 (7.3) | 7.64 (7.1) | 7.64 (7.3) | 7.66–7.62 |
| | 7.44 (7.4) | 7.39 (7.6) | 7.46–7.31 | 7.58 (7.5) | 7.46–7.39 | 7.59 (7.4) |
| | 7.35 (7.3) | 7.32 | | 7.46–7.31 | 7.35 (7.6) | 7.46–7.39 |
| | | | | | | 7.34–7.29 |
| $CMe_3$ | 1.25 | 1.21 | 1.28 | 1.27 | | |
| OH | | | | | 1.70 | 1.70 |

**Table 2** $^{13}$C NMR data (125 MHz; $CDCl_3$) for hydroxyproline derivatives **9**–**11**; $\delta$-values in ppm ($^{19}$F–$^{13}$C coupling constants in Hz are given for **11**)

| | **9** major:minor isomer 2:1 | | **10** major:minor isomer 5:4 | | **11** major:minor isomer 5:4 | |
|---|---|---|---|---|---|---|
| $C^\alpha$ | 58.0 | 57.3 | 57.6 | 57.4 | 57.6 | 57.4 |
| $C^\beta$ | 36.9 | 38.6 | 37.9 | 39.0 | 38.5 | 39.6 |
| $C^\gamma$ | 69.0 | 68.3 | 69.1[c] | 68.2[b,c] | 70.2 | 69.3 |
| $C^\delta$ | 53.3 | 53.8 | 53.3 | 53.8 | 54.7 | 55.4 |
| Fmoc-CH | 47.1[a] | 47.1[a] | 47.1[b] | 47.1[b] | 47.2 | 47.1 |
| Fmoc-$CH_2$ | 68.0 | 67.6 | 68.2[b,c] | 67.8[c] | 67.8 | 68.4 |
| Fmoc-Ar-C | 143.7–120.0 | 144.0–119.9 | 143.8–119.9 | 144.1–119.9 | 143.9–120.0 | 144.1–120.0 |
| $CO_2R$ | 175.6 | 177.6 | 168.8 | 169.0 | 168.6 | 168.8 |
| CON-Fmoc | 156.0 | 154.4 | 154.9 | 154.1 | 154.9 | 154.5 |
| Pfp-C | | | 141.0,[b] 139.8, 137.9[b] | 141.0,[b] 139.8, 137.9[b] | 141.0[b] (254), 139.8 (251), 137.9[b] (255) | 141.0[b] (254), 139.8 (251), 137.9[b] (255) |
| $Me_3CO$ | 74.3[b] | 74.3[b] | 74.5[b] | 74.5[b] | | |
| $CMe_3$ | 28.2[b] | 28.2[b] | 28.1[b] | 28.1[b] | | |

[a] Two distinct peaks. [b] Signal of doubled intensity. [c] May be interchanged vertically.

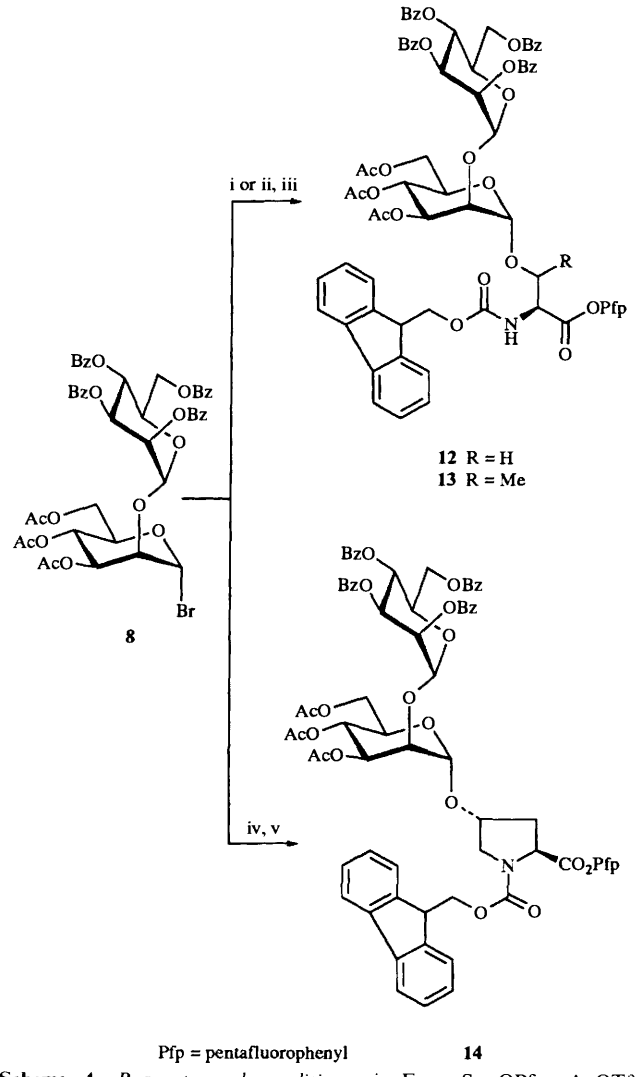
until a significant conversion could be detected by TLC. This appropriate reaction temperature could also be determined visually by the sudden formation of insoluble silver bromide. The successively higher final temperature necessary for completion of the glycosylation of Ser, Thr and Hyp derivatives indicates their putative decreasing reactivity. It is noticeable that the lower yields of the serine and hydroxyproline building blocks **12** and **14** were not associated with the presence of significant amounts of by-products in the reaction mixtures; rather they seem to be inherently more unstable towards purification by VLC on silica gel.

Again, two inseparable isomers of compound **14** were detected by NMR spectroscopy (see Tables 8 and 10 below) and, surprisingly, the isomer ratio (5:4) remained essentially unchanged relative to substrate **11** despite the attachment of the bulky disaccharide moiety at $C^\gamma$. As in compounds **9**–**11**, the α-proton of the major isomer of product **14** was located slightly downfield compared with $H^\alpha$ of the minor isomer. Also, the $^{13}$C NMR data pointed[45] to the *trans* isomer as being the most abundant component.

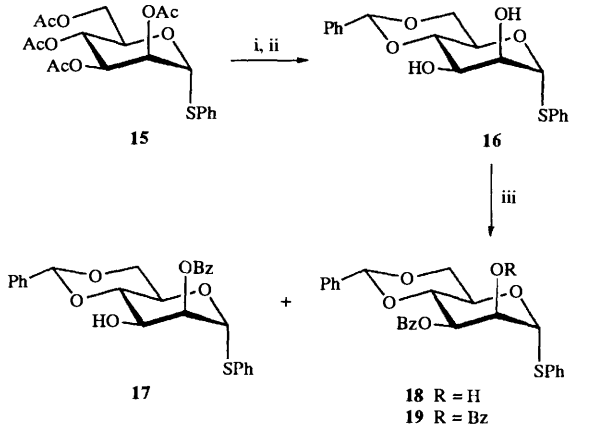
For the synthesis of the α(1,3)-linked mannobioside, acceptor **17** was intended to be coupled to the bromide **5**, since a similarly substituted acceptor, 2-[*p*-(trifluoroacetamido)phenyl]ethyl 2-*O*-acetyl-4,6-*O*-benzylidene-α-D-mannopyranoside, had previously been employed in a Königs–Knorr reaction.[47]

Deacetylation of phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside[48] **15** followed by monobenzylidenation, using α,α-dimethoxytoluene in dimethylformamide (DMF) under toluene-*p*-sulfonic acid (PTSA) catalysis,[49] yielded a complex acetal mixture from which phenyl 4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside **16** was selectively crystallized (~ 25–35%) (Scheme 5). An additional amount of compound **16** (~ 15–25%, always adding up to ~ 50% total yield) was obtained by acid-catalysed equilibration of the mother-liquor and subsequent crystallization. The total yield of ~ 50% is similar to that reported by Kobayashi and co-workers[39,50] (44% overall from D-mannose) using tetrafluoroboric acid as catalyst; however, no experimental details or NMR data were given. The method presented here is also suitable for large-scale preparations as column chromatography is avoided.

Attempts at performing a selective PTC benzoylation[40] of compound **16** gave unpredictable results as the desired compound **17** was obtained in strongly varying yields (30–55%). This problem arises partly from the extreme crystallinity of compound **16** (it precipitates from most solvents, except for DMF, upon cooling to 0 °C) which implies the simultaneous use of large amounts of solvents and careful control of the temperature to avoid precipitation of the educt. Slow addition of benzoyl chloride to the stirred and cooled (just below 0 °C) suspension of compound **16** and tetrabutylammonium hydrogen sulfate (TBAHS) in aq. sodium hydroxide–dichloromethane reduced the tendency for attack at the 3-position, as the low concentration of the highly reactive 2-ionized species of substrate **16** then is matched by a similarly low concentration of benzoylating agent. Purification by VLC on silica gel afforded the desired phenyl 2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside **17** (56%) as the major product whereas the 3-*O*-benzoylated compound **18** and the 2,3-di-*O*-benzoylated compound **19** were obtained in 18.5% and 17% yield, respectively. When compared with the selective benzoylation of the



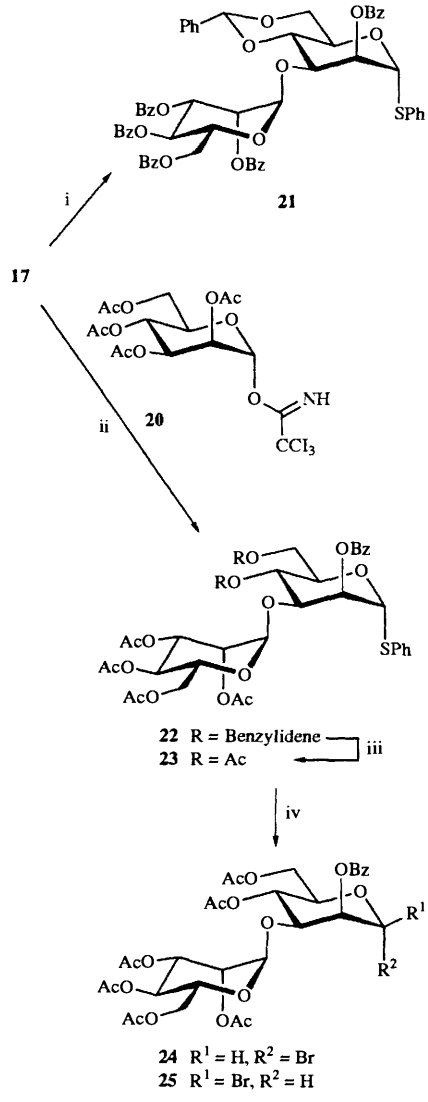

**Scheme 4** *Reagents and conditions:* i, Fmoc-Ser-OPfp, AgOTf, $CH_2Cl_2$, 3 Å MS, −40 to −25 °C, 69%; ii, Fmoc-Thr-OPfp, AgOTf, $CH_2Cl_2$, 3 Å MS, −35 to −20 °C, 76%; iii, 2,4,6-collidine; iv, **11**, AgOTf, $CH_2Cl_2$, 3 Å MS, −40 to −15 °C, 56%; v, 2,6-di-Bu$^t$-4-Me-pyridine




**Scheme 5** *Reagents and conditions:* i, NaOMe–MeOH; ii, Ph-CH(OMe)$_2$, TsOH, DMF, cryst. (MeOH), 50%; iii, BzCl, $Bu_4NHSO_4$, aq. NaOH–$CH_2Cl_2$, 56% **17**

higher reactivity of its 3-OH group are responsible for the poor selectivity. Benzoylation of compound **16** with benzoyl chloride in pyridine–dichloromethane, performed at low temperature (−40 to −20 °C), gave almost exclusively the 3-*O*-benzoylated compound **18** (67%). Furthermore, a lower excess of benzoyl chloride (1.1 mol equiv.) than recommended[40] for the corresponding ethyl thioglycoside (1.5 mol equiv.) was consumed more completely in the PTC reaction (98% *versus* 68% conversion for the ethyl thioglycoside[40]), also pointing to a much higher reactivity of the 3-OH group in compound **16** than in its ethyl thioglycoside analogue. NMR data for compounds **16**–**19** are presented in Tables 3 and 4.

Several attempts at glycosylation of acceptor **17** using bromide **5** as glycosyl donor in the presence of silver trifluoromethanesulfonate (triflate) (−40 °C) in dichloromethane only led to poor yields (25%) of slightly impure disaccharide **21** (Scheme 6). The apparent reluctance to




**Scheme 6** *Reagents and conditions:* i, **5**, AgOTf, $CH_2Cl_2$, 3 Å MS, −40 °C, 25%; ii, TMSOTf, $CH_2Cl_2$, 3 Å MS, −30 °C, 91%; iii, 70% HOAc, 65 °C, then $Ac_2O$–pyridine 20 °C, 94%; iv, $Br_2$, $CH_2Cl_2$, darkness, 91–94%

corresponding ethyl thioglycoside,[40] where 2-*O*-benzoylation predominated (75% yield) and the only by-product isolated was the 2,3-di-*O*-benzoylated compound (13%), it seems likely that a combination of low solubility of compound **16** and a glycosylation of the 3-OH group in differently protected mannosyl acceptors has been reported[51] both under Helferich and Königs–Knorr conditions, but here the trichloroacetimidate method proved successful. Therefore, the trichloroacetimi-

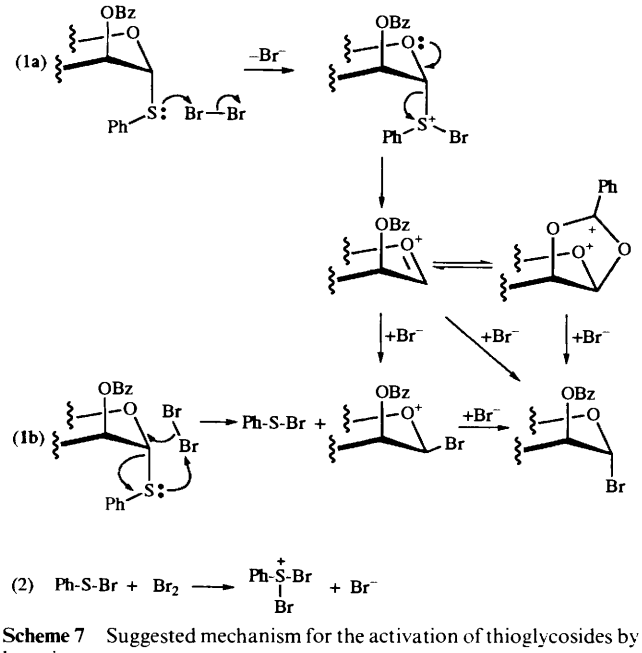
**Table 3** $^1$H NMR data (500 MHz) of benzylidene derivatives **16–19** (in $CDCl_3$ except **16**: $CD_3OD$) chemical shifts in ppm ($J$ in Hz)

| Proton | 16 | 17 | 18 | 19 |
|---|---|---|---|---|
| 1-H | 5.49 (1.5) | 5.68 (1.5) | 5.64 | 5.72 |
| 2-H | 4.18 (3.5, 1.5) | 5.78 (3.6, 1.5) | 4.62 (3.5, 1.5) | 6.01 (3.5) |
| 3-H | 3.95 (10.0, 3.5) | 4.40 (10.0, 3.6) | 5.63 (10.2, 3.5) | 5.86 (10.4, 3.5) |
| 4-H | 4.03 (10.0) | 4.17 (10.0) | 4.41 (10.0) | 4.46 (10.0) |
| 5-H | 4.24 (10.0, 5.0) | 4.49 (10.0, 5.0) | 4.58 (10.0, 5.0) | 4.68 (10.0, 5.0) |
| 6-H$^{eq}$ | 4.13 (10.0, 5.0) | 4.34 (10.4, 5.0) | 4.31 (10.2, 5.0) | 4.38 (10.4, 5.0) |
| 6-H$^{ax}$ | 3.84 (10.0) | 3.95 (10.4) | 3.96 (10.2) | 4.02 (10.4) |
| α-H | 5.62 | 5.73 | 5.67 | 5.73 |
| ArH | 7.57–7.48 | 8.14 | 8.12 | 8.11 |
| | 7.40–7.27 | 7.66–7.50 | 7.64–7.46 | 7.98 |
| | | 7.48–7.33 | 7.40–7.31 | 7.69–7.51 |
| | | | | 7.40–7.34 |

**Table 4** $^{13}$C NMR data (125 MHz) for compounds **16–19**, δ-values in ppm (in $CDCl_3$ except **16**: $CD_3OD$)

| Carbon | 16 | 17 | 18 | 19 |
|---|---|---|---|---|
| C-1 | 91.0 | 87.0 | 88.6 | 87.0 |
| C-2 | 74.2 | 74.1 | 71.2 | 72.5 |
| C-3 | 70.0 | 68.0 | 71.5 | 69.2 |
| C-4 | 80.3 | 79.5 | 76.2 | 76.9 |
| C-5 | 66.5 | 64.7 | 65.2 | 65.3 |
| C-6 | 69.5 | 68.5 | 68.5 | 68.6 |
| C-α | 103.4 | 102.3 | 101.9 | 102.0 |
| *C*OPh | | 165.8 | 165.5 | 165.5 |
| Ar-C | 139.2–127.5 | 137.0–126.3 | 137.0–126.1 | 137.1–126.3 |

date **20** was prepared from 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranose **4** (obtained from orthoester **2**) by reaction with trichloroacetonitrile in dichloromethane containing 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), to give a yield of 97.5%. Omitting the dichloromethane[52] as co-solvent gave a similar yield (92%) of compound **20**, but owing to the low solubility of the crystalline compound **4** in neat trichloroacetonitrile, thus leading to an extended reaction time, the above modified procedure is preferable. Mannosylation of acceptor **17** with trichloroacetimidate **20** using an equimolar amount of trimethylsilyl triflate[51] proceeded very rapidly even at −30 °C; after 15 min[51] only the disaccharide **22** (and a small excess of compound **20**) could be detected by TLC, and compound **22** was isolated in 91% yield. Debenzylidenation of disaccharide **22** in 70% aq. acetic acid and subsequent acetylation afforded the disaccharide **23** in 94% overall yield (Scheme 6). Next, the disaccharide **23** was treated with 1.05 mol equiv. of bromine in dry dichloromethane for 2 h, shielded from light, as earlier reported for the conversion[20] of a protected α(1,6)-dimannosyl thioglycoside into the corresponding α-bromide. However, after purification by VLC on dried silica gel an inseparable mixture of the educt **23** and the α-bromide **24** (in a 2.5:2 ratio), also containing a trace of the putative β-bromide **25**, was obtained. When 1.1 mol equiv. of bromine and an extended reaction time (5 h) were employed, a 10:2:1 mixture of α-bromide **24**, thioglycoside **23** and β-bromide **25** was isolated (no separation by TLC or VLC was observed). Thus, a larger excess (1.2 mol equiv.) of bromine and a reaction time of 5.5 h were required for the optimal conversion of thioglycoside **23** into the pure α-bromide **24**. Therefore, it may be concluded that the activation of the thioglycoside **23** by bromine probably proceeds *via* a series of intermediates such as those depicted in Scheme 7. This explains why the intermediate β-bromide **25** is present in substantial quantities in the product mixture when only a 1.1 mol equiv. of bromine is employed. Additional bromide ions, liberated by reaction (2), Scheme 7, necessary for the otherwise fast equilibration to the more stable α-bromide **24** are thus not available. These observations are in accord with the early work[53,54] of Weygand *et al.* on the conversion of ethyl




**Scheme 7** Suggested mechanism for the activation of thioglycosides by bromine

thioglycosides into bromides. Here inversion of configuration was found to be the case for the glucopyranose derivatives, and the carbocation corresponding to reaction (1a) in Scheme 7 was proposed as intermediate for the α-D-mannopyranose derivative, which gave good yields of the product with retention of configuration. NMR data for compounds **21–24** are given in Tables 5 and 6.

Condensation of glycosyl bromide **24** with $N^2$-Fmoc-Ser-OPfp, $N^2$-Fmoc-Thr-OPfp and $N^2$-Fmoc-Hyp-OPfp **11**, using silver triflate as promoter, afforded the protected, dimannosylated building blocks **26** (72%), **27** (78%) and **28** (66%), respectively (Scheme 8). NMR data for compounds **26–28** are given in Tables 7–10.

**Table 5** $^1$H NMR data (500 MHz) for disaccharides **6**, **8**, **22**–**24** and **31**–**33** in $CDCl_3$, $\delta$-Values in ppm ($J$ in Hz)

| | **6** | **8** | **22** | **23** | **24** | **31** | **32** | **33** |
|---|---|---|---|---|---|---|---|---|
| 1[a]-H | 5.35 (1.8) | 5.28 (1.8) | 5.28 (1.4) | 5.09 | 5.10 (1.7) | 5.17 (1.6) | 5.18 (1.4) | 5.20 (1.4) |
| 2[a]-H | 5.81 (3.2, 2.0) | 5.74 (3.1, 1.9) | 5.37 (2.8, 1.8) | 5.10 | 5.09 (3.1, 1.8) | 5.87 (3.2, 1.8) | 5.86 (3.0, 1.8) | 5.86 (3.2, 1.7) |
| 3[a]-H | 6.09 (10.3, 3.2) | 5.99 (10.1, 3.2) | 5.23[d] | 5.20 (9.4, 2.4) | 5.18 (10.0, 3.1) | 6.04 (10.1, 3.3) | 6.07 (10.1, 3.2) | 6.05 (10.0, 3.2) |
| 4[a]-H | 6.30 (10.2) | 6.08 (10.1) | 5.24[d] | 5.24 (9.7) | 5.22 (9.8) | 6.15 (10.1) | 6.14 (10.0) | 6.22 (10.0) |
| 5[a]-H[c] | 4.89 (3.0) | 4.64 (6.1, 2.1) | 4.19 | 4.08 (6.2, 1.9) | 4.01 (6.0, 2.0) | 4.37 (4.1, 2.2) | 4.36 (4.3, 2.1) | 4.59 (3.0) |
| 6[a]-H | 4.75 (12.1, 2.8) | 4.72 (12.0, 2.1) | 4.30 (12.0, 6.4) | 4.17 (12.1, 6.2) | 4.14 (12.1, 6.0) | 4.49 (12.2, 2.2) | 4.47 (12.1, 2.1) | 4.65 (12.3, 2.4) |
| | 4.49 (12.1, 3.6) | 4.55 (12.0, 6.1) | 4.15 (12.0, 2.0) | 3.99 (12.1, 1.9) | 3.95 (12.1, 2.0) | 4.30 (12.2, 4.2) | 4.30 (12.1, 4.4) | 4.41 (12.3, 3.8) |
| 1[b]-H | 5.93 | 6.72 (1.7) | 5.70 (1.1) | 5.72 | 6.57 (1.3) | 5.85 (1.3) | 6.67 (1.0) | 6.20 |
| 2[b]-H | 4.35 (3.0) | 4.47 (3.0, 1.8) | 5.79 (3.5, 1.2) | 5.72 | 5.65 (3.2, 1.7) | 6.08 (3.2, 1.4) | 6.02 (2.5, 1.0) | 6.14 (3.2) |
| 3[b]-H | 5.26 (9.7, 3.0) | 5.77 (10.0, 3.1) | 4.48 (10.0, 3.6) | 4.28 (9.9, 2.8) | 4.74 (10.0, 3.2) | 5.94 (10.1, 3.2) | 6.36–6.34[d] | 5.81 (10.0, 3.3) |
| 4[b]-H | 5.53 (9.6) | 5.62 (10.0) | 4.30 (9.8) | 5.57 (10.0) | 5.62 (10.1) | 6.24 (10.1) | 6.36–6.34[d] | 6.03 (10.0) |
| 5[b]-H[c] | 3.92 (4.8, 2.4) | 4.24 (4.1, 2.1) | 4.49 (10.0, 5.0) | 4.54 (5.6, 2.3) | 4.21[d] | 4.99 (4.7, 1.7) | 4.65 | 4.30 (5.7, 1.8) |
| 6[b]-H | 4.42 (12.4, 4.9) | 4.40 (12.6, 4.1) | 4.32 (10.2, 5.0) | 4.33 (12.3, 5.6) | 4.35 (12.5, 4.8) | 4.23 (11.0, 4.9) | 4.18 (11.4, 3.7) | 4.25 (10.9, 5.8) |
| | 4.30 (12.4, 2.4) | 4.19 (12.6, 2.1) | 3.95 (10.3) | 4.18 (12.3, 2.3) | 4.21[d] | 3.83 (11.0, 1.8) | 3.88 (11.4, 1.8) | 3.97 (10.9, 1.8) |
| α-H | | | 5.71 | | | | | |
| ArH | 8.10–7.90 | 8.14–7.88 (8 H) | 8.17 | 8.13 | 8.14 | 8.21 | 8.22 | 8.24 |
| | 7.65–7.53 | 7.68–7.55 (3 H) | 7.67 | 7.64 | 7.68 | 8.10–8.05 | 8.10–8.02 | 8.13–8.00 |
| | 7.48–7.39 | 7.51–7.40 (7 H) | 7.58–7.49 | 7.54–7.50 | 7.55 | 7.94–7.69 | 7.94–7.90 | 7.91 |
| | 7.33–7.29 | 7.34–7.29 (2 H) | 7.41–7.34 | 7.37–7.32 | | 7.63–7.30 | 7.64–7.18 | 7.68–7.31 |
| Ac | 2.27, 2.25 | 2.23, 2.22 | 2.23, 2.12, | 2.22, 2.20, 2.19, | 2.23, 2.17, 2.15, | | | |
| | 2.24, 2.12 | 2.13 | 2.05, 1.96 | 2.09, 2.00[e] | 2.14, 1.98, 1.96 | | | |

[a] Designates the non-reducing unit. [b] Designates the reducing unit. [c] For 5-Hs only the two small coupling constants are given. [d] Higher order coupling. [e] Signal with doubled integral.

**Table 6** $^{13}C$ NMR data (125 MHz; $CDCl_3$) for disaccharides **6**, **8**, **21**–**24** and **31**–**33**, $\delta$-values in ppm ($J_{C,H}$ for C-1s in Hz)

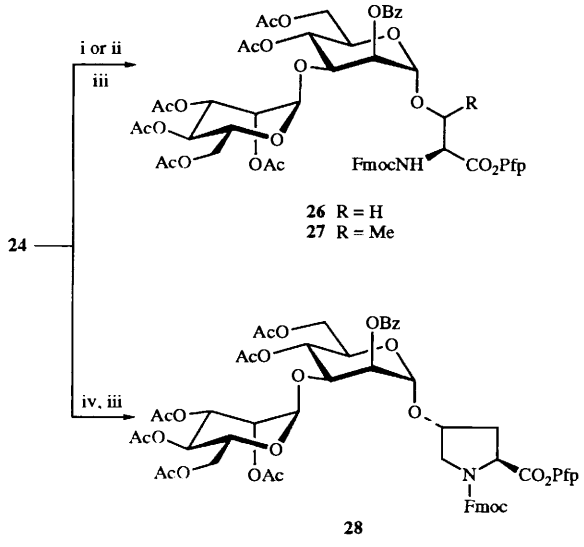
| Carbon | **6** | **8** | **21** | **22** | **23** | **24** | **31** | **32** | **33** |
|---|---|---|---|---|---|---|---|---|---|
| C-1[a] | 98.5 (173.1) | 99.7 (173.4) | 98.9 | 98.7 (177.0) | 99.1 (173.3) | 99.2 (173.2) | 98.0 (173.1) | 98.1 (174.1) | 97.6 (173.5) |
| C-2[a] | 70.7 | 70.4 | 69.9 | 69.1 | 69.7 | 69.6[c] | 70.1 | 70.1[d] | 70.0 |
| C-3[a] | 69.3[c] | 69.1 | 69.7 | 68.7 | 68.3 | 68.2 | 70.2 | 70.1[d] | 70.1 |
| C-4[a] | 66.7 | 67.0 | 66.6 | 66.0 | 65.8 | 65.7 | 66.6 | 66.6 | 66.4 |
| C-5[a] | 69.3[c] | 70.1 | 69.5 | 69.3 | 69.4 | 69.6[c] | 68.9 | 69.0 | 68.8 |
| C-6[a] | 62.4 | 63.2 | 63.0 | 62.7 | 62.3 | 62.3 | 62.5 | 62.4 | 62.4 |
| C-1[b] | 91.0 (161.9) | 84.8 (185.3) | 87.0 | 87.1 (170.0) | 85.6 (170.9) | 83.8 (185.9) | 86.4 (169.3) | 83.8 (186.3) | 77.1 (165.9) |
| C-2[b] | 74.8 | 80.3 | 73.7 | 73.5 | 73.0 | 74.1 | 72.0 | 73.0 | 71.6 |
| C-3[b] | 72.1 | 69.2 | 72.9 | 72.2 | 76.2 | 74.9 | 70.6[c] | 69.2 | 71.8 |
| C-4[b] | 65.9 | 65.4 | 79.1 | 79.1 | 67.5 | 66.3 | 66.9 | 66.0 | 66.2 |
| C-5[b] | 73.3 | 72.9 | 64.9 | 64.9 | 69.6 | 73.1 | 70.6[c] | 73.8 | 78.3 |
| C-6[b] | 61.8 | 61.3 | 68.3 | 68.3 | 62.4 | 61.4 | 67.0 | 65.9 | 66.7 |
| C-α | | | 101.4 | 101.5 (161.1) | | | | | |
| *C*OMe | 171.0, 170.3, 169.3, 168.3 | 170.8, 170.4, 169.2 | | 170.8, 169.8, 169.7, 169.5 | 170.6, 170.5, 170.0, 169.8, 169.6, 169.3 | 170.6, 170.5, 170.0, 169.7, 169.6, 169.3 | | | |
| CO*Me* | 20.9, 20.7, 20.6, 20.5 | 20.7[d], 20.6 | | 20.9, 20.7,[d] 20.6 | 20.8, 20.7, 20.6, 20.5[e] | 20.8, 20.7, 20.6, 20.5[e] | | | |
| *C*OPh | 166.1, 165.5, 165.1, 165.0 | 166.1, 165.6, 165.3, 165.1 | 166.2, 166.0, 165.3, 165.1, 165.0 | 165.9 | 165.6 | 165.4 | 166.0, 165.6,[d] 165.4,[d] 165.2[d] | 165.9, 165.4,[d] 165.3,[d] 165.2[d] | 166.0, 165.5,[d] 165.3,[d] 165.2[d] |
| Ar-C | 133.4–128.2 | 133.7–128.3 | 136.8–126.0 | 136.9–126.0 | 133.7–128.1 | 134.0–128.2 | 133.6–128.2 | 133.7–125.3 | 133.7 128.3 |

[a] Designates the non-reducing unit. [b] Designates the reducing unit. [c] Split signal. [d] Signal of doubled intensity. [e] Signal of an intensity corresponding to more than two carbons.

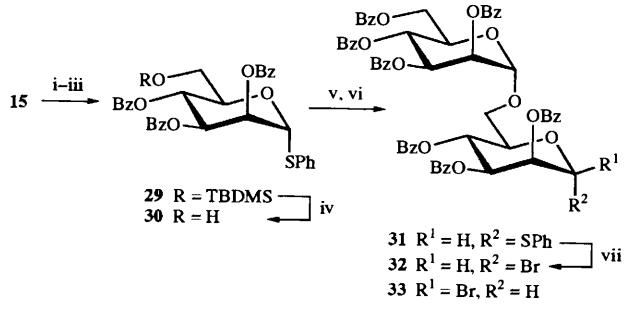
**Table 7** $^1$H NMR data (500 MHz; $CDCl_3$) for protected glycosylated serine and threonine building blocks **12/13**, **26/27** and **34/35**. $\delta$-Values in ppm ($J$ in Hz)

| | 12 | 13 | 26 | 27 | 34 | 35 |
|---|---|---|---|---|---|---|
| 1[a]-H | 5.29 | 5.25 | 5.06 | 5.07 | 5.21 (1.2) | 5.19 (1.5) |
| 2[a]-H | 5.77 (3.1, 1.9) | 5.69 | 5.12 (3.1, 1.9) | 5.10 (3.0, 1.5) | 5.81 | 5.79 (2.8, 1.6) |
| 3[a]-H | 5.98 (10.1, 3.2) | 5.89 (10.0, 2.4) | 5.18 (10.0, 3.3) | 5.18 (10.0, 3.2) | 6.12 (10.2, 2.8) | 6.04 (10.1, 3.1) |
| 4[a]-H | 6.13 (10.0) | 6.05 (10.0) | 5.28 (10.0) | 5.28 (10.0) | 6.20 (10.0) | 6.16 (10.1) |
| 5[a]-H | 4.57 | 4.58 | 3.99 | 3.98 (9.9, 2 × 2.9) | 4.55 | 4.51 |
| 6[a]-H | 4.65 (11.6, 2.0) | 4.55 (12.0, 2.2) | 4.14 (12.0, 4.5) | 4.14 (12.3, 3.5) | 4.67 (12.3, 2.3) | 4.63 (12.1, 2.0) |
| | 4.54 (11.6, 4.8) | 4.48 (12.0, 5.3) | 3.90 (12.0, 1.8) | 3.87 (12.3, 2.3) | 4.42 (12.3, 4.0) | 4.39 (12.1, 4.3) |
| 1[b]-H | 5.17 | 5.27 (2.7) | 5.10 | 5.21 | 5.30 | 5.37 |
| 2[b]-H | 4.21 (2.0) | 4.07 (2.5) | 5.44 | 5.35 | 5.86 | 5.72 (3.0, 1.7) |
| 3[b]-H | 5.40 (9.3, 2.5) | 5.37 (7.7, 2.5) | 4.27 (9.9, 2.5) | 4.28 | 5.99[c] | 5.97 (10.1, 3.2) |
| 4[b]-H | 5.44 (9.3) | 5.34 (8.0) | 5.50 (9.9) | 5.49 (9.8) | 5.99[c] | 6.03 (10.2) |
| 5[b]-H | 4.02 | 3.94 | 4.00 | 4.08 (9.8, 5.6, 2.2) | 4.52 | 4.56 |
| 6[b]-H | 4.34 (12.3, 5.1) | 4.30 (12.3, 5.4) | 4.24 (3.7) | 4.28 (12.2, 5.5) | 4.23 (10.8, 7.8) | 4.22 (10.8, 6.4) |
| | 4.25 (12.3, 2.2) | 4.21 (12.3, 2.0) | | 4.23 (12.2, 2.0) | 3.85 (10.8) | 3.83 (10.8) |
| $H^{\alpha}$ | 4.99 (8.5, 2 × 3.5) | 4.81 (9.7, 2.2) | 5.04 (8.7, 2 × 3.2) | 4.90 (9.7, 2.0) | 5.29 | 5.01 (9.5, 2.2) |
| $H^{\beta}$ | 4.12 (10.8, 3.6) | 4.53 | 4.35 (11.0, 3.0) | 4.62 (6.3, 2.0) | 4.53 | 4.84 (6.4, 2.2) |
| | 4.08 (10.8, 3.0) | | 4.14 | | 4.27 (10.0, 4.4) | |
| $H^{\gamma}$ | | 1.42 (6.3) | | 1.46 (6.3) | | 1.75 (6.4) |
| NH | 6.09 (8.6) | 5.94 (9.0) | 6.07 (8.6) | 5.90 (9.6) | 6.80 (9.2) | 5.91 (9.5) |
| Fmoc-CH | 4.29 (7.0) | 4.20 | 4.29 (6.8) | 4.32 (6.8) | 4.30 (7.4) | 4.37 (7.4) |
| Fmoc-$CH_2$ | 4.49 (7.0) | 4.44 (10.5, 7.2) | 4.56–4.50 | 4.56 (6.8) | 4.52 | 4.57 (10.5, 7.4) |
| | | 4.36 (10.5, 7.2) | | | 4.37 (10.4, 7.8) | 4.50 (10.5, 7.4) |
| ArH | 8.12–7.88 | 8.11–7.84 | 8.15–7.33 | 8.11–7.35 | 8.19–7.89 | 8.16–7.81 |
| | 7.78–7.29 | 7.75–7.25 | | | 7.75–7.16 | 7.88–7.29 |
| Ac | 2.23, 2.21, 2.09 | 2.21, 2.20, 2.11 | 2.20, 2.17, 2.09, 2.07, 1.99, 1.96 | 2.22, 2.16, 2.13, 2.03, 1.98[d] | | |

[a] Designates the non-reducing carbohydrate unit. [b] Designates the reducing carbohydrate unit. [c] Higher order coupling. [d] Signal of doubled integral.




**Scheme 8** *Reagents and conditions:* i, Fmoc-Ser-OPfp, AgOTf, $CH_2Cl_2$, 3 Å MS, −30 to −15 °C, 72%; ii, Fmoc-Thr-OPfp, AgOTf, $CH_2Cl_2$, 3 Å MS, −30 to −20 °C, 78%; iii, 2,6-Bu$^t_2$-4-Me-pyridine; iv, **11**, AgOTf, $CH_2Cl_2$, 3 Å MS, −25 °C, 66%




**Scheme 9** *Reagents and conditions:* i, NaOMe–MeOH; ii, Bu$^t$Me$_2$SiCl, pyridine; iii, BzCl, pyridine; iv, aq. HOAc–THF; v, **5**, AgOTf, $CH_2Cl_2$, 3 Å MS, −45 to −40 °C; vi, 2,4,6-collidine, 86% **31**; vii, $Br_2$, $CH_2Cl_2$, darkness, 83%

The acceptor **30** with a free 6-OH group was prepared from thioglycoside **15** by deacetylation, selective formation of the primary *tert*-butyldimethylsilyl ether (TBDMS ether), followed by benzoylation to compound **29**, which subsequently was hydrolysed as previously described[20] (Scheme 9). However, in the present work the TBDMS ether **29** was obtained in crystalline form, which caused problems in the subsequent hydrolysis step, as it readily precipitated under the earlier reported[20] conditions. Hence, the solvent mixture tetrahydrofuran (THF)–acetic acid–water (1 : 3 : 1) was changed to 3 : 3 : 1 and the amount was increased to keep compound **29** in solution. The isolated yield of the acceptor **30** was 75%.

Coupling of the bromide **5** and the acceptor **30** was performed by the Königs–Knorr method in dichloromethane at −60 to −40 °C which afforded the disaccharide **31** in 86% yield upon repeated purification by VLC on silica gel. Upon treatment with bromine (1.05 mol equiv.) for 3 h, as above, thioglycoside **31** was converted into an anomeric mixture of dimannosyl bromides **32/33** ($\alpha$ : $\beta$ ~ 10 : 1) which were separable by analytical TLC and by preparative VLC to give the $\alpha$-bromide **32** in 74–83% yield. The exact assignment of the $\alpha$- and $\beta$-anomer was determined from the $J_{C\text{-}H}$ coupling of their anomeric carbons, which was 186.3 Hz and 165.9 Hz, respectively (Table 6). Furthermore, MS confirmed compound **33** to contain a bromine atom. Consequently, the reaction of disaccharide **31** with bromine is much faster than that of compound **23**, and an excess of bromine is not required to reach full conversion, but still a mixture of anomeric bromides is obtained.

In the syntheses of building blocks **34** and **35** crude mixtures of anomeric bromides **32** and **33** were coupled directly to $N^{\alpha}$-Fmoc-Ser-OPfp and $N^{\alpha}$-Fmoc-Thr-OPfp, using silver triflate as promoter, in overall yields (from compound **31**) of 74% and 59%, respectively (Scheme 10). Purified $\alpha$-bromide **32** was employed in the coupling with $N^{\alpha}$-Fmoc-Hyp-OPfp **11** which

**Table 8** $^1$H NMR data (500 MHz; $CDCl_3$) for protected glycosylated hydroxyproline building blocks **14**, **28** and **36**. $\delta$-Values in ppm ($J$ in Hz)

| | **14** major : minor isomer 5 : 4 | | **28** major : minor isomer 3 : 2 | | **36** major : minor isomer 5 : 4 | |
|---|---|---|---|---|---|---|
| 1[a]-H | 5.30 | 5.30 | 5.08 | 5.08 | 5.21 | 5.21 |
| 2[a]-H | 5.77 | 5.77 | 5.12 (2.9, 1.7) | 5.12 (2.9, 1.7) | 5.78 | 5.76 (2.8, 1.7) |
| 3[a]-H | 6.00 (10.0, 3.5) | 5.99 (10.0, 3.5) | 5.19 | 5.18 | 6.02 (10.5, 3.0) | 6.04 (10.5, 3.0) |
| 4[a]-H | 6.15 (10.0) | 6.14 (10.0) | 5.26 (9.9) | 5.24 (9.9) | 6.16 (9.9) | 6.18 (9.9) |
| 5[a]-H | 4.60 | 4.60 | 3.98 (9.7, 4.6, 2.2) | 3.98 (9.7, 4.6, 2.2) | 4.48 | 4.51 |
| 6[a]-H | 4.73 (11.7, 2.3) | 4.73 (11.7, 2.3) | 4.10 (12.2, 5.0) | 4.08 (12.2, 5.0) | 4.61 (12.2, 2.2) | 4.66 (12.2, 2.2) |
| | 4.57 (11.7) | 4.57 (11.7) | 3.92 (12.2) | 3.92 (12.2) | 4.38 (12.2, 4.4) | 4.41 |
| 1[b]-H | 5.22 (1.2) | 5.21 (1.2) | 5.20 | 5.20 | 5.33 | 5.31 |
| 2[b]-H | 4.22 (2.4) | 4.18 (2.4) | 5.44 | 5.44 | 5.80 | 5.79 |
| 3[b]-H | 5.38 (9.9, 3.1) | 5.41 (9.8, 3.0) | 4.28 (9.6, 3.0) | 4.28 (9.6, 3.0) | 5.98 (10.2, 3.5) | 6.00 (10.2, 3.5) |
| 4[b]-H | 5.49 (9.9) | 5.50 (9.8) | 5.52 (9.9) | 5.52 (9.9) | 6.08 (10.0) | 6.03 (10.0) |
| 5[b]-H | 4.10–4.03 | 4.10–4.03 | 4.05 (9.9, 6.0, 2.0) | 4.05 (9.9, 6.0, 2.0) | 4.50 | 4.49 |
| 6[b]-H | 4.36 (12.3, 6.0) | 4.38 (12.3, 6.1) | 4.32 (12.1, 5.6) | 4.32 (12.1, 5.6) | 4.23 (11.0, 5.6) | 4.22 (11.0, 5.6) |
| | 4.25 (12.3, 2.2) | 4.25 (12.3, 2.2) | 4.22 (12.1, 2.1) | 4.23 (12.1, 2.1) | 3.83 (11.0) | 3.83 (11.0) |
| H$^\alpha$ | 4.86 (7.7) | 4.76 (7.8) | 4.88 (7.7) | 4.82 (7.7) | 5.08 (7.9) | 5.03 (7.9) |
| H$^\beta$ | 2.77 | 2.77 | 2.77 (13.3, 8.0, 3.3) | 2.81 (13.0, 8.0, 3.7) | 2.99 (13.6, 8.2, 2.5) | 3.02 |
| | 2.35 (13.2, 7.2, 5.4) | 2.39 (13.2, 7.4, 5.3) | 2.45 (13.3, 7.1, 5.4) | 2.51 (13.0, 6.5) | 2.72 (13.6, 7.7, 5.1) | 2.81 (13.5, 7.8, 5.2) |
| H$^\gamma$ | 4.47 | 4.41 | 4.62 | 4.61 | 4.86 | 4.89 |
| H$^\delta$ | 3.75 (11.6, 4.7) | 3.83 (12.2) | 3.87 (11.8, 5.0) | 3.86 | 3.98–3.95 | 4.13 (12.2) |
| | 3.70 (11.6) | 3.67 (12.2, 4.7) | 3.77 (11.8) | | | 3.93 (12.2, 4.1) |
| Fmoc-CH | 4.32 (7.1) | 4.29 (7.0) | 4.35 (7.0) | 4.28 | 4.39 (7.2) | 4.35 (7.0) |
| Fmoc-CH$_2$ | 4.58–4.44 | 4.58–4.44 | 4.57 (10.5, 7.2) | 4.52 (10.5, 6.6) | 4.55 (7.2) | 4.61–4.50 |
| | | | 4.46 (10.5, 7.5) | 4.48 | | |
| ArH | 8.13–7.31 | 8.13–7.31 | 8.15–7.29 | 8.14–7.29 | 8.21–7.25 | 8.21–7.25 |
| Ac | 2.22,[c] 2.21, 2.13[c] | 2.22,[c,d] 2.13[c] | 2.23, 2.17, 2.14, 2.06, 1.99,[c] 1.95[c] | 2.24, 2.16, 2.14, 2.07, 1.99,[c] 1.95[c] | | |

[a] Designates the non-reducing carbohydrate unit. [b] Designates the reducing carbohydrate unit. [c] Split signal. [d] Doubled integral.

**Table 9** $^{13}$C NMR data (125 MHz; $CDCl_3$) for glycosylated Ser/Thr compounds **12**/**13**, **26**/**27** and **34**/**35**. Chemical shifts in ppm ($J_{C,H}$ for C-1s in Hz)

| Carbon | **12** | **13** | **26** | **27** | **34** | **35** |
|---|---|---|---|---|---|---|
| C-1[a] | 99.1[f] (174.1) | 98.1[f] (176.6) | 99.1 (172.9) | 98.8 (174.4) | 97.4 (174.6) | 97.7 (175.0) |
| C-2[a] | 70.5 | 70.3 | 69.6 | 69.6 | 70.4 | 70.3 |
| C-3[a] | 69.3 | 69.3 | 68.5 | 68.5 | 70.2[c,e] | 69.9 |
| C-4[a] | 67.3 | 67.0 | 65.6 | 65.8 | 66.6 | 66.7 |
| C-5[a] | 69.6[c] | 69.4 | 69.4 | 69.4 | 69.1 | 69.0 |
| C-6[a] | 63.2 | 63.0 | 61.9 | 62.0 | 62.5 | 62.6 |
| C-1[b] | 99.5 (173.1) | 100.2 (171.8) | 98.6 (172.5) | 98.4 (173.6) | 98.5 (173.1) | 99.2 (177.0) |
| C-2[b] | 76.2[f] | 75.5[f] | 71.1 | 70.9 | 70.2[e] | 70.2 |
| C-3[b] | 69.6[c] | 69.0 | 75.7 | 75.3 | 69.7 | 69.7 |
| C-4[b] | 66.6 | 67.3 | 66.9 | 67.3 | 66.9 | 67.0[c] |
| C-5[b] | 69.4 | 69.2 | 69.7 | 69.5 | 70.2[c,e] | 70.5 |
| C-6[b] | 62.2 | 62.5 | 62.5 | 62.6 | 66.7 | 67.0[c] |
| *C*OMe | 170.8, 170.3, 169.5 | 170.7, 170.3, 169.3 | 170.5,[c] 170.0, 169.9, 169.6, 169.5 | 170.7, 170.5, 170.1, 169.8, 169.7, 169.4 | | |
| CO*Me* | 20.7[d] | 20.7,[c] 20.6 | 20.8, 20.6,[d] 20.5 | 20.8, 20.6[d,e] 20.4 | | |
| *C*OPh | 166.1, 165.7, 165.2, 165.1 | 166.0, 165.7, 165.1, 165.0 | 165.6 | 165.3 | 166.1, 165.9, 165.7, 165.5, 165.4,[c,e] 165.3 | 166.1, 165.6, 165.5,[c] 165.3[d] |
| Bz Ar-C | 133.6–128.3 | 133.5–128.3 | 133.8–128.7 | 133.7–128.6 | 133.7–128.3 | 133.6–128.3 |
| OCONH | 155.9 | 156.5 | 155.7 | 156.4 | 155.9 | 156.5 |
| *C*O$_2$Pfp | 166.3 | 166.9 | 166.3 | 166.5 | 167.1 | 166.7 |
| C$^\alpha$ | 54.3 | 58.7 | 54.4 | 58.4 | 53.7 | 58.5 |
| C$^\beta$ | 68.9 | 76.6 | 70.1 | 76.8 | 68.1 | 76.8 |
| C$^\gamma$ | | 18.5 | | 18.0 | | 18.7 |
| Fmoc-CH | 47.0 | 47.0 | 47.1 | 47.2 | 47.0 | 47.1 |
| Fmoc-CH$_2$ | 67.6 | 67.4 | 67.5 | 67.5 | 67.9 | 67.9 |
| Fmoc Ar-C | 143.6–120.0 | 143.7–120.0 | 143.5–120.0 | 143.7–120.0 | 143.7–119.9 | 143.7–120.0 |
| Pfp-C | 140.9,[c] 140.0, 138.0[c] | 140.9,[c] 140.0, 138.0[c] | 141.0,[c] 140.0, 138.0[c] | 140.9,[c] 140.0, 138.0[c] | 141.1,[c] 139.9, 138.0[c] | 141.0,[c] 139.9, 137.9[c] |

[a] Designates the non-reducing carbohydrate unit. [b] Designates the reducing carbohydrate unit. [c] Signal of doubled intensity. [d] Signal of an intensity corresponding to more than two carbons. [e] Split signal. [f] Broad signal of low intensity.

afforded building block **36** in 61% yield. NMR data for compounds **34**–**36** are presented in Tables 7–10.

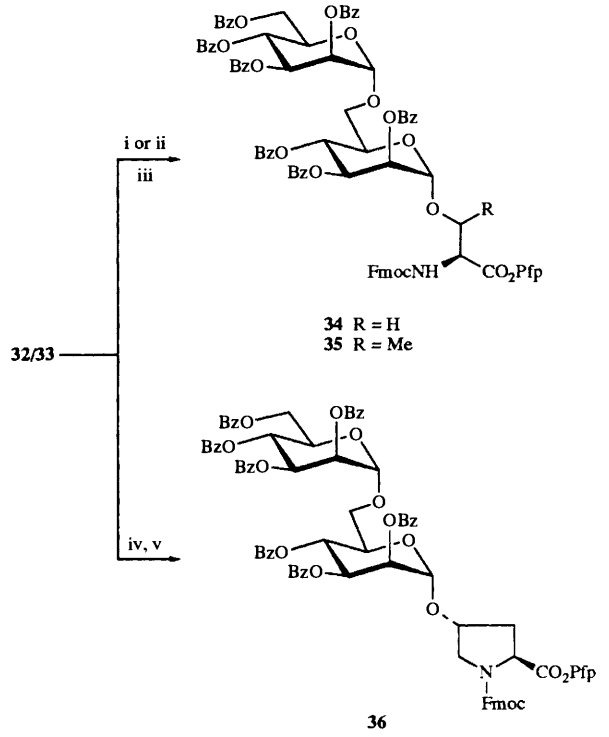
In conclusion, efficient syntheses (Table 11) have been developed for glycosyl amino acids containing 1→2-, 1→3- and 1→6-linked mannose disaccharides ready for incorporation into glycopeptides by solid-phase synthesis using the Fmoc-based continuous-flow method.[55,56]

A reconsideration of the most simple synthetic route to the

**Table 10** $^{13}C$ NMR data (125 MHz; $CDCl_3$) for protected glycosylated hydroxyprolines **14**, **28** and **36**. $\delta$-Values in ppm ($J_{C-H}$ for C-1s in Hz)

| Carbon | **14** major : minor isomer 5 : 4 | | **28** major : minor isomer 3 : 2 | | **36** major : minor isomer 5 : 4 | |
|---|---|---|---|---|---|---|
| C-1[a] | 99.4 (171.9) | 99.2 (174.4) | 99.2[c] (172.5) | 99.2[c] (172.5) | 97.7 (173.4) | 97.5 (172.8) |
| C-2[a] | 70.6 | 70.5 | 69.6[d] | 69.6[d] | 70.6[e] | 70.5[e] |
| C-3[a] | 69.2 | 69.3 | 68.5[c] | 68.5[c] | 69.8[d] | 69.8[d] |
| C-4[a] | 67.4 | 67.3 | 65.5[c] | 65.5[c] | 66.7 | 66.6 |
| C-5[a] | 69.7[c] | 69.7[c] | 69.5[c] | 69.5[c] | 69.2[c] | 69.2[c] |
| C-6[a] | 63.2 | 63.1 | 62.0[c] | 62.0[c] | 62.6[c] | 62.6[c] |
| C-1[b] | 98.6 (170.7) | 97.9 (171.3) | 97.0[c] (171.7) | 97.0[c] (171.7) | 97.3 (171.8) | 96.5 (173.2) |
| C-2[b] | 77.0 | 76.8 | 71.4 | 71.3 | 70.4[e] | 70.4[e] |
| C-3[b] | 69.9 | 69.8 | 76.4 | 76.3 | 69.8[d] | 69.8[d] |
| C-4[b] | 66.4 | 66.5 | 66.8 | 67.0 | 66.8[d] | 67.0 |
| C-5[b] | 69.6 | 69.5 | 69.7 | 69.6[d] | 70.5[e] | 70.6[e] |
| C-6[b] | 62.7 | 62.6 | 62.7 | 62.8 | 66.8[d] | 66.8[d] |
| *C*OMe | 170.8,[e] 170.5, 169.4[e] | 170.8[e], 170.5, 169.4[e] | 170.5, 170.4, 170.0, 169.8, 169.5, 169.4 | 170.5, 170.4, 170.0, 169.8, 169.5, 169.4 | | |
| CO*Me* | 20.8, 20.7, 20.6 | 20.8, 20.7, 20.6 | 20.8, 20.5[d] | 20.8, 20.5[d] | | |
| *C*OPh | 166.0, 165.7, 165.3, 165.1 | 166.0, 165.7, 165.3, 165.1 | 165.7 | 165.6 | 166.1, 165.6[c] 165.5,[d] 165.3[c] | 166.1, 165.6,[c] 165.5,[d] 165.3[c] |
| Bz Ar-C | 133.6–128.3 | 133.6–128.3 | 133.9–128.7 | 133.8–128.7 | 133.7–128.2 | 133.7–128.2 |
| OCONH | 154.6 | 154.0 | 154.6 | 153.9 | 154.7 | 154.2 |
| $CO_2$Pfp | 168.2 | 168.5 | 168.1 | 168.3 | 168.2 | 168.5 |
| $C^{\alpha}$ | 57.8 | 57.4 | 57.7 | 57.4 | 58.0 | 57.7 |
| $C^{\beta}$ | 36.2 | 37.6 | 36.2 | 37.4 | 36.6 | 37.9 |
| $C^{\gamma}$ | 77.2 | 75.1 | 76.7 | 75.5 | 76.6 | 74.4 |
| $C^{\delta}$ | 51.9 | 52.0 | 51.6 | 52.1 | 51.6[e] | 51.6[e] |
| Fmoc-CH | 47.0 | 47.1 | 47.0[c] | 47.0[c] | 47.1 | 47.2 |
| Fmoc-$CH_2$ | 68.0 | 68.4 | 68.1 | 68.5 | 68.1 | 68.5 |
| Fmoc Ar-C | 143.7–120.0 | 144.0–120.0 | 143.6–120.0 | 144.0–120.0 | 143.8–120.0 | 144.1–120.0 |
| Pfp-C | 141.0,[c] 139.7, 137.9[c] | 141.0,[c] 139.7, 137.9[c] | 141.0,[c] 139.8, 137.8[c] | 141.0,[c] 139.8, 137.8[c] | 141.2,[c] 139.6, 137.8[c] | 141.2,[c] 139.6, 137.8[c] |

[a] Designates the non-reducing carbohydrate unit. [b] Designates the reducing carbohydrate unit. [c] Signal of doubled intensity. [d] Signal of an intensity corresponding to more than two carbons. [e] Split signal.




**Scheme 10** *Reagents and conditions:* i, Fmoc-Ser-OPfp, AgOTf, $CH_2Cl_2$, 3 Å MS, −60 to −40 °C, 74%; ii, Fmoc-Thr-OPfp, AgOTf, $CH_2Cl_2$, 3 Å MS, −60 to −30 °C, 59%; iii, 2,4,6-collidine; iv, **11**, AgOTf, $CH_2Cl_2$, 3 Å MS, −40 to −20 °C; v, 2,6-$Bu^t_2$-4-Me-pyridine, 61%

combination of α(1,2)-, α(1,3)- and α(1,6)-linked disaccharide moieties leads to the conclusion that the thioglycoside approach might most conveniently be employed for all three types of disaccharide building blocks, as it proved possible to obtain both 2-OH and 3-OH acceptors by selective benzoylation of compound **16** under different conditions. The trichloroacetimidate method seems to be more efficient than the Königs–Knorr method, exemplified by the synthesis of the α(1,3)-mannobioside **22**. The applications of the building blocks, prepared in the present work, towards the synthesis of glycopeptide templates and libraries are currently under investigation.

## Experimental

### General procedures

TLC was performed on Merck Silica Gel 60 $F_{254}$ aluminium sheets with detection by charring with sulfuric acid, and by UV light when applicable. Mps were measured on a Büchi melting point apparatus and are uncorrected. VLC was performed on Merck Silica Gel 60 H (0.04–0.06 mm), and VLC under anhydrous conditions was performed on predried silica gel (120 °C; > 24 h) with solvents dried over 3 Å molecular sieves. The column size is given as height × diameter (cm). Solvents were purchased from Labscan Ltd. Dichloromethane was distilled from $P_4O_{10}$ (Merck) and kept over 3 Å molecular sieves. Light petroleum was the 60–80 °C fraction. Concentrations were performed under reduced pressure at temperatures below 40 °C. The organic layers were dried over anhydrous $MgSO_4$. Hydrogen bromide in acetic acid (33% w/w) and benzoyl chloride were purchased from Merck. DCCI, TBAHS and 2,6-di-*tert*-butyl-4-methylpyridine were from Fluka. Silver triflate, 2,4,6-trimethylpyridine(2,4,6-collidine), 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) and trichloroacetonitrile were from Aldrich.

$N^{\alpha}$-Fmoc-Ser($Bu^t$)-OPfp, $N^{\alpha}$-Fmoc-Thr($Bu^t$)-OPfp and $N^{\alpha}$-Fmoc-Hyp($Bu^t$)-OH were purchased from Bachem (Bubendorf,

**Table 11** Glycosylation of protected amino acids

| | Serine building blocks | | | Threonine building blocks | | | Hydroxyproline building blocks | | |
|---|---|---|---|---|---|---|---|---|---|
| Disaccharide type | α(1,2) | α(1,3) | α(1,6) | α(1,2) | α(1,3) | α(1,6) | α(1,2) | α(1,3) | α(1,6) |
| Glycosyl donor | **8** | **24** | **32/33** | **8** | **24** | **32/33** | **8** | **24** | **32** |
| Amino acid derivative (mol equiv.) | 1.1 | 1.05 | 1.1 | 1.1 | 1.0 | 0.95 | 1.0 | 1.0 | 1.0 |
| AgOTf (mol equiv.) | 1.3 | 1.15 | 1.3 | 1.3 | 1.15 | 1.15 | 1.2 | 1.15 | 1.2 |
| Reaction time ($t$/h) | 5.5 | 2.75 | 4.0 | 4.25 | 3.0 | 3.0 | 3.25 | 3.25 | 3.75 |
| Reaction temperature ($T$/°C) | −40 to −25 | −30 to −15 | −60 to −40 | −35 to −20 | −30 to −20 | −60 to −30 | −40 to −15 | −40 to −25 | −40 to −20 |
| Product | **12** | **26** | **34** | **13** | **27** | **35** | **14** | **28** | **36** |
| Yield (%) | 69 | 72 | 74 | 76 | 78 | 59 | 56 | 66 | 61 |

Switzerland). Flasks for glycosylations were stored at 120 °C for 12 h. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter, and are given in units of $10^{-1}$ deg cm$^2$ g$^{-1}$. Elemental analyses were carried out at LEO Pharmaceutical Products (Ballerup, Denmark). $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AM 500 MHz spectrometer. Unless otherwise stated the NMR experiments were performed at 300 K in $CDCl_3$. Chemical shifts are given in ppm and referenced to internal $SiMe_4$ ($\delta_H$, $\delta_C$ 0.00). $J$-Values are given in Hz. For all compounds the assignment of $^1$H NMR spectra was based on 1D homonuclear decoupling experiments and 2D homonuclear chemical shift correlation (COSY) spectra. The assignment of $^{13}$C NMR spectra was based on carbon–proton shift-correlation spectra. ES-MS spectra were recorded in the positive mode on a VG Quattro Mass Spectrometer from Fisons.

**1,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranose 3 and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranose 4**

To a solution containing penta-*O*-acetyl-α-D-mannopyranose **1** (40.0 g, 0.10 mol) and molecular sieves (4 Å; 5 g) in dry dichloromethane (50 cm$^3$) was added 5.6 mol dm$^{-3}$ hydrogen bromide in acetic acid (54 cm$^3$, 0.31 mol). The mixture was stirred at room temperature for 1.5 h, poured onto ice (125 g), and the resulting mixture was extracted with dichloromethane (2 × 150 cm$^3$). The organic layer was washed successively with cold water, aq. sodium hydrogen carbonate (twice), and water (each 150 cm$^3$). Drying and evaporation gave a syrup (42.0 g), which was dissolved in anhydrous chloroform (165 cm$^3$; filtered through activated basic alumina). Dry methanol (165 cm$^3$) and 2,6-lutidine (21.7 cm$^3$) and molecular sieves (3 Å; 10 g) were added. Upon storage at room temperature for 2 days, the solution was diluted with chloroform (100 cm$^3$) and washed successively with cold aq. sodium hydrogen carbonate and water (each 200 cm$^3$). Drying and concentration gave a syrup, which was evaporated with toluene (2 × 20 cm$^3$) and then crystallized from diethyl ether–light petroleum (1:1; 150 cm$^3$) on storage overnight at 4 °C. The crystals were washed with cold solvent (50 cm$^3$) and dried in a desiccator to give crude orthoester **2** (26.8 g, 73%) which by NMR spectroscopy was shown to be ~95% pure.

The crude orthoester **2** (26.8 g) was dissolved in acetonitrile (170 cm$^3$) and the solution was cooled to 0 °C. Addition of 10% TFA (aq. 17 cm$^3$) was followed by stirring of the mixture at 0 °C for 3 h. Evaporation gave a syrup which crystallized upon addition of diethyl ether. Upon storage overnight crude crystalline 2-ol **3** (7.55 g, 29%) was obtained. Recrystallization of a large portion (16.7 g) of crude compound **3**, prepared similarly, was performed by dissolution in acetonitrile (50 cm$^3$) and concentration to a syrup, which was dissolved in boiling $Et_2O$–light petroleum (2:1; 450 cm$^3$). Upon storage overnight at 4 °C, the precipitated crystals were filtered off and dried over $P_2O_5$ to yield pure title compound **3** (14.8 g; 27% overall), mp 160–162 °C [lit.,[57] 164–165 °C]; $[\alpha]_D^{21}$ −22.6 (*c* 1.0, $CHCl_3$) [lit.,[57] −23.6 (*c* 1.4, $CHCl_3$)]; $\delta_H$(500 MHz; $CDCl_3$) 5.79 (s, 1-H), 5.39 (t, *J* 9.9, 4-H), 5.04 (dd, *J* 9.9 and 3.0, 3-H), 4.31 (dd, *J* 12.4 and 4.9, 6-H), 4.20 (br s, 2-H), 4.13 (dd, *J* 12.4 and 2.2, 6-H'), 3.78 (ddd, *J* 9.8, 4.9 and 2.2, 5-H) and 2.18, 2.12, 2.09 and 2.05 (4 × 3 H, 4 × Ac); $\delta_C$(125 MHz; $CDCl_3$) 91.6 (C-1), 73.1 (C-5), 72.8 (C-3), 68.4 (C-2), 65.2 (C-4), 61.9 (C-6), 170.7, 170.0, 169.5 and 168.4 (4 × Me*C*O) and 20.8*, 20.7 and 20.6 (4 × CO*Me*) (* two distinct signals).

The mother-liquor was concentrated to a thin syrup (40 g) containing residual TFA (~5%). An aliquot (10 g) of this syrup was fractionated by VLC [5 × 5 cm silica gel; light petroleum–ethyl acetate (5:1 to 2:1)]. The fractions mainly containing 1-ol **4** were evaporated and their residues were crystallized from hexane–ethyl acetate (5:1) to yield title compound **4** (3.43 g, 25%) contaminated with 2% β-anomer and 2% of an unidentified impurity, $\delta_H$(500 MHz; $CDCl_3$) 5.44 (dd, *J* 10.1 and 3.3, 3-H), 5.32 (t, *J* 10.0, 4-H), 5.28 (dd, *J* 3.2 and 1.8, 2-H), 5.26 (dd, *J* 4.0 and 1.8, 1-H), 4.30–4.24 (2 H, m, 5- and 6-H), 4.16 (br d, *J* 12.0, 6-H'), 3.95 (d, *J* 4.1, 1-OH) and 2.18, 2.12, 2.07 and 2.02 (4 × 3 H, 4 × Ac); additional signals for the β-anomer $\delta_H$ 5.23 (t, *J* 10.0, 4-H), 5.10 (dd, *J* 10.1 and 3.4, 3-H) and 5.01 (br s, 1-H); $\delta_C$(125 MHz; $CDCl_3$) 92.1 ($J_{C-H}$ 172.7, C-1), 70.0 (C-2), 68.8 (C-3), 68.4 (C-5), 66.1 (C-4), 62.5 (C-6), 170.9, 170.2, 170.1 and 169.8 (4 × Me*C*O) and 20.8, 20.7 and 20.6 (4 × CO*Me*); additional signal for the β-anomer: $\delta_C$ 92.8 ($J_{C-H}$ 162.3, C-1).

**1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-β-D-mannopyranose 6**

Penta-*O*-benzoyl-α-D-mannose (9.00 g, 12.8 mmol) as a solution in dry dichloromethane (135 cm$^3$) was mixed with 5.6 mol dm$^{-3}$ hydrogen bromide in acetic acid (45 cm$^3$, 252 mmol) and molecular sieves (3 Å; 3.0 g; powdered). After storage overnight at 4 °C, the mixture was diluted with dichloromethane (100 cm$^3$) and poured onto ice–water (100 g). The organic layer was washed successively with cold water, saturated aq. sodium hydrogen carbonate (3 times) and water (each 100 cm$^3$). Drying and evaporation gave the bromide **5** (8.12 g, 96%) as a foam.

The bromide **5** (8.12 g, 12.31 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (1.35 g, 6.57 mmol) and molecular sieves (3 Å, 8.0 g; powdered) were stirred in dry dichloromethane (130 cm$^3$) under Ar at −60 to −40 °C for 75 min. Silver triflate (3.80 g, 14.77 mmol) was added quickly. After stirring of this mixture at −55 to −40 °C for a further 25 min, a solution of the acceptor **3** (2.86 g, 8.21 mmol) in dry dichloromethane (40 cm$^3$) was added. Stirring at −50 to −40 °C was continued for 1.75 h, when 2,4,6-collidine (1.1 cm$^3$, 8.32 mmol) was added. The temperature was slowly raised to 20 °C during 2 h. The mixture was filtered through Celite, which was then washed with dichloromethane (100 cm$^3$). Subsequently, the solution was washed successively with water, aq. sodium thiosulfate, and water (each 250 cm$^3$). Upon drying and evaporation, the residue was dissolved in dry dichloromethane–pyridine (2:1; 45 cm$^3$), benzoyl chloride (4.25 cm$^3$) was added, and the mixture was kept overnight at 4 °C. Concentration with toluene and fractionation by VLC on dried silica gel [two portions, 6 × 5.5 cm, light petroleum to light petroleum–ethyl acetate (2:1)] yielded a fraction of impure disaccharide **6** (0.37 g, 5%) followed

by pure *title compound* **6** (6.10 g, 80%), $[\alpha]_D^{22}$ −49.0 (*c* 1.0, $CHCl_3$); $^1H$ and $^{13}C$ NMR data are presented in Tables 5 and 6, respectively (Found: C, 62.1; H, 4.95. $C_{48}H_{46}O_{19}$ requires C, 62.2; H, 5.0%; M, 926.88).

**$N^2$-(Fluoren-9-ylmethoxycarbonyl)-L-*trans*-hydroxyproline pentafluorophenyl ester 11**

A solution of pentafluorophenol (2.73 g, 14.84 mmol) in dry THF (120 $cm^3$; distilled from sodium) was cooled to −50 °C. After the solution had been stirred under Ar for 10 min, DCCI (2.36 g, 11.42 mmol) was added. The temperature was raised to −20 °C during 30 min, when $N^2$-Fmoc-Hyp(Bu$^t$)-OH **9** (4.80 g, 11.31 mmol) was added. The mixture was stirred at −20 °C for 3 h, when the temperature was allowed to rise to ambient. The mixture was kept overnight at 4 °C. Precipitated dicyclohexylurea was filtered off on Celite and the filtrate was concentrated to a syrup in which TLC [$R_f$ 0.24; light petroleum–ethyl acetate (6:1)] showed the presence of several minor impurities ($R_f$ < 0.2). Upon dissolution in heptane–dichloromethane (3:1; 24 $cm^3$) the crude Pfp ester **10** was loaded onto a VLC column (5 × 6.5 cm), which was eluted (gradient) with heptane to heptane–ethyl acetate (30:1) to give Pfp ester **10** (4.38 g, 67%). $^1H$ and $^{13}C$ NMR data are presented in Tables 1 and 2, respectively (Found: C, 61.8; H, 4.6; N, 2.3. $C_{30}H_{26}F_5NO_5$ requires C, 62.6; H, 4.55; N, 2.4%; M, 575.53).

The above syrup was combined with a similar portion of Pfp ester **10** (7.41 g in all) and was treated with TFA (120 $cm^3$) at room temperature for 1 h. Toluene (50 $cm^3$) was added and the solution was evaporated. The residue was concentrated with toluene (3 × 50 $cm^3$) and then subjected to VLC [10 × 6.5 cm silica gel; hexane to hexane–ethyl acetate (2:1)] which afforded the *unprotected Pfp ester* **11** (5.35 g, 80%) as a foam, $[\alpha]_D^{22}$ −63.5 (*c* 1.1, $CHCl_3$). $^1H$ and $^{13}C$ NMR data are presented in Tables 1 and 2, respectively (Found: C, 59.7; H, 3.7; N, 2.7. $C_{26}H_{18}F_5NO_5$ requires C, 60.1; H, 3.5; N, 2.7%; M, 519.42).

**$N^2$-(Fluoren-9-ylmethoxycarbonyl)-*O*-[3,4,6-tri-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-L-serine pentafluorophenyl ester 12**

Disaccharide **6** (1.51 g, 1.63 mmol) as a solution in dry dichloromethane (10 $cm^3$), 5.6 mol $dm^{-3}$ hydrogen bromide in acetic acid (4.1 $cm^3$, 16.3 mmol) and 3 Å molecular sieves (3.0 g; powdered) were stirred together at room temperature for 1 h. Upon dilution with dichloromethane (50 $cm^3$), the mixture was poured into ice–water (50 $cm^3$). The organic layer was washed successively with cold water, aq. sodium hydrogen carbonate (twice) and water (each 50 $cm^3$), dried and concentrated (oil-pump for 18 h) to yield the bromide **8** (1.41 g, 92%) as a solid. $^1H$ and $^{13}C$ NMR data are presented in Tables 5 and 6, respectively.

The resulting bromide **8** (1.41 g, 1.49 mmol) and $N^2$-Fmoc-Ser-OPfp (0.81 g, 1.64 mmol) were dissolved in dry dichloromethane (35 $cm^3$) and the solution was stirred under Ar over 3 Å molecular sieves (2.5 g; powdered) at −40 °C for 45 min. Silver triflate (0.51 g, 1.96 mmol) was added quickly, and stirring at −40 to −30 °C was continued for 3 h (TLC ~50% conversion) when the temperature was raised to −30 to −25 °C; stirring at this temperature was continued for an additional 2.5 h. 2,4,6-Collidine (0.28 $cm^3$, 2.09 mmol) and dry dichloromethane (20 $cm^3$) were added. After 30 min without cooling, the mixture was filtered ($MgSO_4$–Celite), concentrated and then purified by VLC on dried silica gel (5 × 5.5 cm). Gradient elution with light petroleum to light petroleum–ethyl acetate (2.5:1) gave *title compound* **12** (1.40 g, 69%), $[\alpha]_D^{22}$ −21.0 (*c* 0.9, $CHCl_3$); $^1H$ and $^{13}C$ NMR data are presented in Tables 7 and 9, respectively (Found: C, 61.2; H, 4.3; N, 1.0. $C_{70}H_{58}F_5NO_{22}$ requires C, 61.8; H, 4.3; N, 1.0%; M, 1360.22).

**$N^2$-(Fluoren-9-ylmethoxycarbonyl)-*O*-[3,4,6-tri-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-L-threonine pentafluorophenyl ester 13**

The bromide **8** was prepared as above from disaccharide **6** (1.70 g, 1.83 mmol). The resulting foam of the bromide **8** (1.49 g, 1.57 mmol, 86%) and $N^2$-Fmoc-Thr-OPfp (0.88 g, 1.73 mmol) were dissolved in dry dichloromethane (40 $cm^3$) and the solution was stirred under Ar over 3 Å molecular sieves (3.0 g; powdered) at −50 to −35 °C during 2 h. Silver triflate (0.53 g, 2.06 mmol) was added quickly, and stirring at −35 to −30 °C was continued for 2.5 h (TLC ~50% conversion), when the temperature was raised to −25 to −20 °C. Upon stirring of the mixture for an additional 1.75 h, 2,4,6-collidine (0.295 $cm^3$, 2.20 mmol) was added. After 15 min without cooling, the mixture was filtered ($MgSO_4$–Celite) and the filtrate was concentrated. The residue was subjected to VLC on dried silica gel (5 × 5 cm). Gradient elution with light petroleum to light petroleum–ethyl acetate (2.8:1) afforded *title compound* **13** (1.65 g, 76%) as a solid, $[\alpha]_D^{22}$ −20.6 (*c* 1.1, $CHCl_3$); $^1H$ and $^{13}C$ NMR data are presented in Tables 7 and 9, respectively (Found: C, 62.05; H, 4.4; N, 1.0. $C_{71}H_{60}F_5NO_{22}$ requires C, 62.05; H, 4.4; N, 1.0%; M, 1374.24).

**$N^2$-(Fluoren-9-ylmethoxycarbonyl)-*O*-[3,4,6-tri-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-L-*trans*-hydroxyproline pentafluorophenyl ester 14**

The bromide **8** was prepared as above from disaccharide **6** (1.20 g, 1.29 mmol). The resulting foamy bromide **8** (1.07 g, 1.13 mmol, 87%) and $N^2$-Fmoc-Hyp-OPfp **11** (0.59 g, 1.13 mmol) were dissolved in dry dichloromethane (30 $cm^3$) and the solution was stirred under Ar over molecular sieves (3 Å; 2.0 g) at −40 °C for 45 min. Silver triflate (0.35 g, 1.36 mmol) was added quickly, and the temperature was allowed to rise to −15 °C during 75 min. Stirring of the mixture at −15 °C was continued for 2.5 h, when 2,6-di-*tert*-butyl-4-methylpyridine (0.235 g, 1.14 mmol) was added. After being stirred without cooling for 10 min, the mixture was loaded directly onto a VLC column (4 × 6 cm). Gradient elution with hexane to hexane–ethyl acetate (3.2:1) gave *title compound* **14** (0.88 g, 56%) as a solid, $[\alpha]_D^{22}$ −35.9 (*c* 1.0, $CHCl_3$); $^1H$ and $^{13}C$ NMR data are presented in Tables 8 and 10, respectively (Found: C, 62.2; H, 4.6; N, 1.1. $C_{72}H_{60}F_5NO_{22}$ requires C, 62.4; H, 4.4; N, 1.0%; M, 1386.25).

**Phenyl 4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside 16**

Phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside[48] **15** (13.63 g, 30.9 mmol) was deacetylated with sodium methoxide (1 mol $dm^{-3}$; 3.0 $cm^3$) in methanol (150 $cm^3$). After neutralization (Amberlite IR C-50) and concentration, the resulting syrup was dried overnight (oil-pump) to give phenyl 1-thio-α-D-mannopyranoside (8.21 g, 97%). The synthesis was repeated and the combined material (16.41 g, 60.3 mmol) was stirred with α,α-dimethoxytoluene (9.94 $cm^3$, 66.3 mmol) and PTSA monohydrate (1.32 g) in dry DMF (60 $cm^3$) at 10 mmHg for 5 h. Then the mixture was left at 4 °C for 2 days. Dilution with ethyl acetate (100 $cm^3$) and addition of solid sodium hydrogen carbonate (15.0 g) was followed by stirring of the mixture at room temperature for 5 h; the mixture was then left at 4 °C overnight. Upon addition of ethyl acetate (150 $cm^3$) to dissolve precipitated organic product, the suspension was filtered to remove inorganic solids. After concentration of the filtrate, methanol (1200 $cm^3$) was added to the residue. Slow cooling at 4 °C overnight gave the 4,6-*O*-benzylidene compound **16** (6.41 g, 29.5%). The mother-liquor was concentrated to leave an oil; DMF (25 $cm^3$) and PTSA monohydrate (1.30 g) were added. The mixture was stirred at room temperature for 8 h and left at 4 °C for 2 days. Ethyl

acetate (300 $cm^3$) and solid sodium hydrogen carbonate (15.0 g) were added to the stirred mixture. After 10 min, the mixture was left at 4 °C for 2 days. Dilution with ethyl acetate (200 $cm^3$), filtration, and concentration gave a residue, which crystallized upon addition of methanol (800 $cm^3$) and cooling to 4 °C for 3 days. Filtration gave a second crop of *compound* **16** (4.04 g, 19%). Total yield of product **16** was 48.5%. Recrystallization of a small sample gave needles, mp 182–184 °C; $[\alpha]_D^{27}$ +294.6 (*c* 0.5, acetone); $^1H$ and $^{13}C$ NMR data are presented in Tables 3 and 4, respectively (Found: C, 63.3; H, 5.7; S, 8.75. $C_{19}H_{20}O_5S$ requires C, 63.3; H, 5.6; S, 8.9%; M, 360.43).

**Phenyl 2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-α-D-manno-pyranoside 17**

The dihydroxy compound **16** (1.00 g, 2.77 mmol) was suspended in dichloromethane (80 $cm^3$) containing TBAHS (0.19 g, 0.55 mmol). Upon cooling of the mixture to −3 °C, 5% aq. NaOH (7 $cm^3$) was added. A solution of benzoyl chloride (0.355 $cm^3$, 3.05 mmol) in dichloromethane (5 $cm^3$) was then added during 15 min under vigorous magnetic stirring. After a total of 35 min at −3 to 0 °C, the mixture was combined with a similar batch (same amounts and reaction conditions) prepared simultaneously. The organic layer was washed with water (2 × 100 $cm^3$), dried, and concentrated to ~25 $cm^3$, and was applied to a VLC column (5 × 5 cm). Gradient elution with hexane to hexane–ethyl acetate (4:1) gave successively fractions of the dibenzoylated compound **19** (0.54 g, 17%), the 2-*O*-benzoyl compound **17** (1.45 g, 56%) and the 3-*O*-benzoyl compound **18** (0.48 g, 18.5%).

Crystallization of *compound* **17** from light petroleum–ethyl acetate (20:1) gave needles, mp 150–151 °C; $[\alpha]_D^{27}$ +131.3 (*c* 1.0, $CHCl_3$) (Found: C, 67.4; H, 5.3; S, 6.85. $C_{26}H_{24}O_6S$ requires C, 67.2; H, 5.2; S, 6.9%; M, 464.54). Crystalline *compound* **18** was also obtained from light petroleum–ethyl acetate (20:1), mp 173–174 °C; $[\alpha]_D^{27}$ +148.6 (*c* 1.1, $CHCl_3$) (Found: C, 67.3; H, 5.3; S, 6.85%). Crystalline *compound* **19** was also obtained from light petroleum–ethyl acetate (20:1), mp 141–143 °C; $[\alpha]_D^{27}$ +4.3 (*c* 1.0, $CHCl_3$) (Found: C, 69.9; H, 5.0; S, 5.6. $C_{33}H_{28}O_7S$ requires C, 69.7; H, 5.0; S, 5.6%; M, 568.65). $^1H$ and $^{13}C$ NMR data for compounds **17**–**19** are presented in Tables 3 and 4, respectively.

**Phenyl 3-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-α-D-manno-pyranoside 18**

Compound **16** (0.20 g, 0.56 mmol) was dissolved in dichloromethane–pyridine (1:1; 3 $cm^3$) and the solution was cooled to −40 °C. Benzoyl chloride (0.068 $cm^3$, 0.58 mmol) was added, and during 1 h the temperature was allowed to rise to −20 °C (TLC showed compound **18** to be the predominant product). After being stirred at room temperature for 15 min, the mixture was concentrated with toluene. The residue was fractionated by VLC (2.5 × 3 cm); gradient elution with hexane to hexane–ethyl acetate (5:1) gave dibenzoylated compound **19** (0.024 g, 8%) and the 3-*O*-benzoyl compound **18** (0.173 g, 67%).

**2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl trichloro-acetimidate 20**

2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranose **4** (3.00 g, 8.61 mmol) was dissolved in dry dichloromethane (10 $cm^3$) and the solution was cooled to −20 °C. Trichloroacetonitrile (8.6 $cm^3$, 86.1 mmol) was added, followed by a solution of DBU (0.335 $cm^3$) in dichloromethane (10 $cm^3$), added dropwise by syringe during 10 min. After 1 h the temperature had reached 0 °C, which temperature was maintained for an additional 2 h. The mixture was loaded directly onto a VLC column (5 × 6 cm). Gradient elution with hexane to hexane–ethyl acetate (3:1) gave the trichloroacetimidate **20** (4.14 g, 97.5%) as a syrup, $\delta_H$(500 MHz; $CDCl_3$) 8.82 (s, C=NH), 6.32 (d, *J* 1.9, 1-H), 5.51 (dd, *J* 3.0 and 1.9, 2-H), 5.46 (dd, *J* 9.8 and 3.0, 3-H), 5.44 (t, *J* 9.8, 4-H), 4.32 (dd, *J* 12.2 and 4.7, 6-H), 4.24 (ddd, *J* 9.7, 4.7 and 2.3, 5-H), 4.20 (dd, *J* 12.2 and 2.3, 6-H′) and 2.24, 2.12, 2.11 and 2.05 (4 × 3 H, 4 × Ac).

**Phenyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-1-thio-α-D-mannopyranoside 21**

The bromide **5** was prepared as above from penta-*O*-benzoyl-α-D-mannose (0.315 g, 0.45 mmol). The resulting foamy bromide **5** (0.296 g, 0.45 mmol) and the acceptor **17** (0.136 g, 0.29 mmol) were dissolved in dry dichloromethane (6 $cm^3$) and the mixture was stirred under Ar with molecular sieves (3 Å; 0.3 g; powdered) at −45 to −40 °C for 30 min. Silver triflate (0.144 g, 0.56 mmol) was added quickly, and the mixture was stirred at −40 °C for an additional 1.75 h. 2,4,6-Collidine (0.12 $cm^3$, 0.90 mmol) was added and the temperature was allowed to rise to ambient. Upon dilution with dichloromethane (15 $cm^3$) the mixture was filtered through a layer of $MgSO_4$ and concentrated. The residue was subjected to VLC on dried silica gel (2.5 × 2.5 cm). Gradient elution with light petroleum to light petroleum–ethyl acetate (5:1) gave a ~1:1 mixture of disaccharide **21** and 2,3,4,6-tetra-*O*-benzoyl-β-D-mannose (0.201 g), which was acetylated and then purified by VLC to give a 10:1 mixture of disaccharide **21** and a monosaccharide impurity (0.082 g, 27%). For compound **21**: $\delta_H$(500 MHz; $CDCl_3$) 6.13 (t, *J* 10.0, $4^a$-H), 5.86 (dd, *J* 3.3 and 2.0, $2^a$-H), 5.82 (dd, *J* 10.0 and 3.3, $3^a$-H), 5.62 (d, *J* 2.0, $1^a$-H), 4.76 (dd, *J* 12.5 and 2.5, $6^a$-H), 4.68 (ddd, *J* 10.0, 4.6 and 2.5, $5^a$-H), 4.54 (dd, *J* 12.5 and 4.6, $6^a$-H′) and 5.97 (dd, *J* 3.8 and 1.0, $2^b$-H), 5.77 (d, *J* 1.0, $1^b$-H), 4.63 (dd, *J* 9.8 and 3.8, $3^b$-H), 4.55 (dt, *J* 2 × 10.0 and 5.0, $5^b$-H), 4.44 (t, *J* 9.8, $4^b$-H), 4.38 (dd, *J* 10.2 and 5.0, $6^b$-H), 4.01 (t, *J* 10.2, $6^b$-H′), 5.81 (s, α-H), 8.31, 8.22, 8.06, 7.95 and 7.84 (5 × 2 H, 5 × Bz) and 7.73–7.52 (25 H, ArH). $^{13}C$ NMR data for compound **21** are presented in Table 6.

**Phenyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-1-thio-α-D-mannopyranoside 22**

A dry dichloromethane solution (75 $cm^3$) of the trichloroacetimidate **20** (1.85 g, 3.75 mmol) and the acceptor **17** (1.51 g, 3.26 mmol) was stirred with molecular sieves (3 Å; 10 g; powdered) under Ar for 20 min, when the mixture was cooled to −30 °C. A solution of trimethylsilyl triflate (0.675 $cm^3$, 3.75 mmol) in dry dichloromethane (10 $cm^3$) was added slowly by syringe (during 5 min). Stirring of the mixture at −30 to −25 °C was continued for 15 min, and a solution of diisopropylamine (1.96 $cm^3$, 11.26 mmol) in dichloromethane (5 $cm^3$) was then added. The temperature was allowed to rise to 0 °C during 1 h. Filtration through Celite and concentration (to ~20 $cm^3$) was followed directly by VLC on dried silica gel (5 × 6 cm). Gradient elution with hexane to hexane–ethyl acetate (3:1) yielded *disaccharide* **22** (2.35 g, 91%) as a foam, $[\alpha]_D$ +85.1 (*c* 1.1, $CHCl_3$); $^1H$ and $^{13}C$ NMR data are presented in Tables 5 and 6, respectively (Found: C, 60.3; H, 5.4; S, 3.9. $C_{40}H_{42}O_{15}S$ requires C, 60.45; H, 5.3; S, 4.0%; M, 794.82).

**Phenyl 4,6-di-*O*-acetyl-2-*O*-benzoyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-1-thio-α-D-mannopyranoside 23**

Disaccharide **22** (1.95 g, 2.45 mmol) was heated at 60–65 °C in 70% aq. acetic acid (100 $cm^3$) for 1.5 h. The mixture was concentrated and then was concentrated with toluene (3 × 100 $cm^3$). The residue was acetylated in acetic anhydride–pyridine overnight at 4 °C. Upon concentration with toluene the residue was purified by VLC on dried silica gel (5 × 5 cm). Gradient elution with hexane to hexane–ethyl acetate (2:1) gave the *disaccharide* **23** (1.83 g, 94%) as a foam, $[\alpha]_D$ +61.4 (*c* 1.0, $CHCl_3$); $^1H$ and $^{13}C$ NMR data are presented in Tables 5 and 6, respectively (Found: C, 56.0; H, 5.4; S, 4.0. $C_{37}H_{42}O_{17}S$ requires C, 56.2; H, 5.35; S, 4.05%; M, 790.79).

**4,6-Di-*O*-acetyl-2-*O*-benzoyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl bromide 24**

To a solution of disaccharide **23** (0.200 g, 0.253 mmol) in dry dichloromethane (2 $cm^3$) were added a solution of bromine (0.014 $cm^3$, 0.266 mmol) in dry dichloromethane (2 $cm^3$) and molecular sieves (3 Å; 0.5 g). The mixture was stirred at room temperature for 2 h, shielded from light, followed by filtration through Celite and concentration with toluene (2 × 5 $cm^3$) to give a syrup which was purified directly by VLC on dried silica gel (2.5 × 2.5 cm) using dry solvents. Gradient elution with hexane to hexane–ethyl acetate (2:1) yielded a 2.5:2 mixture of α-bromide **24** and thioglycoside **23** (0.161 g, 83% recovery).

Thioglycoside **23** (0.102 g, 0.129 mmol) was similarly treated with a solution of bromine (0.0075 $cm^3$, 0.142 mmol) in dry dichloromethane for 5 h. Purification by VLC as above afforded a 10:2:1 mixture of α-bromide **24**, thioglycoside **23** and β-bromide **25** (0.090 g, 92%).

To a solution of thioglycoside **23** (0.374 g, 0.473 mmol) in dry dichloromethane (1 $cm^3$) were added molecular sieves (3 Å; 0.75 g) and a solution of bromine (0.0292 $cm^3$, 0.567 mmol) in dry dichloromethane (1 $cm^3$). The mixture was stirred at room temperature for 5.5 h in the dark, and was then loaded directly onto a VLC column (3 × 3 cm). Gradient elution as above gave α-bromide **24** (0.334 g, 93%), with a trace amount of the β-anomer **25** (< 1%); $^1H$ and $^{13}C$ NMR data for α-bromide **24** are presented in Tables 5 and 6, respectively. Additional $^1H$ NMR signals for the β-anomer **25** were observed at $\delta_H$ 6.26 (br s, $1^b$-H) and 4.64 (dd, *J* 10.0 and 3.5, $3^b$-H).

***O*-[4,6-Di-*O*-acetyl-2-*O*-benzoyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-$N^α$-(fluoren-9-ylmethoxycarbonyl)-L-serine pentafluorophenyl ester 26**

Disaccharide **23** (1.45 g, 1.83 mmol) was treated with a solution of bromine (0.113 $cm^3$, 2.20 mmol) in dry dichloromethane (8 $cm^3$), containing molecular sieves (3 Å; 3.0 g) for 6 h in the dark. Direct VLC as above afforded the α-bromide **24** (1.28 g, 1.68 mmol, 92%), which was stirred with a mixture of $N^α$-Fmoc-Ser-OPfp (0.87 g, 1.76 mmol) and molecular sieves (3 Å; 3.5 g) in dry dichloromethane (35 $cm^3$) under Ar at −40 °C for 1 h. Silver triflate (0.49 g, 1.93 mmol) was added quickly, and the temperature was raised to −30 °C. Stirring of the mixture at −30 to −25 °C was continued for 2 h, when the temperature was allowed to rise to −15 °C during a further 45 min. Then 2,6-di-*tert*-butyl-4-methylpyridine (0.35 g, 1.70 mmol) was added. The mixture was stirred without cooling for 15 min, followed by filtration and concentration to ~20 $cm^3$, and the residual solution was loaded directly onto a VLC column (4 × 4.5 cm). Gradient elution with hexane to hexane–ethyl acetate (2:1) yielded *title compound* **26** (1.43 g, 72%), $[α]_D$ +1.3 (*c* 1.0, $CHCl_3$); $^1H$ and $^{13}C$ NMR data are presented in Tables 7 and 9, respectively (Found: C, 56.6; H, 4.8; N, 1.35. $C_{55}H_{52}F_5NO_{22}$ requires C, 56.3; H, 4.5; N, 1.2%; M, 1174.04).

***O*-[4,6-Di-*O*-acetyl-2-*O*-benzoyl-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-$N^α$-(fluoren-9-ylmethoxycarbonyl)-L-threonine pentafluorophenyl ester 27**

The α-bromide **24** was prepared as above from disaccharide **23** (1.50 g, 1.90 mmol). The resulting foamy bromide **24** (1.32 g, 1.73 mmol, 91%) and $N^α$-Fmoc-Thr-OPfp (0.88 g, 1.73 mmol) were dissolved in dry dichloromethane (40 $cm^3$) and the solution was stirred under Ar over molecular sieves (3 Å; 3.0 g) at −40 to −30 °C for 1 h. Silver triflate (0.51 g, 1.98 mmol) was added quickly, and the temperature was allowed to rise to −20 °C during 1 h. Stirring of the mixture at −20 °C was continued for 2 h, when the mixture was cooled to −60 °C and 2,6-di-*tert*-butyl-4-methylpyridine (0.36 g, 1.75 mmol) was added. During 1 h the temperature was allowed to rise to −40 °C, and after 30 min without cooling the mixture was filtered through Celite. Upon concentration to ~20 $cm^3$ the solution was loaded onto a VLC column (4 × 4.5 cm). Gradient elution with hexane to hexane–ethyl acetate (2:1) gave *title compound* **27** (1.62 g, 78.5%), $[α]_D$ −5.9 (*c* 1.0, $CHCl_3$); $^1H$ and $^{13}C$ NMR data are presented in Tables 7 and 9, respectively (Found: C, 56.6; H, 4.8; N, 1.15. $C_{56}H_{54}F_5NO_{22}$ requires C, 56.6; H, 4.6; N, 1.2%; M, 1188.07).

***O*-[4,6-Di-*O*-acetyl-2-*O*-benzoyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-$N^α$-(fluoren-9-ylmethoxycarbonyl)-L-*trans*-hydroxyproline pentafluorophenyl ester 28**

The α-bromide **24** was prepared as above from disaccharide **23** (1.70 g, 2.15 mmol). The resulting foamy bromide **24** (1.55 g, 2.04 mmol, 94.5%) and $N^α$-Fmoc-Hyp-OPfp **11** (1.06 g, 2.04 mmol) were dissolved in dry dichloromethane (40 $cm^3$) and the solution was stirred under Ar over molecular sieves (3 Å; 3.5 g) at −40 °C for 1.25 h. Silver triflate (0.60 g, 2.34 mmol) was added quickly and the temperature was raised to −25 °C during 15 min. Stirring of the mixture at −25 °C was continued for 3 h, when 2,6-di-*tert*-butyl-4-methylpyridine (0.42 g, 2.05 mmol) was added. The mixture was stirred without cooling for 15 min, followed by filtration and concentration to ~20 $cm^3$, and the residual solution was loaded onto a VLC column (4 × 4.5 cm). Gradient elution with hexane to hexane–ethyl acetate (2:1) gave *title compound* **28** (1.61 g, 66%) as a solid, $[α]_D$ −10.6 (*c* 1.05, $CHCl_3$); $^1H$ and $^{13}C$ NMR data are presented in Tables 8 and 10, respectively (Found: C, 57.7; H, 5.0; N, 1.1. $C_{5}$-$H_{54}F_5NO_{22}$ requires C, 57.05; H, 4.5; N, 1.2%; M, 1200.09).

**Phenyl 2,3,4-tri-*O*-benzoyl-6-*O*-*tert*-butyldimethylsilyl-1-thio-α-D-mannopyranoside 29**

TBDMS-ether **29** was prepared as previously described[20] in 82% yield as a syrup which crystallized upon storage. Recrystallization from light petroleum–ethyl acetate (15:1) gave crystals, mp 110–113 °C: $[α]_D^{27}$ −16.4 (*c* 1.0, $CHCl_3$) [lit.,[20] −7.6 (*c* 2.1, $CH_2Cl_2$)].

**Phenyl 2,3,4-tri-*O*-benzoyl-1-thio-α-D-mannopyranoside 30**

TBDMS-ether **29** (6.09 g, 8.71 mmol) was dissolved in THF (4.5 $cm^3$), then acetic acid (13.5 $cm^3$) and water (4.5 $cm^3$) were added to give a suspension containing crystals of compound **29**. After 2 and 4 days additional THF was added (3 $cm^3$ each time) to obtain a clear solution. After stirring of the mixture for 7 days (~50% conversion) additional THF (16 $cm^3$), acetic acid (13.5 $cm^3$) and water (4.5 $cm^3$) were added to dissolve precipitated educt. Even though complete conversion was not reached the solvents were evaporated off after 8 days, and the residue was subjected to VLC. Gradient elution with light petroleum to light petroleum–ethyl acetate (5:1) afforded acceptor **30**[20] (3.80 g, 75%).

**Phenyl 2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-1-thio-α-D-mannopyranoside 31**

The bromide **5** was prepared as above from penta-*O*-benzoyl-α-D-mannose (4.49 g, 6.41 mmol). The resulting foamy bromide **5** (3.90 g, 5.91 mmol, 92%) and the acceptor **30** (2.30 g, 3.94 mmol) were dissolved in dry dichloromethane (35 $cm^3$), and the solution was stirred under Ar with molecular sieves (3 Å; 3.0 g; powdered) at −50 to −40 °C for 1 h. Silver triflate (1.82 g, 7.09 mmol) was added quickly, the mixture was stirred at −45 to −40 °C for 2 h, and 2,4,6-collidine (1.15 $cm^3$, 8.58 mmol) was added. The temperature was slowly raised to ambient. Filtration through Celite, washing with dichloromethane (100 $cm^3$), and concentration of the filtrate gave a foam, which was benzoylated with an excess of benzoyl chloride (3 $cm^3$) in dichloromethane–pyridine (1:1; 30 $cm^3$). Upon storage overnight at room temperature the mixture was diluted with

toluene (100 $cm^3$), filtered, and concentrated to give a syrup, which was subjected to VLC [toluene to toluene–ethyl acetate (80:1)]. Owing to poor separation the obtained residue (6.18 g) was further purified by repeated VLC [light petroleum to light petroleum–ethyl acetate (4:1)] to give *disaccharide* **31** (3.94 g, 86%), $[\alpha]_D$ −12.3 (*c* 1.0, $CHCl_3$); $^1H$ and $^{13}C$ NMR data are presented in Tables 5 and 6, respectively (Found: C, 69.2; H, 4.9; S, 2.6. $C_{67}H_{54}O_{17}S$ requires C, 69.2; H, 4.7; S, 2.8%; M, 1163.22).

**2,3,4-Tri-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-α-D-mannopyranosyl bromide 32**

The disaccharide **31** (2.00 g, 1.72 mmol) was treated with a solution of bromine (0.093 $cm^3$, 1.80 mmol) in dry dichloromethane (5 $cm^3$) containing molecular sieves (3 Å; 1.5 g; powdered). Stirring of the mixture in the dark for 3 h was followed by filtration and concentration with toluene (2 × 50 $cm^3$). The residue was subjected to VLC on dried silica gel (4 × 3 cm). Gradient elution with dry solvents [hexane to hexane–ethyl acetate (3.5:1)] gave successively almost pure α-bromide **32** (1.62 g, 83%) and β-bromide **33** (0.18 g, 9%); $^1H$ and $^{13}C$ NMR data are presented in Tables 5 and 6, respectively. For compound **33**, ES-MS with addition of LiCl: $[M + Li]^+$, 1140.5. $C_{67}H_{49}BrO_{17}$ requires $[M + Li]^+$, *m/z*, 1140.89.

**$N^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-*O*-[2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D- mannopyranosyl)-α-D-mannopyranosyl]-L-serine pentafluorophenyl ester 34**

Disaccharide **31** (2.00 g, 1.72 mmol) was treated with bromine (0.088 $cm^3$, 1.72 mmol) in dry dichloromethane as above. Upon concentration with toluene and lyophilization, the crude bromide **32/33** (2.07 g, theoretical yield: 1.95 g, 1.72 mmol) and $N^{\alpha}$-Fmoc-Ser-OPfp (0.93 g, 1.89 mmol) were dissolved in dry dichloromethane (40 $cm^3$) and the solution was stirred under Ar with molecular sieves (3 Å; 3.0 g) at −60 °C for 1 h. Silver triflate (0.58 g, 2.27 mmol) was added quickly, and stirring at −60 to −40 °C was continued for 1.5 h. After the mixture had been stirred for an additional 2.5 h at −40 °C, *sym*-collidine (0.32 $cm^3$, 2.39 mmol) was added. Upon being stirred without cooling for 45 min, the mixture was filtered through Celite and the filtrate was concentrated. The obtained foam was purified by VLC on dried silica gel (5 × 5 cm). Gradient elution with hexane to hexane–ethyl acetate (3:1) yielded *title compound* **34** (1.97 g, 74%) as a solid, $[\alpha]_D$ −56.3 (*c* 1.0, $CHCl_3$); $^1H$ and $^{13}C$ NMR data are presented in Tables 7 and 9, respectively (Found: C, 65.8; H, 4.3; N, 0.9. $C_{85}H_{64}F_5NO_{22}$ requires C, 66.0; H, 4.2; N, 0.9%; M, 1546.43).

**$N^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-*O*-[2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-L-threonine pentafluorophenyl ester 35**

Disaccharide **31** (1.99 g, 1.71 mmol) was treated with a solution of bromine (0.088 $cm^3$, 171 mmol) in dry dichloromethane (5 $cm^3$) for 2.5 h as above. The crude bromide **32/33** (2.05 g, theoretical yield: 1.94 g, 1.71 mmol) and $N^{\alpha}$-Fmoc-Thr-OPfp (0.82 g, 1.62 mmol) were dissolved in dry dichloromethane (60 $cm^3$) and the solution was stirred under Ar over molecular sieves (3 Å; 5.0 g; powdered) at −60 °C for 1 h. Silver triflate (0.50 g, 1.94 mmol) was added quickly, and stirring of the mixture at −60 to −35 °C was continued for 75 min (TLC: ~ 50% conversion). Then the temperature was raised to −35 to −30 °C and kept within this range for 75 min. Additional $N^{\alpha}$-Fmoc-Thr-OPfp (0.04 g, 0.08 mmol) was added. After a total reaction time of 3 h, diisopropylethylamine (0.405 $cm^3$, 2.33 mmol) was added. Upon being stirred without cooling for 15 min, the mixture was filtered and the filtrate was concentrated. The resulting foam was subjected to VLC on dried silica gel (4 × 6 cm). Gradient elution with hexane to hexane–ethyl acetate (3.5:1) afforded *title compound* **35** (1.58 g, 59%), $[\alpha]_D$ −39.2 (*c* 1.0, $CHCl_3$); $^1H$ and $^{13}C$ NMR data are presented in Tables 7 and 9, respectively (Found: C, 66.1; H, 4.6; N, 1.0. $C_{86}H_{66}F_5NO_{22}$ requires C, 66.2; H, 4.3; N, 0.9%; M, 1560.46).

**$N^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-*O*-[2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-L-*trans*-hydroxyproline pentafluorophenyl ester 36**

The VLC-purified bromide **32** (0.96 g, 0.85 mmol) and $N^{\alpha}$-Fmoc-Hyp-OPfp **11** (0.44 g, 0.85 mmol) were dissolved in dry dichloromethane (35 $cm^3$) and the solution was stirred under Ar with molecular sieves (3 Å; 2.5 g) at −40 °C for 75 min. Silver triflate (0.26 g, 1.02 mmol) was added quickly, and the temperature was allowed to rise to −20 °C during 45 min. Stirring at −20 °C was continued for 3 h and 2,6-di-*tert*-butyl-4-methylpyridine (0.175 g, 0.85 mmol) was added. The temperature was allowed to rise to 0 °C during 2 h. Filtration through Celite was followed by concentration of the filtrate to ~ 20 $cm^3$; this solution was loaded directly onto a VLC column (4 × 4.5 cm). Gradient elution with hexane to hexane–ethyl acetate (3.5:1) gave impure compound **36** (0.34 g) and *title compound* **36** (0.57 g, 43%). Purification of the impure fraction gave an additional amount of compound **36** (0.24 g, 18%). Total yield of *title compound* **36**: 61%; $[\alpha]_D$ −50.6 (*c* 1.0, $CHCl_3$); $^1H$ and $^{13}C$ NMR data are presented in Tables 8 and 10, respectively (Found: C, 66.0; H, 4.3; N, 0.9. $C_{87}H_{66}F_5NO_{22}$ requires C, 66.45; H, 4.2; N, 0.9%; M, 1572.47).

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