Synthesis of Glycosyltyrosine Building Blocks for Solid-Phase Glycopeptide Assembly: Use of Aryl *tert*-Butyl Ethers as Glycosyl Acceptors in Aromatic Glycosylations

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The synthesis of the suitably protected building blocks for solid-phase glycopeptide synthesis, N^{α}-Fmoc-Tyr(Bz_4-β-D-Gal)-OPfp **3**, N^{α}-Fmoc-Tyr(Bz_4-α-D-Gal)-OPfp **4**, N^{α}-Fmoc-Tyr(Bz_3-α-L-Rha)-OPfp **6**, N^{α}-Fmoc-Tyr[Bz_3-α-L-Rha(1→3)-Bz_3-β-D-Gal]-OPfp **18**, and N^{α}-Fmoc-Tyr[Bz_4-α-D-Glc(1→4)-Bz_3-α-DGlc(1→4)-Bz_3-β-D-Glc]-OPfp **20**, are described. The synthesis involves the coupling of the corresponding glycosyl bromides with the aryl *tert*-butyl ether N^{α}-Fmoc-Tyr(Bu^t)-OPfp **1**, using silver trifluoromethanesulfonate as promoter.

In addition to the range of known O-glycosylation sites in naturally occurring glycoproteins,[1] O-glycosylated tyrosine has been identified in glycogenin.[2] Glycogenin, the 38 kDa primer protein for the biosynthesis of glycogen, is gluco- or malto-sylated on the aromatic side-chain of Tyr-194.[2] The anomeric configuration of this glycosidic linkage has not been determined unequivocally but has been proposed to be α.[3] Similarly the presence of a novel O-glycosidic linkage *via* tyrosine has recently been detected on the surface layer (S-layer) glycoprotein of the prokaryotic eubacterium *Clostridium thermohydrosulfuricum* strains.[4] In two of these strains it was established that the phenolic hydroxy group was glycosylated with a linear hexasaccharide through a β-galactosidic bond.[4b,4c]

The currently most efficient approach for the preparation of O-glycopeptides involves the use of protected glycosyl amino acids as building blocks in a stepwise assembly of peptides.[5] In general, the synthesis of the glycosyl amino acid building blocks usually requires a multistep procedure of glycosylation followed by the selective removal of the α-carboxy protecting group, activation of the carboxylic group, and in some cases exchange of the N^{α}-protecting group.[5,6]

Earlier attempts at obtaining glycosylated tryosine involved the synthesis of N^{α}-Z-Tyr(Ac_4-β-D-Glc)-OMe and the glycoside was obtained in 47% yield by reaction of peracetylated sugars in the presence of Lewis acids at elevated temperatures.[7] Horvat *et al.* coupled 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose with N^{α}-Z-Tyr-OBu^t- and N^{α}-Z-Tyr-OBn at 80 °C by activation with a carbodiimide and CuCl, obtaining in most cases an α:β mixture in 40–68% yield.[8,9] The β-glycoside isolated was deblocked at the carboxylic acid and used in a solution-phase synthesis of a glycopeptide. These authors also glycosylated a tetra- and a penta-peptide at the tyrosine hydroxy group; however, yields were only very low. In addition, treatment of N^{α}-Z-Tyr-OMe and N^{α}-Boc-Tyr-OMe with dimeric 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride in dimethylformamide (DMF) at ambient temperature for 24 h led to the α-D-glycoside derivatives in 27 and 25% yield, respectively.[10,11]

An alternative and simplified strategy has recently been published, which involves the direct glycosylation of the active ester derivatives N^{α}-Fmoc-amino acid pentafluorophenyl (Pfp) esters.[12–16] A systematic study of the direct glycosylation of N^{α}-Fmoc-Tyr-OAll and N^{α}-Fmoc-Tyr-OPfp with several glucopyranosyl and maltosyl donors has been published.[17] It was demonstrated that the most convenient glycosylations were obtained when peracylated glycosyl bromides were used with silver trifluoromethanesulfonate in dichloromethane at −10 °C or in acetonitrile at ambient temperature.

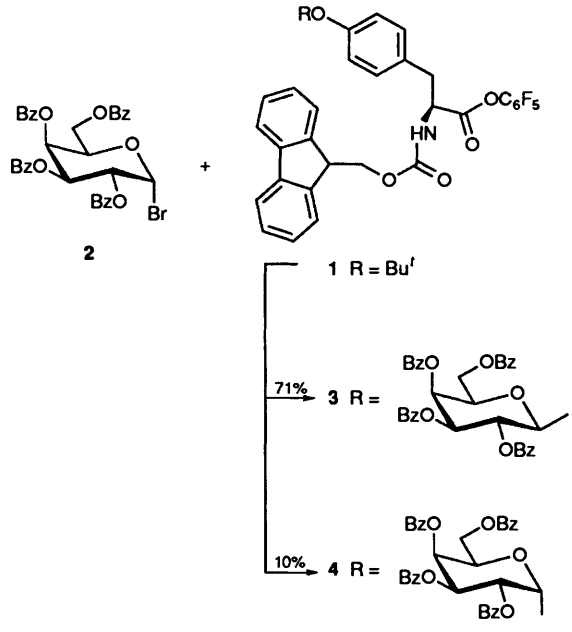
A particular feature associated with aromatic glycosylations, and therefore with the synthesis of tyrosine glycosides, is the lower nucleophilicity of the phenolic hydroxy groups in comparison with aliphatic hydroxy groups. On the other hand, it has been reported that an enhancement of the nucleophilicity of the acceptor hydroxy group (activation) may improve the yields in the glycosylation reactions.[18–22] For example, primary carbohydrate hydroxy groups can be activated by their transformation into trityl ethers.[18,19] Kochetkov *et al.* found that O-*tert*-butyl derivatives of N^{α}-Z-Ser-OMe and N^{α}-Z-Thr-OMe are glycosylated in 51 and 45% yield, respectively, using acetobromoglucose and treatment with silver carbonate followed by addition of toluene-*p*-sulfonic acid and pyridinium perchlorate.[20]

Reaction of acetylated glucopyranosyl bromides in the presence of tin tetrachloride with carbohydrate oxygens activated as tributyltin ethers led to orthoesters.[21] Under similar conditions, benzylated galactopyranosyl halides gave O-glycosides, but with no improvement of the yield.[21,23] Procedures have been reported which involve reaction of the anomeric acetate or trimethylsilyl ethers of the glycosyl donor with the trimethylsilyl ether of the acceptor hydroxy group in the presence of Lewis acid catalysts.[22] These methods are also efficient for stereoselective synthesis of 1,2-*cis*-glycosidic linkages.

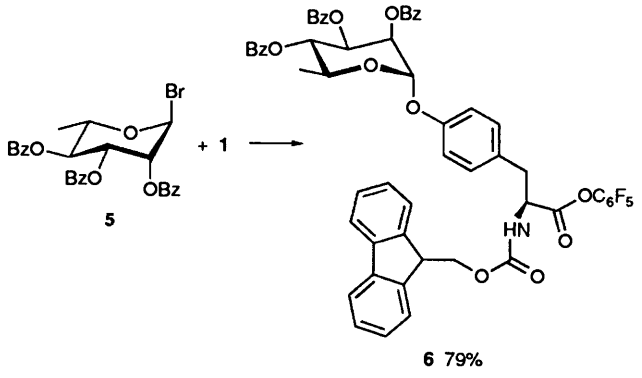
In our recent investigations, N^{α}-Fmoc-Tyr(Bu^t)-OPfp **1** proved to be a better glycosyl acceptor than was N^{α}-Fmoc-Tyr-OPfp for the preparation of N^{α}-Fmoc-Tyr[Ac_4-α-D-Glc-(1→4)-Ac_3-β-D-Glc]-OPfp when using silver trifluoromethanesulfonate (AgOTf) as a promoter.[17] The glycoside was isolated in 81% yield from the Bu^t-derivative compared with a yield of 42% from the derivative with the free hydroxy group. To our knowledge this is the first example of a glycosylation of a *tert*-butyl-protected aromatic hydroxy group in an amino acid derivative. In the present report the general scope of this reaction and its suitability for the preparation of glycosylated tyrosine building blocks for solid-phase peptide synthesis is investigated.

Results and Discussion

First, monosaccharides were studied as glycosyl donors. The reactions were performed in dichloromethane at −10 °C or lower by using AgOTf as a promoter. In a preliminary study



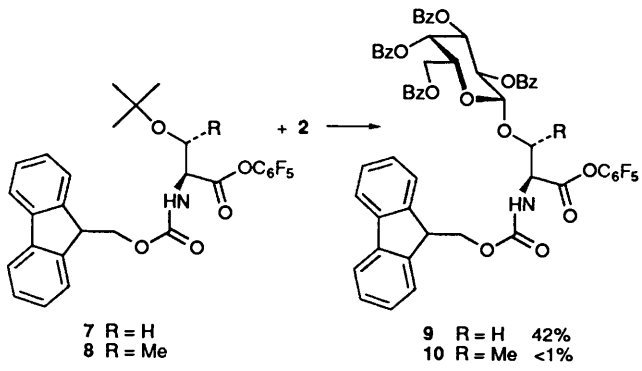
Scheme 1 *Reagents:* AgOTf, CH_2Cl_2



Scheme 2 *Reagents:* AgOTf, CH_2Cl_2

these reaction conditions had proved to be the most efficient for the glycosylation of both N^{α}-Fmoc-Tyr-OPfp and N^{α}-Fmoc-Tyr(Bu^t)-OPfp,[17] **1**. Thus, glycosylation of compound **1** with the benzoylated galactopyranosyl bromide **2** gave the expected 1,2-*trans* product **3** together with a small amount of 1,2-*cis* isomer **4** (Scheme 1). Preparative HPLC afforded the products **3** and **4** in 71% and 10% yield, respectively. Under similar conditions, the more reactive glycosyl donor 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl bromide, **5**, was treated with compound **1**, to give exclusively the 1,2-*trans* glycoside, N^{α}-Fmoc-Tyr(Bz_3-α-L-Rha)-OPfp **6** (Scheme 2). The reaction mixture was kept for 1 h at −20 °C and the product was isolated in 79% yield after preparative HPLC purification.

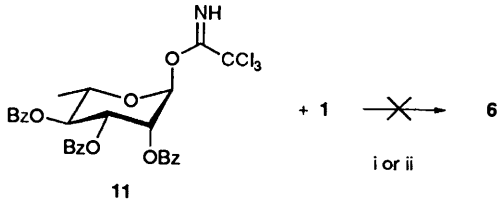
According to Kochetkov *et al.*,[20] the glycosylation of N^{α}-Z-Ser(Bu^t)-OMe and N^{α}-Z-Thr(Bu^t)-OMe proceeds in two steps. First, treatment with silver carbonate led to the formation of an orthoester of the corresponding sugar. However, its conversion into the desired glycosides required further addition of toluene-*p*-sulfonic acid (PTSA), pyridinium perchlorate and catalytic amounts of the corresponding free-hydroxy containing glycosyl acceptors.[20] With this antecedent, we investigated the AgOTf-promoted glycosylation on the aliphatic *tert*-butyl ethers, N^{α}-Fmoc-Ser(Bu^t)-OPfp **7** and N^{α}-Fmoc-Thr(Bu^t)-OPfp **8**. The glycosyl donor **2** was treated with compound **7**. The reaction needed 5 h for completion, according to analytical HPLC, and furnished the glycoside N^{α}-Fmoc-Ser(Bz_4-β-D-Gal)-OPfp **9** in only 42% yield, after preparative HPLC (Scheme 3). Compared



Scheme 3 *Reagents:* AgOTf, CH_2Cl_2

with the previously reported synthesis of compound **9**,[12] the use of the *tert*-butyl ether derivative brought about a lowering of the yield. In addition, compound **10** was not isolated when the benzoylated galactopyranosyl bromide **2** and the threonine derivative **8** were allowed to react under similar conditions. These results are in contrast with those reported which indicated increased reactivity of a secondary compared with a primary trityl ether in trityl-cyanoethylidene condensations.[24] Furthermore, these experiments showed the method to be ineffective for aliphatic glycosylations.

The glycosylation with trichloroacetimidates appears today to be one of the most useful methods for the synthesis of complex oligosaccharides.[25] Therefore, we also tried the glycosylation of compound **1** with compound **11** using both AgOTf and TMSOTf as the promoter (Scheme 4). The reactions were

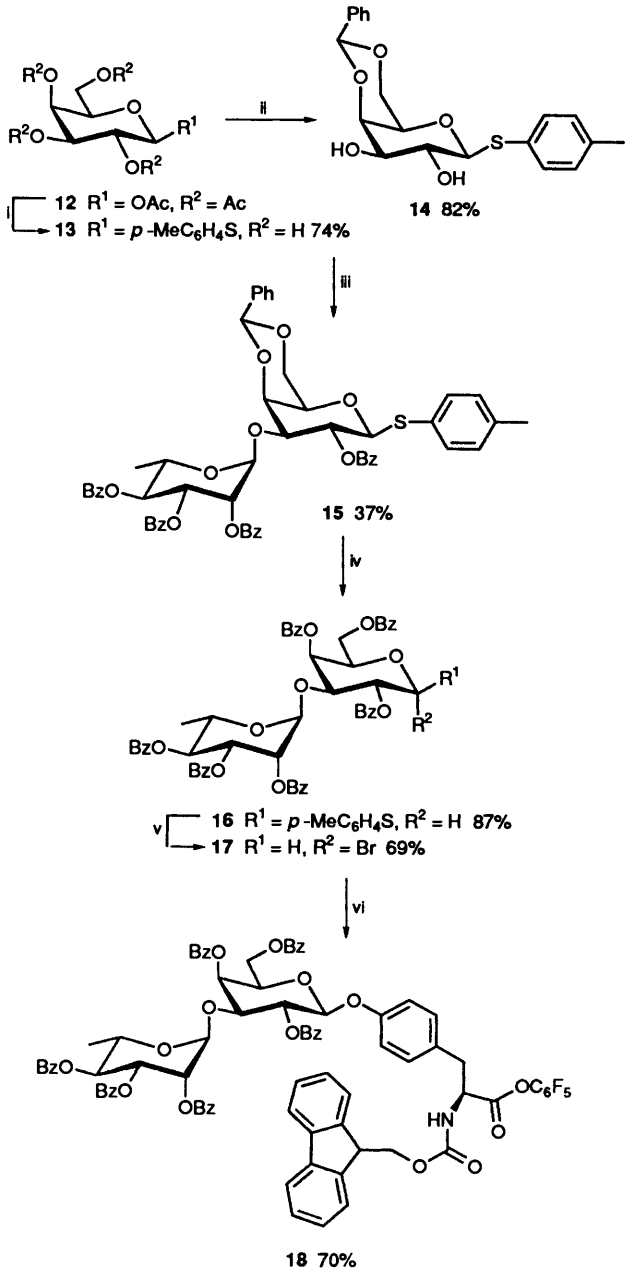


Scheme 4 *Reagents:* i, AgOTf, CH_2Cl_2; ii, TMSOTf, CH_2Cl_2

monitored by TLC, but no glycoside could be detected. The failure of these experiments supported the suggested mechanism of the trichloroacetimidate glycosylation reaction.[26] This mechanism involves a cyclic transition state which requires the participation of a hydrogen bond to the acceptor alcohol, which cannot be formed in compound **1**. At the same time, these results also suggest that the reaction of the glycosyl bromide with the *tert*-butyl ether acceptor proceeded through a nucleophilic attack of the ether oxygen on the oxocarbenium intermediate.

Because excellent yields and good stereoselectivity were observed in the glycosylation with monosaccharides, the study was extended to larger oligosaccharides.

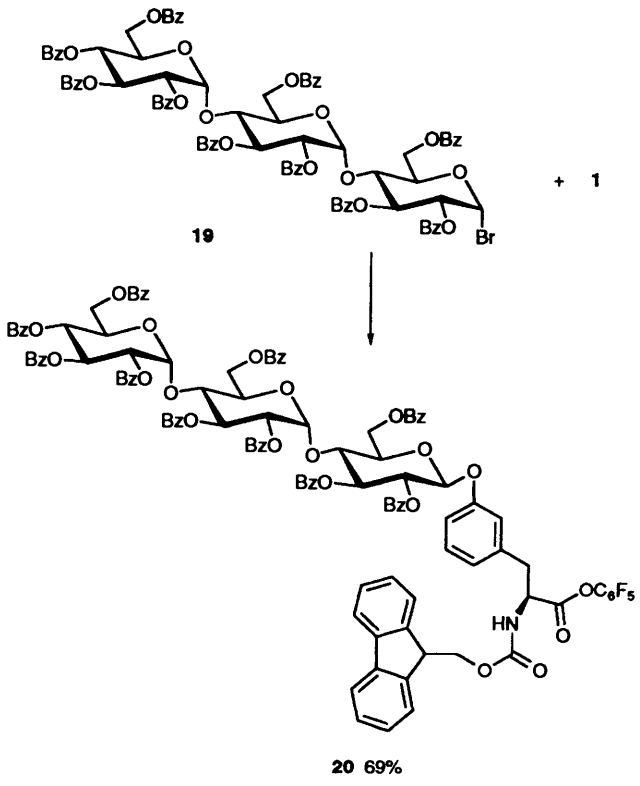
In conjunction with an ongoing project concerning the structural determination and synthesis of glycopeptide–glycoprotein fragments from S-layer glycoprotein[4c] the building block N^{α}-Fmoc-Tyr[Bz_3-α-L-Rha(1→3)-Bz_3-β-D-Gal]-OPfp **18** was synthesized (Scheme 5). Thus, 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose **12** was converted into the thioglycoside **13** in 74% yield, by BF_3:OEt_2-catalysed coupling of *p*-methylbenzenethiol, followed by treatment with sodium methoxide in methanol. Compound **13** then was refluxed with benzaldehyde dimethyl acetal–PTSA in DMF to afford an 82% yield of the 4,6-*O*-benzylidene acetal **14**. The higher reactivity of the 3-hydroxy group of compound **15** favoured the selective glycosylation of the 3-position. Thus, condensation of 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl bromide **5** and the 2,3-diol **14** in the presence of AgOTf in acetonitrile at room temperature



Scheme 5 *Reagents:* i, (*a*) 4-MeC_6H_4SH, BF_3·OEt_2, CH_2Cl_2; (*b*) NaOMe, MeOH; ii, $C_6H_5CH(OMe)_2$, PTSA, DMF; iii, (*a*) **5**, AgOTf, MeCN; (*b*) BzCl, DMAP, pyridine; iv, (*a*) 90% TFA, CH_2Cl_2; (*b*) BzCl, DMAP, pyridine; v, Br_2, CH_2Cl_2; vi, **1**, AgOTf, CH_2Cl_2

gave a mixture, which was benzoylated. Compound **15** could be isolated in 37% yield after silica gel chromatography. Removal of the benzylidene group in compound **15** by treatment with 90% aq. trifluoracetic acid (TFA) and subsequent benzoylation yielded the perbenzoylated 1-thiodisaccharide **16** in 87% yield. Treatment of compound **16** with bromine provided the corresponding bromide **17** in 69% yield after silica gel purification. The coupling of the glycosyl donor **17** with the *tert*-butyl ether **1** was performed in dichloromethane in the presence of AgOTf. The reaction was complete in 1 h at −10 °C and the product, compound **18**, was isolated from HPLC in 70% yield.

Finally, the reaction between decabenzoylmaltotriosyl bromide **19** and the *tert*-butyl ether **1** under similar conditions was investigated. The AgOTf-promoted glycosylation of **19** with the glycosyl acceptor **1** afforded the corresponding glycoside **20** in 69% yield after HPLC (Scheme 6). Attempts to use the even

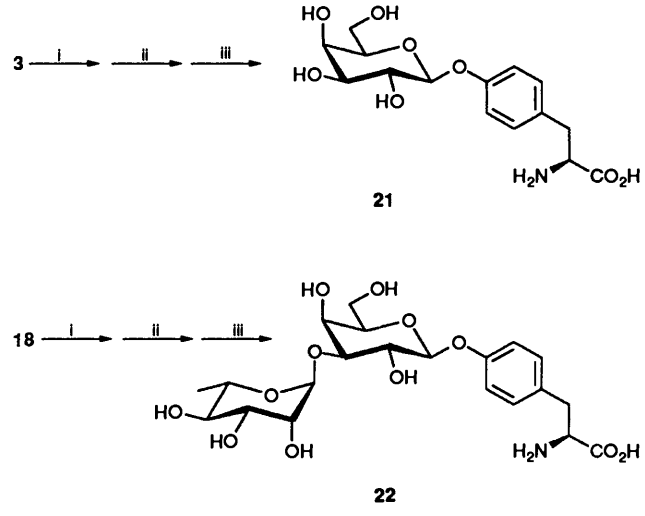


Scheme 6 *Reagents:* AgOTf, CH_2Cl_2

larger perbenzoylated maltoheptosyl bromide as a glycosyl donor were not successful.

The two protected products **3** and **18** were converted into their fully deprotected derivatives **21** and **22**, respectively, by sequential Pfp ester hydrolysis, Fmoc deprotection and transesterification[27] (Scheme 7). The products were characterized by ^1H and ^{13}C NMR spectroscopy.

In conclusion, efficient and convenient stereoselective synthesis of glycosyl tyrosine building blocks for solid-phase glycopeptide synthesis has been presented. The compound N^{α}-Fmoc-Tyr(Bu^t)-OPfp **1**, bearing a *tert*-butyl group on the acceptor oxygen, proved to be a more efficient glycosyl acceptor than the corresponding free-hydroxy derivative in AgOTf-promoted glycosylation reactions with glycosyl bromides.



Scheme 7 *Reagents:* i, HOBt, DMF, water; ii, morpholine; iii, NaOMe, MeOH

However, no improvement was observed by using *tert*-butyl aliphatic ethers as glycosyl acceptors. This fact is probably due to an enhanced nucleophilicity of the ether oxygen electron lone-pairs caused by the *tert*-butyl group. A similar increase in reactivity has recently been demonstrated for tributyltin phenoxides.[28] Simple force-field calculations using the program Insight/Discover (Biosym) indicate that the bulky OBu^t-group forces the lone-pairs out of conjugation with the π-electrons of the aromatic ring. This is further substantiated by the lack of reaction with the otherwise highly reactive trichloroacetimidate glycosyl donors, which suggests that the formation of the oxocarbenium ion is required for the reaction to proceed.

Experimental

General Procedures.—HPLC-grade solvents were purchased from Labscan Ltd. (Dublin, Ireland). Dichloromethane and acetonitrile were distilled from P_4O_{10} and were stored over molecular sieves 3 Å under argon. DMF was distilled by fractional distillation under reduced pressure and kept over molecular sieves 3 Å. Light petroleum was the 60–80 °C fraction. N^{α}-Fmoc amino acids were purchased from Bachem (Bubendorf, Switzerland). TLC was performed on Merck Silica Gel 60 F_{254} aluminium sheets (Darmstadt, Germany) with detection by charring sulfuric acid, and by UV light. Vacuum liquid chromatography[29] (VLC) was performed on Merck Silica Gel 60 H (Darmstadt, Germany). Flasks for glycosylation reactions were stored at 120 °C for 15 h prior to use. Concentrations were carried out at reduced pressure at temperature < 40 °C.

M.p.s were measured on a Büchi melting point apparatus and are uncorrected. Microanalysis was carried out at Leo pharmaceutical products (Ballerup, Denmark). Optical rotations were recorded on a Perkin-Elmer 241 instrument; $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. Electrospray mass spectra (ES-MS) were recorded in the positive mode on a VG Quattro Mass Spectrometer. Preparative purifications by reversed-phase HPLC were carried out on a Waters Delta Prep 3000 equipped with a Waters 991 Photodiode Array detector and using a Waters 1000 Prep Pak module (Flow, 20 cm^3 min^{-1}). Semi-preparative purifications were carried out on a Waters 991 RCM 25 × 10 module with a 25 NV C_{18}(6μ) column (Flow, 10 cm^3 min^{-1}). Analytical HPLC was performed using a Waters RCM 8 × 10 module with a Waters 8 NV C_{18}(4μ) column (Flow, 1 cm^3 min^{-1}). Buffer A was 0.1% aq. TFA and buffer B was 90% acetonitrile, 9.9% water and 0.1% TFA. After HPLC of Pfp-esters the relevant fractions were combined in a separatory funnel and extracted twice with dichloromethane. The combined organic phases were dried over $MgSO_4$, filtered and concentrated to dryness.

^{1}H and ^{13}C NMR spectroscopy was performed on a Bruker AM 500 spectrometer. Unless otherwise indicated the NMR experiments were carried out at 300 K in $CDCl_3$. Chemical shifts are given in ppm and referenced to internal $SiMe_4$ (δ_H, δ_C 0.00). *J* Values are given in Hz (± 0.3 Hz). For all compounds the assignment of ^{1}H NMR spectra was based on 2D homonuclear chemical-shift correlation (COSY) spectra. The assignment of ^{13}C NMR spectra was based on carbon–proton shift-correlation spectra.

N^{α}-*Fmoc-Tyr*(*Bz*$_4$-β-D-*Gal*)-*OPfp* **3**.—Compound **1** (500 mg, 0.80 mmol), AgOTf (410 mg, 1.59 mmol), and molecular sieves (3 Å) were placed in a pre-dried flask in the dark. After evacuation (0.1 Torr) the flask was filled with argon, and dry dichloromethane (20 cm^3) was injected. The suspension was cooled to −10 °C and a solution of the glycosyl bromide **2** (840 mg, 1.27 mmol) in dry dichloromethane (10 cm^3) was injected. After 100 min at −10 °C the suspension was neutralized with diisopropylethylamine (0.271 cm^3) followed by centrifugation. The supernatant was concentrated to dryness, and the residue was redissolved in acetonitrile (10 cm^3) and purified by preparative reversed-phase HPLC (isocratic elution with 75% B for 20 min, then linear gradient of 75–100% B in 50 min followed by isocratic elution with 100% B). This yielded pure β-*glycoside* **3** as a foam (652 mg, 71%); $[\alpha]_D^{25}$ +68.8 (*c* 1, $CHCl_3$) (Found: C, 66.6; H, 4.1; N, 1.3. $C_{64}H_{46}F_5NO_{14}$ requires C, 67.0; H, 4.0; N, 1.2%). ^{1}H and ^{13}C NMR data are presented in Tables 1–4.

N^{α}-*Fmoc-Tyr*(*Bz*$_4$-α-D-*Gal*)-*OPfp* **4**.—The α-*glycoside* **4** (95 mg, 10%) was isolated from the preparation of its anomer **3**; $[\alpha]_D^{25}$ +66.1 (*c* 1, $CHCl_3$) (Found: C, 66.8; H, 4.3%). ^{1}H and ^{13}C NMR data are presented in Tables 1–4.

N^{α}-*Fmoc-Tyr*(*Bz*$_3$-α-L-*Rha*)-*OPfp* **6**.—Compound **1** (93 mg, 0.15 mmol), AgOTf (83 mg, 0.32 mmol), and molecular sieves (3 Å) were placed in a pre-dried flask in the dark. After evacuation (0.1 Torr) the flask was filled with argon, and dry dichloromethane (2 cm^3) was injected. The suspension was cooled to −20 °C and a solution of the glycosyl bromide **5** (175 mg, 0.32 mmol) in dry dichloromethane (2 cm^3) was injected. After 1 h at −20 °C the suspension was neutralized with diisopropylethylamine (0.055 cm^3) followed by filtration through Celite. The solution was concentrated to dryness, and the residue was redissolved in acetonitrile (10 cm^3) and purified by preparative reversed-phase HPLC (linear gradient of 50–100% B in 50 min followed by isocratic elution with 100% B). This yielded pure *title compound* **6** (122 mg, 79%), m.p. 92 °C; $[\alpha]_D^{25}$ +92.0 (*c* 1, $CHCl_3$); 1028.7 $[M + H]^+$ (Found: $C_{57}H_{42}F_5NO_{14}$ requires M, 1027.9). ^{1}H and ^{13}C NMR data are presented in Tables 1–4.

N^{α}-*Fmoc-Ser*(*Bz*$_4$-β-D-*Gal*)-*OPfp* **9**.—Compound **7** (100 mg, 0.19 mmol), AgOTf (96 mg, 0.37 mmol), and molecular sieves (3 Å) were placed in a pre-dried flask in the dark. After evacuation with an oil-pump (0.1 Torr) the flask was filled with argon, and dry dichloromethane (2 cm^3) was injected. The suspension was cooled to −10 °C and a solution of the glycosyl bromide **2** (246 mg, 0.37 mmol) in dry dichloromethane (3 cm^3) was injected. After 5 h at −10 °C the suspension was neutralized with diisopropylethylamine (0.064 cm^3) followed by centrifugation. The supernatant was concentrated to dryness, and the residue was redissolved in acetonitrile (10 cm^3) and purified by preparative reversed-phase HPLC (isocratic elution with 75% B for 20 min, then linear gradient of 75–100% B in 50 min followed by isocratic elution with 100% B). This yielded title compound **9** (82 mg, 42%). NMR data (Tables 1 and 2), m.p. and $[\alpha]_D$-value are in agreement with those reported.[12,30]

p-*Tolyl* 1-*Thio*-β-D-*galactopyranoside* **13**.—To a solution of *p*-methylbenzenethiol (4.66 g, 3.75 mmol) and penta-*O*-acetyl-D-galactopyranose **12** (12.90 g, 3.30 mmol) in dry dichloromethane (260 cm^3) containing molecular sieves (3 Å) was added boron trifluoride–diethyl ether complex (13 cm^3). After 20 h at room temperature the mixture was diluted with dichloromethane (300 cm^3) and filtered through Celite. The organic solution was washed with saturated aq. sodium hydrogen carbonate (3 × 100 cm^3), dried ($MgSO_4$), and concentrated to dryness. The residue was dissolved in 3 mol dm^{-3} methanolic NaOMe. After 1 h at room temperature the solution was neutralized (Amberlite IR C-50) and concentrated to dryness. VLC on silica gel with methanol–chloroform (1 : 9) as the eluent yielded compound **13** (8.00 g, 74%), m.p. 129–130 °C from MeOH; $[\alpha]_D^{25}$ −51.6 (*c* 1, MeOH) (Found: C, 52.9; H, 6.4; S, 10.5. $C_{13}H_{18}O_5S$ requires C, 52.8; H, 6.7; S, 10.1%); ^{1}H NMR and ^{13}C NMR data are presented in Tables 1 and 3.

Table 1 ^{1}H NMR data for carbohydrate units of compounds **3**, **4**, **6**, **9**, **13**–**18**, **20** and **22**; δ-values in ppm (J-values in Hz)

Proton	**3**	**4**	**6**	**9**[a]	**13**[b]	**14**	**15**	**16**	**17**	**18**	**20**	**22**
1-H	5.29 (8.0)	6.10 (3.4)	5.75 (br s)	4.90 (7.8)	4.51 (9.7)	4.49 (9.2)	4.91 (9.9)	4.93 (10.0)	6.04 (2.8)	5.19 (7.8)	5.20 (8.5)	5.11 (7.4)
2-H	6.09 (10.2)	5.91 (10.7)	5.91 (3.4)	5.85 (10.5)	3.58 (9.2)	3.67 (9.2)	5.63 (9.6)	5.73 (9.6)	5.52 (10.1)	6.02 (9.6)	5.48 (10.1)	3.93 (9.9)
3-H	5.69 (3.0)	6.26 (3.3)	6.09 (10.0)	5.72 (3.4)	3.49 (3.0)	3.73 (3.5)	4.11 (3.2)	4.35 (3.2)	4.76 (3.4)	4.38 (2.3)	5.68 (10.1)	3.82 (3.0)
4-H	6.05	6.14	5.81 (10.0)	6.07	3.89	4.24 (0.9)	4.54 (3.2)	5.92	5.57	5.94	4.45	4.09
5-H	4.38 (4.8, 7.4)	4.80 (5.1, 7.6)	4.38 (6.2)	4.38 (6.7, 6.7)	3.53 (6.5, 6.5)	3.58 (1.5, 1.7)	3.67	4.29 (5.1, 7.0)	4.86 (5.3, 6.9)	4.28 (5.0, 6.8)	4.03 (5.9)	3.88
6-H_2	4.51 (11.4), 4.69	4.43 (11.4), 4.62	1.39	4.67 (11.2), 4.70	3.58 (11.4), 3.70	4.06 (12.4), 4.42	4.12 (12.0), 4.49	4.58 (11.7), 4.61	4.59 (11.8), 4.63	4.63 (11.8), 4.65	4.59 (12.0), 4.88	3.78, 3.78
1′-H							5.22	5.19 (1.4)	5.45 (1.6)	5.27 (1.9)	5.62 (3.8)	5.10 (1.0)
2′-H							5.34 (3.2)	5.30 (br s)	5.49 (3.2)	5.38 (2.6)	5.12 (10.0)	4.12 (3.0)
3′-H							5.68 (10.0)	5.53[c]	5.56 (10.0)	5.61 (9.9)	5.96 (8.9)	3.90 (9.8)
4′-H							5.57 (9.9)	5.53[c] (6.0)	5.64 (9.8)	5.58 (10.0)	4.41 (9.6)	3.49 (9.8)
5′-H							4.44 (6.2)	4.31 (6.2)	4.45 (6.2)	4.37 (6.2)	4.44 (3.3)	3.87 (6.0)
6′-H_2							1.20	1.29	1.42	1.35	4.28 (12.5), 4.43	1.31
1″-H											5.79 (3.9)	
2″-H											5.29 (10.5)	
3″-H											6.14 (10.0)	
4″-H											5.70 (10.0)	
5″-H											4.47 (1.9, 3.1)	
6″-H_2											4.66 (12.3), 4.78	
Aromatic protons	7.32–8.17[d]	7.32–8.15[d]	7.31–8.15[d]	7.32–8.15[d]	7.11 (8.1), 7.11	7.14–7.63	7.10–8.09	7.00–8.15	7.10–8.30	7.20–8.40[d]	7.10–8.20[d]	
Other signals					2.3 (Me)	5.54 (CHO_2) 2.40 (Me)	5.58 (CHO_2) 2.41 (Me)	2.36 (Me)				

[a] Refs. 12, 30. [b] Solvent CD_3OD. [c] Very strong $J_{3',4'}$, coupling constant; $\delta_H(C_6D_6)$ 6.08 (1 H, dd, $J_{2',3'}$ 3.4 and $J_{3',4'}$ 10.1, 3′-H) and 6.18 (1 H, t, $J_{2',3'} + J_{3',4'}$ 20.7, 4′-H). [d] Includes Fmoc-aromatic protons.

Table 2 ^{1}H NMR data for aglycone protons of compounds **3**, **4**, **6**, **9**, **18**, **20** and **22**; δ-values in ppm (J-values in Hz)

Proton	3	4	6	9[a]	18	20	22[b]
NH	5.22 (8.5)	5.24 (br s)	5.37 (8.4)		5.20 (8.4)	5.10 (8.5)	
α-H	5.02	4.98 (5.8, 6.2)	5.07 (5.9, 6.3)	4.95 (3.3, 3.4)	4.99 (5.8, 6.7)	4.99 (5.5, 6.9)	3.92
β-H	3.19 (14.1)	3.16 (14.4)	3.27 (14.3)	4.59 (10.1)	3.17 (14.3)	3.12 (14.2)	3.23 (14.2, 4.0)
β'-H	3.30	3.27	3.34	4.09	3.27	2.26	3.07 (8.0)
δ-H	7.03 (8.8)	7.05 (8.1)	7.20		7.02	6.99 (8.0)	7.13 (8.4)
ε-H	7.06	7.14				6.91	7.28
Fmoc-C*H*CH$_2$	4.38 (7.4, 7.5)	4.23 (6.6, 6.7)	4.27 (5.9, 6.3)	4.16 (7.0, 7.0)	4.20 (6.6, 6.9)	4.17 (6.6, 7.4)	
Fmoc-CHC*H*$_2$	4.42 (10.5), 4.52	4.45 (10.6), 4.52	4.50 (10.5), 4.58	4.30 (10.4), 4.70	4.41 (10.6), 4.51	4.32 (10.6), 4.53	

[a] Refs. 12, 30. [b] Solvent D_2O.

Table 3 ^{13}C NMR data for carbohydrate units of compounds **3**, **4**, **6**, **13**–**16**, **18**, **20** and **22**; δ-values in ppm

Carbon	3	4	6	13[a]	14	15	16	18	20	22
C-1	100.2	95.5	95.8	90.7	87.0	85.2	86.4	100.0	99.1	101.4
C-2	69.5	68.8	70.6	71.0	68.7	68.1	69.9	71.2	72.1	70.8
C-3	71.6	68.3	69.9	76.3	73.7	81.9	78.5	77.0	74.9	81.0
C-4	67.9	69.0	71.7	70.4	75.3	75.4	69.9	69.4	73.9	69.0
C-5	71.7	67.8	67.5	80.6	70.0	69.7	75.6	72.1	72.9	76.1
C-6	62.1	62.4	17.6	62.6	69.3	69.4	63.2	62.8	63.0	61.3
C-1′						99.6	99.2	99.2	96.7	103.2
C-2′						70.8	70.4	70.5	70.7	70.9
C-3′						69.6	69.9	69.1	71.7	70.8
C-4′						71.5	71.5	71.5	73.7	72.8
C-5′						67.2	67.5	67.2	69.1	70.0
C-6′						17.6	17.5	17.5	62.3	17.6
C-1″									96.8	
C-2″									70.9	
C-3″									70.0	
C-4″									69.0	
C-5″									70.1	
C-6″									63.0	
Aromatic carbons	117.9–143.6[b]	117.3–143.5[b]	116.9–143.4[b]	130.5, 132.1, 132.9, 138.5	126.5–138.5	126.4–134.5	128.1–133.8	117.8–141.3[b]	117.8–143.3[b]	130.6, 131.6, 117.8, 156.7
CO	165.2, 165.5, 165.9	165.5, 165.6, 165.8, 166.0	165.8, 165.9, 166.1			165.7, 171.8	171.5	164.6, 164.9, 165.1, 165.6, 166.1	164.8, 165.1, 165.3, 165.6, 165.8, 165.9	
Other signals				21.1 (Me)	101.4 (CHO_2) 21.2 (Me)	101.4 (CHO_2) 21.3 (Me)	21.3 (Me)			

[a] Solvent CD_3OD. [b] Includes Fmoc and tyrosine aromatic carbons.

Table 4 ^{13}C NMR data for tyrosine and Fmoc carbons of compounds **3**, **4**, **6**, **18**, **20** and **22**; δ-values in ppm (J-values in Hz)

Carbon	3	4	6	18	20	22
C-α	54.5	54.6	54.6	54.5	54.4	56.7
C-β	37.2	37.0	37.1	37.2	37.2	36.2
Fmoc-CH	47.1	47.1	47.0	47.1	47.1	
Fmoc-CH$_2$	67.3	67.1	67.5	67.2	67.3	
OCONH	156.4	155.8	155.5	156.5	156.3	
*C*O$_2$R	167.9	167.9	167.8	168.0	168.0	182.0
C-F ($J_{C,F}$)[a]	137.8 (244), 139.7 (242), 140.9 (251)	137.9 (246) 141.0 (248)	137.8 (240), 140.9 (250), 142.3 (245)	137.5, 140.5, 142.4	137.9 (250), 141.0 (250)	

[a] These values are the one-bond ^{19}F–^{13}C coupling constants in Hz.

p-Tolyl 4,6-O-*Benzylidene*-1-*thio*-β-D-*galactopyranoside* **14**.—Compound **13** (4.00 g, 1.40 mmol) and benzaldehyde dimethyl acetal (2.34 g, 1.54 mmol) were dissolved in dry DMF (35 cm^3) and PTSA (catalytic amount) was added. The flask was attached to an evaporator and the reaction mixture was kept at 50 °C under rotation at water-pump pressure for 45 min.

Further benzaldehyde dimethyl acetal (0.50 g) was added and the reaction mixture was kept under the same conditions for 1 h, then excess of triethylamine was added until basic on moist pH paper. The solution was concentrated to dryness. VLC on silica gel with ethyl acetate as the eluent yielded *compound* **14** (4.32 g, 82%), m.p. 158 °C (from Et_2O); $[\alpha]_D^{25}$ −71.8 (*c* 1, MeOH) (Found: C, 63.9; H, 5.8; S, 8.8. $C_{20}H_{22}O_5S$ requires C, 64.15; H, 5.9; S, 8.6%); ^1H NMR and ^{13}C NMR data are presented in Tables 1 and 3.

p-*Tolyl* 2-O-*Benzoyl*-4,6-O-*benzylidene*-1-*thio*-3-O-(2,3,4-*tri*-O-*benzoyl*-α-L-*rhamnopyranosyl*)-β-D-*galactopyranoside* **15**.—Compound **14** (335 mg, 0.895 mmol), AgOTf (469 mg, 1.808 mmol), and molecular sieves (3 Å) were placed in a pre-dried flask in the dark. After evacuation (0.1 Torr) the flask was filled with argon, and dry acetonitrile (22 cm^3) was injected. The suspension was stirred at room temperature for 15 min and then a solution of the bromide **5** (962 mg, 1.785 mmol) in (3:2) dry acetonitrile–dichloromethane (23 cm^3) was injected. After 20 h at room temperature the suspension was neutralized with diisopropylethylamine (0.32 cm^3). The mixture was filtered on Celite and the solution was concentrated to dryness. VLC on silica gel with ethyl acetate–toluene (1:15) as eluent gave a mixture [TLC ethyl acetate–light petroleum (1:2), R_F 0.2]. This mixture was dissolved in dry pyridine (5 cm^3) and 4-(dimethylamino)pyridine (DMAP) (catalytic amount) was added. The solution was cooled to 0 °C and benzoyl chloride (1.3 cm^3) was added. The mixture was stirred for 5 h at room temperature, then was cooled with ice and methanol was added dropwise until all solids had dissolved. The solution was diluted with dichloromethane and the organic phase was washed successively with 5% aq. HCl (3 × 100 cm^3) and saturated aq. $NaHCO_3$ (3 × 100 cm^3), dried ($MgSO_4$) and concentrated to dryness. VLC on silica gel with ethyl acetate–toluene (1:15) as eluent gave *title compound* **15** (310 mg, 37%) as a foam; $[\alpha]_D^{25}$ +100.0 (*c* 1, $CHCl_3$) (Found: C, 68.9; H, 5.1; S, 3.3. $C_{54}H_{48}O_{13}S$ requires C, 69.2; H, 5.2; S, 3.4%). ^1H and ^{13}C NMR data are presented in Tables 1 and 3.

p-*Tolyl* 2,4,6-*Tri*-O-*benzoyl*-1-*thio*-3-O-(2,3,4-*tri*-O-*benzoyl*-α-L-*rhamnopyranosyl*)-β-D-*galactopyranoside* **16**.—To a solution of compound **15** (302 mg, 0.32 mmol) in dichloromethane (11 cm^3) at 0 °C was added 90% aq. TFA (0.6 cm^3). After 1 h, the solution was diluted with toluene and the solvent was evaporated off. The residue was dissolved in dry pyridine (10 cm^3) and DMAP (catalytic amount) was added. The solution was cooled to 0 °C and benzoyl chloride (4 cm^3) was added. The mixture was stirred for 5 h at room temperature before being cooled with ice, and then treated dropwise with methanol until all solids had dissolved. The solution was diluted with dichloromethane and the organic phase was washed successively with 5% aq. HCl (3 × 75 cm^3) and saturated aq. $NaHCO_3$ (3 × 100 cm^3), dried ($MgSO_4$) and concentrated to dryness. VLC on silica gel with ethyl acetate–toluene (1:15) as eluent gave *title compound* **16** as a foam (298 mg, 87%); $[\alpha]_D^{25}$ +130.7 (*c* 1, $CHCl_3$) (Found: $[M + H]^+$, 1057.7. $C_{61}H_{52}O_{15}S$ requires M, 1057.15). ^1H and ^{13}C NMR data are presented in Tables 1 and 3.

N^{α}-*Fmoc*-*Tyr*[Bz_3-α-L-*Rha*(1→3)-Bz_3-β-D-*Gal*]-*OPfp* **18**.—To a solution of compound **16** (133 mg, 0.13 mmol) in dry dichloromethane (4 cm^3) containing molecular sieves (3 Å) was added 0.19 mol dm^{-3} bromine in dichloromethane (0.8 cm^3). After 2 h at room temperature, the solution was diluted with toluene and concentrated to dryness. New addition of toluene and concentration were repeated 3 times. VLC on silica gel with ethyl acetate–light petroleum (1:2) yielded compound **17** as a foam (88 mg. 69%); ^1H NMR data are presented in Table 1.

Compound **1** (36 mg, 0.06 mmol), AgOTf (26 mg, 0.10 mmol), and molecular sieves (3 Å) were placed in a predried flask in the dark. After evacuation with an oil-pump the flask was filled wih argon, and dry dichloromethane (0.8 cm^3) was injected. The suspension was cooled to −10 °C and a solution of the glycosyl bromide **17** (88 mg, 0.09 mmol) in dry dichloromethane (1.5 cm^3) was injected. After 1 h at −20 °C the suspension was neutralized (moist pH paper) with diisopropylethylamine (0.023 cm^3) followed by filtration on Celite. The solution was concentrated to dryness, and the residue was redissolved in acetonitrile (10 cm^3) and purified by semi-preparative reversed-phase HPLC (linear gradient of 50–100% B in 120 min followed by isocratic elution with 100% B). This yielded pure title compound **18** as a foam (61 mg, 70%), $[\alpha]_D^{25}$ +84.8 (*c* 1, $CHCl_3$) [Found: *m/z* 1503.1 $(M + H)^+$. $C_{84}H_{64}F_5NO_{20}$ requires M, 1502.4]. ^1H and ^{13}C NMR data are presented in Tables 1–4.

N^{α}-*Fmoc*-*Tyr*[Bz_4-α-D-*Glc*(1→4)-Bz_3-α-D-*Glc*(1→4)-Bz_3-β-D-*Glc*]-*OPfp* **20**.—Compound **1** (45 mg, 0.07 mmol), AgOTf (38 mg, 0.15 mmol), and molecular sieves (3 Å) were placed in a predried flask in the dark. After evacuation with an oil-pump (0.1 Torr) the flask was filled with argon, and dry dichloromethane (3 cm^3) was injected. The suspension was cooled to −10 °C and a solution of the glycosyl bromide **19**[31] (238 mg, 0.15 mmol) in dry dichloromethane (2 cm^3) was injected. After 90 min at −10 °C the suspension was neutralized (moist pH paper) with diisopropylethylamine (0.026 cm^3) followed by filtration on Celite. The solution was concentrated to dryness and the residue was redissolved in acetonitrile (10 cm^3) and purified by preparative reversed-phase HPLC (linear gradient of 50–100% B in 70 min followed by isocratic elution with 100% B). This yielded pure title compound **20** (104 mg, 69%); $[\alpha]_D^{25}$ +57.0 (*c* 1, $CHCl_3$) [Found: 2120.5 $(M + Na)^+$ and 2103.7 $(M + Li)^+$. $C_{118}H_{90}F_5NO_{30}$ requires M, 2097.5]. ^1H and ^{13}C NMR data are presented in Tables 1–4.

4-O-α-D-*Galactopyranosyl*-L-*tyrosine* **21**.—Compound **3** (150 mg, 0.13 mmol) was dissolved in DMF (2 cm^3) and 1-hydroxybenzotriazole (HOBt) (67 mg, 0.42 mmol) and water (0.2 cm^3) were added. The solution was stirred at 20 °C for 18 h until no starting material could be observed by TLC [ethyl acetate–light petroleum (1:3)]. Morpholine (1 cm^3) was added and after 35 min the solution was concentrated to dryness under reduced pressure. The residue was suspended in methanol (2 cm^3) and the suspension was adjusted pH 12 (moist pH paper) by addition of 1 mol dm^{-3} mathanolic sodium methoxide. After 7 h at 20 °C the suspension was neutralized with solid CO_2. It was then concentrated to dryness and the residue was separated by silica gel chromatography [ethyl acetate–methanol–water–acetic acid (6:2:1:1)]. The title compound **21** (33 mg) was isolated as a syrup in 73% yield. The ^1H and ^{13}C NMR spectra of compound **21** have been fully assigned and data have been reported elsewhere.[4c]

4-O-(O-α-L-*Rhamnopyranosyl*-(1→3)-β-D-*galactopyranosyl*)-L-*tyrosine* **22**.—Compound **18** (48 mg, 0.032 mmol) was dissolved in DMF (2 cm^3) and HOBt (3 mg, 0.02 mmol) and water (1 cm^3) were added. The solution was stirred at 20 °C for 24 h. Morpholine (0.5 cm^3) was added and after 35 min the solution was concentrated to dryness under reduced pressure. The residue was dissolved in methanol (2 cm^3) and the solution was adjusted pH 12 (moist pH paper) by addition of 1 mol dm^{-3} methanolic sodium methoxide. After 20 h at 20 °C the solution was neutralized with solid CO_2 and purified by gel filtration on a 1.6 × 80 cm column of Sephadex G-10. The title compound **22** (10.4 mg) was isolated as a syrup, which was characterized by ^1H and ^{13}C NMR spectroscopy as presented in Tables 1–4.

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