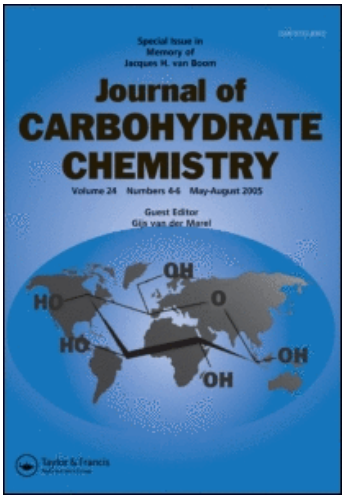


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# SYNTHESIS OF POLYACRYLAMIDE COPOLYMERS CONTAINING (1→6)-BRANCHED (1→3)-β-D-LINKED TRI-AND TETRA-SACCHARIDES RELATED TO SCHIZOPHYLLAN

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## ABSTRACT

The allyl β-glycosides of a trisaccharide *O*-β-D-Glc*p*-(1→3)-*O*-[β-D-Glc*p*-(1→6)]-β-D-Glc*p* and of a tetrasaccharide *O*-β-D-Glc*p*-(1→3)-*O*-[β-D-Glc*p*-(1→6)]-*O*-β-D-Glc*p*-(1→3)-β-D-Glc*p*, corresponding to the branching point or the repeating unit of antitumor (1→6)-branched-(1→3)-β-D-glucans, have been synthesized starting from ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-α-D-glucopyranoside and copolymerized in a radical reaction with acrylamide to obtain polyacrylamide copolymers containing the tri- and tetrasaccharides for immunochemical studies of schizophyllan.

## INTRODUCTION

Fragments having the structures of a branched trisaccharide, *O*-β-D-glucopyranosyl-(1→3)-*O*-[β-D-glucopyranosyl-(1→6)]-D-glucopyranose (**9**), and of a branched tetrasaccharide, *O*-β-D-glucopyranosyl-(1→3)-*O*-[β-D-glucopyranosyl-(1→6)]-*O*-β-D-glucopyranosyl-(1→3)-D-glucopyranose (**25**), occur in many kinds of antitumor (1→3)-β-D-glucans from fungi and lichen.[1,2] We have previously synthesized,[3] using 2,4,6-tri-*O*-acetyl-3-*O*-allyl-α-D-glucopyranosyl bromide as the key intermediate,[4] (1→6)-branched (1→3)-β-D-glucotetraoses (including **25**), which correspond to the three possible

repeating units of schizophyllan, a (1→6)-branched (1→3)-β-D-glucan having antitumor activity.[1] These D-glucotetraoses have been shown to serve as the inhibitors for the precipitation reaction between an antitumor (1→6)-branched (1→3)-β-D-glucan, isolated from the fruiting body of *Volvariella volvacea*, and the antibody specific to the glucan.[5]

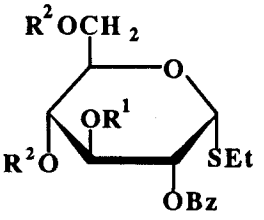
For immunological studies of schizophyllan, we required linear polyacrylamide polymers **31** and **32** with the branches of **9** and **25**, which may be obtainable by radical copolymerization[6,7] of acrylamide with the allyl β-glycosides of **9** and **25**, namely, allyl *O*-β-D-glucopyranosyl-(1→3)-*O*-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside (**17**) and allyl *O*-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)]-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside (**30**). We now report the synthesis of **17** and **30**, using ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-α-D-glucopyranoside[8] (**1**) as the starting material, and the preparation of **31** and **32**.



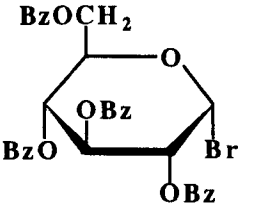
## RESULTS AND DISCUSSION

Condensation of **1** with 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide[9] (**5**) in dichloromethane-toluene in the presence of silver triflate[10] and 2,6-di-*tert*-butyl-4-methylpyridine[11] (DTBMP) gave the (1→3)-β-D-linked disaccharide derivative **6** (86%). Removal of the benzylidene group from **2** by treatment with ethylene glycol-*p*-toluenesulfonic acid[12] in acetonitrile afforded the disaccharide derivative **7** (90%) having free HO-4 and -6 groups. Selective glucosylation of the primary hydroxyl group of **7** with 1.2 mol equiv of **6**, promoted by silver triflate, gave the (1→6)-branched trisaccharide derivative **10** (75%), which was benzoylated to afford the deca-*O*-benzoyl derivative **11**. *O*-Deacetylation of **10** and subsequent acetylation provided the deca-*O*-acetyl derivative **12**.

Treatment of **11** and **12** in dichloromethane with bromine and tetraethyammonium bromide[11,13] gave the corresponding α-bromides **13** (92%) and **15**[3] (84%), respectively, which were coupled with allyl alcohol in toluene in the presence of mercuric cyanide to provide allyl *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-*O*-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2,4-di-*O*-benzoyl-β-D-glucopyranoside (**14**, 85%) and allyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)]-2,4-di-*O*-acetyl-β-D-glucopyranoside (**16**, 81%), respectively. In attempted reactions to obtain **14** and **16**, the trisaccharide thioglycosides **11** and **12** did not react with allyl alcohol in the presence of methyl triflate[14], dimethyl(methylthio)-sulfonium triflate[15] (DMTST) or nitrosyl tetrafluoroborate.[16] Saponification of **14** with methanolic sodium methoxide yielded crystalline trisaccharide allyl β-glycoside **17**, which was identical in all respects to the compound obtained by *O*-deacetylation of **16**. The




| | $R^1$ | $R^2$ |
|---|---|---|
| 1 | H | benzylidene |
| 2 | $BrCH_2CO$ | benzylidene |
| 3 | H | H |
| 4 | H | isopropylidene |

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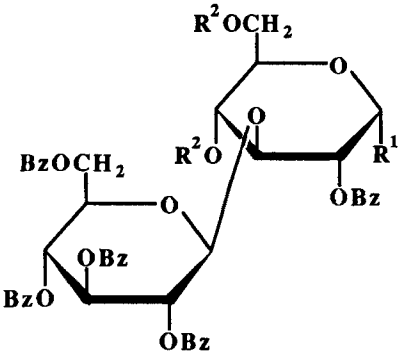
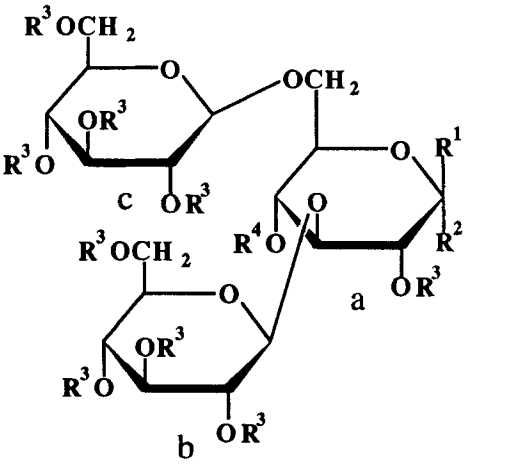



| | $R^1$ | $R^2$ |
|---|---|---|
| 6 | SEt | benzylidene |
| 7 | SEt | H |
| 8 | Br | benzylidene |



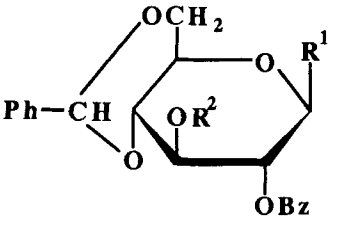

| | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| 9 | -H,OH- | | H | H |
| 10 | H | SEt | Bz | H |
| 11 | H | SEt | Bz | Bz |
| 12 | H | SEt | Ac | Ac |
| 13 | H | Br | Bz | Bz |
| 14 | OAll | H | Bz | Bz |
| 15 | H | Br | Ac | Ac |
| 16 | OAll | H | Ac | Ac |
| 17 | OAll | H | H | H |

sequence (**10** → **12** → **15** → **16** → **17**) may be superior to the one (**10** → **11** → **13** → **14** → **17**) for the preparation of substantial amounts of **17**, since the former requires no chromatographic separation of the products at any steps.

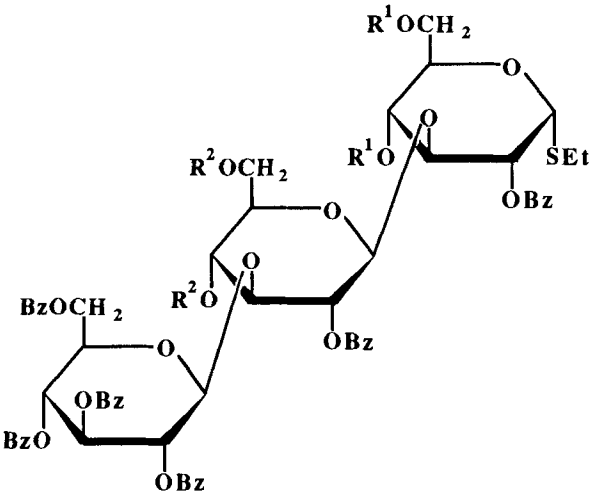
Esterification of **1** with bromoacetyl bromide[17]-pyridine in dichloromethane gave **2**, which was transformed by treatment with chlorine[8,18] into the corresponding crystalline β-chloride **18** (83%). Condensation of **18** with allyl alcohol in the presence of silver triflate-DTBMP afforded **19** (85%), which was *O*-debromoacetylated with thiourea in the presence of 2,6-dimethylpyridine[19] to produce allyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**20**, 93%). However, attempted glycosylations of **20** with the trisaccharide thioglycosides **11** or **12** in the presence of methyl triflate, DMTST or nitrosyl tetrafluoroborate or with the trisaccharide α-glycosyl bromides **13** or **15** in the presence of silver triflate-DTBMP were unsuccessful; no coupling took place. Therefore, an alternative approach to **30** was sought.

Removal of the benzylidene group from **1** with aqueous acetic acid gave **3** (92%) which, on treatment with 2,2-dimethoxypropane-*p*-toluenesulfonic acid in *N*, *N*-dimethylformamide, afforded ethyl 2-*O*-benzoyl-4,6-*O*-isopropylidene-1-thio-β-D-glucopyranoside (**4**, 86%). Silver triflate-promoted glycosylation of **4** with *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranosyl bromide[13] (**8**), prepared (89%) by treatment of **6** with bromine-tetraethylammonium bromide, gave the trisaccharide derivative **21** (82%). The latter was transformed in 73% overall yield into the trisaccharide derivative **24** having HO-4' and -6' unsubstituted, by a sequence involving selective removal of the isopropylidene group with dilute acid (→ **22**), acetylation (→ **23**), and *O*-debenzylidenation. Preferential glucosylation of the primary hydroxyl group of **24** with **5**, as for the reaction of **7** with **5**, afforded the (1→6)-branched tetrasaccharide derivative **26** (72%). Sequential *O*-deacylation of **26** and acetylation gave the trideca-*O*-acetyl thioglycoside **27**. Treatment of **27** with bromine-tetraethylammonium bromide gave the corresponding α-bromide **28**, which was coupled with allyl alcohol in toluene in the presence of mercuric cyanide to give allyl *O*-(2,3,4,6-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→6)]-*O*-(2,4-di-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranoside (**29**, 80%). *O*-Deacetylation of **29** furnished crystalline tetrasaccharide allyl β-glycoside **30**.

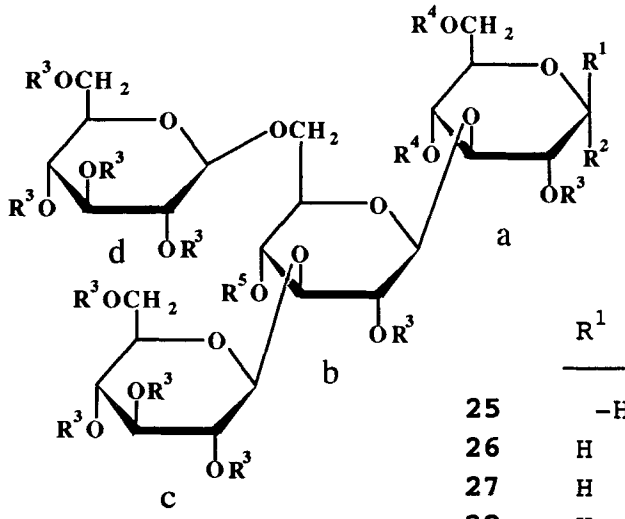
The allyl β-glycosides **17** and **30** were each copolymerized with acrylamide in water in the presence of ammonium persulfate and *N,N,N',N'*-tetramethylethylenediamine[6,7] (TEMED) to provide the copolymers **31** and **32**, which were purified by gel permeation chromatography (GPC) on a column of Bio-Gel P-6. The $^{13}C$ NMR spectra of **31** and **32** contained the signals of the carbons for the carbohydrate moieties, similar to those for the respective monomers **17** and **30**, as well as the signals[7] for $CONH_2$, CH , and $CH_2$ groups



| | $R^1$ | $R^2$ |
|---|---|---|
| **18** | Cl | $BrCH_2CO$ |
| **19** | OAll | $BrCH_2CO$ |
| **20** | OAll | H |

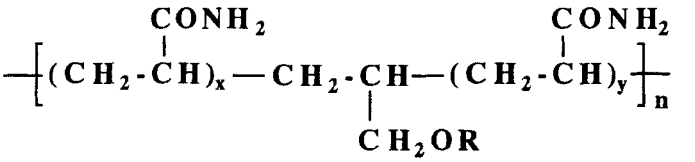


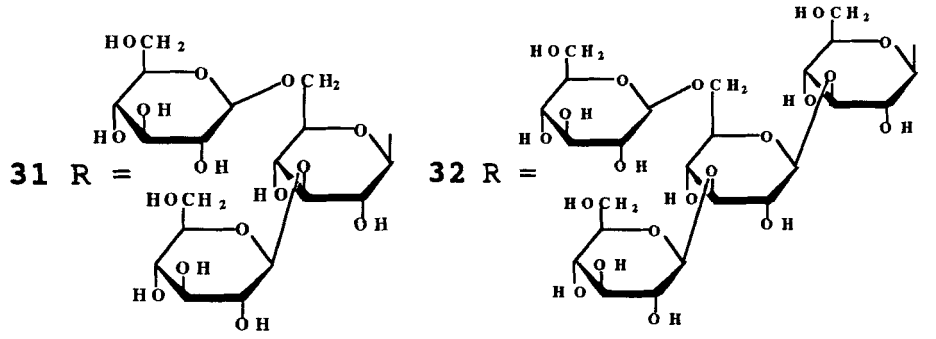
| | $R^1$ | $R^2$ |
|---|---|---|
| **21** | isopropylidene | benzylidene |
| **22** | H | benzylidene |
| **23** | Ac | benzylidene |
| **24** | Ac | H |



| | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ |
|---|---|---|---|---|---|
| **25** | -H,OH- | | H | H | H |
| **26** | H | SEt | Bz | Ac | H |
| **27** | H | SEt | Ac | Ac | Ac |
| **28** | H | Br | Ac | Ac | Ac |
| **29** | OAll | H | Ac | Ac | Ac |
| **30** | OAll | H | H | H | H |

of the non-carbohydrate chain. The carbohydrate contents of **31** and **32** were 40 and 42% (w/w), respectively, as determined by the phenol-sulfuric colorimetric method.[20] The molar ratio of acrylamide to the tri-and tetra-saccharides in **31** and **32** were therefore both calculated to be ~11:1. The weight-average molecular weights, determined by GPC-low-angle laser light-scattering photometer (LALLS), were $13.7 \times 10^4$ and $14.2 \times 10^4$ for **31** and **32**, respectively. The immunochemical study using **31** and **32** is under way.








## EXPERIMENTAL

**General Procedures.** Unless stated otherwise, these were as described.[8] Optical rotations were measured at 20 °C. NMR spectra ($^1H$ at 90 MHz, $^{13}C$ at 22.6 MHz) were recorded with a Hitachi R-90H spectrometer for solutions in $CDCl_3$ and $Me_2SO$-$d_6$ (internal $Me_4Si$) or $D_2O$ (internal sodium 4,4-dimethyl-4-silapentanoate-$d_4$). Molecular weights were determined by GPC (columns; G-3000PW + G-5000PW + G-6000 PW, Tosoh Co. Ltd.)-LALLS ( LS-8000, Tosoh Co. Ltd.).

**Ethyl *O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-α-D-glucopyranoside (6).** A solution of **5** (16.36 g, 24.8 mmol) in $CH_2Cl_2$ (100 mL) was added dropwise over a period of 45 min at 0 °C to a stirred mixture of **1** (8.61 g, 20.7 mmol), silver triflate (7.01 g, 27.3 mmol), DTBMP (3.4 g, 16.6 mmol), and powdered 4A molecular sieve (10 g) in $CH_2Cl_2$ (150 mL) and PhMe (50 mL) under Ar. The mixture was gradually allowed to attain room temperature and then

stirred for 30 min. The insoluble material was collected on a Celite pad and washed with $CH_2Cl_2$, and the combined filtrate and washings were washed successively with aq $Na_2S_2O_3$, aq $NaHCO_3$, and $H_2O$, dried, and concentrated. The residue was subjected to column chromatography (PhMe-EtOAc, 50:1→30:1, stepwise) to give **6** (17.69 g, 86%): $[\alpha]_D$ +63.7° (*c* 1.3, $CHCl_3$); $^{13}C$ NMR ($CDCl_3$) δ 165.9, 165.4, 164.9, 164.8, and 164.0 (C=O), 101.3 and 101.0 (C-1', benzylic C), 82.5 (C-1), 63.1 (C-6'), and 24.2 and 14.75 ($SCH_2CH_3$).

Anal. Calcd for $C_{56}H_{50}O_{15}S$: C, 67.60; H, 5.06. Found: C, 67.85; H, 5.15.

**Ethyl *O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-1-thio-α-D-glucopyranoside (7).** To a solution of **6** (12.54 g, 12.6 mmol) and ethylene glycol (14.0 mL, 0.25 mol) in $CH_3CN$ (200 mL) was added *p*-TsOH·$H_2O$ (0.2 g). The mixture was stirred overnight at room temperature, made neutral with $Et_3N$, concentrated, and extracted with $CH_2Cl_2$. The extract was washed with $H_2O$, dried, and concentrated. Column chromatography (PhMe-EtOAc, 4:1) of the residue gave **7** (10.72 g, 90%): $[\alpha]_D$ +70.9° (*c* 1.2, $CHCl_3$); $^{13}C$ NMR ($CDCl_3$) δ 165.9, 165.5, 164.9, and 164.6 (2 C) (C=O), 101.65 (C-1'), 82.7 (C-1), 81.3 (C-3), 62.5 (C-6), and 23.9 and 14.5 ($SCH_2CH_3$).

Anal. Calcd for $C_{49}H_{46}O_{15}S$: C, 64.89; H, 5.12. Found: C, 64.95; H, 5.20.

**Ethyl *O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-*O*-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2-*O*-benzoyl-1-thio-α-D-glucopyranoside (10).** To a stirred mixture of **7** (6.55 g, 7.2 mmol), silver triflate (2.67 g, 10.4 mmol), DTBMP (0.8 g, 3.9 mmol), and powdered 4A molecular sieve (10 g) in $CH_2Cl_2$ (200 mL) and PhMe (50 mL) at -20 °C was added dropwise during 1 h a solution of **5** (5.72 g, 8.7 mmol) in $CH_2Cl_2$ (80 mL) under Ar. The mixture was allowed to reach 0 °C and then stirred for 1 h. Processing of the mixture as described for the preparation of **6**, followed by column chromatography (PhMe-EtOAc, 30:1→15:1, stepwise) of the residue, afforded **10** (8.04 g, 75%): $[\alpha]_D$ +44.1° (*c* 1.4, $CHCl_3$); $^{13}C$ NMR ($CDCl_3$) δ 165.9-164.4 (C=O), 101.8 and 101.6 (C-1b,c), 82.8 (C-1a), 80.8 (C-3a), 63.1 and 62.8 (C-6b,c), and 23.3 and 14.2 ($SCH_2CH_3$).

Anal. Calcd for $C_{83}H_{72}O_{24}S$: C, 67.11; H, 4.89. Found: C, 67.25; H, 4.77.

**Ethyl *O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-*O*-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2,4-di-*O*-benzoyl-1-thio-α-D-glucopyranoside (11).** Benzoylation of **10** (3.24 g) with benzoyl chloride (0.38 mL) in pyridine (15 mL), followed by column chromatography (PhMe-EtOAc, 50:1) of the residue, gave **11** (3.29 g, 95%): $[\alpha]_D$ +6.5° (*c* 1.8, $CHCl_3$).

Anal. Calcd for $C_{90}H_{76}O_{25}S$: C, 68.00; H, 4.82. Found: C, 68.16; H, 4.92.

**Ethyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-*O*-[2,3,4,6-tetra-**

***O*-acetyl-β-D-glucopyranosyl-(1→6)]-2,4-di-*O*-acetyl-1-thio-α-D-glucopyranoside (12).** A solution of **10** (4.19 g) in dry MeOH (50 mL) and $CH_2Cl_2$ (10 mL) was treated with M NaOMe (1 mL). The mixture was kept overnight at room temperature, made neutral with Amberlite IR-120 ($H^+$) resin, filtered, and concentrated. A solution of the residue in $Ac_2O$-pyridine (20 mL, 1:1 ) was stirred for 1 h at 80 °C, cooled, and concentrated. The last traces of solvents were removed by repeated evaporation of PhMe from the residue, crystallization of which from MeOH gave **12** (2.30 g, 90%): mp 228-230 °C; $[\alpha]_D$ +48.3° (*c* 1.3, $CHCl_3$); $^{13}C$ NMR($CDCl_3$) δ 170.3-168.7 (C=O), 100.7 (2 C, C-1b,c), 80.55 (C-1a), 76.8 (C-3a), 61.8 (2 C, C-6b,c), 20.85-20.3 ($COCH_3$), and 23.6 and 14.4 ($SCH_2CH_3$).

Anal. Calcd for $C_{40}H_{56}O_{25}S$: C, 49.59; H, 5.83. Found: C, 49.65; H, 5.77.

**Allyl *O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-*O*-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2,4-di-*O*-benzoyl-β-D-glucopyranoside (14).** A solution of $Br_2$ (46 μL, 893 μmol) in dry $CH_2Cl_2$ (5 mL) was added at 0 °C to a stirred mixture of **11** (11.19 g, 749 μmol) and 4A molecular sieve (1 g) in $CH_2Cl_2$ (10 mL). After 20 min, $Et_4NBr$ (0.82 g, 1.5 mmol) was added and the mixture was stirred for 2 h at room temperature. The mixture was diluted with $CH_2Cl_2$, filtered through a cotton plug, and the filtrate was washed successively with $H_2O$, aq $NaHCO_3$, and $H_2O$, dried, and concentrated. Column chromatography (hexane-EtOAc, 2:1) of the residue afforded *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-*O*-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2,4-di-*O*-benzoyl-α-D-glucopyranosyl bromide (**13**; 1.10 g, 92%): $[\alpha]_D$ +3.1° (*c* 1.9, $CH_2Cl_2$); NMR ($CDCl_3$) $\delta_H$ 6.44 (d, 1 H, $J_{1,2}$ =3.5 Hz, H-1); $\delta_C$ 101.3 and 101.1 (C-1b,c), and 87.2 (C-1a).

Compound **13** (0.89 g, 553 μmol) was dissolved in a mixture of PhMe (9 mL) and allyl alcohol (0.12 mL, 1.8 mmol) containing $Hg(CN)_2$ (0.2 g, 792 μmol) and 4A molecular sieve (0.5 g). The mixture was stirred overnight at 40 °C and filtered through a Celite layer, which was washed with PhMe. The combined filtrate and washings were washed successively with $H_2O$, aq KI, dried, and concentrated. Column chromatography (PhMe-EtOAc, 15:1) of the residue gave **14** (0.75 g, 85%): $[\alpha]_D$ -24.3° (*c* 1.1, $CHCl_3$); $^{13}C$ NMR ($CDCl_3$) δ 165.8-164.1 (C=O), 133.2 and 116.9 ($CH=CH_2$), 101.3 and 100.5 (C-1b,c), 99.1 (C-1a), and 63.2 and 62.9 (C-6b,c).

Anal. Calcd for $C_{91}H_{76}O_{26}$: C, 68.93; H, 4.83. Found: C, 69.08; H, 4.72.

**Allyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)]-2,4-di-*O*-acetyl-β-D-glucopyranoside (16).** Compound **12** (0.88 g, 939 μmol) was treated in $CH_2Cl_2$ (8 mL) containing 4A molecular sieve (0.5 g) with a solution of $Br_2$ (58 μL, 1.1 mmol) in $CH_2Cl_2$ (5 mL) and $Et_4NBr$ (0.39 g, 1.85 mmol) as described for **11**. Processing of the mixture as described for the

preparation of **13**, followed by crystallization of the residue from $CH_2Cl_2$-$Et_2O$, afforded *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→6)]-2,4-di-*O*-acetyl-α-D-glucopyranosyl bromide (**15**; 0.78g, 84%): mp 192-193 °C; $[\alpha]_D$ +43° (*c* 1.5, $CH_2Cl_2$); lit.[3] 193-195 °C, $[\alpha]_D$ +42°.

Compound **15** (0.55 g, 557 μmol) was dissolved in a mixture of PhMe (8 mL) and allyl alcohol (0.11 mL, 1.6 mmol) containing $Hg(CN)_2$ (0.28 g, 1.1 mmol) and 4A molecular sieve (0.5 g). Processing of the mixture as described for the preparation of **14**, followed by crystallization of the residue from EtOH, afforded **16** (0.44 g, 81%): mp 184-185 °C; $[\alpha]_D$ -35.8° (*c* 1.0, $CHCl_3$); $^{13}C$ NMR ($CDCl_3$) δ 170.25-168.6 (C=O), 133.2 and 117.2 ($CH=CH_2$), 100.7 (2 C, C-1b,c), 99.2 (C-1a), 61.8 (C-6a,b,c), and 20.9-20.3 ($COCH_3$).

Anal. Calcd for $C_{41}H_{56}O_{26}$: C, 51.04; H, 5.85. Found: C, 51.09; H, 5.80.

**Allyl *O*-β-D-Glucopyranosyl-(1→3)-*O*-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside (17).** *O*-Debenzoylation of **14**, as described for **10**, gave **17** (0.17 g, 92%): mp 156-160 °C (from aq EtOH); $[\alpha]_D$ -37.3° (*c* 1.0, $H_2O$); $^{13}C$ NMR ($D_2O$) δ 135.9 and 121.2 ($CH=CH_2$), 105.4 (2 C, C-1b,c), 103.6 (C-1a), 87.1 (C-3a), and 63.4 (3 C, C-6a,b,c).

Anal. Calcd for $C_{21}H_{36}O_{16}$: C, 46.32; H, 6.66. Found: C, 46.27; H, 6.78.

Compound **17** (0.19 g, 95%) was also obtained from **16** (0.35 g) by a similar procedure.

**Ethyl 2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-bromoacetyl-1-thio-α-D-glucopyranoside (2).** A solution of **1** (3.01 g, 7.2 mmol) in $CH_2Cl_2$ (30 mL) containing pyridine (1.2 mL, 14.8 mmol) was cooled to -20 °C, treated with a solution of $BrCH_2COBr$ (0.82 mL, 0.4 mmol) in $CH_2Cl_2$ (10 mL), and kept for 15 min at 0 °C. The mixture was diluted with $CH_2Cl_2$, poured into ice-$H_2O$, and the organic layer was separated, washed successively with cold dil. HCl, aq $NaHCO_3$, and $H_2O$, dried, and concentrated. Column chromatography (PhMe-EtOAc, 50:1) of the residue gave **2** (3.57 g, 92%): mp 120-160 °C (broad, from $Et_2O$-petroleum ether)); $[\alpha]_D$ +145.8° (*c* 1.4, $CHCl_3$); $^{13}C$ NMR ($CDCl_3$) δ 166.1 and 165.2 (C=O), 136.65 and 133.4 (aromatic C-1), 101.5 (benzylic C), 82.8 (C-1), 71.8 and 71.0 (C-2,3), 68.5 (C-6), 62.9 (C-5), 25.0 ($BrCH_2CO$), and 24.4 and 14.75 ($SCH_2CH_3$).

Anal. Calcd for $C_{24}H_{25}BrO_7S$: C, 53.64; H, 4.69. Found: C, 53.57; H, 4.65

**2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-bromoacetyl-β-D-glucopyranosyl chloride (18).** A solution of $Cl_2$ (0.39 g, 5.5 mmol) in $CHCl_3$ (5 mL) was added at 0 °C to a solution of **2** (1.97 g, 3.7 mmol) in $CCl_4$ (10 mL) and $CH_2Cl_2$ (3 mL). After 5 min, the solvents were evaporated and volatile non-carbohydrate by-products[8,18] were removed by repeated evaporation of PhMe. Crystallization of the residue from $Et_2O$-petroleum ether

gave **18** (1.56 g, 83%): mp 157-160 °C; $[\alpha]_D$ -22.8° (*c* 1.4, $CH_2Cl_2$); $^{13}C$ NMR δ 166.1 and 164.7 (C=O), 136.2 and 133.5 (aromatic C-1), 101.4 (benzylic C), 87.9 (C-1), and 24.75 ($BrCH_2CO$).

Anal. Calcd for $C_{22}H_{20}BrClO_7$: C, 51.63; H, 3.94. Found: C, 51.52; H, 3.86

**Allyl 2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-bromoacetyl-β-D-glucopyranoside (19).** A solution of **18** (1.39 g, 2.7 mmol) in $CH_2Cl_2$ (10 mL) was added dropwise at 0 °C to a stirred mixture of allyl alcohol (0.37 mL, 5.4 mmol), silver triflate (0.84 g, 3.3 mmol), DTBMP (0.39 g, 1.9 mmol), powdered 4A molecular sieve (1 g) in $CH_2Cl_2$ (10 mL) and PhMe (5 mL). The mixture was stirred for 1 h at 0 °C and processed as described for the preparation of **6.** Crystallization of the residue from EtOH afforded **19** (1.23 g, 85%): mp 144-145 °C; $[\alpha]_D$ -18.2° (*c* 1.0, $CHCl_3$); $^{13}C$ NMR ($CDCl_3$) δ 166.3 and 164.9 (C=O), 133.1 and 117.5 ($CH=CH_2$), 101.3 (benzylic C), 100.3 (C-1), and 24.9 ($BrCH_2CO$).

Anal. Calcd for $C_{25}H_{25}BrO_8$: C, 56.30; H, 4.72. Found: C, 56.35; H, 4.69.

**Allyl 2-*O*-Benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside (20).** A solution of $(NH_2)_2C=S$ (0.3 g, 3.9 mmol) and 2,6-dimethylpyridine (0.23 mL, 2 mmol) in MeOH (20 mL) was added dropwise at room temperature to a stirred solution of **19** (1.07 g, 2 mmol) in $CH_2Cl_2$ (20 mL). The mixture was stirred for 20 min and then concentrated. A solution of the residue in $CH_2Cl_2$ was washed successively with cold dil. HCl, aq $NaHCO_3$, and $H_2O$, dried, and concentrated. Crystallization of the residue from EtOH-hexane gave **20** (0.77 g, 93%): mp 128-129 °C; $[\alpha]_D$ -41.3° (*c* 1.5, $CHCl_3$); $^{13}C$ NMR ($CDCl_3$) δ, 165.65 (C=O), 133.0 and 117.4 ($CH=CH_2$), 101.7 (benzylic C), and 100.2 (C-1)

Anal. Calcd for $C_{23}H_{24}O_7$: C, 66.98; H, 5.87. Found: C, 67.16; H, 5.94.

**Ethyl 2-*O*-Benzoyl-1-thio-α-D-glucopyranoside (3).** A solution of **1** (3.02 g) in 60% AcOH (50 mL) was heated for 30 min at 90 °C and the solvents were removed by repeated evaporation of PhMe. Crystallization of the residue from $Me_2CO$-MeOH gave **3** (2.19 g, 92%): mp 183-184 °C; $[\alpha]_D$ +176.6° (*c* 1.0, DMF); $^{13}C$ NMR ($Me_2SO$-$d_6$) δ 165.0 (C=O), 133.2 (aromatic C-1), 80.9 (C-1), 60.5 (C-6), and 23.0 and 14.6 ($SCH_2CH_3$).

Anal. Calcd for $C_{15}H_{20}O_6S$: C, 54.86; H, 6.14. Found: C, 54.89; H, 6.10.

**Ethyl 2-*O*-Benzoyl-4,6-*O* -isopropylidene-1-thio-α-D-glucopyranoside (4).** A mixture of **3** (3.52 g), 2,2-dimethoxypropane (7 mL) and *p*-TsOH·$H_2O$ (25 mg) in *N*, *N*-dimethylformamide (20 mL)) was stirred for 3 h at room temperature. Trimethylamine (0.5 mL) was added and the mixture was concentrated. Column chromatography (hexane-EtOAc, 2:1) of the residue afforded **4** (3.40 g, 86%): $[\alpha]_D$ +164.7° (*c* 1.6, $CHCl_3$); NMR ($CDCl_3$) $\delta_H$ 8.09-7.34 (m, 10 H, 2 Ph), 5.71 (d, 1 H, $J_{1,2}$ = 5.9 Hz, H-1), 2.40 (m, 2 H,

$SCH_2CH_3$), 1.52 and 1.45 (2 s, each 3 H, $CMe_2$), and 1.20 (t, 3 H, $SCH_2CH_3$); $\delta_C$ 165.6 (C=O), 99.9 ($Me_2C$), 82.8 (C-1), 29.0 and 19.0 ($Me_2C$), and 24.4 and 14.8 ($SCH_2CH_3$).

Anal. Calcd for $C_{18}H_{24}O_6S$: C, 58.68; H, 6.57. Found: C, 58.76; H, 6.61.

**Ethyl *O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→3)-4,6-*O*-isopropylidene-2-*O*-benzoyl-1-thio-α-D-glucopyranoside (21).** Compound **6** (6.59 g) was treated in $CH_2Cl_2$ (60 mL) containing 4A molecular sieve (2 g) with a solution of $Br_2$ (0.41 mL, 8 mmol) in $CH_2Cl_2$ (20 mL) and $Et_4NBr$ (2.78 g, 13.2 mmol), as described for the preparation of **13**. Column chromatography (hexane-EtOAc, 2:1) of the residue gave *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranosyl bromide[13] (**8**; 5.97 g, 89%): $[\alpha]_D$ +68.5° (*c* 1.9, $CH_2Cl_2$); $^1H$ NMR ($CDCl_3$) δ 6.63 (d, 1 H, $J_{1,2}$ = 3.9 Hz, H-1). The $^{13}C$ NMR spectrum was identical to that reported [13].

A mixture of **4** (1.54 g, 4.2 mmol), silver triflate (1.68 g, 6.5 mmol), DTBMP (0.86 g, 9.2 mmol), and powdered 4A molecular sieve (3 g) in $CH_2Cl_2$ (20 mL) and PhMe (15 mL) was treated with a solution of **8** (5.51 g, 5.4 mmol), as described for the preparation of **6**. Column chromatography (PhMe-EtOAc, 30:1→10:1, stepwise) of the product gave **21** (4.46 g, 82%): $[\alpha]_D$ +56.5° (*c* 1.1, $CHCl_3$); $^{13}C$ NMR ($CDCl_3$) δ 165.8-164.6 (C=O), 101.1, 99.85, 98.7, and 98.1 (C-1',1", benzylic C, $CMe_2$), 82.3 (C-1), 29.5 and 19.4 ($CMe_2$), and 24.2 and 14.7 ($SCH_2CH_3$).

Anal. Calcd for $C_{72}H_{68}O_{21}S$: C, 66.45; H, 5.27. Found: C, 66.60; H, 5.36.

**Ethyl *O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-1-thio-α-D-glucopyranoside (22).** To a solution of **21** (4.19 g) in $Me_2CO$ (50 mL) was added M HCl (0.5 mL). The mixture was stirred for 2.5 h at room temperature, neutralized with solid $NaHCO_3$, and filtered through a Celite layer. The filtrate was concentrated and a solution of the residue in $CH_2Cl_2$ was washed with $H_2O$, dried, and concentrated. Column chromatography (PhMe-EtOAc, 7:3) of the residue afforded **22** (3.53 g, 87%): $[\alpha]_D$ +50° (*c* 1.2, $CHCl_3$); $^{13}C$ NMR ($CDCl_3$) δ 165.8-164.1 (C=O), 101.4 (2 C) and 100.4 (C-1',1", benzylic C), and 23.8 and 14.5 ($SCH_2CH_3$).

Anal. Calcd for $C_{69}H_{64}O_{21}S$: C, 65.71; H, 5.11. Found: C, 65.88; H, 5.22.

**Ethyl *O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-1-thio-α-D-glucopyranoside (23).** Acetylation of **22** (3.56 g) with $Ac_2O$-pyridine (30 mL, 1:1), followed by column chromatography (PhMe-EtOAc, 4:1) of the product, afforded **23** (3.56 g, 96%): $[\alpha]_D$ +50.3° (*c* 1.5, $CHCl_3$); $^{13}C$ NMR ($CDCl_3$) δ 170.4-163.9 (C=O), 101.5 (2 C) and 100.5 (C-1',1", benzylic C), 20.8 ($COCH_3$), and 24.2, and 14.6 ($SCH_2CH_3$).

Anal. Calcd for $C_{73}H_{68}O_{23}S$: C, 65.17; H, 5.09. Found: C, 65.30; H, 4.92.

**Ethyl *O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl- β-D-glucopyranosyl)-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-1-thio-α-D-glucopyranoside (24).** Treatment of **23** (3.27 g) in $CH_3CN$ (35 mL) with ethylene glycol (2.7 mL) and *p*-TsOH·$H_2O$ (15 mg), as described for **6**, followed by column chromatography (PhMe-EtOAc, 2:1) of the product, gave **24** (2.72 g, 89%): $[\alpha]_D$ +46.1° (*c* 1.2, $CHCl_3$); $^{13}C$ NMR ($CDCl_3$) δ 170.3-163.9 (C=O), 101.6 and 100.6 (C-1',1"), 85.7 (C-1), 62.1 (C-6'), 20.6 ($COCH_3$), and 24.2 and 14.6 ($SCH_2CH_3$).

Anal. Calcd for $C_{66}H_{64}O_{23}S$: C, 63.05; H, 5.13. Found: C, 63.20; H, 5.22.

**Ethyl *O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-*O*-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-*O*-(2-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-1-thio-α-D-glucopyranoside (26).** The product obtained by treatment of a mixture of **24** (2.43 g, 1.9 mmol), silver triflate (0.72 g, 2.8 mmol), DTBMP (0.2 g, 973 μmol), and powdered 4A molecular sieve (2 g) in $CH_2Cl_2$ (50 mL) and PhMe (10 mL) with a solution of **5** (1.53 g, 2.3 mmol) in $CH_2Cl_2$ (30 mL), as described for the preparation of **10**, was subjected to column chromatography (PhMe-EtOAc, 6:1→4:1, stepwise) to afford **26** (2.56 g, 72%): $[\alpha]_D$ +23.5° (*c* 1.7, $CHCl_3$); $^{13}C$ NMR ($CDCl_3$) δ 101.4 (3 C, C-1b,c,d), 85.7 (C-1a), 20.6 ($COCH_3$), and 24.1 and 14.6 ($SCH_2CH_3$).

Anal. Calcd for $C_{100}H_{90}O_{32}S$: C, 65.42; H, 4.94. Found: C, 65.59; H, 5.17.

**Ethyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→6)]-*O*-(2,4-di-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (27).** The product obtained by *O*-deacylation of **26** (2.39 g) and subsequent acetylation, as described for the preparation of **12**, was subjected to column chromatography (PhMe-EtOAc, 1:1) to give **27** (1.46 g, 90%): $[\alpha]_D$ +23.7° (*c* 1.3, $CHCl_3$); $^{13}C$ NMR ($CDCl_3$) δ 170.3-168.2 (C=O), 100.9 and 100.55 (2 C) (C-1b,c,d), 81.2 (C-1a) 20.5 ($COCH_3$), and 24.1 and 14.7 ($SCH_2CH_3$).

Anal. Calcd for $C_{52}H_{74}O_{33}S$: C, 49.60; H, 5.92. Found: C, 49.70; H, 5.85.

**Allyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→6)]-*O*-(2,4-di-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (29).** The product obtained by treatment of **27** (1.07 g, 850 μmol) in $CH_2Cl_2$ (12 mL) containing 4A molecular sieve (1 g) with a solution of $Br_2$ (53 μL, 1 mmol) in $CH_2Cl_2$ (5 mL) and $Et_4NBr$ (0.36 g, 1.7 mmol), as described before, was subjected to column chromatography to give *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→6)]-*O*-(2,4-di-*O*-acetyl-β-D-glucopyranosyl) - (1→3) - 2, 4, 6-tri-*O*-acetyl-α-D-glucopyranosyl bromide (**28**; 0.90 g, 83%): $[\alpha]_D$ +34.6° (*c* 0.8, $CH_2Cl_2$); NMR ($CDCl_3$) $\delta_H$ 6.52 (d, 1 H,

$J_{1,2}$ = 3.7 Hz, H-1); $\delta_C$ 170.25-168.15 (C=O), 100.9, 100.6, and 100.5 (C-1b,c,d), 87.2 (C-1a), and 20.8-20.3 ($COCH_3$).

Condensation of **28** (0.75 g, 588 μmol) with allyl alcohol (0.12 mL, 1.8 mmol), as described for the preparation of **14**, followed by column chromatography (EtOAc-PhMe, 3:2) of the product, gave **29** (0.59 g, 80%): $[\alpha]_D$ -44.2° (*c* 1.0, $CHCl_3$); $^{13}C$ NMR ($CDCl_3$) δ 170.5-168.5 (C=O), 133.4 and 117.2 ($CH=CH_2$), 100.9 and 100.2 (2 C) (C-1b,c,d), 99.2 (C-1a), and 20.6-20.45 ($COCH_3$).

Anal. Calcd for $C_{53}H_{72}O_{34}$: C, 50.80; H, 5.79. Found: C, 50.75; H, 5.72.

**Allyl *O*-β-D-Glucopyranosyl-(1→3)-*O*-[β-D-glucopyranosyl-(1→6)]-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside (30).** *O*-Deacetylation of **29** (0.47 g), as described before, gave **30** (0.25 g, 94%): mp 185-186 °C (from aq EtOH), $[\alpha]_D$ -20.2° (*c* 1.2, $H_2O$); $^{13}C$ NMR ($D_2O$) δ 139.95 and 121.3 ($CH=CH_2$), 105.4 (C-1b,c,d), 103.6 (C-1a), 87.9 and 86.8 (C-3a,b), and 63.4 (C-6a,c,d).

Anal. Calcd for $C_{27}H_{46}O_{21}$: C, 45.89; H, 6.56. Found: C, 45.80; H, 6.67.

**Copolymerization of 17 and 30 with acrylamide.** A solution of **17** (50 mg), acrylamide (25 mg) and TEMED (2 μL) in distilled $H_2O$ (0.5 mL) was deaerated at aspirator pressure for 30 min and then flushed with Ar. After addition of a solution of $(NH_4)_2S_2O_8$ (1 mg) in $H_2O$ (10 μL) by injection through a rubber septum, the mixture was kept overnight at room temperature, diluted with $H_2O$ (1 mL), applied to a column of Bio-Gel P-6 (extra fine, 2.5 × 80 cm), and eluted with $H_2O$. Product-containing fractions were combined and concentrated. Lyophilization then gave **31** (36 mg), $[\alpha]_D$ -15.3° (*c* 1.3, $H_2O$).

The copolymer **32** (31 mg) was also obtained by copolymerization of **30** (50 mg) with acrylamide (25 mg) in a similar manner. Compound **32** had $[\alpha]_D$ -11.7° (*c* 1.7, $H_2O$).



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