Syntheses of Benzyl 6-*O*-Sulfo-β-D-glucopyranoside Salts and Their 6*S*-Deuterated Analogues. Conformational Preferences of Their (Sulfonyloxy)methyl Group

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Received June 21, 1989

The syntheses of benzyl 6-*O*-sulfo-β-D-glucopyranoside as the sodium (**7**), lithium (**8**), potassium (**9**), and calcium (**10**) salts in 30% total yield from D-glucose are described. Sulfation of the 6-hydroxy compounds **5** and **17** involved quenching of the reaction mixture with triethylamine and subsequent chromatography on silica gel. This approach resulted in the isolation of the triethylammonium salts of the sulfates **6** and **18** in 92% yield, which represents a significant improvement over earlier procedures. Treatment of **6** with 1.02 M sodium, potassium, or calcium methoxide or lithium ethoxide dissolved in the corresponding alcohol releases the triethylamine and converts the sugar into the deacetylated forms **7**, **9**, **10**, and **8**, respectively. The sodium salt **19**, stereospecifically deuterated at C-6, allowed an unambiguous determination of the two H-6 protons in the ^{1}H NMR spectra. Conformational analysis by NMR spectroscopy of the various sulfates **7**, **9**, **10**, and **8** revealed that neither the sulfate nor the counterion has a significant effect on the conformational equilibrium of the hydroxymethyl group, which is found in solution to be *gt*:*gg* = 38:59.

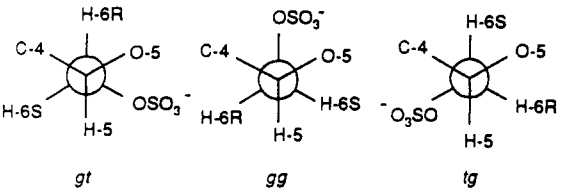
Introduction

6-O-Sulfation of sugars is often found in biologically active oligo- and polysaccharides.[1,2] Heparin, for example, is a highly sulfated glycosaminoglycan which demonstrates regulatory functions in the blood-clotting cascade.[3] A pentasaccharide constituent of heparin has been shown to be responsible for the binding affinity for antithrombin III[4,5] and has subsequently been synthesized.[6,7] N-Type oligosaccharides contain various sugars which may be sulfated in the 6-position.[8,9] O-Linked oligosaccharides, found in the proteoglycans of chondroitin sulfate, were shown to be 6-sulfated.[10] Nevertheless, the influence of sulfate groups on the conformation of an oligosaccharide is not fully understood.[11] Charged, general electrostatic, and nonbonded interactions are possible. The energetics of the sulfate groups in the interaction with a polar carbohydrate are not known. Further, cations are known to influence the molecular conformation of sulfated poly- or oligosaccharides.[1,12,13] As a result, it is necessary to have a simple procedure to prepare sulfated sugars with selected counterions in order to examine this influence of the cations. The usual purification method for sulfated sugars, ion-exchange chromatography, involves a tedious workup procedure which often results in reduced yields. Therefore, we have developed a new access for sulfates with various cations and we have studied the conformational preferences of a freely rotating (sulfonyloxy)methyl group in dependence on a variety of counterions, i.e., sodium, potassium, lithium, and calcium.

The conformational analysis of a hydroxymethyl group can be determined from the interpretation of the coupling constants $J_{H5,H6}$ (Scheme I). There are two hydrogen atoms at the 6-position, and therefore an unequivocal assignment of the resonances belonging to the *pro-S* H-6 and the *pro-R* H-6 in the ^{1}H NMR spectrum must be made. This may be achieved via the stereospecific deuteration of the 6-position followed by a comparison of the spectra of the deuterated with those of the nondeuterated compound.[14]

A variety of syntheses directed toward stereospecifically 6-deuterated glucoses all of which suffer from low diastereoselectivity[15] or long reaction sequences[16,17] have been described in the literature. A major simplification was developed by Ohrui et al., who described a convenient synthesis of hexoses stereospecifically labeled at either 6*S* or 6*R* via a stereospecific photobromination of 1,6-anhydro-β-D-aldopyranose derivatives.[18,19]

Scheme I. Newman Projections of the Three Staggered Conformers of the Rotation of the C-5–C-6 Bond (C5 to the Front)



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(1) Comper, W. D.; Laurent, T. C. *Physiol. Rev.* **1978**, *58*, 255.
(2) Lindahl, U.; Höök, M. *Annu. Rev. Biochem.* **1978**, *47*, 385.
(3) Casu, B. *Adv. Carbohydr. Chem. Biochem.* **1985**, *43*, 51.
(4) Lindahl, U.; Bäckström, G.; Thunberg, L.; Leder, I. G. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 6551.
(5) Choay, J.; Lormeau, J. C.; Petitou, M.; Sinay, P.; Casu, B.; Oreste, P.; Torri, G.; Gatti, G. *Thromb. Res.* **1980**, *18*, 573.
(6) van Boeckel, C. A. A.; Beetz, T.; Vos, J. N.; de Jong, A. J. M.; van Aelst, S. F.; van den Bosch, R. H.; Mertens, J. M. R.; van der Vlugt, F. A. *J. Carbohydr. Chem.* **1985**, *4*, 293.
(7) Petitou, M.; Duchaussoy, P.; Lederman, I.; Choay, J.; Sinay, P.; Jacquinet, J.-C.; Torri, G. *Carbohydr. Res.* **1986**, *147*, 221.
(8) Roux, L.; Holojda, S.; Sundblad, G.; Freeze, H. H. Varki, A. *J. Biol. Chem.* **1988**, *263*, 8879.
(9) Baenziger, J. U.; Green, E. D. *Biochim. Biophys. Acta* **1988**, *947*, 287.
(10) Krusius, T.; Reinhold, V. N.; Margolis, R. K.; Margolis, R. U. *Biochem. J.* **1987**, *245*, 229.
(11) Ferro, D. R.; Provasoli, A.; Ragazzi, M.; Torri, G.; Casu, B.; Gatti, G.; Jacquinet, J. C.; Sinay, P.; Petitou, M.; Choay, J. *J. Am. Chem. Soc.* **1986**, *108*, 6773.
(12) Cael, J. J.; Winter, W. T.; Arnott, S. *J. Mol. Biol.* **1978**, *125*, 21.
(13) Dais, P.; Perlin, A. S. *Carbohydr. Res.* **1987**, *168*, 163.
(14) Rao, V. S.; Perlin, A. S. *Can. J. Chem.* **1983**, *61*, 2688.
(15) Gagnaire, D.; Horton, D.; Taravel, F. R. *Carbohydr. Res.* **1973**, *27*, 363.
(16) Kakinuma, K. *Tetrahedron Lett.* **1977**, 4413.
(17) Kakinuma, K. *Tetrahedron* **1984**, *40*, 2089.
(18) Ohrui, H.; Horiki, H.; Kishi, H.; Meguro, H. *Agric. Biol. Chem.* **1983**, *47*, 1101.

0022-3263/90/1955-0902$02.50/0

Scheme II

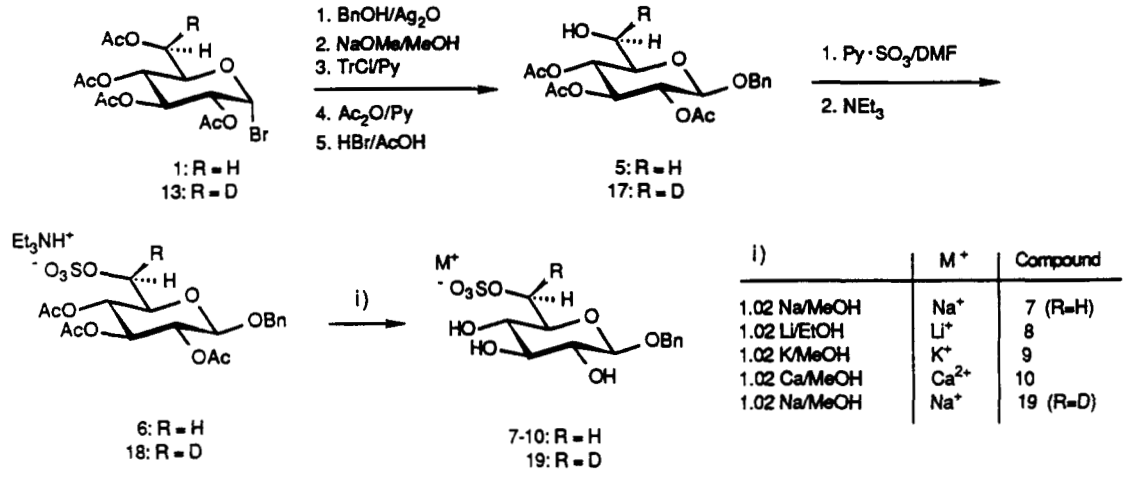

Table I. ^{1}H NMR Chemical Shifts and Coupling Constants of the H-5 and H-6 Protons of β-D-Glucose, 3, and 5–10 and Rotameric Distributions Calculated from the Coupling Constants $J_{H5,H6}$[35]

	δ, ppm			$J_{5,6}$, Hz		rotameric distribution[a]		
	H-5	H-6_{pro-R}	H-6_{pro-S}	H-6_{pro-R}	H-6_{pro-S}	*gt*	*gg*	*tg*
β-D-glucose[b]		3.72	3.90	6.0	2.1	45	53	2
3	3.31	3.56	3.76	5.4	1.8	40	60	−1
7 (Na)	3.50	4.08	4.20	5.3	2.1	38	59	3
8 (Li)	3.50	4.07	4.20	5.4	2.1	39	58	2
9 (K)	3.50	4.07	4.20	5.4	2.1	39	58	2
10 (Ca)	3.50	4.07	4.20	5.4	2.1	39	58	2
5	3.19	3.44	3.59	5.2	2.4	35	59	6
6	3.81	4.15	4.19	5.5	3.1	36	52	12

[a] The sum may deviate from 100% due to round-off errors. [b] Values from ref 36.

Discussion

2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide (**1**) was converted into the benzyl β-glucoside by treatment with benzyl alcohol in the presence of freshly prepared silver oxide under modified Koenigs–Knorr conditions to give benzyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (**2**).[20] Deacetylation led to benzyl β-D-glucopyranoside (**3**) in 70% yield with respect to **1**.[20] Selective protection of the primary hydroxy group of **3** was achieved by tritylation using 2 mol of triphenylmethyl chloride, followed by acetylation to give **4**.[21] Isolation of **4** on silica gel and subsequent cleavage of the 6-O-triphenylmethyl group using HBr in glacial acetic acid improved the yield by 26% over the one-pot reaction[22] and gave 68% of **5** (Scheme II).

Sulfation of benzyl 2,3,4-tri-O-acetyl-β-D-glucopyranoside (**5**) was carried out by using the pyridine–sulfur trioxide complex in absolute *N,N*-dimethylformamide (DMF) and led to a quantitative reaction as determined by TLC. Quenching of the reaction mixture with triethylamine followed by silica gel chromatography using an eluent that contains triethylamine gave the pure triethylammonium salt of the 6-sulfate **6** in 91% yield. This procedure converts the pyridinium salt into the triethylammonium salt, which is stable during column chromatography in a triethylamine-containing eluent and is thus easily purified. Further, the solubility of the triethylammonium sulfate in organic solvents is considerably enhanced over that of the pyridinium counterion (alkali salts are almost insoluble in organic solvents). We found that this procedure avoided many of the problems inherent in earlier studies in which the protected sulfate was prepared as the alkali salt.[23–25]

The triethylammonium salt of benzyl 2,3,4-tri-O-acetyl-6-O-sulfo-β-D-glucopyranoside (**6**) was treated with 1.02 mol of sodium methoxide in absolute methanol. One mole of the sodium methoxide is occupied in the neutralization of the triethylammonium cation forming the free base and the cation of the sulfate whereas the rest, 0.02 mol, is the catalyst for the Zemplen deacetylation. This approach allows the removal of triethylamine during evaporation of the solvent and resulted in a quantitative generation of **7**, the sodium salt of the 6-sulfate. The overall yield for the conversion of **1** to **7** in six steps was 42%. Similarly the triethylammonium salt was converted to the lithium, potassium, and calcium salts **8–10**, respectively, by using lithium in ethanol or potassium or calcium in methanol, respectively. The last step generated the desired metal salt of the sugar sulfate conveniently in quantitative yield while avoiding ion-exchange chromatography.

The stereospecifically deuterated (6*S*)-[6-^{2}H]penta-O-acetyl-α-D-glucopyranoside (**11**) was prepared according to the procedure described by Ohrui et al.[18] Bromination of the exo position of C-6 of 1,6-anhydro-2,3,4-tri-O-benzoyl-β-D-glucopyranose (**12**), subsequent reduction with tri-*n*-butyltin deuteride, and acetolysis gave the stereospecifically deuterated (6*S*)-[6-^{2}H]-1,2,3,4,6-penta-O-acetyl-α-D-glucopyranose (**11**) in 59% overall yield.[18] The degree of deuteration was determined by ^{1}H NMR spectroscopy to be 97%. **11** was converted to the deuterated

(19) Ohrui, H.; Nishida, Y.; Watanabe, M. *Tetrahedron Lett.* **1985**, *26*, 3251.
(20) Fischer, E.; Helferich, B. *Justus Liebigs Ann. Chem.* **1911**, *383*, 68.
(21) Barker, G. R. *Methods. Carbohydr. Chem.* **1963**, *2*, 168.
(22) Iwamoto, R.; Imanaga, Y. *Carbohydr. Res.* **1972**, *24*, 133.
(23) Archbald, P. J.; Fenn, M. D.; Roy, A. B. *Carbohydr. Res.* **1981**, *93*, 177.
(24) Peat, S.; Turvey, J. R.; Clancy, M. J.; Williams, T. P. *J. Chem. Soc.* **1960**, 4761.
(25) Guiseley, K. B.; Ruoff, P. M. *J. Org. Chem.* **1961**, *26*, 1248.

glycosyl bromide **13**. The deuterated sulfate **19** was prepared from **13** by the same procedure described above for compound **5** (Scheme II).

The structure of compound **6** was confirmed by ^1H NMR and ^{13}C NMR as well as 2D (^{13}C,^1H) COSY spectroscopy. The ^1H NMR spectrum shows an ABX system for the protons H-5, *pro-R* H-6, and *pro-S* H-6. The assignment of the prochiral H-6 protons was made by comparison to the deuterated analogue **18**, revealing $\delta_{pro\text{-}R\text{H-}6} = 4.152$, $\delta_{pro\text{-}S\text{H-}6} = 4.188$, and $\delta_{H\text{-}5} = 3.805$ after an analysis of the high-order spin system (Table I). Compared to the precursor **5**, a downfield shift of 0.72 and 0.60 ppm is observed for *pro-R* H-6 and *pro-S* H-6, respectively, which is caused by the deshielding effect of the 6-sulfate group. Similarly, the signal of C-6 of **6**, $\delta_{C\text{-}6} = 65.80$, is shifted downfield by 4.6 ppm in the ^{13}C NMR spectrum whereas the resonance of the adjacent C-5 atom is displaced upfield by 1.9 ppm. These results are in agreement with the general observation of the influence of a sulfate group on the ^{13}C NMR chemical shifts.[23] The analysis of the ^1H NMR and ^{13}C NMR spectra of the unprotected sulfate **7** also shows the influence of the sulfation at C-6. Compared to benzyl β-D-glucopyranoside (**3**), a downfield shift for the signals of the H-6 protons of 0.52 ppm (*pro-R* H-6) and 0.45 ppm (*pro-S* H-6) as well as for the H-5 proton of ca. 0.2 ppm is observed. The ^{13}C NMR spectrum shows a downfield displacement of 5.9 ppm for the C-6 signal and an upfield shift of 2.5 ppm for the C-5 signal. The coupling constant $^1J_{C6,D6} \simeq 22$ Hz is found in most but not all deuterated derivatives. It is not completely clear why the signal of C-6 and the coupling constant $^1J_{C,D}$ are missing only in the ^{13}C NMR spectra of (6*S*)-[6-^{2}H]-1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose and (6*S*)-[6-^{2}H]-1,6-anhydro-2,3,4-tri-*O*-benzoyl-β-D-glucopyranose. Different relaxation characteristics may be responsible for this phenomenon.[26]

The ^1H chemical shifts and the (H,H) coupling constants of the H-5 and H-6 protons are virtually identical for compounds **7–10** (Table I) and indicate that the cation has a negligible influence on the conformation of the (sulfonyloxy)methyl group. Experimental coupling constants $J_{5,6}$ from the heparin polymer exhibit data that are appreciably different from those found here.[27] Rotameric distributions around the C-5–C-6 bond (Table I) were calculated by using the experimentally determined coupling constants $J_{H5,H6}$ as input data for a Karplus-like equation.[28] The results show only a slight difference in the population of the 6-sulfate **7** compared to the analogous unsulfated **3**. This implies that the sulfate group at C-6 has only very little influence on the conformational equilibrium of the C-5–C-6 bond. However, comparing the populations of the hydroxymethyl groups of **3** or **7** to those of β-D-glucose (Table I) indicates that the benzyl aglycon exhibits an effect on the population of the hydroxymethyl or (sulfonyloxy)methyl group by shifting the population equilibrium to a higher percentage of the *gg* conformer (cf. Figure 1). A comparison of the coupling constants and associated populations of **5** with **3** and of **6** with **7** respectively indicates that the protecting group at C-4 also seems to have an influence on the equilibrium of the rotamers at C-5–C-6. Compounds having an *O*-acetyl group at C-4 show a significant population of the *tg* conformer.

In our analysis we find that the preferred conformation of the (sulfonyloxy)methyl group is *gg* and is invariant of the cation. Interestingly, the preferred conformations of the C-5–C-6 bond of potassium β-D-glucopyranose 6-sulfate was determined to be *gt* in a recent X-ray study.[29] The discrepancy may be explained by the strong intermolecular octahedral coordination of the potassium ion in the crystal. In this environment, four different sulfate groups as well as two monosaccharides (via their O-1 or O-5, respectively) participate to coordinate the potassium.

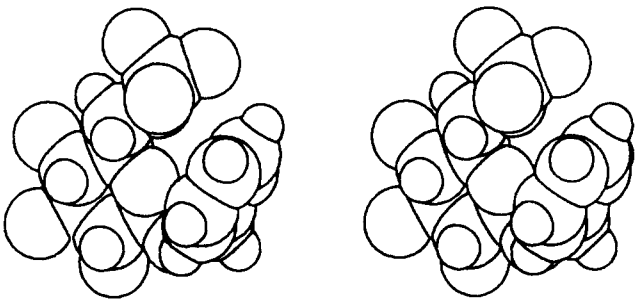

Figure 1. Stereoplot of the CPK model of the calculated global minimum of benzyl 6-*O*-sulfo-β-D-glucopyranoside (*gt* conformation).[34]

Experimental Section

General Methods. ^1H NMR and ^{13}C NMR spectra were recorded with a Bruker WP 80 (^1H, 80.02 MHz; ^{13}C, 20.11 MHz), AM 300 (^1H, 300.013 MHz; ^{13}C, 75.51 MHz), or AM 500 (^1H, 500.137 MHz) spectrometer.

Benzyl 2,3,4-Tri-*O*-acetyl-6-*O*-(triphenylmethyl)-β-D-glucopyranoside (4). A mixture of 8.3 g (30.4 mmol) of **3**[20] and 18 g (64 mmol) of triphenylmethyl chloride in 150 mL of absolute pyridine was stirred at room temperature for 2 days. The reaction mixture was treated with 100 mL of absolute pyridine and then cooled to 0 °C, acetic anhydride (50 mL) was added dropwise, and the reaction mixture was kept overnight at room temperature. The solution was poured into 1 L of ice/water and extracted repeatedly with diethyl ether (5 × 60 mL), and the combined organic layers were washed with aqueous $NaHSO_4$, saturated aqueous $NaHCO_3$, and water. After drying over Na_2SO_4 and concentration in vacuo, a syrup was obtained, which was adsorbed on silica gel. Desorption with petroleum ether/diethyl ether, 3:1, yielded the decomposition products from triphenylmethyl chloride. Further elution with petroleum ether/diethyl ether, 1:1, yielded 14.9 g of microcrystalline **4** (77%): mp 70 °C; $[\alpha]^{20}_D = +1.42°$ ($c = 1.1$ in $CHCl_3$). Elemental anal. Calcd for $C_{38}H_{38}O_9$ (638.71): C, 71.46; H, 6.00. Found: C, 71.57; H, 6.12. ^1H NMR (300 MHz, $CDCl_3$): δ 1.719, 1.962, 2.036 (3 s, OAc), 3.137 (dd, *pro-R* H-6), 3.260 (dd, *pro-S* H-6), 3.535 (ddd, H-5), 4.594 (d, H-1), 4.736 (d, Ha-Bn), 4.964 (d, Hb-Bn), 5.02–5.27 (m, H-2, H-3, H-4), 7.20–7.38 (m, Tr), 7.43–7.50 (m, Bn); $J_{1,2} = 7.6$, $J_{4,5} = 9.5$, $J_{5,6R} = 5.0$, $J_{5,6S} = 2.2$, $J_{6R,6S} = -10.5$, $J_{Ha\text{-}Bn,Hb\text{-}Bn} = -12.3$ Hz. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.18, 2 × 20.44 ($OCOCH_3$), 62.12 (C-6), 68.85 (C-4), 70.18 (C-2), 71.56 (CH_2(Bn)), 73.16 (C-3), 73.43 (C-5), 98.93 (C-1), 127.03, 127.78, 128.45, 128.70 (Ar), 143.60 (quart., C (Tr)), 168.87, 169.28, 170.20 ($OCOCH_3$).

Benzyl 2,3,4-Tri-*O*-acetyl-β-D-glucopyranoside (5). A solution of 14.5 g (22.7 mmol) of **4** in 90 mL of acetic acid was cooled to 0 °C, and a cooled solution of 4.9 mL of HBr/acetic acid (33%) was added. The reaction mixture was stirred for 1.5 min, filtered over a glass suction filter, and washed with 60 mL of cold glacial acetic acid. The filtrate was poured onto a mixture of ice and aqueous $NaHCO_3$. The solution was extracted with dichloromethane (3 × 100 mL), and the combined organic phases were washed with saturated aqueous $NaHCO_3$ and water and dried over Na_2SO_4. After concentration in vacuo, a syrup was obtained, which crystallized from diethyl ether to give 7.9 g of **5** (88%): mp 129–130 °C (lit.[30] mp 132 °C); $[\alpha]^{20}_D = -48.9°$ ($c = 0.8$ in $CHCl_3$)

(26) Koch, H. J.; Perlin, A. S. *Carbohydr. Res.* **1970**, *15*, 403.
(27) Gatti, G.; Casu, B.; Hamer, G. K.; Perlin, A. S. *Macromolecules* **1979**, *12*, 1001.
(28) Gerlt, J. A.; Youngblood, A. V. *J. Am. Chem. Soc.* **1980**, *102*, 7433.
(29) Lambda, D.; Mackie, W.; Sheldrick, B.; Belton, P.; Tanner, S. *Carbohydr. Res.* **1988**, *180*, 183.
(30) Klemer, A.; Bieber, M.; Wilbers, H. *Liebigs Ann. Chem.* **1983**, 1416.

[lit.[30] $[\alpha]^{20}_D$ = −49°]. Elemental anal. Calcd for $C_{19}H_{24}O_9$ (396.39): C, 57.57; H, 6.10. Found: C, 57.49; H, 6.01. ^{1}H NMR (300 MHz, C_6D_6/$CDCl_3$, 1:1): δ 1.763, 1.808, 1.819 (3 s, OAc), 2.185 (dd, 6-OH), 3.187 (ddd, H-5), 3.436 (ddd, *pro-R* H-6), 3.585 (ddd, *pro-S* H-6), 4.403 (d, H-1), 4.485 (d, Ha-Bn), 4.751 (d, Hb-Bn), 5.051 (dd, H-4), 5.150 (dd, H-2), 5.236 (dd, H-3), 7.15–7.25 (m, Ar); $J_{1,2}$ = 7.9, $J_{2,3}$ = $J_{3,4}$ = 9.4, $J_{4,5}$ = 9.8, $J_{5,6R}$ = 5.2, $J_{5,6S}$ = 2.4, $J_{6R,6S}$ = −10.4, $J_{6S,OH}$ = 7.8, $J_{6R,OH}$ = 6.2, $J_{Ha\text{-}Bn,Hb\text{-}Bn}$ = −12.4 Hz. ^{13}C NMR (20.1 MHz, $CDCl_3$): δ 3 × 20.60 ($OCOCH_3$), 61.18 (C-6), 68.72 (C-4), 70.89 (CH_2(Bn)), 71.39 (C-2), 72.73 (C-3), 74.07 (C-5), 99.50 (C-1), 127.66, 127.92, 128.39, 136.91 (Ar), 169.30, 169.97, 170.24 ($OCOCH_3$).

Benzyl 2,3,4-Tri-*O*-acetyl-6-*O*-sulfo-β-D-glucopyranoside Triethylammonium Salt (6). A mixture of 5.0 g (12.6 mmol) of **5** and 4.2 g (26.4 mmol) of pyridine–sulfur trioxide complex in 120 mL of absolute DMF was stirred for 2 days at room temperature until **5** was no longer detectable by TLC. Triethylamine (2.5 mL) was added and the solvent removed in vacuo (approximately 1 Pa). The residue was chromatographed on silica gel, with toluene/ethanol/triethylamine, 3:1:0.05, as eluent. Crystallization from toluene gave 6.6 g of colorless **6** (91%): mp 71–72 °C; $[\alpha]^{20}_D$ = −30.2° (*c* = 0.9 $CHCl_3$). Elemental anal. Calcd for $C_{25}H_{39}O_{12}NS$ (577.64): C, 51.98; H, 6.80; N, 2.42. Found: C, 52.32; H, 6.89; N, 2.38. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.353 (t, CH_3(NEt_3)), 1.983, 1.999, 2.026 (3 s, OAc), 2.351 (s, NH^+), 3.148 (q, CH_2(NEt_3)), 3.805 (ddd, H-5), 4.152 (dd, *pro-R* H-6), 4.188 (dd, *pro-S* H-6), 4.556 (d, H-1), 4.622 (d, Ha-Bn), 4.885 (d, Hb-Bn), 5.015 (dd, H-2), 5.045 (dd, H-4), 5.163 (dd, H-3), 7.22–7.38 (m, Ar); $J_{1,2}$ = 7.9, $J_{2,3}$ = 9.5, $J_{3,4}$ = 9.4, $J_{4,5}$ = 9.4, $J_{5,6S}$ = 3.1, $J_{5,6R}$ = 5.5, $J_{6R,6S}$ = −12.1, $J_{H\text{-}Bn,H\text{-}Bn}$ = −12.2 Hz. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 8.61 (CH_3(NEt_3)), 3 × 20.60 ($OCOCH_3$), 46.47 (CH_2-(NEt_3)), 65.80 (C-6), 68.77 (C-4), 70.54 (CH_2(Bn)), 71.28 (C-2), 72.17 (C-5), 72.98 (C-3), 99.21 (C-1), 127.55, 127.81, 128.35, 136.73 (Ar), 169.35, 169.54, 170.13 ($OCOCH_3$).

Benzyl 6-*O*-Sulfo-β-D-glucopyranoside Sodium Salt (7). Sodium (0.2 g, 8.8 mmol) was added to 400 mL of absolute methanol, and subsequently 5.0 g (8.6 mmol) of **6** was added. After stirring at room temperature for 2 h, the reaction was complete. Neutralization with solid carbon dioxide and evaporation gave a residue, which after three coevaporations with ethyl acetate gave 3.2 g (100%) of white amorphous **7**: mp 255 °C dec; $[\alpha]^{20}_D$ = −35.1° (*c* = 0.74 in H_2O). Elemental anal. Calcd for $C_{13}H_{17}O_9SNa$ (372.32): C, 41.94, H, 4.60. Found: C, 41.62; H, 4.53. ^{1}H NMR (300 MHz, D_2O): δ 3.177 (dd, H-2), 3.28 (dd, H-4), 3.32 (dd H-3), 3.499 (ddd, H-5), 4.080 (dd, *pro-R* H-6), 4.203 (dd, *pro-S* H-6), 4.381 (d, H-1), 4.601 (d, Ha-Bn), 4.780 (d, Hb-Bn), 7.21–7.35 (m, H-Ar); $J_{1,2}$ = 7.9, $J_{2,3}$ = 9.6, $J_{3,4}$ = 8.9, $J_{4,5}$ = 9.7, $J_{5,6R}$ = 5.3, $J_{5,6S}$ = 2.1, $J_{6R,6S}$ = −11.2, $J_{Ha\text{-}Bn,Hb\text{-}Bn}$ = −11.8 Hz. ^{13}C NMR (75.5 MHz, D_2O): δ 67.24 (C-6), 69.49 (C-4), 71.78 (CH_2(Bn)), 73.25 (C-2), 73.95 (C-5), 75.87 (C-3), 101.41 (C-1), 128.73, 128.93, 129.14 (Ar).

Benzyl 6-*O*-Sulfo-β-D-glucopyranoside Lithium Salt (8). Lithium (1.9 mg, 0.27 mmol) in 20 mL of absolute ethanol and 150 mg (0.26 mmol) of **6** were reacted in the same way as described for **7**, to give 92 mg of white amorphous **8**: mp 187 °C dec; $[\alpha]^{20}_D$ = −36.2° (*c* = 0.32 in H_2O). Elemental anal. Calcd for $C_{13}H_{17}O_9SLi$ (356.27): C, 43.83; H, 4.81. Found: C, 43.92; H, 4.87. ^{1}H NMR (500 MHz, D_2O): spectrum identical with that of sodium salt **7**.

Benzyl 6-*O*-Sulfo-β-D-glucopyranoside Potassium Salt (9). Potassium (10.4 mg, 0.27 mmol) in 20 mL of absolute methanol and 150 mg (0.26 mmol) of **6** were reacted in the same way as described for **7**, to give 100 mg of white amorphous **9**: mp 130 °C dec; $[\alpha]^{20}_D$ = −34.1° (*c* = 0.68 in H_2O). Elemental anal. Calcd for $C_{13}H_{17}O_9SK$ (388.43): C, 40.20; H, 4.41. Found: C, 40.02; H, 4.32. ^{1}H NMR (500 MHz, D_2O): spectrum identical with that of sodium salt **7**.

Benzyl 6-*O*-Sulfo-β-D-glucopyranoside Calcium Salt (10). Calcium (5.3 mg, 0.13 mmol) in 20 mL of absolute methanol and 150 mg (0.26 mmol) of **6** were reacted in the same way as described for **7**, to give 95.9 mg of white amorphous **10**: mp 158 °C dec; $[\alpha]^{20}_D$ = −36.6° (*c* = 0.59 in H_2O). Elemental anal. Calcd for $C_{26}H_{34}O_{18}S_2Ca$ (738.74): C, 42.27; H, 4.64. Found: C, 42.15; H, 4.54. ^{1}H NMR (500 MHz, D_2O): spectrum identical with that of sodium salt **7**.

(6*S*)-[6-^{2}H]-2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl Bromide (13). A solution of 9.6 g (26.6 mmol) of **11** in 22 mL of dichloromethane was added dropwise to 120 mL of 33% HBr/acetic acid solution at 0 °C. After complete reaction, the solvent was evaporated at room temperature under reduced pressure. The reaction mixture was poured into 700 mL of ice/water, which was extracted three times with dichloromethane, and the combined extracts were washed with aqueous saturated $NaHCO_3$ and water and subsequently dried with Na_2SO_4. Evaporation of the solvent under reduced pressure at room temperature gave a syrup, which crystallized in white needles after addition of diethyl ether, to yield 9.7 g (89%) of **13**: mp 82 °C; $[\alpha]^{20}_D$ = +196.6° (*c* = 0.7 in $CHCl_3$) (parent ^{1}H isotopomer:[31] yield 84%; mp 88–89 °C; $[\alpha]^{20}_D$ = +198°). Elemental anal. Calcd for $C_{14}H_{18}{}^2HO_9Br$ (412.20): C, 40.79; H, 4.89. Found: C, 40.84; H, 4.81. ^{1}H NMR (80 MHz, $CDCl_3$) (cf. ref 32 for parent ^{1}H isotopomer): δ 2.03, 2.05, 2 × 2.09 (4 s, OAc), 4.15–4.42 (m, H-5, H-6), 4.83 (dd, H-2), 5.15 (dd, H-4), 5.57 (dd, H-3), 6.61 (d, H-1); $J_{1,2}$ = 4.0, $J_{2,3}$ = 9.9, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz. ^{13}C NMR (20.1 MHz, $CDCl_3$): δ 4 × 20.34 ($OCOCH_3$), 60.50 (t, C-6), 67.06 (C-4), 70.05, 70.43, 71.99 (C-2, C-3, C-5), 86.53 (C-1), 169.30, 2 × 169.67, 170.35 ($OCOCH_3$); $^1J_{C,D}$ = 22.1 Hz.

Benzyl (6*S*)-[6-^{2}H]-2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (14). A mixture of 9.5 g (23 mmol) of **13**, 7 g (30 mmol) of silver oxide, 60 mL of benzyl alcohol, and 130 mL of dichloroethane was shaken for 2 days at room temperature in the darkness. The reaction mixture was filtered and evaporated in vacuo to dryness. The residue was dissolved in dichloromethane, washed with aqueous sodium iodide, and subsequently dried over Na_2SO_4. Evaporation under reduced pressure gave crude **14**, which was recrystallized from ethanol/water, 1:1, to give 8.0 g of **14** (72%): mp 90 °C; $[\alpha]^{20}_D$ = −38.3° (*c* = 0.6 in EtOH) (parent ^{1}H isotopomer:[20] yield 72%; mp 96–101 °C; $[\alpha]^{20}_D$ = −49.5°). Elemental anal. Calcd for $C_{21}H_{25}{}^2HO_{10}$ (439.43): C, 57.40; H, 6.19. Found: C, 57.32, H, 6.06. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.98, 2.00, 2.02, 2.10 (4 s, OAc), 3.679 (dd, H-5), 4.260 (dd, H-6), 4.553 (d, H-1), 4.626 (d, Ha-Bn), 4.901 (d, Hb-Bn), 5.066 (dd, H-2), 5.106 (dd, H-4), 5.179 (dd, H-3), 7.25–7.38 (m, Ar); $J_{1,2}$ = 7.7, $J_{2,3}$ = 9.4, $J_{3,4}$ = 9.2, $J_{4,5}$ = 9.4, $J_{5,6}$ = 4.7, $J_{Ha\text{-}Bn,Hb\text{-}Bn}$ = −12.3 Hz. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 4 × 20.60 ($OCOCH_3$), 61.61 (t, C-6), 68.37 (C-4), 70.67 (C-2), 71.22 (C-5), 71.71 (CH_2(Bn)), 72.78 (C-3), 99.21 (C-1), 127.69, 127.97, 128.40 (Ar), 169.23, 169.32, ($OCOCH_3$)); $^1J_{C,D}$ = 21.1 Hz.

Benzyl (6*S*)-[6-^{2}H]-β-D-Glucopyranoside (15). Compound **14** (7.46 g, 17.3 mmol) was stirred in 300 mL of a 0.01 N sodium methylate solution for 1 h at room temperature. The reaction mixture was neutralized with solid carbon dioxide and evaporated under reduced pressure to dryness. Addition of ethyl acetate gave amorphous **15**, which was dried over P_4O_{10} in vacuo at 80 °C, to give 4.5 g of **15** (96%): mp 107 °C; $[\alpha]^{20}_D$ = −43.2° (*c* = 0.8 in H_2O) (parent ^{1}H isotopomer:[20] yield 97%; mp 123–125 °C; $[\alpha]^{20}_D$ = −55.6°). Elemental anal. Calcd for $C_{13}H_{17}{}^2HO_6$ (271.29): C, 57.56; H, 7.06. Found: C, 57.48; H, 7.01. ^{1}H NMR (300 MHz, D_2O): δ 3.14 (m, H-2), 3.20–3.32 (m, H-3, H-4, H-5), 3.541 (d, H-6), 4.348, (d, H-1), 4.579 (d, Ha-Bn), 4.769 (d, Hb-Bn), 7.20–7.40 (m, Ar); $J_{1,2}$ = 7.9, $J_{5,6}$ = 5.4, $J_{Ha\text{-}Bn,Hb\text{-}Bn}$ = −11.6 Hz. ^{13}C NMR (20.1 MHz, D_2O): δ 61.01 (C-6), 70.21 (C-4), 71.90 (CH_2(Bn)), 73.68 (C-2), 2 × 76.38 (C-3, C-5), 101.86 (C-1), 129.02, 129.28, 137.29 (Ar) Hz.

Benzyl (6*S*)-[6-^{2}H]-2,3,4-Tri-*O*-acetyl-6-*O*-(triphenylmethyl)-β-D-glucopyranoside (16). Compound **15** (4.2 g, 15.5 mmol) was converted by the same procedure as described for **4**, to give 8.0 g (12.6 mmol) of **16** (81%): mp 70 °C; $[\alpha]^{20}_D$ = −2.9° (*c* = 0.9 in $CHCl_3$). Elemental anal. Calcd for $C_{38}H_{37}{}^2HO_9$ (639.72): C, 71.35; H, 6.14. Found: C, 71.46; H, 6.07. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.75, 1.98, 2.03 (3 s, OAc), 3.140 (d, H-6), 3.532 (dd, H-5), 4.544 (d, H-1), 4.736 (d, Ha-Bn), 4.964 (d, Hb-Bn), 5.02–5.26 (m, H-2, H-3, H-4), 7.20–7.38 (m, Tr), 7.43–7.50 (m, Bn); $J_{1,2}$ = 7.6, $J_{4,5}$ = 9.6, $J_{5,6}$ = 5.2, $^2J_{Ha\text{-}Bn,Hb\text{-}Bn}$ = −12.3 Hz. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.36, 2 × 20.66 ($OCOCH_3$), 62.09 (t, C-6), 68.83 (C-4), 70.18 (C-2), 71.54 (CH_2(Bn)), 73.15 (C-3), 73.38 (C-5), 98.92 (C-1), 127.03, 127.79, 128.51, 128.70 (Ar), 143.61 (quart, C (Tr)), 168.97, 169.41, 170.38 ($OCOCH_3$); $J_{C,D}$ = 21.1 Hz.

Benzyl (6*S*)-[6-^{2}H]-2,3,4-Tri-*O*-acetyl-β-D-glucopyranoside (17). Compound **16** (7.5 g, 11.7 mmol) was reacted in the same way as described for **5**, to give 4.1 g (10.2 mmol) of **17** (87%): mp 130 °C (lit.[30] mp 132 °C); $[\alpha]^{20}_D$ = −46.6° (*c* = 0.8 in $CHCl_3$) (lit.[30]

(31) Scheurer, P. G.; Smith, F. *J. Am. Chem. Soc.* **1950**, *76*, 3224.
(32) Horton, D.; Turner, W. N. *J. Org. Chem.* **1965**, *30*, 3387.

$[\alpha]^{20}_D = -49°$). Elemental anal. Calcd for $C_{19}H_{23}{}^2HO_9$ (397.40): C, 57.43; H, 6.34. Found: C, 57.52; H, 6.22. ^1H NMR (300 MHz, $C_6D_6/CDCl_3$, 1:1): δ 1.72, 1.78, 1.80 (3 s, OAc), 2.190 (d, 6-OH), 3.159 (dd, H-5), 3.440 (br, H-6), 4.394 (d, H-1), 4.471 (d, Ha-Bn), 4.742 (d, Hb-Bn), 5.072 (dd, H-4), 5.192 (dd, H-2), 5.236 (dd, H-3), 7.15-7.25 (m, Ar); $J_{1,2} = 7.7$, $J_{2,3} = J_{3,4} = 9.2$, $J_{4,5} = 9.5$, $J_{5,6} = 5.1$, $J_{Ha\text{-}Bn,Hb\text{-}Bn} = -12.4$ Hz. ^{13}C NMR (75.5 MHz, $CDCl_3$) (cf. ref 33 for parent ^1H isotopomer): δ 3 × 20.00 ($OCOCH_3$), 60.85 (t, C-6), 68.79 (C-4), 70.60 (CH_2(Bn)), 71.53 (C-2), 72.95 (C-3), 73.99 (C-5), 99.58 (C-1), 127.34-128.32 (m, Ar), 168.87, 169.51, 169.91 ($OCOCH_3$); $J_{C,D} = 21.9$ Hz.

Benzyl (6*S*)-[6-^{2}H]-2,3,4-Tri-*O*-acetyl-6-*O*-sulfo-β-D-glucopyranoside Triethylammonium Salt (18). Compound **17** (3.5 g, 8.8 mmol) was reacted in the same way as described for **6**, to give 4.7 g (8.1 mmol) of compound **18** (92%): mp 73-74 °C; $[\alpha]^{20}_D = -25.0°$ (c = 0.9 in $CHCl_3$). Elemental anal. Calcd for $C_{25}H_{38}{}^2HO_{12}NS$ (578.65): C, 51.89; H, 6.97; N, 2.42. Found: C, 51.47; H, 6.88; N, 2.35. ^1H NMR (300 MHz, $CDCl_3$): δ 1.352 (t, $CH_3(NEt_3)$), 1.982, 2.000, 2.028 (3 s, OAc), 2.351 (s, NH^+), 3.155 (q, $CH_2(NEt_3)$), 3.805 (dd, H-5), 4.137 (d, H-6), 4.558 (d, H-1), 4.625 (d, Ha-Bn), 4.887 (d, Hb-Bn), 5.017 (dd, H-2), 5.045 (dd, H-4), 5.163 (dd, H-3), 7.22-7.38 (m, Ar); $J_{1,2} = 7.9$, $J_{2,3} = 9.5$, $J_{3,4} = 9.4$, $J_{4,5} = 9.5$, $J_{5,6} = 5.5$, $J_{Ha\text{-}Bn,Hb\text{-}Bn} = -12.3$ Hz. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 8.63 ($CH_3(NEt_3)$), 3 × 20.60 ($OCOCH_3$), 46.50 ($CH_2(NEt_3)$), 65.52 (t, C-6), 68.77 (C-4), 70.59 (CH_2(Bn)), 71.31 (C-2), 72.14 (C-5), 73.00 (C-3), 99.24 (C-1), 127.59, 127.85, 128.38, 136.76 (Ar), 169.37, 169.61, 170.17 ($OCOCH_3$); $J_{C,D} = 21.1$ Hz.

Benzyl (6*S*)-[6-^{2}H]-6-*O*-Sulfo-β-D-glucopyranoside Sodium Salt (19). Compound **18** (3.4 g, 5.9 mmol) was reacted in the same way as described for **7**, to give 2.2 g (5.9 mmol) of **19** (100%): mp 255 °C dec; $[\alpha]^{20}_D = -36.7°$ (c = 0.8 in H_2O). Elemental anal. Calcd for $C_{13}H_{16}{}^2HO_9SNa$ (373.33): C, 41.82; H, 4.86. Found: C, 41.67; H, 4.81. ^1H NMR (300 MHz, D_2O): δ 3.157 (dd, H-2), 3.28 (dd, H-4), 3.33 (dd, H-3), 3.476 (dd, H-5), 4.042 (d, H-6), 4.364 (d, H-1), 4.593 (d, Ha-Bn), 4.771 (d, Hb-Bn), 7.21-7.35 (m, H-Ar); $J_{1,2} = 7.9$, $J_{2,3} = 9.5$, $J_{3,4} = 9.0$, $J_{4,5} = 9.7$, $J_{5,6} = 5.3$, $J_{Ha\text{-}Bn,Hb\text{-}Bn} = -11.8$ Hz. ^{13}C NMR (75.5 MHz, D_2O): δ 67.10 (t, C-6), 69.65 (C-4), 71.85 (CH_2(Bn)), 73.37 (C-2), 74.02 (C-5), 76.00 (C-3), 101.52 (C-1), 128.79, 129.04, 129.20, 136.92 (Ar); $J_{C,D} = 22.2$ Hz.

Acknowledgment. We thank Prof. Dr. P. Köll for a generous gift of 1,6-anhydro-β-D-glucopyranose, as well as M. Rundshagen and D. Neemeyer for recording the NMR spectra and the elemental analysis. The Deutsche Forschungsgemeinschaft and the Fond der Chemischen Industrie are thanked for financial support of this work.

Registry No. **3**, 4304-12-5; **4**, 56775-63-4; **5**, 29868-41-5; **6**, 123641-83-8; **7**, 123641-84-9; **8**, 123641-85-0; **9**, 123641-86-1; **10**, 123641-87-2; **11**, 86448-08-0; **13**, 123641-88-3; **14**, 123641-89-4; **15**, 123641-90-7; **16**, 123641-91-8; **17**, 123641-92-9; **18**, 123674-15-7; **19**, 123641-93-0.

Supplementary Material Available: 2D (^{13}C, ^1H) COSY spectrum of **6** (1 page). Ordering information is given on any current masthead page.

(33) Vignon, M. R.; Vottero, J. A. *Tetrahedron Lett.* **1976**, 2445.
(34) Meyer, B. GESA program. *Carbohydr. Res.* In preparation.
(35) Conformational preferences were calculated by using the equation of Gerlt and Youngblood.[28] $2.7x_{gt} + 1.3x_{gg} + 11.7x_{tg} = J_{5,6S}$; $11.5x_{gt} + 1.3x_{gg} + 5.8x_{tg} = J_{5,6R}$; and $x_{gt} + x_{gg} + x_{tg} = 1$.
(36) Nishida, Y.; Ohrui, H.; Meguro, H. *Tetrahedron Lett.* **1984**, *25*, 1575.

Preparation of Carboalkoxyalkylphenylalanine Derivatives from Tyrosine

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Received June 23, 1989

In order to provide the means for the synthesis of peptides incorporating stable and relatively nonpolar mimics of tyrosine phosphates and sulfates, procedures for the conversion of tyrosine derivatives to the corresponding carboalkoxyalkylphenylalanines have been developed. For the synthesis of carboalkoxyethylphenylalanines, a benzyl or benzhydryl ester of *N*-(Boc)tyrosine triflate (**2**) is coupled with an acrylate ester or preferably a 3-(trialkylstannyl)acrylate in the presence of bis(triphenylphosphine)palladium dichloride to give a carboalkoxyethenylphenylalanine derivative. Hydrogenation affords the corresponding *N*-(Boc)carboalkoxyethylphenylalanine. For the preparation of carboalkoxymethylphenylalanines, an ester of **2** is coupled with allyltributyl tin in the presence of bis(triphenylphosphine)palladium dichloride and lithium chloride to give an ester of 4-allylphenylalanine. A two-stage oxidation using ruthenium tetroxide/sodium periodate followed by sodium chlorite in phosphate buffer gives a carboxymethylphenylalanine. Appropriate esterification of the newly formed carboxylic acid and selective deesterification of the α-carboxylate then completes the synthesis of *N*-(Boc)-carboalkoxymethylphenylalanine.

Tyrosine phosphorylation by tyrosine kinases represents an important control point for cell growth and differentiation.[1] In addition, a number of neurohormones and secretory peptides such as gastrin,[2] cholecystokinin,[3] fibronectin,[4] and leucosulfakinin[5] contain a sulfated tyrosine which is necessary for expression of their biological activity. In view of the ionic character and instability of tyrosine phosphates and sulfates,[6] it would be of interest to have access to analogues incorporating less polar and more stable mimics of these groups for structure-activity studies and development of antagonists or agonists of the parent

(1) Krebs, E. G. *Biochem. Soc. Trans.* **1985**, *13*, 813. Hunter, T.; Cooper, J. A. *Ann. Rev. Biochem.* **1985**, *54*, 897. Weinberg, R. A. *Science* **1985**, *230*, 770. Goustin, A. S.; Leof, E. B.; Shipley, G. D.; Moses, H. L. *Cancer Res.* **1986**, *46*, 1015.
(2) Gregory, H.; Hardy, P. M.; Jones, D. S.; Kenner, G. W.; Sheppard, R. C. *Nature* **1964**, *204*, 931.
(3) Mutt, V.; Jorpes, J. E. *Eur. J. Biochem.* **1968**, *6*, 156.
(4) Liu, M.-C.; Lipmann, F. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 34.
(5) Nachamn, R. J.; Holman, G. M.; Haddon, W. F.; Ling, N. *Science* **1986**, *234*, 71. Nachamn, R. J.; Holman, G. M.; Cook, B. J.; Haddon, W. R.; Ling, N. *Biochem. Biophys. Res. Commun.* **1986**, *140*, 357. Nachman, R. J.; Holman, G. M.; Haddon, W. F.; Hayes, T. K. *Peptide Research* **1989**, *2*, 171.
(6) Bettelheim, F. R. *J. Am. Chem. Soc.* **1954**, *76*, 2838.

0022-3263/90/1955-0906$02.50/0