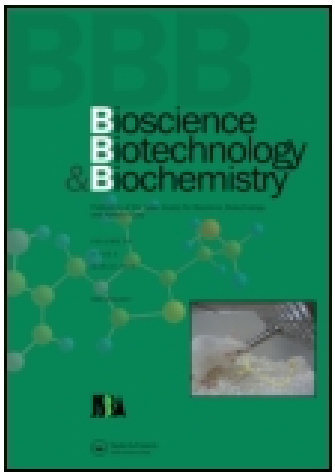

Bioscience, Biotechnology, and Biochemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tbbb20

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Published online: 12 Jun 2014.

To cite this article: Kaori Ozawa, Yukako Taki, Hideharu Ishida, Yukari Yamagata, Shinji Satomura, Makoto Kiso & Akira Hasegawa (1993) Systematic Synthesis of Sulfur-containing p-Nitrophenyl α-Maltopentaoside Derivatives for a Differential Assay of Human α-Amylases, Bioscience, Biotechnology, and Biochemistry, 57:5, 821-828, DOI: 10.1271/bbb.57.821

To link to this article: http://dx.doi.org/10.1271/bbb.57.821

Biosci. Biotech. Biochem., **57** (5), 821–828, 1993

Systematic Synthesis of Sulfur-containing *p*-Nitrophenyl α-Maltopentaoside Derivatives for a Differential Assay of Human α-Amylases

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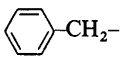
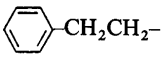
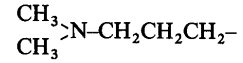
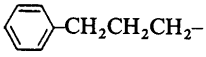
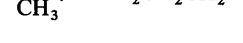
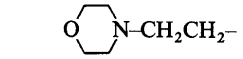
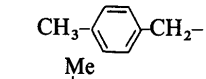
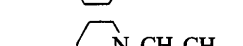
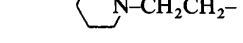
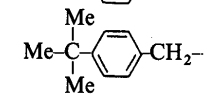
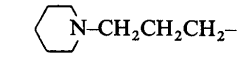
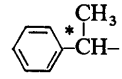
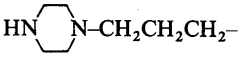
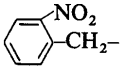
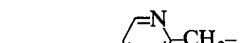
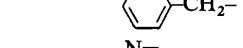
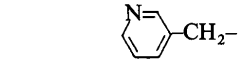
Received December 22, 1992

For use in a differential assay of human α-amylases, a variety of 6^5-*S*-substituted *p*-nitrophenyl α-maltopentaoside derivatives (6–54) were systematically synthesized *via* the key intermediate, *p*-nitrophenyl *O*-(2,3-di-*O*-acetyl-6-*S*-acetyl-4-*O*-benzoyl-6-thio-α-D-glucopyranosyl)-(1→4)-tris[*O*-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→4)]-2,3,6-tri-*O*-acetyl-α-D-glucopyranoside (4), which was easily prepared from *p*-nitrophenyl α-maltopentaoside (G5P) in four steps. The sulfoxide and sulfone derivatives were prepared by oxidizing the corresponding sulfides with *m*-chloroperbenzoic acid.

An assay of the human pancreatic and salivary α-amylases [α-1,4-glucan 4-glucanohydrolase, EC 3.2.1.1; HPA and HSA] in human serum and urine is used for the diagnosis of such diseases as acute pancreatitis and parotitis. Not only a total assay of α-amylases but also a differential assay of HPA and HSA activity have become important for more accurate diagnosis. Some differential assay methods exist using electrophoresis,[1,2] monoclonal antibodies,[3,4] enzyme inhibitors,[5] and synthetic substrates.[6–10] A kinetic method utilizing the difference of substrate specificity between HPA and HSA has particularly been noted because of its simple and rapid application to an automatic assay system. We report here the systematic synthesis of a variety of 6^5-*S*-substituted *p*-nitrophenyl α-maltopentaoside derivatives for use in the differential assay of human α-amylases.

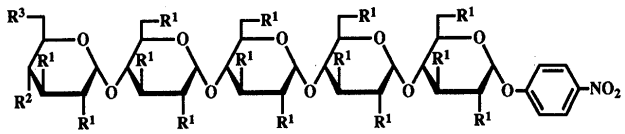
p-Nitrophenyl α-maltopentaoside (G5P, **1**) was treated with benzaldehyde dimethylacetal in the presence of *p*-toluenesulfonic acid monohydrate in *N*,*N*-dimethylformamide (DMF), and the resulting 4^5,6^5-*O*-benzylidene derivative was successively acetylated to give the per-

Table I. Structures of the 6^5-*S*-Substituents

Compound No.	X[a]	Compound No.	X[a]	Compound No.	X[a]
6, 37, 43, 49		**19, 41, 47, 53**	$CH_3(CH_2)_5$–	**28**	
		20, 42, 48, 54	$CH_3(CH_2)_6$–		
7		**21**	$CH_3(CH_2)_{11}$–	**29**	
		22	$CH_3(CH_2)_{17}$–		
8		**23**		**30**	
9, 38, 44, 50		**24**		**31**	
10		**25[c]**		**32**	
11, 12[b]		**26**		**33**	
13		**27**		**34**	
14	CH_3–			**35**	
15, 39, 45, 51	CH_3CH_2–				
16, 40, 46, 52	$CH_3(CH_2)_2$–			**36**	
17	$CH_3(CH_2)_3$–				
18	$CH_3(CH_2)_4$–				

[a] See Fig.
[b] Diastereoisomeric pair.
[c] Diastereoisomeric mixture.

† To whom correspondence should be addressed.
Abbreviations: HPA, human pancreatic α-amylase; HSA, human salivary α-amylase; G5P, *p*-nitrophenyl α-maltopentaoside.



Compound No.	R^1	R^2	R^3
1	OH	OH	OH
2	OAc	—O—Bzd—O—	
3	OAc	OBz	Br
4	OAc	OBz	SAc
5	OH	OH	SNa
6~36	OH	OH	SX
37~42	OH	OH	$S^{*a}(=O)X$
43~48	OH	OH	$S^{*b}(=O)X$
49~54	OH	OH	SO_2X

Fig. Fundamental Structure of the Modified G5P Derivatives.
Bzd, benzylidene; Bz, benzoyl; X, substituents shown in Table I.
*a,*b Chiral sulfoxides.

Table II. Selected Physical Data for the 6^5-*S*-Alkyl and -Aralkyl Derivatives (**7–26**)

Compound No.	Yield (%)	$[\alpha]_D$ (MeOH)	Molecular formula (MW)	Found (calcd.) % of C	H	N
7	65	+168° (*c* 0.21)	$C_{44}H_{63}O_{27}NS$ (1070.03)	49.49 (49.39)	6.20 (5.93)	1.33 (1.31)
8	81	+156° (*c* 0.20)	$C_{45}H_{65}O_{27}NS$ (1084.06)	49.86 (49.83)	6.04 (5.74)	1.29 (1.21)
9	68	+193° (*c* 0.20)	$C_{44}H_{63}O_{27}NS$ (1070.03)	49.36 (49.39)	5.63 (5.93)	1.23 (1.31)
10	79	+182° (*c* 0.19)	$C_{47}H_{69}O_{27}NS$ (1112.12)	50.51 (50.76)	6.21 (6.25)	1.55 (1.26)
11	13[a]	+116° (*c* 0.21)	$C_{44}H_{63}O_{27}NS$ (1070.03)	49.32 (49.39)	6.14 (5.93)	1.57 (1.31)
12	12[a]	+221° (*c* 0.22)	$C_{44}H_{63}O_{27}NS$ (1070.03)	49.23 (49.39)	5.87 (5.93)	1.08 (1.31)
13	70	+148° (*c* 0.20)	$C_{42}H_{66}O_{28}N_2S$ (1084.06)	46.90 (46.66)	5.49 (5.45)	2.54 (2.83)
14	85	+164° (*c* 0.20[b])	$C_{37}H_{57}O_{27}NS$ (979.91)	45.35 (45.21)	5.86 (6.03)	1.43 (1.25)
15	79	+166° (*c* 0.43)	$C_{38}H_{59}O_{27}NS$ (993.94)	46.13 (45.92)	5.86 (5.98)	1.59 (1.41)
16	65	+183° (*c* 0.39)	$C_{39}H_{61}O_{27}NS$ (1007.96)	46.20 (46.47)	5.80 (6.10)	1.34 (1.39)
17	46	+176° (*c* 0.39)	$C_{40}H_{63}O_{27}NS$ (1021.99)	46.99 (47.01)	6.30 (6.21)	1.21 (1.37)
18	62	+172° (*c* 0.45)	$C_{41}H_{65}O_{27}NS$ (1036.02)	47.27 (47.53)	6.48 (6.32)	1.50 (1.35)
19	74	+174° (*c* 0.50)	$C_{42}H_{67}O_{27}NS$ (1050.04)	47.96 (48.04)	6.61 (6.43)	1.29 (1.33)
20	67	+183° (*c* 0.21)	$C_{43}H_{69}O_{27}NS$ (1064.07)	48.34 (48.54)	6.26 (6.54)	1.34 (1.32)
21	50	+163° (*c* 0.19)	$C_{48}H_{79}O_{27}NS$ (1134.21)	50.92 (50.83)	6.89 (7.20)	1.22 (1.23)
22	35	+139° (*c* 0.30)	$C_{54}H_{91}O_{27}NS$ (1218.37)	53.24 (53.23)	7.64 (7.53)	1.04 (1.15)
23	32	+179° (*c* 0.40)	$C_{39}H_{61}O_{27}NS$ (1007.96)	46.66 (46.47)	5.94 (6.10)	1.25 (1.39)
24	31	+179° (*c* 0.40)	$C_{40}H_{63}O_{27}NS$ (1021.99)	46.95 (47.01)	6.23 (6.21)	1.49 (1.37)
25[c]	37	— —	$C_{40}H_{63}O_{27}NS$ (1021.99)	47.06 (47.01)	6.02 (6.21)	1.52 (1.37)
26	45	+179° (*c* 0.46)	$C_{41}H_{65}O_{27}NS$ (1036.02)	47.44 (47.53)	6.42 (6.32)	1.27 (1.35)

[a] See Experimental section.
[b] 1,4-Dioxane–H_2O solvent (5 : 1).
[c] Diastereoisomeric mixture.

Table III. Selected Physical Data for the 6^5-*S*-Aminoalkyl and -Aminoaralkyl Derivatives (**28–36**)

Compound No.	Yield (%)	$[\alpha]_D$ (MeOH)	Molecular formula (MW)	Found (calcd.) % of C	H	N
28	51	+130° (*c* 0.42)	$C_{40}H_{64}O_{27}N_2S$ (1037.01)	46.23 (46.33)	6.38 (6.22)	2.71 (2.70)
29	22	+121° (*c* 0.42)	$C_{41}H_{66}O_{27}N_2S$ (1051.03)	46.98 (46.85)	6.28 (6.33)	2.67 (2.67)
30	69	+125° (*c* 0.72)	$C_{42}H_{66}O_{28}N_2S$ (1079.04)	46.99 (46.75)	6.23 (6.17)	2.56 (2.60)
31	63	+112° (*c* 0.55)	$C_{43}H_{68}O_{27}N_2S$ (1077.07)	48.14 (47.95)	6.44 (6.36)	2.49 (2.50)
32	31	+126° (*c* 0.37)	$C_{44}H_{70}O_{27}N_2S$ (1091.10)	48.44 (48.44)	6.33 (6.47)	2.45 (2.57)
33	25	+138° (*c* 0.22)	$C_{43}H_{69}O_{27}N_3S$ (1092.09)	47.04 (47.29)	6.39 (6.37)	3.65 (3.85)
34	69	+111° (*c* 0.21)	$C_{42}H_{60}O_{27}N_2S$ (1057.00)	47.47 (47.73)	5.53 (5.72)	2.50 (2.65)
35	71	+161° (*c* 0.21)	$C_{42}H_{60}O_{27}N_2S$ (1057.00)	47.65 (47.73)	5.90 (5.72)	2.61 (2.65)
36	87	+167° (*c* 0.21)	$C_{42}H_{60}O_{27}N_2S$ (1057.00)	47.92 (47.73)	5.56 (5.72)	2.51 (2.65)

acetylated $4^5,6^5$-*O*-benzylidene derivative (**2**) in an 85% yield. Treatment of **2** with *N*-bromosuccinimide and barium carbonate in carbon tetrachloride[11] under reflux afforded the 4^5-*O*-benzoyl-6^5-bromo-6^5-deoxy derivative (**3**, 95%). The *SN*2 replacement reaction in **3** with potassium thioacetate was performed in acetone in the presence of Drierite to give the key intermediate, *p*-nitrophenyl *O*-(2,3-di-*O*-acetyl-6-*S*-acetyl-4-*O*-benzoyl-6-thio-α-D-glucopyranosyl)-(1→4)-tris[*O*-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→4)]-2,3,6-tri-*O*-acetyl-α-D-glucopyranoside (**4**) in a 93% yield. Compound **4** was treated with sodium methoxide in methanol to form the 6^5-*S*Na-G5P derivative (**5**) *in situ*, which was then coupled with benzyl, alkyl, and aminoalkyl halides to give a variety of 6^5-*S*-substituted G5P derivatives (**6–36**) in 22–87% yields as shown in the experimental section and Tables II and III.

Treatment of compounds **6**, **9**, **15**, **16**, **19**, and **20** with *m*-chloroperbenzoic acid (MCPBA) in acetic acid gave mixtures of corresponding chiral sulfoxides **37–42** and **43–48** and sulfones **49–54**, which were separated by high-performance liquid chromatography (HPLC). Selected physical data for the 6^5-sulfoxide and -sulfone derivatives are shown in Table IV. When the oxidation was performed with excess MCPBA (more than three equivalents), the corresponding sulfones were obtained almost quantitatively.

The structures of the synthetic compounds were confirmed by their spectral data and elemental analysis. In the ^{1}H-NMR data (see the experimental section and Tables V–X), the chemical shifts of H-6^5 and H-$6'^5$ were most characteristic. For example, the H-6^5 and H-$6'^5$ signals in **3** (the 6^5-Br derivative) were each observed at δ 3.43 ($J_{5^5,6^5}$ = 5.7 Hz, J_{gem} = 13.3 Hz, H-6^5) and δ 3.52 ($J_{5^5,6'^5}$ = 3.3 Hz, J_{gem} = 13.3 Hz, H-$6'^5$). By introducing the *S*-acetyl group in **4**, both the signals were shifted to a higher magnetic field, δ 3.13 ($J_{5^5,6^5}$ = 5.7 Hz, J_{gem} = 13.3 Hz, H-6^5) and δ 3.26 ($J_{5^5,6'^5}$ = 3.3 Hz, J_{gem} = 13.3 Hz, H-$6'^5$), respectively. After introducing a series of *S*-substituents, the signals appeared in a higher magnetic field, δ 2.38–2.72 (H-6^5) and δ 2.83–3.06

Table IV. Selected Physical Data for the 6^5-Sulfoxide and -Sulfone Derivatives (**38–42**, **44–48**, and **50–54**)

Compound No.	Yield (%)	$[\alpha]_D$ (MeOH)	t_R (min)	Molecular formula (MW)	Found (calcd.) % of C	H	N
38[a]	33	+173° (*c* 0.21)	16.0	$C_{44}H_{63}O_{28}NS$ (1086.03)	48.62 (48.66)	6.10 (5.85)	1.50 (1.29)
44[b]	25	+174° (*c* 0.20)	17.3	$C_{44}H_{63}O_{28}NS$ (1086.03)	48.87 (48.66)	5.85 (5.85)	1.01 (1.29)
50	quant.	+91° (*c* 0.077)	17.7	$C_{44}H_{63}O_{29}NS$ (1102.03)	48.15 (47.96)	5.99 (5.76)	1.25 (1.27)
39[a]	28	+250° (*c* 0.040)	10.4	$C_{38}H_{59}O_{28}NS$ (1009.93)	44.99 (45.19)	5.61 (5.89)	1.41 (1.39)
45[b]	32	+150° (*c* 0.080)	10.7	$C_{38}H_{59}O_{28}NS$ (1009.93)	45.28 (45.19)	5.76 (5.89)	1.38 (1.39)
51	quant.	+200° (*c* 0.070)	11.2	$C_{38}H_{59}O_{29}NS$ (1025.93)	44.50 (44.49)	5.91 (5.80)	1.26 (1.37)
40[a]	33	+267° (*c* 0.030)	11.8	$C_{39}H_{61}O_{28}NS$ (1023.96)	45.90 (45.75)	6.11 (6.00)	1.37 (1.37)
46[b]	32	+200° (*c* 0.050)	12.3	$C_{39}H_{61}O_{28}NS$ (1023.96)	45.65 (45.75)	6.16 (6.00)	1.38 (1.37)
52	quant.	+140° (*c* 0.050)	12.9	$C_{39}H_{61}O_{29}NS$ (1039.96)	45.17 (45.04)	5.86 (5.91)	1.25 (1.35)
41[a]	25	+138° (*c* 0.080)	17.1	$C_{42}H_{67}O_{28}NS$ (1066.04)	47.56 (47.32)	6.40 (6.34)	1.27 (1.31)
47[b]	21	+150° (*c* 0.080)	18.3	$C_{42}H_{67}O_{28}NS$ (1066.04)	47.53 (47.32)	6.22 (6.34)	1.49 (1.31)
53	quant.	+156° (*c* 0.077)	18.7	$C_{42}H_{67}O_{29}NS$ (1082.04)	46.35 (46.62)	5.94 (6.24)	1.24 (1.29)
42[a]	30	+173° (*c* 0.043)	19.0	$C_{43}H_{69}O_{28}NS$ (1080.07)	47.68 (47.82)	6.61 (6.44)	1.12 (1.30)
48[b]	27	+163° (*c* 0.040)	20.4	$C_{43}H_{69}O_{28}NS$ (1080.07)	47.79 (47.82)	6.14 (6.44)	1.22 (1.30)
54	quant.	+162° (*c* 0.043)	20.7	$C_{43}H_{69}O_{29}NS$ (1096.07)	46.87 (47.12)	6.31 (6.35)	1.57 (1.28)

[a,b] Chiral sulfoxide pair.

Table V. Selected ^{1}H-NMR (CD_3OD) Data for the 6^5-*S*-Aralkyl Derivatives (**7–13**)

Compound No. (substituent)	Chemical Shift in ppm (coupling constants in Hz)			H-6^5	H-$6'^5$	H-4^5	H-1^5	H-1^3,1^4	H-1^2	H-1^1
7 (Phenethyl)	PhCH_2CH_2– 2.83, broad s —	Ph 7.12–7.28, m —		2.63 dd (7.7, 12.2)	3.01 dd (2.1, 14.3)	3.22 t (9.2)	5.10 d (3.8)	5.13, 5.14 2d (4.6, 4.0)	5.21 d (3.9)	5.66 d (3.7)
8 (3-Phenylpropyl)	$PhCH_2CH_2CH_2$– 1.87, p (7.4) $PhCH_2CH_2CH_2$– 2.65, ~dd (7.4, 13.5)	$PhCH_2CH_2CH_2$– 2.57, t (7.2) Ph 7.10–7.26, m —		2.65 dd (7.4, 13.5)	2.95 dd (~0, 11.9)	3.20 t (9.3)	5.06 d (3.7)	5.12, 5.14 2d (3.7, 3.9)	5.21 d (3.7)	5.67 d (3.7)
9 (*p*-Methylbenzyl)	Me 2.28, s —	Ph 7.05–7.19, m —		2.51 dd (7.9, 11.7)	2.83 dd (3.9, 12.0)	3.19 t (9.3)	5.11 d (3.5)	5.13, 5.15 2d (4.0, 4.0)	5.20 d (3.8)	5.66 d (3.7)
10 {*p*-(*t*-Butyl)-benzyl}	Me_3C 1.29, s —	Ph 7.20–7.39, m —		2.51 dd (8.0, 12.0)	2.86 dd (2.0, 12.5)	3.18 t (9.3)	5.09 d (3.7)	5.13, 5.14 2d (3.5, 3.8)	5.19 d (3.9)	5.65 d (3.7)
11 (α-methylbenzyl[a])	Me 1.50, d (7.1)	CH 4.10, q (7.1)	Ph 7.17–7.30, m —	2.38 dd (8.2, 11.4)	2.78 dd (2.3, 11.9)	3.09 t (9.4)	5.09 d (3.5)	5.12, 5.15 2d (3.7, 3.7)	5.20 d (3.5)	5.66 d (3.3)
12 (α-Methylbenzyl[b])	Me 1.50, d (7.0)	CH 4.06, q (7.0)	Ph 7.18–7.37, m —	2.44 dd (7.0, 12.3)	2.68 dd (2.4, 12.6)	3.21 t (9.2)	5.11 d (3.5)	5.14, 5.14 2d (3.9, 3.5)	5.21 d (3.9)	5.66 d (3.7)
13 (2-Nitrobenzyl)	$–CH_2S–$ 4.13, s — 6-Ph 7.53, dd (1.8, 7.7)	4-Ph 7.44, ~dt (2.2, 7.7) 3-Ph 7.93, dd (1.1, 8.0)	5-Ph 7.58, dt (1.5, 7.7)	2.44 dd (7.0, 12.3)	2.68 dd (2.4, 12.6)	3.21 t (9.2)	5.11 d (3.5)	5.14, 5.14 2d (3.9, 3.5)	5.21 d (3.9)	5.66 d (3.7)

[a,b] Diastereoisomeric pair.

Table VI. Selected ^{1}H-NMR (CD_3OD) Data for the 6^5-*S*-Alkyl Derivatives (**14–22**)

Compound No. (substituent)	Chemical Shift in ppm (coupling constants in Hz)			H-6^5	H-6′^{5}	H-4^5	H-1^5	H-1^3,1^4	H-1^2	H-1^1
14[a] (Methyl)	Me 2.14, s			2.61 dd (7.8, 14.2)	2.93 dd (2.2, 14.1)	3.23 t (9.3)	5.12 d (3.7)	5.14, 5.17 2d (4.0, 4.2)	5.23 d (3.9)	5.69 d (3.7)
15 (Ethyl)	Me 1.22, t (7.3)	Me*CH*$_2$– 2.60, dd (7.3, 13.1)		2.62 dd (8.0, 11.6)	2.97 dd (2.6, 12.0)	3.20 t (9.3)	5.09 d (3.7)	5.13, 5.15 2d (4.7, 4.0)	5.21 d (3.7)	5.66 d (3.7)
16 (Propyl)	Me 0.95, t (7.3)	Me*CH*$_2$CH$_2$– 1.58, dt (7.3, 12.6)	–CH$_2$S– 2.55, t (7.3)	2.59 dd (7.9, 13.6)	2.94 dd (~0, 11.9)	3.20 t (9.2)	5.09 d (3.7)	5.13, 5.15 2d (5.1, 4.0)	5.21 d (3.7)	5.67 d (3.7)
17 (*n*-Butyl)	Me 0.90, t (7.2)	Me(*CH*$_2$)$_2$CH$_2$– 1.35–1.57, m —	–CH$_2$S– 2.57, t (7.2)	2.58 dd (8.0, 11.7)	2.94 dd (~0, 11.7)	3.20 t (9.3)	5.09 d (3.8)	5.13, 5.14 2d (5.1, 4.0)	5.21 d (3.9)	5.66 d (3.7)
18 (*n*-Pentyl)	Me 0.89, t (7.0)	Me(*CH*$_2$)$_3$CH$_2$– 1.25–1.65, m —	–CH$_2$S– 2.56, t (7.2)	2.59 dd (8.1, 11.9)	2.94 dd (~0, 11.5)	3.20 t (9.2)	5.09 d (3.7)	5.13, 5.14 2d (4.6, 4.2)	5.21 d (3.7)	5.67 d (3.7)
19 (*n*-Hexyl)	Me 0.89, t (6.8)	Me(*CH*$_2$)$_4$ CH$_2$– 1.28–1.58, m —	–CH$_2$S– 2.57, t (7.2)	2.59 dd (8.1, 13.9)	2.94 dd (2.2, 14.1)	3.20 t (9.3)	5.09 d (3.7)	5.12, 5.14 2d (4.6, 4.0)	5.20 d (3.7)	5.66 d (3.7)
20 (*n*-Heptyl)	Me 0.90, t (6.6)	Me(*CH*$_2$)$_5$CH$_2$– 1.25–1.56, m —	–CH$_2$S– 2.57, t (7.2)	2.60 dd (8.0, 12.5)	2.95 dd (~0, 12.0)	3.21 t (9.4)	5.08 d (3.7)	5.12, 5.13 2d (4.2, 4.2)	5.20 d (3.7)	5.66 d (3.5)
21 (Dodecyl)	Me 0.94, t (7.7)	Me(*CH*$_2$)$_{10}$CH$_2$– 1.24–1.60, m —	–CH$_2$S– 2.57, t (7.2)	2.60 dd (8.2, 12.5)	2.95 dd (~0, 12.0)	3.21 t (9.2)	5.08 d (3.9)	5.12, 5.13 2d (3.9, 3.7)	5.20 d (3.7)	5.66 d (3.7)
22 (Octadecyl)	Me 0.89, t (7.0)	Me(*CH*$_2$)$_{16}$CH$_2$– 1.28–1.61, m —	–CH$_2$S– 2.57, t (7.2)	2.60 dd (7.9, 12.5)	2.95 dd (2.0, 14.1)	3.21 t (9.3)	5.09 d (3.9)	5.12, 5.14 2d (3.8, 4.2)	5.20 d (3.7)	5.66 d (3.7)

[a] CD_3OD–D_2O solvent (5 : 1).

Table VII. Selected ^{1}H-NMR (CD_3OD) Data for the 6^5-*S*-Alkyl and -Aminoalkyl Derivatives (**23–26** and **28–30**)

Compound No. (substituent)	Chemical Shift in ppm (coupling constants in Hz)				H-6^5	H-6′^{5}	H-4^5	H-1^5	H-1^3,1^4	H-1^2	H-1^1
23 (1-Methylethyl)	2Me 1.23, d (6.6)	–CH– 2.98–3.08, m —			2.62 dd (8.1, 11.8)	2.99 dd (2.5, 12.0)	3.20 t (9.3)	5.09 d (3.7)	5.14, 5.15 2d (4.4, 4.4)	5.22 d (3.7)	5.67 d (3.7)
24 (2-Methylpropyl)	2Me 0.96, d (6.6)	–CH– 1.70–1.84, m —	–CH$_2$S– 2.46, d (7.3)		2.57 dd (7.9, 11.6)	2.94 dd (2.4, 13.9)	3.21 t (9.2)	5.10 d (3.9)	5.13, 5.15 2d (3.7, 4.0)	5.21 d (4.0)	5.66 d (3.7)
25 (1-Methylpropyl)	*Me*CH$_2$– 0.95, 0.96, 2t (7.5, 7.3) –*CH*$_2$CH– 1.50, 1.59, 2m (13.9, 7.0)		–CH(*Me*)– 1.22, d (7.0) –CHS– 2.76, 2.77, 2 sex (6.7, 6.6)		2.61 dd (7.9, 11.3)	2.97 dd (1.8, 13.8)	3.20, 3.21 2t (9.3, 9.2)	5.10 d (3.5)	5.11, 5.15 2d (3.5, 4.0)	5.22 d (3.7)	5.67 d (3.7)
26 (3-Methylbutyl)	2Me 0.88, d (6.7)	–CH*CH*$_2$– 1.35–1.48, m	–*CH*CH$_2$– 1.58–1.70, m	–CH$_2$S– 2.58, t (7.7)	2.59 dd (8.0, 12.8)	2.95 dd (2.0, 13.8)	3.21 t (9.3)	5.09 d (4.0)	5.13, 5.15 2d (3.7, 4.0)	5.21 d (3.7)	5.66 d (3.7)
28 (*N*,*N*-Dimethyl-aminoethyl)	2Me 2.72, s —	–CH$_2$S– 2.91, ~t (7.1)	–N*CH*$_2$CH$_2$– 3.15, ~t (7.8)		2.67 dd (8.0, 12.3)	3.06 dd (~0, 12.3)	3.22 t (9.3)	5.15–5.16 3d —		5.22 d (3.9)	5.67 d (3.7)
29 (*N*,*N*-Dimethyl-aminopropyl)	2Me 2.76, s — –NCH$_2$*CH*$_2$– 3.07, ~t (8.0)		–CH$_2$S– 2.67, ~t (7.1) –N*CH*$_2$– 3.25, ~t (6.8)		2.66 dd (7.6, 12.1)	2.98 dd (~0, 12.3)	3.22 t (9.0)	5.10 d (3.7)	5.14, 5.16 2d (3.9, 4.0)	5.22 d (3.9)	5.67 d (3.7)
30 {2-(Morpholino)ethyl}	O(CH$_2$*CH*$_2$)$_2$N– 2.46–2.50, m —		–CH$_2$CH$_2$S– 2.55–2.77, m —		2.67 dd (6.6, 12.3)	2.99 dd (2.4, 12.1)	3.22 t (9.0)	5.09 d (3.7)	5.12, 5.14 2d (3.9, 3.8)	5.20 d (4.0)	5.66 d (3.7)

(H-6′^{5}). The chemical shifts of H-6^5 and H-6′^{5} from oxidation of the sulfur atom were to a significantly lower magnetic field, which was observed at δ 2.88–2.96 (H-6^5) for sulfoxides **37–42**, and at δ 2.85–2.86 (H-6^5) and δ 3.20–3.22 (H-6′^{5}) for sulfoxides **43–48**.

In conclusion, a variety of novel sulfur-containing *p*-nitrophenyl α-maltopentaoside derivatives (**6–54**) were synthesized by using key intermediate **4**. These G5P derivatives have been employed for a differential assay of human α-amylases, and the results of this application will be published in the near future.

Experimental

Specific rotation values were determined with a Union PM-201 polarimeter at 25°C, and IR spectra were recorded with a Jasco IRA-100 spectrophotometer. ^{1}H-NMR spectra were recorded at 270 MHz with a JEOL JNM-GX 270 spectrometer. Preparative chromatography was performed on silica gel (Wako Pure Chemical Industries, 200 mesh) with the solvent systems specified, concentration being conducted *in vacuo*. High-performance liquid chromatography (HPLC) was performed with Shimadzu Model LC-8A equipment. A column (20 × 250 mm) packed with Wakosil 10C$_{18}$ (reversed phase) was equilibrated with 10% acetonitrile in a 50 mM ammonium acetate solution, and eluted with a linear gradient of acetonitrile from 10 to 60%.

p-Nitrophenyl O-(2,3-di-O-acetyl-4,6-O-benzylidene-α-D-glucopyranosyl)-(1→4)-tris[O-(2,3,6-tri-O-acetyl-α-D-glucopyranosyl)-(1→4)]-2,3,6-tri-O-acetyl-α-D-glucopyranoside (**2**). To a solution of *p*-nitrophenyl α-maltopentaoside (**1**, 5.0 g, 5.27 mmol) in dry DMF (75 ml) were added benzaldehyde dimethylacetal (6 ml, 40.0 mmol) and *p*-toluenesulfonic acid monohydrate (0.75 g). The mixture was stirred overnight at 25°C, and then neutralized with Amberlite IRA 410 (OH$^-$) resin, before the resin was filtered out and washed with methanol. The filtrate and washings were combined and concentrated to a syrup, which was acetylated with acetic anhydride (50 ml) and pyridine (100 ml) for two days at room temperature. After completing the reaction, methanol (30 ml) was added to the mixture at 0°C, which was then concentrated and extracted with dichloromethane. The extract was successively washed with 2 M HCl and water, dried (sodium sulfate), and concentrated. Column chromatography (100:1 dichloromethane–methanol) of the product on silica gel gave **2** (7.28 g, 85%) as an amorphous mass; $[\alpha]_D$ +165° (*c* 0.21, $CHCl_3$); IR ν_{max} (film) cm^{-1}: 3050 (aromatic CH), 2950 (CH), 1750 and 1220 (ester), 1520 and 1360 (NO_2), and 850 and 700 (phenyl); NMR ($CDCl_3$): δ 1.97–2.20 (14s, 42H, 14Ac), 3.62 (t, 1H, $J_{3^5,4^5}=J_{4^5,5^5}=9.3$ Hz, H-4^5), 3.72 (t, 1H, $J_{5^5,6^5}=J_{gem}=10.1$ Hz, H-6^5), 3.81 (dd, 1H, $J_{5^5,6'^5}=4.0$ Hz, $J_{gem}=9.9$ Hz, H-6′^{5}), 3.89–4.08 (m, 8H, H-4$^{2-4}$ and 5$^{1-5}$), 4.10 (t, 1H, $J_{3^1,4^1}=J_{4^1,5^1}=9.9$ Hz, H-4^1), 4.16–4.33 (m, 4H, H-6$^{1-4}$), 4.46–4.60 (m, 4H, H-6′$^{1-4}$), 4.71, 4.72, 4.74 (3dd, 3H, *J*=4.0, 10.3, 4.2, 10.3, 3.8, 10.1 Hz, H-2$^{2-4}$), 4.87 (dd, 1H, $J_{1^5,2^5}=4.1$ Hz, $J_{2^5,3^5}=10.3$ Hz, H-2^5), 4.95 (dd, 1H, $J_{1^1,2^1}=3.7$ Hz, $J_{2^1,3^1}=10.1$ Hz, H-2^1), 5.27 (~d, 2H, *J*=3.9 Hz, H-1^3, 1^4), 5.33 (d, 1H, $J_{1^2,2^2}=4.2$ Hz, H-1^2), 5.37 (d, 1H, $J_{1^5,2^5}=4.2$ Hz, H-1^5), 5.36, 5.38, 5.39 (3t, 3H, *J*=10.7, 9.8, 9.2 Hz, H-3$^{2-4}$), 5.45 (t, 1H, $J_{2^5,3^5}=J_{3^5,4^5}=10.0$ Hz, H-3^5), 5.48 (s, 1H, PhC*H*), 5.73 (t, 1H, $J_{2^1,3^1}=J_{3^1,4^1}=9.3$ Hz, H-3^1), 5.74 (d, 1H, $J_{1^1,2^1}=3.5$ Hz, H-1^1), 7.33–7.45 (m, 5H, *Ph*CH), 7.25 (2d, 2H, *J*=9.3 Hz, *p*-OPhNO$_2$ *o*-Ph proton), 8.25 (2d, 2H, *J*=9.2 Hz, *p*-OPhNO$_2$ *m*-Ph proton).

Anal. Found: C, 52.45; H, 5.16; N, 1.14%. Calcd. for $C_{71}H_{87}O_{42}N$

Table VIII. Selected ^{1}H-NMR (CD_3OD) Data for the 6^5-*S*-Aminoalkyl and -Aminoaralkyl Derivatives (**31–36**)

Compound No. (substituent)	Chemical Shift in ppm (coupling constants in Hz)		H-6^5	H-6′^{5}	H-4^5	H-1^3,1^4,1^5	H-1^2	H-1^1
31 {2-(Piperidino)ethyl}	$CH_2(CH_2CH_2)_2N$– 1.62–1.69, m	$CH_2(CH_2CH_2)_2N$– 1.78–1.87, m	2.72 dd (7.3, 12.3)	3.03 dd (1.8, 12.5)	— — —	5.14–5.17 3d —	5.22 d (3.9)	5.67 d (3.7)
	–CH_2S– 2.93–3.00, m	$CH_2(CH_2CH_2)_2$-NCH_2– 3.14–3.29, m						
32 {3-(Piperidino)propyl}	$CH_2(CH_2CH_2)_2N$– 1.67–1.93, m	–$NCH_2CH_2CH_2$– 2.01–2.07, m	2.67 dd (8.4, 12.1)	2.99 dd (~0, 12.1)	— — —	5.10, 5.12, 5.15 3d (3.5, 3.3, 3.5)	5.21 d (4.0)	5.67 d (3.7)
	–CH_2S– 2.69, ~t (6.7)	$CH_2(CH_2CH_2)_2$-NCH_2– 3.14–3.26, m						
33[a] {3-(Piperazino)propyl}	–CH_2CH_2S– 1.77–1.83, m	HN(CH_2CH_2)$_2$N– 3.19–3.25, m	2.59 dd (7.8, 13.6)	2.98 dd (~0, 12.3)	— — —	5.15, 5.18, 5.19 3d (3.7, 3.3, 3.8)	5.25 d (3.5)	5.71 d (3.5)
	HN(CH_2CH_2)$_2$N$CH_2CH_2CH_2$– 2.61–2.69, m							
34 {(2-Piridyl)methyl}	5-Ph 7.25–7.27, m — 4-Ph 7.78, dt (1.8, 7.7)	3-Ph 7.48, d (7.9) 6-Ph 8.43, ~d (3.9)	2.60 dd (7.6, 12.4)	2.92 dd (2.5, 14.1)	3.21 t (9.1)	5.09, 5.14, 5.15 3d (3.7, 3.8, 3.9)	5.21 d (3.5)	5.66 d (3.7)
35 {(3-Piridyl)methyl}	5-Ph 7.34–7.39, m — 2-Ph 8.48, ~s	4-Ph 7.84, ~d (7.9)	6-Ph 8.38, ~d (4.4)	2.54 dd (7.4, 12.3)	2.84 dd (2.2, 12.3)	3.22 t (9.2)	5.13, 5.14, 5.16 3d (3.7, 3.8, 4.0)	5.21 d (4.0)	5.66 d (3.7)
36 {(4-Piridyl)methyl}	3-Ph 7.42, ~d (5.9)	2-Ph 8.43, ~d (5.7)	2.54 dd (7.1, 12.4)	2.83 dd (~0, 12.4)	3.22 t (9.3)	5.12, 5.14, 5.16 3d (3.7, 3.8, 3.9)	5.21 d (3.9)	5.66 d (3.5)

[a] CD_3OD–D_2O solvent (3:1).

Table IX. Selected ^{1}H-NMR (CD_3OD) Data for the 6^5-Sulfoxide and -Sulfone Derivatives (**38**, **39**, **44**, **45**, **50**, and **51**)

Compound No. (substituent)	Chemical Shift in ppm (coupling constants in Hz)		H-6^5	H-6'^{5}	H-4^5	H-5^5	H-1^3,1^4	H-1^5	H-1^2	H-1^1
38 {(*p*-Methylbenzyl)-sulfinyl[a]}	Me	MePh*CH*$_2$SO–	2.85	—	3.26	4.07	5.13, 5.14	5.23	5.20	5.66
	2.32, s	4.06, 4.20, 2d	dd	—	t	m	2d	d	d	d
	—	(13.0, 13.2)	(7.3, 13.7)	—	(9.7)	—	(4.0, 4.0)	(3.7)	(3.8)	(3.7)
		Ph								
		7.15–7.25, m								
		—								
44 {(*p*-Methylbenzyl)-sulfinyl[b]}	Me	MePh*CH*$_2$SO–	2.82	3.20	3.13	4.05	5.10, 5.13	5.17	5.21	5.66
	2.32, s	3.98, 4.14, 2d	dd	dd	t	m	2d	d	d	d
	—	(13.2, 13.2)	(10.7, 13.4)	(~0, 13.0)	(9.2)	—	(3.8, 3.8)	(3.7)	(3.9)	(3.7)
		Ph								
		7.15–7.24, m								
		—								
50 {(*p*-Methylbenzyl)-sulfonyl}	Me	MePh*CH*$_2$SO$_2$–	—	—	3.11	4.14	5.13, 5.15	5.37	5.21	5.66
	2.34, s	4.38, 4.50, 2d	—	—	t	dt	2d	d	d	d
	—	(14.3, 13.9)	—	—	(9.3)	(2.3, 8.6)	(3.5, 3.7)	(3.3)	(3.9)	(3.7)
		Ph								
		7.31–7.39, m								
		—								
39 (Ethylsulfinyl[a])	Me	Me*CH*$_2$–	2.95	—	—	4.04	5.13, 5.15	5.21		5.66
	1.32, t	2.87, 2.94, 2dd	dd	—	—	dt	2d	~d		d
	(7.5)	(7.7, 12.7, 7.6, 13.3)	(7.3, 12.9)	—	—	(2.1, 8.0)	(4.0, 4.0)	(3.9)		(3.7)
45 (Ethylsulfinyl[b])	Me	Me*CH*$_2$–	2.85	3.22	3.16	4.07	5.11, 5.14	5.18	5.20	5.66
	1.32, t	2.75, 2.92, 2dd	dd	dd	t	m	2d	d	d	d
	(7.5)	(7.2, 11.2, 7.6, 11.2)	(10.8, 11.2)	(~0, 11.2)	(9.0)	—	(3.9, 4.2)	(3.7)	(3.7)	(3.7)
51 (Ethylsulfonyl)	Me	Me*CH*$_2$–	—	—	3.14	4.10	5.12, 5.14	5.25	5.21	5.66
	1.33, t	3.21, dd	—	—	t	m	2d	d	d	d
	(7.4)	(7.4, 12.9)	—	—	(9.3)	—	(3.9, 3.8)	(3.7)	(3.8)	(3.7)

[a,b] Chiral sulfoxide pair.

(1626.44): C, 52.43; H, 5.39; N, 0.86%.

p-Nitrophenyl O-(2,3-di-O-acetyl-4-O-benzoyl-6-bromo-6-deoxy-α-D-glucopyranosyl)-(1→4)-tris[O-(2,3,6-tri-O-acetyl-α-D-glucopyranosyl)-(1→4)]-2,3,6-tri-O-acetyl-α-D-glucopyranoside (**3**). To a solution of **2** (6.74 g, 4.14 mmol) in dry carbon tetrachloride (85 ml) were added *N*-bromosuccinimide (1.03 g, 5.79 mmol) and barium carbonate (3.27 g, 16.6 mmol). The suspension was heated under reflux while stirring for 3 h, and then the precipitate was filtered out and washed with dichloromethane. The filtrate and washings were combined and concentrated. Column chromatography (100:1 dichloromethane–methanol) of the residue on silica gel gave **3** (6.71 g, 95%) as an amorphous mass; $[\alpha]_D$ +176° (*c* 0.20, $CHCl_3$); IR ν_{max} (film) cm^{-1}: 3100 (aromatic CH), 2950 (CH), 1750 and 1240 (ester), 1520 and 1380 (NO_2), and 850 and 710 (phenyl); NMR ($CDCl_3$): δ 1.98–2.19 (14s, 42H, 14Ac), 3.42 (dd, 1H, $J_{5^5,6^5}$=5.9 Hz, J_{gem}=11.5 Hz, H-6^5), 3.50 (dd, 1H, $J_{5^5,6'^5}$=2.4 Hz, J_{gem}=11.4 Hz, H-6'^{5}), 3.85–4.18 (m, 9H, H-4$^{1-4}$ and 5$^{1-5}$), 4.21–4.33 (m, 4H, H-6$^{1-4}$), 4.46–4.58 (m, 4H, H-6'$^{1-4}$), 4.70–4.78 (3dd, 3H, H-2$^{2-4}$), 4.90 (dd, 1H, $J_{1^5,2^5}$=3.9 Hz, $J_{2^5,3^5}$=10.4 Hz, H-2^5), 4.95 (dd, 1H, $J_{1^1,2^1}$=3.9 Hz, $J_{2^1,3^1}$=10.3 Hz, H-2^1), 5.25 (t, 1H, $J_{3^5,4^5}$=$J_{4^5,5^5}$=10.3 Hz, H-4^5), 5.28, 5.29 (2d, 2H, *J*=4.2, 4.5 Hz, H-1^3, 1^4), 5.33 (d, 1H, $J_{1^2,2^2}$=4.0 Hz, H-1^2), 5.38, 5.39, 5.40 (3t, 3H, *J*=9.0, 8.9, 9.1 Hz, H-3$^{2-4}$), 5.48 (d, 1H, $J_{1^5,2^5}$=3.9 Hz, H-1^5), 5.55 (t, 1H, $J_{2^5,3^5}$=$J_{3^5,4^5}$=10.0 Hz, H-3^5), 5.73 (t, 1H, $J_{2^1,3^1}$=$J_{3^1,4^1}$=9.2 Hz, H-3^1), 5.74 (d, 1H, $J_{1^1,2^1}$=3.3 Hz, H-1^1), 7.25 (2d, 2H, *J*=9.3 Hz, *p*-OPhNO$_2$ *o*-Ph proton), 7.46 (t, 2H, *J*=7.6 Hz, PhCO *m*-Ph proton), 7.61 (t, 1H, *J*=7.3 Hz, PhCO *p*-Ph proton), 8.00 (d, 2H, *J*=7.1 Hz, PhCO *o*-Ph proton), 8.24 (2d, 2H, *J*=9.1 Hz, *p*-OPhNO$_2$ *m*-Ph proton).

Anal. Found: C, 50.00; H, 5.22; N, 0.62%. Calcd. for $C_{71}H_{86}O_{42}NBr$ (1705.35): C, 50.01; H, 5.08; N, 0.82%.

p-Nitrophenyl O-(2,3-di-O-acetyl-6-S-acetyl-4-O-benzoyl-6-thio-α-D-glucopyranosyl)-(1→4)-tris[O-(2,3,6-tri-O-acetyl-α-D-glucopyranosyl)-(1→4)]-2,3,6-tri-O-acetyl-α-D-glucopyranoside (**4**). To a solution of **3** (5.97 g, 3.50 mmol) in dry acetone (180 ml) were added Drierite (6 g) and potassium thioacetate (2.40 g, 21 mmol), and the mixture was stirred overnight at 25°C. The Drierite was filtered out and washed with dichloromethane. The filtrate and washings were combined and concentrated, and column chromatography (1:1 ethyl acetate–hexane) of the residue on silica gel gave **4** (5.54 g, 93%) as an amorphous mass; $[\alpha]_D$ +171° (*c* 0.18, $CHCl_3$); IR ν_{max} (film) cm^{-1}: 3100 (aromatic CH), 2950 (CH), 1740 and 1220 (ester), 1700 (SAc), 1520 and 1380 (NO_2), and 850 and 710 (phenyl); NMR ($CDCl_3$): δ 1.89–2.20 (14s, 42H, 14Ac), 2.33 (s, 3H, SAc), 3.13 (dd, 1H, $J_{5^5,6^5}$=5.7 Hz, J_{gem}=13.3 Hz, H-6^5), 3.26 (dd, 1H, $J_{5^5,6'^5}$=3.3 Hz, J_{gem}=13.6 Hz, H-6'^{5}), 3.88–4.07 (m, 8H, H-4$^{2-4}$ and 5$^{1-5}$), 4.10 (t, 1H, $J_{3^1,4^1}$=$J_{4^1,5^1}$=9.5 Hz, H-4^1), 4.24–4.33 (m, 4H, H-6$^{1-4}$), 4.45–4.53 (m, 4H, H-6'$^{1-4}$), 4.70–4.78 (3dd, 3H, H-2$^{2-4}$), 4.87 (dd, 1H, $J_{1^5,2^5}$=3.8 Hz, $J_{2^5,3^5}$=10.3 Hz, H-2^5), 4.95 (dd, 1H, $J_{1^1,2^1}$=3.7 Hz, $J_{2^1,3^1}$=10.3 Hz, H-2^1), 5.18 (t, 1H, $J_{3^5,4^5}$=$J_{4^5,5^5}$=9.9 Hz, H-4^5), 5.28, 5.29 (2d, 2H, *J*=3.7 Hz, 4.0 Hz, H-1^3, 1^4), 5.33 (d, 1H, $J_{1^2,2^2}$=4.0 Hz, H-1^2), 5.36, 5.38, 5.40 (3t, 3H, *J*=10.3, 9.2, 9.3 Hz, H-3$^{2-4}$), 5.39 (d, 1H, $J_{1^5,2^5}$=4.0 Hz, H-1^5), 5.50 (t, 1H, $J_{2^5,3^5}$=$J_{3^5,4^5}$=10.1 Hz, H-3^5), 5.73 (t, 1H, $J_{2^1,3^1}$=$J_{3^1,4^1}$=9.2 Hz, H-3^1), 5.74 (d, 1H, $J_{1^1,2^1}$=3.7 Hz, H-1^1), 7.26 (2d, 2H, *J*=9.2 Hz, *p*-OPhNO$_2$ *o*-Ph proton), 7.46 (t, 2H, *J*=7.5 Hz, PhCO *m*-Ph proton), 7.60 (t, 1H, *J*=7.5 Hz, PhCO *p*-Ph proton), 8.01 (d, 2H, *J*=7.3 Hz, PhCO *o*-Ph proton), 8.25 (2d, 2H, *J*=9.2 Hz, *p*-OPhNO$_2$ *m*-Ph proton).

Anal. Found: C, 51.29; H, 5.49; N, 0.64%. Calcd. for $C_{73}H_{89}O_{43}NS$ (1700.54): C, 51.56; H, 5.28; N, 0.82%.

p-Nitrophenyl O-(6-S-benzyl-6-thio-α-D-glucopyranosyl)-(1→4)-tris[O-α-D-glucopyranosyl-(1→4)]-α-D-glucopyranoside (**6**). To a solution of **4** (500 mg, 0.294 mmol) in dry methanol (10 ml) was added a solution of sodium methoxide (0.294 mmol) in dry methanol (2 ml), and the mixture

Table X. Selected ^{1}H-NMR (CD_3OD) Data for the 6^5-Sulfoxide and -Sulfone Derivatives (**40–42**, **46–48**, and **52–54**)

Compound No. (substituent)	Chemical Shift in ppm (coupling constants in Hz) Me	$-(CH_2)_nCH_2S-$	$-CH_2S-$	H-6^5	H-6'^{5}	H-4^5	H-5^5	H-1^3,1^4	H-1^5	H-1^2	H-1^1
40	1.08	1.74–1.83	2.81–2.89	2.96	—	—	4.06	5.13, 5.14	5.21		5.66
(Propylsulfinyl[a])	t	m	m	dd	—	—	m	2d	~d		d
	(7.4)	—	—	(7.0, 12.3)	—	—	—	(4.4, 4.4)	(3.9)		(3.7)
46	1.09	1.72–1.83	2.70–2.91	2.85	3.22	3.16	4.07	5.11, 5.13	5.19, 5.21		5.66
(Propylsulfinyl[b])	t	m	m	dd	dd	t	m	2d	2d		d
	(7.4)	—	—	(11.5, 12.1)	(1.5, 12, 1)	(9.3)	—	(4.0, 3.9)	(3.9, 4.2)		(3.7)
52	1.06	1.78—1.89	3.15—3.21	—	—	3.13	4.07	5.12, 5.14	5.26	5.21	5.66
(Propylsulfonyl)	t	m	m	—	—	t	m	2d	d	d	d
	(7.4)	—	—	—	—	(9.2)	—	(3.7, 4.0)	(3.7)	(3.9)	(3.7)
41	0.90	1.28–1.77	2.87	2.96	—	—	4.07	5.12, 5.14	5.20		5.66
{(*n*-Pentyl)sulfinyl[a]}	t	m	t	dd	—	—	m	2d	~d		d
	(7.1)	—	(7.5)	(7.1, 11.9)	—	—	—	(3.9, 3.9)	(3.7)		(3.5)
47	0.91	1.28–1.81	2.70–2.89	2.85	3.22	3.16	4.07	5.11, 5.13	5.19, 5.20		5.66
{(*n*-Pentyl)sulfinyl[b]}	t	m	m	dd	dd	t	m	2d	2d		d
	(7.1)	—	—	(10.8, 12.5)	(~0, 11.9)	(9.3)	—	(3.9, 4.2)	(4.0, 4.0)		(3.7)
53	0.91	1.28–1.81	3.16–3.23	—	—	3.13	4.07	5.12, 5.14	5.28	5.21	5.66
{(*n*-Pentyl)sulfonyl}	t	m	m	—	—	t	m	2d	d	d	d
	(6.6)	—	—	—	—	(9.3)	—	(3.7, 3.5)	(3.5)	(3.8)	(3.5)
42	0.90	1.20–1.80	2.87	2.96	—	—	4.07	5.12, 5.14	5.21		5.66
(Heptylsulfinyl[a])	t	m	t	dd	—	—	m	2d	~d		d
	(6.9)	—	(7.7)	(7.3, 11.8)	—	—	—	(4.2, 4.2)	(2.9)		(3.5)
48	0.90	1.28–1.78	2.72–2.89	2.86	3.22	3.16	4.07	5.11, 5.13	5.19, 5.20		5.66
(Heptylsulfinyl[b])	t	m	m	dd	dd	t	m	2d	2d		d
	(6.8)	—	—	(10.9, 12.0)	(1.0, 11.7)	(9.2)	—	(3.8, 3.9)	(3.8, 4.0)		(3.9)
54	0.90	1.28–1.81	3.18–3.25	—	—	3.13	4.07	5.12, 5.13	5.26	5.21	5.66
(Heptylsulfonyl)	t	m	m	—	—	t	m	2d	d	d	d
	(6.7)	—	—	—	—	(9.6)	—	(4.3, 4.3)	(3.1)	(3.7)	(3.7)

[a,b] Chiral sulfoxide pair.

was kept for 7 h at 25°C. After all of the starting material had been converted into **5** (detected by TLC, 4 : 2 : 1 buthanol–ethanol–water), benzyl bromide (0.035 ml, 0.294 mmol) was added, and the mixture was stirred for 1.5 h at 40°C under nitrogen and then concentrated. Column chromatography (methanol) of the residue on Sephadex LH-20 gave **6** (145 mg, 47%) as an amorphous mass; $[\alpha]_D$ +182° (*c* 0.19, MeOH); IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 2900 (CH), 1510 and 1340 (NO_2), and 850 and 700 (phenyl); NMR (CD_3OD): δ 2.52 (dd, 1H, $J_{5^5,6^5}$ = 7.9 Hz, J_{gem} = 11.9 Hz, H-6^5), 2.85 (dd, 1H, $J_{5^5,6'^5}$ = 2.1 Hz, J_{gem} = 11.9 Hz, H-6'^{5}), 3.20 (t, 1H, $J_{3^5,4^5} = J_{4^5,5^5}$ = 8.9 Hz, H-4^5), 4.10 (t, 1H, $J_{2^1,3^1} = J_{3^1,4^1}$ = 8.9 Hz, H-3^1), 5.12 (d, 1H, $J_{1^5,2^5}$ = 4.0 Hz, H-1^5), 5.13, 5.15 (2d, 2H, *J* = 4.6, 4.2 Hz, H-1^3, 1^4), 5.21 (d, 1H, $J_{1^2,2^2}$ = 3.9 Hz, H-1^2), 5.66 (d, 1H, $J_{1^1,2^1}$ = 3.5 Hz, H-1^1), 7.18–7.34 (m, 5H, *Ph*CH_2), 7.33 (2d, 2H, *J* = 9.2 Hz, *p*-OPhNO_2 *o*-Ph proton), 8.21 (2d, 2H, *J* = 9.2 Hz, *p*-OPhNO_2 *m*-Ph proton).

Anal. Found: C, 48.77; H, 5.99; N, 1.15%. Calcd. for $C_{43}H_{61}O_{27}NS$ (1056.01): C, 48.91; H, 5.82; N, 1.33%.

*p-Nitrophenyl O-[6-S-(α-methylbenzyl)-6-thio-α-*D*-glucopyranosyl]-(1→4)-tris[O-α-*D*-glucopyranosyl-(1→4)]-α-*D*-glucopyranosides* (**11** and **12**). To a solution of **4** (500 mg, 0.294 mmol) in dry methanol (10 ml) was added a solution of sodium methoxide (0.294 mmol) in dry methanol (2 ml), the mixture then being processed as described for **6** to give a diastereoisomeric mixture of **11** and **12** (190 mg, 60%). The product was chromatographed by HPLC to give **11** (t_R 19.0 min, 40.6 mg, 13%) and **12** (t_R 19.4 min, 38.4 mg, 12%) as amorphous mass. The physical and ^{1}H-NMR data are shown in Tables II and V.

*Other p-nitrophenyl O-(6-S-alkyl-6-thio-α-*D*-glucopyranosyl)-(1→4)-tris[O-α-*D*-glucopyranosyl-(1→4)]-α-*D*-glucopyranosides* (**7–10** and **13–26**). The title compounds were prepared according to the procedure described for **6**. The yields, physical and ^{1}H-NMR data are shown in Tables II, V, VI, and VII.

*p-Nitrophenyl O-[6-S-(N,N-diethylaminoethyl)-6-thio-α-*D*-glucopyranosyl]-(1→4)-tris[O-α-*D*-glucopyranosyl-(1→4)]-α-*D*-glucopyranoside* (**27**). To a solution of **4** (300 mg, 0.176 mmol) in dry MeOH (10 ml) was added a solution of sodium methoxide (0.440 mmol) in dry methanol (3.8 ml), and the mixture was kept for 1.5 h at 25°C. After all of the starting material had been converted into **5**, 2-chlorotriethylamine hydrochloride (45 mg, 0.264 mmol) was added, and the mixture was stirred for 1 h at 40°C under nitrogen, before being processed as described for **6** to give **27** (133 mg, 71%) as an amorphous mass; $[\alpha]_D$ +127° (*c* 0.62, MeOH); IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 2920 (CH), 1400 (NCH_2), 1520 and 1350 (NO_2), and 870 (phenyl); NMR (CD_3OD): δ 1.26 {t, 6H, J_{CH_3,CH_2} = 7.2 Hz, $(CH_3CH_2)_2NCH_2CH_2S$}, 2.71 (dd, 1H, $J_{5^5,6^5}$ = 7.4 Hz, J_{gem} = 12.5 Hz, H-6^5), 2.91 {m, 2H, $(CH_3CH_2)_2NCH_2CH_2S$}, 3.02 (dd, 1H, $J_{5^5,6'^5}$ = ~0 Hz, J_{gem} = 12.5 Hz, H-6'^{5}), 3.12 {dd, 4H, J_{CH_3,CH_2} = 7.2 Hz, J_{gem} = 12.6 Hz, $(CH_3CH_2)_2NCH_2CH_2S$}, 3.21–3.27 {m, 3H, $(CH_3CH_2)_2NCH_2CH_2S$, and H-4^5), 4.10 (t, 1H, $J_{2^1,3^1} = J_{3^1,4^1}$ = 9.0 Hz, H-3^1), 5.13–5.15 (3d, 3H, H-1$^{3-5}$), 5.20 (d, 1H, $J_{1^2,2^2}$ = 3.9 Hz, H-1^2), 5.66 (d, 1H, $J_{1^1,2^1}$ = 3.7 Hz, H-1^1), 7.31 (2d, 2H, *J* = 9.4 Hz, *p*-OPhNO_2 *o*-Ph proton), 8.22 (2d, 2H, *J* = 9.2 Hz, *p*-OPhNO_2 *m*-Ph proton).

Anal. Found: C, 47.51; H, 6.55; N, 2.63%. Calcd. for $C_{42}H_{68}O_{27}N_2S$ (1065.06): C, 47.36; H, 6.44; N, 2.63%.

Other p-nitrophenyl O-(6-S-aminoalkyl-6-thio-α-D-glucopyranosyl)-(1→4)-tris[O-α-D-glucopyranosyl-(1→4)]-α-D-glucopyranosides (**28–36**). The title compounds were prepared according to the procedure described for **27**. The yields, physical and ^{1}H-NMR data are shown in Tables III, VII, and VIII.

p-Nitrophenyl O-(6-benzylsulfinyl-6-deoxy-α-D-glucopyranosyl)-(1→4)-tris[O-α-D-glucopyranosyl-(1→4)]-α-D-glucopyranosides (**37** and **43**). To a solution of **6** (60 mg, 0.0568 mmol) in acetic acid (5 ml) was added *m*-chloroperbenzoic acid (9.8 mg, 0.0568 mmol) at 0°C in the dark. The mixture was stirred for 1 h at room temperature in the dark and then extracted with water–dichloromethane. The aqueous layer was concentrated, and the residue was chromatographed by HPLC to give **37** (t_R 14.5 min, 13.2 mg, 22%), **43** (t_R 15.3 min, 12.1 mg, 20%), and further-oxidized sulfone **49** (t_R 15.9 min, 6.7 mg, 11%) as an amorphous mass. Unreacted **6** (t_R 18.1 min, 9.9 mg, 17%) was recovered.

37: $[\alpha]_D$ +188° (*c* 0.22, MeOH); IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 2940 (CH), 1520 and 1340 (NO_2), 1030 (SO), and 860 and 700 (phenyl); NMR (CD_3OD): δ 2.88 (dd, 1H, $J_{5^5,6^5}$ = 7.3 Hz, J_{gem} = 12.2 Hz, H-6^5), 4.08 (dt, 1H, $J_{4^5,5^5}$ = $J_{5^5,6^5}$ = 7.8 Hz, $J_{5^5,6'^5}$ = 2.0 Hz, H-5^5), 4.10 (t, 1H, $J_{2^1,3^1}$ = $J_{3^1,4^1}$ = 8.1 Hz, H-3^1), 4.09, 4.25 (2d, 2H, J_{gem} = 13.2, 13.2 Hz, Ph*CH*$_2$SO), 5.13, 5.14 (2d, 2H, *J* = 4.0, 4.8 Hz, H-1^3, 1^4), 5.21 (d, 1H, $J_{1^2,2^2}$ = 3.8 Hz, H-1^2), 5.24 (d, 1H, $J_{1^2,2^2}$ = 3.5 Hz, H-1^5), 5.66 (d, 1H, $J_{1^1,2^1}$ = 3.7 Hz, H-1^1), 7.31 (2d, 2H, *J* = 9.4 Hz, *p*-OPhNO_2 *o*-Ph proton), 7.30–7.35 (m, 5H, *Ph*CH_2SO), 8.22 (2d, 2H, *J* = 9.3 Hz, *p*-OPhNO_2 *m*-Ph proton).

Anal. Found: C, 48.04; H, 5.91; N, 1.13%. Calcd. for $C_{43}H_{61}O_{28}NS$ (1072.01): C, 48.18; H, 5.74; N, 1.31%.

43: $[\alpha]_D$ +167° (*c* 0.20, MeOH); IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 2940 (CH), 1520 and 1340 (NO_2), 1050 (SO), and 860 and 700 (phenyl); NMR (CD_3OD): δ 2.86 (dd, 1H, $J_{5^5,6^5}$ = 10.9 Hz, J_{gem} = 13.2 Hz, H-6^5), 3.13 (t, 1H, $J_{3^5,4^5}$ = $J_{4^5,5^5}$ = 9.4 Hz, H-4^5), 3.20 (dd, 1H, $J_{5^5,6'^5}$ = 1.7 Hz, J_{gem} = 13.2 Hz, H-6'^{5}), 4.02, 4.10 (2d, 2H, J_{gem} = 13.2, 13.2 Hz, Ph*CH*$_2$SO), 4.06 (dt, 1H, $J_{4^5,5^5}$ = $J_{5^5,6^5}$ = 11.5 Hz, $J_{5^5,6'^5}$ = 1.7 Hz, H-5^5), 4.10 (t, 1H, $J_{2^1,3^1}$ = $J_{3^1,4^1}$ = 9.2 Hz, H-3^1), 5.10, 5.13 (2d, 2H, *J* = 3.7, 3.8 Hz, H-1^3, 1^4), 5.18 (d, 1H, $J_{1^5,2^5}$ = 3.8 Hz, H-1^5), 5.21 (d, 1H, $J_{1^2,2^2}$ = 3.7 Hz, H-1^2), 5.67 (d, 1H, $J_{1^1,2^1}$ = 3.5 Hz, H-1^1), 7.32 (2d, 2H, *J* = 9.4 Hz, *p*-OPhNO_2 *o*-Ph proton), 7.31–7.35 (m, 5H, *Ph*CH_2SO), 8.22 (2d, 2H, *J* = 9.2 Hz, *p*-OPhNO_2 *m*-Ph proton).

Anal. Found: C, 48.15; H, 5.44; N, 1.23%. Calcd. for $C_{43}H_{61}O_{28}NS$ (1072.01): C, 48.18; H, 5.74; N, 1.31%.

p-Nitrophenyl O-(6-S-benzylsulfonyl-6-deoxy-α-D-glucopyranosyl)-(1→4)-tris[O-α-D-glucopyranosyl-(1→4)]-α-D-glucopyranoside (**49**). To a solution of **6** (30 mg, 0.0284 mmol) in acetic acid (2.5 ml) was added *m*-chloroperbenzoic acid (14.7 mg, 0.085 mmol) at 0°C in the dark. The mixture was stirred for 1 h at room temperature in the dark, and then worked up as just described to give **49** (t_R 15.9 min, 30 mg, quant.) as an amorphous mass; $[\alpha]_D$ +169° (*c* 0.21, MeOH); IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 2940 (CH), 1520 and 1340 (NO_2), 1300 and 1110 (SO_2), and 850 and 700 (phenyl); NMR (CD_3OD): δ 3.12 (t, 1H, $J_{3^5,4^5}$ = $J_{4^5,5^5}$ = 9.4 Hz, H-4^5), 4.11 (t, 1H, $J_{2^1,3^1}$ = $J_{3',4'}$ = 9.1 Hz, H-3^1), 4.15 (dt, 1H, $J_{4^5,5^5}$ = $J_{5^5,6^5}$ = 9.1 Hz, $J_{5^5,6'^5}$ = 2.0 Hz, H-5^5), 4.44, 4.55 (2d, 2H, J_{gem} = 13.7, 14.1 Hz, Ph*CH*$_2$SO$_2$), 5.14, 5.15 (2d, 2H, $J_{1^3,2^3}$ = $J_{1^4,2^4}$ = 3.3 Hz, H-1^3, 1^4), 5.21 (d, 1H, $J_{1^2,2^2}$ = 3.7 Hz, H-1^2), 5.39 (d, 1H, $J_{1^5,2^5}$ = 3.3 Hz, H-1^5), 5.66 (d, 1H, $J_{1^1,2^1}$ = 3.9 Hz, H-1^1), 7.32 (2d, 2H, *J* = 9.3 Hz, *p*-OPhNO_2 *o*-Ph proton), 7.35–7.46 (m, 5H, *Ph*CH_2SO_2), 8.21 (2d, 2H, *J* = 9.3 Hz, *p*-OPhNO_2 *m*-Ph proton).

Anal. Found: C, 47.22; H, 5.61; N, 1.58%. Calcd. for $C_{43}H_{61}O_{29}NS$ (1088.00): C, 47.47; H, 5.65; N, 1.29%.

Other p-nitrophenyl O-(6-S-alkylsulfinyl-6-thio-α-D-glucopyranosyl)-(1→4)-tris[O-α-D-glucopyranosyl-(1→4)]-α-D-glucopyranosides (**38–42** and **44–48**). The title compounds were prepared according to the procedure described for **37** and **43**. The yields, physical and ^{1}H-NMR data are shown in Tables IV, IX, and X.

Other p-nitrophenyl O-(6-S-alkylsulfonyl-6-deoxy-α-D-glucopyranosyl)-(1→4)-tris[O-α-D-glucopyranosyl-(1→4)]-α-D-glucopyranosides (**50–54**). The title compounds were prepared according to the procedure described for **49**. The yields, physical and ^{1}H-NMR data are shown in Tables IV, IX, and X.

References

1) S. Nørby, *Exp. Cell Res.*, **36**, 663–666 (1964).
2) J. E. Berk, S. Hayashi, R. L. Searcy, and N. C. Hightower, *Am. J. Dig. Dis.*, **11**, 695–701 (1966).
3) K. Ito, M. Katayama, N. Minamiura, and T. Yamamoto, *J. Biochem.*, **97**, 1357–1362 (1985).
4) T. E. Mifflin, D. C. Benjamin, and D. E. Bruns, *Clin. Chem.*, **31**, 1283–1288 (1985); M. Gerber, K. Naujoks, H. Lenz, W. Gerhardt, and K. Wullf, *ibid.*, **31**, 1331–1334 (1985).
5) M. D. O'Donnell, O. FitzGerald, and K. F. McGeeney, *Clin. Chem.*, **23**, 560–566 (1977).
6) K. Omichi and T. Ikenaka, *J. Biochem.*, **94**, 1797–1802 (1983).
7) K. Omichi and T. Ikenaka, *Clin. Chim. Acta*, **138**, 197–203 (1984).
8) K. Omichi and T. Ikenaka, *J. Biochem.*, **100**, 1353–1358 (1986).
9) S. Tokutake, K. Kotani, K. Saito, and N. Yamaji, *Chem. Pharm. Bull.*, **40**, 2531–2536 (1992).
10) K. Ogawa, H. Matsui, and T. Usui, *Biosci. Biotech. Biochem.*, **56**, 1933–1936 (1992).
11) S. Hanessian, *Carbohydr. Res.*, **2**, 86–88 (1966).