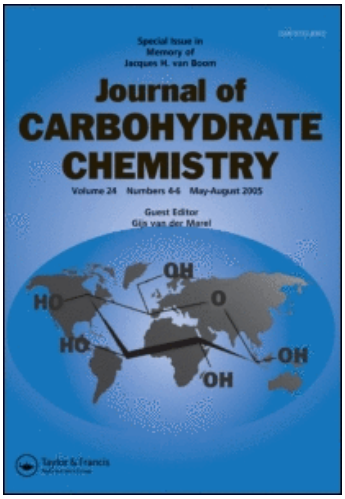

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Peptide Formation by Aminolysis of 1-Thio-β-D-glucopyranosyl Esters of *N*-Acylaminoacids

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PEPTIDE FORMATION BY AMINOLYSIS OF 1-THIO-β-D-GLUCOPYRANOSYL ESTERS OF N-ACYLAMINOACIDS

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ABSTRACT

The susceptibility of the fully acetylated 1-thio-β-D-glucopyranosyl esters of N-protected amino acids toward the amino group of an external amino acid- or peptide-ester was examined in dichloromethane at room temperature and at 40°, respectively. In each case, the aminolysis reaction led to rupture of the C-1 thiolester bond and formation of the corresponding N-acylpeptide ester; the reaction proceeded without racemization of the aglycon chiral centre. Evidence for a remarkably high acylating efficiency of the sugar-amino acid C-1 thiolester bond is presented.

INTRODUCTION

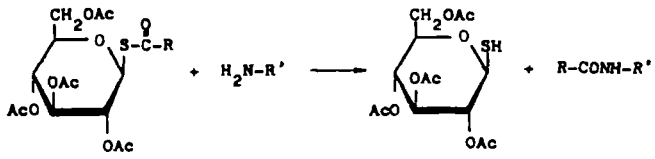
In a previous paper[1] it was shown that the fully acetylated 1-thio-β-D-glucopyranosyl esters of N-protected amino acids and peptides possess a high tendency to undergo S→O and S→N acyl migration involving cleavage of the C-1 thiolester bond and formation

of the corresponding amino acid-ester and -amide derivatives. The thiolester group is the ester function of choice in acyl transfer reactions occurring in biological systems.[2] Thus, the peptide bond formation by thiolester aminolysis represents the crucial reaction step in the non-ribosomal biosynthesis (e.g. gramicidin S) of peptides.[3] The mechanism of hydrolysis of a peptide by cysteine proteases (e.g. papain) involves the formation of tetrasubstituted intermediates also generated in the aminolysis of thiolesters.[4]

We considered it of interest to examine in more detail the reactivity of the above 1-thioglucosyl esters toward amino acids as nucleophiles, and report here data demonstrating a remarkably high acylating efficiency of the sugar-amino acid C-1 thiolester bond.

RESULTS AND DISCUSSION

The susceptibility of the fully acetylated 1-S--(N-acylaminoacyl)-1-thio-β-D-glucopyranoses 1-3 toward the amino group of an external amino-acid-methyl or peptide-methyl ester was examined with 3 molar equivalents of the nucleophile in dichloromethane at room temperature for 4 days. In each case, the reaction led to rupture of the C-1 thiolester bond and formation of tetra-O-acetyl-1-thio-D-glucose and the corresponding N-acylpeptide methyl ester (Scheme 1) which was isolated and characterized. The aminolysis of 2 was practically complete (t.l.c. monitoring) after 2 days, and from an additional experiment performed for 16 h, N-benzyloxycarbonyl-glycyl--glycine methyl ester was isolated in 38% yield. The same treatment of the analogous D-glucopyranosyl esters left them practically unchanged; after 4 days,



	R-C(=O)-	H_2N-R'	R-CONH-R'	Yield (%)
1	Z-Phe	H-Ala-OMe	Z-Phe-Ala-OMe [a]	86
1	Z-Phe	H-Gly-Phe-OMe	Z-Phe-Gly-Phe-OMe	72
2	Z-Gly	H-Gly-OMe	Z-Gly-Gly-OMe [b]	90
3	Z-Ala-Gly	H-Phe-OMe	Z-Ala-Gly-Phe-OMe	75

Z = $PhCH_2OCO$. [a] Lit.[6] [b] Lit.[7]

Scheme 1

the estimated amounts of aminolysis products ranged from < 1 to ~ 5%.[5]

In order to compare the degree of activation of an amino acid linked to HO-1 and HS-1 of β-D-glucopyranose and 1-thio-β-D-glucopyranose, respectively, further reactions were performed at the reflux temperature of dichloromethane (40° C). Under these conditions, the relative reactivities of the C-1 oxygen ester analogues were found[5] to be highly dependent upon the structure of the amino acid nucleophile and the nature of the aglycon side-chain and amino--protecting groups.

Table 1 presents the results of aminolysis reactions carried out with the fully acetylated 1-thio-β-D--glucopyranosyl esters of N-acylphenylalanine (1), -glycine (2), and -alanine (4–9) as the acylating agents and methyl esters of D,L-phenylalanine and glycine as the nucleophiles; for comparison, the reported[5] yields of dipeptide methyl esters formed by aminolysis of the corresponding C-1 oxygen esters are also included. The data provide clear evidence that the

TABLE 1

AMINOLYTIC REACTIVITY OF FULLY ACETYLATED 1-THIO-ß-D-GLUCOPYRANOSYL ESTERS OF N-ACYLAMINO ACIDS IN REFLUXING DICHLOROMETHANE [a]

1-Thio-ß-D-gluco-pyranosyl ester		Nucleophile	Reaction time	Dipeptide isolated		Reported [c] peptide yield (%) for 1-O-acyl analogue after 4 days of reaction
No	Aglycon group	Chemical structure	hours	Chemical structure	Yield [b] (%)	
1	Z-Phe	H-D,L-Phe-OMe	16	Z-Phe-D,L-Phe-OMe	77	no reaction
2	Z-Gly	H-D,L-Phe-OMe	6	Z-Gly-D,L-Phe-OMe [d]	84	50
4	Z-Ala	H-Gly-OMe	16	Z-Ala-Gly-OMe [e]	52	15
5	Z-D-Ala	H-Gly-OMe	16	Z-D-Ala-Gly-OMe	77	33
6	Boc-Ala	H-Gly-OMe	13	Boc-Ala-Gly-OMe [f]	62	50 [g]
7	Boc-D-Ala	H-Gly-OMe	13	Boc-D-Ala-Gly-OMe [h]	70	78 [g]
8	Ac-Ala	H-Gly-OMe	4	Ac-Ala-Gly-OMe [h]	40	93
			13		50	
			23		66	
9	Ac-D-Ala	H-Gly-OMe	4	Ac-D-Ala-Gly-OMe [h]	33	48
			13		38	
			23		66	

[a] All reactions were performed with 2×10^{-2} M thioglucosyl ester and 3 equiv. of nucleophile. [b] Yields refer to the isolated dipeptide derivative and are calculated on the thioglucosyl ester used. [c] Lit.[5]; experimental conditions were identical to those used for C-1 thiol esters. [d] Lit.[8]. [e] Lit.[9]. [f] Lit.[10]. [g] After 16 h of reflux. [h] Lit.[5].

acylating efficiency of the sugar-amino acid C-1 thiolester bond is substantially higher than that of the C-1 oxygen ester bond. Furthermore, in contrast to the D-glucopyranosyl ester series, the thiolesters investigated revealed a surprising uniformity in aminolytic activities to give the respective dipeptide esters in very similar yields.

The results imply that in 1-thio-D-glucopyranosyl ester series, the structures of the nucleophile and aglycon amino acids have only a small effect on the extent of peptide bond formation. Apparently, the sulphur atom with its larger size and lower basicity than oxygen [2] has a by far stronger influence on the reaction rates. According to the generally accepted mechanism for ester[11,12] and thiolester[13,14] aminolysis, one may expect that the departing 1-thio-D--glucopyranosyl moiety represents a much better leaving group than the D-glucosyl moiety, and that the transition state offers less steric hindrance to the reaction in the 1-thio-D-glucopyranosyl-ester than in the D-glucopyranosyl-ester series.

The optical rotation values of the isolated peptide derivatives indicated that the aminolysis of 1-thioglucosyl esters proceeded, as established[5] for their oxygen analogues, without racemization of the aglycon chiral centre. In order to provide a definite confirmation, 2,3,4,6-tetra-O-acetyl-1-S-(N-acetyl-L- and D-alanyl)-1-thio-β-D-glucopyranose (8 and 9) were synthesized from the corresponding N-tert-butoxycarbonyl derivatives 6 and 7, respectively, via deprotection and reacylation of the aglycon amino group. The ^{1}H NMR spectrum of 8 in $CDCl_3$ revealed the methyl doublet (δ 1.41) of alanine at a slightly lower field than the equivalent signal in the spectrum of 9, thus allowing differentiation of the two diastereoisomers.

It should be noted that a direct synthesis of 8 from tetra-O-acetyl-1-thio-D-glucopyranose and N-acetyl-L--alanine by the imidazole-promoted DCC condensation,[1] yielded a partially racemized product in which the L:D ratio of N-acetylalanine was estimated (^{1}H NMR) to be ~ 2:1.

Aminolysis of 8 and 9 with glycine methyl ester afforded Ac-Ala-Gly-OMe and Ac-D-Ala-Gly-OMe, respectively, in high yield (Table 1), thus confirming that under the conditions studied, the aminolysis reaction proceeds with retention of the aglycon amino acid configuration.

EXPERIMENTAL

General Procedures. Column chromatography was performed on Silica Gel (Merck 0.05-0.2 mm) and t.l.c. on Silica Gel 60 (Merck); detection on t.l.c. plates was effected by charring with sulphuric acid, or the chlorine-iodine reagent[15] for peptides. ^{1}H NMR spectra were obtained from a Jeol FX 90 Q FT spectrometer using $(CH_3)_4Si$ (0 ppm) as the internal standard. 2,3,4,6-Tetra-O-acetyl-1-S-(N-acylaminoacyl)-1-thio-β-D--glucopyranoses 1-7 were prepared as described in the previous paper.[1]

Aminolysis reactions. a. To a stirred suspension of the amino acid- , or peptide-, methyl ester hydrochloride (1.5 mmol) in CH_2Cl_2 (25 ml) was added at room temperature the equivalent amount of N-methylmorpholine (0.167 ml) followed by the relevant 1-thioglucosyl ester (1-3, 0.5 mmol). The solution was kept at room temperature for 4 days, whereupon the solvent was removed, the residue was dissolved in EtOAc, and the

organic layer was washed with water, 10% citric acid in water, water, aqueous $NaHCO_3$ and water, dried and concentrated. The residue was passed through a silica gel column with benzene-EtOAc (2:1) to give the corresponding Z-peptide methyl ester in pure form; tetra-O-acetyl-1-thio-D-glucose underwent considerable decomposition during fractionation.

Z-Phenylalanyl-glycyl-phenylalanine Me-ester, obtained by aminolysis of 1 and crystallized from $CHCl_3$-light petroleum, had m.p. 148-150°C, $[\alpha]_D$ +29.4° (c 1, $CHCl_3$).

Anal. Calc. for $C_{29}H_{31}N_3O_6$: C, 67.30; H, 6.04; N, 8.12. Found: C, 67.14; H, 5.95; N, 8.33.

Z-Alanyl-glycyl-phenylalanine Me-ester, obtained by aminolysis of 3 and crystallized from $CHCl_3$-light petroleum, had m.p. 142-144°C, $[\alpha]_D$ +26.5° (c 1, $CHCl_3$).

Anal. Calc. For $C_{23}H_{27}N_3O_6$: C, 62.57; H, 6.16; N, 9.52. Found: C, 62.49; H, 6.26; N, 9.79.

b. Molar ratios of the reactants and the solvent were the same as described above, except that the reaction mixtures were refluxed (bath temp. 40-42°C) for the times indicated in Table 1. The reaction mixtures were worked-up as described above, and the residues were submitted to silica gel chromatography.

Z-Phenylalanyl-D,L-phenylalanine Me-ester, obtained by aminolysis of 1 and crystallized from benzene-light petroleum, had m.p. 119-121°C, $[\alpha]_D$ +8.0° (c 1, $CHCl_3$).

Anal. Calc. for $C_{27}H_{28}N_2O_5$: C, 70.42; H, 6.13; N, 6.08. Found: C, 70.39; H, 6.34; N, 5.93.

Z-D-Alanyl-glycine Me-ester, obtained by aminolysis of 5 and crystallized from $CHCl_3$-light petroleum, had m.p. 91-93°C, $[\alpha]_D$ +22.0° (c 1, MeOH).

Anal. Calc for $C_{14}H_{18}N_2O_5$: C, 57.14; H, 6.17; N, 9.52. Found: C, 57.37; H, 6.13; N, 9.70.

2,3,4,6-Tetra-O-acetyl-1-S-(N-acetyl-L-alanyl)-1--thio-β-D-glucopyranose (8). Compound 6 (535 mg, 1 mmol) was treated with CF_3CO_2H (98%, 2.5 ml) at -10°C for 10 min, whereupon anhydrous ether was added, the solution was concentrated, and traces of CF_3CO_2H were removed by co-distillation with ether. To a solution of the residue in water (75 ml), 20% Ac_2O in acetone (75 ml) was added, the solution was kept overnight at 4°C, and the solvent was removed (0.1 Torr). Crystallization of the residue from $CHCl_3$-light petroleum gave 357 mg (75%) of 8. M.p. 143-144°C, $[\alpha]_D$ -40.0° (c 1, $CHCl_3$). ^1H NMR ($CDCl_3$) δ 2.08 (s, CH_3CO), 2.03 (s, 3 x CH_3CO), 2.00 (s, CH_3CO), 1.41 (d, J 7.25 Hz, CH_3CH).

Anal. Calc. for $C_{19}H_{27}NO_{11}S$: C, 47.80; H, 5.69; N, 2.93; S, 6.72. Found: C, 47.78; H, 5.48; N, 2.78; S, 6.84.

2,3,4,6-Tetra-O-acetyl-1-S-(N-acetyl-D-alanyl)-1--thio-β-D-glucopyranose (9). Treatment of 7 (535 mg) in the same manner as described above afforded the title compound (333 mg, 70%); m.p. 133-135°C ($CHCl_3$-light petroleum), $[\alpha]_D$ +38.5° (c 1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 2.07 (s, CH_3CO), 2.04 (s, CH_3CO), 2.03 (s, CH_3CO), 2.02 (s, CH_3CO), 2.00 (s, CH_3CO), 1.40 (d, J 7.25 Hz, CH_3CH).

Anal. Found: C, 47.86; H, 5.47; N, 2.63; S, 6.53.

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