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KINETICS OF AMINOLYSIS FOR 1-THIO-β-D-GLUCOPYRANOSYL ESTERS OF *N*-ACYLALANINES

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Abstract—The kinetics of aminolysis of 1-thio-β-D-glucopyranosyl esters of *N*-protected alanines (**1**) in dichloromethane, at 26°, by ethyl glycinate, under pseudo-first-order conditions follows the relationship $k_{obsd} = k_2$[H-Gly-OEt]. Significant differences were observed in the rates of dipeptide forming aminolysis for 1-thiolesters **1a-1f**, depending on *N*-acyl substituents and the configuration of alanine.

A great deal of attention has been given to the mechanism of ester aminolysis in aqueous[1] and nonaqueous media.[2] This reaction is of special interest because the proposed tetrasubstituted intermediate(s) involved[3] may also be formed in the acylation of chymotrypsin and other serine proteases by amides, esters, acids etc,[1] as well as in analogous acylation reactions of papain[4] and other cysteine proteases.[5] Data were reported on thionester[6] and thiolester[7] aminolyses, and the extensive mechanistic similarity to the corresponding reactions of oxygen esters was clearly demonstrated.

The tetrahedral intermediate in a rate-determining step was also postulated in aminolyses of activated esters of *N*-protected amino acids with different amino acid esters leading to the peptide bond formation.[8] The effect of peptide substituents on rates of peptide-forming aminolysis reactions,[9] and the influence of steric effects was given particular attention.[10]

We have recently reported that 2,3,4,6-tetra-*O*-acetyl-1-*S*-(*N*-acylaminoacyl)-1-thio-β-D-glucopyranoses (**1**, RCO = *N*-protected amino acid) undergo aminolysis reactions with simple amines, as well as with amino acid or dipetide esters, to form protected peptides and liberate the appropriate 1-thio sugar.[11] These 1-thiolesters were shown to be much more reactive than their oxygen analogs.[12,13] The present work undertakes kinetic studies of the peptide forming aminolysis reaction of 1-thio-D-glucopyranosyl esters **1** in nonaqueous medium.

RESULTS AND DISCUSSION

Fully acetylated 1-thioglucopyranosyl esters **1** (RCO = *N*-acylalanyl residue) undergo reaction with glycine ethyl ester (**2**) to liberate the C-1 free thio sugar **3** and the protected dipeptides **4** (Table 1). Reactions were followed spectrophotometrically at 237 nm (loss of the –S–CO– chromophore) in dichloromethane under conditions of pseudo-first-order kinetics, i.e. with amine in large excess over 1-thiolester. Cumulative absorption bands at 237 nm for reaction products (**3** and **4**) were negligible (~ 5%) when compared with absorption of **1** at the same wavelength. Oxygen analogs of **1** (–O–CO–) do not show any appreciable absorption and could not be investigated under experimental condtions used in this study.

All aminolysis reactions studied in this work obeyed first-order kinetics. The observed pseudo-first-order rate cosntants can be described by the simple equation

$$k_{obsd} = k_2[\text{H-Gly-OEt}]. \quad (1)$$

Plots of k_{obsd} against amine concentration (Fig. 1) give straight lines passing through the origin, indicating that the contribution of the higher terms is negligible under the conditions studied. The second-order rate constants, k_2, for aminolysis reactions are listed in Table 1.

In view of these results and data reported for aminolyses of simple esters,[3] thiolesters,[7] and amino acid esters,[14] a mechanism is proposed for this reaction as presented in Scheme 1. This mechanism involves the reversible formation of a tetrahedral addition intermediate followed by its rate-determining decomposition to products. This leads to the rate expression (eqn 2) which is kinetically equivalent to the observed rate law (eqn 1)

$$-\frac{d[\text{intermediate}]}{dt} = k'K[\text{1-thiolester}][\text{amine}]. \quad (2)$$

As evident from Table 1, compounds **1** belong to the

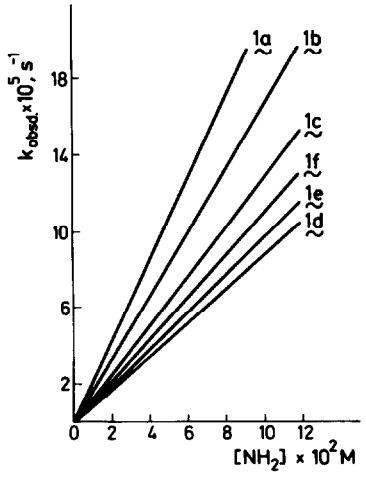
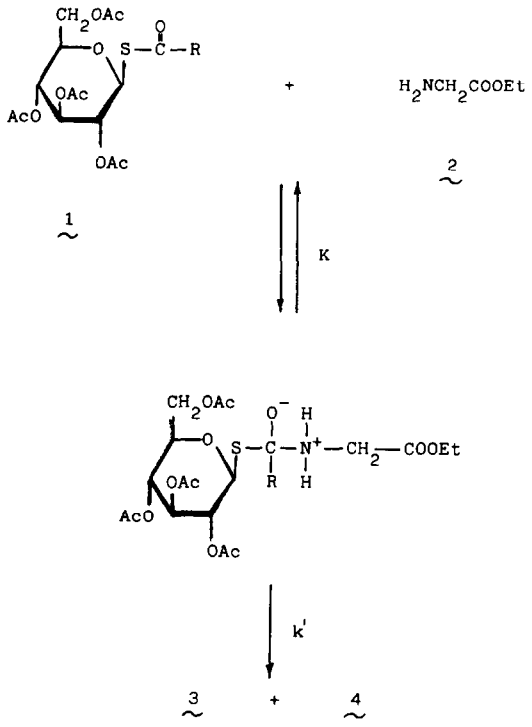


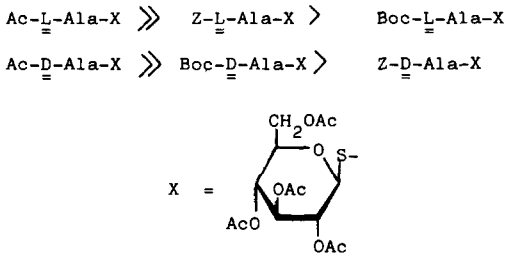
Fig. 1. Plots of k_{obsd} for aminolysis reactions of 1-thiolesters **1a-1f** as a function of glycine ethyl ester (**2**) concentration.



Scheme 1. Proposed mechanism for peptide forming aminolysis reactions of 1-thiolesters **1a-1f**.

two related series of structures; one contains *N*-protected alanines of L-configuration and the other of D-configuration. The realtive reactivities within each series are presented in Scheme 2.

In both series *N*-acetylated (*N*-Ac) 1-thiolesters which contain an amide type of protection, were more



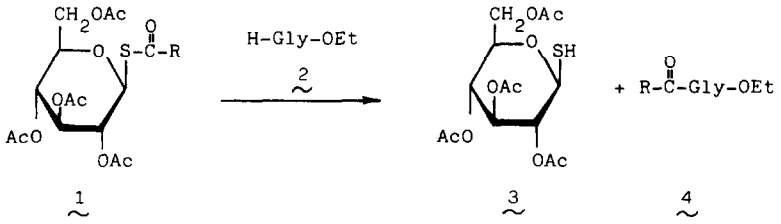
Scheme 2. Order of reactivities in aminolysis reactions of 1-thiolesters **1a-1f**.

reactive than *N*-benzyloxycarbonyl (*N*-Z) or *N*-*t*-butyloxycarbonyl (*N*-Boc) 1-thiolesters with a urethane type of amino acid protection. The difference in reactivities between *N*-benzyloxycarbonyl and *N*-*t*-butyloxycarbonyl 1-thiolesters exists in both series but is much less pronounced than the difference observed for compounds having an amide vs urethane type of protection. Furthermore, the rate of dipeptide forming aminolyses was not the same for 1-thiolesters **1** containing identical *N*-protecting groups, and differing only in the configuration of the amino acid residue. As desribed in Table 1 1-thiolesters **1** having *N*-Ac and *N*-Z protecting groups on L-amino acid residues were more reactive than those belonging to the D-series. In the case of *N*-Boc protected compounds **1** only a slight difference in reactivity was observed, and it was in favor of the D-structure.

These results indicate that the rate of aminolysis for 2,3,4,6-tetra-*O*-acetyl-1-*S*-(*N*-acylalanyl)-1-thio-β-D-glucopyranoses depends on the configuration of the amino acid residue, and on the type of *N*-protection in that part of a molecule.

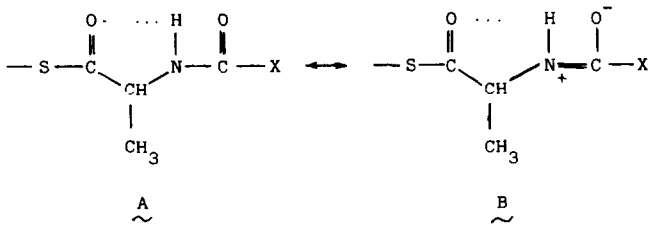
It is well known that amino acid derivatives are capable of forming hydrogen bonds. In fact, such

Table 1. Kinetic parameters for aminolysis reactions of 1-thiolesters **1** with ethyl glycinate **2**.[a]



Compound	R-C(=O)-	$k_2 \times 10^4$, $M^{-1}s^{-1}$	$k_2(L)/k_2(D)$
1a	Ac-L-Ala	21.2 ± 0.4	1.3
1b	Ac-D-Ala	16.7 ± 0.3	
1c	Z-L-Ala[b]	12.9 ± 0.7	1.4
1d	Z-D-Ala	9.1 ± 0.1	
1e	Boc-L-Ala[c]	10.1 ± 0.3	0.9
1f	Boc-D-Ala	11.3 ± 0.3	

[a] In dichloromethane, 26°C; [b] Z = $C_6H_5CH_2OCO$; [c] Boc = $(CH_3)_3COCO$.



A B

Thiolester	X
1a, 1b	CH_3
1c, 1d	OCH_2Ph
1e, 1f	$OC(CH_3)_3$

Scheme 3.

bonds are essential for the secondary structure (α-helices, β-sheets or random coils) of peptides and proteins.[15] Smaller molecules, such as *N*-acetyl amino acid esters, were also studied, and it was concluded that hydrogen bonds exist in those molecules as well. IR data indicated that intramolecular hydrogen bonding occurred between NH and amino acid ester C=O groups.[16,17] Analogous intramolecular hydrogen bonds may be formed in the case of 1-thiolesters **1** (Scheme 3), in which case they could be responsible for different reaction rates in differently *N*-substituted 1-thiolesters. Two forms, **A** and **B**, are possible for compounds **1**. Which one will prevail depends on the character of X (Scheme 3). The negative inductive effects of OCH_2Ph and $OC(CH_3)_3$ groups in urethane type of *N*-protection (**1c**, **1d**, **1e** and **1f**) will favor form **A**, while form **B** will be favored when X in CH_3, as it is in an amide type of *N*-protection (**1a** and **1b**). The NH in **B** may form hydrogen bonds with 1-thiolester C=O more easily than in **A** due to the less basic character of nitrogen in **B**. Such hydrogen bond enhances the electrophilic character of the carbonyl group which then becomes more liable to the nucleophilic attack of amines. The less basic character of the amide nitrogen as compared to the urethane was previously observed and investigated using IR and ^{1}H-NMR techniques.[18] IR spectra of *N*-acetyl 1-thiolesters (**1a** and **1b**) showed NC = O bands at values ($\sim 1680\,cm^{-1}$) which are lower than those for *N*-benzyloxycarbonyl (**1c** and **1d**; $\sim 1720\,cm^{-1}$), and *N*-*t*-butyloxycarbonyl 1-thiolesters (**1e** and **1f**; $\sim 1710\,cm^{-1}$). These data, which are in complete accord with those reported elsewhere,[18] indicate the less double bond character in the amide C=O. That leads to the conclusion that form **B** indeed prevails in the amide type of *N*-protection, and form **A** in the urethane type of *N*-protection of amino acid residues.

^{1}H-NMR spectra of 1-thiolesters **1** are rather complex.[11,13] Only *N*-acetyl 1-thiolesters (**1a** and **1b**) exhibited NH signals which were not overlapped by ring protons belonging to the sugar moiety, and they were found at δ 5.83 ppm for **1a**, and 5.80 ppm for **1b**. Compounds **1c-1f** do not show any signals beyond δ 5.30–5.40 ppm, demonstrating that the urethane NH resonance was shifted uplfield from that of the amide NH. Further data were obtained from ^{1}H-NMR spectra of model compounds. We chose *N*-acetyl, *N*-benzyloxycarbonyl, and *N*-*t*-butyloxycarbonyl L- or D-alanine methyl esters, spectra of which are simplified since there are no sugar ring protons to interfere with other signals. The position of the NH signals was identified in all spectra, and is presented in Table 2. NH in *N*-Ac esters lies at lower field than in *N*-Z or *N*-Boc esters indicating again the more acidic character of the amide NH. Different chemical shifts for amide and urethane NH's was previously observed in larger molecules as well, eg in oligopeptides, urethane NH is easily identified since it is always upfield from other amide NH's in those complex molecules.[15,19]

Obviously, the possibility of intramolecular hydrogen bonds formation does exist in all of the 1-thiolesters studied in this work, but the ease of formation depends on the type of *N*-protection. Type of *N*-protecting groups will, therefore, exert significant influence on rates of aminolysis.

As previously discussed, rates of aminolysis depend on the collapse of the reversibly formed tetrahedral intermediate. Three major bond changes are involved in the aminolysis, ie a C-N amide bond is formed while C-S 1-thiolester and N-H amine bonds are broken (Scheme 1). In the rate-determining transition state, the acyl carbon and the amide nitrogen are tetrahedral. Therefore, variation in rate constants will reflect steric effects in the transition state. It is well known that side chains on amino acid residues,[10] and

Table 2. ^{1}H-NMR chemical shifts for the NH signals of *N*-protected alanine methyl esters.

Compound	Chemical shifts (ppm)[a]
Ac-D-Ala-OMe	6.12
Ac-L-Ala-OMe	6.09
Z-D-Ala-OMe	5.26
Z-L-Ala-OMe	5.26
Boc-D-Ala-OMe	5.03
Boc-L-Ala-OMe	5.06

[a] Determined for 0.1% solutions in CD_2Cl_2.

the active ester group which is directly attached to the reactive centre,[20] influence the rate of aminolysis. We noticed that the configuration of the amino acid residue influences the rate as well. In peptide synthesis racemization often occurs in parallel with the coupling reaction. It was recently observed that the configuration of the activated amino acid residue influences the extent of racemization.[21,22] It is thought that the main cause for that phenomenon lies in the various rates of peptide bond formation in L- and D-amino acid residues.[23] We found that there is no racemization in the peptide forming aminolyses of 1-thiolesters **1**.[11,13] But, our results revealed that the configuration of the thioglycosidically linked amino acid residues in **1** influences the rate of aminolysis. Most probably, steric factors are involved.

In view of the great importance of peptide synthesis, and many complex problems involved in these coupling reactions, kinetic studies seem to be of great significance. They may help in finding more effective coupling reagents, and reaction conditions leading to as little racemization as possible.

EXPERIMENTAL

General procedures. Fully acetylated 1-thio-D-glucopyranosyl esters of *N*-acyl-L- or D-alanine (**1c–1f**) were prepared using the accelerated active ester method.[11] **1e** and **1f** were treated with trifluoroacetic acid, followed by acetylation, to obtain optically pure *N*-acetyl derivatives **1a** and **1b**.[13]

Glycine ethyl ester hydrochloride was prepared by the Boissonnas modification[24] of the Fischer esterification. Dichloromethane (Merck) for kinetic runs was UV grade. Absorbance measurements were determined on a Pye Unicam SP8-TO UV/VIS spectrophotometer. IR spectra were recorded with a Perkin–Elmer 297 spectrometer using NaCl cells (4% solutions of **1a-1f** in CH_2Cl_2). ^{1}H-NMR spectra were obtained from a JEOL FX 90 Q FT spectrometer using $(CH_3)_4Si$ (0 ppm) as the internal standard. CD_2Cl_2 (Merck, spectrophotometric grade) was used as a solvent.

Kinetic procedure. Glycine ethyl ester was liberated from the hydrochloride salt with 33% NaOH according to Goodman and McGahren,[25] stored at −10°, and distilled immediately before use. The concentration of 1-thio-D-glucopyranosyl esters **1** was 5.8×10^{-4} in all runs. The total glycine ethyl ester concentrations varied from 0.0437 to 0.0758 M. The reaction was monitored spectrophotometrically at 237 nm. The decrease in absorption values was taken as a measure of the remaining 1-thiolesters **1**. All measurements were performed in stoppered, thermostatted (26° ± 0.1°), rectangular fused silica cells (d = 1 cm). The reactions were initiated by placing appropriate volumes of **1** and glycine ester solutions in CH_2Cl_2 (thermostatted at 26°) into the spectrophotometric cell. A reference cell with the same amine concentration in CH_2Cl_2 was employed to compensate glycine ethyl ester absorption. Forming products (**3** and **4**) gave absorptions at 237 nm which were less than 5% of the total 1-thiolester absorptions. Reactions were monitored by TLC (Silica Gel 60, Merck), and no products other than **3** and **4** were observed at any time. Owing to a large excess of amine all runs were pseudo-first-order reactions. Aminolyses were followed to 2.0–2.5 half-lives, and infinity points were taken at 10 half-lives. Pseudo-first-order rate constants were obtained for each experiment at fixed amine concentration by fitting the first-order rate equation through unweighted nonlinear regression using the least squares method. Second-order rate constants were obtained from pseudo-first-order rate constants for reactions at different amine concentratons by an unweighted linear regression using the least squares method. In all cases four concentrations (75, 100, 110 and 130 times in excess of the 1-thiolester concentration) of glycine ethyl ester were used.

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