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Metal Ion-Promoted Activation of Amino Acid Esters of Carbohydrates in the Synthesis of Peptides

Horst Kunz* and Richard Kullmann

Institut für Organische Chemie, Universität Mainz, J.-J.-Becher-Weg 18-20, D(W)- 6500 Mainz, Germany

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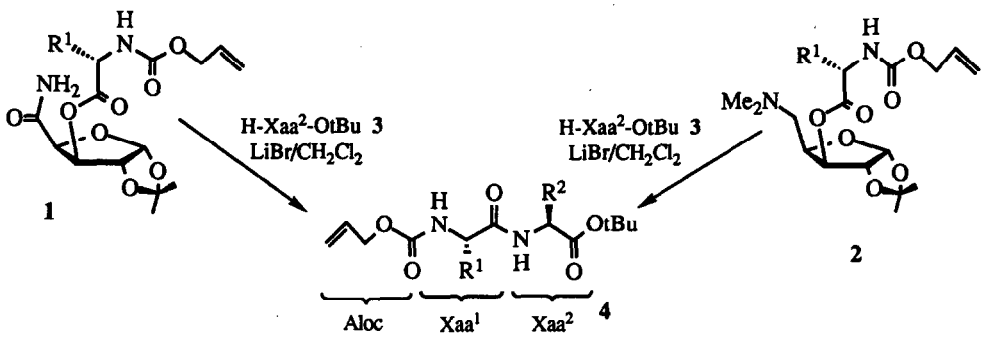
Abstract: Carbohydrate esters **1** and **2** of N-protected amino acids are activated by coordination of metal ions and subjected to aminolysis by amino acid esters **3** to form peptides **4**.

Conventional peptide synthesis is carried out by stepwise chain extension from the C-terminus. Activated N-protected amino acids, e. g. active esters, are required in order to achieve the peptide formation. In contrast, the protein biosynthesis proceeds via chain extension from the N-terminus. Peptide esters of the 5'-terminal ribose of tRNA operate as the acylating agents. Activation by peptidyltransferase and optimal preorientation of the reacting species within the ribosome enable the condensation to occur under physiological conditions. Attempts to simulate the biological peptide formation include intramolecular peptide syntheses[1] in which the amino and the carboxy component were linked to a template. As the carboxy component was applied as an activated aryl[2] or thio ester,[3] intramolecular aminolyses delivered the peptides. Even earlier was shown the enhanced reactivity of amino acyl nucleosides and RNA in saponification reactions.[4] Like the ribose 3'-esters,[4] β-glucopyranose esters of amino acids also show enhanced intrinsic reactivity and were used for peptide formations.[5] A biomimetic peptide synthesis was reported by Letsinger and Klotz,[6] who used a polynucleotide template to which the N-terminally resin-linked peptide ester of a complementary oligonucleotide and the amino acid ester of a second oligonucleotide are assembled.

We here report on a peptide synthesis in which carbohydrate esters of N-protected amino acids serve as the acylating reagents. As these esters neither involve the anomeric center nor contain a reactivity-supporting hydroxyl group, they do not show an enhanced intrinsic reactivity. The method is based on the observation that carbohydrate esters of carboxylic acids display a surprising reactivity in alkaline saponifications.[7,8] Thus, the 3-O-acetyl-1,2-5,6-di-O-isopropylidene-α-D-glucofuranose in sodium hydroxide solution under identical conditions reacts 20 times faster in than isopropyl acetate.[9] Kinetic measurements revealed a low activation entropy responsible for the high reactivity of the carbohydrate ester.[9] The effect is rationalized in terms of an efficient complexation of the sodium ion by the carbohydrate. The alkali ion fixed to the carbohydrate also coordinates the hydroxyl ion, thus, producing an ordered ground state. With other words, the carbohydrate esters become activated by complexation of metal ions.

We now utilized this effect for the development of a biomimetic peptide synthesis. The 1,2-O-isopropylidene-α-D-xyluronic acid amide[10] was condensed with N-allyloxycarbonyl amino acids (Aloc-Xaa-OH)[11] using carbodiimide (DCC), and 4-dimethylamino pyridine (DMAP)[12] to give the 3-O-(N-Aloc)-amino acyl xyluronic amide derivatives **1**. Analogously, 5-deoxy-5-dimethylamino-1,2-isopropylidene-α-D-xylofuranose obtained

from the corresponding 5-O-mesylate[13] and dimethylamine in methanol was reacted with Aloc amino acids to furnish the N-Aloc amino acid esters **2**.



Scheme 1

Both types of carbohydrate amino esters **1** and **2** were used for peptide syntheses with amino acid tert-butyl esters **3**. At room temperature both esters **1** and **2** showed only low or even no reactivity towards the amino components. However, after complexation with lithium bromide (2 equivalents), compounds **1** and **2** smoothly reacted with amino acid esters **3** to form the peptides **4**. In typical experiments, the carbohydrate ester **1** or **2**, respectively, (1.5 mmol) and the amino acid tert-butyl ester **3** (6 mmol) in the presence of lithium bromide (3 mmol) were stirred in dry dichloromethane (12 ml). The formed peptides **4** were isolated from the organic solution after simple washing operations. The carbohydrate alcohols can be re-collected by flash-chromatography. The reaction conditions and the yields of **4** are quoted in Table 1 (reactions of type **1**) and Table 2 (reactions of type **2**).

The results obtained with the xyluronic amide-type esters **1** (Table 1) illustrate that the rate of the peptide forming aminolysis depends upon the bulkiness of both, **1** and **3**. Whereas the glycylglycine derivative was furnished almost quantitatively after 2 days at room temperature, the formation of the valylvaline analogue remained incomplete after 4 days at 40°C. This steric influence was also found for the reactions of the 5-dimethylamino xylofuranose esters **2** (Table 2). In this case, the dipeptide ester (Aloc-Gly-Ala) of type **2** smoothly reacted with amino acid tert-butyl esters **3** (also with H-Val-OtBu) to give the corresponding homologue of **4**. The activating effect of the lithium ion on both types of carboydrate esters **1** and **2** becomes obvious by comparison of the reactions of carbohydrate esters **1** and **2** of Aloc glycine either in presence (first line in Table 1 and 2) or in absence of LiBr (bottom line in Table 1 and 2). For the aminolysis of the Aloc glycine ester of 5-dimethylamino xylofuranose derivative (type **2**) with glycine tert-butyl ester (upper-left corner of Table 2) the rate constants were determined by ^{1}H NMR- and HPLC- measurements. The rate constant of the reaction catalyzed by LiBr (2 equiv.) amounted to $k_{cat} = 2.65 \times 10^{-4} mol^{-1}s^{-1}$, the one of the uncatalyzed reaction was $k_{uncat} = 1.9.10^{-5} mol^{-1}s^{-1}$, giving a factor of acceleration by complexation of the lithium ion of 14. Among other salts proven as catalysts only magnesium bromide showed a similar, but slightly lower effect. Sodium bromide, nickel bromide and copper sulfate showed no effect.

Table 1 : Synthesis of the peptides 4 from 3-O-(N-Aloc-amino acyl)-1,2-O-isopropylidene-α-D-xyluronic acid amide 1 and amino acid esters 3 catalyzed by 2 equivalents of lithium bromide

Xaa^2 / Xaa^1	Gly Yield (t,T)	Ala Yield (t,T)	Phe Yield (t,T)	Val Yield (t,T)
Gly	93 % (2 d 20°C)	88 % (3 d 20°C)	81 % (3 d 20°C, 1 d 40°C)	72 % (3 d 20°C, 1 d 40°C)
Ala	87 % (2 d 20°C)	73% (2 d 20°C, 1 d 40°C)	62 % (2 d 20°C, 2 d 40°C)	57 % (1 d 20°C, 3 d 40°C)
Val	64 % (1 d 20°C, 2 d 40°C)	40 % (1 d 20°C, 2 d 40°C)	35 % (1 d 20°C, 3 d 40°C)	35 % (1 d 20°C, 3 d 40°C)
Gly	21 % [a] (2 d 20°C)	13 % [a] (3 d 20°C)	0 % [a] (3 d 20°C, 1 d 40°C)	0 % [a] (3 d 20°C, 1 d 40°C)

a) Reaction without use of lithium bromide

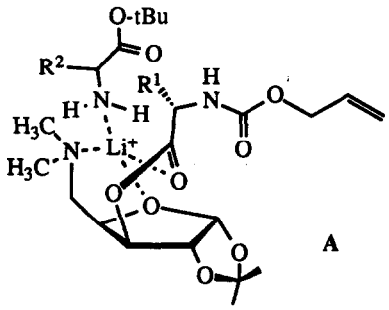
Table 2 : Synthesis of the peptides 4 from 3-O-(N-Aloc-amino acyl)-5-desoxy-5-N,N-dimethyl-amino-1,2-isopropylidene-α-D-xylofuranose 2 and amino acid t-butylester 3 catalyzed by 2 equivalents of lithium bromide

Xaa^2 / Xaa^1	Gly Yield (t,T)	Ala Yield (t,T)	Phe Yield (t,T)	Val Yield (t,T)
Gly	98 % (1 d 20°C)	84 % (1 d 20°C)	85 % (2 d 20°C)	85 % (2 d 20°C)
Ala	96 % (2 d 20°C)	74 % (1 d 20°C, 1 d 40°C)	74 % (1 d 20°C, 1 d 40°C)	79 % (1 d 20°C, 2 d 40°C)
Phe	74 % (2 d 20°C, 1 d 40°C)	71 % (2 d 20°C, 1 d 40°C)	56 % (2 d 20°C, 1 d 40°C)	63 % (2 d 20°C, 1 d 40°C)
Val	61 % (2 d 20°C, 1 d 40°C)	45 % (2 d 20°C, 1 d 40°C)	41 % (1 d 20°C, 1 d 40°C)	28 % (1 d 20°C, 1 d 40°C)
Gly-Ala	84 % (4 d 20°C)	73% (4 d 20°C)	62 % (4 d 20°C)	54 % (4 d 20°C)
Gly	42 % [a] (2 d 20°C)	28 % [a] (3 d 20°C)	21 % [a] (3 d 20°C)	19 % [a] (3 d 20°C)

a) Reaction without use of lithium bromide

According to the results of the alkaline hydrolysis of carbohydrate esters, the activating effect of the lithium ion is rationalized in terms of a complex A in which both, the carbohydrate ester and the amino component are coordinated to the lithium ion. Due to this preorienting effect the aminolysis of the carbohydrate ester is

markedly accelerated. Furthermore, coordination of the lithium ion to the carbohydrate makes the carbohydrate alkohol a better leaving group in comparison to common alkanols.



Scheme 2

This preorientation of the components is efficient only with ions which can coordinate both, nitrogen and oxygen nucleophiles. As a consequence, the peptide formation from the otherwise stable amino acid carbohydrate esters **1** and **2** occurs under very mild conditions. Complexation of lithium ions by carbohydrate functions was also observed in reactions of carbohydrate ester enolates. In these processes, the enhanced leaving group quality of the carbohydrate resulting from its coordination to the lithium ion is responsible for the easy decomposition of the ester enolate to give the corresponding ketene even at -60°C.[14]

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