Papain-Catalyzed Hydrolysis of *N*-Protected Glycosylated Amino Acid Ester

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(Received May 24, 1990)

Synopsis. The selective hydrolysis of α-methyl ester of glycosylated amino acid derivative, Boc-Asn(NAcGlc)-OMe, was achieved by using papain. Optimal conditions for hydrolysis were estimated.

In glycopeptides, carbohydrate moiety affects the conformation[1] or rigidity of the glycopeptides.[2] Incorporation of carbohydrate into peptide chain was achieved to change biological activity. However, chemical synthesis of glycopeptides is problematic due to heavy demands of stereoselective formation of glycosidic bond and protection of functional groups.[3] The glycosylated Asn derivative, N^4-(2-acetamide-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-N^2-(tert-butoxycarbonyl)-L-aspargine methyl ester, Boc-Asn(NAcGlc)-OMe, is a useful intermediate for the synthesis of Asn(NAcGlc) containing peptides. When Boc-Asn(NAcGlc)-OMe is used as an amino component, Boc group is easily and selectively cleaved with HCl/organic solvent. In contrast, in the case when it is used as a carboxyl component, selective saponification of α-methyl ester group without undesired deprotection of the hydroxyl group of carbohydrate moiety is quite difficult. Here, we report on papain-catalyzed selective hydrolysis of backbone ester of Boc-Asn(NAcGlc)-OMe.

Results and Discussion

As shown in Fig. 1, the highest yield of Boc-Asn-(NAcGlc)-OH was achieved by the reaction at pH 6. The yield also depends on the reaction temperature (Fig 2). At 30 °C, yield of 96% is achieved. Thus, the backbone methyl ester is almost quantitatively hydrolyzed with papain. There is little effect of enzyme concentration on the yield (Fig . 3). In conclusion, selective hydrolysis of Boc-Asn(NAcGlc)-OMe can be achieved by using papain. The resulting Boc-Asn-(NAcGlc)-OH is a useful building block for the synthesis of Asn(NAcGlc) containing peptides.

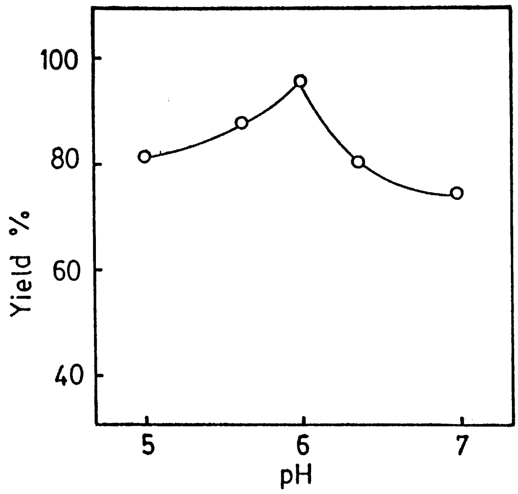


Fig. 1. Effect of pH of the reaction medium on the yield at 30 °C.

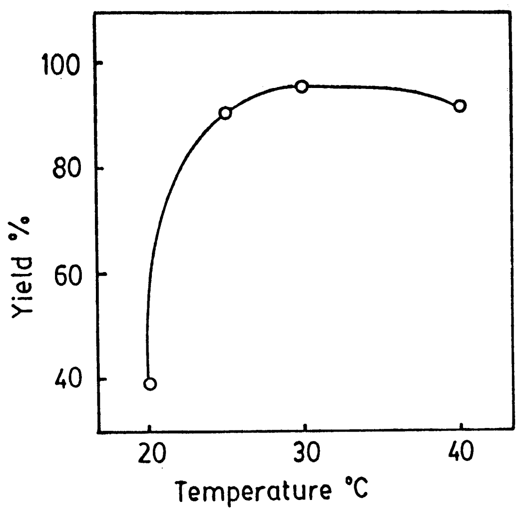
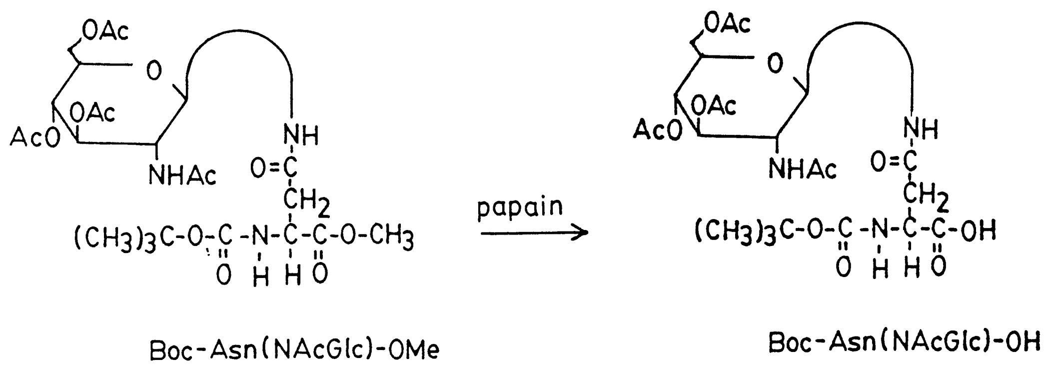


Fig. 2. Effect of reaction temperature on the yield at pH 6.



Scheme 1.

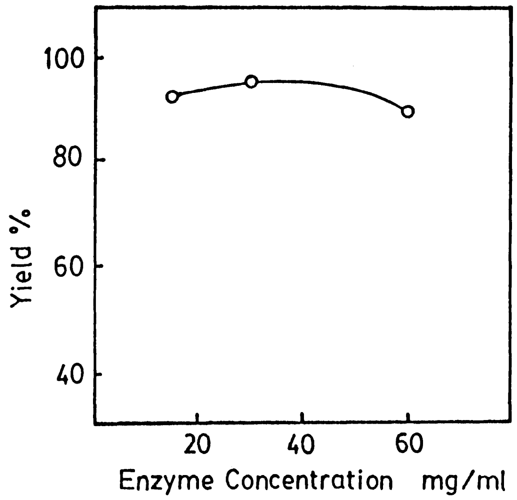


Fig. 3. Effect of enzyme concentration on the yield at 30 °C (pH=6).

Experimental

Melting points are uncorrected. IR spectra (KBr disk) were recorded on a JASCO IR-8000 spectrometer. ^{1}H NMR spectra were recorded on a JEOL GX-270 spectrometer for DMSO-d_6 solution. Synthesis of Boc-Asn(NAcGlc)-OMe is described elsewhere.[4]

Hydrolysis Procedure. We followed the reaction conditions described in a literature,[5] where papain-catalyzed hydrolysis of uncommon amino acid ester has been achieved. Typical hydrolysis procedure is as follows. Boc-Asn(NAcGlc)-OMe (0.2 mmol, 116 mg) was dissolved in McIlvain buffer (pH=6, 10 ml). To the solution was added papain (300 mg) and 2-mercaptoethanol (0.2 ml), and the mixture was stirred overnight. The reaction mixture was acidified with HCl (2 mol dm^{-3}). Desired product was extracted with ethyl acetate, and the organic layer was washed with saturated aq NaCl. The solvent was removed and the crude product was recrystallized from ethyl acetate/petroleum ether. Selective hydrolysis of α-methyl ester was ascertained by ^{1}H NMR analysis of the product, Boc-Asn(NAcGlc)-OH: ^{1}H NMR (DMSO-d_6) δ=1.41 (s, 9H, Boc), 1.76 (s, 3H, NHAc), 1.91 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.99 (s, 3H, OAc), 2.52 (dd, 1H, Asn Cβ H), 2.59 (dd, 1H, Asn Cβ H), 3.79 (m, 1H, C^{5}H) , 3.84 (m, 1H, C^{2}H), 3.94 (dd, 1H, C^{6}H), 4.18 (dd, 1H C^{6}H), 4.26 (m, 1H, Asn Cα H), 4.81 (dd, 1H, C^{4}H), 5.11 (dd, 1H, C^{3}H), 5.15 (dd, 1H, C^{1}H), 6.79 (d, 1H, Asn Nα H), 7.88 (d, 1H, C^{2}NHAC), 8.55 (d, 1H, Asn Nδ H), 13.08 (s, 1H, COOH); IR (KBr), 1537, 1688, 1750 cm^{-1}; mp, 192—193 °C; R_f (MeOH)=0.49.

References

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