66℃ (63.5-65℃)[7]. IR: 3320 cm^{-1} (OH). UV (MeOH) λ_{max} (logε): 217(3.73), 253(2.54), 258(2.63), 264 nm (2.52). ^{1}H-NMR δ (ppm): 1.97 (s; 1H, -OH), 2.85 (d; J=6.0 Hz, 2H, -CH_2-), 4.67 (t; J=6.0 Hz, methine), 6.90-7.33 (m; 10H, aromatic).

1-Ethoxy-1,2-diphenylethane (12)

12 was prepared from **8** with ethanol under reflux as described for **8** to **11**. Purification by column chromatography with ether gave a colourless oil which shows no optical activity. ^{1}H-NMR δ (ppm): 1.03 (t; J=7.5 Hz, 3H, -CH_2-CH_3), 2.70 (d; J=7.5 Hz, 2H, -CH-CH_2-), 3.80 (q; J=7.5 Hz, 2H, -CH_2-CH_3), 4.27 (t; J=7.5 Hz, 1H, -CH-CH_2-), 7.00-7.60 (m; 10H, aromatic).

1-Chloro-1-[2-(β-N-ethoxycarbonyl-N-methyl-aminoethyl)-4,5-dimethoxyphenyl]-1-phenyl-methane (9)

0.14 g (0.5 mmol) **7** in 10 m*l* absolute CH_2Cl_2 were treated with excess ECF under reflux for 3 h. After cooling, the solvent and excess ECF were thoroughly removed *i. vac.*, the remained oily material was purified by column chromatography with $CHCl_3$/ether 1:1 to afford oily **9** which exhibits no optical activity. IR: 1700 cm^{-1} (CO). ^{1}H-NMR δ (ppm): 1.16 (t; J=7.0 Hz, 3H, -CH_2-CH_3), 2.77 (s; 3H, -NCH_3), 2.40-3.67 (m; 4H, -CH_2-CH_2-), 3.73 and 3.83 (2xs; 6H, -OCH_3), 4.07 (q; J=7.0 Hz, 2H, -CH_2-CH_3), 6.07 (s; broad, 1H, H-1), 6.63 (s; 1H, H-6'), 6.90 (s; broad, 1H, H-3'), 7.30 (s; 5H, phenyl).

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Peptide Synthesis with Polymer Bound Active Ester. II[1]. Synthesis of Pyrazolone Resin and Its Application in Acylation Reaction

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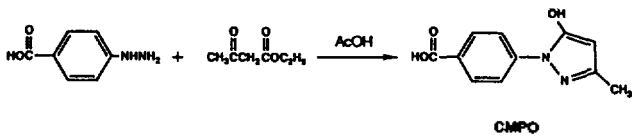
Pyrazolone group containing resin was tested as an acyl carrier in solid phase peptide synthesis. Several kinds of dipeptide derivatives were prepared by aminolysis reactions of Boc-amino acid-pyrazolone resin active ester with various carboxyl protected amino acid derivatives. It was found that the rates of aminolysis reactions were largely dependent on the bulkiness of the amino acid side chains, the carboxyl protecting groups, and the swelling property of the resin. All the dipeptide derivatives were obtained in high yield in 20-30 minutes, and the pyrazolone resin could be reused repeatedly in peptide synthesis without any change of its reactivity.

Introduction

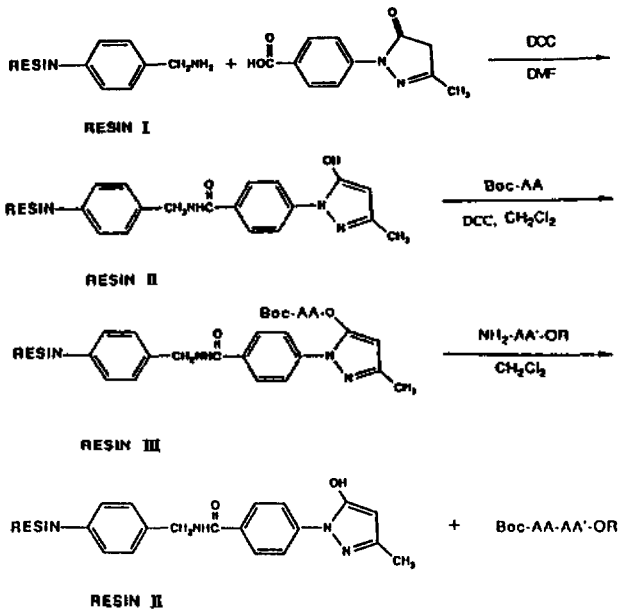
Peptide synthesis using polymers has been a very common method since Merrifield resin was first introduced in 1961[2]. Nowadays, preparation of peptide fragments by polymeric acylating agent, which differs from the original Merrifield's method, has drawn much attention because this method gives several merits such as easy separation of synthetic intermediate and attainment of pure peptide derivatives by simple filtration in the case where excess polymeric acylating agents are used. Various polymeric acylating agents which possess N-hydroxysuccinimide[3], o-nitrophenol[4,5], or 1-hydroxybenzotriazole(HOBt)[6,7] as their functional groups have been reported so far. Recently, we have prepared oximinopyrazole group containing polymeric acylating agents and tested them for the synthesis of various peptide derivatives successfully[1]. During our continuing effort for the development of new polymeric acylating agents, we were interested in pyrazolone active ester[8,9] as the next choice, because pyrazolone active ester has been reported to have an excellent reactivity in solution phase peptide synthesis. So far there has been no report on the application of the pyrazolone functional group in solid phase peptide synthesis. We now wish to report the synthesis of pyrazolone resin and its application as an acylating agent in solid phase peptide synthesis.

Results and Discussion

Aminomethyl resin(Resin I) was prepared from chloromethylpolystyrene 1%-divinylbenzene copolymer by the known



Scheme 1.



Scheme 2.

procedures[10]. 1-(4-Carboxyphenyl)-3-methyl-2-pyrazolin-5-one (CMPO), which possesses an anchoring group to introduce the pyrazolone functionality on to the polymer support, was synthesized from p-hydrazinobenzoic acid and ethyl acetoacetate by modified Knorr's method[11]. Because of the poor solubility of p-hydrazinobenzoic acid in most organic solvents, acetic acid was the only choice for the reaction solvent. As the reaction progressed under refluxing conditions, the reaction mixture was completely dissolved in a short time due to the formation of an intermediate adduct, and after a while, CMPO started to precipitate out as brown solids.

After Resin I(-NH_2 1.40 mmol/g resin) was reacted with 2 equiv. of CMPO and 2.4 equiv. of DCC in DMF, pyrazolone containing polymer support, Resin II, could be obtained as yellow resin. Elemental analysis of nitrogen indicated that the degree of substitution of CMPO in Resin II was 1.10 mmol/g resin.

IR spectrum of Resin II (Figure 1) showed the newly formed amide carbonyl group at 1650 and 1720 cm^{-1}. Picric acid titration[12] of Resin II revealed that 0.10 meq -NH_2/g resin remained unreacted and the coupling yield of CMPO with Resin I was >93%. In order to block the residual amino group, N-acetylation was performed by 10% Ac_2O/pyridine. Besides N-acetylation of the remaining amino group, O-acetylation of pyrazolone enol group was carried out at the same time, which was verified by IR spectrum. Pyrazolone ring carbonyl band at 1720 cm^{-1} disappeared and the newly formed active ester carbonyl band appeared at 1790 cm^{-1} as the pyrazolone carbonyl group was changed into an enol ester group.

To regenerate the enol group, the resin was treated with

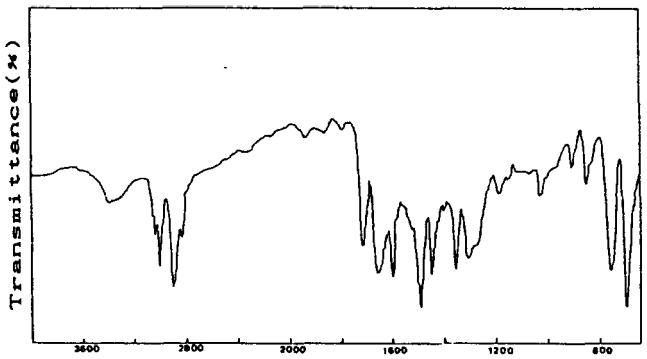


Figure 1. IR Spectrum of Resin II.

excess benzylamine and the result was confirmed by IR spectrum which showed the disappearance of the 1790 cm^{-1} band. As a result of this treatment, N-acetylbenzylamine was released from the resin as well. N-Acetylbenzylamine was isolated and the substitution level of active ester bond(acetyl-Resin II) was calculated to be 0.63 mmol/g resin. This means that N-acetylation reaction of Ac_2O with pyrazolone enol group proceeded in about 60% yield.

Considering the reaction condition with excess Ac_2O and a long reaction time(2 h), the remaining 40% of the pyrazolone functional groups were presumed unavailable for acylation reaction because of steric hindrance of the polymer backbone. As a matter of fact, in subsequent peptide synthetic reactions, although enough reaction time was permitted, the coupling yield of the acyl group to the pyrazolone resin usually fell between 50 and 60% with maximum yield of 63%. Therefore, it can be concluded that about 60% of the total pyrazolone functional groups was available for the acylation reaction.

Polymeric active ester(Resin III) was prepared from the coupling reaction of Resin II with various Boc-amino acids (Boc-Leu, Boc-Phe and Boc-Ser(OBzl)) by DCC. As the coupling reaction progressed, the color of the Resin changed from pale-brown to thick-yellow. The degree of substitution of Resin III was determined by the benzylamine method[3].

Several dipeptides were synthesized by the reaction of Resin III with various C-protected amino acid ester so as to demonstrate the usefulness of the polymeric active ester, Resin III, in peptide synthesis. The progress of the reaction could easily be monitored by the change of resin color or by IR spectrum. As the coupling reaction progressed, the color of the resin changed from yellow to brown, and the carbonyl band at 1790 cm^{-1} completely disappeared in 20-30 minutes. Most of the dipeptide products were obtained as pure solids after crystallization. The reaction times and analytical data for peptide synthesis were summarized in Table 1.

All the results proved that the pyrazolone resin, which showed excellent reactivity in peptide bond formation, could be a good candidate as a new polymer acylating agent. There seem to be an exception, however, as seen in Run 4, which demonstrates that the swelling property of the resin is a very important factor in heterogeneous reaction conditions. When compared with Run 5, Run 4 showed very poor results, even under more favorable reaction conditions, because this particular resin showed poorer swelling property than other

Table 1. Physical Data of Each Coupling Reaction Products with Resin III[a]

Run	Peptide Derivatives[b]	Amino acid Ester: Polymeric active Ester (mol:mol)	Reaction Time (min)	Yield[c] (%)	mp. (℃)	$[\alpha]_D^{25}$ (c=1)	Lit.[16-20] mp. (℃)	Lit.[16-20] $[\alpha]_D^{25}$ (c=1)
1	Boc-Leu-Gly-OMe	1.2:1	20	98	133	−31.4(MeOH)	131	−21.0(DMF)
2	Boc-Leu-Phe-OMe	1.5:1	20	55	80	−29.5(MeOH)	78-79	−28.0(MeOH)
3	Boc-Leu-Val-OMe	1.5:1	20	69	146-147	−45.3(MeOH)	144-147	−44.0(MeOH)
4[d]	Boc-Phe-Gly-OMe	5.5:1	240	89	91	−1.40(MeOH)	–	–
5	Boc-Phe-Gly-OMe	3.0:1	10	95	91	−1.40(MeOH)	–	–
6	Boc-Phe-Phe-OMe	3.0:1	30	89	115	−16.6(MeOH)	114-115	−14.0(MeOH)
7	Boc-Phe-Val-OMe	3.0:1	20	87	119-120	−8.7(DMF)	120-122	−11.0(DMF)
8	Boc-Phe-Leu-OBzl	3.0:1	30	75	81-83	−24.9(MeOH)	82-84	−23.8(MeOH)
9	Boc-Phe-Gly-OPh	0.8:1	20	70	120-121	−4.2(AcOH)	–	–
10	Boc-Ser(OBzl)-Val-OMe	3.0:1	30	97	66-67	−15.4(MeOH)	–	–

[a]The degree of substitution of Resin III was 0.45-0.66 mmol/g resin. [b]Each run was performed at room temperature under identical conditions. [c]All the yields are isolation yields based upon the degree of substitiution of Resin III. [d]Only Run 4 used 2%-divinylbenzene copolymer, whereas the others used 1%-divinylbenzene copolymer.

resins. Table 1 clearly shows that steric effect might play an important role in solid phase peptide synthesis. The highest yields were obtained in the synthesis of Boc-Leu-Gly-OMe(Run 1, 98%) and Boc-Phe-Gly-OMe(Run 5, 95%). Although Run 1 employed less amount of amino acid derivative, Gly-OMe, it gave higher yield than Run 2 and Run 3 did. Run 5 also gave higher yield than Run 6, 7, and 8 did, in spite of shorter reaction time. All these results could be explained by the fact that glycine methyl ester had the least steric hindrance among the various amino acid methyl esters. Comparing Run 2 and 3 or Run 6 and 7, the reactivity of valine derivatives seemed to be higher than that of phenylalanine derivatives in coupling reactions. Run 8 and Run 9 also demonstrate that the bulkiness of the ester group controls the reactivity. The bulkiness of the acyl group, however, did not show any significant effect on the reactivity in peptide bond formation(Run 10). The stabililty of the resin was also tested. Run 1-3 and Run 5-7 were the results of the same resins which were repeatedly acylated 6 times with the previous Boc-amino acid derivatives after each coupling reaction. These results showed that Resin III could be reused repeatedly in peptide synthesis without any change of its reactivity. All these results demonstrated that the polymeric pyrazolone resin(Resin II) can be used successfully with fast reactivity and reusability in solid phase peptide fragment synthesis.

Experimental

IR spectra were recorded on a Jasco DS-710 infrared spectrophotometer using KBr pellets, and UV spectra were taken on a Shimadzu UV-200S double beam spectrophotometer. ^{1}H-NMR spectra were obtained from a Jeol JNM-MH-100 NMR spectrometer. Optical rotations were measured with a Jasco DIP-360 polarimeter, and elemental analyses were performed with Yanaco MT-2 CHN corder. Melting points were measured on a Yanaco MP-S5 and are not corrected. Analytical thin layer chromatography was performed on a silica gel plate(0.25 mm, $60F_{254}$, Merck) with the following solvent systems; A, chloroform/acetic acid(15:1); B, chloroform/methanol(15:1); C, chloroform/methanol(50:1). All solvents were in reagent grade and were purified by methods reported in the literature. Aminomethylpolystyrene(Resin I) was prepared from chloromethylpolystyrene-1%(or 2%) DVB-copolymer(Aldrich, 1 meq. Cl^-/g) according to the literature procedure[10]. The degree of substitution of the various resins were determined by nitrogen analysis. Amino acid methyl, benzyl, and phenyl esters were prepared directly from the corresponding amino acids by the known procedures[13,14] in the form of hydrochloride or p-toluenesulfonate, respectively.

1-(4-Carboxyphenyl)-3-methyl-2-pyrazolin-5-one (CMPO). p-Hydrazinobenzoic acid (3.00 g, 19.72 mmol) and ethyl acetoacetate (2.57 g, 19.72 mmol) were mixed in acetic acid, and then refluxed for 24 hours. After the reaction was completed, the reaction mixture was cooled to room temperature. CMPO was obtained as brown solid, which was filtered and washed thoroughly with methylene chloride in order to remove excess acetic acid. Yield, 3.64 g(85%). mp. 283℃; TLC one spot, Rf 0.86(A); NMR(DMSO-d_6) δ 2.20(s, 3H), 3.82 (s, 0.4H), 5.52(s, 0.8H), 7.75(broad, 0.8H), 8.00-8.30(m, 4H); Anal. Calcd. for $C_{11}H_{10}N_2O_3$(218.21): C, 60.55; H, 4.62; N, 12.84%. Found: C, 60.97; H, 4.39; N, 12.55%.

1-(p-Polystyrylmethylenecarbamoyl)phenyl-3-methyl-2-pyrazolin-5-one(Resin II). Resin I (7.0 g, 1.40 mmol -NH_2/g resin) was swelled with DMF in a reaction vessel[15]. DMF solution of CMPO (4.40 g, 20.0 mmol) and DCC (4.95 g, 24.0 mmol) was then added and shaken for 48 hours. The resin was filtered and washed three times each with DMF, methanol and methylene chloride in turn. The color of the resin changed from white to brown and the picric acid analysis[12] indicated that 0.10 mmol -NH_2/g resin remained unreacted. In order to block the unreacted amino group, the resin was pretreated in pyridine solution of 10% acetic anhydride for 1 minute and then reacted with 60 ml of the same solution for 30 minutes. Again, the resin was filtered and washed three times each with methanol

and methylene chloride respectively. After the resin was swelled with methylene chloride, benzylamine (5 m*l*, 45.9 mmol) was added in order to regenerate the pyrazolone functionality. After one hour of the reaction the resin was washed three times with methylene chloride, and dried in vacuum. IR (KBr pellet), 1650, 1720 cm^{-1}; N, 4.62% (1.10 mmol -CMPO/g resin).

Typical Procedure for the Preparation of Polymeric Active Esters (Resin III). Resin **II** (1.10 mmol -CMPO/g resin) was treated with 2 equiv. of Boc-amino acids and 2.4 equiv. of DCC in methylene chloride and shaken for 6 hours. The resin was washed three times each with methylene chloride, methanol/methylene chloride (1:1), and methylene chloride and dried in vacuum. The degree of substitution of Boc-amino acids was determined by reaction with excess benzylamine followed by back titration with perchloric acid[3]. IR (KBr pellet) 1790 cm^{-1}; Degrees of substitution of Boc-amino acids, 0.55 mmol/g and 0.45 mmol/g (Boc-Leu-Resin **III**); 0.61 mmol/g and 0.51 mmol/g (Boc-Phe-Resin **III**); 0.66 mmol/g (Boc-Ser(OBzl)-Resin **III**).

Typical Procedure for Peptide Synthesis (Boc-Leu-Gly-OMe). Boc-Leu-Resin **III** (2.80 g, 0.55 mmol/g resin) was swelled with methylene chloride (25 m*l*) in the reaction vessel[15]. HCl·Gly-OMe (226 mg, 1.8 mmol) was suspended in methylene chloride (10 m*l*) and neutralized with diisopropylethylamine. The resulting solution was poured into the reaction vessel and the reaction mixture was shaken for 20 minutes at room temperature. The resin was filtered and washed with methylene chloride. The filtrate and the wash were combined. The combined solvent was washed with 10% citric acid (3×20 m*l*) and water (2×20 m*l*) and dried with $MgSO_4$. Evaporation of the solvent gave white solid, which was recrystallized in EtOAc/n-hexane to give white needle crystals, 454 mg (98%, based on the amount of Boc-Leu-Resin **III**).

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