A more critical test of the method was the synthesis of leucylalanylglycylprolylphenylalanylarginine from crystalline phenylalanylarginine prepared by the NCA method. The crude reaction mixture was purified on silica gel H to give a 60% yield of hexapeptide which moved as a single component by tlc, $[\alpha]_{589}$ −62.4° (*c* 1%, 6 *N* HCl), and which showed the following amino acid ratio in its acid hydrolysate: $Leu_{0.99}Ala_{1.01}Gly_{0.98}Pro_{1.00}Phe_{0.98}Arg_{1.01}$. Sequential treatment of the purified hexapeptide with a 5–8% excess of the NCA's of glutamic acid, isoleucine, and proline, respectively, gave a product which was purified on Sephadex to give a 46% yield (based on hexapeptide) of the nonapeptide, $[\alpha]_{589}$ −86.7° (*c* 1%, 6 *N* HCl). The amino acid ratio after acid hydrolysis was: $Pro_{2.04}Ileu_{0.95}Glu_{0.95}Leu_{0.97}Ala_{0.98}Gly_{0.98}Phe_{1.00}Arg_{1.00}$.

Phenylalaninamide, prepared from the NCA by treatment with NH_3, was treated sequentially with the NCA's of aspartic acid, methionine, and tryptophan in a Waring Blendor. The crude product was precipitated at pH 7 and purified on silica gel H to give a 30% yield of the C-terminal[12] tetrapeptide sequence of gastrin characterized as the crystalline hydrochloride, $[\alpha]_{589}$ −17.5° (*c* 2%, methanol), −31.6° (*c* 1%, DMF). The amino acid composition, as determined by acid and LAP hydrolyses, was $Try_{0.95}Met_{1.00}Asp_{1.00}Phe_{1.01}NH_4^+{}_{0.95}$ and $Try_{1.03}Met_{1.05}Asp_{0.94}Phe_{0.99}$, respectively. The LAP cleavage left no peptide fragments detectable by tlc. This tetrapeptide synthesis required a total of about 1 hr and the isolation procedures and crystallization about 3 days.

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Use of Polymers as Chemical Reagents. I. Preparation of Peptides

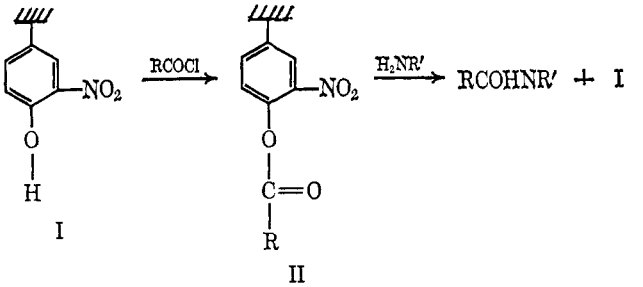
Sir:

Polymers of the type Ⓟ–A containing covalently bound groups A, which readily react with a low molecular weight reagent B, can be used to synthesize compound A–B according to eq 1. To increase yields

$$Ⓟ\text{–}A + B \longrightarrow A\text{–}B + Ⓟ$$

and to facilitate the synthetic procedure insoluble polymers of the above type may be added in large excess to a solution of B in a suitable solvent. At the end of the reaction the insoluble polymer can be removed by filtration or centrifugation. The filtrate which is devoid of A should thus contain only A–B and unreacted B. The most suitable polymers Ⓟ–A to be used as chemical reagents should contain a relatively large amount of A, should show high stability on storage, and should possess the suitable mechanical properties.

To test the possible use of chemically reactive polymers in acylation reactions we prepared insoluble, high molecular weight active polyesters of acetic acid (IIa) and benzoic acid (IIb) by allowing the corresponding chlorides to react with poly-4-hydroxy-3-nitrostyrene cross-linked with 4% divinylbenzene (I) in dimethylformamide (DMF), in the presence of pyridine. The high molecular weight active esters (II) contained approximately 5 mmoles of acyl residues/g of insoluble polymer and could be stored at room temperature in powder form without decomposition. Treating IIa or IIb (1.0 g) in suspension in DMF (15 ml) with 0.5 mmole of tri-L-alanyl-*p*-nitrobenzyl ester (L-Ala_3-PNB) was carried out at room temperature for 4–5 hr. The polymer was removed by centrifugation and the supernatants evaporated to dryness *in vacuo*. The solid residues were found to contain a practically quantitative yield of the corresponding acyl derivatives: acetyl-L-Ala_3-PNB, mp 226°, $[\alpha]^{25}D$ −28.9° (*c* 0.58, DMF); benzoyl-L-Ala_3-PNB, mp 216–218°, $[\alpha]^{25}D$ +2.9° (*c* 0.70, DMF).



I II

II: esters of: acetic acid (IIa); benzoic acid (IIb); Z-L-Phe (IIc); Z-L-Ileu (IId); Z-L-Pro (IIe); N,S-Di-Z-L-Cys (IIf); N-Z-α,-OBz-L-Glu (IIg), where Z = C_7H_7OCO and Bz = C_7H_7

The successful preparation of active esters of N-blocked amino acids of type II enabled their utilization in peptide synthesis. Thus peptides with N- and C-blocked terminal groups were obtained on coupling the insoluble active esters IIc to IIg with desired soluble amino acid or peptide esters containing a free α-amino group. Preferential removal of the N-blocking group from the newly formed peptide enabled the repetition of the coupling reaction with an insoluble active ester of another N-blocked amino acid. Further repetitions of this set of reactions lead obviously to the elongation of the peptide chain and formation of a peptide with a predetermined amino acid sequence.

The insoluble active esters IIc–g were derived from the following corresponding benzyloxycarbonyl amino acid derivatives: benzyloxycarbonyl-L-phenylalanine, benzyloxycarbonyl-L-isoleucine, benzyloxycarbonyl-L-proline, N,S-dibenzyloxycarbonyl-L-cysteine, and N-benzyloxycarbonyl-α-benzyl-L-glutamate, by their coupling with polymer I in DMF by the DCC method.[1,2] The insoluble polymers IIc to IIg contained per gram approximately 1.0–1.5 mmoles of amino acid and could be stored at room temperature without decomposition, similarly to IIa and IIb. In suspension in inert organic solvents polymers IIc to IIg showed chemical behavior similar to that of the cor-

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responding low molecular weight amino acid active esters.

N,S-Di-Z-L-Cys-Gly-OBz was obtained by allowing IIf (1 g containing 1 mmole of cysteine) to react with glycine benzyl ester (0.5 mmole) in DMF (20 ml) with stirring for 5–8 hr at room temperature. The polymer was removed by centrifugation and washed with DMF, and the combined DMF solutions were evaporated to dryness *in vacuo*. The residue was dissolved in wet ethyl acetate and washed with 1 *N* HCl, 5% $NaHCO_3$, and water. On evaporation of the ethyl acetate a chromatographically pure solid product was obtained; yield 98%, based on the amount of glycine benzyl ester employed; mp 116–118°, $[\alpha]^{25}D$ −45.3° (*c* 1.3, DMF) [lit. mp 118–119°,[3] $[\alpha]^{25}D$ −45.5° (*c* 2.0, DMF)[3]]. Z-L-Pro-Gly-OBz was obtained analogously from IIe and glycine benzyl ester; yield 90%, mp 87–88° (lit. mp 88–89°[4]). Z-L-Ileu-(L-Ala)$_5$-PNB was obtained by coupling IId with H_2N-(L-Ala)$_5$-PNB in DMF at room temperature; yield 71%, mp 258–260°, $[\alpha]^{25}D$ −91.1° (*c* 0.34, dichloroacetic acid). An identical peptide was obtained by the DCC method; mp 261–265°, $[\alpha]^{25}D$ −90.5° (*c* 0.46, dichloroacetic acid). N,S-Di-Z-glutathione dibenzyl ester (N-Z-L-Glu-(α-OBz)-L-Cys-(-S-Z)-Gly-OBz), mp 156°, $[\alpha]^{25}D$ −35.5° (*c* 1.0, methanol) [lit. mp 158–159°,[3] $[\alpha]^{25}D$ −35.5° (*c* 1.0, methanol)[3]] was obtained in 85% yield from IIg and S-Z-L-Cys-Gly-OBz. The latter was derived from the N,S-di-Z-L-Cys-Gly-OBz preparation obtained from IIf and Gly-OBz on treatment with HBr in glacial acetic acid. Treating L-alanine *p*-nitrobenzyl ester with excess IIc, removal of the Z-group (by HBr–CH_3COOH) from the dipeptide formed (Z-L-Phe-L-Ala-PNB), neutralization with triethylamine in DMF, and treating the dipeptide ester with a new excess of IIc gave Z-L-Phe-L-Phe-L-Ala-PNB in an over-all 84% yield; mp 159°, $[\alpha]^{25}D$ −26.8° (*c* 0.9, DMF). The same peptide prepared by the DCC method gave mp 160°, $[\alpha]^{25}D$ −26.2° (*c* 1.39, DMF). Z-L-Phe-L-Phe-L-Phe-NH_2 was prepared analogously in 81% yield, mp 227°, $[\alpha]^{25}D$ −31.5° (*c* 0.8, DMF). The same peptide prepared by the DCC method gave mp 226°, $[\alpha]^{25}D$ −32.1° (*c* 1.2, DMF).

A comparison of the method for peptide synthesis described here with that of Merrifield[5] reveals that whereas in the "solid-phase peptide synthesis"[5] it is the peptide which is bound to the insoluble carrier and the N-blocked amino acid active ester is added while in solution, in our case a solution of free peptide ester is added to an insoluble N-blocked amino acid active ester. Furthermore, purification of the intermediate peptides formed during synthesis can be readily effected in our method, since these peptides are liberated into solution. In the Merrifield synthesis, on the other hand, peptide purification can be carried out only after detachment of the final product from the polymeric carrier. In the procedure described here the reaction between peptide ester and active amino acid ester can be carried out in the presence of a large excess of insoluble active ester which can be readily removed at the end of the reaction. It is thus possible to increase yields and to shorten the time of the coupling reaction.

The application of the new method for the synthesis of various low and high molecular weight peptides is under investigation.

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Synthesis of Purine Cyclonucleoside Having a 8,2′-O-Anhydro Linkage

Sir:

Synthesis of various purine cyclonucleosides containing S-anhydro linkages has been reported.[1-3] These compounds were easily desulfurized to afford 2′-deoxyadenosine, 3′-deoxyadenosine (cordycepin), and 5′-deoxyguanosine.

In analogy to similar studies[4] with pyrimidine cyclonucleosides, it would be of interest to investigate the physical and chemical properties of the purine cyclonucleosides containing O-anhydro linkages. Although an earlier attempt[5] to obtain an 8-hydroxypurine nucleoside met with little success, a novel method for the introduction of a hydroxyl group in the 8 position of preformed purine nucleoside has been developed recently in our laboratory.[6]

We wish to report the synthesis of 8,2′-anhydro-8-hydroxy-9-β-D-arabinofuranosyladenine (I), the first purine cyclonucleoside having an O-anhydro linkage. 2′,3′-O-Isopropylideneadenosine (II) was brominated by the method of Holmes, *et al.*,[7] to give 8-bromo-2′,3′-O-isopropylideneadenosine, mp 215–217° (*Anal.* Calcd for $C_{13}H_{16}O_4N_5Br$: C, 40.69; H, 4.18; N, 18.13. Found: C, 40.60; H, 4.35; N, 18.48); ultraviolet λ_{max}^{EtOH} 265 mμ, $\lambda_{max}^{H^+,\ OH^-}$ 264 mμ; R_f(A)[8] 0.60, (B) 0.73. Acetylation of this compound gave 8-bromo-2′,3′-O-isopropylidene-5′-O-acetyladenosine (III), mp 158–159° (*Anal.* Calcd for $C_{15}H_{18}O_5N_5Br$: C, 42.06; H, 4.47; N, 16.38. Found: C, 41.92; H, 4.19; N, 16.71); R_f(B) 0.82. The over-all yield of III from II was *ca.* 30%. Compound III could be obtained also from 2′,3′-O-isopropylidene-5′-O-acetyladenosine[9] by the

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