tridec-4-ene (2b) was prepared as for **2a**, yield 50%.

Anal. Calcd for $C_{20}H_{28}S$: C, 79.94; H, 9.39. Found: C, 80.07; H, 9.60. This product turned out to be diastereomeric free.

NMR (CCl_4) δ 1.00 [s, 9, $C(CH_3)_3$], 1.58 (s, 3, $C{=}CCH_3$), 1.80–2.90 (m, 14, aliphatic H), 6.70–6.95 (AB, 2, ThH).

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Registry No.—**1a**, 62815-66-1; **1b**, 62815-67-2; **2a**, 62815-68-3; **2b**, 62815-69-4; **3a**, 62928-71-6; (*E*)-**5a**, 62815-70-7; (*Z*)-**5a**, 62815-71-8; **5b**, 62815-72-9; **6a**, 62815-73-0; **6b**, 62815-74-1; **7a**, 62815-75-2; **7b**, 62815-76-3; **8a**, 62815-77-4; **8b**, 62815-78-5; **9**, 62815-79-6; **10a**, 62815-80-9; **10b**, 62815-81-0; **11a**, 62815-82-1; **11b**, 62815-83-2; triethyl phosphonoacetate, 867-13-0; 2-acetylthiophene, 88-15-3; 2-pivaloylthiophene, 20409-48-7.

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An Unusual Side Reaction of 1-Succinimidyl Esters during Peptide Synthesis

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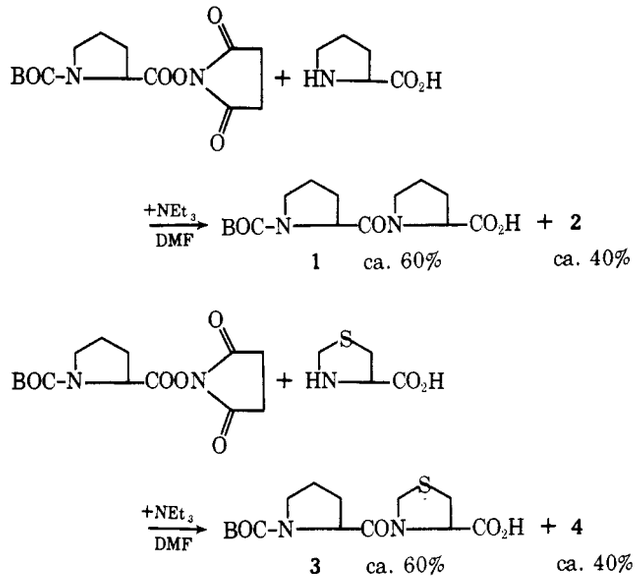
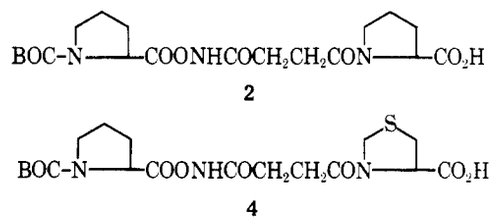
Peptide bond formation mediated through 1-succinimidyl ester couplings in aqueous or nonaqueous solvents is a standard procedure of peptide synthesis.[2] In the present communication we wish to report the observation of a new side reaction of 1-succinimidyl esters.

Coupling of a N-protected amino acid 1-succinimidyl ester with a free amino acid in anhydrous dimethylformamide in the presence of triethylamine results usually in high yields of the dipeptide derivative, as loss through hydrolysis of the activated ester is minimized. As an example, the preparation of *N*-benzyloxycarbonylglycyl-L-proline, containing an 85% ^{13}C-enriched proline residue,[3] is described in the Experimental Section. However, when *N*-tert-butyloxycarbonyl-L-proline 1-succinimidyl ester was coupled under identical conditions with free proline or 4-thiazolidinecarboxylic acid, the expected N-protected dipeptides **1** and **3** were accompanied by secondary products formed in nearly the same amount

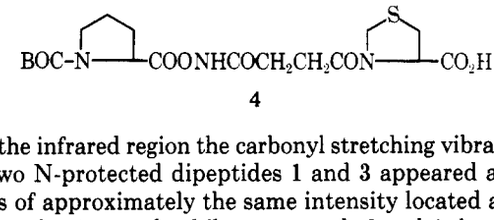


(BOC = *tert*-butyloxycarbonyl)

(contaminants **2** and **4**). Coupling of the activated ester with the sodium salt of proline in an ethanol–water mixture resulted in an important hydrolysis of the ester. The by-products **2** and **4**, which were not chromatographically identical, could not be obtained pure enough for elemental analysis. However, they were obtained free from the corresponding N-protected dipeptides and accompanied only by a trace amount of proline or 4-thiazolidinecarboxylic acid through repeated precipitations. Infrared and NMR spectroscopy as well as mass spectrometry have shown the contaminants to have structures **2** and **4**.



2



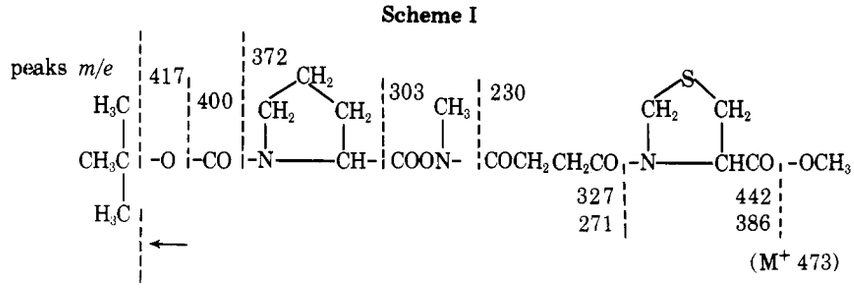
4

In the infrared region the carbonyl stretching vibrations of the two N-protected dipeptides **1** and **3** appeared as three bands of approximately the same intensity located at 1605, 1682, and 1755 cm^{-1}, while compounds **2** and **4** showed two strong absorptions centered at 1605 and 1685 cm^{-1} and a band of medium intensity at 1790 cm^{-1}. The appearance of an absorption at higher frequency (1790 cm^{-1}) is consistent with the presence of a carbonyl group implicated in a *O*-acylhydroxylamine linkage.[4]

The general aspect of the proton NMR spectra of contaminants **2** and **4**, in chloroform solution, was essentially the same as that of the corresponding dipeptides[5] **1** and **3**. In particular, the observation of two singlets at δ 1.40 (smaller) and 1.47 ppm (larger) for compound **2** and at δ 1.43 (smaller) and 1.49 ppm (larger) for compound **4** confirmed the presence of the *tert*-butyloxycarbonyl group. On the other hand, contaminants **2** and **4** presented an additional unresolved peak centered at δ 2.75 ppm, corresponding to four protons, which was absent from the spectrum of **1** and **3**, and which is assigned to the methylene protons of the succinic acid group.

Mass spectra of the methylated (diazomethane) contaminants **2** and **4** confirmed the proposed structure and showed that methylation occurred on the carboxylic acid function and on the nitrogen proton of the *O*-acylhydroxylamine derivatives **2** and **4**. The observed fragmentation of the methylated

Scheme I



contaminant **4** is presented in Scheme I. From the peak at *m/e* 417, corresponding to the thermal decomposition of the *tert*-butyloxycarbonyl group with departure of isobutylene, a normal fragmentation is again observed with peaks at *m/e* 386 and 271. A parallel fragmentation pattern was observed for methylated **4**, which gave a molecular peak at M^+ 455.

O-Acylated hydroxamic acid derivatives have been found to be "activated esters" and have proved useful in peptide synthesis.[6,7] It is therefore surprising that when *N-tert*-butyloxycarbonylproline 1-succinimidyl ester was allowed to react with a twofold excess of proline or 4-thiazolidinecarboxylic acid, contaminants **2** and **4**, which are "activated esters" of hydroxamic acid as shown by the high infrared frequency carbonyl absorption (1790 cm^{-1}), were still formed in the same proportion and did not react further to give the corresponding N-protected dipeptide. It is reassuring to find that, among the activated esters of pivalohydroxamic acid and *N*-benzyloxycarbonyl amino acids,[7] the ester of *N*-benzyloxycarbonylproline is the only one which does not react with amines of amino acids to form a peptide bond. This observation might explain in part why we were able to isolate in both experiments the side products **2** and **4**.

The formation of an intermediate of the type of compounds **2** and **4** was also observed during the coupling of *N-tert*-butyloxycarbonylproline 1-succinimidyl ester with sarcosine. Thin-layer chromatography of the reaction mixture revealed, besides the expected dipeptide and starting materials, a compound developing a characteristic blue color with ninhydrin as do **2** and **4** and giving a positive hydroxamic acid test when sprayed with ferric chloride solution. In no other 1-succinimidyl ester coupling we tried were we able to demonstrate the presence of intermediates of the type of compounds **2** and **4**. Thus, thin-layer chromatography of couplings of sterically hindered amino acid residues, such as the coupling of *N-tert*-butyloxycarbonylproline 1-succinimidyl ester with valine or leucine, or of *N-tert*-butyloxycarbonyl-β-benzylaspartic acid 1-succinimidyl ester with proline, showed the presence of only the starting materials and the corresponding dipeptides. Infrared spectra of the partially purified products, free from interfering 1-succinimidyl ester and hydroxysuccinimide, did not present a carbonyl band at an unusually high frequency.

Experimental Section

Infrared spectra were recorded on a Perkin-Elmer 337 infrared spectrometer in Nujol using sodium chloride plates; ^1H NMR spectra were obtained on a Perkin-Elmer R32 (90 MHz) spectrometer. We are indebted to Dr. B. C. Das who recorded and interpreted mass spectra of methylated compounds **2** and **4**, taken at 200 °C with an AEI mass spectrometer, Model MS9, at the Institut de Chimie des Substances Naturelles in Gif-sur-Yvette. Chromatograms on silica gel plates (Merck) were run in 1-butanol–acetic acid–water, 3:1:1, mixtures.

***N*-Benzyloxycarbonylglycyl-L-proline[8] with ^{13}C-Enriched Proline.**[3] ^{13}C-enriched L-proline (85%, 240 mg, 2 mmol) was suspended in dry dimethylformamide (3 mL), triethylamine (0.28 mL, 2 mmol) was added followed by *N*-benzyloxycarbonylglycine 1-succinimidyl ester (612 mg, 2 mmol), and the reaction mixture was left to stir overnight at room temperature. After dilution with water and acidification to pH 2 with 6 N hydrochloric acid, the product was extracted with ethyl acetate. The organic solution was washed with water, the product extracted with saturated sodium bicarbonate, and the alkaline solution was acidified with hydrochloric acid to pH 5 and evaporated to a small volume. On acidification to pH 2 with 6 N hydrochloric acid the product crystallized out (566 mg, 91%): mp 156–158 °C, lit.[8] mp 158–159 °C. This product was identical to an authentic sample of *N*-benzyloxycarbonylglycyl-L-proline.[8]

***N-tert*-Butyloxycarbonyl-L-prolyl-L-proline[5] (1) and Contaminant 2.** The *N-tert*-butyloxycarbonyl-L-proline 1-succinimidyl ester was coupled with proline and the reaction mixture worked up as described for the preparation of *N*-benzyloxycarbonylglycyl-L-proline, except that the product was precipitated from ethyl acetate by addition of *n*-hexane. From a concentrated ethyl acetate solution of this product, chromatographically pure *N-tert*-butyloxycarbonyl-L-prolyl-L-proline crystallized out (48%). After recrystallization from ethyl acetate, the protected dipeptide (37%) had mp 187–188 °C, lit.[5] mp 186–187 °C; R_f 0.70 on silica gel plates (ninhydrin negative when spotted on cellulose). This product was identical with an authentic sample of *N-tert*-butyloxycarbonyl-L-prolyl-L-proline.[5]

The combined ethyl acetate recrystallization filtrates were highly enriched in contaminant **2** but still contained some protected dipeptide. After evaporation and drying, the oil represented a 40% yield. Repeated trituration in ether, which was accompanied with great losses of material, gave **2** (R_f 0.63) as a solid free from the dipeptide **1** and contaminated by only a trace amount of a slow-moving, ninhydrin-positive material (R_f 0.17). Contaminant **2** gave a positive blue ninhydrin reaction on silica gel and cellulose plates. It gave a positive hydroxamic acid test in the presence of ferric chloride.

Attempts at further purification of compound **2** for analytical purposes or attempts to obtain **2** in larger quantities through column chromatography (Kieselgel 60, Merck; ethyl acetate–methanol, 2:1, or chloroform–methanol, 2:1) resulted in extensive degradation of this compound.

***N-tert*-Butyloxycarbonyl-L-prolyl-L-4-thiazolidinecarboxylic Acid (3) and Contaminant 4.** The protected dipeptide and contaminant **4** were prepared in the same manner as described for the preparation of dipeptide **1**.

The N-protected dipeptide **3** was obtained analytically pure in 32% yield: mp 154–156 °C; $[\alpha]^{22}_D$ −129° (*c* 1.0, $CHCl_3$), R_f 0.72. Anal. Calcd for $C_{14}H_{22}N_2O_5S$: C, 50.9; H, 6.7; N, 8.5; S, 9.7. Found: C, 50.9; H, 6.8; N, 8.4; S, 9.9.

Contaminant **4** had R_f 0.66, ninhydrin positive on silica gel and cellulose plates. It gave a positive hydroxamic acid test in the presence of ferric chloride.

Registry No.—**1**, 15401-08-8; **2**, 62726-56-1; **3**, 62726-57-2; **4**, 62726-58-3; *N-tert*-butyloxycarbonyl-L-proline 1-succinimidyl ester, 3392-10-7; proline, 147-85-3; 4-L-thiazolidinecarboxylic acid, 34592-47-7.

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