The Hydrazide as a Carboxylic-Protecting Group in Peptide Synthesis[1]

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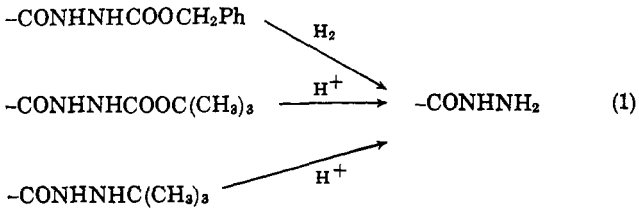
N-Acyl-α-amino acid hydrazides or N-acylpeptide-hydrazides are of use for the formation of peptide bonds either *via* the azides[2] or through oxidative coupling.[3,4] Temporary masking of the hydrazide function to give carboxylic-protected amino acids or peptides has been carried out by conversion to suitably substituted hydrazides such as benzyloxy-carbonyl-,[5] *t*-butyloxycarbonyl-,[6] or tritylhydrazides.[7] Regeneration of the hydrazide group is accomplished by hydrogenolysis in the case of the former, and by acid treatment in the cases of the latter two (eq. 1).

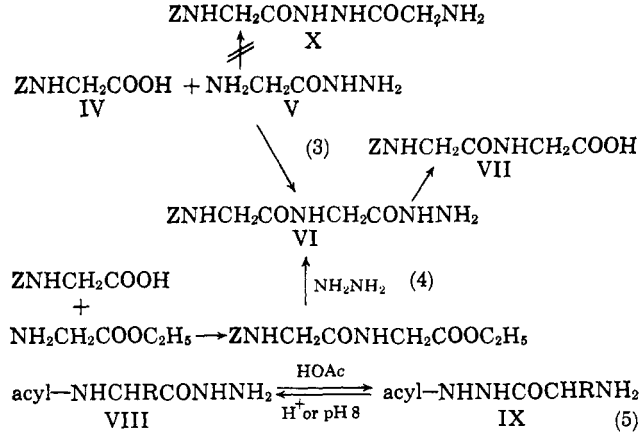


(1)

In an α-amino acid hydrazide I, the hydrazide function is a considerably weaker base than the α-amino, the respective pK_a' being 2.8 ± 0.4 and 7.4 ± 0.3 (in the cases of glycylhydrazide[8] and L-tyrosylhydrazide[9]). We reasoned that in the presence of 1 mole of an N-protected amino acid active ester (or its equivalent) II, there would be preferential attack of the α-amino function of I on the "activated" carbonyl of II, with formation of a peptide bond. The expected product, an N-protected peptide hydrazide III (eq. 2), could be used directly for further extension of the peptide chain.

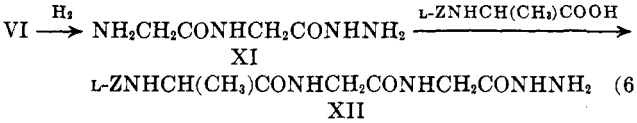
$$\underset{\text{II}}{\text{RNHCHR'COX}} + \underset{\text{I}}{\text{NH}_2\text{CHR''CONHNH}_2} \longrightarrow \underset{\text{III}}{\text{RNHCHR'CONHCHR''CONHNH}_2} \quad (2)$$

We have found that in the case of glycylhydrazide, and using the isoxazolium salt method,[10] the course of reaction envisioned did take place. Thus reaction of benzyloxycarbonylglycine (IV) with glycylhydrazide (V) yielded benzyloxycarbonylglycylglycylhydrazide



(VI) as the sole isolated product (eq. 3). The identity of the product was established by unambiguous synthesis (eq. 4) and by smooth conversion to the corresponding carboxylic acid VII (see below). Brenner, *et al.*,[11] and Kurtz and Niemann[9] have recently described the interconversion of acyl-α-amino acid hydrazides VIII and N-acyl-N'-α-aminoacylhydrazines IX under acidic or basic conditions at elevated temperatures (eq. 5). We believe that, under the conditions we employed for the coupling reaction (eq. 3) (see Experimental), it is unlikely that the peptide hydrazide VI isolated was the result of such a rearrangement. Hence little, if any, of the diacylhydrazine X was produced in the reaction.

Potential use of the method in extension of peptide chains was illustrated by hydrogenation of benzyloxycarbonylglycylglycylhydrazide (VI) to glycylglycylhydrazide (XI) which then yielded benzyloxycarbonyl-L-alanylglycylglycylhydrazide (XII) on being coupled with benzyloxycarbonyl-L-alanine (eq. 6). Chain extension at the carboxyl end may be carried out by oxidative coupling,[3,4] or *via* the azide[2] or the carboxylic acid (*e.g.*, by the isoxazolium[10] or carbodiimide methods[12]). The carboxylic acid may be easily obtained from the hydrazide by oxidation with N-bromosuccinimide or iodine,[3,4,13] a condition under which acid-labile (*e.g.*, *t*-butyloxycarbonyl, O-*t*-butyl) and hydrogenatable groups (*e.g.*, benzyloxycarbonyl) will remain intact.



(6)

Experimental

Melting points were taken on an electrically heated block (Mel-Temp) and were corrected.

Benzyloxycarbonylglycylglycylhydrazide (VI). A.—Benzyloxycarbonylglycylglycine ethyl ester (prepared from benzyloxycarbonylglycine and ethyl glycinate hydrochloride by the carbodiimide method[12]) in ethanol was treated with 2 moles of hydrazine hydrate at room temperature overnight. On filtration and recrystallization from methanol, benzyloxycarbonylglycylglycyl-

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(13) Use of two O's gives the corresponding carboxylic acid,[3,4] while one O results in the formation of a 1,2-diacylhydrazine[4,14]; *e.g.*, $ZNHCH_2CONHNH_2 \rightarrow ZNHCH_2CONHNHCOCH_2NHZ$ (see Experimental).

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hydrazide (VI) was obtained as plates in 79% yield, m.p. 166–167° (lit.[15] m.p. 165–167°).

B.—To 10.4 g. of benzyloxycarbonylglycine (0.05 mole) and 12.7 g. of N-ethyl-5-phenylisoxazolium-3′-sulfonate[10] (0.05 mole) suspended in 200 ml. acetonitrile was added 7.0 ml. of triethylamine (0.05 mole). After being stirred at room temperature for 30 min. the solution became homogeneous, and 4.6 g. of glycylhydrazide[16] (prepared by treatment of ethyl glycinate hydrochloride with hydrazine or by hydrogenation of benzyloxycarbonylglycylhydrazide) was added in one portion. Precipitation of the product occurred after 15 min., and the suspension was stirred at room temperature overnight. On filtration and crystallization from methanol, benzyloxycarbonylglycylglycylhydrazide (VI) was obtained as plates (8.6 g., 62%), m.p. 166–167°, identical (mixture melting point and infrared spectra) with an authentic sample (see above). The mother liquors on working up did not yield any more VI.

Anal. Calcd. for $C_{12}H_{16}N_4O_4$: C, 51.4; H, 5.75; N, 20.0. Found: C, 51.3; H, 5.8; N, 20.2.

Oxidation of Benzyloxycarbonylglycylglycylhydrazide (VI).—The hydrazide (97 mg.) was added during 10 min. to a solution of 123 mg. of N-bromosuccinimide (2 molar equiv.) in water. Benzyloxycarbonylglycylglycine (VII) (68 mg., 74%) was obtained on cooling and filtration. Recrystallized from methanol, it had m.p. 181–181.5 (lit.[17] m.p. 178–179°).

Oxidation of Benzyloxycarbonylglycylhydrazide.—This reaction was carried out under two conditions.

A.—When carried out as in the previously described reaction, benzyloxycarbonylglycine was obtained as the sole product.

B.—To 890 mg. of benzyloxycarbonylglycylhydrazide in 150 ml. of water was added, in small portions with vigorous stirring, N-bromosuccinimide during 15 min. until a yellow color was generated. The weight of N-bromosuccinimide required was 1.11 g. (1.56 molar equiv.). The solid precipitated during the reaction was filtered and crystallized from ethanol to give small needles of 1,2-bis(benzyloxycarbonylglycyl)hydrazine (180 mg.), m.p. 217–219° (lit.[4] m.p. 211–212°).

Anal. Calcd. for $C_{20}H_{22}N_4O_6$: C, 57.95; H, 5.35. Found: C, 57.85; H, 5.6.

Concentration of the filtrate gave needles of benzyloxycarbonylglycine (180 mg.).

Benzyloxycarbonyl-L-alanylglycylglycylhydrazide (XII).—To 2 g. of benzyloxycarbonyl-L-alanine and 12.7 g. N-ethyl-5-phenylisoxazolium-3′-sulfonate[10] suspended in 100 ml. of acetonitrile was added with cooling 7.0 ml. of triethylamine. When the solution became homogeneous a solution of glycylglycylhydrazide (XI) (prepared by hydrogenation of 14.5 g. of benzyloxycarbonylglycylglycylhydrazide in methanol over palladium–charcoal) in 20 ml. of absolute ethanol was added with cooling and stirring. Precipitation of the product occurred at once. Acetonitrile (70 ml.) was added, and the solution was stirred at room temperature overnight. On filtration and crystallization of the solid from ethanol there was obtained 11.0 g. (63%) of small prisms of benzyloxycarbonyl-L-alanylglycylglycylhydrazide (XII) m.p. 200–202°, $[\alpha]^{24}D$ −8° (*c* 0.24, methanol).

Anal. Calcd. for $C_{15}H_{21}N_5O_5$: C, 51.3; H, 6.0; N, 19.9. Found: C, 51.0; H, 6.1; N, 20.1.

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Equilenin 3-Benzyl Ether

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Several methods for the preparation of equilenin and some of its derivatives have been described.[2] The selenium dioxide dehydrogenation of 6-dehydroestrone derivatives described by Djerassi and his co-workers,[2a,b] when used by us, has not given the anticipated yield of equilenin derivatives relatively free of selenium. A modification of their procedures has overcome these objections. 6-Dehydroestrone 3-benzyl ether was dehydrogenated with selenium dioxide in acetic acid, zinc dust was added, and the mixture was heated at 90–100° for 1 hr. Equilenin 3-benzyl ether was isolated from this mixture and was readily purified. Hydrogenolysis removed the benzyl group to yield equilenin.

Experimental

The authors are indebted to Dr. R. T. Dillon and his staff of the analytical division for the analytical data in this paper. Rotations were taken in chloroform at about 1% concentrations at 25 ± 2° unless otherwise stated, and the ultraviolet spectra were observed in methyl alcohol. The n.m.r. spectrum was determined at 60 Mc, in deuteriodimethyl sulfoxide as the solvent and the value reported was in cycles per second as shifts downfield from tetramethylsilane as an internal standard.

6-Dehydroestrone 3-Benzyl Ether.—A mixture of 10 g. (0.037 mole) of 6-dehydroestrone,[2a] 27.5 g. (0.219 mole) of benzyl chloride, 25 g. (0.252 mole) of anhydrous potassium carbonate, and 550 ml. of ethyl alcohol (2B anhydrous) was stirred at reflux for 1.5 hr. The mixture was cooled, diluted with 1 l. of water, and extracted with 1.5 l. of methylene chloride. The extract was washed with 400 ml. of 10% aqueous sodium hydroxide solution, dried over anhydrous sodium sulfate, and filtered; the solvent was removed. Trituration with methyl alcohol (500 ml.) followed by filtration and washing of the solid with methyl alcohol yielded 9.8 g. of crude product, m.p. 135–145°, and 9.3 g. of 6-dehydroestrone 3-benzyl ether (crystallized from methylene chloride–methyl alcohol): m.p. 124–145°, $[\alpha]^{25}D$ −104°, λ_{max} 262 mμ (ε 8600) and 272 mμ (ε 7900), $\lambda_{max}^{CHCl_3}$ 5.75 μ (C=O).

Anal. Calcd. for $C_{25}H_{26}O_2$: C, 83.76; H, 7.31. Found: C, 83.77; H, 7.40.

Equilenin 3-Benzyl Ether.—To a stirred solution of 49 g. (0.136 mole) of 6-dehydroestrone 3-benzyl ether in 950 ml. of hot (90°) acetic acid was added 8.8 g. (0.067 mole) of selenous acid. The mixture was stirred rapidly and heated to 100–110° for 70 min., and cooled to 80°; 65 g. (1.0 mole) of zinc dust was added. The mixture was stirred and heated to 90–100° for 1 hr., and filtered hot; the filter cake was washed with 200 ml. of hot acetic acid. The filtrate was cooled overnight at room temperature and filtered, the orange leaf crystals were washed with methyl alcohol, and the product was air dried to yield 29 g. of crude equilenin 3-benzyl ether, m.p. 168–177°. Crystallization from methylene chloride–methyl alcohol gave 19 g.: m.p. 178–180°; $[\alpha]^{25}D$ +53°; λ_{max} 233 mμ (ε 61,200), 267 (5700), 278 (5300), 289 (3300), 321 (1600), and 336 (2050); λ_{max}^{KBr} 5.72 (C=O), 6.15 (C=C), and 6.23 μ (C=C).

Anal. Calcd. for $C_{25}H_{24}O_2$: C, 84.24; H, 6.79. Found: C, 84.06; H, 6.74.

A second crop 6.5 g., m.p. 165–178° (85% purity from ultraviolet absorption data), was isolated from the mother liquor.

Equilenin.—Equilenin 3-benzyl ether (10 g., 0.028 mole) in 500 ml. of tetrahydrofuran was hydrogenated[3] at room temperature and 1-atm. pressure for 6.5 hr. in the presence of 1.0 g. of Pd–C (5%). The catalyst was removed by filtration, the filtrate was evaporated to dryness *in vacuo*, and the residue was crystallized from 400 ml. of methyl alcohol to yield 6.0 g. (in 2 crops): m.p. 254–256°; $[\alpha]^{25}D$ +90.5° (*c* 1.009, dioxane); λ_{max} 229 mμ (ε 62,400), 269 (4550), 280 (5250), 291 (3700), 326 (2000), and 340 (2400); n.m.r. 40 c.p.s. (CH_3); λ_{max}^{KBr} 3.05 (OH), 5.82 (C=O), and 6.17 and 6.25 μ (C=C).

Anal. Calcd. for $C_{18}H_{18}O_2$: C, 81.17; H, 6.81. Found: C, 81.22; H, 6.91.

The compound was identical with a sample prepared according to the method of Djerassi and co-workers.

(1) To whom communications concerning this paper should be addressed.

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(3) The hydrogenation was carried out by Mr. J. D. Choi of G. D. Searle and Co.