was first suggested by Franzen and Joschek[4] to explain the formation of benzoic acid from the reaction of *o*-bromochlorobenzene with *n*-butyllithium.

Although diphenylacetylene reacts with lithium in ethyl ether to give *cis,cis*-1,2,3,4-tetraphenylbutadiene,[5] the reaction of *p*-chlorodiphenylacetylene with lithium gave only 5% diphenylacetylene and 94% starting material.

Experimental Section

p-Chlorodiphenylacetylene was prepared from *α*-*p*-chloroacetophenone.[6,7]

***n*-Butyllithium and *p*-Chlorodiphenylacetylene.**—*p*-Chlorodiphenylacetylene (25.0 g, 0.118 mol) in 250 ml of anhydrous ethyl ether was added dropwise over a period of 1 hr to a stirred solution under a helium atmosphere of *n*-butyllithium (0.260 mol) in 182 ml of ethyl ether. After stirring for 17 hr at room temperature, the dark solution, which contained a considerable amount of precipitate, was cooled (0°), and 50 ml of ice water was added slowly. An additional 100 ml of water was added and the layers separated. The ethereal solution was dried and concentrated to yield 23.5 g of a dark viscous oil. This oil was dissolved in 50 ml of 2-butanone and poured slowly into 250 ml of rapidly stirring methanol. Filtration gave 3.63 g of a tan polymer: softening point 175–183°; nmr (15% in $CDCl_3$), two broad multiplets at τ 2.25–3.09 and 8.53–9.62 (relative areas 3:1); $\nu_{max}^{CHCl_3}$ 1605 cm^{-1}.

Anal. Found: C, 91.39; H, 6.09; residue, 1.85; mol wt, 2420.

The methanolic filtrate was concentrated and distilled through a small column to give 10.91 g of pale yellow oil, bp 90–135° (0.10 mm.). Vapor phase chromatography (5-ft GE-SF-96, 200°) indicated the presence of diphenylacetylene (27%), *p*-chlorodiphenylacetylene (7%), and *m*- and *p*-*n*-butyldiphenylacetylenes (66%). Redistillation through a spinning-band column yielded 7.0 g (26%) of *m*- and *p*-*n*-butyldiphenylacetylenes: bp 160–161° (0.70 mm.); $n^{26.8}$D 1.6079; λ_{max} 300 mμ (ϵ 33,000), 291 (28,000), 282 (39,000), 275 (31,000), and 267 (27,000); $\nu_{max}^{CHCl_3}$ 2225 cm^{-1}; nmr (20% in CCl_4), τ 2.44–3.04 (9, multiplet), 7.47 (2, triplet, J = 7 cps), 8.33–8.92 (4, multiplet), and 9.10 (3, triplet, J = 6 cps). Vapor phase chromatography showed two overlapping peaks of equal intensity.

Anal. Calcd for $C_{18}H_{18}$: C, 92.26; H, 7.74. Found: C, 91.96; H, 7.93.

Lithium Metal and *p*-Chlorodiphenylacetylene.—The reaction was run in the manner described by Smith and Hoehn.[5] To lithium wire (0.70 g, 0.10 g-atom) suspended in 130 ml of anhydrous ethyl ether under a helium atmosphere, there was added *p*-chlorodiphenylacetylene (21.25 g, 0.1000 mol). After stirring at room temperature for 12 hr, the mixture was heated under reflux for 13 hr. After the mixture cooled to 0°, 75 ml of 95% ethanol was added dropwise and the solution was stirred until all of the lithium metal had reacted. Water (100 ml) was added and the layers separated. The ethereal solution was dried and concentrated to give 20.9 g of solid. Vapor phase chromatography (5-ft GE-SF-96, 200°) indicated that this solid consisted of 94% starting material, 5% diphenylacetylene, and <1% an unidentified compound which had the lowest retention time of the three components.

Registry No.—*p*-Chlorodiphenylacetylene, 5172-02-1; *n*-butyllithium, 109-72-8; *m*-*n*-butyldiphenylacetylene, 19165-50-5; *p*-*n*-butyldiphenylacetylene, 19165-51-6.

(4) V. Franzen and H. Joschek, *Angew. Chem.*, **72**, 564 (1960).

(5) L. E. Smith and H. H. Hoehn, *J. Amer. Chem. Soc.*, **63**, 1184 (1941); H. H. Freedman, G. A. Doorakian and V. R. Sandel, *ibid.*, **87**, 3019 (1965).

(6) D. Y. Curtin and M. C. Crew, *ibid.*, **76**, 3719 (1954).

(7) M. S. Newman and D. E. Reid, *J. Org. Chem.*, **23**, 665 (1958).

The 4-(Methylthio)phenyl and 4-(Methylsulfonyl)phenyl Esters in the Preparation of Peptides and Polypeptides.[1] Synthesis of a Linear O-Depsipeptide

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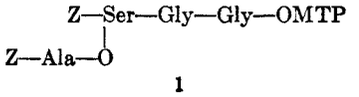
The synthesis of O-depsipeptides requires the condensation of the free carboxyl of a N-protected peptide with a free hydroxyl group. Attempts to form O-depsipeptides with such a terminal hydroxyl group have usually been unsuccessful.[2] For this and other reasons[3] the first bond formed in the synthesis of O-depsipeptides has been the O-peptide ester linkage, such that subsequent condensations then involve the easier amide bond formation. However, using this approach, the use of C-terminal protecting groups which are removed by alkaline treatment, *e.g.*, methyl and ethyl esters, are precluded because the O-peptide ester linkage is even more easily hydrolyzable.

In order to overcome this difficulty inherent in the synthesis of O-depsipeptides we have found that the 4-(methylthio)phenyl (MTP) protective[4] ester and the 4-(methylsulfonyl)phenyl (MSO_2P)[5] activated ester are particularly useful for extending the peptide chain in the presence of an O-peptide ester linkage. For this purpose the synthesis of N-carbobenzoxy-L-seryl-O-(N-carbobenzoxy-L-alanyl)glycylglycine 4-(methylthio)phenyl ester (**1**) is described as an illustration of this approach to the preparation of O-depsipeptides.



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The synthesis commenced with the formation of N-carbobenzoxy-L-serylglycine 4-(methylthio)phenyl ester (**2**) by condensation of N-carbobenzoxy-L-serine and glycine 4-(methylthio)phenyl ester hydrochloride[3] using dicyclohexylcarbodiimide and triethylamine. The mixed-anhydride method was found to be the best procedure to form the O-peptide ester bond. Thus, the anhydride formed from N-carbobenzoxy-L-alanine and ethyl chloroformate was treated with the protected dipeptide MTP ester **2** to give the protected O-depsipeptide MTP ester, N-carbobenzoxy-L-seryl-O-(N-carbobenzoxy-L-alanyl)glycine 4-(methylthio)phenyl ester (**3**). In order to extend the peptide chain it was necessary to convert the protective MTP ester of **3** to its activated MSO_2P counterpart. This activation was achieved by the use oxidative conditions which are more

(1) This is the fourth article in this series; see B. J. Johnson and P. M. Jacobs, *J. Org. Chem.*, **33**, 4524 (1968), for the previous paper.

(2) D. W. Russell, *Quart. Rev.* (London), **20**, 559 (1966).

(3) M. M. Shemyakin, E. I. Vinogradova, M. Yu. Feigina, N. A. Aldonova, Yu. A. Ovchinnikov, and A. A. Kiryushkin, *J. Gen. Chem. USSR*, **34**, 1796 (1964).

(4) B. J. Johnson and P. M. Jacobs, *Chem. Commun.*, 73 (1968).

(5) R. Schwyzer and P. Sieber, *Helv. Chim. Acta*, **41**, 2190 (1958), have reported a few 4-(methylsulfonyl)phenyl esters prepared from 4-(methylsulfonyl)phenol through the diaryl sulfite method.

convenient than those previously reported for this conversion.[6]

To this end the protected O-depsipeptide MTP ester **3** was treated with *m*-chloroperoxybenzoic acid in dioxane for 4 hr to yield N-carbobenzoxy-L-seryl-O-(N-carbobenzoxy-L-alanyl)glycine 4-(methylsulfonyl)-phenyl ester (**4**). Under these mild oxidative conditions the sensitive O-peptide linkage, N-carbobenzoxy-protecting groups, and peptide bonds were not cleaved. The peptide chain was then extended through this MSO_2P-activated ester by reaction of the protected O-depsipeptide **4** with glycine 4-(methylthio)phenyl ester hydrochloride to give the fully protected O-depsipeptide **1**.

It has been shown that the protective 4-(methylthio)-phenyl ester can be converted into the activated 4-(methylsulfonyl)phenyl ester without decomposition of an O-peptide linkage incorporated into the tripeptide to which it was attached. Further coupling through the resulting activated ester was also possible. It is anticipated that this method of protection and then subsequent activation will be of great utility for further synthesis of O-depsipeptides, provided that the amino acid residues of methionine, cysteine, and cystine are not present during the oxidation.

Experimental Section[7]

N-Carbobenzoxy-L-serylglycine 4-(Methylthio)phenyl Ester (2).—To a solution of N-carbobenzoxy-L-serine (3.45 g, 0.0144 mol) in methylene chloride containing 1.5 g of triethylamine was added 3.0 g of DCC and 4.0 g of glycine 4-(methylthio)phenyl ester hydrochloride. The reaction mixture was stirred overnight. The precipitated urea was filtered off, and the solvent was evaporated to give a solid. This solid was dissolved in ethyl acetate and washed with 10% citric acid solution (100 ml), water (two 150-ml portions), sodium bicarbonate solution (100 ml), and water (two 150-ml portions), dried (Na_2SO_4), and evaporated under reduced pressure to give a solid. This material was chromatographed on a column of Silicar CC-7 using chloroform as eluent. The major fraction was crystallized from ethyl acetate–hexane to yield 3.0 g (50%) of the protected dipeptide, mp 136°, $[\alpha]^{25}D$ −6.9° (*c* 1.45, dimethylformamide). *Anal.* Calcd for $C_{20}H_{22}N_2O_6S$: C, 57.4; H, 5.3; N, 6.7. Found: C, 57.2; H, 5.1; N, 6.7.

N-Carbobenzoxy-L-seryl-O-(N-carbobenzoxy-L-alanyl)glycine 4-(Methylthio)phenyl Ester (3).—To a solution of N-carbobenzoxy-L-alanine (1.38 g, 0.006 mol) in 10 ml of dimethylformamide, cooled to 0°, was added 0.6 g of triethylamine and 0.65 g of ethyl chloroformate. The reaction mixture was left at 5° for 20 min and then 2.5 g of N-carbobenzoxy-L-serylglycine 4-(methylthio)-phenyl ester in 10 ml of dimethylformamide was added. The reaction mixture was kept at 4° for 24 hr and then poured into water to give a semisolid. This material was extracted into ethyl acetate and washed with water (two 100-ml portions), dried (Na_2SO_4), and evaporated under reduced pressure to give a solid which was chromatographed on a column of Silicar CC-7 using ethyl acetate as eluent to yield 2.0 g (54%) of the protected O-peptide, mp 156°, $[\alpha]^{25}D$ −13.3° (*c* 0.75, dimethylformamide). *Anal.* Calcd for $C_{31}H_{33}N_3O_9S$: C, 59.7; H, 5.3; N, 6.7. Found: C, 59.5; H, 5.4; N, 6.7.

N-Carbobenzoxy-L-seryl-O-(N-carbobenzoxy-L-alanyl)glycine 4-(Methylsulfonyl)phenyl Ester (3).—To solution of N-carbobenzoxy-L-seryl-O-(N-carbobenzoxy-L-alanyl)glycine 4-(methylthio)phenyl ester (1.5 g, 0.0024 mol) in dioxane was added 1.25 g (3 equiv) of 85% *m*-chloroperoxybenzoic acid. The reaction mixture was left at room temperature for 4 hr and then poured into water (300 ml). The precipitate was collected, dried, and chromatographed on a column of Silicar CC-7 using ethyl acetate as eluent. The major fraction was crystallized from ethyl acetate–hexane to give the protected O-peptide 4-(methylsulfonyl)phenyl ester, 1.2 g (70%), mp 129°, $[\alpha]^{25}D$ −10.5° (*c* 0.95, dimethylformamide). *Anal.* Calcd for $C_{31}H_{33}N_3O_{11}S$: C, 56.8; H, 5.1; N, 6.4. Found: C, 56.9; H, 5.0; N, 6.3.

N-Carbobenzoxy-L-seryl-O-(N-carbobenzoxy-L-alanyl)glycylglycine 4-(Methylthio)phenyl Ester (1).—To a solution of N-carbobenzoxy-L-seryl-O-(N-carbobenzoxy-L-alanyl)glycine 4-(methylsulfonyl)phenyl ester (1.0 g, 0.00153 mol) in 20 ml of dimethylformamide was added 0.16 g of triethylamine and 0.36 g of glycine 4-(methylthio)phenyl ester hydrochloride. The reaction mixture was stirred overnight at room temperature and poured into water. The precipitate was filtered off, dried, and recrystallized from ethyl acetate to yield 0.65 g (63%) of the protected O-peptide 4-(methylthio)phenyl ester, mp 163°, $[\alpha]^{25}D$ −7.7° (*c* 1.3, dimethylformamide). *Anal.* Calcd for $C_{33}H_{36}N_4O_{10}S$: C, 58.2; H, 5.3; N, 8.2. Found: C, 58.0; H, 5.4; N, 8.3.

Registry No.—**1**, 19817-61-9; **2**, 19817-62-0; **3** (thio), 19817-63-1; **3** (sulfonyl), 19817-64-2.

Acknowledgment.—This work was supported by a grant from the National Science Foundation. Thanks are also due to the Crown Zellerbach Corp. for samples of 4-(methylthio)phenol.

(6) B. J. Johnson and E. G. Trask, *J. Org. Chem.*, **33**, 4521 (1968).

(7) All melting points are uncorrected. Analyses were carried out by Dr. S. M. Nagy of Belmont, Mass. Optical rotations were taken on a Carl Zeiss precision polarimeter.

4-Phenylazodiphenylamine, a Novel Reagent for the Determination Grignard Reagent and Its Use in the Preparation of 6α-^{14}C-Methylhydrocortisone

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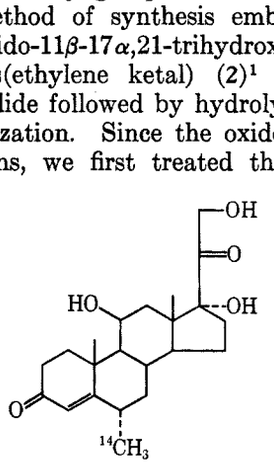
In the course of investigation of the hormonal activity of 6α-methylhydrocortisone (**1**) isotopic labeling of the 6α-methyl group was desired. A direct and economical method of synthesis embodied treatment of 5α,6α-oxido-11β-17α,21-trihydroxypregnane-3,20-dione 3,20-bis(ethylene ketal) (**2**)[1] with ^{14}C-methylmagnesium iodide followed by hydrolysis, dehydration, and isomerization. Since the oxide **2** has three active hydrogens, we first treated these functions with methyl-



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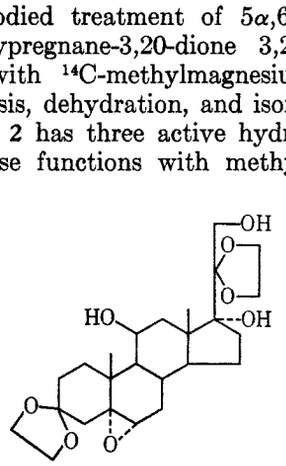

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magnesium iodide and then added ^{14}C-methylmagnesium iodide to react with the oxide function. 4-Phenylazodiphenylamine was used as an internal indicator to follow the course of the addition of methylmagnesium

(1) G. B. Spero, J. L. Thompson, B. J. Magerlein, A. R. Hanze, H. C. Murray, O. K. Sebek, and J. A. Hogg, *J. Amer. Chem. Soc.*, **78**, 6213 (1956); S. Bernstein and R. Littell, *ibid.*, **82**, 1235 (1960).