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.

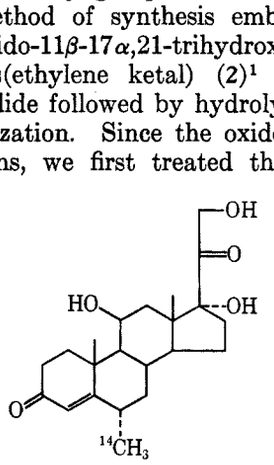
4-Phenylazodiphenylamine, a Novel Reagent for the Determination Grignard Reagent and Its Use in the Preparation of 6α-^{14}C-Methylhydrocortisone

BARNEY J. MAGERLEIN AND WILLIAM P. SCHNEIDER

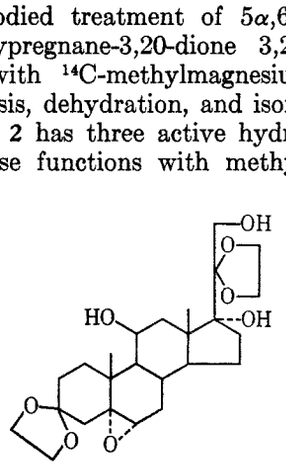
Research Laboratories, The Upjohn Company, Kalamazoo, Michigan 49001

Received January 8, 1969

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-



1



2

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).

iodide to the active hydrogens of oxide **2**. This indicator was previously used in the quantitative determination of lithium aluminum hydride in tetrahydrofuran (THF) by titration with a standardized solution of 1-propanol in benzene.[2] 4-Phenylazodiphenylamine (0.1% solution in benzene) proved to be a highly sensitive test reagent for Grignard Reagent in tetrahydrofuran–ether solutions. This reagent is more convenient than the Gilman reagent,[3] and, moreover, may be used as an *internal* indicator.

4-Phenylazodiphenylamine was also successfully used as the indicator in the quantitative determination of uptake of methylmagnesium iodide by several compounds with active hydrogen atoms. An excess of methylmagnesium iodide was added to the given compound in tetrahydrofuran solution followed by titration with a standardized solution of 1-propanol in benzene using 4-phenylazodiphenylamine as indicator. In this manner 1.0 mmol of sitosterol consumed 0.93 mmol of methylmagnesium iodide; 17β-hydroxy-6,6-dimethylandrostan-3-one, 1.09; hydrocortisone, 3,20-bis(ethylene ketal), 3.1; and 5α,6α-oxido-11β-17α,21-trihydroxypregnane-3,20-dione 3,20-bis(ethylene ketal) (**2**), 2.8–3.1.

In the preparation of 6α-^{14}C-methylhydrocortisone, oxide **2** was treated with methylmagnesium iodide. A slight excess of Grignard Reagent was indicated by 4-phenylazodiphenylamine. Then ^{14}C-methylmagnesium iodide was added. Acid hydrolysis of the resulting 5α-hydroxy-6β-methyl intermediate followed by treatment with alkali yielded 6α-^{14}C-methylhydrocortisone (**1**) in 31.9% yield from oxide **2**.

Experimental Section

Reagents.—Commercial methylmagnesium iodide in ether (Arapahoe Chemical Co.) was determined to be 3.1 *M* by conventional titration. THF was freshly distilled from lithium aluminum hydride. The 0.1 *N* 1-propanol solution in benzene was prepared by adding 0.300 g of 1-propanol, distilled over sodium, to 500 ml of distilled thiophene-free benzene. A 0.1% solution of 4-phenylazodiphenylamine (Eastman Organic Chemicals) in dry benzene was used as indicator.

Titration with Methylmagnesium Iodide.—A 125-ml beaker containing a magnetic stirrer and fitted with a plastic cover containing openings for N_2 inlet and outlet and for the tip of two automatic burets was used as the titration vessel. The system was purged with N_2 and a slow flow of N_2 maintained. A blank determination was then made as follows. THF (25 ml) was pipetted into the beaker followed by 2 ml of methylmagnesium iodide solution and 5 drops of indicator solution. 1-Propanol–benzene was added with stirring until the pink color of the indicator changed to yellow. The titration was then repeated using a sample of 1.0 mmol of steroid which was dissolved in the THF before adding the methylmagnesium iodide. From the volume of standard 1-propanol solution required for the blank less that required for the sample, the millimoles of methylmagnesium iodide consumed were readily calculated.

6α-^{14}C-Methylhydrocortisone (**1**).—^{14}C-Methylmagnesium iodide[4] was prepared in 50 ml of anhydrous ether from 490 mg of magnesium turnings and 2.405 g of ^{14}C-methyl iodide (17.0 mmol with specific activity of 1.77 mCi/mmol).

To a solution of 1.4 g (3 mmol) of 5α,6α-oxido-11β,17α,21-trihydroxypregnane-3,20-dione 3,20-bis(ethylene ketal) (**2**) in 85 ml of THF containing 3 drops of 4-phenylazodiphenylamine solution methylmagnesium iodide was added until a pink color developed. This solution was added to ^{14}C-methylmagnesium iodide prepared above. The ether was distilled and the solution was heated at reflux for 20 hr. Saturated ammonium chloride solution was added to the cooled reaction mixture. The THF was distilled *in vacuo* and the steroid was extracted with benzene. The dried benzene solution was evaporated and the residue was triturated with anhydrous ether. The crystalline residue was dissolved in 80 ml of methanol and 20 ml of 1 *N* HCl. After refluxing for 30 min, the solution was cooled, diluted with 150 ml of methanol, and purged with N_2. With stirring 22.5 ml of 0.1 *N* NaOH was added. After 20 hr at ambient temperature, the solution was neutralized with acetic acid and evaporated *in vacuo*. 6α-^{14}C-Methylhydrocortisone (360 mg, 31.9%), mp 199–205°, 1.3 mCi/mmol, was obtained by recrystallization from ethyl acetate.

Registry No.—**1**, 19886-67-0; 4-phenylazodiphenylamine, 101-75-7.

(2) T. Higuchi and D. A. Zuck, *J. Amer. Chem. Soc.*, **73**, 2676 (1951).
(3) H. Gilman and F. Schulze, *ibid.*, **47**, 2002 (1925).
(4) B. M. Tolbert, *J. Biol. Chem.*, **173**, 205 (1948); H. B. MacPhillamy and C. R. Scholz, *ibid.*, **178**, 37 (1949).