Chemistry of Natural Compounds, Vol. 43, No. 1, 2007

SYNTHESIS OF NEW ESTERS AND AMIDES OF CEPHALOSPORIN G

I. V. Serkov[1] and V. V. Bezuglov[2]

UDC 547.818+547.995

Synthetic methods for cephalosporin G derivatized at the carboxyl were developed. Dinitroglycerine, acetoxymethyl, and choline esters of cephalosporin G and its amide in addition to amides of cephalosporin G with glutamic acid and arginine were synthesized.

Key words: antibiotics, cephalosporin G, amides, esters, dinitroglycerine, acid fluorides.

Cephalosporin-type antibiotics were first isolated in 1961 from the extract of *Cephalosporium acremonium* by E. Abraham and G. Newton [1] and are based on 7-aminocephalosporanic acid. Cephalosporins are widely used in medicine and are similar in structure and mechanism of action to penicillins [2]. Cephalosporin G is also used for enzymatic production of deacetoxycephalosporanic acid [3] and the synthesis of various prodrugs [4]. In the latter instance, a medicinal compound bound to C-3′ of cephalosporin is released in the organism by an enzymatic reaction [4]. Cephalosporins derivatized at the carboxylic acid are much less common. In order to fill this gap, we developed convenient methods for the synthesis of various esters and amides of cephalosporin G.

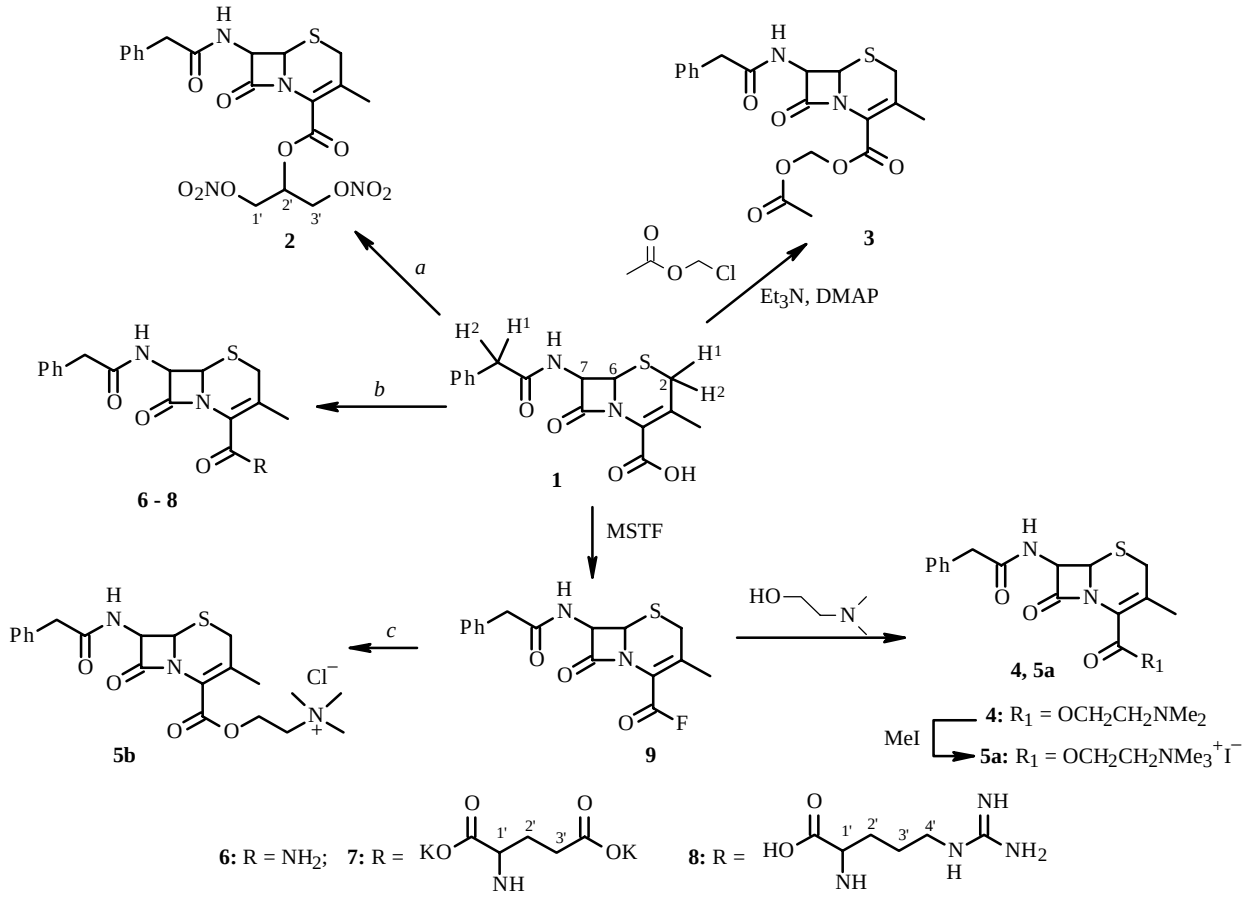
Three groups of alcohols were selected for the synthesis of esters. Glycerine 1,3-dinitrate, a potential medicinal compound that generates nitric oxide, was used to synthesize its ester with cephalosporin G (**2**), which is an alternative to the conjugate of 3-morpholinosydnonimine with cephalosporin that was synthesized earlier [5]. The acetoxymethyl ester (**3**) was synthesized as an example of a derivative that is incorporated into cells and hydrolyzed there. Esters with aminoalcohols were represented by dimethylaminoethanol (**4**) and choline (**5a**, **5b**) derivatives. Amides of cephalosporins are also interesting as starting points for building complex medicinal preparations where amino acids and peptides are used as linkers. We developed methods for the synthesis of cephalosporin G amide (**6**) and the amides of cephalosporin G with glutamic acid (**7**) and arginine (**8**).

Several methods that are usually used for the synthesis of esters and amides were tested. These included: 1) activation of the carboxylic acid by converting it to a mixed anhydride with *i*-butylchloroformate, toluenesulfonylchloride (TsCl), or triisopropylbenzenesulfonylchloride (TPSCl); 2) activation of the carboxylic acid by converting it to an imidazolide that reacts with carbonyl- or oxalyldiimidazole; 3) conversion of cephalosporin to the acid chloride or fluoride; 4) reaction of cephalosporin with alkane halides. Not all methods gave good yields of the final product. In certain instances only traces of the final product were observed using TLC. Based on these experiments, the optimal conditions for giving the best purity and yield of the final products were selected for each compound (see Scheme 1). The glycerine dinitrate ester (**2**) and amides (**6-8**) were synthesized by method 1. The acetoxymethyl ester (**3**) was produced by condensation of cephalosporin with acetoxymethylchloride (method 4) in the presence of Et_3N and dimethylaminopyridine (DMAP).

The choline esters (**5a** and **5b**) were synthesized using two approaches: 1) direct esterification of cephalosporin by choline chloride and 2) esterification of cephalosporin by dimethylaminoethanol with subsequent quaternization into the choline ester. Esterification of cephalosporin with choline chloride was carried out either in the presence of TPSCl or TsCl (method 1) or using carbonyldiimidazole or oxalyldiimidazole (method 2). In the first instance, the reaction was very slow and gave a low yield of the final ester. In the second, the yield of **5b** was satisfactory but separating the product from unreacted choline chloride was difficult.

1) Institute of Physiologically Active Compounds, Russian Academy of Sciences, 142432, Moscow Oblast′, Chernogolovka, Severnyi pr., 1, fax 7495 785 7024, e-mail: serkov@ipac.ac.ru; 2) M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, 117997, Moscow, ul. Miklukho-Maklaya, 16/10, fax 7495 335 7103, e-mail: vvbez@oxylipin.ibch.ru. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 85-88, January-February, 2007. Original article submitted October 16, 2006.

0009-3130/07/4301-0103



a. TPSCl, glycerine 1,3-dinitrate; *b*. *i*-BuOCOCl, Et_3N, RNH_2; *c*. choline chloride or trimethylsilylcholine chloride.

Another method for synthesizing the choline ester consisted of using the acid fluoride of cephalosporin G (**9**) (method 3). It has been reported that SF_4 and dialkylaminotrifluorosulfuranes react with carboxylic acids to give the corresponding acid fluorides [6]. Using morpholinotrifluorosulfurane (MSTF) in the presence of tris-morpholinotrimethyldifluorosilicate produced pure cephalosporin G acid fluoride (**9**), which reacted with choline chloride in the presence of Et_3N. After mixing all reagents, the mixture immediately became dark wine colored. The reaction was complete in several minutes.

Ether precipitated most of the nonpolar side products. Recrystallization produced **5b** that contained ~10% of an unidentified side product, presumably formed by reaction with Et_3N. In order to avoid this impurity, the TMS ester of choline chloride was synthesized for this instance and reacted slowly with cephalosporin G acid fluoride to give **5b** in good yield. The only important drawback of this method was the rapid hydrolysis of choline silyl ester, which contaminated **5b** with starting choline.

The method involving the synthesis in the first step of dimethylaminoethyl ester **4** gave the best results with respect to purity and yield of the choline ester. It was purified by column chromatography over silica gel. Quaternization with methyliodide produced the final choline ester **5a**.

Cephalosporin G amide (**6**) was prepared by several methods. The final amide had to be purified by recrystallization from ethanol or methanol if synthesized through the intermediate acid fluoride (method 3). Considering the very low solubility of the amide in hot alcohol, about 1 L was required to recrystallize 1 g of product. When the mixed anhydride with isobutylchloroformate was reacted with either aqueous ammonia or methanol saturated with gaseous ammonia, the yield of **6** was ~60% (in both instances) and additional purification was not necessary.

As it turned out, the amides of cephalosporin G with glutamic acid (**7**) and arginine (**8**) were practically insoluble in most solvents (except DMSO). This precluded the use of recrystallization to purify the final products. The synthesis of these compounds through the mixed anhydride of cephalosporin G with isobutylchloroformate (method 1) enabled them to be prepared as crystals with >99% purity. Compound (**7**) was isolated as the dipotassium salt. Acidification of an aqueous solution of **7** to pH ~ 7 liberated free glutamic acid through rupture of the amide bond.

Thus, the optimal methods for the synthesis of new esters and amides of cephalosporin G were developed and can be used to prepare analogs of other cephalosporin-type antibiotics.

EXPERIMENTAL

Starting cephalosporin G (Lonza, Switzerland) was recrystallized twice from methanol to produce white crystals with the literature melting point in 65% yield. L-Glutamic acid, L-arginine, 2,4,6-triisopropylbenzenesulfonylchloride (TPS-Cl), *N*,*N*-dimethylaminoethanol, *N*,*N*-dimethylaminopyridine (DMAP), 1,1′-carbonyldimiidazole, oxalyldiimidazole, and isobutylchloroformate were purchased from Fluka (Switzerland); Norit A, from Serva (Germany). Acetoxymethylchoride was synthesized by A. A. Formanovskii (Moscow, Russia). Tris-morpholinotrimethyldifluorosilicate and morpholino-trifluorosulfurane were gratiously supplied by V. E. Pashinnik (Kiev, Ukraine). All solvents were purified by standard methods. Column chromatography used glass columns packed with Kieselgel G60 (Merck, Germany). TLC was performed on Silufol plates (Kavalier, Czech SSR). PMR spectra were recorded on a Bruker WM-500 instrument (Germany). Chemical shifts are given on the δ-scale relative to HMDS. ^{19}F NMR spectra were recorded on a Bruker CXP-200 instrument (Germany). Chemical shifts are given in ppm relative to trifluoroacetic acid.

Cephalosporin G Glycerine 1,3-Dinitrate Ester (2). A solution of cephalosporin G (**1**, 1 g, 3 mmol) in acetone (20 mL) was treated with Et_3N (0.82 mL, 6 mmol), TPS-Cl (1 g, 3.3 mmol), and DMAP (200 mg). The mixture was stirred for 1 h at room temperature, treated with glycerine 1,3-dinitrate (728 mg, 4 mmol), stirred for 4 h at room temperature, diluted with water (100 mL), and extracted with ethylacetate (3 × 150 mL). The organic extract was washed with water and brine, and dried over anhydrous Na_2SO_4. The desiccant was filtered off. The filtrate was evaporated. The solid was recrystallized from methanol to afford **2** (1.02 g, 68%), mp 186-188°C (dec.). PMR spectrum (DMSO-d_6, ppm): 2.04 (3H, s, CH_3), 3.5 (4H, m, $PhCH_2$, H_2-2), 4.80 (4H, m, H_2-1′, H_2-3′), 5.08 (1H, d, H-6), 5.62 (2H, m, H-7, H-2′), 7.279 (5H, m, Ph), 9.1 (1H, d, NH).

Cephalosporin G Acetoxymethyl Ester (3). A solution of **1** (6 g, 18 mmol) in acetone (30 mL) was treated with Et_3N (2.6 mL, 19 mmol) and acetoxymethylchloride (2.6 mL, 19 mmol). The mixture was stirred for 2 h, treated with DMAP (20 mg), stirred for 14 d at room temperature, and filtered. The filtrate was evaporated. The yellow crystalline solid was dissolved in ethylacetate (100 mL), treated with Et_3N (500 µL), washed with water and brine, and dried over anhydrous Na_2SO_4. The desiccant was filtered off. The filtrate was evaporated. The solid was recrystallized from methanol to afford **3** (4.95 g, 67.8%), mp 179-181°C. PMR spectrum ($CDCl_3$, ppm, J/Hz): 2.085 (6H, s, CH_3, $COCH_3$), 3.131 (1H, d, J = 18.5, H^1-2), 3.45 (1H, d, J = 18.5, H^2-2), 3.58 (1H, d, J = 16, $PhCH^1$), 3.64 (1H, d, J = 16, $PhCH^2$), 4.90 (1H, d, J = 5, H-6), 5.74 (1H, dd, J = 5, $J_{(NH)H}$ = 9, H-7), 5.79 (1H, d, J = 6, OCHO), 5.86 (1H, d, J = 6, OCHO), 6.02 (1H, d, $J_{(NH)H}$ = 9, NH), 7.279 (5H, m, Ph).

Cephalosporin G Dimethylaminoethyl Ester (4). A solution of cephalosporin G acid fluoride (2 g, 6 mmol) in acetone (100 mL) was treated with a solution of dimethylaminoethanol (2 mL, 20 mmol) and Et_3N (2 mL) in acetone (10 mL). The mixture was stirred at room temperature for 12 h. The solvent was evaporated. The solid was dissolved in $CHCl_3$ and purified by column chromatography over Al_2O_3 with elution by $CHCl_3$ and $CHCl_3$:CH_3COCH_3 (10:1). Fractions containing the dimethylaminoethyl ester were combined and evaporated to afford **4** (1.4 g, 59%), white crystalline powder. PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 2.057 (3H, s, CH_3), 2.235 [6H, s, $N(CH_3)_2$], 2.53 (1H, m, C<u>H</u>NMe_2), 2.61 (1H, m, C<u>H</u>NMe_2), 3.1 (1H, d, J = 18, H^1-2), 3.43 (1H, d, J = 18, H^2-2), 3.58 (1H, d, J = 16, $PhCH^1$), 3.63 (1H, d, J = 16, $PhCH^2$), 4.28 (2H, m, OCH_2), 4.9 (1H, d, J = 4.5, H-6), 5.73 (1H, dd, J = 4.5, $J_{(NH)H}$ = 9, H-7), 6.06 (1H, d, $J_{(NH)H}$ = 9, NH), 7.283 (5H, m, Ph).

Cephalosporin G Choline Ester Iodide (5a). A solution of cephalosporin G dimethylaminoethyl ester (**4**, 1 g, 2.5 mmol) in $CHCl_3$ (20 mL) was treated with ether (20 mL) and a solution of methyliodide (1 mL, 16 mmol) in ether (5 mL) and stirred at room temperature. The white precipitate that formed after 1 min was filtered off, washed with ether, and dried in a desiccator over P_2O_5 to afford **5** (720 mg, 54%) as cream-colored hygroscopic crystals (for the PMR spectrum, see below).

Cephalosporin G Choline Ester Chloride (5b). A solution of cephalosporin G acid fluoride (**9**, 2 g, 6 mmol) in nitromethane (35 mL) was treated with a warm (35-40°C) solution of choline chloride (130 mg, 0.94 mmol) in nitromethane (15 mL) containing Et_3N (450 µL), stirred for 15 min, and diluted with ether (150 mL). The solvent was decanted. The solid was dissolved in methanol and treated with activated carbon. The carbon was filtered off. The solvent was evaporated. The solid was recrystallized from isopropanol:acetone to afford **5a** (1.4 g, 41%) as yellow hygroscpic crystals. PMR spectrum (CD_3OD, δ, ppm, J/Hz): 2.572 (3H, s, CH_3), 3.406 [9H, s, $N^+(CH_3)_3$], 3.6 (1H, d, J = 18, H^1-2), 3.77 (1H, d, J = 14, $PhCH^1$), 3.8 (1H, d, J = 18, H^2-2), 3.82 (1H, d, J = 14, $PhCH^2$), 3.94 (1H, m, C<u>H</u>N^+Me_3), 3.99 (1H, m, C<u>H</u>N^+Me_3), 4.85 (1H, m, OCH), 4.93 (1H, m, OCH), 5.27 (1H, d, J = 4.5, H-6), 5.87 (1H, d, J = 4.5, H-7), 7.5 (5H, m, Ph).

Cephalosporin G Amide (6). A solution of **1** (3.4 g, 10.2 mmol) in THF (80 mL) was cooled to -35°C and treated with Et_3N (1.42 mL, 10.4 mmol) and isobutylchloroformate (1.42 mL, 10.4 mmol). A precipitate of triethylamine hydrochloride started to form after 1-2 min. The mixture was stirred at this temperature for 25 min and treated with a solution of saturated aqueous ammonia (2 mL) in water (8 mL). Triethylamine hydrochloride dissolved. A white crystalline precipitate began to form after 1 min. The mixture was stirred for 25 min at -15°C. The precipitate was filtered off and washed with methanol, acetone, and ether to afford **6** (2.82 g, 83%), white crystalline powder, mp 258-261°C. Mass spectrum (CCA, *m/z*): 331 $[M]^+$. PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 1.98 (3H, s, CH_3), 3.295 (1H, d, J = 18, H^1-2), 3.46 (1H, d, J = 18, H^2-2), 3.52 (1H, d, J = 13.5, $PhCH^1$), 3.61 (1H, d, J = 13.5, $PhCH^2$), 4.926 (1H, d, J = 5, H-6), 5.49 (1H, dd, J = 5, $J_{(NH)H}$ = 8.5, H-7), 7.28 (5H, m, Ph), 7.47 (2H, d, J = 96.5, NH_2), 9.05 (1H, d, $J_{(NH)H}$ = 8.5, NH).

Cephalosporin G L-Glutamic Acid Amide Dipotassium Salt (7). A solution of **1** (5.1 g, 15.3 mmol) in THF (120 mL) was cooled to -35°C and treated successively with Et_3N (2.2 mL, 16.1 mmol) and isobutylchloroformate (2.2 mL, 16.8 mmol). A precipitate of triethylamine hydrochloride began to form after 1.5-2 min. The mixture was stirred for 40 min, allowing the temperature to rise to 15°C, then diluted with cold water (50 mL) and extracted with ethylacetate (400 mL). The organic layer was separated; washed with saturated $NaHCO_3$ solution, water, and brine; and dried over Na_2SO_4. The desiccant was filtered off. The filtrate was evaporated to afford the solid mixed anhydride of **1** and isobutylformate (5.9 g). The resulting anhydride was dissolved in methanol (270 mL), stirred, and treated in one portion with a mixture of L-Glu (1.68 g, 11.3 mmol) and K_2CO_3 (1.656 g) in water (12 mL). The resulting transparent solution developed a white precicpitate after several minutes and was stirred at room temperature for 50 min. The precipitate was filtered off, washed with methanol and ether, and dried in vacuo to afford **7** (5.92 g, 74%), white crystals, mp 245-246°C (dec.). PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 1.668 (1H, m, H-2′), 1.899 (1H, m, H-2′), 1.917 (3H, s, CH_3), 2.166 (1H, ddd, H-3′), 2.337 (1H, ddd, H-3′), 3.267 (1H, d, J = 18, H^1-2), 3.49 (1H, d, J = 18, H^2-2), 3.50 (1H, d, J = 13, $PhCH^1$), 3.57 (1H, d, J = 13, $PhCH^2$), 4.152 (1H, ddd, H-1′), 4.99 (1H, d, J = 5, H-6), 5.55 (1H, dd, J = 5, $J_{(NH)H}$ = 8.5, H-7), 7.281 (5H, m, Ph), 7.61 (1H, d, $J_{(NH)H}$ = 7, CONH), 9.04 (1H, d, $J_{(NH)H}$ = 8.5, BnCONH).

Cephalosporin G L-Arginine Amide (8). A solution of **1** (3.4 g, 10.2 mmol) in THF (80 mL) was cooled to -35°C and treated successively with Et_3N (1.42 mL, 10.4 mmol) and isobutylchloroformate (1.42 mL, 10.4 mmol). A precipitate of triethylamine hydrochloride began to form after 1.5-2 min. The mixture was stirred for 25 min and treated with a solution of L-arginine (1.83 g, 10.5 mmol) in water (11 mL). Triethylamine hydrochloride dissolved. A white crystalline precipitate began to form after 1 min. the mixture was stirred for 25 min at -15°C. The resulting precipitate was filtered off and washed with methanol, acetone, and ether to afford **8** (3.59 g, 72%), white crystalline powder, mp 242-245°C. PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 1.53 (2H, m, H_2-3′), 1.68 (1H, m, H-2′), 1.75 (1H, m, H-2′), 1.968 (3H, s, CH_3), 3.03 (2H, m, H_2-4′), 3.28 (1H, d, J = 17.5, H^1-2), 3.39 (1H, d, J = 17.5, H^2-2), 3.50 (1H, d, J = 14, $PhCH^1$), 3.57 (1H, d, J = 14, $PhCH^2$), 4.01 (1H, ddd, H-1′), 4.93 (1H, d, J = 4.5, H-6), 5.5 (1H, dd, J = 4.5, $J_{(NH)H}$ = 8.5, H-7), 7.29 (5H, m, Ph), 7.74 (1H, d, $J_{(NH)H}$ = 7.5, CONH), 9.0 (1H, d, $J_{(NH)H}$ = 8.5, BnCONH), 9.1 (1H, m, NH-1′).

Cephalosporin G Acid Fluoride (9). A suspension of tris-morpholinotrimethyldifluorosilicate (1.5 g) in THF (60 mL) at -50°C was stirred, treated with morpholinotrifluorosulfurane (6 mL) and dropwise with **1** (6 g, 18 mmol) in THF (60 mL), stirred for 30 min at -50°C, poured into water (200 mL), and extracted with ethylacetate (500 mL). The organic layer was separated, washed with water and brine, and dried over anhydrous Na_2SO_4. The desiccant was filtered off. The filtrate was evaporated. The solid was dissolved in $CHCl_3$ and purified by column chromatography over silica gel with elution by $CHCl_3$ and $CHCl_3$:CH_3COCH_3 (10:1). Fractions containing the acid fluoride were combined and evaporated. The solid was dissolved in acetone and diluted with three volumes of ether and two volumes of hexane. The resulting white powder was filtered off, washed with ether, and dried to afford **9** (4.5 g, 75%). ^{19}F NMR ($CDCl_3$): -110.9. PMR spectrum ($CDCl_3$, δ, ppm): 2.04 (3H, s, CH_3), 3.45 (4H, m, $PhCH_2$, H_2-2), 5.05 (1H, d, H-6), 5.62 (1H, m, H-7), 7.26 (5H, m, Ph), 9.14 (1H, d, NH).

REFERENCES

1. Yu. A. Ovchinnikov, *Bioorganic Chemistry* [in Russian], Prosveshchenie, Moscow (1987).
2. R. D. Beger, *Drug Discovery Today*, **11**, 429 (2006).
3. J.-L. Pan and M.-J. Syu, *Biochem. Eng. J.*, **23**, 203 (2005).
4. J. W. Grant and T. P. Smyth, *J. Org. Chem.*, **69**, 7965 (2004).
5. X. Tang, T. Cai, and P. G. Wang, *Bioorg. Med. Chem. Lett.*, **13**, 1687 (2003).
6. L. N. Markovskij, V. E .Pashinnik, and A. V. Kirsanov, *Synthesis*, **6**, 787 (1973).