[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF MONTREAL]

Protected Dipeptides Containing Cysteine, Glycine, Phenylalanine, and Tyrosine

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Protected dipeptides have been prepared in which the amino function is blocked with either the tosyl or the phthaloyl group, while the carboxyl group is protected as a benzyl or an ethyl ester.

Coupling is achieved by either the acid chloride or the dicyclohexylcarbodiimide method, in the case of tosylated derivatives, and by the mixed anhydride method with the phthaloylated intermediates.

The apparent interference by the tosyl group with certain peptide-forming reactions is mentioned.

This paper constitutes a preliminary report on some exploratory work carried out in this laboratory in conjunction with a proposed synthesis of a part of the insulin molecule. The work is still in progress, and will be reported in a subsequent paper.

Tosylated derivatives of many amino acids have already been prepared and their desirable properties have been described.[2,3] The advantages of using amino acid benzyl esters have also been discussed.[4] We were therefore led to investigate the concomitant use of both the tosyl and benzyl group in a given dipeptide.

S-Benzyl-L-cysteine (I) was a key intermediate for the synthesis of the cysteine-containing protected dipeptides to be described. This compound, prepared from either L-cysteine hydrochloride monohydrate[5] or L-cystine,[6] was found to have a 190° (lit. m.p. 208–211°,[7] 214°,[6] 222°[8]) was found to be satisfactory for use by its conversion to the following intermediates in good yield (Fig. 1).

In the coupling reactions, the mixed anhydride method was unsuccessful in every case where the tosyl group was in close proximity to the carboxyl group being so activated. Thus III could not be coupled with glycine benzyl ester by this method. The same was true of the attempted condensation of *N*-tosyl-L-tyrosine with *S*-benzyl-L-cysteine ethyl ester. Similarly, III could not be coupled with the sodium salt of L-asparagine, as described by Leach and Lindley[9] in the case of the analogous benzyloxycarbonyl ("carbobenzoxy") derivative. It would seem, therefore, that the tosyl group was interfering with the reaction in some unexplained way. In order to find out if this interference was due to the

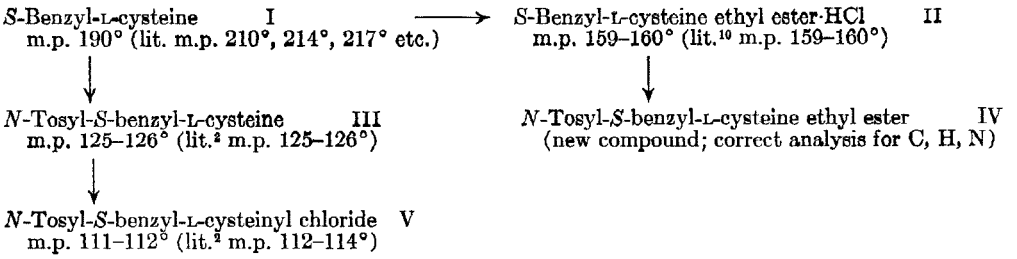

Figure 1

melting point which was invariably lower than that reported in the literature, and not substantially improved by repeated recrystallizations or prolonged drying *in vacuo* at 100°. Despite the melting point discrepancy, the product melting as low as tosyl group alone, or jointly to it and to the *S*-benzyl group on the cysteine residue, or to some adventitious cause, *N*-phthaloyl-L-phenylalanine was coupled by the mixed anhydride method, with glycine ethyl ester, and with *S*-benzyl-L-cysteine ethyl ester. The successful coupling of these residues would appear to substantiate the view that the tosyl group, and not the *S*-benzyl group, impedes mixed anhydride formation in the instances cited.

Another instance of the apparent interference by the tosyl group, encountered in this laboratory,

(1) Holder of a Research Scholarship, Office of Scientific Research (Quebec), 1959–1960, 1960–1961.

(2) J. Rudinger, *Coll. Czech. Chem. Comm.*, **19**, 375 (1954).

(3) J. Honzl and J. Rudinger, *Coll. Czech. Chem. Comm.*, **20**, 1190 (1955).

(4) K. C. Hooper, H. N. Rydon, J. A. Schofield, and G. S. Heaton, *J. Chem. Soc.*, 3148 (1956).

(5) R. A. Gortner and N. F. Hoffmann, *J. Biol. Chem.*, **72**, 433 (1926–1927).

(6) J. L. Wood and V. du Vigneaud, *J. Biol. Chem.*, **130**, 109 (1939).

(7) B. Hegedüs, *Helv. Chim. Acta*, **31**, 737 (1948).

(8) V. du Vigneaud *et al.*, *J. Am. Chem. Soc.*, **81**, 167 (1959).

(9) S. J. Leach and H. Lindley, *Aust. J. Chem.*, **7**, 173 (1954).

was the failure of III to form the corresponding *p*-nitrophenyl ester. Bodanszky and du Vigneaud[11] report that the corresponding *N*-benzyloxycarbonyl compound readily forms the *p*-nitrophenyl ester in high yield. A perusal of the available literature did not reveal any examples of *N*-α-tosyl-*p*-nitrophenyl esters. There has been however, at least one example of the preparation of a tosylated *p*-nitrophenyl ester in which the tosyl group was far removed from the carboxyl group (*cf.* *p*-nitrophenyl *N*-(α)-carbobenzoxy-*N*-(ε)-tosyl lysinate.[12] Thus it would seen that the tosyl group interferes with this reaction only if it is close to the carboxyl group.

The coupling reactions were standard procedures and require little further comment. In those instances where benzyl esters were coupled (see Table I), the dicyclohexylcarbodiimide method[17] was found to be more satisfactory with glycine benzyl ester, whereas the acid chloride method was better when phenylalanine benzyl ester was used.

TABLE I

Amino Acid Sequence[a]	Compound No.	Method of Preparation[b]	Approx. Yield, %
N-Ts-Cy(SBz)-Gly-OBz	XII	1; 2.	60; 87
N-Ts-Cy(SBz)-Phe-OBz	XIII	1; 2.	70; 60
N-Ts-Cy(SBz)-Gly-OC_2H_5	XIVA	2.	66
N-Ts-Cy(SBz)-Gly-OH	XIVB	—	90
N-Ts-Tyr-Cy(SBz)-OC_2H_5	XVA	2	66
N-Ts-Tyr-Cy(SBz)-OH	XVB	—	75
N-Phth-Phe-Gly-OC_2H_5	XVI	3	66
N-Phth-Phe-Cy(SBz)-OC_2H_5	XVII	3	53

[a] Abbreviations: Bz = $C_6H_5CH_2$—; DCC = C_6H_{11}—N=C=N—C_6H_{11}; Ts = *p*-$CH_3C_6H_4SO_2$—; Phth = 1,2-$(CO)_2C_6H_4$. [b] Preparative methods: 1. —COCl + H_2N^- → —CONH—. 2. —COOH + H_2N^- → —CONH. 3. —COOH + $ClCO_2C_2H_5$ + $(C_2H_5)_3N$ + H_2N^- → —CONH—.

EXPERIMENTAL

Melting points have been determined by the capillary tube method, and are uncorrected.

N-Tosyl-S-benzyl-L-cysteine ethyl ester (IV). This product was prepared from *S*-benzyl-L-cysteine ethyl ester hydrochloride[10] (5.5 g.; 0.02 mole) and *p*-toluenesulfonyl chloride (3.8 g.; 0.02 mole) by a procedure paralleling that of Fischer and Lifschitz,[13] and as used for the preparation of *N*-tosyl-L-tyrosine ethyl ester; yield 5.9 g. (75%) as fine needles.

Crystallization of the product was achieved from chloroform–petroleum ether (b.p. 30–52°), and then from alcohol-water. The product melted at 72–73°; $[\alpha]_D^{23}$ +35.6 (*c*, 1.14 in absolute ethanol).

Anal. Calcd. for $C_{19}H_{23}O_4NS_2$: C, 57.99; H, 5.89; N, 3.56. Found: C, 57.84; H, 5.88; N, 3.46.

N-Tosyl-S-benzyl-L-cysteinyl chloride (V). *N*-Tosyl-*S*-benzyl-L-cysteine[14] (10.5 g.; 0.0287 mole) was dissolved in chloroform (120 ml.) and the resulting solution dried over sodium sulfate. The dessicant was filtered and washed with chloroform (100 ml). Thionyl chloride (8 ml.) was added to the combined chloroform solutions. The solution was heated gently, and maintained at reflux for 15 min. After standing for 5 min. at room temperature, the pale yellow solution was treated with petroleum ether (300 ml. b.p. 65–110°; 100 ml., b.p. 30–52°). The product crystallized immediately as colorless flakes or needles, and it was filtered after standing for 1 hr. in the refrigerator; yield, 5 g. (45.5%); m.p. 107–108°, raised to 111–112° by recrystallization from the same solvents (lit.[2] m.p. 112–114°); $[\alpha]_D^{28.8}$ +14.2 (*c*, 1.16 in chloroform).

N-Tosyl-L-tyrosine[13] (VIII). L-Tyrosine was converted to L-tyrosine ethyl ester hydrochloride, m.p. 167–170° (yield quantitative), and thence to *N*-tosyl-L-tyrosine ethyl ester (yield 65.5%), m.p. 111.5–112.5° (lit.[13] m.p. 114°). Saponification of the latter ester gave VIII in 82% yield, m.p. 186–187° (lit.[13] m.p. 187–188°).

N-Phthaloyl-L-phenylalanine (XI). L-Phenylalanine (3.3 g; 0.02 mole) was phthaloylated by the method of Nefkens *et al.*[15]; yield of product, 4.4 g. (74.5%), m.p. 180–181° (lit.[16] m.p. 183–185°) after recrystallization from methanol-water.

N-Tosyl-S-benzyl-L-cysteinyl glycine benzyl ester (XII). A. *From the acid chloride* V. To a vigorously stirring solution of glycine benzyl ester *p*-toluenesulfonate[10] (6.4 g.; 0.02 mole) in chloroform (120 ml.) was added 5% sodium bicarbonate solution (40 ml.). After 0.5 hr. of stirring, the chloroform phase was separated, dried over sodium sulfate, the desiccant filtered, and triethylamine (2.8 ml; 0.02 mole) added. To this solution was added, over about 0.5 hr. a solution of V (7.3 g.; 0.019 mole) in chloroform (50 ml). After 2 hr. of stirring at room temperature, the reaction mixture was washed with water (50 ml.), 5% sodium bicarbonate (35 ml.), water (50 ml.), then dried over sodium sulfate. Petroleum ether (b.p. 30–52°) was added dropwise to the dried chloroform solution, to opalescence; crystallization was induced by scratching, and completed by standing in the refrigerator. The crude product (9.6 g.), was repeatedly recrystallized, to give 6.5 g. (63%) of product as lustrous needles, m.p. 101–102.5°, $[\alpha]_D^{28}$ −10.5 (*c*, 1.74 in chloroform).

B. *By the dicyclohexylcarbodiimide method.*[17] Glycine benzyl ester was prepared *in situ* as in A. To the stirred and cooled solution of the ester was added *N*-tosyl-*S*-benzyl-L-cysteine (7.3 g.). After 5 min. of cooling at −5°, was added 4.1 g. of dicyclohexylcarbodiimide. The reactant mixture was stirred for 0.5 hr. in the cooling bath, then for 3.5 hr. at room temperature. The precipitated dicyclohexylurea was filtered, petroleum ether (b.p. 30–52°) was added, the mixture was seeded with a few crystals of the authentic product, and the total volume of the crystallization liquor brought to about 1 l. with petroleum ether (b.p. 30–52°). The product formed as fine flakes throughout the flask, following an overnight stand in the refrigerator; weight, 8.7 g. (85%), m.p. 99–100.5°, $[\alpha]_D^{28}$ −10.5 (*c*, 1.74 in chloroform).

Anal. Calcd. for $C_{26}H_{28}O_5N_2S_2$ (512.592): C, 60.92; H, 5.51; N, 5.47. Found: C, 60.96; H, 5.48; N, 5.42.

N-Tosyl-S-benzyl-L-cysteinyl-L-phenylalanine benzyl ester (XIII). A. *From the acid chloride.* V. The product was prepared as in the case of the analogous compound XIIA, starting with L-phenylalanine benzyl ester *p*-toluenesulfo-

(10) J. A. Maclaren, W. E. Savige, and J. M. Swan, *Aust. J. Chem.*, **11**, 345 (1958).

(11) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).

(12) M. Bodanszky, J. Meienhofer, and V. du Vigneaud, *J. Am. Chem. Soc.*, **82**, 3195 (1960).

(13) E. Fischer and W. Lifschitz, *Ber*, **48**, 360 (1915).

(14) V. du Vigneaud, M. F. Bartlett, and A. Johl, *J. Am. Chem. Soc.*, **79**, 5572 (1957).

(15) G. H. L. Nefkens, G. I. Tesser, and R. J. F. Nivard, *Rec. trav. chim.*, **79**, 688 (1960).

(16) J. C. Sheehan, D. W. Chapman, and R. W. Roth, *J. Am. Chem. Soc.*, **74**, 3822 (1952).

(17) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).

nate[18] (8.55 g.; 0.02 mole). Addition of petroleum ether (b.p. 30–52°) to the washed reaction mixture, followed by prolonged cooling, gave 8.3 g. (69%) for a product melting between 83–84°. Recrystallization from ethyl acetate–petroleum ether (b.p. 65–110°) raised the melting point of the product to 88–89.5°; $[\alpha]_D^{28}$ +17.5 (*c*, 1.39 in chloroform); yield 6 g. (50%).

Anal. Calcd. for $C_{33}H_{34}O_5N_2S_2$ (602.750): C, 65.75; H, 5.69; N, 4.65. Found: C, 65.65; H, 5.65; N, 4.71.

B. *Using the dicyclohexylcarbodimide method.*[17] The compound was also prepared by the dicyclohexylcarbodiimide method in about a 60% crude yield, m.p. 80–81° (after one crystallization). This melting point agreed reasonably well with that of preparation XIIIA at this stage of purity, and the compound was not purified further, as it was prepared more conveniently by method A.

N-Tosyl-S-benzyl-L-cysteinyl glycine ethyl ester (XIVA). To a stirred suspension of III (7.3 g.; 0.02 mole) in methylene chloride (100 ml.) was added a solution of glycine ethyl ester hydrochloride (2.8 g.; 0.02 mole) in methylene chloride (80 ml.) containing triethylamine (2.8 ml.; 0.02 mole). After 5 min. of cooling in an ice-salt bath were added dicyclohexylcarbodiimide (4.1 g.; 0.02 mole) and dimethylformamide (40 ml.). The reactants were stirred for 0.5 hr. in the cooling bath, then overnight at room temperature. The dicyclohexylurea was filtered, and the filtrate was washed with water (3 × 75 ml.), 5% sodium bicarbonate (75 ml.), water (75 ml.), then dried over magnesium sulfate. Following evaporation of the solvents *in vacuo*, the partly crystalline residue was dissolved in absolute ethanol (50 ml.), with warming, then stored in the refrigerator where the product crystallized; yield, 6 g. (66%), m.p., 112–113°. Recrystallization from ethanol raised the melting point to a constant value of 113–114°, $[\alpha]_D^{29}$ −28.1 (*c*, 1.20 in absolute ethanol).

Anal. Calcd. for $C_{21}H_{26}O_5N_2S_2$ (450.566): C, 55.98; H, 5.82; N, 6.22. Found: C, 55.80; H, 5.67; N, 6.23.

N-Tosyl-S-benzyl-L-cysteinyl glycine (XIVB). Sodium hydroxide (2*N*; 6 ml.) was added dropwise over 20 min. to a stirred and ice-cooled solution of the ester XIVA (2 g.) in acetone (18 ml.). After 40 min. of stirring at room temperature, the reaction mixture was diluted with water (30 ml.), filtered, and then the filtrate was acidified while cooling in ice, to Congo Red with concentrated hydrochloric acid (1.1 ml.). The product crystallized after prolonged refrigeration; weight 1.9 g., m.p. 103–104°. Recrystallization of this product from ethanol-water raised the m.p. to 124–125° (fine needles); $[\alpha]_D^{26}$ −16.3 (*c*, 1.30 in absolute ethanol).

Anal. Calcd. for $C_{19}H_{22}O_5N_2S_2$ (422.514): C, 54.01; H, 5.25; N, 6.63. Found: C, 54.20; H, 5.02; N, 6.55.

N-Tosyl-L-tyrosyl-S-benzyl-L-cysteine ethyl ester (XVA). A solution of *S*-benzyl-L-cysteine ethyl ester (prepared *in situ* from 8.3 g. of II on treatment with 5% sodium bicarbonate as with XIIA in redistilled tetrahydrofuran (50 ml.), was stirred and cooled in an ice-salt bath. To this solution were added VIII (10 g.; 0.03 mole) in redistilled tetrahydrofuran (50 ml.) and then dicyclohexylcarbodiimide (6.2 g.; 0.03 mole). Stirring and cooling the reactants was continued for 4 hr. The precipitated dicyclohexylurea was filtered, and the filtrate was evaporated to dryness *in vacuo*. Ethyl acetate (200 ml.) was added to the oily residue, which was warmed slightly. The suspension was filtered. To the filtrate was added petroleum ether (b.p. 65–110°; 400 ml.) and the product was allowed to crystallize by standing in the refrigerator. There was obtained 8.3 g. of product, m.p. 135–136°.

For analysis, the product was recrystallized from warm ethyl acetate–petroleum ether (b.p. 30–52°), m.p. 139–140° C., $[\alpha]_D^{26}$ −33.8 (*c*, 1.13 in absolute ethanol).

Anal. Calcd. for $C_{28}H_{32}O_6N_2S$ (556.684): C, 60.41; H, 5.79; N, 5.03. Found: C, 60.39; H, 5.90; N, 5.04.

N-Tosyl-L-tyrosyl-S-benzyl-L-cysteine hemihydrate (XVB). Saponification of the ester XVA (12 g.) by the procedure used for XIVB, gave 8.5 g. (75%) of the product, m.p. 117–118° (from ethanol-water). $[\alpha]_D^{28}$ −23.6 (*c*, 1.10 in absolute ethanol).

Anal. Calcd. for $C_{26}H_{28}O_6N_2S_2 \cdot {}^1/_2\ H_2O$ (537.640): C, 58.08; H, 5.44; N, 5.21. Found: C, 57.90; H, 5.63; N, 4.94.

N-Phthaloyl-L-phenylalanyl glycine ethyl ester (XVI). To a solution of XI (2.95 g.; 0.01 mole) in methylene chloride (40 ml.) at 0°, was added triethylamine (1.4 ml.; 0.01 mole) and after 5 min., ethyl chloroformate (0.95 ml.). The reactants were stirred and cooled at 0° for 10 min., and then was added a solution of glycine ethyl ester hydrochloride (1.39 g.; 0.01 mole), and triethylamine (1.4 ml.; 0.01 mole) in methylene chloride (40 ml.). After stirring for 30 min. in the cooling bath, and 1 hr. at room temperature, the reaction mixture was washed with 5% sodium bicarbonate (2 × 25 ml.), then water (25 ml.), and dried over magnesium sulfate. Evaporation of the solvent *in vacuo* gave a crystalline residue, which was recrystallized from boiling absolute ethanol; yield 2.5 g. (66%); m.p. 160–161° (lit.[16,17] m.p. 160.6–161.4°).

N-Phthaloyl-L-phenylalanyl-S-benzyl-L-cysteine ethyl ester (XVII). This compound was prepared exactly as in the case of XVI, from 2.95 g. of XI and 2.77 g. of II; yield 2.7 g. (53%), m.p. 111.5–112.5° (from alcohol-water); $[\alpha]_D^{26}$ −141 (*c*, 1.09 in absolute ethanol).

Anal. Calcd. for $C_{29}H_{29}O_5N_2S$ (516.596): C, 67.42; H, 5.46; N, 5.42. Found: C, 67.47; H, 5.85; N, 5.14.

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(18) L. Zervas, M. Winitz, and J. P. Greenstein, *J. Org. Chem.*, 22, 1515 (1957).