Synthesis of TACK, a New Chloromethyl Ketone Derivative of Arginine[1,2]

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A synthesis is described of TACK, the chloromethyl ketone derivative of N^{α}-tosyl-L-arginine, in which a crystalline intermediate N^{G}-nitro-TACK is found to be converted into TACK with hydrogen fluoride without appreciable impairment of the chloromethyl ketone moiety.

TLCK is an active site-directed reagent for trypsin, first described by Shaw *et al.*[3] Their initial synthesis involves the hydrolytic removal of a benzyloxycarbonyl group from the N^{ε}-protected derivative to lead to the formation of TLCK in a rather low yield. Recently Shaw and Glover[4] have improved the yield greatly by the use of trifluoroacetic acid[5] in this deprotection step. A trifluoroacetic acid-hydrochloric acid mixture was also used by other authors.[6] For the same purpose we tried to use the hydrogen fluoride treatment[7] in which the treatment was found to cause no detectable damage in the chloromethyl ketone moiety of TLCK, affording the reagent in a good yield.[8] This finding prompted us to extend the use of hydrogen fluoride to the synthesis of N^{α}-tosyl-L-arginine chloromethyl ketone (TACK). The only chloromethyl ketone derivative of arginine ever known is *p*-nitrobenzyloxycarbonyl-L-arginine chloromethyl ketone (Z(NO_2)ACK) which has been prepared by Shaw and Glover.[4] Attempts we have made to obtain TACK are as follows:

$$\text{H-Arg(R)-OH} \xrightarrow{\text{Tos-Cl}} \text{Tos-Arg(R)-OH} \xrightarrow{PCl_5}$$

$$\text{Tos-Arg(R)-Cl} \xrightarrow{CH_2N_2} \text{Tos-Arg(R)-CHN}_2 \xrightarrow{\text{HCl}}$$

$$\text{Tos-Arg(R)-CH}_2\text{Cl} \xrightarrow{\text{HF}} \text{Tos-Arg-CH}_2\text{Cl (TACK)}$$

where R is either a tosyl or a nitro group, which is known to be split with hydrogen fluoride.[7,9] When R=NO_2, the penultimate compound Tos-Arg(NO_2)-CH_2Cl was crystalline and was found to be fairly stable. In the case of R=Tos, however, all the intermediates were amorphous and their purification seemed to be of trouble. In this communication we wish to report our TACK synthesis starting from nitroarginine.

TACK, prepared in the present work, was homogeneous in thin-layer chromatography. The chlorine content of TACK hydrochloride has remained unchanged at least for six months, provided that the preparation has been kept dry and refrigerated. The inhibition studies of enzymes with TACK will be described in a separate communication.[10]

Experimental

All melting points were uncorrected.

N^{α}-p-*Toluenesulfonyl*-N^{G}-*nitro*-L-*arginine (I).* Nitroarginine (5.50 g, 25 mmol) was dissolved in 2M sodium hydroxide (37.5 ml), and sodium carbonate (2.65 g, 25 mmol) and acetone (25 ml) were introduced. To this was then added dropwise a solution of *p*-toluenesulfonyl chloride (7.15 g, 37.5 mmol) in acetone (20 ml) at 0 °C. The mixture was stirred at 0 °C for 3.5 hr. After removal of acetone by evaporation *in vacuo*, the aqueous solution was acidified with 4 M hydrochloric acid. The crystalline precipitates which separated were filtered off, washed with water and dried *in vacuo* (8.16 g, 88%). Recrystallization from methanol-water gave the desired product in pure form; wt, 7.39 g (79%), mp 167—169 °C, $[\alpha]_D^{22}+23.6\pm0.7°$ (*c* 1.0, methanol).

Found: C, 42.05; H, 5.20; N, 18.74; S, 8.75%. Calcd for $C_{13}H_{19}N_5O_6S$: C, 41.82; H, 5.13; N, 18.76; S, 8.59%.

N^{α}-p-*Toluenesulfonyl*-N^{G}-*nitro*-L-*arginyl Diazomethane (III).* A solution of I (0.75 g, 2 mmol) in anhydrous tetrahydrofuran (10 ml) was chilled in an ice-salt bath and to this was added phosphorus pentachloride (0.84 g, 4 mmol) in several portions. The mixture was stirred for 60 min during which the temperature was allowed to rise to 0 °C. Anhydrous ether (80 ml) was then introduced to yield sirupy precipitates. The supernatant solution was removed by decantation and the residue was triturated with ether and dried *in vacuo* over sodium hydroxide pellets at 4 °C to give N^{α}-*p-toluenesulfonyl*-N^{G}-*nitro*-L-*arginyl chloride (II)* as amorphous solid (0.47 g, 60%); IR (Nujol) 1790 cm^{-1}. A small sample was dissolved in methanol and this was subjected to tlc (Silica gel GF, E. Mreck) in methanol-chloroform (2 : 8 by vol) to give a single component which was indistinguishable from the authentic methyl ester of I.

The chloride (II) obtained above was dissolved in anhydrous tetrahydrofuran (10 ml) and to this solution was added an ethereal solution of diazomethane at 0 °C until an yellow color persisted. The reaction mixture was stirred at 0 °C for 60 min, after which time anhydrous ether (80 ml) was introduced. The resulting precipitates were filtered off, washed with ether and dried *in vacuo* to give III; wt, 0.36 g (45%, as based on I used), IR (chloroform) 2145 cm^{-1}, $[\alpha]_D^{22}$ $-51.3\pm1.0°$ (*c* 1.0, methanol). The product was used for the subsequent reaction without further purification.

N^{α}-p-*Toluenesulfonyl*-N^{G}-*nitro*-L-*arginyl Chloromethane (IV).* To a 0.25 g-sample of III was added M hydrogen chloride in acetic acid (10 ml) to cause the reaction to occur with the instant evolution of nitrogen. The mixture was kept at room temperature for 60 min, followed by evaporation *in vacuo* at a bath temperature of 35 °C. The residue was then lyophilized from acetic acid. The resulting crude preparation of IV was submitted to a column of silica-gel (0.05—0.2 mm, E. Merck, 25 g) using a mixture of methanol-chloroform (1 : 9 by vol) as a solvent for elution. Tubes 17—30 (5 g/tube) containing the desired product as a main component (as examined by tlc) were collected and evaporated *in vacuo* to give a sirupy residue which crystallized upon addition of chloroform (0.12 g, 49%). Recrystallization from methanol yielded a pure and odorless preparation of IV in an 86% recovery; mp 165—167 °C decomp., $[\alpha]_D^{24}$ $-23.7\pm0.7°$ (*c* 1.0, dimethylformamide), IR (Nujol) 1745 cm^{-1}.

Found: C, 41.64; H, 5.11; N, 17.34; S, 8.02; Cl, 8.80%. Calcd for $C_{14}H_{20}N_5O_5SCl$: C, 41.43; H, 4.97; N, 17.26; S, 7.90; Cl, 8.74%.

N^{α}-p-*Toluenesulfonyl*-L-*arginyl Chloromethane* (N^{G}-*Tosyl*-L-*arginine Chloromethyl Ketone, TACK) Hydrochloride (V).* Compound IV (0.18 g) and anisole (0.2 ml) were put into a reaction vessel (made of fluorinated polyethylene) and hydrogen

fluoride (*ca.* 5 ml) was introduced into the vessel placed in a dry ice-acetone bath. The mixture was stirred at 0 °C for 30 min. After removal of hydrogen fluoride by evaporation *in vacuo* the residue was dissolved in water and the solution was, after washing with ethyl acetate, passed through a column (0.9×10 cm) of Amberlite CG-400 (acetate form); the column was washed with portions of water. The aqueous solutions were combined and M hydrochloric acid (0.5 ml) was added. Lyophilization of the resulting solution gave the desired TACK hydrochloride; wt, 0.18 g (98%), $[\alpha]_D^{25}$ $-21.2\pm0.6°$ (*c*, 1.0, 0.1 M hydrochloric acid), IR (KBr) 1737 cm^{-1}. Tlc revealed that the product was homogeneous to Sakaguchi reagent (Cellulose F, E. Merck, 1-butanol–acetic acid–water (18 : 2 : 5 by vol)).

Found: C, 40.95; H, 5.66; N, 13.13; S, 7.17; Cl, 17.95%. Calcd for $C_{14}H_{22}N_4O_3SCl\cdot HCl\cdot H_2O$: C, 40.48; H, 5.82; N, 13.49; S, 7.72; Cl, 17.07%.

References

1) Presented at the 44th Annual Meeting of the Japanese Biochemical Society, Sendai, Oct. 1971.

2) Abbreviations used in this work are: TLCK, N^{α}-*p*-toluenesulfonyl-L-lysine chloromethyl ketone, N^{α}-*p*-toluenesulfonyl-L-lysyl chloromethane or L-1-chloro-3-tosylamido-7-aminoheptan-2-one; TACK, N^{α}-*p*-toluenesulfonyl-L-arginine chloromethyl ketone, N^{α}-*p*-toluenesulfonyl-L-arginyl chloromethane or L-1-chloro-3-tosylamido-6-guanidinohexan-2-one; Z, benzyloxycarbonyl; Tos, *p*-toluenesulfonyl or tosyl.

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