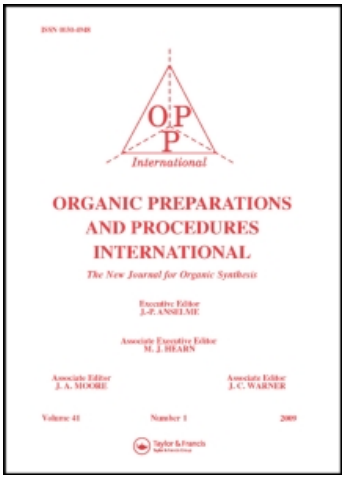

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THIOL ESTERS OF N$^{\alpha}$-TOSYL-L-ARGININE

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THIOL ESTERS OF N^{α}-TOSYL-L-ARGININE

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A series of thiol esters of N^{α}-tosyl-L-arginine was made for study as enzyme substrates. These compounds are useful for determining enzymatic hydrolysis rates, because the liberated thiols can be measured spectrophotometrically using the reagent 5,5'-dithiobis(2-nitrobenzoic acid).[1] Although thioesters of several other blocked amino acids are described in the literature,[2-4] no examples of arginine with the guanidino group blocked or unblocked have been reported. Preliminary work with N^{α}-benzoyl and N^{α}-tosyl-L-arginine using the DCC coupling method[5] gave mixtures of the desired thioesters which were very difficult to purify. Likewise, attempted thioester formation *via* the mixed anhydride method[6] or with tris(ethylthio) borane[7] proved to be unsatisfactory. We discovered that the known but little used method[8] of making blocked arginine acid chloride hydrochloride offered a satisfactory route to the arginine thioesters.

$$N^{\alpha}\text{-Tos-L-Arg-OH} \xrightarrow[2.\ RSH]{1.\ SOCl_2} N^{\alpha}\text{-Tos-L-Arg-SR}$$

In this procedure N^{α}-tosyl-L-arginine was treated directly with thionyl chloride to form the crude acid chloride hydrochloride, and this ether-washed salt was allowed to react with excess mercaptan in DMF to form the thioester hydrochloride. The product was precipitated with anhydrous ether, and purified by silica gel chromatography using acetonitrile-water as the eluant. The final isolation was accomplished by partial concentra-

tion followed by freeze-drying to give a powder. The ethyl, isopropyl, isobutyl and benzyl thioesters were made and were characterized by nmr and by their trypsin-catalyzed hydrolysis. These esters were effective substrates for trypsin, showing relative hydrolysis rates, in decreasing rate order, of benzyl>isobutyl>ethyl>isopropyl. The yield of thiol from the trypsin catalyzed hydrolysis of the above thiol esters as measured by 5,5'-dithiobis-(2-nitrobenzoic acid) was 99-100% except for the isopropyl ester (89%). The high yield of enzymatically cleaved thiol indicates that little if any racemization has occurred in these conversions since only L-amino acids are trypsin substrates.[9]

EXPERIMENTAL

Purifications were followed by tlc on silica using acetonitrile-water (9-1). The nmr spectra were run at 60 MHz in D_2O or CD_3OD solvent.

N^{α}-(p-Toluenesulfonyl)-L-arginine Ethanethiol Ester Hydrochloride.- To N^{α}-(p-toluenesulfonyl)-L-arginine $\cdot 3\ H_2O$[10] (7 g; 0.018 mole) was added 45 ml of $SOCl_2$, and the mixture was promptly cooled to 15-20° and stirred vigorously for about 7 min, during which the solid dissolved and two liquid phases formed. The temperature was lowered to about -15° and slower stirring was continued for 8 min. Anhydrous ether was added and the mixture stirred slowly to precipitate a gum. The cold supernatant solution was decanted, and the residue triturated three times with anhydrous ether. Ethyl mercaptan (20 ml) was added to the crude acid chloride, cooled in a 15° bath, followed by 23 ml of DMF. The mixture was allowed to warm to room temperature, and stirring continued for 45 min. Anhydrous ether (150 ml) was added to precipitate the thiol ester hydrochloride as a gum, the ether solution was decanted, and the gum was treated with CH_2Cl_2 (15 ml). The product was reprecipitated by addition of anhydrous ether and cooling. The residue was transferred to a silica column (3.8 x 33 cm) and the product eluted with 96% acetonitrile-4% water. After collecting an initial

500 ml of eluant, the product was obtained in the next 200 ml. The product was rechromatographed in essentially the same manner to obtain good quality product, which was concentrated in vacuo and freeze-dried to give a 31% yield of a pure white solid. This ethanethiol ester hydrochloride was a hygroscopic powder showing R_f 0.40; $[\alpha]_D^{25}$ -44 (c 1.8, water).

Anal. Calcd. for $C_{15}H_{25}N_4O_3S_2Cl \cdot 1/2\ H_2O$: C, 43.10; H, 6.03; N, 13.40; Cl, 8.48. Found: C, 43.01; H, 6.09; N, 13.36; Cl, 8.62.

The following esters were prepared by procedures closely analogous to that described above.

N^{α}-(p-Toluenesulfonyl)-L-arginine 2-Propanethiol Ester Hydrochloride.- This substance was a solid amorphous, hygroscopic powder, R_f 0.33, $[\alpha]_D^{25}$ -26 (c 2, H_2O).

Anal. Calcd. for $C_{16}H_{27}ClN_4O_3S_2 \cdot 1/2\ H_2O$: C, 44.48; H, 6.53; N, 12.97. Found: C, 44.16; H, 6.42; N, 13.00. This analysis was corrected for 3.53% ash (SiO_2?).

N^{α}-(p-Toluenesulfonyl)-L-arginine 2-Methyl-1-propanethiol Ester Hydrochloride.- This compound was isolated as a crystalline, hygroscopic powder, m.p. 70° dec., R_f 0.35, $[\alpha]_D^{25}$ -29 (c 2, H_2O).

Anal. Calcd. for $C_{17}H_{29}ClN_4O_3S_2 \cdot H_2O$: C, 44.85; H, 6.86; N, 12.31. Found: C, 45.04; H, 6.78; N, 12.42.

N^{α}-(p-Toluenesulfonyl)-L-arginine Phenylmethanethiol Ester Hydrochloride.- The title compound was isolated as a hygroscopic powder after freeze-drying. After careful silica gel chromatography, tlc showed a small amount of N^{α}-tosyl-arginine as an impurity to be present.

Anal. Calcd. for $C_{20}H_{27}ClN_4O_3S_2 \cdot 2\ H_2O$: C, 47.37; H, 6.16; N, 11.08. Found: C, 47.00; H, 5.48; N, 11.42.

Enzymatic Hydrolysis of the Tosylarginine Thiol Esters.- 5,5'-Dithiobis-(2-nitrobenzoic acid) was dissolved in 0.1 M K_2HPO_4 pH 7.4 buffer which was 0.1% in gelatin to make a 1.6 x 10-4 M solution. The thiol ester was

added to make the solution 10^{-4} M in substrate and a trypsin solution (10^{-4} M) added to make the solution about 2.5 x 10^{-7} M in trypsin. The hydrolysis of the thiol ester was followed by the increase in absorbance at 412 nm. The anion of 2-nitro-5-mercaptobenzoic acid has an absorbance of 14,000/mole at 412 nm. A blank containing buffer, Ellman's reagent and substrate showed little or no absorbance at 412 nm. The reaction was complete in 2-5 minutes and showed no change from the initial rate over this time period. The yields of released thiol from the thiol esters were 99-100% except for the isopropyl ester (89%).

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