# Tosylation and Dansylation of Tyrosine Residues of Proteins with 1-Arylsulphonyl-4-dimethylaminopyridinium Salts

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*Summary* 1-Tosyl- and 1-dansyl-4-dimethylaminopyridinium chlorides are easily prepared and may react selectively with tyrosine residues of proteins in aqueous media, leading to stable *O*-arylsulphonates.

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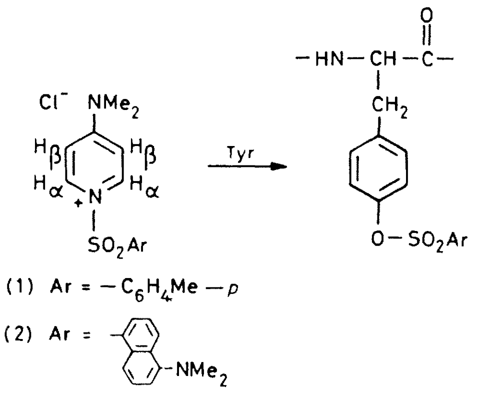
THE arylsulphonylated derivatives of 4-dimethylaminopyridine (DMAP) are easily prepared by adding a 0·1M solution of the arylsulphonyl chloride in dry EtOAc to a stirred solution containing 1 mol. equiv. of DMAP at 0 °C. After filtration and washing, the reagents, obtained in about 90% yield, can be stored in the cold for several months; they had the following properties: tosyl-DMAP$^+$-Cl$^-$ (**1**) (from tosyl chloride): m.p. (decomp.) 124 °C; $\nu_{max}$ (Nujol) 1635 (C=N), 1340, and 1180 cm$^{-1}$ ($SO_2$); $\lambda_{max}$ ($H_2O$) 303 nm (log $\epsilon$ 4·41); $\delta$ ($CDCl_3$) 2·40 (3H, s, ArMe), 3·42 (6H, s, $NMe_2$), 7·95 (2H, d, $H_\beta$), and 8·65 (2H, d, $H_\alpha$); $t_{\frac{1}{2}}$ (hydrolysis at pH 6·0 and 36 °C) 14·5 h; dansyl-DMAP$^+$Cl$^-$ (**2**) (from dansyl chloride): m.p. (decomp.) 95 °C; $\nu_{max}$ (KCl) 1635 (C=N), 1325, 1365, and 1165 cm$^{-1}$ ($SO_2$); $\lambda_{max}$ ($H_2O$): 303 (log $\epsilon$ 4·48) and 350 nm (log $\epsilon$ 3·78); $\delta$ ($CDCl_3$) 2·80 (6H, s, $ArNMe_2$), 3·28 (6H, s, $pyrNMe_2$), 7·40 (2H, d, $H_\beta$), and 8·50 (2H, d, $H_\alpha$); $t_{\frac{1}{2}}$ (hydrolysis at pH 6·0 and 36 °C) 11·0 h.



(1) Ar = $-C_6H_4Me-p$

(2) Ar =

Water-soluble acylated derivatives of DMAP have been found to be useful for the rapid specific chemical modification of protein residues at different pH values [1,2] The 1-tosyl and 1-dansyl derivatives (**1**) and (**2**) show a similar reactivity towards nucleophiles as that of 1-methoxy carbonyl-4-dimethylaminopyridinium chloride *i e* (a) the rates of reaction of amines and phenols are much greater than the rate of hydrolysis at the same pH, (b) the rate of modification of tyrosyl residues is greater than that of the α- and ε-amino groups at pH ⩾ 9 [3]

Modifications of amino-acids using (**1**) were investigated for chains A and B of oxidized bovine insulin and calcitonins † Experiments were performed at pH 10—11 5 in 0 02M lithium carbonate by addition, in three portions, of a 2—3 times excess of reagent at 5 °C in 20 min, amino-acid analysis‡ showed that 70—80% of the Tyr residues were modified, compared with only 0—10% of Lys and 0% of terminal amino residues In the case of human calcitonin the modification of Tyr 12 leads to an important decrease of hypocalcaemic activity The analogous modification of salmon calcitonin (Tyr 22) does not seem to modify the hypocalcaemic activity of this hormone

Dansylations§ with a large excess of (**2**) at pH 3 lead to the slow modification of only Tyr A 19 of bovine insulin, two tyrosine residues of prolactin and four tyrosine residues of bovine serum albumin [4]

At pH 7 the number of Tyr residues modified depends on the amount of excess of (**2**) With a twofold excess of reagent per nucleophilic residue at pH 9 up to 7 dansyl (or tosyl) groups may be introduced in the bovine insulin molecule

Preliminary results on mouse mammary tumour cell membranes have shown that compound (**2**) may be used for the labelling of membranes

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† We have observed that the tosyl derivative of *N* methylimidazole (C Jozefczak G Bram and M Vilkas *C R Acad Sci Ser C* 1970 **271** 553 P Monjoint and M Laloi Diard *Bull Soc Chim Fr* 1973 2357) is much less efficient than (**1**) for the modification of tyrosine residues of proteins

‡ Peptides were hydrolysed in 5 7 M HCl at 110 °C (24 h) Under these conditions the recovery of free tyrosine from the modified residue was found to be about 3—5%

§ U v spectroscopy gives some indication of the selectivity of dansylation $\lambda_{max}$ dansyl OAr 345 $\lambda_{max}$ dansyl NHR 329 nm (N Seiler and M Wiechmann *Z Anal Chem* 1966 **220** 109) For the dansylation of both amino and tyrosine residues with dansyl chloride in heterogeneous media see W R Gray *Methods Enzymol* 1967 **11** 139 H R Horton and D E Koshland *ibid* p 856 C Gros and B Labouesse *Eur J Biochem* 1969 **7** 463

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[2] M Wakselman E Guibe Jampel A Raoult and W D Busse *J Chem Soc Chem Commun* 1976 21
[3] G Guillot Edelheit M Laloi Diard E Guibe Jampel and M Wakselman *J Chem Soc Perkin Trans* 2 1979 1123
[4] E Guibe Jampel and F H Carpenter unpublished results