

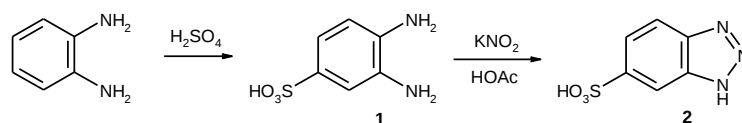
6-SULFOBENZOTRIAZOLE AS A NEW REAGENT FOR AMIDE BOND FORMATION IN AQUEOUS MEDIUM

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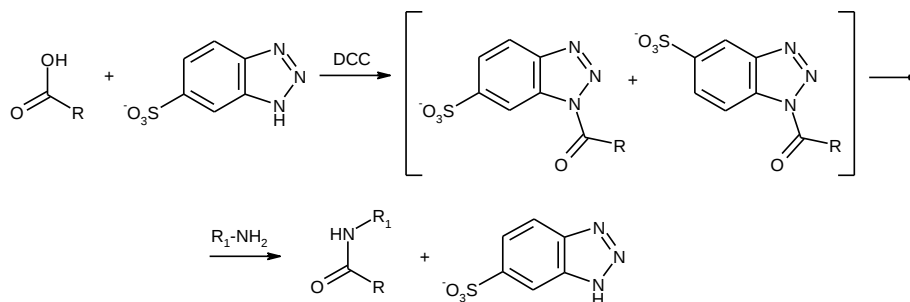
Covalent labeling and conjugation of biomolecules is most commonly performed by active ester approach via the formation of amide bond. But this method requires active esters sufficiently soluble in aqueous or aqueous-organic medium. This can be achieved using the esters containing anionic or cationic functional groups, e.g. sulfonic or ammonium-type functions. Additional requirement for active esters to be used in aqueous conditions is low rate of their hydrolysis at pH 8–8.5 typical for biomolecular coupling reactions. Here we propose the use of sulfobenzotriazolides as new efficient reagents for amide synthesis. N-acylsulfobenzotriazoles are stable in water, and at the same time demonstrate high reactivity towards aliphatic amines due to the presence of a good leaving group that allows amide bond formation under mild reaction conditions in aqueous-organic solutions.

The synthesis of 6-sulfobenzotriazole (Scheme 1) started from the sulfonation of *o*-phenylene diamine with conc. sulfuric acid. Its cyclization to form benzotriazole ring was then performed by diazotization reaction with potassium nitrite and acetic acid. Sulfobenzotriazole was isolated by column chromatography. Ion-exchange chromatography on QAE-Sephadex provided free acid **2**, whereas reverse-phase chromatography on TMS-silica allowed to obtain its triethylammonium salt.



Scheme 1. Synthesis of 6-sulfobenzotriazole.

The mechanism of coupling reaction using 6-sulfobenzotriazole is shown in Scheme 2. Carboxylic acid is first activated with a suitable condensing reagent (for example, N,N'-dicyclohexylcarbodiimide, DCC) in the presence of sulfobenzotriazole salt. Two isomers of water-soluble N-acylsulfobenzotriazole active intermediate can be formed. After the addition of corresponding amine fast nucleophilic substitution reaction (~one hour) results in the smooth formation of amide bond.



Scheme 2. Mechanism of amide formation in the presence of sulfobenzotriazole

We have used sulfobenzotriazole for the introduction of carboxyalkyl linkers into the coumarin dyes to prepare fluorescent labeling reagents (see the accompanying abstract).