NEW WATER-SOLUBLE DERIVATIVES OF 7-HYDROXY-3-HETARYL COUMARINS FOR THE FLUORESCENT LABELING OF BIOMOLECULES

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Fluorescent labeling of biomolecules is widely used in both life sciences and medical diagnostics. We have previously synthesized a number of blue-emitting coumarin-based reagents by the introduction of carboxyalkyl groups into 7-hydroxy-3-hetarylcoumarins, and corresponding 7-methoxy derivatives. They are suitable for the labeling of compounds containing aliphatic amino groups. However, the disadvantage of these derivatives as reagents for the conjugation with biomolecules being typically performed in aqueous medium is their poor solubility in water. Only 7-hydroxycoumarin derivatives are soluble at high pH. Here we propose a new way to increase water solubility of coumarin reagents by their derivatization with 1,3-propanesultone or cysteic acid.


Synthesis of cysteic acid derivatives 3a-d and 4d started with blocking the phenolic hydroxyl of compounds 1a-d with Ac₂O (Scheme, step (i)). Resulting acylated coumarins 2a-d were activated with DCC and HOBt and then condensed with cysteic acid methyl ester in the presence of diisopropylethylamine with subsequent ester hydrolysis by NaOH (ii) in one-flask process. Dye 4d with COOH group on an extended linker was prepared by the activation of 3d with DCC and 6-sulfobenzotriazole followed by the condensation with ε-aminocaproic acid methyl ester and alkaline hydrolysis of methyl ester group (iii). 6-Sulfobenzotriazole synthesis and use for amide bond formation is described in our accompanying abstract.

Sulfopropyl derivatives 5a-d were obtained by alkylating compounds 1a-d with 1,3-propanesultone in the presence of K₂CO₃ (iv). 3-Furylcoumarin reagent 6e was prepared from 5d via its activation with DCC and 6-sulfobenzotriazole followed by condensation with methyl aminocaproate and ester group cleavage by NaOH (v).

Compounds 2a-d were purified by silica column chromatography, whereas reverse-phase chromatography on TMS-silica was used for target dyes containing the sulfonate group. These compounds were obtained as triethylamine and potassium salts and fully characterized by ¹H NMR and LC-MS. Coumarin derivatives 3-6 are well soluble in water and can be used as reagents for the fluorescent labeling of proteins, nucleic acids and other biomolecules.