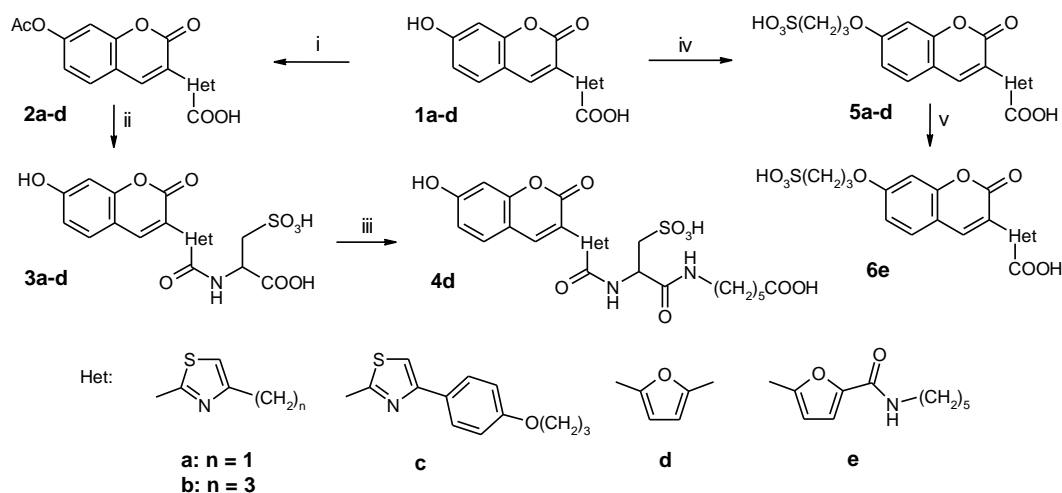


**NEW WATER-SOLUBLE DERIVATIVES OF 7-HYDROXY-3-HETARYL  
COUMARINS FOR THE FLUORESCENT LABELING OF BIOMOLECULES**

*Kuziv Ia. B., Dubey I. Ya.*

Institute of Molecular Biology and Genetics, NAS of Ukraine,  
150 Zabolotnogo str., 03143 Kyiv, Ukraine  
ya.b.kuziv@imbg.org.ua

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 **1** 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.



Scheme. Synthesis of water-soluble derivatives of 3-hetarylcoumarins.

Synthesis of cysteic acid derivatives **3a-d** and **4d** started with blocking the phenolic hydroxyl of compounds **1a-d** with  $\text{Ac}_2\text{O}$  (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  $\epsilon$ -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  $\text{K}_2\text{CO}_3$  (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  $^1\text{H}$  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.