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FORMATION OF CARBOXAMIDES WITH N,N,N',N'-TETRAMETHYL (SUCCINIMIDO) URONIUM TETRAFLUOROBORATE IN AQUEOUS / ORGANIC SOLVENT SYSTEMS

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Abstract: N,N,N',N'-tetramethyl (succinimido) uronium tetrafluoroborate can be employed for an effective and fast formation of carboxamides even in mixed aqueous/organic solvent systems. In this respect it represents an alternative to carbodiimide reagents applied for the same purpose.

One of the standard procedures for the preparation of carboxamides proceeds via activated esters of the carboxy group which react then with an amino group to yield the corresponding carboxamides[1]. The activated esters commonly envisaged for this purpose are e.g. pentachlorophenyl-, p-nitrophenyl-pentafluorophenyl-, and especially the hydroxysuccinimido-esters, which proved to be fairly stable in aqueous solutions[1]. They are usually prepared by the action of dicyclohexylcarbodiimide (DCC).

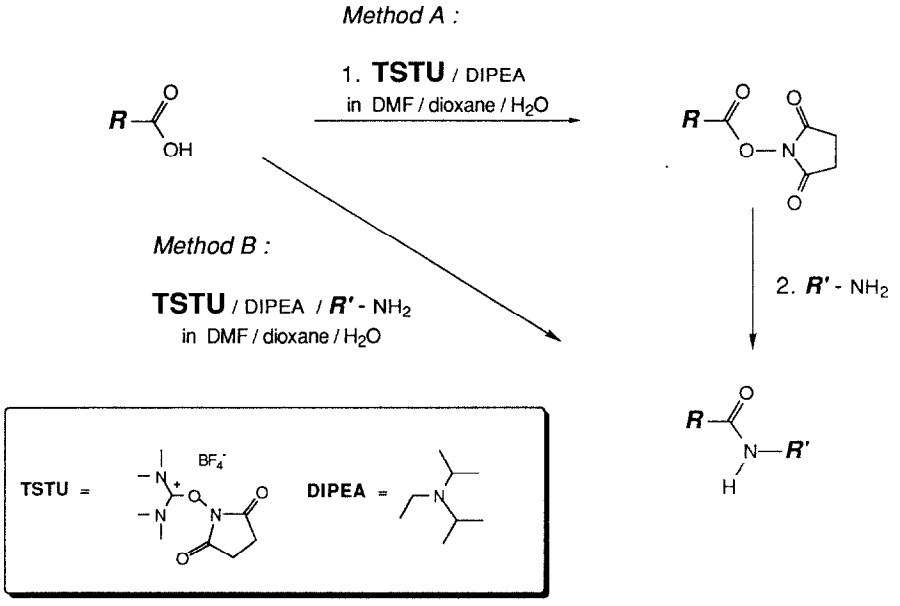
Recently we have reported that N,N,N',N'-tetramethyl (succinimido) uronium tetrafluoroborate (TSTU), a compound which had been used for the hardening of gelatine[2], can also be employed for the transfer of carboxy groups into their corresponding hydroxysuccinimido esters[3,4]. The reaction proceeded very fast and efficiently with only a slight excess of TSTU.

We have applied TSTU to the coupling of bathophenanthroline-ruthenium(II) complexes bearing a carboxy group to 5'-amino-modified oligonucleotides [3]. The reaction was carried out in two steps. Firstly, activation and thereby the transformation to the activated ester was performed in DMF and subsequently, the coupling reaction had to be carried out in a mixture of DMF/dioxane/water in order to ensure good solubility of all the components, i.e. the lipophilic Ru (bathophenathroline) complex and the hydrophilic 5'-amino-modified oligonucleotide.

Now we have found that TSTU is able to transfer carboxy functions into their corresponding hydroxysuccinimido esters even in the presence of water. Furthermore, we realized that with TSTU the coupling reactions between carboxy groups and primary amines can also be directly performed also *in situ* in the presence of water. Thus TSTU represents an useful alternative to the established carbodiimides employed for the same purpose. Problems were encountered if the activation of α-subtituted carboxyl compounds (e.g. α-amino acids) was carried out in the

presence of water. There we recommend a preactivation in a waterfree organic solvent, whereas the subsequent coupling to the amino compound can be performed again in mixed organic / aqueous solvent systems.

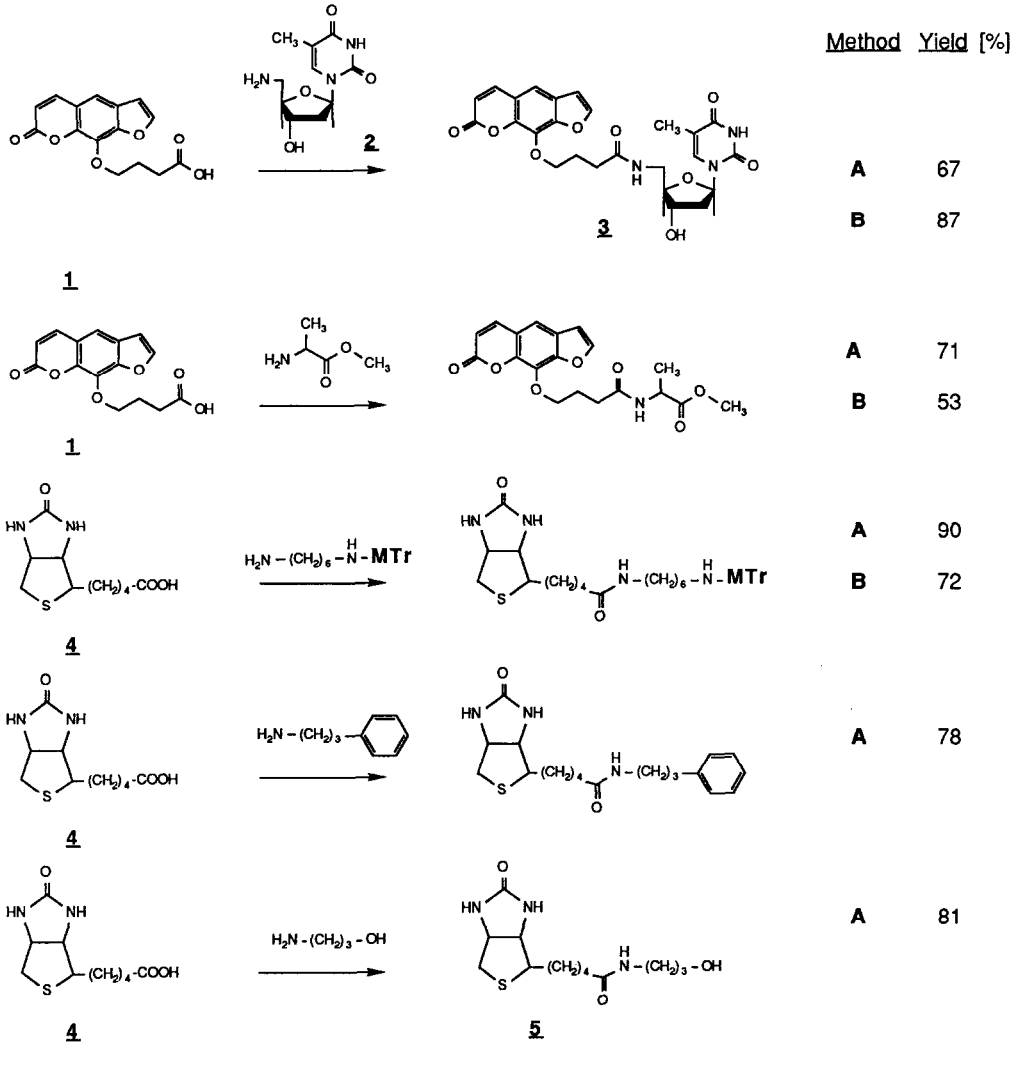
We have prepared a number of different carboxamides. The reactions were either carried out by preforming the hydroxysuccinimido ester with TSTU in a mixture of DMF/dioxane/water in the presence of diisopropyl ethyl amine and then adding the amino component (Method A) or by performing the activation and condensation process directly in the presence of the amino compound (Method B) according to the following *Scheme:*



The reaction of the psoralene derivative **1** with 5'-amino-5'-deoxythymidine (**2**) leading to the carboxamide **3** was evaluated in more detail. When **1** was transferred with TSTU in DMF into the succinimido ester the reaction was complete within 10 min and the pure, activated ester could be isolated in a yield of 66 %. Further reaction of this hydroxysuccinimido ester with 5'-amino-5'-deoxythymidine gave compound **3** in a yield of 61 %. In another experiment the hydroxysuccinimido ester of **1** was prepared *in situ* in a mixture of DMF/dioxane/water in the presence of diisopropylethylamine and then the 5'-amino-5'-deoxythymidine was added leading to **3** in a yield of 67 % (*Scheme 1*; method A). The best yield was obtained in the coupling reaction when it was carried out in the presence of all components according to *Scheme1* (method B) without preactivation of the carboxy group. Pure carboxamide **3** could be isolated in a yield of 87 %. For this reaction only a slight excess of TSTU was used (25 % molar excess with respect to the

carboxy function) and the reaction was complete within 10 min. The other reactions listed in *Table 1* were carried out as indicated by methods A or B. All products were obtained in pure form after crystallization or short column chromatography[5] and were characterized by spectroscopic methods (^{1}H-NMR, MS) and elemental analyses.

Table



In summary we have found that N,N,N',N'-tetramethyl (succinimido) uronium tetrafluoroborate (TSTU) acts as an effective condensing agent to yield carboxamides from the reaction of carboxy compounds with amino components. These reactions proceed even in the presence of water and in a fast and clean manner with only a slight excess of TSTU. The application of TSTU for the formation of carboxamides in mixed aqueous/organic solvent systems could have implications for the attachment of labels to peptides, proteins or amino-modified oligonucleotides as well as for the conjugation of nonpeptidic epitopes to proteins and crosslinking reactions and the attachment of amino or carboxy group-containing compounds to solid support materials via carboxamide bonds.

General procedure

Method A: preparation of **5**: Biotin (122.3 mg, 0.5 mmol) was dissolved in a mixture of 1 ml of DMF, 1 ml of dioxane and 0.5 ml of water. To this solution diisopropylethylamine(257 µl;1.5 mmol) and TSTU (190 mg; 0.63 mmol) were added. After 10 min complete conversion into the corresponding hydroxysuccinimide ester had occurred (TLC) and 57 µl (0.75 mmol) of 3-aminopropanol were added. After a reaction time of 10 min. the reaction mixture was diluted with water and lyophilyzed. Purification by short column chromatography[5] over 25 g of silica and crystallization from CH_2Cl_2/MeOH yielded 124 mg (81%) of pure **5**.

Method B: preparation of **3**:The psoralene derivative **1** (100 mg; 0.35 mmol) and 5'-amino-5'-deoxythymidine (**2** ; 102 mg; 0.42 mmol) were dissolved in a mixture of DMF (1.5 ml), dioxane (3.5 ml) and water (1.5 ml). Diisopropylethylamine (80 µl, 0.47 mmol) and TSTU (130 mg (0.43 mmol) were then added. Complete reaction was observed within 10 min as asessed by TLC. The reaction mixture was diluted with water and extracted several times with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and evaporated yielding 220 mg of crude product. Crystallization from diethyl ether yielded 154 mg (87%) of pure **3** (m.p. 180-182 °C)

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References

1) M.Bodanszky. In "The Peptides", Vol.1(Ed. E.Gross; J.Meienhofer) Academic Press Inc., pp.106-186.

2) Japanese Patent Application 223457,1984; Fuji Photo Film Co.,Ltd.

3) W. Bannwarth, D. Schmidt, R.L. Stallard, C. Hornung, R. Knorr, F. Müller; Helv. Chim. Acta, **71**,2085, 1988

4) R. Knorr, A. Trzeciak, W. Bannwarth, D. Gillessen; Tetrahedron Lett., **30**,1927, 1989.

5) B.J. Hunt, W. Rigby; Chem. Ind. (London), 1868, 1967

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