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A Low-epimerizing Peptide Coupling Reagent Based on the Rearrangement of a Carboxylic-Sulfonic Mixed Anhydride.

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Abstract: A series of peptides has been prepared using an *o*-hydroxybenzenesulfonyl chloride as the condensation reagent. Experimentally, the coupling is a one pot two-steps reaction: formation and aminolysis of a substituted aryl ester. The first step occurs by an elimination-addition reaction involving a sulfoquinone intermediate, followed by an efficient six-membered acyl transfer reaction in the phenolic carboxylic-sulfonic mixed anhydride intermediate. The Young and Anteunis tests show that the degree of epimerization is very low in methylene chloride and acetonitrile.

The racemization-free coupling of segments is still an important problem in modern peptide synthesis. This is due to the easy epimerization (racemization) of the 5-(4*H*)-oxazolone formed by cyclization of an overactivated derivative of an *N*-acyl amino acid.[1,2] The intramolecular capture of a highly activated intermediate, leading to a moderatly activated ester, is one of the most appealing answers for solving this problem: an acyl transfer to a neighboring nucleophile efficiently competes with the participation of the amide function leading to the oxazolone.[2] To apply such a strategy, a series of peptides was prepared using substituted *o*-hydroxybenzenesulfonyl chlorides **1a** and **1b** (Fig. 1) as condensation reagents.[3]

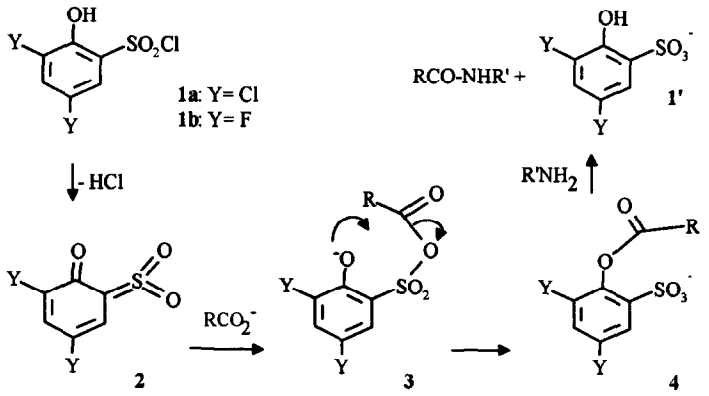

Fig 1: Mechanism of the coupling reaction using reagent 1a or 1b

Sulfoquinones, such as *o*-thioquinone *S,S*-dioxides **2**, are intermediate species in the reactions of *o*- and *p*-hydroxybenzenesulfonyl halides with nucleophiles.[4] T. Zincke first hypothesized the existence of such a type of compounds in the reactions of substituted *p*-hydroxybenzenesulfonyl chlorides in aqueous media.[5] Since then, several studies have been devoted to the formation and the reactivity of these molecules.[6]

It occured to us that the reaction of a sulfoquinone **2**, formed from an *o*-hydroxybenzenesulfonyl halide **1**, with a carboxylate salt in an anhydrous solvent should rapidly lead to a carboxylic-sulfonic mixed anhydride **3**,[7] possessing a neighboring phenolate function (Fig 1). Then, a favorable intramolecular six-membered acyl transfer reaction [8] should give an activated ester **4**, whose aminolysis could give an amide and a water-soluble *o*-hydroxybenzenesulfonic acid by-product **1'**.[10] The hydrophilic ester **4** could also be used for the acylation of amines in aqueous media.[11]

During the preparation of the benzenesulfonyl chlorides **1a** and **1b**, and in order to avoid the formation of *ortho/para* mixtures in the chlorosulfonation reaction,[12] we used 2,4-dihalogenated phenols (Y= Y'= Cl or F) as starting materials. The unknown 3,5-difluoro-2-hydroxybenzenesulfonyl chloride **1b** (m.p. 68.6 °C) was purified by sublimation. The 3,5-dichloro-2-hydroxybenzenesulfonyl chloride **1a** is a commercially available compound. The chlorine substituent being more electron-withdrawing than the fluorine one,[13] the ester **4a** possesses a better leaving group in the aminolysis reaction than its fluorinated counterpart **4b**. However, the fluorinated phenoxide **3b** should be a better nucleophile in the acyl capture reaction than its analog **3a**.

Experimentally, the addition of one equivalent of reagent **1a** or **1b** to a solution of a *N*-protected acid and triethylamine (2 eq) in CH_2Cl_2 or CH_3CN gives a yellow color which rapidly vanishes. The fading is faster with reagent **1b** than with reagent **1a** (1-30 min). If whished, the resulting ester **4** can be isolated. However, addition of the amine to the reaction mixture gives directly the peptide. A series of peptides has been prepared using either reagent **1a** or **1b** (Table 1).[14] Owing to the solubility of the by-products **1'** in the water washes, these peptides have been easily purified. A free hydroxyl function in the amino component did not require protection prior to the reaction (entries 2 and 3). Sterically demanding dipeptide derivatives, such as Boc-Ile-Val-OMe, were formed with good yields (entries 7, 9 and 12). The reported yields are not optimized and refer to analytically pure products. In order to evaluate their optical purity, the homogeneous chromatographic fractions were pooled and *the products were not recrystallized.*

To directly assess the efficiency of reagents **1a** and **1b** for the racemization-free coupling of peptides we concentrated our study on the very sensitive Young test (Table 1, entries 13 and 14).[15b] The coupling of *N*-benzoyl-L-Leucine with glycine ethyl ester, is a reaction in which the formation of the 2-phenyl-5-(4*H*)-oxazolone is more important than in the coupling of usual peptide segments. As judged from this assay, the degree of racemization is very low in CH_2Cl_2 and CH_3CN, particularly with reagent **1a**: 3 % and 4 % respectively, compared to 20 % racemization in CH_2Cl_2 with the DCC/HOBt mixture, widely used for segment condensations.[16, 17] So as to corroborate these results, we used an HPLC/NMR assay. Analyses of the epimeric tripeptide products are easily achieved in a variant of the Anderson test (entry 10), *i. e.* the coupling of Z-Gly-L-Phe with L-Val-OMe (Anteunis test; entries 15 and 16). [18] Compared to DCC (20.8 % and 12 % epimerization in CH_2Cl_2 and CH_3CN, respectively), reagent **1a** leads to less than 1% racemization whereas reagent **1b** gives 5.1 % epimerization in CH_2Cl_2.[19]

Epimerization may also occur through direct α-hydrogen abstraction as in the case of active esters of *N*-protected L-cysteine. [20] *N*-Z-*S*-Bzl-L-Cys-L-Val-OMe was obtained with a good optical purity (Table 1; entry 5). However, in some cases the higher percentages of epimerization observed with reagent **1b** could be due to such an effect, the phenolate of **1'b** being more basic and less hindered than that of **1'a**. [21]

				Reagent 1a			Reagent 1b			lit***	
N°	Acid	Amine	Solvent*	Yield %	m.p. °C	$[\alpha]_{589}$**	Yield %	m.p. °C	$[\alpha]_{589}$**	m.p. °C	$[\alpha]_{589}$
1	Z-Ala	Phe-OMe	A	80	102.6	-13	85	102.5	-13	98-100	-14.1
2	Z-Gly	Tyr-OMe	"	95	51.8	+17	86	53.3	+17	oil	+18.3
3	Z-Gly	Ser-OMe	"	78	95.8	+25	74	95.7	+24	96	+25.5
4	Z-Phe	Gly-OEt	"	88	109.2	-19	89	110.1	-18	110-2	-16.8
5	Z-S-Bzl-Cys	Val-OMe	"	91	76.9	-31	85	76.9	-30	78	-32.1
6	Z-Gly-Phe	Gly-OEt	"	91	118.0	-12	87	118.4	-12	118-9	-12.5
7	Boc-Val	Val-OMe	"	75	165.4	-10	71	165.0	-10	166-7	-9.3
8	Boc-Gly	Pro-OMe	"	93	55.9	-70	89	54.9	-70	66-7	-69
9	Boc-Ile	Val-OMe		94	165.7	-15	88	163.7	-15	166	-15
10	Z-Gly-Phe	Gly-OEt	B	89		-12	79		-11	118-9	-12.5
11	Boc-Gly	Pro-OMe	"	93	56.1	-70	87	55.3	-71	66	-69
12	Boc-Ile	Val-OMe	"	93	165.2	-15	86	164.6	-15	167	-15.4
13	Bz-Leu	Gly-OEt	A	92	156.8	-33[a]	85	154.9	-30[b]	156-7	-34.0
14	Bz-Leu	Gly-OEt	B	85	156.9	-33[c]	75	153.3	-28[d]	156-7	-34.0
15	Z-Gly-Phe	Val-OMe	A	91	105	-17	89	102	-16	98	- 14.1
16	Z-Gly-Phe	Val-OMe	B	96	104	-16					

* A: methylene chloride, B: acetonitrile;

** same solvent and concentration as in the quoted reference;

****Tetrahedron Lett.* **1981**, *22*, 3469 (Z-Ala-Phe-OMe, Z-Phe-Gly-OEt)); ref 9 (Z-Gly-Tyr-OMe, Z-Gly-Ser-OMe, Z-Cys[S-Bzl]-Val-OMe, Z-Gly-Phe-Gly-OEt); *Tetrahedron Lett.* **1985**, *26*, 1341 (Boc-Val-Val-OMe); *J. Chem. Soc. Perkin Trans.1* **1973**, 950 (Boc-Gly-Pro-OMe); *J. Chem. Soc. (C)* **1971**, 2890 ((Boc-Ile-Val-OMe); ref 14b (Bz-Leu-Gly-OEt).

[a] e.e.:97%, [b] e.e.:89%, [c] e.e.:96%; [d] e.e.:82% (reaction with DCC-HOBt or DCC-HOAt in CH_2Cl_2 e.e.:80% or 81%, respectively).

Table 1: Peptide synthesis

Conclusion: owing to the high rates of formation and reaction with carboxylate anions of the *ortho*-thioquinone *S*,*S*-dioxide intermediate **2** ($D_N + A_N$ elimination-addition mechanism) and to the efficiency of the subsequent O→ O six-membered acyl transfer reaction, the formation of the substituted aryl ester **4** is a fast process. Therefore the leakage to the 5-(4*H*)-oxazolone, and thus the epimerization, are reduced.

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