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NEW COUPLING REAGENTS IN PEPTIDE CHEMISTRY

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Abstract: *2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate* **(HBTU)** has been applied as coupling reagent to solid phase peptide synthesis. Furthermore, a general synthetic procedure for new derivatives of different N-hydroxy compounds has been developed. They either act as excellent activating reagents causing low racemization during condensation of peptide segments or are useful tools for the formation of active esters suitable for couplings in mixed aqueous / organic media, respectively.

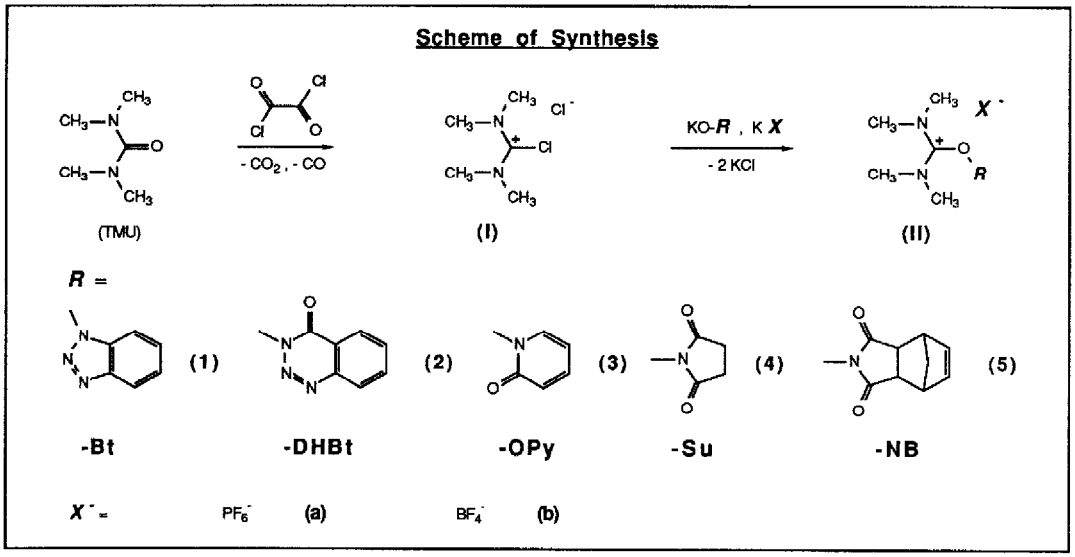
In solid phase peptide synthesis *benzotriazol-1-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate* (BOP) [1] was one of the first reagents for *in situ* formation [2,3] of hydroxybenzotriazolyl esters [4,5]. In a side by side evaluation the superior properties compared to DCC and a number of different other activating reagents could be confirmed [6]. We have successfully used the quite unknown, but excellent activating reagent *2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate* [7] **(HBTU ; II1a)** both in shaker and in continuous flow peptide synthesizers [8]. Couplings proceed smoothly and can even be improved by the addition of 1-hydroxybenzotriazole [4,5] (HOBt). Free aliphatic hydroxyl functions are not affected. During reaction only harmless byproducts are generated which are completely soluble both in water and in organic solvents, a requirement essential for its use in continuous flow systems. Beside HOBt and hexafluorophosphate only tetramethyl urea (TMU) is liberated.

In an improved synthetic procedure for the preparation of tetramethyluronium chloride **(TMU-Cl ; I)** the dangerous phosgene was substituted by oxalyl chloride. For direct formation of **II1a** a new one-pot procedure in anhydrous organic solvents has been established (see: ***Scheme of Synthesis***), which also allows the use of tetrafluoroborate as nonnucleophilic counterion, not possible with the published procedure [7]. Comparative experiments between **HBTU (II1a)** and **TBTU (II1b)** showed that the counterion had no influence in coupling rate or in racemization.

Encouraged by the good results so far a number of new analogs designed for special purposes in peptide chemistry has been prepared following the same route of synthesis # :

2-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate **(TDBTU ; II2b)**, *2-(2-oxo-1(2H)-pyridyl-1,1,3,3-tetramethyluronium tetrafluoroborate* **(TPTU ; II3b)**, *2-succinimido-1,1,3,3-tetramethyluronium tetrafluoroborate* **(TSTU ; II4b)**, and *2-(5-norbornene-2,3-dicarboximido)-1,1,3,3-tetramethyluronium tetrafluoroborate* **(TNTU ; II5b)**.

#**Caution:** reagents **II1** and **II2** may decompose violently when dried at elevated temperatures!



The new derivatives have been used in two different racemization tests and have been compared to other activating reagents using RP-HPLC for separation of the diastereoisomers. With Z-Gly-Phe *(test 1)* best results were obtained using **TPTU (II3b)** / HOBt as activating agents, where we obtained couplings virtually free of racemization (<0.1%). Surprisingly **TMU-Cl (I)** / HOBt also gave good results, its use, however, is very restricted due to its hygroscopicity. **TDBTU (II2b)** showed by far the lowest racemization in the extremely sensitive Bz-Phe coupling *(test 2)*. Under inadequate reaction conditions an opening of the *3,4-dihydro-4-oxo-1,2,3-benzotriazine*-ring may occur [4], generating an activated ester of *2-azido-benzoic acid*, which then acts as an effective blocking reagent for free amino functions. This side reactions is suppressed below 1% when equimolar amounts of **II2b** and no additives are used.

Test 1: **Z-GLy-Phe + Val-OMe ⟶ Z-Gly-(D+L)Phe-Val-OMe**

Activating Reagent	*% D-Isomer*				
	without Additive	*with Additive*			
DCC	36	1.6	(1)	3.0	(2)
HMPT-Cl	35	2.4	(1)		
BOP	4.8	1.2	(1)		
TMU-Cl	22	0.1	(1)		
TBTU	1.4	0.2	(1)		
TDBTU	1.1	0.1	(1) *	0.1	(3) *
TPTU	20	<0.1	(1)	11	(4)
TSTU	26	1.0	(1)	18	(2)
TNTU	28	0.5	(1)	22	(5)

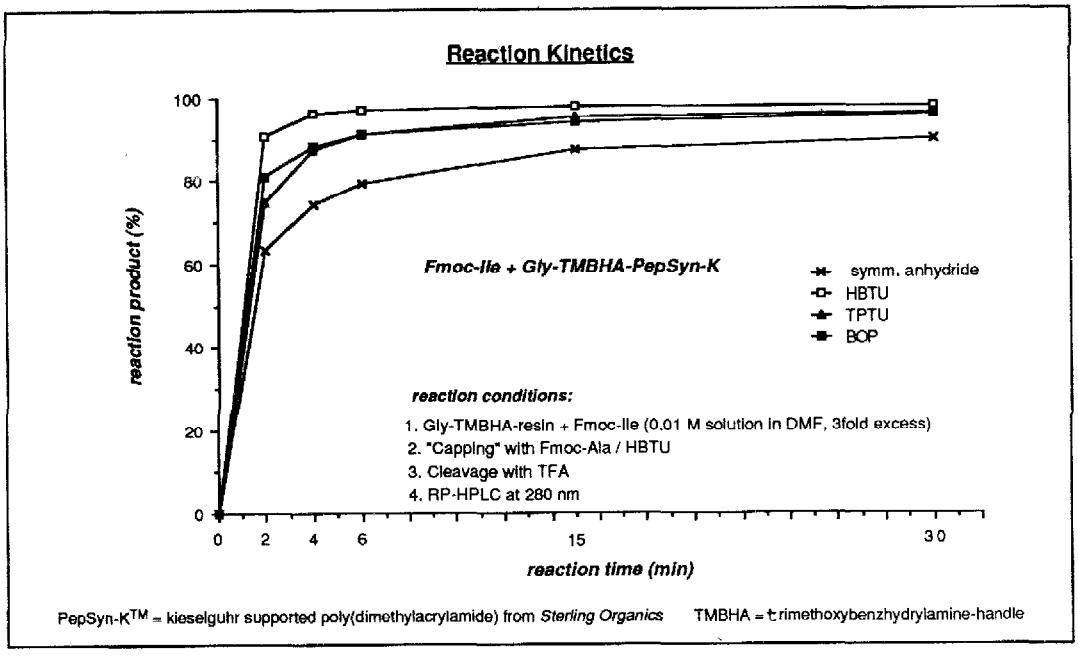
(1) = HOBt (2) = HOSu (3) = HODHBt (4) = HOOPy (5) = HONB

Test 2: **Bz-Phe + Val-OMe ⟶ Bz-(D+L)Phe-Val-OMe**

Activating Reagent	*% D-Isomer*			
	without Additive	*with Additive*		
DCC		40	(1)	
BOP	44	40	(1)	
TMU-Cl		37	(1)	
TBTU	39	36	(1)	
TDBTU	11.2			9.7 (3)*
TPTU		21	(1)	

(1) = HOBt (3) = HODHBt

In a further experiment the reactivities of **II1a** and **II3b** have been compared to BOP and symmetrical anhydrides (see: ***Reaction Kinetics***).



PepSyn-K™ = kieselguhr supported poly(dimethylacrylamide) from *Sterling Organics* TMBHA = trimethoxybenzhydrylamine-handle

1-Hydroxybenzotriazolyl esters are known to be quite unstable in aqueous media due to their high reactivity towards nucleophiles. Taking advantage of the excellent uronium leaving group the corresponding derivatives for direct introduction of -OSu and -ONB esters, **II4b** and **II5b** , have also been prepared (see: ***Scheme of Synthesis***). These reagents have been used successfully for different amide bond formations in

*not recommended : the use of additives forces ring-opening of **II2**

mixed aqueous / organic solvents , *i.e.* coupling of labels to proteins, conjugation of peptidic and nonpeptidic epitopes to carrier proteins, and coupling of labels to aminofunctionalized DNA [9].

Conclusions

TBTU (II1b) and **HBTU (II1a)** are ideally suited coupling reagents for solid-phase peptide synthesis

TPTU (II3b) / HOBt is the reagent of choice for segment condensations

TDBTU (II2b) suppresses racemization best, but is recommended only in critical cases because of the danger of side reactions.

TSTU (II4b) and **TNTU (II5b)** are useful tools for the *in situ* formation of -OSu and -ONB esters and subsequent coupling in aqueous solutions.

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