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Side reactions of onium coupling reagents BOP and HBTU in the synthesis of silica polymer supports

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Summary. Onium activating reagents BOP and HBTU have been used for the polymer linker elongation via the coupling of COOH function with amino group. Possible side reactions accompanying linker synthesis in the presence of BOP and HBTU and their effect on the coupling yields have been studied in model experiments. The results demonstrate that BOP is more stable in the reaction medium than HBTU, with equal coupling efficiency in regular condensation reactions. HBTU can react with amino polymer decreasing coupling yield, whereas it is not the case for BOP. It is thus recommended to avoid the interaction of HBTU reagent with amino polymer before the addition of carboxylic component. Short preactivation of the amino acid component with onium reagents increases the yield of subsequent coupling reaction.

Key words: polymer supports, coupling reagents, phosphonium salts, uronium salts, side reactions.

Introduction. We have recently reported the application of phosphonium and uronium activating reagents to the preparation of silica polymer supports for solid phase oligonucleotide synthesis [1]. Both elongation of the polyamide linkers and attachment of nucleosides to the polymers were performed in the presence of onium coupling reagents BOP and HBTU originally developed for peptide synthesis. However, the issue of possible side processes associated with these reagents has not been addressed in that paper. Here we report on the study of side reactions of onium salts in the silica functionalisation.

Materials and methods. 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) and anhydrous 1-hydroxybenzotriazole (HOBT) were purchased from Aldrich, Fmoc-glycine, piperidine and N,N-diisopropylethylamine (DIPEA) were from Fluka, 1-methylimidazole (MeIm) was obtained from Merck. Other reagents and solvents were from domestic suppliers. DIPEA and piperidine were distilled from NaOH, dimethylformamide and MeIm were distilled in vacuum. Absolute acetonitrile was prepared by distillation over P_2O_5 and then from CaH_2. Fmoc group loading was determined by the treatment of carefully weighed polymer sample with 20 % piperidine solution in DMF for 15 min and measuring UV absorbance at 302 nm, according to [2]. Spectral measurements were performed with Specord UV-Vis spectrophotometer (Carl Zeiss Jena, Germany).

Silochrom-2 silica was functionalised by aminopropyltriethoxysilane in ethanol as previously described [3, 4] to provide aminopropyl polymer **1** containing 125 μmol/g of the amino group. For the linker elongation, 1 g of the silica **1**, Fmoc-glycine (445 mg, 1.5 mmol), BOP (663 mg, 1.5 mmol), HOBT (203 mg, 1.5 mmol) and DIPEA (870 μl, 5 mmol) were agitated in DMF (10 ml) for 3 hours at ambient temperature. The polymer was filtered off, washed with DMF (3x10 ml) and CH_3CN (3x10 ml) and treated with a solution of

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acetic anhydride (940 µl, 10 mmol) and MeIm (800 µl, 10 mmol) in 10 ml of dry CH_3CN with occasional swirling for 30 min to block unreacted amino groups. The silica was filtered off, washed with CH_3CN (3x10 ml), methanol (3x10 ml), chloroform (3x10 ml) and dried. Fmoc protecting group was removed from the polymer **2** by the treatment with 20 % piperidine in DMF (10 ml) for 15 min, and the polymer was washed with DMF (5x10 ml), methanol (3x10 ml) and ether (3x10 ml). The amine content in the polymer **3** determined by the Fmoc test was 89 µmol/g. Amino polymer **3** with terminal glycine residue was used for the model coupling reactions with Fmoc-Gly under various conditions. Each coupling experiment was performed on 50 mg of the support **3** with 0.1 mmol of Fmoc-Gly, BOP or HBTU and HOBT, and 0.3 mmol of DIPEA in 1 ml DMF. Four variants of the coupling reaction were investigated:

1) BOP or HBTU was added to the mixture of amino polymer, Fmoc-Gly, HOBT and DIPEA (normal reaction); normal reactions in the absence of HOBT or the base were also carried out;

2) polymer was treated with BOP (HBTU) in DMF in the presence of HOBT and the base for 5 or 30 min, followed by the addition of Fmoc-Gly;

3) BOP (HBTU) was treated with HOBT and the base in DMF for 5 or 30 min, and then the solution was added to the mixture of polymer and Fmoc-Gly;

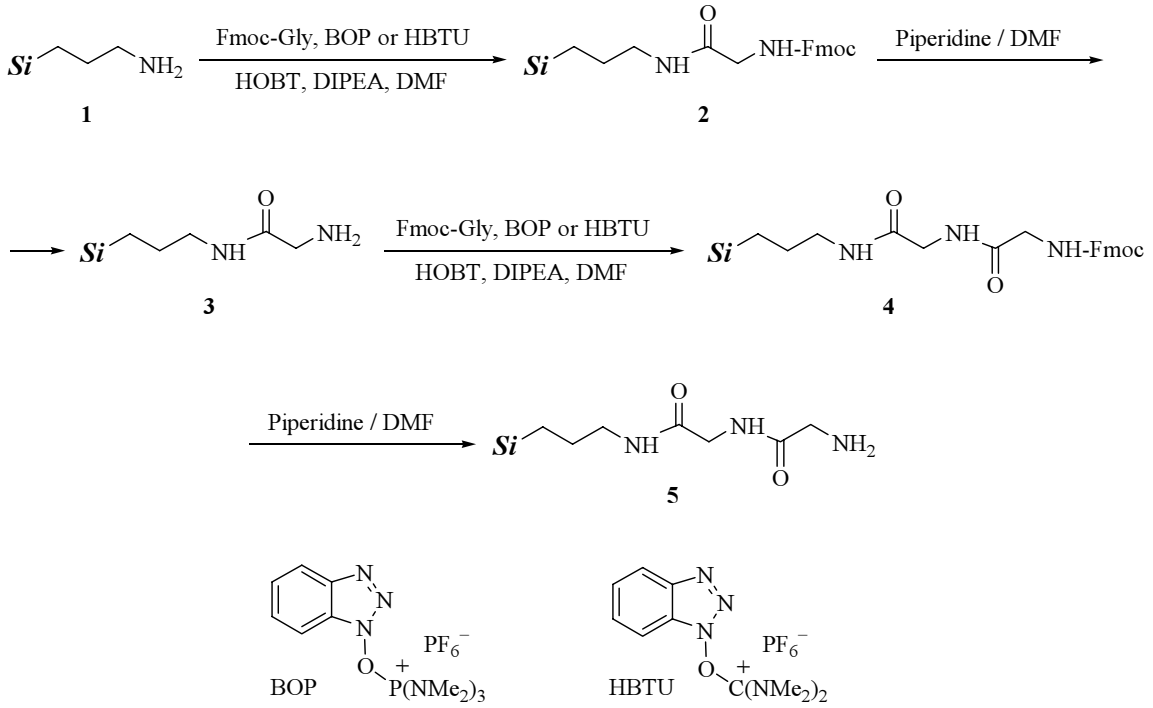
4) Fmoc-Gly was pre-activated for 5 or 30 min with BOP (HBTU) in DMF in the presence of HOBT and DIPEA, and the reaction mixture was added to the polymer.

Subsequent couplings were carried out for 2 hours to result in the polymer **4**. The polymer support was filtered off, washed with DMF (3x2 ml), methanol (3x2 ml) and ether (3x2 ml). Reaction yields based on the initial loading of glycine were determined by Fmoc test via piperidine treatment of polymer samples [2].

Results and discussion. The yield and the purity of oligonucleotides synthesized on polymer supports strongly depend on the length and structure of the linker between the polymer surface and nucleoside. Usually polymer linkers are introduced by covalent attachment of carboxylic acids (as activated esters or in the presence of carbodiimides or arylsulfonylchlorides) to amino groups on polymers [3—6]. The use of activated esters requires an additional step of their preparation, whereas coupling reactions in the presence of common activating reagents should be performed under strictly anhydrous reaction conditions. A number of stable and non-hygroscopic phosphonium and uronium reagents is currently widely used as activators in coupling reactions in peptide synthesis [2, 7—9]. Since the first onium coupling reagents, BOP [10] and HBTU [11], were developed a long time ago, the reaction of N-protected amino acid with N-terminal amino group on the polymer support has become a standard method of solid phase peptide synthesis. Onium reagents have been also applied to oligonucleotide chemistry, including linker synthesis and nucleoside attachment to solid supports [12—15] and oligonucleotide functionalisation, conjugation or labeling [16—19].

We have recently prepared silica supports for oligonucleotide synthesis. Glycyl-glycyl-succinyl and succinyl-ethylenediamine-succinyl linkers were introduced into the Silochrom-2 silica using onium activating reagents, phosphonium salt BOP or uronium salt HBTU in the presence of 1-hydroxybenzotriazole additive [1]. Nucleoside coupling to the polymer was performed via BOP or HBTU activation as well. In the course of out work on the synthesis of solid phase supports we have noticed that coupling reaction yields varied depending on the order of addition of reagents to the polymer. It was an indication that some yield-decreasing side reactions could occur during the carboxy component coupling to amino group. In this paper we present experimental data concerning possible side reactions of onium reagents.

Polymer support for the study of coupling reaction yields was prepared from aminopropyl silica Silochrom-2 [4] by adding one glycine residue (Scheme 1). We have preferred to check coupling yields at the polymer **3** instead of **1** just to decrease possible interference of the shorter linker and the closer proximity of the reaction center to polymer surface in the latter case. The capping with acetic anhydride was used to block the remaining amino groups to eliminate their participation in the next coupling reaction. Glycine loading was determined by the standard Fmoc chemistry, via the cleavage of the Fmoc



Scheme 1. Synthesis of the silica polymer support containing Gly-Gly linker.

protecting group by piperidine treatment [2, 8, 20—21]. Absorbance maximum for the deprotection product, piperidine adduct of dibenzofulvene, is observed at 302 nm. The polymer support **3** containing 89 μmol/g of amino groups was used for the experiments on the coupling efficiency. The condensation reaction yields for onium salts were determined for the coupling of N-protected glycine (Fmoc-Gly) to the amino polymer **3**. The addition of Fmoc-Gly to the polymer was performed with BOP or HBTU activating reagent in the presence of HOBT and the base under various reaction conditions. Model experiments were designed to investigate the coupling efficiency of onium reagents and the extent of side reactions. Equimolar amounts of Fmoc-Gly, HOBT and coupling reagents were used for the coupling, with 3 eq. of the base. Regular coupling reactions have been studied, along with reactions involving the pretreatment of the polymer with onium reagents, or the BOP (HBTU) reagents treatment with HOBT and the base before starting the coupling reaction. Another type of coupling was performed with amino acid component preactivated with onium reagents. The coupling yields were determined by measuring the UV absorption at 302 nm of the solution resulted from the Fmoc deprotection in the first cycle and comparing it with the second Fmoc cleavage. So there was no need to use an extinction coefficient of Fmoc deprotection product (ε = 7800 in 20 % piperidine/DMF [2, 8]) to obtain the yields via the direct NH_2 content measurement. Coupling yields are presented in the Table.

The first step of coupling reaction is an activation of the carboxy function with onium reagent followed by the nucleophilic attack of amino component at activated carboxylic group to form amide bond. The proposed reaction mechanism of the phosphonium reagents involves rapid formation of the reactive acyloxyphosphonium intermediate **6** that is then transformed into oxybenzotriazole active ester **7** (Scheme 2). Both **6** and **7** can react with nucleophiles, **6** reportedly being more active [7, 9, 22—24]. We were not able to find a literature on the mechanism of acid activation by uronium (carbonium) salts. However, oxybenzotriazole esters are also formed in the presence of uronium salts [7, 9], so the intermediate formation of corresponding acyloxyuronium derivative similar to the phosphonium intermediate **6** can be suggested.

In the regular coupling reactions, the BOP reagent was somewhat less efficient than HBTU under the same reaction conditions (coupling yields 84 and 87 %, respectively). The coupling

Table

The effect of reaction conditions on the yield of Fmoc-Gly coupling to the polymer ***3***

Coupling reagent	Coupling reaction yield, %								
	Normal coupling reaction[1]			Polymer pretreatment, min[2]		Reagent pretreatment, min[3]		Amino acid pretreatment, min[4]	
	Regular	-HOBT	-DIPEA	5	30	5	30	5	30
BOP	84	79	<1	82	77	83	81	87	82
HBTU	87	80	<1	78	54	80	68	88	80

[1]*Coupling reagent was added to the mixture of polymer, Fmoc-Gly, HOBT, DIPEA (regular reaction), or the same mixture except HOBT (-HOBT) or the base (-DIPEA).*

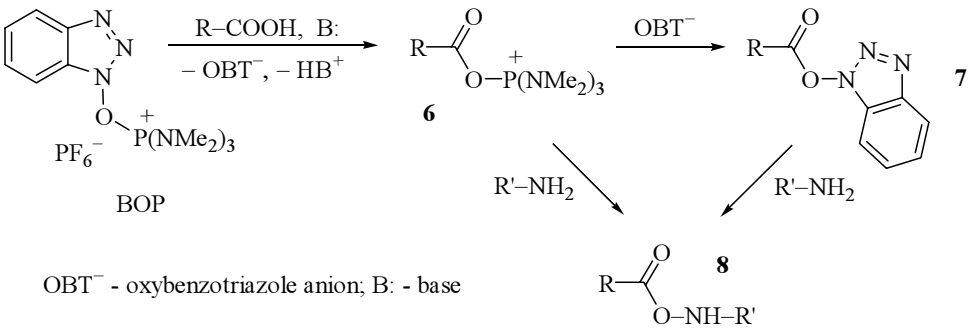
[2]*Polymer was treated with coupling reagent, HOBT and DIPEA, and then Fmoc-Gly was added.*

[3]*Coupling reagent was treated with HOBT and DIPEA, and then added to the mixture of polymer and Fmoc-Gly.*

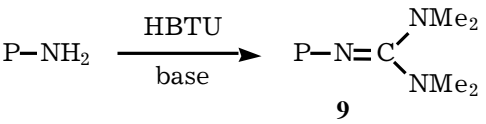
[4]*Fmoc-Gly was treated with coupling reagent, HOBT and DIPEA, and then added to polymer.*

efficiency of onium reagents in the absence of HOBT additive was lower for 5—7 %. No coupling was observed in the absence of DIPEA, so the presence of the base is essential for the condensation, both for the formation of the amino acid anion and for the basic catalysis of the reaction of polymeric NH_2 group with active intermediate **6** or **7**. The presence of HOBT additive accelerates the transformation of acyloxonium intermediates like **6** into activated esters **7**. Oxybenzotriazole esters **7** are known to be relatively stable [8, 9, 25], and they react with amines efficiently even in aqueous solutions [18]. HOBT esters can be quite easily analysed by chromatography [7, 23], whereas acyloxyphosphonium intermediates **6** were detected by ^{31}P NMR only for sterically hindered acids at low temperatures [24].

The reaction yield after the polymer treatment with onium reagents was decreased for HBTU. This can result from the reaction of HBTU with N-terminal amino group of the polymer linker in the absence of COOH component. HBTU is able to react with amines with formation of tetramethylguanidine derivative **9** resulted from the transfer of the tetramethyluronium moiety from HBTU to the amino group of the polymer [7, 26] (Scheme 3). While carboxylic component is absent, the amount of the free polymer NH_2 groups decreases in time, and guanidine formation quenches the coupling reaction with activated carboxylic acid. Indeed, the treatment of the support **3** with HBTU/HOBT/DIPEA for 5 and 30 min followed by the addition of Fmoc-Gly resulted in the subsequent coupling reaction yields 78 and 54 %, respectively, so the prolonged treatment of amino component with HBTU strongly decreases the yield. It seems that similar yield-decreasing side reaction blocking polymer amino groups does not proceed to a significant extent with BOP. The formation of the side products may somewhat limit HBTU usefulness for the polymer synthesis.



Scheme 2. Mechanism of the coupling reaction catalysed by BOP.



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P – polymer support

Scheme 3. Guanidine formation upon amino polymer treatment with HBTU.

An additional factor in the poor HBTU efficiency in this case could be also the reported lower stability of HBTU in the solution in the presence of the base as compared to BOP [7], although this factor alone can hardly result in such a big difference. To evaluate the stability of onium reagents in the solution, BOP and HBTU were kept in DMF in the presence of HOBT and the base for 5 and 30 min, and then introduced into the coupling reaction. Indeed, the reagent pretreatment with HOBT/DIPEA resulted in significant decrease of the coupling yields, much more pronounced in the case of HBTU (68 % after 30 min pretreatment). Lower stability of the uronium reagent is in agreement with its higher coupling activity.

Comparing the results obtained for coupling reagents pretreatment with those involving polymer pretreatment (which obviously included reagent pretreatment as well), one can discriminate between the effects of the reagent decomposition and the side reaction at amino group. The results suggest that BOP is more stable in the reaction medium, and it does not react with polymeric NH_2 group as HBTU does, which is in accordance with published data [2, 7—9]. After 30 min pretreatment of the polymer with onium reagents, the coupling yields decreased for 33 % with HBTU and only for 7 % with BOP. Although side reactions at amino group are not supposed to be really dangerous in normal coupling reactions, it is recommended however to avoid the addition of the onium coupling reagents to amino polymer in the absence of COOH component.

On the other hand, short preactivation of the carboxylic component with onium reagents prior to the addition to the amino polymer improved the coupling reaction yield, more noticeably for BOP (for 3 %). In this case, activated ester of carboxylic acid **7** is formed in advance and then directly react with NH_2 component, with side reaction of the amine with coupling reagent to occur to a lesser extent. The simple and convenient procedure consisting of the preactivation of the acid with coupling reagent for 5 min followed by the addition of the reaction mixture to the amino component is more reliable and provide better reaction yields than «regular» reaction with *in situ* activation. The results indicate that HOBT active ester is formed quickly and is sufficiently stable in the reaction solution. Perhaps it is more stable than phosphonium (uronium) derivatives of the amino acid. Fast transformation of the onium coupling reagents into relatively stable activated esters can prevent the decomposition of HBTU and BOP in the reaction mixture. Nevertheless, the prolonged preactivation of Fmoc-Gly (30 min) resulted in the noticeable decrease of the coupling yield and should therefore be avoided.

The results presented here for the solid phase condensation may somewhat differ from those observed for the homogeneous coupling reactions in the solution peptide synthesis. It should be noted that the reaction rates on the polymer surfaces are lower, and the diffusion of reagents to the reaction centers may be relatively slow. In this case coupling reagent has more chances to decompose in the reaction medium or undergo the reaction with aminoalkyl group of the polymer.

Concerning the nucleotide attachment to the support in the presence of onium salts, the same side reactions including reagents decomposition in the solution or their reaction with NH_2 groups can occur when nucleoside succinates are being coupled to the polymer aminolinker. It was shown that nucleosides with free amino groups, especially cytidine, can be acylated with HOBT esters of amino acids [27]. We have also observed the acylation of heterocyclic amino groups of deprotected oligonucleotides in their conjugation with carboxy porphyrin derivatives activated by BOP-HOBT system [18]. However, when protected nucleoside reacts with carboxylated polymer, there is no reaction center at nucleoside to interact with onium salts. So possible effect of onium coupling reagents on nucleosides can be generally excluded in this case.

In conclusion, the coupling of N-protected amino acid to amino polymer was used to study the reaction efficiency of onium activating reagents, HBTU or BOP in the presence of HOBT, under various conditions. It was shown

that in normal reaction both phosphonium and uronium coupling reagents provide similar efficiency. The presence of HOBT additive is favorable for the efficient condensation reactions. However, the treatment of the amino polymer with uronium reagent HBTU in the absence of COOH component resulted in significant yield decrease of the subsequent coupling due to the reagent reaction with polymer amino group, whereas BOP treatment did not result in the substantial yield decrease. HBTU more rapidly degraded in the solution than BOP under the reported reaction conditions. Thus, phosphonium reagent BOP appears to be more reliable and less sensitive to the variations of synthetic procedures than HBTU activating reagent. Addition of the coupling reagent to amino support before the carboxylic component should be excluded, especially for HBTU. At the same time, short preactivation of the amino acid component with onium coupling reagents before the addition to the polymer improved the coupling yield and can therefore be recommended for the practical use, including peptide synthesis applications.

Abbreviations: BOP — benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; DIPEA — diisopropylethylamine; DMF — dimethylformamide; Fmoc — 9-fluorenylmethoxycarbonyl; Gly — glycine; HBTU — 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBT — 1-hydroxybenzotriazole.

Побічні реакції онієвих конденсуючих реагентів BOP та HBTU в синтезі полімерних носіїв на основі силікагелю

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Резюме. Онієві активуючі реагенти BOP та HBTU було використано для елонгації полімерного лінкера шляхом конденсації карбоксильних та аміногруп. У модельних експериментах було вивчено побічні реакції, що супроводжують синтез лінкера в присутності BOP та HBTU, та їх вплив на вихід реакції конденсації. Результати показали, що в реакційному середовищі BOP стійкіший від HBTU за приблизно однакової ефективності в нормальних реакціях конденсації. HBTU, на відміну від BOP, здатний реагувати з амінополімером. Рекомендовано уникати взаємодії HBTU з полімером перед додаванням кислотного компонента реакції. Коротка попередня активація амінокислоти онієвим реагентом підвищує вихід реакції конденсації.

Ключові слова: полімерні носії, конденсуючі реагенти, фосфонієві солі, уронієві солі, побічні реакції.

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