Uronium salts from polymeric *N*-hydroxysuccinimide (P-HOSu) as new solid-supported peptide coupling reagents

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Dedicated to Professors E. A. Rúveda and Roberto A. Rossi on occasion of their 70th and 60th birthdays, respectively

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Abstract

The new polymeric uronium salts P-TSTU and P-HSTU have been prepared from polymeric *N*-hydroxysuccinimide (P-HOSu) and used as solid-supported reagents for peptide coupling reactions. The P-HOSu liberated can be recovered by simple filtration and reused for the preparation of new reagents.

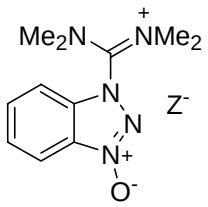
Keywords: Uronium salts, peptides, polymers, coupling reagents

Introduction

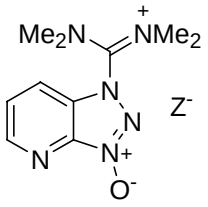
The formation of the amide bond is the crucial step in the synthesis of an impressive array of organic compounds of biological interest,[1] the most representative being peptides and peptoids although being present in oligocarbamates, oligoamides, β-lactams, polyenamides, benzodiazepines, diketopiperazines, and hydantoins.

The activation of carboxylic acids for the formation of the amide bond is usually carried out using the so-called peptide coupling reagents.[2] During the last few years, a plethora of these coupling reagents has appeared in the literature,[2] improving older coupling methods. Amongst these nowadays-preferred peptide coupling reagents are the aminium/uronium derivatives, which have become popular because of their higher efficiency and low tendency towards racemization of the amino acid or peptide residues. These salts are usually prepared by reaction of a compound of the type XOH with a halouronium salt from a urea such as tetramethylurea (TMU). Examples of these common aminium/uronium salts are the 1-hydroxybenzotriazole derivatives **1a**[3a] (TBTU) and **1b**[3b] (HBTU), the 7-azabenzotriazole derivatives **2a** (TATU) and **2b** (HATU),[4] the 3-hydroxy-3,4-dihydro-1,2,3-benzotriazin-4-one derivatives **3a**[5a] (TDBTU) and **3b**[5b]

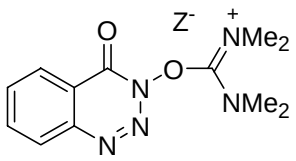
(HDTU) the 1-hydroxy-1,2-dihydro-2-pyridinone derivatives **4a**[5a] (TPTU) and **4b**[5c] (HPTU), the 1-hydroxysuccinimide derivatives **5a**[5a] (TSTU) and **5b**[5d] (HSTU), and the norbornene dicarboximido derivative **6**[5a] (TNTU), almost all of them commercially available.



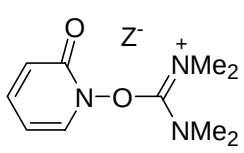
1a (TBTU): Z = BF_4
1b (HBTU): Z = PF_6



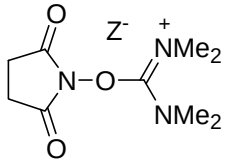
2a (TATU): Z = BF_4
2b (HATU): Z = PF_6



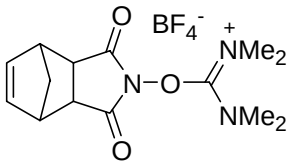
3a (TDBTU): Z = BF_4
3b (HDTU): Z = PF_6



4a (TPTU): Z = BF_4
4b (HPTU): Z = PF_6



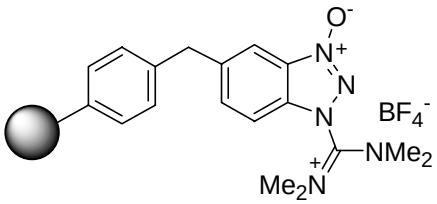
5a (TSTU): Z = BF_4
5b (HSTU): Z = PF_6



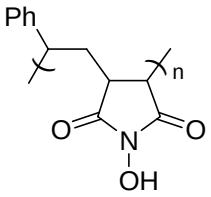
6 (TNTU)

On the other hand, the development of new polymer-supported reagents for organic synthesis is receiving increasing attention nowadays both for easy recycling and for the simplification of conventional workup procedures.[6] In this context, and in connection with our project on the development of new solid-supported reagents applicable to peptide chemistry, we have prepared polystyrene-bound P-TBTU (**7**) as an efficient, although rather expensive, polymer-bound peptide coupling reagent.[7] In addition, we have recently developed a co-polymer of polystyrene and maleic anhydride containing the *N*-hydroxysuccinimide moiety (P-HOSu, **8**)[8] which can be easily obtained from an inexpensive commercially available polymer and used as a recoverable racemization-reducing additive for the dicyclohexylcarbodiimide (DCC)-mediated coupling of amino acids. This P-HOSu (**8**) has also been used for the preparation of polymeric reagents for the protection of amino groups with the 9-fluorenylmethoxycarbonyl (Fmoc),[9] 2,7-di-*tert*-butyl-9-fluorenylmethoxycarbonyl (Dtb-Fmoc),[10] allyloxycarbonyl (Alloc)[11] and propargyloxycarbonyl (Proc)[11] groups. Furthermore, ammonium salts obtained from P-HOSu (**8**) have been employed as amino releasing and racemization-lowering reagents in carbodiimide-mediated amidations.[12] In all cases, these polymeric reagents are rather soluble in polar organic solvents and the P-HOSu (**8**) can be easily removed from the reaction mixture by simple filtration and reused. Using these antecedents, we envisaged that P-HOSu (**8**) could be suitable for the preparation of new and economical polymeric uronium salts related to TSTU (**5a**) and its hexafluorophosphate analogue HSTU (**5b**), therefore receiving the acronyms P-TSTU (**9a**) and P-HSTU (**9b**). The use of these new reagents would allow the easy separation of the P-

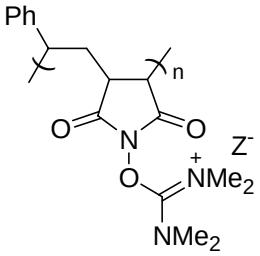
HOSu (**8**) liberated after the coupling reaction, something especially valuable when working at small scale.



7 (P-TBTU)



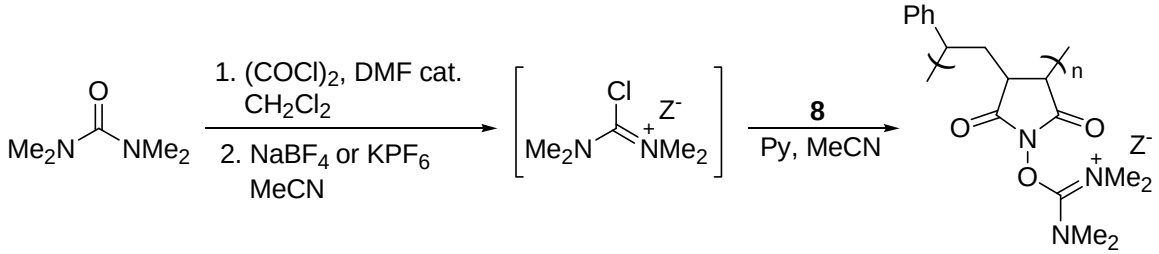
8 (P-HOSu)



9a (P-TSTU): Z = BF_4
9b (P-HSTU): Z = PF_6

Results and Discussion

P-HOSu (**8**) was prepared by reaction of commercially available poly(styrene-*alt*-maleic anhydride) with an 50% aqueous solution of hydroxylamine according to the reported method.[8] The activity of the prepared P-HOSu (**8**) was determined to be 1.0 mmol g^{-1} by the assay of the OH group content according to the synthesis of the *N*-isopropylacetamide.[13] The P-HOSu (**8**) obtained reacted with 4 equiv of the chlorouronium tetrafluoroborate **11a** or hexafluorophosphate **11b** in the presence of pyridine as base for 24 h at room temperature in acetonitrile as solvent affording P-TSTU (**9a**) or P-HSTU (**9b**) as white solids after precipitation with hexane and filtration after (Scheme 1). The reaction progress between P-HOSu (**8**) and **11** was followed by IR spectroscopy, and considered complete after the total disappearance of the broad O-H stretching band at 3437 cm^{-1}. The obtained polymers showed a new strong IR band at *ca*. 1710 cm^{-1} which could be assigned to a C=N in an uronium salt.[14] The chlorouronium salts **11** were obtained from tetramethylurea (**10**) by reaction with oxalyl chloride in the presence of a catalytic amount of DMF, followed by anion exchange with sodium tetrafluoroborate or potassium hexafluorophosphate (Scheme 1).[15]



10

11a: Z = BF_4
11b: Z = PF_6

9a: Z = BF_4
9b: Z = PF_6

Scheme 1. Synthesis of P-TSTU (**9a**) and P-HSTU (**9b**).

The P-TSTU (**9a**) and P-HSTU (**9b**) prepared were used as solid-supported peptide coupling reagents. Thus, differently *N*-protected amino acids reacted with amino acid ester hydrochlorides in the presence of 1.1 equiv of P-TSTU (**9a**) or P-HSTU (**9b**) and pyridine as organic base for 24 h (TLC) at 50 ºC in MeCN as solvent, yielding the corresponding peptides (Table 1), which were pure after workup according to their ^{1}H NMR spectra. The use of other organic bases such as triethylamine or room temperature gave place to lower yields.

Table 1. Peptides prepared using P-TSTU (**9a**) and P-HSTU (**9b**) as coupling reagents

Entry	Reagent	Peptide	Yield (%)[a]	Mp (ºC)[b]	$[\alpha]_D^{25}$ [b] (solvent)
1	**9a**	BocGly-PheOEt	65	oil	-24 (*c* 1, EtOH)
2	**9b**	BocGly-PheOEt	41	oil	-24 (*c* 1, EtOH)
3	**9a**	BocAla-PheOEt	63	100-101	-8 (*c* 1, EtOH)
4	**9b**	BocAla-PheOEt	35	100-101	-8 (c 1, EtOH)
5	**9a**	BocVal-PheOEt	59	117-118	-23 (c 1, EtOH)
6	**9b**	BocVal-PheOEt	31	117-118	-23 (c 1, EtOH)
7	**9a**	CbzVal-PheOEt	50	157-158	-9 (c 0.7, EtOH)
8	**9a**	BocAib-ValOMe	23	110-111	+19 (c 1, EtOH)
9	**9a**	CbzGlyPhe-ValOMe[c]	55		-40 (*c* 1, MeOH)
10	**9b**	CbzGlyPhe-ValOMe[c]	32		-40 (*c* 1, MeOH)

[a] Isolated yield based on the starting amino acids. [b] Measured from the crude peptide. [c] Anteunis' test[16]: 50:1 diastereomer ratio (^{1}H NMR, 500 MHz).

In all cases, the isolated yields of the peptides obtained were higher using P-TSTU (**9a**) than P-HSTU (**9b**) (Table 1). The coupling of an sterically hindered residue such as BocValOH afforded moderate yields with P-TSTU (**9a**) (Table 1, entry 5). However, when the coupling reaction was performed with both sterically hindered amino acid residues, the yield was rather low (Table 1, entry 8). The extent of possible racemization was checked by measuring the epimerization degree on the tripeptide generated by coupling CbzGlyPheOH and ValOMe (Anteunis's test)[16] (Table 1, entries 9 and 10). The epimerization was low, the final tripeptide obtained as a 10:0.2 mixture of CbzGly-L-Phe-ValOMe and CbzGly-D-Phe-ValOMe diastereoisomers detected by ^{1}H NMR (500 MHz) analysis, both for P-TSTU (**9a**) or P-HSTU (**9b**). This value was considerably lower than the 26% of the D-isomer reported when using TSTU.[5a]

P-HOSu (**9**) was quantitatively recovered pure, once the coupling reaction was finished, by precipitation with hexane, filtration and washing with diluted HCl. The recovered P-HOSu (**7**) was employed for the preparation of new reagents **9a** and **9b**.

We conclude that P-TSTU (**9a**) and P-HSTU (**9b**) are new promising polymeric *N*-hydroxysuccinimide (P-HOSu)-derived uronium salts which can be employed, especially P-TSTU (**9a**), as solid-supported peptide coupling reagents, the P-HOSu liberated being easily separated and reused.

Experimental Section

General Procedures. All reactions carried out during the preparation of the uronium salts were moisture-protected using a drying tube with calcium chloride. Chlorouronium tetrafluoroborate (**11a**) or hexafluorophosphate (**11b**) were obtained according to the reported procedure.[15] P-HOSu (**8**) was prepared as previously described.[8] Reagents and solvents from commercial suppliers were used as provided. Analytical TLC was performed with Schleicher & Schuell F1400/LS silica gel plates and the spots were visualized with UV light at 254 nm or by charring with 0.3% ninhydrin. Melting points are uncorrected. IR spectra were recorded with a Nicolet 510 P-FT and only structurally important peaks are indicated. NMR spectra were measured with a Bruker AC-300 or DRX-500 using TMS as internal standard. Optical rotations were measured with a Jasco DIP-1000 polarimeter.

Synthesis of polymeric reagents P-TSTU (9a) and P-HSTU (9b). A solution of the chlorouronium salts[15] **11a** or **11b** (40 mmol) in MeCN (40 mL) was added to a suspension of P-HOSu[8] (**7**, loading = 1.0 mmol g^{-1}) (10 g, 10 mmol) and pyridine (2.4 mL, 30 mmol) in MeCN (20 mL). The mixture was stirred at 80 ºC for 24 h and cooled at rt, followed by addition of hexane (30 mL). The solid was filtered and washed with MeCN/hexane mixtures (3 x 50 mL, 2/3 v/v) and dried at 100ºC under vacuum (0.1 Torr) affording 10.3 g of P-TSTU (**9a**) or P-HSTU (**9b**) as white solids. P-TSTU (**9a**): IR (KBr) ν 1708 (C=N) and 1063 cm^{-1} (BF_4^-). P-HSTU (**9b**): IR (KBr) ν 1711 (C=N) and 844 cm^{-1} (PF_6^-).

Coupling reactions using reagents P-TSTU (9a) and P-HSTU (9b). General procedure. To a solution of the N-protected amino acid (1 mmol) and the aminoester hydrochloride (1 mmol) in MeCN (15 mL) was added pyridine (160 μL, 2 mmol) and P-TSTU (**9a**) or P-HSTU (**9b**) (1.1 mmol). The heterogeneous mixture was stirred at 50 ºC for 24 h and hexane (10 mL) was added. The resulting solid consisted of P-HOSu (**7**) which was recovered by filtration and washing with 1M HCl (20 mL). The resulting filtrate was treated with saturated NaCl (50 mL) and extracted with AcOEt (3 x 10 mL). The combined organics were washed with water (5 x 10 mL), dried (Na_2SO_4) and evaporated (15 Torr) affording pure (NMR) peptides (Table 1).

BocGly-PheOEt.[17] ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (t, *J* = 7.1 Hz, 3H), 1.44 (s, 9H), 3.10 (m, 2H), 3.70 (m, 2H), 4.11 (q, *J* = 7.1 Hz, 2H), 4.85 (m, 1H), 5.58 (br s, 1H), 7.11 (br s, 1H), 7.14-7.26 (m, 5H).

BocAla-PheOEt.[18] ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (t, *J* = 7.1 Hz, 3H), 1.30 (d, *J* = 6.7 Hz, 2H), 1.43 (s, 9H), 3.10 (m, 2H), 4.11 (q, *J* = 7.1 Hz, 2H), 4.81 (m, 1H), 5.49 (br s, 1H), 7.01 (br s, 1H), 7.12-7.24 (m, 5H).

BocVal-PheOEt.[19] ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (d, *J* = 6.7 Hz, 3H), 0.92 (d, *J* = 6.7 Hz, 3H), 1.16 (t, *J* = 7.1 Hz, 3H), 1.44 (s, 9H), 2.06 (m, 1H), 3.05 (m, 2H), 4.02 (m, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 4.84 (m, 1H), 5.24 (br s, 1H), 7.04 (br s, 1H), 7-16-7-28 (m, 5H).

CbzVal-PheOEt.[20] ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.80 (d, *J* = 6.7 Hz, 3H), 0.85 (d, *J* = 6.7 Hz, 3H), 1.20 (t, *J* = 7.1 Hz, 3H), 2.01 (m, 1H), 3.04 (m, 2H), 3.92 (m, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 4.81 (m, 1H), 5.04 (s, 2H), 5.24 (br s, 1H), 7.04 (br s, 1H), 7.14-7.26 (m, 10H).
BocAib-ValOMe.[21] ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (d, *J* = 6.8 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H), 1.50 (s, 15H), 2.16 (m, 1H), 3.72 (s, 3H), 4.52 (m, 1H), 4.93 (br s, 1H), 7.02 (br s, 1H).
CbzGly-L-Phe-ValOMe.[16] ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.75 (d, *J* = 6.6 Hz, 3H), 0.78 (d, *J* = 6.6 Hz, 3H), 1.96 (m, 1H), 2.94 (m, 2H), 3.57 (s, 3H), 3.77 (d, *J* = 5.5 Hz, 2H), 4.34 (dd, *J* = 8.0, 5.5 Hz, 1H), 4.75 (m, 1H), 5.01 (s, 2H), 5.75 (t, *J* = 5.5 Hz, 1H), 6.86 (d, *J* = 8.0 Hz, 1H), 7.04-7.26 (m, 11H).

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