S-(2-Pyrimidinyl)- and *S*-(2-(4,6-dimethylpyrimidinyl))-1,1,3,3-tetramethylthiouronium hexafluorophosphates: novel reagents for in situ peptide coupling

Philip Garner,* Özge Şeşenoğlu and H. Ümit Kaniskan

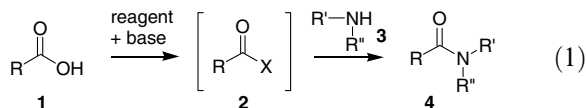*Department of Chemistry, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, OH 44106-7078, USA*

This paper is dedicated to Professor Miklos Bodanszky on the occasion of his 90th birthday and in
appreciation of his lasting contributions to the field of peptide chemistry

Abstract—Two new reagents for in situ peptide coupling based on the 2-mercaptopyrimidine core have been developed. The readily prepared thiouronium salts were found to promote both peptide and segment coupling efficiently with low racemization/epimerization levels.
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Because of the importance of both natural and unnatural peptides as structurally defined molecular scaffolds, there is continued interest in new and effective ways to construct the peptide bond.[1] One line of research has been concerned with the development of in situ peptide coupling reagents that both activate the carboxylic acid component and facilitate amide bond formation in one pot. A working mechanism for this transformation (Eq. 1) starting from carboxylic acid **1** involves the formation of an active ester **2** (X = leaving group) followed by nucleophilic acyl substitution with amine **3** to give the amide **4**. Two general classes of in situ peptide coupling reagents have emerged from these studies (Fig. 1). The reagents are exemplified by the uronium salt **5** (HBTU, Y = CH$_2$)[2] and phosphonium salt **6** (PyBOP, Y = CH$_2$),[3] both of which were based on the peptide coupling additive HOBt.[4]

Figure 1. HOAt and HOBt-based peptide coupling reagents.

stereochemical integrity of the α-carbon.[5] An analogous phosphonium reagent **8** (PyAOP, Y = N) was reported the next year.[6] While originally formulated as uronium salts, the structures of HBTU and HATU were later reformulated as guanidinium salts **5b** (*N*-HBTU) and **7b** (*N*-HATU) based on X-ray crystallographic data.[7] A deliberate synthesis of *O*-HATU (**7a**) was recently reported.[8] Based on the limited number of direct comparisons (Ref. 8), *O*-HATU appears to be a more effective peptide coupling reagent than *N*-HATU but it is not commercially available.

The design of *N*-HATU was based on the hypothesis that the nitrogen atom at C-7 would accelerate peptide coupling by facilitating intramolecular acylation of the amine component via the hydrogen bonded ensemble **9** (Fig. 2). It was argued that the seven-membered ring transition state (TS) would be favored over the possible alternative six-membered TS (H-bonding to N-2)

$$\underset{\textbf{1}}{\overset{O}{\underset{R}{\bigvee}}OH} \xrightarrow{\substack{\text{reagent} \\ + \text{base}}} \left[\underset{\textbf{2}}{\overset{O}{\underset{R}{\bigvee}}X} \right] \xrightarrow[\textbf{3}]{\overset{R'-NH}{\underset{R''}{}}} \underset{\textbf{4}}{\overset{O}{\underset{R}{\bigvee}}N{\overset{R'}{\underset{R''}{}}}} \qquad (1)$$

A major advance came in 1993, when Carpino reported the effectiveness of the in situ peptide coupling reagent **7** (HATU, Y = N) in terms of both chemical yield and
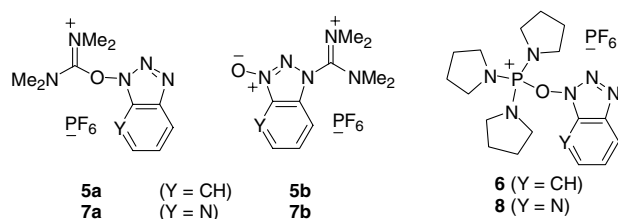
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* Corresponding author. Tel.: +1 216 368 3696; fax: +1 216 368 3006; e-mail: ppg@case.edu
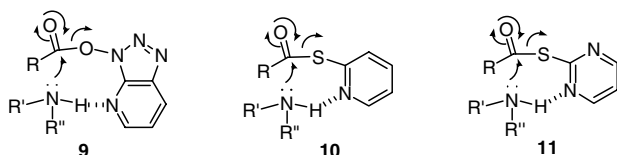
Figure 2. Proposed activated complexes.

because the former better accommodated a more linear H-transfer.[9] The enhanced rate could also be attributed to the increased acidity of 7-amino-1-hydroxybenzo-triazole (HOAt, $pK_a = 8.7$ in DMSO).[10] However, sub-sequent studies with *N*-HATU isomers underscored the special influence of the 7-amino group.[11] Because of its effectiveness, *N*-HATU has become the reagent of choice for difficult peptide couplings. Unfortunately, *N*-HATU is an expensive reagent and this has limited its use to some extent. The cost of *N*-HATU can be traced to its 7-aza-1-hydroxybenzotriazole (HOAt) core, which is prepared by a multistep synthesis.[12] With this back-ground in mind, we explored a new class of in situ peptide coupling reagents based on the inexpensive 2-mercaptopyrimidine core.

This study actually began during our development of *S*-(2-pyridinyl)-1,1,3,3-tetramethylthiouronium hexafluoro phosphate (HPTT) for the synthesis of 2-pyridinethiol esters.[13] At the time, the possibility that this reagent might also be used for in situ peptide coupling was con-sidered. The use of preformed 2-pyridinethiol active esters for peptide synthesis had been reported by Loyd and Young in 1968.[14] Shortly thereafter, Mukaiyama described an in situ variant of this reaction in conjunction with his oxidation–reduction condensation technology using 2,2′-dipyridyldisulfide and triphenylphosphine.[15] In a significant application, Kurokawa and Ohfune successfully used 2-pyridinethiol esters for peptide cou-pling to silylated α-aminoacids in their total synthesis of echinocandin D.[16] It can be argued that, in these reac-tions the peptide bond forming step proceeded via a pre-organized complex akin to **10**.[17] While HPPT did promote in situ peptide coupling, it was clearly inferior to *N*-HATU in terms of the reaction rate and racemiza-tion level when applied to a stringent test case.

Mechanistic considerations suggested to us that the overall rate of peptide coupling might be enhanced if the 2-pyridinethiol moiety ($pK_a = 9.81$ in H_2O)[18] of HPTT were replaced with a 2-pyrimidinethiol moiety ($pK_a = 7.01$ in H_2O).[19] Such a replacement would not only make the active ester carbonyl more electrophilic but also cause the tetrahedral intermediate to collapse faster. This substitution would also preclude the possi-bility of an unproductive thiol ester conformation (plac-ing the carbonyl anti to the heterocyclic nitrogen), thus enhancing the rate of coupling on entropic grounds. Finally, 2-mercaptopyrimidine is commercially available at a cost that is less than 1/10 of HOAt. The first hint of the benefit of 2-pyrimidinethiol esters for peptide cou-pling can actually be found (as a single example) in the Lloyd and Young full paper cited above. A more exten-sive description of the use of preformed 2-pyrimidineth-

iol active esters for peptide coupling was subsequently reported in a patent by Japanese workers but this work was apparently never followed up on.[20]

Two new coupling reagents, *S*-(2-pyrimidinyl)-1,1,3,3-tetramethylthiouronium hexafluorophosphate (**14**) and *S*-(4,6-dimethyl-2-pyrimidinyl)-1,1,3,3-tetramethylthio-uronium hexafluorophosphate (**16**) were prepared by routes analogous to the one we had developed for HPTT (Eq. 2). Thus, the known chloroformamidinium salt **12**[21] was combined with 2-mercaptopyrimidine **13** and 4,6-dimethyl-2-mercaptopyrimidine **15** in the presence of triethylamine to give the thiouronium hexafluorophos-phate salt **14** (mp 84–87 °C) in 65% yield and the corre-sponding dimethyl analog **16** (mp 100–102 °C) in 94% yield, respectively. The molecular structures of these compounds and their purity were established by ^1H and ^{13}C NMR spectroscopy as well as combustion analy-sis. Both of these compounds are air stable, non-hygro-scopic crystalline solids.[22]
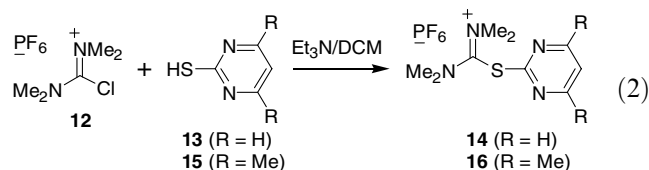
To evaluate the potential of **14** as a peptide coupling reagent, we examined its use for the synthesis of three dipeptides:[23] Cbz-Phe-Val-OMe, Cbz-Gly-Phe-OMe, and Cbz-Phg-Pro-NH$_2$.[24] We also used these reagents for the assembly of four tripeptides by segment cou-pling: Cbz-Phe-Val-Ala-OMe, Cbz-Phe-Val-Pro-NH$_2$, Cbz-Gly-Phe-Val-OMe, and Cbz-Gly-Phe-Pro-NH$_2$. These particular test cases were chosen primarily be-cause they had been previously synthesized using *N*-HATU. In this way, a direct comparison between these reagents could be made in terms of reaction rate and racemization/epimerization levels. With the exception of reagent used, we followed the general reaction condi-tions and workup procedure of Carpino. The initial cou-plings were effected by combining equimolar quantities of the carboxylic acid, coupling reagent, and the free amine (or its HCl salt), in the presence of 2 (or 3) equiv-alents of diisopropylethylamine (DIEA) in DMF. As indicated by the data in Table 1, reagent **14** is an effec-tive peptide and segment coupling reagent. When com-pared to *N*-HATU, the only significant differences occurred with two of the tripeptides (entries 2 and 6) and with the very stringent test case involving the stereo-chemically labile amino acid Cbz-Phg-OH (entry 7), where the degree of racemization was higher.[25]

We next set out to optimize the reaction conditions to reduce the level of racemization observed during the coupling of Cbz-Phg-OH to H-Pro-NH$_2$ (Table 2). Since longer reaction times did not lead to increased levels of racemization, it was hypothesized that α-deprotonation was occurring at the active ester stage and was related to the base strength of DIEA ($pK_a = 10.1$).[26] Reducing the amount of DIEA to one equivalent did not help

Table 1. Coupling reactions[23] with reagent **14**

Entry	Peptide	Base (equiv)	Crude yield (%)	DL or LDL (%)
1	Cbz-Phe-ξ-Val-OMe	DIEA (3)	93	2.7 ± 0.1
2	Cbz-Phe-Val-ξ-Ala-OMe	DIEA (3)	79	6.2 ± 0.1
3	Cbz-Phe-Val-ξ-Pro-NH$_2$	DIEA (2)	86	4.1 ± 0.15
4	Cbz-Gly-ξ-Phe-OMe	DIEA (3)	quant.	—
5	Cbz-Gly-Phe-ξ-Val-OMe	DIEA (3)	69	2.7 ± 0.1
6	Cbz-Gly-Phe-ξ-Pro-NH$_2$	DIEA (2)	61	4.3 ± 0.1
7	Cbz-Phg-ξ-Pro-NH$_2$	DIEA (2)	95	22.0 ± 1

Table 2. Optimization studies with Cbz-Phg-Pro-NH$_2$

Entry	Reagent	Base (equiv)	Additive (equiv)	Crude yield (%)	DL (%)
1	**14**	DIEA (1)	—	84	21.0
2	**14**	TMP (1)	—	87	11.7
3	**14**	—	—	51	9.6
4	**14**	DIEA (1)	2-Mercaptopyrimidine (1)	85	31.0
5	**16**	DIEA (1)	—	86	8.8
6	**16**	TMP (1)	—	82	9.1

(entry 1). However, switching to the weaker base 2,4,6-collidine (TMP) ($pK_a = 7.43$) cut racemization in half (entry 2). A similar level of racemization was observed without any added base but the yield of product was lower (entry 3). The fact that racemization actually increased in the presence of an extra equivalent of 2-mercaptopyrimidine (entry 4) suggested that this by-product actually contributed to the racemization problem. This reasoning led us to try the dimethylated reagent **16** since 4,6-dimethyl-2-mercaptopyrimidine was expected to be a more sterically hindered, kinetically weaker base. Indeed, the use of reagent **16** with 1 equivalent of either DIEA or 2,4,6-collidine brought the level of racemization down to 9% while retaining a high conversion (entries 5 and 6). While this improvement was gratifying, reagent **16** still did not outperform *N*-HATU in terms of reaction rate and racemization level. The former difference was clearly seen with a very hindered dipeptide. *N*-HATU mediated coupling of Cbz-Aib-OH and H-Aib-OMe produced Cbz-Aib-Aib-OMe in 56% yield. However, the reaction stalled at the active ester stage when either **14** or **16** was used.[27] These results may underscore the necessity of a geometrically favored H-bond between amine and active ester in TS.

In conclusion, we have developed a new type of reagent for in situ peptide coupling based on the inexpensive 2-mercaptopyrimidine core. The resulting thiouronium salts **14** and **16** are easy to prepare and, in favorable instances, may provide a cost effective alternative to *N*-HATU and *N*-HBTU.

Acknowledgements

Supplementary data

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4. Reagent abbreviations used: HOAt = 7-aza-1-hydroxy-benzotriazole; HOBt = *N*-hydroxybenzotriazole; PyAOP = 7-azobenzotriazolyoxytris(pyrrolidino)phosphonium hexafluorophosphate; PyBOP = 1-benzotriazolyoxytris(pyrrolidino)phosphonium hexafluorophosphate; *N*-HBTU = *N*-[(1*H*-benzotriazol-1-yl)-(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; *N*-HATU = *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide.
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22. *S*-(2-Pyrimidinyl)-1,1,3,3-tetramethylthiouronium hexafluorophosphate (**14**): Tetramethylchloroformamidinium hexafluorophosphate (5.0 g, 0.018 mol)[13] and 2-pyrimidinethiol (2.9 g, 0.018 mol) were dissolved in 10 mL of freshly distilled DCM. Triethylamine (2.5 mL, 0.018 mol) was added dropwise at rt (a white smoke evolved) and the reaction mixture was stirred for 10 min. It was then concentrated under reduced pressure and the resulting crude orange oil was crystallized from a mixture of 3:1 MeOH–*i*-PrOH to give white crystals, which were washed with *i*-PrOH and then Et_2O to give **14** as a white solid (4.17 g). 65% yield, mp = 84–87 °C; ^1H NMR (300 MHz, DMSO-d_6) δ 3.24 (s, 12H), 7.53 (t, $J = 4.9$ Hz, 1H), 8.81 (d, $J = 4.9$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 44.1, 120.0, 159.0, 164.8, 169.3; HRMS (FAB+) *m/z* calcd for $C_9H_{15}N_4S$ 211.1012, found 211.1003; Anal Calcd for $C_9H_{15}F_6N_4PS$: C, 30.34; H, 4.24; N, 15.73. Found: C, 30.29; H, 4.43; N, 15.51.

S-(4,6-Dimethyl-2-pyrimidinyl)-1,1,3,3-tetramethylthiouronium hexafluorophosphate (**16**) A similar procedure using 4,6-dimethyl-2-pyrimidinethiol gave **16** as a white solid (7.59 g). 94% yield, mp = 100–102 °C; ^1H NMR (600 MHz, $CDCl_3$) δ 2.45 (s, 6H), 3.33 (s, 12H), 6.98 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.5, 43.9, 118.9, 163.6, 169.2, 169.7; HRMS (FAB+) *m/z* calcd for $C_{11}H_{19}N_4S$ 239.1325, found 239.1312; Anal Calcd for

$C_{11}H_{19}F_6N_4PS$: C, 34.38; H, 4.98; N, 14.58. Found: C, 34.39; H, 5.12; N, 14.40.

23. General procedure for peptide couplings:
To 0.25 mmol of the acid, 0.25 mmol of amine (or amine salt), and 0.50 (or 0.75) mmol base in 2 (or 3) mL of DMF was added 0.25 mmol of coupling reagent at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and at rt for 1.5 h for dipeptides and 3 h for tripeptides. The mixture was diluted with 50 mL of EtOAc, and extracted with 1 N HCl (2×20 mL), satd $NaHCO_3$ (2×20 mL), brine (2×20 mL), and dried over $MgSO_4$. The solvent was removed under vacuum and crude peptide was directly analyzed by HPLC unless otherwise indicated. Solid NaCl was added to break up emulsions formed during the extractions. According to NMR and HPLC analysis no more than 3% (w/w) of 2-mercaptopyrimidine dimer was detected in crude products prepared with reagent **14**. All of the peptides synthesized in this paper are known. The NMR data obtained were consistent with that reported previously.[28–31]
24. Abbreviations used: Cbz = (benzyloxy)carbonyl; Phe = (*S*)-phenylalanine; Val = (*S*)-valine; Ala = (*S*)-alanine; Gly = glycine; Phg = (*S*)-phenylglycine; Pro = (*S*)-proline; Aib = 2-aminoisobutyric acid.
25. Literature values: Cbz-Phe-Val-Ala-OMe: 82% yield, 1.9% LDL (*N*-HATU + DIEA, Ref. 29a), 82% yield, 6.0% LDL (*N*-HBTU + DIEA, Ref. 29a); Cbz-Phe-Val-Pro-NH_2: 81% yield, 12.7% LDL (*N*-HATU + DIEA, Ref. 31b), 5.9% LDL (*N*-HATU + TMP-DIEA, Ref. 8), ~10% LDL (*O*-HATU + DIEA, Ref. 8), 3.4% LDL (*O*-HATU + TMP-DIEA, Ref. 8), 90% yield, 27.4% LDL (*N*-HBTU + DIEA, Ref. 31b), 20.6% LDL (*N*-HBTU + TMP-DIEA, Ref. 8), 10.3% LDL (*O*-HBTU + TMP-DIEA, Ref. 8); Cbz-Gly-Phe-Pro-NH_2: 86% yield, 0.8% LDL (*N*-HATU + DIEA, Ref. 31b), 85% yield, 5.9% LDL (*N*-HBTU + DIEA, Ref. 31b); Cbz-Gly-Pro-NH_2 81% yield, 6.3% LDL (*N*-HBTU + DIEA, Ref. 31b). In our hands, *N*-HATU gave 96% yield, 2.6% LDL for Cbz-Gly-Phe-Val-OMe and 94% yield, 3.1% DL for Cbz-Phg-Pro-NH_2.
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