Pergamon

Tetrahedron Letters 41 (2000) 2463–2466

TETRAHEDRON LETTERS

Polymer-bound TBTU as a new solid-supported reagent for peptide synthesis

Rafael Chinchilla, David J. Dodsworth, Carmen Nájera * and José M. Soriano

Departamento de Química Orgánica, Universidad de Alicante, Apartado 99, 03080 Alicante, Spain

Received 29 November 1999; revised 17 January 2000; accepted 25 January 2000

Abstract

A new polymer-supported TBTU (P-TBTU) has been prepared from polystyrene-bound 1-hydroxybenzotriazole (P-HOBT) and used efficiently as a solid-supported reagent for peptide-coupling reactions, and is even effective with wet solvents. The P-HOBT can be recovered by simple filtration and reused for the preparation of new P-TBTU.

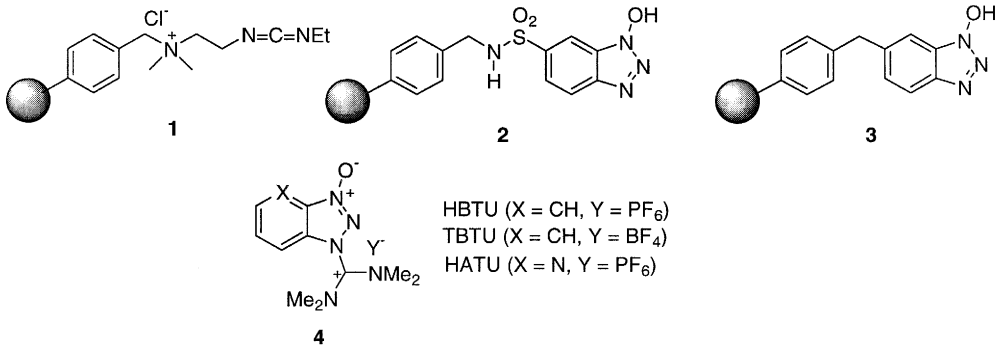
Keywords: aminium salts; peptides; polymers; coupling reagents.

The development of new polymer-supported reagents for organic synthesis is receiving increasing attention nowadays both for easy recycling ('green' chemistry) and for the simplification of conventional workup procedures.[1] For the creation of the amide bond in peptide-forming reactions, polymeric carbodiimide **1**[2] (P-EDC) and 1-hydroxybenzotriazole (P-HOBT), supported on normal cross-linked polystyrene beads **2**[3] or on macroporous polystyrene **3**,[4] have been used as activating agents in the presence of PyBrOP (bromotrispyrrolidinophosphonium hexafluorophosphate) or DCC (dicyclohexylcarbodiimide), respectively. P-HOBT **3**[5] has also been used in peptide synthesis[6,7] with DCC as the coupling agent, initially yielding polymeric active esters which are finally coupled with the corresponding amino acid ester.

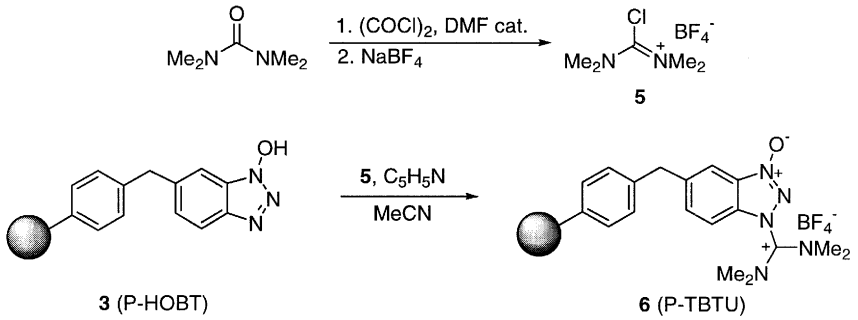
In peptide-coupling chemistry, tetramethylurea-derived aminium salts **4** from 1-hydroxybenzotriazole (HOBT) such as HBTU and TBTU,[8] or from 1-hydroxy-7-azabenzotriazole (HOAt) such as HATU,[9] are well-established reagents due to their efficiency and the low degree of undesirable racemization compared to the use of classical carbodiimide-coupling methods. In this context, and in connection with our project on the development of new reagents for peptide coupling and amide formation,[10] our aim was to design a polymer-bound HOBT-tetramethylurea-derived salt of the type **4** (X=CH) as a suitable reagent for the direct formation of the amide bond in peptides. This solid-supported reagent would allow the easy recovery of the immobilized HOBT by filtration once the reaction was finished.

* Corresponding author. Fax: +34 96 5903548; e-mail: cnajera@ua.es (C. Nájera)

0040-4039/00/$ - see front matter
PII: S0040-4039(00)00180-5



P-HOBT **3** was prepared from polystyrene–2% divinylbenzene copolymer resin (200–400 mesh, Fluka) according to the reported method.[6] The activity of the prepared P-HOBT was determined to be 0.25 mmol g^{-1} based on the synthesis of the *N*-isopropylacetamide.[5a] This polymer-supported HOBT **3** reacted with 4 equiv. of the chlorouronium tetrafluoroborate **5** in the presence of pyridine as the base for 24 h at room temperature in acetonitrile as the solvent (Scheme 1). The uronium salt **5** was obtained from tetramethylurea by reaction with oxalyl chloride in the presence of a catalytic amount of DMF, followed by anion exchange with sodium tetrafluoroborate (Scheme 1).[10] Due to its lower cost, the tetrafluoroborate counter-anion was chosen instead of the hexafluorophosphate, which is also frequently used in peptide-coupling reagents.



Scheme 1.

The reaction progress between P-HOBT and **5** was followed by IR spectroscopy, and considered complete after the total disappearance of the broad O–H stretching band at 3450 cm^{-1}. The resulting polymer **6** was filtered and washed thoroughly and successively with methanol, acetonitrile and ether, and dried at 60°C in vacuo (0.1 torr). The obtained resin showed a new strong IR band at 1670 cm^{-1} which could be assigned to an aminium C=N,[11] and its elemental analysis showed an increase of ca. 10% in nitrogen content, relative to the starting P-HOBT. As this new solid-supported reagent can be seen as a polymer-supported TBTU, it was given the acronym P-TBTU, following the common uronium nomenclature. The aminium representation for **6**, instead of the isomeric uronium, has been arbitrarily depicted according to the reported aminium crystalline structure for HBTU.[12]

The P-TBTU thus prepared was used as a solid-supported peptide-coupling reagent. Differently, *N*-protected amino acids reacted with amino acid ester hydrochlorides in the presence of P-TBTU and an organic base for 24 h (TLC) at rt yielding the corresponding dipeptides (Table 1), which were pure according to their ^1H NMR spectra after workup. Various solvents were used (Table 1, entries 1–3) and acetonitrile was finally chosen. Moreover, pyridine and triethylamine were tested as bases (Table 1, entries 3 and 4), the first one affording higher yields.

The isolated yields of the peptides obtained were good (Table 1, entries 1–7) and almost no difference

Table 1
Peptides prepared using P-TBTU as coupling reagent[a]

Entry	Peptide[b]	Solvent	Base	Yield (%)[c,d]
1	BocGly-PheOEt	CH_2Cl_2	pyridine	80
2	BocGly-PheOEt	DMF	pyridine	70
3	BocGly-PheOEt	MeCN	pyridine	82 (95)
4	BocGly-PheOEt	MeCN	Et_3N	71
5	BocAla-PheOEt	MeCN	pyridine	75
6	BocVal-PheOEt	MeCN	pyridine	73
7	CbzVal-PheOEt	MeCN	pyridine	70 (82)
8	BocVal-AibOMe[e]	MeCN	pyridine	37 (53)
9	BocAib-ValOMe[e]	MeCN	pyridine	35
10	Cbz*N*MeVal-ValOMe[e]	MeCN	pyridine	15
11	BzLeu-GlyOEt[e,f]	MeCN	pyridine	63[g]
12	CbzGlyPhe-ValOMe[e,h]	MeCN	pyridine	62[i]

[a] Reactions were performed at rt for 24 h. [b] The formed bond is indicated. [c] Isolated pure peptides (^{1}H NMR). [d] In parenthesis yields obtained using TBTU (3h, rt, MeCN, Py). [e] The reaction was performed at 50°C. [f] Young's test: $[\alpha]^{25}_D$ −24 (*c* 3.1, EtOH). Lit. [14] $[\alpha]^{20}_D$ −34 (*c*, 3.1, EtOH) [g] 35% at rt. [h] Anteunis's test: No epimerization was observed (^{1}H NMR, 500 MHz). [i] 44% at rt.

was observed when protecting groups such as Boc or Cbz were employed (Table 1, compare entries 6 and 7). However, when sterically hindered amino acid derivatives, such as those from 2-aminoisobutyric acid (Aib), were employed in the coupling reaction, the isolated yields were only ca. 10% after 1 day at rt. In these cases (Table 1, entries 8 and 9), heating the reaction to 50°C was necessary to increase the final yield. The same increase in the reaction temperature was used when coupling an *N*-methylated residue, although even under these conditions the isolated yield was very low (Table 1, entry 10). In order to compare all these results with those obtained when using TBTU, some coupling reactions were performed using this reagent under similar reaction conditions (see Table 1, entries 3, 7 and 8). The reaction times were longer when using P-TBTU rather than TBTU, as expected, when coupled with polymer-supported reagents. Moreover, the isolated yields when using P-TBTU were slightly lower than with TBTU.

The extent of possible racemizations produced using P-TBTU as a peptide-coupling reagent was checked by measuring the epimerization degree on the tripeptide generated by coupling CbzGlyPheOH and ValOMe (Anteunis' test).[13] No epimerization was detected in the final peptide by ^{1}H NMR (500 MHz) analysis, irrespective of whether the reaction was performed at rt or 50°C (Table 1, entry 12), the spectrum showing just one diastereomer. Moreover, only 15% of the racemized D-isomer of the dipeptide generated by coupling L-BzLeuOH and GlyOEt (Young's test) was obtained, as measured by comparison of its optical rotation value with that obtained from the literature (Table 1, entry 11).[14] This level of racemization, especially in Young's test, is lower than those achieved using TBTU as coupling agent.[15] Even compared with HATU, P-TBTU gave similar low epimerization in Anteunis' test and also a lower amount of racemization in Young's test.[15]

The hydrolytic stability of this new coupling reagent is remarkable. Thus, when the coupling reaction between BocGlyOH and PheOEt was performed using MeCN/water (5%) as solvent, 75% of BocGly/PheOEt was isolated which is not a very different yield than when using technical grade (99%) MeCN (Table 1, entry 3). However, when TBTU was used under the same reaction conditions (MeCN and 5% water), only 20% yield of the corresponding dipeptide was obtained.

After each of the performed coupling reactions, the solid-supported P-HOBT was recovered by simple filtration, showing identical IR spectra to that originally used. Using this recovered P-HOBT, new P-TBTU was prepared and was employed in coupling reactions, showing a decrease in the isolated yield of the final peptide of just ca. 5%.

We concluded that P-TBTU is a promising new solid-supported peptide-coupling reagent with high hydrolytic stability which allows for the recovery of the P-HOBT and affords results comparable to TBTU. Studies on the influence of the use of other polymers for supporting TBTU, and the preparation of other solid-supported aminium/uronium salts for peptide-coupling reactions are now underway.[16]

Acknowledgements

We thank the Dirección General de Enseñanza Superior e Investigación Científica (project no. 1FD97-0721) of the Ministerio de Educación y Cultura (MEC) and the Conselleria de Cultura Educació i Ciència of the Generalitat Valenciana (project no. GV99-33-1-02) for financial support. We also thank Luxembourg Industries (PAMOL) Ltd. for a generous gift of reagents.

References

1. For recent reviews on polymer-supported chemistry, see: (a) Hermkens, P. H. H.; Ottenheijm, H. C. J.; Rees, D. C. *Tetrahedron* **1996**, *52*, 4527–4554. (b) Hermkens, P. H. H.; Ottenheijm, H. C. J.; Rees, D. C. *Tetrahedron* **1997**, *53*, 5643–5678. (c) Brown, R. *Contemp. Org. Synth.* **1997**, 216–237. (d) Früchtel, J. S.; Jung, G. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 17–42. (e) Gravert, D. J.; Janda, K. D. *Chem. Rev.* **1997**, *97*, 489–509. (f) Brown, R. C. D. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3293–3320. (g) Brown, A. R.; Hermkens, P. H. H.; Ottenheijm, H. C. J.; Rees, D. C. *Synlett.* **1998**, 817–827. (h) Wenworth Jr., R.; Janda, K. D. *Chem. Commun.* **1999**, 1917–1924. (i) Lorsbach, B. A.; Kurth, M. J. *Chem. Rev.* **1999**, *99*, 1549–1581. (j) James, I. W. *Tetrahedron* **1999**, *55*, 4855–4946.
2. Desai, M. C.; Stephens Stramiello, L. M. *Tetrahedron Lett.* **1993**, *34*, 7685–7688.
3. Pop, I. E.; Déprez, B. P.; Tartar, A. L. *J. Org. Chem.* **1997**, *62*, 2594–2603.
4. (a) Dendrinos, K. G.; Jeong, J.; Huang, W.; Kalivretenos, A. G. *Chem. Commun.* **1998**, 499–500. (b) Huang, W.; Kalivretenos, A. G. *Tetrahedron Lett.* **1995**, *36*, 9113–9116.
5. Polymer **3** has also been used for the synthesis of *N*-hydroxysuccinimide esters: (a) Dendrinos, K. G.; Kalivretenos, A. G. *Tetrahedron Lett.* **1998**, *39*, 1321–1324; and for the protection of amides as carbamates with the Cbz, Fmoc and Boc groups: (b) Dendrinos, K. G.; Kalivretenos, A. G. *J. Chem. Soc., Perkin Trans 1* **1998**, 1463–1464.
6. Kalir, R.; Warshawsky, A.; Fridkin, M.; Patchornik, A. *Eur. J. Biochem.* **1975**, *59*, 55–61.
7. Mokotoff, M.; Zhao, M.; Roth, S. M.; Shelley, J. A.; Slavoski, J. N. Kouttab, N. M. *J. Med. Chem.* **1990**, *33*, 354–360.
8. König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788–798.
9. Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397–4398.
10. Bailén, M. A.; Chinchilla, R.; Dodsworth, D. J.; Nájera, C. *J. Org. Chem.* **1999**, *64*, 8936–8939.
11. The same strong band was observed in a IR spectrum of TBTU.
12. Abdelmoty, I.; Albericio, F.; Carpino, L. A.; Foxman, B. M.; Kates, S. A. *Lett. Pept. Sci.* **1994**, *1*, 57–67.
13. Van der Auwera, C.; Van Damme, S.; Anteunis, M. J. O. *Int. J. Pept. Protein. Res.* **1987**, *29*, 464–471.
14. Williams, M. W.; Young, G. T. *J. Chem. Soc.* **1963**, 881–889.
15. TBTU: Anteunis' test, 1.1% D-isomer. Young's test, 39% D-isomer (Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927–1930). HATU: Anteunis' test, no epimerization. Young's test, 20% D-isomer (Ref. 10).
16. In a typical coupling reaction, to a solution of the *N*-protected amino acid (0.25 mmol) in the corresponding solvent (7 mL) was added P-TBTU (1.2 g) and the organic base (0.5 mmol). The heterogeneous mixture was stirred for 5 min and the aminoester hydrochloride (0.25 mmol) was added, stirring the suspension for 1 day at rt or 50°C (see Table 1). The resulting P-HOBT was recovered by filtration and washed with EtOAc (50 mL). The filtrate was washed with saturated NaCl (20 mL), 2N HCl (3×10mL), saturated $NaHCO_3$ (3×10 mL) and water (3×10 mL). The organic layer was dried ($NaSO_4$) and the solvent was evaporated (15 torr) affording the corresponding peptide.