Pergamon

Tetrahedron Letters 42 (2001) 4487–4489

TETRAHEDRON LETTERS

Polymer-bound *N*-hydroxysuccinimide as a solid-supported additive for DCC-mediated peptide synthesis

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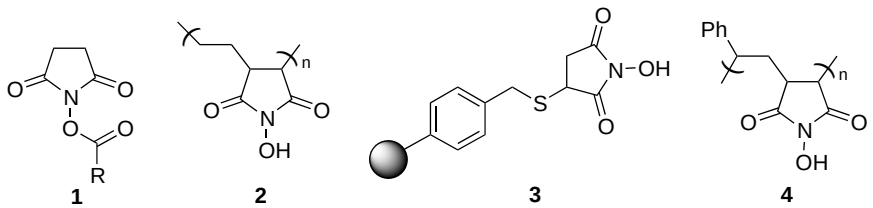
Received 12 March 2001; revised 7 May 2001; accepted 9 May 2001

Abstract—Polymer-supported *N*-hydroxysuccinimide (P-HOSu) has been prepared from a styrene/maleic anhydride co-polymer and used as an easily removable activating and racemization-reducing additive for the peptide coupling of amino acid derivatives using DCC.

The use of 1,3-dicyclohexylcarbodiimide (DCC) in the presence of *N*-hydroxysuccinimide (HOSu) or other HOX compounds as racemization-reducing additives is a classical method for peptide bond formation, which involves the formation of active esters,[1] derivative **1** being an example of one of them prepared from HOSu. These activated species usually subsequently undergo smooth coupling reactions with amines for the formation of amide bonds, and have been widely used in both solution and solid-phase peptide synthesis.[2] Isolated HOSu-derived active esters have been employed, for instance, for the preparation of leucyldemethylblasticidin S as part of a study of self-resistance mechanisms of *Streptomyces* bacteria,[3] or for the synthesis of a novel family of hairpin cyclic peptides.[4] In addition, they have been used in the final step of the synthesis of the immunomodulator galactosylceramide AGL-537,[5] and for the preparation of biotinylated ligands for asymmetric hydrogenations,[6] tetramine calixarenes as selective extractants of actinide ions,[7] or molecular umbrellas.[8] Typically, the formation of isolated or in situ formed active HOSu-derived esters (**1**) is achieved by the use of DCC as the initial coupling agent,[1,2] although a uronium salt such as the commercially available 2-succinimido-1,1,3,3-tetramethyluronium tetrafluoroborate (TSTU)[9] or HOSu-derived carbonates or phosphates have also been used.[1] In addition, HOSu esters have been isolated via transesterification reactions mediated by polystyrene-supported 1-hydroxybenzotriazole-derived esters.[10]

Few examples of solid-supported HOSu esters have been reported in the literature. Thus, polymer-bound HOSu from a poly(ethylene-co-*N*-hydroxymaleimide) polymer **2** cross-linked with aliphatic[11] or aromatic diamines[12] have been prepared using DCC, and employed for the synthesis of amides and peptides. More recently, supported HOSu (**3**) has been prepared from rather expensive Merrifield and ArgoPore™ resins and used for the formation of resin-bound active HOSu esters from labeling reagents such as fluorescein, coumarin, acridinium and biotin, followed by coupling with amines such as striol, thyroxine, phenytoin and desipramine haptens for clinical immunoassays.[13] In most cases, these HOSu-supported reagents were used for the formation of the peptide bond through an isolated polymeric succinimidyl ester.

In this context, we thought of using a supported HOSu of type (**2**) as an additive for the DCC-mediated direct synthesis of peptides. This polymeric reagent would promote the acylation step and would allow a facile



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Keywords: amino acids; coupling reagents; polymers; peptides.

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0040-4039/01/$ - see front matter
PII: S0040-4039(01)00763-8

purification and recovery by simple filtration. Thus, polymer-supported HOSu [P-HOSu (**4**)] was obtained by the reaction of commercially available poly(styrene-*alt*-maleic anhydride)[14] with an 50% aqueous solution of hydroxylamine at 90°C for 1 day. After addition of hexane and acidification with HCl, the resulting P-HOSu (**4**) was filtered and dried in vacuo. This final polystyrene copolymer was easy to handle in different solvents and presented good mechanical stability, the cross-linking with diamines, as in the case of poly(ethylene-co-*N*-hydroxymaleimide) (**2**)[11,12] was not found to be necessary.

The loading of P-HOSu (**4**) was determined by the assay of the OH group content.[15] Thus, treatment of 450 mg of P-HOSu with acetic anhydride, subsequent filtration and washing of the acetylated polymer and further reaction with *N*-isopropylamine afforded 67 mg of the corresponding *N*-isopropylacetamide, which indicated an activity for the P-HOSu of 1.5 mmol g^{-1}.

The obtained P-HOSu (**4**) was employed as an additive in the DCC-mediated peptide coupling reaction between Boc, Cbz and Fmoc-protected amino acids and amino acid esters. The results obtained for different peptide couplings using P-HOSu, and the comparable reaction using HOSu and no additive, are presented in Table 1. The base added to the reaction mixture exerted a slight influence on the final yield. Thus, when pyridine was used in the reaction with P-HOSu, the dipeptide BocGly-PheOEt was obtained in 98% yield (Table 1, entry 1), however, when triethylamine was used a 90% yield was isolated (Table 1, entry 3). The reaction was normally performed at room temperature, except in the case of sterically hindered amino acids, which required warming of the reaction mixture to 40°C in order to achieve acceptable yields. Filtration and washing of the reaction mixture allowed the separation of the P-HOSu, which was then reused without appreciable loss of activity.

The presence of P-HOSu generally increased the isolated yield of the obtained peptide, as shown for instance in the synthesis of BocGly-PheOEt and BocAla-ValOMe in 98 and 95% isolated yields, respectively (Table 1, entries 1 and 8). When only DCC was employed, however, the yields were 70 and 68%, respectively (Table 1, entries 2 and 5). The effect of P-HOSu was, in general, similar to that of adding free HOSu, although in some cases P-HOSu afforded better yields (Table 1, compare entries 1 and 2 and entries 8 and 9). When difficult couplings using sterically hindered α,α-dialkylamino acids such as α-aminoisobutyric acid (Aib) were performed, the process was clearly favored when adding P-HOSu to the DCC-mediated reaction. Thus, BocAib-ValOMe was isolated in 63% yield when using P-HOSu/DCC as compared with only 40% yield when using DCC (Table 1, entries 13 and 14). In addition, hindered *N*-methylated amino acids such as Cbz*N*-MeValOH could also be coupled using this methodology (Table 1, entry 16). The use of diisopropylcarbodiimide (DIC) instead of DCC as coupling agent afforded similar or even slightly lower results.

The extent of racemization employing this additive was examined by using Anteunis' test[16] (the coupling of CbzGlyPheOH and ValOMe, Table 1, entries 17 and 18). When Anteunis' tripeptide was prepared using

Table 1. Peptides prepared by DCC-mediated coupling using P-HOSu (**4**) or HOSu as additives

Entry	Peptide[a]	Additive	*T* (°C)	*t* (h)	Base	Yield (%)[b,c]
1	BocGly-PheOEt	P-HOSu	25	6	Pyridine	98
2	BocGly-PheOEt	HOSu	25	6	Pyridine	85 (70)
3	BocGly-PheOEt	P-HOSu	25	6	Et_3N	90
4	CbzGly-GlyOMe	P-HOSu	25	7	Pyridine	94
5	CbzGly-GlyOMe	HOSu	25	7	Pyridine	96 (93)
6	CbzGly-AlaOMe	P-HOSu	25	7	Pyridine	83
7	CbzGly-AlaOMe	HOSu	25	7	Pyridine	94 (85)
8	BocAla-ValOMe	P-HOSu	25	7	Pyridine	95
9	BocAla-ValOMe	HOSu	25	7	Pyridine	83 (68)
10	FmocAla-GlyOEt	P-HOSu	40	24	Pyridine	47
11	FmocAla-GlyOEt	HOSu	40	24	Pyridine	58 (42)
12	BocVal-AibOMe	P-HOSu	40	24	Pyridine	65
13	BocAib-ValOMe	P-HOSu	40	24	Pyridine	63
14	BocAib-ValOMe	HOSu	40	24	Pyridine	70 (40)
15	CbzAib-ValOMe	P-HOSu	40	24	Pyridine	59
16	Cbz*N*MeVal-ValOMe	P-HOSu	40	24	Pyridine	56
17	CbzGlyPhe-ValOMe[d]	P-HOSu	25	7	Pyridine	90[e]
18	CbzGlyPhe-ValOMe[d]	HOSu	25	7	Pyridine	88[f] (84[g])

[a] The formed bond is indicated.
[b] Isolated pure peptides (^{1}H NMR).
[c] In parenthesis yields obtained without any additive under the same reaction conditions.
[d] Anteunis's test.[16]
[e] A 18:1 mixture of epimers was detected (^{1}H NMR).
[f] A 25:1 mixture of epimers was detected (^{1}H NMR).
[g] A 9:1 mixture of epimers was detected (^{1}H NMR).

DCC without any additive, the observed degree of epimerization was 9:1, as measured by ^{1}H NMR (Table 1, entry 18, footnote g), whereas when the reaction was performed in the presence of P-HOSu the level of epimerization was reduced to 18:1 (^{1}H NMR) (Table 1, entry 17, footnote e). By way of comparison, a 25:1 mixture was detected adding HOSu (Table 1, entry 18, footnote f).

We conclude that the economical, simple and easily recoverable polymer-supported HOSu represents a convenient additive which improves the applicability of the DCC-peptide coupling methodology. Further studies on the use of this polymeric HOSu for the preparation of other polymeric HOSu-derived reagents are now underway.

In a typical peptide coupling reaction using P-HOSu (**4**), a suspension of the protected amino acid (0.5 mmol), aminoester hydrochloride (0.5 mmol), P-HOSu (367 mg, 0.55 mmol of active HOSu), DCC (103 mg, 0.5 mmol) and pyridine (122 μL, 1.5 mmol) in MeCN (15 mL) was stirred at rt or 40°C for the time indicated in Table 1. Hexane (10 mL) was added and the resulting precipitate was washed with hexane/AcOEt, 1/1 (10 mL) and filtered. The filtered solid consisted of P-HOSu, which was recovered almost quantitatively. To the filtrate was added saturated NaCl (20 mL) and the mixture was extracted with AcOEt (3×10 mL). The organic layer was washed with 2 M HCl (3×10 mL), saturated $NaHCO_3$ (3×10 mL) and water (3×10 mL). The solvent was dried (Na_2SO_4) and evaporated (15 Torr) affording the corresponding crude peptides.

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.

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