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Ammonium salts from polymer-bound *N*-hydroxysuccinimide as solid-supported reagents for EDC-mediated amidations

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Abstract—New ammonium and alkylammonium salts derived from a polymeric *N*-hydroxysuccinimide (P-HOSu) have been prepared and used for the amidation of carboxylic acids and amino acids mediated by 1-ethyl-3-(3'-dimethylamino-propyl)carbodiimide hydrochloride (EDC). These polymer-supported ammonium salts afforded the corresponding amides in good yield, without detectable α -racemization and with easy recovery of the P-HOSu after the amidation reaction, being especially suitable for the amidation of Fmoc-protected amino acids. © 2002 Published by Elsevier Science Ltd.

The synthesis of amides from the corresponding carboxylic acids is a basic transformation in organic synthesis,¹ being particularly interesting for the synthesis of amino acid amides, useful for the preparation of peptide amides. Thus, the C-terminals of many biologically active peptides bear a primary amide, some examples being the muramyl peptides, which are sleep factors which stimulate slow-wave sleep,^{2a} a considerably number of hormones including secretin,^{2b} calcitonin,^{2c} thyroliberin^{2d} and luliberin,^{2e} antimicrobials such as protegrins^{2f} or fungicides.^{2g} There are also frequent examples of *N*-alkylated active amide peptides,³ such as HIV and FIV protease inhibitors^{4a} or analogs of enkephalins with opioid activity.^{4b} In addition, changing the C-terminal carboxy function of a biologically active peptide into a peptidyl *N*-alkylamide often leads to analogs with more favorable or prolonged activity.^{4c} Therefore, the development of new procedures for the easy, clean and racemization-free amidation of carboxylic acids with application to amino acids seems desirable.

The preparation of amides by addition of ammonia or amines to carboxylic acid derivatives such as acyl halides, anhydrides or esters can be troublesome when other sensitive functionalities are present, due to the basic and nucleophilic character of these nitrogenated compounds. This is something especially serious when base-sensitive protected amino acids (i.e. Fmoc-amino

acids) are the starting materials for the synthesis of amidated peptides.

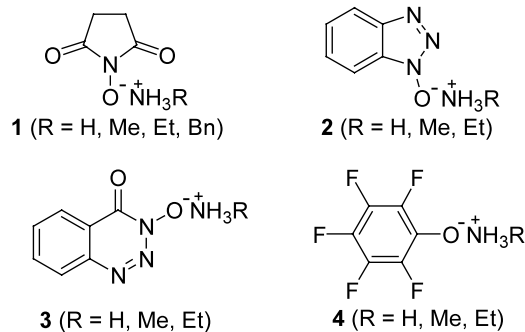
The use of carbodiimides such as dicyclohexylcarbodiimide (DCC) for the activation of a carboxylic acid together with the addition of an amine is a frequently used procedure for the amidation reaction in spite of known problems related to formation and removal of urea by-products. For example, high dielectric solvents, necessary for dissolving many starting materials, favors the formation of *N*-acylureas.⁵ Furthermore, for protected amino acids, the activation is accompanied generally by high levels of racemization.⁵ All these undesirable processes can be avoided or diminished by the addition of certain additives such as *N*-hydroxysuccinimide (HOSu), 1-hydroxybenzotriazole (HOBT) or 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one (HOObt).⁵ Recently, other coupling reagents such as PyBOP,^{6,7} HBTU,^{6,7} HOTT^{6,8} and TOTT^{6,8} have been used for the activation of the carboxylic acid function and trapping of in situ-generated ammonia.

An interesting combination of amine-releasing and racemization-lowering reagents are the ammonium and alkylammonium salts of HOSu **1**,⁹ which show higher solubility in organic solvents than ammonium chloride or amine chlorhydrates. These salts **1** are easily prepared by mixing HOSu and the corresponding amine and reacts, in the presence of DCC, with protected amino acids and peptide acids affording the corresponding epimerization-free primary and secondary amides. Reagent **1** (R=H) has also recently been employed in the DCC-mediated amidation in high yield

Keywords: amidation; ammonium salts; polymers; *N*-hydroxysuccinimide.

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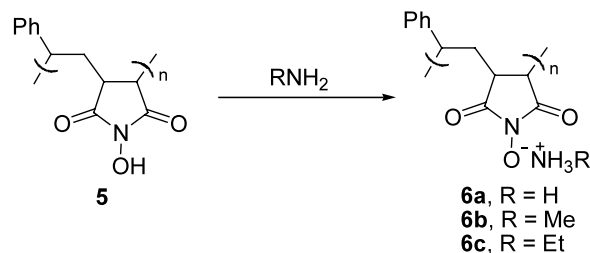
of a polymerization-sensitive amino acid bearing an acrylamide moiety.¹⁰ Amidations mediated by DCC or diisopropylcarbodiimide (DIC) using the more expensive corresponding salts from HOBT **2**,¹¹ HOObt **3**^{9a} and pentafluorophenol **4**¹² have been reported.



At the same time, the development of polymer-supported reagents applicable to organic synthesis is receiving enormous attention in the expanding field of 'green chemistry' because of easy recycling and the simplification of conventional work-up procedures.¹³ For example, polymer-supported HOBT¹⁴ and polymeric HOSu¹⁵ have been employed for the creation of the amide bond. In this context, and in connection with our project on the development of new solid-supported reagents applicable to peptide chemistry such as P-TBTU,¹⁶ we have recently developed a co-polymer containing the *N*-hydroxysuccinimide moiety (P-HOSu, **5**).¹⁷ This polymer can be easily obtained by reaction of aqueous hydroxylamine with inexpensive commercially available poly(styrene-*alt*-maleic anhydride) and has been employed as a recoverable racemization-reducing additive for the DCC-mediated coupling of amino acids.¹⁷ In addition, P-HOSu (**5**) has also been used for the preparation of polymer-bound amino-protecting reagents such as Fmoc-P-OSu¹⁸ or 2,7-di-*tert*-butyl-Fmoc-P-OSu.¹⁹

With these antecedents, we envisaged that P-HOSu (**5**) could be suitable for the preparation of polymer-supported ammonium or alkylammonium salts which would be effective as mild amine-releasing and also racemization-lowering agents in carbodiimide-mediated amidation reactions. This solid-supported reagent would allow the easy separation of the P-HOSu (**5**) once the amidation reaction is finished, which could be particularly valuable when working on a small scale. Ammonium, methylammonium or ethylammonium salts **6** were obtained by addition of aqueous solutions of ammonia, methylamine or ethylamine, respectively, to P-HOSu (**5**),¹⁷ followed by filtration of the precipitate, washing and drying in vacuo in an oven (Scheme 1). The loading of **6** was estimated in ca. 1 mmol/g according to the loading of starting P-HOSu (**5**).²⁰

These salts were employed in the amidation reaction of carboxylic acids and amino acids mediated by 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (EDC). The selection of this carbodiimide was made according to the convenient generation of easily remov-



Scheme 1.

able water-soluble urea by-products.²¹ Thus, reaction of ammonium salts **6** and EDC with carboxylic acids or Cbz-, Fmoc- and Boc-protected amino acids in refluxing methanol for 1 day, afforded the corresponding crude pure primary and secondary amides in good yields after filtration and aqueous work-up (Table 1). The residue obtained after the filtration step consisted of P-HOSu (**5**), which can be re-used for the preparation of new **6**.

Amidation reactions took place with aliphatic and aromatic carboxylic acids (Table 1, entries 1–6) and no by-products were observed even if functional groups sensitive to ammonolysis were present. Thus, no Michael addition product was detected in the reaction with an α,β -unsaturated acid such as cinnamic acid (Table 1, entry 5), only the pure amide being isolated. In addition, no nucleophilic substitution reaction was observed when the reaction was performed with a haloacid such as ϵ -bromocaproic acid (Table 1, entry 6). Differently protected α -amino acids were also amidated (Table 1, entries 7–14) in good yields. Thus, side-chain protecting groups such as benzyl esters remained intact as in the case of the reaction of Cbz-Tyr(Bn)-OH (Table 1, entry 7), the reaction being also successful when Cbz- (Table 1, entry 7), Boc- (Table 1, entries 11–14) or base-sensitive Fmoc-protected amino acids (Table 1, entries 8–10) were used. In all cases, no racemization of the final amide was observed, comparing detected and reported optical rotation values (Table 1, entries 7 and 8 and 11–13) and using chiral HPLC analysis.²² Even a very hindered amino acid such as Boc-protected α -aminoisobutyric acid (Aib) afforded an acceptable yield of the corresponding pure amide (Table 1, entry 14). As comparison we prepared the non-polymeric *N*-hydroxysuccinimide-derived ammonium salt **1** (R = H)^{9a} and performed the amidation of Fmoc-Leu-OH under the same reaction conditions. This reaction afforded 87% of a crude containing the corresponding amide and ca. 30% of 9-methylene-9H-fluorene which is the residual product from partial deprotection of the Fmoc-group, the polymeric counterpart **6a** affording 83% of the pure amide **6a** (Table 1, entry 8). Furthermore, when the amidation reaction of Fmoc-Leu-OH was performed in the absence of **6a**, but using 20% aqueous ammonia and EDC in methanol as solvent, ca. 60% of Fmoc-deprotection was detected. A similar 50% of deprotection was observed under the same reaction conditions, but using in situ generated ammonia from ammonium chloride and diisopropylethylamine.

Table 1. Preparation of amides employing polymeric salts **6**

Entry	Acid	Reagent	Amide	Yield ^a (%)	Mp ^b (°C)	$[\alpha]_D^{25,b}$
1	PhCO ₂ H	6a	PhCONH ₂	78	130–131 (128–129) ^c	
2	PhCO ₂ H	6b	PhCONHMe	74	78–79 (76–78) ^d	
3	PhCO ₂ H	6c	PhCONHEt	84	64–65 (60) ^e	
4	2-ClC ₆ H ₄ CO ₂ H	6a	2-ClC ₆ H ₄ CONH ₂	75	141–142 (142–143) ^c	
5	(<i>E</i>)-PhCH=CHCO ₂ H	6a	(<i>E</i>)-PhCH=CHCONH ₂	86	148–149 (146–148) ^c	
6	BrCH ₂ (CH ₂) ₄ CO ₂ H	6a	BrCH ₂ (CH ₂) ₄ CONH ₂	87	92–93 (94–95) ^c	
7	CbzTyr(Bn)OH	6a	CbzTyr(Bn)NH ₂	79	183–184 (184–186) ^c	–7.8 [–7.1 (<i>c</i> 1, acetone)] ^c
8	FmocLeuOH	6a	FmocLeuNH ₂	83	191–192 (181–183) ^c	–13.8 [–13.5 (<i>c</i> 1, EtOH)] ^c
9	FmocLeuOH	6b	FmocLeuNHMe	73	181–182	–16.4 (<i>c</i> 1, EtOH)
10	FmocLeuOH	6c	FmocLeuNH ₂ Et	67	185–186	–20.5 (<i>c</i> 1, EtOH)
11	BocLeuOH	6a	BocLeuNH ₂	79	150–151 (150–152) ^f	–13.0 [–11.9 (<i>c</i> 1, MeOH)] ^f
12	BocLeuOH	6b	BocLeuNHMe	73	120–121 (118–120) ^f	–15.1 [–15.3 (<i>c</i> 1, MeOH)] ^f
13	BocLeuOH	6c	BocLeuNH ₂ Et	75	105–106 (102–105) ^f	–40.4 [–39.1 (<i>c</i> 1, MeOH)] ^f
14	BocAibOH	6a	BocAibNH ₂	54	170–171 (167–169) ^c	

^a Isolated pure crude compounds (¹H, ¹³C NMR) after work up.^b For crude products. In parenthesis or square brackets, reported values.^c Ref. 8.^d Ref. 23.^e Ref. 24.^f Ref. 9a.

We conclude that the new polymeric *N*-hydroxysuccinimide ammonium salts **6** are appropriate reagents for the synthesis of primary and *N*-alkylated amides from carboxylic acids and amino acids using EDC as activating agent. This mild amidation system affords particularly good results with base-sensitive Fmoc-protected amino acids, even better than using the non-polymeric counterpart, allowing the preparation of racemization-free pure crude amides in good yields and with easy recycling of the residual P-HOSu (**5**).

Procedure for the synthesis of polymeric salts **6**

6a: P-HOSu^{17,20} (5 g, 5 mmol) was suspended in MeOH (80 mL) and a 20% aqueous solution of ammonia (4.7 mL, 50 mmol). The mixture was stirred vigorously for 1 day, and the residue was filtered and washed with cold mixtures of MeOH/toluene. The resulting solid was dried in vacuo at 70°C.

6b: P-HOSu^{17,20} (5 g, 5 mmol) was suspended in MeOH (80 mL) and a 40% aqueous solution of methylamine (4.4 mL, 50 mmol) and the mixture was stirred vigorously for 1 day. Water (50 mL) was added and the resulting solid was filtered, washed with water and dried in vacuo at 70°C.

6c: P-HOSu^{17,20} (5 g, 5 mmol) was suspended in MeOH (80 mL) and a 70% aqueous solution of ethylamine (4.1 mL, 50 mmol) was added and the mixture was stirred vigorously for 1 day. The solvent was evaporated in vacuo to almost dryness and toluene (50 mL) was added. The solid was filtered, washed with toluene and dried in vacuo at 70°C.

A typical procedure for amidation using **6**

A suspension of the carboxylic acid (1 mmol), the corresponding polymeric salt **6** (1.2 g, 1.2 mmol) and EDC (200 mg, 1.05 mmol) in MeOH (20 mL) was refluxed for 1 day. The solvent was evaporated and the residue was triturated with a mixture of water (20 mL) and AcOEt/hexane (30 mL, 2/1 v/v). The solid was filtered and washed with water, and a mixture of AcOEt/hexane (2/1 v/v), and consisted of P-HOSu (**5**). The filtrate was washed with 5% NaHCO₃ (3×20 mL) and water (20 mL) and the organics were dried over Na₂SO₄. The solvent was evaporated affording pure crude amides.

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References

- (a) March, J. *Advanced Organic Chemistry*; John Wiley & Sons: New York, 1992; pp. 416–425; (b) *Comprehensive Organic Synthesis*; Trost, B. M., Ed.; Pergamon Press: Oxford, 1991; Vol. 6, pp. 381–417.
- (a) Brown, R. *Behav. Brain. Res.* **1995**, *69*, 85–90; (b) Guchi, K.; Mochizuki, T.; Inoue, T.; Yanaihara, C.;

- Naruse, S.; Nokihara, K.; Forssmann, W. G.; Mutt, V.; Kanno, T.; Yanaihara, N. *Pept. Chem.* **1986**, 191–196; (c) Lee, Y. S.; Lee, H. J.; Pozdnyakov, P. I.; Samukov, V. V.; Kim, H. J. *J. Pept. Res.* **1999**, *54*, 328–335; (d) Fletcher, A. R.; Jones, J. H.; Ramage, W. I.; Stachulski, A. V. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2261–2267; (e) Seyer, R.; Aumelas, A.; Caraty, A.; Rivaille, P.; Castro, B. *Int. J. Pept. Protein Res.* **1990**, *35*, 465–472; (f) Tamamura, H.; Murakami, T.; Horiuchi, S.; Sugihara, K.; Otaka, A.; Takada, W.; Ibuka, T.; Waki, M.; Yamamoto, N. *Chem. Pharm. Bull.* **1995**, *43*, 853–858; (g) Marner, F.-J.; Moore, R. E.; Hirotsu, K.; Clardy, J. *J. Org. Chem.* **1977**, *42*, 2815–2819.
3. Songster, M. F.; Barany, G. *Methods Enzymol.* **1997**, *289*, 126–174.
4. (a) Le, V.-D.; Mak, C. C.; Lin, Y.-C.; Elder, J. H.; Wong, C.-H. *Bioorg. Med. Chem.* **2001**, *9*, 1185–1195; (b) Alsina, M. A.; Sole, N.; Mestres, C.; Busquets, M. A.; Haro, I.; Garcia Anton, J. M. *Int. J. Pharm.* **1991**, *70*, 111–117; (c) Meisenbach, M.; Echner, H.; Voelter, W. *Chem. Commun.* **1997**, 849–850.
5. Recent review: Albericio, F.; Chinchilla, R.; Dodsworth, D. J.; Nájera, C. *Org. Prep. Proc. Int.* **2001**, *33*, 203–303.
6. PyBOP: *O*-(benzotriazol-1-yl)-tris(pyrrolidino)phosphonium hexafluorophosphate. HBTU: *O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate. HOTT: *S*-(1-oxido-2-pyridinyl)-1,1,3,3-tetramethyluronium hexafluorophosphate. TOT: *S*-(1-oxido-2-pyridinyl)-1,1,3,3-tetramethyluronium tetrafluoroborate.
7. Wang, W.; McMurray, J. S. *Tetrahedron Lett.* **1999**, *40*, 2501–2504.
8. Bailén, M. A.; Chinchilla, R.; Dodsworth, D. J.; Nájera, C. *Tetrahedron Lett.* **2000**, *41*, 9809–9813.
9. (a) Somlai, C.; Szókán, G.; Baláspiri, L. *Synthesis* **1992**, 285–287; (b) Somlai, C.; Szókán, G.; Penke, B. *Synthesis* **1995**, 683–686.
10. Wang, J.; Meersman, F.; Esnouf, R.; Froeyen, M.; Busson, R.; Heremans, K.; Herdewijn, P. *Helv. Chim. Acta* **2001**, *84*, 2398–2408.
11. (a) Bajusz, S.; Ronai, A. Z.; Szekely, J. I.; Graf, L.; Dunai-Kovacs, Z.; Berzetei, I. *FEBS Lett.* **1977**, *76*, 91–92; (b) Zhang, L.; Rapp, W.; Goldammer, C.; Bayer, E. In *Innovation Perspect. Solid Phase Synth. Collect. Pap.*; Epton, R., Ed.; Mayflower Worldwide: Birmingham, 1994; pp. 717–722 [*Chem. Abstr.* **123**:170153].
12. Low, M.; Rill, A.; Kisfaludy, L. In *Peptides 1984*; Ragnarsson, U., Ed.; Almquist & Wiksell: Stockholm, 1985; pp. 267–270 [*Chem. Abstr.* **103**:178613].
13. 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) Früchtel, J. S.; Jung, G. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 17–42; (c) Hermkens, P. H. H.; Ottenheijm, H. C. J.; Rees, D. C. *Tetrahedron* **1997**, *53*, 5643–5678; (d) Brown, R. *Con-temp. Org. Synth.* **1997**, 216–237; (e) Gravert, D. J.; Janda, K. D. *Chem. Rev.* **1997**, *97*, 489–509; (f) Shuttleworth, S. J.; Allin, S. M.; Sharma, P. K. *Synthesis* **1997**, 1217–1239; (g) Lorschbach, B. A.; Kurth, M. J.; Brown, R. C. D. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3293–3320; (h) Hermkens, P. H. H.; Ottenheijm, H. C. J.; Rees, D. C. *Synlett* **1998**, 817–827; (i) Wenworth, R., Jr.; Janda, K. D. *Chem. Commun.* **1999**, 1917–1924; (j) Brown, A. R. *Chem. Rev.* **1999**, *99*, 1549–1581; (k) James, I. W. *Tetrahedron* **1999**, *55*, 4855–4946; (l) Shuttleworth, S. J.; Allin, S. M.; Wilson, R. D.; Nasturica, D. *Synthesis* **2000**, 1035–1074; (m) Ley, S. V.; Baxendale, I. R.; Bream, R. N.; Jackson, P. S.; Leach, A. G.; Longbottom, D. A.; Nesi, M.; Scott, J. S.; Storer, R. I.; Taylor, S. J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3815–4195.
14. (a) Kalir, R.; Warshawsky, A.; Fridkin, M.; Patchornik, A. *Eur. J. Biochem.* **1975**, *59*, 55–61; (b) Chen, S.-T.; Chang, C.-H.; Wang, K.-T. *J. Chem. Res. (S)* **1991**, 206–207; (c) Huang, W.; Kalivretenos, A. G. *Tetrahedron Lett.* **1995**, *36*, 9113–9116; (d) Dendrinis, K. G.; Jeong, J.; Huang, W.; Kalivretenos, A. G. *Chem. Commun.* **1998**, 499–500; (e) Albericio, F.; Cases, M.; Alsina, J.; Triolo, S. A.; Carpino, L. A.; Kates, S. A. *Tetrahedron Lett.* **1997**, *38*, 4853–4856; (f) Pop, I. E.; Déprez, B. P.; Tartar, A. L. *J. Org. Chem.* **1997**, *62*, 2594–2603.
15. (a) Laufer, D. A.; Chapman, T. M.; Marlborough, D. I.; Vaidya, V. M.; Blout, E. R. *J. Am. Chem. Soc.* **1968**, *90*, 2696–2698; (b) Fridkin, M.; Patchornik, A.; Katchalski, E. *Biochemistry* **1972**, *13*, 466–471; (c) Akiyama, M.; Shimizu, K.; Narita, M. *Tetrahedron Lett.* **1976**, *13*, 1015–1018; (d) Adamczyk, M.; Fisfpaugh, J. R.; Mattingly, P. G. *Tetrahedron Lett.* **1999**, *40*, 463–466; (e) Barrett, A. G. M.; Cramp, S. M.; Roberts, R. S.; Zecri, F. *Org. Lett.* **2000**, *2*, 261–264.
16. Chinchilla, R.; Dodsworth, D. J.; Nájera, C.; Soriano, J. M. *Tetrahedron Lett.* **2000**, *41*, 2463–2466.
17. Chinchilla, R.; Dodsworth, D. J.; Nájera, C.; Soriano, J. M. *Tetrahedron Lett.* **2001**, *42*, 4487–4489.
18. (a) Chinchilla, R.; Dodsworth, D. J.; Nájera, C.; Soriano, J. M. *Tetrahedron Lett.* **2001**, *42*, 7579–7581; (b) Chinchilla, R.; Dodsworth, D. J.; Nájera, C.; Soriano, J. M.; Yus, M., ES, 2001, P200101169.
19. Chinchilla, R.; Dodsworth, D. J.; Nájera, C.; Soriano, J. M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1817–1820.
20. The loading of P-HOSu (**5**) was 1.0 mmol/g (determined by the assay of the OH group content: Ref. 14d).
21. Pottorf, R. S. In *Encyclopedia of Reagents for Organic Synthesis*; Paquette, L. A., Ed.; John Wiley & Sons: Chichester, 1995; pp. 2430–2432.
22. HPLC determinations were performed using a Chiralpak AD chiral column (hexane/iPrOH, 8:2 v/v).
23. Aldrich Catalogue.
24. Stenberg, V. I.; Singh, S. P.; Narain, N. K. *J. Org. Chem.* **1977**, *42*, 2244–2246.