Pergamon

Tetrahedron Letters 40 (1999) 463–466

TETRAHEDRON LETTERS

Preparation and Use of *N*-Hydroxysuccinimidyl Active Ester Resins

Maciej Adamczyk,* Jeffrey R. Fishpaugh and Phillip G. Mattingly
Abbott Laboratories, Divisional Organic Chemistry, 100 Abbott Park Road, D-9NM AP-20, Abbott Park, IL 60064
Received 12 October 1998; accepted 9 November 1998
Abstract: *Syntheses of solid-phase active esters derived from new N-hydroxysuccinimidyl resins **5a** and **5b** are described. Their practical utility is illustrated in the ready formation of amides in high yield and high purity.*

The purification of organic compounds has been greatly simplified by use of resins that sequester the desired product or undesired reaction by-products. We have recently reported the utility of resin-supported carbodiimides that function in the latter mode. Solutions of pure active esters,[1] amides[2] and thioesters[3] were prepared from carboxylic acids where the urea by-product of the reaction remained bound to the resin. In these cases, excess resin-bound carbodiimide could be used to ensure high conversion to the desired product and the only purification that was needed was removal of the spent resin by filtration. Active esters prepared by this method were of high purity and could be used directly to prepare a variety of useful bioconjugates. However, these bioconjugate preparations had to be further purified to remove by-products of the acylation reaction, i.e., unreacted active ester, free carboxylic acid from hydrolysis of the active ester and *N*-hydroxysuccinimide (NHS). To overcome this disadvantage, preparation of resin-supported active esters became a primary objective. Such resins would ideally be free flowing powders with a relatively high loading rate (~1 mmol/g resin), swell efficiently in a variety of solvents, and have good mechanical stability. Their reactivity would be sufficient to readily acylate amines, yet have sufficient stability to permit isolation.

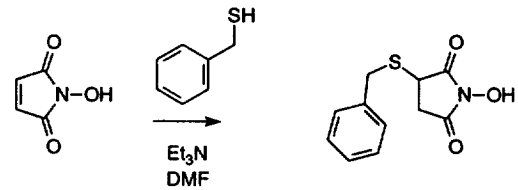
Initially, we considered active esters based on the commercially available Kaiser oxime resin,[4] but our early experiments indicated that the low reactivity of *O*-acyl oximes with amines would be problematic. On the other hand, active esters using the recently reported hydroxybenztriazole resins were too reactive, displaying poor stability in DMF.[5] NHS esters were expected to be of intermediate reactivity. There were several literature reports of active esters based on polymeric NHS. Fridkin, et al.,[6] cross-linked poly(ethylene-*co*-maleic anhydride) with a variety of aliphatic diamines then converted the remaining succinic anhydrides into NHS groups by reaction with hydroxylamine. Andreev, et al.,[7] followed a similar course using aromatic diamines as the cross-linking reagent. Both groups prepared peptides using these materials. In our hands, these reagents were amorphous powders that had a tendency to form a gummy mass in DMF. Akiyama's group[8] prepared active esters from protected amino acids and *N*-hydroxymaleimide (**1**), then polymerized the *N*-acyloxymaleimide with styrene and divinylbenzene to arrive at the polymeric NHS active ester. This approach required the preparation and purification of the active ester *before* incorporation into the polymer, which partially defeated the purpose of solid-supported synthesis. We thought it would be much simpler to incorporate the NHS group into a well-defined and commercially available resin, then use it to form an active ester, thus maintaining all the inherent

PII: S0040-4039(98)02425-3

advantages offered by solid-supported synthesis.

By analogy to the Michael addition of thiols to maleimide, *N*-hydroxymaleimide (**1**, Aldrich) was expected to react with a thiol resin to generate the desired NHS resin. Since the addition of thiols to **1** had not yet been demonstrated, a solution-phase model reaction was first carried out (Scheme 1). Benzyl mercaptan added to **1** to give the expected Michael product (**2**).

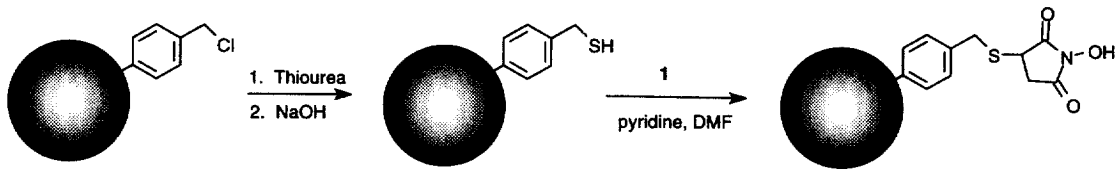
Scheme 1. *Reaction of benzyl mercaptan with N-hydroxymaleimide (1)*



1 **2**

Two commercially available chloromethylpolystyene resins, the traditional, inexpensive Merrifield resin (**3a**) and a new rigid, highly cross-linked resin, ArgoPore™-Cl (**3b**) were converted to their respective thiol resins by reaction with thiourea followed by basic hydrolysis of the *S*-alkyl isothiourea intermediate.[9] The conversion to thiol resins (**4a, b**) was quantitative based on solid-phase NMR[10] and elemental analysis.[11] An excess of *N*-hydroxymaleimide (**1**, 700–800 mol%) in the presence of pyridine in DMF was used to quantitatively (based on NMR and elemental analysis) cap the solid-phase thiols and afford the NHS resins **5a, b.**

Scheme 2. *Synthesis of NHS resins (7, 8)*

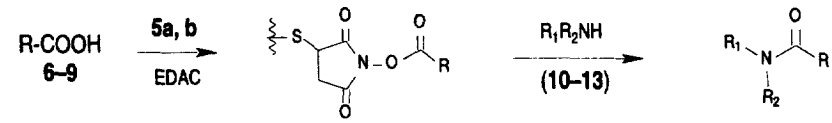


3a, b **4a,b** **5a,b**

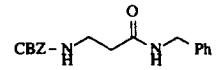
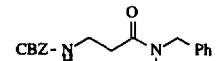
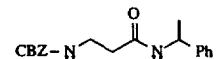
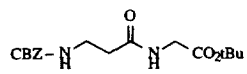
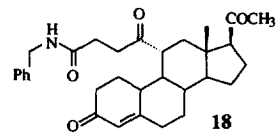
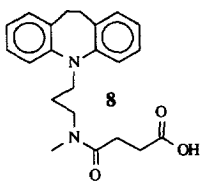
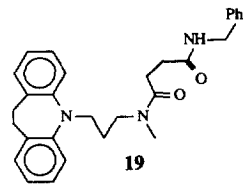
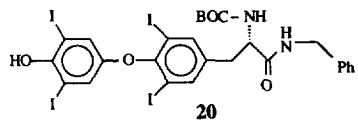
a = Merrifield resin b = ArgoPore™ resin

With the NHS resins in hand, a series of resin-bound active esters were prepared (Table) by acylation with the listed carboxylic acids (300 mol%) in the presence of ethyl 3-(dimethylamino)propylcarbodiimide (EDAC). After washing/drying *in vacuo*, these resins were coupled with amines (limiting) to form the amides noted in the Table. Purification of the products entailed only removal of the spent resin by filtration or centrifugation and evaporation of the solvent. It was necessary to conduct the reaction of the Merrifield-based NHS resin in chloroform/dimethylformamide (1:1) to ensure sufficient swelling, while the ArgoPore™ material could be used in dimethylformamide alone. Both resins performed similarly when *N*-Cbz-β-alanine (**6**) was the carboxylic acid (entries 1/2 and 3/4). Excellent yields of amide were obtained from resin **6b** using primary,

Table. *Preparation of amides via NHS resins*



6-9 a, b 14–20

Entry	R-COOH	Resin (5)[a]	Amine	Amide	% Yield[b]
1	*N*-CBZ-β-ALA (6)	a	$Ph\text{-}CH_2\text{-}NH_2$ (10)	 14	94
2	6	b	10		99
3	6	a	$Ph\text{-}CH_2\text{-}NH\text{-}CH_3$ (11)	 15	97
4	6	b	11		95
5	6	b	$Ph\text{-}CH(CH_3)\text{-}NH_2$ (12)	 16	91
6	6	a	$H_2N\text{-}CH_2\text{-}CO_2t\text{-}Bu$ (13)	 17	98
7	Progesterone-11-hemisuccinate (7)	a	$Ph\text{-}CH_2\text{-}NH_2$ (10)	 18	89
8	 8	a	$Ph\text{-}CH_2\text{-}NH_2$ (10)	 19	89
9	*N*-BOC-L-Thyroxine (9)	b	$Ph\text{-}CH_2\text{-}NH_2$ (10)	 20	95

[a]Resin **5a** used in 1:1 chloroform:dimethylformamide, resin **5b** used in dimethylformamide only. [b]Yields were not optimized.

branched primary, and secondary amines (entries 2, 5 and 4, respectively). The non-polar substrates, the steroidal acid progesterone 11-hemisuccinate (**7**) and a tricyclic antidepressant hemisuccinate derivative (**8**) along with the polar substrate *N*-BOC-L-thyroxine (**9**) were readily transformed into their corresponding benzyl

amides **18**, **19** and **20** in high yield (entries 7-9) via their respective solid-phase active esters.

In conclusion, the solid phase *N*-hydroxysuccinimide resins **5a** and **b** were prepared from commercially available chloromethyl polystyrene resins by a highly efficient and convenient route that maintained the physical and mechanical properties of the parent resins. The NHS resins could be efficiently acylated with carboxylic acids **6–9** to afford the corresponding solid-phase active esters **6–9a,b**. The active ester resins displayed all the advantages normally seen in solid-phase synthesis: convenience, ease of use and no need for purification.

General Procedures: NHS resin 5: Pyridine (1.2 mL, 15 mmol) was added to a heterogeneous mixture of thiomethyl resin (**4**, 2.5 mmol), *N*-hydroxymaleimide (**1**, 566 mg, 5.0 mmol) and DMF (40 mL) at room temperature under a nitrogen atmosphere. The reaction mixture was stirred for 24 h at room temperature, 4 h at 55 °C, cooled and filtered to afford crude NHS resin which was washed with 2 x 40 mL DMF, 2 x 25 mL distilled water and 3 x 25 mL methanol. The resin was dried *in vacuo* for 20 hours to afford resin **5** (96%).

Active Esters 6–9a,b. Ethyl 3-(dimethylamino)propylcarbodiimide (EDC, 124 mg, 0.65 mmol) was added to a heterogeneous mixture of carboxylic acid (0.60 mmol), **5a** (300 mg, ~0.20 mmol) in 4.0 mL of a 1:1 mixture of chloroform and DMF or **5b** (200 mg, ~0.20 mmol) in 4.0 mL of DMF, the reaction mixture was stirred overnight at room temperature under a nitrogen atmosphere. Removed liquid from mixture by centrifugation, washed crude active ester resin 2 × 5 mL DMF/H_2O (1:1), 1 × 5 mL DMF and 2 × 5 mL THF. Dried *in vacuo* to afford desired solid supported active ester resin.

Amides from Solid Phase NHS Esters. Amine (0.028 mmol) was added to a suspension of active ester resin (~0.175 mmol) in 4 mL of chloroform/DMF (1:1, resin derived from **5a**) or 4 mL of DMF (resin derived from **5b**); the reaction mixture was stirred overnight, then the spent resin was removed by filtration or centrifugation. The resin was washed with DMF (2 × 2 mL) and the combined solutions were evaporated *in vacuo*. Residue was dissolved in either chloroform or DMF, filtered to remove any finely ground resin, then evaporated *in vacuo*. All amides had satisfactory ^{1}H NMR, ESI mass spectral data and were >95% pure by analytical HPLC [Waters analytical column (μBondapak), λ = 254 nm, 2.0 mL/min, eluent = acetonitrile/0.05% aqueous trifluoroacetic acid].

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