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Convenient Preparation of *N*-Maleoyl Amino Acid Succinimido Esters using *N*-Trifluoroacetoxysuccinimide

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Abstract: One-pot cyclization and esterification of readily available maleamic acid derivatives using *N*-trifluoroacetoxysuccinimide provide a convenient and cost-effective route to a variety of useful *N*-maleoyl amino acid *N*-hydroxysuccinimido esters.

Keywords: heterobifunctional linker, maleimide, succinimido ester, *N*-trifluoroacetoxysuccinimide

Heterobifunctional cross-linking reagents have a wide range of important applications in bioorganic and materials chemistry. Among the various derivatives currently available, the succinimido esters (OSu esters) of *N*-maleoyl-amino acids **1** have proved particularly popular and versatile, providing an amine-reactive and thiol-reactive grouping, disposed within an aliphatic or aromatic skeleton. The presence of two such complementary reactive moieties within the same molecule has often been exploited for the preparation of chimeric protein derivatives via side chain-selective, and

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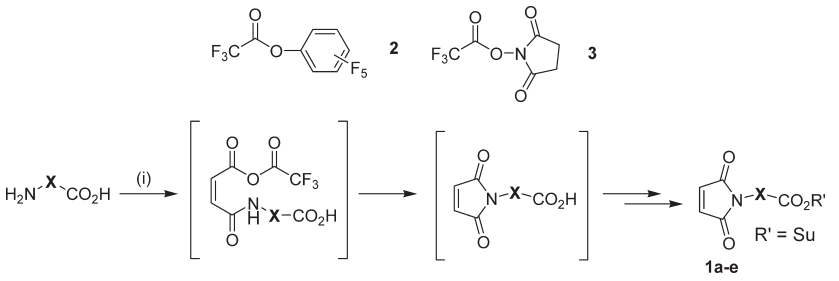
sometimes site-selective, conjugation of synthetic peptides,[1] antibodies,[2] or fluorescent labels[3] to various proteins, as well as in numerous other novel applications in biological chemistry[4–7] and nanoelectronic research.[8] Existing methods for the synthesis of *N*-maleoyl active ester derivatives include conversion of amino acids to *N*-maleoyl amino acids by treatment with *N*-methoxy carbonylmaleimide,[9] or cyclization of preformed maleamic acids [*N*-(3-carboxyacroyl) amino acids], followed by diimide-mediated esterification.[10,11] Such approaches are typically low yielding and tend to involve cumbersome workup procedures and chromatographic purification of intermediates. Indeed, although several *N*-maleoyl OSu esters are commercially available, they are both costly and may contain small but significant quantities of impurities such as dicyclohexylurea (DCU).

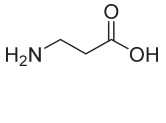
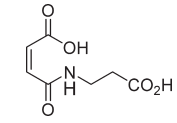
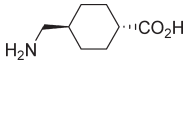
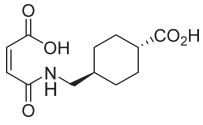
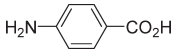
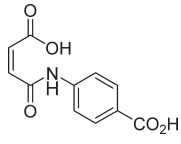
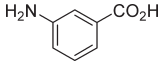
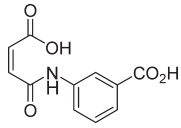
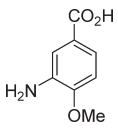
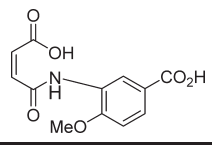
Nielsen and Buchardt[12] have reported a one-pot preparation of these derivatives via in situ DCC/HOSu-mediated cyclization and esterification of an intermediate maleamic acid. In our hands, and as noted elsewhere,[13] this proved problematic, thus prompting us to look for alternative means to effect this overall transformation. Because pure maleamic acids can be obtained in almost quantitative yield without recourse to chromatography,[10] we reasoned that splitting the approach of Nielsen and Buchardt into two discrete steps might prove advantageous [i.e., amic acid formation, then cyclization and esterification). However, aside from largely ineffective diimide reagents, there are comparatively few alternative reagent combinations that may be employed for the concurrent cyclization and esterification steps. Adamcyk[13] has reported the one-pot synthesis of 4-(*N*-maleimido methyl)cyclohexane-1-carboxylic acid pentafluorophenyl ester using the commercially available reagent pentafluorophenyl trifluoroacetate **2**, which presumably first cyclizes the precursor maleamic acid via an in situ mixed trifluoroacetic anhydride, then mediates esterification of the amino acid carboxyl via a second such mixed anhydride species. The analogous reagent, *N*-trifluoroacetoxy succinimide **3**, which fitted our requirements, was originally developed as a coupling agent for peptide chemistry[14] and has since proved effective for other one-pot acylation or esterification reactions,[15,16] but no application to the preparation of *N*-maleoyl amino acid succinimido esters had been previously reported. Compound **3** is conveniently prepared by the reaction of trifluoroacetic acid anhydride and HOSu,[14] and is stable in storage for several months at 4°C. We found that treatment of the isolated maleamic acids corresponding to five previously reported heterobifunctional linkers[8,11,17] with **3** (5 eq) and *sym*-collidine (2 eq) in DMF solution for 10 h led to a highly efficient, gram-scale conversion to the desired *N*-maleoyl succinimidyl esters **1a–e** (Scheme 1). Concentration of the reaction mixture in vacuo, followed by suspension of the residue in chloroform and successive washing with 5% $NaHCO_3$ and water, gave rise to almost pure products upon drying and evaporation of the organic solution. Recrystallized products were obtained in yields of 65–89%, representing a substantial



Scheme 1. Reagents and conditions: (i) **3**, *sym*-collidine, DMF, o/n.

increase in both yield and efficiency relative to previously reported methods (Table 1). The choice of base proved critical; for example, less sterically encumbered amines such as *N,N*-diisopropylethylamine gave rise to significant amounts of colored by-products upon overnight reaction, which could not be readily removed by crystallization.

Table 1. Preparation of *N*-maleoyl amino acid succinimido esters

Amino acid	Maleamic acid	Yield of *N*-maleoyl ester derivative	Mp	Lit. mp
		68% (**1a**)	158–161	160–163[8]
		89% (**1b**)	170.5–173.5	165–167[11]
		80% (**1c**)	205–207	194–195[11]
		75% (**1d**)	175–178	175–177[17]
		65% (**1e**)	179–182	164–166.5[17]

In conclusion, we have devised a convenient and cost-effective new procedure for the large-scale preparation of several important *N*-maleoyl active esters using *N*-trifluoroacetoxysuccinimide. This should increase the scope for using these heterobifunctional linkers in many of the potential applications cited previously. We are currently exploring the effectiveness of *N*-trifluoroacetoxysuccinimide in mediating the formation of various other maleimide derivatives from the corresponding maleamic acids.

EXPERIMENTAL

Melting points were determined on an Electrothermal IA9000 series digital melting-point apparatus using open capillaries and are quoted uncorrected. NMR spectra were recorded in $CDCl_3$ on a Bruker Avance DPX 300 FT-spectrometer operating at 300 MHz (^{1}H) or 75 MHz (^{13}C).

Preparation of Maleamic Acids

Maleamic acids were prepared as described by Rich et al.,[10] by treatment of a suspension of the amino acid (1 eq) in glacial acetic acid with an acetic acid solution of maleic anhydride (1 eq) at room temperature. After stirring at room temperature overnight, the suspension was filtered and the precipitate was washed well with ether and dried in vacuo. The crude maleamic acids were obtained in essentially quantitative yield and were used without further purification.

Typical Procedure for Cyclization and Esterification using *N*-Trifluoroacetoxysuccinimide

A cooled (0°C) and stirred solution of *N*-(carboxyacroyl)-4-(aminomethyl)-cyclohexane-1-carboxylic acid (1.36 g, 5.33 mmol) in anhydrous DMF (20 mL) was treated with *sym*-collidine (1.48 mL, 11.22 mmol). After 30 min, a solution of *N*-trifluoroacetoxysuccinimide (5.64 g, 26.7 mmol) in DMF (5 mL) was added. Cooling of the stirred solution continued for 10 h, whereafter it was allowed to attain room temperature overnight. The solution was concentrated under reduced pressure, and the residue was resuspended in chloroform. The organic solution was washed successively with 1 M hydrochloric acid, 5% sodium hydrogen carbonate, and water and then dried (Na_2SO_4). Concentration of the solution under reduced pressure gave an off-white powder, which was recrystallized from CH_2Cl_2–hexane to give **1b** as a white powder (1.59 g, 89%).

Data

1a: ^{1}H NMR ($CDCl_3$, δ, ppm) 2.81 (4H, s, 2 × CH_2), 3.01 (2H, t, $J = 6.9$ Hz, CH_2CO$_2$), 3.93 (2H, t, $J = 6.9$ Hz, CH_2N), 6.73 (2H, s, 2 × =CH). ^{13}C NMR ($CDCl_3$, δ, ppm) 25.93, 30.12, 33.38, 134.69, 167.37, 169.09, 170.46.

1b: ^{1}H NMR ($CDCl_3$, δ, ppm) 1.00–2.61 (10H, m, cyclohexyl CH_2, CH), 2.81 (4H, s, 2 × CH_2), 3.38 (2H, d, $J = 6.9$ Hz, NCH_2), 6.70 (2H, s, 2 × =CH). ^{13}C NMR ($CDCl_3$, δ, ppm) 25.98, 28.40, 29.70, 36.47, 40.80, 43.79, 134.32, 169.54, 170.99, 171.37.

1c: ^{1}H NMR ($CDCl_3$, δ, ppm) 2.91 (4H, s, 2 × CH_2), 6.90 (2H, s, 2 × =CH), 7.63 (2H, d, $J = 8.7$ Hz, Ar), 8.23 (2H, d, $J = 8.7$ Hz, Ar). ^{13}C NMR ($CDCl_3$, δ, ppm) 26.08, 124.16, 125.61, 131.87, 134.90, 137.70, 161.55, 169.05, 169.53.

1d: ^{1}H NMR ($CDCl_3$, δ, ppm) 2.91 (4H, s, 2 × CH_2), 6.89 (2H, s, 2 × =CH), 7.60–7.82 (2H, m, Ar), 8.12–8.18 (2H, m, Ar). ^{13}C NMR ($CDCl_3$, δ, ppm) 26.07, 126.69, 128.10, 130.04, 130.17, 132.41, 134.80, 161.49, 169.25, 169.44.

1e: ^{1}H NMR ($CDCl_3$, δ, ppm) 2.89 (4H, s, 2 × CH_2), 3.90 (3H, s, OCH_3), 6.87 (2H, s, 2 × =CH), 7.10 (1H, d, $J = 8.9$ Hz, Ar), 7.90 (1H, d, $J = 2.2$ Hz, Ar), 8.22 (1H, dd, $J = 8.8$, 2.2, Ar). ^{13}C NMR ($CDCl_3$, δ, ppm) 26.06, 56.82, 112.43, 118.06, 120.79, 133.34, 134.31, 135.03, 161.05, 169.38, 169.59.

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