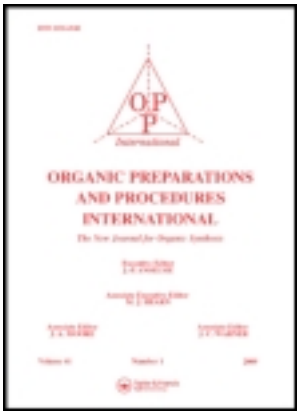

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One-pot Synthesis of Succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC)

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One-pot Synthesis of Succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC)

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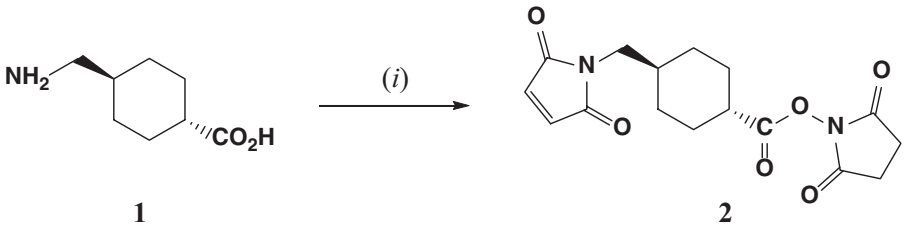
Succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (2, SMCC) has found utility in numerous areas of chemistry and biotechnology. The well-established chemoselective reactivity of SMCC has promoted its use as a heterobifunctional linker in immumoassays,[1] radio-labeling for tumor imaging,[2–4] therapeutic agent delivery,[5,6] the immobilization of oligonucleotides on glass surfaces,[7] and more recently, in boron neutron capture therapy.[8] The high demand for SMCC has resulted in numerous methods for its preparation, although to date, a concise, high-yielding synthesis has not been reported.

SMCC was first synthesized by Yoshitake and co-workers in a three-step process that hinged on an acetic acid/sodium acetate promoted cyclization, and provided the desired product in 21% overall yield from *trans*-4-(aminomethyl)cyclohexane carboxylic acid (**1**).[9] In 1991, Nielsen and Buchardt reported the formation of SMCC through the one-pot, DCC promoted esterification/cyclization of *trans*-4-(aminomethyl)cyclohexane carboxylic acid (**1**) in 75% yield.[10]



1 **2**

i.) Maleic anhydride, trifluoroacetic anhydride, *N*-hydroxysuccinimide, *sym*-collidine, DMF, 0°C to rt, 92%

However, the method of Nielsen and Buchardt[10] was unable to produce analogous aliphatic substrates were not obtainable in reasonable yields. Adamczyk and Johnson

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later reported difficulty in reproducing the procedures of Yoshitake and Nielsen, isolating pure SMCC in only 15–20% yield.[11] These undesirable results prompted the development of pentafluorophenyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (FMCC)[11] as a replacement for SMCC in the synthesis of a heterobifunctional linker.[12] Paterson and Eggleston also cited problems with the previously reported method of Nielsen and Buchardt, but were able to prepare SMCC using *N*-trifluoroacetoxy succinimide (TFA-NHS) as an esterification reagent.[13] TFA-NHS has been known for some time,[14] and was previously used for the formation of *N*-trifluoroacetyl amino acid succinimidyl esters.[15] Paterson and Eggleston accomplished the one-pot cyclization/esterification of the isolated *N*-maleamic acid of *trans*-4-(aminomethyl)cyclohexane carboxylic acid (**1**). This method provided SMCC in an 89% overall yield, however, the *N*-maleamic acid intermediate and the TFA-NHS reagent have to be isolated prior to use, resulting in a three-step overall process.

During the optimization of the synthesis of a heterobifunctional linker of which SMCC is an intermediate, we discovered conditions for the *in situ* generation of TFA-NHS. A 1:1 mixture of commercially available trifluoroacetic anhydride and *N*-hydroxysuccinimide promoted the conversion of carboxylic acids to succinimidyl esters and the cyclization of *N*-maleamic acids to *N*-maleamides. The *in situ* formation of TFA-NHS was applied to the synthesis of SMCC, and as observed by Nielsen and Buchardt,[10] the *N*-maleamic acid could be generated *in situ*. Also, in accordance with the findings of Paterson and Eggleston,[13] *sym*-collidine was determined to be the optimal base. After aqueous work-up and trituration with diethyl ether, SMCC was reproducibly isolated in >90% yield and >99% purity on ~15 g scale.

Experimental Section

^{1}H NMR spectra were recorded at ambient temperature at either 300 MHz or 400 MHz on Varian Mercury, Mercury Plus, Unity Plus or 400-MR spectrometers. Deuterated solvents were used as received from Sigma-Aldrich and data is reported in δ from TMS as an internal standard. ^{13}C NMR were recorded at ambient temperature at 100 MHz on a Varian 400-MR spectrometer and data are reported as δ from TMS as an internal standard. High resolution mass spectra were acquired using an Agilent G6220A Time-of-Flight Mass Spectrometer equipped with a dual electrospray ion source operated in the positive ion mode. Mass correction was accomplished using two internal reference masses. HPLC was performed on a Waters Alliance 2696 with a Waters 2996 Photodiode Array Detector and Symmetry C18 (4.6 × 150 mm) analytical column, 98:2 0.1% aqueous TFA/acetonitrile ramped to 0:100 over 20 minutes, 1 mL/min, 230 nm. HPLC samples were prepared as 1 mg in 1 mL of DMF. Melting points were obtained using an Electrothermal Mel-Temp® melting point apparatus and are uncorrected. All reactions, unless specified, were performed under an atmosphere of nitrogen in glassware that had been flame-dried under vacuum. Unless otherwise noted, all reagents were commercially obtained. *N,N*-Dimethylformamide and diethyl ether were purchased as anhydrous grade from Sigma-Aldrich and used directly from the bottle.

Succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC)

A suspension of *trans*-4-(aminomethyl)cyclohexane carboxylic acid (**1**) (7.86 g, 50.0 mmol) and maleic anhydride (4.90 g, 50.0 mmol) in DMF (250 mL, 0.20 M) in a 1 L round bottom flask was stirred at rt for 6 h. The resulting colorless solution was cooled to 0°C as *sym*-collidine (13.9 mL, 105 mmol) was added dropwise (Flask A). In a separate flask (Flask B), a solution of *N*-hydroxysuccinimide (23.0 g, 200 mmol) in DMF (250 mL, 0.8 M) in a 500 mL round bottom flask was stirred at 0°C as trifluoroacetic anhydride (27.8 mL, 200 mmol) was added dropwise. The reaction mixture was stirred for 10 min, and *sym*-collidine (26.4 mL, 200 mmol) was added dropwise. After stirring for 10 min, the solution in Flask B was added by positive-pressure cannula to Flask A over a period of 1–4 h. Both flasks were kept at 0°C for the duration of the addition. After addition was complete, the ice bath was removed and the reaction mixture warmed to rt overnight. The reaction mixture was diluted with CH_2Cl_2 (300 mL) and 1 M HCl (250 mL). The layers were separated and the organic layer was washed with 1 M HCl (2 × 250 mL). The resulting organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated *in vacuo* to afford a yellow solid. The solid was triturated with Et_2O (3 × 200 mL) to afford SMCC as a white solid (15.31 g, 92%): HPLC *t*R 10.4 min, 99.2%, mp 163–165 °C (*lit*.[16] 165–167°C). Spectral data was identical to those reported in literature:[13] ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.71 (s, 2H), 3.39 (d, $J = 7.0$ Hz, 2H), 2.82 (s, 4H), 2.58 (tt, $J = 12.2$, 3.6 Hz, 1H), 2.16 (m, 2H), 1.79 (m, 2H), 1.73 (m, 1H), 1.54 (m, 2H), 1.06 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.1, 170.7, 169.3, 134.2, 43.6, 40.6, 36.3, 29.5, 28.2, 25.8; HRMS (ESI) *m/z* calcd for $C_{16}H_{18}N_2O_6$ 334.11649, found 334.11748.

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