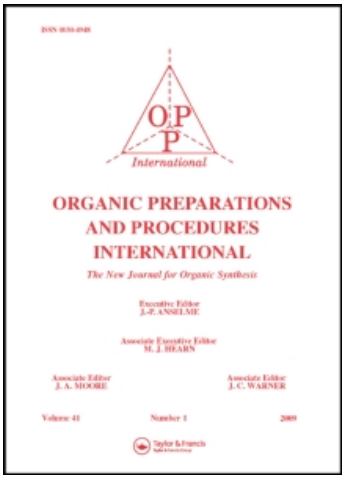

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SYNTHESIS OF PENTAFLUOROPHENYL-4-(*N*-MALEIMIDOMETHYL) CYCLOHEXANE-1-CARBOXYLATE (FMCC)

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IR (KBr): 3000 (m), 2940 (w), 2840 (s), 1460 (m), 1410 (s), 1375 (s), 1290 (s), 1260 (s), 1215 (s), 1120 (s), 1044 (s), 760 (w), 700 (s), 640 (m) cm^{-1}; ^1H NMR: δ 3.60 (q, 4H, CH_2) ; ^{19}F NMR: δ -64.50 (t, 6F, CF_3).

$(CF_3CH_2)_2Te_2$ (4), bp. 60°/2 torr, yield 1.90 g (90%).

Anal. Calcd. for $C_4H_4F_6Te_2$: C, 11.40; H, 0.95; Te, 60.59. Found: C, 11.40; H, 0.92; Te, 60.39

IR (KBr): 2980 (s), 2940 (s), 2880 (s), 1470 (s), 1415 (s), 1370 (s), 1275 (s), 1255 (s), 1200 (s), 1100 (s), 1040 (s), 780 (w), 650 (sh), 630 (s), 508 (w) cm^{-1}; ^1H NMR: δ 4.10 (q, 4H, CH_2); ^{19}F NMR: δ -66.00 (t, 6F, CF_3).

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SYNTHESIS OF PENTAFLUOROPHENYL-4-(*N*-MALEIMIDOMETHYL) CYCLOHEXANE-1-CARBOXYLATE (FMCC)

Submitted by (03/11/93)

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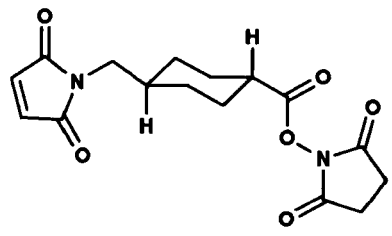
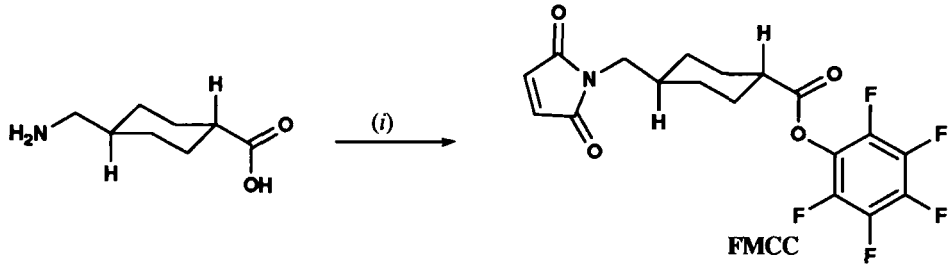
Current research in our laboratory necessitated the synthesis of succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), or an equivalent analogue. SMCC is a heterobifunctional cross-linking reagent with a maleimido functionality linked to a succinimidyl active ester and has been used extensively in a variety of recent biotechnological endeavors. For example, SMCC has been used to couple enzymes to antibodies for the development of immunoassays,[1] linkage of toxins to antibodies or cell-specific protein ligands for targeted delivery of therapeutic agents,[2] and coupling of radiolabels to antibodies for tumor imaging.[3] Unfortunately, our attempts to synthesize

SMCC by the methods of Yoshitake *et al.*[4] and Nielsen *et al.*[5] met with only limited success. Isolation of the final product by recrystallization was exceedingly troublesome and yields of pure product were poor (15-20%). Replacement of the succinimidyl active ester of SMCC with a pentafluorophenyl ester was an attractive alternative for synthesis because pentafluorophenyl active esters are known for ease of purification by crystallization, while also exhibiting excellent coupling characteristics: high reactivity toward amines, low tendency toward side-reactions, and low rates of racemization.[6]



SMCC

Synthesis of previously undescribed in the literature pentafluorophenyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (FMCC) was achieved by a one-pot reaction in which *trans*-4-(aminomethyl)cyclohexanecarboxylic acid is reacted with maleic anhydride to form the maleamido acid which is then reacted *in situ* with 2.5 equivalents of pentafluorophenyl trifluoroacetate, simultaneously forming the maleimide and pentafluorophenyl ester. The pure product was easily isolated in 70% yield by extraction, followed by recrystallization from EtOAc/Hexane.



i) 1. Maleic anhydride 2. Pentafluorophenyl trifluoroacetate, diisopropylethylamine

EXPERIMENTAL SECTION

Maleic anhydride, *trans*-4-(aminomethyl)cyclohexanecarboxylic acid, pentafluorophenyl trifluoroacetate, *N,N*-diisopropylethylamine, and *N,N*-dimethylformamide were purchased from Aldrich. Methylene chloride, ethyl acetate, and hexane were purchased from Fisher Scientific. Magnesium sulfate was purchased from EM Science. ^{1}H NMR spectrum was obtained on a Varian Gemini 300 NMR spectrometer, mass spectra on a Nermag 3010 instrument, and melting point on Thomas melting point apparatus.

Pentafluorophenyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (FMCC).- To a stirred solution of maleic anhydride (312 mg, 3.18 mmol) in 4 mL DMF was added *trans*-4-(aminomethyl)-cyclohexanecarboxylic acid (500 mg, 3.18 mmol). After stirring for 6 hours, under N_2, the reaction solution was cooled to 0°, and diisopropylethylamine (1.38 mL, 7.95 mmol) was added, followed by a solution of pentafluorophenyl trifluoroacetate (1.37 mL, 7.95 mmol) dissolved in 2 mL DMF. The reaction was then warmed to room temperature, stirred for 16 hrs, under N_2, then poured into 30 mL H_2O and extracted with CH_2Cl_2 (2 x 30 mL). The CH_2Cl_2 extracts were combined, dried over $MgSO_4$,

and the solvent was removed *in vacuo*. The resulting residue was recrystallized from 12 mL of EtOAc/Hexane (70/30) to afford 898 mg (70%) of the desired product as a colorless solid, mp. 157-158°. ^{1}H NMR ($CDCl_3$): δ 6.72 (s, 2H), 3.41 (d, 2H), 2.57-2.65 (m, 1H), 2.18 (d, 2H), 1.48-1.84 (m, 5H), 1.07-1.16 (m, 2H); MS (DCI, NH_3): m/z $(M + NH_4)^+$ 421.

Anal. Calcd. for $C_{18}H_{14}NO_4F_5$: C, 53.61; H, 3.50; N, 3.47. Found: C, 53.47; H, 3.49; N, 3.44

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