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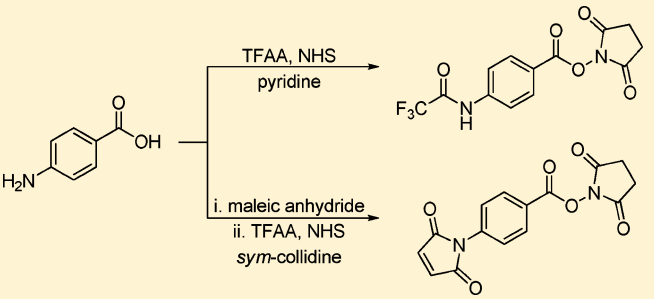
In Situ Formation of *N*-Trifluoroacetoxy Succinimide (TFA-NHS): One-Pot Formation of Succinimidyl Esters, *N*-Trifluoroacetyl Amino Acid Succinimidyl Esters, and *N*-Maleoyl Amino Acid Succinimidyl Esters

Nicholas M. Leonard* and Jarmila Brunckova

ADD Organic Operations (Department 04KA, Building AP-8B) Diagnostics Division, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, Illinois 60064-6016, United States

Supporting Information

ABSTRACT: A method for the in situ formation of *N*-trifluoroacetoxy succinimide (TFA-NHS) and its application in the formation of succinimidyl esters is presented. The developed method provides *N*-trifluoroacetyl and *N*-maleoyl amino acid succinimidyl esters from a variety of amino acids using a one-pot, high-yielding protocol. Investigations into the formation of an *N*-maleoyl amino acid succinimidyl ester supported the proposal of a revised reaction mechanism, and contributed to the optimization of the reaction conditions.



Succinimidyl esters are an essential functional group in organic synthesis. Because their stability upon isolation and their reactivity with various nucleophiles, succinimidyl esters have been utilized in peptide synthesis,[1] bioconjugations,[2] natural product synthesis,[3] intramolecular amide formation,[4] the synthesis of combinatorial libraries,[5] DNA sequencing,[6] and molecular imaging.[7] In addition, *N*-maleoyl amino acid succinimidyl esters are used extensively as heterobifunctional linkers in diagnostic immunoassays.[8]

While there are a number of methods for the formation of succinimidyl esters,[3c,9] the most commonly utilized is the treatment of a carboxylic acid with a carbodiimide and *N*-hydroxysuccinimde. The use of carbodiimides on large scale, however, is impractical because of the creation of stoichiometric waste and the requirement of toxic or expensive reagents. *N*,*N*′-Dicyclohexylcarbodiimide (DCC), the most commonly used carbodiimide, is a low melting solid that is difficult to handle and a known sensitizer.[10] In addition, the dicyclourea byproduct of the coupling reaction is often difficult to separate from the desired ester or the subsequent reaction mixtures. Among commercially available carbodiimide reagents, 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride (EDAC) is an attractive alternative to DCC, as it forms a water-soluble urea byproduct that can be removed by aqueous workup. However, the use of EDAC is undesirable in large scale pharmaceutical applications as it creates toxic aqueous waste streams.[11] Succinimidyl ester formation without carbodiimides can be accomplished with activated derivatives of *N*-hydroxysuccinimide, and the use of the carbonate,[12] oxalate,[13] phosphonate,[14] and uronium salts[15] of *N*-hydroxysuccinimide have been reported.[3c,9] However, these NHS derivatives are not commercially available and need to be synthesized and isolated prior to use, adding an additional step to the overall formation of the desired succinimidyl ester.[16]

N-Trifluoroacetoxy succinimide (TFA-NHS) has also been used for the synthesis of succinimidyl esters. While the preparation and use of this reagent has been known for some time,[17] TFA-NHS is not commercially available, and except for the work of Rao and co-workers (Scheme 1),[5] has been used sparingly for the formation of succinimidyl esters.[2,18] Also, TFA-NHS has to be prepared and isolated prior to use, and current conditions requiring excess trifluoroacetic anhydride (TFAA) in refluxing benzene[17] or toluene[19] are undesirable for large scale processes.

N-Maleoyl amino acid succinimidyl esters are frequently used as heterobifunctional linkers in bioconjugation chemistry.[7a,8] Buchardt and Nielsen reported the synthesis of *N*-maleoyl amino acid succinimidyl esters through the DCC promoted cyclization/activation of an in situ formed *N*-maleamic acid (Scheme 1).[20] This method, however, is plagued by low yields and the failure of chained aliphatic substrates to provide the desired products. As demonstrated by Eggleston and Paterson, TFA-NHS conditions can also provide *N*-maleoyl amino acid succinimidyl esters, but optimal results are obtained when the *N*-maleamic acids are isolated prior to use (Scheme 1).[21] The need for isolation of the TFA-NHS reagent and the *N*-maleamic acid results in a three-reaction process for the formation of *N*-maleoyl amino acid succinimidyl esters. Because of the current limitations in structural diversity and step

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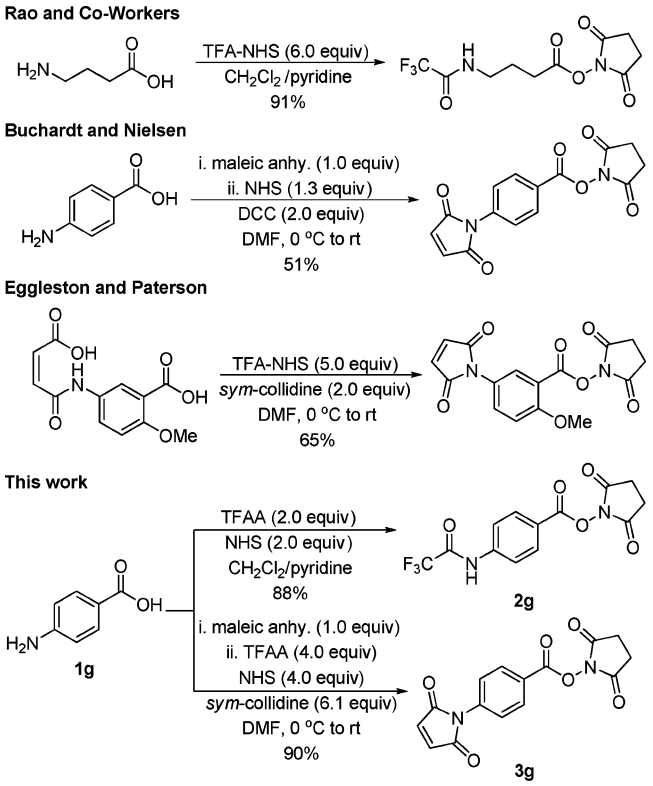
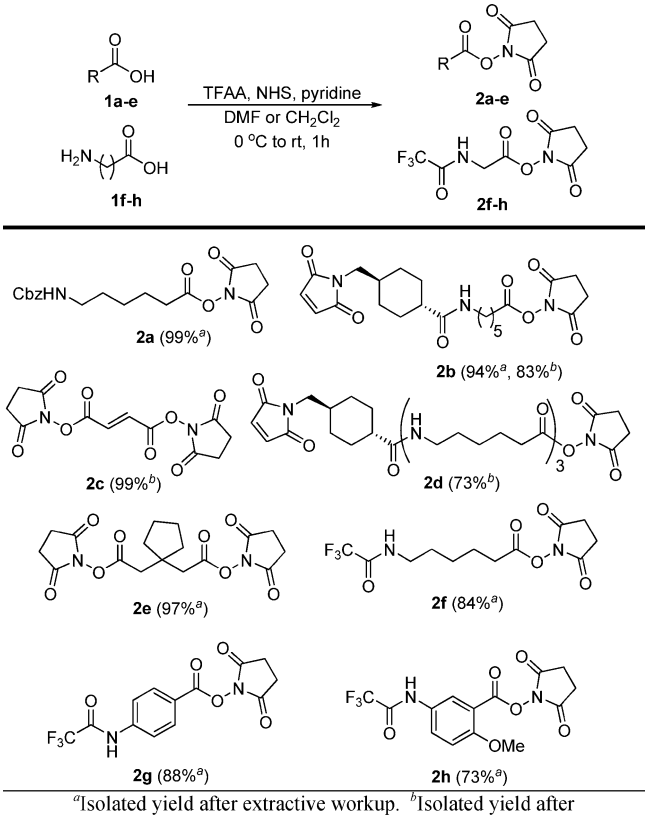
Scheme 1. Available Methods for the Formation of Nitrogen-Functionalized Amino Acid Succinimidyl Esters



Scheme 2. Formation of Succinimidyl Esters and *N*-Trifluoroacetyl Amino Acid Succinimidyl Esters



[a]Isolated yield after extractive workup. [b]Isolated yield after concentration of the reaction mixture and washing of the solids with ethyl acetate.

economy, a more efficient method for the formation of *N*-maleoyl amino acid succinimidyl esters would find utility in the synthesis of heterobifunctional linkers for bioconjugation chemistry.

While optimizing the synthesis of a heterobifunctional linker, conditions for the in situ generation of TFA-NHS were discovered. A 1:1 mixture of commercially available trifluoroacetic anhydride (TFAA) and *N*-hydroxysuccinimide (NHS) was observed to convert carboxylic acids to succinimidyl esters and promote the formation of *N*-maleamides (Scheme 1). Herein, we report the use of in situ formed TFA-NHS as a general method for the single-flask formation of succinimidyl esters, *N*-trifluoroacetyl amino acid succinimidyl esters, and *N*-maleoyl amino acid succinimidyl esters in high yield from commercially available (or readily available) amino acid starting materials.

A variety of carboxylic acids were subjected to reaction conditions with NHS, TFAA, and pyridine (Scheme 2). Unless otherwise specified, 2 equiv of NHS and TFAA were required, and all products were obtained in high purity without chromatography. Depending on substrate solubility, the reaction solvent was DMF or a 2:1 mixture of dichloromethane/pyridine.[22] Heterobifunctional linkers **2b**[8a] and **2d**[8b−d] were isolated in good yield, and an alternative workup involving concentration of the reaction mixture and trituration of the resulting residue could be implemented, thereby eliminating the need for aqueous workup. Treatment of fumaric acid and tetramethylene glutaric acid provided the corresponding succinimidyl esters **2c** and **2e** in excellent yield. The presence of two carboxylic acid functionalities in these substrates required the use of five equivalents of NHS, TFAA, and pyridine. Fumaric acid succinimidyl ester **2c** was isolated after concentration of the reaction mixture and trituration of the resulting residue with ethyl acetate.[16]

N-Trifluoroacetyl amino acid succinimidyl esters were also synthesized with in situ generated TFA-NHS. Upon treatment of 6-aminocaproic acid with 2 equiv of TFAA and NHS, the one-pot protection of the amine and activation of the carboxylic acid provided **2f** (Scheme 2). Amino-substituted benzoic acid derivatives also provided *N*-trifluoroacetyl amino acid succinimidyl esters **2g** and **2h** in good yield. Rao and co-workers reported the formation of *N*-trifluoroacetyl amino acid succinimidyl esters to require 6 equiv of preformed and isolated TFA-NHS (Scheme 1).[5]

The in situ formation of TFA-NHS was further developed for the formation of *N*-maleoyl amino acid succinimidyl esters (Scheme 3). Initial reactions were carried out with isolated *N*-maleamic acids, however, it was soon discovered that the *N*-maleamic acids could be formed in situ. The optimized process provides *N*-maleoyl amino acid succinimidyl esters **3f−j** from readily available amino acids (**1f−j**) in a single reaction flask (Scheme 3). Four equivalents of TFAA and NHS are required for complete conversion, and *sym*-collidine was found to be the optimal base.[21] This transformation provides efficient access to costly commercially available *N*-maleoyl amino acid succinimidyl esters **3f**[23] and **3i**,[24] which serve as heterobifunctional linkers in bioconjugate chemistry.[7a,8a] The described in situ TFA-NHS-mediated method provides access to products that were not obtainable with the method of Buchardt and Nielsen[20] (Scheme 1), and allows for the one-pot formation of products which previously required the three-step process of Eggleston and Paterson[21] (Scheme 1).

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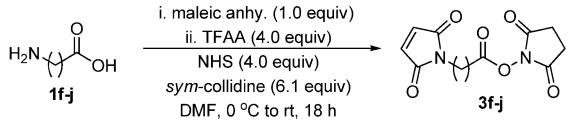
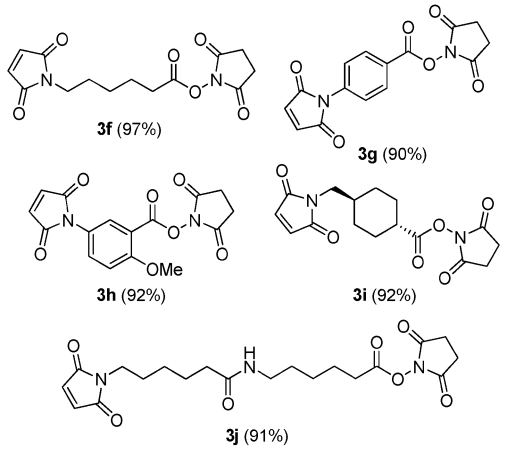
Scheme 3. Formation of *N*-Maleoyl Amino Acid Succinimidyl Esters

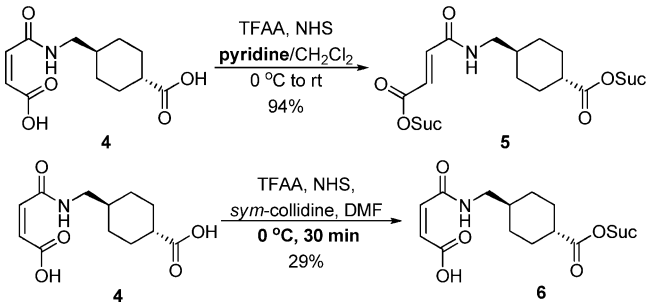




Isolated yields are reported.

Choice of base was found to have a significant impact on the reaction outcome. When CH_2Cl_2/pyridine was used as the solvent for the attempted transformation of *N*-maleamic acid **4** to **3i**, isomerization product **5** was isolated as a brown solid in 94% yield (Scheme 4). Eggleston and Paterson reported the use

Scheme 4. Isolation of Isomerization Product 5 and Intermediate 6

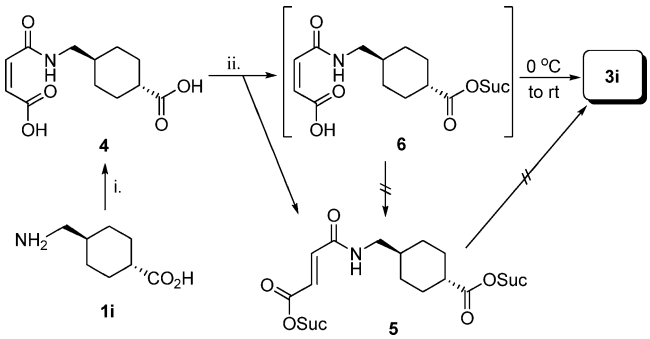


of less sterically hindered bases to facilitate the formation of colored byproducts that could not be separated from the desired product.[21] It is plausible that these observed impurities contain the *E*-isomer of the *N*-maleamic acid used in the reaction.

The isolation of a reaction intermediate provided further insight into the mechanism of the reaction. It was initially assumed that the reaction proceeded via the mechanism proposed by Eggleston and Paterson, in which the mixed anhydride of the *N*-maleamic acid and TFA cyclizes to provide an *N*-maleoyl carboxylic acid. Subsequent esterification of the pendent carboxylic acid with an additional equivalent of TFA-NHS would then provide the *N*-maleoyl amino acid succinimidyl ester.[21] However, when the formation of **3i** was monitored by HPLC, an intermediate was repeatedly observed which required overnight stirring at room temperature for full conversion.[22] Considering that the formation of succinimidyl esters with in situ formed TFA-NHS were all complete within one hour at 0 °C (Scheme 2), it was unlikely that the observed intermediate was the *N*-maleoyl carboxylic acid proposed by Eggleston and Paterson.[21] When the cooled (0 °C) reaction mixture of *N*-maleamic acid **4** was quenched 30 min after the addition of TFAA/NHS, the long-lived intermediate was isolated and determined to be *N*-maleamic acid succinimidyl ester **6** (Scheme 4).[22] Further monitoring by HPLC confirmed the immediate formation of both isomerization byproduct **5** and intermediate **6** upon addition of TFAA/NHS. Also, the amount of **5** was not observed to fluctuate over the course of the reaction, suggesting that **5** does not form through the isomerization of intermediate **6**.[22]

The HPLC monitoring data and characterization of compounds **5** and **6** supported the proposal of a revised reaction mechanism. Treatment of amino acid **1i** with maleic anhydride provides *N*-maleamic acid **4**. Subsequent addition of TFAA, NHS and *sym*-collidine promotes the rapid formation of isomerization byproduct **5** and intermediate **6** (Scheme 5).

Scheme 5. Proposed Reaction Mechanism for the Formation of *N*-Maleoyl Amino Acid Succinimidyl Ester 3i[a]



[a]Reagents and conditions: (i) maleic anhydride, DMF, rt, 6 h; (ii) TFAA (4 equiv), NHS (4 equiv), *sym*-collidine (6.1 equiv), DMF, 0 °C to rt.

Once the addition is complete, the reaction mixture consists of primarily **6**, and the amount of **5** remains constant. Sterically hindered esterification of the *N*-maleamic carboxylic acid of **6** is slow at 0 °C, so warming of the reaction mixture to room temperature facilitates esterification and cyclization to form **3i**.

Elucidation of the revised reaction mechanism facilitated the optimization of the reaction conditions. In early studies, variation in the amount of base, temperature, and method of TFA-NHS addition provided the desired product (**3i**) contaminated with 2−10% of isomerization byproduct **5**. Once the reactive pathways of the reaction were understood, it was discovered that addition of *sym*-collidine to in situ formed **4**, followed by slow addition of a solution of TFAA, NHS, and *sym*-collidine to the reaction mixture at 0 °C limits the formation of **5**. The optimized procedure allows for the reproducible isolation of **3i** containing less than 1% of isomerization byproduct **5** in >90% yield on 15 g scale.

In conclusion, a method utilizing the in situ formation of TFA-NHS for the synthesis of succinimidyl esters has been presented. This method was shown to facilitate the one-pot formation of *N*-trifluoroacetyl and *N*-maleoyl amino acid succinimidyl esters in good yield and high purity. In addition, the characterization of a byproduct and a reactive intermediate in the formation of *N*-maleoyl amino acid succinimidyl ester **3i**

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supported the proposal of a revised reaction mechanism, which facilitated the optimization of the reaction conditions.

EXPERIMENTAL SECTION

6-((1*r*,4*r*)-4-((2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)methyl)-cyclohexanecarboxamido)hexanoic Acid (1b). To a solution of **3i** (6.69 g, 20.0 mmol) in DMF (200 mL, 0.15 M) was added 6-aminocaproic acid (3.94 g, 30.0 mmol). The resulting mixture was stirred at rt for 16 h. The reaction mixture was filtered and the filtrate was concentrated in vacuo to afford a white solid. The solid was triturated with water (2 × 200 mL) and filtered. The filter cake was dried at 50 °C under house vacuum for 16 h to provide **1b** as a white solid (6.37 g, 91%): mp 145−148 °C; ^{1}H NMR (400 MHz, DMSO) δ 7.64 (t, J = 5.6 Hz, 1H), 7.01 (s, 2H), 3.23 (d, J = 7.1 Hz, 2H), 2.98 (dd, J = 12.6, 6.7 Hz, 2H), 2.18 (t, J = 7.4 Hz, 2H), 1.99 (tt, J = 12.0, 3.3 Hz, 1H), 1.73−1.57 (m, 4H), 1.57−1.42 (m, 3H), 1.41−1.30 (m, 2H), 1.30−1.17 (m, 4H), 0.88 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 174.7, 174.4, 171.3, 134.3, 43.8, 43.1, 38.1, 36.1, 33.6, 29.4, 28.4, 28.6, 25.9, 24.2; HRMS (ESI) m/z calcd for $C_{18}H_{26}N_2O_5$ 350.18482, found 350.18417.

Benzyl 6-(6-(Benzyloxycarbonylamino)hexanamido)-hexanoate (7). To a suspension of *N*-benzyloxycarbonyl-6-amino-hexanoic acid (10.0 g, 37.69 mmol) and 6-amino-hexanoic acid benzyl ester toluene-4-sulfonic acid[25] (14.83 g, 37.69 mmol) in CH_2Cl_2 (145 mL, 0.26 M) was sequentially added hydroxybenzotriazole (6.11 g, 45.23 mmol), *N*,*N*-diisopropylethylamine (16.4 mL, 94.23 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (8.67 g, 45.23 mmol). After complete addition, the reaction mixture became a clear solution and was stirred at rt for 18 h. The reaction mixture was diluted with CH_2Cl_2 (150 mL) and 1 N HCl (300 mL). The layers were separated and the organic phase was washed with 1 N HCl (3 × 300 mL), 1 N NaOH (3 × 200 mL), and saturated aqueous NaCl (2 × 150 mL). The resulting organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo to provide **7** as a white solid (16.94 g, 95%): mp 74−75 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (m, 10H), 5.62 (br s, 1H), 5.10 (s, 2H), 5.08 (s, 2H), 4.92 (br s, 1H), 3.20 (m, 4H), 2.35 (t, J = 7.4 Hz, 2H), 2.13 (t, J = 7.4 Hz, 2H), 1.64 (m, 4H), 1.50 (m, 4H), 1.33 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.3, 172.7, 156.4, 136.6, 135.9, 128.5, 128.4, 128.1, 128.1, 128.0, 127.9, 66.5, 66.1, 40.7, 39.1, 36.4, 34.0, 29.6, 29.2, 26.3, 26.2, 25.1, 24.4; HRMS (ESI) m/z calcd for $C_{27}H_{36}N_2O_5$ 468.26242, found 468.26400.

6-(6-Aminohexanamido)hexanoic Acid (1j). To a solution of **7** (0.870 g, 1.86 mmol) in MeOH/CH_2Cl_2 (20 mL:20 mL) was added Pd/C (200 mg). The resulting suspension was stirred under 50 psi of hydrogen. After 18 h, the mixture was filtered through Celite, and the filtrate was concentrated in vacuo to provide a **1j** as a white solid (0.450 g, 99%): mp 174−176 °C; ^{1}H NMR (400 MHz, CD_3OD/D_2O) δ 3.52 (dt, J = 3.2, 1.6 Hz, 1H), 3.38 (t, J = 6.7 Hz, 2H), 3.17 (t, J = 7.6 Hz, 2H), 2.44 (t, J = 7.3 Hz, 2H), 2.37 (t, J = 7.4 Hz, 2H), 1.94−1.81 (m, 4H), 1.81−1.68 (m, 4H), 1.64−1.48 (m, 4H); ^{13}C NMR (101 MHz, CD_3OD/D_2O) δ 183.6, 176.6, 40.4, 40.2, 38.7, 36.5, 29.5, 27.8, 27.4, 26.8, 26.4, 26.1; HRMS (ESI) m/z calcd for $C_{12}H_{24}N_2O_3$ 244.17869, found 244.17926.

Representative Procedure A: Preparation of Succinimidyl Esters and *N*-Trifluoroacetyl Amino Acid Succinimidyl Esters. To a cooled (0 °C) mixture of *Z*-ε-Ahx−OH (**1a**) (0.265 g, 1.00 mmol), *N*-hydroxysuccinimide (0.230 g, 2.00 mmol) and pyridine (0.32 mL, 4.00 mmol) in CH_2Cl_2 (5 mL, 0.20 M) was added trifluoroacetic anhydride (0.28 mL, 2.00 mmol). After addition was complete, the ice bath was removed and the reaction was stirred at rt for 1 h. The reaction mixture was diluted with CH_2Cl_2 (15 mL) and 1 M HCl (20 mL). The layers were separated and the organic layer was washed with 1 M HCl (2 × 20 mL) and $NaHCO_3$ (2 × 20 mL). The resulting organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo to afford **2a** as a clear oil (0.360 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39−7.26 (m, 5H), 5.09 (s, 2H), 4.94 (br s, 1H), 3.20 (m, 2H), 2.79 (s, 4H), 2.60 (t, J = 7.2 Hz, 2H), 1.76 (m, 2H), 1.54 (dt, J = 13.5, 6.7 Hz, 2H), 1.44 (dt, J = 9.7, 6.8 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.1, 168.4, 156.4, 136.6, 128.4, 128.0, 66.5, 40.6, 30.7, 29.3, 25.6, 25.5, 24.1; HRMS (ESI) m/z calcd for $C_{18}H_{22}N_2O_6$ 362.14779, found 362.14953.

Succinimidyl Ester 2b. Representative procedure **A** was followed using **1b** (6.35 g, 18.6 mmol), *N*-hydroxysuccinimide (4.29 g, 37.3 mmol), trifluoroacetic anhydride (5.18 mL, 37.3 mmol), and pyridine (3.00 mL, 37.3 mmol) in DMF (93 mL, 0.20 M) to afford **2b** as a white solid (7.85 g, 94%). Alternative workup: Concentration of the reaction mixture and trituration of the resulting residue with EtOAc (2 × 100 mL) afforded **2b** as a white solid (6.91 g, 83%). Spectral data for ^{1}H NMR was identical to those reported in literature.[8b] ^{13}C NMR has not been previously reported: ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.7, 171.0, 169.2, 168.4, 133.9, 45.1, 43.6, 38.7, 36.3, 30.8, 29.8, 28.8, 25.6, 25.6, 24.1.

Succinimidyl Diester 2c. Representative procedure **A** was followed using fumaric acid (**1c**) (0.116 g, 1.00 mmol), *N*-hydroxysuccinimide (0.575 g, 5.00 mmol), trifluoroacetic anhydride (0.70 mL, 5.00 mmol), and pyridine (0.40 mL, 5.00 mmol) in DMF (5.0 mL, 0.20 M). Alternative workup: Concentration of the reaction mixture and trituration of the resulting residue with EtOAc (2 × 10 mL) afforded **2c** as a white solid (0.308 g, 99%). Spectral data was identical to those reported in literature.[16]

Succinimidyl Ester 2d. Representative procedure **A** was followed using **1d**[8d] (3.95 g, 6.85 mmol), *N*-hydroxysuccinimide (3.15 g, 27.4 mmol), trifluoroacetic anhydride (3.81 mL, 27.4 mmol), and pyridine (5.54 mL, 68.5 mmol) in DMF (275 mL, 0.025 M). Alternative workup: Concentration of the reaction mixture and trituration of the resulting residue with EtOAc (2 × 275 mL) afforded **2d** as a white solid (3.35 g, 73%). Spectral data was identical to those reported in literature.[8d]

Succinimidyl Diester 2e. Representative procedure **A** was followed using 3,3-tetramethyleneglutaric acid (**1e**) (0.186 g, 1.00 mmol), *N*-hydroxysuccinimide (0.575 g, 5.00 mmol), and trifluoroacetic anhydride (0.70 mL, 5.00 mmol) in 2:1 CH_2Cl_2/pyridine (6.0 mL, 0.17 M) to afford **2e** as a white solid (0.370 g, 97%): mp 72−75 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.90 (s, 4H), 2.83 (s, 8H), 1.75 (s, 8H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.1, 166.7, 43.4, 38.5, 37.9, 25.6, 23.8; HRMS (ESI) m/z calcd for $C_{17}H_{20}N_2O_8$ 380.12197, found 380.12287.

***N*-Trifluoroacetyl Amino Acid Succinimidyl Ester 2f.** Representative procedure **A** was followed using 6-aminocaproic acid (**1f**) (0.131 g, 1.00 mmol), *N*-hydroxysuccinimide (0.230 g, 2.00 mmol), trifluoroacetic anhydride (0.28 mL, 2.00 mmol), and pyridine (0.16 mL, 2.00 mmol) in DMF (4.0 mL, 0.25 M) to afford **2f** as a colorless oil which solidified upon standing (0.271 g, 84%). Spectral data was identical to those reported in literature.[26]

***N*-Trifluoroacetyl Amino Acid Succinimidyl Ester 2g.** Representative procedure **A** was followed using 4-aminobenzoic acid (**1g**) (0.137 g, 1.00 mmol), *N*-hydroxysuccinimide (0.230 g, 2.00 mmol), and trifluoroacetic anhydride (0.28 mL, 2.00 mmol) in 2:1 CH_2Cl_2/pyridine (6.0 mL, 0.17 M) to afford **2g** as a white solid (0.303 g, 88%): mp 220−222 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.74 (br s, 1H), 8.16 (d, J = 8.9 Hz, 2H), 7.98 (d, J = 8.9 Hz, 2H), 2.90 (s, 4H); ^{13}C NMR (101 MHz, DMSO) δ 170.4, 161.1, 155.0 (q, J = 37.6 Hz), 142.7, 131.4, 121.0, 120.8, 115.5 (q, J = 288.7 Hz), 25.6; HRMS (ESI) m/z calcd for $C_{13}H_9N_2O_5F_3$ 330.04636, found 330.04792.

***N*-Trifluoroacetyl Amino Acid Succinimidyl Ester 2h.** Representative procedure **A** was followed using 2-methoxy-5-amino-benzoic acid (**1h**) (0.167 g, 1.00 mmol), *N*-hydroxysuccinimide (0.230 g, 2.00 mmol), and trifluoroacetic anhydride (0.28 mL, 2.00 mmol) in 2:1 CH_2Cl_2/pyridine (6.0 mL, 0.17 M) to afford **2h** as a white solid (0.264 g, 73%): mp 217−219 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.41 (br s, 1H), 8.29 (d, J = 2.7 Hz, 1H), 8.02 (dd, J = 9.2, 2.8 Hz, 1H), 7.37 (d, J = 9.2 Hz, 1H), 3.91 (s, 3H), 2.89 (s, 4H); ^{13}C NMR (101 MHz, DMSO) δ 170.4, 159.9, 157.2, 154.5 (q, J = 37.1 Hz), 129.1, 129.0, 124.0, 115.7 (q, J = 288.4 Hz), 113.9, 113.3, 56.4, 25.6; HRMS (ESI) m/z calcd for $C_{14}H_{11}N_2O_6F_3$ 360.05692, found 360.05622.

Representative Procedure B: Preparation of *N*-Maleoyl Amino Acid Succinimidyl Esters. A suspension of *trans*-amino-methylcyclohexane carboxylic acid (**1i**) (7.86 g, 50.0 mmol) and

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maleic anhydride (4.90 g, 50.0 mmol) in DMF (250 mL, 0.20 M) was stirred at rt for 6 h. The resulting clear 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) 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 it was stirred 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 **3i** as a white solid (15.31 g, 92%): HPLC *t*R 10.4 min, 99.2%, mp 158−160 °C (lit.[27] 165−167 °C). Spectral data was identical to those reported in literature:[21] ^{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.

***N*-Maleoyl Amino Acid Succinimidyl Ester 3f.** Representative procedure **B** was followed to afford **3f** as a clear oil (300 mg, 97%). Flask A: 6-Aminocaproic acid (**1f**) (0.131 g, 1.00 mmol), maleic anhydride (0.098 g, 1.00 mmol), DMF (5 mL, 0.2 M), and *sym*-collidine (0.28 mL, 2.10 mmol). Flask B: *N*-Hydroxysuccinimide (0.460 g, 4.00 mmol), trifluoroacetic anhydride (0.56 mL, 4.00 mmol), *sym*-collidine (0.53 mL, 4.00 mmol), and DMF (5 mL, 0.8 M). Spectral data was identical to those reported in literature.[7a]

***N*-Maleoyl Amino Acid Succinimidyl Ester 3g.** Representative procedure **B** was followed to afford **3g** as a white solid (0.283 g, 90%). Flask A: 4-Aminobenzoic acid (**1g**) (0.137 g, 1.00 mmol), maleic anhydride (0.098 g, 1.00 mmol), DMF (5 mL, 0.2 M), and *sym*-collidine (0.28 mL, 2.10 mmol). Flask B: *N*-Hydroxysuccinimide (0.460 g, 4.00 mmol), trifluoroacetic anhydride (0.56 mL, 4.00 mmol), *sym*-collidine (0.53 mL, 4.00 mmol), and DMF (5 mL, 0.8 M): mp 195−197 °C (lit.[5] 194−195 °C). Spectral data was identical to those reported in literature.[7a,21]

***N*-Maleoyl Amino Acid Succinimidyl Ester 3h.** Representative procedure **B** was followed to afford **3h** as a white solid (0.113 g, 92%). Flask A: 2-Methoxy-5-aminobenzoic acid (**1h**) (0.060 g, 0.36 mmol), maleic anhydride (0.035 g, 0.36 mmol), DMF (5 mL, 0.07 M), and *sym*-collidine (0.10 mL, 0.76 mmol). Flask B: *N*-Hydroxysuccinimide (0.116 g, 1.44 mmol), trifluoroacetic anhydride (0.20 mL, 1.44 mmol), *sym*-collidine (0.19 mL, 1.44 mmol), and DMF (1.8 mL, 0.8 M): mp 182−184 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 2.6 Hz, 1H), 7.58 (dd, J = 9.0, 2.7 Hz, 1H), 7.12 (d, J = 9.0 Hz, 1H), 6.86 (s, 2H), 3.96 (s, 3H), 2.89 (s, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.4, 169.3, 160.1, 159.7, 134.5, 133.8, 130.6, 123.9, 114.9, 113.1, 56.7, 25.9; HRMS (ESI) m/z calcd for $C_{16}H_{12}N_2O_7$ 344.06445, found 344.06577.

***N*-Maleoyl Amino Acid Succinimidyl Ester 3j.** Representative procedure **B** was followed to afford **3j** as a white solid (0.384 g, 91%). Flask A: **1j** (0.244 g, 1.00 mmol), maleic anhydride (0.098 g, 1.00 mmol), DMF (5 mL, 0.2 M), and *sym*-collidine (0.28 mL, 2.10 mmol). Flask B: *N*-Hydroxysuccinimide (0.460 g, 4.00 mmol), trifluoroacetic anhydride (0.56 mL, 4.00 mmol), *sym*-collidine (0.53 mL, 4.00 mmol), and DMF (5 mL, 0.8 M): mp 72−74 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.68 (s, 2H), 5.78 (br s, 1H), 3.50 (t, J = 7.2 Hz, 2H), 3.25 (dd, J = 12.6, 6.5 Hz, 2H), 2.85 (s, 4H), 2.63 (t, J = 7.1 Hz, 2H), 2.16 (t, J = 7.5 Hz, 2H), 1.78 (q, J = 7.2 Hz, 2H), 1.70−1.50 (m, 6H), 1.46 (m, 2H), 1.30 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.0, 171.0, 169.4, 168.6, 134.2, 39.1, 37.8, 36.6, 31.0, 29.0, 28.4, 26.5, 25.9, 25.8, 25.3, 24.4; HRMS (ESI) m/z calcd for $C_{20}H_{27}N_3O_7$ 421.18490, found 421.18582.

Carboxylic Acid 4. A suspension of *trans*-aminomethylcyclohexane carboxylic acid (**1i**) (2.00 g, 20.4 mmol) and maleic anhydride (3.20 g, 20.4 mmol) in MeCN (102 mL, 0.20 M) was stirred at rt for 16 h. The suspension was filtered, and the resulting solid was washed with MeCN (2 × 50 mL). The solid was dried under high vacuum to afford **4** as a white solid (4.46 g, 86%): HPLC *t*R 7.0 min, 98.0%, mp 192−194 °C; ^{1}H NMR (400 MHz, DMSO) δ 9.11 (t, J = 5.4 Hz, 1H), 6.43 (d, J = 12.5 Hz, 1H), 6.24 (d, J = 12.5 Hz, 1H), 3.04 (t, J = 6.3 Hz, 2H), 2.13 (tt, J = 12.1, 3.5 Hz, 1H), 1.90 (m, 2H), 1.74 (m, 2H), 1.52−1.36 (m, 1H), 1.26 (m, 2H), 0.95 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 176.7, 165.5, 165.4, 132.9, 131.8, 45.2, 42.4, 36.6, 29.3, 28.2; HRMS (ESI) m/z calcd for $C_{12}H_{17}N_1O_5$ 255.11067, found 255.11031.

Succinimidyl Ester 5. To a cooled (0 °C) solution of **4** (0.255 g, 1.00 mmol), *N*-hydroxysuccinimide (0.575 g, 5.00 mmol), and pyridine (1.60 mL, 20.0 mmol) in CH_2Cl_2 (10 mL, 0.10 M) was added trifluoroacetic anhydride (0.70 mL, 5.00 mmol). The resulting orange reaction mixture was warmed to rt over 24 h. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and 1 M HCl (20 mL). The layers were separated and the organic layer was washed with 1 M HCl (2 × 20 mL) and saturated aqueous $NaHCO_3$ (2 × 20 mL). The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo to provide **5** as a brown solid (0.424 g, 94%): HPLC *t*R 9.3 min, 81.0%, mp 93−95 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 (d, J = 15.5 Hz, 1H), 6.99 (d, J = 15.5 Hz, 1H), 6.41 (t, J = 5.9 Hz, 1H), 3.26 (t, J = 6.4 Hz, 2H), 2.87 (s, 4H), 2.83 (s, 4H), 2.60 (tt, J = 12.2, 3.4 Hz, 1H), 2.18 (m, 2H), 1.89 (m, 2H), 1.66−1.48 (m, 3H), 1.08 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.6, 169.2, 168.9, 162.4, 160.8, 140.8, 124.3, 45.6, 40.4, 36.9, 29.3, 28.1, 25.6, 25.6; HRMS (ESI) m/z calcd for $C_{20}H_{23}N_3O_9$ 449.14343, found 449.14471.

Succinimidyl Ester 6. To a cooled (0 °C) solution of **4** (0.638 g, 2.50 mmol) and *N*-hydroxysuccinimide (1.15 g, 10.0 mmol) in DMF (15 mL, 0.17 M) was added *sym*-collidine (1.33 mL, 10.0 mmol). The reaction mixture was stirred at 0 °C for 10 min, and trifluoroacetic anhydride (1.40 mL, 10.00 mmol) was added dropwise. The resulting reaction mixture was stirred at 0 °C for 30 min. The reaction mixture was diluted with $CHCl_3$ (50 mL) and 1 M HCl (50 mL). The layers were separated and the organic layer was washed with 1 M HCl (2 × 50 mL). The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo to provide a white solid. The white solid was triturated with chloroform (25 mL) to provide **6** as a white solid (0.258 g, 29%): HPLC *t*R 8.8 min, 98.4%, mp 186−187 °C; ^{1}H NMR (400 MHz, DMSO) δ 14.99 (br s, 1H), 9.08 (t, J = 5.6 Hz, 1H), 6.44 (d, J = 12.5 Hz, 1H), 6.24 (d, J = 12.5 Hz, 1H), 3.07 (t, J = 6.3 Hz, 2H), 2.81 (s, 4H), 2.70 (tt, J = 12.1, 3.5 Hz, 1H), 2.01 (m, 2H), 1.80 (m, 2H), 1.51 (m, 1H), 1.44 (m, 2H), 1.07 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 170.9, 170.2, 165.5, 165.4, 132.5, 132.0, 44.9, 39.6, 36.1, 28.7, 28.0, 25.5; HRMS (ESI) m/z calcd for $C_{16}H_{20}N_2O_7$ 352.12705, found 352.12723.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

HPLC chromatograms and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: Nicholas.Leonard@abbott.com

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