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Process Development of the Soft HDAC Inhibitor SHP-141 – Acylation of Methyl Paraben and Suberyl Hydroxamic Acid Formation

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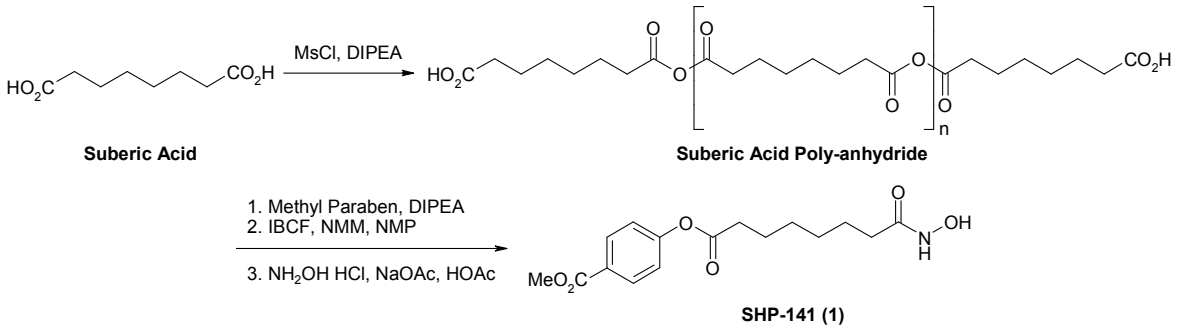

ABSTRACT

SHP-141 (**1**) is a hydroxamic acid-based inhibitor of histone deacetylate enzymes (HDACs) which is under development for the treatment of cutaneous T-cell lymphoma (CTCL). The original synthesis of **1** involved five synthetic steps beginning with suberic acid monomethyl ester. Final deprotection of the *O*-benzyl hydroxamate moiety using hydrogen and palladium catalyst mandated the use of metal scavengers to reduce palladium levels to within ICH guidance. Owing to the sensitivity of **1** towards self-condensation and the potential for N-O bond cleavage under hydrogenolytic conditions, we developed an alternative route to **1** which avoids Pd-mediated hydrogenation and prolonged metal scavenger treatment. This two-step process employs readily-available suberic acid and methyl paraben and has successfully delivered multiple kilograms of **1** for clinical use. Importantly, crude **1** was stabilized for recrystallization in acetonitrile (ACN) solution by the addition of 0.1% citric acid and 4% water. Additionally, the filtration and drying of suitably-sized aggregates of **1** with high purity (100 area%) was accomplished via temperature cycling of the **1**/ACN solution.

KEYWORDS: SHAPE, SHP-141, HDAC, Suberic acid, Methyl paraben, FBRM

INTRODUCTION

SHP-141 (**1**, Figure 1), a "soft" inhibitor of certain histone deacetylase enzymes or HDACs, was invented by scientists at Dana-Farber Cancer Institute for the treatment of cutaneous T-cell lymphoma (CTCL).[1] A topical formulation of SHP-141 allows HDAC inhibition at the site of action (i.e., skin) while the "soft" ester linkage is cleaved by plasma esterases to generate methyl paraben (**2**) and suberic acid hydroxamic acid (**3**), thus limiting the adverse effects associated with systemic HDAC inhibition.[2] Topical SHP-141 gel is currently being studied in a randomized Phase 2 clinical trial for the treatment of early-stage CTCL.[1]

[Figure 1]

The initial clinical manufacturing route is described in Scheme 1. Beginning with suberic acid monomethyl ester (**4**), the acid chloride (**5**) was formed but not isolated. Instead, **5** was directly reacted with *O*-benzyl hydroxylamine to afford the *O*-protected hydroxamic acid (**6**). LiOH-mediated saponification of the ester group and EDC-mediated coupling of **7** with **2** afforded penultimate intermediate **8** which was converted to **1** following palladium-catalyzed removal of the *O*-benzyl protective group.

[Scheme 1]

This first generation synthetic route presented some key challenges for commercial manufacturing. Firstly, the final protective group removal with 10% Pd/C required prolonged metal scavenger treatment (Smopex® 243) to reduce palladium levels to <10 ppm. Stability studies of **1** indicated partial degradation in a variety of organic solvents (*infra*) thus protracted manipulations of **1**-containing solutions were a concern. In addition, literature precedent suggested that hydrogenation over certain palladium catalysts might afford N-O bond cleavage

leading to amide formation (not shown).[3] Having observed the product of N-O bond cleavage of **8** in our early development work, we were reluctant to rely on a chemical transformation which might vary with scale, reaction time, and catalyst lot. Specifically, we worried that the formation of this structurally-similar amide (not shown) at the final step might greatly complicate the purification of **1**.[4]

RESULTS AND DISCUSION

Our second generation design is outlined retrosynthetically in Figure 2. We reasoned that **1** could be prepared via the coupling of carboxylic acid **9** with hydroxylamine or a hydroxylamine equivalent.[5] Several methods are reported for the conversion of carboxylic acids (or, an activated species derived thereof) to hydroxamic acids. Carboxylic acid **9** would, in turn, come from the reaction of **2** with cyclic suberic anhydride (**10**).

[Figure 2]

Suberic anhydride (**10**) is reported to form when suberic acid (**11**) is heated with acetic anhydride (Ac_2O).[6-9] In our hands, after 90 min at 150 °C, excess Ac_2O was removed by vacuum distillation and co-evaporation with *o*-xylene. Suberic anhydride reacted with **2** (1.1 eq) and triethylamine (TEA, 1.1 eq) in THF (5 vol) at 20 °C to form a mixture of **9** and suberic acid bis-(methyl paraben) ester (**12**, Scheme 2). After 3 h, water and MTBE were added and **12** was removed by filtration. The layers were separated and the basic aqueous phase was cooled to 0 °C, acidified with conc. HCl (pH 2-3), and filtered to provide crude **9** as a white-colored solid.

[Scheme 2]

The formation of **12** suggested that **9** (or its TEA salt) was further reacting with **10** to afford linear suberyl anhydride species (not shown) that could then react with **2**. High performance liquid chromatographic (HPLC) analysis suggested that **10** was, in fact, comprised of a mixture of suberyl poly-anhydrides and not a single cyclic suberyl anhydride species as shown. With the goal of controlling suberyl poly-anhydride formation, we investigated other activating agents. In the event, **11** in THF (5 vol) was reacted with TEA (1.1 eq) and activating agent (1.1 eq, Table 1) at -5 °C. After 3 h, **2** (1.1 eq) and TEA (2.2 eq) were added and the reaction mixture was processed as previously described. Owing to its low cost and ease of handling, methanesulfonyl chloride (MsCl) was selected for further optimization.

[Table 1]

Mechanistically, treatment of **11** with MsCl in the presence of TEA (1 eq) would initially form methanesulfonyl suberate (**13**) and/or bis-methanesulfonyl suberate (**14**) together with TEA·HCl salt (1 eq, Scheme 3, equation 1). However, as soon as **13** (or, **14**) formed, reaction with a second equivalent of **11** (or, triethylammonium suberate) would give rise to linear suberyl anhydride (**15**) (Scheme 3, equation 2). Repeating this process, i.e., **15** + **13**, would afford higher order suberyl poly-anhydrides (not shown). Ultimately, **11** would be entirely consumed in this process forming a distribution of suberyl poly-anhydrides. Notably, any cyclic suberic anhydride (**10**) which might form is subject to ring opening by **11** (Scheme 3, equation 3) or **13** (not shown) to propagate poly-anhydride formation.

[Scheme 3]

Chromatographic analysis of the Stage 1a reaction mixture provided support for the formation of suberyl poly-anhydrides (Figure 3A). Each of the observed peaks in the chromatogram

represented a unique suberyl poly-anhydride species which were differentiated only by the number of incorporated suberic acid monomers. No methanesulfonyl-containing poly-suberates were detected by mass spectral analysis. Notably, formation of each anhydride linkage would release one equivalent of methanesulfonic acid (MsOH, *infra*).

[Figure 3]

The Stage 1b reaction of suberyl di-anhydride (**16**) with **2** under basic conditions illustrates the formation of both **9** and **12** (Figure 4). This reaction required excess TEA (or, DIPEA) to scavenge MsOH from the previous step (*supra*) as well as to deprotonate **2**. Reaction of **2** at the "internal" carbonyl group (Path A) affords an equivalent of **11** together with the methyl paraben ester of suberyl anhydride (**17**) which undergoes further reaction (Path C) to provide two equivalents of **9**. Alternatively, **17** can react with **2** to generate **11** plus **12** (Path D). When **2** reacts at the "external" carbonyl group of **16**, both **9** and **15** are formed (Path B); **15** would further react to provide **9** and **11** (not shown). Thus, this mechanistic interpretation suggests that **9** will be formed in no greater than 50% yield and that both **11** and **12** will be by-products in the reaction. Because of the low cost and ready availability of **11** and **2**, we reasoned that this limitation on the theoretical yield of **9** would be acceptable on commercial scale.

[Figure 4]

This two-step process requires sufficient base to neutralize both the HCl (from MsCl) and MsOH (via anhydride formation), i.e., twice the equivalents of MsCl used in Step 1a, plus the amount of base required to deprotonate **2** used in Step 1b. We performed a series of experiments to evaluate the required quantity and order of addition of reagents (Table 2). Comparable yields of isolated **9** were observed when the first charge of organic base (DIPEA) was added in a single portion (Table 2, Entry 1) or divided between Step 1a and Step 1b (Table 2, Entry 2). However,

when DIPEA (2 eq) was added in one portion on 40 g-scale (i.e., suberic acid), the heterogeneous reaction mixture became too thick to stir owing to precipitation of both DIPEA·HCl and DIPEA·MsOH salts (Table 2, Entry 3). Employing an initial charge of DIPEA (1.3 eq), the reaction mixture was warmed to 50 °C following **2**/DIPEA (1.75 eq) addition (Table 2, Entry 4). After ca. 16 h (i.e., overnight) at 50 °C and standard work-up, **9** was isolated in 42% yield.

[Table 2]

Recognizing that the addition of solid **2** and the slow filtration of **12** might present problems during commercial manufacturing, we added **2** as a THF (2 vol) solution and developed an alternative work-up procedure for **9** (Table 2, Entry 5). To this end, when the reaction was deemed complete, the reaction mixture (Step 1b) was diluted with toluene (5 vol), water (10 vol) and DIPEA (1.0 eq) at 50 °C. After 20 min, the layers were separated and the warm toluene layer was extracted with DIPEA/water to remove additional **9**. The combined aqueous phase was then cooled and acidified (pH 2-3) with conc. HCl. The resultant solid was collected by filtration and processed as before to provide **9** in 37% yield. This process was successfully demonstrated beginning with 7.5 kg of **11** (Table 2, Entry 6). Residual **11** and **2** were conveniently removed by suspending crude **9** in 1:1 MeOH/water at ambient temperature. Following filtration, the wet cake was washed with 1:1 MeOH/water and dried *in vacuo* for 12 h at 35-45 °C to provide 5.2 kg (39%, 100 area%) of **9** as a white-colored solid.

To establish the reaction time course, we monitored the formation of **9** and **12** at 50 °C by HPLC analysis. As shown in Figure 3B, the reaction was complete between 5 and 14 h.

Interestingly, the rates of formation for both **9** and **12** were nearly superimposable which suggested a common reaction pathway.

For the preparation of **1**, we activated **9** using isobutylchloroformate (IBCF) in the presence of *N*-methylmorpholine (NMM) followed by the addition of 50% aqueous NH_2OH (Scheme 4). We chose 1-methyl-2-pyrrolidinone (NMP) as solvent owing to its miscibility with water. Both the activation of **9** with IBCF/NMM (to afford **18**) and the addition of aqueous NH_2OH to **18**/NMP were exothermic reactions and thus required low temperature, i.e., ≤ -10 °C, and efficient mixing. SHP-141 (**1**) was partly unstable under these conditions giving rise to oligomeric species **19** and **20** as prominent degradants – the former being the product of **1** self-condensation (with loss of **2**) while the latter partly arose via the reaction of **1** with unreacted **18**. The rate of formation of **19** and **20** accelerated with increasing temperature and concentration of **1**. When minimal agitation was applied, the symmetric anhydride (**21**) was observed in >10 area% by HPLC analysis.

[Scheme 4]

Not surprisingly, **1** was unstable in the presence of excess NH_2OH owing to sensitivity of the *O*-acylated methyl paraben moiety. In addition, **1** degraded under strongly acidic conditions (pH <3). We observed, however, that slightly acidic (pH 4-6) solutions of **1** had improved stability. We therefore attempted to maintain the reaction pH at 5.5-6.5 throughout the addition of aqueous NH_2OH/NMP by employing acetic acid as a buffering agent.

To this end, a pre-cooled (<10 °C) solution of 50% aqueous NH_2OH (1.05 eq) and HOAc (2.0 eq) in NMP (1 vol based on **9**) was added slowly to a cooled (-20 °C) solution of **18**/NMP [NB: prepared by adding IBCF (1.1 eq) and NMM (1.1 eq) to **9** (20 g, 1.0 eq) in NMP (5 vol) at -20 °C.] The reaction mixture was warmed to 10 °C. After 16 h, the product (**1**) was precipitated by

the addition of water (15 vol) then collected by filtration, washed with water, and air-dried (Table 3, Entry 1). To facilitate a final, polish filtration, crude **1** was dissolved in THF containing 1% HOAc (5 vol), filtered and then re-precipitated by the addition of water (20 vol). The solid was collected by filtration, washed with water and dried *in vacuo* at 40 °C to afford 17.0 g (81%) of **1** as white-colored needles (92.1 area%; **20**: 7.0 area%).

To minimize adventitious formation of **20**, we reasoned that the inverse addition of **18**/NMP to the NH_2OH/water/NMP solution would result in less **18** being available to react with **1**. Application of the inverse addition resulted in reduced levels of **20** compared to the control conditions (5.3 area% vs. 0.76 area%) when analyzed prior to the first water addition (Table 3, Entries 1 and 2) and afforded **1** in good yield with excellent chemical purity (Table 3, Entry 2). Notably, in the two aforementioned reactions, the amount of **20** increased to 7.0 area% and 1.4 area%, respectively, after the precipitated **1** was allowed to stir overnight prior to final filtration. We further modified the process by employing $NH_2OH{\cdot}HCl$ as the hydroxylamine source (Table 3, Entry 3). Differential scanning calorimetric (DSC) analysis of 50% aqueous NH_2OH and buffered (NaOAc/HOAc) $NH_2OH{\cdot}HCl$ solutions showed a higher onset temperature (115 °C vs. 102 °C, respectively) and lower decomposition energy (ΔH = -66 vs. -1654 J/g, respectively) for the latter condition.

[Table 3]

As described in Figure 5A, four acetate-based buffers were prepared (pH range: 5.1 to 6.1), combined with $NH_2OH{\cdot}HCl$, and independently evaluated using the standard Stage 2b reaction conditions. Notably, the pH 5.1 buffer was exhausted at ca. 60-70% addition of **18**/NMP as evidenced by the drop in pH (Figure 5B, Entry 1). Since free NH_2OH has a pKa of 5.95, this drop in pH likely resulted in less free NH_2OH being available for reaction with **18** (*infra*). The

remaining three buffer systems maintained a constant pH throughout the addition of the **18**/NMP solution (Figure 5B, Entries 2-4).

[Figure 5]

We monitored the four reaction profiles by HPLC analysis. Following the standard protocol (Stage 2a), **18** was formed with high chemical purity in each of the four reactions (Table 4). These results demonstrated that when reaction pH was maintained at 5.5-6.5, **1** was afforded in ≥90 area% chemical purity while minimizing the formation of **20**.

[Table 4]

The final solid form of **1** is not pharmaceutically important as **1** is dissolved then formulated as a gelled solution for topical application. As described previously, the addition of water to the Stage 2b reaction mixture afforded solid **1** as fine particles which presented problems during filtration, i.e., slow filtration rate. Thus, to help with this final processing step, we investigated alternative polymorphic forms of **1** in order to identify a more easily filterable solid form.

A set of 13 organic solvents was investigated for the recrystallization of **1** using both slow cooling (20 °C/h) and fast cooling (4 °C) procedures (Supplemental Table S1); the dried, isolated solids were then examined by X-ray powder diffraction (XRPD). Previously, we observed that **1** was unstable in acetonitrile (ACN) and other polar organic solvents for ≥4 h at >45 °C but could be partly stabilized with <1% HOAc or citric acid (Supplemental Table S2). Thus, for this investigation, we added 0.2% HOAc (v/v) to the solvents in order to stabilize these **1**-containing solutions. From this analysis, we identified two unique polymorphic forms of **1** (Table 5). The XRPD patterns of **1** Form A and **1** Form B are shown in Supplemental Figure S1. Microscopic analysis of **1** Form A revealed birefringent aggregates while **1** Form B existed predominantly as needle-shaped crystals (Supplemental Figure S2). Disappointingly, all attempts to generate

larger crystals of either **1** Form A or **1** Form B were unsuccessful (data not shown). We therefore opted to improve the aggregate particle size of **1** Form A via the addition of a suitable co-solvent.

[Table 5]

We compared the combination of isopropyl acetate (IPAc), methyl *tert*-butyl ether (MTBE) or toluene with water for the Stage 2b precipitation process. A mixture of purified **1** (10 g, ≥95 area%) in NMP (8.5 vol), water (1.1 vol), and HOAc (1 vol) was cooled to 0 °C. The described co-solvent (4 vol) was added either before or before-and-after the addition of water (20 vol) causing the precipitation of **1** (Table 6). The precipitated **1** was collected by filtration, washed with water, MTBE, air-dried, then analyzed by HPLC. The recovery of **1** from all the experiments was ca. 90%. When toluene was employed as co-solvent, pea-sized aggregates of **1** formed within a toluene/water/NMP emulsion which allowed an improved filtration rate of **1** compared to material precipitated from water only (ca. 1 h on 25 g-scale), IPAc/water or MTBE/water processes (Table 6, Entries 1-5). On larger scale, however, a slower filtration rate was observed perhaps due to disaggregation of **1** during the MTBE washes resulting in a more compressed filter cake (Table 6, Entries 6-8). When pre-cooled (0-8 °C) 12% NaCl/water (brine) was used in combination with toluene, a complete aqueous/organic phase separation was observed and **1** was obtained as an easily-filterable granular solid (Table 6, Entry 9). A comparison of pre-cooled 8%, 10%, and 12% brine suggested that the most granular form of **1** was obtained using toluene and 8% brine (data not shown). Importantly, the application of toluene and 8% brine exhibited reduced degradation of **1** over time as compared to IPAc/water (Supplemental Table S3).

[Table 6]

Owing to the temperature sensitivity of **1**-containing solutions, initial attempts to purify **1** focused on low temperature (<40 °C) trituration of crude **1** in organic solvents such as DCM or MTBE. Although these processes were effective at removing **20** and other related impurities, they were less able to purge oligomeric species **19**. To develop a more effective purification process, we explored the solvent systems first identified in the polymorphic characterization of **1** (Supplemental Tables S1 and S2). For purification at Stage 3, crude **1** (5 g) was added to ACN containing 0.1% wt/v citric acid (12-15 vol) with or without water and the heterogeneous mixture was warmed to 50 °C. After 2 h, the clear solution was cooled to ambient temperature and maintained for 16 h. The precipitated solid was collected by filtration, washed with ACN (2 × 5 vol) and dried *in vacuo* at 40 ± 5 °C. Analyses (HPLC) of the collected solids and filtrates are presented in Table 7.

[Table 7]

These results suggested that higher percentages of water in ACN, although providing material with high purity, could negatively impact the recovery of **1** (Table 7, Entry 3). Gratifyingly, however, there was no impact on the purity or recovery of **1** when the heating time was extended to 6 h (Table 7, Entry 4). Based on these observations, crude **1** (44 g, 91.4 area%) was treated with 0.1% citric acid/4% water in ACN (12 vol) at 45 °C to provide purified **1** (52% recovery, 99.6 area%). Despite the modest recovery for this process (50-70%), the excellent purity and solution stability of **1** allowed us to explore the final isolation conditions.

To optimize the particle size of purified **1** in advance of filtration, we turned to Focused Beam Reflectance Measurement (FBRM) technology to understand how particle size might vary across temperature cycles. Acetonitrile containing **1**, 0.1% citric acid and 4% water were placed into a reactor containing an FBRM probe (FBRM® D600L, Mettler-Toledo) and the heterogeneous

mixture was stabilized at 23 °C. The mixture was then subjected to four temperature cycles while monitoring particle size distribution (Table 8). The data from Cycles 2 and 3 suggested that larger particles might arise via a more rapid cooling phase (Supplemental Figure S3). Application of a modified temperature cycle (see: Experimental) consistently provided **1** Form A in >99 area% purity by HPLC analysis. In order to improve the recovery of **1**, we lowered the final isolation temperature to 10 °C. On 10 g-scale, this modification afforded a 72% recovery of **1** Form A (100 area%).

[Table 8]

CONCLUSIONS

In summary, a robust three stage process was developed for the synthesis and purification of **1** from inexpensive starting materials (Scheme 5). We successfully demonstrated the chemistry and process on kg-scale and applied this process for the cGMP preparation of >10 kg of **1** for clinical use. Key modifications include: i. the implementation of a hot toluene extraction to separate **12** from **9**·DIPEA salt (Stage 1b); ii. the inverse addition of **18** to a buffered (pH 5-6) hydroxylamine solution, i.e., NH_2OH·HCl (1.3 eq)/NaOAc (1.3 eq)/HOAc (5.6 eq, Stage 2b); iii. the precipitation of **1** from the Stage 2b reaction mixture using pre-cooled 8% brine/toluene to increase the particle size and improve the filtration rate of crude **1**; iv. the recrystallization of crude **1** using 4% water in ACN containing 0.1% citric acid (Stage 3); and, v. the incorporation of temperature cycling at Stage 3 to generate **1** Form A of sufficient size to facilitate final isolation at 10 °C.

[Scheme 5]

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR spectra were obtained at 500 and 125 MHz, respectively, using a Bruker Avance spectrometer using tetramethylsilane as the internal standard. Reactions were routinely performed under a nitrogen atmosphere using standard glassware and high purity, commercial-grade solvents. LC/MS analysis was performed on a Thermo-Fisher MSQ Plus instrument with a Gemini 5 μ C6-Phenyl 110 Å column (50 × 4.60 mm) using standard gradient conditions (A: water containing 0.1% HOAc v/v; B: acetonitrile containing 0.1% HOAc v/v); MS data were acquired with ESI, positive ionization; UV detection at 254 nm unless otherwise noted. Compound purifications were performed using either flash silica gel chromatography or preparative HPLC (Varian Prostar) in normal phase (SiO_2, EtOAc/hexane, 250 × 41.4 mm) or reversed-phase (C18, 100 Å, 60 μ, 250 × 41.4 mm). Abbreviations: ACN, acetonitrile; $CDCl_3$, chloroform-*d*; DIPEA, diisopropylethylamine; DMSO, dimethylsulfoxide; eq, equivalents; g, grams; HCl, hydrochloric acid; h, hours; HOAc, acetic acid; IBCF, isobutyl chloroformate; kg, kilograms; min, minutes; MeOH, methanol; MHz, megahertz; mL, milliliters; mol, moles; MS, mass spectrum; MsCl, methanesulfonyl chloride; MTBE, methyl *tert*-butyl ether; NaOAc, sodium acetate; NMM, *N*-methylmorpholine; NMP, 1-methyl-2-pyrrolidinone; NMR, nuclear magnetic resonance; ppm, parts per million; THF, tetrahydrofuran; vol, volumes.

*Octanedioic acid mono-(4-methoxycarbonyl-phenyl) ester (**9**).* To a solution of Suberic Acid (7.5 kg, 43.0 mol) in THF (30 L, 4.0 vol) as added methanesulfonyl chloride (4.9 kg, 1.0 eq) at 15-20 °C followed by DIPEA (7.2 kg, 1.3 eq). The resulting light yellow-colored solution was agitated for 1 h at 15-25 °C. Methyl Paraben (6.9 kg, 1.05 eq) in THF (15 L, 2 vol) was added at 15-25 °C followed by a second portion of DIPEA (9.7 kg, 1.75 eq). The reaction mixture was then heated to 50 °C. After 20 h, a third portion of DIPEA (5.6 kg, 1.0 eq) was added followed

by the addition of toluene (37.5 L, 5 vol) and water (75.0 L, 10 vol). After 20 min, agitation was stopped and the layers were separated. The organic layer was washed once with water (37.5 L, 5 vol) containing DIPEA (2.8 kg, 0.5 eq). The combined aqueous layers were cooled to 15-25 °C and the pH was adjusted to 2.5 using conc. HCl (~7 L) which resulted in the precipitation of **9** as a white-colored solid. The thick suspension of **9** was filtered through standard weave, polypropylene cloth and washed three times with water (37.5 L, 5 vol). The wet cake was conditioned overnight and 1:1 MeOH:Water (42 L, 5.6 vol) was added. After stirring for 1 h, the suspension was filtered through standard weave, polypropylene cloth, washed three times with 1:1 MeOH:Water (21 L, 2.8 vol) then dried *in vacuo* at 35-45 °C to afford 5.2 kg (39%) of **9** as a white-colored solid (98.8 area%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.98 (br s, 1H), 8.01 (d, J = 8.75 Hz, 2H), 7.28 (d, J = 8.75 Hz, 2H), 3.86 (s, 3H), 2.60 (t, J = 7.35 Hz, 2H), 2.21 (t, J = 7.35 Hz, 2H), 1.64 (app quint, J = 7.30 Hz, 2H), 1.52 (app quint, J = 7.30 Hz, 2H), 1.34 (m, 4H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 174.3, 171.3, 165.4, 154.1, 130.6, 127.0, 122.1, 52.1, 33.5, 33.3, 28.1, 27.9, 24.2, 24.0 ppm. HRMS (ESI), *m/z* 309.1187 [(M+H), calcd for $C_{16}H_{21}O_6$: 309.1338].

*4-(7-Hydroxycarbamoyl-heptanoyloxy)-benzoic acid methyl ester (**1**).* To a solution of $NH_2OH{\cdot}HCl$ (0.70 kg, 1.25 eq), water (2.5 L, 1 vol) and HOAc (2.71 kg, 5.6 eq) was added NaOAc (0.87 kg, 1.30 eq) and NMP (9.38 L, 3.75 vol). The heterogeneous mixture (i.e., thin slurry) was cooled to ca. -10 °C and stirred overnight.

To a solution containing **9** (2.50 kg, 1 eq) in NMP (10 L, 4 vol) at -3 °C was added IBCF (1.22 kg, 1.10 eq) followed by NMM (0.86 kg, 1.05 eq). The resultant suspension of **17**/NMP was added to the pre-formed hydroxylamine solution over 55 min at ca. -10 °C. Toluene (10 L, 4 vol) was added to the reaction mixture followed by the addition over 70 min of pre-cooled (~6 °C),

8% NaCl/water (37 L, 15 vol). After 30 min, the solid was collected by filtration through standard weave, polypropylene cloth and washed with water (3 × 8.5 L, 3 × 3.4) then MTBE (3 × 8.5 L, 3 × 3.4 vol). The wet cake was conditioned overnight then dried *in vacuo* at room temperature to provide 1.97 kg (75%) of **1** as a white-colored solid (99.8 area%).

A solution containing crude **1** (1.97 kg) and citric acid (26 g, 0.11% based on ACN) in 4% water/ACN (25.6 L, 13 vol) was heated to 45 °C. The slightly hazy solution was filtered through a 1.2 μm filter cartridge and the filtrate was warmed to 45 °C to dissolve any precipitated solid then cooled to 30 °C over 30 min. Upon reaching 30 °C, the solution was warmed to 35 °C and maintained for 30 min. The solution was cooled to 10 °C over 6 h and the resultant suspension was stirred at 10 °C. After 16 h, the solids were collected by filtration, washed three times with ACN (3 × 2 vol ACN) and dried *in vacuo* at room temperature to provide **1** Form A (1.49 kg, 76% recovery) as a white-colored solid (100 area%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.34 (s, 1H), 8.67 (s, 1H), 8.02 (d, J = 8.75 Hz, 2H), 7.29 (d, J = 8.75 Hz, 2H), 3.86 (s, 3H), 2.60 (t, J = 7.40 Hz, 2H), 1.96 (t, J = 7.40 Hz, 2H), 1.64 (m, 2H), 1.51 (m, 2H), 1.36 (m, 2H), 1.28 (m, 2H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.4, 169.1, 165.5, 154.2, 130.7, 127.1, 122.2, 52.2, 33.4, 32.2, 28.2, 28.0, 24.9, 24.1 ppm. HRMS (ESI), *m/z* 324.0960 [(M+H), calcd for $C_{16}H_{22}NO_6$: 324.1447].

ASSOCIATED CONTENT

SUPPORTING INFORMATION

The supporting information contains the chemical characterization of **12**, **19**, and **20**. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

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Author Contributions

All authors contributed to the writing or editing of this manuscript. All authors contributed to the design, synthesis, or analysis of the chemistry described in this manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

2-MeTHF, 2-methyltetrahydrofuran; ACN, acetonitrile; Ac_2O, acetic anhydride; $CDCl_3$, chloroform-*d*; CTCL, cutaneous T-cell lymphoma; DCM, dichloromethane; DIPEA, diisopropylethylamine; DMSO, dimethylsulfoxide; DSC, differential scanning calorimetry;

DVS, dynamic vapor sorption; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; EtOAc, ethyl acetate; EtOH, ethanol; eq, equivalents; FBRM, focused beam reflectance measurement; g, grams; HCl, hydrochloric acid; HDAC, histone deacetylase enzyme; h, hours; HOAc, acetic acid; HPLC, high performance liquid chromatography; IBCF, isobutyl chloroformate; *i*-PrOH, isopropanol; IPAc, isopropyl acetate; kg, kilograms; LiOH, lithium hydroxide; min, minutes; MEK, methyl ethyl ketone; MeOH, methanol; MHz, megahertz; MIBK, methyl isobutyl ketone; mL, milliliters; mol, moles; MS, mass spectrum; MsCl, methanesulfonyl chloride; MsOH, methanesulfonic acid; MTBE, methyl *tert*-butyl ether; NaOAc, sodium acetate; *n*-BuOH, normal butanol; ND, not detected; NMM, *N*-methylmorpholine; NMP, 1-methyl-2-pyrrolidinone; NMR, nuclear magnetic resonance; Pd/C, palladium on carbon catalyst; ppm, parts per million; RH, relative humidity; RM, reaction mixture; THF, tetrahydrofuran; TEA, triethylamine; vol, volumes; v/v, volume-to-volume; wt/v, weight-to-volume; XRPD, X-ray powder diffraction

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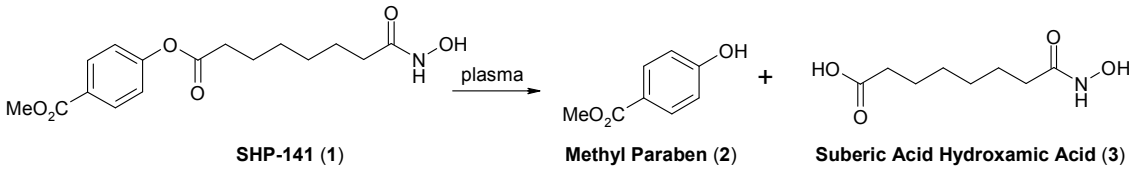


Figure 1. Enzymatic deactivation of SHP-141 (1) by plasma esterases

Scheme 1. First Generation Synthesis of SHP-141 (1)

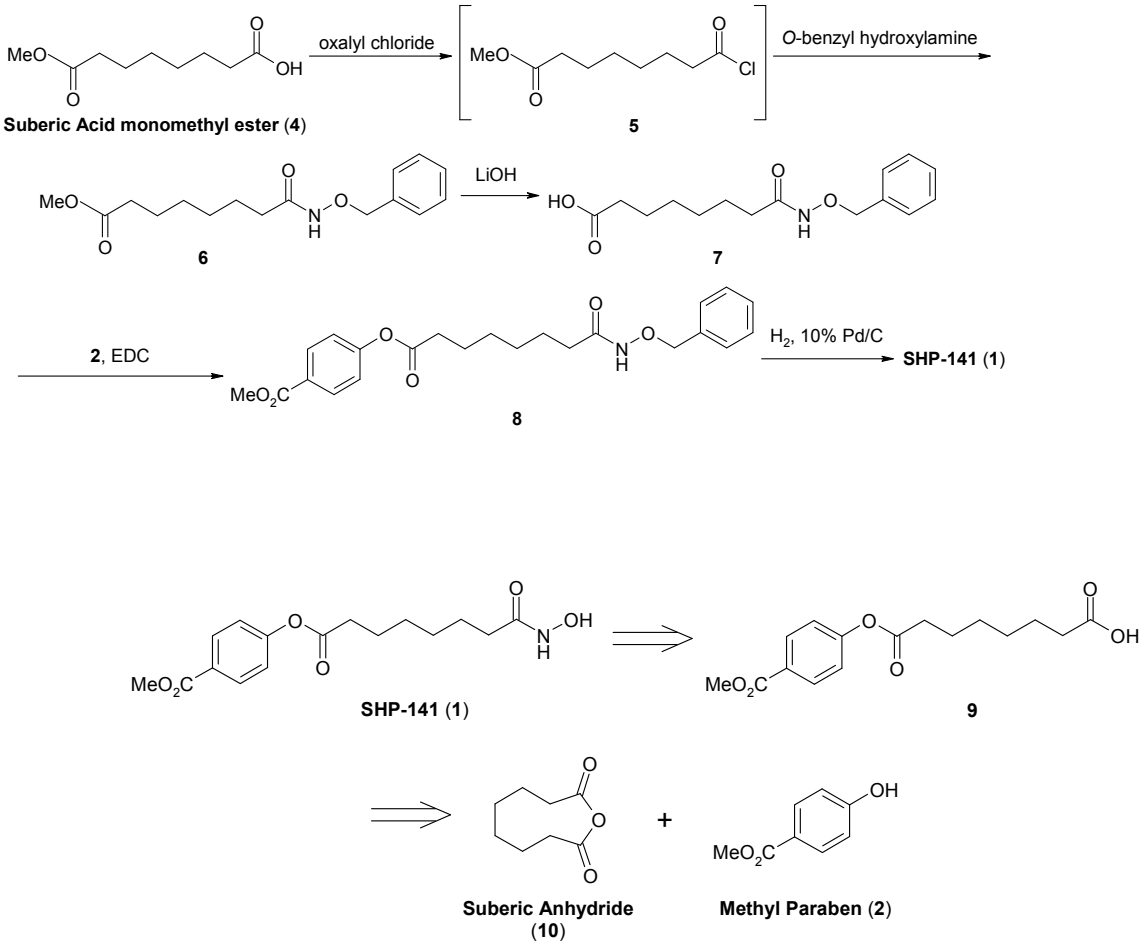


Figure 2. Retrosynthetic Analysis of 1. The Second Generation Approach

Scheme 2. The Reaction of Methyl Paraben (2) with Suberic Anhydride (10)

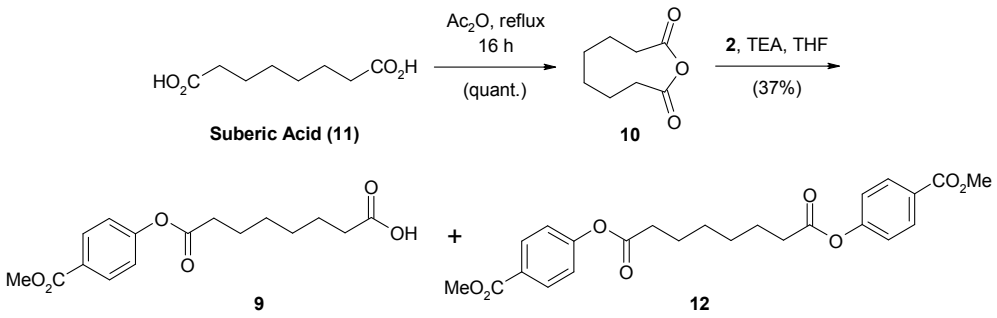
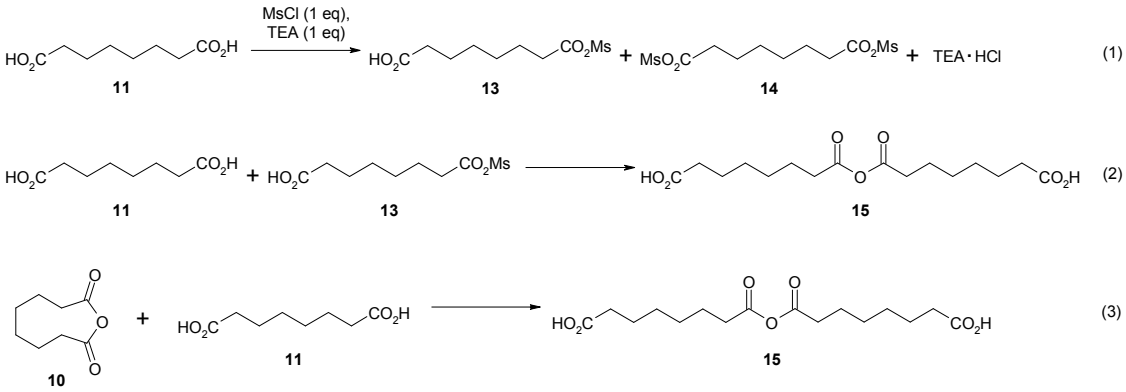


Table 1. The Synthesis of 9 using Selected Activating Agents

Entry	Activating Agent	9 (Isolated Yield, %)
1	MsCl	37
2	Ms_2O	39
3	Ac_2O	23
4	Me_3CCOCl	27

Scheme 3. Stage 1a: The Formation of Linear Suberyl Anhydride and Poly-anhydrides



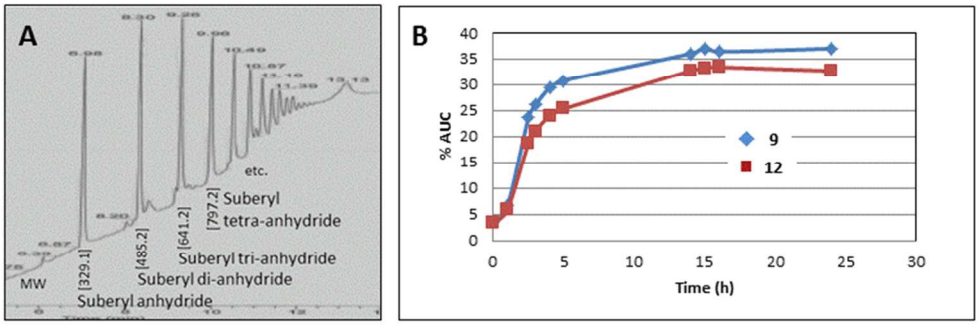


Figure 3. HPLC Analysis of the Stage 1a Reaction Mixture (λ = 210 nm) (A); and, the Formation of 9 and 12 during Stage 1b (B)

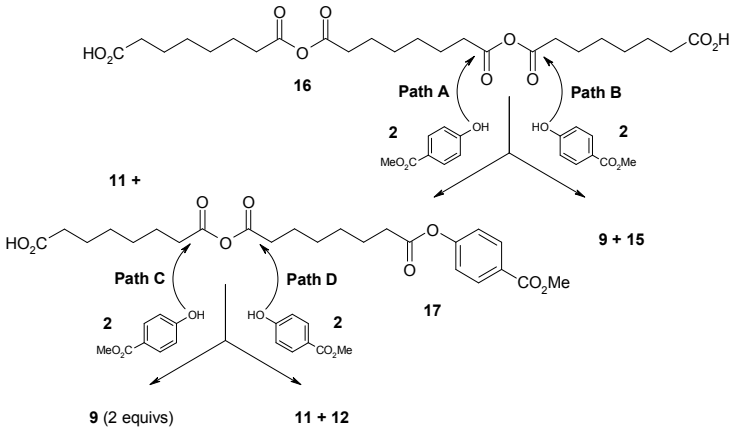


Figure 4. Stage 1b: The Reaction of 2 with Suberyl Poly-anhydrides

Table 2. Results for the Formation of 9 by Varying the Quantity of Base Added at Stage 1a and Stage 1b

Entry	11, g	Step 1a: 11 + MsCl		Step 1b: Suberyl Anhydrides + 2		Total DIPEA (eq)	Yield,[1] %
		MsCl (eq)	DIPEA (eq)	2 (eq)	DIPEA (eq)		
1	20	1.0	2.1	1.1	1.1	3.2	40
2	10	1.0	1.3	1.1	1.8	3.1	42
3	40	1.0	2.0	1.1	na	na	na[2]
4	40	1.0	1.3	1.05	1.75	3.05	42[3]
5	70	1.0	1.3	1.05	1.75	3.05	39[3,4]
6	7500	1.0	1.3	1.05	1.75	3.05	39[3,4,5]

General procedure: Suberic acid (**11**, 1 eq) was dissolved in anhydrous THF (5 vol) containing DIPEA. The mixture was cooled to 0 °C and MsCl was added. After 1 h, solid **2** and DIPEA were added and the mixture was warmed to ambient temperature. After 16 h, water (20 vol), MTBE (5 vol), and DIPEA (1.0 eq) were added. After 30-60 min, **12** was removed by vacuum filtration. The biphasic filtrate was separated and the **9**-containing aqueous layer was washed with MTBE (5 vol), cooled to 0 °C, and acidified (pH 2-3) with conc. HCl. Crude **9** was collected by filtration then suspended in MeOH (2 vol) for 1 h at ambient temperature. Water (2 vol) was added and the heterogeneous mixture was stirred for 1 h and filtered. The wet cake was washed with 1:1 MeOH/water and dried under vacuum at 50 °C to afford **9** as a white-colored solid. [1] Yield of **9** based on **11**. [2] Reaction mixture was too thick to stir at Step 1a; therefore, the reaction was discontinued. [3] Following **2** addition, reaction mixture heated to 50 °C for 16 h. [4] Methyl Paraben (**2**) added as a THF (2 vol) solution. Modified work-up using toluene extraction of **12** (*supra*). [5] Crude **9** triturated in 1:1 MeOH/water (*supra*). na, not applicable.

Scheme 4. Stage 2: The Formation of 18 and Its Reaction with 50% Aqueous NH_2OH Containing HOAc

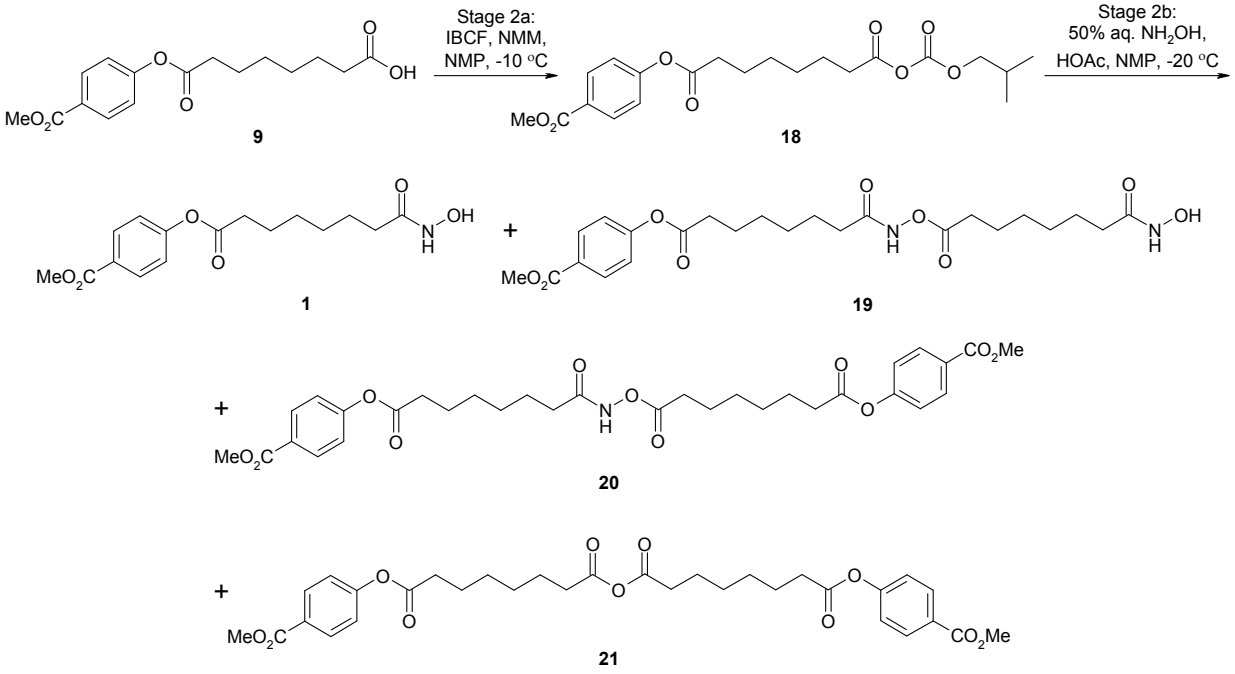


Table 3. A Comparison of the Effects of Acetic Acid or Acetate Buffer on Stage 2b

Entry	9 (g)	IBCF (eq)	NMM (eq)	NH_2OH Source (eq)		HOAc (eq)	NaOAc (eq)	HPLC Analysis[2] (area%)		Yield (Purity, area%)
				Aq.	HCl			1	20	
1[1]	20	1.1	1.1	1.05	0	2.0	0	91.6	5.3	81% (92.1)[3]
2	20	1.1	1.1	1.2	0	2.2	0	91.7	0.76	73% (98.6)[4]
3	20	1.1	1.1	0	1.2	1.2	1.2	94.7	ND	75% (99.2)[5]

General procedure: Carboxylic acid **9** (20 g, 1 eq) was dissolved in anhydrous NMP (5 vol). The solution was cooled to -5 °C and IBCF and NMM were added. After 0.5 h, the thin suspension was transferred to an addition funnel (**18**: >95 area%). The reactor was charged with the described NH_2OH source and NMP (1.1 vol based on **9**). After cooling to -10 °C, the **18**/NMP solution was added over 24 min then the reaction mixture was warmed to 10 °C. After 16 h, water (19 vol) was added and the resultant solid was collected by filtration, washed (water) and air-dried. Crude **1** was dissolved in THF containing 1% HOAc (5 vol) to facilitate a polish filtration step. Water (20 vol) was added over 1 h and the resulting suspension was aged (0.33 h), filtered, washed with water, and dried to constant weight under vacuum at 40 °C to afford **1** (15.3 g) as a white, needle-like solid. [1] Addition of hydroxylamine to **18**/NMP; see text. [2] HPLC analyses performed prior to first water addition. [3] **20**: 7.0 area%. [4] **20**: 1.4 area%. [5] **20**: 0.4 area%. ND, not detected.

A

Entry	$NH_2OH{\cdot}HCl$ (eq)[1]	NaOAc (eq)[1]	HOAc (eq)[1]	pH
1	1.2	1.0	5.1	5.1
2	1.2	1.2	5.1	5.5
3	1.5	1.5	2.0	6.1
4	1.3	1.3	5.6	5.5

[1]Based on **9** (1 eq).

B

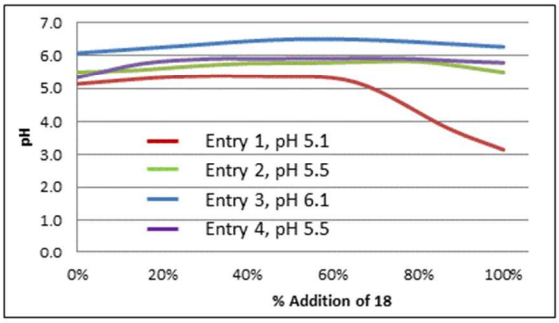


Figure 5. The pH of Several Prepared Hydroxylamine/Acetate Solutions (A); and, the Effect on pH when Adding 18/NMP to Hydroxylamine/Acetate Solutions (B)

Table 4. Effect of Hydroxylamine/Acetate Solution on Stage 2b Reaction Mixtures (HPLC)

Entry	HPLC Analysis of Stage 2a RM (area%)		pH Range[1]	HPLC Analysis of Stage 2b RM (area%)[2]			
	18	9		1	9	18	20
1	96.9	2.3	3.2-5.4	69.7	4.91	22.4	2.96
2	95.7	2.1	5.5-5.8	92.3	2.78	ND	0.62
3	96.5	2.0	6.1-6.5	91.6	2.63	ND	0.11
4	94.5	3.9	5.5-6.1	89.9	4.67	ND	0.50

RM, Reaction Mixture. [1] pH range throughout addition of **18**/NMP to buffered $NH_2OH{\cdot}HCl$ solution. [2] HPLC analysis performed prior to water addition. ND, Not Detected.

Table 5. Physical Characterization of 1 Form A and 1 Form B

XRPD Pattern	DSC (°C)	Onset Decomposition Temperature*	Moisture Sorption Analysis§			Microscopy
			60% RH	90% RH‡	Post-DVS XRPD	
1 Form A	114.2 °C (endotherm); 183.7 °C (exotherm)	185.5 °C	0.06 wt%	0.004 wt%	Form A	Birefringent, round- and needle-shaped particles. Particle size: 20-100 μm.
1 Form B	109.2 °C (endotherm); 184.4 °C (exotherm)	166.2 °C	0.3 wt%	0.2 wt%	Form B	Birefringent, needle-shaped particles. Particle size: 10-50 μm.

XRPD, X-ray powder diffraction; DSC, differential scanning calorimetry; RH, relative humidity. * Determined by thermogravimetric analysis (TGA). § Determined by dynamic vapor sorption (DVS). ǂ The slightly lower wt% moisture measured at 90% RH is likely related to system error of the internal balance.

Table 6. The Effect of Co-solvent on Filtration Rate of Crude 1

Entry[1]	Co-Solvent	Volume, Pre-/Post-(mL)[2]	Water (mL)	Aging Time	Filter/Wash Time (min)[3]	HPLC Analysis (area%)		Recovery (%)
						1	20	
1	IPAc	0/40	200	16 h	16	96.8	3.2	90
2	IPAc	10/30	200	16 h	30	98.2	1.9	94
3	MTBE	10/30	200	16 h	35	95.7	4.3	90
4	toluene	10/30	200	16 h	5	99.5	0.5	88
5	toluene	40/0	200	20 min	5	99.1	0.9	95[7]
6	toluene	120/0	610	2 min	20[4]	97.1	2.9	94
7	toluene	120/0	610	6 min	35	97.4	2.6	96
8	toluene	120/0	610	1 h	35	96.7	3.3	94
9	toluene	120/0	450[5]	2 h	<1 min[6]	98.1	1.7	90

[1] Entries **1**-**6** were performed on 10 g-scale; entries **7**-**9** were performed on 30 g-scale. [2] Listed as pre-/post-addition of water. [3] The suspensions were aged for the listed time at ambient temperature prior to filtration. After filtration, the solids were washed with water (3 × 50 mL) the MTBE (2 × 50 mL). [4] Prior to MTBE washing, wet cake dimensions were 4.0 cm (height, H) × 9.5 cm (diameter, D); post-washing dimensions: 1.5 cm H × 9.5 cm D. [5] Pre-cooled 12% brine. [6] Filter time excludes washes. [7] **1** Form B.

Table 7. Stage 3 Recrystallization of Crude 1[1]

Entry	Citric Acid[2]	Water[3]	Time (h)	Recovery (%)	Solid Purity (area%)				Filtrate Purity (area%)			
					MP	1	19	20	MP	1	19	20
1	0.1	0	2	78	ND	98.4	1.0	0.5	4.2	61.0	12.7	22.0
2	0.1	1	2	80	ND	99.1	0.9	ND	2.9	64.5	13.0	19.6
3	0.1	5	2	64	ND	100	ND	ND	2.1	61.8	20.4	14.6
4	0.1	5	6	68	ND	100	ND	ND	3.1	62.0	15.5	18.7

[1] Crude **1** purity: 96.7 area% **1**, 2.2 area% **19**, 1.1 area% **20**. [2] wt/v% ACN. [3] v/v% ACN. ND, Not Detected.

Table 8. Temperature Cycles Applied for the Recrystallization of Crude 1.

Cycle 1		Cycle 2		Cycle 3		Cycle 4	
Time (h)	Temp (°C)	Time (h)	Temp (°C)	Time (h)	Temp (°C)	Time (h)	Temp (°C)
0	23	0	20	0	20	0	20
1	45	1	45	1	45	1	45
2	45	1.5	45	1.5	45	1.5	45
2.5	40	2.5	30	2.5	30	2	35
3.5	40	3.5	30	3.0	40	3	35
7.5	20	7.5	20	9.0	20	8	10
11.5	20	11.5	20	na	na	na	na

na, not applicable.

Scheme 5. Final Synthetic Process for the Manufacture of 1