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Solution Phase Preparation of Highly Pure Amide Mixtures via In-Situ Chlorotrimethylsilane Protection and Activation

Koc-Kan Ho [a b], Nai Fang Wang [a], Christine Lercara [a], Doris C. O'Toole [a], Douglas M. Achan [a], Edmund A. Vuocolo [a] & Andrea Leone-Bay [a]

[a] Emisphere Technologies Inc., 15 Skyline Dr., Hawthorne, NY, 10532

[b] Pharmacopeia, Inc., 101 College Road East, Princeton, NJ, 08540

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SOLUTION PHASE PREPARATION OF HIGHLY PURE AMIDE MIXTURES VIA IN-SITU CHLOROTRIMETHYLSILANE PROTECTION AND ACTIVATION

Koc-Kan Ho,[+] Nai Fang Wang, Christine Lercara, Doris C. O'Toole, Douglas M. Achan, Edmund A. Vuocolo, Andrea Leone-Bay.*

Emisphere Technologies Inc., 15 Skyline Dr., Hawthorne, NY 10532.

Abstract. Coupling of 4-(4-aminophenyl)butyric acid **1** with acyl halides in both organic and aqueous media were found to produce large amount of oligomeric materials. By using an *in situ* chlorotrimethylsilane protection/activation procedure, these oligomers were suppressed completely and the desired 4-(4-acylaminophenyl)butyric acids **3** were obtained in good yield and high purity. The method was also extended to a parallel synthesis of a three component mixture. ^{1}H-NMR of the mixture indicated that each component was formed in a nearly stoichiometric quantity.

Synthesis on a solid support, in conjunction with the "split/pool" strategy to produce combinatorial libraries has become an important technique in the drug discovery process.[1] One advantage of using solid phase synthesis is that reaction yields can be maximized by the addition of excess reagents, driving the reactions to completion. Because the product is linked to a solid support, the unreacted reagents can be easily removed by washing. The disadvantages in polymer-

*To whom correspondence should be addressed.

[+]Current address: Pharmacopeia, Inc., 101 College Road East, Princeton, NJ 08540.

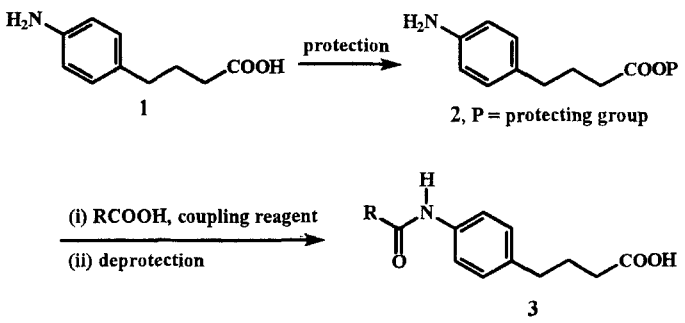
supported synthesis are the high cost of the resins and the processing problems associated with handling large quantities of solid. Thus, this methodology is not practical for producing multi-gram quantities of materials. Alternatively, traditional liquid-phase reactions can provide larger quantities of compounds. In the case of the synthesis of mixtures (parallel synthesis),[2] optimization of the associated chemistry to near quantitative yield under stoichiometric conditions is required because the use of excess reactants is problematic. Purification steps such as phase extraction or column chromatography to remove the excess unreacted reagents are difficult. More importantly, the kinetics of the individual reactions under non-stoichiometric conditions can give mixtures in which the components are not produced in an equimolar ratio. This concentration difference may introduce bias in subsequent screening of the compounds. Therefore, the solution-phase preparation of mixtures is restricted to high yield reactions such as carbamate formation from alcohols and isocyanates.[2]

As part of our oral drug delivery program,[3] large-scale syntheses of pure compounds with the general structure **3** were required. In order to expedite our *in vivo* screening program, the preparation of mixtures with the general structure **3** in multi-gram quantities was also needed. Herein we report a chlorotrimethylsilane-mediated, amide bond-forming procedure that allows not only the preparation of pure compounds **3** but also the solution-phase synthesis of highly pure amide mixtures.

Compounds represented by structure **3** can be prepared by coupling of an acid component (i.e. RCOOH) with the protected 4-(4-aminophenyl)butyric acid

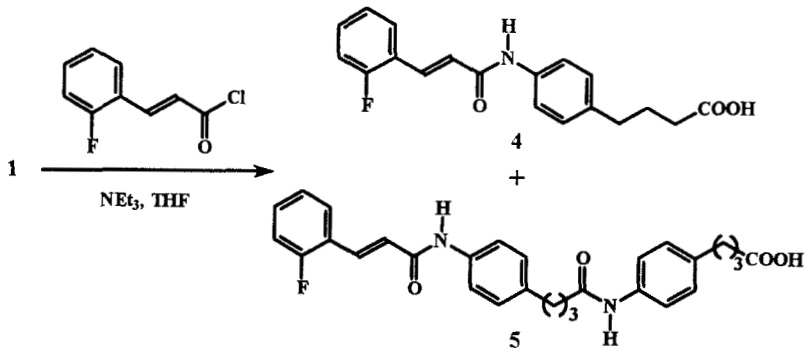
derivative **2** (Scheme 1). However, this route is not cost effective because it requires: (i) protection of 4-(4-aminophenyl)butyric acid **1**; (ii) removal of excess coupling reagent or by-products generated from the coupling reagent; and (iii) deprotection after coupling.

Scheme 1

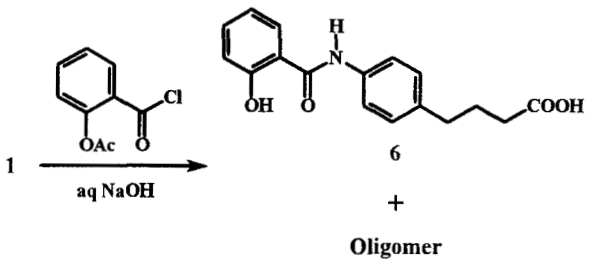


Condensation of 4-(4-aminophenyl)butyric acid **1** with an acyl chloride is the most obvious approach to compound **3**. We have investigated this reaction in both organic and aqueous media. When the unprotected 4-(4-aminophenyl)-butyric acid was reacted with 2-fluorocinnamyl chloride (Scheme 2), large quantities of oligomer **5** were observed. Similarly, the reaction of *O*-acetyl-salicyloyl chloride with 4-(4-aminophenyl)butyric acid in aqueous base (Schotten-Baumann reaction, Scheme 3) gave a mixture of compound **6** and the oligomer. This suggests that the free acid groups of **4** and **6** were activated by the acyl halides and further condensed with 4-(4-aminophenyl)butyric acid. This type of side reaction is well documented in the preparation of *N*-Fmoc-amino

Scheme 2



Scheme 3



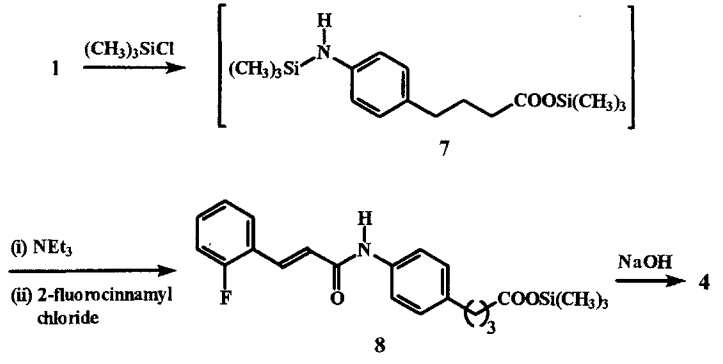
acids from unprotected natural amino acids.[4] Additionally, the reduced nucleophilicity of the anilino function in **1** increases the probability of reaction between the carboxylates of **4** and **6** with the acyl electrophiles, which leads to oligomer formation.

The failure of the above reactions to produce the desired products cleanly and in good yield indicated that complete elimination of the diamide by-product required protection of the free carboxylic acid. The trimethylsilyl ester was selected as the acid protecting group.[5] These esters are easily prepared by

reaction of the acids with chlorotrimethylsilane and are readily hydrolyzed under mild conditions.[6] Although trimethylsilyl esters have been used widely for carboxylate protection in the preparation of *N*-Fmoc amino acids,[4] *N*-trityl amino acids,[6] and tribenzyloxycarbonyl-arginine,[7] they have not been reported with anilines. We chose to investigate this procedure for the preparation of compounds **3**.

4-(4-Aminophenyl)butyric acid **1** was treated with chlorotrimethylsilane in dichloromethane at reflux to afford the ester **7** (Scheme 4), which was then reacted with the 2-fluorocinnamyl chloride. Following an aqueous work up, analysis by reversed- phase HPLC showed the crude reaction mixture to be a single component. Thus, compound **4** was formed exclusively, and the oligomer **5** was completely suppressed. This procedure proved to be very effective in eliminating oligomer formation via *in situ* protection of the acid and possibly

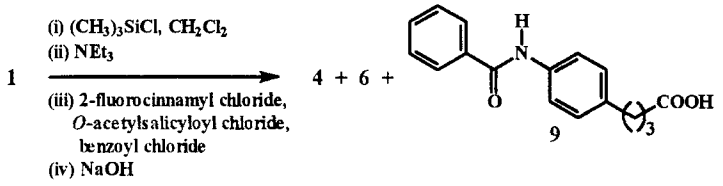
Scheme 4



nucleophilicity enhancement of the aniline by forming a trimethylsilyl amine intermediate 7.[8] A similar process was used to produce **6** in multi-kilo quantities.

This protection/activation process was next demonstrated in the context of a parallel synthesis by preparing a mixture containing three components. Thus, three equivalents of 4-(4-aminophenyl)butyric acid **1** were reacted with one equivalent each of 2-fluorocinnamyl chloride, *O*-acetylsalicyloyl chloride, and benzoyl chloride (Scheme 5). Following an aqueous work up, three major peaks

Scheme 5



were observed by reversed-phase HPLC. The retention times of these peaks are identical to those of the reference compounds (i.e. **4**, **6**, **9**), which were prepared and characterized separately. The results of ^{1}H-NMR analyses indicated that the compounds are present in near-stoichiometric quantities.

The trimethylsilylchloride procedure previously used to prepare *N*-Fmoc amino acids was successfully applied to amide formation from an aniline substrate. This method facilitated the preparation of compound **3** in good yield and with high purity. We have also demonstrated that the method is useful for

the preparation of gram quantities with mixtures of high purity. Each component of the mixture is formed in a nearly stoichiometric amount.

Experimental. Melting points are uncorrected. NMR spectra were measured using a Bruker AM300 (300 MHz) spectrometer (^{1}H at 300 MHz, ^{13}C at 75 MHz). ^{1}H and ^{13}C chemical shifts are reported in δ ppm relative to DMSO as an internal standard (2.49 ppm and 39.5 ppm, respectively). The carbon multiplicities are assigned via DEPT sequence experiments. Thin-layer chromatography was performed on Whatman MK6F plates, and compounds were detected by UV, I_2, or ninhydrin staining. Reversed-phase HPLC was performed with a Vydac Protein & Peptide (C_{18}, 250 x 4.6 mm, 5 μm particle size, 300 A pore size) column and a UV detector (220 nm). The sample was eluted with 0.1% TFA in water (Mobile phase A) and 0.1 % TFA in 50 % acetonitrile/water (Mobile phase B) (flow rate, 1 mL/min; gradient, 0 % to 100 % B over 20 min and 100 % B throughout). Microanalytical data was obtained from Robertson Microlit Laboratory, Inc. (Madison, NJ). 4-(4-Aminophenyl)butyric acid was synthesized using a published procedure[9] or purchased from commercial sources. Other starting materials were purchased from commercial sources and used without further purification.

2-Fluorocinnamyl chloride. A catalytic amount of DMF and thionyl chloride (14.27 mL, 190.7 mmoles, 1.3 equiv) was added to a solution of 2-fluorocinnamic acid (25 g, 150.5 mmoles, 1 equiv) in dichloromethane (215 mL).

The mixture was refluxed under argon for 1 h. The solvent and excess thionyl chloride were removed by evaporation. The solid residue was recrystallized from warm hexanes to afford 2-fluorocinnamyl chloride (18.02 g, 67%) as a colorless solid: mp < 37 °C.

Scheme 2. A 500-mL three-neck round bottom flask was fitted with a magnetic stirrer, an addition funnel, and kept under argon atmosphere. A mixture of 4-(4-aminophenyl)butyric acid (15.0 g, FW 179.2, 83.70 mmoles, 1.00 equiv), triethylamine (18.63 g, FW 101.19, 184.14 mmol, 2.2 equiv), and a catalytic amount of DMAP in THF (190 mL) was added to the flask, and cooled in an ice bath. A solution of 2-fluorocinnamyl chloride (18.29 g, FW 198.6, 92.07 mmoles, 1.10 equiv) in THF (60 mL) was charged to the addition funnel and added dropwise over 1 h. The reaction was stirred in the ice bath for 1 h and at ambient temperature for 1 h. The reaction was poured into a mixture of ice (200 g) and 2 M sulfuric acid (200 mL). A brown solid formed, and was collected by filtration. The brown solid is a mixture containing 4-[4-(2-fluorocinnamyl)aminophenyl]butyric acid **4** and oligomer **5**.

Scheme 3. A 1-L round bottom flask fitted with a magnetic stirrer was charged with 4-(4-aminophenyl)butyric acid (50.0 g, FW 179.2, 0.28 moles, 1.17 equiv) and 2 M aqueous sodium hydroxide (300 mL). Finely ground *O*-acetylsalicyloyl chloride (47.7 g, FW 198.6, 0.24 moles, 1.00 equiv) was added portionwise over 1 h to the above stirring solution. After the addition, the reaction was stirred for 2.5 h at ambient temperature, and the pH of the solution

was kept at *ca* 10 by addition of 10 M sodium hydroxide. The solution was then acidified with 1 M hydrochloric acid to pH 1.0, and a pale pink solid formed. The solid was filtered, washed with 1 M hydrochloric acid (3 x 100 mL), water (100 mL), and air dried. The brown solid is a mixture containing 4-(4-salicyloylaminophenyl)butyric acid **6** and the oligomeric material.

4-[4-(2-Fluorocinnamyl)aminophenyl]butyric acid 4 (Scheme 4). A 250-mL three neck round bottom flask equipped with a magnetic stirrer and a reflux condenser was charged with a suspension of 4-(4-aminophenyl)butyric acid (5.0 g, FW 179.2, 28 mmoles, 1.00 equiv) in dichloromethane (70 mL). Chlorotrimethylsilane (6.03 g, FW 108.6, 56 mmoles, 2.00 equiv) was added in one portion, and the mixture was refluxed under argon for 1 h. The reaction was allowed to cool to room temperature and was placed in an ice bath (internal temperature < 10 °C). The reflux condenser was replaced with an addition funnel containing triethylamine (4.3 g, FW 101.2, 42 moles, 1.50 equiv). The triethylamine was added dropwise over 15 min. The funnel was replaced by another addition funnel containing a solution of 2-fluorocinnamyl chloride (5.17 g, FW 184.59, 28 mmoles, 1.00 equiv) in dichloromethane (10 mL). The solution was added dropwise over 30 min. The reaction was stirred in the ice bath for 30 min and at ambient temperature for 1 h. The dichloromethane was evaporated to give a brown oil. An ice-cold solution of 2 M sodium hydroxide (100 mL) was added to the oil, and the mixture was stirred for 2 h to afford a clear, brown solution. The solution was acidified with 2 M sulfuric acid (80 mL)

to afford a light brown solid, which was collected by filtration. Reversed-phase HPLC indicated that the solid contains mainly compound **4** . The solid was recrystallized from 50 % acetone-water (v/v) to give 4-[4-(2-fluorocinnamyl)aminophenyl]butyric acid **4** (7.33g, 80 %) as a tan solid: mp 168-169 °C; ^{1}H-NMR (300 mHz, DMSO-d_6) δ 12.08 (1H, s), 10.27 (1H, s), 7.48 (4H, m), 7.44 (1H, m), 7.28 (2H, m), 7.14 (2H, d, J = 8.38 Hz), 6.95 (1H, d, J = 15.85 Hz), 2.54 (2H, t, J = 7.30 Hz), 2.20 (2H, t, J = 7.37 Hz), 1.77 (2H, m). Anal. Calcd for $C_{19}H_{18}FNO_3$: C, 69.71; H, 5.54; N, 4.28. Found: C, 69.82; H, 5.64; N, 4.20.

Oligomer 5. An analytical sample was prepared by the reaction in Scheme 2, followed by recrystallization from 70% ethanol/water (3x): mp 146-149 °C; ^{1}H-NMR (300 mHz, DMSO-d_6) δ 12.08 (1H, br s), 10.26 (1H, s), 9.82 (1H, s), 7.65 (4H, m), 7.46 (3H, m), 7.29 (2H, m), 7.12 (4H, m), 6.95 (1H, d, J = 15.86), 2.53 (4H, m), 2.29 (2H, t, J = 7.25 Hz), 2.18 (2H, t, J = 7.53 Hz), 1.87 (2H, m), 1.75 (2H, m). Anal. Calcd for $C_{29}H_{29}FN_2O_4$: C, 71.30; H, 5.98; N, 5.73. Found: C, 71.10; H, 5.97; N, 5.74.

4-(4-Salicyloylaminophenyl)butyric acid 6. 4-(4-Aminophenyl)butyric acid (50 g) was reacted with *O*-acetylsalicyloyl chloride (55.60 g) according to Scheme 4 to give compound **6** as tan needles (65.7 g, FW 299.3, 78 %): mp 174-176 °C; ^{1}H-NMR (300 mHz, DMSO-d_6) δ 12.00 (2H, 2 overlapped broad s), 10.35 (1H, s), 7.97 (1H, m), 7.61 (2H, d, J = 8.44 Hz), 7.42 (1H, m), 7.18 (2H, d, J = 8.44 Hz), 6.96 (2H, m), 2.56 (2H, t, J = 7.36 Hz), 2.21 (2H, t, J = 7.41 Hz),

1.79 (2H, m). Anal. Calcd for $C_{17}H_{17}NO_4$: C, 68.22; H, 5.72; N, 4.68. Found: C, 68.27; H, 5.70; N, 4.62.

Preparation of mixtures (Scheme 5). A 100-mL three neck round bottom flask equipped with a magnetic stirrer and a reflux condenser was charged with a suspension of 4-(4-aminophenyl)butyric acid (1.78 g, FW 179.2, 9.92 mmoles, 1.00 equiv) in dichloromethane (25 mL). Chlorotrimethylsilane (2.16 g, FW 108.6, 19.84 mmoles, 2.00 equiv) was added in one portion, and the mixture was heated to reflux for 1 h under argon. The reaction was allowed to cool to rt and was placed in an ice bath (internal temperature < 10 °C). The reflux condenser was replaced with an addition funnel containing triethylamine (1.51 g, FW 101.2, 14.88 mmoles, 1.50 equiv). The triethylamine was added dropwise over 15 min. The funnel was replaced by another addition funnel containing a solution of 2-fluorocinnamyl chloride (0.56 g, FW 184.59, 3.05 mmoles, 0.307 equiv), *O*-acetylsalicyloyl chloride (0.66g, FW 198.61, 3.34 mmol, 0.337 equiv) and benzoyl chloride (0.50g, FW 140.57, 3.53 mmol, 0.356 equiv) in dichloromethane (10 mL). The solution was added dropwise over 20 min. The reaction was stirred in the ice bath for 30 min and at ambient temperature for 12 h. The dichloromethane was evaporated to give a brown oil. An ice-cold solution of 2 M sodium hydroxide (25 mL) was added, and the mixture was stirred for 2 h. The solution was acidified with 2 M hydrochloric acid (30 mL) and extracted with ethyl acetate (2 x 40 mL). The combined organic layers were decolorized with activated charcoal (0.5 g), dried over sodium sulfate, filtered,

and evaporated to give a light brown solid (3.00 g). Three major components were observed by reversed-phase HPLC and their retention times are identical to those of the reference samples, which were prepared separately. ^{1}H-NMR shows all the chemical shifts for each compound. Each gram of this sample contains approximately 0.33g of individual component. An analytical sample of compound **9** was obtained from a literature procedure.[3c]

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