A Simple Method for the Synthesis of Long-Chain Alkyl Esters of Amino Acids

Christopher L. Penney, Pravinkant Shah, and Silvio Landi*

Connaught Research Institute, Willowdale, Ontario, Canada M2R 3T4

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A practical one step procedure is described for the synthesis of long-chain alkyl esters of amino acids. A number of octadecyl (stearyl) amino acids were made in moderate to high overall yields of 40–90% via methanesulfonic acid catalyzed esterification in an octadecanol melt. No special anhydrous conditions or N-protected amino acids were required and so in the case of lower product yields the synthesis was still desirable relative to alternative methodologies. Further, simpler routes gave lower yields. A correlation was observed between reactant hydrophilicity and product yields.

Long-chain 18-carbon esters of amino acids are of medicinal value. *N*-Stearoyl amino acids possess nutritional potential[1] and industrial utility,[2,3] while stearyl amino acids are effective immunoadjuvants for vaccine production[4] and allergy desensitization therapy.[5] Their usefulness is enhanced by the fact that they are simple molecules made of biocompatible components. In the case of the stearyl tyrosine hydrochloride adjuvant,[4,5] synthesis was achieved by hydrogen chloride gas catalyzed esterification of L-tyrosine and octadecanol. However, the product yields were only about 10%.

Esterification of amino acids is an important process for which a number of established methods have been described[6,7] and are still being developed to allow reaction under a variety of conditions.[8–10] However, many of these methods rely on the alcohol component to be present as the solvent and therefore are not readily applicable with long chain solid alcohols. Two potential high yield approaches would be reaction of an N-protected amino acid and alkanol in the presence of coupling agent (e.g., carbodiimide and 4-(dimethylamino)pyridine catalyst)[11] or alkyl halide.[12,13] Nonetheless, these methods do not represent the most practical industrial approach as they require expensive N-protected amino acids (more so in the case of cesium salts), subsequent deprotection, and anhydrous reaction conditions and reagents, they can lead to racemization via N-protected intermediates,[11] and product yields are not always high. Therefore, we sought to retain the advantages of a direct acid-catalyzed esterification but modified the procedure so as to improve reaction yield.

The original synthesis of stearyl tyrosine hydrochloride was undertaken[4,5] as shown in path a, Table II. This method does not employ an organic solvent but instead the solid alcohol is melted and the gas catalyst bubbled through the reaction. However, the alcohol is not present as in the normal solvent excess (e.g., for Fisher esterification) and tyrosine exhibits poor solubility properties. It was therefore reasoned that tyrosine ethyl ester would display better solubility and so under similar conditions, transesterification was undertaken. The yield, after conversion to free base and reconversion to the hydrochloride (Table II), was about 50%. While the conversion to the free base may appear unnecessary in path a, as both the crude and final products are hydrochloride salts, it was necessary in order to remove unreacted material.

Although the yield was significantly improved, it was thought that it could be further increased. Of the available esterification procedures for amino acids, it was noted that sulfonic acid catalysis was not among the most commonly employed. Sulfonic acids are more often used as esterification catalysts for organic acids, one recent relevant example being the preferred use of methanesulfonic acid for the synthesis of isostearyl benzoate.[14] Toluenesulfonic acid has found limited use in the synthesis of amino acid esters (e.g., with benzyl alcohol[15] or polyethylene glycol[16]). However, it was decided that methanesulfonic acid as a neat, anhydrous, portable liquid merited examination. Interestingly, as far back as 1964, it has been cited as a preferred acid catalyst for ester hydrolysis.[17] Both transesterification, with reactant ethyl esters, and esterification were studied. The product yields, along with other properties, are recorded in Table I. In all cases examined, the product yield via esterification was 10–20% greater than for the corresponding transesterification. However, for stearyl tyrosine adjuvant,[4,5] both methods gave a significantly improved yield over any reaction by using hydrogen chloride gas as a catalyst. This could at least be partly attributed to the fact that methanesulfonic acid improved the solubility of the reactants relative to hydrogen chloride. Further, by product formation was reduced so that unreacted octadecanol was recovered as an off-white crystalline solid instead of a dark, waxy semisolid as was usually observed with hydrogen chloride gas. Dioctadecyl ether formation was reduced and octadecyl chloride eliminated; both were identified (by mass spectrometry) when hydrogen chloride was employed as catalyst.

In Table I, it may be noted that the amino acids are divided into two groups: nonpolar and polar. While the conditions for synthesis of the crude methanesulfonate salt are identical for both groups, conversion to the free base

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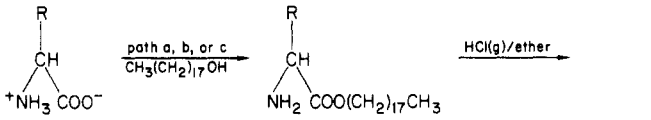
Table I. Percent Yield from Reactant Amino Acids and Some Properties of Product Stearyl Amino Acid Hydrochlorides

amino acid	mp, °C	$[\alpha]_D$, deg (DMF)	yield of stearyl ester[a] (hydrochloride): trans-esterification	yield of stearyl ester[a] (hydrochloride): esterification	solubility,[a] mg/mL	hydrophilicity value
		Nonpolar				
tyrosine	171–173	+12.5 (c 1)	70%	90%	<0.01	−2.3
phenylalanine	101–103	+25.0 (c 2)	70%	90%	<0.01	−2.5
leucine	81–83	+5.0 (c 2)	73%	86%	<0.01	−1.8
valine	71–73	+5.5 (c 2)	55%	71%	0.02	−1.5
β-alanine	105–106 (crystals)			82%	0.05	
glycine	108–109 (crystals)		50%	65%	<0.01	0
		Polar				
proline	90–91 (crystals)			55%	0.10	−0.5 ± 1.0
lysine	165 dec 100 sample translucent			40%	5.00	+3.0

[a] All elemental analysis for C, H, and N were within ±0.3% of the theoretical. All products were characterized by ^{1}H NMR and IR spectroscopy and gave spectra consistent with product structure. [b] Solubility in distilled water determined experimentally.

Table II. Synthesis of Long-Chain $C_{18}H_{37}$ Amino Acids[a]



amino acid	path	reactn conditn
		Nonpolar
tyrosine (R = $CH_2C_6H_4OH$)	a	(1) HCl(g), Δ (2) $NaOH/H_2O$
glycine (R = H)[b]	b	(1) CH_3SO_3H, Δ (2) $NaHCO_3/H_2O$ phase reactn, 18 h, room temp
		Polar
lysine (R = $(CH_2)_4NH_3^+$	c	(1) CH_3SO_3H, Δ
proline (R = $(CH_2)_3$ (α-imino acid)		(2) $NaOH/H_2O$–CH_3OH solutn reactn, $^1/_2$ h, room temp

[a] Path a as described in the literature for stearyl tyrosine hydrochloride;[4,5] path b as developed for amino acids with nonpolar side chains; path c as developed for amino acids with polar side chains. [b] Glycine (R = H) to tyrosine (R = $CH_2C_6H_4OH$) see Table I.

(paths b and c, Table II) is different for each group. When stearyl proline was synthesized by paths b (for nonpolar amino acids) and c (for polar amino acids), the product yields were 30% and 55%, respectively. The lower yield was attributed to mechanical loss since with the polar amino acids, the crude methanesulfonate salt and free base were hygroscopic. For both amino acid groups, product yield did not rigorously depend upon reaction temperature or time. For example, in the case of tyrosine, proline, and lysine, a temperature increase of 15 °C had no significant effect on product yield. The low yield of stearyl lysine could not be increased by the use of other acid catalysts. Use of sulfanilic acid gave no product, sulfuric acid caused dehydration of octadecanol, and toluenesulfonic acid yielded the final product contaminated with octadecanol.

Interestingly, a possible correlation was observed between apparent hydrophilicity of the reactant amino acid and product yield. In Table I, for comparison "hydrophilicity values"[18,19] are presented beside the product yield. Briefly, these values are defined so that the most hydrophilic region of a protein could be identified (as potential immunological or antigenic determinants). As they are meant for protein analysis, a value is not reported for β-alanine.

As regards to purity of the final product, it is worthwhile to note that compounds of analytical purity were obtained in all cases, except stearyl β-alanine, without any chromatography or crystallization. However, crystallization of stearyl-β-alanine hydrochloride from methanol–ether gave analytically pure product. Stearyl proline was isolated as a crystalline material, and stearyl glycine could be crystallized from methanol. In our hands, stearyl amino acids with hydrophobic side chains could not be crystallized. With the exception of stearyl glycine, the crude stearyl amino acid product was contaminated with a small amount of octadecanol and unreacted amino acid but this was virtually eliminated by conversion to the free base, and the product precipitated from an etheral solution as the hydrochloride salt. In the case of stearyl glycine, the octadecanol present in the crude product was significant (approximately 1 equiv of alcohol for every 2 of product).

In conclusion, methanesulfonic acid is a valuable catalyst for the synthesis of long-chain alkyl (stearyl) amino acid esters. When this reagent is used, products of analytical purity may be prepared in moderate to excellent yield in a simple manner. The reaction requires no special or anhydrous conditions, solvents, or expensive reagents. The method is therefore ideally suited for large scale operations.

Experimental Section

Melting points were determined on a Gallenkamp apparatus and are uncorrected. For TLC precoated silica gel sheets, with fluorescent indicator, and cellulose sheets from Eastman were used. Visualization was accomplished with iodine, UV light where applicable, and ninhydrin spray (Sigma). Optical rotations were determined with a 2-dm tube with an Adam Hilger Model 70 polarimeter. Values are for samples soluble enough to permit measurement in spectrograde dimethylformamide dried over Linde 3Å molecular sieves.[20] IR spectra were recorded with a Beckman TR 4 IR spectrophotometer. UV spectra were recorded with an Unicam SP-1800 spectrophotometer. Proton magnetic resonance spectra were recorded with a Varian T-60 and/or XL-200 spectrometer at McGill University, Montreal, Quebec. Chemical shifts (δ) are reported with reference to tetramethylsilane. Elemental analysis of stearyl amino acid hydrochloride salts was performed by Guelph Chemical Laboratories Ltd., Guelph, Ontario.

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Synthesis of Stearyl Tyrosine Hydrochloride by Esterification of Nonpolar Amino Acids. To L-tyrosine (8 g, 44 mmol) and octadecanol (30 g, 111 mmol) in a 122 °C oil bath was added over 5 min methanesulfonic acid [4.0 mL, 62 mmol (all other nonpolar amino acids required 3.0 mL (46 mmol))]. The reaction was stirred at 124–129 °C for 2 and $^1/_2$ h. The hot brown solution containing a few insoluble particles was poured cautiously into 800 mL of chilled ether (dry ice–acetone bath). The resultant suspension was stirred 2 h at room temperature and filtered and the insoluble solid was thoroughly washed with ether (3 × 300 mL) and dried in vacuo. The yield of crude methanesulfonate salt was 23.0 g, mp 125–127 °C. TLC using the same system as for the final product indicated a good purity ester with small amounts of tyrosine and octadecanol. Removal of solvent from the combined etheral filtrate and washings gave 22.0 g of lusturous solid, which TLC (chloroform) indicated to be mostly octadecanol combined with a nonpolar byproduct.

To 1.5 L of fresh 0.1 M sodium hydrogen carbonate was added over 10 min crushed (mesh 20) crude ester (21.0 g). The suspension was stirred 18 h at room temperature and filtered and the solid dried in vacuo. The yield of crude base was 17.0 g: mp 79–81 °C (lit. mp 80–83 °C); ^{1}H NMR ($CDCl_3$ and $CDCl_3$–D_2O) was as reported.[21]

To crude, crushed (mesh 20) free base (15.0 g) was added 1 L of ether and this was stirred for 20 min with gentle heating via a warm water bath. The translucent solution was filtered giving a greyish insoluble material (0.4 g tyrosine) and a clear filtrate with a small amount of free base precipitate. This precipitate was redissolved, and HCl gas was bubbled through the solution for 10 min. The thick, white slurry was again filtered and the solid dried in vacuo. The yield of white hydrochloride was 15.0 g (90% from tyrosine): mp 171–173 °C; R_f silica (3:1 petroleum ether–acetone) 0.50; R_f silica (ethyl acetate) 0.55; $[\alpha]_D$ +12.5 °(*c* 1, DMF); λ_{max}^{MeOH} 278 nm (ϵ 1.7 × 10^3); ν 1730 (C=O), 1235 cm^{-1} (C-O); ^{1}H NMR (Me_2SO-d_6–$CDCl_3$) 0.9 (t, 3 H, CH_3), 1.1–1.8 (m, 32 H, $(CH_2)_{16}$), 3.1 (m, 2 H, $PhCH_2$), 4.1 (m, 3 H, OCH_2 + CH), 6.7 and 7.0 (2 d, 4 H, Ph), 8.6 (m, 3 H, NH_3^+).

Anal. Calcd for $C_{27}H_{48}NO_3Cl$: C, 68.98; H, 10.29; N, 2.98. Found: C, 68.97; H, 10.16; N, 2.84; S, 0.57. [No catalyst carry over to product.]

By Transesterification. Reaction was as above except that ethyl tyrosine hydrochloride (40 mmol) replaced tyrosine to give a 70% product yield; chromatographic and spectroscopic properties were as above; mp 170–172 °C.

Anal. Found: C, 68.88; H, 10.46; N, 2.69; S, 0.19.

Synthesis of Stearyl Lysine Hydrochloride by Esterification of Polar Amino Acids. To L-lysine monohydrochloride (8 g, 44 mmol) and octadecanol (30 g, 111 mmol) in a 122 °C oil bath was added over 5 min methanesulfonic acid [5.8 mL, 90 mmol for this dicationic amino acid (all other polar amino acids required 3.0 mL (46 mmol))]. The reaction was stirred at 124–129 °C for 2 and $^1/_2$ h and was worked up as above for crude stearyl tyrosine methanesulfonate salt, to give a crude, potentially hygroscopic product. All of the material was stirred in a gently warmed mixture of water (300 mL) and methanol (50 mL). The misty solution was filtered and to the filtrate was added over 20 min sodium hydroxide [50 mL, 2 N (25 mL for all other polar amino acids)] after which a thick white solution, pH ~12, was observed. To this was added ether (200 mL), and the whole solution stirred 5 min and transferred to a separatory funnel. The aqueous layer was again extracted with ether (2 × 200 mL) after which it became clear. The etheral portions were combined, dried with anhydrous sodium sulfate, and filtered to give a clear filtrate. HCl gas was bubbled through the free base solution for 10 min, after which the hazy solution was stored at 4 °C for 2 h and filtered, and the solid dried in vacuo. The yield of white hydrochloride was 8.2 g (40% from lysine): mp >165 °C dec (with sample shrinking slightly and translucent at 100 °C); R_f cellulose (*n*-propanol) 0.88; ν 1730 (C=O), 1220 cm^{-1} (C-O); ^{1}H NMR (DMSO-d_6) 0.9 (t, 3 H, CH_3), 1.0–1.9 (m, 38 H, $(CH_2)_{16}$ + $(CH_2)_3$), 2.8 (m, 2 H, $N^{\epsilon}CH_2$), 4.0 (m, 1 H, CH), 4.2 (t, 2 H, OCH_2), 8.4 (m, 3 H, NH_3^+).

Anal. Calcd for $C_{24}H_{52}N_2O_2Cl_2$: C, 61.13; H, 11.12; N, 5.94. Found: C, 61.14; H, 11.23; N, 6.05; S, 0.08.

The yield and purity of stearyl lysine was not significantly changed when the reaction was repeated under milder conditons: 1 and $^1/_2$ h at 114–119 °C. However, with *p*-toluenesulfonic acid as catalyst (90 mmol, 15.5 g of anhydrous acid prepared by heating in vacuo, 17.1 g of monohydrate) the product was significantly contaminated: R_f silica (chloroform) 0.50, octadecanol, 0.00, stearyl lysine: mp >152 °C dec (with sample translucent at 91 °C).

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Registry No. Stearyl tyrosine hydrochloride, 77229-76-6; stearyl phenylalanine hydrochloride, 95362-52-0; stearyl leucine hydrochloride, 95362-53-1; stearyl valine hydrochloride, 95362-54-2; stearyl β-alanine hydrochloride, 95362-55-3; stearyl glycine hydrochloride, 59404-67-0; stearyl proline hydrochloride, 36261-57-1; stearyl lysine hydrochloride, 95362-56-4; L-tyrosine, 60-18-4; L-phenylalanine, 63-91-2; L-leucine, 61-90-5; L-valine, 72-18-4; β-alanine, 107-95-9; glycine, 56-40-6; L-lysine monohydrochloride, 657-27-2; ethyl tyrosine hydrochloride, 4089-07-0; ethyl phenylalanine, 3081-24-1; ethyl leucine, 2743-60-4; L-proline, 147-85-3; ethyl valine, 17431-03-7; ethyl glycine, 459-73-4; stearyl tyrosine methanesulfonate salt, 95362-57-5; stearyl tyrosine, 73393-27-8; octadecanol, 112-92-5; *p*-toluenesulfonic acid, 104-15-4; methanesulfonic acid, 75-75-2.

(21) The assignment of OCH_2 and $PhCH_2$ at 3.0 and 4.2[5] should be reversed as evidenced by the spectrum of octadecanol, OCH_2 at 3.6, all other stearyl amino acids, OCH_2 at 4.1–4.2, and the ethyl ester of *N*-acetyltyrosine with OCH_2 at 4.1.