Polysilylated Coenzyme A for a High-Yielding Preparation of Very Lipophilic Acyl Coenzymes A in Anhydrous Organic Solvents

Karine Lucet-Levannier,† Jean-Paul Lellouche,† and Charles Mioskowski*,†,‡

Service des Molécules Marquées, CEA, CE-Saclay
Département de Biologie Cellulaire et Moléculaire
F-91191 Gif-sur-Yvette, France
Faculté de Pharmacie, Université Louis Pasteur
Laboratoire de Synthèse Bio-Organique associé au CNRS
74 route de Rhin BP 24, F-67401 Illkirch, France

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The membrane-bound elongase complex recently purified and characterized from *Allium porrum* microsomes[1,2] catalyzes the two-carbon chain elongation described by the equation **1** → **2** in Scheme 1.

By analogy with the multistep enzymatic sequence admitted for mammalian elongases,[3−5] putative functionalized acyl coenzymes A (acyl-CoAs) **3**, **4**, and **5** can be written as plausible intermediates of each discrete step of this elongation.

In order to prove this postulated sequence, the syntheses of **3**, **4**, and **5** but also the rational design of specific lipophilic inhibitors interfering with each discrete step would be an interesting approach. Since this particular elongase complex involves lipophilic long chain acyl coenzymes A as the unique type of substrates and/or products, an efficient and general preparation of these very lipophilic acyl-CoAs is required.

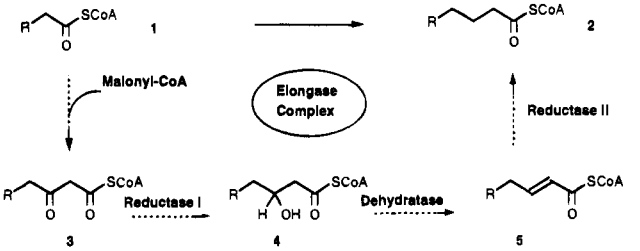
The purpose of this work is to describe a new synthetic preparation of acyl-CoAs particularly well-suited for those containing highly lipophilic long-chain acyl residues based on the key transformation CoA → polysilylated CoA.

In the current state of knowledge, a great variety of enzymatic[6−8] and chemical[9−24] syntheses of coenzyme A (CoA) thioesters of fatty acids are currently available. Nevertheless, they suffer obviously from inherent limitations such as (1) the availability and substrate specificities of the involved thiokinases and (2) the necessary use of a water−organic solvent binary mixture (usually tetrahydrofuran or acetone) during the coupling of CoA **6** and variously activated acids **7** as acylating reagents (see the left part of Scheme 2). This whenever possible cosolubilization of the highly hydrophilic **6** but also the often underestimated chemical reactivity of **7** toward water present in the condensation medium most likely account for the erratic and/or medium range yields in desired acyl-CoAs **8**.

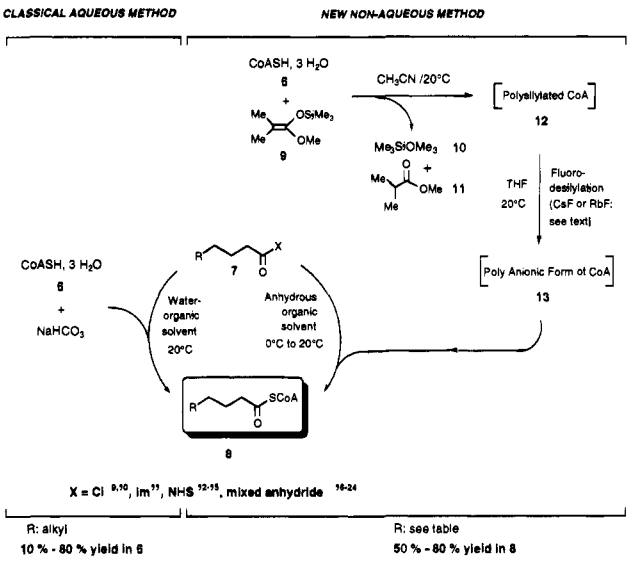
These former limiting factors could be greatly neglected provided that the condensation step of **7** with an appropriate chemically modified *lipophilic derivative* of **6** could be performed in an *anhydrous organic solvent*. Such a chemical temporary modification of **6** forms the basis of our new preparation of **8**. The two *one-pot* consecutive reactions **6** → **12** (silylation of **6**) and **12** → **8** via **13** (generation of the polyanion **13** and condensation step with **7**) can be performed under very mild conditions. The right part of Scheme 2 illustrates our subject and can be described as (1) silylation of **6** and (2) generation of the polyanion **13** and condensation step with **7**.

Routine coupled GC/MS analysis of highly hydrophilic polar compounds derivatized by trimethylsilylation is a common strategy that benefits from the general protective and interesting

Scheme 1. Multistep Enzymatic Sequence Catalyzed by the *Allium porrum* Microsomal Elongase Complex Starting with **1** (R = $C_{16}H_{33}$)



Scheme 2. Synthetic Scheme Describing the Nonaqueous Method of Preparation of **8** Compared with the Classical Aqueous One



† CEA, CE-Saclay.
‡ Université Louis Pasteur.

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Table 1. Standard Experimental Conditions for Preparation of Various Functionalized acyl-CoAs [a]

entry	substrate	acylating meth[b]	acyl-CoA[c]	yield[d] (%)
1	$CH_3(CH_2)_{16}CO_2X_S$[f]	A	**1**	60
2	$CH_3(CH_2)_{16}CO_2X_P$[f]	B	**1**	85
3	$CH_3(CH_2)_{18}CO_2X_S$	A	**2**	80
4	$CH_3(CH_2)_{18}CO_2X_S$	C	**2**	80
5	$CH_3(CH_2)_{18}CO_2X_S$	B	**2**	80
6	$CH_3(CH_2)_{18}CO_2X_P$	D	**2**	80
7	$CH_3(CH_2)_{18}CO_2X_P$	E	**2**	70
8	$CH_3(CH_2)_{16}(E)CH{=}CFCO_2X_S$	A	**14**	60
9	$CH_3(CH_2)_{16}(E)CH{=}CFCO_2X_P$	B	**14**	70
10	$CH_3(CH_2)_{16}CH(OH)CH_2CO_2X_S$	A	**15**	80
11	$CH_3(CH_2)_{16}(Z)CH{=}CHCO_2X_S$	A	**16**	50
12	$CH_3(CH_2)_{16}C(OCH_2CH_2CH_2O)CH_2CO_2X_S$	A	**17**	60
13	$CH_3(CH_2)_{16}C(OCH_2CH_2CH_2O)CH_2CO_2X_P$	B	**17**	70
14	$CH_3(CH_2)_{14}(Z)CH{=}CFCHOHCH_2CO_2X_S$	A	**18**	70
15	$CH_3(CH_2)_{15}CHFCH(OH)CH_2CO_2X_S$[e]	A	**19**	75
16	$CH_3(CH_2)_{15}CHFCH(OH)CH_2CO_2X_P$[e]	A	**19**	75

[a] The reaction was carried out in 2 mL of dry THF starting from 1.5 equiv of **7** per 1 equiv of **6**. The condensation of **13** and **7** is performed at 20 °C during 4 h. [b] Method A: CsF/DCH-18-C-6 (10% molar). Method B: RbF/DCH-18-C-6 (10% molar). Method C: CsF/TDA-1 (20% molar). Method D: RbF/TDA-1 (20% molar). Method E: CsF/ultrasound. [c] These acyl coenzymes A are HPLC homogeneous.[28] [d] Yield of isolated compound purified by preparative HPLC. [e] 1/1 erythro/threo mixture. [f] X_S = succinimido. X_P = phthalimido.

solubilization properties inferred to silylated derivatives.[25] In our case, we discovered that **6** (trihydrate form, 1.0 equiv, 90% purity, Sigma) can be mildly and efficiently silylated by dimethylketene methyl trimethylsilyl acetal **9**[26,27] (25.0 equiv) in anhydrous acetonitrile (1 mL, 20 °C, 14 h). After reaction, the *homogeneous medium* is concentrated under vacuum (0.1 mmHg, 1 h), affording the silylated coenzyme A **12** as a colorless oil. It is worthy of note that excess **9** and volatile byproducts **10** and **11** are eliminated as well. **12**, as a *lipophilic equivalent of* **6**, can be perfectly dissolved in most common organic solvents, even in very apolar ones like tetrahydrofuran, 1,2-dimethoxy ethane, chloroform, dichloromethane, and hexane. **9** was the only neutral silylating reagent tested which greatly reduced the oxidation of **6** to its disulfide (<5%) and which proved to be compatible with the highly functionalized **6** without damage. In spite of the lability of the trimethylsilyl (TMS) groups, high-field ^1H and ^{13}C NMR spectroscopic analyses as well as FAB-MS experiments (positive ions, *m*-$NO_2C_6H_4CH_2OH$ matrix) performed on **12** suggest a structure in which at least *seven functional groups* are silylated ($[M]^+$ = 1273) compared with the *eleven* potentially reactive groups contained in **6**.

Desilylation of **12** in anhydrous THF under solid−liquid phase transfer conditions using CsF or RbF (25.0 equiv)/dicyclohexyl-18-crown-6 (DCH-18-C-6, 10% molar) or TDA-1 (tris(3,6-dioxaheptyl)amine, 20% molar) as the source of fluoride anion slowly generates the polyanion **13** in the medium. **13** is then added to a THF solution of the activated acids **7**, the formation of acyl coenzymes A **8** being monitored by HPLC.[28] Remarkably, when nBu_4NF is used, yields in **8** are lower by 15−20%. Much experimental work emphasizing variations in reaction time, solvents, and temperature of the condensation step, **6**/**7** ratios, and modes of activation of **7** (nature of the X group) allowed us to propose a set of standard experimental conditions optimizing the yields in octadecanoyl/eicosanoyl coenzymes A **1** and **2** chosen as model compounds (see Table 1, entries 1−7).

Several points are particularly relevant and critical for success, such as (1) the use of solid−liquid phase transfer conditions during the condensation step and (2) the choice of the activating group X = succinimidooxy or phthalimidooxy in **7**. Also noteworthy is the beneficial use of ultrasound instead of crown ether without any noticeable decrease in the yield of **8**.[29] Moreover, when compared to the aqueous method, only a slight excess of activated acids relative to CoA (1.0−1.5 equiv) is used during the coupling, which is valuable for highly functionalized activated acids.

Interestingly, these conditions were also revealed to be efficient for the synthesis of the functionalized acyl-CoAs **14**−**19** in good yields (see lower part of table, entries 8−16). Obviously, although only applied to the preparation of acyl coenzymes A, we suggest that the silylated derivative **12** and the polyanion **13** will find further interesting applications.[30]

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Supporting Information Available: Typical experimental procedures for the synthesis of **7**, **12**, **13**, and acyl coenzymes A **1**, **2**, and **14**−**19**, FAB-MS spectral data for **2** and **14**−**19**, and the English version of the French patent (ref 30) (2 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(29) Quite interestingly, coupling methods A−D and E give comparable yields in **8** although involving respectively a "naked" polyanion **13** or **13** wrapped about Cs^+/Rb^+ ions. A mechanistic alternative would involve a preferential desilylation of the silylated thiol function of **12** followed by coupling with **7** and subsequent sequential deprotections of the other trimethylsilylated groups in **8**.
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