Journal of Visualized Experiments

Synthesis of esters via a greener Steglich esterification in acetonitrile

--Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE58803R1
Full Title:	Synthesis of esters via a greener Steglich esterification in acetonitrile
Keywords:	chemistry; Steglich esterification; carbodiimide coupling; green chemistry; cinnamyl ester derivatives; butyric ester derivatives; hexanoic ester derivatives; decanoic ester derivatives
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Additional Information:	
Question	**Response**
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TITLE:
Synthesis of Esters via a Green Steglich Esterification in Acetonitrile

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KEYWORDS:
Chemistry, Steglich esterification, carbodiimide coupling, green chemistry, cinnamyl ester derivatives, butyric ester derivatives, hexanoic ester derivatives, decanoic ester derivatives

SHORT ABSTRACT:
A modified Steglich esterification reaction was used to synthesize a small library of ester derivatives with primary and secondary alcohols. The methodology uses a non-halogenated and greener solvent, acetonitrile, and enables product isolation in high yields without the need for chromatographic purification.

LONG ABSTRACT:
The Steglich esterification is a widely-used reaction for the synthesis of esters from carboxylic acids and alcohols. While efficient and mild, the reaction is commonly performed using chlorinated or amide solvent systems, which are hazardous to human health and the environment. Our methodology utilizes acetonitrile as a greener and less hazardous solvent system. This protocol exhibits rates and yields that are comparable to traditional solvent systems and employs an extraction and wash sequence that eliminates the need for the purification of the ester product via column chromatography. This general method can be used to couple a variety of carboxylic acids with 1° and 2° aliphatic alcohols, benzylic and allylic alcohols, and phenols to obtain pure esters in high yields. The goal of the protocol detailed here is to provide a greener alternative to a common esterification reaction, which could serve useful for ester synthesis in both academic and industrial applications.

INTRODUCTION:
Ester compounds are widely used for the applications such as flavor compounds, pharmaceuticals, cosmetics, and materials. Commonly, the use of carbodiimide coupling reagents is used to facilitate an ester formation from a carboxylic acid and an alcohol[1]. For example, in the Steglich esterification, dicyclohexylcarbodiimide (DCC) is reacted with a

carboxylic acid in the presence of 4-dimethylaminopyridine (DMAP) to form an activated acid derivative, generally in a chlorinated solvent system or dimethylformamide (DMF)[2–4]. The activated acid derivative then undergoes a nucleophilic acyl substitution with an alcohol to form the ester product, which is usually purified via chromatography. The Steglich esterification enables mild coupling of large, complex carboxylic acids and alcohols, including sterically hindered secondary and tertiary alcohols[2,5,6]. The goal of this work is to modify the standard Steglich esterification protocol to provide a greener synthetic option for this common esterification reaction.

One important aspect in the design of new synthetic methodology is to seek to minimize the use and formation of hazardous substances. The Twelve Principles of Green Chemistry[7] can be used to provide a guideline for creating safer syntheses. Some of these include the prevention of waste generation (Principle 1) and the use of safer solvents (Principle 5). In particular, solvents account for 80-90% of the non-aqueous mass of the materials in pharmaceutical manufacturing[8]. Thus, modifying a protocol to use a less hazardous solvent can make a large impact on the greenness of an organic reaction.

Steglich esterification reactions often use anhydrous chlorinated solvent systems or DMF; however, these solvents are of concern for both the environment and human health. Dichloromethane (CH_2Cl_2) and chloroform ($CHCl_3$) are probable human carcinogens, and DMF has reproductive toxicity concerns[9]. In addition, CH_2Cl_2 is ozone depleting[10]. Thus, a less hazardous solvent for the Steglich esterification would be of great utility. While there are not yet green replacements for polar aprotic solvents, acetonitrile is recommended as a greener replacement for CH_2Cl_2, $CHCl_3$, and DMF[9]. Acetonitrile is currently produced as a byproduct in acrylonitrile manufacturing; however, a green synthesis of acetonitrile from biomass on an academic scale has been reported[11], and potential options for the reuse and recovery from waste streams are being investigated[12]. Acetonitrile has previously been used as a greener solvent alternative for carbodiimide coupling reactions in solid-phase peptide synthesis to form amide linkages[13]. The use of acetonitrile as a solvent system for Steglich esterifications has been demonstrated[14–21]; however, these methods have not focused on the green aspect of the solvent and also employ additional purification via column chromatography.

Reducing the need for column chromatography as a purification step also minimizes hazardous solvent waste[8]. In addition to using a less hazardous reaction solvent, the methodology enables the isolation of highly pure product without the need for chromatography. The traditionally used dicyclohexylcarbodiimide (DCC) coupling reagent is substituted with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC). The basic amine functional group on this reagent enables the reaction byproducts and any residual reagents to be removed via acidic and basic wash steps.

The protocol presented herein can be used with a variety of acid and alcohol partners (**Figure 1**). It was used to synthesize a small library of cinnamyl ester derivatives using primary, secondary, benzyl, and allyl alcohols and phenols[22]. Additionally, the rate of the esterification reaction in acetonitrile is comparable to that in the chlorinated and DMF solvent systems,

without a need to dry or distill the acetonitrile prior to the reaction[22]. Esters synthesized from tertiary alcohols have not been isolated, which is currently a limitation of the methodology compared to the traditional Steglich esterification in chlorinated solvent[23]. In addition, other acid-labile groups could be affected by the acid wash steps, potentially necessitating column chromatography for purification after acetonitrile removal. Despite these limitations, the reaction is a facile and general method for the synthesis of esters in high yields using a range of both alcohol and carboxylic acid components. The use of a greener solvent system and high purity without the need for chromatography steps make this protocol an attractive alternative to a traditional Steglich esterification.

PROTOCOL:

Caution: Consult Safety Data Sheets (SDSs) prior to the use of the chemicals in this procedure. Use appropriate personal protective equipment (PPE) including splash goggles, lab coat, and nitrile or butyl gloves as many of the reagents and solvents are corrosive or flammable. Carry out all reactions in a fume hood. It is unnecessary to dry glassware or to use a nitrogen atmosphere for this protocol.

1. Carbodiimide Coupling Reaction for Primary Alcohols

1.1. In a 50 mL round bottom flask, combine *(E)*-cinnamic acid (151 mg, 1.02 mmol, 1.2 equiv), DMAP (312 mg, 2.91 mmol, 3 equiv), and EDC (244 mg, 1.28 mmol, 1.5 equiv). Add acetonitrile (15 mL) and 3-methoxybenzyl alcohol (98 μL, 0.85 mmol, 1 equiv) to the mixture along with a stir bar.

CAUTION: Acetonitrile is a flammable solvent.

1.2. Clamp the flask in a 40 °C water bath and stir the reaction.

Note: If the reaction involves an aromatic alcohol, monitor the reaction for the loss of the alcohol via thin-layer chromatography (TLC) using 1:3 ethyl acetate/hexane. The reaction is complete when the alcohol spot is no longer visible on the TLC plate by irradiation with a UV lamp.

2. Extraction Workup

2.1. Once the reaction is complete as indicated by TLC or after 45 min, remove the acetonitrile under reduced pressure using a rotary evaporator to obtain a crude solid.

Note: Please see additional resources for the information regarding the use of a rotary evaporator[24,25].

2.2. To the residue, add diethyl ether (20 mL) and 1 M HCl (20 mL). Swirl the flask to dissolve the residue into the solvent layers.

CAUTION: Diethyl ether is a highly flammable solvent.

Note: To decrease the solvent hazard, ethyl acetate can be used in place of the diethyl ether; however, there is a greater potential for emulsion formation during the extraction and wash steps.

2.3. Pour the solution into a separatory funnel. Rinse the evaporating flask with additional diethyl ether (5 mL) and add the rinse to the separatory funnel.

2.4. Gently shake the separatory funnel to extract the product into the ether layer, venting periodically. Allow the layers to separate, and then remove the aqueous layer by draining it out from the bottom of the funnel into an Erlenmeyer flask or beaker.

Note: Please see additional resources for the information regarding extractions and the use of a separatory funnel[24,25].

3. Washing Procedure

3.1. To the organic layer remaining in the separatory funnel, add 1 M HCl (20 mL) and gently shake the separatory flask, venting periodically. Allow the layers to separate, and then remove the aqueous layer by draining it out from the bottom of the funnel into an Erlenmeyer flask or beaker.

3.2. Repeat the washing procedure with saturated sodium bicarbonate solution (2×20 mL) and then with saturated sodium chloride solution (20 mL).

3.3. Pour the organic layer out from the top of the separatory funnel into a clean Erlenmeyer flask, dry the layer with magnesium sulfate, and gravity filter the solution through filter paper into a massed evaporation flask.

Note: Please see additional resources for information regarding extractions and the use of magnesium sulfate as a drying agent[24,25].

3.4. Remove the diethyl ether solvent under reduced pressure using a rotary evaporator.

3.5. Analyze a sample of the product by ^{1}H and ^{13}C NMR spectroscopy in $CDCl_3$ and by mass spectrometry.

Note: Please see additional resources for the information regarding the preparation of samples for NMR analysis[24,25].

4. Carbodiimide Coupling Reaction for Secondary and Electron-deficient Alcohols

4.1. In a 50 mL round bottom flask, combine *(E)*-cinnamic acid (151 mg, 1.02 mmol, 1.2 equiv), DMAP (312 mg, 2.91 mmol, 3 equiv), and EDC (244 mg, 1.28 mmol, 1.5 equiv). Add acetonitrile (15 mL) and diphenylmethanol (157 mg, 0.85 mmol, 1 equiv) to the mixture along with a stir bar.

CAUTION: Acetonitrile is a flammable solvent.

4.2. Clamp the flask and stir the reaction at room temperature for 24 h. Insert an air condenser into the flask neck to minimize solvent evaporation.

4.3. Follow the extraction workup and washing procedure described in Steps 2-3 above.

5. Carbodiimide Coupling Reaction for Long-Chain or Hydrophobic Carboxylic Acids

5.1. In a 50 mL round bottom flask, combine decanoic acid (146 mg, 0.85 mmol, 1 equiv), DMAP (312 mg, 2.91 mmol, 3 equiv), and EDC (244 mg, 1.28 mmol, 1.5 equiv). Add acetonitrile (15 mL) and diphenylmethanol (157 mg, 0.85 mmol, 1 equiv) to the mixture along with a stir bar.

CAUTION: Acetonitrile is a flammable solvent.

5.2. Clamp the flask and stir the reaction at room temperature for 24 h. Insert an air condenser into the flask neck to minimize solvent evaporation. If a primary alcohol is used, stir the reaction in a water bath at 40 °C for 1 h.

5.3. Follow the extraction workup and washing procedure described in Steps 2-3 above.

REPRESENTATIVE RESULTS:

Using the modified Steglich esterification in acetonitrile followed by an acid-base extraction workup, 3-methoxybenzyl cinnamate (**8**) was obtained as a light-yellow oil (205 mg, 90% yield) without the need for column chromatography. ^{1}H and ^{13}C NMR spectra are presented in **Figure 2** to confirm the structure and to indicate purity.

Compounds **9**-**17** were synthesized using a similar protocol (**Figure 3**) with yields of 77-90%. All compounds were analyzed by ^{1}H and ^{13}C NMR spectroscopy and high-resolution mass spectrometry (HRMS) and found to be of similar purity to 3-methoxybenzyl cinnamate by NMR analysis. Tabulated data for compounds **8**-**17** is reported in **Table 1**.

Slight changes to the general protocol for primary alcohols were made to obtain optimal yields and purity for compounds **12**-**17**. Secondary alcohol reactions were run for 24 h at room temperature to allow the reaction to go to completion[22]. For the decanoic acid reactions, using 1.2 equivalents of carboxylic acid to 1 equivalent of alcohol for both primary and secondary alcohols yielded esters with a decanoic acid impurity (**Figure 4**). The long-chain acid is not soluble in the basic aqueous wash layers and remains in the organic layer. Other hydrophobic

acids could behave similarly. This issue was solved by used a 1:1 molar ratio of decanoic acid to alcohol, which yielded pure ester products. A slightly longer reaction time (60 min) was required for the primary alcohol reaction to go to completion for the 1:1 molar ratio reaction.

FIGURE AND TABLE LEGENDS:

Figure 1. General reaction scheme. The general scheme for the reaction involves the coupling of a carboxylic acid and an alcohol, which is facilitated using a carbodiimide coupling reagent (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, or EDC) and 4-dimethylaminopyridine (DMAP) in acetonitrile. To demonstrate the reaction breadth, esters were formed using various acids (**1**-**5**) with either a primary (**6**) or secondary (**7**) alcohol.

Figure 2. ^{1}H and ^{13}C NMR spectra for 3-methoxybenzyl cinnamate (8). The ^{1}H NMR spectrum (**A**) and ^{13}C NMR spectrum (**B**) of 3-methoxybenzyl cinnamate are shown with the product structure. Corresponding assignments are denoted on each spectrum and were confirmed using ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, and ^{1}H-^{13}C HMBC 2D NMR experiments. Spectra were obtained after solvent removal; no additional purification steps were used. The purity of this compound is representative of all reactions tested.

Figure 3. Ester structures synthesized using the methodology. Five acids (**1-5, Figure 1**) were combined with either a primary or secondary alcohol (**6** and **7**, respectively, **Figure 1**). The ester structures (**8-17**) are shown along with the percent yield for the reaction. Reactions were monitored for loss of the alcohol by TLC (1:3 ethyl acetate/hexane). Primary alcohol reactions were run at 40 °C in a water bath for 45 min for esters **8**-**11** and 60 min for ester **12**. Secondary alcohol reactions were run for 24 h at room temperature. For decanoic acid reactions (**12** and **17**), 1 molar equivalent of carboxylic acid to alcohol was used instead of 1.2 equivalents.

Figure 4. ^{1}H NMR spectra for diphenylmethyl decanoate (17) using 1:1.2 and 1:1 molar equivalents of alcohol to carboxylic acid. (**A**) Decanoic acid (1.2 equiv, top, or 1 equiv, bottom) was reacted with diphenylmethanol (1 equiv), EDC (1.5 equiv), and DMAP (3 equiv) in acetonitrile. The reactions were stirred at room temperature for 24 h and then the ester was isolated via the extraction and wash protocol. Residual decanoic acid remains in the product when the carboxylic acid is used in excess, as it is not soluble in the basic aqueous layer. The signal at 2.35 ppm shown in the inset indicates residual carboxylic acid in the product sample. (**B**) The use of a 1:1 ratio of carboxylic acid to alcohol enables a clean isolation of the ester, indicated by the loss of the signal at 2.35 ppm.

Table 1. Tabulated data for compounds 8-17. Chemical shifts (δ) are reported in ppm, and coupling constants (*J*) are reported in hertz (Hz). Signals are reported as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), or combinations of the above. HRMS data are reported as *m*/*z*.

DISCUSSION:

The methodology presented here was developed to minimize the hazards from solvent associated with a traditional Steglich esterification by using a greener solvent system and by

reducing the need for column chromatography[8,9]. Comparable reaction yields and rates can be achieved with the use of acetonitrile in place of dry chlorinated solvents or DMF[22].

Several key steps enable the efficient purification of product without the need for chromatography. After the reaction, the acetonitrile is first removed by rotary evaporation. The removal of solvent is essential, as acetonitrile is miscible with water and will affect the partitioning of reaction components during the extraction and wash steps. Basic impurities, including DMAP, EDC, and urea byproducts, are then removed with the acid wash steps. Any residual carboxylic acid is removed during the basic wash steps. Thus, all reagents and impurities can be removed, leaving ester in the organic layer. Subsequent drying and solvent removal led to high yields of pure ester products.

Adjustments of the protocol were needed to obtain high yields of ester product for the use of secondary alcohols or very hydrophobic carboxylic acids. The rate of the reaction for secondary or electron deficient alcohols is slower than that of primary alcohols, so it is necessary to either increase the reaction temperature (60 °C) or to run the reaction for longer time periods at room temperature. In addition, we found that excess carboxylic acid cannot be used if the acid is insoluble in the saturated sodium bicarbonate wash solution. For long chain carboxylic acids, such as decanoic acid, the reaction mixture should have a 1:1 ratio of alcohol to carboxylic acid reagents to avoid a carboxylic acid impurity in the final product.

Various carboxylic acids and alcohol partners can be used in the formation of esters, shown here and in previous work[22]. However, esters of tertiary alcohols have not been isolated with the current methodology. As the ability to couple carboxylic acids to sterically hindered tertiary alcohols is a common application of the Steglich esterification[23], the inability to obtain esters with tertiary alcohols is a limitation of the current methodology. We are pursuing NMR kinetics studies to investigate the mechanism and constraints of this reaction both in acetonitrile-d_3 and chloroform-*d*. In the future, we hope to adapt the method to enable the synthesis of esters with tertiary alcohols.

In summary, this work describes a greener Steglich esterification protocol that can be utilized for the synthesis of esters of various carboxylic acids with primary, secondary, benzylic and allylic alcohols and phenols. The methodology provides a less hazardous alternative to a common esterification reaction.

ACKNOWLEDGMENTS:
This research was supported by Siena College and the Center for Undergraduate Research and Creative Activity. We thank Dr. Thomas Hughes and Dr. Kristopher Kolonko for helpful conversations, Ms. Allycia Barbera for early stage work on this methodology, and the Siena College Stewart's Advanced Instrumentation and Technology (SAInT) Center for instrumentation resources.

DISCLOSURES:
The authors have nothing to disclose.

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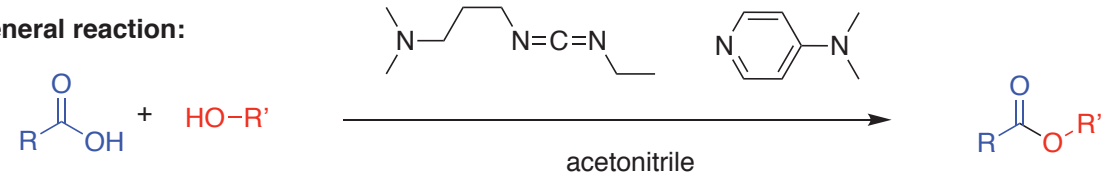
Figure 1

General reaction:



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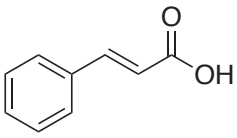

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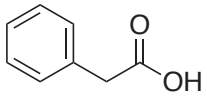

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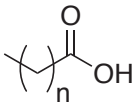
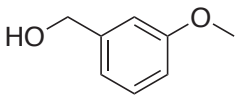


n = 2, 4, 8

3-5

Representative alcohols:



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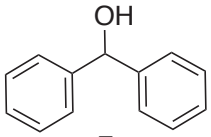

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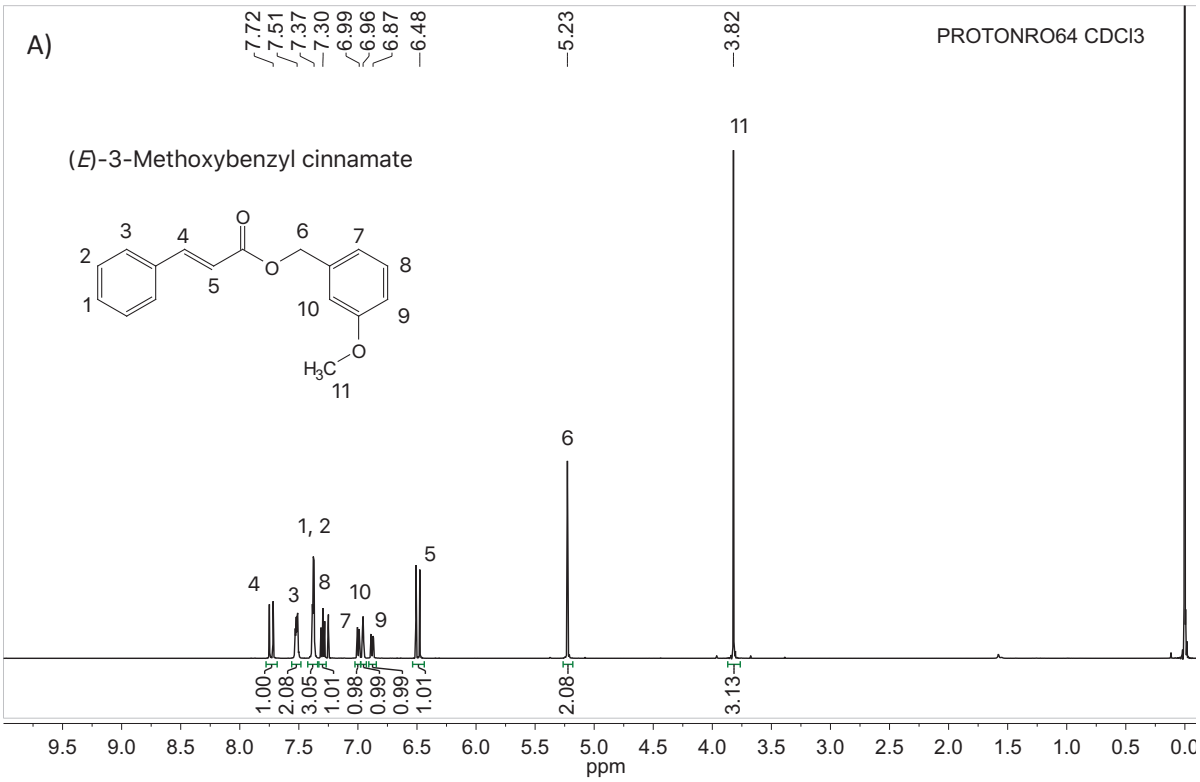
Figure 2



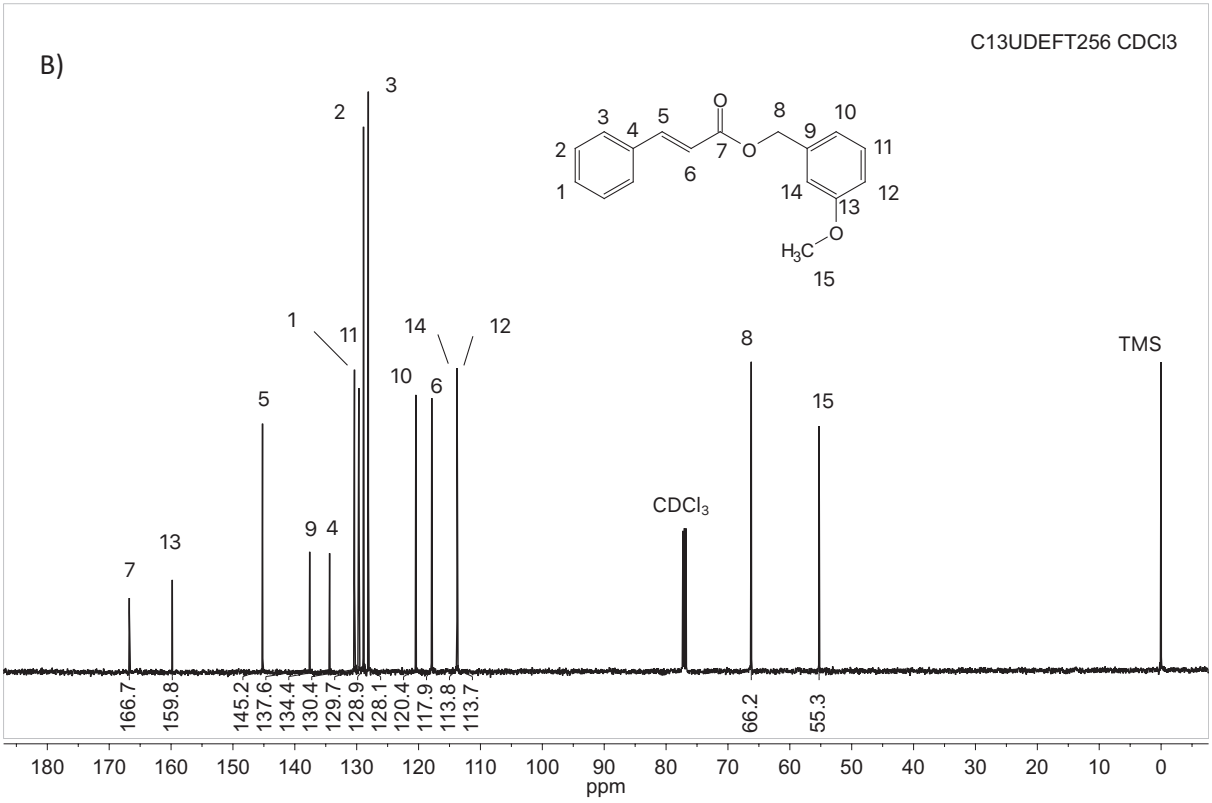


Figure 3

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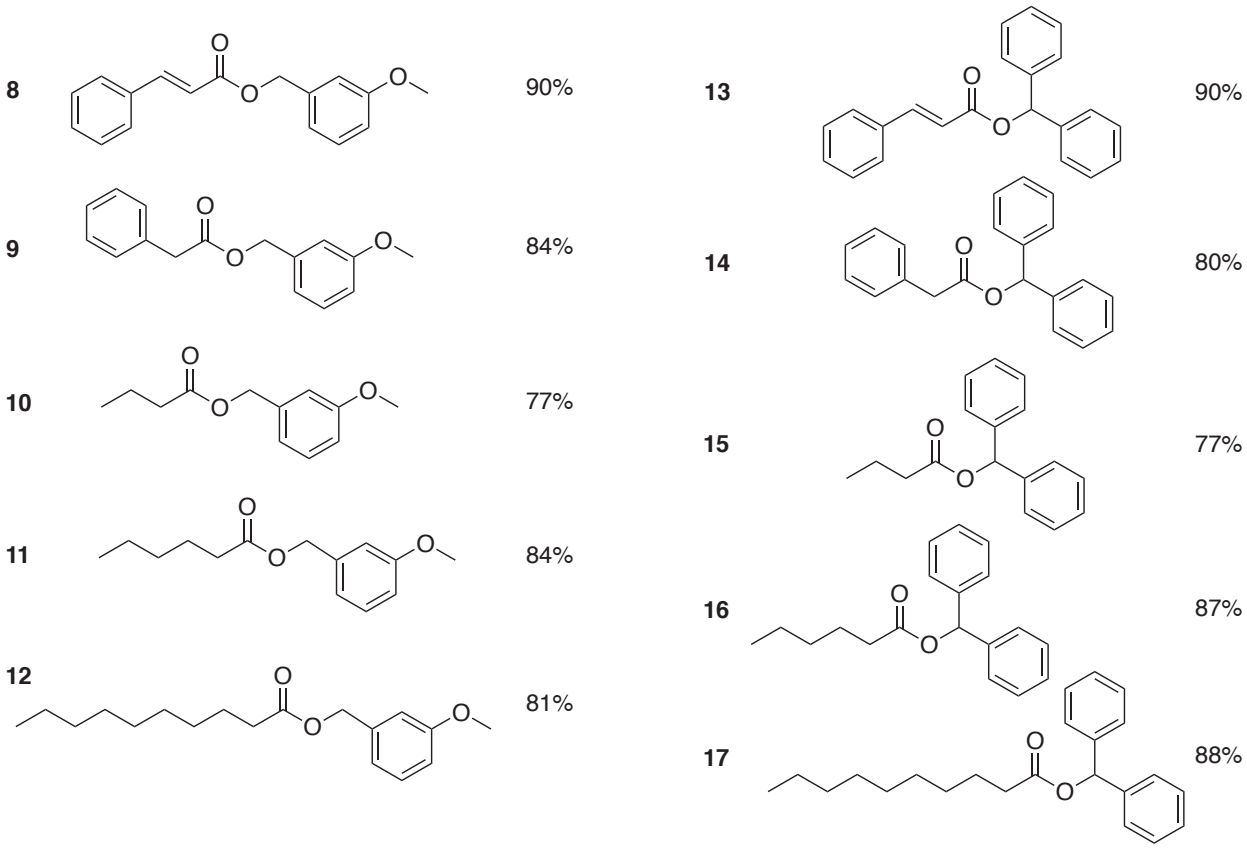

Figure 4

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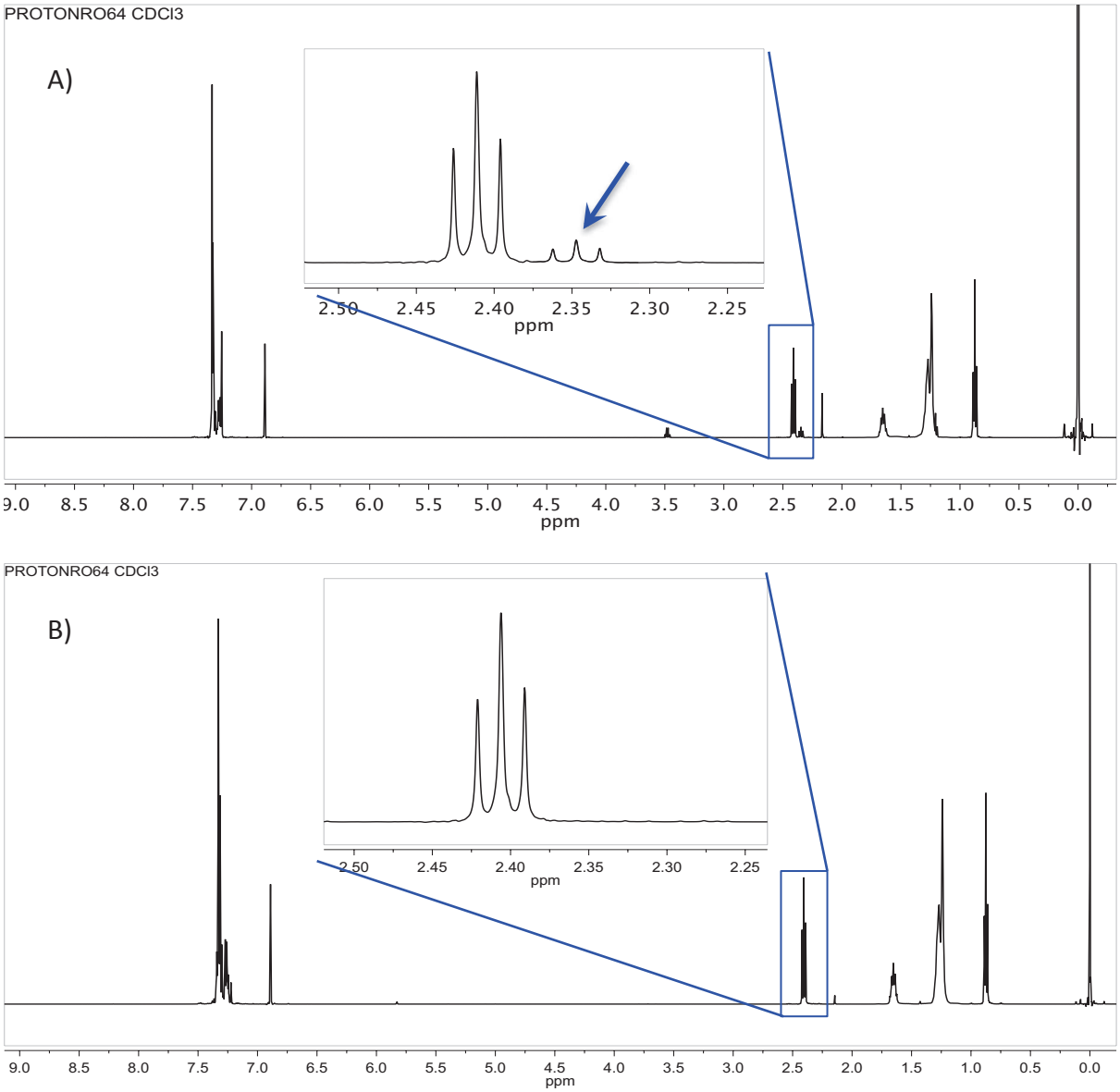

Table 1 Click here to access/download;Table;Table 1.xlsx

Compound	R_f (1:3 EtOAc/hex); appearance	^{1}H NMR (500 MHz, $CDCl_3$)	^{13}C NMR (126 MHz, $CDCl_3$)	HRMS
3-methoxybenzyl cinnamate (**8**)	3-methoxybenzyl alcohol R_f = 0.27; Product R_f = 0.61; light yellow oil	δ 7.77 (d, *J* = 16.0 Hz, 1H), 7.60 – 7.50 (m, 2H), 7.49 – 7.36 (m, 3H), 7.33 (t, *J* = 7.8 Hz, 2H), 7.03 (ddd, *J* = 7.4, 1.5, 0.8 Hz, 1H), 7.02 – 6.97 (m, 1H), 6.91 (ddd, *J* = 8.3, 2.6, 1.0 Hz, 1H), 6.53 (d, *J* = 16.0 Hz, 1H), 5.26 (s, 2H), 3.86 (s, 3H)	δ 166.8, 159.8, 145.2, 137.6, 134.4, 130.4, 129.7, 128.9, 128.1, 120.4, 117.9, 113.8, 113.7, 66.2, 55.3	ESI calcd for $C_{17}H_{16}O_3$ $(M+Na)^+$ 291.0992, found 291.0993
3-methoxybenzyl phenylacetate (**9**)	R_f = 0.57; light yellow oil	δ 7.35 – 7.27 (m, 5H), 7.25 (t, *J* = 8.6 Hz, 1H), 6.89 (ddd, *J* = 7.4, 1.5, 0.8 Hz, 1H), 6.85 (ddd, *J* = 8.3, 2.6, 1.0 Hz, 1H), 6.83 – 6.81 (m, 1H), 5.11 (s, 2H), 3.77 (s, 3H), 3.68 (s, 2H)	δ 171.3, 159.8, 137.4, 133.9, 129.6, 129.3, 128.6, 127.1, 120.2, 113.9, 113.3, 66.4, 55.2, 41.4	ESI calcd for $C_{16}H_{16}O_3$ $(M+Na)^+$ 279.0992, found 279.0990
3-methoxybenzyl butyrate (**10**)	R_f = 0.68; colorless oil	δ 7.27 (t, *J* = 7.7 Hz, 1H), 6.93 (ddd, *J* = 7.5, 1.6, 0.8 Hz, 1H), 6.90 – 6.88 (m, 1H), 6.86 (ddd, *J* = 8.2, 2.6, 1.0 Hz, 1H), 5.09 (s, 2H), 3.81 (s, 3H), 2.35 (t, *J* = 7.4 Hz, 2H), 1.68 (h, *J* = 7.4 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H).	δ 173.5, 159.8, 137.7, 129.6, 120.3, 113.7, 113.6, 65.9, 55.2, 36.2, 18.5, 13.7	ESI calcd for $C_{12}H_{16}O_3$ $(M+Na)^+$ 231.0992, found 231.0991
3-methoxybenzyl hexanoate (**11**)	R_f = 0.74; colorless oil	δ 7.27 (d, *J* = 7.7 Hz, 1H), 6.93 (ddd, *J* = 7.5, 1.6, 0.6 Hz, 1H), 6.90 – 6.88 (m, 1H), 6.85 (ddd, *J* = 8.2, 2.6, 0.9 Hz, 1H), 5.09 (s, 2H), 3.81 (s, 3H), 2.35 (t, *J* = 7.5 Hz, 2H), 1.74 – 1.56 (m, 2H), 1.39 – 1.25 (m, 4H), 0.89 (t, *J* = 7.1 Hz, 3H)	δ 173.6, 159.8, 137.7, 129.6, 120.3, 113.7, 113.6, 65.9, 55.2, 34.3, 31.3, 24.7, 22.3, 13.9	ESI calcd for $C_{14}H_{20}O_3$ $(M+Na)^+$ 259.1305, found 259.1304
3-methoxybenzyl decanoate (**12**)	R_f = 0.71; colorless oil	δ 7.27 (t, *J* = 7.9 Hz, 1H), 6.93 (ddd, *J* = 7.5, 1.6, 0.6 Hz, 1H), 6.90 – 6.87 (m, 1H), 6.88 (ddd, *J* = 8.3, 2.5, 0.6 Hz, 1H), 5.09 (s, 2H), 3.80 (s, 3H), 2.35 (t, *J* = 7.6 Hz, 2H), 1.76 – 1.52 (m, 2H), 1.42 – 1.12 (m, 12H), 0.88 (t, *J* = 7.0 Hz, 3H)	δ 173.7, 159.8, 137.7, 129.6, 120.3, 113.7, 113.6, 65.9, 55.2, 34.3, 31.9, 29.4, 29.3, 29.2, 25.0, 22.7, 14.1	ESI calcd for $C_{18}H_{28}O_3$ $(M+Na)^+$ 315.1931, found 315.1931
diphenylmethyl cinnamate (**13**)	diphenylmethanol R_f = 0.47; Product R_f = 0.66; white solid	δ 7.79 (d, *J* = 16.0 Hz, 1H), 7.60 – 7.54 (m, 2H), 7.46 – 7.36 (m, 11H), 7.35 – 7.30 (m, 2H), 7.05 (s, 1H), 6.60 (d, *J* = 16.0 Hz, 1H)	δ 166.0, 145.4, 140.3, 134.4, 130.4, 128.9, 128.5, 128.2, 127.9, 127.2, 118.0, 77.0	ESI calcd for $C_{22}H_{18}O_2$ $(M+Na)^+$ 337.1199, found 337.1191
diphenylmethyl phenylacetate (**14**)	R_f = 0.66; light yellow oil	δ 7.35 – 7.19 (m, 15H), 6.87 (s, 1H), 3.72 (s, 2H)	δ 170.4, 140.1, 133.8, 129.4, 128.6, 128.5, 127.9, 127.1, 127.0, 77.2, 41.7	ESI calcd for $C_{21}H_{18}O_2$ $(M+Na)^+$ 325.1199, found 325.1201

diphenylmethyl butyrate (**15**)	R_f = 0.72; light yellow oil	δ 7.37 – 7.30 (m, 10H), 7.29 – 7.25 (m, 2H), 6.89 (s, 1H), 2.40 (t, *J* = 7.5 Hz, 2H), 1.69 (h, *J* = 7.4 Hz, 2H), 0.93 (t, *J* = 7.4 Hz, 3H)	δ 172.6, 140.4, 128.5, 127.8, 127.1, 76.6, 36.5, 18.5, 13.7	ESI calcd for $C_{17}H_{18}O_2$ $(M+Na)^+$ 277.1199, found 279.1197
diphenylmethyl hexanoate (**16**)	R_f = 0.76; light yellow oil	δ 7.36 – 7.29 (m, 8H), 7.29 – 7.24 (m, 2H), 6.89 (s, 1H), 2.41 (t, *J* = 7.5 Hz, 2H), 1.72 – 1.60 (m, 2H), 1.36 – 1.21 (m, 4H), 0.87 (t, *J* = 7.0 Hz, 3H)	δ 172.8, 140.4, 128.5, 127.8, 127.1, 76.6, 34.6, 31.3, 24.6, 22.3, 13.9	ESI calcd for $C_{19}H_{22}O_2$ $(M+Na)^+$ 305.1512, found 305.1509
diphenylmethyl decanoate (**17**)	R_f = 0.76; light yellow oil	δ 7.35 – 7.29 (m, 8H), 7.29 – 7.23 (m, 2H), 6.89 (s, 1H), 2.41 (t, *J* = 7.5 Hz, 2H), 1.71 – 1.59 (m, 2H), 1.33 – 1.18 (m, 12H), 0.87 (t, *J* = 7.0 Hz, 3H)	δ 172.8, 140.4, 128.5, 127.9, 127.1, 76.6, 34.6, 31.9, 29.4, 29.3, 29.1, 25.0, 22.7, 14.1	ESI calcd for $C_{23}H_{30}O_2$ $(M+Na)^+$ 361.2138, found 361.2150

Name of Material/ Equipment	Company	Catalog Number
Note: All commercially available reagents and solvents were used as received without further purification.		
trans -cinnamic acid	Acros Organics	158571000
butyric acid	Sigma-Aldrich	B103500
hexanoic acid	Sigma-Aldrich	153745-100G
decanoic acid	Sigma-Aldrich	21409-5G
phenylacetic acid	Sigma-Aldrich	P16621-5G
3-methoxybenzyl alcohol	Sigma-Aldrich	M11006-25G
diphenylmethanol	Acros Organics	105391000
chloroform-*d*	Acros Organics	166260250
hexane	BDH Chemicals	BDH1129-4LP
ethyl acetate	Sigma-Aldrich	650528
diethyl ether	Fisher Scientific	E138-500
acetonitrile	Fisher Scientific	A21-1
4-dimethylaminopyridine	Acros Organics	148270250
magnesium sulfate	Fisher Scientific	M65-3
hydrochloric acid, 1 M	Fisher Scientific	S848-4
sodium chloride	BDH Chemicals	BDH8014
sodium bicarbonate	Fisher Scientific	S25533B
1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride	Chem-Impex	00050
thin layer chromatography plates	EMD Millipore	1055540001

Comments/Description

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Caution: corrosive
Caution: corrosive

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99.8% with 1% v/v tetramethylsilane, Caution: toxic
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Caution: flammable
Caution: flammable
ACS Certified, >99.5%, Caution: flammable
Caution: toxic

Caution: corrosive

Caution: skin and eye irritant
aluminum backed sheets

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Author(s):	Andrew B. Lutjen, Mackenzie A. Quirk, and Erin M. Kolonko*

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CORRESPONDING AUTHOR

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Signature:	E. M. Kolonko
Date:	07/19/2018

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August 13, 2018

Dr. Bing Wu, Review Editor
Journal of Visualized Experiments
1 Alewife Center Suite 200
Cambridge MA 02140

Dear Dr. Wu,

I am submitting a revised version of the manuscript JoVE58803 entitled "Synthesis of esters via a greener Steglich esterification in acetonitrile" for consideration for publication in the *Journal of Visualized Experiments*.

We appreciate the comments and suggestions from the peer and editorial reviews and have amended the manuscript to address the issues raised. A summary of the comments and the specific changes that we have made to the manuscript is provided on the accompanying pages.

We thank you for the opportunity to contribute a revision to the *Journal of Visualized Experiments*.

Sincerely,

Erin M. Kolonko
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Department of Chemistry and Biochemistry
Siena College
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(518)-782-6956

Response to reviewer major and minor concerns for JoVE58803 "Synthesis of esters via a greener Steglich esterification in acetonitrile"

Editorial comments:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Response: We have thoroughly proofread the manuscript for spelling and grammar issues, to the best of our knowledge.

2. Please rephrase the Long Abstract to more clearly state the goal of the protocol.

Response: We have added a specific statement of the goal to the Long Abstract. (Lines 37-38)

3. Please rephrase the Introduction to include a clear statement of the overall goal of this method.

Response: We have added a specific statement of the goal to the introduction. (Lines 51-53)

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Response: We have removed commercial language regarding instrumentation and replaced the information with generic terms.

5. Please revise the protocol (lines 96-105, etc.) to contain only action items that direct the reader to do something (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible.

Response: We have revised the "General remarks" section to use only imperative tense. (Lines 105-109)

6. 2.1.1: Please mention how complete reaction is confirmed by TLC.

Response: We have added a line in the protocol note to more clearly explain when a complete reaction by TLC is determined. (Lines 122-124)

7. 3.1.5: Please add more details here about how to prepare an NMR sample and how to perform the NMR experiments. Alternatively, add references to published material specifying how to perform the protocol action.

Response: We have added a reference for preparing an NMR sample to the protocol. (Line 176)

8. Because Table 1 shows data from mass spectrometry, please mention in the protocol that mass spectrometry is performed on the products.

Response: We have added the recommended statement. (Line 173)

9. Lines 190-198: Please consider describing such modifications in the protocol.

Response: We have added Sections 4-5 to the protocol to describe the specific modifications to the protocol. (Lines 179-207)

10. References: Please do not abbreviate journal titles. Please include page numbers for all journal references.

Response: We have corrected the reference section as requested.

Reviewer #2:

Major Concerns:

If the major thrust of the paper is extending the reaction utility to ACN solvent, then I would like to see a better review of what has been done. I find the references somewhat lacking. The use of carbodiimide procedure published by Steglich in 1978 has been cited over 900 times since then. A more comprehensive review of the utility of the reaction and the potential benefit of acetonitrile as a solvent would strengthen the manuscript. The following papers could be referenced:

Nat. Prod. Rep., 2015,32, 605-632 "Ester coupling reactions - an enduring challenge in the chemical synthesis of bioactive natural products"

Org. Biomol. Chem., 2015,13, 2393- 2398 "Peptide synthesis beyond DMF: THF and ACN as excellent and friendlier alternatives"

Response: We agree that these additions will strengthen the manuscript. In the introduction, we have added additional statements regarding the Steglich esterification in general and the use of acetonitrile for this reaction, along with corresponding references. (Lines 45-52 and 72-76, References 4-6, 13-21)

Minor Concerns:

The major utility of the Steglich esterification over other methods is the ability to generate esters from carboxylic acids in the presence of acid labile moieties. For example, tertiary alcohols not accessible by Fischer esterification can be made by the Steglich using CH2Cl2; however, this remains a limitation in this modification (as the authors point out). In addition, the acid work up minimizes the potential for a broader utility and scope. This submission adds a few representative acids to generate a small library of esters that may be of interest to some above and beyond to the work already reported in the Biorg Med Chem paper.

Response: We agree that there are limitations to the method in regard to tertiary alcohols (described previously in the text) and potentially to other acid-labile groups. We have added a

statement of the potential limitation regarding acid-labile groups for the acid wash steps in the introduction. (Lines 92-94). While this could limit the utility of the wash sequence in lieu of column chromatography for some compounds, it does not limit the use of acetonitrile as a greener reaction solvent.

Reviewer #3:

Minor Concerns: Will this protocol succeed for dicarboxylic acid esterification. The author can try and report reactions of succinic acid and 1.3 -acetone dicarboxylic acid

Response: We are interested in this application and plan to investigate the possibility in the future. We feel, however, that it is outside the scope of the current protocol.