**TITLE:**

Synthesis of Esters via a Green Steglich Esterification in Acetonitrile

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**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 alcohol1. 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 alcohols2,5,6. 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 Chemistry7 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 manufacturing8. 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 (CH2Cl2) and chloroform (CHCl3) are probable human carcinogens, and DMF has reproductive toxicity concerns9. In addition, CH2Cl2 is ozone depleting10. 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 CH2Cl2, CHCl3, and DMF9. Acetonitrile is currently produced as a byproduct in acrylonitrile manufacturing; however, a green synthesis of acetonitrile from biomass on an academic scale has been reported11, and potential options for the reuse and recovery from waste streams are being investigated12. Acetonitrile has previously been used as a greener solvent alternative for carbodiimide coupling reactions in solid-phase peptide synthesis to form amide linkages13. The use of acetonitrile as a solvent system for Steglich esterifications has been demonstrated14–21; 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 waste8. 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 phenols22. 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 reaction22.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 solvent23. 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. 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. 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.

1. **Extraction Workup**
   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 evaporator24,25.

* 1. 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.

* 1. 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. 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 funnel24,25.

1. **Washing Procedure**
   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.
   2. Repeat the washing procedure with saturated sodium bicarbonate solution (2 × 20 mL) and then with saturated sodium chloride solution (20 mL).
   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 agent24,25.

* 1. Remove the diethyl ether solvent under reduced pressure using a rotary evaporator.
  2. Analyze a sample of the product by 1H and 13C NMR spectroscopy in CDCl3 and by mass spectrometry.

Note: Please see additional resources for the information regarding the preparation of samples for NMR analysis24,25.

1. **Carbodiimide Coupling Reaction for Secondary and Electron-deficient Alcohols**
   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.

* 1. 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.
  2. Follow the extraction workup and washing procedure described in Steps 2-3 above.

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

* 1. 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.
  2. 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. 1H and 13C 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 1H and 13C 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 completion22. 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. 1H and 13C NMR spectra for 3-methoxybenzyl cinnamate (8).** The 1H NMR spectrum (**A**) and 13C NMR spectrum (**B**) of 3-methoxybenzyl cinnamate are shown with the product structure. Corresponding assignments are denoted on each spectrum and were confirmed using 1H-1H COSY, 1H-13C HSQC, and 1H-13C 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. 1H 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 () 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 chromatography8,9. Comparable reaction yields and rates can be achieved with the use of acetonitrile in place of dry chlorinated solvents or DMF22.

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 work22. 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 esterification23, 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.

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**DISCLOSURES:**

The authors have nothing to disclose.

**REFERENCES:**

1. Williams, A., Ibrahim, I.T. Carbodiimide chemistry: recent advances. *Chemical Reviews*. **81** (6), 589–636 (1981).

2. Höfle, G., Steglich, W., Vorbrüggen, H. 4-Dialkylaminopyridines as Highly Active Acylation Catalysts. [New synthetic method (25)]. *Angewandte Chemie International Edition in English*. **17** (8), 569–583 (1978).

3. Neises, B., Steglich, W. Simple Method for the Esterification of Carboxylic Acids. *Angewandte Chemie International Edition in English*. **17** (7), 522–524 (1978).

4. Tsvetkova, B., Tencheva, J., Peikov, P. Esterification of 7-theophyllineacetic acid with diethylene glycol monomethyl ether. *Acta pharmaceutica (Zagreb, Croatia)*. **56** (2), 251–7 (2006).

5. Tsakos, M., Schaffert, E.S., Clement, L.L., Villadsen, N.L., Poulsen, T.B. Ester coupling reactions – an enduring challenge in the chemical synthesis of bioactive natural products. *Natural Product Reports*. **32** (4) (2015).

6. Morales-Serna, J. *et al.* Using Benzotriazole Esters as a Strategy in the Esterification of Tertiary Alcohols. *Synthesis*. **2010** (24), 4261–4267 (2010).

7. Anastas, P., Eghbali, N. Green Chemistry: Principles and Practice. *Chemical Society Reviews*. **39** (1), 301–312 (2010).

8. Constable, D.J.C., Jimenez-Gonzalez, C., Henderson, R.K. Perspective on solvent use in the pharmaceutical industry. *Organic Process Research and Development*. **11** (1), 133–137 (2007).

9. Byrne, F.P. *et al.* Tools and techniques for solvent selection: green solvent selection guides. *Sustainable Chemical Processes*. **4** (1), 7 (2016).

10. Hossaini, R., Chipperfield, M.P., Montzka, S.A., Rap, A., Dhomse, S., Feng, W. Efficiency of short-lived halogens at influencing climate through depletion of stratospheric ozone. *Nature Geoscience*. **8** (3) (2015).

11. Corker, E.C., Mentzel, U. V, Mielby, J., Riisager, A., Fehrmann, R. An alternative pathway for production of acetonitrile: ruthenium catalysed aerobic dehydrogenation of ethylamine. *Green Chemistry*. **15** (4), 928–933 (2013).

12. McConvey, I.F., Woods, D., Lewis, M., Gan, Q., Nancarrow, P. The Importance of Acetonitrile in the Pharmaceutical Industry and Opportunities for its Recovery from Waste. *Organic Process Research & Development*. **16** (4), 612–624 (2012).

13. Jad, Y.E. *et al.* Peptide synthesis beyond DMF: THF and ACN as excellent and friendlier alternatives. *Organic & Biomolecular Chemistry*. **13** (8), 2393–2398 (2015).

14. Williams, J. *et al.* Quantitative method for the profiling of the endocannabinoid metabolome by LC-atmospheric pressure chemical ionization-MS. *Analytical Chemistry*. **79** (15), 5582–5593 (2007).

15. Benmansour, F. *et al.* Discovery of novel dengue virus NS5 methyltransferase non-nucleoside inhibitors by fragment-based drug design. *European Journal of Medicinal Chemistry*. **125**, 865–880 (2017).

16. Maier, W., Corrie, J.E.T., Papageorgiou, G., Laube, B., Grewer, C. Comparative analysis of inhibitory effects of caged ligands for the NMDA receptor. *Journal of Neuroscience Methods*. **142** (1), 1–9 (2005).

17. Schwartz, E. *et al.* Water soluble azido polyisocyanopeptides as functional β-sheet mimics. *Journal of Polymer Science Part A: Polymer Chemistry*. **47** (16), 4150–4164 (2009).

18. Hangauer, M.J., Bertozzi, C.R. A FRET-Based Fluorogenic Phosphine for Live-Cell Imaging with the Staudinger Ligation. *Angewandte Chemie International Edition*. **47** (13), 2394–2397 (2008).

19. Hsieh, P.-W., Chen, W.-Y., Aljuffali, I., Chen, C.-C., Fang, J.-Y. Co-Drug Strategy for Promoting Skin Targeting and Minimizing the Transdermal Diffusion of Hydroquinone and Tranexamic Acid. *Current Medicinal Chemistry*. **20** (32), 4080–4092 (2013).

20. Moretto, A. *et al.* A Rigid Helical Peptide Axle for a [2]Rotaxane Molecular Machine. *Angewandte Chemie International Edition*. **48** (47), 8986–8989 (2009).

21. Hanessian, S., McNaughton-Smith, G. A versatile synthesis of a β-turn peptidomimetic scaffold: An approach towards a designed model antagonist of the tachykinin NK-2 receptor. *Bioorganic & Medicinal Chemistry Letters*. **6** (13), 1567–1572 (1996).

22. Lutjen, A.B., Quirk, M.A., Barbera, A.M., Kolonko, E.M. Synthesis of (E)-cinnamyl ester derivatives via a greener Steglich esterification (In Press). *Bioorganic & Medicinal Chemistry*. (2018).

23. Wang, Z. *Steglich Esterification*. *Comprehensive Organic Name Reactions and Reagents*. doi: 10.1002/9780470638859. John Wiley & Sons, Inc. Hoboken, NJ, USA. (2010).

24. Padias, A.B. *Making the Connections: A How-To Guide for Organic Chemistry Lab Techniques*. Hayden McNeil. Plymouth, MI. (2011).

25. Zubrick, J.W. *The Organic Chem Lab Survival Manual: A Student’s Guide to Techniques, 10th edition*. John Wiley & Sons. (2015).