The Synthesis of Medium-Chain-Length β -Hydroxy Esters via the Reformatsky Reaction

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Abstract The synthesis of medium-chain-length β -hydroxy esters in good yield via the Reformatsky reaction is described. This work will be used as the basis for further investigation of hydroxyalkanoate polymers as potential feedstock for biofuel production.

Key words Reformatsky reaction, biosynthesis, polyhydroxyalkanoate, biofuel, 3-hydroxy esters, *Pseudomonas putida*

There has been recent interest in developing alternate sources of biofuels that can be used as a replacement for fossil fuels. One of the most prevalent biofuels is biodiesel produced by the methanolysis of long-chain (C16–C20) fatty acid triacyl glycerides that are the major constituents of plant oils.¹ However, biodiesel production results in several by-products, such as glycerol and free fatty acids, which cannot be used as fuels and are of otherwise low value. It has been recently demonstrated that these waste by-products can be used as feedstock for the bacteria *Pseudomonas putida* LS46.² This strain of *P. putida* can efficiently convert these by-products into a variety of medium-chain-length polyhydroxyalkanoates (mcl-PHAs – Scheme 1), with chain lengths of 6 to 14 carbons. These bioester polymers can be considered of higher value than the biodiesel waste by-products. For example, polyhydroxyalkanoate (PHA) has been investigated as a feedstock for biodegradable plastics and other products.³

Scheme 1 The production of polyhydroxyalkanoates from glycerol or other feedstock by *P. putida* LS46

We are interested in investigating the chemical conversion of PHA into other value-added products, such as ‘drop-in’ biofuels. For example the methanolysis of PHA results in a 3-hydroxymethyl ester with the chain length, and resulting chemical and physical properties, which is dependent on the chemical composition of the feedstock polymer. We decided to investigate 3-hydroxymethyl and -ethyl esters with a full range of chain lengths to determine the optimal of carbon atoms for downstream conversion to biofuel. However, optimizing growth conditions for *P. putida* to produce PHA with a specific chain length is time-consuming and expensive. In addition, although the free acids are commercially available their average cost (~\$10/mg) requires the development of a more economical synthesis. Therefore, a synthetic methodology was developed that would provide a convenient and economical access to a series of 3-hydroxy esters of the required chain length (C4–C12).

We decided to investigate the use of the Reformatsky reaction for this purpose as it is one of the most useful methods for the formation 3-hydroxy esters.⁴ The Reformatsky reaction can be carried out in aqueous neutral conditions, in contrast to the alkaline conditions required for aldol condensations or the dry inert conditions required when using Grignard reagents. The Reformatsky reaction has been extended to a large variety of substrates⁵ and an asymmetric version has even been developed.⁶ It was decided that this reaction would offer an attractive approach to synthesize our desired compounds as a series of aldehyde precursors are commercially available as is both ethyl and methyl bromoacetate. Here, we report the synthesis of a series of 3-hydroxymethyl and -ethyl esters in good yields using the Reformatsky reaction.

The Reformatsky reaction was used to generate the β -hydroxy esters (Table 1) reported here. The reactions were carried out using wet THF as solvent since it had been previously reported that the use of wet THF in the Reformatsky

reaction produces significantly better yields with aliphatic aldehydes than anhydrous THF.⁷ Preliminary method development reactions were conducted on a small scale (2 mmol) with the product yields ranging from 42 to 81%. Slow addition of $\text{BF}_3 \cdot \text{OEt}_2$ via syringe pump was also used.

Table 1 Synthesis of C6 to C12 β -Hydroxy Esters

1-8

Product	R ¹	R ²	Yield (%)
1	(CH ₂) ₂ Me	Me	90
2	(CH ₂) ₂ Me	Et	90
3	(CH ₂) ₄ Me	Me	85
4	(CH ₂) ₄ Me	Et	86
5	(CH ₂) ₆ Me	Me	92
6	(CH ₂) ₆ Me	Et	93
7	(CH ₂) ₈ Me	Me	94
8	(CH ₂) ₈ Me	Et	95

In order to produce an amount of each 3-hydroxy ester sufficient for testing as potential biofuels we decided to optimize the reaction at a larger scale. However, due to the exothermic nature of the Reformatsky reaction, scale-up often requires specialized equipment and reaction conditions.^{8,9} We therefore decided to optimize our reaction at the 0.1 mole scale as it was felt that this would prevent run-away reactions, which may occur at larger scales. Initial attempts at this scale involved the formation of the zinc enolate through slow addition of bromoacetate to a mixture of $\text{BF}_3 \cdot \text{OEt}_2$ -activated zinc. This was followed by slow addition of a solution of the aldehyde to this mixture. Although run-away reactions did not occur, a mixture of products was observed irrespective of addition rate. All attempts to optimize the reaction through changing order and rate of addition of reagents resulted in complex mixtures with low yields of desired product.

As part of the optimization process, we discovered that it was not necessary to activate the zinc granules with $\text{BF}_3 \cdot \text{OEt}_2$ if they were suspended in refluxing THF. Therefore, in order to minimize side products, the following procedure was developed. To a refluxing solution of THF were added zinc (2 equiv), aldehyde, and bromoacetate (2 equiv) in quick succession. **WARNING:** This reaction requires venting as the mixture immediately undergoes rapid solvent boiling, which requires extensive condensation. However, under these conditions the reaction is complete in 20 minutes with high yields (85–95%). The yields of the 3-hydroxy esters produced by the Reformatsky reactions under these conditions are summarized in Table 1.

In order to complete our library of molecules for biofuel testing, the 3-hydroxy esters were hydrolyzed to the corresponding free acids under basic conditions. These results are summarized in Table 2. These free acids are oils or solids at room temperature and therefore may not be suitable as biofuels. However, they may serve as useful substrates for deoxygenation reactions in the downstream conversion of the *P. putida* polyhydroxyalkanoates.

Table 2 Saponification of β -Hydroxy Esters to β -Hydroxy Acids

9-12

Product	R ¹	Yield (%)
9	(CH ₂) ₂ Me	72
10	(CH ₂) ₄ Me	81
11	(CH ₂) ₆ Me	92
12	(CH ₂) ₈ Me	86

In summary, we have synthesized a series of 3-hydroxy esters with carbon chain lengths of 6–12 carbons using the Reformatsky reaction. This method has been optimized for an intermediate scale of 100 mmol, which prevents run-away reactions and the formation of side products. This intermediate scale also provides an amount of product that is sufficient for evaluating these molecules as potential biofuels. This testing is underway and will be reported on when complete.

All ¹H NMR and ¹³C NMR spectra were obtained using a Bruker AC 300 in CDCl₃ unless otherwise stated. IR spectra were obtained by ATR on a Bruker Alpha FT-IR using a thin film formed by solvent evaporation.

Reformatsky Reaction; General Procedure

THF (200 mL) was added to an oven-dried 500 mL round-bottomed flask equipped with a condenser 30 cm in length and left open to the atmosphere. The THF was used directly as purchased without any further drying or purification. The solvent was then rapidly stirred using a magnetic stir bar and brought to reflux (66 °C) on a sand bath. The condenser was then temporarily raised and 6.5 g (0.1 mol, 2 equiv) of Zn granules were added to the hot solvent. This was followed by the rapid addition of the aldehyde (0.1 mol) and the bromoacetate (as either the methyl or ethyl ester) (0.2 mol, 2 equiv) into the reaction mixture. The condenser was immediately reattached to the round-bottomed flask at which point rapid boiling occurred. The reaction was allowed to rapidly reflux for 1 min at which point the reaction vessel was removed from heat and allowed to cool to r.t. with stirring. The reaction was observed to be complete by TLC (eluent: hexanes–EtOAc, 80:20) within 20 min for all substrates. Excess THF was removed under vacuum and the resultant brown oil was dissolved in hexanes and quenched with H₂O to form a yellow precipitate. The mixture was filtered and the hexane layer was washed with H₂O (100

mL), aq 1 M HCl (2 × 50 mL), H₂O (100 mL) and brine (50 mL), and dried (Na₂SO₄). Removal of hexanes under vacuum furnished the products **1–8** in yields ranging from 86 to 95% (Table 1).

Methyl 3-Hydroxyhexanoate (1)

Yield: 13.14 g (90%, 90 mmol); clear yellow oil.

IR (film): 3468w, 2957m, 1724s, 1437m, 1166s, 1122m, 993m, 847w cm⁻¹.

¹H NMR (300 MHz, CDCl₃): δ = 0.91 (t, *J* = 7.3 Hz, 3 H), 1.42 (m, 4 H), 2.38 (dd, *J* = 9.1, 16.9 Hz, 1 H), 2.49 (dd, *J* = 4.2, 16.3 Hz, 1 H), 2.90 (br s, 1 H), 3.69 (s, 3 H), 3.99 (m, 1 H).

¹³C NMR (75 MHz, CDCl₃): δ = 14.0, 18.7, 38.7, 41.2, 51.8, 67.8, 173.5.

Ethyl 3-Hydroxyhexanoate (2)

Yield: 14.4 g (90%, 90 mmol); clear yellow oil.

IR (film): 3459w, 2960w, 1718s, 1465w, 1372m, 1166s, 1017s, 847w, 733w cm⁻¹.

¹H NMR (300 MHz, CDCl₃): δ = 0.91 (t, *J* = 7.0 Hz, 3 H), 1.25 (t, *J* = 7.6 Hz, 3 H), 1.41 (m, 4 H), 2.37 (dd, *J* = 9.1, 16.7 Hz, 1 H), 2.47 (dd, *J* = 3.4, 17.3 Hz, 1 H), 2.96 (d, *J* = 3.78 Hz, 1 H), 3.99 (m, 1 H), 4.14 (q, *J* = 7.0 Hz, 2 H).

¹³C NMR (75 MHz, CDCl₃): δ = 14.0, 14.2, 18.7, 38.7, 41.4, 60.7, 67.8, 173.2.

Methyl 3-Hydroxyoctanoate (3)

Yield: 14.79 g (85%, 85 mmol); clear yellow oil.

IR (film): 3458w, 2929m, 1724s, 1437m, 1164s cm⁻¹.

¹H NMR (300 MHz, CDCl₃): δ = 0.88 (t, *J* = 6.3 Hz, 3 H), 1.29 (m, 8 H), 2.40 (dd, *J* = 8.8, 16.9 Hz, 1 H), 2.51 (dd, *J* = 3.8, 15.6 Hz, 1 H), 3.70 (s, 3 H), 3.99 (m, 1 H).

¹³C NMR (75 MHz, CDCl₃): δ = 14.1, 22.7, 25.2, 31.8, 36.6, 41.2, 51.8, 68.1, 173.6.

Ethyl 3-Hydroxyoctanoate (4)

Yield: 16.17 g (86%, 86 mmol); clear yellow oil.

IR (film): 3432w, 2931m, 1718s, 1372m, 1162s, 1026s, 732w cm⁻¹.

¹H NMR (300 MHz, CDCl₃): δ = 0.88 (t, *J* = 6.7 Hz, 3 H), 1.27 (m, 11 H), 2.39 (dd, *J* = 8.82, 16.5 Hz, 1 H), 2.50 (dd, *J* = 3.9, 15.8 Hz, 1 H), 2.90 (s, 1 H), 3.99 (m, 1 H), 4.17 (q, *J* = 7.0 Hz, 2 H).

¹³C NMR (75 MHz, CDCl₃): δ = 14.1, 14.3, 22.7, 25.3, 31.8, 36.6, 41.4, 60.8, 68.2, 173.2.

Methyl 3-Hydroxydecanoate (5)

Yield: 18.58 g (92%, 92 mmol); clear yellow oil.

IR (film): 3478w, 2925s, 2855m, 1726s, 1437m, 1163s, 1056m, 991s, 723w cm⁻¹.

¹H NMR (300 MHz, CDCl₃): δ = 0.87 (t, *J* = 6.5 Hz, 3 H), 1.42 (m, 12 H), 2.40 (dd, *J* = 8.8, 16.3 Hz, 1 H), 2.51 (dd, *J* = 3.3, 16.4 Hz, 1 H), 2.84 (br s, 1 H), 3.71 (s, 3 H), 3.99 (m, 1 H).

¹³C NMR (75 MHz, CDCl₃): δ = 14.2, 22.7, 25.6, 29.3, 29.6, 31.9, 36.6, 41.2, 51.8, 68.2, 173.6.

Ethyl 3-Hydroxydecanoate (6)

Yield: 20.1 g (93%, 93 mmol); clear yellow oil.

IR (film): 3496w, 2927m, 1720m, 907s, 728s, 647m cm⁻¹.

¹H NMR (300 MHz, CDCl₃): δ = 0.87 (t, *J* = 6.7 Hz, 3 H), 1.27 (m, 15 H), 2.38 (dd, *J* = 9.0, 16.3 Hz, 1 H), 2.50 (dd, *J* = 3.5, 16.1 Hz, 1 H), 2.95 (br, 1 H), 3.99 (m, 1 H), 4.16 (q, *J* = 7.0 Hz, 2 H).

¹³C NMR (75 MHz, CDCl₃): δ = 14.2, 14.3, 22.7, 25.6, 29.3, 29.6, 31.9, 36.6, 41.4, 60.8, 68.2, 173.2.

Methyl 3-Hydroxydodecanoate (7)

Yield: 21.62 g (94%, 94 mmol); clear yellow oil.

IR (film): 3468w, 2923s, 2853m, 1726s, 1437m, 1170s, 732m cm⁻¹.

¹H NMR (300 MHz, CDCl₃): δ = 0.88 (t, *J* = 5.9 Hz, 3 H), 1.28 (m, 16 H), 2.45 (dd, *J* = 16.2, 18.8 Hz, 1 H), 2.47 (dd, *J* = 13.2, 16.2 Hz, 1 H), 2.99 (br, 1 H), 3.70 (s, 3 H), 4.00 (m, 1 H).

¹³C NMR (75 MHz, CDCl₃): δ = 14.1, 22.7, 25.5, 29.3, 29.5, 29.5, 29.6, 31.9, 36.6, 41.2, 51.7, 68.0, 173.5.

Ethyl 3-Hydroxydodecanoate (8)

Yield: 23.18 g (95%, 95 mmol); clear yellow oil.

IR (film): 3496w, 2927m, 1720m, 907s, 728s, 647m cm⁻¹.

¹H NMR (300 MHz, CDCl₃): δ = 0.87 (t, *J* = 7.0 Hz, 3 H), 1.26 (m, 19 H), 2.43 (dd, *J* = 15.9, 19.0 Hz, 1 H), 2.45 (dd, *J* = 13.5, 16.6 Hz, 1 H), 3.05 (br, 1 H), 3.99 (m, 1 H), 4.16 (q, *J* = 7.0 Hz, 2 H).

¹³C NMR (75 MHz, CDCl₃): δ = 14.1, 14.2, 22.7, 25.5, 29.3, 29.5, 29.5, 29.6, 31.9, 36.6, 41.4, 60.6, 68.0, 173.1.

Saponification of Esters; General Procedure

To a 50 mL round-bottomed flask fitted with a stir bar was added hexane (5 mL) and the respective 3-hydroxymethyl ester (1 mmol). This mixture was heated to 50 °C at which point sat. KOH in MeOH (0.5 mL) was added. This led to the instant formation of a precipitate. The mixture was then stirred vigorously at 60 °C for 30 min, then removed from heat, and the solvents were evaporated under reduced pressure to provide the product as a potassium salt. This residue was dissolved in distilled H₂O (10 mL) and extracted with CHCl₃ (3 × 10 mL). The aqueous layer was collected and acidified with concd HCl to pH <1. Et₂O (20 mL) was added to the aqueous acidic solution and this solution was stirred vigorously for 1 h. The organic and aqueous layers were separated the aqueous layer was extracted with Et₂O (2 × 20 mL). The combined organic layers were dried (Na₂SO₄), filtered, and evaporated under reduced pressure to give the corresponding product, which was used without further purification (Table 2).

3-Hydroxyhexanoic Acid (9)

Yield: 95 mg (72%, 0.72 mmol); clear oil.

IR (film): 3390br, 2959m, 1704s, 1407m, 1174m, 1123m, 1017m, 845m cm⁻¹.

¹H NMR (300 MHz, CDCl₃): δ = 0.90 (t, *J* = 7.30 Hz, 3 H), 1.43 (m, 4 H), 2.43 (dd, *J* = 8.76, 16.4 Hz, 1 H), 2.53 (dd, *J* = 4.57, 16.3 Hz, 1 H), 4.03 (m, 1 H), 7.20 (br, 1 H).

¹³C NMR (75 MHz, CDCl₃): δ = 13.9, 18.7, 38.6, 41.2, 68.0, 177.4.

3-Hydroxyoctanoic Acid (10)

Yield: 130 mg (81%, 0.81 mmol); yellow oil.

IR (film): 3312br, 2929m, 1707s, 1374w, 1238m, 1044m, 933w, 872w, 608w cm⁻¹.

¹H NMR (300 MHz, CDCl₃): δ = 0.88 (t, *J* = 6.76 Hz, 3 H), 1.30 (m, 8 H), 2.46 (dd, *J* = 9.01, 16.3 Hz, 1 H), 2.56 (dd, *J* = 4.21, 16.0 Hz, 1 H), 4.03 (m, 1 H), 6.42 (br, 1 H).

^{13}C NMR (75 MHz, CDCl_3): δ = 14.1, 22.7, 25.2, 31.7, 36.5, 41.2, 68.2, 177.9.

3-Hydroxydecanoic Acid (11)

Yield: 173 mg (92%, 0.92 mmol); white solid; mp 57.5 °C.

IR (film): 3534w (H_2O), 3036br, 2920s, 2848m, 1679s, 1439m, 1221s, 907m, 710w, 544w cm^{-1} .

^1H NMR (300 MHz, CDCl_3): δ = 0.87 (t, J = 6.61 Hz, 3 H), 1.27 (m, 12 H), 2.45 (dd, J = 8.92, 16.6 Hz, 1 H), 2.56 (dd, J = 3.84, 16.4 Hz, 1 H), 4.03 (m, 1 H), 6.68 (br, 1 H).

^{13}C NMR (75 MHz, CDCl_3): δ = 14.2, 22.7, 25.5, 29.3, 29.5, 31.9, 36.6, 41.2, 68.2, 178.0.

3-Hydroxydodecanoic Acid (12)

Yield: 186 mg (86%, 0.86 mmol); white solid; mp 74 °C.

IR (film): 3534w (H_2O), 2952br, 2913s, 2847m, 1680s, 1469w, 1441w, 1216m, 866w, 548m cm^{-1} .

^1H NMR (300 MHz, acetone- d_6): δ = 0.87 (t, J = 6.34 Hz, 3 H), 1.29 (m, 16 H), 2.36 (dd, J = 8.02, 15.6 Hz, 1 H), 2.45 (dd, J = 4.66, 15.6 Hz, 1 H), 2.80 (br, 1 H), 3.97 (m, 1 H).

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Supporting Information

Supporting information for this article is available online at <http://dx.doi.org/10.1055/s-0034-1379479>. Included are ^1H and ^{13}C NMR spectra.

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