

## Research Note

SYNTHESIS OF NORBORNENE FURYL ESTERS  
AND THEIR ENZYMATIC KINETIC  
RESOLUTION BY PIG LIVER ESTRASE (PLE)

M. Mamaghani and A. Namazi

*Department of Chemistry, Faculty of Science, Guilan University, P.O. Box 1914, Rasht,  
Islamic Republic of Iran*

## Abstract

The reaction of furylacrylic acid with cyclopentadiene in toluene gave the expected Diels-Alder endo- and exo-adducts in ~1:1 ratio. The adducts were separated by iodolactonization method to provide 3-endo-(2'-furyl) bicyclo [2.2.1] hept-5-ene-2-exo-carboxylic acid and 3-exo-(2'-furyl) bicyclo [2.2.1] hept-5-ene-2-endo-carboxylic acid. Esterification of these acids by MeI/HMPA gave the expected methyl esters. Kinetic resolution of racemic endo- and exo-ester adducts by PLE (phosphate buffer, pH, 8) provided the related optically active isomers.

Enzymes have been used widely in organic synthesis and in recent years, several reviews have expressed the great importance of this methodology [1-4]. One of the enzymes which has received much current interest is PLE [5]. This hydrolase has been employed as a chiral catalyst in the kinetic resolution of norbornene type mono and diesters [1,4]. These rigid systems have proven to be extremely useful synthon in the synthesis of natural products [6-10] and pharmaceutical agents [11-13].

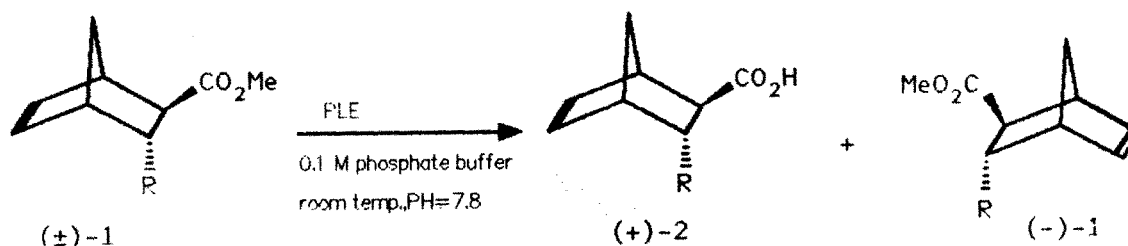
In our previous work, in order to examine the possible charge transfer type interactions in PLE hydrolysis, aromatic substituted norbornene esters were synthesized and their PLE hydrolysis were studied [14] (Scheme 1). This research revealed that the presence of phenyl and p-nitrophenyl substituent in the endo-position of exo-norbornene esters (1), on the basis of the reaction time

(Table 1), make them poor substrate for PLE. In order to evaluate the effect of furyl substituents in the PLE hydrolysis of these systems, racemic methyl 3-endo-(2'-furyl) bicyclo [2.2.1] hept-5-ene-2-exo-carboxylate (3) and methyl 3-exo-(2'-furyl) bicyclo [2.2.1] hept-5-ene-2-endo-carboxylate (4) were synthesized and subjected to PLE hydrolysis.

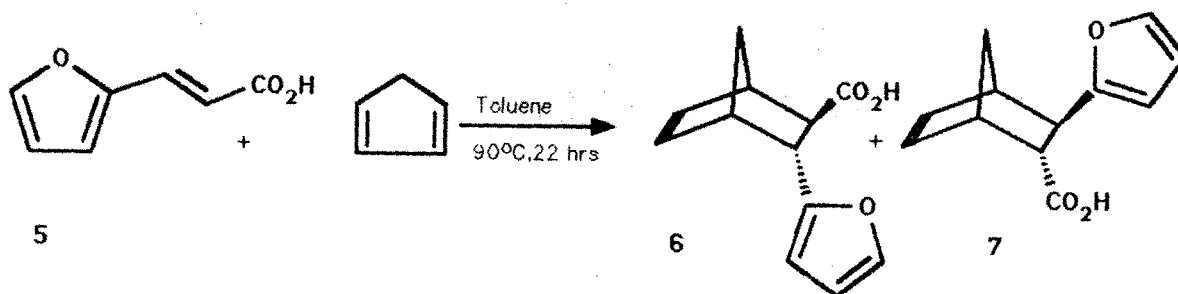
The related acids of target molecules (3) and (4) were prepared through a Diels-Alder reaction of furylacrylic acid and freshly prepared dicyclopentadiene in toluene at 90°C (Scheme 2). After 22 h reflux at this temperature, examination of the resultant reaction product by TLC (EtOAc/hexane, 1:3) showed the presence of endo- and exo-adducts along with a trace of unreacted furylacrylic acid.

The acid adducts (6&7) were separated by an iodolactonization method [15] at pH=8 (Scheme 3). The reaction was complete in 30 minutes and 3-exo-(2'-furyl) bicyclo [2.2.1] heptane carbolactone (8) was

**Keywords:** PLE; Kinetic resolution; Hydrolysis; Norbornene



Scheme 1



Scheme 2

recovered from the aqueous solution by centrifuge. Reduction of the resultant carbolactone with zinc powder and glacial acetic acid (rt., 3<sup>1/2</sup>h) provided the expected endo-acid (7) in 73% yield. The further structural evidence for the endo-acid (7) was also provided by the transformation of the acid to the related methyl ester which was carried out using Shaw's method [16] with MeI/HMPA.

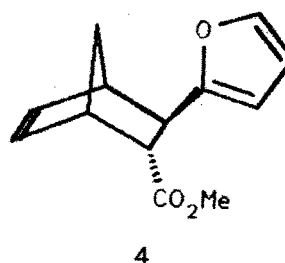
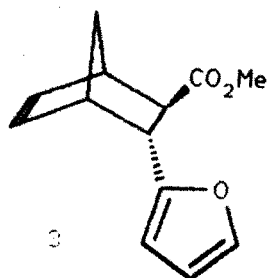
The aqueous solution from iodolactonization reaction produced the exo-acid contaminated with unreacted

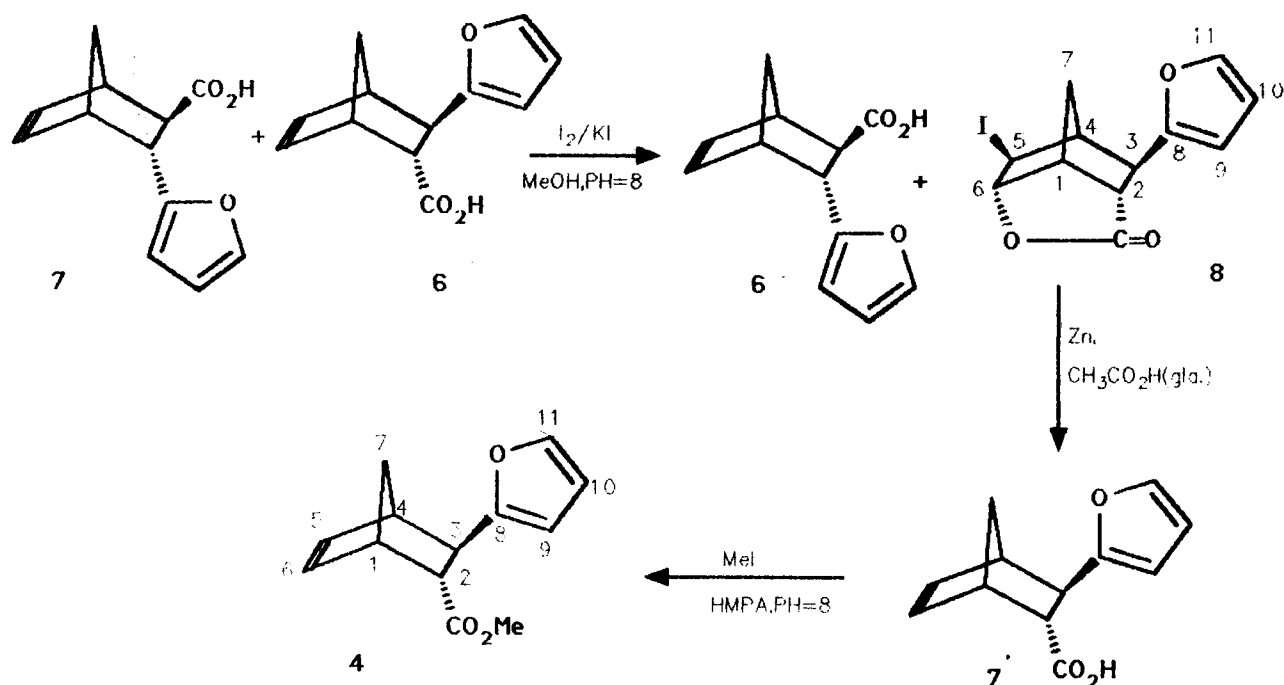
furylacrylic acid. This mixture was transformed to the related methyl ester by the standard method (MeI/HMPA) [16]. Attempts to purify the exo-ester by column chromatography failed. Therefore, an enzymic method using PLE was employed. PLE hydrolysis of mixed esters provided the pure exo-ester. In this reaction, methyl furylacrylate is hydrolysed much faster than exo-ester. The structure of exo-ester adduct was confirmed by spectroscopic methods.

( $\pm$ )-Methyl 3-exo-(2'-furyl) bicyclo [2.2.1] hept-5-

Table 1

R	con%	rt	Acid		Ester	
			c.y%	ee%	c.y%	ee%
C <sub>6</sub> H <sub>5</sub> -	50%	12 days	39	61.5	46.7	59.5
p-O <sub>2</sub> N-C <sub>6</sub> H <sub>4</sub> -	33%	16 days	31.6	10.66	67.3	4.47





Scheme 3

ene-2-endo-carboxylate (4) was subjected to enzymic hydrolysis using PLE as a chiral catalyst (Scheme 4). At 50% conversion the reaction provided the related (+)-endo acid (7) in 48% yield and unreacted (-)-endo ester (4), in 37% yield. The structure of both endo-acid and ester were reconfirmed by comparison of their spectroscopic data with those of racemic counterparts.

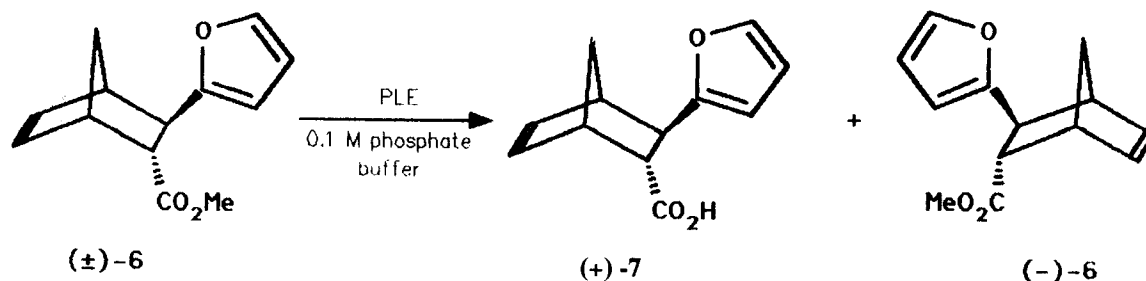
The same methodology was applied to the resolution of ( $\pm$ )-methyl 3-endo-(2'-furyl) bicyclo [2.2.1] hept-5-ene-2-exo-carboxylate (3). When the reaction was stopped at 50% conversion, (+)-exo-carboxylic acid (6), and unreacted (-)-exo-ester (3) were formed in 35% and 30% yield, respectively.

The structure of the exo-acid was established by the spectroscopic methods. The spectroscopic data (IR &  $^1H$ NMR) of the (-)-exo-ester were quite consistent with those of racemic ester.

In this research facile hydrolysis of furyl derivatives of trans norbornene esters by PLE makes them a good substrate for PLE. However, on the basis of these results and our studies on the PLE hydrolysis of aromatic substituted norbornene mono esters (Table 1), it seems the size of aromatic substituents may play a crucial role in this hydrolysis. Furthermore, ease of hydrolysis of exo-ester (2h) relative to the endo-isomer (36h), confirms once again previously reported stereochemical requirements in PLE hydrolysis of these systems [17, 18].

### Experimental Section

Melting points were measured with Electro Thermal and are uncorrected. IR spectra were determined on a Shimadzu IR-470 spectrometer.  $^1H$ NMR spectra were recorded on a Jeol, 60 PMX (60 MHz) and Bruker AC,



Scheme 4

FT-NMR (80 MHz) in deuteriochloroform ( $\text{CDCl}_3$ ) with tetramethylsilane (TMS). For Mass spectra, a Varian spectrometer 5970 (EI) was used. A thin layer chromatography (TLC) was carried out on Merk Kieselgel 60H ASTM 35-70. Flash chromatography was carried out at pressure of ca.1.5 bar using Merk Kieselgel 60H F<sub>254</sub> Art No 7730. PLE was purchased from Sigma.

**3-Endo-(2'-furyl) bicyclo [2.2.1] hept-5-ene-2-exo-carboxylic-acid (6) and 3-exo-(2'-furyl) bicyclo [2.2.1] hept-5-ene-2-endo-carboxylic acid (7)**

Freshly cracked cyclopentadiene (5 ml) in toluene (15 ml) was added to a solution of furylacrylic acid (1.8 g, 13 mmol) [19] in toluene (50 ml) at 90°C for 12 h. The reaction mixture was stirred for another 10 h at this temperature. After cooling to room temperature, a solution of NaOH 5% was added until pH = 10. The resultant aqueous phase was separated by a separatory funnel and acidified by HCl 5% to pH=4-5. This solution was extracted by ether (3 ×15 ml) and the ethereal solution was dried ( $\text{MgSO}_4$ ) and evaporated *in vacuo* to provide the expected products (6) and (7), contaminated with a trace of furylacrylic acid (2.25 g). The presence of unreacted furylacrylic acid was confirmed by TLC (EtOAC/Hexane 1:1) and <sup>1</sup>HNMR. This mixture was separated by the iodolactonization method.

**Separation of 3-exo-(2'-furyl) bicyclo [2.2.1] hept-5-ene-2-endo-carboxylic acid (7) and 3-endo-(2'-furyl) bicyclo [2.2.1] hept-5-ene-2-exo-carboxylic acid (6)**

A mixture of acid adducts (6) and (7) (4g) was neutralized to pH=8 by means of a 20% solution of NaOH and a 5% aqueous solution of  $\text{NaHCO}_3$ . To this mixture, methanol (10 ml) was added and then from a stock solution of iodine in water [ $\text{I}_2$  (2g) + KI (4 g) +  $\text{H}_2\text{O}$ (12 ml)] was added (10 ml) at room temperature and the resultant mixture was stirred for 30 minutes at this temperature. An off-white solid was precipitated immediately after the addition of the iodine solution. The solid was separated by centrifuge and washed with a solution of  $\text{Na}_2\text{S}_2\text{O}_4$  (2x10 ml) and dried under vacuo to provide the new 3-exo-(2'-furyl) bicyclo [2.2.1] heptane carbolactone (1.36 g, 4.12 mmol), mp=87°C; IR (KBr): 3100 (m), 2950 (m), 1780, 1790 (vs), 1190 (s), 1160 (m), 1150 (s), 1000 (vs); <sup>1</sup>HNMR:  $\delta$  (ppm) 7.3 (d, J=1.8 Hz, 1H), 6.3 (dd, J=1.8, 3.3 Hz, 1H), 6.1 (d, J=3.3 Hz, 1H), 5.2 (m, 1H), 4(d, J=2.4 Hz, 1H), 3.3 (m, 2H), 2.9 (m, 2H), 2.3 (m, 2H); Mass, m/e(%): 218 (M<sup>+</sup>, 9), 153 (7), 152 (34), 121 (22), 66(7).

The aqueous solution from iodolactonization was acidified with HCl 5% (pH=4-5) and extracted by  $\text{CHCl}_3$  (3x20 ml). The organic phase was separated and dried ( $\text{MgSO}_4$ ). The solvent was removed to give 3-endo-(2'-

furyl)-bicyclo [2.2.1] hept-5-ene-2-exo-carboxylic acid contaminated with a trace of furylacrylic acid (2.5 g).

**Methyl 3-endo-(2'-furyl)bicyclo [2.2.1] hept-5-ene-2-exo-carboxylate (3) and methyl (2'-furyl) acrylate**

To a mixture of 3-endo-(2'-furyl) bicyclo [2.2.1] hept-5-ene-2-exo-carboxylic acid and furylacrylic acid (1.5 g) obtained from iodolactonization reaction, hexamethylphosphoramide (HMPA)(15 ml) was added. By careful addition of a 25% solution of NaOH, pH was adjusted to 8-9. The reaction mixture was stirred at room temperature for 1 h. MeI (1.38 ml, 22.06 mmol) was added and stirred continuously for 18 h at the same temperature. The mixture was extracted with ether (3x15 ml), the organic phase washed with a 5% solution of NaOH (2x10 ml), then water (2x15 ml) and dried by  $\text{MgSO}_4$ . Evaporation of the solvent gave the expected methyl esters (0.9 g). These Methyl esters, due to their close R<sub>f</sub>, could not be separated by column chromatography. Therefore, an enzymic method was employed.

To a suspension of mixed methyl ester (3) and methylfurylacrylate (0.52 g) in 0.1 M phosphate buffer (25 ml), pH=8, 30°C, PLE (80 $\mu$ l) was added. The pH was kept at 8 by a continuous addition of a 0.1M NaOH solution. After consumption of 8 ml NaOH (1 h), examination of the mixture (EtOAC/hexane 1:3) showed that all of the methylfurylacrylate had been hydrolyzed. Therefore, pH increased to 10 by the addition of a 20%  $\text{Na}_2\text{CO}_3$  and the resultant mixture was extracted by  $\text{CHCl}_3$  (3x25 ml). The organic phase was separated and dried ( $\text{MgSO}_4$ ). The solvent was evaporated to produce 3-endo-(2'-furyl)bicyclo[2.2.1]hept-5-ene-2-exo-carboxylate (0.37g, 1.69 mmol); IR( $\text{CCl}_4$ ): 3050 (w), 1735 (vs), 1664(w), 1280-1140(s) $\text{cm}^{-1}$ ; <sup>1</sup>HNMR:  $\delta$ (ppm) 7.3 (d, J=1.8 Hz, 1H), 6-6.4 (m, 4H), 3.62 (s, 3H), 2.8-3.34 (m, 4H), 1.4-1.9 (m, 2H); Mass, m/e(%): 218 (M<sup>+</sup>, 6), 153 (7), 152(34), 121(24), 66 (8).

**3-Exo-(2'-furyl)bicyclo[2.2.1] hept-5-en-2-endo-carboxylic acid (7)**

To a solution of 3-exo-(2'-furyl) bicyclo[2.2.1] heptane carbolactone (0.33 g, 1 mmol) in glacial acetic acid (1 ml), zinc powder (0.4 g) was added. The reaction mixture was stirred at room temperature for 3<sup>1/2</sup>h and the resultant mixture was filtered and washed with hot water (3x5 ml). The filtrate was extracted with ether (3x8 ml), dried ( $\text{MgSO}_4$ ), and evaporated. Recrystallization from water gave 3-exo-(2'-furyl)bicyclo[2.2.1]hept-5-ene-2-endo-carboxylic acid (0.15 g, 0.74 mmol, 73.5%), mp=120-122°C; IR(KBr):3400-2400(s), 1690(vs), 1640 (w), 1420 (m), 1280 (s), 1240(s), 1200(s)  $\text{cm}^{-1}$ ; <sup>1</sup>HNMR:  $\delta$ (ppm) 9.66 (s, 1H), 7.33 (d, J=1.7 Hz, 1H), 6-6.36 (m, 4H), 3 - 3.33 (m, 4H), 1.33-1.86 (m, 2H).

**Methyl 3-exo-(2'-furyl)bicyclo[2.2.1]hept-5-ene-2-endo-carboxylate (4)**

To a solution of 3-exo-(2'-furyl)bicyclo[2.2.1]hept-5-ene-2-endo-carboxylic acid (1.5 g, 7.35 mmol) in HMPA (15 ml), a 25% solution of sodium hydroxide was added (pH=8-9). The reaction mixture was stirred for 1 h and methyl iodide (1.38 ml, 22 mmol) was added and stirred continuously for 24h. The reaction mixture was extracted with ether (3x15 ml) and etherial solution washed by a 5% solution of NaOH (2x10 ml) and water (3x15 ml), respectively. The organic phase was separated and dried (MgSO<sub>4</sub>). Removal of the solvent produced the desired product (0.84g, 3.85 mmol, 54%) as a viscos oil; IR(neat): 3100(m), 1740 (vs), 1640 (m), 1170-1200 (s)cm<sup>-1</sup>; <sup>1</sup>HNMR δ(ppm) 7.6 (d, J=1.7 Hz, 1H), 6.3-6.6 (m, 4H), 3.9 (s,3H), 3.2-3.6 (m,4H), 1.6-2.26 (m,2H); Mass (EI), m/e (%): 218 (M<sup>+</sup>, 9), 153 (7), 152(34), 121 (22), 66(7).

**PLE hydrolysis of methyl 3-exo-(2'-furyl)bicyclo[2.2.1] hept-5-ene-2-endo-carboxylate (4)**

Racemic methyl 3-exo-(2'-furyl)bicyclo[2.2.1]hept-5-ene-2-endo-carboxylate (0.62 g, 2.84 mmol) was suspended in a 0.1 M phosphate buffer solution (pH=8, 25 ml) and incubated with PLE (150μl) at 30°C. The pH was kept constant by the continuous addition of 0.1M NaOH. At 50% coversion (36h), the reaction was stopped by adding a 20% sodium carbonate solution until the pH was 10. The aqueous solution was extracted with chloroform (3x20 ml). The combined organic layers were dried (MgSO<sub>4</sub>) and concentrated to yield optically active unreacted ester (-)-methyl 3-exo-(2'-furyl)bicyclo[2.2.1]hept-5-ene-2-endo-carboxylate (0.23g, 1.05 mmol, 37%), [α]<sub>D</sub><sup>30</sup> = -91.67°C (c=0.6, CHCl<sub>3</sub>). The spectroscopic data (IR & <sup>1</sup>HNMR) of the product was quite consistent with those of racemic compound.

The aqueous layer was acidified with 5% HCl to pH=4 and extracted with chloroform (3x20 ml). The chloroform layer was dried (MgSO<sub>4</sub>) and concentrated at vacuo to give the optically active (+)-3-exo-(2'-furyl)-bicyclo[2.2.1]hept-5-ene-2-endo-carboxylic acid (0.28 g, 1.37 mmol, 48.5%), [α]<sub>D</sub><sup>30</sup> = +50°C(c=0.4, CHCl<sub>3</sub>), mp=122°C (for spectroscopic data see racemic endo-acid).

**PLE hydrolysis of (±)-methyl 3-endo-(2'-furyl)bicyclo[2.2.1] hept-5-ene-2-exo-carboxylate (3)**

The standard procedure was used for the hydrolysis of (±)-methyl 3-endo-(2'-furyl)bicyclo[2.2.1]hept-5-ene-2-exo-carboxylate (0.37 g, 1.69 mmol) in a 0.1 M phosphate buffer by PLE (100μl). The reaction was stopped at 50%

conversion to provide unreacted optically active (-)-methyl 3-endo-(2'-furyl)bicyclo[2.2.1]hept-5-ene-2-exo-carboxylate (0.1 g, 0.51 mmol, 30%), [α]<sub>D</sub><sup>30</sup> = -33° (c=0.91, CHCl<sub>3</sub>) [the IR & <sup>1</sup>HNMR spectra were the same as (3)] and optically active (+)-3-endo-(2'-furyl)bicyclo[2.2.1] hept-5-ene-2-exo-carboxylic acid (0.12g, 0.58 mmol, 35%), [α]<sub>D</sub><sup>30</sup> = +48.42°C (c=1.96, CHCl<sub>3</sub>), mp=82-84°C; IR(KBr): 3500 -2400 (s), 1700 (vs), 1640(w), 1580(w), 1440(s), 1200(s)cm<sup>-1</sup>; <sup>1</sup>HNMR:δ (ppm) 7.25 (d, J=1.7 Hz, 1H), 6.05(m, 2H), 5.9 (m, 2H), 3.7(t, J=4 Hz, 1H), 3.2(s, 2H), 2.4(d, J=4 Hz, 1H), 1.4-1.95 (m, 2H).

**Acknowledgements**

We are grateful to the Research Council of Guilar University for their financial support.

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