***SHORT PAPER***

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# Enzymatic hydrolysis of 4′-(1-chloro-2-acetoxyethyl)-benzo-15-crown-5-ether and 4′-acetoxyethyl-benzo-15-crown-5-ether; a facile synthesis of the optically active chlorohydrin analogue incorporating crown ether†

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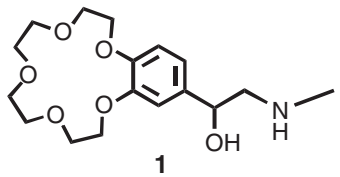
Optically active chlorohydrin analog incorporating crown ether was synthesised by enzymatic hydrolysis using *Candida Antarctica* lipase in high enantiomeric excess.

**Keywords**: crown ether, lipase, hydrolysis, enantiomeric excess



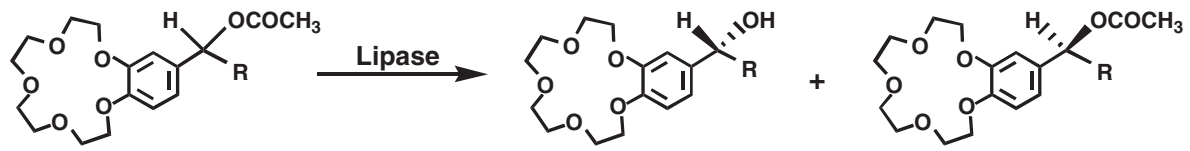
**1**

The endohydrophilic/exolipophilic structure of the crown ethers permits lipophilisation of alkali metal ions. As synthetic analogues of the well-known ionophores, neutral ligands of the crown ether type have contributed greatly to our understanding of selective ion transport processes in biological membranes. Furthermore, crown compounds are of great interest in view of their pathological and physiological properties.[1] Medicinal substances including crown ether inhibit the growing of bacteria and virus disease.[2] Vogtle *et al*. reported that the synthesis of papaverine[3a] and adrenaline[3b] crown ethers combining with benzo-15-crown-5 ether (**1:** adrenaline type crown ether). Recently we reported that racemic large secondary alcohol, 4′-hydroxyethyl-benzo-15-crown-5-ether was kinetically resolved in high optical yield by asymmetric transformation with CAL (*Candida Antarctica* lipase) and *Pseudomonas Cepacia* lipase.[4] The synthesis for this type of chiral crown ether unit is an important task due to the very useful as a chiral synthon.

In this work we report the preparation of chlorohydrin analog incorporating crown ether (±)-**3** by enzymatic hydrolysis. In addition we also report the enzymatic hydrolysis of phenylethanol type crown compounds (±)-**2** (Scheme 1).

The crown compound (±)-**3** was prepared from benzo-15-crown-5-ether by Friedel–Crafts reaction with chloroacethyl chloride,[5] followed by reduction with $NaBH_4$ according to the literature procedure[6] and then acetylated with acetic anhydride–pyridine at room temperature for 4h.

In a typical hydrolysis experiment, enzymes were added to a solvent containing substrate (±)-**2** and (±)-**3**, and resulting mixture was incubated at 30°C under reciprocal shaking. Among the commercially available enzymes,[7] only CAL was found to accelerate hydrolysis of crown compounds (±)-**2** and (±)-**3** to give the corresponding alcohols (*R*)-**4** in toluene and (*R*)-**5** in water in excellent yield and e.e.(enantiomeric excess) values, respectively (Table 1, Entry 3, 7). When a water was used as a solvent with enzyme, poor results were obtained with respect to e.e. value of the hydrolysis product from crown substrate (±)-**2** (Table 1, Entry 2). It was found that, in pure water, hydrolysis of crown compounds (±)-**2** occurred without enzyme (Table 1, Entry 1). This non-enzymatic hydrolysis may be responsible for the low e.e. values. In contrast crown compound (±)-**3** was hydrolysed by CAL in pure water without non-enzymatic hydrolysis, although the long reaction time (92 h) was required. It seems that former type of crown compound was easily hydrolysed probably due to its strong solubility to the water. The results are summarised in the Table 1. All the enzymatically hydrolysed products had an *R* configuration. The e.e. values obtained by the kinetic resolutions were determined by means of HPLC. The *E* values were calculated according to the literature.[8] The absolute configurations of the product alcohol (*R*)-**4** and the remaining ester (*S*)-**2**, were determined by comparison of the optical data




(±)-**2** : R = -$CH_3$
(±)-**3** : R = -$CH_2Cl$

(R)-**4** : R = -$CH_3$
(R)-**5** : R = -$CH_2Cl$

(S)-**2** : R = -$CH_3$
(S)-**3** : R = -$CH_2Cl$

**Scheme 1** Enzymatic hydrolysis of(±)-**2** and (±)-**3**.

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† This is a Short Paper, there is therefore no corresponding material in *J Chem. Research (M).*

**Table 1** Enzymatic hydrolysis of crown ether derivatives (±)-**2** and (±)-**3** using lipase

| | | | | | Product alcohol | | | | Remaining ester | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Entry | Substrate[a] | Lipase[b] | Solvent | Reaction time/h | Yield/%[c] | conf.[a] | e.e./%[d] | $[\alpha]^{25}_{D}$[e] | Yield/%[c] | conf.[a] | e.e./%[d] | $[\alpha]^{25}_{D}$[e] | *E*[f] |
| 1 | (±)-**2** | None | Water | 3 | 40 | - | 0 | - | 60 | - | 0 | - | 1 |
| 2 | (±)-**2** | CAL | Water | 0.4 | 37 | *(R)*-**4** | 59 | +10.8 | 33 | *(S)*-**2** | >99 | –71.3 | 5 |
| 3 | (±)-**2** | CAL | Toluene | 92 | 35 | *(R)*-**4** | 94 | +17.2 | 40 | *(S)*-**2** | >99 | –71.3 | 54 |
| 4 | (±)-**2** | CAL | Acetone | 50 | 39 | *(R)*-**4** | 80 | +14.6 | 47 | *(S)*-**2** | 72 | –51.8 | 15 |
| 5 | (±)-**2** | CAL | THF | 50 | | | | No Conversion | | | | | |
| 6 | (±)-**3** | None | Water | 150 | | | | No Conversion | | | | | |
| 7 | (±)-**3** | CAL | Water | 53 | 24 | *(R)*-**5** | >99 | +25.7 | 35 | *(S)*-**3** | 73 | –41.3 | 270 |
| 8 | (±)-**3** | CAL | Toluene | 150 | | | | No Conversion | | | | | |
| 9 | (±)-**3** | CAL | Acetone | 150 | | | | No Conversion | | | | | |
| 10 | (±)-**3** | CAL | THF | 85 | | | | No Conversion | | | | | |

[a](±)-**2**: (±)-4′-acetoxyethyl-benzo-15-crown-5-ether, (±)-**3**: (±)-4′-(1-chloro-2-acetoxyethyl)-benzo-15-crown-5-ether, (*R*)-**4**: (*R*)-4′-hydroxyethyl-benzo-15-crown-5-ether, (*R*)-**5**: (*R*)-4′-(1-chloro-2-hydroxyethyl)-benzo-15-crown-5-ether
[b]CAL = Lipase from *Candida Antarctica*.
[c]Isolated yield.
[d]Determined by HPLC (Daicel chiralcel OD column (n-hexane:2-propanol = 9 : 1)).
[e]Optical rotations were measured in chloroform at 25 °C
[f]Calculated from $E = \ln[1-c(1+e.e._{product})]/\ln[1-c(1-e.e._{product})]$ according to literature.[8]

with those reported in the literature[4] and product alcohol (*R*)-**5** and the remaining ester (*S*)-**3**, the latter of which was converted into the alcohol, were determined by $^1$H NMR (500 MHz) $CDCl_3$, from the diastereomeric differences in chemical shifts made by the methyl group in (*S*)-O-methylmanderate esters.[9]

We thus conclude that however, the obtained product alcohol (*R*)-**4** was poor results in water solvent due to an acceleration of non-enzymatic hydrolysis, remaining ester (*S*)-**2** was obtained in excellent e.e. values. Using toluene as a reaction solvent, CAL was found to accelerate hydrolysis and transesterification[4] of phenylethanol type crown compounds (±)-**2** and corresponding ester in excellent yield and e.e. values. Furthermore, CAL was an excellent catalyst for the hydrolysis of chlorohydrin analog incorporating crown ether (±)-**3**. Considering the broad substrate specificity of lipases, this approach is expected to be synthetically useful for asymmetric preparation of optically pure crown ether type chlorohydrin derivatives. Further work is under way to synthesize of adrenaline derivatives as a medicinal property combining with benzo-15-crown-5 ether **1**.

## Experimental

CAL (Novozyme 435®, lipase from *Candida Antarctica*) was obtained a gift from Novo Nordisk Bio Industry Ltd. Melting points were taken using a Gallenkamp melting point apparatus. Infrared spectra were taken with a HORIBA FT-710 spectrometer. $^1$H-NMR spectra were recorded at room temperature on a Varian INOVA 500 instrument. Chemical shifts are denoted in δunits (ppm), relative to tetramethylsilane (TMS) as internal standard or relative to residual solvent peaks; *J* values are given in Hz. Mass spectra were obtained using a JEOL JMS-AX505HA mass spectrometer. Optical rotations were measured with a JASCO DIP-340 digital polarimeter. Enantiomeric excess (e.e.) value of the product was determined by chiral HPLC analysis with a Daicel chiralcel OD column with hexane - 2-propanol (9:1) as mobile phase at a flow rate of 0.5 ml/min. UV detection at 278 nm was used for quantification at ambient temperature.

*Synthesis of* (±)-**2:** Racemic crowned ester (±)-**2** was prepared from 4′-hydroxyethyl-benzo-15-crown-5-ether. (±)-4′-hydroxyethyl-benzo-15-crown-5-ether was synthesised from benzo-15-crown-5-ether by the procedure reported[10] and then acetylated as follows. Acetic anhydride (18.1 g, 17.8 mmol) was added slowly to a solution of 4′-hydroxyethyl-benzo-15-crown-5-ether[4] (18.4 g, 59 mmol) in dry pyridine (150 ml), and the mixture was stirred under nitrogen at room temperature for 4h. The reaction mixture was diluted with water (100 ml) and extracted with two 100-ml portions of chloroform. The organic extract was washed with 6N HCl and water. After drying over sodium sulfate, the solvent removal under reduced pressure gave a residue which was chromatographed on silica gel in ethyl acetate/hexane (80:20), yielding the expected product (±)-**2** (16.6 g, 80%) as a reddish yellow oil; $^1$H NMR (500 MHz, $CDCl_3$) δ7.27–6.85 (m, 3H, Cp), 5.82 (q, 1H, *J*=3Hz, CH), 4.18–4.11 (m, 4H, –O–$CH_2$–$CH_2$–), 3.92–3.88 (m, 4H, –O–$CH_2$–$CH_2$–), 3.89 (s, 8H, –O–$CH_2$–$CH_2$–), 2.05 (s, 3H, $COCH_3$), 1.51 (d, 3H, *J*=6.5Hz, $CH_3$), IR ν(neat), cm$^{-1}$: 1727, 1214 (CO), 1141 (C–O–C), 941, 860 (1,2,4-substituted benzene). Anal. calc. for $C_{18}H_{26}O_7$, C, 61.00; H, 7.39. Found; C, 60.98; H, 7.35.

*Synthesis of* (±)-**3:** Acetylated chlorohydrin derivative (±)-**3** was prepared from corresponding crown ether, 4′-(1-chloro-2-hydroxyethyl)-benzo-15-crown-5-ether. (±)- 4′-(1-chloro-2-hydroxyethyl)-benzo-15-crown-5-ether was prepared by Friedel-Crafts reaction of benzo-15-crown-5-ether with chloroacethyl chloride,[5] followed by reduction with $NaBH_4$[6] and then acetylated by acetic anhydride described above. **;**(±)-**3**: colourless crystal, 87 %; m.p. 84–87°C, $^1$H NMR (500 MHz, $CDCl_3$) δ6.90–6.82 (m, 3H, Cp), 5.89 (dd, 1H, *J*=3.4, 4.8 Hz, CH), 4.18–4.11 (m, 4H, –O–$CH_2$–$CH_2$–), 3.94–3.88 (m, 4H, –O–$CH_2$–$CH_2$–), 3.76 (s, 8H, –O–$CH_2$–$CH_2$–), 3.72 (d, 2H, $CH_2$), 2.13 (s, 3H, $COCH_3$), IR ν(KBr), cm$^{-1}$: 1743, 1230 (CO), 1141 (C–O–C), 941, 860 (1,2,4-substituted benzene). Anal. calc. for $C_{18}H_{25}O_7Cl$, C, 55.60; H, 6.48. Found; C, 55.72; H, 6.50.

*Lipase-catalysed hydrolysis*: As a typical example is described below: To a solution of (±)-**2** (233 mg, 0.6 mmol) in 10 ml of the chosen solvent, CAL (233 mg) was added and the suspension was incubated at 30°C under reciprocal shaking (about 150 cycles min$^{-1}$). The reaction was stopped by filtration of the enzyme, and the filtrate was extracted with chloroform, dried over sodium sulfate, and evaporated to dryness. The product (*R*)-**3** and remaining substrate (*S*)-**2** were separated by column chromatography on silica gel using ethyl acetate/hexane (50:50) as eluent. The first elution afforded the remaining substrate (*S*)-**2** and the second elution gave the product alcohol (*R*)-**3**. Others were similarly examined and the results were summarised in Table 1.

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