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Enzymatic Generation of Planar Chirality in the (Arene)Cr(CO)3 Series: Experimental Results and Modelling Studies

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Abstract: Hydrolysis using pig liver esterase of (benzene-1,2-diacetic ester)Cr(CO)3 complexes generates the half-ester in high enantiomeric purity. The high enantioselectivity may be rationalised through substrate interaction with the Jones active site model of pig liver esterase.

INTRODUCTION

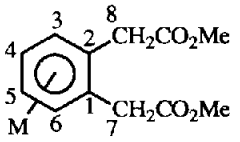
The use of biotransformation methods for the synthesis of enantiomerically pure organic compounds continues to increase[1]. Though differing in the type of chirality (planar as opposed to carbon centred) and in their sensitivity to oxidation and compatibility with aqueous media, enzymatic methods are also finding increasing application to the synthesis of enantiomerically pure organometallic complexes.[2] (Arene)Cr(CO)3 complexes continue to attract interest as synthetic intermediates, and biotransformation methods based on esterification[3] or reduction[4] have been used in the kinetic resolution of several substrates. We wish to report here our full results[5] on the enzymatic generation of planar chirality through hydrolytic desymmetrization of a *meso* substrate. Lipase catalysed acylation of (1,2-benzenedimethanol)Cr(CO)3 has been used to generate planar chirality with e.e. values of 80-90 %,[3c] and recently, generation of planar chirality through Pd-catalysed cross coupling has been reported,[6] though with low enantiomeric excess. We describe also molecular modelling studies of substrate recognition by the active site of pig liver esterase (PLE) which provide some insight into the high enantioselectivity observed in this system.

RESULTS AND DISCUSSION

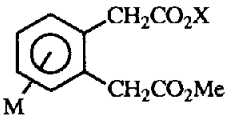
The diester complexes **1** and **2** were prepared by standard methods from the free ligands and $Cr(CO)_6$. For enzymatic hydrolysis, we have found that the cheaper crude extract, pig liver acetone powder, gives excellent results.[7] Controlled hydrolyses of **1** and **2** using PLE proceeds cleanly with consumption of one mole of NaOH to provide the half esters **3** and **4** in high yield. In common with other carboxy substituted (arene)Cr(CO)3 complexes, **3** and **4** are of limited solution stability and were converted into the enantiomeric

diesters **7a,b** by treatment with ethyl or methyl chloroformate respectively. This reaction presumably proceeds through formation of mixed anhydrides which undergo facile CO_2 elimination to give **5a,b**. In organic systems, such facile elimination is particularly facilitated by the presence of electron withdrawing substituents β to the carbonyl.[8] In this respect, the electron withdrawing nature of the $Cr(CO)_3$ group is well established.[9]

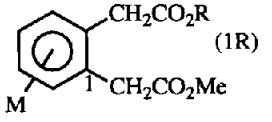
Analysis by chiral HPLC reveals e.e. values of 94 and 99 % for **5a,b** respectively, by comparison with a racemic sample. The absolute configuration has been determined from a single crystal study of the menthyl derivative **6** prepared from reaction of **3** with (-)-menthyl chloroformate (Figure 1, Table 1). This complex may also be used for the estimation of enantiomeric purity, since an additional set of four well defined doublet resonances due to the μ-CH_2 protons are observed in material prepared from racemic **3**. No kinetic resolution occurs on reaction of **3** with (-)-menthyl chloroformate over long reaction times. Specific rotations are relatively small, and the CD spectrum of **6** reveals only small Δε values in the region of the spectrum attributable to d-d transitions.



1 M = $Cr(CO)_3$

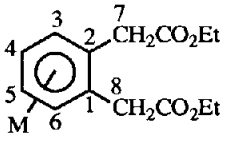


3 X = H



5a R = Et

6 R = (1R',2S',5R') menthyl



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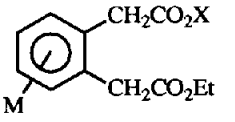
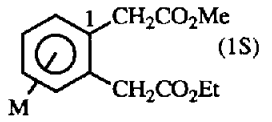
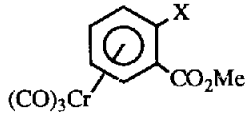


4 X = H



5b

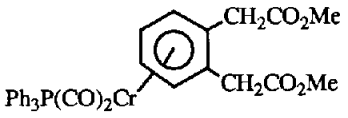
To further understand the high enantioselectivity of this reaction, we have investigated modelling of the substrate-enzyme interaction using as a basis the site model proposed recently for PLE[10] and structures of **1** and the **inactive** substrates **7** to **10**.



7 X = CO_2Me

8 X = Me

9 X = OMe

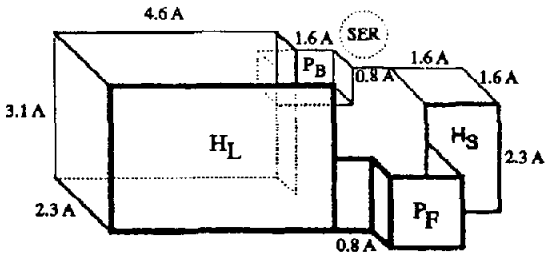


10

The structures of **1** and **10** have been determined during this work, and important features of the metal coordination geometry are given in Figure 1 and Table 1. The ester conformation of the inactive substrate **8** has been modelled using as a basis the structure of the $Cr(CO)_2(+)$ - PPh_2(neomenthyl) derivative.[11] Despite several attempts, we have been unable to grow satisfactory single crystals of **7**. Modelling of this substrate

was achieved through attachment of a Cr(CO)3 fragment to the MOPAC energy minimised structure of the free ligand.[12]

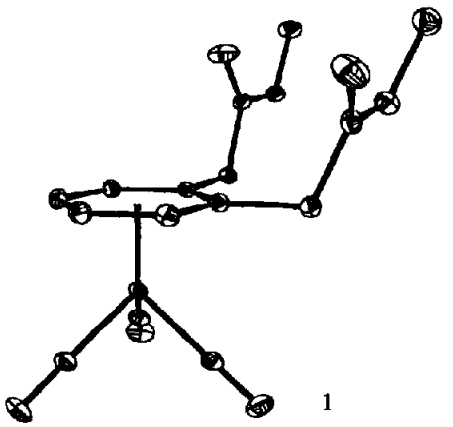
The Jones model[10] of the active site of PLE is based on an arrangement of five cubic regions of space, as illustrated in **12**. H_L and H_S represent two hydrophobic zones of volumes 33 $Å^3$ and 5.5 $Å^3$ respectively. These pockets may accommodate weakly polar heteroatoms such as halogen and ether or ketal oxygen if necessary. Two other sectors (P_B and P_F) accept strongly polar or hydrophilic fragments. The most common role of P_F is to bind the eventual non-hydrolysed ester group of diesters. In contrast to P_F, P_B is too polar to accept hydrophobic moieties. It interacts well with hydrogen donors and a variety of alcohol, ether and carbonyl functionalities. The essential catalytic region involves the serine residue at P_B which initiates hydrolysis by attack at the reactive ester group. To be hydrolysed, the carbon atom of the methyl ester being hydrolysed must be placed within an sp^3 carbon-oxygen bond length of the serine sphere so that it may readily proceed to the tetrahedral intermediate required in serine protease-catalysed hydrolyses.



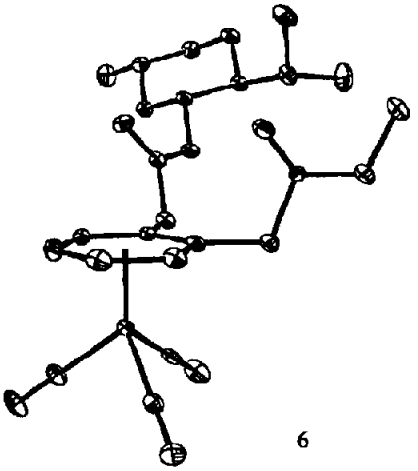
(12)

Modelling shows that in all cases, the smaller hydrophobic site H_S is too small to accept the (arene)Cr(CO)3 residue. Using the larger H_L site, as shown in **13**, substrate **1** fits appropriately into the active site in the orientation which leads to the observed absolute configuration. For **1**, it is of interest to note that the structure observed in the solid state lacks a plane of symmetry. While both β-ester carbons are perpendicular to the ring (dihedral angle with ring = 88/101 °), the ester substituent at C8 is twisted such as to maintain approximate coplanarity of the two ester moieties (interplanar angle = 18 °). The staggered orientation of the Cr(CO)3 unit relative to the ring is that preferred for an o-disubstituted complex in which the substituents have identical or nearly identical properties,[14] but is also rotated slightly from mirror plane symmetry. The loss of symmetry in the menthyl complex **6** is even greater. Twisting of the β-ester carbons away from the perpendicular (dihedral angle with ring = 104/111 °) and twisting about both CH_2CO_2Me bonds generates an interplanar angle between the ester groups of 129 °. Though the orientation of the Cr(CO)3 group remains staggered, a unique carbonyl now bisects the angle **between** the ester substituents. Thus, flexibility in both substituent and Cr(CO)3 conformation may be important in maximising selectivity in the enzyme-substrate interaction.

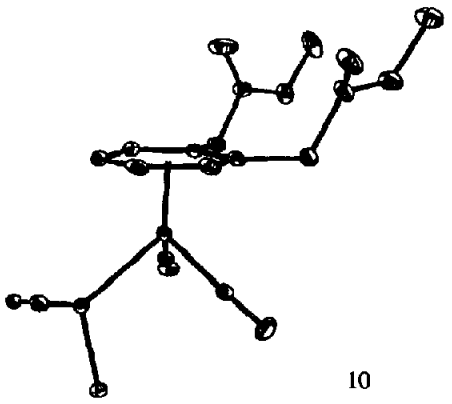
Figure 1. Molecular Structures of 1, 6 and 10



1



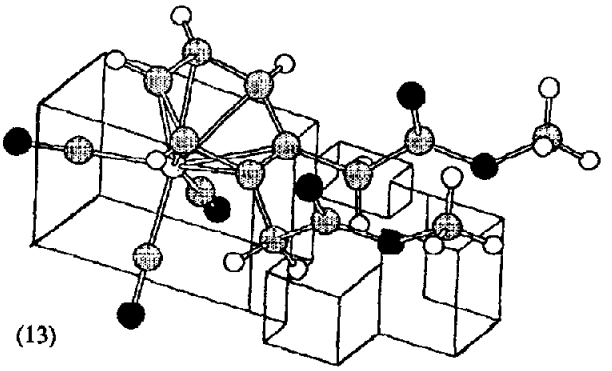
6



10

Table 1 Important Geometrical Data

	1	6	10
Cr-C (ring) (av) (Å)	2.22	2.21	2.18
Cr-CO (av) (Å)	1.84	1.84	1.81
Cr-P (Å)	-	-	2.343
CO-Cr-CO (av) (°)	88.7	89.0	91.5
CO-Cr-P (av) (°)	-	-	89.4



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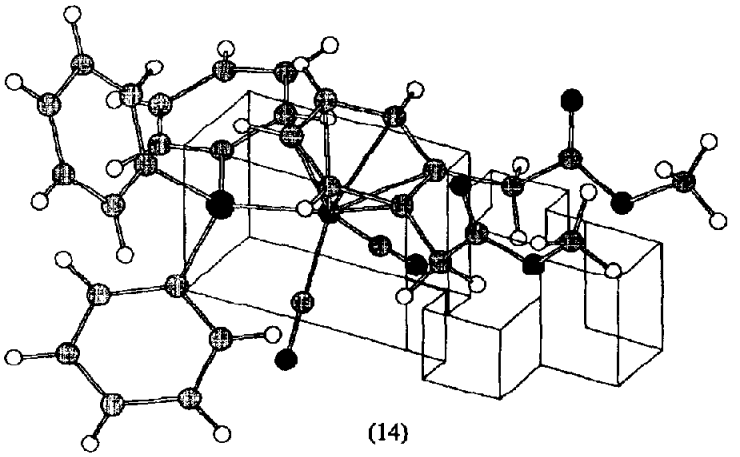


(14)

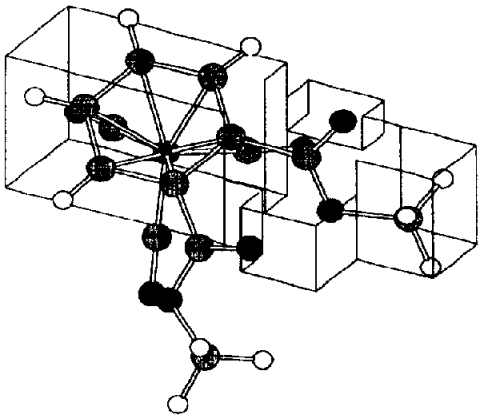
In the phosphine derivative **10**, the staggered orientation of the $Cr(CO)_2L$ moiety is maintained, with PPh_3 furthest from the ester substituents. While the configuration of the ester groups remains essentially the same as the tricarbonyl, it is clear from **14** that the sterically demanding PPh_3 ligand precludes access to the hydrophobic H_L site.

O-disubstituted substrates lacking the spacer CH_2 group such as **8**, **9** and the *meso* complex **7** do not undergo hydrolysis on treatment with PLE.[15] The fit of the **modelled** substrate **7** to the active site is shown in **15**. The twist of the molecule in H_L which is required to present an ester face rather than edge to the serine residue is precluded by the steric demands of the other ester substituent. As shown in **16**, a similar rationale may be applied to substrate **8**, where the deviation of the ester group from coplanarity with the arene ring is less marked.

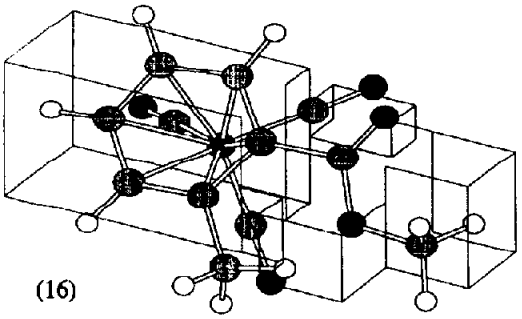
Finally, it may be noted that the *meta* substrate **17** does undergo hydrolysis with PLE with poor but **opposite** enantioselectivity.[2c]

Increased reactivity and opposite enantioselection (though weak) may possibly result from a side-on fit of the molecule into the active site [shown in **19**] in which the *meta* methyl group projects from the open face of H_L and the ester moiety is presented face on to the serine residue at a distance of about 1.1 - 1.2 Å. Such

an orientation is not possible for an *ortho* substituted complex due to interaction of the *ortho* substituent with the H_L/P_B interface.



(15)



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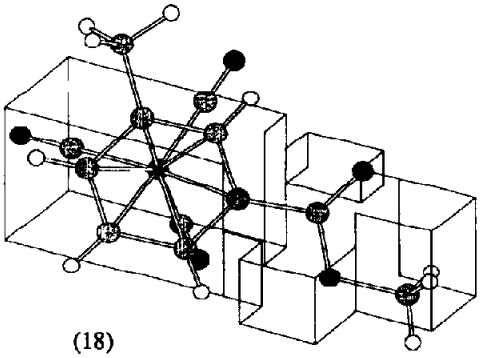
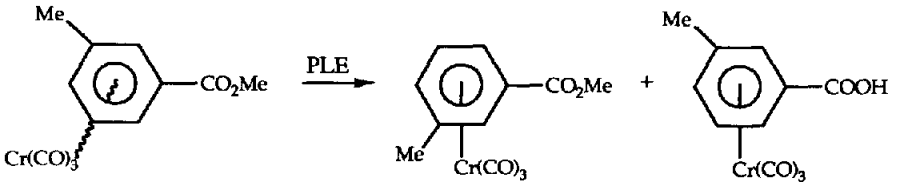


(18)



17 **17**(1S) 6 % e.e. **18**(1R) 22 % e.e.

EXPERIMENTAL

^1H NMR spectra were obtained on a JEOL 270 spectrometer; chemical shifts are relative to TMS. Optical purities were determined using a Chiralcel O.J. column on a Beckman System Gold apparatus. Reactions involving organometallic compounds were conducted under nitrogen. The free benzene-1,2-diacetic esters were prepared by acid catalysed esterification of benzene-1,2-diacetic acid.[18] All $Cr(CO)_3$ complexes were obtained by the standard Pauson procedure.[19] Pig liver acetone powder (Sigma L8251) was obtained commercially. Spectral and analytical data for new complexes are given below.

1 M.p.: 96-97 °C; microanalysis: (calc) C-50.3, H-3.91 %, (found) C-50.4, H-3.70 %; infrared (hexane): 1979, 1911, 1903 cm^{-1}; ^1H NMR (C_6D_6) : 4.53 (dd, H3/6, J_{3-4} = 4.8, J_{3-5} = 2.9), 4.31 (dd, H4/5), 3.10, 2.70 (d, H7/8, Jvic = 16.0), 3.24 (s, CO_2Me).

2 M.p.: 71-72 °C; microanalysis: (calc) C-52.8, H-4.67 %, (found)C- 52.6, H-4.40 %; infrared (hexane): 1980, 1912, 1904 cm^{-1}; ^1H NMR (C_6D_6): 4.66 (dd, H3/6, J_{3-4} = 4.9, J_{3-5} = 2.8), 4.42 (dd, H4/5), 3.20, 2.80 (d, H7/8, Jvic = 16.1), 3.89, 0.95 (q,t, $CO_2CH_2CH_3$, J = 7.1).

7 M.p. 61-62 °C; microanalysis: (calc) C-47.3, H-3.03 %, (found) C-47.5 %, H-2.98 %; infrared (hexane): 1995, 1931 cm^{-1}; ^1H NMR (C_6D_6): 4.98 (dd, H3/6, J_{3-4} = 4.8, J_{3-5} = 2.7), 4.16 (dd, H4/5), 3.36 (s, CO_2Me).

(a) Preparation of racemic 3, 4 and 5

Complex **1** (1.6 g, 4.5 mmol) was dissolved in methanol (30 ml). To this was added a solution made from addition of potassium (0.27 g, 4.8 mmol) to water (10 ml). The mixture was stirred overnight and then diluted with water (150 ml). The precipitated hemiester **3** is obtained (0.95 g, 61%) after extraction with ethyl acetate and removal of solvent. The racemic diester **5** was obtained by dissolution of **3** (0.74 g, 2.1 mmol) in thf (15 ml) followed by sequential addition at -10 °C of NEt3 (1.2 equivalents) and ethylchloroformate (1 equivalent). After warming to room temperature and stirring for 2 hours, the mixture was filtered through silica. After removal of thf, the residue was purified by preparative tlc (eluant 5:3 diethylether/pentane to give **5** (0.7 g, 83%) which was crystallized from diethylether/heptane. M.p.: 90-91 °C; microanalysis: (calc) C-51.6, H-4.30 %, (found) C-51.4, H-4.10 %; infrared (hexane): 1977, 1910, 1904 cm^{-1}; ^1H NMR (C_6D_6): 4.57 (m, H3/6), 4.32 (m, H4/5), 3.15 (intensity 2H), 2.75, 2.73 (d, H7/8, all J_{vic} = 16.1), 3.26 (s, CO_2Me), 3.86, 0.93 (q, t, $CO_2CH_2CH_3$, J = 7.1).

(b) Enzymatic hydrolysis of 1 and 2

PLE (1.3 g) was added as a powder to a solution of **1** (1 g, 2.8 mmol) in 10 ml of a 9:1 water/methanol mixture previously adjusted to pH 7.2 with 2M NaOH. The pH was maintained at 7.2 during hydrolysis by addition of 2M NaOH from an automatic burette. After cessation of reaction at half-hydrolysis, the solution was acidified with 0.1 M HCl and extracted with ethyl acetate to give **3** (0.8 g, 85 %). Treatment with ethyl chloroformate as described in (a) gave complex **5** of 94 % e.e. ($[\alpha]_D$ + 11.3, c = 7.1 x 10^{-3}, ethyl acetate). Similar treatment of **3** with (-)-menthyl chloroformate gave **6** as a single diastereoisomer. M.p.: 112-113 °C; microanalysis: (calc) C-59.8, H-6.22 %, (found) C-59.5, H-6.01 %; infrared (hexane): 1979, 1911, 1903 cm^{-1}; $[\alpha]_D$ - 22.5, c = 0.54, acetone; CD: λ_{max} ($\Delta\varepsilon$) 320 (+ 0.4), 280 (0), c = 1 x 10^{-3}, CH_3CN; ^1H NMR (C_6D_6): 4.2 - 4.8 (m, H3-6), 3.11, 2.77 (d, H7 or 8, J_{vic} = 16.2), 3.28, 2.88 (d, H7 or 8, J_{vic} = 16.4), 3.30 (s, CO_2Me), 4.78, 0.89, 0.92 (m, d, d, $CHMe_2$, J = 7.0), 0.76 (d, Me, J = 6.5), 0.7 - 2.0 (m, ring menthyl).

Enzymatic hydrolysis of **2**, followed by subsequent reaction of **4** with methyl choroformate gave the opposite enantiomer of **5** in 99 % e.e. ($[\alpha]_D$ -11.4, c = 7.1 x 10^{-3}, ethyl acetate).

(c) Preparation of 10

Complex **1** (1.0 g, 3.3 mmol) and PPh_3 (0.86 g, 3.3 mmol) were dissolved in heptane (200 ml) and irradiated for 2 hours using a 90 W medium pressure mercury lamp. Evaporation of solvent followed by purification by preparative tlc (5:3 diethyl ether/pentane) gave **10** as a red solid (0.53 g, 30 %). M.p.: 155 - 157 °C; microanalysis: (calc) C-64.9, H-4.93%, (found) C-65.1, H-4.66 %; infrared (CH_2Cl_2): 1885, 1829 cm^{-1}. ^1H NMR (C_6D_6): 4.48 (m, H3/6), 4.29 (m, H4/5), 3.56, 3.03 (d, H7/8, J_{vic} = 16.2).

(d) Crystallographic Data

Structures were solved by direct methods (SHELXS-86)[20] and refined by full matrix least squares (SHELXL-93).[21] Data were corrected for Lorentz and polarisation effects. Hydrogen atoms were included in calculated positions with thermal parameters 30 % greater than the atom to which they were attached. The absolute configuration of **6** was determined by the Flack absolute structure parameter which was close to zero. The non-hydrogen atoms were refined anisotropically. All calculations were performed on a VAX 6610 computer. The ORTEX program was used to obtain the drawings.[22] Full listings of crystallographic data (collection details, data reduction and refinement, atomic coordinates, thermal parameters, positional parameters, calculated and observed structure factors, bond lengths and angles) have been deposited at the Cambridge Crystallographic Data Centre.

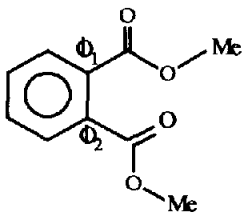
1 Monoclinic, space group $P2_1/c$, a = 13.518(3), b = 8.7570(10), c = 14.596(3) Å, β = 112.838(9)°, Z=4, R_1= 0.0381 for 208 parameters and 2943 observed reflections.

6 Orthorhombic, space group $P2_12_12_1$, a = 7.612 (3), b = 12.425 (2), c = 25.487(6) Å, Z = 4, R_1 = 0.0949 for 294 parameters and 3298 observed reflections.

10 Triclinic, space group $P\bar{1}$, a = 9.241(1), b = 9.873(1), c = 16.274(1)Å, α = 107.702(7), β = 95.502 (6), γ = 94.032 (7) °, Z = 2, R_1 = 0.0446 for 370 parameters and 3609 observed reflections.

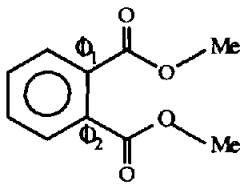
References

1. For reviews on the use of PLE in organic systems, see (a) Jones, J.B. *Pure Appl. Chem.*, **1990**, *62*, 1445-1448; (b) Tamm, C. *Pure Appl. Chem.*, **1992**, *64*, 1187-1191; (c) Zhu, L.M.; Tedford, M.C. *Tetrahedron*, **1990**, *46*, 6587-6611; (d) Ohno, M.; Otsuka, M. *Organic Reactions*, **1989**, *37*, 1-55.
2. For reviews, see (a) Howell, J.A.S.; Jaouen, G. *Trends in Organic Chemistry*, **1993**, *4*, 669-695; (b) Ryabov, A.D. *Angew. Chem., Int. Ed. Engl.*, **1991**, *30*, 931-941; (c) Bergbreiter, D.E.; Momongan, M. *Biochem. Biotech.*, **1992**, *32*, 55-72; (c) Yamazaki, Y.; Hosono, K. *Ann. N.Y. Acad. Sci.*, **1990**, *613*, 738-746.
3. (a) Nakamura, K.; Ishihara, K.; Ohno, A.; Uemura, M.; Nishimura, H.; Hayashi, Y. *Tetrahedron Lett.*, **1990**, *31*, 3603-3604; (b) Yamazaki, Y.; Hosono, K. *Tetrahedron Lett.*, **1990**, *31*, 3895-3896; (c) Yamazaki, Y.; Hosono, K. *Agric. Biol. Chem.*, **1990**, *54*, 3357-3361; (d) Yamazaki, Y.; Morohashi, N.; Hosono, K. *Biotechnology Lett.*, **1991**, *13*, 81-86; (e) Baldoli, C.; Maiorana, S.; Carrea, G.; Riva, S. *Tetrahedron: Asymmetry*, **1993**, *4*, 767-772.
4. (a) Yamazaki, Y.; Hosono, K. *Agric. Biol. Chem.*, **1988**, *52*, 3239-3240; (b) Yamazaki, Y.; Uebayasi, M.; Hosono, K. *Eur. J. Biochem.*, **1989**, *184*, 671-680; (c) Yamazaki, Y.; Hosono, K. *Tetrahedron Lett.*, **1989**, *30*, 5313-5314; (d) Gillois, J.; Jaouen, G.; Buisson, D.; Azerad, R. *J. Organomet. Chem.*, **1989**, *367*, 85-93; (e) Top, S.; Jaouen, G.; Baldoli, C.; Del Buttero, P.; Maiorana, S. *J. Organomet. Chem.*, **1991**, *413*, 125-135.
5. For a preliminary communication, see Malezieux, B.; Jaouen, G.; Salaün, J.; Howell, J.A.S.; Palin, M.G.; McArdle, P.; Cunningham, D.; O'Gara, M.; *Tetrahedron: Asymmetry*, **1992**, *3*, 375-376.
6. Uemura, M.; Nishimura, H.; Hayashi, T. *Tetrahedron Lett.*, **1993**, *34*, 107-110.
7. For a recent organic example, see Basavaiah, D; Rama Krishna, P.; Bharathi, T.K. *Tetrahedron: Asymmetry*, **1995**, *6*, 439-454.
8. Windholz, T.B. *J. Org. Chem.*, **1960**, *25*, 1703-1708.
9. See, for example, Hegedus, L.S. *Transition Metals in the Synthesis of Complex Organic Molecules*, University Science Books, Mill Valley, California, 1994, pp. 307-322.
10. Toone, E.J.; Werth, M.J.; Jones, J.B. *J. Am. Chem. Soc.*, **1990**, *112*, 4946-4952.
11. Jaouen, G.; Howell, J.A.S.; Tirvengadum, M.C.; McArdle, P.; Cunningham, D. *J. Organomet. Chem.*, **1989**, *370*, 51-64.
12. MOPAC energy minimisation of the ester conformation of dimethyl o-phthalate yields **11a** as an energy minimum, though **11b** is only 0.4 kcal higher. The key feature of both is a significant twist of the ester substituents out of the arene plane. Two crystal structures of related compounds indeed demonstrate the **11a** conformation.(13)



11a

$\Phi_1 = 49°$, $\Phi_2 = 34°$



11b

$\Phi_1 = 37°$, $\Phi_2 = 53°$

Coordination of $Cr(CO)_3$ may alter ϕ_1/ϕ_2 values, or change the order of stability of **11a/11b**.

13(a) Hunger, J.; Wolff, C.; Tochtermann, W.; Peters, E.M.; von Schneri, H.G. *Chem. Ber.*, **1986**, *119*, 2698-2722; (b) Bocelli, G.; Grenier-Loustalot, M.F. *Acta Cryst.*, **1986**, *42C*, 379-381.

14. Muetterties, E.L.; Bleeke, J.R.; Wucherer, E.J.; Albright, T.A. *Chem. Rev.*, **1982**, *82*, 499-525.

15. Similar observations have been reported by other workers; see reference 2(c). For purely organic substrates, few examples of PLE hydrolyses of benzoate esters have been reported,[16] though hydrolysis of phenylacetate esters is well established.[17] We have confirmed that the free ligand of **7** is indeed an active substrate for PLE.

16(a) Lettenberg, C; Gautier, A.; Ohleyer, E. *Appl. Biochem. Biotechnol.*, **1992**, *37*, 43-52; (b) Baraldi, P.G.; Bazzarini, R.; Manfredini, S.; Simoni, D.; Robins, M.J. *Tetrahedron Lett.*, **1993**, *34*, 3177-3180.

17. Barton, P.; Laws, A.D.; Page, M.I. *J. Chem. Soc., Perkin Trans.* 2, **1994**, 2021-2029.

18. De Luca, C.; Inesi, A.; Rampasso, L. *J. Chem. Soc., Perkin Trans.* 2, **1983**, 1821-1830.

19. Mahaffy, C.A.L.; Pauson, P.L. *Inorg. Synth.*, **1990**, *28*, 137-140.

20. Sheldrick, G.M. *Acta Cryst.*, **1990**, *A46*, 467-473.

21. Sheldrick, G.M. SHELXL-93, A Computer Program for Crystal Structure Determination, University of Gottingen, 1993.

22. McArdle, P. *J. Appl. Cryst.*, **1994**, *27*, 438-442.

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