High Acylation Rates and Enantioselectivity with Cyclodextrin Complexes of Rigid Substrates

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Abstract: Previous work in these laboratories has shown that the acylation of β-cyclodextrin by *p*-nitrophenyl 3-*trans*-ferrocenylpropenoate is an excellent model for the first step in the serine protease catalyzed hydrolysis of esters. Saturation kinetics were observed, and rate accelerations on the order of 10^6 were attained. We report herein that improvement in the rate acceleration can be realized by freezing out residual rotational degrees of freedom in the acylation transition state. Partial immobilization of the acylate side chain has been accomplished by bridging to the ferrocene nucleus, resulting in a nearly 10-fold increase in the rate acceleration. Furthermore, a high enantioselectivity in the acylation of β-cyclodextrin by this bridged substrate has been observed with a 20-fold rate difference for the two enantiomers. The absence of a differential solvent deuterium isotope effect is offered as evidence that the enantioselectivity is not due to differential hydrogen bonding in the transition state (general-acid catalysis). The determination of the absolute configuration of the fast enantiomer together with the known configuration of β-cyclodextrin has allowed the postulation of a geometric basis for the observed enantioselectivity.

Introduction

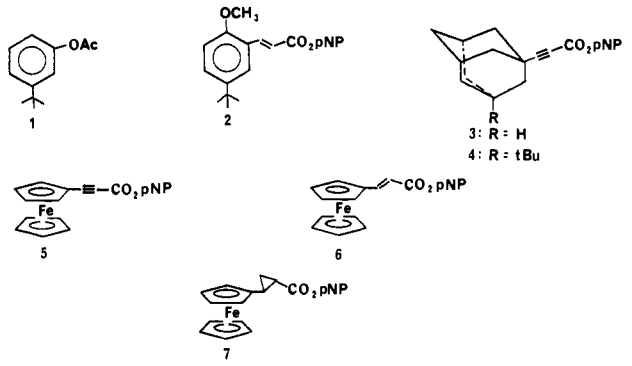
In studies of cyclodextrins (cycloamyloses) as enzyme models,[1] particular interest has attended those processes in which a substrate binds into the cyclodextrin molecular cavity and then undergoes reaction with one of the cyclodextrin hydroxyls.[2-6] The velocity of such an intracomplex process can be compared with that of a suitable intermolecular reaction as an indication of the effect of complexing on reaction rates. Enzymes achieve much of their catalytic efficiency by molecular complexing which brings the substrate into good proximity to the catalytic groups. Thus if molecular complexing by cyclodextrins is to be the basis of catalysis by artificial enzymes constructed on the cyclodextrin framework,[7] it is important to learn how to achieve accelerations of enzyme-like magnitude for at least some reactions of cyclodextrin molecular complexes.

Enzymes at kinetic saturation typically achieve rate accelerations of 10^5–10^{10} for their catalyzed reactions compared with uncatalyzed substrate reactions under the same pH and temperature conditions. However, until recently the maximum acceleration of a process promoted by complexing with an unmodified cyclodextrin was only 250-fold, for acylation of β-cyclodextrin by *m-tert*-butylphenyl acetate (**1**).[3] The rate of acylation within the complex, which is first order in hydroxide ion, is compared with the rate of acylation of solvent in the absence of cyclodextrin, a process which is also first order in hydroxide ion.

We have recently described[6] a study in which we attempted to optimize the geometry of the substrate for such intracomplex acylations. Molecular models suggested that for the best previous cases the substrate would be better bound than would be the transition state, while to achieve fast reactions the converse situation should obtain. We were able to improve the rate ratio for intracomplex acylation relative to simple hydrolysis, $k_{complex}/k_{un}$, by two kinds of approach. In one, we modified the cyclodextrin cavity by adding an intrusive floor, so as to bring the position of the bound substrate closer to that of the bound transition state. This meant that less binding energy need be lost on reaction; as expected this led to an improvement in $k_{complex}/k_{un}$. Larger effects were observed from changing the nature of the substrate.

Derivatives of *m-tert*-butylcinnamic acid *p*-nitrophenyl ester (**2**) showed improved acylation rates on complexing; *p*-nitrophenyl esters of adamantanepropiolic acid (**3**) and of *tert*-butyladamantanepropiolic acid (**4**) were better yet, but the largest accelerations were seen with some ferrocene derivatives. The *p*-nitrophenyl esters of ferrocenylpropiolic acid (**5**) and of ferrocenylacrylic acid (**6**) showed very large accelerations, with reported $k_{complex}/k_{un}$'s of 140 000 and 750 000, respectively. The improvement of this rate ratio by over 3 orders of magnitude brought it into the region characteristic of enzymes with moderate effectiveness, specifically of α-chymotrypsin.

The general sequence of effectiveness of these substrates **1** through **6** was as expected from molecular models.[6] For a



transition state of acylation resembling the tetrahedral intermediate the best binding occurs if the chain carrying the acyl group is essentially perpendicular to the axis of the bulky hydrophobic group of the substrate. Such perpendicularity is present in the ferrocene compounds such as **5**, in contrast to a larger angle in the meta-substituted phenyl rings of substrates **1** and **2**. However, it was not clear from molecular models why the ferroceneacrylic acid derivative (**6**) was better than the propiolate (**5**). In the ferroceneacrylate ester the side chain has two single bonds which, on rotation, generate a variety of conformations. By contrast, with the acetylenic side chain of **5** only one of the single bonds generates new conformations on rotation. Since in the transition state for acylation most of this nominal free rotation is frozen, one would expect that a greater loss of freedom in the reaction of **6** with cyclodextrin would make it a less effective substrate than **5**. The actual result was opposite to this expectation.

Conjugation effects should tend to freeze the acrylate (**6**) into a preferred conformation (**6'**) in which the π systems of the ester, of the vinyl group, and of the ferrocene system are parallel. Molecular models show that this geometry is actually the one

(1) For a review, cf.: Bender, M. L.; Komiyama, M. "Cyclodextrin Chemistry"; Springer-Verlag: New York, 1977.
(2) Heinrich, N.; Cramer, F. *J. Am. Chem. Soc.* **1965**, *87*, 1121–1126.
(3) VanEtten, R. L.; Sebastian, J. F.; Clowes, G. A.; Bender, M. L. *J. Am. Chem. Soc.* **1967**, *89*, 3242–3253, 3253–3262.
(4) van Hooidonk, C.; Breebart-Hansen, J. *Recl. Trav. Chim. Pay-Bas* **1970**, *89*, 289–294. van Hooidonk, C.; Groos, C. *Ibid.* **1970**, *89*, 845–851.
(5) Siegel, B.; Breslow, R. *J. Am. Chem. Soc.* **1975**, *97*, 6869–6870.
(6) Breslow, R.; Czarniecki, M. F.; Emert, J.; Hamaguchi, H. *J. Am. Chem. Soc.* **1980**, *102*, 762–770.
(7) For our approaches to this, see: (a) Breslow, R.; Doherty, J.; Guillot, G.; Lipsey, C. *J. Am. Chem. Soc.* **1978**, *100*, 3227–3229; Breslow, R.; Hammond, M.; Lauer, M. *Ibid.* **1980**, *102*, 421–422; Breslow, R.; Bovy, P.; Lipsey Hersh, C. *Ibid.* **1980**, *102*, 2115–2117.

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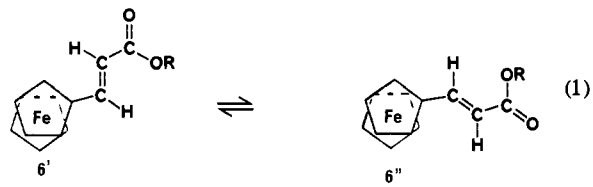
needed for reaction of the ester with a cyclodextrin hydroxyl group, so if this conformation were strongly frozen in by conjugation effects in the substrate, it would be less surprising that **6** is a more effective substrate than is **5**. Conjugation effects in **5** will also tend to hold the ester group in the plane of the cyclopentadienyl ring, of course, but if both **5** and **6** have essentially the correct geometry in their preferred conformations the slight rate advantage seen for **6** could reflect other small geometric differences.

Some evidence for this idea that **6** is largely frozen in the correct conformation comes from our examination of the corresponding cyclopropane derivative (**7**). We find that $k_{complex}/k_{un}$ is decreased by almost 3 orders of magnitude when a rigid vinyl group is replaced by a rigid cyclopropane ring. Models show that **7** can adopt the correct conformation for acylation of a cyclodextrin hydroxyl in the complex, but in the absence of strong conjugation effects it does not have to. Thus rotational freedom is lost on proceeding from bound starting material to bound transition state (to bound intermediate to bound product) with the resultant lower rate. However, NMR studies of **6** at room temperature show AA'BB' symmetry in the protons of the substituted ferrocenyl ring, suggesting conformational averaging (e.g., between conformations **6'** and **6''**) on the NMR time scale (see eq 1). While freezing



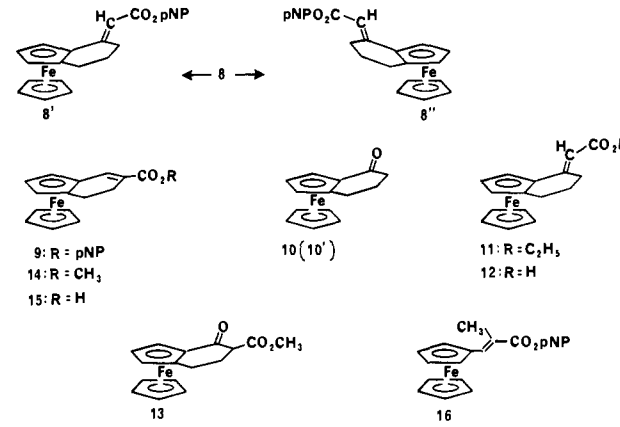
(1)

out an already partially hindered rotation does not have kinetic consequences as large as those for freezing out a really free rotation during reaction,[8] we felt that further improvement in $k_{complex}/k_{un}$ could be attained with substrates related to **6** in which the conformation is more fully locked. Accordingly, we have modified structure **6** into two fused-ring versions, viz., **8** and **9**. One of these structures **9** was inferior to **6** as a substrate, but the other (**8**) was the best substrate we have examined so far. Furthermore, both **8** and **9** are D,L mixtures (as is the **6'**,**6''** mixture of conformers). We find that chiral β-cyclodextrin shows an enantiomeric preference with both **8** and **9**.

Results

Reaction of 1,2-ferrocenocyclohexen-3-one (**10**)[9] under Reformatsky conditions afforded the reported mixture of *E* and *Z* isomers of the (carbomethoxy)methylene derivative (**11**).[10] Saponification and careful crystallization yielded the pure *E* acid (**12**), which was converted in the normal way to the *p*-nitrophenyl ester (**8**). Carbomethoxylation of **10** afforded **13** as an epimeric mixture which was converted to **14** by reduction and dehydration. This was saponified to the carboxylic acid **15**, which was converted to the *p*-nitrophenyl ester (**9**). Because of the rate results with **8**, we also prepared the *p*-nitrophenyl ester of (*E*)-3-ferrocenyl-2-methylpropenoate (**16**) by a straightforward sequence starting with a Wittig reaction on ferrocenylcarboxaldehyde.

As described in the Experimental Section, we have determined rate constants for acylation of β-cyclodextrin by these substrates in aqueous dimethyl sulfoxide solution with the same techniques we have described previously.[6] All compounds showed Michaelis–Menten kinetics, so we could measure both the Michaelis constant K_m for the complex, which we will refer to as a dissociation constant, and a rate constant $k_{complex}$ for acyl transfer from substrate to cyclodextrin in the complex at the operating pH. To



compare this with the rate of hydrolysis of the substrate at the same pH, we have adopted a different procedure from that we used previously.[6]

The enormous accelerations seen here make it somewhat impractical to measure k_{un} and $k_{complex}$ under the same experimental conditions. For example, at a pH at which **8** is reacting with cyclodextrin in a matter of seconds the $t_{1/2}$ for simple hydrolysis would be 8 months. Thus we perform the simple hydrolysis at a high pH, essentially the same pH (Table I) for all substrates. Since the simple hydrolyses, but not the cyclodextrin reactions, had shown buffer catalysis,[6] we achieve this high pH with KOH. Thus the relative magnitudes of k_{un} for different substrates are well established.

For the cyclodextrin acylation reactions the lower pH involved is attained with buffers. We had shown,[6] and confirm in these studies, that $k_{complex}$ in this pH region is first order in [OH^-], as determined by the pH meter. We had also previously shown,[6] and confirm in these studies, that the simple hydrolysis of such substrates is also first order in [OH^-]. Thus the only remaining problem is to relate the rates in these different pH regions, which is trivial if the readings of "pH" have the normal relationship to hydroxide concentration.

The pH meter was operated in the millivolt mode, as suggested[11] for such situations, but there is still a question of whether the glass electrode responds correctly to hydroxide concentration at these high pHs. We find that this is no problem for pHs which read in the 11–12 region, in which borate buffers of various ratios show the same increments in pH for pure H_2O or H_2O/Me_2SO solvent. However, changing a KOH concentration from 0.020 to 0.100 M in our H_2O/Me_2SO solution led to an observed pH change from 15.20 to 15.77, corresponding to a 3.7-fold rather than 5-fold change in hydroxide ion. With tetrabutylammonium hydroxide a change from 0.02 to 0.10 M in H_2O/Me_2SO led to a change in the pH reading 15.15 to 15.77, a 4.2-fold rather than 5-fold change in hydroxide ion. Thus at high pHs the pH meter may somewhat underestimate the [OH^-] concentration.[12]

Since this limited problem is coming in only at the highest pHs, we extrapolate the data for simple hydrolysis at high pH down to a calculated value of k_{un} at the pH of the cyclodextrin reaction by simply using the pH readings for the two sets of conditions. If there is an error from the glass electrode at high pH, the result of this procedure is a slight *underestimate* of $k_{complex}/k_{un}$ for the entire series but no change in relative values among compounds. The data are listed in Table I. In Table I we also list a set of $k_{complex}$'s and of k_{un}'s all calculated for pH 10.00. These can be used for readier comparisons among different compounds.

Our data for the ferroceneacrylate ester **6** differ slightly from those we had reported previously.[6] The values of $k_{complex}$ at a given pH and of K_m are both a bit higher as the result of careful repurification of the cyclodextrin, but the largest change is an increase in the value of k_{un} derived by our new procedure. The

(8) Page, M. I. *Chem. Soc. Rev.* **1973**, *2*, 295–323. See especially p 305.
(9) Preparation: Rinehart, K. L.; Curby, R. J.; Sokol, P. E. *J. Am. Chem. Soc.* **1957**, *79*, 3420–3424; Rinehart, K. L.; et al. *Ibid.* **1962**, *84*, 3263–3269. Resolution: Schlogl, K.; Fried, M.; Falk, H. *Monatsh. Chem.* **1964**, *95*, 576–597. Absolute configuration: Schlogl, K.; Falk, H. *Angew. Chem.* **1964**, *76*, 570.
(10) Rosenblum, M.; Abbate, F. W. *J. Am. Chem. Soc.* **1966**, *88*, 4178–4184. Although this is not reported, we find that the product is a mixture.
(11) Recommended by the manufacturer specifically for determining high pHs in media containing organic solvents.
(12) It is clear that this is not only a cation effect on the electrode, since some error is seen even with an organic cation.

Table I. Kinetic and Binding Constants at 30.0 °C

substrate	$k_{complex}$,[a,f] $s^{-1} \times 10^3$	pH[a]	$k_{complex}$ corrected to pH 10.00, $s^{-1} \times 10^3$	K_m,[b,f] mM	k_{un},[c,f] $s^{-1} \times 10^3$	pH[c]	k_{un} corrected to pH 10.00, $s^{-1} \times 10^8$	$k_{complex}/k_{un}$	k_{fast}/k_{slow}[e]
ferroceneacrylate 6	167 ± 7	10.24	96	8.8 ± 0.5	41.4 ± 0.6	15.19	26.7	360 000	
exocyclic ester 8	5.27 ± 0.23	10.17	3.56	4.6 ± 0.3	3.56 ± 0.03	15.20	2.25	160 000	
	107 ± 9	10.17	72.3	3.8 ± 0.5				3 200 000	20
endocyclic ester 9	13.6 ± 0.4	10.94	1.56	4.5 ± 0.2	27.8 ± 1.2	15.26	15.3	10 000	
	87.6 ± 7.2	10.94	10.1	3.8 ± 0.5				66 000	6.6
α-methylacrylate 16	38.7 ± 1.4	11.34	1.77	7.0 ± 0.4	32.4 ± 0.5	15.22	19.5	9 100	

[a] Pseudo-first-order rate constant for acylation of β-cyclodextrin by fully bound substrate in Me_2SO/H_2O at the "pH" indicated, as measured with a glass electrode. [b] Dissociation constant of the substrate-cyclodextrin complex. [c] Pseudo-first-order rate constant for hydrolysis of the substrate in the absence of cyclodextrin at the "pH" indicated. [d] k_{un} corrected by use of the pH meter readings. Cf. Discussion. [e] Relative values of $k_{complex}$ for the fast and slow enantiomers. [f] All errors are standard deviations.

result, as Table I shows, is that we now estimate a value of $k_{complex}/k_{un}$ of 360 000 for **6** rather than the 750 000 reported previously.[6] If this is correct and does not reflect the possible systematic error discussed above, the new value still makes **6** the best known substrate before the present work.

The observations with **8** were striking. Two different pseudo-first-order rate processes were seen for acylation of β-cyclodextrin, and as Table I shows, the fast reaction, corresponding to 50% of the substrate, had 20 times the rate of the second process which consumes the other 50%. Of course "**8**" is a D,L mixture of **8′** and **8″**. This is also true of the cyclohexenone **10**, which has been resolved and had its absolute configuration determined.[9] We carried out the partial resolution of **10**, as described,[9] to obtain material with $[\alpha]^{23}_D$ of +390 ± 10°, representing a 67% enantiomeric excess of configuration **10′**. We then converted this to **8′**, which proved to be dextrorotatory with $[\alpha]^{23}_D$ of +1260 ± 110°. Reaction of the original **8′**,**8″**, D,L mixture with β-cyclodextrin for the first fast reaction period, then isolation, afforded unreacted **8** enriched in the levorotatory isomer, with $[\alpha]^{23}_D$ of the recovered material of −1240 ± 30°. Thus the fast enantiomer of **8** is **8′**, while the slow one is **8″**.

Related behavior was seen for the endocyclic olefin **9**, which is also a D,L mixture. It also showed a fast reaction for 50% of the material and a slower reaction for the other 50%. However, in this case the enantiomeric selectivity ratio was only 6.6; we have not determined the absolute configuration of the preferred isomer. The most striking result was that both enantiomers of **9** were slower than was **6** or **8**. As will be seen in the Discussion section, this apparently reflects a steric hindrance problem for **9**.

Discussion

As the data in Table I show, freezing the rotation of one of the single bonds in the side chain of **6** by building in a ring leads to a 9-fold increase in the value of $k_{complex}/k_{un}$ for **8′**. The value of 3 200 000 for **8′** may even be a slight underestimate, as indicated in our discussion of the calculation of k_{un}. In addition, as we have pointed out previously,[5,6] the rates of reactions in the interior of enzymes, in which the medium perhaps resembles our H_2O/Me_2SO mixed solvent, are typically compared against uncatalyzed reaction rates in pure H_2O. We have observed[5] an acceleration of 25-fold for the hydrolysis of *p*-nitrophenyl esters with the same buffer in H_2O/Me_2SO compared with pure H_2O. Thus when this additional factor is applied, the $k_{complex(H_2O/Me_2SO)}/k_{un(H_2O)}$ is ca. 80 000 000 for **8′**, almost a 10^8 acceleration.

The improvement of **8′** over **6** may contain a factor of 2 from statistics. Since **8′** is much better than its enantiomer **8″**, it is possible that **6** also reacts preferentially in only one of its enantiomeric conformations **6′**. The rest of the improvement must come from freezing out some of the residual free rotation in **6**. Obviously any rotation about the single bond to the ferrocene nucleus has been frozen out, but the rotational barrier at the single bond to the ester group has probably also been increased. There is a 12-fold decrease in k_{un} on converting **6** to **8** which must represent better conjugation in **8**, with the resulting better electron donation and decreased reactivity in simple hydrolysis. This decreased reactivity of **8** for electronic reasons is also seen in $k_{complex}$ of **8′** and **8″**, although for **8′** the geometric improvement almost compensates this effect. Since freezing out even one free single-bond rotation typically[13] increases intramolecular reaction rates by 10^2, the modest $k_{complex}/k_{un}$ improvement in **8′** compared with **6** confirms our suggestion that **6** does indeed have significant rotational barriers already.

Compound **9** might have been expected to resemble **8** in freezing one single bond entirely and helping to freeze the second one. However, it does not show the strong decrease in k_{un} characteristic of improved conjugation in **8**. Most striking, **9** is actually a poorer substrate than is **6** (or **8**). Molecular models suggested that in the presumably preferred enantiomer the ring CH_2 group on carbon 2 of the acrylate ester chain interferes sterically with β-cyclodextrin. This interpretation was confirmed by the data on **16**, an open-chain acrylate with a CH_3 group in the offending position. As Table I shows, this methyl substitution slows k_{un} by only 27% but decreases $k_{complex}$ by over 50-fold. In fact comparing cyclic compound **9** with its open-chain analogue **16**, we see (Table I) changes in $k_{complex}/k_{un}$ similar to those we have already discussed when **8** is compared with **6**.

One of our most striking observations is the large enantiomeric preference with **8**. The rate ratio of 20 for reaction of **8′** and **8″** represents the largest chiral discrimination observed with carboxylic esters and cyclodextrins,[14] exceeded only by rate ratios for α-cyclodextrin phosphonylations or phosphorylations, by D,L mixtures[4] in which attack occurs directly at the asymmetric atom. Since we know the absolute configuration of the reactive enantiomer **8′**, and of course of β-cyclodextrin, one might hope to furnish a detailed molecular description of this enantiomeric selectivity.

Schematically, the cyclodextrin absolute configuration can be represented as shown in Figure 1. Unfortunately, there is no easy way to determine whether the C-2 hydroxyl or the C-3 hydroxyl is the one which attacks the substrate first. As we have described previously[6] for substrate **6**, we find with **8** that the isolated product is, by NMR, a mixture of the C-2 acylated and C-3 acylated compounds. This might in principle mean that either one can attack first, but more likely it simply represents the result of the well-known[15] equilibration of sugar esters with neighboring hydroxyls under basic conditions after the reactive hydroxyl is acylated. We believe that this reactive hydroxyl is one at C-2, by analogy to its demonstrated[16] greater reactivity toward methylation (in which subsequent equilibration does not occur). If this is the case, then as Figure 1 shows the approach to C-2 by **8′** permits bulky parts of the substrate to fit into the space between sugar residues. With the less reactive enantiomer **8″** these bulky sections of substrate would interfere with the C-3 hydroxyl during an attack by the alkoxide ion at C_2.

(13) See examples in: Kirby, A. J.; Fersht, A. R. *Prog. Bioorg. Chem.* **1971**, *1*, 1–80, and similar reviews.
(14) Reference 1, Chapter VII.
(15) Haines, A. *Adv. Carbohydr. Chem. Biochem.* **1976**, *33*, 101–107.
(16) Casu, B.; Reggiani, M.; Gallo, G. G.; Vigevani, A. *Tetrahedron* **1968**, *24*, 803–810. Cf.: A. Pinter.

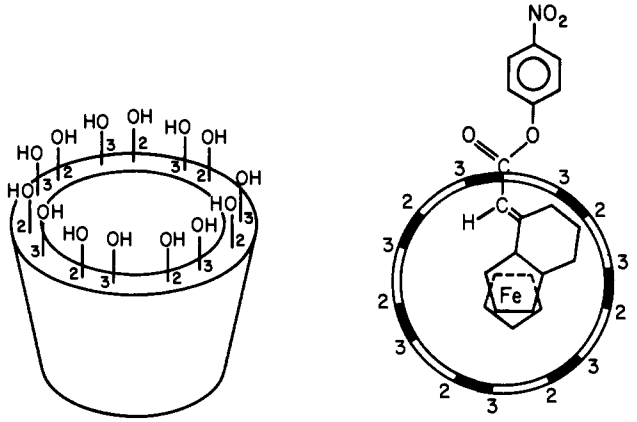


Figure 1. A schematic drawing showing the counterclockwise arrangement of the C-2, C-3 hydroxyls in the individual glucose units of β-cyclodextrin and the presumed orientation of the more reactive enantiomer 8′ when bound into the cyclodextrin cavity. Models suggest that attack by an ionized C-2 hydroxyl could be faster with 8′ than with its mirror image 8″, as observed, because there is more space for the bulky leaving group *between* glucose residues. There are also differences in possible hydrogen bonds from other hydroxyl groups, but the absence of a deuterium isotope effect on the enantiomeric selectivity seems to rule this out as contributing to the high enantioselectivity of the acylation reaction.

We considered the possibility that the enantiomeric selectivity might reflect hydrogen bonding. That is, during attack by a C-2 alkoxide on the substrate carbonyl it might be possible with 8′ to stabilize the developing tetrahedral intermediate by hydrogen bonding from a C-3 hydroxyl which might be less effective with the enantiomer 8″. To check this point we examined the relative reactivities of β-cyclodextrin with 8′ and 8″ in D_2O/Me_2SO rather than H_2O/Me_2SO. In the deuterated solvent one would expect weaker hydrogen bonding effects from the exchanged hydroxyl groups, but the enantiomeric selectivity was still 19.3 (vs. 20.3 with H_2O/Me_2SO). Thus the enantiomeric selectivity is apparently not related to hydrogen bonding differences. It must instead reflect geometric differences in clockwise vs. counterclockwise approach of substrate to the reactive cyclodextrin group, as Figure 1 suggests.

One might wonder whether 8′ is now the optimal substrate for cyclodextrin acylation or whether still further improvements in $k_{complex}/k_{un}$ can be achieved. We believe that further improvements are possible. Thus not all the degrees of freedom are fully frozen out in 8′ which must eventually be frozen in the transition state for reaction. Furthermore, when 8′ binds into the cyclodextrin cavity, it should be free to spin in the cavity,[17] so freezing out that motion will also cost entropy of activation. Finally, molecular models suggest that substrates such as **6** and **8** lose almost no binding on proceeding to the transition state, but in an ideal substrate the transition state would be *better* bound than the substrate.[18] Thus the almost 10^8 acceleration we have invoked for 8′ is probably still not the limit attainable by careful tailoring of the geometry of the substrate to the demands of the cyclodextrin acylation reaction.

Experimental Section

Aqueous buffers were prepared with deionized water. The dimethyl sulfoxide used in the kinetic runs was MCB omnisolve grade. β-Cyclodextrin (CPC) was recrystallized from water and dried overnight at 80 °C (0.05 torr) just prior to use. 1,2-Ferrocenocyclohexen-3-one was prepared as described in the literature.[9] Column chromatography was carried out by using Merck Silica Gel 60 and Fisher neutral alumina. All other materials were standard reagent grade.

NMR spectra were measured in $CDCl_3$ solution with tetramethylsilane (Me_4Si) as an internal reference standard by using a 90-MHz Perkin-Elmer R-32 instrument. IR spectra were recorded on a Perkin-Elmer 621 grating spectrophotometer. A Cary 118 spectrophotometer was used both for recording UV/vis absorption spectra and for kinetics. All reported melting points are uncorrected. Microanalyses were performed by Galbraith Laboratories. Low-resolution mass spectra were recorded on Finnigan 3300 mass spectrometer with a 6000 series data system. pH measurement was accomplished with a Radiometer PHM 63 pH meter equipped with a Radiometer GK2321C combination electrode.

Kinetics. Reaction buffers were prepared by adding 4 volumes of 10 mM aqueous potassium dihydrogen phosphate/sodium hydroxide buffer (pH 6.5–7.6) to 6 volumes of dimethyl sulfoxide. The resulting solutions had pHs ranging from 10.1 to 11.4 as determined with the glass electrode. β-Cyclodextrin solutions (0.9–6.0 mM) were prepared with this buffer and stored under nitrogen. Substrate solutions (3–4 mM in dimethyl sulfoxide) were stored in the dark.

A kinetic run was initiated by equilibrating 1.00 mL of β-cyclodextrin solution to 30.0 ± 0.2 °C in the spectrophotometer chamber. A 10-μL sample of substrate solution was injected (to make the solution 30–40 μM in substrate) and the absorbance at 410 nm monitored as a function of time. After a suitable interval (10 half-lives), the final absorbance was measured. The pH of the solution throughout the run was found to remain constant to within ±0.02 unit.

In the case of substrates showing monophasic kinetics, data from the first 4–5 half-lives were fitted to a simple exponential by using a standard nonweighted least-squares routine. The resulting rate constants showed standard deviations of less than 1%. For substrates exhibiting biphasic kinetics, the method of subtraction[19] was employed. The rate constants for the slow phase generally showed standard deviations less than 1%. The rate constants for the fast phase showed standard deviations less than 2%.

The maximal rate constants ($k_{complex}$) and binding constants (K_m) were extracted from rates measured at eight different concentrations of β-cyclodextrin by using the method of Eadie.[20]

To measure the β-cyclodextrin-catalyzed rates in D_2O/Me_2SO, we prepared the following reaction buffer: 50 mL of pH 6.55 10 mM aqueous potassium dihydrogen phosphate/sodium hydroxide was lyophilized and the residue redissolved in deuterium oxide to make 50 mL. This buffer (4 volumes) was added to 6 volumes of dimethyl sulfoxide, giving a mixed-solvent buffer with a measured pH of 10.42 (not corrected for deuterium).

All uncatalyzed rates were measured in a solution prepared by adding 4 volumes of aqueous 0.020 M potassium hydroxide to 6 volumes of dimethyl sulfoxide under nitrogen. The kinetics were performed and data analyzed as described above, and rate constants showing standard deviations of less than 1% were obtained. The rate constants shown in the table represent the average of three duplicate runs. The pH was observed to hold to within ±0.04 unit throughout the run.

(*E*)-3-(Carboxymethylene)-1,2-ferrocenocyclohexene (12). A mixture (5:1) of (*E*)- and (*Z*)-3-(carbethoxymethylene)-1,2-ferrocenocyclohexenes[10] (1.35 g, 4.16 mmol) was dissolved in ethanol (35 mL), and aqueous 6 N sodium hydroxide (35 mL) was added. The mixture was stirred at room temperature in the dark for 23.5 h and then partitioned between methylene chloride and water. The aqueous layer was acidified with concentrated HCl and extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate and evaporated to afford a partially crystalline mixture (2:1) of the *E* and *Z* acids. Recrystallization from benzene afforded exclusively the desired *E* acid (677 mg, 56%) as red-orange microcrystals: mp 165.5–166 °C; NMR δ 6.01 (b s, 1), 4.61 (m, 1), 4.38 (m, 2), 4.10 (s, 5), 3.71 (m, 1), 2.9–1.8 (m, 5); IR (KBr) 3300–2200, 1664, 1591, 1103, 1001 cm^{-1}; mass spectrum (CI) *m/e* 297; UV/vis ($CHCl_3$) 254 (4.06), 304 (4.14), 361 (3.17), 477 (3.98) nm. Anal. ($C_{16}H_{16}O_2Fe$): C, H.

(*E*)-3-(Carboxymethylene)-1,2-ferrocenocyclohexene *p*-Nitrophenyl Ester (8). 3-(Carboxymethylene)-1,2-ferrocenocyclohexene (592 mg, 2.00 mmol), *p*-nitrophenol (306 mg, 2.20 mmol), dicyclohexylcarbodiimide (454 mg, 2.20 mmol), and *p*-(dimethylamino)pyridine (24.3 mg, 0.2 mmol) were stirred in 30 mL of dry ether in the dark at room temperature for 3 h. Evaporation of the solvent followed by column chromatography on Activity III alumina (100 g) with benzene as the solvent afforded 479 mg (57.5%) of the ester. Recrystallization from carbon tetrachloride gave red microcrystals: mp 137–139.5 °C; NMR δ 8.28 (d, 2, *J* = 9 Hz), 7.34 (d, 2, *J* = 9 Hz), 6.14 (b s, 1), 4.64 (b s, 1), 4.44 (b s, 2), 4.15 (s, 5), 3.69 (m, 1), 2.9–1.8 (m, 5); IR (KBr) 1727, 1614, 1518, 1349, 1104, 997 cm^{-1}; mass spectrum (CI), *m/e* 418; UV/vis ($CHCl_3$) 313 (4.40), 370 sh, 495 (3.25), 265 (4.19) nm. Anal. ($C_{22}H_{19}NO_4Fe$): C, H, N.

4-(Carbomethoxy)-1,2-ferrocenocyclohexen-3-one (13). A 50% oil dispersion of sodium hydride (0.576 g, 12 mmol) was washed with dry

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hexane under nitrogen to remove the oil. Dimethyl carbonate (14.0 mL) that had been distilled from calcium hydride was added followed by solid 1,2-ferrocenocyclohexen-3-one (1.02 g, 4.00 mmol) and 10 μL of methanol, and the mixture was refluxed under nitrogen for 2.5 h. The reaction mixture was quenched with methanol (1 mL) followed by acetic acid (2 mL) and partitioned between chloroform and water. The organic layer was washed with aqueous sodium bicarbonate followed by water and then dried over anhydrous magnesium sulfate and concentated to an oil. Column chromatography on silica gel (100 g) with chloroform/methanol (20:1) as the eluting solvent afforded the product (1.25 g, 100%) as a semisolid consisting of a mixture of epimers (5:2 by NMR): NMR δ 4.89 (m, 1), 4.54 (m, 2), 4.29 and 4.19 (each s, 5), 3.85 and 3.69 (each s, 3), 3.22 (m, 1), 3.0–2.1 (m, 4); IR (neat film) 1739, 1670, 1109, 1005 cm^{-1}; mass spectrum (CI), *m/e* 313.

4-(Carbomethoxy)-1,2-ferrocenocyclohexa-1,3-diene (14). A solution of sodium borohydride (1.69 g, 44.5 mmol) in methanol (40 mL) was added to a solution of 4-(carbomethoxy)-1,2-ferrocenocyclohexen-3-one (1.39 g, 4.47 mmol) in methanol (60 mL), and the mixture was stirred for 10 min at room temperature. The mixture was cooled to 0 °C and quenched with acetic acid (12 mL). Concentrated HCl (30 mL) was added and the mixture warmed to room temperature and stirred for 10 min. The reaction mixture was partitioned between water and ether, and the organic layer was washed with aqueous sodium bicarbonate followed by water and dried over anhydrous magnesium sulfate. Concentration to a red oil followed by column chromatography on Activity IV neutral alumina (200 g) with ether/pentane (1:5) as the eluting solvent gave 806 mg (61%) of the desired product as a red oil: NMR δ 7.51 (m, 1), 4.25 (b s, 3), 4.12 (s, 5), 3.77 (s, 3), 3.0–2.2 (m, 4); IR (neat film) 1699, 1612, 1109, 1002 cm^{-1}; mass spectrum (CI), *m/e* 297.

4-Carboxy-1,2-ferrocenocyclohexa-1,3-diene (15). 4-(Carbomethoxy)-1,2-ferrocenocyclohexa-1,3-diene (712 mg, 2.41 mmol) was dissolved in ethanol (25 mL), aqueous 6 N sodium hydroxide (25 mL) was added, and the solution was stirred at room temperature in the dark for 18 h. The solution was partitioned between methylene chloride and water, and the aqueous layer was acidified with concentrated HCl and extracted with methylene chloride. The organic extract was dried over anhydrous sodium sulfate and evaporated to afford the red crystalline acid (615 mg) in 90% yield. Recrystallization from 2-propanol gave brilliant red microcrystals: mp 187.5–188.5 °C; NMR δ 7.68 (b s, 1), 4.30 (b s, 3), 4.15 (s, 5), 3.0–2.2 (m, 4); IR (KBr) 3300–2200, 1656, 1597, 1105, 1003 cm^{-1}; mass spectrum (CI), *m/e* 283; UV/vis 259 (4.02), 309 (4.08), 373 (3.10), 486 (2.99) nm. Anal. ($C_{15}H_{14}O_2Fe$): C, H.

4-Carboxy-1,2-ferrocenocyclohexa-1,3-diene *p*-Nitrophenyl Ester (16). 4-Carboxy-1,2-ferrocenocyclohexa-1,3-diene (346 mg, 1.23 mmol), *p*-nitrophenol (188 mg, 1.35 mmol), dicyclohexylcarbodiimide (280 mg, 1.36 mmol), and *p*-(dimethylamino)pyridine (15.1 mg, 0.12 mmol) were stirred in dry ether (20 mL) at room temperature in the dark for 3.5 h. Evaporation followed by column chromatography on silica gel (50 g) with methylene chloride as the solvent gave 407 mg (82%) of the crystalline ester. Recrystallization from ether afforded violet-red crystals: mp 145–147 °C; NMR δ 8.27 (d, 2, *J* = 9 Hz), 7.83 (b s, 1), 7.36 (d, 2, *J* = 9 Hz), 4.36 (s, 3), 4.18 (s, 5), 3.1–2.2 (m, 4); IR (KBr) 1719, 1605, 1515, 1342, 1104, 1003 cm^{-1}; mass spectrum (CI), *m/e* 404; UV/vis 270 (4.16), 317 (4.27), 380 sh, 501 (3.24) nm. Anal. ($C_{21}H_{17}NO_4Fe$): C, H, N.

Ethyl (*E*)-3-Ferrocenyl-2-methylpropenoate. Ferrocenecarboxaldehyde[21] (0.857 g, 4.00 mmol) and (carbethoxyethylidene)triphenylphosphorane (3.62 g, 10.0 mmol) were dissolved in chloroform (100 mL), and the solution was refluxed in the dark for 19 h. The reaction mixture was concentrated to a dark oil and passed down a short column of silica gel (25 g) with methylene chloride as the solvent to remove excess reagent and product triphenylphosphine oxide. Chromatography on a long column of silica gel (120 g) with ether/pentane (1:7) as the eluting solvent cleanly separated the product isomers, affording the *Z* isomer (69.5 mg, 6%) as a red oil and the *E* isomer (1.05 g, 88%) as an orange crystalline solid. Recrystallization of the desired *E* isomer from hexane afforded lustrous orange plates: mp 62–63.5 °C; NMR δ 7.44 (b s, 1), 4.48 (m, 2), 4.34 (m, 2), 4.23 (q, 2, *J* = 7 Hz), 4.13 (s, 5), 2.01 (b s, 3), 1.32 (t, 3, *J* = 7 Hz); IR (KBr) 1702, 1631, 1104, 1001 cm^{-1}; mass spectrum (CI), *m/e* 299; UV/vis 248 (4.17), 294 (4.19), 353 (3.23), 462 (2.94) nm. Anal. ($C_{16}H_{18}O_2Fe$): C, H.

(*E*)-3-Ferrocenyl-2-methylpropenoic Acid. Ethyl (*E*)-3-ferrocenyl-2-methylpropenoate (752 mg, 2.52 mmol) was dissolved in ethanol (25 mL), aqueous 6 N sodium hydroxide (25 mL) was added, and the solution was stirred at room temperature in the dark for 16 h. The solution was partitioned between methylene chloride and water, and the aqueous layer was acidified with concentrated HCl and extracted with methylene chloride. The organic extract was dried over anhydrous sodium sulfate and evaporated to afford the red crystalline acid (668 mg, 98%). Recrystallization from ether afforded brick red crystals: mp 170.5–172.5 °C; NMR δ 7.63 (b s, 1), 4.56 (m, 2), 4.42 (m, 2), 4.18 (s, 5), 2.16 (b s, 3); IR (KBr) 3300–2200, 1649, 1617, 1600, 1106, 1007 cm^{-1}; mass spectrum (CI), *m/e* 271; UV/vis 248 (4.15), 295 (4.18), 357 (3.23), 467 (2.97) nm. Anal. ($C_{14}H_{14}O_2Fe$): C, H.

***p*-Nitrophenyl (*E*)-3-Ferrocenyl-2-methylpropenoate (16).** (*E*)-3-Ferrocenyl-2-methylpropenoic acid (379 mg, 1.40 mmol), *p*-nitrophenol (214 mg, 1.54 mmol), dicyclohexylcarbodiimide (318 mg, 1.54 mmol), and *p*-(dimethylamino)pyridine (17.1 mg, 0.14 mmol) were stirred in dry ether (20 mL) at room temperature in the dark for 3.5 h. Evaporation followed by column chromatography on silica gel (60 g) with methylene chloride as the eluting solvent gave 433 mg (79%) of the crystalline ester. Recrystallization from 2-propanol afforded briliant red plates: mp 130–132 °C; NMR δ 8.30 (d, 2, *J* = 9 Hz), 7.75 (b s, 1), 7.36 (d, 2, *J* = 9 Hz), 4.59 (m, 2), 4.47 (m, 2), 4.21 (s, 5), 2.13 (s, 3); IR (KBr) 1717, 1623, 1526, 1345, 1107, 1012 cm^{-1}; mass spectrum (CI), *m/e* 392; UV/vis 262 (4.22), 303 (4.39), 365 (3.40), 476 (3.21) nm. Anal. ($C_{20}H_{17}NO_4Fe$): C, H, N.

Preparation of (+)-(*E*)-3-(Carboxymethylene)-1,2-ferrocenocyclohexene *p*-Nitrophenyl Ester (8′). The partial resolution of ferrocenocyclohexen-3-one was carried out as described by Schlogl, Fried, and Falk.[9b] After recrystallization from ether, a material with $[\alpha]^{23}D = +390 \pm 10°$ representing a 67% enantiomeric excess was obtained.

Subjection to the Reformatsky reaction as detailed by Rosenblum and Abbate[10] gave dextrorotatory 3-(carbethoxymethylene)-1,2-ferrocenocyclohexene. Saponification and esterification as described above for the racemic material provided optically active (*E*)-3-(carboxymethylene)-1,2-ferrocenocyclohexene *p*-nitrophenyl ester. The material exhibited $[\alpha]^{23}D = +1260 \pm 110°$ after recrystallization from ether.

Isolation of Optically Active (*E*)-3-(Carboxymethylene)-1,2-ferrocenocyclohexene *p*-Nitrophenyl Ester (8″) after Partial Reaction of the Racemic Material with β-Cyclodextrin. β-Cyclodextrin (215.4 mg) was dissolved in 15 mL of pH 6.67 10 mM aqueous potassium dihydrogen phosphate/sodium hydroxide buffer. Then 30.2 mg of racemic (*E*)-3-(carboxymethylene)-1,2-ferrocenocyclohexene *p*-nitrophenyl ester was dissolved in 22.5 mL of dimethyl sulfoxide, and the two solutions were placed in a 30 °C water bath. After thermal equilibration, the dimethyl sulfoxide solution was poured into the aqueous solution and the mixture was swirled in the water bath. After exactly 50 s, the reaction was quenched with 0.25 mL of concentrated hydrochloric acid. The reaction mixture was poured into 35 mL of water and extracted with 2 × 75 mL of ether. The pooled ether extracts were washed with 75 mL water, 5 × 75 mL of saturated aqueous sodium bicarbonate, and, finally, 75 mL of water. After being dried over anhydrous magnesium sulfate, evaporation afforded a small amount of red solid. Chromatography of the material on a column of silica gel (10 g) with methylene chloride as the eluting solvent gave pure (by TLC) unreacted starting ester (15.9 mg). Recrystallization from ether yielded red crystals of levorotatory ester with $[\alpha]^{23}D = -1240 \pm 30°$.

Preparation and Kinetics of *p*-Nitrophenyl 2-*trans*-Ferrocenylcyclopropanecarboxylate (7). The *p*-nitrophenyl ester 7 was prepared from the known 2-*trans*-ferrocenylcyclopropanecarboxylic acid[21] by the method used to prepare ester 15.

Preliminary kinetic parameters for this ester were obtained before the techniques detailed in this paper were devised by using the methods described in the previous paper from these laboratories.[6] At 30 °C, pH 11.56 in 60/40 Me_2SO/water, $k_{complex}$, K_m and k_{un} (including buffer catalysis) were determined to be 0.018 s^{-1}, 7.9 nM, and 6.3 × 10^{-5} s^{-1}, respectively. The rate acceleration $k_{complex}/k_{un}$ is thus 290.[22]

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