unreactive under the conditions used for ester aminolysis. Also, in the reaction of di-*n*-propylamine and the 2,4-dinitrophenyl trimethylacetate there is no detectable di-*n*-propyl-2,4-dinitrophenylaniline produced.

Acknowledgment. This work was supported in part by a grant from the National Science Foundation.

Registry No. *p*-Nitrophenyl acetate, 830-03-5; *p*-nitrophenyl propionate, 1956-06-5; *p*-nitrophenyl valerate, 1956-07-6; *p*-nitrophenyl trimethylacetate, 4195-17-9; *p*-nitrophenyl *tert*-butylacetate, 22406-32-2; *n*-butylamine, 109-73-9; isopropylamine, 75-31-0; *sec*-butylamine, 13952-84-6; *tert*-butylamine, 75-64-9; propionoyl chloride, 79-03-8; valeroyl chloride, 638-29-9; *tert*-butylacetoyl chloride, 7065-46-5.

Optimization of Metallocene Substrates for β-Cyclodextrin Reactions

Ronald Breslow,* George Trainor,[1] and Akihiko Ueno[2]

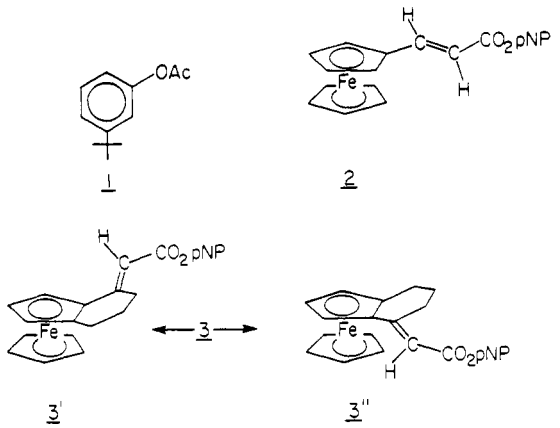
Contribution from the Department of Chemistry, Columbia University, New York, New York 10027. *Received September 7, 1982*

Abstract: The *p*-nitrophenyl ester of (*E*)-ruthenoceneacrylic acid reacts in a complex to acylate β-cyclodextrin, but with a poorer binding and rate constant than for the corresponding ferrocene derivative. The *p*-nitrophenyl ester of (*E*)-3-(carboxymethylene)-1,2-ferrocenocyclopentene is a mixture of two enantiomers. One enantiomer acylates β-cyclodextrin 5 900 000 times as fast in aqueous Me_2SO as it hydrolyzes under the same conditions (1.5×10^8 times as fast as hydrolysis in pure water at the same pH); the other enantiomer is 62-fold slower. These are the largest accelerations and enantiomeric selectivities known for such reactions. Ferrocene-1,3-diacrylate esters react with β-cyclodextrin in processes in which the first and second acylation reveal important geometric effects. β-Cyclodextrin 6-tosylate is used to block unproductive binding and clarify the structural effects.

In the imitation of enzymatic reactions by simpler components, there are two types of studies of interest. One group comprises intramolecular reactions, such as catalyses by neighboring groups. These help establish the magnitudes of rate effects one can expect when the functional groups of an enzyme and a substrate are held firmly in the correct geometry for reaction; intramolecular processes are also direct models for some reactions that involve covalent enzyme–substrate species, as in the hydrolysis of acyl-enzyme intermediates in peptidase and esterase reactions with intramolecular catalysis by the enzyme. The second class of enzyme model studies involves intermolecular catalysis, which imitate the reaction of independent enzyme and substrate molecules. As with enzymes themselves, such models are much more effective if the catalyst and substrate associate in some way so the intermolecular process can occur within a complex.

The cyclodextrins have excited much interest as the basis for enzyme models that can complex substrates.[3] α-Cyclodextrin (cyclohexaamylose) and β-cyclodextrin (cycloheptaamylose) are readily available, and in water or in polar organic solvents they bind hydrophobic organic segments of appropriate geometry with good affinities. Many catalysts have been constructed by attachment of appropriate functional groups to cyclodextrins, and the unmodified molecules themselves have also been studied as enzyme models. In one general approach, the cyclodextrin hydroxyls act as nucleophiles to react with bound substrates such as esters.[4,5] Such reactions produce acylated cyclodextrin, so they are not catalytic. However, acylation of a cyclodextrin hydroxyl within a complex is a partial model, but lacking the other catalytic groups, for the acylation steps of such serine proteases as chymotrypsin.

The results of such studies showed interesting geometric selectivities, but poor rate accelerations. By comparison of the deacylation of a substrate such as *m-tert*-butylphenyl acetate (**1**)



within a β-cyclodextrin complex with the rate of hydrolysis of the substrate under the same conditions in the absence of β-cyclodextrin, acyl transfer to a cyclodextrin hydroxyl was accelerated by a little more than 10^2 by the proximity effect within the complex.[5] Proximity effects in intramolecular processes[6] can lead to rate effects of 10^{10}; such large effects are generally invoked for enzyme–substrate complexes to explain enzymatic velocities. Thus it was important to see whether really large rate accelerations could be achieved in some intracomplex reactions, or whether unknown factors still separated the great effectiveness of enzymatic processes from the rates available in model systems. For this reason we have instituted a program of optimization for cyclodextrin acylation reactions.

Our first studies[7] showed that previously examined substrates had incorrect geometries for fast reaction. That is, the substrates fit the cyclodextrin cavities well, but molecular models suggested that much of the binding must be lost in the transition state for acyl transfer. When we modified the cyclodextrin by building in an intrusive floor, so the cavity was well defined and shallower, the *m-tert*-butylphenyl acetate reaction was improved by an order

(1) NIH Postdoctoral Fellow, 1979–1981.
(2) On leave from the Pharmaceutical Instutitue, Tohoku University, 1981–1982.
(3) For reviews, cf.: Bender, M. L.; Komiyama, M. "Cyclodextrin Chemistry"; Springer-Verlag: New York, 1977. Tabushi, I. *Acc. Chem. Res.* **1982**, *15*, 66.
(4) Heinrich, N.; Cramer, F. *J. Am. Chem. Soc.* **1965**, *87*, 1121.
(5) van Etten, R. L.; Sebastian, J. F.; Clowes, G. A.; Bender, M. L. *J. Am. Chem. Soc.* **1967**, *89*, 3242–3253, 3253–3262.
(6) Cf.: Kirby, A. J.; Fersht, A. R. *Prog. Bioorg. Chem.* **1971**, *1*, 1–80.

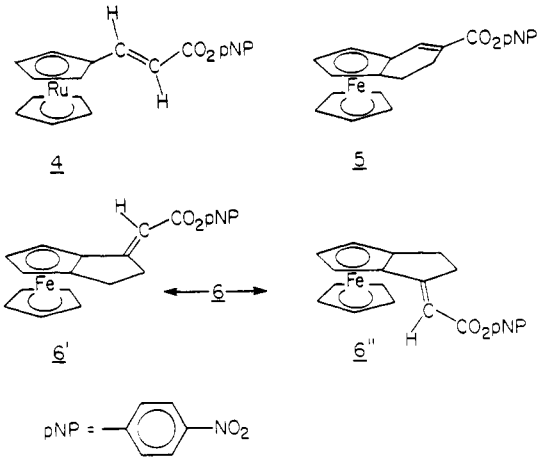
0002-7863/83/1505-2739$01.50/0 © 1983 American Chemical Society

of magnitude since the substrate binding geometry was now closer to that of the transition state.[7] Even more striking effects were obtained by modifying the substrate geometry itself. In the optimum case examined in our earliest study, the *p*-nitrophenyl ester of ferroceneacrylic acid (**2**) showed a rate acceleration of more than 10^5 in comparison with acyl transfer to β-cyclodextrin with hydrolysis under the same conditions.

This work was extended by further improvement in substrate geometry, in which the flexible acrylate ester side chain was incorporated into a fused ring.[8] Substrate **3** showed an acceleration of 3.2×10^6 for one of its enantiomers; it also showed a 20-fold enantiomeric selectivity. However, we pointed out that **3** is undoubtedly still not the optimal substrate. Improvements might be expected if some additional degrees of freedom are frozen out, particularly the freedom of **3** to spin within the cyclodextrin cavity. Perhaps most important, molecular models suggested that **3** would still be somewhat better bound by β-cyclodextrin than would be the transition state for acylation, while for optimum acceleration the transition state should bind better than the substrate.[9] We now wish to describe further studies of cyclodextrin acylations that explore some of these possible improvements.

Results

As a simple extension of the work of ferroceneacrylates, we prepared the corresponding ruthenocene derivative **4**. The kinetic



data, listed in Table I, show that the substitution of ruthenium for iron, which changes the geometry (ruthenocene is ca. 10% thicker than ferrocene) and the electronic properties of the system, leads to poorer binding and poorer rate. The K_m dissociation constant for the complex is larger for ruthenium, and the value of $k_{complex}/k_{un}$ is less. Thus we focused further work on ferrocene derivatives.

$$\text{cyclodextrin} + \text{substrate} \rightleftharpoons \text{complex}$$

$$\text{dissociation constant} = K_m$$

$$\text{complex} + OH^- \rightleftharpoons \text{cyclodextrin anion} \rightarrow \text{acylated product}$$

$$\text{rate of acylation at a given pH} = k_{complx}[\text{complex}]$$

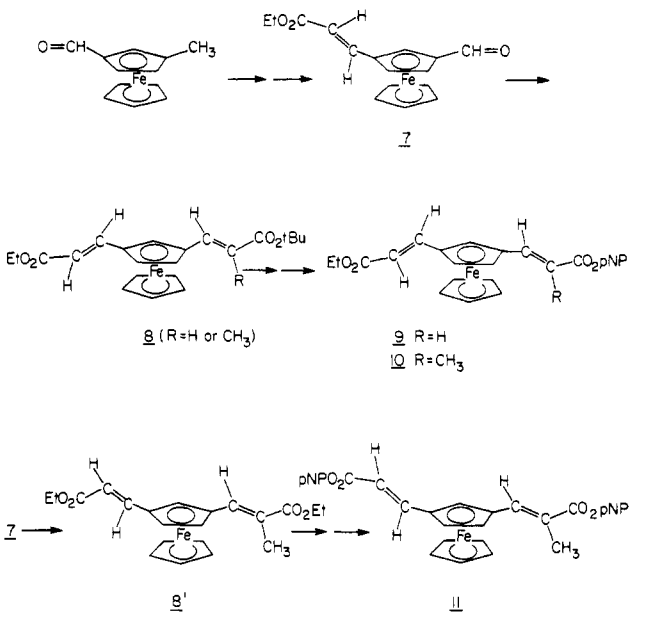
$$\text{substrate} + OH^- \rightarrow \text{hydrolyzed product}$$

$$\text{rate of hydrolysis at a given pH} = k_{un}[\text{substrate}]$$

We had found that one enantiomer, **3'**, was the best substrate so far, but that the other enantiomer, **3''**, was far inferior. Furthermore **5**, an analogue of **3** with an endocyclic double bond, was also a relatively poor substrate. This all suggested that the rates in these systems are affected by subtle geometric factors, so we decided to examine compound **6**. Reformatszky synthesis from the known[10] 1,2-ferrocenocyclopenten-3-one and ethyl bromoacetate, followed by dehydration, afforded the corresponding ethyl ester. This was converted in the normal fashion to **6**, which proved to be the best substrate yet examined. As the data in Table I show, the two enantiomers of **6** both have normal dissociation constants K_m, but the values of $k_{complex}$ differ by a factor of 62, a very large enantiomeric preference. With the rather low k_{un} for **6**, the ratio of 5 900 000 for $k_{complex}/k_{un}$ for one of the enantiomers is the highest value yet observed.

Several of the questions about possible improvements seemed answerable if we could prepare ferrocene-1,3-diacrylate esters. These two-armed esters were prepared from 3-methylferrocenecarboxaldehyde[11] by the sequence in Scheme I. After Wittig reaction, oxidation with MnO_2 afforded the aldehyde ester **7**. This on a second Wittig reaction produced the diester **8**; at each Wittig stage the *E* ester was carefully purified to remove the *Z* isomer. The three *p*-nitrophenyl esters **9**, **10**, and **11** were then examined in reaction with β-cyclodextrin in aqueous Me_2SO, as described earlier[7,8] (cf. Experimental Section). Each ester **9**, **10**, and **11** exists as a DL mixture, so in every case we saw two rate processes, each consuming 50% of the substrate, which we assign to the fast and slow enantiomer. In addition, substrate **11** has two different reactive ester functions and showed a total of four overlapping rate processes. The rates were extracted from the experimental curves of optical density vs. time by appropriate computer deconvolution. The data are listed in Table I.

Scheme I[a]



[a] Only one enantiomer is shown.

In some cases it became desirable to add an intrusive floor on the primary side of the cyclodextrin, so as to clarify the significance of some of the results. We had produced such floors earlier[7,12] by hepta substitution of β-cyclodextrin, but in this case we simply used β-cyclodextrin 6-tosylate. The compound was stable under our reaction conditions, and the data on this compound in Table I showed that the tosylate group did indeed block and intrude into the primary face of the cyclodextrin cavity.

Discussion

It is not clear why the ferrocene-based system **2** is slightly better than the ruthenoceneacrylate ester **4**. The binding of **4** is 10–20%

(7) (a) Breslow, R.; Czarniecki, M. F.; Emert, J.; Hamaguchi, H. *J. Am. Chem. Soc.* **1980**, *102*, 762. (b) Czarniecki, M. F.; Breslow, R. *Ibid.* **1978**, *100*, 771.
(8) Breslow, R.; Trainor, G. *J. Am. Chem. Soc.* **1981**, *103*, 154.
(9) In recent unpublished work in collaboration with Professor W. le Noble, volume of activation studies are consistent with this conclusion.
(10) Shirafuji, T.; Odaira, A.; Yamamoto, Y.; Nozaki, H. *Bull. Chem. Soc. Jpn.* **1972**, *45*, 2884.
(11) Falk, H.; Haller, G.; Schlögl, K. *Monatsh. Chem.* **1967**, *98*, 592.
(12) Emert, J.; Breslow, R. *J. Am. Chem. Soc.* **1975**, *97*, 670.

Table I. Kinetic and Binding Constants at 30 °C, pH 10.0[a]

substrate	host[b]	$k_{complex}$,[c] $s^{-1} \times 10^3$	K_m,[d] mM	k_{un},[e] $s^{-1} \times 10^8$	$k_{complex}/k_{un}$
ferroceneacrylate **2**	CD	94 ± 5	7.5 ± 0.5	28.3 ± 0.8	332 000
ferroceneacrylate **2**	t-CD	39 ± 1.4	2.2 ± 0.2	28.3 ± 0.8	138 000
ruthenocene ester **4**	CD	91 ± 9	11.6 ± 1.3	38.9 ± 1.2	234 000
cyclopentenyl ester **6′**	CD	92 ± 14	5.7 ± 1.2	1.58 ± 0.9	5 900 000
cyclopentenyl ester **6″**	CD	1.49 ± 0.15	4.7 ± 0.7	1.58 ± 0.9	95 000
diacrylate ester **9**	CD	32.2 ± 0.3	0.97 ± 0.04	56.3 ± 2.3	57 000
diacrylate ester **9**	CD	6.22 ± 0.20	1.53 ± 0.05	56.3 ± 2.3	11 000
diacrylate ester **9**	t-CD	190 ± 28	9.5 ± 1.9	56.3 ± 2.3	340 000
diacrylate ester **9**	t-CD	45.7 ± 15.7	14.9 ± 6.2	56.3 ± 2.3	81 000
methacrylate ester **10**	CD	0.70 ± 0.02	0.83 ± 0.12	42.1 ± 0.9	1 700
methacrylate ester **10**	CD	0.19 ± 0.01	0.89 ± 0.11	42.1 ± 0.9	450
methacrylate ester **10**	t-CD	1.05 ± 0.01	1.46 ± 0.04	42.1 ± 0.9	2 500
methacrylate ester **10**	t-CD	0.32 ± 0.01	1.82 ± 0.12	42.1 ± 0.9	760
acrylate methacrylate **11**	CD	52.1 ± 1.6	1.63 ± 0.13	70.5 ± 15.0	74 000
acrylate methacrylate **11**	CD	15.3 ± 1.3	4.44 ± 0.60	70.5 ± 15.0	22 000
acrylate methacrylate **11**	CD	0.82 ± 0.03			
acrylate methacrylate **11**	CD	0.073 ± 0.006			
acrylate methacrylate **11**	t-CD	55 ± 19[g]	12 ± 5	70.5 ± 15.0	78 000
acrylate methacrylate **11**		0.82 ± 0.06			
acrylate methacrylate **11**		0.22 ± 0.03			

[a] In 60% Me_2SO–40% H_2O v/v. Rate constants extrapolated to "pH 10.0", as measured with a glass electrode. All errors are standard deviations. [b] CD is β-cyclodextrin, t-CD is 6-tosyl-β-cyclodextrin. [c] Pseudo-first-order rate constant for acylation of the cyclodextrin by fully bound substrate. [d] Dissociation constant of the substrate–cyclodextrin complex. [e] Pseudo-first-order rate constant for hydrolysis of the substrate in the absence of cyclodextrin. [f] Reported[7] values of 96, 8.8, 26.7, and 360 000 for these constants. [g] The two rate processes were not resolved in this case.

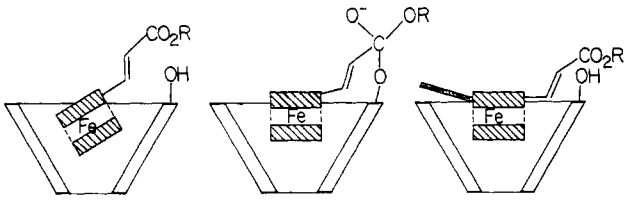
weaker, so apparently the greater thickness of ruthenocene does not lead to a better interaction with the cyclodextrin cavity. The rate constant for acylation in the complex $k_{complex}$ is almost the same for the two, with a slight advantage to **2**, even though the ruthenium compound shows a 50% larger uncatalyzed rate k_{un} because of electronic differences. Geometric factors lead to poorer binding for the starting material and even more so for the transition state in the ruthenium series, so we have not examined ruthenocene derivatives further.

Although the two enantiomeric cyclopentenoferrocene derivatives **6′** and **6″** have dissociation constants K_m that are similar to those of the cyclohexenoferrocene analogues **3**, the rate constants respond in opposite directions to this small geometric change. The rate constant for reaction with cyclodextrin increases by 30% for the more reactive enantiomer of **6** compared with **3**, but decreases by more than 50% for the slow enantiomer. Presumably the fast enantiomer of **6** is **6′**, by analogy with our finding that **3′** is the fast enantiomer in that case. The rate of simple hydrolysis of **6** is only two-thirds that of **3**, suggesting that the more nearly coplanar **6** is deactivated by slightly better conjugation of the ferrocene with the ester group.

Molecular models do not clearly suggest the origin of the improvement in $k_{complex}$ for **6′** compared with **3′**, or even the 62-fold difference in $k_{complex}$ for **6′** and **6″**. However, the result is that **6′** is the best substrate known so far for a cyclodextrin reaction. The ratio of 5 900 000 for $k_{complex}/k_{un}$ for **6′** is almost twice that for **3′**. As we have pointed out previously,[7,8] the value of k_{un} in our medium is higher than it would be in water solution, and for another nitrophenyl ester, which was soluble in both media, we had found[13] a 24-fold increase in k_{un} when the reaction was performed in 60% Me_2SO–water rather than in pure water. Thus in the current case the ratio of $k_{complex}$ in Me_2SO–water to k_{un} in water for **6′** would be ca. 1.5×10^8. This ratio is related to that normally invoked for enzymes, in which reaction in the protein interior, with both proximity and medium effects, is compared with the uncatalyzed reaction in water. In our case the enzyme interior is partially mimicked by use of Me_2SO–water as solvent. Regardless of the merits of this solvent extrapolation, the observed cyclodextrin acceleration by 5.9×10^6 and enantiomeric selectivity of 62-fold are substantial effects resulting from improved substrate geometry.

As we have discussed earlier, ferrocene derivatives such as **2**, **3**, and **6** can bind into the cavity of β-cyclodextrin with a geometry close to that required for reaction. On conversion to the tetrahedral intermediate for acylation by addition of a cyclodextrin hydroxyl to the carbonyl group, only a small lifting[9] of the ferrocene system is required from its position of deepest penetration in the cyclodextrin–substrate complex. However, optimum rates should result if the transition state is *better* bound than the substrate. Thus we wanted to construct new compounds, related to our best substrates, in which such better binding occurs. For ease of synthesis we decided to try to optimize the rate for ferroceneacrylate **2** rather than for fused-ring systems such as **6**, even though **6** is almost 20-fold better as a substrate.

Molecular models show that the ferrocene system of **2** can go deeply into the cyclodextrin cavity by a slight tipping (**12**) which



12 **13** **14**

permits the side chain to rest above the secondary hydroxyls. On conversion to the tetrahedral intermediate (**13**), the ferrocene system rises a bit out of the cavity as the tipped system straightens out. We hoped to prevent such deep penetration of the edge of the ferrocene unit remote from the side chain by attaching a projection to that remote edge (**14**). We have used a similar approach to improve the reaction rate of a cinnamate ester with cyclodextrin by attaching an additional short chain to prevent the substrate from binding too deeply.[7]

The simplest such projection is another acrylate ester chain. As we have described,[7,8] the conjugation between ferrocene and an acrylate ester tends to force such a chain to be rigid and coplanar with the cyclopentadienyl system. Thus we set out to synthesize derivatives of ferrocene-1,3-diacrylic acid.

3-Methylferrocenecarboxaldehyde was obtained by formylation of methylferrocene, and separation from isomers by careful chromatography.[11] Oxidation of the methyl group is complicated by the oxidizability of the ferrocene system, but an appropriate sequence was devised. As shown in Scheme I, the aldehyde group was converted to an ethyl acrylate chain, and the methyl group was then oxidized to formyl with MnO_2. This was then converted to a *tert*-butyl acrylate chain, and the *tert*-butyl group was se-

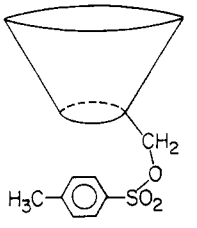
(13) Siegel, B.; Breslow, R. *J. Am. Chem. Soc.* **1975**, *97*, 6869.

lectively removed. The resulting acid was converted to the *p*-nitrophenyl ester group, yielding substrate [9]. In related ways the substrates **10** and **11** were also prepared. All these compounds were prepared as the pure *E* isomers, but they are of course still mixtures of enantiomers. Thus in the kinetic studies **9** and **10** all showed *two* processes that liberate *p*-nitrophenoxide ion, because of the different reactivities of their enantiomers. Substrate **11** showed *four* acylation rates, because of the different reactivities of its two ester groups and the presence of two enantiomers. Reaction of **11** with β-cyclodextrin produces novel cyclodextrins, capped and plugged on the secondary side.

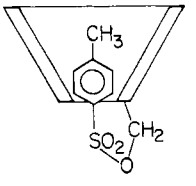
The comparison of **9** with the single-chain substrate **2** led to a surprise. The value of k_{un} for **9** was twice that for **2**, as might be expected because of the substitution in **9** of an electron-attracting group. However, the two enantiomers of **9** showed reactions with β-cyclodextrin with $k_{complex}$ only 33% and 6% of the value for **2**. Thus the hoped for improvement was not present.

A clue to the problem is seen in the values of K_m for compounds **9** and **2**. The binding of the two enantiomers of **9** is 9-fold and 6-fold stronger than that of **2**, which is curious if the extra side chains in **9** prevent deep binding of the ferrocene systems. Of course the extra side chain could be contributing some binding interaction of its own and **9** might still have the correct geometry for reaction. We thought it likelier that we are dealing with an alternate nonproductive complex, with very different geometry from that needed for acylation. We have seen such a situation previously[7] with an adamantane derivative that bound strongly so as to put a side chain right through the cyclodextrin activity, in a completely unreactive geometry. In the previous case we blocked this nonproductive binding, and restored normal reactivity, by the addition of a flexible intrusive floor to the cyclodextrin.[7] A similar approach proved effective here.

In polar solutions of β-cyclodextrin 6-tosylate (**15**) the toluene ring should insert partly into the cavity (**16**). In this geometry



15



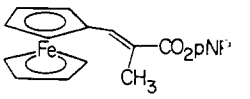
16

substrates would be expected to bind less deeply and only into the secondary face of cyclodextrin. Table I shows that this tosylation of β-cyclodextrin led to an acylation reaction with **2** that was 2.5 times slower than with simple cyclodextrin but with 4.0 times stronger substrate binding. As we had seen before with an intrusive floor,[7] the new hydrophobic surface helps to bind **2** better but moves it up to a geometry that is a little too high for optimal reaction (a nonintrusive floor[7] had given the better binding of *2 without* slowing the rate). The results for **9′** and **9″** were very different.

Both **9′** and **9″** were bound 10-fold more *weakly* by the tosylated cyclodextrin. They were even more weakly bound than was **2**, consistent with our idea that the extra projection in **9** should hinder full binding. Thus it seems clear that without the tosyl floor cyclodextrin binds **9** in some more stable alternate geometry. With the geometrical adjustment produced by the tosyl floor, **9′** and **9″** acylation rates increased by 6-fold and 7-fold, respectively. This is expected if the new geometry is productive and the alternate, without the floor, was not.

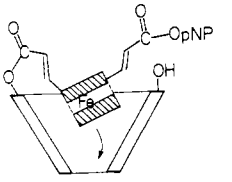
The overall result is that **9′** is more than four times as reactive as is **2** toward 6-tosylcyclodextrin, with the $k_{complex}/k_{un}$ more than twice as large for **9′** as for **2**. Thus the hoped for improvement in **9** from the extra projection is actually present, if small. However, **2** is still the better substrate for unmodified β-cyclodextrin.

Related effects are seen with methacrylate substrate **10**, but the changes are smaller. Tosylation of β-cyclodextrin increases the rate and decreases the binding for both enantiomers **10′** and **10″**, but to a lesser extent than in **9**. The rate constants $k_{complex}$ for **10′** and **10″** are smaller because of steric hindrance from the methyl group, as we had reported earlier for the analogous **17**.



17

This difference in reactivity of an acrylate and a methacrylate ester let us examine the two successive reactions of **11** (**11′** and **11″**). The first acylation by the acrylate chain produces a covalent link, so the second acylation is intramolecular. We considered the possibility that this second acylation could be accelerated by two factors: (1) various degrees of freedom in a cyclodextrin complex would be frozen out by the covalent link, including the freedom to spin in the cavity, and (2) the first ester link could act as a hinge to permit deep binding of the ferrocene unit only in the transition state rather than in the starting material (**18**). Both of these effects should favor reaction.

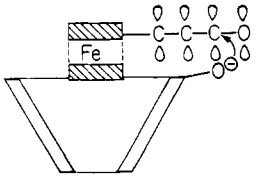


18

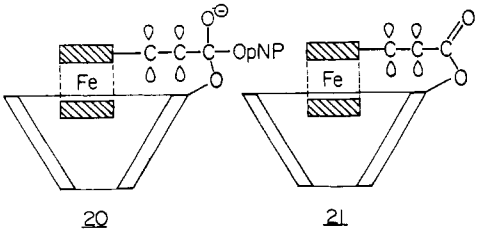
The results in Table I show that these effects, if present, were overcome by other factors. The first acylations by **11′** and **11″** were faster than those by **9′** and **9″**, reflecting electronic factors in part, but the second acylations by **11′** and **11″** were similar to those by the closely analogous **10′** and **10″**. Thus in this case the simple intracomplex reactions are about as good as those of the intramolecular analogues.

Since the substrate modifications we have described here have improved the cyclodextrin acylation reactions only modestly, one might wonder whether substrates such as **6** are the best that can be devised. Further improvements in rate still seem possible. For one thing, even in our best substrate **6** it is likely that the substrate is bound more strongly into the cavity than is the transition state, as evidenced by the comparison of substrates **9** and **2** with β-cyclodextrin tosylate. Substrates in which the transition-state geometry is much more favorable for binding remain to be explored. Another factor relates to the geometric changes that accompany reaction.

In all the substrates, **2**, **3**, **4**, **5**, **6**, **9**, **10**, and **11**, the ester carbonyl is held coplanar with the cyclopentadiene ring by conjugation, in an ideal geometry for attack of a cyclodextrin alkoxide from underneath (**19**) to form the tetrahedral intermediate (**20**).



19



20 21

However, forward decomposition of this tetrahedral intermediate

will produce a cyclodextrin ester (**21**) in which the acrylate conjugated system is badly twisted. This may explain our finding[14] that maximum accelerations $k_{complex}/k_{un}$ for any given acyl group are seen with the best leaving groups. With a good leaving group the transition state occurs on the path from **19** to **20**, but with a poorer leaving group it may occur between **20** and **21** and be destabilized by the loss of conjugation that develops. The twisting shown in **21** may also affect the second acylation step with substrate **11**, in which such an ester link is present.

An optimal substrate would thus be one with a geometric double minimum for the carboxyl group. For attack by the nucleophilic oxygen the carboxyl must be in one plane, but it must end up in a perpendicular plane in the product (or the nucleophile must move into the original plane). Whether or not such optimal substrates can be produced, the accelerations already achieved make it clear that complexes with well-defined geometry can show the accelerations needed for true enzyme models.[15,17]

Experimental Section

Aqueous buffers were prepared with deionized water. The dimethyl sulfoxide used in the kinetics runs was MCB omnisolv grade. β-Cyclodextrin (CPC) was recrystallized 3 times from water and dried overnight at 80 °C under reduced pressure.

NMR spectra were measured in $CDCl_3$ solution by using a 90-MHz Perkin-Elmer R-32 instrument. IR spectra were recorded on a JASCO IRA-1 spectrophotometer. A Beckman UV-5 spectrophotometer was used for kinetics. All reported melting points are uncorrected. Mass spectra were recorded on a Finnigan 3300 mass spectrometer with a 6000 series data system (CI) and on a Ribermag R 10-10 mass spectrometer with a pdp8a system (CI, NH_3). pH measurement was performed with a Radiometer PHM 63 pH meter equipped with a Radiometer GK2321C combination electrode.

Kinetics. Reaction buffers were prepared by adding four volumes of 10 mM aqueous potassium dihydrogen phosphate/sodium hydroxide buffer (pH's 6.39 and 6.74) to six volumes of dimethyl sulfoxide. The resulting solutions had pH's of 10.00 and 10.42, respectively, as determined with the glass electrode. β-Cyclodextrin or tosylated β-cyclodextrin solutions (1.5–10 mM) were prepared with this buffer just prior to use. Substrate solutions (4–6 mM in dimethyl sulfoxide) were stored in the dark. A kinetic run was initiated by equilibrating 1.00 mL of catalyst solution to 30 ± 0.1 °C in the spectrophotometer chamber. A 10-μL sample of substrate solution was injected (to make the solution 40–60 μM in substrate) and the absorbance at 410 nm monitored as a function of time.

Both mono- and biphasic kinetics were solved with appropriate computer programs. In the case of the first acylation with **11**, several values close to the absorbance at half-completion of the reaction were used for calculation, and the values that gave the best fit between experimental and calculated curves were adopted. The kinetics were usually done at pH 10.00, but the pH 10.42 solution was used for the slow reaction observed in the case of **10** and the second acylation of **11** and extrapolated to pH 10.00. The maximal rate constants ($k_{complex}$) and binding constants (K_m) were obtained from Lineweaver–Burk plots.

All uncatalyzed rates were measured in a solution prepared by adding four volumes of aqueous 0.00524 M potassium hydroxide to six volumes of dimethyl sulfoxide under nitrogen. The pH of the solution, 14.46, was obtained from reading in the millivolt mode, and the kinetic results were extrapolated to pH 10.0. The rate for the uncatalyzed reaction of **11** was obtained by calculation based on a computer program for consecutive reactions. The data for the other substrates were fitted to a simple exponential for a first-order reaction over 10 half-lives. The rate constants shown in Table I represent the average of three runs.

***p*-Nitrophenyl (*E*)-3-Ruthenocenylpropenoate.** (*E*)-3-Ruthenocenylpropenoic acid[16] (211 mg, 0.701 mmol), *p*-nitrophenol (108 mg, 0.776 mmol), dicyclohexylcarbodiimide (159 mg, 0.771 mmol), and 4-(dimethylamino)pyridine (88 mg, 0.072 mmol) were stirred in methylene chloride (10.5 mL) for 2 h. Evaporation followed by column chromatography on silica gel (30 g) with methylene chloride as the eluting solvent gave 231 mg (78%) of the ester. Recrystallization from 2-propanol gave yellow needles: mp 173–177 °C; NMR δ 8.27 (d, 2, *J* = 9, Hz), 7.69 (d, 1, *J* = 15.5 Hz), 7.33 (d, 2, *J* = 9 Hz), 6.13 (d, 1, *J* = 15.5 Hz), 4.93 (t, 2, *J* = 2 Hz), 4.77 (t, 2, *J* = 2 Hz), 4.60 (s, 5); IR (KBr) 1718, 1607, 1514, 1341, 1100, 998 cm^{-1}; mass spectrum (CI), *m/e* 424; UV/vis 299 (4.40), 370 (3.73) nm. Anal. Calcd for $C_{19}H_{15}NO_4Ru$: C, 54.03; H, 3.58; N, 3.32. Found: C, 53.82; H, 3.57; N, 3.28.

(*E*)-3-(Carbomethoxymethylene)-1,2-ferrocenocyclopentene. 1,2-Ferrocenocyclopenten-3-one[10] (135 mg, 0.562 mmol), zinc powder (82 mg, 1.26 mmol; prewashed with acid, water, methanol, and ether and then oven-dried), and methyl bromoacetate (0.11 mL, 1.16 mmol) were refluxed under nitrogen in a dry solvent consisting of 3 mL of benzene and 3 mL of ether. After 1 h, the solid residue was removed by filtration. *p*-Toluenesulfonic acid (0.28 g) was added and the mixture was refluxed for 10 min. The organic solution was washed with water, dried over $MgSO_4$, and then evaporated. Chromatography on a long column of silica gel (60 g) with benzene as the solvent gave the *Z* ester (14.6 mg) as a red oil and the desired *E* ester (66.5 mg) as a partially crystalline red oil. Elution of the column with benzene/ethyl acetate (1:1) allowed the recovery of 53.8 mg of starting material. The yields of *E* and *Z* ester based on a 60% conversion of starting material were 67% and 15%, respectively. The *Z* ester slowly isomerized to a mixture of *Z* and *E* ester on standing. The *E* ester showed the following spectroscopic properties: NMR δ 5.90 (t, 1, *J* = 2.5 Hz), 4.42 (t, 2, *J* = 2 Hz), 4.34 (t, 1, *J* = 2.5 Hz), 4.03 (s, 5), 3.73 (s, 3), 3.55 (m, 2), 2.82 (d, d, d, 1, *J* = 15.5, 6.5, 3.5 Hz), 2.60 (d, d, d, 1, *J* = 15.5, 8.5, 6); IR (KBr) 1684, 1623, 1104, 995 cm^{-1}; mass spectrum (CI) 297; UV/vis 254 (4.18), 304 (4.26), 350 sh, 475 (2.95) nm.

(*E*)-3-(Carboxymethylene)-1,2-ferrocenocyclopentene. (*E*)-3-(Carboxymethoxymethylene)-1,2-ferrocenocyclopentene (273 mg, 0.922 mmol) was dissolved in 9 mL of ethanol and 9 mL of aqueous 6 N sodium hydroxide was added. The mixture was stirred at room temperature in the dark for 40 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 Na_2SO_4 and evaporated to afford the acid (260 mg) in 100% yield. Recrystallization from methylcyclohexane afforded a brick-red microcrystalline solid: mp 171.5–173.5 °C dec; NMR δ 5.92 (t, 1, *J* = 2 Hz), 4.46 (t, 2, *J* = 2.5 Hz), 4.38 (t, 1, *J* = 2.5 Hz), 4.40 (s, 5), 3.56 (m, 2), 2.84 (d, d, d, 1, *J* = 15.5, 6.5, 3.5), 2.62 (d, d, d, 1, *J* = 15.5, 7.5, 6.0); IR (KBr) 3300–2200, 1652, 1597, 1105, 999 cm^{-1}; mass spectrum (CI) 283; UV/vis 254 (4.10), 304 (4.17), 360 sh, 479 (2.91) nm. Anal. Calcd for $C_{15}H_{14}FeO_2$: C, 63.86; H, 5.00. Found: C, 61.81; H, 4.98.

(*E*)-3-(Carboxymethylene)-1,2-ferrocenocyclopentene *p*-Nitrophenyl Ester. (*E*)-3-(Carboxymethylene)-1,2-ferrocenocyclopentene (122 mg, 0.43 mmol), *p*-nitrophenol (65 mg, 0.47 mmol), dicyclohexylcarbodiimide (97 mg, 0.47 mmol), and *p*-(dimethylamino)pyridine (12 mg, 0.10 mmol) were stirred in methylene chloride (7 mL) for 2 h. Evaporation followed by resolution of the complex reaction mixture on a column of silica gel (50 g) with methylene chloride as the eluting solvent gave the red crystalline ester (73 mg) in 42% yield. Recrystallization from 2-propanol afforded lustrous red leaves: mp 180.5–183.5 °C; NMR δ 8.28 (d, 2, *J* = 9 Hz), 7.36 (d, 2, *J* = 9 Hz), 6.09 (t, 1, *J* = 2 Hz), 4.53 (d, 2, *J* = 2 Hz), 4.45 (t, 1, *J* = 2.5 Hz), 4.08 (s, 5), 3.58 (m, 2), 2.88 (d, d, d, 1, *J* = 15.5, 6.0, 4.5), 2.66 (d, d, d, 1, *J* = 15.5, 7.5, 7.0), IR (KBr) 1718, 1606, 1509, 1341, 1113, 997 cm^{-1}; mass spectrum (CI) 404; UV/vis 264 (4.19), 315 (4.43), 370 sh, 493 (3.17) nm. Anal. Calcd for $C_{21}H_{19}FeNO_4$: C, 62.24; H, 4.73; N, 3.46. Found: C, 62.58; H, 4.30; N, 3.41.

(*E*)-3-Methyl-1-(3-ethoxy-3-oxo-1-propenyl)ferrocene. 3-Methylferrocenecarboxaldehyde[11] (0.346 g, 1.52 mmol), separated from the other isomers by alumina chromatography (Brockman IV) with ether/hexane (1:10), and (carbethoxymethylene)triphenylphosphorane (3.10 g, 8.90 mmol) were stirred in methylene chloride (12 mL) at room temperature in the dark for 2 days. Evaporation followed by column chromatography on silica gel with ether/hexane (1:5) as the eluting solvent afforded the product (0.349 g, 77%) as a red oil: NMR δ 1.35 (t, 3, *J* = 7 Hz), 2.04 (s, 3), 4.11 (s, 5), 4.25 (q, 2, *J* = 8 Hz), 4.38 (m, 3), 5.99 (d, 1, *J* = 15 Hz), 7.52 (d, 1, *J* = 16 Hz); IR (neat film) 1708, 1628 cm^{-1}; mass spectrum (CI), *m/e* 299.

(*E*)-3-Formyl-1-(3-ethoxy-3-oxo-1-propenyl)ferrocene. The above compound (895 mg, 3.0 mmol) was dissolved in toluene (90 mL), and activated MnO_2 (805 mg), which was azeotropically treated with benzene before use, was added. The mixture was stirred at 90 °C in the dark for 4 h and then filtered. Evaporation followed by column chromatography on silica gel with methylene chloride as the solvent gave 190 mg (20%) of the desired product as a red oil: NMR δ 1.33 (t, 3, *J* = 7 Hz), 4.26 (q, 2, *J* = 7 Hz), 4.29 (s, 5), 4.88 (s, 1), 4.99 (s, 1), 5.13 (s, 1), 6.17 (d, 1, *J* = 16 Hz), 7.54 (d, 1, *J* = 16 Hz), 9.99 (s, 1); IR (neat film) 1708, 1678, 1630 cm^{-1}; mass spectrum (CI), *m/e* 313.

(*E*,*E*)-3-[3-(*tert*-Butoxy)-3-oxo-1-propenyl]-1-(3-ethoxy-3-oxo-1-propenyl)ferrocene. The above aldehyde (50 mg, 0.160 mmol) and ((*tert*-butoxycarbonyl)methylidene)triphenylphosphorane (150.6 mg, 0.4 mmol) were refluxed in benzene (4 mL) in the dark for 4 h. Evaporation of the solvent followed by column chromatography on silica gel with

(14) Cf. the results for an imidazole leaving group in ref 7b. Other work with anilides of various of our ferroceneacrylates confirms this picture.

(15) Support of this work by the N.I.H. is gratefully acknowledged.

(16) Kamiyama, S. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 909.

methylene chloride gave 49.2 mg (75%) of the desired product as a red oil: NMR δ 1.32 (t, 3, J = 7 Hz), 1.52 (s, 9), 4.14 (s, 5), 4.22 (q, 2, J = 7 Hz), 4.66 (b, s, 2), 4.77 (s, 1), 6.02 (d, 2, J = 15 Hz), 6.07 (d, 2, 15 Hz), 7.40 (d, 2, J = 16 Hz), 7.51 (d, 2, J = 16 Hz), IR (neat film) 1708, 1608, 1590, 1514, 1344, 1118 cm^{-1}; mass spectrum (CI, NH_3), m/e 411 (100) ($M{\cdot}H^+$), 428 (82) ($M{\cdot}NH_4^+$).

(*E*,*E*)-3-[(4-Nitrophenoxy)-3-oxo-1-propenyl]-1-(3-ethoxy-3-oxo-1-propenyl)ferrocene (9). The above diester (49.2 mg, 0.12 mmol) was stirred in trifluoroacetic acid (2 mL) for 0.5 h. After evaporation of trifluoroacetic acid, 4 mL of benzene was added followed by evaporation of the solution. This treatment with benzene was repeated several times. Without further purification, the acid product was used for the next reaction. The acid, *p*-nitrophenol (20.9 mg, 0.15 mmol), dicyclohexylcarbodiimide (30.9 mg, 0.15 mmol), and *p*-(dimethylamino)pyridine (1.5 mg, 0.012 mmol) were stirred in 2 mL of methylene chloride in the dark at room temperature for 5 h. Evaporation of the solvent followed by column chromatography on silica gel with methylene chloride as the solvent afforded 28 mg (49% from the diester) of the ester. Recrystallization from carbon tetrachloride gave red crystals: mp 156.5–157.5 °C; NMR δ 1.33 (t, 3, J = 7 Hz), 4.20 (s, 5), 4.24 (q, 2, J = 7 Hz), 4.77 (br s, 2), 4.87 (s, 1), 6.12 (d, 2, J = 16 Hz), 6.25 (d, 2, J = 16 Hz), 7.36 (d, 2, J = 9 Hz), 7.53 (d, 1, J = 16 Hz), 7.78 (d, 1, J = 16 Hz), 8.29 (d, 2, J = 9 Hz); IR (KBr) 1708, 1608, 1590, 1514, 1344, 1118 cm^{-1}; mass spectrum (CI, NH_3), m/e 476 ($M{\cdot}H^+$). Anal. Calcd for $C_{24}H_{21}NO_6Fe$: C, 60.64; H, 4.46; N, 2.95. Found: C, 60.86; H, 4.40; N, 2.96.

(*E*,*E*)-3-[2-(*tert*-Butoxy)carbonyl-1-propenyl]-1-(3-ethoxy-3-oxo-1-propenyl)ferrocene. The aldehyde ester **7** (212 mg, 0.679 mmol) and ((*tert*-butoxycarbonyl)ethylidene)triphenylphosphorane (592 mg, 1.52 mmol) were stirred in methylene chloride (25 mL) at room temperature in the dark for 15 h. Concentration to a red oil followed by column chromatography on silica gel with methylene chloride gave 242 mg (84%) of the desired product as a red oil: NMR δ 1.33 (t, 3, J = 7 Hz), 1.53 (s, 9), 2.01 (s, 3), 4.14 (s, 5), 4.25 (q, 2, J = 6 Hz), 4.66 (s, 2), 4.90 (s, 1), 6.11 (1, d, J = 16 Hz), 7.33 (1, s), 7.57 (1, d, J = 15 Hz); IR (neat film) 1695 (broad), 1630 cm^{-1}; mass spectrum (CI), m/e 425.

(*E*,*E*)-3-(2-Carboxy-1-propenyl)-1-(3-ethoxy-3-oxo-1-propenyl)-ferrocene. The above diester (191 mg, 0.45 mmol) was stirred in trifluoroacetic acid (5 mL) for 2 h. Evaporation, followed by column chromatography on silica gel with methylene chloride/methanol (20:1), gave 122 mg (74%) of the crystalline acid: mp 300 °C; NMR δ 1.32 (t, 3, J = 7 Hz), 2.14 (s, 3), 4.15 (s, 5), 4.23 (q, 2, J = 7 Hz), 4.72 (s, 2), 4.84 (s, 1), 6.12 (d, 1, J = 16 Hz), 7.55 (d, 1, J = 16 Hz), 7.57 (s, 1); IR (KBr) 1710 (broad), 1630 (broad), 1380 cm^{-1}; mass spectrum (CI), m/e 369.

(*E*,*E*)-3-[2-(4-Nitrophenoxy)carbonyl-1-propenyl]-1-(3-ethoxy-3-oxo-1-propenyl)ferrocene (10). The above acid (61.1 mg, 0.166 mmol), *p*-nitrophenol (25.4 mg, 0.183 mmol), dicyclohexylcarbodiimide (37.7 mg, 0.183 mmol), and *p*-(dimethylamino)pyridine (2.0 mg, 0.017 mmol) were stirred in 7 mL of methylene chloride in the dark at room temperature for 5 h. Evaporation of the solvent followed by column chromatography on silica gel with methylene chloride as the solvent afforded 43.4 mg (53%) of the ester as red crystals: mp 130.5–131 °C; NMR δ 1.34 (t, 3, J = 7 Hz), 2.17 (s, 3), 4.18 (s, 5), 4.23 (q, 2, J = 7 Hz), 4.76 (s, 2), 4.86 (s, 1), 6.11 (d, 2, J = 16 Hz), 7.34 (d, 2, J = 10 Hz), 7.55 (d, 1, J = 16 Hz), 7.65 (s, 1), 8.30 (d, 2, J = 9 Hz); IR (KBr) 1722, 1615, 1589, 1516, 1345 cm^{-1}; mass spectrum (CI), m/e 490.

(*E*,*E*)-3-(2-Ethoxycarbonyl-1-propenyl)-1-(3-ethoxy-3-oxo-1-propenyl)ferrocene. The aldehyde **7** (219 mg, 0.70 mmol) and (carbethoxyethylidene)triphenylphosphorane (1.016 g, 2.8 mmol) were dissolved in methylene chloride (15 mL) and the solution was stirred at room temperature in the dark for 4 h. Evaporation of the solvent followed by column chromatography with methylene chloride as the solvent gave the product (265 mg, 95%) as a red oil: NMR δ 1.33 (t, 6, J = 7 Hz), 2.05 (s, 3), 4.14 (s, 5), 4.25 (q, 4, J = 7 Hz), 4.66 (s, 2), 4.81 (s, 1), 6.10 (d, 1, J = 16 Hz), 7.42 (s, 1), 7.56 (d, 1, J = 16 Hz); IR (neat film) 1700 (broad), 1360 (broad) cm^{-1}; mass spectrum (CI), m/e 397.

(*E*,*E*)-3-(2-Carboxy-1-propenyl)-1-(2-carboxyethenyl)ferrocene. The above diester (210 mg, 0.53 mmol) was dissolved in ethanol (9.4 mL); aqueous 6 N sodium hydroxide (9.4 mL) was added, and the solution was stirred at room temperature in the dark for 24 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 product (152 mg, 84%). Recrystallization from methylene chloride afforded red crystals: mp 217.5–218 °C; NMR δ 2.08 (s, 3), 4.19 (s, 5), 4.77 (s, 2), 4.90 (s, 1), 6.14 (d, 1, J = 15 Hz), 7.61 (s, 1), 7.72 (d, 1, J = 15 Hz); IR (KBr) 1678, 1622 cm^{-1}; mass spectrum (CI), m/e 341.

(*E*,*E*)-3-[2-(4-Nitrophenoxy)carboxyl-1-propenyl]-1-[3-(4-nitrophenoxy)-3-oxo-1-propenyl]ferrocene (11). The above dicarboxylic acid (68 mg, 0.2 mmol); *p*-nitrophenol (30.6 mg, 0.22 mmol), dicyclohexylcarbodiimide (45.4 mg, 0.22 mmol), and *p*-(dimethylamino)pyridine (2.4 mg, 0.02 mmol) were stirred in 8 mL of methylene chloride in the dark at room temperature for 3 h. Column chromatography on silica gel with methylene chloride as the solvent afforded 73 mg (63%) of the product. Recrystallization from benzene and *n*-hexane afforded red crystals: mp 192–193 °C; NMR δ 2.18 (s, 3), 4.24 (s, 5), 4.83 (s, 2), 4.96 (s, 1), 6.30 (d, 1, J = 15 Hz), 7.36 (d, 4, J = 9 Hz), 7.70 (s, 1), 7.83 (d, 1, J = 15 Hz), 8.29 (d, 4, J = 9 Hz); IR (KBr) 1725, 1607, 1587, 1513, 1338, 1116 cm^{-1}; mass spectrum (CI, NH_3) 600 (100) ($M{\cdot}NH_4^+$), 583 (52) ($M{\cdot}H^+$). Anal. Calcd for $C_{29}H_{22}N_2O_8Fe$: C, 59.80; H, 3.82; N, 4.81. Found: C, 59.81; H, 3.93; N, 4.95.

Registry No. 2, 85116-52-5; **4**, 85185-85-9; **6**, 85248-80-2; **6′**, 85185-87-1; **6″**, 85248-79-9; **7**, 85185-89-3; **8** (R = H), 85185-90-6; **8** (R = CH_3), 85202-32-0; **8′**, 85202-33-1; **9**, 85185-91-7; **10**, 85185-94-0; **11**, 85185-97-3; CD, 7585-39-9; t-CD, 67217-55-4; (*E*)-3-(carbomethoxymethylene)-1,2-ferrocenocyclopentene, 85185-86-0; 1,2-ferrocenocyclopenten-3-one, 82796-15-4; (*Z*)-3-(carbomethoxymethylene)-1,2-ferrocenocyclopentene, 85248-78-8; (*E*)-3-(carboxymethylene)-1,2-ferrocenocyclopentene, 85185-95-1; 3-methylferrocenecarboxaldehyde, 12214-96-9; (*E*)-3-methyl-1-(3-ethoxy-3-oxo-1-propenyl)ferrocene, 85185-88-2; (*E*,*E*)-3-(carboxyethenyl)-1-(3-ethoxy-3-oxo-1-propenyl)-ferrocene, 85185-92-8; (*E*,*E*)-3-(2-carboxy-1-propenyl)-1-(3-ethoxy-3-oxo-1-propenyl)ferrocene, 85185-93-9; (*E*,*E*)-3-(2-carboxy-1-propenyl)-1-(2-carboxyethenyl)ferrocene, 85185-96-2; methylbromoacetic acid, 96-32-2; ((*tert*-butoxycarbonyl)methylidene)triphenylphosphorane, 35000-38-5; ((*tert*-butoxycarbonyl)ethylidene)triphenylphosphorane, 56904-86-0; (carbethoxyethylidene)triphenylphosphorane, 5717-37-3; carbethoxymethylenetriphenylphosphorane, 1099-45-2.

(17) **Note Added in Proof**: In a recent paper Cram and Katz [Cram, D. J.; Katz, H. E. *J. Am. Chem. Soc.* **1983**, *105*, 135] estimated a rate factor of 10^{11} by which a *p*-nitrophenyl ester of an amino acid acylates a bound host molecule, compared with the rate for acylation of an unbound analogue, in $CHCl_3$ solution with a tertiary amine buffer. This comparison includes a binding constant of over 10^9 as part of the rate factor, so it is not calculated on the same basis as are $k_{complex}/k_{un}$ in our work. The Cram–Katz system, in $CHCl_3$, actually acylates rather slowly compared with known rates of hydrolysis of *p*-nitrophenyl esters in H_2O with such buffers. We find (Labelle, M., unpublished work) that their substrate undergoes "uncatalyzed" hydrolysis, in H_2O with their buffer, ca. 500 times faster than their reported acyl transfer rate. Thus on our basis the Cram–Katz system shows a $k_{complex}/k_{un}$ smaller than 10^{-2}.