Table I. Degree of Racemization during Peptide Bond Formation

Component activated[a,b]	Condensed with[a,b]	% D-L or L-D in product with coupling agent[c] CDI	K	DCC	EDC	Methyl resonance[d] L-L	D-L or L-D
For-L-Ala	L-Phe-OMe	<3	<3	<3	<3	80.5	74.3
For-L-Phe	L-Ala-OMe	<3	<3	<3	<3	79.5	72.5
Ac-L-Ala	L-Phe-OMe	<3	<3	35	27	79.0	71.5
Ac-L-Phe	L-Ala-OMe	16	6	50	41	81.0	73.5
Bz-L-Ala	L-Phe-OMe	19	<3	..	..	84.5	79.5
Bz-L-Phe	L-Ala-OMe	35	<3	..	..	79.5	74.5
Z-Gly-L-Ala	L-Phe-OMe	<3	5	10	12	78.0	71.0
Z-Gly-L-Phe	L-Ala-OMe	<3	9	17	17	77.5	70.5
Z-Gly-Gly-L-Phe	L-Ala-OMe	10	15	25	20	81.5	72.0

[a] For = formyl; all other abbreviated designations of compounds follow IUPAC-IUB rules. [b] The optical purity of the starting materials was verified by gas–liquid partition chromatography: N-acetyl and N-benzoyl compounds were converted to the menthyl ester derivatives, while the N-formyl compounds were hydrolyzed and analyzed as the N-trifluoroacetyl-L-prolyl peptide esters; the methyl ester compounds were assayed similarly.[7] [c] The limit of measurement was generally 3%, although in some cases a more accurate value was obtained by duplicate procedures. [d] All spectra were determined on a Varian A-60 spectrometer with the center of gravity of the chemical shift given in hertz downfield from tetramethylsilane (J = 7.2 ± 0.3 Hz). The compounds were dissolved in deuteriochloroform (deuterioethanol for the tetrapeptide).

nuclear magnetic resonance (nmr) spectra.[14a] The methyl doublet signal in an L–L (or D–D) compound was at lower field than the equivalent signal for the D–L (or L–D) analog due to deshielding and offered a potentially convenient means for the quantitative analysis of such mixtures. We have now employed this technique to examine the influence of several coupling agents and N-acyl protecting groups on the extent of racemization during peptide synthesis.

In a typical experiment, N-acetyl-L-phenylalanine (0.207 g, 1 mmole), carbonyldiimidazole (0.162 g, 1 mmole), and methylene chloride or acetonitrile (5 ml) was stirred at −5° for 1 hr. The hydrochloride of L-alanine methyl ester (0.139 g, 1 mmole) and triethylamine (0.14 ml, 1 mmole) were added and the solution was kept at 0° for 15 hr. After suitable washings, the solution was dried and evaporated; the solid residue was redissolved in deuteriochloroform and the solution was used for nmr analysis.

It is evident (Table I) that carbonyldiimidazole (CDI) and 2-ethyl-5-phenylisoxazolium-3′-sulfonate (K) are preferable here to dicyclohexycarbodiimide (DCC) and 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide (EDC). Optical purity is lower in the peptides derived from N-benzoyl- and N-acetylamino acids, but the N-formyl group is noted as a potentially useful amino protecting group.[14b] The strongly electron-withdrawing N-acyl functions obviously cause a decrease of steric homogeneity in the products, while the phenylalanyl derivatives seem more prone to racemize than the alanyl derivatives.

Among the advantages of the nmr procedure are convenience, generality, and rapidity, while sensitivity is comparable to previous methods. As a test, an artificial mixture of N-acetylalanylphenylalanine methyl ester (L–L, 9.862 mg; D–L, 0.201 mg) was readily resolved at 100 MHz with the aid of a computer of average transients. At 60 MHz both sets of methyl doublets are separable in the racemic N-benzoyl and N-benzyloxycarbonyl peptides but overlap to give a triplet in the corresponding N-acetyl and N-formyl peptides. Area integration is simplified in this latter pattern, which can be internally standardized by the presence of the acetyl or methyl ester singlets. Nmr is extensively used for the gathering of kinetic data, and it is suggested that such an approach in combination with the method outlined here may have immediate application to work concerned with the mechanisms of racemization in amino acids,[15] peptides,[16–18] and active esters.[19,20]

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Acknowledgment. We wish to thank the National Aeronautics and Space Administration (NsG 81-60) and the National Institutes of Health (AM 10671-01A1) for the support of this work.

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Enzyme–Substrate Interaction by Nuclear Magnetic Resonance

Sir:

We should like to report the study of enzyme–substrate (or inhibitor) interaction by nmr techniques using the changes in chemical shifts of hydrogen or fluorine atoms of the substrate (or inhibitor) exchanging rapidly between solution and the active site of the enzyme.[1] The magnitude and direction of such shifts can potentially give a detailed understanding of the manner in which various parts of a substrate interact with the active site of an enzyme. If δ is the observed change in

(1) This method has also recently been applied to lysozyme: E. W. Thomas, *Biochem. Biophys. Res. Commun.*, 24, 611 (1966); M. A. Raftery, F. W. Dahlquist, S. Parsons, and S. I. Chan, unpublished observations.

Table I[a]

	Δ, cps	K_s
N-Acetyl-D-*p*-fluorophenylalanine	-83 ± 5[b]	$(6 \pm 2) \times 10^{-3}$
N-Acetyl-D-*m*-fluorophenylalanine	-85 ± 7[b]	$(11 \pm 5) \times 10^{-3}$
N-Acetyl-D-phenylalanine (acetyl hydrogens)	~10[c]	$\sim 10^{-3}$

[a] $E^0 \cong 3 \times 10^{-3}$ *M*; $S^0 = (1\text{–}8) \times 10^{-2}$ *M*. [b] At 56.4 Mcps; 95% confidence limits. [c] At 60 Mcps.

chemical shift, Δ the total change in chemical shift between the substrate in solution and in the ES complex, S^0 the initial substrate concentration, E^0 the initial enzyme concentration, and K_s the dissociation constant for the enzyme–substrate complex, eq 1 can be derived (assuming $\delta \ll \Delta$).

$$\frac{1}{\delta} = \frac{K_s + S^0}{\Delta E^0} \qquad (1)$$

This technique has been applied to a study of the interaction of chymotrypsin with N-acetyl-DL-*p*-fluorophenylalanine and N-acetyl-DL-*m*-fluorophenylalanine (by observation of the fluorine resonance), and with

The ^{19}F resonance of N-acetyl-DL-*p*-fluorophenylalanine at pD 6 in deuterium oxide–citrate buffer at 40° appears as a AA'BB'X multiplet. Addition of chymotrypsin results in a separation into two overlapping multiplets corresponding to the individual D and L isomers. Addition of pure D or L isomers shows that the observed separation is due to a shift to lower field of the ^{19}F resonance of the D isomer; the L isomer is bound much less strongly by the enzyme and shows no significant change in chemical shift at these concentrations. Figure 1 shows three typical spectra, in the presence of lysozyme (the same spectrum is observed in the absence of enzyme), where a separation of 6 cps is evident, and where a separation of 10 cps is present. The reciprocals of the observed shifts ($1/\delta$) are plotted against S^0 in

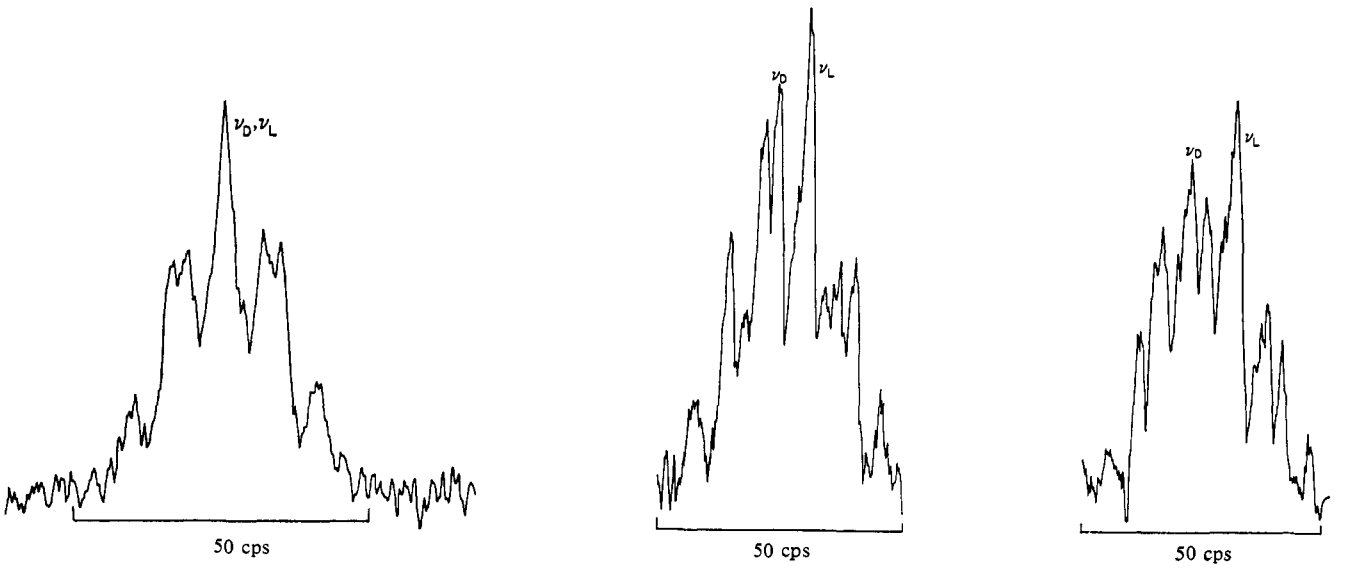

Figure 1. N-Acetyl-DL-*p*-fluorophenylalanine (50 cps; pD 6): (left) 0.042 *M*, in the presence of 0.003 *M* lysozyme, shift 0 cps, (middle) 0.083 *M*, in the presence of 0.003 *M* α-chymotrypsin, shift 6 cps; (right) 0.042 *M*, in the presence of 0.003 *M* α-chymotrypsin, shift 10 cps.

N-acetyl-D- and -L-phenylalanine by observation of the acetyl hydrogen resonance. Use of fluorine as a probe has the advantage that ^{19}F chemical shifts are larger and considerably more sensitive to environmental effects than ^{1}H chemical shifts.[2] None of the changes in nmr spectra to be discussed subsequently are observed when either lysozyme or ribonuclease is added as the enzyme component nor are there any changes in the nmr spectra of the fluorine compounds with diisopropylphosphoryl-chymotrypsin in which the active serine is phosphorylated.

(2) W. Emsley, J. Feeney, and L. H. Sutcliffe, "High Resolution Magnetic Resonance Spectroscopy," Vol. 2, Pergamon Press, New York, N. Y., 1966.

Figure 2. The analogous plot for N-acetyl-D-*m*-fluorophenylalanine is shown in Figure 3.

Similar results are obtained for the ^{1}H resonances of the acetyl group of N-acetyl-D-phenylalanine and N-acetyl-L-phenylalanine, although the observed shifts are much smaller. The results are summarized in Table I.

The shifts are in each case to lower fields and can plausibly be interpreted as evidence that the aromatic ring and acetyl group bind in a hydrophobic pocket at the active site. Changes in the fluorine chemical shift of this magnitude caused by different solvent environments have been reported.[3,4] These conclusions am-

(3) D. F. Evans, *J. Chem. Soc.*, 877 (1960).

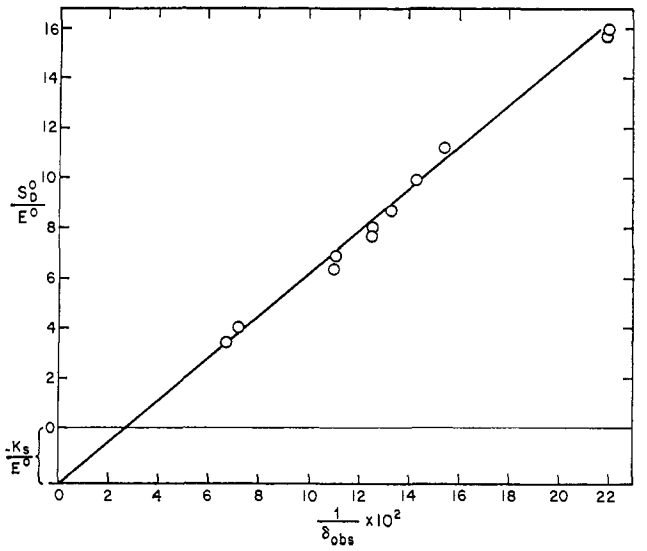

Figure 2. Plot of S_D^0/E^0 *vs.* $1/\delta_{obsd}$ for N-acetyl-*p*-fluorophenylalanine; $E^0 = 0.003$ *M* in pD 6 citrate. Shift of D isomer.

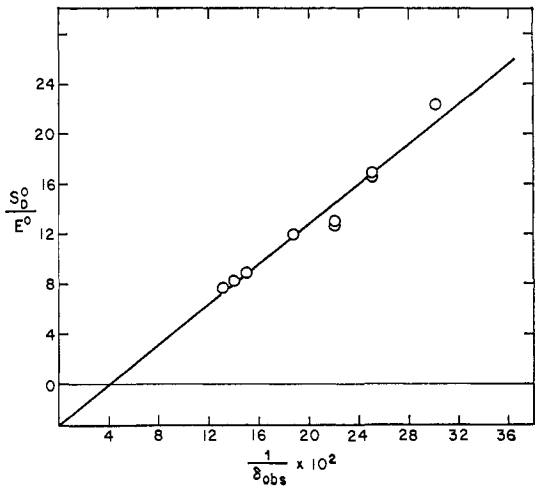

Figure 3. Plot of S_D^0/E^0 *vs.* $1/\delta_{obsd}$ for N-acetyl-*m*-fluorophenylalanine; $E^0 = 0.003$ *M* in pD 6 citrate. Shift of D isomer.

plify previously suggested models for the binding site[5,6] and should also be compared with the recently published map at 2-A resolution of tosyl-α-chymotrypsin.[7]

The dissociation constant for other substances which compete with N-acetyl-D-*m*-fluorophenylalanine for the active site can also be determined by this method. The appropriate expression is then

$$\frac{1}{\delta} = \frac{1}{\Delta E^0}\left(K_s + S_s^0 + \frac{K_s}{K_I} S_I^0\right)$$

where K_s and S_s^0 refer to the constants and concentrations of N-acetyl-D-*m*-fluorophenylalanine and K_I and S_I^0 to those of the competing substance. For N-acetyl-D-tryptophan and N-acetyl-L-tryptophan values of approximately 4×10^{-3} are obtained, in fair agreement with that of $(2 \pm 1) \times 10^{-3}$ obtained by equilibrium dialysis.[8]

In these calculations we have assumed that chymotrypsin is present as the monomer. Though there is evidence for dimerization at concentrations lower than those used here,[9] there is also evidence that in the presence of substrate[10] the dimer dissociates. In our work the substrate is present in excess and virtually all the enzyme should be present as the ES complex.

Acknowledgment. We wish to thank Mr. F. W. Dahlquist and Professors M. A. Raftery and S. I. Chan for useful discussions, Mr. G. Thompson and G. Swanson for early experimental assistance, and the U. S. Public Health Service for financial support (GM-10218).

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(6) B. Zerner and M. L. Bender, *ibid.*, **86**, 3669 (1964), and subsequent papers.

(7) B. W. Matthews, P. B. Sigler, R. Henderson, and D. M. Blow, *Nature*, **214**, 652 (1967).

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(9) M. S. N. Rao and G. Kegeles, *J. Am. Chem. Soc.*, 80, 5724 (1958).

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Silylation of Ferrocene by Chloro- and Aminosilanes under Friedel–Crafts Conditions

Sir:

Halosilanes have not been known to undergo reactions analogous to Friedel–Crafts alkylation of aromatic compounds.[1-3] Indeed, the formation of siliconium ions by heterolytic cleavage of silicon–halogen bonds with Lewis acids is virtually unknown.[4-7] Triphenylsiliconium ion, derived from the iodo- and bromosilanes but not the chlorosilane, has been detected only as the bipyridyl-stabilized ion.[8]

We report here that ferrocene can be silylated by chloro- and aminosilanes under Friedel–Crafts conditions. This constitutes the first successful use of halosilanes in reactions analogous to Friedel–Crafts alkylation and provides evidence that siliconium ion, probably in the form of a ferrocene-complexed intermediate, can be generated *via* cleavage of silicon–halogen bonds under these conditions. Some of our results are listed in Table I.

The observed reactivity is in part the result of enhancement of the catalytic activity of aluminum chloride by complexing with donor species, *viz.*, the aminosilane or triethylamine. The concept of a dimolar aluminum chloride complex of a tertiary amine, proposed as a catalytic species for aromatic alkylation,[9] has been found applicable to phosphorus(III) and

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(3) F. A. Drahowzal in "Friedel-Crafts and Related Reactions," Vol. II, Part 1, G. A. Olah, Ed., Interscience Publishers, London, 1964, p 466, and references therein.

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