suggesting that the solution structure of the mutant protein may differ from that of the wild type.[12] In addition, a number of amino acid replacements remote from Pro 117, both within the active site[12] and elsewhere,[9] alter the equilibrium between the conformational forms revealed by the H_ϵ protons. These last two observations suggest the desirability of additional information about the presence of a cis prolyl peptide bond in SNase in solution.

We have obtained additional evidence for the presence of a cis prolyl peptide bond involving Pro 117 in wild-type SNase by using samples of wild type and the P117G mutant biosynthetically labeled with [4-^{13}C]proline.[13,14] Previous studies have shown that the ^{13}C NMR chemical shift of the labeled 4-carbon in a cis X-prolyl peptide bond is about 3 ppm upfield of the labeled carbon in a trans peptide bond.[15] We have used proton detection methodology[16] to observe the ^{13}C resonances in the labeled proteins. ^{1}H-^{13}C heteronuclear chemical shift spectra of the labeled samples of wild-type SNase and its P117G mutant obtained at 40 °C in the absence of the active-site ligands Ca^{2+} and pdTp are reproduced in Figure 1. The intense correlations at ^{13}C chemical shifts of 28 ppm in both spectra are characteristic of proline residues in trans peptide bond geometries. Prolines in cis peptide bond geometries are expected to be approximately 3 ppm upfield of these correlations.[15] Although several correlations associated with natural abundance ^{13}C nuclei are present in these spectra (as evidenced by spectra obtained on unlabeled samples), the correlation having a ^{13}C shift of 25.7 ppm and a ^{1}H shift of 2.1 ppm in the spectrum of wild-type SNase is associated with the labeled proline and, therefore, can be assigned to proline in a cis peptide bond geometry. The correlations associated with prolines in trans peptide bond geometries in P117G show small changes in dispersion in both the ^{13}C and ^{1}H dimensions, but it is clear that the correlation associated with proline in a cis peptide bond geometry is missing. Our experiments do not allow a quantitative measure of the position of the cis/trans equilibrium but reveal that the cis isomer predominates. Thus, the proline residue participating in a cis peptide bond is Pro 117.

These experiments provide persuasive experimental evidence that a cis X-prolyl peptide bond predominates in solution. Independent confirmation of our assignment of the cis prolyl residue to Pro 117 is detailed in the accompanying communication, in which sequence-specific assignments are used to detect and assign the cis geometry to the Lys_{116}-Pro_{117} peptide bond in the presence of Ca^{2+} and pdTp.[18] Thus, we conclude that, in solution both in the presence and absence of ligands and in the crystalline state[11] in the presence of ligands, this X-prolyl peptide bond exists predominantly in the cis geometry.

Our observation does not identify which structural feature(s) are responsible for the multiple conformations which are apparent via the resonances of the resolved histidine H_ϵ protons. For example, the X-prolyl peptide isomerization may serve to slow the rate of interconversion between two conformations that differ in hydrogen-bonding networks or steric interactions rather than the isomerization itself being the driving force for two conformations.[7,10]

Acknowledgment. We thank Dr. Dennis A. Torchia and Professor Paul E. Young for a generous sample of [4-^{13}C]proline and Dr. Torchia for sharing his experimental results during the course of this work. The P117G mutant was generously provided by Professor Robert O. Fox. This research was supported by NIH GM-34573 to J.A.G. and P.H.B. and NSF DMB 88-06744 to P.H.B.

Supplementary Material Available: One-dimensional ^{13}C NMR spectra of [4-^{13}C]proline-labeled SNase and P117G in the absence of Ca^{2+} and pdTp (2 pages). Ordering information is given on any current masthead page.

Lack of Inhibition in the Cleavage of *p*-Nitrophenyl Acetate by β-Cyclodextrin: Evidence for the Absence of Aryl Group Inclusion in the Transition State for Esterolysis

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The cleavage of phenyl acetates by cyclodextrins (CDs)[1] in aqueous base is generally believed to take place from an ester·CD complex in which the aryl group of the ester is included in the cavity of the CD.[1-4] Thus, other species that bind to the CD should inhibit the reaction by competition.[2a,5] Such is the case for the cleavage of *m*-nitrophenyl acetate (mNPA) by α-CD[2a] and, as described below, by β-CD. In contrast, the cleavage of *p*-nitrophenyl acetate (pNPA) by β-CD *is not strongly retarded* by various potential inhibitors (PIs), and in some cases the reaction is actually *faster*!

Studies of the inhibition of mNPA cleavage[6] gave dissociation constants (K_i) in good agreement with values determined by other methods (Table I), but comparable studies with pNPA did not. Experiments with fixed [PI] and varying [β-CD] showed that the cleavage of pNPA is faster with added 1-butanol; it is not inhibited (Figure 1). Also, addition of 1-hexanol or cyclohexanol brings about rate increases, suggesting that there is a reaction between alcohols and the pNPA·CD complex. To obtain rate constants for this reaction, we carried out experiments with a fixed, high [CD] and varying [PI].[7] As shown by the example in Table II, values of k_{obsd} are very different from those expected for simple inhibition (k_{inh}). Analysis of such data was based on the following approach.

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(16) The pulse sequence used was

^{1}H: 90°-τ- -180°-$t_{1/2}$- -τ-acquisition

^{13}C: -90°-$t_{1/2}$- -90°- -decoupling

with the appropriate phase cycling.[17] The data was obtained by using a Varian XL-400 NMR spectrometer in conjunction with a Nalorac proton detection probe. ^{13}C NMR chemical shifts were referenced with respect to DSS. ^{1}H NMR chemical shifts were referenced with respect to H^2HO, which in turn was referenced to DSS.
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(6) Inhibition studies followed the method of Van Etten et al.[2a] Cleavage of mNPA (0.2 mM) in a 0.2 M phosphate buffer (pH 11.6) containing β-CD (1 mM) and various concentrations of PI was monitored by UV-visible spectrophotometry, with stopped-flow mixing.[3]
(7) Cleavage of pNPA (0.05 mM) in a 0.2 M phosphate buffer (pH 11.6) containing β-CD (10 mM) and various concentrations of PI was monitored by UV-visible spectrophotometry, with stopped-flow mixing.[3]

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Table I. Dissociation Constants of β-CD·PI Complexes and Constants for the Cleavage of pNPA by β-CD in the Presence of Potential Inhibitors (PI)[a]

inhibitor	K_d, mM	K_i, mM	k_a, M^{-1} s^{-1} (CD·S + PI →)	k_b, M^{-1} s^{-1} (CD·PI + S →)
n-BuOH	60[b]	56 ± 0.4	7.7	55
n-HexOH	4.6[b]	4.4 ± 0.2	39	22
c-HexOH	2.0[b]	1.8 ± 0.2	57	13
n-$BuSO_3^-$		89 ± 6	2.8	31
n-$HexSO_3^-$	5.6[c]		52	37
n-$PenCO_2^-$	~9[d]	16 ± 1	6.5	13
n-$HepCO_2^-$	~1.6[d]	1.2 ± 0.5	116	18
c-$HexCO_2^-$		4.6 ± 0.3	64	37
ClO_4^-	50[e]	48 ± 15	2.4	15

[a] In water, at 25 °C. Values of K_d are from the literature; K_i were obtained from the inhibition of mNPA cleavage.[6] The constants k_a and k_b are for the processes in eq 3 and 6. [b] Matsui, Y.; Mochida, K. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 2808. Reference 4. [c] Satake, I.; Yoshida, S.; Hayakawa, K.; Meda, T.; Kusumoto, Y. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 3991. [d] Estimated from a graph in the following: Ono, K.; Tokuda, M.; Murakami, K. *Polymer Reprints, Jpn.* **1979**, *28*, 1302. [e] Mochida, K.; Kagita, A.; Matsui, Y.; Date, Y. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 3703.

Table II. Rate Constants for the Cleavage of pNPA by β-CD in the Presence of 1-Butanol as Potential Inhibitor (PI)[a]

$[PI]_0$, mM	[PI], mM	[CD], mM	k_{obsd}, s^{-1}	k_{inh}, s^{-1}	k_{corr}, s^{-1}
0	0	10	0.403	0.403	0.660
40	36.08	6.08	0.469	0.331	0.979
60	55.04	5.04	0.485	0.304	1.125
80	74.30	4.30	0.491	0.282	1.254
100	93.74	3.74	0.498	0.264	1.388

[a] At 25 °C, pH 11.6.[7] The actual [PI] and [CD] were calculated, as described.[9] As [CD] decreases, the amount of pNPA bound to β-CD decreases from 56 to 32%; the expected rate constants are "k_{inh}". The slope of k_{corr} vs [PI] affords k_a (see eq 5).

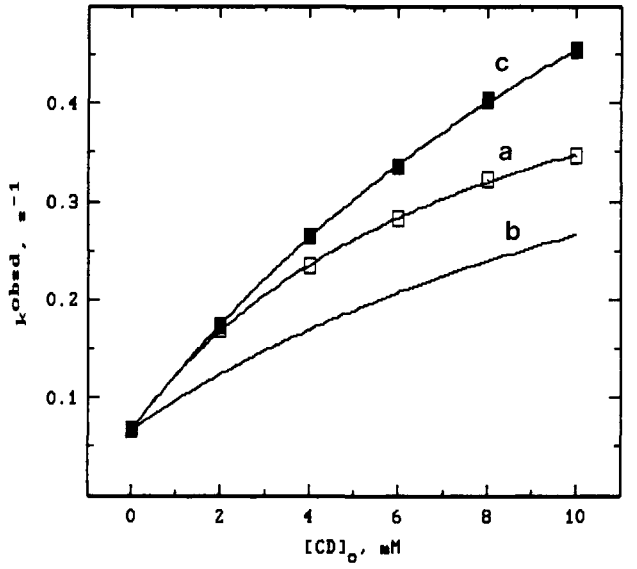


Figure 1. Effect of 1-butanol on rate constants for the cleavage of pNPA in the presence of β-CD at pH 11.6: (a) no 1-butanol; (b) expected for inhibition by 60 mM 1-butanol; (c) observed data for 60 mM 1-butanol.

Consider cleavage in the medium (eq 1), by β-CD (eq 2), and by a process involving the ester·CD complex and the PI (eq 3):

$$S \xrightarrow{k_u} \text{products} \quad (1)$$

$$S + CD \underset{K_s}{\rightleftharpoons} CD{\cdot}S \xrightarrow{k_c} \text{products} \quad (2)$$

$$CD{\cdot}S + PI \xrightarrow{k_a} \text{products} \quad (3)$$

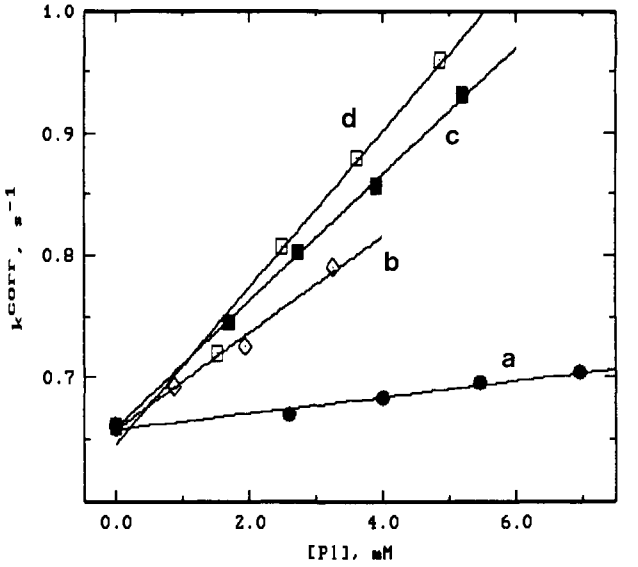
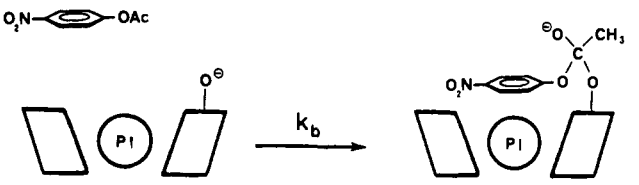


Figure 2. Dependence of k_{corr} (eq 5) for the cleavage of pNPA in 10 mM β-CD on [PI], where [PI] has been corrected for complexation with β-CD.[9] The data shown are for PI = (a) hexanoate ion; (b) 1-hexanol; (c) hexanesulfonate ion; (d) cyclohexanecarboxylate ion. Similar plots were obtained for the other PI in Table I, and their slopes afforded values of k_a (eq 5).

Scheme I



Observed rate constants should have the form of eq 4,[8] rearrangement of which gives eq 5. Thus, k_{corr} should vary linearly

$$k_{obsd} = \frac{k_u K_s + (k_c + k_a[PI])[CD]}{K_s + [CD]} \quad (4)$$

$$k_{corr} = \{k_{obsd}(K_s + [CD]) - k_u K_s\}/[CD] = k_c + k_a[PI] \quad (5)$$

with [PI], as in Figure 2, and from the slopes we obtain values of k_a (Table I).[9] Besides alcohols, which might react via their anions,[10] an adherence to eq 5 was found with much less nucleophilic ions: alkane sulfonate, alkanoate, and perchlorate (Table I).

Values of k_a vary approximately as $1/K_i$ (Table I), suggesting that PI is bound to β-CD during the reaction. Perhaps, therefore, the reaction is better viewed as

$$CD + PI + S \underset{K_i}{\rightleftharpoons} CD{\cdot}PI + S \xrightarrow{k_b} \text{products} \quad (6)$$

Values of k_b (Table I)[11] are close to one another, and only slightly smaller than the rate constant for the reaction of pNPA with β-CD alone (83 M^{-1} s^{-1}), under the same conditions. This important observation is consistent with ester cleavage, which involves acyl transfer to an ionized CD hydroxyl group,[1-4] taking place with the aryl group of pNPA *outside* the CD cavity, so that other species may occupy the cavity (Scheme I). The fact that the substrate pNPA binds to β-CD (K_s = 7.9 mM) is irrelevant with respect to its cleavage; substrate binding and transition-state

(8) Note: *if* $k_a = 0$, then eq 4 reduces to $k_{obsd} = (k_u K_s + k_c[CD])/(K_s + [CD])$, which is the form for normal behavior.[3]

(9) Use of eq 5 requires true values of [CD] and [PI]; these were calculated from $[CD] = \{-B + (B^2 + 4K_i[CD]_0)^{1/2}\}/2$, where $B = ([PI]_0 + K_i - [CD]_0)$, and $[PI] = [PI]_0 - ([CD]_0 - [CD])$, e.g., Table II.

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(11) The processes in eq 3 and 6 are kinetically equivalent, so that $k_a/K_s = k_b/K_i$. Thus, $k_b = k_a K_i/K_s$.

binding are distinct processes, which are not necessarily related.[3,5,12]

From correlation analysis of rate data using steric parameters, Matsui et al.[3] concluded that the substituents of *p*-X-phenyl acetates are probably not inside the cavity of β-CD during the transition state for acyl transfer. The present results provide direct evidence for this conclusion.

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Chemoselective Synthesis of Allyltrimethylsilanes by Cross-Coupling of Vinyl Triflates with Tris((trimethylsilyl)methyl)aluminum Catalyzed by Palladium(0)†

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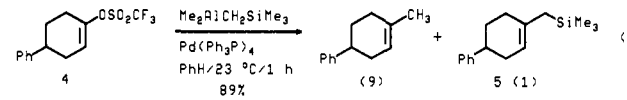
The palladium(0)-catalyzed cross-coupling of organostannanes with vinyl triflates recently developed by Stille[1] constitutes an especially valuable olefin synthesis, in part due to the easy access of the triflate from carbonyl precursors.[2] The organostannane, the organometallic component of this reaction, derives its special utility from its stability and chemoselectivity[3] vis-à-vis, for example, the more typical Grignard,[4] organozinc, or alane reagents in this regard.[5] Using tetrakis((trimethylsilyl)methyl)tin[6] [Sn-$(CH_2SiMe_3)_4$] in conjunction with a vinyl triflate should then provide an elegant route to allyltrimethylsilanes. However, in our hands, and as noted by Stille in the context of aryl triflates,[8] this stannane does not function in the cross-coupling reaction with vinyl triflates. In this communication, we now report that tris((trimethylsilyl)methyl)aluminum (**1**),[9] $(Me_3SiCH_2)_3Al$, conveniently generated in situ from commercially available ((trimethylsilyl)methyl)lithium and aluminum trichloride, functions as an unusually chemoselective alane reagent in the palladium(0)-catalyzed cross-coupling of vinyl triflates to give allyltrimethylsilanes as shown in Table I.

We were prompted to undertake this study from a need for substantial quantities of 2-((trimethylsilyl)methyl)-2-cyclohexen-1-one (**2**), previously reported[10] only as a component of a mixture, after five steps and in less than 10% yield. Thus, we reasoned that **2** might be quickly fashioned from triflate **3**, available from 1,2-cyclohexanedione (Tf_2O, Et_3N, CH_2Cl_2, −78 °C, 91%), and $Sn(CH_2SiMe_3)_4$. However, enone triflate **3**, as well as triflate **4**, derived from 4-phenylcyclohexanone (81%),[2e] did not react with this stannane when the Stille protocol[1] was used.

Reasoning that a sluggish transmetalation step in the catalytic cycle[1b] might explain the reluctance of the (trimethylsilyl)methyl (Me_3SiCH_2) group to be delivered via initial Sn → Pd transfer, we considered more reactive metals (MgX, ZnX, AlR_2) in this regard. However, Me_3SiCH_2MgCl[11] is not expected to be compatible with the enone function in **2** or **3**, and the corresponding organozinc chloride reagent results in only 25% conversion of **4** to **5** (2 equiv, 5 mol % $NiCl_2$·dppp, THF, 65 °C, 12 h). Although an aluminum reagent seemed attractive,[12] chemoselectivity vis-à-vis the enone remained in doubt[13] due to the reactivity of this functionality toward alanes.[14]

Nevertheless, we chose ((trimethylsilyl)methyl)dimethylaluminum ($Me_2AlCH_2SiMe_3$) as our trimethylsilylmethylating reagent, which was prepared in situ from 1 equiv each of Me_3SiCH_2Li and Me_2AlCl.[15] From eq 1, it can be seen that triflate **4** gives a 9:1 mixture of olefins using $Me_2AlCH_2SiMe_3$ in conjunction with a highly active in situ generated Pd(0) catalyst[16] wherein CH_3 transfers in preference to CH_2SiMe_3.



From Table I it can be seen that $(Me_3SiCH_2)_3Al$ (**1**), generated in situ by addition of 3 equiv of Me_3SiCH_2Li (1 M in pentane) to 1 equiv of $AlCl_3$ suspended in 1,2-dichloroethane, reacts with a variety of vinyl triflates under mild conditions, catalyzed by in situ formed tetrakis(triphenylphosphine)palladium(0) [Pd-$(PPh_3)_4$][16] in benzene, to give good yields of the corresponding allyltrimethylsilanes. The reaction proceeds with a remarkably high degree of chemoselectivity. As seen in entries 1 and 4–8, enone, enoate, ester, allyl alcohol, and aryl bromide functionalities remain intact even in the presence of excess **1**. That only one of the three Me_3SiCH_2 groups in **1** is transferred is supported by the fact that less that 1 equiv of **1** results in incomplete reaction, and typically 1.2–1.4 equiv gives the best results. Although we did not explore the use of neat **1**,[9] the hazard of dealing with this reagent coupled with the need for LiCl (typically 3 equiv) in the vinyl triflate/Pd(0) oxidative addition step[1] favors the current protocol.

The expected stereospecificity of the title reaction was addressed and confirmed by using the pure (*E*)-vinyl triflate **6** (52%) derived from ethyl acetoacetate (Tf_2O, Et_3N, CH_2Cl_2, −78 °C; 2.5:1 *E*/*Z*). Under the typical conditions, only the (*E*)-allylsilane isomer **7** is

† Dedicated to the memory of Professor J. K. Stille.

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