Acknowledgments. This work was supported by a grant from the Research Corporation. The authors thank Dr. Robert Riddle for recording the NMR spectra and Dr. Roger Upham for obtaining the mass spectral data.

References and Notes

(1) Recipient of Faculty Research Award 143 from the American Cancer Society.
(2) S. Masamune, P. A. Rossy, and G. S. Bates, *J. Am. Chem. Soc.*, **95**, 6452–6454 (1973).
(3) S. Masamune, G. S. Bates, and P. E. Georghiou, *J. Am. Chem. Soc.*, **96**, 3686–3688 (1974).
(4) D. H. R. Barton and S. W. McCombie, *J. Chem. Soc., Perkin Trans. 1*, 1574–1585 (1975).
(5) D. H. R. Barton and R. V. Stock, *J. Chem. Soc., Perkin Trans. 1*, 1773–1776 (1975).
(6) M. Y. H. Wong and G. R. Gray, *Tetrahedron Lett.*, 1617–1620 (1977).
(7) H. Zinner, H. Brandner, and G. Rembarz, *Chem. Ber.*, **89**, 800–805 (1956).
(8) N. K. Kochetkov and B. A. Dmitriev, *Izv. Akad. Nauk SSSR, Otd. Khim. Nauk*, 1262–1267 (1962).
(9) H. Zinner, E. Wittenburg, and G. Rembarz, *Chem. Ber.*, **92**, 1614–1617 (1959).
(10) T. van Es, *Carbohydr. Res.*, **32**, 370–374 (1974).
(11) D. Horton and J. D. Wander, *Carbohydr. Res.*, **13**, 33–47 (1970).
(12) B. Berrang, D. Horton, and J. D. Wander, *J. Org. Chem.*, **38**, 187–192 (1973).
(13) F. A. Carey and A. S. Court, *J. Org. Chem.*, **37**, 1926–1929 (1972).
(14) V. M. Micovic and M. L. J. Mihailovic, *J. Org. Chem.*, **18**, 1190–1200 (1953).
(15) M. Fetizon and M. Jurion, *J. Chem. Soc., Chem. Commun.*, 382–383 (1972).
(16) W. F. J. Huurdeman and H. Wynberg, *Tetrahedron Lett.*, 3449–3452 (1971).
(17) V. S. Waravdekar and L. D. Saslaw, *J. Biol. Chem.*, **234**, 1945–1950 (1959).
(18) D. G. Lance and J. K. N. Jones, *Can. J. Chem.*, **45**, 1533–1538 (1967).
(19) R. U. Lemieux, L. Anderson, and A. H. Connor, *Carbohydr. Res.*, **20**, 59–72 (1971).
(20) The chemical shifts assigned to H-1 of the β-pyranose forms of 2-deoxy-D-*erythro*-pentose and its 2(*S*)-deuterio isomer are in error in ref 21. These authors assigned that resonance at τ 4.97 (δ 5.03), in disagreement with the value of δ 5.31 previously reported by Lemieux et al.[19] and the value of δ 5.27 reported herein.
(21) B. Radatus, M. Yunker, and B. Fraser-Reid, *J. Am. Chem. Soc.*, **93**, 3086–3087 (1971).
(22) Free-energy calculations based on interaction energies of nonbonded substituents (S. J. Angyal, *Aust. J. Chem.*, **21**, 2737–2746 (1968)) indicate that both the α- and β-pyranose forms should strongly prefer the $^4C_1$ conformation.
(23) R. E. Deriaz, W. G. Overend, M. Stacey, E. G. Teece, and L. F. Wiggins, *J. Chem. Soc.*, 1879–1883 (1949).
(24) E. Breitmaier, G. Jung, and W. Voelter, *Chimia*, **26**, 136–139 (1972).
(25) The assignments of ^{13}C resonances are derived from ref 23. The actual chemical shifts reported herein are slightly different from those reported in ref 23 owing to the use of a different internal standard. The spectra are otherwise identical.
(26) The ^{13}C resonances listed are those containing deuterium substitution. The spectrum is otherwise identical with the parent 2-deoxyaldose.
(27) P. A. Levene and T. Mori, *J. Biol. Chem.*, **83**, 803–816 (1929).

Complexation of 4-Biphenylcarboxylate by Cyclohexaamylose. A Conductometric and ^{13}C Nuclear Magnetic Resonance Spectrometric Analysis

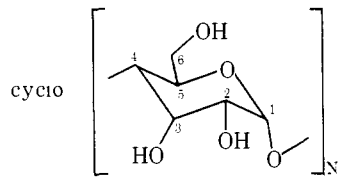
Robert I. Gelb, Lowell M. Schwartz, Christopher T. Murray, and Daniel A. Laufer*

Contribution from the Department of Chemistry, University of Massachusetts at Boston, Boston, Massachusetts 02125. *Received November 9, 1977*

Abstract: Conductometric and ^{13}C NMR spectrometric analyses of aqueous solutions containing variable concentrations of cyclohexaamylose and lithium or sodium salts of the 4-biphenylcarboxylate anion indicate formation of a ternary complex composed of two cyclohexaamylose molecules and a single 4-biphenylcarboxylate ion. Conductometric measurements of corresponding equilibrium constants at five temperatures between 20 and 45 °C yielded values of -28 ± 2 kcal mol^{-1} and -72 ± 6 cal mol^{-1} deg^{-1} for the standard enthalpy and entropy changes, respectively, of the complexation reaction. Chemical shifts of cyclohexaamylose and 4-biphenylcarboxylate carbons in the complex are discussed in terms of a bitoroidal ternary model.

Introduction

Cyclohexaamylose, commonly named α-cyclodextrin, is a member of the homologous cycloamylose (I) series of cyclic



I

oligomers which consists of 6–12 α-1,4-linked D-glucopyranosyl residues.[1] The prefix terminology α, β, γ····cyclodextrin refers to degrees of cyclic oligomerization where N = 6, 7, 8···, respectively. α-Cyclodextrin (N = 6) is known to bind molecules and ions in the interior cavity of its torus-shaped molecule. The resultant inclusion complexes have been investigated by various spectral methods and these have been reviewed.[2]

Published studies[2b] of the binding of a large number of substrates by cycloamyloses have generally assumed a 1:1 stoichiometry in the complexation reaction. However, there are some examples of two cycloamylose:one substrate stoichiometries involving methyl orange,[3,4] orange I,[5] 6-*p*-toluidinylnaphthalene 2-sulfonate (TNS),[6] *n*-heptane,[7] and cyclohexane.[7] Complexes with 3:1 stoichiometries have been suggested with palmitoyl Co-A substrate.[8]

The diversity of these systems and the relative absence of structural and thermodynamic information regarding higher complexes led us to further study the reaction of α-cyclodextrin with multifunctional substrates. We selected the 4-biphenylcarboxylate anion and investigated the interactions between its polar and nonpolar sites with the various zones of the α-cyclodextrin cavity. As shown in studies of related complexations,[9,10] structural information of this kind may be readily provided by ^{13}C NMR spectrometry. On the other hand, 4-biphenylcarboxylate is an ionic substrate, and its concentration may be conveniently monitored by conductometry at dilution levels inaccessible to ^{13}C NMR spectrometry. We therefore measured stoichiometric, thermodynamic, and structural

0002-7863/78/1500-3553$01.00/0

Table I. Least-Squares Slope and Intercept Values for Line Segments in Figure 1

Carbon	Line segment $< a/V^0F_{Cy} <$		Intercept, ppm	Slope	Stoichiometric coefficient n
1	0.0	0.4	0.002 ± 0.011[a]	1.182 ± 0.056[a]	2.06 ± 0.15
1	0.8	1.6	0.562 ± 0.014	0.029 ± 0.011	
3	0.0	0.4	0.006 ± 0.006	0.651 ± 0.027	1.98 ± 0.15
3	0.8	1.6	0.334 ± 0.011	0.002 ± 0.009	

[a] The uncertainties represent standard errors.

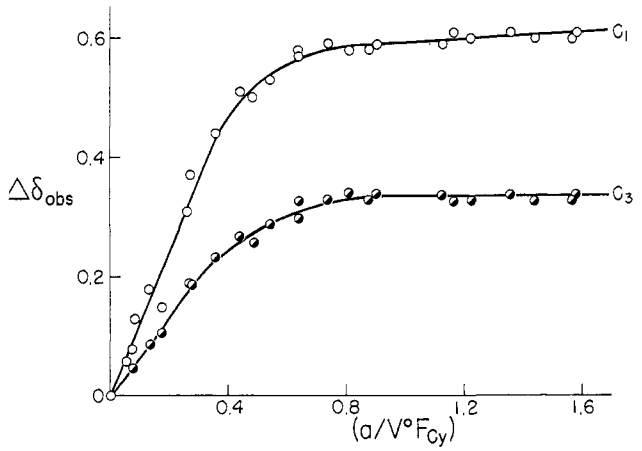


Figure 1. Chemical shift displacements of C_1 and C_3 carbons of Cy upon addition of A^-.

parameters of the complexes by the complementary methods of conductometry and ^{13}C NMR spectrometry.

^{13}C NMR Stoichiometric Analysis

Because several of the ^{13}C NMR resonances of α-cyclodextrin (Cy) are markedly displaced on complexation with 4-biphenylcarboxylate (A^-), measurement of these chemical shifts should provide a means for determining the overall stoichiometry of the complex. In order to do this we first examined the ^{13}C NMR spectrum of a D_2O solution containing 0.05 F Cy, the line assignments of which are known.[11] Our chemical shift data are consistent with those previously reported. In a separate series of experiments, the resonance lines were found to be independent of Cy concentration between 0.005 and 0.100 F to within ±0.02 ppm. These data indicate that Cy molecules do not aggregate in the absence of substrates, in agreement with a previously published conclusion.[7] Next, small portions of Na^+A^- were added stepwise to the 0.05 F Cy sample and the spectrum was recorded. In this fashion we obtained chemical shift data with solutions containing up to 0.08 F A^-, which was the apparent solubility limit. No additional peaks other than those ascribed to Cy and A^- were observed. The data were analyzed as follows.

Consider the formation of a single complex Cy_nA^-. Under rapid exchange conditions the observed chemical shift of a given Cy resonance, δ_{obsd}, may be taken as the weighted average of the complexed and free Cy resonances.

$$\delta_{obsd}\{[Cy] + n[Cy_nA^-]\} = \delta_0[Cy] + n\delta_n[Cy_nA^-] \quad (1)$$

δ_0 and δ_n represent the chemical shift of a given carbon resonance in pure Cy and Cy_nA^-, respectively. The stoichiometric coefficients which multiply the concentration of complex reflect the fact that n mol of Cy is bound in 1 mol of the complex, so that n mol of the carbon atoms giving rise to a particular resonance are bound in each mol of the complex as well. A fixed quantity of Cy was employed in our experiments, so that

$$V\{[Cy] + n[Cy_nA^-]\} = F_{Cy}V^0 \quad (2)$$

where F_{Cy} denotes the initial concentration of Cy in V^0 mL and V is the solution volume after adding a portion of Na^+A^-.

As a, the number of moles of Na^+A^-, increases, the chemical shifts δ_{obsd} are increasingly displaced from δ_0 as more Cy is complexed. If the complex is fairly strong, we expect the variation of δ_{obsd} with a to change markedly when the stoichiometric equivalence point is reached. Before this equivalence point there is an excess of Cy, i.e., $na < V^0F_{Cy}$ and all A^- is complexed. Consequently, $[Cy_nA^-] = a/V$. Substitution into eq 1 and 2 yields the displacements

$$\Delta\delta_{obsd} = \frac{na}{V^0F_{Cy}}\Delta\delta_n;\ na \leq V^0F_{Cy} \quad (3)$$

where $\Delta\delta_{obsd}$ is $(\delta_{obsd} - \delta_0)$, $\Delta\delta_n$ is $(\delta_n - \delta_0)$, and it is understood that one such equation applies to each carbon resonance line. At the equivalence point $na = V^0F_{Cy}$, all Cy is complexed, and so $\delta_{obsd} = \delta_n$ or $\Delta\delta_{obsd} = \Delta\delta_n$. After the equivalence point, excess Na^+A^- has no effect on complexed Cy and so $\Delta\delta_{obsd}$ remains fixed at $\Delta\delta_n$.

$$\Delta\delta_{obsd} = \Delta\delta_n;\ na \geq V^0F_{Cy} \quad (4)$$

As a result, a plot of $\Delta\delta_{obsd}$ vs. a/V^0F_{Cy} consists of two linear segments which intersect where $1/n = a/V^0F_{Cy}$. These plots are shown in Figure 1 and the results of a least-squares treatment of each line segment are given in Table I. Simultaneous solution of the line equations representing the C_1 and C_3 carbons yielded n values of 2.06 ± 0.15 and 1.98 ± 0.15, respectively. The line parameter uncertainties represent standard errors and were obtained from the least-squares analysis and the n-value uncertainties were calculated from these by propagation of variance procedures. An estimate of the maximum probable error in n was made on the basis of the following estimated error limits: ±0.02 ppm in $\Delta\delta_{obsd}$, ±2% in Cy concentration, ±0.5% in purity of Na^+A^-, and ±2% due to weighing and transfer errors. The results were n = 2.06 ± 0.3 based on the C_1 data and n = 1.98 ± 0.3 based on the C_3 data.

Conductometric Determination of Equilibrium Constants

The formation of Cy complexes with 4-biphenylcarboxylate anions must reduce the mobility of the ion through an aqueous solution. This would be reflected by a decrease in the specific conductance of a solution of A^- upon addition of Cy. Thus, the conductance of solutions containing varying concentrations of Cy and the substrate should provide a means of determining the equilibrium constants of the complexation reaction. We chose to measure such solutions under the conditions of a conductometric titration where a solution containing 0.100 F Cy was added to a solution containing ca. 0.01 F substrate as the lithium salt Li^+A^-. The conductance $(1/R)$ of such a solution is related to the ionic composition by the equation

$$1/R = \frac{1}{1000\theta}\{[Li^+]\lambda_{Li^+} + [A^-]\lambda_0 + [CyA^-]\lambda_1 + [Cy_2A^-]\lambda_2\} \quad (5)$$

where θ is the conductance cell constant, [j] is the molar concentration of species j, and λ_j denote the corresponding

equivalent ionic conductances at the ionic strength and temperature of the solution. In addition to conservation equations for substrate

$$F_{LiA} = [Li^+] = [A^-] + [CyA^-] + [Cy_2A^-] \quad (6)$$

and for Cy

$$F_{Cy} = [Cy] + [CyA^-] + 2[Cy_2A^-] \quad (7)$$

we assume that the solution composition is specified by two stepwise complex formation equilibria

$$K_1 = \frac{[CyA^-]\gamma_1}{[Cy][A^-]\gamma_{Cy}\gamma_0} \quad (8)$$

and

$$K_2 = \frac{[Cy_2A^-]\gamma_2}{[Cy][CyA^-]\gamma_{Cy}\gamma_1} \quad (9)$$

In these equations F_{LiA} and F_{Cy} are the total analytical concentrations at any point during the titrations and γ_{Cy} and γ_n represent activity coefficients for free Cy and for Cy_nA^- molecules, respectively. We assumed that $\gamma_{Cy} = 1.00$ because Cy is uncharged and that the singly charged ionic activity coefficients are related to ionic strength I by the Debye-Hückel equation

$$\log \gamma_n = \frac{-A\sqrt{I}}{1 + Ba_n\sqrt{I}} \quad (10)$$

where A and B are temperature-dependent parameters taken from the Robinson and Stokes[12] tabulation and that the ion-size parameters are 0.75, 1.6, and 1.8 nm for a_0, a_1 and a_2, respectively. The equivalent conductance values λ_j in eq 5 also depend on ionic strength and since this quantity never exceeds 0.01 M, we assume that an extended form of the Onsager relationship[12]

$$\lambda_j = \lambda_j^0 - \frac{(\alpha + \beta\lambda_j^0)\sqrt{I}}{1 + Ba_n\sqrt{I}} \quad (11)$$

is a valid approximation. λ_j^0 is the equivalent conductance at infinite dilution and α and β are temperature-dependent parameters again taken from Robinson and Stokes.[12] Although λ^0_{Li+} is known,[12] the other λ_j^0 values are not. We determine λ_0^0 (for uncomplexed A^- ion) from the conductance measurement of the initial point of the titration. Since this solution contains no Cy, the two terms in eq 5 corresponding to CyA^- and Cy_2A^- are zero, the conductance depends only on Li^+ and A^- ions, and the contribution due to Li^+ is known. Equation 5 is solved for λ_0 and then eq 11 for λ_0^0. These results at various temperatures are given in Table II. We determined λ_2^0 for Cy_2A^- in a similar manner but by a separate experiment measuring the conductance of a solution prepared with a F_{Cy}/F_{LiA} ratio (~10:1) sufficiently large that we could expect nearly quantitative conversion of A^- to Cy_2A^-. Assuming complete conversion, the λ_2^0 value was calculated and this was taken as an initial estimate in an iterative procedure. When K_1 and K_2 were determined, we could calculate the small residual concentrations of A^- and CyA^- in this solution and by compensating for the conductance of these species could refine the λ_2^0 value and then iteratively refine K_1 and K_2.

The remaining unknown parameters in the set of eq 5-11 are K_1, K_2, and λ_1^0, but since these equations are highly coupled, nonlinear, and overdetermined, it is impossible to solve for any of the unknowns explicitly and we used a nonlinear regression method attributable to Gauss.[13] Briefly, this method linearizes the model eq 5-11 around a set of initial guesses of the unknown parameters and thereby replaces these parameters with unknown "parameter corrections". The "parameter corrections" are determined by a least-squares solution minimizing the sum of squared deviations between the experimental conductances and the conductances predicted by the linearized model equations. The initial parameter guesses are then adjusted by the "parameter corrections" and these new parameter values serve as the guesses for the next iteration seeking further "parameter corrections". The iteration converges to the minimum sum of squared deviations when all the "parameter corrections" are sufficiently small. At this point the set of parameter values are accepted as those which satisfy the original model equations in the least-squares sense. We wrote a digital computer program to carry out this Gauss iteration and to permit interaction with the user through a terminal at each step of the way. This feature enabled us to guide the convergence by resetting initial guesses or by holding certain parameters constant as needed and to enter refined λ_2^0 values as K_1 and K_2 changed as explained above.

Table II. Conductometric and Equilibrium Parameters of Cy_nA^- Complexes

Temp, °C	λ_2^0	λ_1^0	λ_0^0	K_1K_2, 10^5M^{-2}	Fit,[a] %
	mho cm^2 eq^{-1}				
20	11.9	17	23.1	1.3	0.08
25	13.4	17	25.8	0.87	0.08
30	14.6	22	29.6	0.25	0.08
37	17.5	28	33.5	0.12	0.44
45	20.0	30	39.6	0.032	0.26

[a] Relative root mean square deviation of calculated from experimental conductances.

Before discussing the results of these calculations, one further complication remains, namely, the addition of Cy to the solution causes a substantial increase in the viscosity,[14] which causes a decrease in all of the λ values and the measured conductance. It has been found experimentally that λ^0 values are proportional to the reciprocal of the viscosity, and further, the values of α and β in eq 11 are related to the viscosity in the same way. We were able to account for the variation in viscosity by means of a "blank titration" technique in which a standard solution of NaCl was titrated with Cy under conditions identical with those that would later be employed to titrate the A^- solutions. These data were compared in turn to conductance values obtained from a "titration" of standard NaCl with pure water. Each set of data was suitably corrected for the solvent conductance (1-2 μmho cm) and a small impurity conductance in the Cy reagent. The ratios of the corrected conductance values obtained when NaCl was titrated with water compared to the titration of NaCl with Cy yielded a viscosity correction which was found to be linear with volume of added Cy solution in the concentration range of the experiment (up to 0.016 F). The values were smoothed by a least-squares analysis before they were applied to the Cy-A^- titration data. As an example, the specific conductance data obtained in a series of titrations follow: (a) titration of 0.01 F NaCl with H_2O, 2 mL added, 1023.6 μmho cm; (b) titration of 0.01 F NaCl with 0.1 F Cy, 2 mL added, 1017.5 μmho cm; (c) titration of water with 0.1 F Cy, 2 mL added, 2.78 μmho cm; (d) water "blank", 1.61 μmho cm; (e) titration of Li^+A^- with 0.1 F Cy, 424.9 μmho cm. Appropriate combination of the above conductances, namely (a − d)/(b − c), yielded a "vicosity correction" of 1.0072 which along with the other data yielded a least-squares equation for the viscosity correction of $\eta/\eta_0 = 1.0000\ (\pm 0.0005) + 0.0035\ (\pm 0.0001)v$ where v is the volume in milliliters of a 0.1 F Cy solution added to a 50-mL sample solution. The uncertainties represent standard errors. The viscosity correction predicted by this equation for an appropriate v was then used to correct the A^- conductance, and in the case above a value of 425.1 μmho cm was obtained.

While the unity intercept values and linearity of the correction equation seemed to indicate that the calculation was

Table III. K_1K_2 ^{13}C NMR Data[a]

	$F_{A^-} \times 10^3$	$\Delta\delta_{obsd}$	Log (K_1K_2)
C_1 data	7.62	0.27	4.12
$\Delta\delta_2$ = 0.75 ppm	15.9	0.32	3.95
	24.4	0.38	3.96
C_3 data	7.62	0.15	4.10
$\Delta\delta_2$ = 0.43 ppm	15.9	0.19	4.00
	24.4	0.23	4.05

[a] $F_{Cy} = 4.97 \times 10^{-3}$ M in D_2O at 30 °C.

a valid one, it remained for us to verify that no complexes were formed between Cy and lithium, sodium, or chloride ions. We obtained the ^{13}C NMR spectra of 0.05 F Cy solutions containing from 0 to 0.5 F NaCl and, in a separate series of experiments, 0–0.05 F LiCl. The resonances observed for each of the six nonequivalent carbon atoms in the Cy moiety were identical to within the reproducibility of measurement (±0.01 to ±0.02 ppm) over the entire concentration range of added salts. This is in contrast to chemical shift displacement of about 1 ppm observed in Cy complexes with other substrates. Assuming that possible complexes with these inorganic ions would result in similar chemical shift changes in Cy resonances and that we would detect a displacement of chemical shift of 0.05 ppm, the formation constants of any 1:1 Cy complexes with Na^+ or Cl^- would be smaller than ~0.1 and less than ~1 for complexation with Li^+. Our conclusions regarding the sodium and chloride ions are in agreement with previously published reports.[14,15] Since our conductometric measurements utilized solutions containing less than 0.01 M of these inorganic ions, the presence of such complexes, if they exist at all, cannot have had an appreciable effect on our measurements.

Application of analytical and computational procedures described above to conductometric titrations at five temperatures between 20 and 45 °C led to λ_0^0, λ_1^0, λ_2^0, and K_1K_2 parameter sets which are listed in Table II. We found that equally good fits were obtained over a range of K_1 and K_2 parameter values at any given temperature. In other words, the equations and experimental data could not be solved for unique values of K_1 and K_2. However, for any particular set of suitable parameters the K_2/K_1 ratio was much greater than unity and the K_1K_2 product was virtually invariant at a given temperature. A plot of ln (K_1K_2) vs. $1/T$ was found to be linear.

These results indicate that the ternary complex (Cy_2A^-) is the only detectable product under our experimental conditions, i.e., the concentrations of the binary complex (CyA^-) were below the conductometric detection limit.

Thermodynamic Parameters of Complex Formation

Using the well-known thermodynamic relationship

$$\ln (K_1K_2) = -(\Delta H^\circ/R)(1/T) + \Delta S^\circ/R \qquad (12)$$

we plotted ln (K_1K_2) vs. $1/T$ data points corresponding to entries listed in Table II. The resultant linear plot led to a least-squares equation ln (K_1K_2) = (14 100 ± 900)($1/T$) − (36 ± 3), where the parameter uncertainties are standard errors. The slope and intercept of this line yielded values for the standard enthalpy and entropy changes, respectively, of ternary complex formation at 20–45 °C. These are given below.

$$\Delta H^\circ = -28 \pm 2 \text{ kcal mol}^{-1}$$

$$\Delta S^\circ = -72 \pm 6 \text{ cal mol}^{-1} \text{ deg}^{-1}$$

Confirmation of K_1K_2 Value by ^{13}C NMR

To confirm our conductometric K_1K_2 determination by an independent method, we measured the Cy ^{13}C NMR chemical shifts in dilute solutions of Cy and A^- where only a fraction of the Cy is complexed. $f \equiv \Delta\delta_{obsd}/\Delta\delta_2$ varies from zero when Cy in solution is uncomplexed to unity when Cy is totally complexed, and hence this ratio may be taken as the fraction of Cy bound in the complex, provided that the concentration of binary complex CyA^- is negligible as the conductometric study seems to indicate. When the two equilibrium equations 8 and 9 are combined with Cy and A^- conservation equations, the result expressed in terms of f is

$$K_1K_2 = \frac{f(\gamma_2/\gamma_0)}{(1-f)^2(2F_{A^-} - fF_{Cy})F_{Cy}} \qquad (13)$$

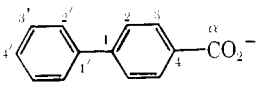
again assuming that the concentration of CyA^- is negligible. We do not know the activity coefficient ratio γ_2/γ_0 in the D_2O solvent used for the ^{13}C NMR solutions, but since γ_2 and γ_0 both refer to singly charged ions and Cy_2A^- has a larger effective diameter than A^-, the Debye-Hückel activity coefficient correlation would predict $\gamma_2 > \gamma_0$, but the ratio γ_2/γ_0 should nevertheless be near unity.

Equation 13 can be employed to yield K_1K_2 values from our $\Delta\delta_{obsd}$ data once the value of $\Delta\delta_2$ is known, since f is the ratio of these displacements and all the other terms in the equation are known experimental variables. Values of $\Delta\delta_2$ for the C_1 carbon of Cy were estimated under conditions where f is close to unity, namely, solutions where high concentrations of Cy and excess A^- were present. These corresponded to mole-ratio data given in Figure 1 where $0.8 < (a/V^0F_{Cy}) < 1.6$. $\Delta\delta_2$ for C_1 was taken as 0.60 ppm. This estimate along with $\Delta\delta_{obsd}$ values listed in the third column of Table III gave three initial estimates of K_1K_2 from C_1 data and these were averaged. In order to refine the estimates of K_1K_2 and $\Delta\delta_2$, an iterative calculation employed the average initial K_1K_2 value and eq 13 to solve for values of f where the concentrations of Cy and A^- were large (f near 1). These f values along with $\Delta\delta_{obsd}$ (near 0.60 ppm for C_1) yielded new estimates of $\Delta\delta_2$ and the calculation of K_1K_2 was repeated using an average of these. The iteration was continued until successive values of $\Delta\delta_2$ were within 0.005 ppm. The procedure above was used to analyze C_3 data as well and the results are given in Table III.

We made estimates of the uncertainties in our data that led to a maximum probable error of ±0.12 in our value of log (K_1K_2) = 4.03. All of our values fall well within the range. Our ^{13}C NMR derived value of log (K_1K_2) = 4.03 pertains to the D_2O solvent system used in these measurements and differs somewhat from the value of log (K_1K_2) = 4.40 at 30 °C in the H_2O solvent employed for conductance measurements. This disparity seems consistent with the different solvating properties of D_2O and H_2O. In addition, the essential constancy of the ^{13}C NMR derived equilibrium constant indicates that the Cy_2A^- complex dominates in D_2O as well as H_2O solvent.

Structure of the Cy_2A^- Complex

We shall attempt to deduce structural information about the Cy_2A^- complex by examining the relative $\Delta\delta_2$ values of carbons on both Cy and A^- within the complex. To distinguish these ^{13}C NMR resonances we introduce a superscript notation. For example, superscript (Cy1) refers to the C_1 carbon of α-cyclodextrin (I, N = 6) and superscript (A^-2') denotes the $C_{2'}$ carbon of 4-biphenylcarboxylate (II). The subscripts obsd, 0, and 2 on δ and $\Delta\delta$ retain their meanings.



II

The fraction f of complexed Cy may be expressed in terms of the resonance displacements $\Delta\delta_{obsd}/\Delta\delta_2$ for any particular Cy carbon atom. The equality of f in terms of Cy1 to f in terms of Cy2–Cy6 permits us to calculate $\Delta\delta^{(Cy)}$ values for all Cy

Table IV. Cy and A^- Chemical Shifts[a] and Their Displacements[b] in Complex Cy_2A^-

Cy C	$\delta_0^{(Cy)}$[c]	$\Delta\delta_2^{(Cy)}$[d]	A^- C	$\delta_0^{(A^-)}$[e]	$\Delta\delta_2^{(A^-)}$
1	102.37	0.75	1	144.12	0.35 ± 0.06
2	72.72	0.39	2	128.10[f]	−1.5 ± 0.08[f]
3	74.31	0.43	3	130.17[g]	2.8 ± 0.05[g]
4	82.22	0.08	4	136.32	0.9 ± 0.07
5	73.02	0.12	1′	140.92	1.8 ± 0.10
6	61.45	0.00	2′	127.72[f]	−1.6 ± 0.08[f]
			3′	130.54[g]	1.4 ± 0.1[g]
			4′	129.12	−1.4 ± 0.1
			α	176.33	−3.3 ± 0.05

[a] δ_0, parts per million downfield from Me_4Si. [b] $\Delta\delta_2 = \delta_2 - \delta_0$. Negative signs indicate upfield displacement. [c] Each entry represents the average of five measurements of 0.050 M Cy solutions. These had standard deviations of 0.01–0.02 ppm. [d] Each entry represents the average of several measurements with a standard deviation of about ±0.02 ppm. [e] Average of four solutions containing 0.02–0.06 F Na^+A^- or Li^+A^-. [f,g] Assignments may be reversed.

carbons from the equation

$$\Delta\delta_2^{(Cy)} = \Delta\delta_{obsd}^{(Cy)}\,(\Delta\delta_2^{(Cy1)}/\Delta\delta_{obsd}^{(Cy1)}) \qquad (14)$$

$\Delta\delta_{obsd}^{(Cy)}$ data were obtained in the mole-ratio experiments and the displacement $\Delta\delta_2^{(Cy1)} = 0.75$ ppm was determined as described in the previous section. The results are given in the third column of Table IV.

$\Delta\delta_2^{(A^-)}$ values required two further ^{13}C NMR experiments. First, $\delta_0^{(A^-)}$ chemical shifts for all A^- carbons were determined by measuring ^{13}C NMR resonances of four pure Na^+A^- and Li^+A^- solutions in the concentration range 0.02–0.06 F. The average of these four is given in the fifth column of Table IV. Standard deviations calculated from the four solutions for each carbon were within the expected ±0.02 ppm precision of our measurements. The observed resonances were assigned to specific carbons of the A^- ion using a number of comparative criteria: (1) symmetry factors, (2) relative relaxation rates of protonated and nonprotonated carbons,[16] (3) published shifts of 4-biphenylcarboxylic acid,[17] and (4) best fit of measured *p*-NO_2 substituent effects in the 4′-nitro-4-biphenylcarboxylate/4-biphenylcarboxylate system with corresponding effects in the 4-nitrobiphenyl/biphenyl[17] system.

Second, $\delta_{obsd}^{(Cy)}$ and $\delta_{obsd}^{(A^-)}$ chemical shifts were recorded simultaneously for all carbons in four solutions each 0.050 F in Cy and 0.000, 0.026, 0.052, and 0.077 F in Na^+A^-. $\Delta\delta_2^{(A^-)}$ values were calculated from these as follows. The fraction of complexed A^- in solution is

$$\frac{[Cy_2A^-]}{F_{A^-}} = \frac{\Delta\delta_{obsd}^{(A^-)}}{\Delta\delta_2^{(A^-)}} \qquad (15)$$

Similarly, the fraction of complexed Cy is

$$\frac{2[Cy_2A^-]}{F_{Cy}} = \frac{\Delta\delta_{obsd}^{(Cy)}}{\Delta\delta_2^{(Cy)}} \qquad (16)$$

In the same solution, equating $[Cy_2A^-]$ yields

$$\Delta\delta_2^{(A^-)} = \frac{2F_{A^-}}{F_{Cy}}\,\Delta\delta_2^{(Cy)}\left(\frac{\Delta\delta_{obsd}^{(A^-)}}{\Delta\delta_{obsd}^{(Cy)}}\right) \qquad (17)$$

Substitution of displacements $\Delta\delta_{obsd}^{(A^-)}$ and $\Delta\delta_{obsd}^{(Cy1)}$ recorded for each of the three solutions containing Na^+A^- (0.026–0.077 F) and Cy (0.050 F) along with $\Delta\delta_2^{(Cy1)} = 0.75$ ppm in eq 17 led to three $\Delta\delta_2^{(A^-)}$ values for each of the nine nonequivalent A^- carbons. These were averaged, and the resulting set of $\Delta\delta_2^{(A^-)}$ displacements is given in the sixth column of Table IV.

Since the ^{13}C NMR data are recorded under rapid exchange conditions, the $\Delta\delta_2^{(Cy)}$ parameters represent the average perturbation induced by two distinct binding sites of the 4-biphenylcarboxylate ion on the Cy components of the complex. They also reflect Cy·Cy interactions within the complex. The Cy displacements show little or no perturbation at the C_5 and C_6 carbons (0.12 and 0.00 ppm, respectively), whereas the C_2 and C_3 carbons are significantly perturbed. (0.39 and 0.43 ppm, respectively). The latter secondary carbon atoms are located on the larger rim of Cy, and we define it here as the "head" of the molecule. We consider the pattern of C_2,C_3 vs. C_5,C_6 displacements as evidence favoring an antiparallel, "head to head" association of the two Cy moieties in Cy_2A^-.

We assembled a space-filling model of a Cy_2A^- complex with this orientation. In this model the A^- ligand is coaxially occluded in a zone defined by the duplicate bands of C_5 methine groups. The model suggests that the points of closest approach among the two Cy and one A^- constituents would result in perturbations at the C_3, C_2, and, to a lesser extent, the C_5 carbons of Cy. The observed $\Delta\delta_2^{(Cy)}$ displacements are 0.43, 0.39, and 0.12 ppm, respectively. On the other hand, the C_6 methylene bands are located on the "tails" of the "head to head" model. These sites are remote from the A^- ion and no perturbation would be expected there. The observed $\Delta\delta_2^{(Cy6)}$ displacement is 0.00 ppm. The C_1 carbons of Cy are significantly deshielded ($\Delta\delta_2^{(Cy1)} = 0.75$ ppm) and this may be in part reflective of macrocyclic conformational changes associated with the complexation[11] as well as direct interaction with the substrate.

As shown in Table IV, carbon atoms in both phenyl moieties of the A^- anion are substantially perturbed as expected from the Cy_2A^- model. The carboxylate carbon is most perturbed ($\Delta\delta_2^{(A^-\alpha)} = -3.3$ ppm), clearly reflecting the transfer of a charged group from D_2O solution into a less polar cavity defined by the two Cy moieties. Perturbations of phenyl carbons are expected to be delocalized and attenuated by charge relay within and across the rings by resonance, π polarization, and field effects. A detailed interpretation of $\Delta\delta_2^{(A^-)}$ values does not seem possible at this time. We note, however, that the pattern of alternating signs of $\Delta\delta_2^{(A^-)}$ values is generally consistent with induced chemical shift displacements due to ring substitution in aromatic systems.[17,18]

Discussion

Results of conductometric and ^{13}C NMR spectrometric analyses of solutions containing α-cyclodextrin and 4-biphenylcarboxylate ions are consistent with exclusive formation of a ternary complex

$$2Cy + A^- \overset{K_1K_2}{\rightleftharpoons} Cy_2A^- \qquad (18)$$

where $\log (K_1K_2)$ is 4.40 in aqueous solution at 30 °C. We have measured an average $\Delta H° = -14$ kcal/mol of Cy compared with typical values of −9.6 to −11.6 kcal mol^{-1} in binary complexes.[2b,15] This is a somewhat more negative value per mol of Cy than previously reported. It may be due to formation

of intermacrocyclic hydrogen bonds between Cy constituents within the complex.

It is difficult to make direct comparison between our $\Delta S°$ value of -72 cal mol^{-1} deg^{-1} with the available $\Delta S°$ values for binary complexes since these values refer to reactions of neutral species with α-cyclodextrin. However, most of the entropy changes reported for substrates structurally similar to 4-biphenylcarboxylate lie between about -10 and -30 cal mol^{-1} deg^{-1}.[2b,15] On the basis of $\Delta S°$ per mol of Cy, the ternary complex appears to be more ordered than comparable binary complexes, which would be expected from a ternary model involving extensive intermacrocyclic hydrogen bonding as well as a less mobile substrate.[10] The large negative standard entropy change associated with reaction 18 may be accounted for by a substantial reduction in the number of rotational and vibrational degrees of freedom available to macrocyclic components of ternary vs. binary complexes.

We have proposed a "head to head" Cy_2A^- model to account for observed ^{13}C NMR resonances of the complex relative to unbound species. In this model hydrogen bonding between C_2 and C_3 hydroxy groups of one Cy moiety and corresponding C_2 and C_3 groups of the other seems plausible.

It is noteworthy that formation of a Cy_2A^- complex involves the transfer of a charged carboxylate group from an aqueous solvation sphere into a Cy cavity surrounded by methine groups and glycosidic oxygens. Since the Cy cavity is generally regarded as less polar than an aqueous environment, this process might be expected to be unfavorable. The measured $\Delta H°$ and $\Delta S°$ values seem to indicate that this effect is compensated for by additional interactions presumably involving Cy moieties of the ternary complex. It is also possible that complexation of the carboxylate anion does not appreciably influence the $\Delta H°$ and $\Delta S°$ values because the anion may retain part of its solvation sphere in the complex.

The surprising stability and exclusive formation of the 2:1 complex containing the 4-biphenylcarboxylate substrate has led us to explore complex formation with other bifunctional substrates. We are also studying effects of functional group substitution in α-cyclodextrin on the complexation reaction. In view of the sustained interest in cycloamylose derivatives as enzyme models,[2c,19,20] more information concerning the factors involved in binary vs. ternary complex formation would seem useful.

Experimental Section

Chemicals. Lithium 4-biphenylcarboxylate (LiBPC) was prepared from commercial samples of 4-biphenylcarboxylic acid (0.063 mol) and lithium carbonate (0.035 mol) which were dissolved in hot distilled water (ca. 400 mL) and boiled for about 10 min. The hot solution was filtered and cooled. The first two crops were combined and recrystallized twice from distilled water leading to 7.5 g (60%) of clear white plates. The concentration of a LiBPC stock solution was determined by potentiometric titration with standard 0.1 F HCl solution and by a second method involving treatment with excess standard 0.1 F HCl followed by potentiometric back-titration with standard 0.02 F NaOH solution. The end points were estimated from a Gran plot of the titration data.

The sodium salt (NaBPC) was prepared from 4-biphenylcarboxylic acid (0.047 mol) which was treated with 70 mL of 1 F NaOH. The mixture was heated to about 60 °C and 10-mL aliquots of methanol were added until the solution became homogeneous. Filtration and cooling led to NaBPC which was recrystallized twice from hot 0.01 F NaOH solution. The final sample crystallized as white flakes in about 50% yield and did not melt at 290 °C. Portions of this sample were titrated with 0.1 F HCl or treated with excess 0.1 F HCl and back-titrated with 0.02 F NaOH as described above for the lithium salt.

Solutions containing commercial samples of Cy·4H$_2$O were analyzed by ^{13}C NMR spectrometry using high signal/noise conditions. The resultant spectra included non-Cy resonances corresponding to no more than ca. 2% of the sample peaks. These samples were used in subsequent experiments without further purification.

^{13}C NMR Analyses. Pulsed Fourier transform ^{13}C NMR measurements were recorded on a Varian CFT-20 spectrometer operating at 20.00 MHz. Parameters were set in the following ranges: spectral width 3–4 kHz, pulse width 6–18 μs (26–77° tip angle), acquisition time 0.15–1.02 s, pulse delay 0.00–2.00 s, data points 1.2–8.2K, completed transients 0.8–160K. All ^{13}C NMR spectra were proton decoupled and were obtained at an ambient temperature of 30 ± 2 °C in 10-mm tubes. Chemical shifts were measured in parts per million downfield from external Me_4Si with a reproducibility ranging from ±0.01 to ±0.02 ppm.

^{13}C NMR analyses of Cy/D_2O solutions were typically carried out in the following way. An aliquot (8.00 mL) of a Cy stock solution was pipetted into the NMR tube and the spectrum was recorded. The sample was titrated with microweighed portions of NaBPC, NaCl, or LiCl and the tube was spun in the spectrometer probe for at least 5 min prior to data acquisition. This procedure was followed at each titration point to ensure thermal equilibration and solvation of added salt. ^{13}C NMR spectra of Cy (0.005–0.100 F), NaBPC (0.024 F), and LiBPC (0.02–0.06 M) D_2O solutions were recorded in separate series of experiments.

Conductometric Analyses. Conductances were measured with a General Radio type 1608-A impedance bridge along with Yellow Springs Instrument Co. type 3403 or Beckman dip cells. The temperature was regulated with a MGW Lauda constant temperature circulating bath and monitored by a digital thermometer to ±0.1 °C.

The following conductometric titrations were carried out at each temperature (20.0, 25.0, 30.0, 37.0, and 45.0 °C): (1) A NaCl solution (50 mL, 0.0100 F) was titrated with 10 mL of distilled water in 1-mL aliquots. (2) A NaCl solution (50 mL, 0.0100 F) was titrated with 10 mL of 0.100 M Cy solution in 1-mL aliquots. (3) Distilled water (50 mL) was titrated with 10 mL of 0.100 F Cy solution in 1-mL aliquots. (4) A LiBPC solution (50 mL, 0.0089 F) was titrated with 10 mL of distilled water in 0.5-mL aliquots. (5) A LiBPC solution (50 mL, 0.0089 F) was titrated with 10 mL of 0.100 F Cy solution in 0.5-mL aliquots. In addition to these titrations, conductances of 10-mL solutions containing (6) 0.0100 F NaCl, (7) 0.0089 F LiBPC, and (8) distilled water were measured at each of the above temperatures before and after addition of excess Cy (1.00 g). Conductances were recorded 3–5 min after addition of each aliquot following the establishment of equilibrium conditions in the dip cell.

Acknowledgments. We thank Professor Elkan R. Blout for providing access to the CFT-20 spectrometer at the Department of Biological Chemistry, Harvard Medical School. We thank Professor John D. Roberts for sponsoring one of us (D.A.L.) as a research fellow at the Division of Chemistry and Chemical Engineering, California Institute of Technology, June–Dec 1974. This work was supported in part by a research grant from the National Institute of General Medical Sciences of the National Institutes of Health, U.S. Public Health Service (GM-19645).

References and Notes

(1) (a) A. O. Pulley and D. French, *Biochem. Biophys. Res. Commun.*, **5**, 11 (1965); (b) D. French, A. O. Pulley, J. A. Effenberger, M. A. Rougvie, and M. Abdullah, *Arch. Biochem. Biophys.*, **11**, 153 (1965).
(2) (a) D. J. Wood, F. E. Hruska, and W. Saenger, *J. Am. Chem. Soc.*, **99**, 1735 (1977); (b) J. H. Fendler and E. J. Fendler, "Catalysis in Micellar and Macromolecular Systems", Academic Press, New York, N.Y., 1975; (c) R. J. Bergeron, *J. Chem. Educ.*, **54**, 204 (1977).
(3) N. H. Hennrich and F. Cramer, *J. Am. Chem. Soc.*, **87**, 1121 (1965).
(4) K. Harata, *Bull. Chem. Soc. Jpn.*, **49**, 1493 (1976).
(5) A. Harada, M. Furue, and S. Nozakura, *Macromolecules*, **9**, 701 (1976).
(6) H. Kondo, H. Nakatani, and K. Hiromi, *J. Biochem. (Tokyo)*, **79**, 393 (1976).
(7) A. Wishnia and S. J. Lappi, *J. Mol. Biol.*, **82**, 77 (1974).
(8) R. Bergeron, Y. Machida, and K. Bloch, *J. Biol. Chem.*, **250**, 1223 (1975).
(9) R. Bergeron and M. A. Channing, *Bioorg. Chem.*, **5**, 437 (1976).
(10) J. P. Behr and J. M. Lehn, *J. Am. Chem. Soc.*, **98**, 1743 (1976).
(11) P. Colson, H. J. Jennings, and I. C. P. Smith, *J. Am. Chem. Soc.*, **96**, 8081 (1974).
(12) R. A. Robinson and R. H. Stokes, "Electrolyte Solutions", 2nd ed, Butterworths, London, 1965.
(13) Y. Bard, "Nonlinear Parameter Estimation", Academic Press, New York, N.Y., 1974, Chapter 5.
(14) J. F. Wojcik and R. P. Rohrbach, *J. Phys. Chem.*, **79**, 2251 (1975).
(15) E. A. Lewis and L. D. Hansen, *J. Chem. Soc., Perkin Trans. 2*, 2081

(1973).
(16) G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists", Wiley, New York, N.Y., 1972, Chapter 2.
(17) E. M. Schulman, K. A. Christensen, D. M. Grant, and C. Walling, *J. Org. Chem.*, **39**, 2686 (1974).
(18) W. F. Reynolds and G. K. Hamer, *J. Am. Chem. Soc.*, **98**, 7296 (1976).
(19) J. Emert and R. Breslow, *J. Am. Chem. Soc.*, **97**, 670 (1975).
(20) M. Komiyama and M. L. Bender, *J. Am. Chem. Soc.*, **99**, 8021 (1977).

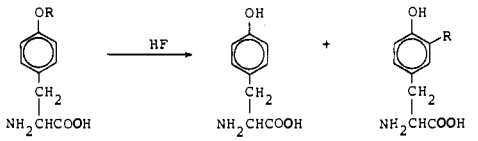
Tyrosine Protecting Groups: Minimization of Rearrangement to 3-Alkyltyrosine during Acidolysis[1,2]

M. Engelhard and R. B. Merrifield*

Contribution from The Rockefeller University, New York, New York 10021.
Received September 19, 1977

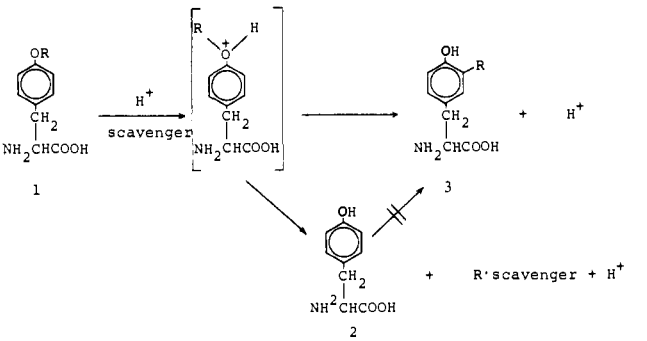
Abstract: In an effort to avoid the rearrangement of tyrosine ethers to 3-alkyl derivatives that occurs during acidolysis with hydrogen fluoride four new tyrosine protecting groups were designed and tested under conditions used in peptide synthesis. *O*-Isobornyltyrosine and *O*-[1-(5-pentamethylcyclopentadienyl)ethyl]tyrosine gave no rearrangement product. However, they were far too labile toward acidolysis in 50% TFA/CH_2Cl_2 to be used in the standard procedures of peptide synthesis, although they might be valuable in different schemes. The other two derivatives, *O*-isopropyltyrosine and *O*-cyclohexyltyrosine, both were sufficiently stable in acid and underwent minimal rearrangement. The cyclohexyl group was readily cleaved from Tyr(c-Hex) under standard cleavage conditions with HF (0 °C, 30 min) and is therefore an especially suitable protecting group for peptide synthesis. Its value was demonstrated by a synthesis of angiotensin II in which fully active hormone was obtained in high yield without the formation of significant amounts (<0.3%) of the cyclohexyltyrosyl rearrangement product.

Many side chain protecting groups used in peptide synthesis are cleaved in a final deprotection step by acidolysis. For the protection of the phenolic side chain of tyrosine several groups with such properties are available.[3] However, none is entirely satisfactory for prolonged stepwise peptide synthesis which is, for example, the usual strategy in solid-phase peptide synthesis.[4] Some of these protecting groups are too sensitive to acids[5] or to nucleophiles[6] and others undergo rearrangements during deprotection in strong acids to form 3-alkyltyrosine (**3**) by-products.[7]



Tyrosine benzyl ether (Tyr(Bzl)) has frequently been used in combination with N^{α}-Boc protection for stepwise syntheses employing selective acidic deprotection of the N^{α} group, after coupling of each Boc amino acid. The degree of selectivity of Boc removal by trifluoroacetic acid was greatly improved by the introduction of derivatives containing electron-withdrawing halogen substituents such as *m*-bromobenzyltyrosine[8] or 2,6-dichlorobenzyltyrosine.[7] The latter substitution also reduced the 3-alkylation side reaction in the final deprotection stage from levels of 10–70% to about 5%. While this level of rearrangement is tolerable for small peptides containing few tyrosines, it is unsatisfactory for large peptides with many tyrosine residues.

It is now well established that the acid-catalyzed rearrangement of aryl ethers to ring-substituted phenols proceeds via both intermolecular and intramolecular pathways.[9] To minimize or avoid the alkylation reaction several points must be considered. On the one hand it is possible to suppress the intermolecular pathway by scavengers such as anisole[7] or benzene[9] which compete with starting material and reaction products for the alkylating species. However, it is more difficult to interfere with the intramolecular pathway. Thus, the solvent



and the acid used for the cleavage reaction have only a small effect on the magnitude of the alkylation side reaction.[7,9] Data derived from the acidolysis of benzyltyrosine in trifluoroacetic acid or hydrogen fluoride suggest that the intramolecular pathway is less favored in the presence of HF.[7] However, the nature of the leaving group should have more influence on the intramolecular reaction. For example, benzyl phenyl ether is rapidly converted by aluminum bromide in chlorobenzene to a mixture of phenol and *o*-benzylphenol,[10] whereas *sec*-butyl phenyl ether is cleaved to both ortho and para products.[9,11] This indicates a dominant intramolecular pathway in the case of the cleavage of the benzyl ether whereas in the latter example both intra- and intermolecular reactions occur.[9,11] It is also expected that a group yielding upon acidolysis a cation that could rearrange to a neutral, nonalkylating product would have the desired properties:

$$\text{Tyr(R)} \xrightarrow{\text{HF}} \text{Tyr} + \text{R}^+ \rightarrow \text{R} + \text{H}^+$$

Finally, it should be noted that steric effects should also play a role on the ratio of the intra- and intermolecular reactions. These data suggest the suitability of alkyl ethers for the protection of the phenolic hydroxyl of tyrosine. This is supported by the fact that H-Tyr(Bu-*t*)-OH shows less than 0.05% rearrangement during HF acidolysis.[12] However, this protecting group is much too labile[5] to be used in prolonged stepwise

0002-7863/78/1500-3559$01.00/0