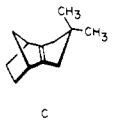
Table V. p_y Coefficients at C(1) of the Diene Subunits (Lowest Occupied π Orbital) in Various Isodicyclopentadiene Congeners and Experimental Diels-Alder Cycloaddition Ratios

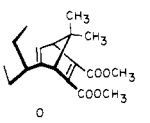
		product ratios (above-plane:below-plane)[a]				
compd	p_y coeff	PM	BQ	benzyne	DMAD	MTAD
2	−0.016				35:65	50:50
36	−0.014	0:100[b]	0:100	0:100	0:100	100:0
37	−0.014	[100:0][c]			30:70	100:0
1	−0.007	54:46	61:39		32:68	50:50
3	+0.018	100:0		100:0	100:0	

[a] PM = *N*-phenylmaleimide; BQ = *p*-benzoquinone; DMAD = dimethyl acetylenedicarboxylate; MTAD = *N*-methyltriazolinedione. [b] Green, K. E., unpublished results. [c] The adduct obtained in this example possesses Alder stereochemistry and cannot strictly be compared with the anti-Alder products formed in the other examples (see text).

package[36] was used to obtain the best possible orientation for fragment C of the molecule. The coordinates for this structural subunit were derived from an earlier study.[30b] The best orientation was then used as



C



D

input to MULTAN 80[37] as a correctly oriented fragment. The C map with the highest combined figure of merit (3.00) also had a fairly high residual 23.7, but it clearly revealed the presence of fragment D. The two mixing carbon atoms were then located on an electron density map.

All full-matrix least-squares refinements were done in the SHELX-76 system.[38] After isotropic refinement of the non-hydrogen atoms converged (R = 0.123), one cycle of anisotropic refinement and a difference electron density mpa revealed the positions of the majority of the hydrogen atoms. The hydrogen atoms were then included in the model as fixed contributions with C-H = 1.00 Å and $B_H = B_{C(iso)} + 1.0$ Å^2. The final refinement cycle with anisotropic non-hydrogen atoms resulted in agreement indices of R = 0.087 and R_w = 0.061 (based on F) for the 1544 intensities with $F_o^2 > 0.5\sigma(R_o^2)$ and 217 variables. A final structure factor calculation on the intensities with $F_o^2 > 3\sigma(R_o^2)$ yielded an R index of 0.057. The final difference electron density map contained maximum and minimum peak heights of 0.41 and −0.45 e Å^{-3}. Scattering factors for the carbon and oxygen atoms[39a] and for the hydrogen atom[39b] were taken from the usual sources.

The crystallographic details for **32** are compiled in Table I and relevant bond lengths and bond angles in Tables II and III, respectively. Selected least-squares planes are included in Table IV.

Acknowledgment. Financial support for the research conducted at The Ohio State University was provided by the National Cancer Institute (Grant CA-12115). The work in Heidelberg was supported by the Fonds der Chemischen Industrie and Deutsches Forschungsgemeinschaft.

Registry No. **1**, 93255-09-5; **2**, 93255-10-8; **3**, 93255-11-9; **6**, 70705-73-6; **12**, 36439-88-0; *exo*-**13**, 93255-12-0; *endo*-**13**, 93379-66-9; **14**, 93255-30-2; **15**, 7333-67-7; **16**, 765-46-8; **17**, 26154-22-3; **18**, 93255-13-1; **19**, 93255-14-2; **19** (diol), 93255-31-3; **20**, 93255-15-3; **21**, 93255-16-4; **22**, 93255-17-5; **23**, 93379-60-3; **24**, 93255-18-6; **25**, 93255-19-7; **26**, 93379-61-4; **27**, 93255-20-0; **28**, 93255-21-1; **29**, 93379-62-5; **30**, 93255-22-2; **31**, 93379-63-6; **32**, 93255-23-3; **33**, 93379-64-7; **34**, 93255-24-4; **35**, 93379-65-8; **38**, 93255-25-5; **39**, 93255-26-6; **40**, 93255-27-7; **41**, 93255-28-8; **42**, 93255-29-9; DMAD, 762-42-5; PTAD, 4233-33-4; phenyl(tribromomethyl)mercury, 3294-60-8; *p*-benzoquinone, 106-51-4; benzyne, 462-80-6; anthranilic acid, 118-92-3; isoamyl nitrite, 110-46-3; *N*-phenylmaleimide, 941-69-5.

Supplementary Material Available: Stereodrawing of the unit cell, final positional and thermal parameters, and observed and calculated structure factors for **32** (12 pages). Ordering information is given on any current masthead page.

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Syntheses of Calcium-Selective, Substituted Diaza-Crown Ethers: A Novel, One-Step Formation of Bibracchial Lariat Ethers (BiBLEs)

Vincent J. Gatto and George W. Gokel*

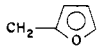
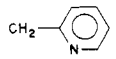
Contribution from the Department of Chemistry, Univeristy of Maryland, College Park, Maryland 20742. *Received May 29, 1984*

Abstract: Ten N,N-disubstituted derivatives of 4,13-diaza-18-crown-6 (**7**) have been prepared, three of which exhibit Ca^{2+}, over either Na^+ or K^+, selectivity. This selectivity has been achieved by utilizing polar, yet uncharged, donor groups such as carbethoxymethyl. Several of these compounds have been prepared by a novel, one-step reaction of aliphatic primary amines with triethylene glycol diiodide. The compounds prepared by this method include examples having the following substituents on nitrogen: benzyl, 2-methoxybenzyl, 2-hydroxyethyl, allyl, 2-furylmethyl, and 2-pyridylmethyl. Isolated yields of pure product for the one-step reaction were in the range 26 ± 4%. The corresponding derivatives of **7** having 2-methoxyethyl, carbethoxymethyl, carboxymethyl, and 2-hydroxybenzyl sidearms were also prepared so that cation selectivity as a function of structure could be assessed. Homogeneous stability constants (K_s) for the association between the various ligands and Na^+, K^+, and Ca^{2+} were determined and are reported. Significant Ca^{2+} selectivity is achieved for the first time in these systems which should retain a high degree of binding dynamics since the donor groups of primary interest in this study are non-ionizable.

During the past decade, there has been enormous synthetic activity in the macrocyclic polyether area. Several thousand novel and interesting structures have been prepared as part of this effort.[1] Although it is quite difficult to characterize all classes of these

0002-7863/84/1506-8240$01.50/0

Table I. One-Step Synthesis of Bibracchial Lariat Ethers: N,N′-Disubstituted 4,13-Diaza-18-crown-6 Derivatives[a]

compd no.	R	concn.[b]	M^{+}[c]	time[d]	yield, %	mp, °C	$M_r(m/e)$
1	$PhCH_2$[e]	0.42	Na	30	29	80–81	442
2	$2\text{-}MeOC_6H_4CH_2$	0.33	Na	24	30	86–87	502
3	$CH_2CH{=}CH_2$	0.25	Na	22	26	45–46	342
3	$CH_2CH{=}CH_2$	0.25	K	25	25	45–46	342
4		0.22	Na	22	27	34–36[g]	422
5	$HOCH_2CH_2$	0.13	Na	24	28	130–132[f]	nd
6		0.22	Na	25	22	208–210[f]	nd

[a] Reactions were conducted under N_2 in MeCN at reflux temperature with 1 equiv of diiodide and 5 equiv of anhydrous metal carbonate per 1 equiv of amine. [b] Molarity of diiodide. [c] Cation of metal carbonate. [d] In hours. [e] This compound was reported (Wester, N.; Voegtle, F. *J. Chem. Res., Miniprint* **1978**, 4856–4863) as having a melting point of 80 °C. [f] Isolated first as the NaI complex for which the melting point is given. [g] H. Tsukube has prepared this compound by alkylation and reports a melting point of 37–38 °C. We thank him for informing us of this result prior to publication.

compounds, some of the major ones are simple monocyclic crowns,[2] cryptands,[3] spherands,[4] and cavitands,[5] and recently our own efforts have focussed on the compounds we refer to as "lariat ethers."[6] Lariat ethers are designed to mimic naturally occurring ionophores by presenting a cation with a three-dimensional, intramolecular array of binding sites as do the cryptands or spherands. In this way, we hoped to achieve a somewhat higher level of cation binding than generally observed with simple, monocyclic crown ethers. By placing the additional binding sites on sidearms attached to the macroring, we hoped to retain the high degree of flexibility and dynamics characteristic of ionophores like valinomycin.[7]

In several recent papers, we have reported our efforts to prepare lariat ethers having macrorings of various sizes and sidearms bearing a variety of donor group(s). We have shown that (a) cation binding is, in many cases, enhanced for lariat ethers relative to simple monocyclic crown ethers,[6] (b) the donor group bearing sidearm participates intramolecularly in the cation binding process both in solution[8] and in the solid state,[9] and (c) some of the lariats exhibit highly dynamic behavior, as judged by ^{13}C NMR T_1 relaxation time and ^{23}Na NMR linewidth studies.[10] We have also made an effort to clarify the relationship between cation selectivity and hole-size diameters since the lariat ethers cannot be understood in simple, monocyclic terms. The results of the latter study[11] suggested that the "hole-size relationship" is, at best, somewhat oversimplified.

The next logical step in the overall effort was to try to adjust cation selectivity by varying factors other than hole size. Altering hole size is effective for nonflexible or relatively inflexible compounds like cryptands, cavitands, and spherands. Unfortunately, when the hole size is structurally restricted, the binding dynamics usually follow suit and reduce the effectiveness of cation transport. We therefore sought to examine other variables in an effort to achieve Ca^{2+} selectivity while retaining dynamic behavior. This has resulted in the synthesis of N-pivot lariat ethers having two arms which we term "bibracchial lariat ethers", borrowing from the Latin *bracchium* meaning "arm". We abbreviate by using the acronym "BiBLEs".

Results and Discussion

The results obtained thus far suggest that a three-dimensional arrangement of donor groups is important for effective binding of cations, including Ca^{2+}. In addition, polar donor groups appear to enhance Ca^{2+} selectivity. In order to confirm these general indications, we have prepared several lariat ethers having two arms, each containing polar, but not charged, donor groups. Our choice of N-pivot compounds results from previous studies which have shown that crown ethers having arms joined at a macroring carbon are relatively inflexible and exhibit both weak and solvent-dependent cation binding.[6c,d] In contrast, when the donor group containing sidearm is attached to a nitrogen rather than carbon pivot, binding constants are found to be quite substantial.[6e] ^{13}C NMR relaxation time and ^{23}Na NMR linewidth measurements indicate that the nitrogen-pivot compounds are more dynamic complexing agents than the corresponding carbon-pivot compounds. The sidearm donor groups in the N-pivot series contribute more to overall cation binding than is the case for the C-pivot compounds.[10] In order to determine how two arms, rather than one, would contribute to the overall binding, we have prepared diaza compounds **1–11** and determined their homogeneous stability constants (K_s) with Na^+, K^+, and Ca^{2+} cations in anhydrous methanol solution.[11]

The parent compound in this study is 4,13-diaza-18-crown-6 (**7**). It has been prepared by several different methods over the years.[12] Likewise, derivatives of **7** possessing ether, hydroxyl, amide, ketone carbonyl, ester carbonyl, carboxyl, amine, and phthalimide donor groups have all been reported. These two-armed compounds have usually been prepared by alkylation or acylation of **7**.[13] In general, the syntheses have been accomplished by multistep procedures, some of which involve complex experimental manipulations. The method described here is a novel and simple, one-step procedure by which a variety of symmetrical, N,N′-disubstituted derivatives of **7** can be prepared. *N,N′*-Dibenzyl-4,13-diaza-18-crown-6, which is readily obtained by this procedure, can be converted into the parent diamino crown (**7**)

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in high yield. This obviates the need for executing the difficult and, in our experience, sometimes dangerous preparations of triglycolic acid.[14]

Synthesis by One-Step Cyclization. We have found that when primary aliphatic amines are heated with 1,2-bis(2-iodoethoxy)ethane (**13**), in MeCN solution containing anhydrous Na_2CO_3 or K_2CO_3, symmetrical *N,N'*-disubstituted derivatives of 4,13-diaza-18-crown-6 (**7**, see eq 1) are formed in good yield. Reaction conditions and yields for a variety of compounds prepared by using this method are recorded in Table I.

$$R\text{-}NH_2 + I\text{-}CH_2(CH_2OCH_2)_2CH_2\text{-}I \xrightarrow[CH_3CN,\ 83°]{Na_2CO_3} \underset{\mathbf{13}}{} \quad \underset{\mathbf{1\text{-}6}}{\text{R-N, N-R macrocycle}} \qquad (1)$$

Compounds **2**, **3**, and **4** were isolated as free ligands (path a) after column chromatography and recrystallization. Compound **1** was also isolated as the free ligand. Column chromatography was avoided for this compound by recrystallizing the crude product from Me_2CO:dioxane (1:1). This afforded the NaI complex of **1**. The complex was dissolved in $CHCl_3$, washed with H_2O, dried, and reduced in vacuo. The residue was crystallized from hexanes to yield pure **1** as white crystals. This crystallization procedure was attempted on compound **2** but was not successful. When *p*-anisidine was reacted with 1,2-bis(2-iodoethoxy)ethane under the same conditions used for the aliphatic amines, *N*-(*p*-methoxyphenyl)monoaza-9-crown-3 (**12**) was the only cyclization product isolated after column chromatography (see eq 2).

$$CH_3O\text{-}C_6H_4\text{-}NH_2 + 13 \xrightarrow[CH_3CN,\ 83°]{Na_2CO_3} \underset{12\ d,\ 13\%}{} \quad \underset{\mathbf{12}}{\text{N, OCH}_3} \qquad (2)$$

Not only is the 9-membered ring the exclusive cyclization product of this reaction, its formation is much slower than the usual rate of dimer formation. Aniline nitrogen is less nucleophilic than aliphatic nitrogen and therefore reacts more slowly with the primary iodide. It appears that once monoalkylation occurs, cyclization to a nine-membered ring is preferred. Note that since heteroatoms occupy every third ring position, the nine-membered crown ring is not as difficult to form as the carbocyclic analogues are known to be.

Two compounds, bis(2-hydroxyethyl) ether **5** and bis(2-pyridylmethyl) ether **6** were isolated as their NaI complexes [**5**·NaI and **6**·NaI] after column chromatography and recrystallization.

$$\text{R-N, Na}^+\text{, N-R}\ \ I^- \qquad \mathbf{5},\ R = CH_2CH_2OH \qquad \mathbf{6},\ R = CH_2\text{-(2-pyridyl)}$$

Syntheses by Alkylation or Acylation of 7. Because of experimental and manipulative difficulties, compounds **8–11** could not be prepared by using this new cyclization reaction. These compounds were required for comparative purposes and were prepared from 4,13-diaza-18-crown-6 (**7**) by the methods described in eq 2. Compound **7** was obtained from **1** by hydrogenolysis over 10% Pd on C in 92% yield after recrystallization from hexanes. The bis(carbethoxymethyl) derivative, **9**, was prepared by alkylation of **7** with ethyl bromoacetate (Na_2O_3 base) in refluxing MeCN. Aqueous hydrolysis of a pure sample of **9** as previously described by Kulstad and Malmsten[12c] afforded **10** in 81% yield after recrystallization. These workers have previously prepared compound **8** in 29% yield by alkylation of **7** with methoxyethyl *p*-toluenesulfonate.[12c] We have found that acylation of **7** with methoxyacetyl chloride followed by diborane reduction of the bisamide affords **8** as a transparent oil in 76% overall yield. Finally, compound **11** was prepared by alkylation of **7** with *o*-chloromethylphenyl acetate (Na_2CO_3 base) in refluxing MeCN. The bis-acetate hydrolyzed during alumina chromatography and **11** was obtained in 85% overall yield.

Table II. Homogeneous Stability Constants for Bibracchial Lariat Ethers[a]

	R	log K_s Na^+	K^+	Ca^{2+}
1	$PhCH_2$	2.72	3.38	2.79
2	2-MeO-$C_6H_4CH_2$	3.65	4.94	3.27
5	$HOCH_2CH_2$	4.87	5.08	6.02
7	H	~1.5	~1.8	na
8	$CH_3OCH_2CH_2$	4.75	5.46	4.48
9	$EtOCOCH_2$	5.51	5.78	6.78
10	$HOOCCH_2$	na	~1.8	na
11	2-HO-$C_6H_4CH_2$	2.40	2.59	2.95

[a] Measured in absolute MeOH at 25.0 ± 1.0 °C with a Corning 476210 electrode for Na^+ and Ca^{2+}, a Corning 476220 electrode for K^+, and an Orion Model 501 "Ionalyzer" meter.

$$PhCH_2NH_2 + 13 \longrightarrow \underset{\mathbf{1},\ 29\%}{PhCH_2\text{-N, N-CH}_2Ph} \qquad \xrightarrow[10\%\ Pd/C]{H_2,\ EtOH} \underset{\mathbf{7},\ 92\%}{HN, NH} \xrightarrow[or\ acylation]{alkylation} \mathbf{7} \rightarrow \mathbf{11} \qquad (3)$$

Binding Data. Compounds **2** and **11** cannot be directly compared with compounds **5**, **8**, **9**, or **10**. The latter compounds possess secondary donor groups two carbon atoms removed from the macroring. Compounds **2** and **11** possess donor groups three carbon atoms removed from the macroring. Keeping these structural differences in mind, several interesting trends can be gleaned from the binding data (Table II). First, the presence of secondary donor groups on the sidearms enhances binding for all of the cations studied. This result is expected on the basis of previous results with monoaza crown ethers. The flexibility of the present compounds allows effective donor group interactions from the ring. When combined with solvation from two sidearm donor groups, the ligand–cation interaction is strong. Note that as donor group polarity increases,[15] the Na^+ and Ca^{2+} binding constants increase. Compound **8**, with an ether group in each sidearm, has a lower binding constant for Na^+ and Ca^{2+} than **5** which possesses the more polar hydroxyl donor group on each sidearm. The same trend is observed when comparing **5** and **9**.

The conclusions drawn above can be understood in terms of ion–dipole interactions. Since Ca^{2+} is more charge dense than Na^+, the former should be more strongly solvated by polar donor groups. This expectation is supported by an increase in Ca^{2+}/Na^+ selectivity as the donor groups become more polar.[15] Note that **8** has a higher binding constant with Na^+ than it does with Ca^{2+}. Compound **5** shows a slight increase in Na^+ binding when compared to **8**, but the Ca^{2+} binding is increased by more than one order of magnitude. This trend continues for **8** and **9**. The increase in Na^+ binding when an ether function is replaced by ester carbonyl is less than an order of magnitude. Calcium cation binding for the same compounds increases more than two orders of magnitude. These results suggest that diazacrown ethers with polar groups interact more strongly with the cation than with

(14) The preparation of triglycolic acid to which we refer [Lehn, J.-M. United States Patent 3 888 887, June 10, 1975] involves nitric acid oxidation of triethylene glycol. Although this procedure generally works as published, conditions must be controlled very carefully to avoid having the reaction become too vigorous for the vessel in which it is conducted to contain it.

(15) The polarity of the donor groups was assessed by considering the sidearms as whole molecules [e.g., $-CH_2COOEt$ considered to be ethyl acetate]. The dielectric constants of the molecules increase in the following order: $CH_3OCH_2CH_3$, $HOCH_2CH_3$, EtO–CO–CH_3.

solvent, decreasing their solvent dependence.

The binding constants of **7**, **10**, and **11** may seem unusually low, considering the arguments just presented. Compound **7** is capable of forming strong, N–H hydrogen bonds, especially in a protic solvent like methanol. The lack of any sidearm further reduces the binding ability. Compound **10** is a bis-amino acid crown ether and probably exists as a zwitterion under neutral conditions. Obviously, if the nitrogens of the crown cavity are protonated, complexation will be severely reduced. Finally, compound **11** is a bisphenol whose hydroxyl groups may hydrogen bond to the ring nitrogen atoms. Such interactions must necessarily reduce binding strength with cations. The actual extent of intramolecular hydrogen bonding in these structures is currently under investigation.

Summary

We have shown that ligand selectivity for Ca^{2+} cations over either Na^+ or K^+ can be achieved by using bibracchial lariat ethers of rational design. The two-armed diazacrowns have been used so that three-dimensional binding can be achieved but flexibility and binding dynamics will be retained. Our studies show that polar donor groups like ester carbonyl strongly favor Ca^{2+} over either Na^+ or K^+ but less polar groups like ethers favor K^+. We have prepared several of the structures required for this study by a novel, one-step cyclization reaction reported here for the first time which proves both effective and reasonably versatile.

Experimental Section

General. ^1H NMR spectra were recorded on a Varian EM-360 or XL-100 spectrometer in $CDCl_3$ solvent and Me_4Si as internal standard. IR spectra were recorded on a Perkin-Elmer 281 spectrophotometer. Mass spectra were recorded on a Du Pont 21742 or Hitachi RMU7E spectrometer. Elemental analyses were performed at the University of Maryland. Gas-chromatographic analyses were performed on a Varian Model 920 gas chromatograph with a thermal conductivity bridge detector equipped with a 5 ft × 0.25 in. 1.5% OV-101 column on 100/120 mesh Chromosorb G. Helium was used as the carrier gas, and the flow rate was ca. 60 mL/min. Thin-layer chromatographic analyses were conducted by using precoated TLC plastic sheets purchased from EM Reagents (aluminum oxide 60 F_{254} and silica gel 60 F_{254}). All compounds were judged to be single substances by either TLC or GC before conducting binding experiments.

Binding Studies. The binding constants for Na^+, K^+, and Ca^{2+} were determined in absolute methanol with use of ion selective electrodes. A Corning 476210 sodium ion selective electrode was used for sodium and a Corning 476220 monovalent cation electrode was used for potassium. A Ag/AgCl electrode was used as a reference electrode. Emf measurements were made with an Orion Model 701A "Ionalyzer" millivolt meter. The apparatus was contained in a drybox and the temperature was maintained at 25.0 ± 1.0 °C with use of di-*n*-butyl phthalate as a heat-transfer solvent. The binding studies for Ca^{2+} were done in competition with sodium. The binding constants were calculated by following a procedure described earlier.[11]

Synthesis. 1,2-Bis(2-iodoethoxy)ethane (**13**) was prepared by the method of Kulstad and Malmsten.[12c] *o*-Chloromethylphenyl acetate was prepared by the method of Zawadowski.[17]

***N,N'*-Dibenzyl-4,13-diaza-18-crown-6 (1).** To a vigorously stirred solution containing 1,2-bis(2-iodoethoxy)ethane (93.0 g, 0.25 mol) and Na_2CO_3 (125 g, 1.2 mol) in refluxing CH_3CN (400 mL) was added a solution of benzylamine (27.0 g, 0.25 mol) in CH_3CN (200 mL). After being heated at reflux for 30 h, the mixture was cooled, filtered, and concentrated in vacuo. The crude solid product was dissolved in a refluxing solution of acetone:dioxane (1:1) and allowed to crystallize. The precipitated crystals (mixture of NaI and NaI complex of **1**) were dried and dissolved in a minimum amount of water. The aqueous solution was extracted with $CHCl_3$ (3 × 100 mL). The combined organic phases were dried ($MgSO_4$), concentrated in vacuo, and crystallized (hexanes) to afford 15.7 g (29%) of a white solid (mp 80–81 °C) with physical properties indentical with those reported for **1**.[16]

***N,N'*-Bis(2-methoxybenzyl)-4,13-diaza-18-crown-6 (2).** The synthesis of this compound was analogous to that of **1**. Chromatography (aluminum, 1.5% 2-propanol:hexanes) followed by recrystallization (hexanes) provided **2** (18.9 g, 30%) as a white solid (mp 86–87 °C): ^1H NMR ($CDCl_3$) δ 2.84 (t, 8 H, CH_2N), 3.62–3.80 (m, 26 H, CH_3O, CH_2O and benzyl), 6.80–7.50 (m, 8 H, aromatic); IR (KBr) 1600, 1590, 1490, 1460, 1440, 1240, 1120, 1060, 720 cm^{-1}; mass spectrum M^+, 502. Anal. Calcd for $C_{28}H_{42}N_2O_6$: C, 66.89; H, 8.44; N, 5.57. Found: C, 66.70; H, 8.70; N, 5.24%.

***N,N'*-Diallyl-4,13-diaza-18-crown-6 (3).** A solution of allylamine (0.70 g, 12 mmol), 1,2-bis(2-iodoethoxy)ethane (3.8 g, 10 mmol), and Na_2CO_3 (5.3 g, 50 mmol) in MeCN (40 mL) was heated (sealed tube) for 22 h. The reaction was cooled, filtered, and concentrated in vacuo. The residue was taken up in $CHCl_3$ (25 mL), and the organic phase was washed with water (25 mL). The organic phase was dried ($MgSO_4$) and concentrated in vacuo. Chromatography (alumina, 1% 2-propanol:hexanes) followed by recrystallization (hexanes) yielded **3** (0.44 g, 26%) as a white crystalline solid (mp 45–46 °C): ^1H NMR ($CDCl_3$) δ 2.80 (t, 8 H, CH_2N), 3.18 (d, 4 H, CH_2 allyl), 3.66 (t and s, 16 H, CH_2O) 5.02–5.24 (m, 4 H, $C{=}CH_2$), 5.60–6.13 (m, 2 H, $HC{=}C$); IR (KBr) 3060, 2920, 1820, 1640, 1490, 1470, 1450, 1410, 1350, 1330, 1295, 1260, 1240, 1120 (s), 1060 (s), 990, 970, 960, 940, 900, 875, 840, 810 cm^{-1}; mass spectrum M^+, 342. Anal. Calcd for $C_{13}H_{34}N_2O_4$: C, 63.11; H, 10.03; N, 8.18. Found: C, 63.13; H, 10.20; N, 8.22.

***N,N'*-Bis(2-furanylmethyl)-4,13-diaza-18-crown-6 (4).** The synthesis of this compound was analogous to that of **1**. Chromatography (alumin, 30% ethyl acetate:hexanes) followed by recrystallization (hexanes) afforded **4** (0.55 g, 27%) as a white crystalline solid (mp 34–36 °C): ^1H NMR ($CDCl_3$) δ 2.80 (t, 8 H, CH_2N), 3.60 (s and t, 16 H, CH_2O), 3.70 (s, 4 H, furfuryl), 6.10–6.31 (m, 4 H, furan H), 7.34 (d, 2 H, 5-furan H); IR (neat) 2920, 2860, 1500, 1450, 1360, 1125 (s), 1030, 1020, 730 cm^{-1}; mass spectrum M^+, 422. Anal. Calcd for $C_{22}H_{34}N_2O_6$: C, 62.53; H, 8.13; N, 6.63. Found: C, 62.92; H, 8.40; N, 6.75.

***N,N'*-Bis(2-hydroxyethyl)-4,13-diaza-18-crown-6 (5).** A solution of ethanolamine (12.8 g, 0.2 mol), 1,2-bis(2-iodoethoxy)ethane (74.0 g, 0.2 mole, and Na_2CO_3 (106.0 g, 1.0 mol) in MeCN (1.5 L) was stirred vigorously at reflux temperature for 24 h. The reaction was cooled, filtered, and concentrated in vacuo. The remaining residue was combined with $CHCl_3$ (100 mL), filtered, and concentrated in vacuo. Chromatography (silica gel 60, 3% 2-propanol:$CHCl_3$) followed by recrystallization (THF) afforded **5**·NaI (14.1 g, 28%) as a white crystalline solid (mp 130–132 °C): ^1H NMR (D_2O) δ 2.47 and 2.57 (t and t, 12 H, CH_2N), 3.44 and 3.50 (t and s, 20 H, CH_2O), 4.50 (s, 2 H, OH); IR (KBr) 3400 (s), 3360 (s), 2980, 2910, 2820, 1480, 1470, 1450, 1365, 1360, 1280, 1150, 1130, 1110 (s), 1090 (s), 1080, 940, 875 cm^{-1}. Anal. Calcd for $C_{16}H_{34}N_2O_6NaI$: C, 38.40; H, 6.86; N, 5.60. Found: C, 38.31; H, 7.10; N, 5.46. Distillation of the above solid (Kugelrohr apparatus, bp 194–200 °C (0.1 mm)) provided pure **5** (100%) as a colorless oil with physical properties identical with those reported.[13c]

***N,N'*-Bis(2-pyridylmethyl)-4,13-diaza-18-crown-6·NaI (6·NaI).** The synthesis of this compound was analogous to that for **5**·NaI. Chromatography (silica gel 60, 5% methanol/$CHCl_3$) followed by recrystallization (2-propanol) provided **6**·NaI (2.6 g, 22%) as a white solid (mp 208 °C dec): ^1H NMR ($CDCl_3$) δ 2.85 (t, 8 H, CH_2N), 3.39 (s, 8 H, OCH_2CH_2O), 3.59 (t, 8 H, CH_2O), 3.85 (s, 4 H, benzyl), 7.08–7.82 (m, 6 H, pyridine H), 8.33–8.44 (m, 2 H, 6-pyridine H); IR (KBr) 2880, 2820, 1590, 1575, 1475, 1435, 1355, 1310, 1270, 1135, 1110 (s), 1075, 1000, 940, 920, 820, 800, 770 cm^{-1}. Anal. Calcd for $C_{24}H_{36}N_4O_4NaI$: C, 48.48; H, 6.12; N, 9.43. Found: C, 48.27; H, 5.91; N, 9.42.

4,13-Diaza-18-crown-6 (7). Compound **1** (17.1 g, 40 mmol), freshly recrystallized from EtOH, 10% Pd/C catalyst (1.7 g), and absolute EtOH (200 mL) were shaken in a Parr series 3900 hydrogenation apparatus at 60 psi H_2 pressure and 25 °C for 24 h. The mixture was filtered and concentrated in vacuo to yield, after recrystallization from hexanes, 9.7 g (92%) of a white solid (mp 114–115 °C) with physical properties identical with those reported for **7**.[12a]

***N,N'*-Bis(methoxymethylcarbonyl)-4,13-diaza-18-crown-6.** To a vigorously stirred solution containing methoxyacetyl chloride (3.4 g, 31.0 mmol) in benzene (50 mL) was slowly added a solution containing 4,13-diaza-18-crown-6 (3.7 g, 14.1 mmol) and triethylamine (3.1 g, 31.0 mmol) in benzene (50 mL). After addition the reaction was stirred at room temperature for 1 h. The reaction was concentrated in vacuo and taken up in 100 mL of $CHCl_3$. The organic phase was washed first with 25 mL of 1 N HCl, then with 25 mL of water, and finally with 25 mL of 1 N NaOH. The organic phase was dried (Na_2SO_4) and concentrated in vacuo to yield the title compound (4.6 g, 80%) as a white solid (mp 94–95 °C): ^1H NMR ($CDCl_3$) δ 3.33 (s, 6 H, CH_3), 3.61–3.68 (m, 24 H, CH_2N and CH_2O), 4.12 (s, 4 H, $COCH_2O$); IR (KBr) 2960, 2890, 2870, 2820, 1655 (s), 1470, 1460, 1440, 1410, 1370, 1350, 1320, 1310, 1280, 1250, 1230, 1200, 1145, 1115 (s), 1110 (s), 1040, 1020, 980, 930, 915, 870, 815, 800 cm^{-1}. Anal. Calcd for $C_{18}H_{34}N_2O_8$: C, 53.13; H, 8.45; N, 6.89. Found: C, 52.96; H, 8.69; N, 6.90.

***N,N'*-Bis(2-methoxyethyl)-4,13-diaza-18-crown-6. (8).** N,N'-Bis-(methoxymethylcarbonyl)-4,13-diaza-18-crown-6 (4.3 g, 10.6 mmol) was added at once to a 1.0 M solution of diborane in THF (84 mL) at 0 °C.

(16) Wester, N.; Voegtle, F. *J. Chem. Res., Miniprint* **1978**, 4856.
(17) Zawadowski, T. *Rocz. Chem.* **1968**, *42*, 297.

The reaction was brought to room temperature and stirred for 18 h. Excess diborane was destroyed by cautious addition of water until there was no further evolution of hydrogen. The reaction was concentrated in vacuo and the residue added to 100 mL of 6 N HCl. The solution was heated at reflux (1 h), cooled, and neutralized with NaOH pellets. The aqueous phase was extracted with CH_2Cl_2 (3 × 100 mL). The organic phase was dried ($MgSO_4$) and concentrated in vacuo. A bulb-to-bulb distillation (Kugelrohr apparatus, 144–146 °C (0.05 mm)) of the residue afforded **8** (3.7 g, 97%) as a transparent oil with physical properties identical with those reported.[12c]

***N,N'*-Bis(carbethoxymethyl)-4,13-diaza-18-crown-6 (9).** A solution of 4,13-diaza-18-crown-6 (6.0 g, 23 mmol), ethyl bromoacetate (3.4 g, 50 mmol), and Na_2CO_3 (5.4 g, 51 mmol) in MeCN (100 mL) was heated at reflux for 24 h. The reaction was then cooled, filtered and concentrated in vacuo. The residue was taken up in $CHCl_3$ (100 mL) and washed with H_2O (100 mL). The organic phase was dried ($MgSO_4$) and concentrated in vacuo. A bulb-to-bulb distillation (Kugelrohr apparatus, 195–197 °C (0.18 mm)) of the residue afforded **9** (9.2 g, 92%) as a transparent oil with physical properties identical with those already reported.[12c]

***N,N'*-Bis(carboxymethyl)-4,13-diaza-18-crown-6 (10).** A solution of **9** (3.0 g, 6.9 mmol) in water (22 mL) was heated at reflux temperature for 48 h. The reaction was cooled and concentrated in vacuo. Ethanol (40 mL) was added to the residue, and the mixture was left to stand overnight. The resulting crystals were filtered and dried in vacuo (100 °C (0.1 mm)) for 2 h to afford **10** (2.1 g, 81%) as a white solid (mp 173–175 °C) with physical properties identical with those reported.[12c]

***N,N'*-Bis(2-hydroxybenzyl)-4,13-diaza-18-crown-6 (11).** A solution of 4,13-diaza-18-crown-6 (3.00 g, 11.4 mmol), *o*-chloromethylphenyl acetate (4.69 g, 25.4 mmol), and Na_2CO_3 (2.69 g, 25.4 mmol) in MeCN (50 mL) was heated at reflux temperature for 20 h. The reaction was cooled, filtered, and concentrated in vacuo. The residue was taken up in $CHCl_3$ (100 mL) and washed with H_2O (100 mL). The organic phase was dried ($MgSO_4$) and concentrated in vacuo. The resulting yellow oil was identified as N,N'-bis(2-acetoxybenzyl)-4,13-diaza-18-crown-6: ^1H NMR ($CDCl_3$) δ 2.28 (s, 6 H, CH_3), 2.77 (t, 8 H, CH_2N), 3.59 (m, 20 H, CH_2O and benzyl), 6.80–7.50 (m, 8 H, aromatic); IR (neat) 3040, 2880, 1770 (s), 1495, 1460, 1375, 1215 (s), 1180, 1120, 1045, 920, 755 (s) cm^{-1}. Chromatography of the crude bis-acetate (alumina, 75% Et_2O:hexanes) resulted in acetate hydrolysis and afforded **11** (4.60 g, 85%) as a white solid (mp 120–122 °C): ^1H NMR ($CDCl_3$) δ 2.83 (t, 8 H, CH_2N), 3.62–3.79 (m, 20 H, CH_2O and benzyl), 6.56–7.36 (m, 8 H, aromatic), 9.90 (br s, 2 H, hydroxyl); IR (KBr) 3100 (br), 2980, 2960, 2920, 2840, 1620, 1590, 1490, 1260, 1250, 1150, 1130, 1120 cm^{-1}. Anal. Calcd for $C_{26}H_{38}N_2O_6$: C, 65.79; H, 8.09; N, 5.90. Found: C, 66.08; H, 8.35; N, 5.68.

***N*-(4-Methoxyphenyl)monoaza-9-crown-3 (12).** A solution of *p*-anisidine (1.2 g, 10 mmol), 1,2-bis(iodoethoxy)ethane (3.7 g, 10 mmol), and Na_2CO_3 (5.3 g, 50 mmol) in MeCN (45 mL) was heated at reflux temperature for 12 days. The reaction was cooled, filtered, and concentrated in vacuo. The residue was taken up in $CHCl_3$ (50 mL) and washed with H_2O (50 mL). The organic phase was dried with $MgSO_4$ and concentrated in vacuo to yield, after column chromatography (alumina, 4% EtOAc:hexanes), 0.3 g (13%) of the title compound as a yellow oil: ^1H NMR ($CDCl_3$) δ 3.42–3.83 (m, 15 H, CH_2O and CH_2N and CH_3O), 6.50–6.90 (m, 4 H, aromatic): IR (neat) 2900, 2860, 1615, 1510 (s), 1460, 1350, 1260, 1240, 1190, 1130, 1110, 1040, 1000, 930, 860, 810 cm^{-1}; mass spectrum M^+, 237. Anal. Calcd for $C_{13}H_{19}NO_3$: C, 65.79; H, 8.09; N, 5.90. Found: C, 65.70; H, 8.30; N, 5.60.

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Registry No. **1**, 69703-25-9; **2**, 93000-66-9; **3**, 93000-67-0; **4**, 90633-85-5; **5**, 69930-74-1; **5**·NaI, 87249-10-3; **6**·NaI, 93000-65-8; **7**, 23978-55-4; **8**, 72911-99-0; **9**, 62871-83-4; **10**, 72912-01-7; **11**, 88104-28-3; **12**, 93000-70-5; **13**, 36839-55-1; $PhCH_2NH_2$, 100-46-9; MeO-*o*-$C_6H_4CH_2NH_2$, 6850-57-3; CH_2=$CHCH_2NH_2$, 107-11-9; $NH_2(CH_2)_2$-OH, 141-43-5; $MeOCH_2C(O)Cl$, 38870-89-2; $BrCH_2C(O)OEt$, 105-36-2; $CH_3C(O)O$-*o*-$C_6H_4CH_2Cl$, 15068-08-3; MeO-*p*-$C_6H_4NH_2$, 104-94-9; Na^+, 17341-25-2; K^+, 24203-36-9; Ca^{2+}, 14127-61-8; 2-furanmethanamine, 617-89-0; *N,N'*-bis[(methoxymthyl)carbonyl]-4,13-diaza-18-crown-6, 93000-68-1; *N,N'*-bis(2-acetoxybenzyl)-4,13-diaza-18-crown-6, 93000-69-2; 2-pyridinemethanamine, 3731-51-9.

Dynamics at the Active Site of N^2-Acetyl-N^1-(4-fluorobenzyl)carbazoyl-α-chymotrypsin

J. T. Gerig* and S. J. Hammond

Contribution from the Department of Chemistry, University of California, Santa Barbara, Santa Barbara, California 93106. *Received March 26, 1984*

Abstract: N^2-Acetyl-N^1-(4-fluorobenzyl)carbazoyl-α-chymotrypsin is a catalytically inactive protein which should closely resemble the acylated enzyme intermediate formed during reaction of chymotrypsin with substrates derived from phenylalanine. Fluorine and deuterium nuclear relaxation in this carbazoylated enzyme and a specifically deuterated analogue have been examined. Analysis of the spin–lattice relaxation data (observed at several radio frequencies) and $^{19}F[^1H]$ nuclear Overhauser effects indicate that rotation of the aromatic ring of the carbazoyl group in the protein is slow relative to overall protein tumbling. However, observed fluorine line widths are larger than those predicted from this analysis. Experiments are described which suggest that the excess line widths are not due to heterogeneity of the protein, and exchange of the fluoroaromatic ring between environments characterized by different fluorine chemical shifts is proposed as a possible explanation. Fluorine NMR studies indicate that the acylated enzyme is resistant to loss of tertiary structure (denaturation) near the active site when the protein is dissolved in 8 M urea.

The enzyme α-chymotrypsin cleaves amide and ester bonds by a mechanism which involves formation of an acylenzyme intermediate at the active site serine-195 residue. *N*-Acetyl-L-phenylalanine ethyl ester (**I**) is an excellent substrate for chymotrypsin and the acylenzyme that it forms is very rapidly hydrolyzed.[1] Kurtz and Niemann showed that when the methine group of **I** is substituted by a nitrogen atom, giving a carbazoic acid derivative (**II**), the resultant molecule binds at the enzyme active site but does not acylate the serine; that is, **II** is a competitive inhibitor of the enzyme.[2] Elmore and Smyth found that an aryl ester of the same carbazoic acid (**IIIa**) is sufficiently reactive to acylate chymotrypsin and that the acylenzyme which is formed does not readily undergo hydrolysis.[3] They used this reaction

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