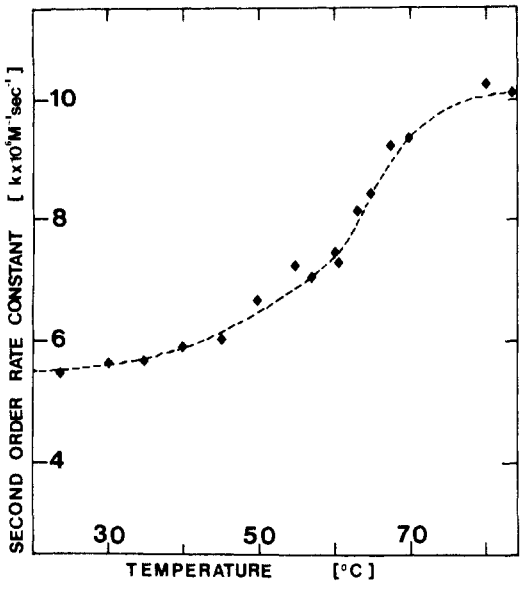

Figure 3. Temperature variation of the second-order rate constant obtained with pure $(C_{18}OCH_2)_8PcH_2$: λ_{ex} = 532 nm.

plot of the second-order rate constant k versus temperature (Figure 3) shows that k increases with increasing temperature: it is (5.5 ± 1.0) × 10^6 M^{-1} s^{-1} at 25 °C and (10.0 ± 1.0) × 10^6 M^{-1} s^{-1} at 84 °C. The inflection point at 62 °C corresponds to the crystal → liquid crystal transition. Thus triplet-triplet annihilation is twice as efficient in the liquid crystal than in the crystal.

The minimum number of absorbed mole-photons necessary to obtain second-order decay kinetics has been determined from laser energy variation experiments. Assuming a triplet yield of 0.14,[8,11] the minimum triplet molar fraction needed is 0.0070 at 25 °C and 0.0067 at 77 °C. As triplet exciton migration occurs only via a short range (<15 Å) interaction[12a] and because in the examined phases the intercolumnar distance is 36 Å,[13] such an interaction is possible only for the molecules belonging to the same column. The average distance between the macrocyles inside the column being 4.5 Å,[14] the exciton path length can be calculated: 640 Å at 25 °C, 670 Å at 77 °C. The exciton diffusion coefficient for one-dimensional energy migration[12b] is found to be respectively 16 × 10^{-6} cm^2 s^{-1} and 10 × 10^{-6} cm^2 s^{-1} at 25 °C and 77 °C. Like the second-order decay rate constant, the diffusion coefficient is higher in the liquid crystalline phase than in the crystalline one by a factor 1.6, consistent with a more efficient exciton migration in the liquid crystal than in the crystal.

The exciton path length, as expected, is higher than the one determined for singlet excitons (100–200 Å) in similar systems,[5] but it remains low compared to the triplet exciton path lengths determined for organic single crystals in which typical values are of the order of a few microns.[12a] It is tempting to assign the shortening of the triplet exciton path length to an exciton trapping when the one-dimensional way is interrupted, i.e., when the continuity of the column breaks down. Therefore the path length determined in the columnar phases should be correlated with the column length: the columns would be made of 140–150 phthalocyanine molecules. The column length determined by this method is expected to be higher than the coherence length given by X-ray diffraction,[15] because distortions of the column axis are allowed as far as the one-dimensional migration is preserved.

These experimental results have been treated on the basis of mean values. A statistical treatment taking into account the distribution of the various parameters is in progress.

Registry No. 1, 112839-59-5.

Supplementary Material Available: Sample and laser pulse characteristics, boundary conditions for a second-order decay kinetics, and determination of the exciton diffusion coefficient (A) (1 page). Ordering information is given on any current masthead page.

(11) This value is an upper limit for the $(C_{18}OCH_2)_8PcH_2$ columnar phases because they are nonoutgassed.
(12) Birks, J. B. *Photophysics of the Aromatic Molecules*; Wiley: New York, 1971, (a) p 544 and (b) p 532.
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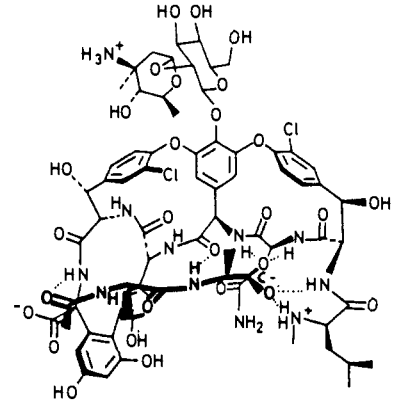
Carboxylic Acid Complexation by a Synthetic Analogue of the "Carboxylate-Binding Pocket" of Vancomycin

Nalin Pant and Andrew D. Hamilton*

Department of Chemistry, Princeton University
Princeton, New Jersey 08544
Received November 3, 1987

Vancomycin[1] is a clinically important antibiotic that disrupts bacterial cell wall biosynthesis by binding to the terminal D-Ala-D-Ala sequence of one of the peptidoglycan precursors.[2] The active complex **1** involves six hydrogen bonds between vancomycin



1

and the dipeptide substrate[3] (bold lines in **1**). In addition nonbonded interactions between the alanine methyl groups and hydrophobic regions of the antibiotic may account for its strong substrate- and stereospecificity.[1b,3,4] Five of the six hydrogen bonds in **1** are found near the right-hand ring of the antibiotic, and they form a binding pocket for the carboxylate region of the terminal D-alanine.[3]

In an effort[5] to determine the minimal functional unit of vancomycin we have synthesized an analogue **2** of the right hand ring and shown that it binds to carboxylic acids by a similar mechanism to the antibiotic. Two key structural features of this model[5] are an N-terminal amino group[3] and a bulky substituent on the central amino acid,[6] both of which are thought to play important roles in binding.[3]

Receptor **2** was prepared in six steps from amino acid starting materials.[7] Protection of 3,5-dinitro-L-tyrosine as its *tert*-but-

* Address correspondence to this author at Department of Chemistry, University of Pittsburgh, Pittsburgh, PA 15260.

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(2) Perkins, H. R. *Pharmac. Ther.* **1982**, *16*, 181.
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(6) This substituent may be important in generating the bound conformation of the macrocycle: Williams, D. H., personal communication.
(7) The present paper describes a model (**2**) based on D-leucine and L-tyrosine that has an essentially enantiomeric relationship to the right hand ring of vancomycin (L-asparagine, D-tyrosine).

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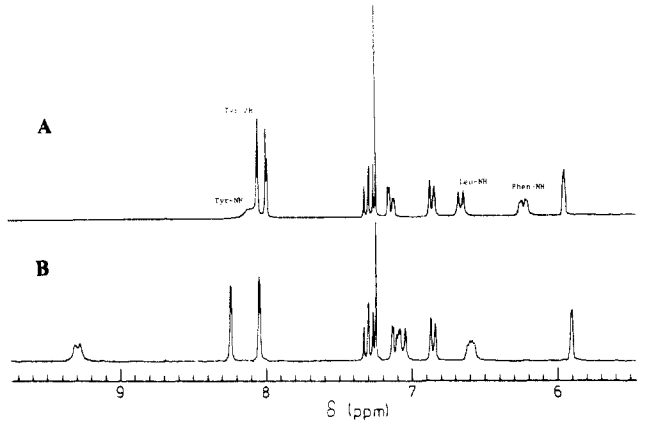
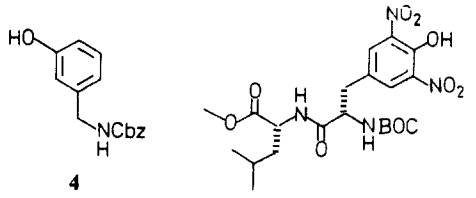


Figure 1. Downfield region of ^{1}H NMR of A: **2** and B: a 1:1 mixture of **2** and cyanoacetic acid. In $CDCl_3$ at 25 °C.

oxycarbonyl derivative followed by reaction with D-leucine methyl ester hydrochloride (CH_2Cl_2, Et_3N, BOP[8]) gave dipeptide **3** in 68% yield. Tosylation of **3** (tosyl chloride, pyridine) and in situ



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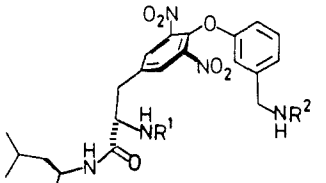
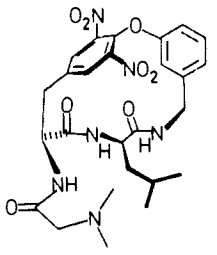


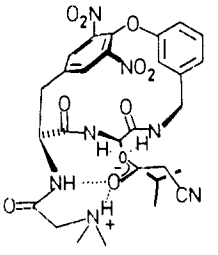
5; R^1 = BOC, R^2 = Cbz, R^3 = Me
6; R^1 = H, R^2 = Cbz, R^3 = Me
7; R^1 = $COCH_2N(CH_3)_2$, R^2 = Cbz, R^3 = Me
8; 2HCl.R^1 = $COCH_2N(CH_3)_2$, R^2 = H, R^3 = H

reaction with phenol **4**[5] (pyridine, 90 °C) gave diphenyl ether **5** in 24% yield. Mild acid deprotection (10% TFA–CH_2Cl_2, 0 °C, 4 h) of **5** gave amine **6** which was reacted with dimethylglycine acid chloride to form tripeptide **7** in 59% yield. The final steps involved acid deprotection (HCl–TFA, 1:2) of **7** to amino acid **8** followed by cyclization (BOP,[8] CH_2Cl_2, 5 days) to provide, after silica gel chromatography (CH_2Cl_2:MeOH eluent), macrocycle **2**[9] in 10% overall yield from **7**.

The ^{1}H NMR of **2** showed an upfield shifted singlet at 5.95 due to the benzylamine-2 proton[5] and a pair of doublets at 8.05 and 7.99 ppm from the tyrosine-2,6 protons reflecting restricted rotation of this ring in the cyclic peptide (Figure 1A). Addition of 1 equiv of cyanoacetic acid (pK_a = 2.44)[10] to a $CDCl_3$ solution of **2** resulted in marked changes in its ^{1}H NMR spectrum (Figure



2



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1B).[11] These changes are consistent with an association which involves proton transfer from acid to amine and complexation of the carboxylate anion by multiple hydrogen bonding to amide groups on the receptor (as shown in **9**). Most notably, downfield shifts were seen in the resonances of the CH_3 and CH_2 groups adjacent to the amine (0.35 and 0.66 ppm, respectively) and the amide-NHs of the benzylamine (0.38 ppm), leucine (0.22 ppm), and tyrosine (1.16 ppm) groups. In addition, the resonance at 8.05 (due to the Tyr-aromatic proton inside the binding pocket) shifted downfield substantially (0.18 ppm). Similar spectral changes are seen with trifluoroacetic acid (pK_a = 0.23)[10] and difluoroacetic acid (pK_a = 1.24);[10] however, acetic acid (pK_a = 4.75)[10] caused little change. The existence of a pK_a threshold (≈3–4) strongly suggests that proton transfer to form the carboxylate–ammonium ion pair is a key step in the recognition of carboxylic acids by **2**. However, the NMR changes are not simply due to protonation effects. Titration of a $CDCl_3$ solution of **2** with picric acid (pK_a = 0.29)[10] caused only those shifts expected from protonation of the free amine[12] with minor changes in the rest of the spectrum.

The formation of a complex of structure **9** is further supported by the change in multiplicity of the cyanoacetic acid CH_2 resonance from a singlet to two doublets (AB system) on complexation.[13] This is due to the previously enantiotopic CH_2 becoming diastereotopic (δ_A 3.25, δ_B 3.16, J_{AB} = 18 Hz) upon association with the chiral binding site formed by **2**.[14] In addition, an *inter*molecular NOE is observed between the methylene protons of the substrate and those protons on the inside of the peptide cavity (all three amide-NHs, one tyrosine-aromatic proton). Significantly, only the tyrosine resonance at 8.23 is enhanced in the NOE experiment confirming its position directly above the bound substrate. A plot of chemical shift changes against $NCCH_2CO_2H$ concentration showed clear saturation behavior, and from this an association constant of 580 M^{-1} was estimated.[15]

We are presently incorporating elements of the left-hand side of vancomycin into **2** to increase the number of receptor–substrate interactions and to allow the stereospecific complexation of peptide carboxylates.

Acknowledgment. We thank the National Institutes of Health (GM 35208) for financial support of this work and M. Baum (Princeton) and D. H. Williams (Cambridge) for helpful discussions.

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(9) **2**: ^{1}H NMR ($CDCl_3$) 8.15 (1 H, br, Tyr-NH), 8.04 (1 H, d, J = 2 Hz, Tyr-2H), 7.99 (1 H, d, J = 2 Hz, Tyr-6H), 7.28 (1 H, t, J = 8 Hz, phen-5H), 7.13 (1 H, dd, J = 8, 2 Hz, phen-4H), 6.85 (1 H, d, J = 7.5 Hz, phen-6H), 6.65 (1 H, d, J = 9 Hz, Leu-NH), 6.25 (1 H, m, phen-NH), 5.94 (1 H, s, phen-2H), 5.08 (1 H, m, Tyr-CH), 5.00 (1 H, dd, J = 16, 10 Hz, phen-CH_2), 4.35 (1 H, m, Leu-C*H*NH), 3.70 (2 H, m, phen-CH_2, Tyr-CH_2), 3.13, 3.04 (2 H, 2d, J = 17 Hz, NCH_2), 2.85 (1 H, dd, J = 14, 4 Hz, Tyr-CH_2), 2.41 (6 H, s, NCH_3), 1.55 (1 H, m, Leu-C*H*CH_3), 1.44 (2 H, m, Leu-C*H*$_2$CH), 0.94 (3 H, d, J = 6 Hz, Leu-CH_3), 0.90 (3 H, d, J = 6 Hz, Leu-CH_3); MS, M$^+$ 556.2274 $C_{26}H_{32}N_6O_8$ requires 556.2281.

(10) *Dissociation Constants of Organic Acids and Bases*; Kortüm, G., Vogel, W., Andrussow, K., Eds.; IUPAC-Butterworths: London, 1961.

(11) **2**: $NCCH_2CO_2H$ (1:1), ^{1}H NMR ($CDCl_3$) 9.29 (1 H, d, J = 7.8 Hz, Tyr-NH), 8.23 (1 H, d, J = 2 Hz, Tyr-2H), 8.03 (1 H, d, J = 2 Hz, Tyr-6H), 7.29 (1 H, t, J = 8 Hz, phen-5H), 7.11 (1 H, dd, J = 8, 3 Hz, phen-4H), 7.06 (1 H, d, J = 9 Hz, Leu-NH), 6.85 (1 H, d, J = 7.5 Hz, phen-6H), 6.59 (1 H, d, J = 4 Hz, phen-NH), 5.90 (1 H, s, phen-2H), 5.09 (1 H, m, Tyr-CH), 4.81 (1 H, dd, J = 16, 8 Hz, phen-CH_2), 4.43 (1 H, m, Leu-C*H*NH) 3.91 (1 H, dd, J = 16, 4 Hz, phen-CH_2), 3.80, 3.70 (2 H, 2d, J = 10 Hz, NCH_2), 3.64 (1 H, dd, J = 14, 5 Hz, Tyr-CH_2), 3.24 (2 H, s, $NCCH_2CO_2$), 2.96 (1 H, dd, J = 14, 3 Hz, Tyr-CH), 2.76 (6 H, s, NCH_3), 1.52 (1 H, m, Leu-C*H*CH_3), 1.46 (2 H, m, Leu-C*H*$_2$CH), 0.89 (6 H, m, Leu-CH_3).

(12) Large downfield shifts of $(CH_3)_2NCH_2$ resonances and small downfield shift (0.2 ppm) of tyrosine amide NH.

(13) Due to fast exchange conditions, this effect is seen when [substrate]/[**2**] is less than 0.5.

(14) For an example of this effect with chiral crown ethers and alkylammonium substrates, see: Laidler, D. A.; Stoddart, J. F. *Tetrahedron Lett.* **1979**, 453.

(15) Using nonlinear least-squares analysis. When the acid is present in large excess there is evidence (particularly in the Tyr-NH resonance) of higher order associations.