Novel Inhibitors of Prolyl 4-Hydroxylase. 5.[1] The Intriguing Structure–Activity Relationships Seen with 2,2′-Bipyridine and its 5,5′-Dicarboxylic Acid Derivatives

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Received January 11, 1993•

Members of a series of 2,2′-bipyridines have been synthesized and tested as inhibitors of prolyl hydroxylase (EC 1.14.11.2). The structure–activity relationships seen with [2,2′-bipyridine]-5-carboxylic acid (4) closely resemble those of pyridine-2-carboxylic acid (2). Accordingly, [2,2′-bipyridine]-5,5′-dicarboxylic acid (11, IC_{50} = 0.19 μM) is the most potent inhibitor of its type yet reported. However, 2,2′-bipyridines lacking a 5-carboxylate are poor inhibitors. These contrasting structure–activity relationships are discussed in terms of net anionic charge, iron chelation, and the availability of alternative putative binding modes at a single binding site in each catalytic subunit.

Introduction

Background. We have already described[2] in detail our interest in the inhibition of prolyl hydroxylase (EC 1.14.11.2) [procollagen-L-proline, 2-oxo-glutarate:oxygen oxidoreductase (4-hydroxylating)]:[3] it is of therapeutic interest because the enzyme is unavoidably involved[4] in the biosynthesis of the collagen deposited during life-threatening fibroses. During these fibrotic states the involved organ or tissue becomes unbearably congested with inappropriately large amounts of collagen.

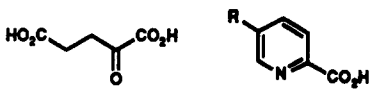
Inhibition of prolyl hydroxylase should hinder the undesirable accumulation of newly synthesized collagen in fibrotic tissue by diverting collagen biosynthesis into a degradative pathway. However, inhibition of prolyl hydroxylase might also produce toxic consequences by interfering both with normal collagen turnover and with the biosynthesis of other vital collagenous molecules. Therefore, clinical evaluation of the net benefit of a prolyl hydroxylase inhibitor in the treatment of life-threatening fibrotic conditions would be very desirable.[5,6] However, no agents are available[7] that would be suitable for such an investigation.

Enzyme Mechanism. It seems probable that the hydroxylation is carried out by an iron(IV) oxo species and that this species is generated by oxidative decarboxylation of 2-ketoglutarate (1) in the coordination shell of enzyme-bound iron(II). The exact molecular details of the enzyme reaction are not known with certainty, but the active-site chemistry proposed by Hanauske-Abel and Guenzler[8] and summarized in Scheme I provides a sound basis for work in this area.

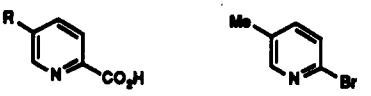
This mechanistic hypothesis emphasises the availability of binding sites for 2-ketoglutarate (1) in the region of the iron(II) held at the catalytic site of the enzyme. The iron(II) is assumed to form a 5-membered chelate with the planar, anionic, bidentate keto carboxylate ligand while a lipophilic spacer presents the distal carboxylate to an unspecified anion binding site on the enzyme.

Enzyme Inhibition. We have previously reported[1] the inhibitory activity against prolyl hydroxylase of a diverse series of 2-substituted pyridines, which are structurally similar to the known[9] weak inhibitor pyridine-2-carboxylic acid (2). One of this series is 2,2′-bipyridine (2,2′-bipyridyl, 3), which was already known[10,11] to be an inhibitor of prolyl hydroxylase and which has long been used[12] to prevent the hydroxylation of collagenous proteins during the isolation from eggs of the natural substrate for prolyl hydroxylase.

Our analysis of the structure–activity relationships known[1,9–15] for inhibition of prolyl hydroxylase with 2-substituted pyridyl chelating agents indicated that 2,2′-bipyridine (3) (IC_{50} = 34 μM) is a good inhibitor of prolyl hydroxylase relative to pyridine-2-carboxylic acid (2) (IC_{50} = 112 μM) and that appropriate modification of the structure might lead us to far more potent inhibitors. In

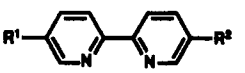


(1)



(2) R = H
(19) R = $CONHC_6H_4$-4-OEt
(20) R = $CONHSO_2Ph$
(22) R = CO_2H

(10)



No.	R^1	R^2
3	H	H
4	CO_2H	H
5	$CONHC_6H_4$-4-OEt	H
6	$CONHSO_2Ph$	H
7	Me	H
8	COCl	H
9	Me	Me
11	CO_2H	CO_2H
12	CO_2Et	CO_2Et
13	$CONHC_6H_4$-4-OEt	$CONHC_6H_4$-4-OEt
14	$CONHSO_2Ph$	$CONHSO_2Ph$
15	COCl	COCl
16	$CONHC_6H_4$-4-OEt	CO_2H
17	COCl	CO_2Et
18	CO_2H	CO_2Et
21	$CONHC_6H_4$-4-OEt	CO_2Et

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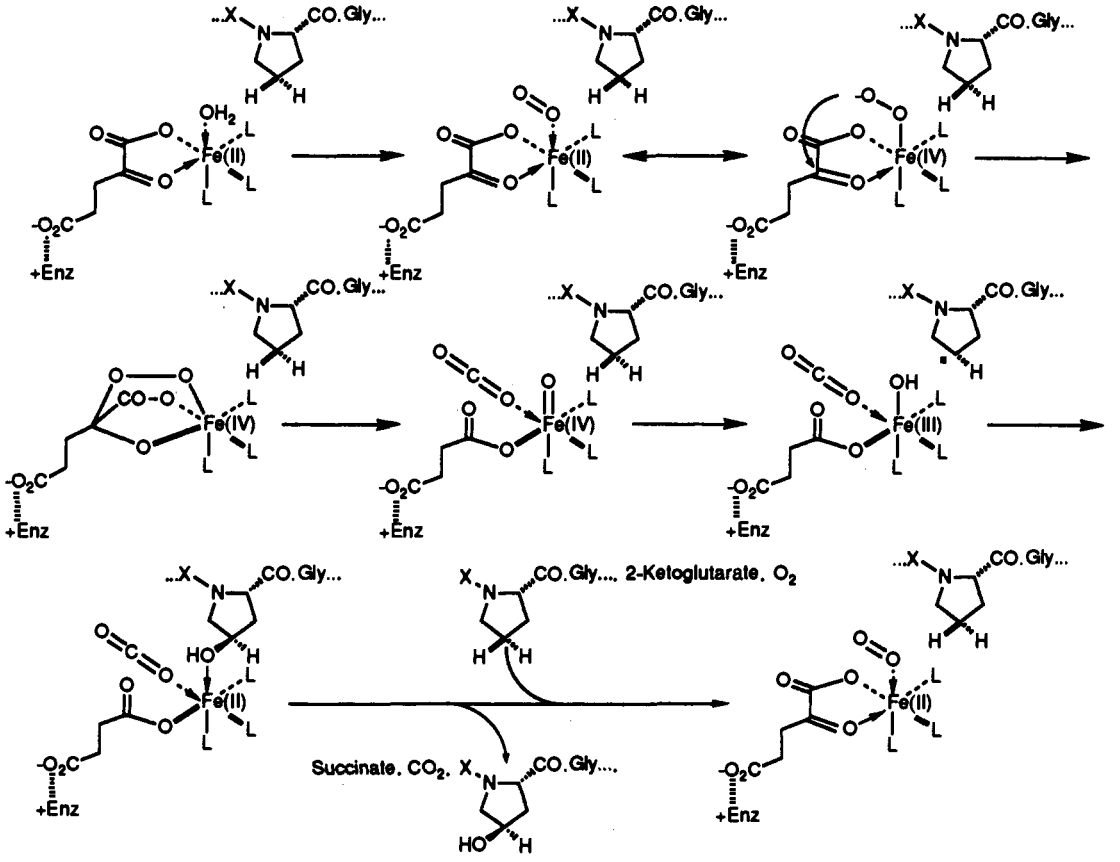
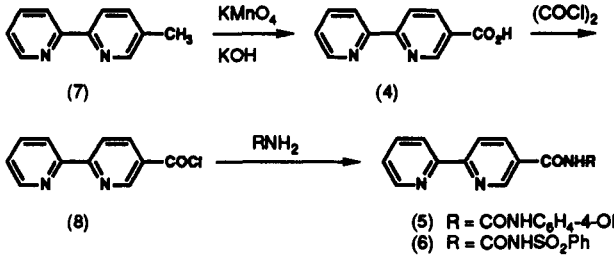
• Abstract published in *Advance ACS Abstracts*, November 1, 1993.

0022-2623/93/1836-3853$04.00/0

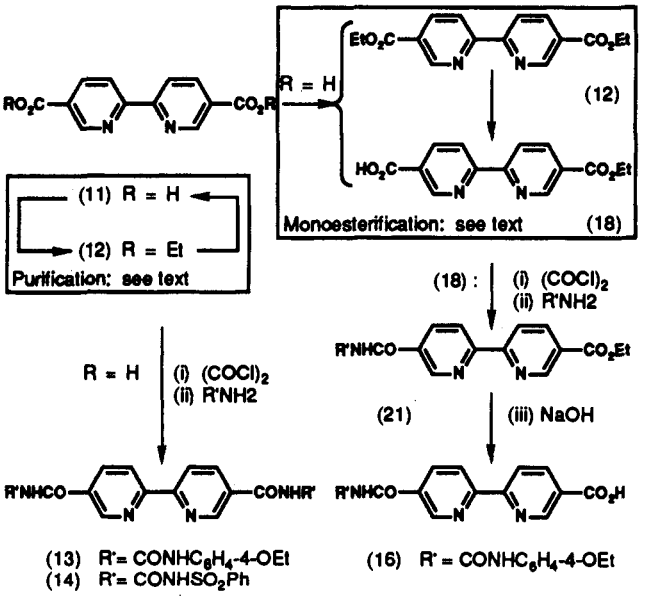
Scheme I. Catalytic Cycle of Oxidative Decarboxylation of 2-Ketoglutarate during the 4-Hydroxylation of Peptidylproline (X = Peptidyl)



Scheme II



Scheme III



particular, we were interested to determine whether [2,2′-bipyridine]-5-carboxylic acid (**4**) and the corresponding 5-anilides (e.g. **5**) and 5-acyl sulfonamides (e.g. **6**) would follow the structure–activity relationship determined for pyridine-2-carboxylic acid (**2**) and be more potent inhibitors than any that were then known. It was also of interest to investigate whether the symmetrical 5,5′-analogues would exhibit the increase in potency that would be expected on purely statistical grounds or whether the effects of symmetrical substitution would betray the asymmetry of the binding site.

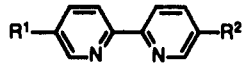
We now describe some unexpected results observed in this series of 5-mono- and 5,5′-disubstituted 2,2′-bipyridine derivatives that contains potent novel inhibitors of prolyl hydroxylase and that was discovered at Zeneca Pharmaceuticals (formerly ICI Pharmaceuticals) during our search for clinically effective inhibitors of excessive collagen deposition.

Chemistry

The preparation of 5-monosubstituted 2,2′-bipyridine derivatives is summarized in Scheme II: 5-methyl[2,2′-bipyridine] (**7**)[16] was oxidized with alkaline potassium permanganate to the 5-carboxylic acid **4**;[17,18] reaction of the corresponding acid chloride **8** with the appropriate amine gave the target amides **5** and **6**.

Symmetrical 5,5′-disubstituted 2,2′-bipyridine derivatives were prepared by an analogous route (Scheme III). 5,5′-Dimethyl[2,2′-bipyridine] (**9**), made from 2-bromo-5-methylpyridine (**10**) using the *in situ* coupling method of Tiecco and co-workers,[19] was oxidized to [2,2′-bipyridine]-5,5′-dicarboxylic acid (**11**), which was purified as its diethyl ester **12**.[20,21] The bis-*N*-(4-ethoxyphenyl)- and bis-*N*-(phenylsulfonyl)amides **13** and **14** were then each prepared from the corresponding bis(acid chloride) **15**.

Table I. Synthetic, Analytical, and Biochemical Data for 2,2′-Bipyridine Inhibitors of Prolyl Hydroxylase



no.	R^1	R^2	adduct	yield, %	method	anal.	mp, °C	solvent[a]	IC_{50}, μM[b]	*n*
2	pyridine-2-carboxylic acid				–[c]				111.5	3
3	H	H			–[c]				34.2	2
4	H	$CO_2^-K^+$	$1.00H_2O$	21	A.(i)	C,H,N[d]	254–6 dec	A	13.2	3
5	H	$CONHC_6H_4$-4-OEt		45	A.(ii)	C,H,N	211–2	B	(13%)[e]	1
6	H	$CONHSO_2Ph$		29	A.(ii)	C,H,N	244–6	C	33.8	2
11	CO_2H	CO_2H		34[f]	A.(i), B.(i).(a), C	C,H,N	>300[g]	D	0.185	2
13	$CONHC_6H_4$-4-OEt	$CONHC_6H_4$-4-OEt	$0.25H_2O$	14	A.(ii)	C,H,N	>300	E	(13%)[h]	1
14	$CONHSO_2Ph$	$CONHSO_2Ph$		42	A.(ii)	C,H,N,S	>300	F	5.04	2
16	$CONHC_6H_4$-4-OEt	CO_2H	$0.25H_2O$	14	B.(iii)	C,H,N	>300	F	0.32	2
18	CO_2H	CO_2Et		62[i]	B.(i)	C,H,N[j]	267–8	G		
21	$CONHC_6H_4$-4-OEt	CO_2Et	$0.25H_2O$		B.(i)–(ii)	C,H,N[k]	220–3	H		
22	pyridine-2,5-dicarboxylic acid				–[c]				5.18	4

[a] A = HOAc; B = precipitated from reaction mixture with dichloromethane (DCM) and washed with DCM; C = methanol; D = precipitated from basic solution with HOAc–concentrated HCl–H_2O (2:1:1); E = DMSO; F = DMF/HOAc; G = ethanol; H = DCM. [b] Geometric mean of *n* determinations. [c] Aldrich Chemical Co. [d] C, 51.3; H, 4.2; N, 10.6; H_2O, 7.5. $C_{11}H_7N_2KO_2 \cdot H_2O$ requires C, 51.5; H, 3.5; N, 10.9; H_2O, 7.0. [e] Percent inhibition at 50 μg/mL (ca. 150 μM). [f] Overall yield from 5,5′-dimethyl-2,2′-bipyridyl (ref 20 and references therein) for oxidation (88%), esterification (68%), and saponification (56%). [g] Literature[21] mp >360 °C. [h] Percent inhibition at 100 μg/mL (ca. 200 μM). [i] Overall from diacid 11; 90% from diester 12. [j] C, 61.9; H, 4.4; N, 9.8. $C_{14}H_{12}N_2O_4$ requires C, 61.8; H, 4.4; N, 10.3. [k] C, 66.7; H, 5.5; N, 11.4. $C_{22}H_{21}N_3O_4 \cdot 0.25H_2O$ requires C, 66.7; H, 5.5; N, 10.6.

The key intermediate in the preparation of the unsymmetrical monoacid monoanilide **16** was the mono(ethyl ester) mono(acid chloride) **17**. The corresponding monoester monoacid **18** was available by partial esterification of [2,2′-bipyridine]-5,5′-dicarboxylic acid (**11**). The diester **12** that was also formed in this process could be easily separated and partially saponified to complete the conversion into the monoacid monoester **18**. The monoacid monoanilide **16** was then prepared by acylation of 4-ethoxyaniline with the monoester mono(acid chloride) **17** followed by saponification of the ester group (Scheme III).

Results

The potencies of prolyl hydroxylase inhibitors in this series are listed in Table I. Disappointingly, although [2,2′-bipyridine]-5-carboxylic acid (**4**) was a significantly more potent inhibitor of prolyl hydroxylase than was 2,2′-bipyridine (**3**) (IC_{50}s of 13 and 34 μM, respectively), it was better by only half an order of magnitude or less. More surprising was the effect of the amides **5** and **6**: the 5-acyl sulfonamide **6** was only about as active as 2,2′-bipyridine (**3**), and the 5-anilide **5** was not demonstrably active.

In these respects this series appears different from the pyridine-2-carboxylates, where introduction of the 5-carboxylate improved potency by over an order of magnitude,[9] conversion of the 5-carboxylate into an anilide (as in e.g. **19**) maintained potency,[15] and its conversion into an acyl sulfonamide (as in e.g. **20**) improved potency yet again[14] (IC_{50}s = 112, 5.2, 5.0, and 1.1 μM, respectively).

In sharp contrast to the results observed with the monosubstituted derivatives, [2,2′-bipyridine]-5,5′-dicarboxlic acid (**11**) was a potent inhibitor of prolyl hydroxylase (IC_{50} = 0.19 μM). Although this compound benefits from the addition of a 5-carboxylic acid to [2,2′-bipyridine]-5-carboxylic acid (**4**), the anticipated 10-fold improvement[9] was far exceeded in this symmetrically disubstituted derivative. Once again the equivalence of anilides and carboxylic acids already observed at the 5-position of pyridine-2-carboxylic acids[15] was not apparent in the symmetrical amide analogues **13** and **14** of the diacid **11**. However, there is some internal consistency since the rank order of potency already seen in the monosubstituted derivatives is still apparent: the bis(acyl sulfonamide) **14** was about 25-fold less potent than the bis(carboxylic acid), and the bisanilide **13**, like the 5-anilide **5**, was not demonstrably active.

Even more remarkable was the activity of the unsymmetrical mono(carboxylic acid) monoanilide **16**: with an IC_{50} = 0.32 μM, this bipyridine was close to the most potent member of this series and apparently shows a fine disregard for any linear dependence of activity on structural factors! Only with this compound was the anticipated[9,14,15] dependence of activity on structure seen.

Discussion

Many factors may need to be invoked to explain the activity of this series of inhibitors of prolyl hydroxylase. The differences in the net negative charge of some of the different inhibitors, the role of both kinetic and thermodynamic aspects of iron chelation,[11] and the availability of alternative putative binding modes for the inhibitor at the enzyme active site must all be taken into account. Even so, it is not easy to reconcile the structure–activity relationships of this 2,2′-bipyridine series with a model of competitive inhibition at a single binding site.

The view that is most consistent with the existing model[8,9,15] is that it is [2,2′-bipyridine]-5-carboxylic acid (**4**) that is the "parent" of this series: the pronounced and similar enhancements to potency produced by either of the additional 5′-carboxy- and 5′-[(4-ethoxyphenyl)amino]-carbonyl groups then have parallels in the pyridine-2-carboxylic acid series. Previous results have indicated that there is a binding site on the enzyme at which an anilide moiety such as [(4-ethoxyphenyl)amino]carbonyl is as acceptable as a carboxylate group and that this site probably has H-bond-donating character.[15] The behavior of the 5′-substituents of [2,2′-bipyridine]-5-carboxylic acid is thus readily understood. However, it is clear that there is also a site at which acyl sulfonamide and anilide are not acceptable as alternatives to carboxy, an observation also previously seen with the pyridine-2-carboxylic acid series.[1]

In this interpretation the activity of 2,2′-bipyridine (**3**) is the greatest anomaly. However, under the conditions of the assay (20 μM ferrous iron), inhibitors that are only effective at concentrations of ca. 10–60 μM show no real preference for binding enzyme-bound ferrous iron rather than free ferrous iron. 2,2′-Bipyridine (**3**) (IC_{50} = 34 μM) clearly falls squarely into this class of inhibitor whereas

[2,2′-bipyridine]-5,5′-dicarboxylic acid (11) (IC_{50} = 0.19 μM) is clearly a much more potent inhibitor of prolyl 4-hydroxylase that must be forming interactions with the enzyme that it is not able to form with ferrous iron in solution. Indeed, there is not enough of the more potent inhibitors 11 and 16 present to complex even a small fraction of the iron present in solution in the assay. [2,2′-Bipyridine]-5-carboxylic acid (4) is an inhibitor of intermediate potency (IC_{50} = 13 μM) that may form significantly strong interactions with the enzyme but in which the incremental effect of the added 5-carboxylate is still somewhat masked by the overwhelming effect of chelation to an excess of free ferrous iron.

It is interesting to speculate how the enzyme manages to accomodate inhibitors with such a wide range of size, shape, and charge. The current view[8] is that there are three potential ligand sites available for enzyme substrates and inhibitors (Scheme I), giving rise to six distinguishable binding modes for unsymmetrical bidentate ligands—more if the variable nature of the ligand at the remaining site is taken into account—and three binding modes for symmetrical bidentate ligands. In reality there may be very significant differences in the interactions made by different "competitive" inhibitors. Unfortunately, the kinetic and thermodynamic complexities posed by the strong ferrous iron ligands in the presence of the ferrous iron added to the enzyme assay would make it difficult to tease out meaningful K_i values. We have not even attempted to show that the inhibition of prolyl 4-hydroxylase by compounds of this series is strictly competitive with 2-ketoglutarate and so the interpretation of the different interactions made by the different inhibitors discovered in this work must remain speculative.

One interesting feature of this series is that the two examples of potent (avian) prolyl hydroxylase inhibitors that it contains are not inhibitors of collagen production by cultured chick embryo fibroblasts.[22] Since there is no interspecies difference, the problem must be that the local free concentration of inhibitor in the enzyme compartment—the cisternae of the rough endoplasmic reticulum[4]—cannot be maintained at high enough levels for even these compounds to be effective. All reversible and clinically effective agents have to maintain adequate local drug activities at the target tissue: even though their effects as prolyl hydroxylase inhibitors cannot be evaluated *in vivo* the results with these potent members of this series of 2,2′-bipyridines highlights the importance of ensuring satisfactory drug pharmacokinetics. Until these problems can be overcome, the members of the 2,2′-bipyridine series will remain interesting biochemical tools rather than drug candidates.

Conclusions

The results of this study clearly show that structure–activity relationships of prolyl hydroxylase inhibitors may not always be directly transferred from one series to another even when they are both based on pyridines. As already described for derivatives of pyridine-2,5-dicarboxylic acid,[14,15] there appear to be two separate carboxylate binding sites: one selective for anionic groups and the other able to accommodate anilides as well as anions. Using these interactions we have been able to develop novel inhibitors of prolyl 4-hydroxylase such as [2,2′-bipyridine]-5,5′-dicarboxylic acid (11) (IC_{50} = 0.19 μM) that are far more potent that previously known members of this series. However, the lack of linearity seen in the structure–activity relationships strongly suggests that the possibility of alternative binding modes at the same binding site should be considered whenever attempting to design new and more potent inhibitors of prolyl 4-hydroxylase.

Experimental Section

The following general procedures were followed unless otherwise stated: Melting points were determined on a Büchi melting point apparatus and are uncorrected. ^{1}H-NMR spectra were obtained on a Bruker AC250, Bruker AM200, Varian EM390, or JEOL FX90Q instrument using dilute solutions in [$^2H_6$]DMSO (DMSO-d_6), [^{2}H]$CHCl_3$ ($CDCl_3$), or [$^2H_5$]pyridine (C_5D_5N) with tetramethylsilane as an internal standard. ^{1}H-NMR spectra were obtained for all isolated intermediates as well as for final products and were always consistent with the structural assignments. Column chromatography was carried out by gravity filtration using Merck Art. 7734 Kieselgel 60. Extracts in water-immiscible solvents were dried using anhydrous magnesium sulfate, and organic solvents were evaporated under reduced pressure using a Büchi R110 rotary evaporator. Elemental analyses were carried out under the direction of Mr. B. Crooks in the analytical section of Zeneca Pharmaceuticals at Alderley Park. Petroleum ether refers to the fraction boiling between 60–80 °C; water refers to distilled water. 5-Methyl[2,2′-bipyridine] (7)[16] and [2,2′-bipyridine]-5,5′-dicarboxylic acid (11)[21] were made by standard procedures. 2-Bromo-5-methylpyridine (10) was purchased from Aldrich Chemical Co.

Biological Testing. The inhibitory potency of the compounds against prolyl 4-hydroxylase was determined using the indirect assay described by Cunliffe, Franklin, and Gaskell,[23] in which the conversion of the labeled cofactor 2-ketoglutarate into labeled succinate is measured. In experiments with inhibitors the preequilibrated enzyme reaction mixture was added to a solution of the inhibitor before the reaction is started by addition of 2-ketoglutarate. All points were determined in duplicate, and IC_{50} values were obtained from six-point dose–response curves by interpolation. The logarithmic standard deviation estimated from the eight sets of replicate data (N = 20, ϕ = 12) was σ = 0.14. Accordingly, results for compound$_i$ and compound$_j$ of group sizes i and j, respectively, are significantly different ($p < 0.05$) if their larger ratio exceeds 2.02 (i = 2, j = 2), 1.90 (2, 3), or 1.84 (2, 4) [$t_{(95\%,\phi=12)}$ = 2.18].

A. *N*-(4-Ethoxyphenyl)[2,2′-bipyridine]-5-carboxamide (5). (i) [2,2′-Bipyridine]-5-carboxylic Acid (4).[17] A mixture of 5-methyl[2,2′-bipyridine] (7)[16] (1.0 g, 5.9 mmol) and a solution of potassium hydroxide (0.35 g, 6.3 mmol) in water (50 mL) was heated under reflux and treated with potassium permanganate (finely ground; 0.9 g, 5.7 mmol). When the purple color had faded a second portion of potassium permanganate (0.9 g, 5.7 mmol) was added. The mixture was heated under reflux until the purple color had again faded. The mixture was filtered, the filter cake was washed with hot water, and the combined filtrate and washings were acidified with acetic acid–concentrated hydrochloric acid–water (20:10:10) and then evaporated to dryness. The residual solid was recrystallized from acetic acid and washed with ether to give the potassium salt of [2,2′-bipyridine]-5-carboxylic acid (4): 0.31 g, 21%; mp 254–256 °C dec; ^{1}H NMR (DMSO-d_6) δ 7.60 (ddd, J = 7.5 Hz, J = 5 Hz, J = 1.5 Hz, 1 H), 8.10 (dt, J = 2.5 Hz, J = 7.5 Hz, 1 H), 8.35–8.65 (m, 3 H), 8.77 (br d, J = 5 Hz, 1 H), 9.18 (br s, 1 H). Anal. ($C_{11}H_9KN_2O_3 \cdot H_2O$) C, H, N.

(ii) *N*-(4-Ethoxyphenyl)[2,2′-bipyridine]-5-carboxamide (5). (a) Acid Chloride 8. A suspension of the potassium salt of [2,2′-bipyridine]-5-carboxylic acid monohydrate (4) (0.125 g, 0.49 mmol) in thionyl chloride (10 mL) was heated under reflux for 45 min and then evaporated to dryness. The residue was suspended in dry toluene and reevaporated twice, dissolved in dichloromethane (50 mL), and filtered under argon through a plug of cotton-wool to give a solution of the acid chloride 8.

(b) Anilide 5. The solution of the acid chloride 8 was stirred and treated dropwise with a solution of 4-ethoxyaniline (0.10 mL, 1.07 g, 0.78 mmol) and triethylamine (0.25 mL, 0.18 g, 1.8

mmol) in dichloromethane (5 mL). The mixture was left overnight and the precipitated product was isolated by filtration and washed with dichloromethane to give *N*-(4-ethoxyphenyl)-[2,2′-bipyridine]-5-carboxamide (**5**): 0.089 g, 57%; mp 211–212 °C; ^{1}H NMR (DMSO-d_6) δ 1.37 (t, J = 8 Hz, 3 H), 4.05 (q, J = 8 Hz, 2 H), 6.95 (d, J = 8 Hz, 2 H), 7.4–7.65 (m, 1 H), 7.72 (d, J = 8 Hz, 2 H), 8.01 (t, J = 7.5 Hz, 1 H), 8.35–8.65 (m, 3 H), 8.75 (d, J = 5 Hz, 1 H), 9.03 (s, 1 H), 10.35 (br s, 1 H). Anal. ($C_{19}H_{17}N_3O_2$) C, H, N.

B. 5′-[[(4-Ethoxyphenyl)amino]carbonyl][2,2′-bipyridine]-5-carboxylic Acid (16). (i) 5′-(Ethoxycarbonyl)[2,2′-bipyridine]-5-carboxylic Acid (18). (a) Partial Esterification. A mixture of [2,2′-bipyridine]-5,5′-dicarboxylic acid (11)[21] (10.78 g, 44.2 mmol), ethanol (absolute; 250 mL), and concentrated sulfuric acid (27 mL) was heated under reflux until only traces of the diacid remained undissolved. The mixture was filtered to remove unreacted diacid 11, poured into iced water (1 L), and refiltered. The precipitate (P) was partitioned between ethyl acetate and saturated aqueous sodium bicarbonate (A). The organic phase (O) was washed with saturated aqueous sodium bicarbonate solution (A), water (A), and brine (A). The four aqueous phases A were all combined, filtered, and acidifed with concentrated HCl. The precipitate was isolated by filtration to give the first fraction of the monoester 18, 0.79 g, 6.6%. The organic phase O was dried and evaporated, and the residue was recrystallized from ethanol to give diethyl [2,2′-bipyridine]-5,5′-dicarboxylate (12): 8.15 g, 61%; mp 146–147 °C (lit. mp 148–149 °C).[24]

(b) Partial Saponification. A stirred solution of diester 12 (3.5 g, 11.7 mmol) in dichloromethane (100 mL) was treated dropwise at room temperature under argon with a solution of potassium hydroxide (0.65 g, 11.6 mmol) in ethanol (20 mL). A white precipitate formed, and the mixture was diluted with dichloromethane (50 mL) to facilitate stirring, which was continued overnight. The mixture was evaporated to dryness, and the residue was processed as was precipitate P (above) to yield monoester 18, 2.41 g, and diester 12, 0.66 g, which was resaponified in dichloromethane (40 mL) using potassium hydroxide (0.15 g) in ethanol (5 mL) to yield a further sample of monoester 18, 0.45 g; overall yield from diester 2.86 g, 90%, overall yield from diacid 62%. A sample of monoester 18 (5.0 g) recrystallized from ethanol gave 5′-(ethoxycarbonyl)[2,2′-bipyridine]-5-carboxylic acid (18): 4.0 g, recovery 80%; mp 267–268 °C; ^{1}H NMR (DMSO-d_6) δ 1.37 (t, J = 7 Hz, 3 H), 4.39 (d, J = 7 Hz, 2 H), 8.43 (t, J = 2.5 Hz, 1 H), 8.47 (t, J = 2.5 Hz, 1 H), 8.41–8.48 (dd + dd, 2 H), 8.54–8.60 (d + d, J = 8 Hz, J = 8 Hz, 2 H), 9.19 (br s, 1 H). Anal. ($C_{14}H_{12}N_2O_4$) C, H, N.

(ii) Ethyl 5′-[[(4-Ethoxyphenyl)amino]carbonyl][2,2′-bipyridine]-5-carboxylate (21). (a) Acid Chloride 17. Prepared from 5′-(ethoxycarbonyl)[2,2′-bipyridine]-5-carboxylic acid (18) (0.2 g, 0.74 mmol) and thionyl chloride (10 mL) by method A.-(ii).(a)

(b) Anilide 21. The solution of the acid chloride 17 was stirred and treated dropwise with a solution of 4-ethoxyaniline (0.26 mL, 0.29 g, 2.02 mmol) and triethylamine (0.50 mL, 0.36 g, 3.6 mmol) in dichloromethane (5 mL). The mixture was left overnight, and the precipitated product was isolated by filtration, recrystallized from dichloromethane, and then purified by column chromatography on silica gel using 1% triethylamine in petroleum ether–ethyl acetate (50:50) to give ethyl 5′-[[(4-ethoxyphenyl)-amino]carbonyl][2,2′-bipyridine]-5-carboxylate (**21**): 0.070 g, 24%; mp 220–223 °C; ^{1}H NMR (C_5D_5N) δ 1.27 (t + t, J = 7 Hz, J = 7 Hz, 6 H), 3.95 (q, J = 7 Hz, 2 H), 4.36 (q, J = 7 Hz, 2 H), 7.07 (d, J = 8 Hz, 2 H), 8.05 (d, J = 8 Hz, 2 H), 8.45 (dd, J = 7.5 Hz, J = 2.5 Hz, 1 H), 8.65–8.80 (m + pyridine, [3 H]), 9.46 (d, J = 2.5 Hz, 1 H), 9.66 (d, J = 1.5 Hz, 1 H), 11.24 (s, 1 H). Anal. ($C_{22}H_{21}N_3O_4$·0.25H_2O) C, H, N.

(iii) 5′-[[(4-Ethoxyphenyl)amino]carbonyl][2,2′-bipyridine]-5-carboxylate (21) [prepared from monoester 18 (0.39 g, 0.10 mmol as described in B.(i) and (ii) above] and sodium hydroxide (0.1 g, 0.10 mmol) in water–DMF (ca. 10:1; 25 mL) was heated on a steam bath for 6 h. The mixture was filtered, and the retained solids were washed with water. The combined filtrate and washings were acidified with glacial acetic acid–concentrated hydrochloric acid–water (20:10:10), and the precipitated product was isolated by filtration and recrystallized from DMF–acetic acid to give 5′-[[(4-ethoxyphenyl)amino]carbonyl][2,2′-bipyridine]-5-carboxylic acid (**16**): 0.074 g, 14.2% (overall from 18); mp >300 °C; ^{1}H NMR (DMSO-d_6; 120 °C) δ 1.22 (t, J = 7 Hz, 3 H), 4.08 (q, J = 7 Hz, 2 H), 6.95 (d, J = 9 Hz, 2 H), 7.69 (d, J = 9 Hz, 2 H), 8.35–8.65 (m, 4 H), 9.23 (br s, 2 H), 10.16 (br s, 1 H). Anal. ($C_{20}H_{17}N_3O_4$·0.25H_2O) C, H, N.

C. Regeneration of [2,2′-Bipyridine]-5,5′-dicarboxylic Acid (11)[21] from Its Purified Diethyl Ester (12). A suspension of recrystallized diethyl [2,2′-bipyridine]-5,5′-dicarboxylic acid (**12**) (prepared in an overnight reaction by method B.(i).(a); 3.38 g, 11.27 mmol) in a solution of sodium hydroxide (0.71 g, 17.8 mmol) in water (75 mL) and ethanol (25 mL) was heated under reflux for 3 h, cooled to room temperature, and then filtered. The filtrate was acidified with acetic acid–concentrated hydrochloric acid–water (20:10:10), and the precipitated product was isolated by filtration and air-dried to give [2,2′-bipyridine]-5,5′-dicarboxylic acid (**11**): 1.55 g, 56%; mp >300 °C (lit.[21] mp >360 °C). Anal. ($C_{12}H_8N_2O_4$) C, H, N.

Acknowledgment. We gratefully acknowledge the contributions of Dr. H. Tucker and R. I. Dowell to many useful discussions. We thank Dr. T. J. Franklin, C. J. Cunliffe, and M. Hitchen for providing the biological data, and we thank Dr. L. Furlong for detailed guidance in its statistical evaluation.

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