Bipyridine Cardiotonics: The Three-Dimensional Structures of Amrinone and Milrinone[1]

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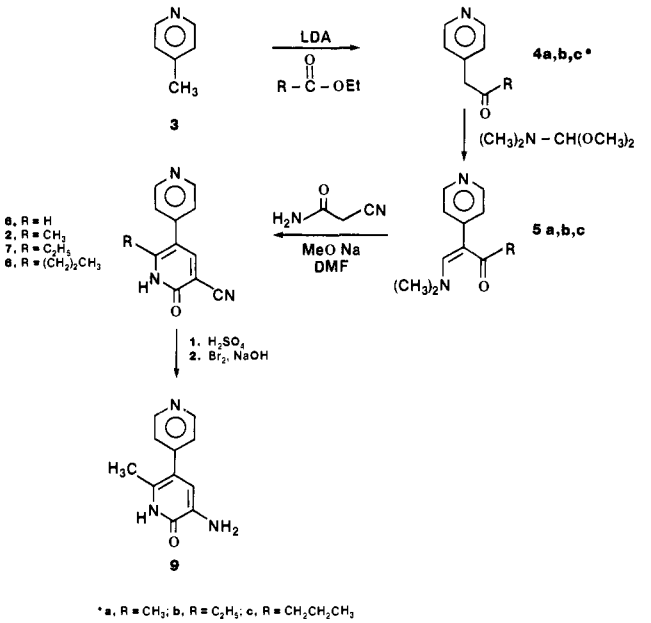
The cardiotonic drug milrinone (1,6-dihydro-2-methyl-6-oxo-[3,4'-bipyridine]-5-carbonitrile) is superior to its analogue amrinone (5-amino-[3,4'-bipyridin]-6(1*H*)-one) by virtue of its greater potency and reduced side effect profile. We confirmed initial reports on the potencies of milrinone and amrinone and found that after intravenous administration to phenobarbital anesthetized dogs, the drugs had cumulative inotropic ED_{50}'s of 37 and 1891 $\mu g/kg$, respectively; relative effects on heart rate and blood pressure were comparable. There are two structural differences between amrinone and milrinone: (1) milrinone has a pyridone 2-methyl substituent and (2) the pyridone 5-amino substituent of amrinone is replaced with a nitrile in milrinone. We confirmed structure–activity studies that indicated that the 2-methyl substituent appears to be primarily responsible for the dramatic difference in the potencies of amrinone and milrinone. A plausible explanation for the effect of the methyl substituent is an altered molecular topology resulting from its steric interaction with the 3',5'-hydrogen atoms. Consequently, we probed the three-dimensional structures of these two compounds by X-ray crystallography. The dihedral angle between the planes formed by the two aromatic rings of amrinone was 1.3°. In marked contrast, the corresponding angle for milrinone was 52.2°. Moreover, ^1H NMR studies revealed conformational differences in solution. Whereas the 2-methyl substituent undoubtedly produces some electronic and hydrophobic perturbations in the bipyridine cardiotonic series, the most significant effect, from a global viewpoint, is the altered molecular topology.

Although diuretics and vasodilators are widely used to ameliorate symptoms of congestive heart failure, cardiac glycosides still represent the cornerstone of therapy.[2] This is despite their relatively weak inotropic efficacy[3] when compared to intravenous inotropes such as dopamine and dobutamine and their narrow therapeutic index.[4] Development of orally and chronically effective digitalis replacements has received considerable attention over the past decade. Of particular interest is a new class of drugs that displays both inotropic and peripheral vasodilator activities; these dual activities enhance cardiac output by simultaneously augmenting cardiac contractility and reducing impedence to ventricular ejection.[5]

Amrinone (5-amino-[3,4'-bipyridin]-6(1*H*)-one) was the progenitor of this new class of cardiotonics.[6-8] This bipyridine cardiotonic has stimulated considerable research to define its mechanism of action, pharmacology, and role in the management of congestive heart failure. Although results of short-term clinical studies were promising,[9-14]

Scheme I



long-term investigations with the oral form of the drug revealed that it was associated with a variety of adverse effects.[15-27] These included thrombocytopenia, fever,

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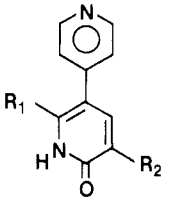
Table I. Structures and Properties of Bipyridine Cardiotonics



no.	R_1	R_2	formula	mp, °C	solvent	anal.
2 (milrinone)	Me	CN	$C_{12}H_9N_3O$	>300[a]	DMF/H_2O	C, H, N
6	H	CN	$C_{11}H_7N_3O$	>300[b]	DMF/H_2O	C, H, N
7	Et	CN	$C_{13}H_{11}N_3O$	287–290 dec[c]	DMF/H_2O	C, H, N
8	*n*-Pr	CN	$C_{13}H_{13}N_3O$	232–234[d]	DMF/H_2O	C, H, N
9	Me	NH_2	$C_{11}H_{12}N_3O$	283–285 dec[e]	DMF/H_2O	C, H, N

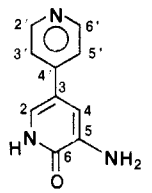
[a] Lit. mp (ref 35) >300 °C. [b] Lit. mp (ref 38) >300 °C. [c] Lit. mp (ref 35) > 300 °C. [d] Lit. mp (ref 35) 232–234 °C. [e] Lit. mp (ref 35) >300 °C.

Table II. Biological Activities of Bipyridine Cardiotonics

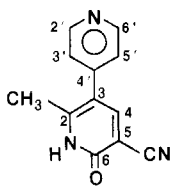
	anesthetized dog[a]				
no.	ED_{50} for contractility: μg/kg, iv	increase in HR	decrease in MAP	*n*	PDEase III inhibition: IC_{50}, μM
1 (amrinone)	1891 ± 239	13 ± 2	16 ± 2	4	150 (95–230)[b]
2 (milrinone)	37 ± 9	14 ± 4	10 ± 3	3	12 (8.3–18)
6	3134 ± 598	12 ± 1	7 ± 1	3	380 (245–590)
7	120 ± 26	19 ± 3	12 ± 5	2	12 (8.1–19)
8	748 ± 124	25 ± 11	20 ± 2	2	38 (33–44)
9	55 ± 10	9 ± 1	10 ± 2	3	31 (15–63)

[a] ED_{50}'s were determined by least-square linear regression analysis and are reported as the mean ± SEM of experimental values. Heart rate (beats/minute) and mean arterial blood pressure (mmHg) values are those recorded at the inotropic ED_{50}'s. Control values were as follows: contractility, 50-*g* tension; heart rate (HR), 142 ± 5 beats/min; mean arterial blood pressure (MAP), 104 ± 4 mmHg. [b] Values in parentheses are 95% confidence limits derived from regression analysis of the logit transformation as described in ref 51.

anorexia, abdominal pain, nausea, emesis, and some indications of increased mortality. Because of the unacceptably high incidence of severe side effects, studies with the oral form of the drug were terminated; however, the intravenous form is now available for the acute management of refractory heart failure.



Amrinone, 1



Milrinone, 2

Milrinone (1,6-dihydro-2-methyl-6-oxo-[3,4′-bipyridine]-5-carbonitrile),[28,29] an analogue of amrinone, is currently undergoing extensive clinical evaluation. Milrinone is reported to be superior to amrinone by virtue of its 20–50-fold greater potency and reduced propensity to produce side effects.[30–34]

We reasoned that a plausible explanation for the increased potency of milrinone is an altered molecular topology resulting from an interaction of the 2-methyl substituent with the 3′,5′-hydrogen atoms. Consequently, we probed the three-dimensional structures of amrinone and milrinone by X-ray crystallography and ^1H NMR spectroscopy. We also conducted some highly focused structure–activity relationship (SAR) studies to confirm previously reported data[35] that indicated that the 2-methyl substituent, rather than the 5-nitrile moiety, was primarily responsible for the increased potency of milrinone.

Results and Discussion

Chemistry. Most of the milrinone and amrinone analogues synthesized in these studies were prepared by minor modification of the general procedures of Lesher[35,36] (Scheme I). In each case 4-picoline (3) was metalated with LDA and then treated with the appropriate ester to pro-

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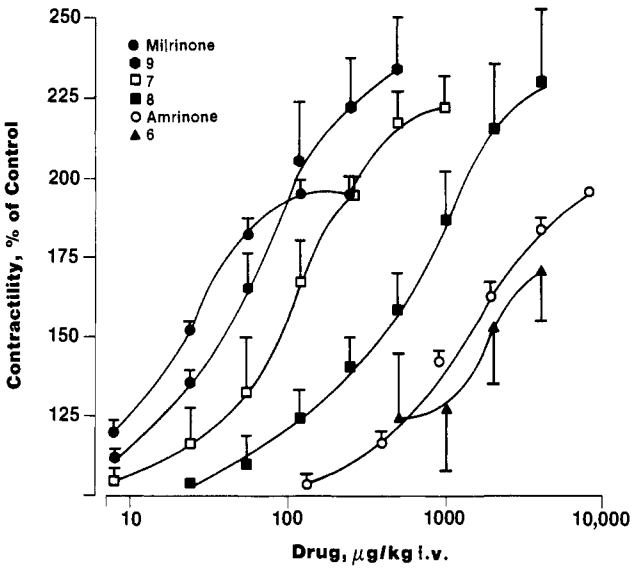

Figure 1. Dose-dependent effects of bipyridine cardiotonics in phenobarbital-anesthetized dogs. Drugs were administered at 5-min intervals and peak responses were recorded. Each point is the mean ± SEM of experimental values. Control contractility was 50-*g* tension.

vide the pyridyl ketones **4a-c**. Reaction with dimethylformamide dimethyl acetal followed by treatment with cyanoacetamide in the presence of base produced the pyridylpyridones **2**, **7**, and **8**. Partial hydrolysis of milrinone to the 5-carboxamide followed by a Hoffman reaction provided the 5-amino analogue of milrinone (**9**).[37] The 5-cyano analogue of amrinone (**6**) was prepared from 4-picoline according to the reported procedure.[38] Physical properties of these compounds are compiled in Table I.

Pharmacology. Instrumented phenobarbital anesthetized dogs were used to quantitate the inotropic potencies of these compounds. Intravenous administration of 1891 μg/kg amrinone resulted in a 50% increase in contractility that was accompanied by a 13 beat/min increase in heart rate and a 16 mmHg decrease in mean arterial blood pressure (Table II, Figure 1). Inotropic ED_{50}'s of **6**, **8**, **7**, **9**, and milrinone (**2**) were 3134, 748, 120, 55, and 37 μg/kg, respectively (Figure 1, Table II). These data indicate milrinone is 51-fold more potent than amrinone, which agrees with the results of other investigators.[28,39] There are two structural differences between amrinone and milrinone: (1) milrinone has a pyridone 2-methyl substituent and (2) the pyridone 5-amino substituent of amrinone is replaced with a nitrile substituent in milrinone. The data of Figure 1 and Table II clearly indicate that the substituent difference at the 5-position of the pyridone ring is not the principal reason for the dramatic differences in the inotropic activities of amrinone and milrinone. The cyano analogue of amrinone (**6**) was 65% less potent than amrinone (ED_{50}'s = 3134 and 1891 μg/kg, respectively), whereas milrinone was 46% more potent than **9**, the amino analogue of milrinone. Thus, whether the amine–nitrile interchange makes the bipyridine cardiotonic more or less active is compound dependent and appears to be unpredictable; in any event the magnitude of the potency change

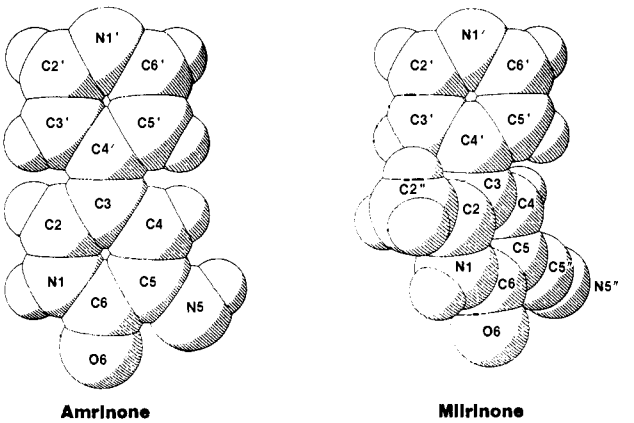

Figure 2. Computer-generated space-filling representations of amrinone and milrinone. These were produced from three-dimensional X-ray crystallographic coordinates.

is 2-fold or less. Thus, the 2-methyl substituent of milrinone appears to be primarily responsible for its 51-fold potency advantage relative to amrinone. These studies confirmed those of Lesher and co-workers,[35] who demonstrated that incorporation of an alkyl substituent at the 2-position of the pyridone ring led to a 10–100-fold increase in inotropic potency, regardless of the nature of the 5-position substituent.

The mechanism of action of several recently discovered noncatecholamine, nonglycoside cardiotonic agents appears to involve, at least in part, the selective inhibition of the low K_m, cyclic AMP specific phosphodiesterase (PDEase III) that is present in myocardial cells.[40,41] Consequently, we investigated the ability of these amrinone analogues to inhibit PDEase III (Table II). All analogues were inhibitors of PDEase III, and a relationship between inotropic activity and PDEase inhibition was apparent. Milrinone was 13-fold more potent as a PDEase III inhibitor than amrinone (IC_{50}'s = 12 and 150 μM, respectively). However, milrinone was only 2.5 times more potent than the amino analogue of milrinone (**9**, IC_{50} = 31 μM), indicating that the methyl group of milrinone, rather than the cyano substituent, is the most important structural difference between amrinone and milrinone relative to PDEase III inhibition. The correlation between in vitro PDEase III IC_{50}'s and in vivo inotropic ED_{50}'s was highly significant ($r = 0.98$, $p < 0.01$). Bristol and co-workers have reported a similar correlation involving a variety of inotropes from different chemical classes.[42]

X-ray Studies. To determine whether this pronounced difference in potency might result from alterations in molecular topology induced by the 2-methyl substituent of milrinone, X-ray crystallography was employed to map the three-dimensional structures of these bipyridine cardiotonics. The dihydrochloride salt of amrinone crystallized from ethanol as yellow prisms in the monoclinic space group $P2_1$. A total of 962 unique reflections were measured. The structure was solved by direct methods and refined to a final $R = 0.0696$. Milrinone hydrochloride crystallized from ethanol/water as orange prisms in the orthorhombic space group $Pna2_1$. Intensities of 754 unique reflections were measured. The structure was solved by direct methods and refined to a final $R = 0.0947$.

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Table III. ^1H and ^{13}C NMR Assignments (ppm) for Bipyridine Cardiotonics

amrinone (1)			9		6			milrinone (2)	
C no.	^1H	^{13}C	C no.	^1H	C no.	^1H	^{13}C	C no.	^1H
2	7.25	119.08	2		2	8.41	140.7	2	
3		115.9	3		3		115.7	3	
4	6.89	108.0	4	6.51	4	8.72	147.8	4	8.19
5		144.4	5		5		104.5	5	
6		157.3	6		6		160.0	6	
2′	8.60	149.9	2′	8.60	2′	8.60	150.0	2′	8.63
3′	7.50	119.3	3′	7.39	3′	7.74	120.4	3′	7.44
4′		138.8	4′		4′		142.9	4′	
5′	7.50	119.3	5′	7.39	5′	7.74	120.4	5′	7.44
6′	8.60	149.9	6′	8.60	6′	8.60	150.0	6′	8.63
			2-Me	2.15	5-CN		116.4	2-Me	2.33

Three-dimensional structures are depicted in Figure 2, using computer-generated space-filling representations. As can be readily discerned, dihedral angles between the planes formed by the aromatic rings of amrinone and milrinone are dramatically different. In the crystal state, amrinone is virtually a planar molecule, with a dihedral angle of 1.3°; this high degree of molecular planarity presumably allows significant conjugation between the two pyridine rings. In marked contrast, the corresponding dihedral angle of milrinone is 52.2°, reflecting the steric interaction between the methyl substituent and the 3′,5′-hydrogen atoms which skews the aromatic rings from planarity.

NMR Studies. These X-ray crystallographic data provided evidence for dramatic conformational differences between these two bipyridines in the solid state. However, the conformation of biaryls in the solid state is not always predictive of conformation in solution. For example, biphenyl is planar in the crystalline state whereas in solution the deviation from planarity is estimated to be 23°;[43] consequently we employed ^1H NMR to determine whether conformational differences between amrinone and milrinone occur in solution.

The ^1H NMR spectra of amrinone (1), **9**, **6**, and milrinone (**2**) are displayed in Figure 3, panels A, B, C, and D, respectively. As shown in panel A the AX pattern of the pyridine ring protons of amrinone occurs as doublets at 8.60 (C-2′, C-6′) and 7.50 (C-3′, C-5′) ppm with a coupling constant of 5.83 Hz. The resonances of the C-2 and C-4 pyridone protons appear at 7.25 and 6.89 ppm with a typical meta coupling constant of 2.26 Hz. If the solution conformation of the 2-methyl analogue of amrinone (**9**) mimics that of milrinone (**2**) in the crystalline state, then its C-4 proton should be partially in the shielding zone of the pyridine ring anisotropic current and resonate further upfield than the corresponding proton in amrinone. Thus, the chemical shift of the C-4 proton can be used as a conformational reporter for this pair of analogues. The same reasoning applies to milrinone and the 5-cyano analogue of amrinone (**6**).

To calculate the magnitude of the upfield shift produced by methyl-induced topological perturbation, the crucial C-2 and C-4 resonances of amrinone (1) and **6** had to be rigorously assigned. Thus, ^1H and ^{13}C NMR spectra of 1 and **6** were fully assigned by employing ^1H to ^1H spin–spin decoupling, ^1H to ^1H difference NOE, selective ^1H to ^{13}C decoupling, and 2D NMR analysis of long-range (3 bond) ^{13}C to ^1H couplings.[44] The ^1H NMR spectra of **9** and **2** were unambiguously assigned by inspection. A complete summary of the ^1H and ^{13}C chemical shift assignments is presented in Table III.

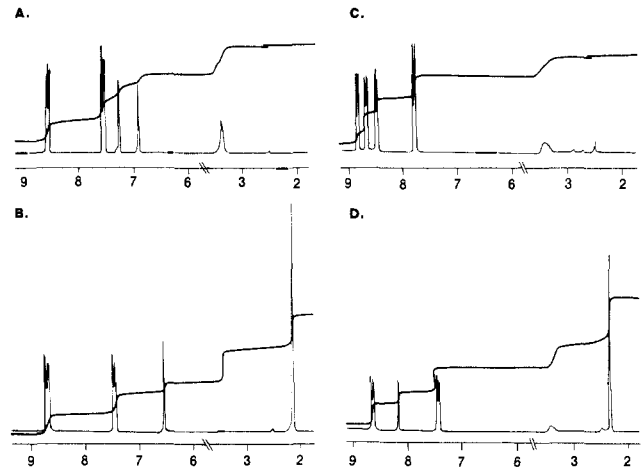

Figure 3. ^1H NMR spectra of amrinone, **9**, **6**, and milrinone, (panels A, B, C, and D, respectively). Spectra were obtained in Me_2SO-d_6 on a Bruker WM-270 spectrometer in the FT mode.

Difference NOE was used to tentatively assign resonances for the C-2 and C-4 protons of amrinone. When the pyridone NH of amrinone (11.8 ppm) was irradiated, a NOE was observed at the 7.25 ppm proton resonance, thus assigning the proton of C-2. Spin–spin decoupling then demonstrated that the 6.89 ppm proton was meta coupled to the C-2 proton, thus assigning it as the C-4 proton. To confirm this NOE assignment we demonstrated ^1H to ^{13}C coupling from the pyridone NH to C-2 by obtaining a gated ^{13}C spectrum; addition of D_2O should eliminate C–NH coupling. Slight sharpening was indeed seen at the 119.08 ppm carbon, whereas no change was observed at the 108.0 ppm carbon. Therefore, these resonances represent carbons 2 and 4, respectively. Selective ^1H to ^{13}C decoupling revealed that the 7.25 and 6.89 ppm protons were bonded to carbons 2 (119.08 ppm) and 4 (108.0 ppm), respectively. With the C-4 proton resonance of amrinone assigned, we could then calculate the magnitude of the anisotropically induced shift in **9**. As shown in Figure 3 (panel B) and Table III, the singlet of the C-4 proton of **9** resonates at 6.51 ppm, a 0.38 ppm upfield shift relative to the corresponding resonance of amrinone.[45]

Assigning the spectra of **6** was straightforward. Two-dimensional analysis revealed that the cyanocarbon at

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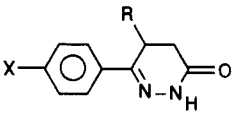
(45) Although these NOE and ^1H to ^{13}C coupling experiments support the reported C-2 and C-4 assignments, they are not incontrovertible due to the small magnitude of the NOE and peak sharpening; however, if our C-2 and C-4 assignments were reversed, it would simply increase the magnitude of the upfield shift from 0.38 to 0.74 ppm.

116.4 ppm is 3-bond coupled to the 8.72 ppm proton; thus the 8.72 and 8.41 ppm resonances correspond to the C-4 and C-2 protons, respectively. The C-4 proton of milrinone (**2**) resonates at 8.19 ppm, which represents a 0.53 ppm upfield shift relative to the C-4 proton of **6**. Thus, these NMR data strongly suggest there are conformational differences in solution between the methylated and unmethylated amrinone analogues.[46]

Conclusions

Our SAR studies confirmed a previous report[35] that indicated that the cyano–amino interchange in bipyridine cardiotonics cannot be responsible for the 51-fold difference in inotropic potency between amrinone and milrinone. Therefore, the predominant portion of the difference is contributed by the methyl substituent at C-2 of milrinone. The same effect of a similarly disposed methyl group has been noted in the dihydropyridazinone cardiotonics CI-914 and CI-930[42] (**12** and **13**, respectively) and platelet aggregation inhibitors **14** and **15**.[47] The methyl-substituted



12: X = 1-imidazolyl, R = H (CI-914)
13: X = 1-imidazolyl, R = Me (CI-930)
14: X = CH_3CONH, R = H
15: X = CH_3CONH, R = Me

analogues are, in each case, considerably more potent than their desmethyl analogues, and Bristol et al.[42] attributed this substituent effect to a methyl-sized lipophilic pocket in the active site of PDEase III. The methyl-induced increase in activity of bipyridine cardiotonics could result from similar hydrophobic interactions, from electronic effects of the methyl group, from altered molecular topology, or a combination of these three factors. However, the electronic effects of the pyridone substituents appear to be relatively unimportant as evidenced by the interchangability of the cyano and amino substituents. Hydrophobic and topological effects of the C-2 methyl substituent are difficult to dissect experimentally, but the most significant, from a global viewpoint, is the altered topology. Furthermore, as the methyl group is extended to ethyl or propyl, inotropic activity decreases significantly (Table II), indicating that hydrophobicity (at least beyond $\pi = 0.56$) can be a deleterious feature of substituents at C-2 of bipyridine cardiotonics.

These studies demonstrated that there are dramatic crystal-state conformational differences between amrinone and milrinone. Furthermore, ^{1}H NMR data indicated that these differences also occur in solution. Highly focused SAR studies indicated that the methyl substituent, which produces conformational perturbations, rather than the cyano substituent, is the cardinal reason for the increased potency of milrinone as a PDEase III inhibitor and as a positive inotrope.

(46) It could be argued that the upfield shift of the C-4 resonance in the methyl-substituted analogues results solely from inductive effects of the methyl group rather than topological perturbations. However, the upfield shift produced by the inductive effects of the meta methyl substituent would be expected to be no more than 0.09 ppm, assuming the substituent parameters of a methyl group on a pyridine and benzene are similar. See: ref 43; pp 42–43.

(47) Thyes, M.; Lehmann, H. D.; Gries, J.; Konig, H.; Kretzschmar, R.; Kunze, J.; Lebkucher, R.; Lenke, D. *J. Med. Chem.* **1983**, *26*, 800.

Experimental Section

Methods. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are not corrected. Proton magnetic resonance (^{1}H NMR) spectra were obtained with a Bruker WM-270 spectrometer. ^{13}C NMR spectra were obtained with a Bruker WM-270 spectrometer operated at 67.9 MHz; 2D NMR spectra were obtained with a Bruker WM-250 instrument. Solutions were prepared in either Me_2SO-d_6 or Me_2SO-d_6 containing D_2O; the sample temperature during data acquisition was ambient (~23 °C). Difference NOE (nuclear Overhauser effect) spectra were obtained by substracting free induction decays accumulated with the decoupler off-resonance from similar accumulations with particular resonances irradiated, followed by Fourier transformation of the difference signals. The procedure was not optimized for maximum NOE measurement; the usual irradiation period was 2.0 s, followed by a preaccumulation delay of 0.03 s. Two-dimensional NMR studies were conducted as described by Bax.[44] Chemical shifts are reported in ppm downfield from a tetramethylsilane internal standard (δ scale). The ^{1}H NMR data are presented in the form: (solvent in which spectra were taken), δ value of signal (peak multiplicity, integrated numbers of protons, coupling constant (if any), and assignment). Mass spectra were recorded from a Varian MAT CH-5 spectrometer at the ionization voltage expressed in parentheses. Only the peaks of high relative intensity or of diagnostic importance are presented in the form: m/e (intensity relative to base peak). Microanalytical data were provided by the Physical Chemistry Department of the Lilly Research Laboratories; only symbols of elements analyzed are given and they were within 0.4% of theoretical values unless indicated otherwise.

Except where noted, a standard procedure was used for product isolation. This involved quenching by addition to water, filtration or exhaustive extraction with a solvent (washing of extract with aqueous solutions, on occasion), drying over an anhydrous salt, and evaporation of solvent under reduced pressure. Particular solvents, aqueous washes (if needed), and drying agents are mentioned in parentheses after the phrase "product isolation".

Amrinone (free base) was obtained from Sterling-Winthrop. Other bipyridine cardiotonics used in this study were synthesized by procedures outlined by Lesher.[35,36,38] Hydrochloride salts were formed by adding an excess of ethanol that was saturated with hydrogen chloride to an ethanol solution of the drug.

X-ray Crystallography. The dihydrochloride salt of amrinone crystallized as the monohydrate from ethanol as yellow prisms in the monoclinic space group $P2_1$, with two molecules in a unit cell having the dimensions a = 16.810 (9) Å, b = 6.860 (2) Å, c = 10.787 (7) Å, and β = 150.85 (2)°; calculated density was 1.426 g cm^{-3}. Intensities of 962 unique reflections with 2θ less than 116.0° were measured on a four-angle diffractometer using monochromatic copper radiation. Positions of the atoms were obtained by interpretation of an E map phased by the direct methods routine SOLV of the SHELXTL program.[48] The structure was refined by the least-squares method with anisotropic temperature factors for all atoms except hydrogens, which were included at calculated positions with isotropic temperature factors. The final R factor was 0.0696 for 851 observed reflections. Tables V–IX (see supplementary material paragraph) show the atomic coordinates, bond lengths, bond angles, anisotropic temperature factors, and hydrogen atom coordinates, respectively.

The hydrochloride salt of milrinone crystallized from ethanol/water as orange prisms in the orthorhombic space group $Pna2_1$ with four molecules in a unit cell having dimensions a = 7.588 (2) Å, b = 10.743 (5) Å, c = 14.026 (5) Å. The calculated density was 1.439 g cm^{-3}. Intensities of 754 unique reflections with 2θ less than 116.0° were measured on a four-angle diffractometer using monochromatic copper radiation. Positions for most atoms were determined by interpretation of an E map phased by the direct methods routine SOLV of the SHELXTL program;[48] the remainder were located from successive E maps. The structure was refined by the least-squares method with anisotropic temperature factors for all atoms except hydrogens, which were included at calculated positions with isotropic temperature factors. The final R factor was 0.0947 for 520 observed reflections. Tables

(48) Purchased from Nicolet, originator: Sheldrick, G. M.

X-XIV (see supplementary material paragraph) show the atomic coordinates, bond lengths, bond angles, anisotropic temperature factors, and hydrogen atom coordinates, respectively.

Syntheses. The synthetic methods used in this study are illustrated by the preparation of **4b**, **5b**, **7**, and **9**.

1-(4-Pyridyl)-2-butanone (4b). *n*-Butyllithium (280 mL of a 1.15 M solution in hexane, 322 mmol) was added to a solution of diisopropylamine (34.2 g, 339 mmol) in 300 mL of THF with cooling by an ice/ethanol bath; temperature was not allowed to rise above 20 °C during the addition. The reaction was stirred 15 min at 0 °C, whereupon 4-picoline (30 g, 322 mmol) in 130 mL of THF was added in a dropwise fashion resulting in a deep red solution. After addition was complete, 4 mL of HMPA was added, and the reaction was stirred for 30 min at 0 °C. Ethyl propanoate (32.9 g, 322 mmol) in 130 mL of THF was added in a dropwise fashion over a period of 45 min, and then the reaction was warmed to room temperature and stirred for 90 min. The reaction was cooled to 0 °C whereupon acetic acid (38 mL, 660 mmol) was added. Solvent was removed in vacuo, and the residue was dissolved in water and extracted with chloroform. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated in vacuo to provide 44 g of a dark oil. Preparative HPLC (silica gel, 0–60% ethyl acetate in hexane gradient) afforded 14.5 g (30%) of **4b** as a yellow oil with: ^{1}H NMR ($CDCl_3$) δ 1.07 (t, 3, J = 8 Hz, CH_3), 2.5 (q, 2, J = 8 Hz, CH_2CH_3), 3.71 (s, 2 H, Ar CH_2), 7.13 (d, 2 H, J = 6 Hz, Ar H β to N), 8.53 (d, 2 H, J = 6 Hz, Ar H α to N). This intermediate was used in the following reaction without further characterization.

1,6-Dihydro-2-ethyl-6-oxo-[3,4′-bipyridine]-5-carbonitrile (7). A solution of 1-(4-pyridyl)-2-butanone (**4b**; 14 g, 94 mmol) and dimethylformamide dimethyl acetal (37 g, 310 mmol) in 140 mL of DMF was heated at 50–65 °C for 4.5 h and then cooled to ca 5 °C overnight. Removal of solvent in vacuo provided 20.4 g of **5b** as a dark oil that was homogeneous by TLC: ^{1}H NMR (Me_2SO-d_6) δ 0.92 (t, 3, J = 7 Hz, CH_2CH_3), 2.36 (q, 2, J = 7 Hz, CH_2CH_3), 2.70 (s, 6, NCH_3), 7.1 (d, 2, J = 5.4 Hz, Ar H β to N), 7.6 (s, 1, =$CHN(CH_3)_2$, 8.44 (d, 2, J = 5.4 Hz, Ar H α to N); mass spectrum (70 eV), m/e (rel intensity) 204 (46, M^+), 175 (100), 132 (53). This intermediate was used in the following reaction without further characterization.

A mixture of the unpurified enamine **5b** (5 g, 24.5 mmol), 2-cyanoacetamide (2.26 g, 27 mmol), and sodium methoxide (2.8 g, 52.5 mmol) in 50 mL of DMF was heated to 90–100 °C for 2.5 h, cooled, and maintained at ca. 5 °C overnight. Solvent was removed in vacuo, and the residue was dissolved in 100 mL of pH 7 phosphate buffer. Hydrochloric acid was used to adjust the pH to 6 and the solution was cooled to 5 °C. The precipitate was filtered and recrystallized from DMF/water to provide 2.5 g (45% for two steps) of **7** as a light tan solid with mp 287–290 °C dec. (lit.[35] mp >300 °C). Anal. ($C_{13}H_{11}N_3O$) C, H, N.

1,6-Dihydro-2-methyl-6-oxo-[3,4′-bipyridine]-5-carboxamide.[37] Concentrated sulfuric acid (3.7 mL, 66 mmol) was added to milrinone (**2**; 1 g, 4.7 mmol), and the solution was heated on a steam bath for 15 min. The solution was then cooled to 0 °C, whereupon concentrated ammonium hydroxide and ice were added to bring the volume to ca. 75 mL and the pH to ca. 10. The resulting precipitate was filtered and dried to provide 1.04 g (95%) of product. Analytical HPLC (1% ammonium acetate in 15% acetonitrile/water) indicated a purity of 98.9%. The product displayed the following: ^{1}H NMR (Me_2SO-d_6) δ 2.36 (s, 3, CH_3), 7.43 (d, 2, J = 5.4 Hz, Ar H β to N), 8.23 (s, 1, Ar H of pyridone ring), 8.63 (d, 2, J = 5.4 Hz, Ar H α to N); mass spectrum (70 eV), m/e (rel intensity) 229 (100, M^+), 213 (71), 184 (77). This intermediate was used in the following reaction without further characterization.

5-Amino-2-methyl-[3,4′-bipyridin]-6(1*H*)-one (9).[37] A mixture of 1,6-dihydro-2-methyl-6-oxo-[3,4′-bipyridine]-5-carboxamide (1.0 g, 4.4 mmol) and 1 N sodium hydroxide (27.5 mL, 27.5 mmol) was heated on a steam bath to achieve homogeneity. The solution was cooled to 0 °C and bromine (270 μL, 5.2 mmol) was added in a dropwise fashion. The reaction was heated on a steam bath for 3 h, filtered, acidified to pH 7 with 1 N hydrochloric acid, and cooled to ca. 5 °C for 2 h. The precipitate was filtered and recrystallized from 10% DMF/water to yield 500 mg (57%) of product with mp 283–5 °C dec. A small portion was recrystallized again from DMF/water to provide the analytical sample: mp 283–285 °C dec (lit.[35] mp >300 °C). Anal. ($C_{11}H_{11}N_3O$) C, H, N.

Pharmacological Methods. Experiments in Anesthetized Dogs. Male beagle dogs (8.0–20.0 kg) were anesthetized with sodium thiopental (15 mg/kg, iv) and maintained with sodium phenobarbital (100 mg/kg, iv). A positive pressure pump provided ventilation through a cuffed endotracheal tube (18 strokes/min, 20 mL/kg per stroke) and a heating pad maintained the body temperature at 37–38 °C. Both vagus nerves were sectioned through an incision in the neck. Cardiovascular measurements were made as previously described.[49] Test compounds were dissolved and injected in deionized water. Doses were given at 5-min intervals.

Preparation and Assay of Isozymes of Canine Cardiac Phosphodiesterase (PDEase). Fractionation of the three major isozymes of PDEase from canine ventricular muscle was carried out essentially as described by Thompson et al.[50] PDEase activity was assayed by minor modification of the method described by Thompson et al.[50] IC_{50}'s and 95% confidence limits (Table II) were calculated as previously described.[51] Precise details on the fractionation and assay are provided as supplementary material.

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Registry No. 1, 60719-84-8; 1 dihydrochloride, 100683-16-7; **2**, 78415-72-2; **2** hydrochloride, 100683-17-8; **4b**, 6304-20-7; **5b**, 78504-62-8; **6**, 62749-26-2; **7**, 78504-63-9; **8**, 80047-22-9; **9**, 80047-27-4; 4-picoline, 108-89-4; ethyl propanoate, 105-37-3; dimethylformamide dimethyl acetal, 4637-24-5; 2-cyanoacetamide, 107-91-5; 1,6-dihydro-2-methyl-6-oxo-[3,4<<spn-bipyridine]-5-carboxamide, 80047-24-1.

Supplementary Material Available: Atomic coordinates, bond lengths, bond angles, anisotropic temperature factors and hydrogen atom coordinates for amrinone (Tables V-IX) and milrinone (Tables X-XIV). Experimental details for fractionation and assay of phosphodiesterase (7 pages). Ordering information is given on any current masthead page.

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