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# Structure–Activity Relationships of Inotropic Bipyridines: Crystallographic Analysis of Four Milrinone Analogues

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## Abstract

1,6-Dihydro-2′-methyl-6-oxo-[3,4′-bipyridine]-5-carbonitrile (I), $C_{12}H_9N_3O$, $M_r$ = 211.23, monoclinic, $C2/c$, $a$ = 13.152 (2), $b$ = 8.987 (2), $c$ = 16.538 (3) Å, $\beta$ = 95.60 (1)°, $V$ = 1945.4 (5) Å$^3$, $Z$ = 8, $D_x$ = 1.442 Mg m$^{-3}$, $\lambda$(Mo $K\alpha$) = 0.71073 Å, $\mu$ = 0.090 mm$^{-1}$, $F$(000) = 880, $T$ = 90 K, $R$ = 0.066 for 1997 observed reflections. 1,6-Dihydro-2,2′-dimethyl-6-oxo-[3,4′-bipyridine]-5-carbonitrile (II), $C_{13}H_{11}N_3O$, $M_r$ = 225.25, triclinic, $P\bar{1}$, $a$ = 6.704 (1), $b$ = 6.846 (1), $c$ = 13.273 (3) Å, $\alpha$ = 81.31 (2), $\beta$ = 85.28 (2), $\gamma$ = 66.02 (2)°, $V$ = 550.0 (2) Å$^3$, $Z$ = 2, $D_x$ = 1.360 Mg m$^{-3}$, $\lambda$(Mo $K\alpha$) = 0.71073 Å, $\mu$ = 0.084 mm$^{-1}$, $F$(000) = 236, $T$ = 90 K, $R$ = 0.056 for 2301 observed reflections. 5-Amino-2-methyl-[3,4′-bipyridin]-6(1*H*)-one (III), $C_{11}H_{11}N_3O.HCl.2H_2O$, $M_r$ = 273.72, triclinic, $P\bar{1}$, $a$ = 7.200 (2), $b$ = 10.695 (2), $c$ = 17.556 (4) Å, $\alpha$ = 77.20 (2), $\beta$ = 87.94 (2), $\gamma$ = 83.58 (2)°, $V$ = 1310.0 (7) Å$^3$, $Z$ = 4, $D_x$ = 1.388 Mg m$^{-3}$, $\lambda$(Mo $K\alpha$) = 0.71073 Å, $\mu$ = 0.293 mm$^{-1}$, $F$(000) = 576, $T$ = 291 K, $R$ = 0.067 for 4921 reflections. 5-Amino-2′-methyl-[3,4′-bipyridin]-6(1*H*)-one (IV), $C_{11}H_{11}N_3O$, $M_r$ = 201.23, monoclinic, $P2_1/c$, $a$ = 9.5350 (9), $b$ = 14.310 (2), $c$ = 7.4961 (8) Å, $\beta$ = 105.280 (1)°, $V$ = 986.7 (4) Å$^3$, $Z$ = 4, $D_x$ = 1.355 Mg m$^{-3}$, $\lambda$(Mo $K\alpha$) = 0.71073 Å, $\mu$ = 0.085 mm$^{-1}$, $F$(000) = 424, $T$ = 160 K, $R$ = 0.047 for 1732 reflections. There is a broad range of molecular conformations for these bipyridines, as reflected by the bipyridine torsion angle C(2)—C(1)—C(1′)—C(2′). The largest angles are observed for the two 2′-methyl milrinone analogues (I) and (II), which have values of 144.2 (2) and 74.3 (2)°, respectively, compared with that of 42.3° observed for milrinone. The addition of either a 2- or 2′-methyl to amrinone causes the almost coplanar parent structure to adopt twist angles of 38.5 (2) and −36.8 (2)° for molecules 1 and 2 of (III), respectively, and 59.4 (3)° for (IV). A common feature of these structures is a hypercentric distribution of their normalized structure factors. Ring–ring stacking interactions observed in these structures contribute to the hyperparallelism observed in some crystal lattices or the creation of superlattices by the addition of noncrystallographic symmetry in other structures. These bipyridine molecules form a network of hydrogen bonds with the keto O or pyridine N atoms acting as acceptors and the pyridone 3-N or 5-amino group acting as donors. Each of the two independent bipyridinium cations of (III) form alternate layers in the lattice and are involved in a two-dimensional network of hydrogen bonds. Biochemical activity, as measured by the stimulatory effect on rabbit myocardial membrane $Ca^{2+}$-ATPase activity, reveals that only (II) and (III) have appreciable activity with 49 and 57% of enzyme stimulation by milrinone, respectively.

## 1. Introduction

Milrinone (1,6-dihydro-2-methyl-6-oxo-[3,4′-bipyridine]-5-carbonitrile) and amrinone (5-amino-[3,4′-bipyridin]-6(1*H*)-one), its parent bipyridine, are members of a class of oral nonglycosidic, noncatecholamine cardiac positive-inotropic agents developed for the treatment of congestive heart failure (Baim, McDowell, Cherniles, Monrad, Parker, Edelson, Braunwald & Grossman, 1983; Pastelin, Mendez, Kabela & Farah, 1983). Although the mechanism of the inotropic effect is not well understood, it has been shown to involve cAMP phosphodiesterase (Young & Ward, 1988) and/or altered intracellular $Ca^{2+}$ compartmentalization (Mylotte, Cody, Davis, Davis, Blas & Schoenl, 1985). Milrinone is approximately 30 times more potent than amrinone (Baim *et al.*, 1983). In addition, structure–function data indicate that the 2-methyl substituent of milrinone, rather than the 5-cyano group, is responsible for its increased potency (Robertson, Krushinski, Pollock & Hayes, 1988). Other studies have shown that milrinone, but not amrinone, stimulates $Ca^{2+}$-ATPase activity in rabbit myocardial membranes in a manner similar to that observed for thyroid hormones (Mylotte *et al.*, 1985; Rudinger, Mylotte, Davis, Davis & Blas, 1984). In studies utilizing rabbit skeletal muscle sarcoplasmic reticulum membranes, structure–activity correlations and computer modeling revealed that the presence of the 2-methyl and 5-cyano groups of milrinone were responsible for its thyromimetic activity (Warnick, Davis, Davis, Cody, Galindo & Blas, 1993).

To evaluate the molecular features required for the activity of these bipyridines in heart enzyme systems,

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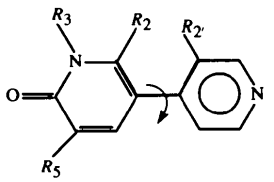
and to understand differences in their thyromimetic potential, structural studies of a series of milrinone derivatives have been carried out (Cody, 1987; Cody, Suwin'ska & Wojtczak, 1991; Cody & Wojtczak, 1991*a*,*b*).




| | $R_{2'}$ | $R_2$ | $R_3$ | $R_5$ |
|---|---|---|---|---|
| (I) | $CH_3$ | H | H | CN |
| (II) | $CH_3$ | $CH_3$ | H | CN |
| (III) | H | $CH_3$ | H | $NH_2$ |
| (IV) | $CH_3$ | H | H | $NH_2$ |

These data reveal that the parent inotrope, amrinone, has greater conformational flexibility than the more potent agent, milrinone (Cody, 1987). As part of this program, we report the crystal structures of four bipyridine derivatives: 1,6-dihydro-2′-methyl-6-oxo-[3,4′-bipyridine]-5-carbonitrile (I) and 1,6-dihydro-2,2′-dimethyl-6-oxo-[3,4′-bipyridine]-5-carbonitrile (II), new milrinone analogues substituted in the pyridine ring, as well as two methylated amrinone analogues: 5-amino-2-methyl-[3,4′-bipyridin]-6(1*H*)-one (III) and 5-amino-2′-methyl-[3,4′-bipyridin]-6(1*H*)-one (IV). Biochemical activity, as measured by the stimulation of myocardial membrane $Ca^{2+}$-ATPase activity, indicates that milrinone is the most potent stimulator; and of these bipyridine analogues, only the 2′-methylmilrinone (II) and the 2-methylamrinone analogue (III) have appreciable activity with 49 and 57% to the stimulation by milrinone, respectively (Cody, Wojtczak, Davis & Davis, 1990). These data suggest that the presence of an *ortho*-methyl group gives rise to a twist conformation and is an important feature for activity.

## 2. Methods

Milrinone, amrinone and analogues were kindly provided by Sterling–Winthrop Research Institute (Rensselaer, NY, and Collegeville, PA). Crystal structures of two 2′-methylated analogues of the inotropic agent milrinone, as well as two methylated analogues of amrinone were determined (Table 1). A Syntex *P*3 diffractometer and Nb-filtered Mo $K\alpha$ radiation ($\lambda$ = 0.71073 Å) was used in an $\omega$–$2\theta$ scan mode to collect the diffraction data from all crystals. Cell parameters were determined by least-squares refinement of 50 reflections, $16.59 < 2\theta < 27.72°$, for (I), 50 reflections, $20.21 < 2\theta < 29.83°$, for (II), 25 reflections, $21.21 < 2\theta < 27.91°$, for (III), and 44 reflections, $20.25 < 2\theta < 29.94°$, for (IV). No significant intensity variation for standards was detected. Absorption and extinction corrections were not applied. Direct methods using *MULTAN*78 (Main, Hull, Lessinger, Germain, Declercq & Woolfson, 1978) and *NQEST* (De Titta, Edmonds, Langs & Hauptman, 1975) were used to solve these structures. A detailed analysis of *E* statistics revealed a hypercentric distribution for structures (II), (III) and (IV) (Table 2). Further analysis of *E* statistics showed that the *h*0*l* reflections in (III) and, all data, *hkl* and *h*0*l* reflections for (IV) have hypercentric distributions. Hypercentric *E* distributions can result from the presence of pseudosymmetry or hypersymmetry in the lattice. Pseudosymmetry causes a partial disappearance of the inversion center or the addition of noncrystallographic symmetry elements. On the other hand, hypersymmetry as a result of hypercentrosymmetry or a regular repetition of subunits at a given interval along a straight line or noncrystallographic molecular symmetry can give rise to a superlattice. Hyperparallelism and hypercentrosymmetry influence the distribution of all classes of reflections, whereas the presence of noncrystallographic symmetry elements is reflected in hypercentric distributions of corresponding classes of reflections (Giacovazzo, 1980). The function $\Sigma w(|F_o| - |F_c|)^2$ was minimized and $w = 1/\sigma^2(F)$ weights were used for (I) and (II), and $w = 1$ for (III) and (IV). For all structures the non-H atoms were refined anisotropically. In (I) the H-atom positions and thermal parameters were refined and the final range of C—H distances was 0.89–1.03 Å, with N—H = 0.86 Å, while in (II) positional parameters were refined and thermal parameters were calculated as being one unit greater than $B_{eq}$ of a non-H atom. The range of C—H distances was 0.94–0.99 Å and the N—H distance was 0.95 Å. In (III) the H-atom positions were calculated from geometry and set at 1.08 Å and the N—H range was 0.86–0.96 Å. The water and amino hydrogens were located on a $\Delta\rho$ electron-density map; the thermal parameters were calculated as for (II) and H-atom parameters were not refined. In (IV) all H-atom positions were calculated from geometry and not refined, with $B_{eq}$ calculated as for (II). The N—H distances ranged between 0.90 and 0.92 Å. Atomic scattering factors were taken from *International Tables for X-ray Crystallography* (1974, Vol. IV). Other programs used: data processing programs described by Blessing (1989), Enraf–Nonius package (Enraf–Nonius, 1979) run on a DEC VAX8600.

## 3. Results and discussion

A summary of crystallographic data collection and structure refinement details is given in Table 1 and the molecules are shown in Fig. 1. Atomic coordinates and equivalent isotropic thermal parameters for these four

Table 1. *Unit-cell parameters and refinement results for* (I), (II), (III) *and* (IV)

| | (I) | (II) | (III) | (IV) |
|---|---|---|---|---|
| Formula | $C_{12}H_9N_3O$ | $C_{13}H_{11}N_3O$ | $C_{11}H_{12}N_3O^+Cl^-.2H_2O$ | $C_{11}H_{11}N_3O$ |
| Solution | $C_3HO$ | MeOH | EtOH/$H_2O$/HCl | EtOH/$H_2O$ |
| Crystal size (mm) | 0.1 × 0.2 × 0.3 | 0.07 × 0.1 × 0.5 | 0.2 × 0.2 × 0.48 | 0.2 × 0.22 × 0.4 |
| Space group | $C2/c$ | $P\bar{1}$ | $P\bar{1}$ | $P2_1/c$ |
| $a$ (Å) | 13.152 (2) | 6.704 (1) | 7.200 (2) | 9.5350 (9) |
| $b$ (Å) | 8.987 (2) | 6.846 (1) | 10.695 (2) | 14.310 (2) |
| $c$ (Å) | 16.538 | 13.273 (3) | 17.556 (2) | 7.4961 (8) |
| $\alpha$ (°) | 90.0 | 81.31 (2) | 77.20 (2) | 90.0 |
| $\beta$ (°) | 95.60 (1) | 85.28 (2) | 87.94 (2) | 105.280 (1) |
| $\gamma$ (°) | 90.0 | 66.02 (2) | 83.58 (2) | 90.0 |
| $V$ (Å$^3$) | 1945.4 (5) | 55.0 (2) | 1310.0 (7) | 986.7 (4) |
| $Z$ | 8 | 2 | 4 | 4 |
| $D_x$ (Mg m$^{-3}$) | 1.442 | 1.360 | 1.388 | 1.355 |
| $\lambda$(Mo $K\alpha$) (Å) | 0.71073 | 0.71073 | 0.71073 | 0.71073 |
| $\mu$ (mm$^{-1}$) | 0.090 | 0.084 | 0.293 | 0.085 |
| $F$(000) | 880 | 236 | 576 | 424 |
| $2\theta_{max}$ (°) | 55 | 57 | 55 | 55 |
| No. of reflections measured | 3942 | 2791 | 7878 | 3406 |
| No. of reflections observed | 1997 | 2301 | 4921 | 1732 |
| No. of variables | 180 | 187 | 325 | 136 |
| $T$ (K) | 90 | 90 | 291 | 160 |
| $R$ | 0.066 | 0.056 | 0.067 | 0.047 |
| $wR$ | 0.056 | 0.050 | 0.063 | 0.045 |
| $S$ | 2.364 | 2.385 | 1.012 | 0.661 |
| $\Delta\rho_{max}$ (e Å$^{-3}$) | 0.58 | 0.56 | 0.67 | 0.32 |
| $\Delta\rho_{min}$ (e Å$^{-3}$) | −0.42 | −0.56 | −0.82 | −0.40 |

$R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$; $wR = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|]^{1/2}$; $S = [\Sigma w(|F_o| - |F_c|)^2/(n-m)]^{1/2}$.

Table 2. *Distributions in* $\langle(E^2-1)^3\rangle$ *for bipyridines*

Theoretical values for the average $(E^2-1)^3$ distributions for centric and hypercentric structures are 8.00 and 26.00, respectively.

| Structure | Class of reflections | | | | |
|---|---|---|---|---|---|
| | All | $hkl$ | $0kl$ | $h0l$ | $hk0$ |
| (II) | 20.149 | 21.765 | 10.146 | 19.353 | 10.041 |
| (III) | 15.982 | 13.794 | 14.770 | 29.744 | 8.446 |
| (IV) | 33.559 | 36.157 | 12.174 | 27.182 | 9.445 |

structures are presented in Tables 3–6* and selected geometical parameters are listed in Tables 7 and 8. The bipyridine conformation is characterized by the torsion angle C(2)—C(1)—C(1′)—C(2′).

* Lists of structure factors, anisotropic thermal parameters and H-atom coordinates have been deposited with the IUCr (Reference: BK0018). Copies may be obtained through The Managing Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

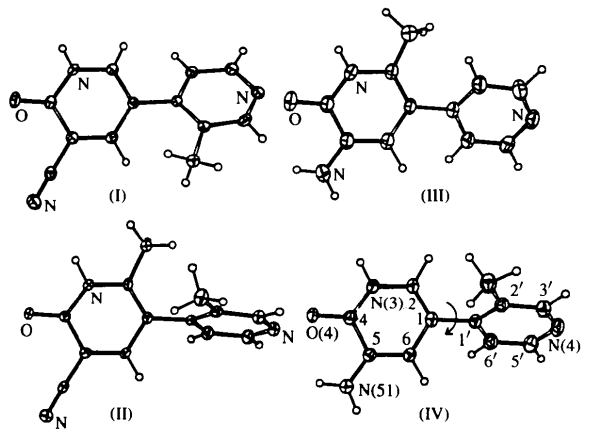



Fig. 1. The molecular conformation of (I), (II), (III) and (IV), with the torsion angle C(2)—C(1)—C(1′)—C(2′) highlighted in (IV).

Table 3. *Fractional atomic coordinates* (×10$^4$) *and equivalent isotropic thermal parameters* (Å×10$^2$) *with e.s.d.'s in parentheses for* (I)

$B_{eq} = (8\pi^2/3)\sum_i\sum_j U_{ij}a_i^*a_j^*\mathbf{a}_i.\mathbf{a}_j$.

| | $x$ | $y$ | $z$ | $B_{eq}$ |
|---|---|---|---|---|
| C(1) | 6271 (2) | 2826 (2) | 3794 (1) | 134 (5) |
| C(2) | 6298 (2) | 3590 (3) | 3078 (1) | 145 (5) |
| N(3) | 6297 (1) | 2897 (2) | 2360 (1) | 155 (5) |
| C(4) | 6313 (2) | 1373 (3) | 2247 (1) | 154 (5) |
| O(4) | 6359 (1) | 825 (2) | 1572 (1) | 207 (4) |
| C(5) | 6275 (2) | 553 (2) | 2995 (1) | 145 (5) |
| C(51) | 6274 (2) | −1046 (3) | 2941 (1) | 165 (5) |
| N(52) | 6280 (2) | −2321 (2) | 2911 (1) | 226 (5) |
| C(6) | 6255 (2) | 1256 (3) | 3727 (1) | 140 (5) |
| C(1′) | 6294 (2) | 3641 (2) | 4576 (1) | 136 (5) |
| C(2′) | 5757 (2) | 3190 (3) | 5222 (1) | 149 (5) |
| C(21′) | 5091 (2) | 1842 (3) | 5222 (1) | 190 (6) |
| C(3′) | 5830 (2) | 4096 (3) | 5915 (1) | 169 (5) |
| N(4′) | 6362 (2) | 5343 (2) | 5999 (1) | 172 (5) |
| C(5′) | 6870 (2) | 5768 (3) | 5386 (1) | 180 (6) |
| C(6′) | 6870 (2) | 4958 (3) | 4675 (1) | 158 (5) |

As shown in Table 7, the pyridone ring geometry of molecule (I) has a quinoid-like structure, while molecule (II) has a benzene-like resonance structure. In addition, the C—N distances in the pyridine ring of molecule (I) are shorter than the others in this series. The effects of these resonance structures are also reflected in the hydrogen bonding and stacking patterns of (I).

The C(2′)—C(3′) distances observed in (I), (II) and (IV) are significantly longer than those found in (III) and milrinone (Cody, 1987). The geometry of (II) is less distorted than observed for (I), milrinone (Cody, 1987) and 2-methyl-[3,4′-bipyridin]-6(1*H*)-one (Cody & Wojtczak, 1991*a*), since the molecular conformation with both rings almost perpendicular minimizes the repulsive interactions between the methyl substituents

Table 4. *Fractional atomic coordinates* ($\times 10^4$) *and equivalent isotropic thermal parameters* (Å$\times 10^2$) *with e.s.d.'s in parentheses for* (II)

$B_{eq} = (8\pi^2/3)\sum_i\sum_j U_{ij}a_i^*a_j^*\mathbf{a}_i.\mathbf{a}_j$.

| | x | y | z | $B_{eq}$ |
|---|---|---|---|---|
| C(1) | 4874 (3) | 8758 (3) | 8074 (1) | 120 (5) |
| C(2) | 7107 (3) | 7840 (2) | 8185 (1) | 123 (5) |
| C(21) | 8741 (3) | 7910 (3) | 7365 (1) | 162 (5) |
| N(3) | 7935 (2) | 6876 (2) | 9122 (1) | 119 (4) |
| C(4) | 6742 (3) | 6698 (2) | 9998 (1) | 118 (5) |
| O(4) | 7671 (2) | 5666 (2) | 10807 (1) | 155 (3) |
| C(5) | 4424 (3) | 7755 (3) | 9886 (1) | 119 (4) |
| C(51) | 3052 (3) | 7706 (3) | 10765 (1) | 135 (5) |
| N(52) | 1915 (3) | 7678 (2) | 11459 (1) | 196 (5) |
| C(6) | 3547 (3) | 8733 (3) | 8941 (1) | 125 (5) |
| C(1′) | 3827 (3) | 9779 (3) | 7070 (1) | 130 (5) |
| C(2′) | 3830 (3) | 8548 (3) | 6320 (1) | 143 (5) |
| C(21′) | 4964 (4) | 6134 (3) | 6440 (2) | 210 (6) |
| C(3′) | 2685 (3) | 9661 (3) | 5437 (1) | 174 (5) |
| N(4′) | 1618 (3) | 11803 (2) | 5253 (1) | 187 (5) |
| C(5′) | 1647 (3) | 12942 (3) | 5981 (1) | 187 (5) |
| C(6′) | 2701 (3) | 12013 (3) | 6895 (1) | 168 (5) |

Table 5. *Fractional atomic coordinates* ($\times 10^4$) *and equivalent isotropic thermal parameters* (Å$\times 10^2$) *with e.s.d.'s in parentheses for* (III)

$B_{eq} = (8\pi^2/3)\sum_i\sum_j U_{ij}a_i^*a_j^*\mathbf{a}_i.\mathbf{a}_j$.

| | x | y | z | $B_{eq}$ |
|---|---|---|---|---|
| C(1) | 2537 (4) | 1439 (3) | 60 (2) | 225 (7) |
| C(2) | 2045 (4) | 560 (3) | 656 (2) | 233 (7) |
| C(21) | 1366 (5) | 645 (3) | 1462 (2) | 315 (9) |
| N(3) | 2115 (4) | −648 (2) | 500 (1) | 251 (6) |
| C(4) | 2617 (4) | −978 (3) | −190 (2) | 256 (8) |
| O(4) | 2653 (4) | −2118 (2) | −260 (1) | 370 (7) |
| C(5) | 3086 (4) | 50 (3) | −820 (2) | 234 (7) |
| N(51) | 3625 (4) | −277 (2) | −1518 (2) | 313 (7) |
| C(6) | 3044 (5) | 1257 (3) | −683 (2) | 249 (7) |
| C(1′) | 2548 (4) | 2874 (3) | 146 (2) | 226 (7) |
| C(2′) | 2048 (5) | 3901 (3) | −487 (2) | 280 (8) |
| C(3′) | 2126 (5) | 5153 (3) | −419 (2) | 305 (8) |
| N(4′) | 2668 (4) | 5401 (2) | 253 (2) | 291 (7) |
| C(5′) | 3174 (5) | 4444 (3) | 868 (2) | 316 (9) |
| C(6′) | 3141 (5) | 3178 (3) | 825 (2) | 283 (8) |
| C(1*) | 2439 (4) | 8270 (3) | 4969 (2) | 224 (7) |
| C(2*) | 2934 (4) | 9257 (3) | 4378 (2) | 234 (7) |
| C(21*) | 3635 (5) | 9186 (3) | 3575 (2) | 329 (9) |
| N(3*) | 2855 (4) | 10459 (2) | 4539 (1) | 248 (6) |
| C(4*) | 2335 (4) | 10783 (3) | 5228 (2) | 253 (7) |
| O(4*) | 2293 (4) | 11919 (2) | 5306 (1) | 368 (7) |
| C(5*) | 1874 (4) | 9746 (3) | 5855 (2) | 246 (7) |
| N(51*) | 1335 (4) | 10058 (3) | 6559 (2) | 332 (8) |
| C(6*) | 1914 (4) | 8542 (3) | 5711 (2) | 249 (7) |
| C(1′*) | 2444 (4) | 6929 (3) | 4887 (2) | 241 (7) |
| C(2′*) | 1924 (5) | 6604 (3) | 4201 (2) | 319 (9) |
| C(3′*) | 1924 (5) | 5329 (3) | 4169 (2) | 356 (10) |
| N(4′*) | 2385 (4) | 4392 (2) | 4795 (2) | 336 (8) |
| C(5′*) | 2861 (5) | 4656 (3) | 5467 (2) | 338 (9) |
| C(6′*) | 2914 (5) | 5918 (3) | 5525 (2) | 294 (8) |
| Cl(1) | 6938 (2) | 2999 (1) | 2272 (1) | 413 (3) |
| Cl(2) | 1578 (2) | 6882 (1) | 1892 (1) | 433 (3) |
| O(*W*1) | 7800 (5) | 7746 (3) | 2708 (2) | 494 (9) |
| O(*W*2) | 3472 (5) | 2660 (3) | 3426 (2) | 604 (10) |
| O(W3) | 5544 (5) | 5835 (3) | 2636 (3) | 751 (14) |
| O(*W*4) | 519 (5) | 4131 (3) | 2683 (2) | 696 (12) |

* Molecule 2 in asymmetric unit (III).

and the neighboring rings, and the ring angles at C(1) and C(1′) are closer to 120° than those found in other structures. Methylation in either the pyridone or pyridine ring influences the ring geometry around the methylated

Table 6. *Fractional atomic coordinates* ($\times 10^4$) *and equivalent isotropic thermal parameters* (Å$\times 10^2$) *with e.s.d.'s in parentheses for* (IV)

$B_{eq} = (8\pi^2/3)\sum_i\sum_j U_{ij}a_i^*a_j^*\mathbf{a}_i.\mathbf{a}_j$.

| | x | y | z | $B_{eq}$ |
|---|---|---|---|---|
| C(1) | −2103 (2) | 3918 (1) | 3811 (3) | 178 (5) |
| C(2) | −2054 (2) | 4633 (1) | 2630 (3) | 192 (5) |
| N(3) | −1000 (2) | 4629 (1) | 1688 (2) | 185 (4) |
| C(4) | 28 (2) | 3959 (1) | 1837 (3) | 173 (5) |
| C(5) | 23 (2) | 3209 (1) | 3127 (3) | 173 (5) |
| C(6) | −1036 (2) | 3204 (1) | 4065 (3) | 178 (5) |
| O(4) | 923 (2) | 3985 (1) | 856 (2) | 207 (4) |
| N(51) | 1064 (2) | 2538 (1) | 3287 (3) | 243 (5) |
| C(1′) | −3227 (2) | 3868 (1) | 4859 (3) | 181 (5) |
| C(2′) | −4715 (2) | 3839 (1) | 3988 (3) | 194 (5) |
| C(3′) | −5660 (2) | 3800 (2) | 5128 (3) | 223 (5) |
| N(4′) | −5252 (2) | 3762 (1) | 6978 (3) | 250 (5) |
| C(5′) | −3822 (3) | 3760 (2) | 7771 (3) | 258 (6) |
| C(6′) | −2783 (2) | 3819 (2) | 6787 (3) | 225 (5) |
| C(21′) | −5328 (2) | 3819 (2) | 1915 (3) | 248 (6) |

Table 7. *Bond lengths* (Å) *for* (I), (II), (III) *and* (IV) *with e.s.d.'s in parentheses*

| | (I) | (II) | (III) Molecule 1 | (III) Molecule 2 | (IV) |
|---|---|---|---|---|---|
| C(1)—C(2) | 1.372 (3) | 1.379 (2) | 1.374 (4) | 1.375 (4) | 1.362 (3) |
| C(1)—C(6) | 1.415 (3) | 1.398 (2) | 1.428 (4) | 1.425 (4) | 1.419 (3) |
| C(1)—C(1′) | 1.484 (3) | 1.494 (2) | 1.470 (4) | 1.473 (4) | 1.488 (3) |
| C(2)—N(3) | 1.341 (3) | 1.363 (2) | 1.374 (4) | 1.370 (4) | 1.372 (3) |
| C(2)—C(21) | — | 1.489 (3) | 1.500 (5) | 1.496 (5) | — |
| N(3)—C(4) | 1.382 (3) | 1.374 (2) | 1.360 (4) | 1.361 (4) | 1.355 (3) |
| C(4)—C(5) | 1.445 (3) | 1.432 (2) | 1.437 (4) | 1.437 (4) | 1.445 (3) |
| C(4)—O(4) | 1.228 (3) | 1.251 (2) | 1.250 (4) | 1.249 (4) | 1.266 (3) |
| C(5)—C(6) | 1.369 (3) | 1.384 (2) | 1.362 (4) | 1.363 (4) | 1.374 (3) |
| C(5)—C(51) | 1.439 (3) | 1.430 (2) | — | — | — |
| C(51)—N(52) | 1.147 (3) | 1.150 (2) | — | — | — |
| C(5)—N(51) | — | — | 1.379 (4) | 1.382 (4) | 1.364 (3) |
| C(1′)—C(2′) | 1.397 (3) | 1.398 (3) | 1.404 (4) | 1.400 (5) | 1.397 (3) |
| C(1′)—C(6′) | 1.406 (3) | 1.395 (2) | 1.396 (5) | 1.396 (4) | 1.397 (3) |
| C(2′)—C(3′) | 1.401 (3) | 1.395 (2) | 1.378 (5) | 1.377 (5) | 1.397 (3) |
| C(2′)—C(21′) | 1.495 (4) | 1.501 (2) | — | — | 1.509 (3) |
| C(3′)—N(4′) | 1.321 (3) | 1.338 (2) | 1.345 (5) | 1.336 (4) | 1.339 (3) |
| N(4′)—C(5′) | 1.321 (3) | 1.336 (3) | 1.342 (4) | 1.337 (5) | 1.336 (3) |
| C(5′)—C(6′) | 1.382 (3) | 1.385 (2) | 1.376 (5) | 1.381 (5) | 1.385 (4) |

position, decreasing the value of the *exo* angle on either C(2) or C(2′) atoms (Table 8). The steric hindrance resulting from such methylation is then minimized by an increase in C(2)—C(1)—C(1′) or C(2′)—C(1′)—C(1) angles. Methylation at the 2′ position of the pyridine ring results in a smaller value for the C(1′)—C(2′)—C(3′) angle for (I), (II) and (IV) (Table 8) compared with 119.7 (1)° in milrinone (Cody, 1987) or 119.8 (1)° in its 2-methyl analogue (Cody & Wojtczak, 1991*a*). These changes in geometry are consistent with the patterns observed from the analysis of the variance of monosubstituted benzene rings, which indicate that much of the benzene ring distortions are related to the $\sigma$ electronegativity or $\pi$-donor/acceptor character of the substituent (Domenicano, Murray-Rust & Vaciage, 1983).

Methylation at the 2-position of the pyridone ring in these inotropic bipyridines causes the molecule to deviate from planarity. The torsion angle values found in methylated analogues, milrinone (Cody, 1987) and two independent molecules of (III) [45.2 (2), −38.5 (4)

Table 8. *Bond angles* (°) *for* (I), (II), (III) *and* (IV) *with e.s.d.'s in parentheses*

| | | | (III) | | |
|---|---|---|---|---|---|
| | (I) | (II) | Molecule 1 | Molecule 2 | (IV) |
| C(2)—C(1)—C(6) | 115.6 (2) | 118.3 (2) | 118.8 (3) | 118.7 (2) | 118.8 (2) |
| C(2)—C(1)—C(1′) | 120.4 (2) | 122.7 (2) | 123.1 (3) | 123.8 (2) | 122.1 (2) |
| C(6)—C(1)—C(1′) | 124.0 (2) | 119.0 (2) | 118.0 (3) | 117.5 (2) | 119.0 (2) |
| C(1)—C(2)—N(3) | 122.3 (2) | 119.1 (2) | 117.2 (2) | 117.3 (2) | 119.1 (2) |
| C(1)—C(2)—C(21) | — | 125.1 (2) | 128.0 (3) | 128.1 (3) | — |
| C(21)—C(2)—N(3) | — | 115.7 (2) | 114.7 (3) | 114.5 (2) | — |
| C(2)—N(3)—C(4) | 125.5 (2) | 126.0 (2) | 126.6 (3) | 126.6 (2) | 124.9 (1) |
| N(3)—C(4)—O(4) | 121.6 (2) | 120.8 (2) | 121.0 (3) | 121.1 (2) | 121.0 (1) |
| N(3)—C(4)—C(5) | 112.8 (2) | 114.5 (2) | 116.3 (3) | 116.2 (2) | 116.8 (1) |
| O(4)—C(4)—C(5) | 125.6 (2) | 124.7 (3) | 122.7 (3) | 122.7 (2) | 122.1 (1) |
| C(4)—C(5)—C(6) | 121.8 (2) | 120.4 (2) | 118.6 (3) | 118.6 (2) | 118.6 (1) |
| C(4)—C(5)—C(51) | 117.1 (2) | 118.4 (2) | — | — | — |
| C(4)—C(5)—N(51) | — | — | 116.8 (3) | 117.0 (2) | 116.8 (1) |
| C(51)—C(5)—C(6) | 121.1 (2) | 121.2 (2) | — | — | — |
| N(51)—C(5)—C(6) | — | — | 124.5 (3) | 124.4 (2) | 124.5 (2) |
| C(5)—C(51)—N(52) | 178.8 (2) | 178.6 (2) | — | — | — |
| C(5)—C(6)—C(1) | 121.9 (2) | 121.6 (2) | 122.4 (3) | 122.5 (2) | 121.6 (2) |
| C(1)—C(1′)—C(2) | 123.7 (2) | 121.8 (2) | 119.8 (3) | 123.2 (3) | 122.6 (2) |
| C(1)—C(1′)—C(6′) | 118.7 (2) | 119.3 (2) | 122.6 (3) | 119.5 (3) | 119.0 (2) |
| C(2)—C(1′)—C(6′) | 117.3 (2) | 118.8 (2) | 117.5 (3) | 117.3 (3) | 118.4 (2) |
| C(1′)—C(2′)—C(3′) | 117.2 (2) | 117.0 (2) | 120.0 (3) | 120.1 (3) | 117.0 (2) |
| C(1′)—C(2′)—C(21′) | 124.9 (2) | 122.9 (2) | — | — | 123.5 (2) |
| C(21)—C(2′)—C(3′) | 117.5 (2) | 120.1 (2) | — | — | 119.5 (2) |
| C(2′)—C(3′)—N(4′) | 124.8 (2) | 125.0 (2) | 120.4 (3) | 120.5 (3) | 125.2 (2) |
| C(3′)—N(4′)—C(5′) | 117.8 (2) | 116.8 (2) | 121.3 (3) | 121.5 (3) | 116.4 (2) |
| N(4′)—C(5′)—C(6′) | 122.8 (2) | 123.4 (2) | 120.5 (3) | 120.2 (3) | 123.5 (2) |
| C(1′)—C(6′)—C(5′) | 119.7 (2) | 119.1 (2) | 120.4 (3) | 120.3 (3) | 119.3 (2) |

and 36.8 (4)°, respectively] reflect a similar influence of methylation on the conformation in these structures. The increased twist of these methylated molecules compared with the more planar nonmethylated parent compounds suggests a major contribution of steric hindrance to the molecular conformation. The influence of the 2-methyl group on the molecular conformation is even greater for molecules not substituted in position 5 of the pyridone ring. The torsion angle found in 2-methyl-3,4′-bipyridin-6(1H)-one is 58.8 (2) and 55.9 (2)° for two polymorphs, respectively (Cody & Wojtczak, 1991*a*).

The effect of a 2′-methyl substituent on the molecular conformation in (I) is similar to that of the 2-methyl observed for milrinone (Cody, 1987), although the orientation of the 2′-methyl differs greatly from the other 2′-methyl analogues. The molecular twist caused by a 2′-methyl group in (IV) is one of the largest found for monomethylated derivatives in the series, and is similar to that of the 2-methyl analogue (Cody & Wojtczak, 1991*a*). In (II), the only derivative methylated in both 2- and 2′- positions, the steric hindrance was minimized by the adoption of an almost perpendicular conformation of the molecule (Fig. 1).

### 3.1. *Crystal packing*

As illustrated in Table 9 and Figs. 2–5, the bipyridine molecules form a network of hydrogen bonds with the 4-keto or 4′-N of the pyridine ring acting as acceptors and the 3-NH or 5-amino group acting as donors. All bipyridine inotropic agents reported here also participate in stacking interactions involving both bipyridine rings, with ring–ring distances varying from 3.3 to 3.5 Å. This results in a hyperparallelism of the ring layers in some crystal lattices, and the addition of noncrystallographic symmetry elements in others which is reflected in the hypercentric distributions in the average $\langle(E^2 - 1)^3\rangle$ observed for (II), (III) and (IV) (Table 1). A similar pattern was also observed in the two polymorphs of the 2-methyl derivative (Cody & Wojtczak, 1991*a*).

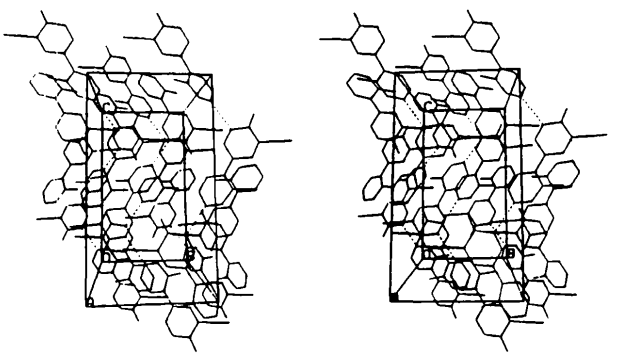


Fig. 2. Stereo packing diagram of (I); hydrogen bonds are indicated as broken lines.

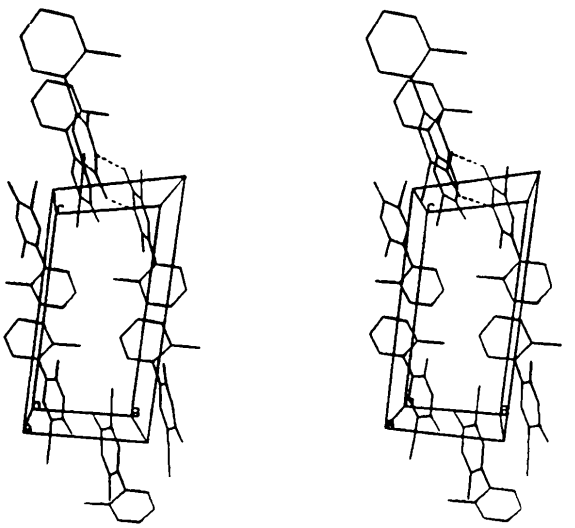


Fig. 3. Stereo packing diagram of (II); hydrogen bonds are indicated as broken lines.

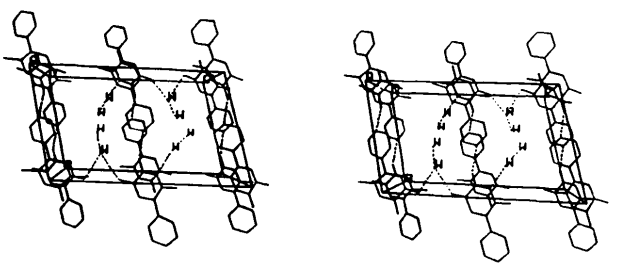


Fig. 4. Stereo packing diagram of (III).HCl.2$H_2O$; hydrogen bonds are indicated as broken lines.

As illustrated in Fig. 2, the crystal packing of (I) indicates that the molecules form layers with ring stacking and hydrogen bonding between the pyridine N and the pyridone ring N—H (Table 9). Of the bipyridines studied, this motif has been observed only in the structure of amrinone which has four independent conformers in the asymmetric unit (Cody, 1987). The 4-keto function does not participate in any intermolecular interactions, as observed in the other bipyridine structures. In (II) both rings are involved in stacking interactions and the packing can be described as layers of parallel rings (Fig. 3). The difference in hydrogen-bonding patterns between (I) and (II) (Table 9) is reflected in the hypersymmetry observed in the data and its higher density (Table 1). The packing of the two independent molecules in (III) (Fig. 4) is made up of repeating and alternate layers of the two molecules parallel to the (001) plane and are separated by layers of water molecules and chloride ions. The two independent molecules are related by a noncrystallographic center of symmetry $(\frac{1}{4},0,\frac{1}{4})$ and a noncrystallographic twofold axis. The solvent layers do not reproduce this pseudosymmetry and result in a superlattice structure. The orientation of both independent cations in (III) and the similarity of their conformations results in a noncrystallographic center of symmetry relating them in the crystal lattice. In the structure of (IV), the hypercentric values observed for the general reflections suggests hypercentrosymmetry or hyperparallelism. An analysis of the packing of (IV) (Fig. 5) reveals stacking interactions [C(2′)—C(3′) 3.443 (3) Å] between pyridine rings that are almost perpendicular to the $2_1$ screw axis along the *b* axis and indicates that hyperparallelism is the reason for the observed *E* distributions.

These structural data reveal conformational flexibility, which is influenced by substituent patterns in both the pyridone and pyridine rings of this series of inotropic bipyridines, as the most active analogues are those with 2-methyl and 5-cyano substituents. Although the effect on conformation by the 2′-methyl substituent is similar to that of the 2-methyl group, the effect on $Ca^{2+}$-ATPase stimulatory activity is less pronounced as bipyridines with 2-methyl and 5-cyano substituents are more active (Mylotte *et al.*, 1985; Cody *et al.*, 1990).

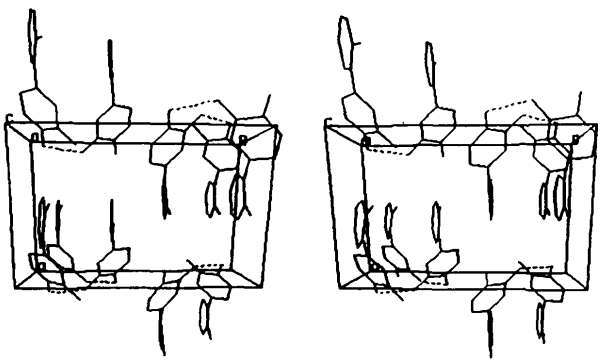


Fig. 5. Stereo packing diagram of (IV); hydrogen bonds are indicated as broken lines.

Table 9. *Hydrogen-bond geometry in bipyridine analogues*

| *D*···*A* | *D*—H | H···*A* | *D*···*A* | ∠*D*—H···*A* | Symmetry code |
|---|---|---|---|---|---|
| (I) | | | | | |
| N(3)···N(4′) | 0.87 (3) | 1.89 (3) | 2.759 (2) | 174 (3) | $x, 1-y, z-\frac{1}{2}$ |
| (II) | | | | | |
| N(3)···O(4) | 0.95 (2) | 1.80 (2) | 2.747 (2) | 176 (2) | $2-x, 1-y, 2-z$ |
| (III) | | | | | |
| N(51)···O(*W*1) | 0.95 | 2.24 | 3.126 (4) | 155 | $1-x, 1-y, -z$ |
| N(4′)···O(4) | | | 2.605 (3) | | $x, y+1, z$ |
| N(4′*)···O(4*) | | | 2.605 (3) | | $x, y-1, z$ |
| N(3*)···O(*W*2) | 0.86 | 1.93 | 2.773 (4) | 168 | $x, y+1, z$ |
| N(51*)···O(*W*1) | 0.90 | 2.21 | 3.047 (5) | 154 | $1-x, 2-y, 1-z$ |
| O(*W*1)···Cl(2) | | | 3.171 (4) | | $1+x, y, z$ |
| O(*W*2)···Cl(1) | 0.91 | 2.25 | 3.159 (4) | 173 | $x, y, z$ |
| O(*W*2)···Cl(2) | | | 3.165 (4) | | $x, y, z$ |
| O(*W*3)···O(*W*1) | | | 2.776 (5) | | $x, y, z$ |
| O(*W*4)···Cl(1) | 0.93 | 2.21 | 3.139 (4) | 173 | $-1+x, y, z$ |
| O(*W*4)···Cl(2) | | | 3.127 (4) | | $x, y, z$ |
| O(*W*4)···O(*W*2) | | | 2.691 (5) | | $x, y, z$ |
| (IV) | | | | | |
| N(3)···O(4) | 0.92 | 1.84 | 2.766 (2) | 175 | $-x, 1-y, -z$ |
| N(51)···O(4) | 0.90 | 2.04 | 2.934 (2) | 168 | $x, \frac{1}{2}-y, \frac{1}{2}+z$ |

* Molecule 2 in asymmetric unit (III).

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