3-Amino-6,6′-bis(methoxycarbonyl)-2,2′-bipyridine, a Model for the Central Chelation Unit of Streptonigrin†

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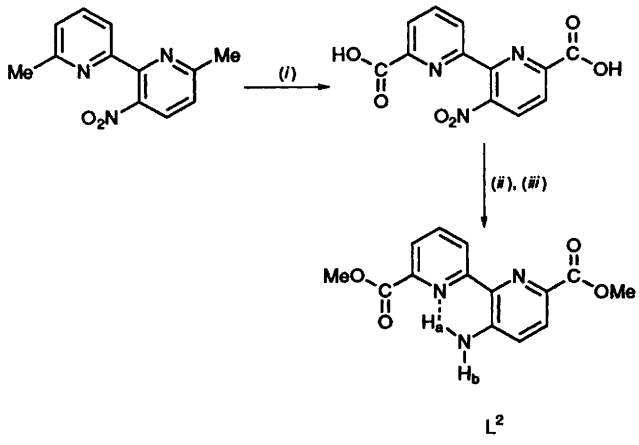
The synthesis, crystal structure and solution conformation of 3-amino-6,6′-bis(methoxycarbonyl)-2,2′-bipyridine (L^2) have been determined. The crystal structure was refined to a residual of 0.046 for 2218 independent observed reflections. The crystals are monoclinic, space group $P2_1/c$, a = 9.229(2), b = 10.342(2), c = 13.650(2) Å, β = 90.92(2)°. The two pyridyl rings are almost coplanar as a consequence of an intramolecular hydrogen bond between the amino group and the pyridyl nitrogen in the adjacent ring. The same conformation is observed in solution. The effect of solvent, temperature and concentration of zinc(II) on the types of complexes formed by 3-amino-6,6′-dimethyl-2,2′-bipyridine (L^1) and L^2 was studied. For L^1 the 2:1 complex is more stable than the 1:1 complex. Substantial stabilization of the 1:1 complex of L^2 occurs due to co-ordination of the ester groups at the 6,6′ positions. The relevance of these results to the structure and properties of the antitumour drug streptonigrin is discussed.

Streptonigrin is a highly functionalized 7-aminoquinoline-5,8-dione which is active against a range of human cancers.[1–6] The activity of the drug is believed to arise from DNA strand scission *via* a reductive mechanism involving metal ions. A range of transition-metal ions interact with streptonigrin,[7–13] and it has been suggested that their involvement in the mechanism of action of the drug is either by direct complexation with streptonigrin or reduced streptonigrin[9,12–17] and/or catalysing the production of hydroxyl radical.[12,14–19] Zinc(II)–streptonigrin complexes appear to have unique properties compared with other transition metal–streptonigrin complexes. Reduction of the quinone ring of streptonigrin is inhibited by zinc(II) and this effect has been suggested to be due to a zinc-assisted tautomeric shift.[7] A stable zinc(II)–streptonigrin–DNA complex has also been reported,[13] suggesting that zinc(II) acts as a delivery agent for streptonigrin to DNA.

We have determined the solution conformation of streptonigrin in [$^2H_8$]tetrahydrofuran,[20] and have reported the synthesis and co-ordination chemistry of 3-amino-6,6′-dimethyl-2,2′-bipyridine (L^1), a model for the central bipyridyl site in streptonigrin.[21] In solution, L^1 co-ordinates to Cu^I, Cd^{II} and Zn^{II} through the bipyridyl nitrogens. In view of the biological importance of zinc–streptonigrin complexes, we have attempted to identify the metal-binding sites of streptonigrin with zinc(II) in solution. Variable-temperature NMR spectroscopy showed an equilibrium between multiple complexes on the NMR time-scale and full assignment of the complexes has not been possible. In order to assist in the interpretation of NMR data and ascertain the role of the carboxyl groups on metal binding, we now report the metal-binding properties of a second streptonigrin analogue, 3-amino-6,6′-bis(methoxycarbonyl)-2,2′-bipyridine (L^2). Owing to the particular importance of Zn^{II} in the biological activity of streptonigrin, this study focused on determination of the relative amounts and stabilities of the zinc(II) chelates of L^2 in solution.

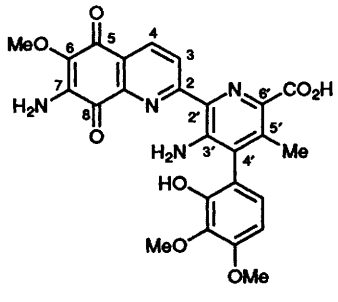
Results and Discussion

Synthesis of 3-Amino-6,6′-bis(methoxycarbonyl)-2,2′-bipyridine.—3-Amino-6,6′-bis(methoxycarbonyl)-2,2′-bipyridine L^2 was prepared as shown in Scheme 1. Oxidation of 6,6′-dimethyl-3-nitro-2,2′-bipyridine with chromium(VI) oxide in concentrated sulfuric acid[22] afforded crude 6,6′-dicarboxy-3-nitro-2,2′-bipyridine in good yield. Purification of the diacid was difficult due to poor solubility and hence it was immediately



L^2

Scheme 1 (*i*) H_2SO_4, CrO_3, 0 °C; (*ii*) $SOCl_2$, MeOH; (*iii*) H_2, Pd–C, EtOH



Streptonigrin

† *Supplementary data available* (No. SUP 57066, 6 pp.): chemical shifts. See Instructions for Authors, *J. Chem. Soc., Dalton Trans.*, 1995, Issue 1, pp. xxv–xxx.

Table 1 Positional parameters for L^2

Atom	x	y	z
O(1)	0.0989(3)	−0.0840(3)	0.7881(2)
O(2)	0.0340(3)	−0.2211(3)	0.9065(2)
O(3)	0.6748(4)	0.4297(3)	1.1464(2)
O(4)	0.7942(3)	0.5263(3)	1.0258(2)
N(1)	0.2762(3)	0.0435(3)	0.9175(2)
N(2)	0.5278(3)	0.2808(3)	1.0090(2)
N(3)	0.4204(4)	0.1496(4)	0.7688(3)
C(1)	−0.0477(5)	−0.2948(5)	0.8338(4)
C(2)	0.1046(4)	−0.1167(4)	0.8729(3)
C(3)	0.1859(4)	−0.0480(4)	0.9511(3)
C(4)	0.1731(4)	−0.0753(4)	1.0501(3)
C(5)	0.2544(5)	−0.0045(4)	1.1161(3)
C(6)	0.3467(4)	0.0899(4)	1.0830(3)
C(7)	0.3563(4)	0.1108(4)	0.9812(3)
C(8)	0.4592(4)	0.2078(4)	0.9419(3)
C(9)	0.4892(4)	0.2179(4)	0.8395(3)
C(10)	0.5973(5)	0.3066(4)	0.8140(3)
C(11)	0.6655(4)	0.3798(4)	0.8832(3)
C(12)	0.6282(4)	0.3654(4)	0.9811(3)
C(13)	0.6975(4)	0.4410(4)	1.0601(3)
C(14)	0.8651(5)	0.6081(5)	1.0968(3)

Table 2 Bond lengths (Å) and angles (°) for L^2

O(1)–C(2)	1.206(5)	O(2)–C(1)	1.453(6)
O(2)–C(2)	1.345(5)	O(3)–C(13)	1.205(5)
O(4)–C(13)	1.344(5)	O(4)–C(14)	1.436(5)
N(1)–C(3)	1.346(5)	N(1)–C(7)	1.329(5)
N(2)–C(8)	1.338(5)	N(2)–C(12)	1.334(5)
N(3)–C(9)	1.347(5)	C(2)–C(3)	1.477(6)
C(3)–C(4)	1.388(5)	C(4)–C(5)	1.374(6)
C(5)–C(6)	1.377(6)	C(6)–C(7)	1.410(5)
C(7)–C(8)	1.488(5)	C(8)–C(9)	1.433(5)
C(9)–C(10)	1.403(6)	C(10)–C(11)	1.358(6)
C(11)–C(12)	1.393(5)	C(12)–C(13)	1.471(6)
C(1)–O(2)–C(2)	115.9(4)	C(13)–O(4)–C(14)	116.7(3)
C(3)–N(1)–C(7)	119.1(4)	C(8)–N(2)–C(12)	119.8(4)
O(1)–C(2)–O(2)	122.6(4)	O(1)–C(2)–C(3)	124.9(4)
O(2)–C(2)–C(3)	112.5(4)	N(1)–C(3)–C(2)	113.7(4)
N(1)–C(3)–C(4)	122.5(4)	C(2)–C(3)–C(4)	123.8(4)
C(3)–C(4)–C(5)	118.4(4)	C(4)–C(5)–C(6)	119.8(4)
C(5)–C(6)–C(7)	118.8(4)	N(1)–C(7)–C(6)	121.4(4)
N(1)–C(7)–C(8)	117.9(4)	C(6)–C(7)–C(8)	120.6(4)
N(2)–C(8)–C(7)	115.5(4)	N(2)–C(8)–C(9)	122.0(4)
C(7)–C(8)–C(9)	122.4(4)	N(3)–C(9)–C(8)	124.4(4)
N(3)–C(9)–C(10)	119.6(4)	C(8)–C(9)–C(10)	116.1(4)
C(9)–C(10)–C(11)	121.0(4)	C(10)–C(11)–C(12)	119.3(4)
N(2)–C(12)–C(11)	121.8(4)	N(2)–C(12)–C(13)	115.8(4)
C(11)–C(12)–C(13)	122.3(4)	O(3)–C(13)–O(4)	122.1(4)
O(3)–C(13)–C(12)	125.7(4)	O(4)–C(13)–C(12)	112.1(4)

converted into the soluble diester by treatment with thionyl chloride followed by methanol. 6,6′-Bis(methoxycarbonyl)-3-nitro-2,2′-bipyridine was cleanly reduced using hydrogenation to give L^2 in 92% yield. The alternate route to L^2 whereby the nitro group was initially reduced using tin(II) chloride and concentrated hydrochloric acid, followed by esterification with methanol afforded a reduced yield compared with the procedure outlined in Scheme 1.

The proton and quaternary carbon NMR spectra of L^2 were assigned using proton decoupling and long-range carbon–proton decoupling experiments optimized for three-bond coupling.[23] A crystal structure of L^2 was obtained (Fig 1). Selected bond angles and lengths and positional parameters are summarized in Tables 1 and 2. The molecule adopts a nearly planar conformation with the two pyridyl rings in a *trans* orientation and rotated by 9° with respect to each other. The near coplanarity of the pyridyl rings is promoted by an

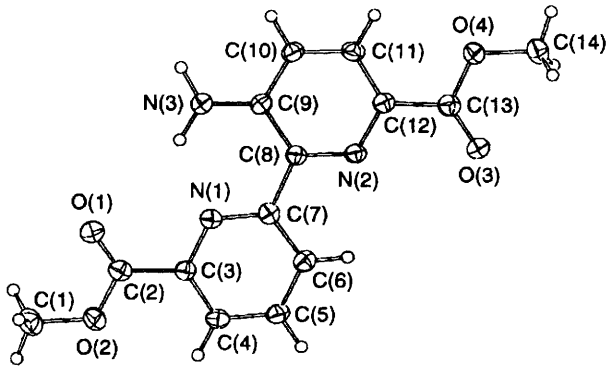


Fig. 1 An ORTEP depiction and numbering scheme of L^2

intramolecular hydrogen bond between a proton on the amine group, N(3), and the pyridyl nitrogen, N(1) [N(1) ··· N(3) 2.68, N(1) ··· H(1N) 1.97 Å]. There is also a weak intermolecular hydrogen bond between the other amine proton and the carbonyl oxygen, O(3).

Titrations of Zinc(II) *with* L^1.—A series of titration experiments with zinc(II) and L^1 and L^2 were carried out in several solvents (CD_3CN, [$^2H_8$]tetrahydrofuran, CD_3NO_2) and the resultant complexes were studied by variable-temperature NMR spectroscopy. We previously reported that L^1 forms a bipyridyl complex with Zn^{II} at room temperature.[21] In order to identify the exact species in solution, additional titration experiments and variable-temperature studies were carried out on this complex.

Compound L^1 forms two complexes in solution, the 1:1 complex $[ZnL^1]^{2+}$ **1a** and the 1:2 complex $[ZnL^1_2]^{2+}$ **1b**. The relative stabilities of **1a** and **1b** are dependent on solvent, temperature and the number of equivalents of zinc(II) trifluoromethanesulfonate (triflate) added to the solution. Complexes **1a** and **1b** were assigned on the basis of the chemical shifts of the methyl groups at positions 6 and 6′. For **1b**, the methyl resonances are shifted upfield relative to L^1, while for **1a** they are shifted downfield. The latter slight downfield shift is consistent with the metal co-ordination.[24] In contrast, in **1b** the methyl groups at positions 6, 6′ are shielded by the aromatic rings of the second ligand molecule (see **2a, 2b** in Fig. 2).

In CD_3CN, complex **1b** is the only species observed in the range 200–300 K and up to 5–10 equivalents of zinc(II). The 1:1 complex **1a** was only observed in the presence of a large excess of zinc(II) (18.0 equivalents); under these conditions the relative amounts of the two complexes present were **1a**:**1b** = 0.09:1.0 at 236 K, while at higher temperatures (300 K) the amount of **1a** increased, **1a**:**1b** = 1.0:1.0.

In [$^2H_8$]tetrahydrofuran, a strong co-ordinating solvent, addition of an excess of Zn^{II} (18.0 equivalents) to a solution of L^1 was necessary for complexation. Under these conditions, at room temperature, a slightly broadened spectrum was obtained consistent with exchange between L^1 and complex **1a**. At low temperature (236 K) only a small amount of **1b** was detected. These results are consistent with competitive co-ordination of [$^2H_8$]tetrahydrofuran with Zn^{II} which destabilizes **1b** compared with the same experiment in CD_3CN.

Titrations of Zinc(II) *with* L^2.—A similar set of experiments were carried out with L^2. A typical set of spectra in CD_3CN are shown in Fig. 2. As in the case of L^1, formation of two complexes, **2a** and **2b**, was observed. However, the relative stabilities of these compared with the corresponding complexes **1a** and **1b** were markedly different.

Addition of 0.5 equivalent of Zn^{II} to a CD_3CN solution of L^2 at 236 K afforded exclusively **2b**, which was assigned to the 2:1 complex, in which two bipyridyl groups chelate to the zinc(II) [Fig. 2(*b*)]. Addition of 1.0 equivalent afforded a

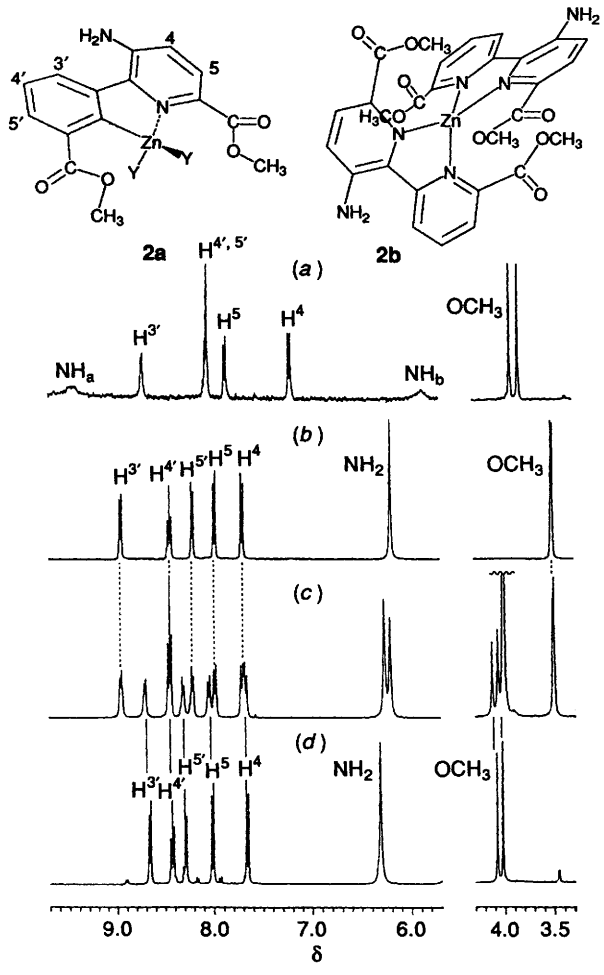
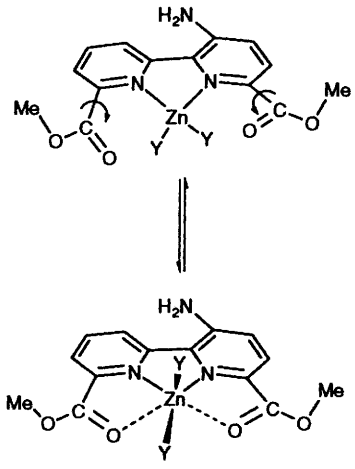


Fig. 2 Proton NMR (CD_3CN, 400 MHz) spectra at 236 K of (*a*) L^2, (*b*) L^2 + 0.5 equivalent $Zn(CF_3SO_3)_2$, giving **2b**, (*c*) L^2 + 1.0 equivalent $Zn(CF_3SO_3)_2$ giving **2a**:**2b** = 45:55 and (*d*) L^2 + 18.0 equivalents $Zn(CF_3SO_3)_2$ giving **2a**

mixture of **2b** and a new complex **2a** [Fig. 2(*c*)], and in the presence of an excess of Zn^{II}, **2a** was the only complex detected [Fig. 2(*d*)].

The differences in chemical shifts of the protons in complexes **2a** and **2b** (see SUP 57066), and the relative stabilities of these complexes compared with the analogous complexes formed by L^1, **1a** and **1b** respectively, provide clear evidence that the ester side-chains are involved in co-ordination with the metal centre in **2** (Scheme 2). First, while the 1:1 complex **2a** is present in a significant amount (≈50%) in CD_3CN and 1.0 equivalent of Zn^{II}, **1a** is not observed under identical conditions, *i.e.* in the absence of co-ordinating groups at the 6 and 6′ positions L^1 forms almost exclusively the 2:1 complex **1b**. Comparison of the chemical shifts of the protons in **2a** and **2b** supports these assignments. For the 1:1 complex **2a**, the methoxyl resonances shift downfield (0.10–0.27 ppm), relative to L^2 [Fig. 2(*d*)] as would be expected upon co-ordination of the ester side-chains. However, a similar downfield shift is observed for the methyl resonances of **1a** compared with L^1 and is attributed to metal–bipyridine co-ordination. In contrast, the methoxyl resonances shift upfield for **2b** (0.28–0.41 ppm), due to the ring-current effect of the second bipyridine ligand. Similar upfield shifts of protons and substituents at the 6 and 6′ positions of polymeric 2,2′-bipyridine metal complexes are well documented.[25,26] As zinc(II) stabilizes a variety of co-ordination geometries, it is likely that the ester groups are bound (and unbound, in rapid equilibrium) to the metal centre in **2b** to give a five- or six-co-ordinate complex (*e.g.* Scheme 2). Co-ordination of the methoxy



Scheme 2 Representation of binding of methoxyl oxygens to metal in complex **2**; Y = $CF_3SO_3^-$, solvent *etc.*

oxygens cannot be ruled out and ligands denoted Y (Scheme 2) may be vacant, solvent, triflate or L^2. The ^{13}C NMR spectra of the ligand and complexes were recorded but did not allow unambiguous assignment of which oxygen(s) were involved in complexation.

With the exception of $H^{3'}$, the aromatic protons in complexes **2a** and **2b** shift downfield relative to L^2 (0.11–0.69 ppm) due to complexation. The nearly coplanar geometry of the pyridyl rings in **2** places $H^{3'}$ and the amino group proximal, and as a consequence the environment (and hence chemical shift) of $H^{3'}$ is particularly sensitive to the interplanar angle.* Hence, if the geometries of the bipyridyl rings in **2a** and **2b** are similar, one would expect similar chemical shifts for $H^{3'}$ in each case. The slight upfield shift of $H^{3'}$ in **2a** (0.03–0.08 ppm), *versus* the large downfield shift of $H^{3'}$ in **2b** (0.20–0.27 ppm), compared with L^2, suggests that the interplanar angles are different in these two complexes.

For streptonigrin it has been suggested that complexation may occur at the aminopyridyl site rather than as a bipyridyl ligand. There was no evidence of zinc(II) binding to the 3-amino group in L^2. Complexation results in breaking of the hydrogen bond in L^2, and as a result the amino resonances of complex **2** appear upfield [Fig. 2(*b*)–2(*d*)] compared to those of H_a and H_b in L^2 [Fig. 2(*a*)]. At 236 K, the resonances of the amino protons in **2a** and **2b** shifted downfield by 0.18–0.44 ppm, relative to the non-hydrogen-bonded proton H_b of the amino group in L^2 (Scheme 1, Fig.2). For comparison, titrations of zinc(II) triflate into an CD_3CN solution of aniline at low temperature showed a downfield shift of 1.2 ppm of the amino resonance on complexation, *i.e.* the amino protons in **2** would be expected to resonate much further downfield of H_b if complexation involving the amino group occurred.

Effect of the Counter Ion.—Most studies were carried out using the triflate as counter ion due to the excellent solubility of the resultant complexes in a range of solvents. With this counter ion (1.0 equivalent), at room temperature, exchange between **2a** and **2b** is fast on the NMR time-scale and broadened resonances were observed in the NMR spectra. Hence assignments were made at low temperatures (*e.g.* Fig. 2). In contrast, with the less substitution-labile chloride counter ion, only signals due to the 1:1 complex **2a** were observed on titration of zinc(II) chloride (0.1–12.0 equivalents) at room temperature.

* In the crystal structure[21] of $[(CdL^1Cl_2)_2]$ an interplanar angle of 17.9° was observed; the twisting about the biaryl bond is largely due to the interaction of the amino group with the adjacent ring.

Effect of Solvent on Complex Stability.—Of particular importance to this study is the effect of solvents on the stabilities and structures of the complexes formed between L^2 and zinc(II). The solution conformation of streptonigrin was determined in [$^2H_8$]tetrahydrofuran, a polar solvent which allows the exchangeable protons to be observed on the NMR time-scale.[20] Metal complexes of streptonigrin have been studied in alcohol and aqueous solutions.[7–13]

Table 3 summarizes the effect of solvent and concentration of zinc(II) on the relative stabilities of the 1:1 and 2:1 complexes formed by L^1 and L^2. Solvents of higher donor number,[27] compete more strongly with L^2 for co-ordination to the zinc(II), thus lowering the stability of the complexes in that solvent. The high donor number of tetrahydrofuran reflects a strong interaction between the solvent and zinc(II). Hence, an excess of zinc(II) (2.0 equivalents) was required before any complexation was observed for both L^1 and L^2. In contrast, stoichiometric amounts of zinc(II) were required to give the corresponding complexes in CD_3CN. These results reflect the weaker interactions of CD_3CN with zinc(II), as is expected from the lower donor number of CD_3CN and the inability of zinc(II) to undergo d_π bonding with CD_3CN.

The relative stabilities of the complexes varied with temperature in all solvents studied. When the zinc(II) concentration was held constant the population of the 1:2 complex increased as the temperature was lowered.

Table 3 Effects of temperature, solvent and zinc(II) concentration on complexation of L^1 and L^2*

Solvent	Equivalents of $Zn(O_3SCF_3)_2$	T/K	L^1:**1a**:**1b**	L^2:**2a**:**2b**
CD_3CN	0.5	236	9:0:91	0:0:100
	1.0	236	0:0:100	0:45:55
	18.0	236	0:9:91	0:91:9
C_4D_8O	0.5	230		100:0:0
	1.0	230		84:8:8
	18.0	230	0:91:9	
		218		0:38:62
CD_3NO_2	0.5	248		30:0:70
	1.0	248	0:0:100	0:62:38
	saturated solution	248	0:0:100	0:75:25

* Ratios determined by integration of spectra.

Conclusion

This study establishes the importance of co-ordinating groups at the 6 and 6′ positions of 3-amino-2,2′-bipyridines in determining the types of complexes formed in solution. 3-Amino-6,6′-bis(methoxycarbonyl)-2,2′-bipyridine contains the key co-ordinating sites that are also present in the minimum subunit of streptonigrin required for biological activity.[28] Our results suggest that in streptonigrin, the oxygen substituents at positions 8 and 6′ have a significant role in stabilizing zinc(II)–streptonigrin complexes. As most studies of streptonigrin with zinc(II) have been carried out in polar solvents and a large excess of zinc, our results suggests that under these conditions the major species present is the 1:1 bipyridyl complex analogous to **2a**.

Experimental

Melting points were determined on a Reichert heating stage and are uncorrected. Ultraviolet spectra were recorded on a Hitachi 150–20 spectrophotometer, infrared spectra on a Perkin-Elmer 1600 FT-IR spectrometer. The NMR spectra were recorded on a Bruker AMX400 or an AC200 spectrometer. The temperature of the spectrometer probe was calibrated by the shift difference of methanol resonances in the ^1H NMR spectrum.[29] The ^1H and ^{13}C NMR spectra of L^2 were assigned using C–H correlation experiments (see SUP 57066). Spectra were recorded in the solvent indicated, locked on solvent deuterium and referenced to residual solvent protons. Mass spectra were recorded on an AEI MS-902 spectrometer at 70 eV (*ca.* 1.12×10^{-17} J).

Syntheses.—*6,6′-Dicarboxy-3-nitro-2,2′-bipyridine.* 6,6′-Dimethyl-3-nitro-2,2′-bipyridine[21] (517 mg, 2.26 mmol) was dissolved in concentrated sulfuric acid (7 cm^3). The yellow solution was cooled to 0 °C and chromium(VI) oxide (1.35 g, 13.6 mmol) added over 1 h. The red-orange reaction mixture was allowed to warm to room temperature, heated at 75 °C for 4 h and then stirred at room temperature for 12 h. The solution was poured onto an ice–water mixture and the green-white precipitate separated by centrifugation. The solid was collected, suspended in the minimum volume of water, basified with potassium hydroxide and filtered. The filtrate was acidified with hydrochloric acid to pH >3. The resultant fine white precipitate was isolated by centrifugation and washed with methanol affording pure 6,6′-dicarboxy-3-nitro-2,2′-bipyridine as a fine white powder (576 mg, 98%), m.p. >260 °C; $\tilde{\nu}_{max}/cm^{-1}$ 3482s, 3095w, 2923s, 2853m, 2460w, 1944w, 1687s, 1582w, 1541w and 1461w (KBr disc); δ_H[$(CD_3)_2SO$, 200 MHz] 8.26 (4 H, m) and 8.61 (1 H, d, J 8.2 Hz); *m/z* 289 ($M^{\bullet+}$, 73), 272 (15), 259 (23), 245 (100), 243 (11), 227 (44), 213 (20), 169 (26), 153 (59), 141 (21), 126 (29), 122 (10), 114 (21), 104 (11), 100 (18.4), 77 (26), 51 (21), 45 (16), 39 (15) and 32 (22%).

6,6′-Bis(methoxycarbonyl)-3-nitro-2,2′-bipyridine. 6,6′-Dicarboxy-3-nitro-2,2′-bipyridine (85.0 mg, 0.294 mmol) was dissolved in freshly distilled thionyl chloride (20 cm^3) and held at reflux for 8 h under nitrogen. The thionyl chloride was removed under reduced pressure leaving the diacid chloride which was treated with dry methanol (50 cm^3) and held at reflux for 10 h. The solvent was removed *in vacuo* affording the crude product as a yellow solid. Recrystallization from light petroleum (b.p. 60–80 °C) gave 6,6′-bis(methoxycarbonyl)-3-nitro-2,2′-bipyridine (90 mg, 97%) as white crystals; $\tilde{\nu}_{max}/cm^{-1}$ 3433m, 3079m, 2954m, 2925m, 2849m, 1740s, 1725s, 1582s, 1548s, 1444s, 1424s, 1389s, 1374s, 1310s and 1257s (KBr disc); δ_H($CDCl_3$, 200 MHz) 3.99 (3 H, s, CH_3), 4.06 (3 H, s, CH_3), 8.07 (1 H, dd, J 7.77, $H^{4'}$), 8.26 (3 H, m, $H^{3',5',5}$) and 8.39 (1 H, d, J 7.66 Hz, H^4); *m/z* 317 ($M^{\bullet+}$, 24), 301 (5), 287 (8), 271 (3), 259 (100), 227 (30), 213 (5), 169 (5), 153 (7), 152 (7), 141 (4), 126 (5), 77 (3) and 57 (3%).

3-Amino-6,6′-bis(methoxycarbonyl)-2,2′-bipyridine (L^2). Crude 6,6′-bis(methoxycarbonyl)-3-nitro-2,2′-bipyridine (590 mg, 1.86 mmol) was dissolved in dry methanol (500 cm^3). Palladium(II) on charcoal (10%, 60 mg) was added and the solution was stirred at 50 °C under hydrogen for 15 h. The product precipitated as a yellow solid during the hydrogenation. The solvent was removed *in vacuo*, the solid dissolved in chloroform (500 cm^3) and filtered through Celite. The solvent was removed *in vacuo*, the crude product was suspended in diethyl ether, held at reflux for 4 h and filtered off. The precipitate was recrystallized from 95% chloroform–5% pentane affording pure 3-amino-6,6′-bis(methoxycarbonyl)-2,2′-bipyridine (L^2) (486 mg, 92%), m.p. 241 °C (Found: C, 58.9; H, 4.8; N, 14.5. $C_{14}H_{13}N_3O_4$ requires C, 58.5; H, 4.6; N, 14.6%); λ_{max}(log ε) ($CHCl_3$) 354 (1.92) and 292 nm (2.09); $\tilde{\nu}_{max}/cm^{-1}$ 3387s (NH_2), 3247m (NH_2), 2958w, 1738s, 1719s, 1602s, 1584s, 1476w, 1434m, 1388m, 1309s, 1253s, 1192s and 1131s (KBr disc); δ_H (CD_3CN, 200 MHz) 3.87 (3 H, s, OCH_3), 3.96 (3 H, s, OCH_3), 7.22 (1 H, d, J 8.8, H^4), 7.50 (2 H, br s, NH_2), 7.88 (1 H, d, J 8.6, H^5), 8.07 (2 H, m, $H^{4',5'}$) and 8.76 (1 H, dd, J 6.1, 3.3, $H^{3'}$); ($CDCl_3$, 400 MHz) 3.97 (3 H, s, CH_3), 4.01 (3 H, s, CH_3), 7.09 (1 H, d, J 8.6, H^4), 7.17 (2 H, br s, NH_2), 7.96 (1 H, d, J 8.6, H^5), 7.98 (1 H, dd, J 7.8, $H^{4'}$), 8.07 (1 H, dd, J 7.8, 0.8, $H^{5'}$) and 8.90 (1 H, dd, J 7.8, 0.8 Hz, $H^{3'}$); δ_C($CDCl_3$,

400 MHz) 53.0, 53.5 (C^8, $C^{8'}$), 124.3 (C), 124.5 (C), 126.6 (C), 127.7 (C), 135.4 (C^2), 136.2 (C^6), 138.4 (C), 145.5, 147.6 ($C^{2'}$, $C^{6'}$), 159.0 (C^3), 166.0 ($C^{7'}$) and 166.4 (C^7); *m/z* 287 ($M^{\cdot+}$, 100), 254 (33), 229 (30), 226 (33), 196 (17), 169 (44), 168 (42), 141 (20), 114 (17) and 52 (12%).

NMR Titration Experiments.—The NMR samples of L^1 and L^2 were prepared as 50 and 11–21 mmol dm^{-3} solutions (CD_3CN, [$^2H_8$]tetrahydrofuran and CD_3NO_2) respectively. Hygroscopic salts [zinc(II) triflate and chloride] were dried under high vacuum at 100 °C for 3 d prior to use. They were titrated by addition of a solution of the salt or by addition of solid to the NMR tube (0.5–18 equivalents depending on the solubility of the salt) and vortexing of the solution. Both methods gave the same results.

X-Ray Crystallography.—For diffractometry the crystal was mounted on a glass fibre with cyanoacrylate resin. Lattice parameters at 21°C were determined by least-squares fits to the setting parameters of 25 independent reflections, measured and refined on an AFC-7R four-circle diffractometer employing graphite-monochromated Cu-Kα radiation. Intensity data were collected in the range $1 < \theta < 60°$. Data reduction and application of Lorentz, polarization, absorption and decomposition corrections were carried out using the TEXSAN system.[30]

The structure was solved by direct methods using SHELXS 86.[31] Hydrogen atoms were included at calculated sites with fixed isotropic thermal parameters. All other atoms were refined anisotropically. Full-matrix least-squares methods were used to refine an overall scale factor, positional and thermal parameters. Neutral atom scattering factors were taken from Cromer and Waber.[32] Anomalous dispersion effects were included in F_c;[33] the values for $\Delta f'$ and $\Delta f''$ were those of Creagh and McAuley.[34] The values for the mass-attenuation coefficients were those of Creagh and Hubbell.[35] All calculations were performed using TEXSAN[30] and plots were drawn using ORTEP.[36]

Additional material available from the Cambridge Crystallographic Data Centre comprises H-atom coordinates and thermal parameters.

Crystal data. $C_{14}H_{13}N_3O_4$, *M*, 287.27, monoclinic, space group $P2_1/c$, $a = 9.229(2)$, $b = 10.342(2)$, $c = 13.650(2)$ Å, $\beta = 90.92(2)°$, $U = 1302.7(5)$ Å^3, D_c ($Z = 4$) = 1.46 g cm^{-3}, μ(Cu-Kα) = 9.24 cm^{-1}, $\lambda = 1.5418$ Å, $F(000) = 600$, pale yellow plate 0.19 × 0.13 × 0.05, $T_{max,min} = 0.997, 0.844$, $N = 2218$, $N_o = 1107$ [$I > 2\sigma(I)$], $R = 0.046$, $R' = [\Sigma w(|F_o| - |F_c|)^2/\Sigma wF_o^2]^{\frac{1}{2}}$ 0.049, $w = 1/[\sigma^2(F_o)]$.

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