Electronic Supplementary Information

Bipyridyl Ligands as Photoactivatable Mono- and Bis-Alkylating Agents Capable of DNA Cross-Linking.

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General Procedures. The [2,2']bipyridinyl-5,5'-diol, has been prepared according to published procedures.[1] The other reagents (of commercial origin) were distilled or re-crystallized before use. For the irradiations, spectroscopic grade solvents were used as received. Before irradiation all the solutions were purged with argon for 5 minutes. For the preparative irradiations a merry go-round reactor supporting four 15w lamps was used.

^{1}H and ^{13}C NMR spectra were recorded with a 300 MHz spectrometer, and the chemical shifts are reported relative to TMS. The structures of the new compounds were deduced from the results of ^{1}H, ^{13}C, and DEPT-135, when carbon type assignments were included. Reverse preparative column chromatography was conducted on LiChroprep RP-18 (40-63 μm), eluent H_2O : MeOH 7:3 + TFA 1%. Reaction products were also separated and quantified analytically by reverse-phase (Intersil ODS-2, 5 μm, column dimension : ϕ = 4.6 mm, length = 250 mm, ϕ = 10.0 mm, length = 250 mm, Micro-Column) HPLC chromatography using a variable-wavelength detector.

6,6'-Bis-dimethylaminomethyl-[2,2']bipyridinyl-5,5'-diol (**5**). N,N-dimethylmethyleneiminium chloride (130 mg, 1.39 mmol) and K_2CO_3 (47 mg, 0.35 mmol) were added to a solution of [2,2']bipyridinyl-5,5'-diol (87 mg, 0.46 mmol), in anhydrous CH_2Cl_2 (2.5 ml). The resulting suspension was heated to reflux under nitrogen for 6 hrs. The solution was filtered off and the solid was treated twice with methanol (10 ml). After filtration, evaporation of the solvent gave 133 mg (0.44 mmol, yield 96%) of **5** as colourless crystals. Mp> 216°C (dec.). ^{1}H-NMR (CD_3OD): δ 3.07 (s, 12 H), 4.57 (s, 4 H), 7.43 (d, 2 H, J = 8.7 Hz), 8.46 (d, 2 H, J = 8.7 Hz). ^{13}C-NMR (CD_3OD): 44.6 (CH_3), 58.5 (CH_2), 123.7 (CH), 125.1 (CH), 138.2 (C), 148.6 (C), 153.8 (C).

Photochemical Reactions in methanol. Irradiations have been carried out on 100 ml solution of **5** (10^{-3} M) in methanol in the absence and in the presence of morpholine and L-amino esters ($5x10^{-2}$ M)., dividing the solution in 10 ml Pyrex tubes. In more detail, a solution of amine **5** with no nucleophiles, and in presence of morpholine, L-proline methyl or *t*-butyl esters were capped after being flushed with argon for 5 min and externally irradiated by means of four 15 W phosphor-coated lamps (center of emission 310 nm) for 60 min in a merry-go-round apparatus, affording the corresponding adducts **6**, **7**, **9** and **10** in almost quantitative yield. The irradiated solutions were evaporated under reduced pressure and the products **6**, **7**, **9** and **10** were purified by reverse preparative column chromatography [LiChroprep RP-18 (40-63μm), eluent H_2O : MeOH 7:3 + TFA 1%]. After chromatography trifluoroacetate anion exchange by HCl 0.1M (2 ml) addition gave the adducts **7**, and **9** as chloride salts.

6,6'-Bis-methoxymethyl-[2,2']bipyridinyl-5,5'-diol (**6**). Pale yellow crystals. Mp 124-126°C. ^{1}H-NMR (CD_3OD): δ 3.65 (s, 6 H), 4.85 (s, 4 H), 7.68 (d, 2 H, J = 8.5 Hz), 8.25 (d, 2 H, J = 8.5 Hz). ^{13}C-NMR (CD_3OD): 60.0 (CH_3), 69.3 (CH_2), 123.6 (CH), 128.9 (CH), 139.5 (C), 144.9 (C), 155.4 (C).

6,6'-Bis-morpholin-4-ylmethyl-[2,2']bipyridinyl-5,5'-diol (**7**) **2HCl**. Pale yellow crystals. Mp> 176°C (dec.). ^{1}H-NMR (D_2O): δ 3.46 (m, 4 H), 3.96 (m, 4 H), 4.62 (s, 4 H), 7.58 (d, 2 H, J = 8.8 Hz), 8.12 (d, 2 H, J = 8.8 Hz). ^{13}C-NMR (CD_3OD): 52.0 (CH_2), 55.4 (CH_2), 63.5 (CH_2), 124.0 (CH), 126.3 (CH), 135.2 (C), 145.3 (C), 153.0 (C).

6,6'-Bis-L-prolineOMe-4-ylmethyl-[2,2']bipyridinyl-5,5'-diol (9) 2HCl. Pale yellow crystals. Mp> 194°C (dec.). ^{1}H-NMR (CD_3OD): δ 2.21-2.42 (m, 4 H), 2.60 (m, 2 H), 2.92 (m, 2 H), 3.50 (m, 2 H), 3.79 (m, 2 H), 3.87 (s, 6H), 4.55 (m, 2 H), 4.72 (AB system, 4H), 7.38 (d, 2 H, J = 8.7 Hz), 8.36 (d, 2 H, J = 8.7 Hz). ^{13}C-NMR (CD_3OD): δ 24.1, 29.8, 52.0, 57.4, 58.8, 68.7, 124.0, 125.2, 138.3, 148.5, 154.2, 169.4.

6,6'-Bis-L-prolineO*t*Bu-4-ylmethyl-[2,2']bipyridinyl-5,5'-diol (10) 2CF_3COOH. Pale yellow crystals. Mp> 189°C (dec.). ^{1}H-NMR (CD_3OD): δ 1.42 (s, 18 H) 2.00-2.32 (m, 6 H), 2.60 (m, 2 H), 3.50 (m, 2 H), 3.93 (m, 2 H), 4.50 (m, 2 H), 4.68 (AB system, 4H), 7.38 (d, 2 H, J = 8.7 Hz), 8.36 (d, 2 H, J = 8.7 Hz). ^{13}C-NMR (CD_3OD): δ 24.1, 28.4, 29.9, 56.0, 57.4, 68.7, 86.2, 119.3 (*q*, CF_3COO), 124.0, 125.2, 138.3, 148.5, 154.2, 161.8 (*q*, CF_3COO),169.4.

Photochemical Reactions in water. The typical procedure for the photochemical reaction 6,6'-bis-dimethylaminomethyl-[2,2']bipyridinyl-5,5'-diol (**5**) in aqueous solution with morpholine, glycine, and L-proline is as follows.

Glycine (0.751 g, 10 mmol) and **5** (40 mg, 0.132 mmol) were dissolved in 100 ml of phosphate buffered solution (pH 8). The solution was poured into 10 Pyrex tubes, flushed with argon for 5 min, and then externally irradiated by means of four 15 W phosphor-coated lamps (center of emission 310 nm) for 1.5-3 hrs in a merry-go-round apparatus. After this time the solution was evaporated under vacuum. Column chromatography (LiChroprep RP-18 (40-63 μm), eluent H_2O : MeOH 8:2 TFA 1%) of the crude and trifluoroacetate exchange by chloride anion, as described above gave the adduct **8** as hydrochloride salt (39 mg, 0.090 mmol, 68% yield).

({6'-[(Carboxymethyl-amino)-methyl]-5,5'-dihydroxy-[2,2']bipyridinyl-6-ylmethyl}-amino)-acetic acid (**8**) **2HCl.** Pale yellow crystals. Mp>136°C (dec.) ^{1}H-NMR (CD_3OD): δ 3.93 (s, 4 H), 4.57 (s, 4 H), 7.61 (d, 2 H, J = 8.5 Hz), 8.11 (d, 2 H, J = 8.5 Hz). ^{13}C-NMR (CD_3OD): 45.2, 47.9, 123.6, 127.1, 136.3, 142.0, 152.9, 169.8.

6,6'-Bis-L-proline-4-ylmethyl-[2,2']bipyridinyl-5,5'-diol (**11**) **2HCl. 11** has been identified by

comparison with an authentic sample obtained by deprotection of the *t*But-ester **10** according the following procedure:

10 as bis-trifluoroacetate salt (24 mg, 0.030 mmol) has been dissolved into 0.5 ml of a TFA:CH_2Cl_2:Et_3SiH=1:2.5:0.2 solution and stirred at r.t. for 30 min. The solution was evaporated under reduced pressure and HCl 0.1M (2 ml) was added to the residue. After solvent evaporation **11** (15 mg, 0.029 mmol, 97% yield) was recovered as chloride salts.

Pale yellow crystals. Mp> 184°C (dec.). ^{1}H-NMR (CD_3OD): δ 2.16-2.32 (m, 6 H), 2.64 (m, 2 H), 3.56 (m, 2 H), 4.00 (m, 2 H), 4.59 (m, 2 H), 4.86 (s, 4H), 7.58 (d, 2 H, J = 8.5 Hz), 8.39 (d, 2 H, J = 8.5 Hz). ^{13}C-NMR (CD_3OD): δ 24.1, 29.9, 55.5, 57.4, 61.1, 68.7, 124.4, 126.2, 138.0, 146.5, 154.5, 171.7.

6-N3-deoxycytidinyl-6'-dimethylaminomethyl-1-ylmethyl-[2,2']bipyridinyl-5,5'-diol (**12**) **2HCl**.

Deoxycytidine monohydrate (0.981 g, 4 mmoli) and **5** (45 mg, 0.150 mmoli) were dissolved in 100 ml of phosphate buffered solution (pH 7.5), and then purged with nitrogen (after poring into 10 Pyrex tubes), and irradiated for 3 hrs. After this time the solution was evaporated under vacuum. Column chromatography (LiChroprep RP-18 (40-63μm), eluent H_2O : MeOH 7:3 TFA 1%) of the crude and trifluoroacetate anion exchange by HCl 0.1M addition gave the adduct **12** as hydrochloride salt (9 mg, 0.016 mmol, 11% yield). Pale yellow solid. Mp> 198°C (dec.). ^{1}H-NMR (D_2O): δ 2.31 (m, 1H), 2.38 (m, 1H), 2.92 (s, 6H), 3.63-3.85 (m, 2H), 4.01 (m, 1H), 4.38 (m, 1H), 4.48 (s, 2 H), 5.42 (s, 2H), 6.12 (t, *J* = 6.5 Hz, 1H), 6.35 (d, J = 7.9 Hz, 1H), 7.40 (d, J = 8.7 Hz, 1H), 7.63 (d, J = 8.7 Hz, 1H), 7.85 (d, J = 8.7 Hz, 1H), 8.10 (d, J = 8.7 Hz, 1H), 8.15 (d, J = 7.9 Hz, 1H).

DNA cross-linking experiments. Plasmid pBR322 (0.5 μg/sample) was mixed with increasing amounts (2.5, 5, 10, 20, 40, 80 μM) of compounds **5** and **11**, in phosphate buffer (50 mM, pH 7.5). A saturated water solution of 4,5',8-trimethylpsoralen was used to get a 0.5 nM concentration of drug in the control samples. Reaction mixtures were irradiated at 320 nm for 5 min at 120 W at room temperature. Irradiated solutions were added of alkaline agarose gel loading buffer (50 mM NaOH, 1

mM EDTA, 3% Ficoll, 0.02% bromophenol blue) and loaded on a 1% alkaline agarose gel containing 50 mM NaOH and 1 mM EDTA. Gels were run in 50 mM NaOH and 1 mM EDTA at 100 mA for 18 h and stained with ethidium bromide (0.5 μg/mL) for 30 min and subsequently washed in water for 10 min. Stained gels were visualized in Gel-Doc 1000 (Bio-Rad, Italy) and DNA bands were quantified by Quantity One software (Bio-Rad).

Reference

1) Y. Fukuda, S. Seto, H. Furuta, H. Ebisu, Y. Oomori, S. Terashima *J. Med. Chem.* **2001**, *44,* 1396.