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# Synthesis of 2H,9H-naphtho[2,3-b:7,6-b′]dipyran-2,9-diones as potential DNA-reactive agents[☆]

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## Abstract

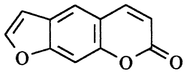
New 2H,9H-naphtho[2,3-b:7,6-b′]dipyran-2,9-diones have been synthesized. The tetracyclic derivatives were obtained by two different synthetic pathways, both having, as a common intermediate, the 3,6-dihydroxynaphthalene-2,7-dicarboxaldehyde.

*Keywords:* Nucleic acids; Intercalation; Naphthopyrone; Tetracyclo; Carbon suboxide

## 1. Introduction

Planar aromatic or partially aromatic structures have always been regarded as able to intercalate between the base pairs of the DNA double helix [1–3]. This holds true for a number of important drugs such as the anticancer agents of the anthracenedione structure type [4].

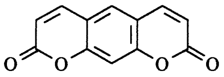
Other interesting compounds derived from psoralen (**I**)



(I)

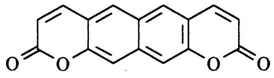
have been investigated during the last two decades due to their ability to form photocycloadducts between the double bonds of the furan and pyran ring and the double bond of the pyrimidine bases of the DNA, when irradiated with UV-A light This produced valuable photochemotherapeutic agents; including 8-MOP and TMP presently in clinical use [5–7].

Further research in the field of photoactive compounds lead to some symmetrical 2H,8H-benzo[1,2-b;5,4-b′]dipyran-2,8-dione derivatives, compound **II** is an example, that have been tested for their ability to intercalate between the B-DNA base pairs and possibly exhibit photoreactivity properties [8–11].



(II)

To further investigate the steric and electronic factors affecting the DNA-binding and photobinding process we planned to synthesize the following new tetracyclic compound **III**:



(III)

This may represent a probe for particular spatial arrangements of the nucleic acids as it must interact with DNA having distinct geometry from that interzacting with the tricyclic parent compound **II**. This molecule is in fact too large to intercalate into the classical B-structure with the same orientation as **II**, but can be made to recognize other, non canonical, features of DNA. The synthetic pathway is shown in Scheme 1.

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[☆] Dedicated to Professor Antonio Maccioni

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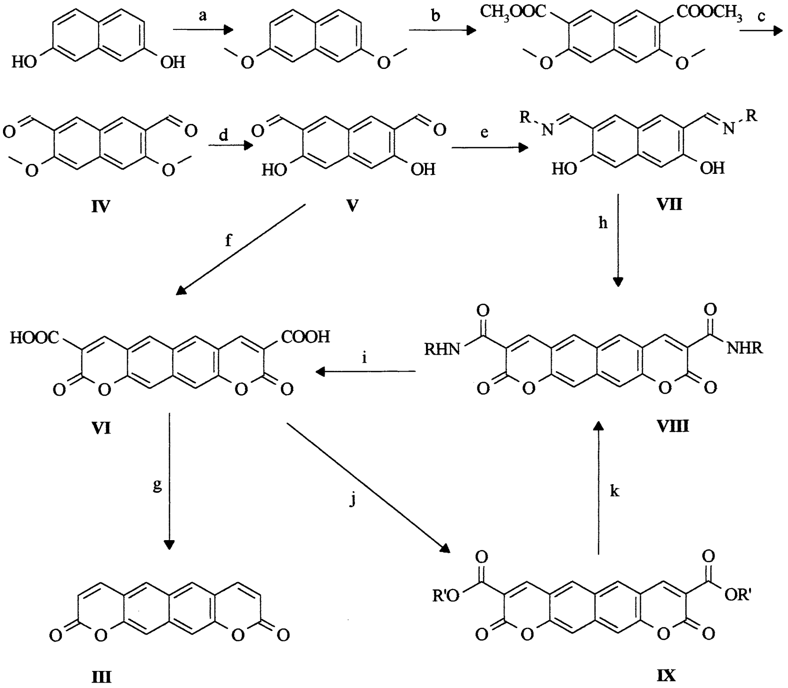


Scheme 1. **a**, DMS, $K_2CO_3$, acetone, reflux, 2 h; **b**, 1. $n$-BuLi, $Et_2O$, $CO_2$, − 70 to r.t., overnight. 2. $CH_3OH$, MSA, reflux; **c**, $NaAlH_2(OCH_2CH_2OCH_3)_2$-morpholine, toluene, − 10°C; **d**, $AlCl_3$, $CH_2Cl_2$, r.t., overnight; **e**, R–$NH_2$, ethanol–AcOH, reflux, 4 h; **f**, 1. ethyl cyanoacetate, KOH, $H_2O$, r.t., overnight. 2. HCl, reflux, 30′; **g**, Δ; **h**, $C_2O_3$, dioxane–acetonitrile, − 70°C to r.t., 120 h; **i**, KOH 20%, ethanol, reflux; **j**, R–OH, DCC, THF, reflux, 24 h; **k**, R–$NH_2$, THF, reflux, overnight.

## 2. Experimental

Melting points were determined by a Kofler apparatus and are uncorrected. FT-IR spectra were recorded on a Perkin–Elmer system 2000 spectrophotometer using KBr mulls. $^1H$ NMR were recorded on a Varian Unit 300 or on a Varian Gemini 200. EI mass spectra were obtained using a QMD 1000 (Fison Instruments) at 70 eV. Elemental analysis (CHN) were within 0.40% of the calculated values and were performed on a Carlo Erba 1016 elemental analyser.

Reagents and solvents were used as purchased without further purification. 2,7-Dimethoxynaphthalene-3,6-dicarboxylic acid dimethyl ester and carbon suboxide were obtained by known literature procedures [12–16].

M.p. are in °C, IR frequencies in $cm^{-1}$, $^1H$ NMR chemical shifts ($\delta$) in ppm and coupling constants ($J$) in Hz.

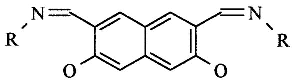
### *2.1. 2,7-Dimethoxynaphthalene-3,6-dicarboxaldehyde (IV)*

Toluene, 25 ml, and sodium bis(2-methoxyethoxy)-aluminium hydride 65% in toluene, 21 ml (71 mmol), were pooled together and cooled at − 10°C. At this temperature a solution of morpholine, 6.7 ml (77 mmol), in toluene was dropped in. The resulting reaction mixture was left at − 10°C for 30 min and then a solution of 2,7-dimethoxynaphthalene-3,6-dicarboxylic acid dimethyl ester, 8.7 g (29 mmol), in 80 ml of toluene was dropped in. The cooling bath was removed and the reaction left at room temperature overnight. Water was added to quench the reaction and the mixture was made acidic to dissolve the aluminium hydroxide formed. The product was extracted in ethyl acetate and the organic phase dried and concentrated. A total of 3.9 g of **IV**, (56%) were obtained. M.p. 232–235°C; IR 1680, 1620, 1260, 1320, 1180, 1130, 1100, 1020, 940, 890, 850, 800; $^1H$ NMR ($CDCl_3$) 3.97 (s, 6H, $OCH_3$), 7.03 (s, 2H, H1 and H8), 7.20 (s, 2H, H4 and H5), 10.44 (s, 2H, CHO).

### *2.2. 2,7-Dihydroxynaphthalene-3,6-dicarboxaldehyde (V)*

Aluminium trichloride, 9 g (70 mmol), and dichloromethane, 20 ml, were stirred for 2 h at room temperature and then 2,7-dimethoxynaphthalene-3,6-dicarboxaldehyde, 3.2 g (15 mmol), was added. The reaction mixture was stirred for 3 h at room temperature and then quenched in ice–HCl and extracted in ethyl acetate. The organic phase was dried over magnesium sulfate and evaporated under reduced pressure. The residue was crystallized from ethanol to give **V** pure; 2.6 g (80%). M.p. 217–220°C; IR 3240, 1680, 1630, 1570, 1260, 1240, 1150, 1090, 1020, 980, 950, 920, 830, 800; $^1H$ NMR (DMSO-$d_6$) 7.09 (s, 2H, H1 and

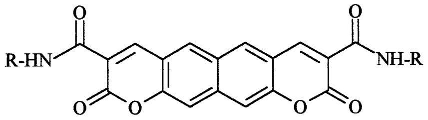
Table 1



(VII)

| Comp. | R | Yield (%) | M.p. (°C) | $M^+$ | IR (cm$^{-1}$) | $^1$H NMR (DMSO-d$_6$) |
|---|---|---|---|---|---|---|
| **a** | OH | 65 | 170–175 | 246 | 3450, 3320, 2860, 1620, 1540, 1535, 1315, 1240, 1090, 1000, 970, 895 | 6.9–7.8 (m, 4H, arom), 8.0 (s, 2H, CH=), 9.5 (s, 2H, NHOH), 10.2 (s, 2H, ArOH) |
| **b** | $NH_2$ | 68 | 184–187 | 244 | 3400, 3320, 3250, 2720, 1650, 1530, 1320, 1300, 1230, 1080, 1000, 980, 870, 760, 700 | 4.3 (s, 2H, $NH_2$), 6.9–7.8 (m, 4H, arom), 8.0 (s, 2H, CH=), 10.2 (s, 2H, ArOH) |
| **c** | $(CH_2)_2NEt_2$ | 54 | 192–197 | 412 | 3320, 3280, 1675, 1520, 1350, 1310, 1240, 1210, 1100, 1050, 980, 940, 850, 770, 690 | 1.1 (t, 12H, $CH_3$, $J = 8.7$, 1.4–1.8 (mm, 8H, $CH_2$), 4.1 (q, 8H, $CH_2$, $J = 8.7$), 6.9–7.8 (m, 4H, arom), 8.0 (s, 2H, CH=), 10.1 (s, 2H, ArOH) |
| **d** | $OCH_3$ | 80 | 178–180 | 274 | 3400, 3270, 3100, 1680, 1550, 1510, 1340, 1320, 1210, 1200, 1110, 1000, 970, 810, 740 | 4.0 (s, 6H, $OCH_3$), 6.9–7.8 (m, 4H, arom), 8.0 (s, 2H, CH=) |

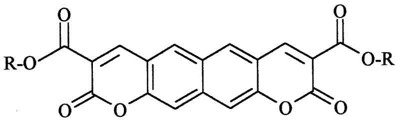
Table 2



(VIII)

| Comp. | R | Yield (%) | M.p. (°C) | $M^+$ | IR (cm$^{-1}$) | $^1$H NMR (DMSO-d$_6$) |
|---|---|---|---|---|---|---|
| **a** | OH | 95 | 205–210 | 382 | 3327, 2927, 2854, 2364, 1690, 1628, 1578, 1537, 1312, 1245, 1089, 893 | 6.9–7.8 (m, 4H, arom), 8.0 (s, 2H, OH), 8.3 (s, 2H, CH=), 9.5 (s, 2H, NH) |
| **b** | $NH_2$ | – | 215–221 | 380 | 3753, 3678, 3327, 1680, 1626, 1579, 1378, 1346, 1272, 1245, 1187, 1089, 1048, 893 | 4.8 (s, 4H, $NH_2$), 6.9–7.8 (m, 4H, arom), 8.3 (s, 2H, CH=), 9.5 (s, 2H, NH) |
| **c** | $(CH_2)_2NEt_2$ | – | 215–216 | 548 | 3500, 3480, 1700, 1680, 1650, 1610, 1580, 1370, 1290, 1250, 1170, 1080, 1040, 850 | 1.1 (t, 12H, $CH_3$, $J = 8.7$, 1.4–1.8 (m, 8H, $CH_2$), 4.1 (q, 8H, $CH_2$, $J = 8.7$), 6.9–7.9 (m, 4H, arom), 8.3 (s, 2H, CH=), 9.0 (s, 2H, NH) |
| **d** | $OCH_3$ | – | 190–205 | 410 | 3823, 3653, 3630, 1690, 1627, 1578, 1461, 1312, 1272, 1186, 1051, 893 | 4.0 (s, 6H, $OCH_3$), 6.8–7.9 (m, 4H, arom), 8.2 (s, 2H, CH=), 9.1 (s, 2H, NH) |

Table 3



**(IX)**

| Comp. | R | M.p. (°C) | $M^+$ | IR (cm$^{-1}$) | $^1$H NMR (DMSO-d$_6$) |
|---|---|---|---|---|---|
| **a** | 1-(4-nitrophenyl) | >300 | 594 | 1760, 1630, 1250, 1150, 1090, 980, 970, 860, 810 | 6.9–8.2 (m, 12H, arom),<br>7.3 (s, 2H, CH=) |
| **b** | 1-(2,4,6-trichlorophenyl) | 176–180 | 711 | 1760, 1630, 1230, 1170, 1080, 1000, 970, 850 | 6.9–8.4 (m, 8H, arom),<br>7.3 (s, 2H, CH=) |

H8), 7.19 (s, 2H, H4 and H5), 10.30 (s, 2H, CHO); 10.97 (s, 2H, OH).

### 2.3. *2H,9H-naphtho[2,3-b:7,6-b′]dipyran-2,9-dione* ***(III)***

2,7-Dihydroxynaphthalene-3,6-dicarboxaldehyde, 2.1 g (10 mmol), sodium hydroxide in water (20% w/v), 20 ml, and ethyl cyanoacetate, 3.4 g (30 mmol), were mixed and stirred at room temperature overnight. Hydrochloric acid, 180 ml, was added and the reaction mixture refluxed for 30 min and then cooled. The solid 2H,9H-naphtho[2,3-b:7,6-b′]dipyran-2,9-dione-3,8-dicarboxylic acid (**VI**) was filtered and crystallized from acetic acid. M.p. 239–242°C; IR 3300, 1750, 1630, 1240, 1160, 1080, 1000, 980, 870, 810; $^1$H NMR (DMSO-d$_6$) 7.91 (s, 2H, H11 and H12), 8.66 (s, 2H, H5 and H6), 8.83 (s, 2H, H4 and H7); mass 352 ($M^+$). The diacid was then decarboxylated in refluxing diphenyl ether or by sublimation at 250°C and 0.02 mmHg, to yield **III**; 0.49 g (30%). M.p. > 300°C; IR 1750, 1630, 1230, 1150, 1040, 1000, 980, 860, 800; $^1$H NMR (DMSO-d$_6$) 6.58 (d, 2H, $J = 9.50$, H3 and H8), 7.95 (s, 2H, H11 and H12), 8.22 (d, 2H, $J = 9.50$, H4 and H7), 8.48 (s, 2H, H5 and H6); mass (EI) 264 ($M^+$), 253, 236, 224, 208, 196, 181, 165, 151.

The diacid **VI** was also obtained by hydrolysis of compounds **VIII a–d** in refluxing 20% aqueous potassium hydroxide.

### 2.4. *General procedure for the synthesis of compounds* ***VII a–d***

To 2,7-dihydroxynaphthalene-3,6-dicarboxaldehyde, 1.1 g (5 mmol), dissolved in a mixture of 20 ml of ethanol and 0.2 ml of glacial acetic acid, 10 mmol of the appropriate $H_2N–R$ were added and the solution refluxed 4 h. The solvent was removed under reduced pressure and the residual oil dissolved in hexane and crystallized by addition of methanol. Data are shown in Table 1.

### 2.5. *General procedure for the synthesis of compounds* ***VIII a–d***

Carbon suboxide (3.2 mmol) was added over 1 h at − 70°C to a stirred solution of **VII a–d** (1.6 mmol) dissolved in a mixture of 50 ml of anhydrous 1,4-dioxane and 30 ml of acetonitrile. Then the reaction was left to stir at 0°C for 5 h and at room temperature for 120 h. The solvent was evaporated under reduced pressure and the residue treated with diisopropyl ether. The resulting solid was crystallized from methanol. Data are shown in Table 2.

### 2.6. *General procedure for the synthesis of compounds* ***IX a, b***

To a solution of **VI**, 1 g (2.8 mmol), and 1.2 g (5.8 mmol) of 1,3-dicyclohexylcarbodiimide, in 100 ml of warm anhydrous THF a solution of 5.6 mmol of the phenol derivative, in 90 ml of anhydrous THF was added slowly. The resulting mixture was refluxed 24 h and cooled. The urea was filtered off and the THF evaporated under reduced pressure. The residue dissolved in a solution of acetone and lead acetate. The starting material is recovered as lead salt while the mother liquor is concentrated under reduced pressure and the residue crystallized from methanol. Data are shown in Table 3.

## 3. Results and discussion

### 3.1. *Synthetic strategy*

The synthetic work started from the reported preparation of 7-hydroxy-4′-methyl naphtho(2,3;6′,5′)-α-pyrone [17], but it was soon realized that any synthetic pathway using an electrophile to extend the tricyclic system to the target compound **III** would not work. This is because the β position on the naphthalene ring was less nucleophilic than the α position. So, the 2,7-dimethoxynaphthalene-3,6-dicarboxilic acid dimethyl ester was chosen as suitable starting compound [18,19], as the substitution pattern in the naphthalene ring was the one we needed. It was reduced by deactivated sodium bis(2-methoxyethoxy)aluminium hydride to the dicarboxaldehyde and this method gave better results than the reduction to the alcohol with DIBAL and the subsequent oxidation with manganese dioxide or the Rosenmund reduction in our hands. To hydrolize the ethers aluminium chloride was preferred, but pyridinium chloride or hydrobromic acid were also suitable.

To cyclize the obtained 2,7-dihydroxynaphthalene-3,6-dicarboxaldehyde to the desired tetracyclic linear target **III** two different pathways were followed. First a Knoevenagel type reaction was attempted with ethyl cyanoacetate as the active methylene compound. The reaction product was the 2H,9H-naphtho[2,3-b:7,6-b′]-dipyran-2,9-dione-3,8-dicarboxylic acid (**VI**), that was thermally decarboxylated to the desired 2H,9H-naphtho[2,3-b:7,6-b′]dipyran-2,9-dione.

The reaction of carbon suboxide with aromatic azomethines, oximes and hydrazones bearing an *ortho* -hydroxy, -amino, mercapto substituent to give, respectively, benzopyran, quinoline and benzothiopyran derivatives has been described previously [16]. A mechanism for this reaction has also been proposed [15]. By this synthetic pathway few amide derivatives have been obtained, but the reaction seems quite flexible. The amides were then hydrolized to the carboxylic acid **VI**. Other useful intermediates were the active esters **IX**.

### 3.2. Interaction with DNA targets

Attempts were made to characterize the interaction of the tetracyclic compound **III** with nucleic acids. Unfortunately, its exceedingly low solubility in aqueous media, where any binding to DNA must be logically performed, prevented us from any meaningful investigation on the matter. Indeed derivatization of the novel compounds with polar side chain groups is required before an assessment can be made on the potential of the new tetracyclic system as a DNA-reactive structure.

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