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Porphyrinyl-Nucleosides Containing Fluorinated Nucleobases

Leszek Czuchajowski*, Anna Palka, Matthew Morra and Vinay Wandrekar
Department of Chemistry, University of Idaho, Moscow, ID 83844, U.S.A.

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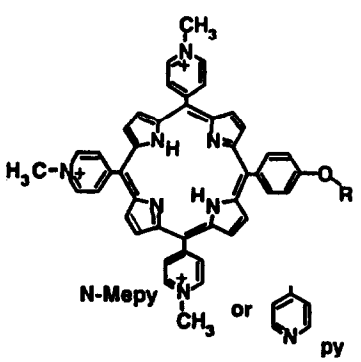
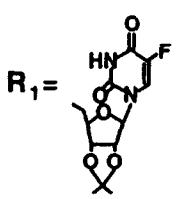
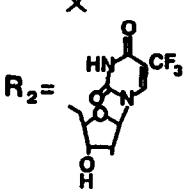
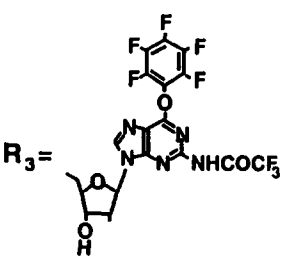
Abstract: Porphyrins were synthesized in which a meso-p-phenylene-O- bridge joins the porphine core with 5'-C of 5-fluorouridine (protected), 5-CF_3-thymidine or 2-N-trifluoroacetamidato-6-O-pentafluorophenyl-2'-deoxyguanosine.

The successful synthesis of water-insoluble and then of water-soluble porphyrinyl-nucleosides containing adenosine, uridine and thymidine[1,2,3] and the strong tumoricidal activity of some of them against human malignant melanoma[4] prompted the authors to synthesize porphyrinyl-nucleosides containing fluorine in the non-porphyrinyl structural unit. Expectations for their characteristic bioactivity were based on the established behavior of some fluorinated nucleosides: the antiviral/anti-HIV activity of particular fluorinated 2'-deoxy-uridines[5] and -thymidines,[6] antitumor activity of 5-fluoro-pyrimidine nucleosides[7,8] and their properties as anti-cancer prodrugs.[9] Some porphyrins have also shown anti-cancer properties.[10,11] Besides preparing new interesting models for biological tests,[12] the authors wanted to investigate how the presence of fluorine or fluorine-containing groups in a nucleobase influences the already described condensations of porphyrins with nucleosides.[1-4]









1 N-Mepy R=R_1 2 N-Mepy R=R_2 3 N-Mepy R=R_3

1a py R=R_1 2a py R=R_2 3a py R=R_3

1Co
2Co
3Co

With this in mind, the following nucleosides were used as coreagents with the porphyrin: 5-fluoro-(2',3'-0-isopropylidene)-uridine (R_1), 5-trifluoromethyl thymidine (R_2) and 2-N-trifluoroacetamido-6-0-pentafluorophenyl)-2'-deoxyguanosine (R_3). The porphyrin was represented by meso-tri-4-pyridyl-p-hydroxyphenylporphyrin (PHPP),[2-4] or its N-methyl-(4-pyridinium)-derivative which contributed to the formation, respectively, of the water-insoluble and water-soluble porphyrinyl-(fluorinated)-nucleosides in question. These were the meso-tri(N-methyl-4-pyridinium)porphyrinyl-p-phenylene-5'-0-(2',3'-0-isopropylidene)-5-fluorouridine **1**, and the respective non-N-methylated compound **1a**; the meso-tri(N-methyl-4-pyridinium)porphyrinyl-p-phenylene-5'-0-(5-trifluoromethyl)thymidine **2**, and the respective non-N-methylated compound **2a**; and the meso-tri(N-methyl-4-pyridinium)-porphyrinyl-p-phenylene-5'-0-(2-N-trifluoroacetamido-6-0-pentafluorophenyl)-2'-deoxyguanosine **3**, and the respective non-N-methylated compound **3a**.

Condensation between the OH group of PHPP and fluorinated 5'-0-tosyl-nucleosides R_1, R_2 or R_3 proceeded for 48 h in DMF in the presence of sodium hydride and cesium carbonate at 58-60° C.[3,4] To obtain **1a** and **1**, 5-fluorouridine was used as a 2',3'-isopropylidene derivative.[13] During tosylation of the latter which was carried in pyridine,[7] a 10% aqueous solution of $CdCl_2$ was used rather than a saturated solution. This enabled us to obtain the cadmium pyridine complex as an emulsion from which it was easy to extract the tosylated compound with ethyl acetate. Yield of **1a**, 26.6%; yield of **1** in N-methylation of **1a**, 63.5%. To obtain **2a**, the 5-trifluoromethyl thymidine was tosylated in the same manner as the protected 5-fluoro uridine: yield of tosylation, 50.6%; yield of **2a**, 8.4%; yield of N-methylation to **2**, 58.8%. To obtain **3a**, 2-amino-2'-deoxyguanosine was treated with trifluoroacetic anhydride and then with pentafluorophenol in pyridine:[14] yield of (2-N-trifluoroacetamido-6-0-pentafluorophenyl)-2'-deoxyguanosine (PFDG), 36%. The reaction proceeded smoothly: 3',5',6-trifluoroacetyl guanosine and the respective 6-pyridinium complex[14] did not appear in the products (compare also Ref. 15). The tosyl derivative of PFDG (yield 30%) was used for condensation with PHPP. The raw product was purified by column chromatography on silica gel with $CHCl_3$ used first as an eluent, followed by $CHCl_3/CH_3OH$ 25:10; yield 18.7%; yield of N-methylation to **3**, 58.2%.

$$\underset{\text{(PHPP)}}{py_3\text{-PORPH-}C_6H_4\text{-OH}} \xrightarrow[NaH,Cs_2CO_3]{\text{5'-OTs-nucleoside } (R_{1,2,3})} \underset{\textbf{(1a, 2a, 3a)}}{py_3\text{-PORPH-}C_6H_4\text{OR}} \xrightarrow[CH_3NO_2]{CH_3I} \underset{\textbf{(1, 2, 3)}}{(\text{N-Mepy})_3\text{-PORPH-}C_6H_4\text{-OR}}$$

Metallation of the porphyrinyl-(fluorinated)nucleosides, **1**, **2** and **3**, by cobalt(II) was performed on their pyridinium tri-iodide salts, which were formed during N-methylation of **1a**, **2a** and **3a** with CH_3I-CH_3NO_2. Metallation in a refluxing methanol solution of $CoCl_2 \cdot 6H_2O$ resulted in the chloride salts of **1Co**, **2Co** and **3Co** (accompanied by remarkable shifts of the Soret bands, 426 to 441, 413 to 432 and 421 to 437 nm, respectively). They could not be purified on the silica gel or Florisil column even with methanol as an eluent; purification has been accomplished, however, on a basic alumina column. Yields of the tri-chloride salts were **1Co** 28%, **2Co** 71% and **3Co** 70%. The

cobalt derivatives seemed particularly attractive because the respective derivatives of non-fluorinated porphyrinyl-nucleosides, especially of porphyrinyl-thymidines, were found to be active against malignant melanoma cells. (For Co-II-5,10-porphyrinyl-dithymidine the inhibition of the growth of malignant cells reached 95%.[12])

The spectral data are in compliance with the expected structures.

1a. MS(FAB) 924 m/z $(M+1)^+$; ^{1}H NMR (DMSO-d_6), δ ppm: 9.04 (d, 4H, py), 8.86 (s, 8H, β-pyr), 8.25 (m, 8H, py), 8.12 (m, 2H, ar), 7.40 (d, 1H, H-6), 7.30 (m, 2H, ar), 5.95 (s, 1H, H-1'), 5.12 (m, 2H, H-2', H-3'), 4.6 (m, 1H, H-4'), 4.1-4.2 (m, 2H, H-5'), 1.68 (s, 3H, ipr), 1.45 (s, 3H, ipr), -3.00 (s, 2H, por); UV-vis (CH_3OH), λ_{max} nm: 252, 305.5, 412(S), 514.5, 549.5, 589, 644.

1. ^{1}H NMR (CD_3OD), δ ppm: 9.4 (d, 6H, py), 8.96 (bs, 8H, β-pyr), 8.92 (bs, 6H, py), 8.12 and 7.22 (dd, 4H, ar), 5.75 (bs, 1H, H-1'), 5.1 (d, 1H, H-3'), 5.0 (s, 1H, H-2'), 4.8 (s, 9H, N-CH_3), 4.41 (bs, 1H, H-4'), 4.36 and 3.9 (bs, 2H, H-5'), 1.6 (s, 3H, ipr), 1.4 (s, 3H, ipr), -2.90 (s, 2H, por); UV-vis (CH_3OH) λ_{max} nm: 200, 219, 259, 426(S), 519, 555.5, 592, 649.

1Co. UV-vis (CH_3OH) λ_{max} nm: 227.5, 441(S), 553.

2a. MS(FAB) 913 m/z $(M+1)^+$; ^{1}H NMR (DMSO-d_6), δ ppm: 9.04 (m, 4H, py), 8.9 (m, 8H, β-pyr), 8.30 (m, 4H, py), 8.0 (m, 4H, ar), 7.5 (s, 4H, ar), 4.35 (t, 1H, H-3'), 4.01 (m, 1H, H-5'), 3.4 (m, 1H, H-4'), 2.55 (m, 1H, H-2'); UV-vis (CH_3OH) λ_{max} nm: 239.5, 304.5, 367, 418.5(S), 514, 548.5, 589, 642.

2. ^{1}H NMR (DMSO-d_6) δ ppm: 9.48 (d, 6H, py), 9.25 (d, 5H, py), 9.0 (s, 1H, py), 8.8 (s, 8H, β-pyr), 7.88 (s, 4H, ar), 7.6 (s, 1H, H-6), 5.92 (m, 1H, H-1'), 4.8 (s, 9H, N-CH_3), 4.35 (s, 1H, H-3'), 4.0 (d, 1H, H-5'), 3.9 (s, 1H, H-4'), 2.55-2.6 (m, 1H, H-2"), 2.2 (s, 1H, H-2'); UV-vis (CH_3OH) λ_{max} nm: 205, 236.5, 243.5, 413(S), 505, 542.5, 578.

2Co. UV-vis (CH_3OH) λ_{max} nm: 200, 219.5, 432(S), 554.5.

3a. MS(FAB), m/z: 1042 (M - C_4F_3O, the latter representing a fragment resulting from removal of C_2F_2 as one of the possibilities[17]), 648 [porphyrinyl unit with O-CH_2(5')], 493 [nucleoside unit without -CH_2(5') and -CF_3], 396 [nucleoside unit without -CH_2(5') and -OC_6F_5], 307 (porphyrine core); ^{1}H NMR (CD_3OD), δ ppm: 9.04 (d, 4H, py), 8.9 (s, 1H, H-8), 8.86 (s, 8H, β-pyr), 8.25 (m, 8H, py), 8.0 (m, 2H, ar), 7.4 (m, 2H, ar), 6.28 (t, 1H, H-1'), 5.38 (m, 1H, 3'-OH), 4.1 (m, 1H, H-3'), 3.90 (m, 1H, H-4'), 2.8 (m, 1H, H-2'), 2.38 (bs, 1H, H-2"); UV-vis (CH_3OH) λ_{max} nm: 190-200, 291, 402.5(S), 500, 535, 578, 635.

3. ^{1}H NMR (CD_3OD) δ ppm: 9.1 (d, 4H, py), 8.9 (s, 1H, H-8), 8.7 (d, 8H, β-pyr), 8.20 (bs, 8H, py), 7.9 (m, 2H, ar), 7.2 (m, 2H, ar), 6.2 (t, 1H, H-1'), 4.7 (s, 9H, N-CH_3), 4.2 (m, 1H, H-3'), 3.7 (m, 1H, H-4'), 2.8 and 2.38 (2H, H-2', H-2"); ^{19}F NMR (CD_3OD), δ ppm: 166.53 (2F ortho), 167.7 (2F meta), 173.97 (1F para); UV-vis (CH_3OH), λ_{max} ppm: 211, 421(S), 521, 542, 588, 642.

3Co. UV-vis (CH_3OH), λ_{max} nm: 200, 217.5, 437(S), 553.5

Comparison of the yields of **1** and **2** with the non-fluorinated analogs shows a facilitation of the condensation step with porphyrin when the fluorinated nucleosides were applied (**1**, 26.5% vs 17%; **2**, 8.4% vs 4%). The water soluble porphyrinyl-fluorinated guanosine **3** represents a first known porphyrinyl-guanosine system. Previous attempts to attach the non-fluorinated guanosine were unsuccessful under the conditions applied to other porphyrinyl nucleosides. Compounds 3 and 3a can be used for easy generation of porphyrinyl-guanosines containing 6-O-methyl- or 6-

amino- substituents,[16] which would give them a chance, along with other porphyrinyl-nucleosides, to modify the oligonucleosides. Biological tests, including toxicity in vivo and in vitro, are now in progress with very encouraging preliminary results.[12]

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