A New Method for the Synthesis of 5-Fluorouracil Prodrugs

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Abstract: Bromo-tris(dimethylamino)-phosphonium-hexafluorophosphate (BROP) is a particularly suitable reagent for synthesising some 5-fluorouracil prodrugs in a one step reaction. Several acids, especially retinoic acid, were bound to the N_1 of 5-fluorouracil, with yields of about 50% after purification.

Key words: N_1-retinoyl-5-fluorouracil, BROP, artificial nucleosides, prodrug

Proliferative vitreoretinopathy (PVR) is characterised by a rapid and uncontrolled proliferation of ocular cells within the vitreous body of the eye, frequently resulting in retinal detachment.[1] Silicone oil is often used to treat mechanical retinal detachment, but in the case of PVR, a reproliferation is common.[2] The injection of an antitumor agent would stop the proliferation and the silicone oil could be both a vehicle for this agent and effective against the retinal detachment. Another difficulty is the length of the treatment; to be effective the antitumor agent must be present in the vitreous body for at least 6 weeks. Pharmacological studies have shown that 5-fluorouracil (FU) **1** inhibits the growth of rabbit fibroblasts both *in vitro* and *in vivo* as well as being capable of reducing experimental intraocular proliferation in rabbits.[3] However, FU is insoluble in silicone oil and is eliminated very rapidly following intravitreal injection. So, in patients, repeated injections would be necessary, which is uncomfortable. Other investigations showed that retinoic acid (RA) inhibits retinal cell proliferation. The antimetabolite effect of RA in a rabbit model of PVR has already been proved.[4]

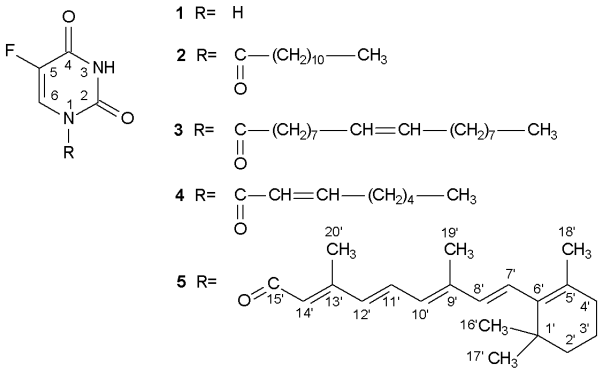
We thus chose to prepare a prodrug containing both FU and RA, i.e. retinoyl-FU **5**. This lipophilic compound should slowly liberate the two active compounds, thus maintaining a therapeutic intraocular level of FU and RA in the vitreous body for several days or weeks.

Before preparing **5** from RA, which is difficult to handle because of its rapid isomerisation in the presence of light and temperature and its sensitivity to water traces, three model compounds were synthesised:

– N_1-lauroyl-FU **2**: a prodrug with a long alkyl chain,

– N_1-oleyl-FU **3**: a prodrug with a long unsaturated alkyl chain,

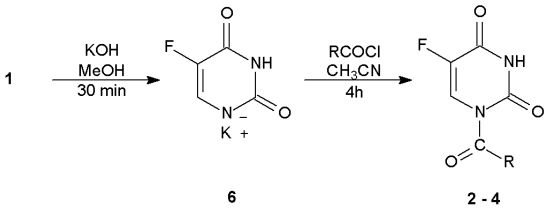
– N_1-octenoyl-FU **4**: a prodrug with a long unsaturated alkyl chain conjugated with the carbonyl group (Figure 1).



Structures of 5-fluorouracil and prodrugs synthesised.

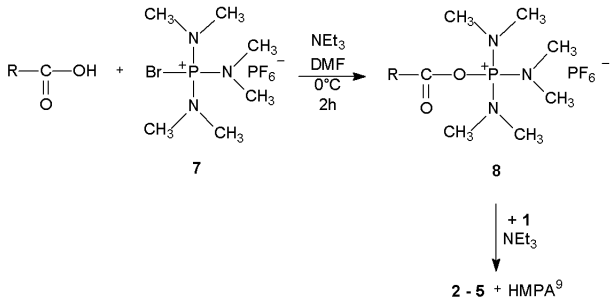
Figure 1

We first used a known method for the preparation of N_1-acyl-FU compounds with a saturated alkyl chain[5a] (Scheme 1). The acid chloride was prepared by a known method, using oxalyl chloride, then added to a solution of FU potassium salt **6** in acetonitrile.[6] The corresponding N_1-acyl-FU was purified by chromatography. All compounds were characterised with ^{1}H NMR, ^{13}C NMR, mass spectrometry and elemental microanalysis.[7] Since the N_1 position of FU is more reactive than the N_3 position towards acyl chlorides, the preferred formation of N_1-alkyl or N_1-alkenyl carbonyl derivatives was expected. Nevertheless, the N_1 substitution was established from the ^{1}H NMR chemical shift of H_6, which is ≈8.2-8.3 ppm ($CDCl_3$) for N_1-substituted derivatives and ≈7.2-7.3 ppm for N_3-substituted derivatives.[5] Compounds **2-4** were obtained (Table 1), but we were unable to get retinoyl-FU **5** by this method.



Scheme 1

To synthesise this product, we tried without success some classical coupling agents, such as DCC in the presence of HOBt or 2-mercaptothiazoline. However, bromotris(dimethylamino)-phosphonium-hexafluorophosphate (BROP) **7**, which has been described for the coupling of N-methyl-aminoacids[8] and disubstituted amines, allowed us to get compounds **2-5** (Table 1), according to the mechanism described in scheme 2. The reaction of the carboxylic acid and FU **1** in the presence of BROP and triethylamine in DMF gave the desired products **2-5**.[6] BROP was added last to avoid as far as possible the formation of anhydride. Since this agent is very reactive, we made the coupling at 0°C to prevent the formation of the N_3 substituted prodrugs.



Scheme 2

The purification was the most difficult step, because of the lack of stability of compounds **2-5** towards silica acidity and their different solubility in solvents. Table 1 shows that the two methods led to compounds **2-4** in comparable yields.

Table 1 Yields obtained with both methods described in schemes 1 and 2 after purification of the compounds.

Compound	**2** [%]	**3** [%]	**4** [%]	**5** [%]
Method 1	41	41	50	/
Method 2	41	39	50	44

Method 1: carboxylic acid (1.5 eq), oxalyl chloride (7.5 eq), dry toluene, RT, 1 h; FU (1.02 eq), KOH (1 eq), MeOH, RT, 30 min; acid chloride formed, potassium salt of FU, acetonitrile, 0°C, 4 h.
Method 2: carboxylic acid (1 eq), FU (1.5 eq), BROP (1.5 eq), NEt_3 (3 eq), dry DMF, 0°C, 2 h.

The moderate yields can be explained by the low stability of the prodrugs. The N_1-CO bond hydrolyses[5a] very easily in water and in basic medium, so this makes the reaction and the purification difficult. The reactions have to be done under nitrogen, all solvents must be anhydrous, the silica gel for chromatography has to be dried and the products have to be stored under nitrogen. However, only the BROP method led to compound **5**. Moreover, the one step reaction is shorter and easier to run. BROP is thus an excellent reagent for coupling poorly reactive amines or amides and acids.

References and Notes

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(6) Typical experimental procedure for both methods:
Method 1: in a first step, reaction of a suitable acid (1.5 mmol) with oxalyl chloride (7.5 mmol) in dry toluene (20 ml) for 1 hour led to the desired acid chloride. At the same time, stirring FU (1.02 mmol) with potassium hydroxyde (1 mmol) in methanol (5 ml) for 30 minutes gave the potassium salt of FU. In a second step, the acid chloride was added dropwise to a solution, refrigerated at 0°C, containing the white residue of FU potassium salt in acetonitrile (10 ml). The mixture was then stirred at room temperature for 4 hours.
Method 2: the carboxylic acid (1 mmol) and FU (1.5 mmol) were dissolved in anhydrous DMF (35 ml), and stirred at 0°C. Triethylamine (3 mmol) and BROP (1.5 mmol) were added. The mixture was then stirred at 0°C for 2 hours.
(7) a) N_1-lauroyl-5-fluorouracil **2**: the different salts, FU and anhydride were separated on a small dry silica gel chromatography column. The remaining acid was removed by dissolving it in cyclohexane. ^{1}H NMR (400 MHz, $CDCl_3$): 0.85 (3H, t, J 6.8 Hz, CH_3), 1.23 (16H, m, $(CH_2)_8$-CH_3), 1.69 (2H, app quin, J 7.3 Hz, CH_2-$(CH_2)_8$-CH_3), 3.09 (2H, t, J 7.3 Hz, O=C-CH_2), 8.27 (1H, d, $^3J_{H\text{-}F}$ 6.6 Hz, H_6), 9.2 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 14.1 (s, CH_3), 22.6, 24.4, 28.9, 29.28, 29.29, 29.4, 29.54, 29.56, 31.9, 39.0 (10s, $(CH_2)_{10}$-CH_3), 121.8 (d, $^2J_{C\text{-}F}$ 36.7 Hz, C_6), 141.3 (d, $^1J_{C\text{-}F}$ 244.0 Hz, C_5), 147.8 (s, C_2), 156.8 (d, $^2J_{C\text{-}F}$ 28.4 Hz, C_4), 172.0 (s, CH_2-C=O). MS (FAB$^+$, MNBA) m/z 313 [MH]$^+$, 625 [2MH]$^+$, 131 [FU+H]$^+$, 183 (loss of one FU molecule). Anal. for $C_{16}H_{25}N_2O_3F$: C, 61.51; H, 8.07; N, 8.97. Found: C, 61.46; H, 7.95; N, 8.98. White solid: mp 92°C.
b) N_1-oleyl-5-fluorouracil **3**: since it is very unstable on a silica gel column, the compound was purified by a liquid/ liquid extraction in heptane/ acetonitrile. ^{1}H NMR (400 MHz, $CDCl_3$): 0.88 (3H, t, J 6.6 Hz, CH_3), 1.29 (20H, m, $(CH_2)_4$-CH_2-CH=CH-$(CH_2)_6$-CH_3), 1.72 (2H, app quin, J 7.0 Hz, CH_2-CH_2-C=O), 2.02 (4H, m, CH_2-CH=CH-CH_2), 3.12 (2H, t, J 7.3 Hz, CH_2-C=O), 5.35 (2H, m, CH=CH), 8.29 (1H, d, $^3J_{H\text{-}F}$ 6.6 Hz, H_6), 9.2 (1H, s, NH).^{13}C NMR (100 MHz, $CDCl_3$): 14.1 (s, CH_3), 22.7, 24.4, 27.17, 27.23, 28.9, 29.1, 29.25, 29.3 (2C), 29.5, 29.7, 29.8, 31.9 (12s, $(CH_2)_5$-CH_2-CH=CH-CH_2-$(CH_2)_6$-CH_3), 39.1 (s, CH_2-C=O), 121.8 (d, $^2J_{C\text{-}F}$ 36.6 Hz, C_6), 130.1 and 129.7 (2s, CH=CH), 141.3 (d, $^1J_{C\text{-}F}$ 244.0 Hz, C_5), 147.9 (s, C_2), 157.0 (d, $^2J_{C\text{-}F}$ 28.4 Hz, C_4), 172.1 (s, CH_2-C=O). MS (FAB$^+$, MNBA) m/z 395 [MH]$^+$, 417 [MNa]$^+$, 265 (loss of one FU molecule). Viscous oil.
c) N_1-octenoyl-5-fluorouracil **4**: the different salts, FU and anhydride were separated on a small dry silica gel chromatography column. The remaining acid was removed by dissolving it in cyclohexane. ^{1}H NMR (400 MHz, $CDCl_3$): 0.90 (3H, t, J 6.6 Hz, CH_3), 1.33 (4H, m, $(CH_2)_2$-CH_3), 1.52

(2H, app quin, J 7.2 Hz, *CH*$_2$-CH$_2$-CH=), 2.34 (2H, app q, J 7.2 Hz, *CH*$_2$-CH=), 7.08 (1H, dt, $^3J_{H\text{-}Htrans}$ 15.2 Hz, $^4J_{H\text{-}H}$ 1.5 Hz, =CH-C=O), 7.33 (1H, td, J 6.8 Hz, $^3J_{H\text{-}Htrans}$ 15.2 Hz, CH$_2$-*CH*=), 8.23 (1H, d, $^3J_{H\text{-}F}$ 6.6 Hz, H_6), 8.7 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 13.9 (s, CH_3), 22.4, 27.5, 31.3, 33.1 (4s, *(CH$_2$)$_4$*-CH_3), 121.8 (s, =*CH*-C=O), 122.5 (d, $^2J_{C\text{-}F}$ 36.5 Hz, C_6), 141.3 (d, $^1J_{C\text{-}F}$ 243.1 Hz, C_5), 147.8 (s, C_2), 156.0 (s, CH$_2$-*CH*=), 156.5 (d, $^2J_{C\text{-}F}$ 28.4 Hz, C_4), 164.0 (s, =CH-*C*=*O*). MS (FAB$^+$, MNBA): m/z 255 $[MH]^+$ ($\rightarrow$ MS/MS 131, 125), 509 $[2M+H]^+$, 531 $[2M+Na]^+$. Anal. for $C_{12}H_{15}N_2O_3F$: C, 56.68; H, 5.95; N, 11.02. Found: C, 56.83; H, 6.04; N, 10.98. White solid: mp 77°C.

d) N_1-retinoyl-5-fluorouracil **5**: the different salts, FU and anhydride were separated in the dark, on a small dry silica gel chromatography column. The remaining acid was removed by dissolving it in cyclohexane. ^{1}H NMR (400 MHz, $CDCl_3$): 0.97 (6H, s, CH_3 16', CH_3 17'), 1.40 (2H, m, $H_{2'}$), 1.55 (2H, m, $H_{3'}$), 1.64 (3H, s, CH_3 18'), 1.95 (2H, s, $H_{4'}$), 1.97 (3H, s, CH_3 19'), 2.35 (3H, s, CH_3 20'), 6.10 (1H, d, $^3J_{H\text{-}Htrans}$ 15.9 Hz, $H_{8'}$), 6.13 (1H, d, J 11.3 Hz, $H_{10'}$ or $H_{12'}$), 6.30 (1H, d, $^3J_{H\text{-}Htrans}$ 15.9 Hz, $H_{7'}$), 6.37 (1H, d, $^3J_{H\text{-}Htrans}$ 15.0 Hz, $H_{12'}$ or $H_{10'}$), 6.78 (1H, s, $H_{14'}$), 7.13 (1H, dd, J 11.3 Hz, $^3J_{H\text{-}Htrans}$ 15.0 Hz, $H_{11'}$), 8.14 (1H, d, $^3J_{H\text{-}F}$ 6.8 Hz, H_6), 8.46 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 12.0 (s, $C_{19'}$), 14.6 (s, $C_{20'}$), 18.1 (s, $C_{3'}$), 20.8 (s, $C_{18'}$), 28.0 (2C, s, C_{16}, $C_{17'}$), 32.2 (s, $C_{2'}$), 33.3 (s, $C_{1'}$), 38.6 (s, $C_{4'}$), 117.4 (s, $C_{14'}$), 122.1 (d, $^2J_{C\text{-}F}$ 36.8 Hz, C_6), 128.4 (s, $C_{10'}$ or $C_{12'}$), 129.1 (s, $C_{7'}$), 129.7 (s, $C_{5'}$), 133.4 (s, $C_{11'}$), 133.9 (s, $C_{12'}$ or $C_{10'}$), 136.0 (s, $C_{8'}$), 136.6 (s, $C_{6'}$ or $C_{9'}$), 140.1 (d, $^1J_{C\text{-}F}$ 242.1 Hz, C_5), 141.2 (s, $C_{9'}$ or $C_{6'}$), 146.7 (s, C_2), 155.5 (d, $^2J_{C\text{-}F}$ 27.8 Hz, C_4), 158.5 (s, $C_{13'}$), 161.9 (s, $C_{15'}$). MS (FAB$^+$, MNBA) m/z 435 $[MNa]^+$, 131 $[FU+H]^+$, 283 (loss of one FU molecule). Orange solid: mp 151°C.

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(9) Using another coupling reagent, bromo-tris-pyrrolidino-phosphonium-hexafluorophosphate, can prevent the formation of HMPA, which is very toxic. The by-product formed is tris-pyrrolidinophosphoramide and the yields are close to those obtained with BROP.

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