## Mitsunobu Alkylation of Cancerostatic 5-Fluorouridine with (2E)-10-Hydroxydec-2-enoic Acid, a Fatty Acid from Royal Jelly with Multiple Biological Activities

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Dedicated to Prof. Dr. Zygmunt Kazimierczuk, Warsaw, on the occasion of his 70th birthday

5-Fluorouridine (1) – a nucleoside antimetabolite with strong cancerostatic properties – was protected *i*) at the 2'- and 3'-OH groups with a heptan-4-ylidene residue and *ii*) at the 5'-OH group with a (4-methoxyphenyl)(diphenyl)methyl residue. This fully protected compound, **3**, was submitted to a *Mitsunobu* reaction with the *N*-hydroxysuccinimide (NHS) ester, **5**, of (2*E*)-10-hydroxydec-2-enoic acid (**4**) which gave nucleolipid **6**. The latter was detritylated with  $Cl_2CHCOOH$  to yield the co-drug **7** as NHS ester.

**Introduction.** – The transport of a 'small molecule' drug across a target cell membrane can occur by four different mechanisms: *i*) by passive transport, *ii*) by carrier-mediated passive transport, *iii*) by active transport, and *iv*) by corpuscular absorption (phagocytosis or pinocytosis). If a pharmacologically active compound is not taken up by an active transport mechanism, its internalization into a target cell depends strongly on its lipophilicity. This is usually characterized by its partition coefficient *P* between octan-1-ol and H<sub>2</sub>O; these data are often given as  $\log_{10}P_{ow}$  values [1].

According to '*Lipinski*'s Rule of Five', the  $\log_{10}P_{ow}$  value should be  $\leq 5$  to exhibit a good oral bioavailability [2][3]. In a series of articles, we reported the synthesis of hybrid molecules of both, canonical nucleosides, such as uridine, 5-methyluridine, thymidine, and inosine, as well as of nucleoside antimetabolites, *e.g.*, 5-fluorouridine and 6-azauridine, with various kinds of lipids [4–11].

In this article, we describe the preparation of a conjugate between 5-fluorouridine (1) [12-22] and (2E)-10-hydroxydec-2-enoic acid (HDEA; 4), a fatty acid from royal jelly which is fed to the queen bee [23]. The fatty acid HDEA exhibits several outstanding biomedical properties. For example, it increases the generation of neurons and decreases that of astrocytes from neural stem/progenitor cells (NSCs) which have self-renewal capacity and multipotent activity to differentiate into neurons, astrocytes, and oligodendrocytes during development. This observation suggests that royal jelly contains compounds that differently influence neuronal and/or glial lineages [24], and that HDEA is one of such compounds of royal jelly that facilitates neurogenesis by NSCs similar to docosahexaenoic acid.

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Moreover, HDEA has been reported to show immune-modulatory properties [25], antitumor activity [26], collagen production-promoting activity [27], as well as antibiotic activity [28], a finding which is not really surprising, since royal jelly would otherwise be an excellent substrate for bacterial growth. Very recently, *Herdewijn* and co-workers used  $\alpha$ - and  $\beta$ -OH as well as  $\alpha$ -amino fatty acids for the synthesis of nucleolipids with different nucleobases and different sugar moieties and investigated these new co-drugs for their anti-HIV activities [29–31].

**Results and Discussion.** – In an earlier study, we have shown that a *Mitsunobu* alkylation of a pyrimidine nucleoside occurs only then swimmingly and without formation of many side products, if all glyconic OH groups are protected (*Scheme*) [7][8].

Therefore, 5-fluorouridine (1) was first reacted with heptan-4-one to the O-2',3'-heptylidene derivative, 2, which was subsequently protected at the 5'-OH group with a (4-methoxyphenyl)(diphenyl)methyl (MMTr) residue to give 3.

In the following, HDEA (4) was reacted with *N*-hydroxysuccinimide (NHS) to give NHS ester **5**. Compound **5** was then submitted to a *Mitsunobu* reaction with **3** to give the highly lipophilic nucleolipid **6**. The latter was detritylated with 4% Cl<sub>2</sub>CHCOOH solution in CH<sub>2</sub>Cl<sub>2</sub> (10 min, r.t.). Upon this reaction, several by-products were formed so that **7** could be isolated only in moderate yield (11%). The latter compound represents a novel co-drug combining two molecules with completely different pharmacological activities. Further reactions of **7** with a fluorescent label at the NHS ester moiety, as well as phosphitylation at 5'-position, will be published elsewhere.

The *Figure* displays the stepwise increase of lipophilicity of the compounds during the synthesis of 7 - expressed in terms of  $\log_{10}P_{\text{ow}}$  values.

All novel substances were characterized by <sup>1</sup>H- and <sup>13</sup>C-NMR spectroscopy, using DEPT-135 spectra for the assignment of <sup>13</sup>C resonances, as well as by ESI-MS.

**Conclusions.** – Only a fully protected nucleoside, such as **3**, can be regioselectively N-alkylated at the base with an  $\omega$ -OH fatty acid NHS ester under *Mitsunobu* conditions.

The authors gratefully acknowledge the financial support of the *BBraun AG* (Melsungen, Germany), as well as the *Bundesministerium für Wirtschaft* (FKZ KF 2369401 S B9 and FKZ 2369501 S B9). We thank Prof. Dr. *Uwe Beginn* (Organic Materials Chemistry, University of Osnabrück) for excellent laboratory facilities. Moreover, we thank Mrs. *Marianne Gather-Steckhan* for NMR spectra and Dr. *Stefan Walter* for ESI-MS.

## **Experimental Part**

General. All chemicals were purchased from Sigma–Aldrich (Deisenhofen, Germany) or TCI– Europe (Zwijndrecht, Belgium). Solvents were of laboratory grade and were distilled before use. Compounds **2** and **3** were prepared according to [9]; they were identical in all respects to authentic samples. Thin-layer chromatography (TLC): aluminum sheets, silica gel 60  $F_{254}$  (SiO<sub>2</sub>; 0.2 mm; Merck, Germany). Column chromatography (CC): SiO<sub>2</sub> 60 (2 × 20 cm). <sup>1</sup>H- and <sup>13</sup>C-NMR spectra: AMX-500 (Bruker, Rheinstetten, Germany; 500.14, and 125.76 MHz, resp.); in (D<sub>6</sub>)DMSO;  $\delta$  in ppm rel. to Me<sub>4</sub>Si as internal standard, J in Hz. ESI-MS: Bruker Daltonics Esquire HCT instrument (Bruker Daltonics,

1308



*a*) Heptan-4-one, (EtO)<sub>3</sub>CH, 4м HCl in 1,4-dioxane, DMF. *b*) MMTrCl, pyridine. *c*) NHS, DCC, DMF. *d*) Benzene, DIAD, Ph<sub>3</sub>P. *e*) 4% Cl<sub>2</sub>CHCOOH in CH<sub>2</sub>Cl<sub>2</sub>.



Leipzig, Germany); ionization with 2% aq. HCOOH soln.; in m/z.  $\log_{10}P_{ow}$  Values were calculated using the http://eadmet.com/de/physprop.php site with ePhysChem that contains ALOGPS v.3.0.

*1-[[*(2E)-*10-Hydroxydec-2-enoyl]oxy]pyrrolidine-2,5-dione* (**5**). HDEA (**4**; 745 mg, 4 mmol) and NHS (461 mg, 4 mmol) were dissolved in DMF and cooled to 5°. Then, *N*,*N*'-dicyclohexylcarbodiimide (DCC; 824 mg, 4 mmol) was added, and the mixture was stirred for 24 h at ambient temp. Precipitated 1,3-dicyclohexylurea was filtered off, and the filtrate was evaporated to dryness and subsequently dried *in vacuo*. The residue was suspended in CH<sub>2</sub>Cl<sub>2</sub>, residual 1,3-dicyclohexylurea was filtered off, and the filtrate was evaporated to dryness and subsequently dried *in vacuo*. The residue was suspended in CH<sub>2</sub>Cl<sub>2</sub>, residual 1,3-dicyclohexylurea was filtered off, and the filtrate was evaporated to dryness. Product **5** was crystallized from <sup>1</sup>PrOH. Yield: 590 mg (52%). *R*<sub>f</sub> (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 98:2) 0.36. <sup>1</sup>H-NMR: 7.25 (*dt*, <sup>3</sup>*J*(8,7)=15.8, <sup>3</sup>*J*(8,9)=7.0, H–C(8)); 6.19 (*d*, <sup>3</sup>*J*(7,8)= 15.8, H–C(7)); 4.27 (*t*, <sup>3</sup>*J*(HO,15)=2.5, OH); 3.39–3.36 (*m*, CH<sub>2</sub>(15)); 2.82 (*s*, CH<sub>2</sub>(3,4)); 2.31 (*Ψq*, <sup>3</sup>*J*(9,8)=7.0, <sup>3</sup>*J*(9,10)=3.3, CH<sub>2</sub>(9)); 1.48–1.40 (*m*, CH<sub>2</sub>(13,14)); 1.29 (br. *s*, CH<sub>2</sub>(10, 11, 12)). <sup>13</sup>C-NMR: 170.19 (C(2,5)); 161.34 (C(6)); 156.57 (C(7)); 115.11 (C(8)); 60.60 (C(15)); 32.39 (C(9)); 31.97 (C(14)); 2.851, 26.90, 25.37, 25.35 (4 CH<sub>2</sub>). ESI-MS: 283.59 ([*M*+H]<sup>+</sup>, C<sub>14</sub>H<sub>21</sub>NO<sub>5</sub><sup>+</sup>; calc. 283.14), 567.18 ([2*M*+H]<sup>+</sup>).

5-Fluoro-5'-O-[(4-methoxyphenyl)(diphenyl)methyl]-3-{(8E)-10-oxo-10-[(2,5-dioxopyrrolidin-1yl)oxy]dec-8-en-1-yl]-2',3'-O-(1-propylbutylidene)uridine (6). Ph<sub>3</sub>P (262 mg, 1 mmol) was added to a clear soln. of 3 (750 mg, 1.2 mmol) and 5 (280 mg, 1 mmol) in THF (7 ml) under stirring at ambient temp. Then, the mixture was warmed to 45° for 2 min. Subsequently, diisopropylazodicarboxylate (DIAD; 200 mg, 1 mmol) was added, and the mixture was stirred overnight at r.t. After evaporation of the solvent, the residue was dissolved in PrOH, and the soln. was cooled to  $-20^{\circ}$ , whereupon a slightly yellowish solid precipitated. Yield: 890 mg (97%). Rf (CH2Cl2/MeOH 98:2) 0.76. <sup>1</sup>H-NMR: 8.17 (d, <sup>3</sup>J(6,F)=5.0, H–C(6)); 7.66–7.54 (m, H–C(8"")); 7.41–7.15 (m, 4 H–C(3""), 4 H–C(4""), 2 H–C(5""), 2 H-C(7''); 6.86–6.82 (*m*, 2 H–C(8'')); 6.19 (*d*,  ${}^{3}J(9''', 8''') = 15.0$ , H–C(9''')); 5.81 (br. *s*, H–C(1')); 5.06-4.97 (m, H-C(2')); 4.77-4.63 (m, H-C(3')); 4.26-4.17 (m, H-C(4')); 3.72 (s, Me(10''')); 3.69-3.61 (*m*, CH<sub>2</sub>(1''')); 3.13-3.04 (*m*, CH<sub>2</sub>(5')); 2.82 (br. *s*, CH<sub>2</sub>(12''', 13''')); 2.37-2.24 (*m*, CH<sub>2</sub>(7''')); 1.70-1.59  $(m, \operatorname{CH}_2(2'')_{endo}); 1.53-1.41 \ (m, \operatorname{CH}_2(2'')_{exo}); 1.39-1.11 \ (m, 2 \ \operatorname{CH}_2(3''), \ \operatorname{CH}_2(2'''-6'''')); 0.91 \ (t, t)$  $J_{4''_{endo}} = 7.5, \text{ Me}(4''_{endo}); 0.84 (t, {}^{3}J(4''_{exo}, 3''_{exo}) = 7.5, \text{ Me}(4'')_{exo}).$  <sup>13</sup>C-NMR: 170.17  $(C(11''',14''')); 161.34 (C(10''')); 158.13 (C(9'')); 156.49 (C(8''')); 156.30 (d, {}^{2}J(F,4)=26.4, C(4));$ 148.67 (C(2)); 144.02, 138.36 (2 C(2''')); 142.74 (d,  ${}^{1}J(F,5) = 303.0$ , C(5)); 134.72 (C(6''')); 131.41, 131.33, 129.83, 128.68, 128.58, 127.87, 127.77, 127.68, 126.81, 126.75 (10 C (MMTr)); 126.24 (d, <sup>2</sup>J(F,6)=34.0,

C(6)); 116.56 (C(1'')); 115.11 (C(9''')); 113.10, 113.05 (2 C(8''')); 93.83 (C(4')); 86.54 (C(1')); 85.86 (C(1''')); 83.75 (C(2')); 80.95 (C(3')); 64.32 (C(5')); 54.88 (C(10''')); 40.97 (C(1''')); 38.64 (C(2'')\_{endo}); 38.51 (C(2'')\_{exo}); 31.95 (C(7''')); 28.34 (C(5''')); 28.25 (C(6''')); 26.88 (C(4''')); 26.52 (C(2''')); 26.03 (C(3''')); 25.32 (C(12''',13''')); 16.87 (C(3'')\_{endo}); 16.20 (C(3'')\_{exo}); 14.01 (2 C(4'')). ESI-MS: 936.3 ([M + Na]<sup>+</sup>, C<sub>51</sub>H<sub>62</sub>FN<sub>3</sub>O <sup>+</sup><sub>1</sub>; calc. 912.05).

5-Fluoro-3-{(8E)-10-oxo-10-[(2,5-dioxopyrrolidin-1-yl)oxy]dec-8-en-1-yl]-2',3'-O-(1-propylbutylidene)uridine (7). Compound 6 (400 mg, 0.44 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (9 ml). After addition of 4% Cl<sub>2</sub>CHCOOH soln. in CH<sub>2</sub>Cl<sub>2</sub> (61), the mixture was stirred at r.t. for 15 min and then neutralized by addition of 5% aq. NaHCO<sub>3</sub> soln. The org. layer was separated, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and evaporated to dryness. CC (SiO<sub>2</sub> 60; CH<sub>2</sub>Cl<sub>2</sub>/MeOH 98:2) gave 7 (50 mg, 11%) after evaporation of the solvent and drying in vacuo. R<sub>f</sub> (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 98:2) 0.24. <sup>1</sup>H-NMR: 8.22 (d, <sup>2</sup>J(F,6) = 7.0, H–C(6)); 7.27–7.24 (m,  $H-C(8''''); 6.19 (d, {}^{3}J(9''', 8'''') = 16.0, H-C(9''''); 5.88 (s, H-C(1')); 4.91-4.90 (m, H-C(2')); 4.77-4.77 (m, H-C(2$  $(m, H-C(3')); 4.15-4.14 (m, H-C(4')); 3.79-3.74 (m, CH_2(1''')); 3.66-3.51 (m, CH_2(5')); 2.83 (s, CH_2($  $CH_2(12''', 13''')); 2.33-2.27 (m, CH_2(7''')); 1.68-1.64 (m, CH_2(2'')_{endo}); 1.55-1.35 (m, CH_2(2'')_{exo}); 1.55-1.35 ($  $CH_2(2''', 6''')$ ; 1.35–1.18 (*m*, 2  $CH_2(3'')$ ,  $CH_2(3'''-5''')$ ); 0.92 (*t*,  ${}^{3}J(4''_{endo}, 3''_{endo}) = 7.0$ ,  $Me(4'')_{endo}$ ); 0.87  $(t, {}^{3}J(4''_{exo}, 3''_{exo}) = 7.0, Me(4'')_{exo})). {}^{13}C-NMR: 169.49 (C(11''', 14''')); 161.63 (C(10''')); 156.30 (C(8'''));$ 155.29 (C(4)); 149.64 (C(2)); 142.60 (d, <sup>1</sup>J(F,5)=325.0, C(5)); 124.93 (C(6)); 118.50 (C(1")); 115.63 (C(9''')); 96.33 (C(4')); 87.26 (C(1')); 84.62 (C(2')); 80.66 (C(3')); 63.08 (C(5')); 42.17 (C(1''')); 39.52 (C(1')); 63.08 (C(5')); 42.17 (C(1''')); 39.52 (C(1')); 42.17 (C(1''')); 42.17 (C(1'')); 42.17 (C(1'')) $(C(2'')_{endo}); 39.48 (C(2'')_{exo}); 33.03 (C(7''')); 29.18 (C(5''')); 29.07 (C(6'''); 28.93 (C(4''')); 27.70 (C(2'''));$ 27.53 (C(3''')); 26.84 (C(12''')); 25.85 (C(13''')); 17.70 (C(3'')\_{endo}); 17.10 (C(3'')\_{exo}); 14.51 (C(4'')\_{endo}); 14.47 (C(4'')<sub>exo</sub>). ESI-MS: 624.26 ( $[M+H]^+$ , C<sub>30</sub>H<sub>43</sub>FN<sub>3</sub>O<sup>+</sup><sub>10</sub>; calc. 624.29).

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Received February 10, 2015