Supplementary material

Synthesis and Structure-Activity Relationships of Uracil Nucleotide Derivatives and Analogs as Agonists at Human $P2Y_2$, $P2Y_4$, and $P2Y_6$ Receptors

*Ali El-Tayeb,[§] Aidong Qi,[#] and Christa E. Müller[§]**

[§]Pharmaceutical Institute, Pharmaceutical Chemistry Poppelsdorf, University of Bonn, Kreuzbergweg 26, D-53113 Bonn, Germany, [#]Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, USA.

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Table 1. Elemental analyses for compounds **6a, 6b, 6c, 6e, 6f, 6g, 18, 21g, 23e, 23f, 23g.**

Compd.	Formula	Calculated			Found		
		C	H	N	C	H	N
6a	$C_9H_{12}N_2O_5S.0.25\ H_2O$	40.79	4.72	10.57	40.97	4.69	10.27
6b	$C_{10}H_{14}N_2O_6$	46.51	5.46	10.85	46.25	5.18	10.49
6c	$C_{17}H_{18}N_2O_7$	56.35	5.01	7.73	55.69	4.98	7.62
6e	$C_9H_{12}N_2O_7.0.5\ H_2O$	40.11	4.82	10.40	40.39	5.15	9.02
6f	$C_{10}H_{14}N_2O_6$	46.51	5.46	10.85	46.12	5.99	10.49
6g	$C_{12}H_{18}N_2O_6$	50.35	6.34	9.79	49.95	6.35	9.60
18	$C_{30}H_{24}N_2O_8S.0.75\ H_2O$	61.42	4.35	4.77	61.40	4.52	4.43
21g	$C_7H_{10}N_2O_2$	54.54	6.54	18.17	54.27	6.55	18.00
23e	$C_{30}H_{24}N_2O_{10}.0.5\ H_2O$	61.90	4.29	4.81	62.08	4.31	4.44
23f	$C_{31}H_{26}N_2O_9.0.5\ H_2O$	64.18	4.65	4.83	64.49	4.55	4.58
23g	$C_{33}H_{30}N_2O_9$	66.21	5.05	4.68	65.69	5.06	4.62

Chemical synthesis of nucleosides

The uracil derivatives were converted to the activated trimethylsilyl ethers[1] and subsequently reacted, according to a modified Hilbert-Johnson[2] procedure in the presence of a Friedel-Crafts[3] catalyst (a Lewis acid), with 2,3,5-tri-*O*-acetyl- or benzoyl-protected 1-acetyl-β-D-ribose to afford the benzoylated or acetylated nucleosides. Deprotection by sodium methylate in methanol[4] led to formation of the desired nucleosides. The syntheses of the base-modified uracil derivatives and analogs are depicted in schemes 1 and 3.

2-Thiouracil (**16**) was silylated with hexamethyldisilazane (HMDS) in the presence of a catalytic amount of trimethylsilyl chloride or ammonium sulfate, respectively. Silylated 2-thiouracil was condensed with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose in dichloroethane catalyzed by tin(IV) chloride leading to the formation of **18** (Scheme 1). The silyl groups were removed by hydrolysis using a saturated aqueous sodium hydrogen carbonate solution. This process is characterized by the formation of an emulsion and/or suspension due to the formation of tin(IV) oxide hydrate. The use of potassium hydrogen carbonate showed a certain advantage over the use of sodium hydrogen carbonate.[5] Repeated filtration of the formed emulsion over silica gel was efficiently used to destroy the emulsion and to remove tin(IV) oxide hydrate affording **18**. The benzoylated nucleoside **18** was deprotected via transesterification using sodium methylate solution in methanol, and the benzoic acid methyl ester formed was removed by extraction with diethyl ether. The aqueous layer containing the product was treated with ion exchange resin (proton form), followed by subsequent lyophilization to yield 2-thiouridine (**6a)**.[6]

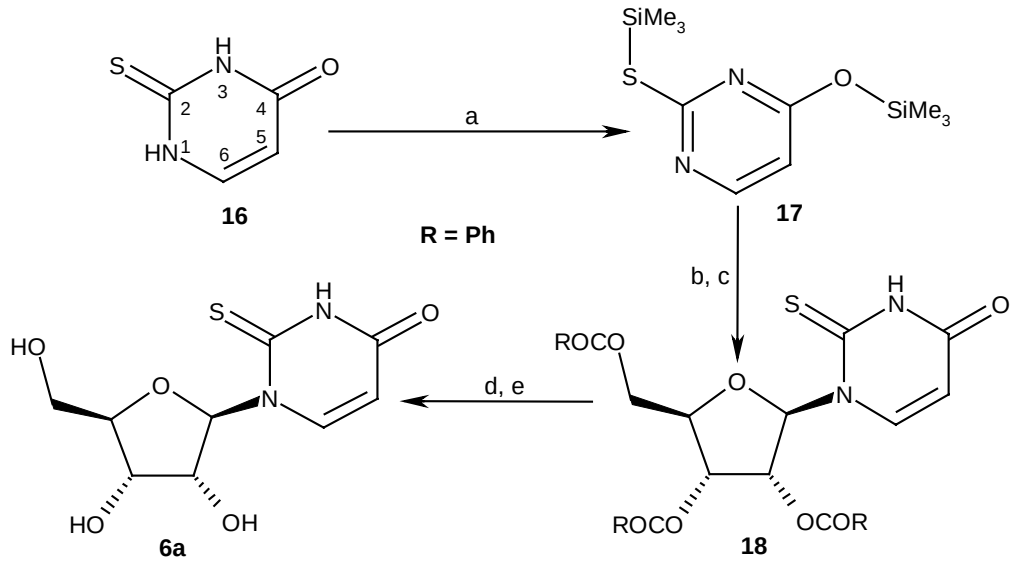
N3-substituted uridine derivatives were synthesized from uridine by alkylation with ar(alkyl) halogenides in the presence of potassium carbonate as a base using a mixture of equal amounts of acetone and N,N-dimethylformamide as a solvent. N3-Phenacyluridine[7] (**6c**, Scheme 2) was obtained in 82% yield after purification on a silica gel column. In contrast, the purification of N3-methyluridine[8] (**6b**) proved to be a relatively difficult due to its nearly same degree of hydrophilicity as the starting compound uridine; the reported procedure using chloroform : ethylacetate : methanol (5 : 4 : 1) for silica

gel chromatography followed by recrystallization from methanol and ethyl acetate / petroleum ether mixtures[9] was not successful in our hands. We finally succeeded by using a mixture of dichloromethane : methanol (9 :1) as eluent for silica gel chromatography, followed by crystallization from acetone and subsequently from n-hexane.[8]

6-Propyluracil (**21g**) was obtained by reaction of commercially available 6-propyl-2-thiouracil (**20**) with chloroacetic acid in water for 5 h at reflux conditions affording **21g** in 86% yield (Scheme 3).[10]

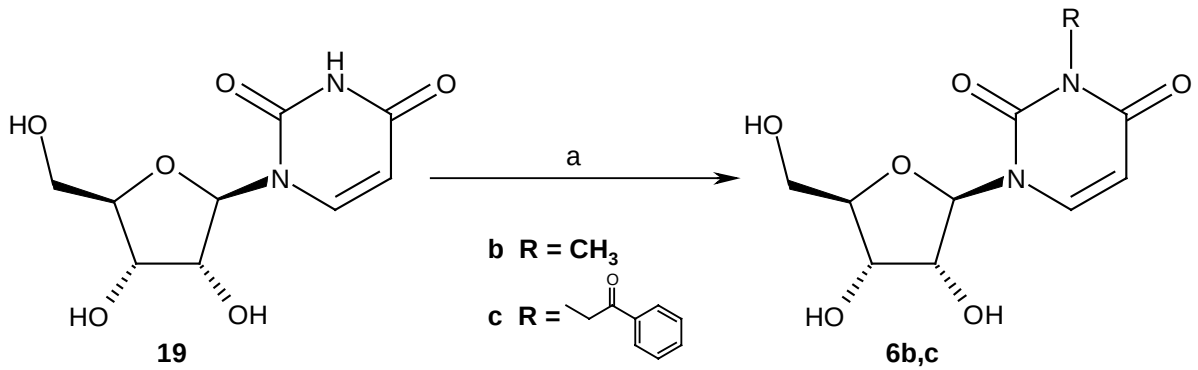
Synthesis of 6-substituted uracil nucleosides is characterized, due to steric and electronic factors, by the formation of both N1- and N3- as well as N1,N3-bis-glycosylated products. The regioselectivity has been described to be solvent-dependent; with a less polar solvent such as 1,2-dichloroethane yielding the N3-riboside as the main product (68%) while the uridine-analogous N1-riboside (13%) and the bis-riboside (3%) are the minor products.[11] The product ratios are changed in case of a more polar solvent such as acetonitrile, which affords the N3- (43%) and the N1-riboside (41%) in equal amounts, while the bis-riboside is only obtained in 3% yield.[11] In the present study we focussed our attention on isolating and purifying the N3-riboside using dichloroethane as a solvent (Scheme 3). The uracil derivatives **21e-g** were refluxed with hexamethyldisilazane and after the reaction mixture had become a clear solution containing **22e-g**, tin(IV) chloride was added as a Lewis acid and the reaction with the ribose derivative was performed in dichloroethane as previously reported [11-13] affording the protected nucleosides **23e-g**. After deprotection with sodium methylate in methanol,[4,14] or ammonia in methanol,[15] respectively, the corresponding nucleosides **6e-g** were obtained. The traces of N1-ribosides and N1,N3-bis-riboside which formed during the reaction were removed by silica gel column chromatography. Structures could be confirmed by ^{1}H NMR spectra.[11,12]

Scheme 1. Synthesis of 2-thiouridine (**6a**)[a]



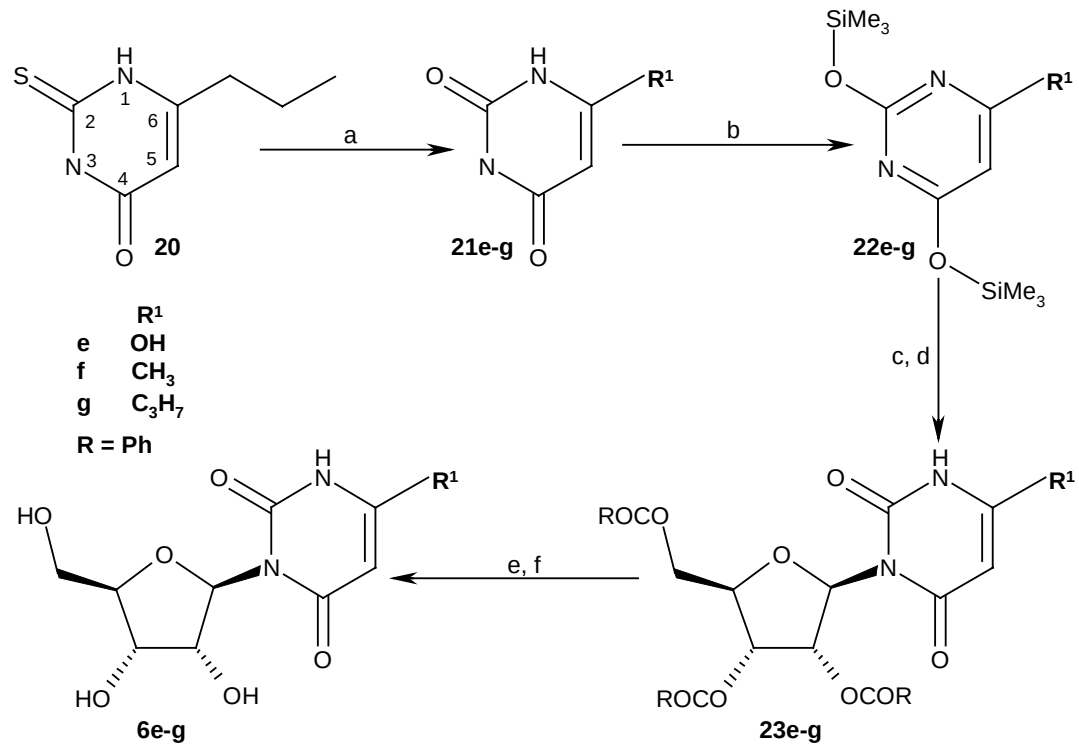
[a]Reagents, Conditions and Yields: (a) HMDS, trimethylsilyl chloride or $(NH_4)_2SO_4$, reflux, 5 h (98% **17**) (b) $SnCl_4$, dichloroethane, 1-*O*-acetyl-tri-*O*-benzoyl-*β*-D-ribofuranose, rt, 5h (c) H_2O, $NaHCO_3$, overnight (81% **18**) (d) $NaOCH_3$, methanol, rt, 3h (e) DOWEX 50 x 8 proton form (93% **6a**).

Scheme 2. Synthesis of N3-substituted uridine derivatives (**6b,c**)[a]



[a]Reagents, Conditions and Yields: (a) K_2CO_3, DMF/acetone 1:1, 50-60 °C (70% **6b,** 82% **6c**).

Scheme 3. Synthesis of 6-substituted uridine derivatives (**6e-g**)[a]



[a]Reagents, Conditions and Yields: (a) chloroacetic acid, 5h (86% **21g**) (b) HMDS, trimethylsilyl chloride or $(NH_4)_2SO_4$, reflux 1-6 h (96-98% **22e-g**) (c) $SnCl_4$, dichloroethane, 1-*O*-acetyl-tri-*O*-benzoyl-*β*-D-ribofuranose, rt, 5h (71% **23e,** 80% **23f,** 87% **23g**) (d) H_2O, $NaHCO_3$, overnight (e) $NaOCH_3$, methanol, rt, 3h (f) DOWEX 50 x 8 proton form (75% **6e,** 92% **6f,** 95% **6g**).

Experimental procedures

General procedure of silylation

A suspension of the uracil derivative (10 mmol) in 30 ml of 1,1,1,3,3,3-hexamethyldisilazane (HMDS) and 1 ml of trimethylsilyl chloride, or a few crystals of $(NH_4)_2SO_4$, respectively, was refluxed for ca. 7 hours until a clear solution was obtained. The solution was allowed to cool, and the excess of HMDS was removed under reduced pressure. The silylated uracil derivative was kept under argon until further reaction. A small sample of the oily syrup was dissolved in $CDCl_3$ and checked for complete silylation by NMR spectroscopy. Yield: ca. 98%.[14]

General procedure of nucleoside formation

Silylated uracil derivative (5 mmol) kept under argon was dissolved in 50 ml of 1,2-dichloroethane, and 1.43 g (0.65 ml, 5.5 mmol) of $SnCl_4$ (10% excess) was added to the solution with vigorous stirring, 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (4.4 g, 8.8 mmol) in 50 ml 1,2-dichloroethane was added dropwise to yield a slightly yellowish solution. The mixture was stirred at rt for 7 h, the reaction was controlled by TLC (dichloromethane : methanol = 9 : 1). The solution was poured into a saturated aqueous $NaHCO_3$ solution under vigorous stirring and then allowed to stand overnight. The suspension was filtered over silica gel and the gel was washed twice with 50 ml ethyl acetate each, and twice with 100 ml of chloroform each. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered over silica gel and evaporated to dryness. The residue was crystallized from ethanol to give a white powder or crystals.[14]

General procedure for deprotection

Benzoylated or acetylated nucleoside derivative (1.2 mmol) was dissolved in a mixture of 20 ml of absolute CH_3OH and 3.9 ml of 5% methanolic $NaOCH_3$ solution. The solution was stirred at rt for 3-8 h and completion of the reaction was determined by TLC (dichloromethane : methanol = 3 : 1). Neutralization of the solution was achieved by adding DOWEX-50 WX-8 ion exchange resin (proton form) previously washed with CH_3OH. After filtering the resin off, the CH_3OH was evaporated and 20 ml of water was added. The benzoic acid (or acetic acid) methyl ester was extracted 2 times with 30 ml diethyl ether each and the water fraction was lyophilized to give the deprotected nucleoside.[14]

General procedure for the preparation of 3-substituted uridine derivatives (6 b and c)

Uridine (1.47 g, 6 mmol), anhydrous K_2CO_3 (1.41 g, 10.2 mmol), phenacyl chloride. or methyl iodide, respectively (15 mmol), were dissolved in a mixture of DMF and acetone (1 : 1, 16 ml) and refluxed in an oil bath for 4 h at 120 °C (**6c**), or at 60 °C (**6b**). The acetone was evaporated and 70 ml of ice was added followed by extraction with ethyl acetate. The organic phase was dried over anhydrous sodium sulfate. The product was purified by coloumn chromatography using dichlormethane : methanol (20 : 1) in case of compound **6c**, or dichloromethane : methanol (9 :1) for **6b**. The latter compound had to be further purified by recrystallization from a mixture of methanol, ethyl acetate, and petroleum ether (1 : 2 : 9).[7,8]

Synthesis of compound 21g

A suspension of 1.87 g (11 mmol) of 6-propyl-2-thiouracil (**20**) in 20 ml of water was refluxed for 5 h with 1.98 g (21 mmol) of chloroacetic acid. After all H_2S was evaporated as detected by a filter paper impregnated with lead acetate, the reaction mixture was allowed to cool. The precipitated product was isolated by filtration and after drying, the product was recrystallized from ethanol.[10]

NMR spectral data of final products

6a: 2-Thio-1-β-D-ribofuranosyl(3*H*)pyrimidine-2,4-dione. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 3.60(m, 2H), 3.88 (m, 1H), 3.95 (q, 1H, J = 4.64 Hz), 4.04 (q, 1H, J = 4.38 Hz), 5.07 (d, 1H, J = 4.99 Hz), 5.2 (t, 1H, J = 4.47 Hz), 5.37 (d, 1H, J = 5.26 Hz), 5.95 (d, 1H, J = 8.15 Hz), 6.53 (d, 1H, J = 3.68 Hz), 8.14 (d, 1H, J = 8.15 Hz), 12.50 (s, 1H). ^{1}H-NMR (500 MHz, DMSO-d_6 + D_2O) δ 3.55 (m, 2H), 3.88 (m, 1H), 3.93 (t, 1H, J=5.39 Hz), 4.01 (t, 1H, J=4.60 Hz), 5.94 (d, 1H, J = 8.15 Hz), 6.52 (d, 1H, J = 3.94 Hz), 8.09 (d, 1H, J = 8.15 Hz). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 60.03, 69.12, 74.71, 84.80, 92.74, 106.56, 141.13, 159.73, 176.53.

6b: 3-Methyl-1-β-D-ribofuranosylpyrimidine-2,4-dione. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 3.14 (s, 3H), 3.52 (m, 2H), 3.84-3.85 (q, 1H, J = 3.77 Hz), 3.94 (q, 1H, J = 4.73 Hz), 4.0 (q, 1H, J = 4.91 Hz), 5.03 (m, 2H), 5.32 (d, 1H, J = 4.99 Hz), 5.74 (d, 1H, J = 7.89 Hz), 5.89 (d, 1H, J = 4.99 Hz), 7.92 (d, 1H, J = 8.15 Hz). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 27.35, 60.78, 69.77, 73.86, 84.92, 89.06, 100.83, 139.10, 151.09, 162.22.

6c: 3-Phenacyl-1-β-D-ribofuranosylpyrimidine-2,4-dione. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 3.66 (m, 2H), 3.87 (m, 1H), 3.99 (t, 1H, J = 4.86 Hz), 4.07 (t, 1H, J = 4.86 Hz), 5.09 (m, 1H), 5.12 (m, 1H), 5.39 (d, 1H, J = 4.99 Hz), 5.35 (s, 2H), 5.81 (d, 1H, J = 4.47 Hz), 5.87 (d, 1H, J = 8.15 Hz), 7.60-8.09 (m, 6H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 47.14, 60.86, 69.85, 73.87, 85.08, 89.13, 100.94, 128.14-134.20, 139.92, 150.87, 161.74, 192.57.

6e: 1-β-D-ribofuranosyl(3*H*)pyrimidine-2,4,6-trione. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 3.38 (m, 5H), 4.04 (t, 1H, J = 6.44 Hz), 4.33 (bs, 4H), 5.88 (d, 1H, J = 3.15 Hz), 11.35 (s, 1H). ^{1}H-NMR (500 MHz, DMSO-d_6 + D_2O) δ 3.36 (m, 5H), 4.03 (t, 1H, J = 6.44 Hz), 4.33 (m, 1H), 5.85-5.86 (d, 1H, J = 3.15 Hz). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 56.19, 62.40, 70.07, 71.58, 84.33, 88.01, 151.12, 166.18, 166.90.

6f: 6-Methyl-3-β-D-ribofuranosyl(1*H*)pyrimidine-2,4-dione. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 2.01 (s, 3H), 3.38 (m, 2H), 3.50 (m, 1H), 3.64 (m, 3H), 4.07 (t, 1H, J = 6.31 Hz), 4.45 (m, 1H), 5.43 (s, 1H), 6.02 (d, 1H, J = 3.94 Hz), 11.08 (s, 1H). ^{1}H-NMR (500 MHz, DMSO-d_6 + D_2O) δ 2.00 (s, 3H), 3.37 (m, 2H), 3.55 (m, 1H), 4.05 (t, 1H, J = 6.18 Hz), 4.44 (m, 1H), 5.43 (s, 1H), 6.00 (d, 1H, J = 3.94 Hz). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 18.15, 62.52, 70.34, 71.01, 84.49, 87.38, 98.69, 151.13, 152.41, 162.90.

6g: 6-Propyl-3-β-D-ribofuranosyl(1*H*)pyrimidine-2,4-dione. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 0.86 (t, 3H, J = 7.36Hz), 1.51 (sixt, 2H, J = 7.45), 2.24 (t, 2H, J = 7.49), 3.38 (m, 2H), 3.57 (m, 1H), 3.65 (m, 1H), 4.08 (m, 1H), 4.47 (bs, 3H), 5.43 (d, 1H, J = 1.84), 6.03 (d, 1H, J = 3.68), 11.04 (s, 1H). ^{1}H-NMR (500 MHz, DMSO-d_6 + D_2O) δ 0.84 (t, 3H, J = 7.36), 1.48 (sixt, 2H, J = 7.45), 2.23 (t, 2H, J = 7.62), 3.37 (m, 2H), 3.55 (m, 1H), 4.05 (t, 1H, J = 6.31), 4.45 (m, 1H), 5.45 (s, 1H), 6.01 (d, 1H, J = 3.94Hz). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 13.36, 20.32, 35.52, 62.50, 70.33, 70.97, 84.49, 87.40, 98.05, 151.30, 155.78, 162.99.

17: 2-Trimethylsilylthio-4-trimethylsilyloxypyrimidine. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.33 (s, 9 H), 0.44 (s, 9 H), 6.3 (d, 1H, J = 5.67 Hz), 8.1 (d, 1H, J = 5.83 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 0.25, 0.10, 106.0, 145.3, 157.64, 167.49.

18: 2-Thio-1-(2´,3´,5´-tri-O-benzoyl-β-D-ribofuranosyl)(3*H*)pyrimidine-2,4-dione. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 4.72 (d, 2H, J = 4.72 Hz), 4.85 (q, 1H, J = 5.14 Hz), 5.83 (t, 1H, J = 6.14 Hz), 5.8-5.9 (m, 1H), 5.97 (d, 1H, J = 8.19 Hz), 7.22 (d, 1H, J = 4.72 Hz), 7.4-8.0 (m, 16H), 12.7 (s, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 63.63, 70.23, 73.88, 79.50, 90.19, 107.36, 128.54-134.05, 140.56, 159.43, 164.55, 164.65, 165.58, 176.16.

21g: 6-Propyl(1*H*,3*H*)pyrimidine-2,4-dione. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 0.85 (t, 3H, J = 7.40 Hz), 1.54 (sixt, 2H, J = 7.46 Hz), 2.23 (t, 2H, J = 7.56 Hz), 5.29 (s, 1H, C5-H), 10.72 (s, 1H), 10.82 (s, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 13.33, 20.39, 33.74, 98.18, 151.86, 156.47, 164.33.

22e: 2,4,6-Tris(trimethylsilyloxy)pyrimidine. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.30 (s, 27 H), 5.68 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 0.40, 0.46, 89.86, 162.48, 171.28.

22f: 6-Methyl-2,4-bis(trimethylsilyloxy)pyrimidine. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.29 (s, 9 H), 0.30 (s, 9 H), 2.25 (t, 3H, J = 7.62 Hz), 6.06 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 0.26, 20.23, 102.13, 162.94, 169.94, 173.97.

22g: 6-Propyl-2,4-bis(trimethylsilyloxy)pyrimidine. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.27 (s, 9 H), 0.28 (s, 9 H), 0.83 (t, 3H, J = 7.36 Hz), 1.62 (sixt, 2H, J = 7.54 Hz), 2.42 (t, 2H, J = 7.62 Hz), 6.06 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 0.23, 13.65, 21.62, 39.36, 102.13, 162.94, 169.94, 173.97.

23e: 1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)(3*H*)pyrimidine-2,4,6-trione. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 3.72 (s, 2H), 4.49 (q, 1H, J = 6.40 Hz), 4.65 (m, 2H), 6.30 (m, 2H), 6.44 (d, 1H, J = 1.57 Hz), 7.3-7.9 (m, 15H, aromatic), 11.5 (s, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 56.19, 63.54, 70.08, 74.01, 77.95, 85.22, 128.65-133.99, 150.99, 164.62, 164.84, 165.60.

23f: 6-Methyl-3-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)(1*H*)pyrimidine-2,4-dione. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 2.06 (d, 3H, J = 0.52 Hz), 4.49-4.53 (m, 1H), 4.65 (m, 2H), 5.54 (d, 1H, J = 0.78 Hz), 6.06 (m, 2H), 6.46 (d, 1H, J = 1.57 Hz), 7.35-7.96 (m, 15H), 11.33 (s, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 19.06, 64.31, 71.42, 74.36, 78.74, 85.95, 99.32, 129.43-134.73, 151.56, 153.99, 163.04, 165.51, 165.65, 166.34.

23g: 6-Propyl-3-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)(1*H*)pyrimidine-2,4-dione. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 0.89 (t, 3H, J = 7.49 Hz), 1.55 (sixt, 2H, J = 7.54 Hz), 2.30 (t, 2H, J = 7.62 Hz), 4.51 (q, 1H, J = 6.13 Hz), 4.65 (m, 2H), 5.54 (d, 1H, J = 1.05 Hz), 6.07 (m, 2H), 6.47 (s, 1H), 7.35-7.97 (m, 15H), 11.29 (s, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 13.37, 20.30, 33.65, 63.61, 70.68, 73.66, 78.01, 85.95, 97.93, 128.70-133.98, 150.98, 156.57, 162.42, 164.76, 164.90, 165.60.

Table 2: Purity of the synthesized nucleotides using two different HPLC systems.

Compound	HPLC System 1[a]		HPLC System 2[b]	
	Retention time (min)	%Purity	Retention Time (min)	%Purity
9a	2.76	98.2	4.8[c]	99.0
9b	3.3	97.8	1.33[d]	97.4
9c	3.4	97.5	4.3[c]	97.1
9d	3.3	97.3	3.8[c]	96.8
9e	3.28	100	4.6[c]	99.8
9f	2.9	98.1	1.3[d]	97.3
9g	3.9	99.0	4.2[c]	98.3
10a	4.8	96.4	1.43[d]	96.6
10b	4.7	99.0	1.46[d]	99.3
10c	5.7	97.1	2.3[d]	97.6
10d	4.9	98.0	3.9[c]	98.4
10f	4.11	97.3	1.36[d]	96.4
10g	5.6	98.7	4.8[c]	99.0
11a	5.4	96.7	1.67[d]	96.3
11b	8.5	97.0	1.73[d]	96.6
11c	8.1	99.0	2.61[d]	98.6
11d	8.1	98.9	1.54[d]	98.6
11e	7.4	97.1	1.46[d]	96.7
11f	5.5	98.7	1.6[d]	98.1
11g	7.8	99.0	1.66[d]	99.2
12	3.2	97.5	4.6[c]	97.1
13	3.2	99.1	4.29[c]	99.5
15	4.3	96.8	1.27[d]	97.0

[a] The column was eluted with a solvent gradient of 0-10% of acetonitrile in 50 mM aq. NH_4HCO_3 buffer for 15 min at a flow rate of 5 ml/min using am RP-HPLC column (Knauer 20 mm ID, Eurospher-100 C18). UV absorption was detected at 254 nm.

[b] Elution was performed with a gradient of water : methanol (containing 2 mM of NH_4CH_3COO) from 90 : 10 to 0 : 100 for 40 min at a flow rate of 250 µl /min. UV absorption was scanned from 190-400 nm using a diode array detector. For quantitative analysis the UV absorption of the products was detected at 254 nm (Agilent 1100 DAD G 1315B).

[c] Using a Phenomenex 150 x 2.00 mm 4µ-polar- RP-HPLC 80 A column.

[d] Using a Phenomenex Luna 50 x 2.00 mm 3µ C18 HPLC column.

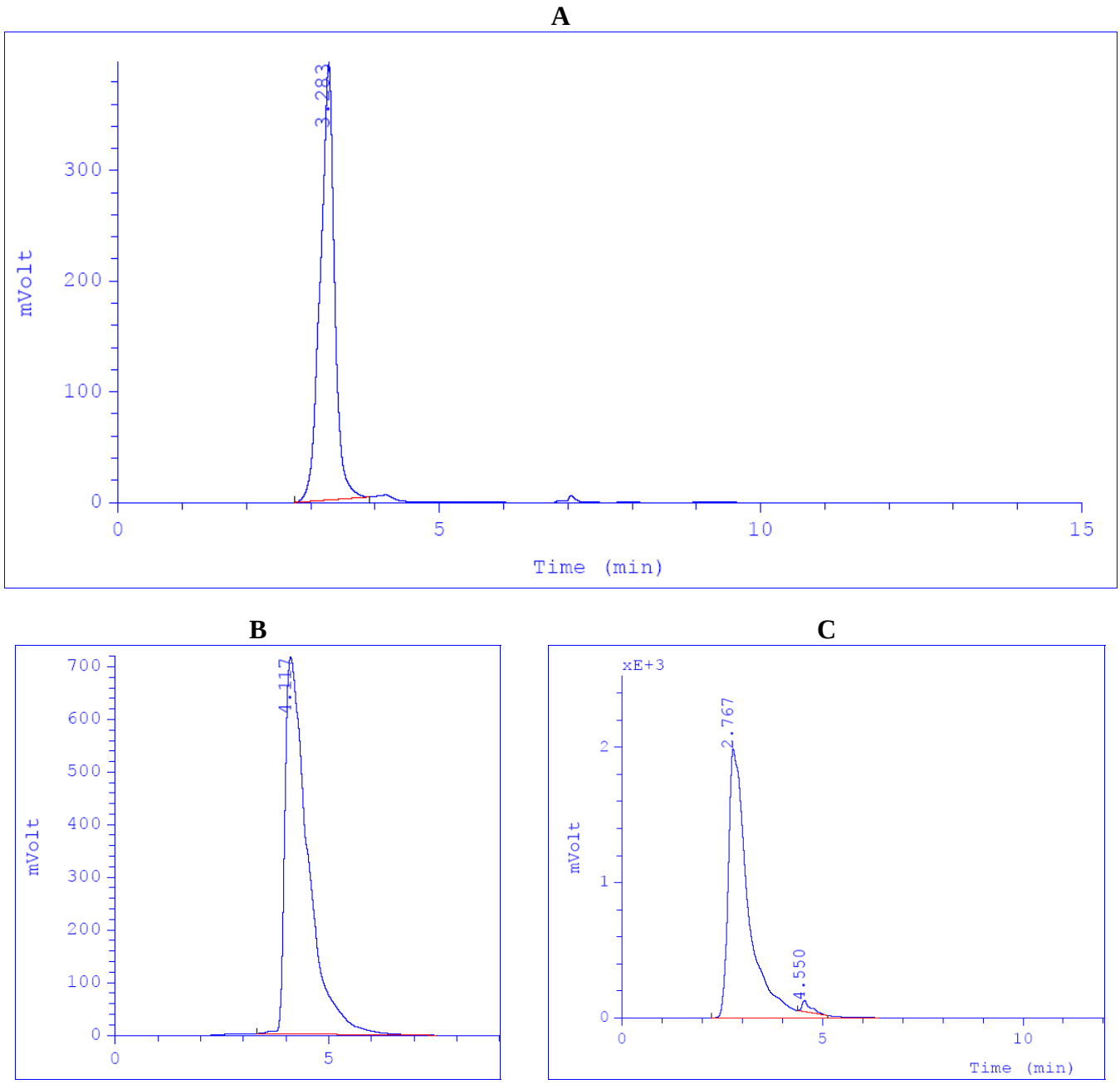


Figure 1. Selected HPLC chromatograms of test compounds (**A. 9e**, **B. 10f, C. 9a**) in HPLC system 1.

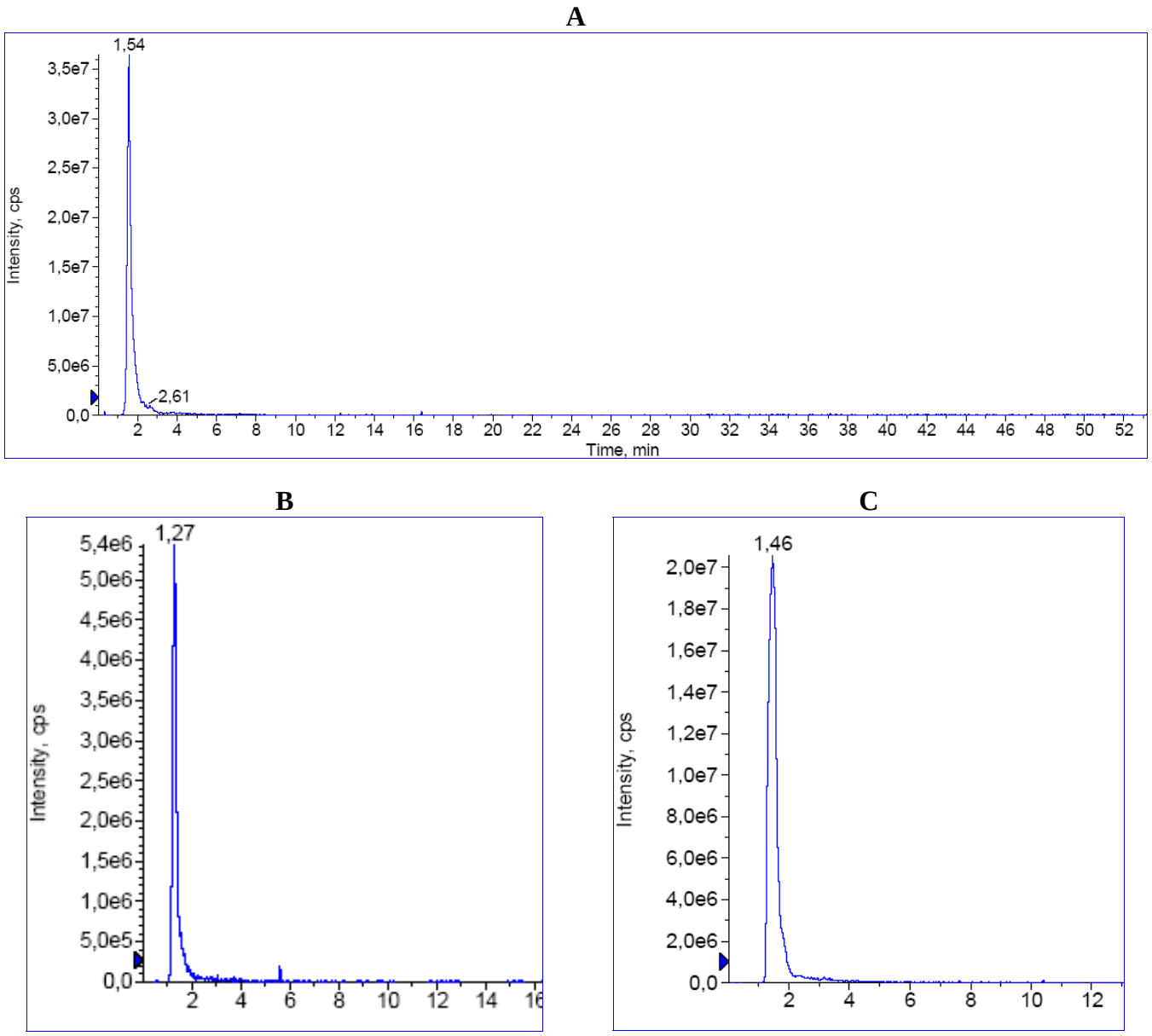


Figure 2. Selected HPLC chromatograms of test compounds (**A. 11d, B. 15, C. 10b**) in HPLC system 2.

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