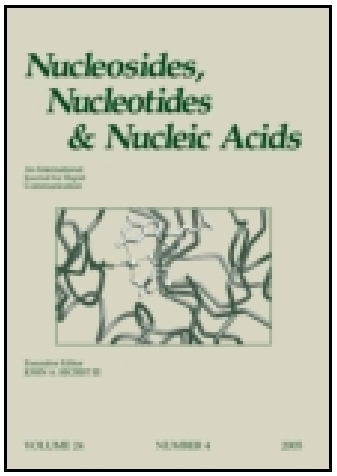


Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn20

Synthesis of Novel 5′-Uridine-Head Amphiphiles as Model for DNA Molecular Recognition

Matteo Tiecco [a], Pietro Di Profio [a], Raimondo Germani [a] & Gianfranco Savelli [a]

[a] CEMIN-Excellence Centre on Innovative Nanostructured Materials, Department of Chemistry, University of Perugia, Perugia, Italy

Published online: 20 Oct 2009.

To cite this article: Matteo Tiecco, Pietro Di Profio, Raimondo Germani & Gianfranco Savelli (2009) Synthesis of Novel 5′-Uridine-Head Amphiphiles as Model for DNA Molecular Recognition, Nucleosides, Nucleotides and Nucleic Acids, 28:10, 911-923, DOI: 10.1080/15257770903306625

To link to this article: http://dx.doi.org/10.1080/15257770903306625

Nucleosides, Nucleotides and Nucleic Acids, 28:911–923, 2009

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770903306625

Taylor & Francis
Taylor & Francis Group

SYNTHESIS OF NOVEL 5′-URIDINE-HEAD AMPHIPHILES AS MODEL FOR DNA MOLECULAR RECOGNITION

Matteo Tiecco, Pietro Di Profio, Raimondo Germani, and Gianfranco Savelli

CEMIN–Excellence Centre on Innovative Nanostructured Materials, Department of Chemistry, University of Perugia, Perugia, Italy

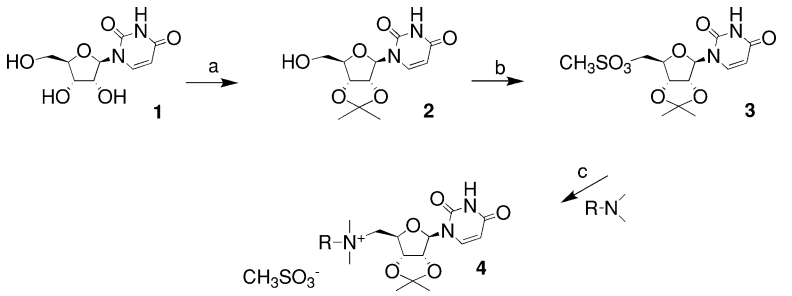
□ *Here we describe uridine functionalization in the 5′ position, which provides new classes of cationic and nonionic amphiphiles specifically designed as DNA transfection agents. The synthetic procedures developed to obtain the cationic uridine-head surfactants prevented intramolecular cyclization that occurs when uridine is functionalized in this position without using protecting groups in the uracil.*

Keywords DNA transfection agents; uridine-head amphiphiles; uracil

Surfactants play important roles in studies of interaction with biomacromolecules: e.g. stabilization and super-activation of enzymes[1] and DNA interaction and super-coiling effects.[2] Synthetic approaches are focused on preparing novel surfactant structures functionalized for specific roles.

Recent publications[3] have focused on the synthesis of amphiphilic nucleosides and nucleotides that are functionalized primarily in the base and in 2′ and 3′ hydroxyl groups of the ribose. These amphiphiles have interesting supramolecular properties in water, organic, and mixed solvents because they form vesicles, helices, wormlike aggregates, fibers, and gels. However, nucleosidic or nucleotidic amphiphiles specifically designed to interact with DNA for transfection are still lacking considering the efficiency of amphiphiles in this process.[4] The few available reports focus on amphiphiles with only the base involved in interactions because the ribose is functionalized in a way that strongly reduces the capability of recognition of the headgroup.[5] Unfortunately, when functionalized in the 5′ position,

Received 1 December 2008; accepted 18 May 2008.
Support of this work by Ministero dell'Università e Ricerca (PRIN 2006).
Address correspondence to Gianfranco Savelli, CEMIN–Excellence Centre on Innovative Nanostructured Materials, Department of Chemistry, Via Elce di Sotto n°8-I 06100 Perugia, University of Perugia, Italy. E-mail: savelli@unipg.it



SCHEME 1 a) p-toluenesulphonic acid, 2,2-dimethoxypropane, room temperature, 1.5 hours; b) CH_3SO_2Cl, TEA, 0°C, 16 hours; c) R = $C_{12}H_{25}$, reflux, 24 hours.

uridine can easily undergo intramolecular cyclization process that is sometimes the main reaction path.[6] For this reason we developed synthetic routes to novel compounds functionalized in 5′ position that are more effective. Here we describe alternative approaches that prevent intramolecular cyclization and may prove useful not only for uridine amphiphiles synthesis, but also for generic functionalization in the 5′ position of uridine.

All our synthetic procedures start from 2′,3′-*O*-isopropylidene uridine (**2**) that is prepared from commercially available uridine (**1**). Our method improves on reported procedures[7] by decreasing reaction times and increasing yields (Scheme 1). To synthesize the first class of cationic compounds (which contains an ammonium group bound to the 5′ position) we functionalized **2** with mesyl group in the 5′ position. Reaction with dimethyldodecylamine provided the ammonium salt (Scheme 1).

Nucleophilic substitution of the mesyl group in the 5′ position of **3** was very hard to reproduce and yields were low, ranging from 0 to about 30% under different experimental conditions. The dominant process is uridine intramolecular cyclization, as illustrated in Figure 1. That is triggered by deprotonation by the amine. In fact, methyl didodecylamine, which is more basic, gives only the cyclization product in this reaction. This well-known intramolecular process[6] is an obstacle in oligo-nucleoside synthesis, that is favoured by polar solvents and high temperatures. It occurs with a wide range of leaving groups and bases, even in the 2′ and 3′ positions. Uridine conformation is another major factor in this process. By rotation of the

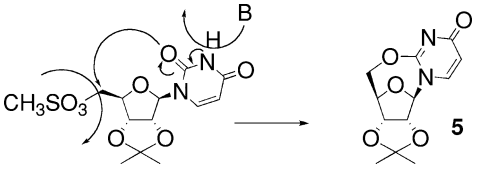


FIGURE 1 Uridine intramolecular cyclization process.

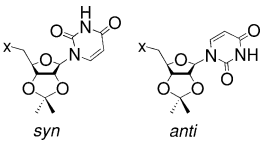
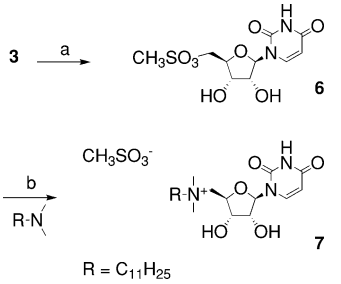


FIGURE 2 *syn* and *anti* conformations of uridine.

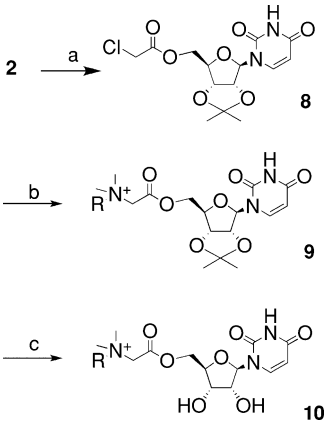
C-N (1-1′) bond, uridine assumes two limit conformations: *syn* and *anti* (Figure 2). Although uridine exists in both conformations, NOESY ^{1}H-NMR data[8] show that uridine 2′,3′-*O*-isopropylidene exists mainly in the *syn* conformation (99:1) due to steric hindrance, the cyclization occurs from the *syn* conformation. This unwanted product competing reaction was reduced by removing the protecting group before mesyl substitution to increase the population of the reactive *anti* conformation (Scheme 2).

When the same reaction was conducted on deprotected uridine, yields were much higher and the process was reproducible. At reflux, only ~10% of cyclization product was obtained. At a lower temperature (65°C) with a longer reaction time (7 days) no cyclization product was observed. To separate the ammonium salt from the cyclization product, the product mixture was treated with $NaClO_4$ and then passed over a Cl^- ion exchange to give the chloride salt. To obtain further evidence that removing the protecting group to prevent cyclization is an efficient process, we repeated the same reaction with methyl didodecylamine. On the deprotected mesyl substrate (**6**) no product, neither the cyclization nor the substitution, was observed even when the temperature was raised to 160°C and DMF was used instead of CH_3CN, probably because of lower amine nucleophilicity. Because the same reaction on the protected 5′-mesyl uridine (**3**) led only to the cyclization product, these results shows that removal of 2′,3′-*O*-isopropylidene to prevent cyclization is efficacious.

Another approach to obtain an ammonium salt via nucleophilic substitution and preventing unwanted cyclization reactions is to functionalize the uridine with a group that can be substituted by a tertiary amine at lower temperatures. With this approach we synthesized a second class of cationic



SCHEME 2 a) HCl (10%), room temperature, 12 hours; b) 65°C, 1 week.



SCHEME 3 a) Chloroacetyl chloride, Py, -10°C, 2 hours; b) dimethyldodecylamine (R = C12H25), 40°C, 24 hours; c) DOWEX monosphere 650L resin, room temperature, 12 hours.

products with the ammonium bound in the 5′ position with an ester spacer (**10**) (Scheme 3).

2′,3′-*O*-isopropylidene uridine (**2**) was functionalized with chloroacetyl chloride to obtain 5′-*O*–chloroacetyl-2′,3′-*O*-isopropylidene uridine (**8**).[9] Reaction with dimethyldodecylamine gave an amphiphilic ammonium salt (**9**). Because the reaction temperature was low (40°C), no cyclization occurs and the isopropylidene group does not need to be removed to prevent cyclization. The final step is removal of the protecting group with an acid resin such as DOWEX monosphere 650 L. At higher temperatures (reflux) cyclization product (**5**) and a carboxy-betaine were also obtained (Figure 3).

This intramolecular process is somewhat different from that of the other class of cationic compounds. In this case amine substitutes chloride and then the amine excess that is needed to obtain the substitution product promotes cyclization by acting as a base. This compound (**10**) is base-labile and thermo-labile. We also observed degradation with mild sonication in water or organic solvents.

The last class of uridine-head compounds are nonionic compounds. Scheme 4 illustrates the procedures that provide these novel nonionic amphiphilic uridine derivatives.

Functionalization in the 5′ position was performed using lauroyl chloride[10] and *p*-octyloxybenzoyl chloride. Isopropylidene was removed

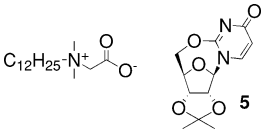
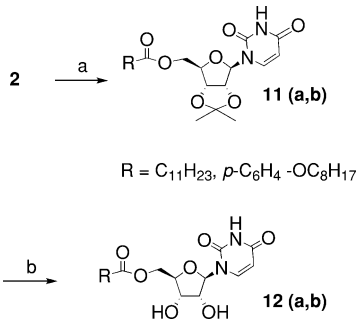


FIGURE 3 The cyclization product and the carboxy-betaine obtained refluxing 5′-*O*-chloroacetyl-2′,3′-isopropilidene uridine and dimethyldodecylamine.



SCHEME 4 a) Py, lauroyl chloride (**11a**), p-octyloxybenzoylchloride (**11b**), room temperature, 24 hours; b) HCl (10%), room temperature, 12 hours (**12a**), 14 hours (**12b**).

with HCl to obtain very good yields of 5′-lauroyl uridine and 5′-*p*-octyloxybenzoyl uridine (90% for **11a** -80% for **11b** and 87% for **12**). Because uridine was functionalized with these substrates at room temperature, no cyclization products were observed.

In summary, we functionalized uridine in the 5′ position to give novel uridine amphiphilic compounds. In the procedures developed to obtain the two cationic surfactants, the methods prevented unwanted cyclization products. Interestingly, the amide in uracil position 3 did not require protection even though, given the role of this portion in the cyclization process (Figure 1), this might have appeared to be the most efficient way of preventing cyclization.

In the procedure for the first cationic amphiphile (**7**), removing the protecting group in the uridine increased yields and ensured reproducibility of nucleophilic substitution of the mesyl in 5′ position even at high temperatures that favour cyclization.[6] In the synthesis of the second cationic product (**10**), we functionalized uridine as a quaternary ammonium, by using chloroacetyl chloride that yielded the nucleophilic substitution product at temperatures of about 40°C. The synthetic procedures for the nonionic compounds (**11** and **12**) gave very good yields.

These novel compounds are currently being used on DNA interaction studies to evaluate their capacity to super-coil DNA and to assess their transfection efficiency.

EXPERIMENTAL

Unless otherwise specified, all reactions were carried out under anhydrous conditions with freshly distilled reagents and solvents or commercially available anhydrous solvents and under nitrogen atmosphere. Melting points were determined on Barloworld Scientific Stewart (Dunmow, UK) SMP3 apparatusand are uncorrected. Gas-chromatography analyses were performed on an Agilent 6850 Series II Network GC instrument (Agilent

Technologies, Santa Clara, CA, USA). TLC separations were performed on silica gel on TLC-cards on aluminium foils. ^{1}H-NMR and ^{13}C-NMR were registered on a Bruker 200 MHz and on a Bruker 400 MHz instruments. ESI-MS experiments were obtained on a QUATTRO triplequadrupole mass spectrometer (Micromass, Manchester, UK), operating in the positive and negative ion mode and equipped with a Z-spray electrospray source. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) spectra were measured on a Voyager DE-PRO MALDI-TOF mass spectrometer (Applied Biosystems, Foster City, CA, USA) operating in the reflection positive ion mode. MALDI mass spectra were calibrated through external calibration using an Applied Biosystems standard calibration mixture.

2′,3′-*O*-isopropylidene-uridine (2)

Uridine (**1**) (3 g, 12.3 mmol), 2,2-dimethoxypropane (20 mL, 0.1626 mol), and *p*-toluenesulfonic acid (100 mg, 0.525 mmol) were dissolved in dry acetone (100 mL) and stirred at room temperature for 1.5 hour. The mixture was dried under vacuum at room temperature to give a spongy yellow raw; the crude product was left in this condition for about 4 hours. Amberlyst A-21 basic resin was then added with 20 mL of dry acetone. The resin was filtered and the mixture dried under vacuum. The product was crystallized from dichloromethane (DCM) at −20°C to give white crystals. Yield 90% (3.15 g); m.p. = 165–168°C.

^{1}H-NMR (200 MHz, CD_3OD) $\delta = 1.3$ (s, 3H, CH_3 isop.), 1.5 (s, 3H, CH_3 isop.), 3.8 (m, 2H, CH_2 in 5′), 4.2 (m, 1H, H in 4′), 4.8 (m, 2H, H in 2′ and 3′), 5.7 (d, 1H, A of AMX, $J_{AX} = 8$ Hz, H in 5), 5.9 (m, 1H, H in 1′), 7.8 (d, 1H, M of AMX, $J_{AX} = 8$ Hz, H in 6).

^{13}C-NMR (200 MHz, CD_3OD) $\delta = 25.7$ (isop.), 27.7 (isop.), 63.2 (5′), 82.4 (2′), 86.0 (3′), 88.6 (4′), 94.3 (1′), 102.8 (5), 115.4 (ipso isop.), 144.1 (6), 152.4 (ipso 2), 166.4 (ipso 4).

2′,3′-*O*-isopropylidene-5′-mesyl-uridine (3)

2′,3′-*O*-isopropylidene-uridine (**2**) (1.314 g, 4.62 mmol) was dissolved in anhydrous DCM (35 mL) with TEA (1 mL, 7.17 mmol). At the temperature of 0°C, CH_3SO_2Cl (0.4 mL, 5.17 mmol) was added dropwise. After 16 hours, the mixture was washed with water and ice. The organic phases were dried with anhydrous Na_2SO_4 and evaporated under vacuum: a pale yellow solid was obtained. Yield 90% (1.50 g,); m.p. = 108–110°C.

^{1}H-NMR (200 MHz, $CDCl_3$) $\delta = 1.4$ (s, 3H, CH_3 isop.), 1.6 (s, 3H, CH_3 isop.), 3.0 (s, 3H, mesyl), 4.4 (m, 1H, H in 4′), 4.5 (m, 2H, CH_2 in 5′), 4.9 (m, 1H, H in 2′), 5.0 (m, 1H, H in 3′), 5.6 (m, 1H, H in 1′), 5.7 (d, 1H, A of AMX, $J_{AX} = 8$ Hz, H in 5), 7.2 (d, 1H, M of AMX, $J_{AX} = 8$ Hz, H in 6), 8.7 (sbr, 1H, NH in 3).

^{13}C-NMR (200 MHz, CD_3OD) $\delta = 24.6$ (isop.), 26.6 (isop.), 37.6 (mesyl), 70.8 (5′), 82.7 (2′), 85.8 (3′), 86.7 (4′), 96.3 (1′), 103.1 (5), 115.7 (ipso isop.), 145.1 (6), 152.1 (ipso 2), 166.4 (ipso 4).

2′,3′-*O*-isopropylidene-5′-dimethyldodecyl Ammonium-uridine Mesylate (4)

2′,3′-*O*-isopropylidene-5′-mesyl-uridine (**3**) (1.61 g, 4.44 mmol) was dissolved in anhydrous CH_3CN (30 mL) with freshly distilled dimethyldodecylamine (1.8 mL, 6.49 mmol), the mixture was refluxed for 24 hours. The solvent was evaporated by vacuum and the resultant yellow oil washed many times with anhydrous diethyl ether. The solid was crystallized in ethyl acetate at -20°C. Yield 28% (0.72 g,); m.p. = 184–186°C.

^{1}H-NMR (400 MHz, CD_3OD) δ = 0.9 (t, 3H, CH_3-R), 1.3 (m, 18H, $(CH_2)_9$), 1.4 (s, 3H, CH_3 isop.), 1.5 (s, 3H, CH_3 isop.), 1.8 (m, 2H, CH_2 in β at N^+), 2.7 (s, 3H, $CH_3SO_3^-$), 2.9 (ss, 6H, $N^+(CH_3)_2$), 3.1 (m, 2H, R-CH_2-N^+), 4.3 (m, 1H, H in 2′), 4.6 (m, 1H, H in 3′), 5.0 (m, 1H, H in 4′), 5.1 (dd, 2H, CH_2 in 5′), 5.7 (m, 1H, H in 1′), 6.1 (d, 1H, A of AMX, $J_{AX} = 8$ Hz, H in 5), 7.9 (d, 1H, M of AMX, $J_{AX} = 8$ Hz, H in 6).

^{13}C-NMR (400 MHz, CD_3OD) δ = 14.6 (CH_3 chain), 24.9 (isop.), 26.6 (isop.), 23.9, 25.9, 27.6, 30.4-30.9 (CH_2- chain), 33.2 (mesyl), 39.7 (5′), 44.6 (α at N^+), 59.3 ($N^+(CH_3)_2$), 76.7 (3′), 83.2 (4′), 86.7 (2′), 100.6 (1′), 110.5 (5), 114.3 (ipso isop.), 145.8 (6), 160.0 (ipso 2), 175.0 (ipso 4).

Didodecylmethylamine

Didodecylamine (3.1 g, 8.76 mmol) was dissolved in 40 mL of ethanol at 40°C and HCOOH (1.6 mL, 42.4 mmol) was added dropwise. After 30 minutes, HCOH (1.6 mL, 23.2 mmol) was added dropwise and the temperature was raised to 82°C for 2 hours. After cooling to room temperature aqueous 10% NaOH solution was added to quench the reaction. DCM was added and the organic phase was separated and evaporated to obtain a pale yellow liquid. This liquid was purified on a silica gel column, eluent EtOH. Yield 74% (2.38 g); GC > 99%.

^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.8 (t, 6H, $2CH_3$), 1.1 (m, 36H, -$(CH_2)_{18}$-), 1.3 (m, 4H, $2CH_2$ in β to N), 2.1 (s, 3H, CH_3), 2.2 (t, 4H, CH_2-N).

Cyclonucleoside (5)

2′,3′-*O*-isopropylidene-5′-mesyl-uridine (**3**) (0.955 g, 2.64 mmol) was dissolved in anhydrous CH_3CN (40 mL) with didodecylmethylamine (1.4 g, 3.81 mmol), and refluxed for 6 days. The solvent was evaporated by vacuum and the resultant yellow oil washed many times with diethyl ether. The crude

product was recrystallized from diethyl ether/ethyl acetate (50/50) to give white crystals. Yield 60% (0.45 g); m.p. = decomposes at T ≥ 230°C.

^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.3 (s, 3H, CH_3 isop.), 1.5 (s, 3H, CH_3isop.), 4.1 (m, 1H, H in 3′), 4.4 (m, 1H, H in 2′), 4.6 (m, 1H, H in 4′), 4.9 (m, 2H, CH_2 in 5′), 5.3 (m, 1H, H in 1′), 6.0 (d, 1H, A of AMX, J_{AX} = 8 Hz, H in 5), 7.2 (d, 1H, M of AMX, J_{AX} = 8 Hz, H in 6).

Anal. Calcd for $C_{12}H_{16}N_2O_5$: N 9.99, C 55.71, H 5.75; found N 10.12, C 56.00, H 5.98.

5′-Mesyl-uridine (6)

2′3′-*O*-isopropylidene-5-mesyl-uridine (**3**) (1.504 g, 4.15 mmol) was dissolved in MeOH (7 mL) at room temperature; a water solution of HCl (10%) was added dropwise. After 12 hours the mixture was dried under vacuum, extracted with MeOH, filtered, the filtrate was dried under vacuum, and then treated with P_2O_5 under vacuum for 3 days to remove the residual H_2O. Yield 85% (1.137 g,); amorphous.

^{1}H-NMR (200 MHz, CD_3OD) δ = 3.0 (s, 3H, mesyl), 4.2 (m, 2H, CH_2 in 5′), 4.3 (m, 1H, H in 4′), 4.5 (m, 1H, 2′), 4.6 (5.5 m, 1H, 3′), 5.9 (m, 1H, H in 1′), 5.7 (d, 1H, A of AMX, J_{AX} = 8 Hz, H in 5), 7.7 (d, 1H, M of AMX, J_{AX} = 8 Hz, H in 6).

^{13}C-NMR (200 MHz, CD_3OD) δ = 37.5(mesyl), 70.3 (5′), 70.4 (3′), 74.9 (2′), 83.0 (4′), 91.7 (1′), 103.1 (5), 142.6 (6), 152.4 (ipso 2), 166.2 (ipso 4).

Uridine-5′-dimethyldodecyl Ammonium Mesylate (7)

5-mesyl-uridine (**6**) (1.00 g, 3.10 mmol) was dissolved in anhydrous CH_3CN (40 mL) with dimethyldodecylamine (1.29 mL, 4.65 mmol), treated at 65°C for 1 week, and dried under vacuum to give a yellow oil. This crude product was washed many times with Et_2O giving a semi-solid material that was crystallized from Et_2O/MeOH (75/25) giving white, highly hygroscopic crystals. Yield 75% (1.24 g); m.p. = 154–156°C.

^{1}H-NMR (200 MHz, CD_3OD) δ = 0.8 (t, 3H, CH_3-R), 1.3 (m, 18H, $(CH_2)_9$), 1.8 (m, 2H, CH_2 in β at N^+), 2.7 (s, 3H, mesyl), 3.2 (ss, 6H, $N^+(CH_3)_2$), 3.4 (m, 2H, R-CH_2-N^+), 3.7 (m, 1H, H in 2′), 3.8 (m, 1H, H in 3′), 4.1 (m, 1H, H in 4′), 4.3 (m, 1H, H in 5′), 5.7 (d, 1H, A of AMX, J_{AX} = 8 Hz, H in 5), 5.8 (m, 1H, H in 1′), 7.7 (d, 1H, M of AMX, J_{AX} = 8 Hz, H in 6).

^{13}C-NMR (400 MHz, CD_3OD) δ = 14.7 (CH_3 chain), 23.9-33.3 (-CH_2-chain), 39.7 (mesyl), 52.6 (5′), 52.7 (α at N^+), 66.7 ($N^+(CH_3)_2$), 66.9 ($N^+(CH_3)_2$), 73.4 (2′), 73.6 (3′), 78.1 (4′), 95.7 (1′), 103.5 (5), 144.2 (6), 152.1 (ipso 2), 166.1 (ipso 4).

MALDI-TOF MS: *m/z* $[M+H]^+$ calcd for $C_{23}H_{42}N_3O_5$ 440.32 found 440.34.

ESI-MS: *m/z* $[M+H]^+$ calcd for $C_{23}H_{42}N_3O_5$ 440.32 found 440.50.

Uridine 2′,3′-*O*-isopropylidene-5′-*O*-chloroacetyl (8)

Uridine 2′,3′-*O*-isopropylidene (**2**) (1.49 g, 5.24 mmol) was dissolved in anhydrous DCM (30 mL) with pyridine (0.85 mL, 10.5 mmol), cooled to −10°C, and chloroacetyl chloride (0.83 mL, 10.4 mmol) was added dropwise with magnetic stirring. After 2 hours, cold water saturated of NaCl was added, the organic phase washed many times with water, dried with anhydrous Na_2SO_4, and evaporated under vacuum. The crude product was crystallized from DCM/Et_2O (60/40) at -20°C. Yield 72% (1.36 g); m.p. = 139–141°C.

^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.4 (s, 3H, CH_3 isop.), 1.6 (s, 3H, CH_3 isop.), 4.1 (s, 2H, $ClCH_2CO$), 4.4 (m, 1H, H in 4′), 4.5 (m, 2H, CH_2 in 5′), 4.9 (m, 1H, H in 2′), 5.1 (m, 1H, H in 3′), 5.6 (m, 1H, H in 1′), 5.8 (d, 1H, A of AMX, J_{AX} = 8 Hz, H in 5), 7.3 (d, 1H, M of AMX, J_{AX} = 8 Hz, H in 6), 9.7 (sbr, 1H, NH in 3).

^{13}C-NMR (200 MHz, $CDCl_3$) δ = 24.9 (isop.), 26.7 (isop.), 40.4 (Cl-CH_2), 65.3 (5′), 80.6 (4′), 84.2 (2′), 85.0 (3′), 95.0 (1′), 102.4 (5), 114.4 (ipso isop.), 142.4 (6), 150.4 (ipso 2), 163.3 (ipso 4), 166.6 (ipso acetyl).

Uridine 2′,3′-*O*-isopropylidene-5′-O-Acetyl-dimethyl Dodecyl Ammonium Chloride (9)

Uridine 2′,3′-*O*-isopropylidene-5′-*O*-chloroacetyl (**8**) (3.18 g, 8.83 mmol) was dissolved in anhydrous CH_3CN (40 mL) with dimethyldodecylamine (3 mL, 10.8 mmol) and warmed at 40°C for 24 hours. Removal of the solvent under vacuum to gave a pale yellow oil. The crude product was washed many times with diethyl ether giving a noncrystalline sticky solid. Yield 70% (3.55 g); amorphous.

^{1}H-NMR (200 MHz, CD_3) δ = 0.9 (t, 3H, CH_3-R), 1.3 (m, 20H, $(CH_2)_{10}$-), 1.4 (s, 3H, CH_3 isop.), 1.6 (s, 3H, CH_3 isop.), 1.8 (m, 2H, CH_2 in β at N^+), 3.3 (m, 6H, CH_3-N^+), 3.5 (m, 2H, CH_2 in α at N^+), 3.6 (m, 1H, H in 3′), 3.8 (m, 2H, H in 2′), 3.9 (m, 2H, CO-CH_2-N^+), 4.3 (H in 4′), 4.4 (m, 2H, CH_2 in 5′), 5.7 (d, 1H, A of AMX, J_{AX} = 8 Hz, H in 5), 5.9 (m, 1H, H in 1′), 7.8 (d, 1H, M of AMX, J_{AX} = 8 Hz, H in 6).

^{13}C-NMR (200 MHz, CD_3OD) δ = 12.5 (CH_3- chain), 25.7 (CH_3 isop), 28.3 (CH_3 isop), 21.8, 22.7, 23.7, 25.4, 28.6, 28.8, 31.2 (-CH_2- chain), 49.8 (-$\underline{C}H_2$-CH_2-N^+), 50.3 (R-CH_2-N^+), 60.4, 61.2 (CH_3-N_+), 63.7 (N^+-$\underline{C}H_2$-CO), 65.1 (5′), 80.3 (3′), 83.9 (2′), 86.5 (4′), 92.2 (1′), 100.8 (5), 113.2 (ipso isop), 141.9 (6), 151.1 (ipso 2), 164.8 (ipso N^+-CH_2-$\underline{C}O$), 165.0 (ipso 4).

Uridine-5′-*O*-Acetyl-dimethyl Dodecyl Ammonium Chloride (10)

Uridine 2′,3′-O-isopropylidene-5′-O-acetyl-dimethyl dodecyl ammonium chloride (**9**) (3.55 g, 6.18 mmol) was dissolved in MeOH at room temperature, and DOWEX monosphere 650L (H^+) resin beads (~8 g) were added,

stirred at room temperature overnight, passed through a column containing the same resin, and dried under vacuum to give a slightly yellow solid. Yield 88% (2.90 g); m.p. = 168–171°C.

^{1}H-NMR (200 MHz, CD_3OD) δ = 0.9 (t, 3H, CH_3-R), 1.3 (m, 20H, $(CH_2)_{10}$-), 1.7 (m, 2H, CH_2 in β at N^+), 3.3 (s, 6H, CH_3-N^+), 3.6 (m, 2H, CH_2 in α at N^+), 3.7 (m, 2H, H in 2′), 3.8 (s, 2H, N^+CH_2CO), 4.1 (m, 2H, H in 3′), 4.2 (m, 2H, CH_2 in 5′), 4.4 (m, 1H, H in 4′), 5.7 (d, 1H, A of AMX, J_{AX} = 8 Hz, H in 5), 5.9 (m, 1H, H in 1′), 8.0 (d, 1H, M of AMX, J_{AX} = 8 Hz, H in 6).

^{13}C-NMR (200 MHz, CD_3OD) δ = 14.7 (CH_3 chain), 23.8, 27.4, 30.3, 30.9, 33.2, 52.6 (-CH_2- chain), 54.0 (R-CH_2-N^+), 62.2, 62.4 (CH_3-N_+), 66.8 (N^+-$\underline{C}H_2$-CO), 67.2 (5′), 71.4 (3′), 75.5 (2′), 86.5 (4′), 90.8 (1′), 103.0 (5), 143.0 (6), 152.7 (ipso 2), 166.5 (ipso N^+-CH_2-$\underline{C}O$), 166.7 (ipso 4).

MALDI-TOF MS: *m/z* $[M+H]^+$ calcd for $C_{25}H_{44}N_3O_7$ 498.63 found 498.48.

ESI-MS: *m/z* $[M+H]^+$ calcd for $C_{25}H_{44}N_3O_7$ 498.63 found 498.52.

2′,3′-O-isopropylidene-5′-lauroyl-uridine (11a)

2′3′-*O*-isopropylidene-uridine (**2**) (2.87 g, 10.1 mmol) was dissolved with anhydrous pyridine (1 mL, 12.4 mmol) in dry DCM/THF (70/30) at room temperature. Lauroyl chloride (2.6 mL, 11.2 mmol) was added dropwise with magnetic stirring for 24 hours. The solvent was removed under vacuum to give a yellow oil that was dissolved in Et_2O, the organic phase was washed with water, dried with anhydrous Na_2SO_4, and dried under vacuum. The crude product was purified by column chromatography (SiO_2, Et_2O), yielding a sticky solid. Yield 90% (4.24 g); amorphous.

^{1}H-NMR (200 MHz, $CDCl_3$) δ = 0.6 (t, 3H, R-CH_3), 1.0 (m, 18H, -CH_2-), 1.1 (s, 3H, CH_3 isop.), 1.3 (s, 3H, CH_3 isop.), 2.0 (t, 2H, CH_2CO), 4.0 (m, 1H, H in 4′), 4.1 (m, 2H, CH_2 in 5′), 4.5 (m, 1H, H in 2′), 4.7 (m, 1H, H in 3′), 5.4 (m, 1H, H in 1′), 5.5 (d, 1H, A of AMX, J_{AX} = 8 Hz, H in 5), 7.0 (d, 1H, M of AMX, J_{AX} = 8 Hz, H in 6), 8.7 (sbr, 1H, NH in 3).

^{13}C-NMR (200 MHz, CD_3OD) δ = 14.6 (CH_3- chain), 23.9 (CH_3- isop), 27.6 (CH_3- isop), 14.6-33.2 (-CH_2- chain), 35.0 (R-$\underline{C}H_2$-CO), 65.3 (5′), 82.9 (3′), 86.0 (2′), 86.5 (4′), 95.8 (1′), 104.8 (5), 115.5 (ipso isop), 144.6 (6), 152.0 (ipso 2), 166.3 (ipso 4), 170.8 (ipso R-CO).

2′,3′-O-isopropylidene-5′-*p*-octyloxybenzoyl-uridine (11b)

2′3′-*O*-isopropylidene-uridine (**2**) (1.72 g, 6.16 mmol) was dissolved in anhydrous pyridine (25 mL) at room temperature. *p*-Octyloxybenzoyl chloride (2.6 mL, 11.2 mmol) was added dropwise and the solution was stirred for 24 hours. The pyridine was removed under vacuum, the resultant

yellow raw was dissolved in Et_2O and washed with aqueous HCl (10%). The organic phase was dried with anhydrous Na_2SO_4 and Et_2O removed under vacuum. The product was purified by column chromatography (SiO_2, Et_2O) giving a white sticky solid. Yield 80% (2.54 g); amorphous.

^{1}H-NMR (200 MHz, CD_3OD) δ = 0.9 (t, 3H, CH_3-R), 1.3 (m, 12H, -$(CH_2)_6$-), 1.4 (s, 3H, CH_3 isop.), 1.6 (s, 3H, CH_3 isop.), 1.8 (m, 2H, O-CH_2-CH_2-), 4.0 (t, 2H, O-CH_2), 4.5 (m, 1H, H in 4′), 4.5 (m, 2H, CH_2 in 5′), 5.0 (m, 1H, H in 2′), 5.1 (m, 1H, H in 3′), 5.6 (d, 1H, A of AMX, J_{AX} = 8 Hz, H in 5), 5.8 (m, 1H, H in 1′), 7.0 (m, 2H, Ar), 7.6 (d, 1H, M of AMX, J_{AX} = 8 Hz, H in 6), 7.9 (m, 2H, Ar).

^{13}C-NMR (200 MHz, CD_3OD) δ = 14.6 (CH_3- chain), 25.6 (CH_3- isop), 27.2 (CH_3- isop), 23.8, 24.3, 27.6, 30.4, 30.5, 33.1 (-CH_2- chain), 65.6 (5′), 69.5 (O-CH_2-), 82.9 (3′), 86.2 (2′), 86.8 (4′), 95.9 (1′), 102.8 (5), 115.5 (ipso isop), 115.5 (Ar), 123.0 (ipso Ar-CO), 132.8 (Ar), 144.2 (6), 152.1 (ipso 2), 165.0 (ipso Ar-O), 166.3 (ipso 4), 170.8 (ipso Ar-CO).

5′-lauroyl-uridine (12a)

2′,3′-O-isopropylidene-5′-lauroyl-uridine (**11a**) (4.2 g, 9.0 mmol) was dissolved in acetone at room temperature (50 mL), aqueous HCl (10%) was added until the reaction ended as shown by TLC. The reaction required 40 mL of HCl (10%) over about 12 hours. The product was insoluble in this mixture, and collected by filtration giving white crystals. Yield 87% (3.34 g); m.p. = 138–140°C.

^{1}H-NMR (200 MHz, DMSO-d_6) δ = 0.9 (t, 3H, R-CH_3), 1.2 (m, 16H, -$(CH_2)_9$-), 1.5 (t, 2H, CH_2-CH_2-CO), 2.3 (t, 2H, CH_2-CO), 3.6 (m, 1H, H in 2′), 3.9 (m, 2H, CH_2 in 5′), 4.2 (m, 1H, H in 4′), 5.2 (m, 1H, H in 3′), 5.5 (m, 1H, H in 2′), 5.7 (d, 1H, A of AMX, J_{AX} = 8 Hz, H in 5), 5.8 (m, 1H, H in 1′), 7.6 (d, 1H, M of AMX, J_{AX} = 8 Hz, H in 6), 11.3 (sbr, 1H, H in 3).

^{13}C-NMR (200 MHz, DMSO-d_6) δ = 12.6 (CH_3- chain), 22.1, 24.4, 28.7, 29.0, 31.3, (-CH_2- chain), 33.3 (R-CH_2-CO), 63.6 (5′), 69.7 (3′), 72.7 (2′), 81.0 (4′), 88.6 (1′), 101.9 (5), 140.7 (6), 150.6 (ipso 2), 164.0 (ipso 4), 172.7 (ipso R-CO).

ESI-MS: calcd for $C_{21}H_{34}N_2O_7$ 426.51, *m/z* $[M+H]^+$ found 427.50; *m/z* $[M-H]^-$ found 425.32.

5′-*p*-octyloxybenzoyl-uridine (12b)

2′,3′-O-isopropylidene-5′-p-octyloxybenzoyl-uridine (**11b**) (0.78 g, 1.51 mmol) was dissolved in 5 mL of acetone; aqueous HCl (10%) added until the reaction was complete as shown by TLC. The 25 mL of HCl 10% was added over about 14 hours. The insoluble product was collected by filtration giving white crystals. Yield 87% (0.63 g); m.p. = 156–159°C.

^{1}H-NMR (200 MHz, DMSO-d_6) δ = 0.9 (t, 3H, R-CH_3), 1.3 (m, 10H, (-CH_2)5-), 1.7 (m, 2H, O-CH_2-CH_2-), 4.1 (t, 2H, O-CH_2-CH_2-), 4.2 (m, 1H, H in 4′), 4.4 (m, 2H, CH_2 in 5′), 5.3 (m, 1H, H in 2′), 5.6 (m, 1H, H in 3′), 5.7 (d, 1H, A of AMX, J_{AX} = 8 Hz, H in 5), 5.8 (m, 1H, H in 1′), 7.1 (m, 2H, Ar), 7.6 (d, 1H, M of AMX, J_{AX} = 8 Hz, H in 6), 7.9 (m, 2H, Ar), 11.4 (sbr, 1H, H in 3).

^{13}C-NMR (200 MHz, DMSO-d_6) δ = 12.6 (CH_3- chain), 22.1, 25.4, 28.7, 31.2 (-CH_2- chain), 63.9 (O-CH_2-), 67.9 (5′), 69.7 (3′), 72.8 (2′), 81.1 (4′), 89.0 (1′), 101.9 (5), 114.5 (Ar), 121.3 (ipso Ar-CO), 131.4 (Ar), 140.8 (6), 150.5 (ipso 2), 162.8 (ipso Ar-O), 163.0 (ipso 4), 165.2 (ipso Ar-CO).

ESI-MS: calcd for $C_{24}H_{34}N_2O_7$ 476.52, *m/z* $[M+H]^+$ found 477.53; *m/z* $[M-H]^-$ found 475.36.

REFERENCES

1. a) Spreti, N.; Mancini, M.V.; Germani, R.; Di Profio, P.; Savelli, G. Substrate effect on a-chymotrypsin activity in aqueous solutions of "big-head" ammonium salts. *J. Mol. Catal. B* **2008**, 50, 1–6; b) Alfani, F.; Cantarella, M.; Spreti, N.; Germani, R.; Savelli, G. α-chymotrypsin superactivity in cetyltrialkylammonium bromide-rich media. *Appl. Biochem. Biotechnol.* **2000**, 88, 1–15; c) Spreti, N.; Alfani, F.; Cantarella, M.; D'Amico, F.; Germani, R.; Savelli, G. a-Chymotrypsin superactivity in aqueous solutions of cationic surfactants. *J. Mol. Catal.* **1999**, 6, 99–110.
2. a) Wang, Y.; Dubin, P.L.; Zhang, H. Interaction of DNA with cationic micelles: Effects of micelle surface charge density, micelle shape, and ionic strenght on complexation and DNA collapse. *Langmuir*, **2001**, 17, 1670–1673; b) Sun, X.; Cao, E.; Liu, D.; Bai, C. The divalent cation-induced DNA condensation studied by atomic force microscopy and spectra analysis. *Inor, Chem. Comm.*, **2002**, 5, 181–186; c) Bhattacharya, S.; Mandal, S.S. Evidence of interlipidic ion-pairing in anion-induced DNA release from cationic amphiphile-DNA complexes. Mechanistic implications in transfection. *Biochemistry*, **1998**, 37, 7764–7777.
3. a) Moreau, L.; Barthèlèmy, P.; El Maataoui, M.; Grinstaff, M. W. Supramolecular assemblies of nucleoside phosphocholine amphiphiles. *J. Am. Chem.. Soc.* **2004**, 126, 7533–7539; b) Giorgi, T.; Lena, S.; Marianini, P.; Cremonini, M.; Masiero, S.; Pieraccini, S.; Rabe, J.; Samorì, P.; Spada, G.; Gottarelli, G. Supramolecular helices via self-assembly of 8-oxoguanosines. *J. Am. Chem.. Soc.* **2003**, 125, 14741–14749; c) Bombelli, F.; Berti, D.; Pini, F.; Keiderling, U.; Baglioni, P. Flexibility of dilauroyl-phosphatidyl-nucleoside wormlike micelles in acqueous solutions. *J. Phys. Chem. A* **2004**, 108, 16427–16434.
4. Goracci, L.; Germani, R.; Savelli, G.; Bassani, D. Hoechst 33258 as a pH-Sensitive Probe to Study the Interaction of Amine Oxide Surfactants with DNA. *ChemBiochem* **2005**, 6, 197–203.
5. a) Shiruda, P.; Kumar, V.; Ganesh, K. Chimeric peptide nucleic acids incorporating (2S,5R)-aminoethyl pipecolyl units: synthesis and DNA binding studies. *Tetrahedron Lett.* **2004**, 45, 3085–3088; b) Barthèlèmy, P.; Prata, C.; Filocamo, S.; Maynor, B.; Hashmi, N.; Lee, S.; Grinstaff, M. W. Supramolecular assemblies of DNA with neutral nucleoside amphiphiles. *Chem. Comm.*, **2005**, 1261–1263.
6. a) Mitsunobu, O. The use of diethyl azodicarboxylate and triphenylphosphine in synthesis and transformation of natural products. *Synthesis*, **1981**, 1–28; b) Amarnanth, V.; Broom, D. Chemical Synthesis of Oligonucleotides. *Chem. Rev.* **1977**, 77, 183–217.
7. a) Shaikh, N. S.; Bhor, S.; Gajare, A.; Deshpande, V.; Wakharkar, R. Mild and facile procedure for clay-catalyzed acetonide protection and deprotection of N(Boc)-amino alcohols and protection of 1,2-diols. *Tetrahedron Lett.* **2004**, 45, 5395–5398; b) Jung, K.; Hohl, R.; Wiemer, A.; Wiemer, D. Synthesis of phosphonate derivatives of uridine, cytidine, and cytosine arabinoside. *Bioorg. Med. Chem.*, **2000**, 8, 2501–2509; c) Johnson, D.; Widlanski, T. Facile deprotection of O-Cbz-protected nucleosides by hydrogenolysis: an alternative to O-benzyl ether-protected nucleosides. *Org. Lett.* **2004**, 6, 4643–4646.

8. a) Rosemeyer, H.; Tòth, G.; Golankiewicz, B.; Kazimierczuk, Z.; Bourgeois, W.; Ktretschmer, U.; Muth, H.; Seela, F. Syn-anti conformational analysis of regular and modified nucleosides by 1D ^{1}H NOE difference spectroscopy: a simple graphical method based on conformationally rigid molecules. *J. Org. Chem.* **1990**, 55, 5784–5790; b) Schirmer, R.; Davis, J.; Noggle, J.; Hart, P. Conformational analysis of nucleosides in solution by quantitative application of the nuclear overhauser effect. *J. Am. Chem.. Soc.* **1972**, 94, 2561–2572.
9. Cook, A.; Maichuk, D. Use of chloroacetic anhydride for the protection of nucleoside hydroxyl groups. *J. Org. Chem.* **1969**, 35, 1940–1943.
10. Ozaki, S.; Akiyama, T.; Ike, Y.; Mori, H.; Hoshi, A. Synthesis and antitumor activity of 5′-O-acyl-fluorouridines. *Chem. Pharma, Bull.* **1989**, 37, 3405–3408.