# Accepted Manuscript


Versatile synthesis of 2′-amino-2′-deoxyuridine derivatives with a 2′-amino group carrying linkers possessing a reactive terminal functionality

Andrzej Gondela, Mateusz D. Tomczyk, Łukasz Przypis, Krzysztof Z. Walczak

PII: S0040-4020(16)30719-0

DOI: 10.1016/j.tet.2016.07.061

Reference: TET 27957

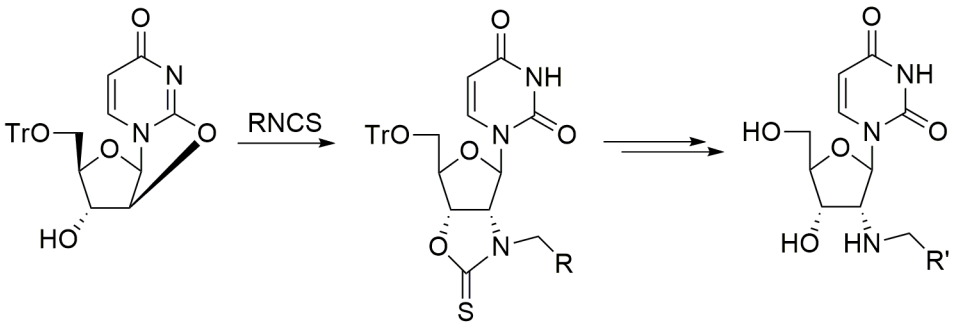
To appear in: *Tetrahedron*

Received Date: 20 February 2016

Revised Date: 8 July 2016

Accepted Date: 25 July 2016

Please cite this article as: Gondela A, Tomczyk MD, Przypis E, Walczak KZ, Versatile synthesis of 2′-amino-2′-deoxyuridine derivatives with a 2′-amino group carrying linkers possessing a reactive terminal functionality, *Tetrahedron* (2016), doi: 10.1016/j.tet.2016.07.061.




R = $(CH_2)_nCOOMe$, $(CH_2)_nNHBoc$; n = 1-6
R' = $(CH_2)_nCOOH$; $(CH_2)_nNH_2$; n = 1-6

# Versatile synthesis of 2'-amino-2'-deoxyuridine derivatives with a 2'-amino group carrying linkers possessing a reactive terminal functionality

Andrzej Gondela[a,b], Mateusz D. Tomczyk[a], Łukasz Przypis[a], Krzysztof Z. Walczak[a]*

*Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Silesian University of Technology, B. Krzywoustego 4, 44-100 Gliwice, Poland*

Keywords: 2,2'-anhydrouridine, 2'-amino-2'-deoxyuridine, isothiocyanates, ring opening, fluorophores

**Abstract:** 2,2'-Anhydrouridine has been successfully converted into the appropriate 2'-amino-2'-deoxyuridine derivatives in a reaction with isothiocyanates obtained from amino acids or α,ω-diaminoalkanes. The initially formed oxazolidine-2-thione ring is cleaved under basic conditions into the corresponding 2'-amino(substituted)-2'-deoxyuridine derivatives. The implemented additional terminal functionality in the substituent attached to the 2'-amino group allows further modifications with e.g. fluorophore moiety.

## 1. Introduction

Since the discovery of puromycin, an amino nucleoside that inhibits protein synthesis, the activity of nucleosides possessing an amino group in the sugar moiety is well documented and several methods of their synthesis have been developed. The activity of 2'-amino-2'-deoxynucleosides against bacteria, viruses and mycoplasma was ascertained in both the purine and pyrimidine series.[1-6] The analogues of 2'-amino-2'-deoxyuridine (2AU) containing a 3'- or 2'-hydroxyamino group are cytotoxic to murine leukaemia L1210 cells.[7] Novel ribozymes containing 2AU instead of uridine were more active in *in vitro* ligation of oligodeoxynucleotides.[8-11]

[b] Present address: Selvita S.A., Bobrzyńskiego 14, 30-348 Kraków, Poland. E-mail: agondela@gmail.com *Corresponding author: Krzysztof Z. Walczak, Tel.:+4832 2371308; Fax: +4832 2372094. E-mail: Krzysztof.Walczak@polsl.pl

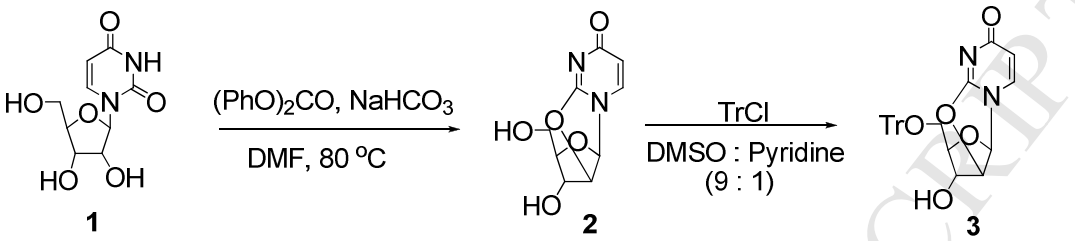
The analogues of 2AU 5'-diphosphate were considered to be agonists of the human P2Y6 receptor, a member of the G-protein coupled receptors.[12-14] Another practical application of amino nucleosides related to the presence of an amino group, is their usage as a conjugation site for the construction of linkers in oligonucleotides chemistry.[15,16] Using this strategy the 2-furanyl moiety,[17,18] amino acids,[19] porphyrins [20] and coumarin [21] were incorporated into the sugar ring of 2AU and applied as components of antisense oligonucleotides able to improve drug efficacy by enhancement of resistance to chemical and enzymatic degradation.[22,23] Due to the importance of 2'-amino-2'-deoxynucleosides as potent therapeutics and reactants, several paths for their synthesis have been developed. Aside from the synthesis methods based on modifications of the monosaccharide molecule before coupling with the nucleobase, there are three independent methods for the synthesis of 2'-amino-2'-deoxynucleosides exploiting the transformation of uridine and its derivatives. The first one relies on substitution of a leaving group by the phthalimide ion, and subsequent cleavage with hydrazine.[10,24] In another approach, 2,2'-anhydrouridine upon treatment with sodium azide, afforded the 2'-azido-2'deoxy-derivative. Catalytic reduction of the azido group gave the desired 2'-aminoderivative.[25-28] As both of these reactions occur with inversion of configuration on the atom bearing the leaving group the starting derivatives should possess the D-*arabino* configuration, which in the course of reaction is inverted into D-*ribo*. In the third method the 3'-OH group of 2,2'-anhydrouridine, under treatment with an excess of trichloroacetonitrile, afforded 3'-*O*-trichloroacetimidate, which under heating in the presence of catalytic amounts of trimethylamine easily formed an intramolecular cyclic product, oxazoline.[29,30] The oxazoline when treated with ethanolic sodium hydroxide initially forms the 2'-*N*,3'-*O*-oxazolidin-2-one intermediate, which on prolonged heating gave 2'-amino-2'-deoxyuridine in 70-80% yield.[30]

In this report we present another approach involving the use of 5'-*O*-trityl-2,2'-anhydrouridine, which under basic conditions, upon treatment with the appropriate isothiocyanates (prepared from the methyl esters of amino acids or mono-protected α, ω-diaminoalkanes), afforded the corresponding tetrahydrofuro[3,4-*d*]-oxazole-2(3*H*)-thione derivatives. We explored two ways for the preparation of the final products. In the first one, tetrahydrofuro[3,4-*d*]-oxazole-2(3*H*)-thiones were transformed by a ring opening reaction into *N*-substituted 2'-amino-2'-deoxy-5'-*O*-trityluridine derivatives under basic conditions. In the case of amino acid derivatives under applied conditions, the methyl ester group is also hydrolysed. The opposite sequence of reactions was applied in the case of *N*-Boc derivatives; the trityl protection was done prior to the oxazole-2(3*H*)-thione ring cleavage. In both cases the yield of desired 2'-amino(substituted)-2'-deoxyuridine is satisfactory.

## 2. Results and discussion

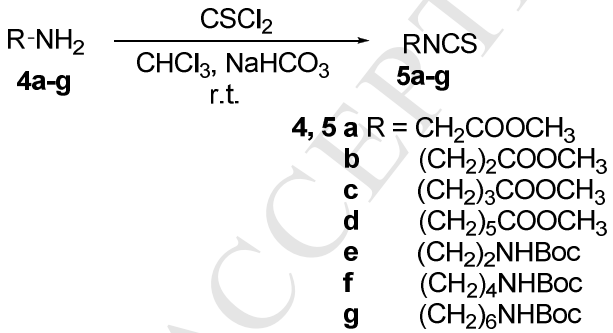
The starting 2,2'-anhydrouridine **2** was prepared according to the known method[31] avoiding to use of HMPA. Commercially available uridine was reacted with diphenyl carbonate in the presence of sodium bicarbonate in DMF solution at 80 $^{o}$C. Reaction occurred smoothly (2 h to complete) and **2** was isolated in 90% yield (Scheme 1). For the protection of the 5'-hydroxyl group, the trityl group was selected due to its stability under basic conditions. Tritylation was performed according to known reported method,[32] with slight modification. The reaction was carried out in a mixture of anhydrous DMSO and pyridine in the ratio 9:1 (v:v) at room temperature. The 5'-*O*-trityl-2, 2'-anhydrouridine **3** was obtained in 73% yield. At the same time in parallel experiments the second reactants, isothiocyanates derived from the compounds possessing a primary amino group, were synthesized. There are several methods for the transformation of primary amines into isothiocyanates. Usually carbon disulphide[33-34] or thiophosgene[35] is used as a source of thiocarbonyl group. As the amino group donors we selected methyl esters of amino acids and α, ω-diaminoalkanes. In the latter, one of the amino

groups was protected as the *N*-Boc derivative. Initially amino acids were reacted with methanol in the presence of thionyl chloride giving the methyl esters as their hydrochlorides in the crystal form.[36] Mono protected NH-*Boc* α, ω-diaminoalkanes were received from commercial sources.




Scheme 1. Synthesis of 5'-*O*-trityl-2,2'-anhydrouridine

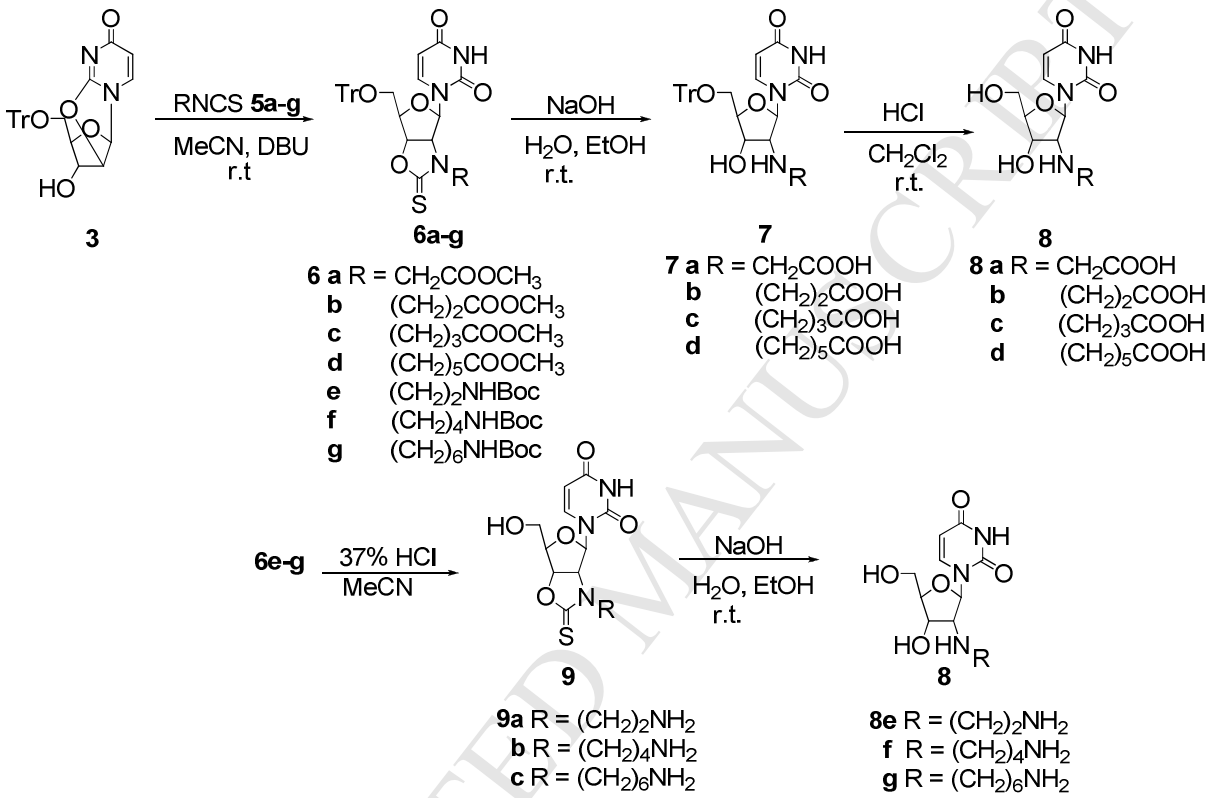
The preparation of isothiocyanates was performed in a two-phase system. A suspension of the selected amino compound **4a-g** in chloroform and an aqueous saturated solution of sodium bicarbonate, was treated with an excess of thiophosgene (1.5 eq.) at room temperature (Scheme 2). The progress of reaction was monitored by TLC (30% EtOAc: *n*-hexane) and after disappearance of the amino compounds the reaction mixture was diluted with cold water.




Scheme 2. Synthesis of isothiocyanates derived from amino compounds

Work up and purification on silica gel gave the appropriate isothiocyanates **5a-g** in yields of 58%-75% and with sufficient purity for the next step. The reaction of 2, 2'-anhydrouridine **3** with isothiocyanates **5a-g** was carried out in a solution of acetonitrile in the presence of 1,8-

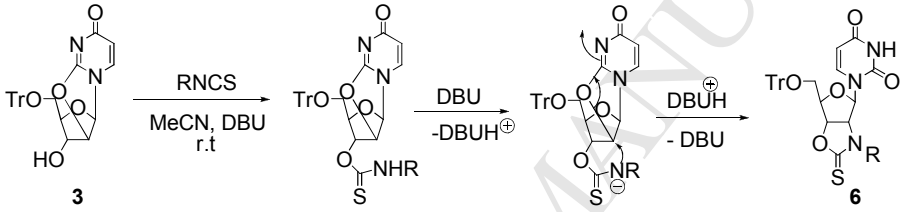
diazabicyclo[5.4.0]undec-7-ene (DBU) as a base (Scheme 3). The isothiocyanates and base were used in a twofold excess with respect to anhydrouridine **3**. The reaction was carried out at room temperature and finished after disappearance of the uridine substrate (TLC, 5% MeOH:$CHCl_3$).



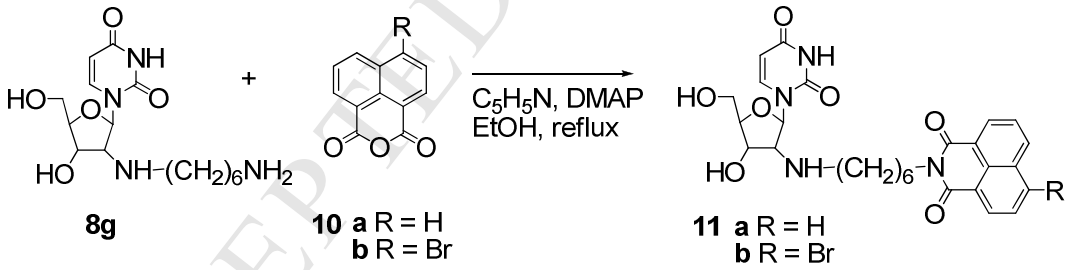

Scheme 3. Synthesis of 2'-(*N*-substituted)amino-2'-deoxyuridine derivatives

Purification using column chromatography (20% MeOH:$CH_2Cl_2$) gave the desired tetrahydrofuro[3, 4-*d*]-oxazole-2(3*H*)-thione derivatives **6a-g** in yields of 54-87%. The key step of the synthetic pathway involved cleavage of the [3, 4-*d*]-oxazole-2(3*H*)-thione ring in uridine derivative **6**. In the case of compounds **6a-d,** having a methyl alkanoate back bone, the oxazole-2(3*H*)-thione ring was easily opened when treated with an aqueous solution of sodium hydroxide at elevated temperature. Under these conditions the ester group present on the 2'-*N*-alkyl terminal position was also cleaved and **7** were obtained in satisfactory yields of 65-73%. Deprotection of the 5'-hydroxyl group was carried out at room temperature under

acidic conditions giving the final products **8a-d** in good yield. For compounds **6e-g** we explored another approach for the preparation of the final products. According to our observation the oxazole-2(3*H*)-thione ring in **6** is resistant under acidic conditions at room temperature. This fact permits removal of both protecting groups, carbamate present in the *N*-Boc fragment of **6,** and the 5'-*O*-trityl ether simultaneously. When **6e-g** was treated with hydrochloric acid in acetonitrile solution at room temperature, both *N*-Boc and trityl protecting groups were removed and **9a-c** derivatives were isolated in good yield. The final products **8e-g** were obtained by oxazole-2(3*H*)-thione ring opening in the presence of base in yields of 88% - 92% (Scheme 3).




Scheme 4. Formation of tetrahydrofuro[3, 4-*d*]-oxazole-2(3*H*)-thione derivatives




Scheme 5. Introduction of fluorophore unit into 2'-aminofunctionalized uridine

The formation of **6** can be explained as a result of reaction sequences initiated by the reaction of the 3'-OH group of the anhydrouridine with isothiocyanate. The initially formed thiocarbamate is deprotonated by DBU resulting in intramolecular nucleophilic attack on the carbon C-2' of anhydrouridine. The *erythro* configuration of the 3'-OH group in **3** determines the side of attack by the deprotonated thiocarbamate unit and subsequent formation of the [3,4-*d*]-oxazole-2(3*H*)-thione ring (Scheme 4). A similar intermediate, namely

tetrahydrofuro[3,4-*d*]oxazol-2(3*H*)-one was postulated in the reaction of 2,2'-anhydrouridine with *O*-benzyl hydroxylamine in the presence of *N*,*N*'-carbonyldiimidazole.[29] The resulting 3'-*O*-(benzyloxyamino)carbonyl derivative was converted into the corresponding 2'-(benzyloxyamino)-2'-*N*,3'-*O*-carbonyl derivative under treatment with catalytic amounts of DBU.

Finally we explored the usefulness of the obtained uridine derivatives **8** in the synthesis of conjugates with a common fluorophore (Scheme 5).[37,38] Thus **8g** reacted with benzo[*de*]isochromene-1,3-dione **10a** and its 6-bromo derivative **10b** at elevated temperature to give the corresponding imide conjugates **11** in very good yield.

## 3. Conclusion

We have devised a new approach for the synthesis of 2'-amino-2'-deoxyuridine derivatives where the amino group bears a linker with an additional functional group located on the terminal carbon atom, either a carboxylic or amino group. By the choice of the order of deprotection steps is possible to selectively remove the trityl and N-Boc protecting groups and retain the oxazolidine-2-thione ring. Under basic conditions the oxazolidine-2-thione ring is cleaved together with the ester group present in the 2'-NH-linker, whereas the 5'-*O*-trityl and *N*-Boc groups are retained. The terminal functional group can be used for further functionalization of the uridine e.g. conjugation with a fluorophore unit.

## 4. Experimental

### 4.1.General

All reagents and solvents were of analytical grade, obtained from commercial suppliers and used without further purification except for $CH_2Cl_2$, which was distilled prior to use and MeCN purified by distillation from $P_2O_5$ and dried through storage over activated 3A molecular sieves. Anhydrous pyridine was dried over KOH pellets. Anhydrous $Et_3N$ was

dried through storage over activated 4A molecular sieves. All reactions were monitored by TLC using silica-gel-coated aluminium plates with a fluorescence indicator ($SiO_2$ 60, $F_{254}$) and were visualized by UV light. Column chromatography was performed using silica gel packed columns (particle size 0.040-0.063 mm, Merck). $^1$H NMR spectra were recorded on a Varian 600 MHz System or Agilent 400 MHz Spectrometer; $^{13}$C NMR spectra were recorded at 150 MHz or 100 MHz, respectively. Chemical shifts were measured relative to residual non-deuterated solvent resonances. Melting points were determined using a Boethius M HMK hot-stage apparatus. IR spectra were recorded on Nicolet 6700 FT-IR Spectrometer (Thermo Scientific). High-resolution electrospray ionisation mass spectroscopy (ESI-MS) experiments were performed using a Waters Xevo G2 QTOF instrument equipped with an injection system (cone voltage 50 V; source 120 °C).

2, 2'-Anhydrouridine **2**[31] and 5'-*O*-trityl-2, 2'anhydrouridine **3**[32] was obtained in yields of 89% and 75%, respectively. Methyl esters of selected amino acids were prepared according to literature data.[36] *N*-Boc-1,4-butanediamine and *N*-Boc-1,6-hexanediamine were purchased from Sigma-Aldrich.

### 4.2. Typical procedure for preparation of isothiocyanates 5a-g

To a mixture of sodium bicarbonate solution (10 mL, sat. aq.) and $CH_2Cl_2$ (10 mL) the hydrochloride of amino acid methyl ester **5a-d** or *N*-Boc-mono-protected α,ω-diaminoalkane (15mmol) **4e-g** was added. The reaction mixture was cooled down to 0 $^o$C and a solution of thiophosgene (15 mmol) in $CH_2Cl_2$ (5mL) was added dropwise while stirring. After 30-60 min. TLC indicated a total disappearance of substrate (TLC, 30% EtOAc/*n*-hexane). The organic layer was separated and the aqueous layer was extracted with $CH_2Cl_2$ (3x10 mL). The organic extracts were collected together, dried ($Na_2SO_4$) and the solvent was evaporated under diminished pressure. The residual oil was purified on silica gel (30% EtOAc/*n*-hexane) and afforded the desired isothiocyanates as viscous yellow oils.

*4.2.1. Methyl 2-isothiocyanatoacetate* **5a**.[39] Yield 58% (1.24 g); $\delta_H$ (600 MHz, $CDCl_3$) 3.84 (s, 2H), 4.26 (s, 3H); $\delta_C$ (150 MHz, $CDCl_3$) 166.6 (C=O), 138.4 (C=S), 53.2, 46.3; MS (ESI) $[M+H]^+$ calcd for $C_4H_6NO_2S$=132.0119, Found 132.0126; $R_f$ (30% EtOAc/*n*-$C_6H_{14}$) 0.52.

*4.2.2. Methyl 3-isothiocyanatopropanoate* **5b**.[39] Yield 59% (1.28 g); $\delta_H$ (600 MHz, $CDCl_3$) 2.71 (t, 6.6 Hz, 2H), 3.75 (s, 3H), 3.82 (t, *J* 6.6 Hz, 2H); $\delta_C$ (150 MHz, $CDCl_3$)170.3, 126.9, 52.2, 40.8, 34.4; MS (ESI) $[M+H]^+$ calcd for $C_5H_8NO_2S$=146.0276, Found=146.0281; $R_f$ (30% EtOAc/*n*-$C_6H_{14}$) 0.49.

*4.2.3. Methyl 4-isothiocyanatobutanoate* **5c**.[40] Yield 75% (1.79 g); $\delta_H$ (600 MHz, $CDCl_3$) 2.00-2.05 (m, 2H), 2.48 (t, *J* 7.2 Hz, 2H), 3.64 (t, *J* 6.6 Hz, 2H), 3.70 (s, 3H); $\delta_C$ (150 MHz, $CDCl_3$) 172.5, 130.5, 51.7, 44.3, 30.5, 25.1; MS (ESI) $[M+Na]^+$ calcd for $C_6H_9NO_2SNa$= 182.0252, Found=182.0250; $R_f$ (30% EtOAc/*n*-$C_6H_{14}$) 0.47.

*4.2.4. Methyl 6-isothiocyanatohexanoate* **5d**. Yield 71% (1.99 g); FT IR [ATR,ν $cm^{-1}$] 2048, 1746, 436, 1208, 1150, 1054; $\delta_H$ (600 MHz, $CDCl_3$) 1.43-1.48 (m, 2H), 1.65-1.75 (m, 4H), 2.40 (t, *J* 7.8 Hz, 2H), 3.53 (t, *J* 6.6 Hz, 2H), 3.68 (s, 3H); $\delta_C$ (150 MHz, $CDCl_3$) 173.7, 128.0, 51.6, 44.9, 33.7, 26.1, 26.0, 24.1; MS (ESI) $[M+Na]^+$ calcd for $C_8H_{13}NO_2SNa$=210.2491, Found=210.2490; $R_f$ (30% EtOAc/*n*-$C_6H_{14}$) 0.46.

*4.2.5. t-Butyl 2-isothiocyanatoethylcarbamate* **5e.**[41] Yield 63% (1.91g), white solid; Mp 63-64 $^oC$ [65 $^oC$][41]; FT IR [ATR,*ν* $cm^{-1}$] 2932, 2095, 1687, 1510, 1165; $\delta_H$ (400 MHz, $CDCl_3$) 1.46 (s, 9H), 3.41-3.36 (m, 2H), 3.65 (t, *J* 5.7 Hz, 2H), 4.93 (br. s, 1H); $\delta_C$ (100 MHz, $CDCl_3$) 155.6, 132.3, 80.1, 45.4, 40.8, 28.3; MS (ESI) $[M+ H]^+$ calcd for $C_8H_{15}N_2O_2S$=203.0854, Found=203.0850; $R_f$ (5% MeOH/$CHCl_3$) 0.56.

*4.2.6. t-Butyl 4-isothiocyanatobutylcarbamate* **5f**.[42] Yield 60% (2.1 g) white semisolid; $\delta_H$ (600 MHz, $CDCl_3$) 1.45 (s, 9H), 1.58-1.65 (m, 2H), 1.70-1.77 (m, 2H), 3.16 (q, *J* 9.6 Hz, 2H),

3.59 (t, *J* 9.6 Hz, 2H), 4.63 (br s, 1H); $\delta_C$ (150 MHz, $CDCl_3$) 155.6, 132.3, 80.1, 45.4, 40.8; MS (ESI) $[M+ H]^+$ calcd for $C_{10}H_{19}N_2O_2S$=231.1167, Found=231.1158; $R_f$ (5% MeOH/$CHCl_3$) 0.62.

*4.2.7. t-Butyl 6-isothiocyanatohexylcarbamate* **5g**.[42] Yield 72% (2.8 g) white semisolid; $\delta_H$ (600 MHz, $CDCl_3$) 1.31-1.54 (m, 15H), 1.67-1.74 (m, 2H), 3.13 (q, *J* 6.4 Hz, 2H), 3.51 (t, *J* 6.4 Hz, 2H), 4.59 (br s, 1H); $\delta_C$ (150 MHz, $CDCl_3$) 155.9, 129.8, 79.0, 44.9, 40.3, 29.8, 29.8, 28.8, 26.2, 25.9; MS (ESI) $[M+H]^+$ calcd for $C_{12}H_{23}N_2O_2S$=259.1480, Found=259.1493; $R_f$ (5% MeOH/$CHCl_3$) 0.74.

### 4.3. Typical procedure for preparation of compounds 6a-g

To a suspension of 5'-*O*-trityl-2,2'-anhydro-β-D-uridine **3** (1.7 mmol) in anhydrous MeCN (5 mL), isothiocyanates **5a-g** (3.4 mmol) and DBU (3.4 mmol) were added under argon. After completion of the reaction (TLC, 5% MeOH/$CHCl_3$) the reaction mixture was washed with an aqueous solution of citric acid (10%, 2 x 10 mL). The aqueous layer was extracted with EtOAc (3x5 mL). The combined organic layers were washed with $H_2O$ (5 mL), dried ($Na_2SO_4$) and concentrated. Purification of the crude product by column chromatography (5% MeOH/$CHCl_3$) gave the compound **6a-6g** as a white solid.

*4.3.1. Methyl 2-((3aR,4R,6R,6aS)-4-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-thioxo-6-(trityloxymethyl)tetrahydrofuro[3,4-d]oxazol-3(2H)-yl)acetate* **6a**. Yield 87% (0.89 g); Mp 147-149 $^oC$; FT IR [ATR, $\nu$ $cm^{-1}$] 2953, 1679, 1541, 1448, 1251, 1208, 1000; $\delta_H$ (600 MHz, $CDCl_3$) 3.52-3.54 (dd, *J* 11.4, 3.0 Hz, 1H), 3.60-3.63 (dd, *J* 11.4, 4.2 Hz, 1H), 3.70 (s, 3H), 3.89-3.95 (m, 2H), 4.56-4.62 (m, 1H), 4.83-4.86 (m, 1H), 5.41 (d, *J* 7.8 Hz, 1H), 5.45-5.46 (m, 1H), 6.06 (d, *J* 1.8 Hz, 1H), 7.27-7.36 (m, 15H), 7.67 (d, *J* 7.8 Hz, 1H), 9.25 (s, 1H); $\delta_C$ (150 MHz, $CDCl_3$) 188.0, 168.3, 163.1, 150.32, 142.7, 139.2, 128.5, 128.2, 127.7, 102.7,

90.5, 87.9, 86.2, 80.9, 71.6, 62.1, 52.7, 48.4; MS (ESI) $[M+H]^+$ calcd for $C_{32}H_{30}N_3O_7S$= 600.1804, Found =600.1802; $R_f$ (5% MeOH/$CHCl_3$) 0.40.

*4.3.2. Methyl 3-((3aR,4R,6R,6aS)-4-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-thioxo-6-(trityloxymethyl)tetrahydrofuro[3,4-d]oxazol-3(2H)-yl)propanoate* **6b.** Yield 54% (0.56 g); Mp 106-107 $^oC$; FT IR [ATR, $\nu$ $cm^{-1}$] 2925, 1683, 1447, 1258, 1202, 1001; $\delta_H$ (600 MHz, $CDCl_3$) 2.73-2.77 (m, 2H), 3.05-3.30 (m, 1H), 3.53-3.62 (m, 1H), 3.69 (s, 3H), 3.91-3.96 (m, 1H), 4.22-4.26 (m, 1H), 4.43-4.45 (m, 1H), 4.94-4.96 (m, 1H), 5.40 (d, *J* 7.8 Hz, 1H), 5.44-5.46 (m, 1H), 6.12 (d, *J* 1.8 Hz, 1H), 7.28-7.38 (m, 15H), 7.67 (d, *J* 7.8 Hz, 1H), 9.23 (s, 1H); $\delta_C$ (150 MHz, $CDCl_3$)186.6, 172.9, 163.0, 150.2, 143.0, 139.9, 128.7, 128.30, 128.8, 103.4, 90.4, 87.9, 85.6, 80.9, 71.2, 62.5, 52.3, 42.7, 31.5; MS (ESI) $[M+H]^+$ calcd for $C_{33}H_{32}N_3O_7S$= 614.1961, Found=614.1960; $R_f$ (5% MeOH/$CHCl_3$) 0.45.

*4.3.3. Methyl 4-((3aR,4R,6R,6aS)-4-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-thioxo-6-(trityloxymethyl)tetrahydrofuro[3,4-d]oxazol-3(2H)-yl)butanoate* **6c**. Yield 69% (0.73 g); FT IR [ATR, $\nu$ $cm^{-1}$] 2927, 1683, 1446, 1256, 1201, 1003; $\delta_H$ (600 MHz, $CDCl_3$) 2.00-2.05 (m, 2H), 2.43-2.46 (m, 2H), 3.52 (dd, *J* 10.8, 3.6 Hz, 1H), 3.60 (dd, *J* 10.8, 3.0 Hz, 1H), 3.66 (s, 3H), 4.10-4.17 (m, 2H), 4.43-4.45 (m, 1H), 4.75 (dd, *J* 9.0, 1.8 Hz, 1H), 5.38 (dd, *J* 9.0, 4.8 Hz, 1H), 5.42 (dd, *J* 8.4, 2.4 Hz, 1H), 6.05 (d, *J* 2.4 Hz, 1H), 7.26-7.30 (m, 15H), 7.69 (d, *J* 7.8 Hz, 1H), 9.45 (s, 1H); $\delta_C$ (150 MHz, $CDCl_3$) 186.8, 173.7, 163.1, 150.2, 142.9, 139.9, 128.7, 128.3, 127.8, 103.2, 90.3, 87.9, 86.1, 80.6, 70.3, 62.4, 52.0, 46.5, 31.1, 21.4; MS (ESI) $[M+H]^+$ calcd for $C_{34}H_{34}N_3O_7S$ =628.2117, Found=628.2115; $R_f$ (5% MeHO/$CHCl_3$) 0.64.

*4.3.4. Methyl 6-((3aR,4R,6R,6aS)-4-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-thioxo-6-(trityloxymethyl)tetrahydrofuro[3,4-d]oxazol-3(2H)-yl)hexanoate* **6d.** Yield 81% (0.89 g); Mp 65-66 $^oC$; FT IR [ATR, $\nu$ $cm^{-1}$] 2929, 1685, 1448, 1255, 1207, 1006; $\delta_H$ (600 MHz, $CDCl_3$) 1.39-1.40 (m, 2H), 1.65-1.69 (m, 4H), 2.31-2.34 (m, 2H), 3.55-3.60 (m, 4H), 3.65 (s, 3H),

4.42-4.44 (m, 1H), 4.62-4.64 (dd, *J* 9.0, 2.4 Hz, 1H), 5.39-5.40 (m, 2H), 6.06-6.07 (d, *J* 1.8 Hz, 1H), 7.27-7.37 (m, 15H), 7.70 (d, *J* 1.8 Hz, 1H), 9.55 (s, 1H); $\delta_C$ (150 MHz, $CDCl_3$) 186.5, 174.0, 163.0, 150.1, 142.8, 139.6, 128.6, 128.2, 127.7, 103.2, 89.8, 87.9, 86.0, 79.9, 70.5, 62.17, 51.6, 47.0, 33.8, 26.0, 25.6, 24.4; MS (ESI) $[M+H]^+$ calcd for $C_{36}H_{38}N_3O_7S$= 656.2430, Found = 656.2430; $R_f$ (5% MeOH/$CHCl_3$) 0.65.

*4.3.5. t-Butyl 2-((3aR,4R,6R,6aS)-4-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-thioxo-6-(trityloxymethyl)tetrahydrofuro[3,4-d]oxazol-3(2H)-yl)ethylcarbamate* **6e**. Yield 65% (0.74 g); FT IR [ATR, $\nu$ $cm^{-1}$] 2934, 1686, 1449, 1254, 1159; $\delta_H$ (400 MHz, DMSO-$d_6$) 1.34 (s, 9H), 3.17-3.22 (m, 2H), 3.26 (dd, *J* 10.4, 4.4 Hz, 1H), 3.31-3.33 (br, 1H), 3.40-3.47 (m, 2H), 3.98-4.04 (m, 1H), 4.30 (dt, *J* 6.4, 4.4 Hz, 1H), 5.02 (d, *J* 8.0 Hz, 1H), 5.26 (dd, *J* 8.8, 4.8 Hz, 1H), 5.60 (dd, *J* 8.0, 2.0 Hz, 1H), 6.00 (d, *J* 2.4 Hz, 1H), 6.94 (t, *J* 6.0 Hz, 1H), 7.20 (d, *J* 8.0 Hz, 1H), 7.25-7.40 (m, 15H); $\delta_C$ (100 MHz, DMSO-$d_6$) 186.2, 163.1, 155.6, 150.1, 143.3, 142.2, 128.2, 127.9, 127.1, 102.1, 91.1, 86.3, 85.1, 82.2, 77.9, 68.3, 63.4, 46.3, 36.5, 28.1; HRMS (ESI) $[M+H]^+$ calcd for $C_{36}H_{39}N_4O_7S$=671.2539, Found=671.2534; $R_f$ (5% MeOH/$CH_2Cl_2$) 0.65.

*4.3.6. t-Butyl 4-((3aR,4R,6R,6aS)-4-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-thioxo-6-(trityloxymethyl)tetrahydrofuro[3,4-d]oxazol-3(2H)-yl)butylcarbamate* **6f**. Yield 87% (1.03 g); Mp 109-110 $^oC$; FT IR [ATR, $\nu$ $cm^{-1}$] 2938, 1684, 1446, 1258, 1157,740; $\delta_H$ (600 MHz, DMSO-$d_6$) 1.36-1.39 (m, 11H), 1.53-1.63 (m, 2H), 2.88-2.97 (m, 2H), 3.28 (dd, *J* 10.2, 4.2 Hz, 1H), 3.42 (dd, *J* 10.2, 6.6 Hz, 1H), 3.83-3.88 (m, 2H), 4.30 (dt, *J* 6.6, 4.8 Hz, 1H), 4.97(dd, *J* 8.4, 2.4 Hz, 1H), 5.32 (dd, *J* 8.4, 4.8 Hz, 1H), 5.57 (d, *J* 7.8 Hz, 1H), 6.00 (d, *J* 1.8 Hz, 1H), 6.78 (t, *J* 5.4 Hz, 1H), 7.28-7.40 (m, 15H), 7.72 (d, *J* 7.8 Hz, 1H), 11.46 (s, 1H); $\delta_C$ (150 MHz, DMSO-$d_6$) 185.6, 163.0, 155.5, 150.2, 143.2, 141.9, 128.1, 127.9, 127.1, 101.9,

90.7, 86.3, 85.2, 81.6, 77.3, 68.1, 63.2, 46.0, 40.0, 28.2, 26.7, 22.5; MS (ESI) $[M+H]^+$ calcd for $C_{38}H_{43}N_4O_7S$=698.2774, Found=698.2770; $R_f$ (5% MeOH/$CH_2Cl_2$) 0.43.

4.3.7. *t-Butyl 6-((3aR,4R,6R,6aS)-4-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-thioxo-6-(trityloxymethyl)tetrahydrofuro[3,4-d]oxazol-3(2H)-yl)hexylcarbamate* **6g**. Yield 76% (0.82 g); Mp 89-90 $^oC$; FT IR [ATR, $\nu$ $cm^{-1}$] 2936, 1686, 1447, 1256, 1159, 748; $\delta_H$ (600 MHz, DMSO-$d_6$) 1.22-1.26 (m, 4H), 1.34-1.37 (m, 11H), 1.53-1.63 (m, 2H), 2.88 (dd, *J* 13.6, 6.6 Hz, 2H), 3.27 (dd, *J* 10.4, 4,1 Hz, 1H), 3.35 (dd, *J* 10.4, 6.6 Hz, 1H), 3.43-3.47 (m, 1H), 3.84 (ddd, *J* 13.8, 9.5, 6.8 Hz, 1H), 4.30 (dd, *J* 6.6, 4.1 Hz, 1H), 4.96 (dd, *J* 8.4, 2.4 Hz, 1H), 5.30 (dd, *J* 8.4, 4.1 Hz, 1H), 5.57 (dd, *J* 7.8, 2.2 Hz, 1H), 6.00 (d, *J* 2.2 Hz, 1H), 6.73 (t, *J* 5.4 Hz, 1H), 7.27-7.40 (m, 15H), 7.70 (d, *J* 7.8 Hz, 1H), 11.45 (d, *J* 2.2 Hz, 1H); $\delta_C$ (150 MHz, DMSO-$d_6$) 185.3, 163.1 , 155.6, 150.2, 143.3, 141.8, 128.2, 128.0, 127.2, 102.1, 90.5, 86.4, 85.0, 81.6, 77.3, 68.1, 63.2, 46.3, 39.8, 29.3, 28.3, 25.9, 25.8, 25.2; MS (ESI) $[M+H]^+$ calcd for $C_{40}H_{47}N_4O_7S$=727.3165, Found=727.3164; $R_f$ (5% MeOH/$CH_2Cl_2$) 0.42.

**4.4. Preparation of 2'-amino-2'-deoxyuridine derivatives 7a-d and 8e-g**

Compound **6a-d** or **9a-c** (0.5 mmol) was dissolved in aqueous ethanol (10 mL EtOH, 2 mL $H_2O$) containing NaOH (10 mmol). The reaction mixture was refluxed for two hours (TLC, 10% MeOH:$CHCl_3$). After disappearance of substrate, the reaction mixture was cooled down, portioned between EtOAc (50 mL) and $H_2O$ (20 mL) and neutralized with 20% aq. HCl. The organic layer was separated, and the aqueous solution was extracted with EtOAc (2 x 10 mL). The organic extracts were combined, dried ($MgSO_4$) and the volatiles were removed under diminished pressure. Purification of the residue on silica gel using gradient MeOH (10% to 50% in $CH_2Cl_2$) gave the product **7a-d** or **8e-g** as a white semisolid.

*4.4.1. 2-((2R, 3R, 4S, 5R)-2-(2, 4-Dioxo-3, 4-dihydropyrimidin-1(2H)-yl)-4-hydroxy-5-(trityloxymethyl)tetrahydrofuran-3-ylamino)ethanoic acid* **7a**. Yield 66% (0.18 g); FT IR

[ATR, $\nu$ $cm^{-1}$] 3058, 1686, 1245, 1112, 1078, 763; $\delta_H$ (600 MHz, DMSO-$d_6$) 3.13-3.17 (m, 2H), 3.21-3.48 (m, 5H), 4.07 (d, *J* 3.0 Hz, 1H), 4.26-4.32 (m, 2H), 5.36 (d, *J* 8.0 Hz, 1H), 7.23-7.54 (m, 15H), 5.80 (s, 1H), 7.74 (d, *J* 8.0 Hz, 1H), 11.41 (s, 1H); $\delta_C$ (150 MHz, DMSO-$d_6$) 163.43, 151.06, 143.81, 140.77, 128.74, 128.44, 127.61, 101.88, 87.71, 86.92, 83.85, 74.07, 68.76, 64.11, 63.86, 55.33, 49.58; MS (ESI) $[M+H]^+$ calcd for $C_{30}H_{30}N_3O_7$=544.2084, Found=544.2082; $R_f$ (10% MeOH/$CH_2Cl_2$) 0.22.

*4.4.2. 3-((2R,3R,4S,5R)-2-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-hydroxy-5-(trityloxy methyl)tetrahydrofuran-3-ylamino)propanoic acid* **7b**. Yield 65% (0.18 g); FT IR [ATR, $\nu$ $cm^{-1}$] 3058, 1683, 1563, 1448, 1405, 1270, 1082, 772; $\delta_H$ (600 MHz, DMSO-$d_6$) 2.14-2.31 (m, 4H), 2.72 (t, *J* 6.2 Hz, 2H), 3.16-3.22 (m, 3H), 3.32 (dd, *J* 10.5, 4.9 Hz, 1H), 3.99 (d, *J* 3.6 Hz, 1H), 4.20-4.26 (m, 2H), 5.36 (d, *J* 8.1 Hz, 1H), 7.23-7.46 (m, 15H), 7.66 (d, *J* 8.1 Hz, 1H), 11.45 (s, 1H); $\delta_C$ (150 MHz, DMSO-$d_6$) 171.70, 170.79, 158.48, 151.24, 148.29, 136.13, 135.86, 135.04, 109.47, 94.86, 94.31, 91.45, 76.53, 71.80, 71.59, 52.00, 44.56; MS (ESI) $[M+H]^+$ calcd for $C_{31}H_{32}N_3O_7$=558.2240, Found=558.2238; $R_f$ (10% MeOH/$CH_2Cl_2$) 0.26.

*4.4.3. 4-((2R,3R,4S,5R)-2-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-hydroxy-5-(trityloxy methyl)tetrahydrofuran-3-ylamino)butanoic acid* **7c**. Yield 73% (0.21 g); FT IR [ATR, $\nu$ $cm^{-1}$] 3058, 1686, 1557, 1447, 1404, 1270, 1082, 747; $\delta_H$ (600 MHz, DMSO-$d_6$) 1.56-1.66 (m, 2H), 2.16-2.25 (m, 2H), 2.56-2.58 (m, 2H), 3.17-3.25 (m, 2H), 3.33 (dd, *J* 10.5, 4.8 Hz, 1H), 4.00 (dd, *J* 7.9, 4.0 Hz, 1H), 4.18 (dd, *J* 5.1, 4.4 Hz, 1H), 5.35 (d, *J* 8.1 Hz, 1H), 5.71 (d, *J* 6.2 Hz, 1H), 7.24-7.43 (m, 15H), 7.67 (d, *J* 8.1 Hz, 1H), 11.34 (s, 1H); $\delta_C$ (150 MHz, DMSO-$d_6$) 162.8, 150.5, 143.3 140.3, 128.2, 127.9, 127.1, 101.5, 87.0, 86.4, 83.7, 68.2, 63.54, 63.2, 46.6, 31.7, 25.0; MS (ESI) $[M+H]^+$ calcd for $C_{32}H_{34}N_3O_7$=572.2397, Found=572.2395; $R_f$ (10% MeOH/$CH_2Cl_2$) 0.26.

*4.4.4. 6-((2R, 3R, 4S, 5R)-2-(2, 4-Dioxo-3, 4-dihydropyrimidin-1(2H)-yl)-4-hydroxy-5-(trityl oxymethyl)tetrahydrofuran-3-ylamino)hexanoic acid* **7d**. Yield 71% (0.21 g); FT IR [ATR, $\nu$ $cm^{-1}$] 3059, 1687, 1549, 1447, 1270, 1083, 765; $\delta_H$ (600 MHz, DMSO-$d_6$) 1.26 (dt, *J* 14.1, 7.1 Hz, 2H), 1.35-1.43 (m, 4H), 1.45-1.57 (m, 4H), 2.18 (t, *J* 7.3 Hz, 2H), 2.56 (dd, *J* 11.6, 5.2 Hz, 1H), 3.16-3.35 (m, 3H), 3.90-4.05 (m, 1H), 4.11-4.22 (m, 1H), 5.37 (d, *J* 8.0 Hz, 1H), 5.71 (d, *J* 5.2 Hz, 1H), 7.28-7.41 (m, 15H), 7.67 (d, *J* 8.0 Hz, 1H), 11.35 (s, 1H); $\delta_C$ (150 MHz, DMSO-$d_6$) 174.34, 162.83, 150.54, 143.33, 140.35, 128.23, 127.93, 127.14, 101.55, 87.52, 86.42, 83.75, 68.22, 63.57, 47.11, 33.61, 26.15, 24.37; MS (ESI) $[M+H]^+$ calcd for $C_{34}H_{38}N_3O_7$=600.2710, Found=600.2708; $R_f$ (10% MeOH/$CH_2Cl_2$) 0.31.

**4.5. Preparation of 8a-d and 9a-c**

Protected derivative of uridine **7a-d** or **6e-g** (0.6 mmol) was dissolved in $CH_2Cl_2$ (10 mL) and treated with concentrated HCl (0.3 mL, d=1.18 g/mL, 3.3 mmol) at room temperature. After completion of the reaction (TLC, 1% $Et_3N$ in 50%MeOH/$CH_2Cl_2$), $H_2O$ (10 mL) was added and the reaction mixture was neutralized with saturated $NaHCO_3$ solution. The water layer was washed with $CH_2Cl_2$ (2 x 3 mL) and the organic layer discarded. The water was removed under diminished pressure and the residue was dissolved in minimal amounts of MeOH and purified by column chromatography (1% $Et_3N$ in 50%MeOH/$CH_2Cl_2$).

*4.5.1. 2-((2R, 3R, 4S, 5R)-2-(2, 4-Dioxo-3, 4-dihydropyrimidin-1(2H)-yl)-4-hydroxy-5-(hydro xylmethyl)tetrahydrofuran-3-ylamino)acetic acid* **8a**. Yield 93% (0.17 g) white semisolid; FT IR [ATR, $\nu$ $cm^{-1}$] 3224, 1687, 1581, 1396, 1271, 1094, 814; $\delta_H$ (600 MHz, $D_2O$) 3.84 (dd, *J* 12.7, 4.0 Hz, 1H), 3.90 (dd, *J* 12.7, 3.1 Hz, 1H), 3.92-3.96 (m, 1H), 4.00-4.05 (m, 1H), 4.24 (dd, *J* 6.7 Hz, 6.0 Hz, 1H), 4.31-4.34 (m, 1H), 4.67 (dd, *J* 6.0, 3.1 Hz, 1H), 5.97 (d, *J* 8.1 Hz, 1H), 6.37 (d, *J* 6.7 Hz, 1H), 7.92 (d, *J* 8.1 Hz, 1H); $\delta_C$ (150 MHz, $D_2O$) 169.23, 165.95,

151.78, 141.08, 103.06, 86.80, 85.66, 68.31, 62.06, 60.75, 47.22; MS (ESI) $[M+H]^+$ calcd for $C_{11}H_{16}N_3O_7$=302.0988, Found=302.0986; $R_f$ (30% MeOH/$CH_2Cl_2$) 0.28.

*4.5.2. 3-((2R,3R,4S,5R)-2-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-hydroxy-5-(hydroxy methyl)tetrahydrofuran-3-ylamino)propanoic acid* **8b**. Yield 89% (0.17 g) white semisolid; FT IR [ATR, $\nu$ $cm^{-1}$] 3229, 1682, 1575, 1396, 1273, 1107, 1052, 815; $\delta_H$ (600 MHz, $D_2O$) 2.53-2.62 (m, 2H), 3.15-3.25 (m, 2H), 3.85 (dd, *J* 12.6, 4.2 Hz, 1H), 3.91 (dd, *J* 12.6, 3.6 Hz, 1H), 3.92-3.94 (m, 1H), 4.29-4.31 (m, 1H), 4.62 (dd, *J* 6.0, 3.0 Hz, 1H), 5.98 (d, *J* 8.4 Hz, 1H), 6.20 (d, *J* 6.6. Hz, 1H), 7.95 (d, *J* 8.4 Hz, 1H); $\delta_C$ (150 MHz, $D_2O$) 170.3, 168.7, 154.5, 144.0, 105.5, 89.1, 71.1, 65.3, 63.6, 46.7, 36.6; MS (ESI) $[M+H]^+$ calcd for $C_{12}H_{18}N_3O_7$=316.1144, Found=316.1142; $R_f$ (30% MeOH/$CH_2Cl_2$) 0.29.

*4.5.3. 4-((2R,3R,4S,5R)-2-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-hydroxy-5-(hydroxy methyl)tetrahydrofuran-3-ylamino)butanoic acid* **8c**. Yield 82% (0.16 g) white semisolid; FT IR [ATR, $\nu$ $cm^{-1}$] 3240, 1674, 1545, 1409, 1271, 1104, 1050, 815; $\delta_H$ (600 MHz, $D_2O$) 1.96 (dt, *J* 13.8, 7.0 Hz, 2H), 2.33 (t, *J* 7.0 Hz, 2H), 2.98-3.17 (m, 2H), 3.83 (dd, *J* 12.6, 4.2 Hz, 1H), 3.86 (dd, *J* 12.6, 3.6 Hz, 1H), 3.96-3.98 (t, *J* 6.4 Hz, 1H), 4.27 (dd, *J* 6.4, 3.1 Hz, 1H), 4.61 (dd, *J* 5.6, 2.8 Hz, 1H), 5.97 (d, *J* 8.1 Hz, 1H), 6.26 (d, *J* 7.1 Hz, 1H), 7.91 (d, *J* 8.1 Hz, 1H); $\delta_C$ (150 MHz, $D_2O$) 184.4, 168.6, 154.4, 143.9, 105.7, 89.1, 88.3, 71.0, 64.6, 63.6, 49.6, 37.4, 25.7; MS (ESI) $[M+H]^+$ calcd for $C_{13}H_{20}N_3O_7$=330.3137, Found=330.3135; $R_f$ (30% MeOH/$CH_2Cl_2$) 0.32.

*4.5.4. 6-((2R, 3R, 4S, 5R)-2-(2, 4-Dioxo-3, 4-dihydropyrimidin-1(2H)-yl)-4-hydroxy-5-(hydroxy methyl)tetrahydrofuran-3-ylamino)hexanoic acid* **8d**. Yield 95% (0.20 g) white semisolid; FT IR [ATR, $\nu$ $cm^{-1}$] 3259, 1686, 1448, 1271, 1079, 812; $\delta_H$ (600 MHz, $D_2O$) 1.39-1.41 (m, 2H), 1.58-1.64 (m, 2H), 1.72-1.80 (m, 2H), 2.21 (t, *J* 7.4 Hz, 2H), 3.10-3.19 (m, 2H), 3.85 (dd, *J* 12.6, 4.0 Hz, 2H),3.89 (dd, *J* 12.6, 3.2 Hz, 1H), 4.11 (dd, *J* 7.1, 6.0 Hz, 1H), 4.30-

4.33 (m, 1H), 4.66 (dd, *J* 6.0, 2.6 Hz, 1H), 5.98 (d, *J* 8.1 Hz, 1H), 6.35 (d, *J* 7.1 Hz, 1H), 7.92 (d, *J* 8.1 Hz, 1H); $\delta_C$ (150 MHz, $D_2O$) 182.8, 165.9, 151.7, 144.2, 103.2, 86.8, 85.4, 68.4, 61.8, 60.9, 47.0, 36.6, 25.5, 25.4, 25.0; MS (ESI) $[M+H]^+$ calcd for $C_{15}H_{24}N_3O_7$= 358.1614, Found: 358.1614; $R_f$ (30% MeOH/$CH_2Cl_2$) 0.30.

*4.5.5. 1-((2R,3R,4S,5R)-3-(2-Aminoethylamino)-4-hydroxy-5-(hydroxymethyl)tetrahydro furan-2-yl)pyrimidine-2,4(1H,3H)-dione* **8e**. Yield 91% (0.15 g) white semisolid; FT IR [ATR, $\nu$ $cm^{-1}$] 3348, 1680, 1566, 1387, 1261; $\delta_H$ (600 MHz, $D_2O$) 2.89-2.95 (m, 2H), 3.28-3.33 (m, 2H), 3.84 (dd, *J* 12.6, 4.2 Hz, 1H), 3.90 (dd, *J* 12.6, 3.6 Hz, 1H), 3.95-3.98 (m, 2H), 4.27-4.30 (m, 2H), 4.60 (dd, *J* 6.0, 3.0 Hz, 1H), 5.97 (d, *J* 8.4 Hz, 1H), 6.22 (d, *J* 6.6. Hz, 1H), 7.98 (d, *J* 8.4 Hz, 1H); $\delta_C$ (150 MHz, $D_2O$) 169.8, 166.7 , 153.2, 143.7, 105.2, 89.0, 712.0, 65.1, 63.2, 48.7, 43.6; MS (ESI) $[M+H]^+$ Calcd for $C_{11}H_{19}N_4O_5$ =287.1355, Found=287.1355; $R_f$ (40% MeOH/$CH_2Cl_2$) 0.20.

*4.5.6. 1-((2R,3R,4S,5R)-3-(4-Aminobutylamino)-4-hydroxy-5-(hydroxymethyl)tetrahydro furan-2-yl)pyrimidine-2,4(1H,3H)-dione* **8f**. Yield 88% (0.17 g) white semisolid; FT IR [ATR, $\nu$ $cm^{-1}$] 3346, 1678, 1565, 1384, 1260; $\delta_H$ (600 MHz, $D_2O$) 1.38-1.44 (m, 2H), 1.54-1.58 (m, 2H), 2.61 (t, *J* 7.2 Hz, 2H), 3.03-3.11 (m, 2H), 3.83 (dd, *J* 12.6, 4.2 Hz, 1H), 3.88 (dd, *J* 12.6, 4.2 Hz, 1H), 3.96-3.97 (m, 2H), 4.28-4.29 (m, 2H), 4.61-4.64 (m, 1H), 5.97 (d, *J* 8.4 Hz, 1H), 6.24 (d, *J* 7.2 Hz, 1H), 7.92 (d, *J* 8.4 Hz, 1H); $\delta_C$ (150 MHz, $D_2O$) 168.5, 156.0, 144.1, 105.8, 89.6, 88.4, 71.2, 65.0, 63.9, 47.7, 44.2, 36.1, 26.7; MS (ESI) $[M+H]^+$ calcd for $C_{13}H_{23}N_4O_5$=315.1668, Found=315.1669; $R_f$ (40% MeOH/$CH_2Cl_2$) 0.22.

4.5.7. *1-((2R,3R,4S,5R)-3-(6-Aminohexylamino)-4-hydroxy-5-(hydroxymethyl)tetrahydro furan-2-yl)pyrimidine-2,4(1H,3H)-dione* **8g**. Yield 92% (0.19 g) white semisolid; FT IR [ATR, $\nu$ $cm^{-1}$] 3340, 1674, 1561, 1388, 1264; $\delta_H$ (600 MHz, $D_2O$) 1.32-1.39 (m, 2H), 1.42-1.46 (m, 2H), 1.62-1.66 (m, 4H), 2.58-2.62 (m, 2H), 3.05-3.12 (m, 2H), 3.80 (dd, *J* 12.6, 4.2

Hz, 1H), 3.86 (dd, *J* 12.6, 4.2 Hz, 1H), 3.92 (dd, *J* 12.6, 3.0 Hz, 1H), 4.28-4.31 (m, 2H), 4.61 (dd, *J* 6.0, 3.0 Hz, 1H), 5.95 (d, *J* 7.8 Hz, 1H), 6.23 (d, *J* 7.2 Hz, 1H), 7.92 (d, *J* 7.8 Hz, 1H); $\delta_C$ (150 MHz, $D_2O$)168.7, 154.5, 144.2, 105.7, 89.4, 88.3, 71.2, 64.9, 63.8, 46.1, 44.9, 29.4, 27.5, 26.5, 26.2; MS (ESI) $[M+H]^+$ calcd for $C_{15}H_{27}N_4O_5$=343.1981, Found=343.1979; $R_f$ (40% MeOH/$CH_2Cl_2$) 0.25.

*4.5.8. 1-((3aR,4R,6R,6aS)-3-(2-Aminoethyl)-6-(hydroxymethyl)-2-thioxohexahydrofuro[3,4-d]oxazol-4-yl)pyrimidine-2,4(1H,3H)-dione* **9a**. Yield 86% (0.17 g) white semisolid; FT IR [ATR, $\nu$ $cm^{-1}$] 2938, 1682, 1631, 1489, 1447, 1266, 1102, 997; $\delta_H$ (600 MHz, DMSO) 2.69-2.75 (m, 1H), 2.78-2.83 (m, 1H), 3.50-3.56 (m, 3H), 3.65 (dd, *J* 11.4, 4.8 Hz, 1H), 3.68 (dd, *J* 11.4, 4.8 Hz, 1H), 3.72 (ddd, *J* 14.4, 7.8, 6.6 Hz, 1H), 4.06 (ddd, *J* 14.4, 8.4, 6.0 Hz, 1H), 4.18 (dd, *J* 9.0, 4.2 Hz, 1H), 4.93 (dd, *J* 9.0, 3.0 Hz, 1H), 5.25 (dd, *J* 9.0, 4.2 Hz, 1H), 5.69 (d, *J* 7.8 Hz, 1H), 6.00 (d, *J* 3.0 Hz, 1H), 7.76 (d, *J* 7.8 Hz, 1H), 11.46 (d, *J* 1.8 Hz, 1H); $\delta_C$ (150 MHz, DMSO) 185.9, 171.4, 163.4, 150.4, 141.7, 101.9, 90.5, 86.5, 82.2, 68.8, 60.5, 51.6, 42.2, 30.2; MS (ESI) $[M+H]^+$ calcd for $C_{12}H_{17}N_4O_5S$=329.0919, Found=329.0918; $R_f$ (20% MeOH/$CH_2Cl_2$) 0.10.

*4.5.9. 1-((3aR,4R,6R,6aS)-3-(4-Aminobutyl)-6-(hydroxymethyl)-2-thioxohexahydrofuro[3,4-d]oxazol-4-yl)pyrimidine-2,4(1H,3H)-dione* **9b**. Yield 89% (0.19 g) white solid; Mp 113-114 °C; FT IR [ATR, $\nu$ $cm^{-1}$] 2933, 1683, 1629, 1489, 1448, 1266, 1102, 998; $\delta_H$ (600 MHz, DMSO-$d_6$) 1.32 (qn, *J* 7.2 Hz, 2H), 1.52-1.56 (m, 4H), 2.53-2.55 (m, 3H), 3.49 (ddd, *J* 13.8, 9.0, 4.8 Hz, 1H), 3.66 (dd, *J* 12.0, 4.8 Hz, 1H), 3.69 (dd, *J* 12.0, 4.8 Hz, 1H), 3.86 (ddd, *J* 13.8, 9.6, 6.6 Hz, 1H), 4.19 (dd, *J* 9.0, 4.8 Hz, 1H), 4.44 (s, 1H), 4.92 (dd, *J* 9.0, 2.4 Hz, 1H), 5.27 (dd, *J* 8.4, 4.2 Hz, 1H), 5.67 (d, *J* 7.8 Hz, 1H), 5.97 (d, *J* 2.4 Hz, 1H), 7.76 (d, *J* 7.8 Hz, 1H); $\delta_C$ (150 MHz, DMSO-$d_6$) 185.8, 163.6, 150.6, 141.3, 102.1, 90.2, 86.6, 81.8, 68.3, 60.5,

46.4, 40.9, 29.8, 22.9; MS (ES) $[M+H]^+$ calcd for $C_{14}H_{21}N_4O_5S$= 357.1232, Found=357.1230; $R_f$ (20% MeOH/$CH_2Cl_2$) 0.12.

*4.5.10. 1-((3aR,4R,6R,6aS)-3-(6-Aminohexyl)-6-(hydroxymethyl)-2-thioxohexahydrofuro[3,4-d]oxazol-4-yl)pyrimidine-2,4(1H,3H)-dione* **9c**. Yield 85% (0.20 g) white solid; Mp: 99-100 °C; FT IR [ATR, $\nu$ $cm^{-1}$] 2934, 1682, 1632, 1489, 1449, 1261, 1100, 998; $\delta_H$ (600 MHz, DMSO-$d_6$) 1.22-1.31 (m, 6H), 1.42 (qn, *J* 7.2 Hz, 2H), 1.51-1.64 (m, 4H), 2.63 (t, *J* 7.2 Hz, 2H), 3.48 (ddd, *J* 13.8, 9.0, 4.8 Hz, 1H), 3.66 (dd, *J* 12.0, 4.8 Hz, 1H), 3.70 (dd, *J* 12.0, 4.8 Hz, 1H), 3.85 (ddd, *J* 13.8, 9.6, 6.6 Hz, 1H), 4.19 (dd, *J* 9.0, 4.8 Hz, 1H), 4.91 (dd, *J* 8.4, 2.4, 1H), 5.29 (dd, *J* 8.4, 4.2 Hz, 1H), 5.68 (d, *J* 7.8 Hz, 1H), 5.99 (d, *J* 2.4 Hz, 1H), 7.78 (d, *J* 7.8 Hz, 1H); $\delta_C$ (150 MHz, DMSO) 185.6, 163.6, 150.6, 141.0, 102.0, 90.0, 86.4, 81.7, 68.3, 60.3, 46.3, 45.6, 29.9, 25.7, 25.6, 25.1; MS (ESI) $[M+H]^+$ calcd for $C_{16}H_{25}N_4O_5S$= 385.1545, Found=385.1544; $R_f$ (20% MeOH/$CH_2Cl_2$) 0.13.

**4.6. Conjugation with anhydride of 1, 8-naphthalenedicarboxylic acid**

A mixture of benzo[*de*]isochromene-1,3-dione **10a**, **b** (0.3 mmol) and **8g** (0.1 g, 0.26 mmol) in anhydrous ethanol (5 mL) containing pyridine (0.2 mL, 2.5 mmol) and 4-*N*,*N*-dimethyl-pyridine (0.1g, 0.8 mmol) was refluxed under argon for 18 h and afterwards evaporated to dryness. The traces of pyridine were removed by co-evaporation with anhydrous toluene (2 x 5 mL). The residual oil was purified on silica gel packed column using a mixture of 10% MeOH/$CHCl_3$ as an eluent.

*4.6.1. 2-(6-((2R,3R,4S,5R)-2-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-3-ylamino)hexyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione* **11a**. Yield 93% (0.13 g) yellowish solid; Mp 176-177 ºC; FT IR [ATR, $\nu$ $cm^{-1}$] 3382, 2941, 1690, 1652, 1589, 1345, 1264, 1238, 1054, 779; $\delta_H$ (600 MHz, DMSO-$d_6$) 1.22 (s, 1H), 1.29-1.35 (m, 2H), 1.35-1.43 (m, 2H), 1.58-1.66 (m, 2H), 2.47-2.52 (m, 2H), 2.53-2.59 (m, 1H),

3.17 (dd, *J* 6.5, 5.7 Hz, 1H), 3.53-3.62 (m, 2H), 3.87 (dd, *J* 6.4, 3.4 Hz, 1H), 3.99-4.06 (m, 2H), 4.11 (dd, *J* 5.2, 2.7 Hz, 1H), 5.08 (t, *J* 4.9 Hz, 1H), 5.42 (s, 1H), 5.65 (d, *J* 8.0 Hz, 1H), 5.72 (d, *J* 7.1 Hz, 1H), 7.87 (dd, *J* 8.2, 7.2 Hz, 3H), 7.91 (d, *J* 8.1 Hz, 1H), 8.45 (dd, *J* 8.4, 1.0 Hz, 2H), 8.49 (dd, *J* 7.3, 1.1 Hz, 2H), 11.29 (s, 1H); $\delta_C$ (150 MHz, DMSO-$d_6$) 163.3, 162.9, 150.7, 140.6, 134.2, 131.2, 130.6, 127.2, 127.1, 122.0, 101.7, 86.6, 85.9, 68.4, 63.6, 61.1, 47.1, 39.5, 29.4, 27.4, 26.3, 26.2; MS (ESI) $[M+H]^+$ calcd for $C_{27}H_{31}N_4O_7$= 523.2192, Found=523.2190; $R_f$ (10% MeOH/$CHCl_3$) 0.15.

*4.6.2. 6-Bromo-2-(6-((2R,3R,4S,5R)-2-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-3-ylamino)hexyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione* **11b**. Yield 97% (0.15 g) yellowish solid; Mp 109-110 $^oC$; FT IR [ATR, $\nu$ $cm^{-1}$] 3391, 2940, 1694, 1622, 1589, 1346, 1264, 1238, 1054, 779; $\delta_H$ (600 MHz, DMSO-$d_6$) 1.22 (s, 1H), 1.25-1.35 (m, 2H), 1.35-1.42 (m, 2H), 1.61 (dt, *J* 14.6, 7.3 Hz, 2H), 2.47-2.53 (m, 2H), 2.53-2.59 (m, 1H), 3.13-3.21 (m, 1H), 3.55 (dd, *J* 11.9, 3.2 Hz, 1H), 3.59 (dd, *J* 11.9, 3.7 Hz, 1H), 3.87 (dd, *J* 6.3, 3.4 Hz, 1H), 3.97-4.04 (m, 2H), 4.11 (dd, *J* 5.1, 2.7 Hz, 1H), 5.08 (s, 1H), 5.42 (s, 1H), 5.64 (d, *J* 8.0 Hz, 1H), 5.72 (d, *J* 7.1 Hz, 1H), 7.90 (d, *J* 7.1 Hz, 1H), 7.98 (dd, *J* 8.5, 7.3 Hz, 1H), 8.20 (d, 1H, *J* 7.8 Hz, 1H), 8.31 (d, *J* 7.8 Hz, 2H), 8.52 (dd, *J* 8.5, 1.1 Hz, 1H), 8.55 (dd, *J* 7.3, 1.1 Hz, 1H), 11.28 (s, 1H); $\delta_C$ (150 MHz, DMSO-$d_6$) 162.9, 162.7, 162.7, 150.7, 140.6, 132.5, 131.5, 131.2, 130.9, 129.7, 129.0, 128.7, 128.2, 122.7, 121.9, 101.7, 86.6, 85.9, 68.4, 63.6, 61.1, 47.1, 39.6, 29.4, 27.3, 26.3, 26.2; MS (ESI) $[M+H^+]^+$ calcd for $C_{27}H_{30}BrN_4O_7$=601.1298; 603.1277 Found=601.1296; 603.1275; $R_f$ (10% MeOH/$CHCl_3$) 0.13.

**5. References**

1. Iwai, Y., Nakagawa, A.; Nagai, A.; Matsuyama, K.; Takahashi, Y.; Matsushita, M.; Hirano, A.; Omura, S. *J. Antibiot.* **1979**, *32*, 1367.

2. Okawa, N., Nakayama, H.; Ikeda, K.; Furihita, K.; Shimazu, A.; Otake, N.; Yonehara, Y. *Agric.Biol.Chem. (Tokyo)*, **1980**, *44*, 1671.
3. Taguchi, F.; Imatani, Y.; Nagaki, D.; Nakagawa, A.; Omura, S. *J. Antibiot*. **1981**, *34*, 313.
4. Utagawa, T.; Morisawa, H.; Yamanaka, S.; Yamazaki, A.; Hirose, Y. *Agric. Biol. Chem*., **1985**, *49*, 2711.
5. Sharma, R. A.; Bobek, M., Bloch, A. *J. Med. Chem*., **1975**, *18*, 955.
6. Lin, T. S.; Zhang, X. H.; Wang, Z. H.; Prusoff, W. H. *J. Med. Chem*., **1988**, *31*, 484.
7. Ogawa, A.; Tanaka, M.; Sasaki, T.; Matsuda, A. *J. Med. Chem*. **1998**, *41*, 5094.
8. Vasil'eva, S. V.; Abramova, T. V.; Ivanova,T. M.; G. V. Shishkin, G. V.; Sil'nikov, V. N. *Russ. J. Bioorg. Chem.* **2004**, *30*, 234.
9. Scherr, M.; Klebba, Ch.; Haener, R.; Ganser, A.; Engels, J. W. *Bioorg. Med. Chem. Lett*. **1997**, *7*, 1791.
10. Das, S. R.; Fong, R; Piccirilli, J. A. *Curr. Opinion Chem. Biol*. **2005**, *9*, 585.
11. Teramoto, N.; Imanishi, Y.; Ito, Y. *J. Bioactive and Compatible Polymers* **2000**, *15*, 297.
12. Besada, P.; Shin, D. H.; Costanzi, S.; Ko, H.; Mathe´, Ch.; Gagneron, J.; Gosselin, J. G.; Maddileti, S.; Harden, T. K.; Jacobson, K. A. *J. Med. Chem*. **2006**, *49*, 5532.
13. Ivanov, A. A.; Ko, H.; Cosyn, L.; Maddileti, S.; Besada, P.; Ingrid Fricks, I.; Costanzi, S.; Harden, T. K.; Van Calenbergh, S.; Jacobson, K. A. *J. Med. Chem*. **2007**, *50*, 1166.
14. Ko, H.; Carter, R. L.; Cosyn, L.; Petrelli, R.; de Castro, S.; Besada, P.; Zhou, Y.; Cappellacci, L.; Palmarisa Franchetti, P.; Grifantini, M.; Van Calenbergh, S.; Harden, T.; Jacobson, K. A. *Bioorg. Med. Chem*. **2008**, *16*, 6319.
15. Winkler, J.; Urban, E.; Losert, D.; Wacheck, V.; Pehamberger, H.; Noe, Ch. R. *Nucleic Acids Res*. **2004**, *32*, 710.
16. Ozaki, H.; Momiyama, S.; Yokotsuka, K.; Sawai, H. Tetrahedron Lett. **2001**, *42*, 677.
17. Halila, S.; Velasco, T.; De Clercq, P.; Madder, M. *Chem. Commun*. **2005**, 936.

18. de Beeck, M. O.; Madder, A. *J. Am. Chem. Soc*. **2011**, *133*, 796.

19. Matulic-Adamic, J.; Beigelman, L.; Dudycz, L. W.; Gonzalez, C.; Usman, N.; *Bioorg. Med. Chem. Lett*. **1995**, *5*, 2721.

20. Sitaula, S.; Reed, S. M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 850.

21. Mitsui, T.; Nakano, H.; Yamana, K. *Tetrahedron Lett.* **2000**, *41*, 2605.

22. Kalra, N.; Parlato, M. C.; Parmarb, V. S.; Wengel, J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3166.

23. Bugaut, A.; Bathany, K.; Schmitter, J. M.; Raynera, B. *Tetrahedron Lett*. **2005**, *46*, 687.

24. Karpeisky, A.; Sweedler, D.; Haeberli, P.; Read, J.; Jarvis, K.; Beigelman, L. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 3345.

25. Walcher, G.; Pfleiderer, W. *Helv. Chim. Acta*, **1996**, *79*, 1067.

26. Ikehara, M.; Maruyama, T.; Miki, H. *Tetrahedron Lett*. **1976**, *17*, 4485.

27. Ranganathan, R. *Tetrahedron Lett.* **1977**, *18*, 1291.

28. Verheyden, P. H.; Wagner, D.; Moffatt, J. G. *J. Org. Chem*. **1971**, *36*, 250.

29. McGee, D. P. C.; Sebesta, D. P.; O'Rourke, S. S.; Martinez, R. L.; Jung, M. E.; Pieken, W. A. *Tetrahedron Lett*. **1996**, *37*, 1995.

30. McGee, D. P. C.; Vaughn-Settle, A.; Vargeese, Ch. Zhai, Y. *J. Org. Chem*. **1996**, *61*, 781.

31. Sebesta, D. P.; O'Rourke, S. S.; Martinez, R. L.; Pieken, W. A.; McGee, D. P. C. *Tetrahedron* **1996**, *52*, 14385.

32. Faul, M. M.; Huff, B. E.; Dunlap, S. E.; Frank, S. A.; Fritz, J. E.; Kaldor, S. W.; LeTourneau, M. E.; Staszak, M. A.; Ward, J. A.; Werner, J. A.; Winneroski, L. L. *Tetrahedron* **1997**, *53*, 8085.

33. Henrik Munch, H.; Hansen, J. S.; Pittelkow, M.; Christensen, J. B.; Ulrik Boas, U. *Tetrahedron Lett*. **2008**, *49*, 3117.

34. Zhang, H.; Liu, R. Q.; Liu, K. Ch.; Li, Q. B.; Li, Q. Y.; Liu, S. Z. *Molecules* **2014**, *19*, 13631.

35. Ulatowski, F. Jurczak, J. *J. Org. Chem*. **2015**, *80*, 4235.

36. Li, J.; Sha, Y. *Molecules* **2008**, *13*, 1111.

37. Hwang, J.T.; Greenber, M. M. *Organic. Lett*., **1999**, *1*, 2021.

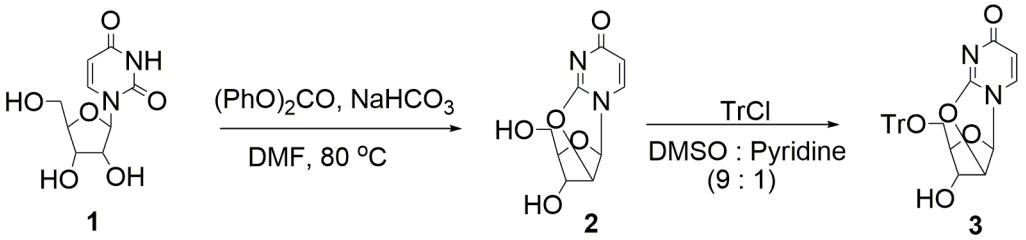
38. Kawai, K.; Kawabata, K.; Tojo, S.; Majima, T. *Bioorg. Med. Chem. Lett.,* **2002**, *12*, 2363.

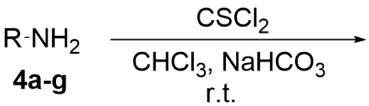
39. Lebedev, A. V.; Lebedeva, A. B.; Sheludyakov, V. D.; Ovcharuk, S. N.; Kovaleva, E. A.; Ustinova, O. L. *Russ. J. Gen. Chem.,* **2006**, *76*, 1069.

40. Garmaise, D. L.; Schwartz, R.; McKay, A. F. *J. Am. Chem. Soc*., **1958**, *80*, 3332.

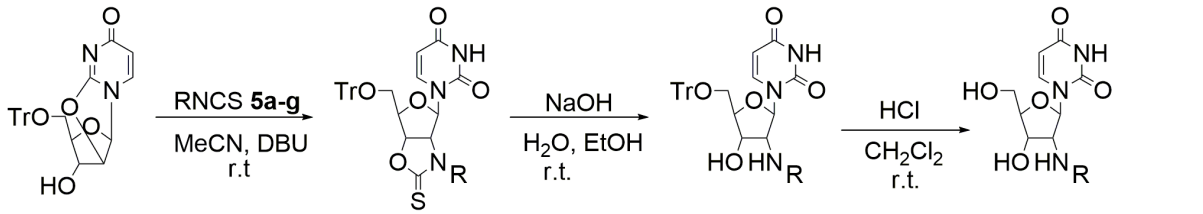
41. Sureshbabu, V. N.; Naik, S. A.; Hemantha, H. P.: Narendra,N.; Das, U.; Guru Row T. N., *J. Org. Chem*. **2009**, *74*, 5260.

42. Galcera, C. M-O.; Roubert, P.; Sidhu, A.; Thurieau, Ch. PCT/FR2002/004055, 2004.






**4, 5 a** R = $CH_2COOCH_3$
**b** $(CH_2)_2COOCH_3$
**c** $(CH_2)_3COOCH_3$
**d** $(CH_2)_5COOCH_3$
**e** $(CH_2)_2NHBoc$
**f** $(CH_2)_4NHBoc$
**g** $(CH_2)_6NHBoc$

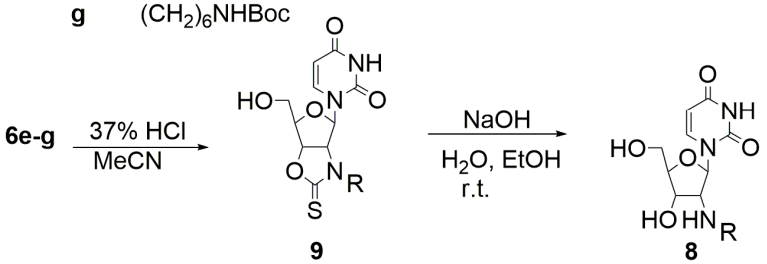



**3** **6a-g** **7** **8**

**6 a** R = $CH_2COOCH_3$
**b** $(CH_2)_2COOCH_3$
**c** $(CH_2)_3COOCH_3$
**d** $(CH_2)_5COOCH_3$
**e** $(CH_2)_2NHBoc$
**f** $(CH_2)_4NHBoc$
**g** $(CH_2)_6NHBoc$

**7 a** R = $CH_2COOH$
**b** $(CH_2)_2COOH$
**c** $(CH_2)_3COOH$
**d** $(CH_2)_5COOH$

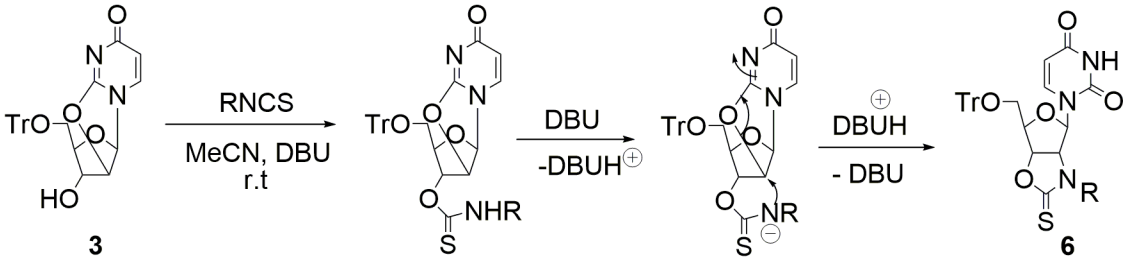
**8 a** R = $CH_2COOH$
**b** $(CH_2)_2COOH$
**c** $(CH_2)_3COOH$
**d** $(CH_2)_5COOH$



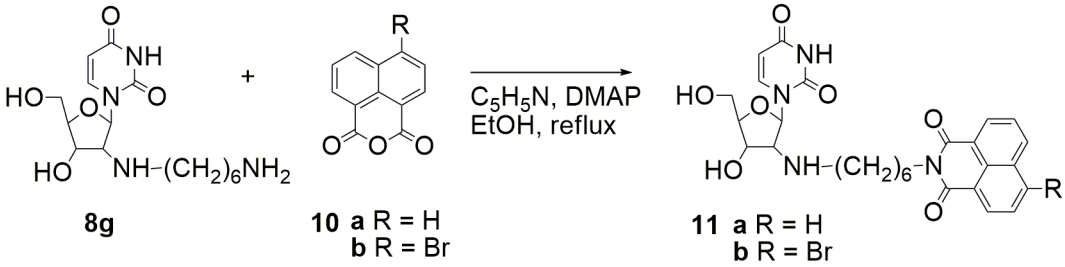

**9** **8**

**9a** R = $(CH_2)_2NH_2$
**b** R = $(CH_2)_4NH_2$
**c** R = $(CH_2)_6NH_2$

**8e** R = $(CH_2)_2NH_2$
**f** R = $(CH_2)_4NH_2$
**g** R = $(CH_2)_6NH_2$




**3** **6**




**8g**

**10** **a** R = H
**b** R = Br

**11** **a** R = H
**b** R = Br