Tetrahedron 66 (2010) 8450–8457

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Michael versus retro-Michael reaction in the regioselective synthesis of N-1 and N-3 uracil adducts

Sławomir Boncel, Maciej Mączka, Krzysztof Z. Walczak *

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

ARTICLE INFO

Article history:
Received 21 April 2010
Received in revised form 4 August 2010
Accepted 23 August 2010
Available online 27 August 2010

Keywords:
5-Substituted uracil derivatives
Acrylic derivatives
Acyclic nucleosides
Regioselectivity
Michael-type addition
retro-Michael reaction

ABSTRACT

By controlling the temperature or reaction time in the base-catalysed Michael-type addition of 5-substituted uracil derivatives we were able to synthesise N-1 or N-3 uracil adducts using methyl acrylate and acrylonitrile as acceptors. The mechanism of this chemical inequivalence was established using ^{1}H NMR spectroscopic studies. The investigations revealed that formation of the N-1 adduct was achievable under kinetically controlled conditions irrespective to the type of the base used (TEA, DBU). In turn, synthesis of the N-3 adducts proceeded from the initially formed N-1,N-3 diadduct via a retro-Michael reaction which dominates at elevated temperature or prolonged reaction time.

1. Introduction

One of the important aspects in a synthesis of acyclic pyrimidine nucleosides is the intentional and effective introduction of substituents into a required position of the nucleobase ring. Due to the important role of many 1-glycosyluracils in biochemical processes,[1,2] mainly N-1 uracil glycosides have been studied in the pursuit of novel anticancer and antiviral drugs (Fig. 1). For example, 1-cyanomethyl 5-halogenouracils (A) were active against P-388/s, FM-3A/s and U-937/s cell lines.[3] On the other hand, several N-3 mono- and N-1,N-3 disubstituted uracil derivatives are also biologically active. Not N-1 (B) but N-3 regioisomer of β-alloxazine (C) (bearing latent uracil subunit) has the hydrogen-bonding characteristic of β-D-thymidine and can be used in the construction of fluorescent nucleosides.[4] *N*-3-carboxyalkyl Willardine derivatives (D) are known as AMPA and kainate receptor antagonists.[5]

The N3-position of the uracil ring has been exploited in conjugates with anticancer agents like Paclitaxel or Vinblastine.[6] 3-Carboranyl-β-D-thymidine derivatives are widely used in neutron capture therapy as a part of anticancer therapy.[7]

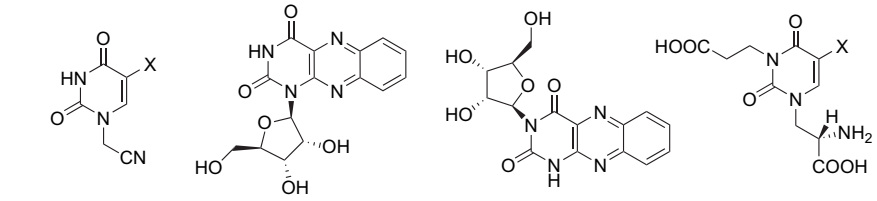


Fig. 1. Examples of N-1 and N-3 substituted uracil derivatives of medicinal importance.

* Corresponding author. Tel.: +48 32 237 17 92; fax: +48 32 237 20 94; e-mail address: Krzysztof.Walczak@polsl.pl (K.Z. Walczak).

0040-4020/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2010.08.059

The most common strategies for the alkylation of uracil towards acyclic nucleosides involve reaction of appropriate alkylating agents (alkyl halides, oxiranes) with an activated uracil ring. Activation under basic conditions results in production of a mixture of all *N*- or *O*-monosubstituted and frequently disubstituted derivatives, whereas activation via initial O-persilylation furnishes N-1 as the main and N-1,N-3 bis-alkylated derivatives as side products. Activation by the presence of an electron-withdrawing group (NO_2, CN, etc.) at the 5-position of uracil ring enables regioselective N-1 alkylation via the ANRORC (Addition of Nucleophile, Ring Opening, Ring Closure) reaction.[8,9] Strategies leading to selective synthesis of N-3 alkylated uracils included selective N-1 deprotection of bis-Cbz-protected 5-fluoro-1,3-bis(hydroxymethyl) uracils[10] and N-1 Boc-protection, subsequent N-3 alkylation and deprotection.[11] In another approach a rearrangement of *N*-4-carboxyethylcytosines for the synthesis of *N*-3-carboxyethyluracils was applied.[12] A detailed summary of these methods as well as a rationale behind N-1 versus N-3 alkylation of ambident uracil was published very recently.[13]

Another synthetic pathway for the synthesis of *N*-alkylated uracils and their precursors is the Michael-type addition (in this case aza-Michael reaction) of uracil anions to acceptors possessing activated double bonds, e.g., acrylic derivatives. Contrary to the above-mentioned methods, this reaction emerges as a very convenient one since it limits the number of products to mainly N-1 monoadducts and N-1,N-3 diadducts. Despite the fact that this attribute has generated many synthetic approaches towards the formation of an exocyclic C–N bond,[14–19] the number of reports on the controllable N-1 versus N-3 Michael-type addition is limited.[20,21] Experimental[22,23], and theoretical[24] studies confirmed the favourable formation of N-1 adducts, which were also thermodynamically more stable.

Continuing our research interest in the synthesis of acyclic nucleosides we have examined a mechanism of the N-1 versus N-3 alkylation of 5-substituted uracils via Michael-type addition. Basing on our previous research[25] and the aforementioned theoretical premises, we employed basicity of a deprotonating agent and kinetic parameters of the reaction (time and temperature) as key parameters to synthesise either N-1 or N-3 adducts. We used triethylamine (TEA) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as readily accessible deprotonating agents of different basicity and poor nucleophilicity. By tracing the course of reaction kinetic pathway by ^{1}H NMR spectroscopic studies we expected to establish the mechanism of regioselective synthesis of N-1 and N-3 uracil Michael adducts.

2. Results and discussion

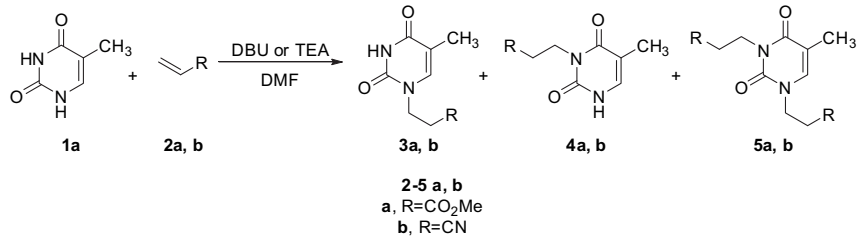
2.1. ^{1}H NMR spectroscopic studies of the Michael-type addition

We performed semi-kinetic studies of Michael-type addition of thymine anion (Michael donor) generated from thymine (**1a**, 1 equiv) in anhydrous DMF using various amounts (1.0 and 2.0 equiv) of deprotonating agent—DBU or TEA (Table 1). Mono- and/or dianions of thymine, after achievement of equilibrium (10 min), were reacted with an appropriate Michael acceptor—methyl acrylate (**2a**) or acrylonitrile (**2b**), used in different proportions (0.5 and 1.0 equiv) (Scheme 1).

Table 1
Semi-kinetic ^{1}H NMR spectroscopic studies of the Michael-type addition of thymine (**1a**) to methyl acrylate (**2a**) and acrylonitrile (**2b**)

Entry	DA	Acceptor (**2**)	D	DA	A	Temp [°C]	Yield[a] [%]		
							N-1	N-3	Diadduct
							3a	**4a**	**5a**
1	DBU	Methyl acrylate (**2a**)	1	1	1	20	86	0	7
2			1	2	1		91	0	7
3			1	1	0.5		49	0	0
4			1	1	1	80	61	13	25
5			1	2	1		52	32	16
6			1	1	0.5		45	1	3
7	TEA		1	1	1	20	86	0	0
8			1	2	1		90	0	0
9			1	1	0.5		45	0	0
10			1	1	1	80	98	0	0
11			1	2	1		100	0	0
12			1	1	0.5		49	0	0
Entry	DA	Acceptor (**2**)	D	DA	A	Temp [°C]	Yield[a] [%] N-1	N-3	Diadduct
							3b	**4b**	**5b**
13	DBU	Acrylonitrile (**2b**)	1	1	1	20	46	0	45
14			1	2	1		38	0	58
15			1	1	0.5		43	0	4
16			1	1	1	80	7	0	89
17			1	2	1		0	25	74
18			1	1	0.5		32	0	17
19	TEA		1	1	1	20	84	0	0
20			1	2	1		88	0	0
21			1	1	0.5		43	0	0
22			1	1	1	80	96	0	0
23			1	2	1		98	0	0
24			1	1	0.5		47	0	0

[a] Yield calculated on thymine.



Scheme 1. Reaction of thymine with methyl acrylate and acrylonitrile under various conditions involved in the ^{1}H NMR spectroscopic semi-kinetic studies.

Table 2
Conditions of semi-kinetic studies enabling observation of reversibility of Michael-type addition of thymine (**1a**, 1 equiv) and 5-bromouracil (**1b**, 1 equiv) to methyl acrylate (**2a**, 2 equiv) in the presence of DBU (1 equiv) and corresponding yields of products calculated by ^{1}H NMR spectroscopy

Entry	Donor (1 equiv)	Time [h]	Yield [%]		
			N-1 (**3a**)	N-3 (**4a**)	N1,N3 (**5a**)
1	Thymine (**1a**)	0.25	19	0	1
2		+1.25	76	0	22
3		+1.25 (at 60 °C)	35	40	25
			N-1 (**3c**)	N-3 (**4c**)	N1,N3 (**5c**)
4	5-Bromouracil (**1b**)	2 (+0.5 at 80 °C)	69	30	0
5		144	5	95	0
6		336	0	100	0

All the reactions were performed for 2 h. After this time, reactions were quenched by neutralisation of the basic catalyst with concentrated hydrochloric acid, and volatiles were removed by distillation under reduced pressure. The samples after each reaction course were dissolved in DMSO-d_6 and ^{1}H NMR spectra were acquired. Areas in the aliphatic region of ^{1}H NMR spectra served for further quantitative analyses. These areas ranging from 4.08 to 3.78 ppm and revealing signals from methylene groups

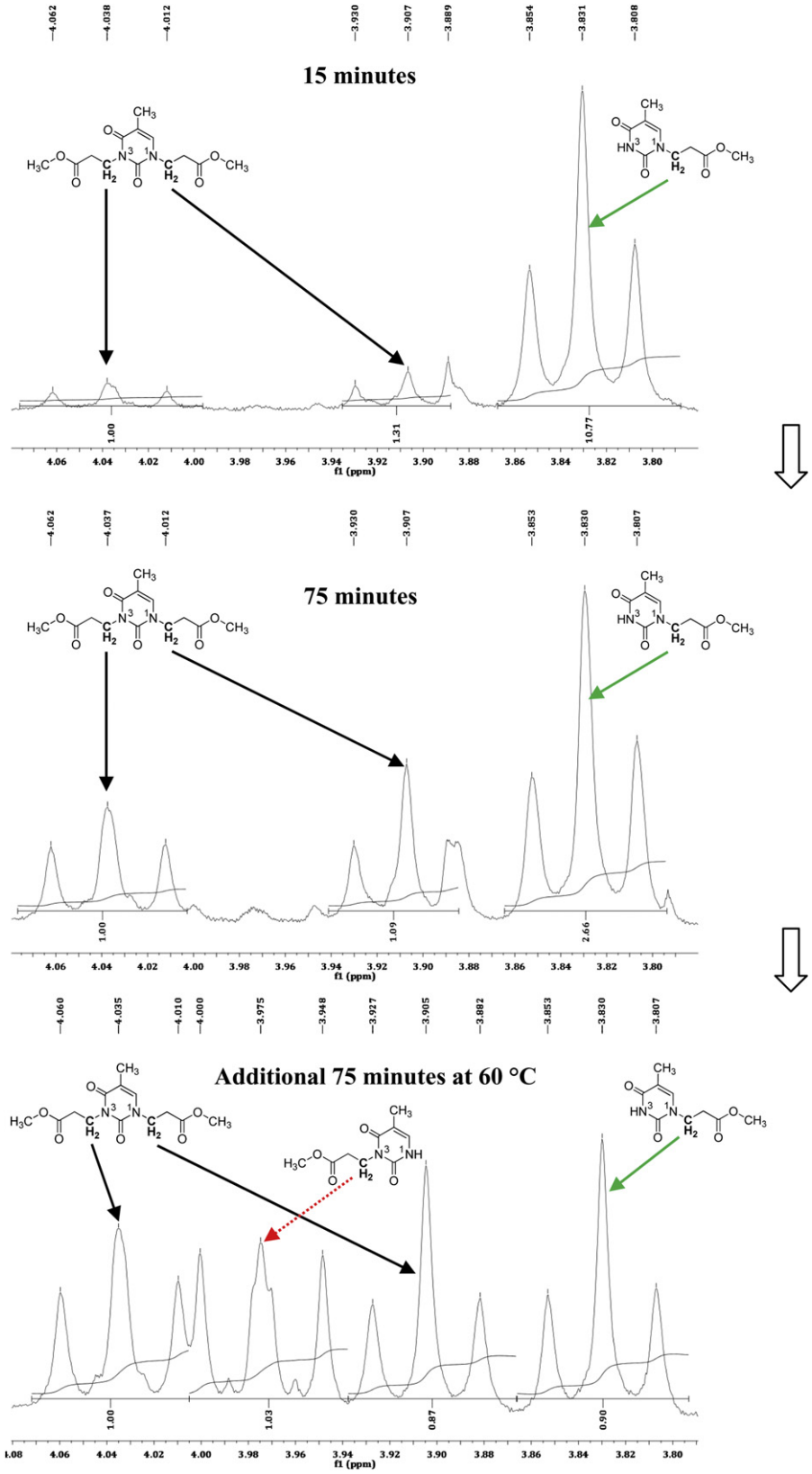


Fig. 2. Quantitative ^{1}H NMR study of Michael-type addition of thymine (**1a**) to methyl acrylate (**2a**, 2 equiv) in the presence of DBU (1 equiv).

directly bonded to N-1 or N-3 nitrogen atoms of the thymine ring allowed to calculate molar fractions [%] of all Michael-products: N-1 adduct (**3a**), N-3 adduct (**4a**) and N-1,N-3 diadduct (**5a**) (Table 1).

As the studies revealed, addition of thymine (**1a**) to methyl acrylate (**2a**) in the presence of DBU at ambient temperature results in a formation of N-1 adduct (**3a**) as the main product irrespective of the ratio of reactants and catalyst (entries 1–3). At elevated temperature (entries 4–6), a significant increase in the amounts of both N-3 adduct (**4a**) and diadduct (**5a**) was evident. Moreover, when the amount of base was doubled in relation to stoichiometric ratio of reactants (entry 5), an increase in the yield of N-3 adduct at the cost of diadduct formation was observed. On the other hand, using TEA as a deprotonating agent led exclusively to N-1 addition regardless of all variations in temperature and proportions of reactants/catalyst (entries 7–12). Being encouraged by these results, we extended the studies for acrylonitrile (**2b**) and the same trends were observed in the case of DBU (entries 13–18). At ambient temperature, the content of diadduct (**5b**) in a post-reaction mixture was higher as compared to the reaction of thymine with methyl acrylate. The main difference occurs in the reaction at elevated temperature (entries 16–18). Under these conditions, both bis-cyanoethylation and N-1 decyanoethylation of diadduct (**5b**) became more considerable reactions. In the case of a double molar excess of DBU (entry 17), a complete decay of N-1 adduct (**3b**) was observed. The excessive amount of deprotonating agent appeared then as essential for the retro-Michael reaction of the diadduct. In turn, an identical behaviour to methyl acrylate was observed in the case of reactivity of thymine towards acrylonitrile in the presence of TEA (entries 19–24)—neither diadduct (**5b**) nor N-3 adduct (**4b**) was obtained under these conditions. It became evident that TEA is not capable of formation either N-1,N-3 diadduct or N-3 adduct under the above conditions.

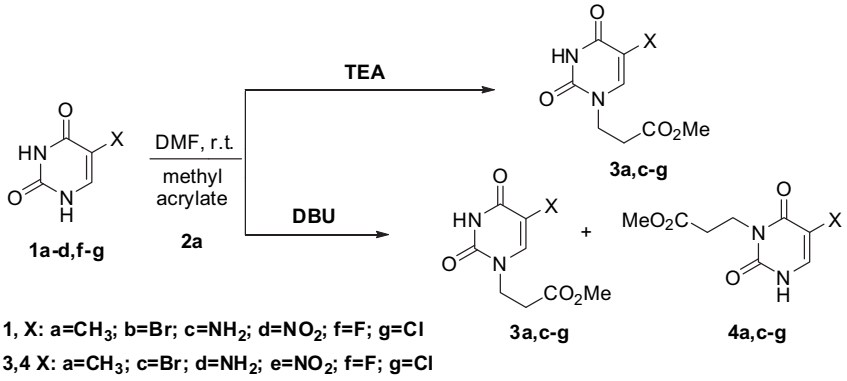
Experiments under conditions guaranteeing observation of reversibility of Michael-type addition (donor/base/acceptor=1:1:2, ambient and then elevated temperature 60/80 °C) were further performed (Table 2).

These studies revealed that N-1 adduct (**3a**) was the product of a kinetically controlled Michael-type addition of thymine (**1a**) to methyl acrylate (**2a**). In a single experiment, at prolonged time or at elevated temperature in the reaction course primarily N-1,N-3 diadduct (entry 2) and afterwards N-3 adduct (entry 3) were formed, particularly in the presence of an excess of DBU. A sequence of ^{1}H NMR spectra as a function of time and temperature (as depicted in Table 2) is presented in Fig. 2. Magnified sections of these spectra δ: (4.08–3.78 ppm) provided finely separated peaks as, initially three, and in the last stage four triplets ($^3J_{H-H}$=6.9–7.8 Hz) deriving from β-methylene protons (*N*–CH_2CH_2CO) from particular adducts. In this area signals from these protons are located at δ=3.83, 3.98, and 4.04, 3.91 ppm for N-1 adduct (**3a**), N-3 adduct (**4a**) and diadduct (**5a**), respectively. This observation is in an excellent accordance with ^{1}H NMR spectra of pure compounds (see Experimental part). Summarising, prolonged reaction time of thymine with methyl acrylate in the presence of DBU at ambient temperature leads to an increase in the yield of N-1,N-3 diadduct at the cost of N-1 adduct. By increasing the temperature, due to the retro-Michael reaction diadduct transforms into the N-3 and not the N-1 adduct.

In the case of the reaction of 5-bromouracil (**1b**) with methyl acrylate (**2a**) three separated experiments were conducted. In these experiments it was evident that either increased temperature (entry 4) or prolonged time of reaction course at ambient temperature (entries 5 and 6) led to N-3 adduct (**3c**) as the main product.

2.2. Other 5-substituted uracils as Michael donors

Basing on our preliminary research[25,26] and optimised conditions obtained in the above presented manner, we have confirmed the formerly observed tendency in regioselectivity for other 5-substituted uracils (**1a**–**d**) (Scheme 2, Table 3).



Scheme 2. Synthesis of various 5-substituted N-1 and N-3 Michael-type uracil adducts in the presence of different bases as catalysts.

A use of TEA enabled us to obtain exclusively N-1 Michael-type adducts for the all DMF-soluble 5-substitued uracils in excellent yields when column chromatography was used for isolation of the products. In the case of 5-aminouracil (**1c**) DBU emerged as an excellent catalyst. Specifically in this case, N-1 adduct (**3c**) was isolated in high yield as the sole product. In the case of TEA, poor solubility of Michael donor was relevant, and the same product (**3c**) was obtained only in 3% yield comparing to 78% in the case of DBU where donor solubility was enhanced but not completed. Here, N-1 product was formed in the absence of both N-3 adduct (**4c**) and N-1,N-3 diadduct (**5c**). Moreover, during the reaction course, the amine group retained unreactive towards methyl acrylate. Generally, we have confirmed at this point that prolonged time of the reaction or elevated temperature in the presence of DBU substantially change regioselectivity into N-3 adduct.

2.3. Identification of regioisomers

A straightforward distinction between the N-1 and N-3 regioisomers was possible by means of NMR spectroscopy since the signals from the β-methylene groups (directly bound to the ring) in the N-3 isomers always occur at higher chemical shift in comparison to the N-1 isomers. We have also found that in the N-3 adduct (**4a**) the coupling constant between the H-1 and H-6 protons is observable and equals 5.2 Hz. Furthermore, UV/VIS spectra of N-1 (**3c**) and N-3 (**4c**) regioisomers recorded at extreme values of pH (pH=0 and pH=14)

Table 3
N-1 and N-3 regioselective Michael-type addition of 5-substituted uracils to methyl acrylate at ratio of donor/base/acceptor=1:1:2; Reactions were run at ambient temperature for 2 h, then heated and kept at 80 °C for 0.5 h for DBU; For TEA as a deprotonating agent—reactions were performed at ambient temperature; The isolated yields (calculated on 5-substituted uracil) after workup described in Experimental part

No.	X	pK_{a1} (DMF)	DA	N^1 yield [%]	N^3 yield [%]
				3a,c–g	**4a,c–g**
1a	CH_3	15.5	TEA	57	0
			DBU	0	57[a]
			DBU	64	36
1b	Br	12.7	TEA	98	0
			DBU	70	30
1c	NH_2	16.0	TEA	3	0
			DBU	78	0
1d	NO_2	9.1	TEA	35[b]	0
			DBU	27	0
1f	F	12.4	TEA	90	0
			DBU	36	64
1g	Cl	12.5	TEA	88	0
			DBU	64	36

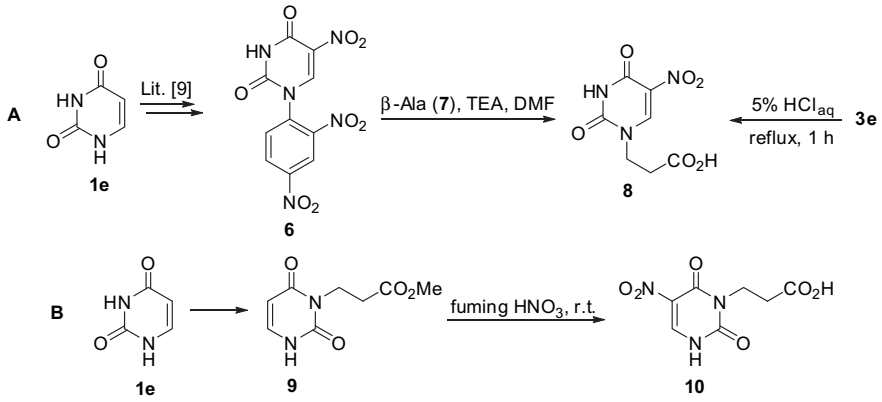
[a] Time of reaction 24 h for DBU at ambient temperature and 1 h at 80 °C, subsequently quenched.
[b] This product was obtained in 54% yield in the absence of deprotonating agent.

Molar absorption coefficients (ε) were equal to 7600 and 6900 [$dm^3\ mol^{-1}\ cm^{-1}$] for N-1 and N-3 adduct, respectively.

5-Nitrouracil (**1d**)—the most acidic derivative in the whole set of examined uracils, irrespective of the type of base, afforded only N-1 adduct (**3e**). An unequivocal confirmation of its structure (**3e**) was performed based on the independent synthetic pathway (Scheme 3).

A reaction of 1-(2,4-dinitrophenyl)-5-nitrouracil (**6**) with β-alanine (**7**) (running according to the ANRORC mechanism) carried out using previously published procedure[9] (Scheme 3, A) furnished an acid derivative (**8**). This product possessed identical spectral and physical properties (^1H and ^{13}C NMR spectra, mixed melting point) with (**8**) obtained via acidic hydrolysis of **3e** according to a known procedure.[28]

The N-3 regioisomer (**10**) was obtained in good yield via simultaneous nitration and hydrolysis[29] of uracil N-3 adduct (**9**) using white fuming nitric acid (Scheme 3, B). Moreover, **3e** subjected itself to the reaction with methyl acrylate under both basic and neutral conditions did not afford the expected diadduct, even in the prolonged reaction course.



Scheme 3. A—Structure verification of N-1 adduct (**3e**) of 5-nitrouracil via ANRORC reaction; B—alternative synthesis of acidic derivative of 5-nitrouracil N-3 adduct (**10**).

also allowed to identify them since a strong bathochromic effect at pH=14 for N-1 uracil anion of N-3 adduct caused by an enhanced delocalisation of negative charge over a larger molecular area was present (Fig. 3). Maximum of absorption for N-3 adduct shifted from 276 nm to 299 nm (+23 nm). A similar shift was not observable in the case of the N-1 adduct. These results are in accordance with previous UV/VIS studies of N-1 and N-3 substituted uracil derivatives.[27]

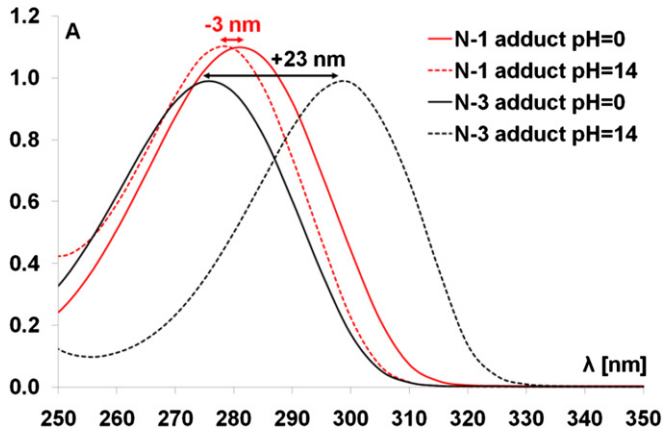


Fig. 3. UV/VIS spectra of N-1 (**3c**) and N-3 (**4c**) adducts of 5-bromouracil recorded at extreme values of pH (pH=0 and pH=14, 1 M HCl_{aq} and 1 M $NaOH_{aq}$, respectively), $c=1.4\times10^{-4}$ M.

2.4. Discussion

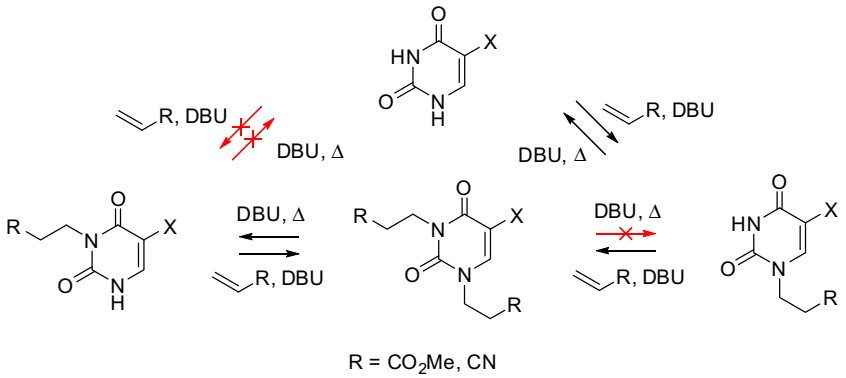
In order to categorically establish the mechanism of regioselective synthesis of N-1 and N-3 adducts we performed separately a reaction of N-1 (**3a**), N-3 (**4a**) adducts and diadduct (**5a**) under the identical retro-Michael reaction conditions (Table 4).

Table 4
Susceptibility of regioisomeric adducts of thymine (**3a** and **4a**) and diadduct (**5a**) to retro-Michael reaction under the identical conditions—expressed by the composition of the reaction mixture calculated by ^1H NMR quantitative analysis

Adduct	DBU	Temp [°C]	Time [min]	[mol %]			
				1a	**3a**	**4a**	**5a**
N-1 (**3a**) (1 equiv)	1 equiv	60	150	80	20	0	0
N-3 (**4a**) (1 equiv)				0	0	100	0
N-1,N-3 (**5a**) (1 equiv)				0	0	85	15

In these experiments both regioisomers and N-1,N-3 diadduct (1 equiv each) were heated in DMF solution in the presence of DBU (1 equiv) for 150 min. Under these conditions, N-3 adduct (**4a**) emerged as entirely unreactive in retro-Michael reaction and was isolated from the post-reaction mixture in 100% yield. In turn, N-1 adduct (**3a**) substantially underwent elimination of methyl acrylate and, apart from the unreacted substrate, thymine (**1a**) was isolated in 80% yield. As it was predictable, the N-1,N-3 diadduct treated in the same manner underwent an elimination leading selectively to

the N-3 adduct. These results confirm that N-3 regioisomers as thermodynamically more stable than their N-1 counterparts are selectively formed from N-1,N-3 diadducts via retro-Michael reaction. As a summary, the course of the competitive, reversible and subsequent reactions is presented in Scheme 4.



Scheme 4. Regioselective synthesis of N-1 (**3a**,**c**–**g**) and N-3 (**4a**,**c**–**g**) uracil adducts in the presence of DBU as a deprotonating agent; R=CO_2Me (**2a**), CN (**2b**).

A significant difference of basicity of TEA and DBU (in water and DMF) used as deprotonating agents corresponds to the fact that only DBU as a stronger base is capable of both double deprotonation of uracil ring and induction of elimination of methyl acrylate (Table 5).

Table 5
Values of pK_{a1} determined experimentally for deprotonating agents and uracil donors used in this work

	Deprotonating agent		Donor	
	TEA	DBU	Thymine (**1a**)[a]	5-Bromouracil (**1b**)[a]
pK_{a1} (Water)	10.75[30]	12[31,32]	9.8[33]	8.1[33]
pK_{a1} (DMF)	9.2[34]	12.8[b,35]	15.5[33]	12.7[33]

[a] Macroscopic dissociation constants K_{a1} for 5-substituted uracil derivatives comprise two microscopic constants $k_{a1(N1-H)}$ and $k_{a1(N3-H)}$ related by following formula: $K_{a1}=k_{a1(N1-H)}+k_{a1(N3-H)}$.

[b] Value obtained by extrapolation from linear relation between pK_a values in water and DMF for the set of *N*-centred organic bases related by an equation: $pK_{a(DBU)}=0.9463pK_{a(water)}+1.4154$.

Thus, for both cases in the initial stage of the Michael-type addition a formation of solely N-1 adduct as a product of kinetic control is observed. In the prolonged time of reaction or at elevated temperature, an amount of diadduct increases and, in the presence of a strong base, retro-Michael reaction dominates and N-3 regioisomer is formed. Hauser's rule[36] as an explanation of behaviour of uracil dianions may be safely eliminated as it claims that the more basic (and frequently more nucleophilic) anions—in this case the N-3 anion—would form N-3 adducts under kinetically controlled conditions. Here, in the presence of a double molar excess of DBU and 1 equiv of the Michael acceptor only the N-1 adduct and, subsequently, diadduct were formed. Moreover, steric effects are negligible in the retro-Michael reaction as in the previous studies ethoxide anion was applied in the selective synthesis of N-3 adducts via similar synthetic procedure.[20,21] Furthermore, the more acidic 5-substituted uracil derivative is, the weaker and less nucleophilic its conjugated base (anion) is. This behaviour was demonstrated by a decreased reactivity of 5-nitrouracil towards methyl acrylate in the presence of a deprotonating agent. Also, 5-nitrouracil N-1 adduct (**3e**) was inactive when treated with methyl acrylate in the presence of DBU.

3. Conclusions

Using a semi-kinetic ^{1}H NMR spectroscopy investigation, we have unequivocally established the mechanism of Michael-type addition of 5-substitued uracils to acrylic-type acceptors. The reactions lead to N-1 or N-3 adducts depending on the basicity of the deprotonating agent, temperature and time of the reactions. These results allow the synthesis of N-1 acyclic nucleosides in high regioselectivity in the presence of a deprotonating agent of a broad range of basicity as well as N-3 uracil derivatives via N-1,N-3 diadduct route, however in the presence of strong base and at increased temperature or prolonged time of reaction. Limitation of the synthetic pathway towards N-3 adducts is acidity of the uracil donor what was evident for 5-nitrouracil. Nevertheless, these products can be obtained via another synthetic strategy.

4. Experimental part

4.1. General remarks

NMR spectra were recorded at 300 MHz for ^{1}H NMR and 75.5 MHz for ^{13}C NMR on a Varian Inova 300 MHz in DMSO-d_6 solution if not indicated else; δ values are in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard. Elemental analyses were obtained using a Perkin–Elmer 240C apparatus. Mass spectrum was recorded at ESI TOF ionisation on Ionspec 4.7 T Ultima FTMS FT-ICR MS Fourier Transform Ion Cyclotron Resonance Mass Spectrometer. FT-IR spectra acquisitions were carried out in a Bruker Tensor 27 Infrared Spectrometer. All used reagents were purchased from Aldrich or Alfa Aesar. TLC 60 F_{254} plates and silica gel 60 (0.040–0.063 mm) were purchased from Merck. Melting points were measured at Boetius apparatus and are uncorrected. DMF was distilled prior to use and stored over molecular sieves 4 Å.

All the reactions in the semi-kinetic ^{1}H NMR spectroscopic studies were performed for 2 h. After this time, reactions were quenched by neutralisation of the basic catalyst with concentrated hydrochloric acid, and volatiles were removed by distillation under reduced pressure. The samples after each reaction course (of 50 μL each) were dissolved in 0.6 mL of DMSO-d_6 and ^{1}H NMR spectra were acquired (256 scans) using Varian Unity INOVA XL-300 spectrometer at 300 MHz. The workup of ^{1}H NMR spectra was performed by means of Mestrec® version 4.5.6.0.

4.2. Michael-type addition of 5-substituted uracils (1a–d) to methyl acrylate (2a)

4.2.1. General procedure. To a solution of 5-substituted uracil derivative (**1a**–**d**) (5 mmol) in anhydrous DMF (10 mL) deprotonating

agent (TEA or DBU) (1 mmol) was added while stirring. After 5 min methyl acrylate (**2a**) (10 mmol) was added dropwise. Stirring was continued until the consumption of limiting reactant was achieved (24 h for TEA, 2 h for DBU, TLC, 5% MeOH/$CHCl_3$, vol). In the case of DBU a reaction flask was transferred into an oil bath, heated and kept at 80 °C for 30 min. Subsequently, base was neutralised by adding conc. HCl_{aq}, solvent was evaporated under reduced pressure using rotary evaporator and the residue was purified on chromatographic column using 5% MeOH/$CHCl_3$ (vol) as eluting system.

*4.2.1.1. Methyl 3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1 (2H)-yl)propanoate (**3a**).* Colourless crystals, mp 134–136 °C. ^{1}H HMR ($CDCl_3$): δ=9.50 (s, H-3, 1 H), 7.21 (d, $^4J_1$=0.6 Hz, H-6, 1H), 3.97 (t, $^3J_1$=6.0 Hz, >NCH_2, 2H), 3.71 (s, OCH_3, 3H), 2.79 (t, $^3J_1$=6.0 Hz, CH_2CO, 2H), 1.91 (d, $^4J_1$=0.6 Hz, CH_3, 3H). ^{13}C NMR: δ=172.0, 164.5, 151.1, 141.7, 110.4, 52.2, 45.1, 33.0, 12.4. $C_9H_{12}N_2O_4$ (212.20): calcd C 50.94, H 5.70, N 13.20; found C 51.21, H 5.65, N 12.99. IR (KBr pellet): ν=3163 (N–H), 3099, 3038, 2957 (CH_3), 2831, 1720 (C=O), 1690 (C=O), 1660 (C=O), 1466, 1437, 1373, 1354, 1327, 1286, 1207 (C–O–C), 1138 (C–O) cm^{-1}.

*4.2.1.2. Methyl 3-(5-methyl-2,6-dioxo-2,3-dihydropyrimidin-1 (6H)-yl)propanoate (**4a**).* Colourless crystals, mp 112–114 °C. ^{1}H HMR: δ=10.93 (s, H-1, 1H), 7.29 (d, $^3J_1$=5.2 Hz, H-6, 1H), 4.02 (t, $^3J_1$=7.5 Hz, >NCH_2, 2H), 3.59 (s, OCH_3, 3H), 2.52 (t, $^3J_1$=7.5 Hz, CH_2CO, 2H), 1.78 (s, CH_3, 3H). ^{13}C NMR: δ=172.0, 164.5, 151.1, 141.7, 110.4, 52.2, 45.1, 33.0, 12.4. $C_9H_{12}N_2O_4$ (212.20): calcd C 50.94, H 5.70, N 13.20; found C 50.71, H 5.62, N 13.07. IR (KBr pellet): ν=3140 (N–H), 2975 (CH_3), 2263, 1832, 1768 (C=O), 1640 (C=O), 1608, 1496, 1448, 1368, 1304, 1224 (C–O–C), 1144 (C–O), 1080 cm^{-1}.

*4.2.1.3. Dimethyl 3,3′-(5-methyl-2,4-dioxopyrimidine-1,3 (2H,4H)-diyl)dipropanoate (**5a**).* Post-reaction mixtures from the semi-kinetic studies (Table 1) were combined and separated using column chromatography and 5% MeOH/$CHCl_3$ (vol) as eluting system. White semi-solid. ^{1}H NMR: δ=7.59 (s, H-6, 1H), 4.04 (t, $^3J_1$=7.5 Hz >N^3-CH_2, 2H), 3.91 (t, $^3J_1$=6.9 Hz >N^1-CH_2, 2H), 3.61 (s, OCH_3, 3H), 3.58 (s, OCH_3, 3H), 2.71 (t, $^3J_1$=6.9 Hz >N^1–CH_2CH_2, 2H), 2.52 (t, $^3J_1$=7.5 Hz >N^1-CH_2CH_2, 2H), 1.80 (s, CH_3, 3H). $C_{13}H_{18}N_2O_6$ (298.29): calcd C 52.34, H 6.08; N 9.39; found C 52.12, H 6.00; N 9.66. IR (KBr pellet): ν=3140, 2936 (CH_3), 2222, 1784, 1639 (C=O), 1608 (C=O), 1496, 1448, 1368, 1272, 1208 (C–O–C), 1128 (C–O) cm^{-1}.

*4.2.1.4. Methyl 3-(5-bromo-2,4-dioxo-3,4-dihydropyrimidin-1 (2H)-yl)propanoate (**3c**).* Colourless crystals, mp 158–160 °C ^{1}H NMR: δ=11.77 (br s, H-3, 1H), 8.19 (s, H-6, 1H), 3.90 (t, $^3J_1$=6.9 Hz, >NCH_2, 2H), 3.61 (s, OCH_3, 3H), 2.72 (t, $^3J_1$=6.9 Hz, CH_2CO, 2H); ^{13}C NMR: δ=171.2, 159.7, 150.2, 145.8, 94.3, 51.6, 44.2, 32.5. $C_8H_9BrN_2O_4$ (277.07): calcd C 34.68, H 3.27, N 10.11; found C 34.51, H 3.13, N 9.94. IR (KBr pellet): ν=3152 (N–H), 3098, 3020, 2959 (CH_3), 2876, 2833, 1736 (C=O), 1701 (C=O), 1659 (C=O), 1614, 1518, 1466, 1421, 1381,1350, 1261, 1202 (C–O–C), 1177, 1142 (C–O), 1070, 1049 (C–Br) cm^{-1}.

*4.2.1.5. Methyl 3-(5-bromo-2,6-dioxo-2,3-dihydropyrimidin-1 (6H)-yl)propanoate (**4c**).* Colourless crystals, mp 153–155 °C ^{1}H NMR: δ=11.59 (br s, H-1, 1H), 7.97 (s, H-6, 1H), 4.01 (t, $^3J_1$=6.9 Hz, >NCH_2, 2H), 3.58 (s, OCH_3, 3H), 2.55 (t, $^3J_1$=6.9 Hz, CH_2CO, 2H); ^{13}C NMR: δ=171.5, 159.3, 152.5, 138.7, 97.1, 52.1, 38.0, 31.9. ESI MS (*m*/*z*): M^++Na=301.01 (100%), 299.02 (98%); calcd M^++Na. 300.06. $C_8H_9BrN_2O_4$ (277.07): calcd C 34.68, H 3.27, N 10.11; found C 34.57, H 3.33, N 10.06. IR (KBr pellet): ν=3207, 3175 (N–H), 3084, 2993, 2949, 1898, 1734 (C=O), 1713 (C=O), 1676 (C=O), 1649 (C=O), 1620, 1495, 1441,1364, 1340, 1300, 1269, 1200 (C–O–C), 1140 (C–O), 1099, 1016 (C–Br) cm^{-1}.

*4.2.1.6. Methyl 3-(5-amino-2,4-dioxo-3,4-dihydropyrimidin-1 (2H)-yl)propanoate (**3d**).* Redish crystals, mp 136–137 °C (110 °C crystal phase transition). ^{1}H HMR: δ=11.31 (s, H-3, 1H), 6.80 (s, H-6, 1H), 4.13 (br s, NH_2, 2H), 3.81 (t, $^3J_1$=6.6 Hz, >NCH_2, 2H), 3.61 (s, OCH_3, 3H), 2.66 (t, $^3J_1$=6.6 Hz, CH_2CO, 2H). ^{13}C NMR: δ=171.4, 161.2, 149.0, 122.7, 120.4, 51.6, 43.8, 32.8. $C_8H_{11}N_3O_4$ (213.19): calcd C 45.07, H 5.20, N 19.71; found C 45.18, H 5.46, N 19.94. IR (KBr pellet): ν=3400 (NH_2), 3329, 3200 (N–H), 2957, 2791, 1736 (C=O), 1699 (C=O), 1641 (C=O), 1487, 1435, 1385, 1356, 1327, 1281, 1209 (C–O–C), 1178, 1144 (C–O), 1072, 1038 cm^{-1}.

*4.2.1.7. Methyl 3-(5-nitro-2,4-dioxo-3,4-dihydropyrimidin-1 (2H)-yl)propanoate (**3e**).* Pale yellow needles, mp 151–153 °C. ^{1}H HMR: δ=12.03 (s, H-3, 1H), 9.26 (s, H-6, 1H), 4.07 (t, $^3J_1$=6.6 Hz, >NCH_2, 2H), 3.61 (s, OCH_3, 3H), 2.78 (t, $^3J_1$=6.6 Hz, CH_2CO, 2H). ^{13}C NMR: δ=171.2, 155.0, 151.4, 149.3, 124.6, 51.6, 45.3, 31.9. $C_8H_9N_3O_6$ (243.17): calcd C 39.47, H 3.63, N 17.32; found C 39.51, H 3.73, N 17.28. IR (KBr pellet): ν=3180 (N–H), 2999 (CH_3), 1716 (C=O), 1699 (C=O), 1635 (C=O), 1533 (asym NO_2), 1470, 1408, 1366 (sym NO_2), 1311, 1252, 1207 (C–O–C), 1190, 1163 (C–O) cm^{-1}.

*4.2.1.8. Methyl 3-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1 (2H)-yl)propanoate (**3f**).* Colourless crystals, mp 149–150 °C. ^{1}H HMR: δ=11.80 (s, H-3, 1H), 8.06 (d, $^3J_{H,F}$=7.2 Hz, H-6, 1H), 3.86 (t, $^3J_1$=6.9 Hz, >NCH_2, 2H), 3.61 (s, OCH_3, 3H), 2.72 (t, $^3J_1$=6.9 Hz, CH_2CO, 2H). ^{13}C NMR: δ=171.3, 157.5 (d, $^2J_{C,F}$=25.5 Hz), 149.6, 139.4 (d, $^1J_{C,F}$=227.3 Hz), 130.6 (d, $^2J_{C,F}$=33.5 Hz), 51.6, 44.2, 32.3. $C_8H_9FN_2O_4$ (216.17): calcd C 44.45, H 4.20, N 12.96; found C 44.57, H 4.00, N 12.98. IR (KBr pellet): ν=3171 (N–H), 3078, 3049, 2968, 2841, 1739 (C=O), 1717 (C=O), 1663 (C=O), 1477, 1448, 1416, 1354, 1337, 1259 (C–F), 1215 (C–O–C), 1171 (C–O), 1130, 1065, 1038, 669 (C–F) cm^{-1}.

*4.2.1.9. Methyl 3-(5-fluoro-2,6-dioxo-2,3-dihydropyrimidin-1 (6H)-yl)propanoate (**4f**).* Colourless crystals, mp 114–115 °C. ^{1}H HMR: δ=11.15 (s, H-1, 1H), 8.45 (d, $^3J_{H,F}$=5.7 Hz, H-6, 1H), 4.01 (t, $^3J_1$=7.5 Hz, >NCH_2, 2H), 3.59 (s, OCH_3, 3H), 2.56 (t, $^3J_1$=7.5 Hz, CH_2CO, 2H). ^{13}C NMR: δ=171.1, 157.2 (d, $^2J_{C,F}$=25.0 Hz), 149.7, 139.4 (d, $^1J_{C,F}$=264.8 Hz), 125.1 (d, $^2J_{C,F}$=31.5 Hz), 51.6, 40.3, 31.5. $C_8H_9FN_2O_4$ (216.17): calcd C 44.45, H 4.20, N 12.96; found C 44.29, H 4.06, N 13.11. IR (KBr pellet): ν=3180 (N–H), 3086, 2963 (CH_3), 2930, 2853, 2716, 1739 (C=O), 1719 (C=O), 1705 (C=O), 1660 (C=O), 1508, 1450, 1383, 1356, 1256 (C–F), 1217 (C–O–C), 1161 (C–O), 1105, 1028, 667 (C–F) cm^{-1}.

*4.2.1.10. Methyl 3-(5-chloro-2,4-dioxo-3,4-dihydropyrimidin-1 (2H)-yl)propanoate (**3g**).* Colourless crystals, mp 148–149 °C. ^{1}H HMR: δ=11.82 (s, H-3, 1H), 8.13 (s, H-6, 1H), 3.90 (t, $^3J_1$=6.9 Hz, >NCH_2, 2H), 3.61 (s, OCH_3, 3H), 2.73 (t, $^3J_1$=6.9 Hz, CH_2CO, 2H). ^{13}C NMR: δ=171.2, 159.5, 143.5, 105.8, 51.6, 44.3, 32.2. $C_8H_9ClN_2O_4$ (232.62): calcd C 41.31, H 3.90, N 12.04; found C 41.43, H 3.95, N 12.14. IR (KBr pellet): ν=3020 (N–H), 2883 (CH_3), 2843, 1745 (C=O), 1714 (C=O), 1630 (C=O), 1464, 1435, 1396, 1352, 1333, 1286, 1217 (C–O–C), 1177 (C–O), 1078 (C–Cl), 1032 cm^{-1}.

*4.2.1.11. Methyl 3-(5-chloro-2,6-dioxo-2,3-dihydropyrimidin-1 (6H)-yl)propanoate (**4g**).* Colourless crystals, mp 125–125.5 °C. ^{1}H HMR: δ=11.62 (s, H-1, 1H), 7.93 (s, H-6, 1H), 4.04 (t, $^3J_1$=7.2 Hz, >NCH_2, 2H), 3.59 (s, OCH_3, 3H), 2.57 (t, $^3J_1$=7.2 Hz, CH_2CO, 2H). ^{13}C NMR: δ=171.8. 159.9, 150.9, 139.1, 106.4, 52.2, 37.5, 32.1. $C_8H_9ClN_2O_4$ (232.62): calcd C 41.31, H 3.90, N 12.04; found C 41.27, H 4.11, N 11.92. IR (KBr pellet): ν=3211 (N–H), 3175, 3090, 2993, 2951, 1741 (C=O), 1733 (C=O), 1666 (C=O), 1495, 1441, 1364, 1340, 1302, 1269, 1202, 1148, 1101 (C–Cl), 1030 cm^{-1}.

4.3. Michael-type addition of thymine (1a) to acrylonitrile (2b) in the kinetic studies

Post-reaction mixtures from the semi-kinetic studies (Table 1) were combined and separated using column chromatography and 5% MeOH/$CHCl_3$ (vol) as eluting system.

*4.3.1. 3-(5-Methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)propanenitrile (**3b**).* The adduct of thymine (**1a**) to acrylonitrile (**2b**) was obtained and possessed physicochemical and spectral data in total accordance with those published elsewhere.[25]

*4.3.2. 3-(5-Methyl-2,6-dioxo-2,3-dihydropyrimidin-1(6H)-yl)propanenitrile (**4b**).* Colourless crystals, mp 165–166 °C. ^{1}H HMR: δ=11.04 (d, $^3J_1$=5.4 Hz, H-1, 1H), 7.36 (dd, $^4J_1$=1.2 Hz, $^3J_1$=5.4 Hz, H-6, 1H), 4.03 (t, $^3J_1$=6.9 Hz, >NCH_2, 2H), 2.83 (t, $^3J_1$=6.9 Hz, CH_2, 2H), 1.79 (d, $^4J_1$=1.2 Hz, CH_3, 3H). ^{13}C NMR: δ=163.5, 151.0, 136.8, 118.4, 107.1, 35.3, 15.5, 12.3. $C_8H_9N_3O_2$ (179.18): calcd C 53.63, H 5.06, N 23.45; found C 53.49, H 5.11, N 23.72. IR (KBr pellet): ν=3020 (N–H), 2971 (CH_3), 2820, 2253 (CN), 1751 (C=O), 1656 (C=O), 1601, 1485, 1336, 1160, 1112, 1016 cm^{-1}.

4.4. Synthesis of acidic subunits

*4.4.1. 3-(5-Nitro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)propanoic acid (**8**).* 1-(2,4-Dinitrophenyl)-5-nitrouracil (**6**) (1 mmol) and DMF (2 mL) were added to a solution of β-alanine (**7**) (2 mmol) in water (1 mL). Subsequently TEA (2 mmol) was added and the reaction mixture was stirred for 24 h (TLC). Afterwards the next portion of TEA (1 mmol) was added and the reaction was continued for next 24 h. TLC revealed total consumption of limiting reactant. Volatiles were removed under reduced pressure using rotary evaporator and solid filtered off, washed with ethyl acetate (3×5 mL) followed by water (2×3 mL). The light yellow solid was recrystallised from water giving 0.18 g (78%) white needles. All the properties were in total accordance with references.[28] Mp 247–249 °C. $C_7H_7N_3O_6$ (229.15): calcd C 36.69, H 3.08, N 18.34; found C 36.70, H 3.02, N 18.51. IR (KBr pellet): ν=3207, 3099 (O–H), 2966, 2943, 2804, 1711 (C=O), 1652 (C=O), 1526 (asym NO_2), 1462, 1406, 1373, 1325 (sym NO_2), 1252, 1237, 1190, 1108, 1094, 1067 cm^{-1}.

*4.4.2. 3-(5-Nitro-2,6-dioxo-2,3-dihydropyrimidin-1(6H)-yl)propanoic acid (**10**).* To **9**[25] (0.25 mmol) was added HOKO (1 mL) and acetic anhydride (1 mL). The reaction was monitored using TLC. After 3 days additional portion of HOKO (0.5 mL) was added. In next 2 days the reaction was completed and the post-reaction mixture was poured on ice (6 g) and then, nearly clear solution was adjusted to pH=3 with solid $NaHCO_3$. The solution was extracted with ethyl acetate (4×8 mL). Organic layer was dried with anhydrous Na_2SO_4 and evaporated under reduced pressure giving 0.040 g (70%) white crystalline solid. Mp 213–215 °C. ^{1}H HMR: δ=12.28 (br s, H-3, OH, 2H), 8.92 (s, H-6, 1H), 3.99 (t, $^3J_1$=7.8 Hz, >NCH_2, 2H), 2.49 (t, $^3J_1$=7.8 Hz, CH_2CO, 2H). ^{13}C NMR: δ=172.0, 154.8, 149.9, 146.6, 124.9, 36.5, 31.4. $C_7H_7N_3O_6$ (229.15): calcd C 36.69, H 3.08, N 18.34; found C 36.57, H 3.27, N 18.19. IR (KBr pellet): ν=3222, 1753, 1736 (C=O), 1692 (C=O), 1510 (asym NO_2), 1492, 1372, 1329 (sym NO_2), 1261, 1190, 1170 cm^{-1}.

Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2010.08.059.

References and notes

1. Woods, D. D. *Br. J. Exp. Pathol.* **1940**, *21*, 74.
2. Fildes, P. *Lancet* **1940**, *1*, 955.
3. Kim, J. C.; Dong, E.-S.; Park, J. I.; Bae, S.-D.; Kim, S.-H. *Arch. Pharmacal Res.* **1994**, *7*, 480.
4. Wang, Z.; Rizzo, C. J. *Org. Lett.* **2000**, *2*, 227.
5. Dolman, N. P.; More, J. C. A.; Alt, A.; Knauss, J. L.; Troop, H. M.; Bleakman, D.; Collingridge, G. L.; Jane, D. E. *J. Med. Chem.* **2006**, *49*, 2579.
6. Aspland, S. E.; Ballatore, C.; Castillo, R.; Desharnais, J.; Eustaquio, T.; Goelet, P.; Guo, Z.; Li, Q.; Nelson, D.; Sun, C.; Castellino, A. J.; Newman, M. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5194.
7. Tjarks, W.; Tiwari, R.; Byun, Y.; Narayanasamy, S.; Barth, R. F. *Chem. Commun.* **2007**, 4978.
8. Gondela, A.; Walczak, K. *Carbohydr. Res.* **2005**, *340*, 1379.
9. Gondela, A.; Walczak, K. *Tetrahedron: Asymmetry* **2005**, *16*, 2107.
10. Nagase, T.; Seike, K.; Shiraishi, K.; Yamada, Y.; Ozaki, S. *Chem. Lett.* **1988**, 1381.
11. Jaime-Figueroa, S.; Zamilpa, A.; Guzmán, A.; Morgans, D. J., Jr. *Synth. Commun.* **2001**, *31*, 3739.
12. Ueda, T.; Fox, J. J. *J. Org. Chem.* **1964**, *29*, 1762.
13. Boncel, S.; Gondela, A.; Walczak, K. *Synthesis* **2010**, *10*, 1573.
14. Paegle, R. A.; Lidak, M. Y.; Shvachkin, Y. P. *Khim. Geterotsikl. Soedin.* **1966**, *2*, 316.
15. Cai, Y.; Wu, J. Y.; Wang, N.; Sun, X.-F.; Lin, X.-F. *Chin. Chem. Lett.* **2004**, *15*, 594.
16. Cai, Y.; Sun, X.-F.; Wang, N.; Lin, X.-F. *Synthesis* **2004**, 671.
17. Xu, J.-M.; Qian, C.; Liu, B.-K.; Wu, Q.; Lin, X.-F. *Tetrahedron* **2007**, *63*, 986.
18. Peshakova, L.; Kalcheva, V. *Z. Chem.* **1990**, *30*, 438.
19. Peshakova, L.; Kalcheva, V.; Madzhova, L. *Izv. Khim.* **1991**, *24*, 91.
20. Nováček, A.; Lissnerová, M. *Collect. Czech. Chem. Commun.* **1968**, *33*, 1003.
21. Nováček, A. *Collect. Czech. Chem. Commun.* **1971**, *36*, 1964.
22. Kimura, E.; Kitamura, H.; Koike, T.; Shiro, M. *J. Am. Chem. Soc.* **1997**, *119*, 10909.
23. Luyten, I.; Pankiewicz, K. W.; Watanabe, K. A.; Chattopadhyaya, J. *J. Org. Chem.* **1998**, *63*, 1033.
24. Crippa, S.; Di Gennaro, P.; Lucini, R.; Orlandi, M.; Rindone, B. *Gazz. Chim. Ital.* **1993**, *123*, 197.
25. Boncel, S.; Walczak, K. *Lett. Org. Chem.* **2006**, *3*, 534.
26. Boncel, S.; Osyda, D.; Walczak, K. *Beilstein J. Org. Chem.* **2007**, *3*, 40.
27. Cleaves, H. J., II. *Astrobiology* **2002**, *2*, 403.
28. Boncel, S.; Walczak, K. *Nucleosides, Nucleotides Nucleic Acids* **2009**, *28*, 103.
29. Boncel, S.; Mączka, M.; Koziol, K. K. K.; Motyka, R.; Walczak, K. Z. *Beilstein J. Org. Chem.* **2010**, *6*, 34.
30. Perrin, D. D. *Dissociation Constants of Organic Acids and Bases*; Butterworths: London, 1965; Supplement 1972.
31. Granitza, D.; Beyermann, M.; Wenschuh, H.; Haber, H.; Carpino, L. A.; Truranb, G. A.; Bienerta, M. *J. Chem. Soc., Chem. Commun.* **1995**, *21*, 2223.
32. McGrier, P. L.; Solntsev, K. M.; Miao, S.; Tolbert, L. M.; Miranda, O. R.; Rotello, V. M.; Bunz, U. H. F. *Chem.—Eur. J.* **2008**, *14*, 4503.
33. Mikstis, U. J.; Smolova, N. T.; Veveris, A. V. *Zh. Anal. Khim.* **1977**, *32*, 362.
34. Kolthoff, I. M.; Chantooni, M. K., Jr.; Smagowski, H. *Anal. Chem.* **1970**, *42*, 1662.
35. Rived, F.; Canals, I.; Bosch, E.; Rosés, M. *Anal. Chim. Acta* **2001**, *439*, 315.
36. Hauser, C. R.; Harris, T. M. *J. Am. Chem. Soc.* **1958**, *80*, 6360.