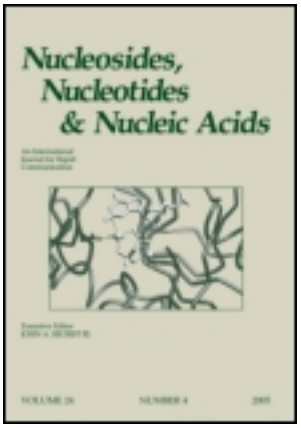

Nucleosides, Nucleotides and Nucleic Acids

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

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Version of record first published: 14 Feb 2009

To cite this article: Slawomir Boncel & Krzysztof Walczak (2009): Novel Acyclic Amide-Conjugated Nucleosides and Their Analogues, Nucleosides, Nucleotides and Nucleic Acids, 28:2, 103-117

To link to this article: http://dx.doi.org/10.1080/15257770902736467

Nucleosides, Nucleotides and Nucleic Acids, 28:103–117, 2009

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770902736467

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NOVEL ACYCLIC AMIDE-CONJUGATED NUCLEOSIDES AND THEIR ANALOGUES

Slawomir Boncel and Krzysztof Walczak

Department of Organic Chemistry, Biochemistry and Biotechnology, Silesian University of Technology, Gliwice, Poland

□ *An effective one-step synthesis of new amide-conjugated nucleosides and their analogues, in the presence of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) as the condensing agent, is presented. Substrate subunits carrying carboxylic group were obtained by acidic hydrolysis of Michael-type adducts of various 5-substituted uracil derivatives to methyl acrylate. Amine substrate was synthesized by reduction of 1-(2′-cyanoethyl)thymine with sodium borohydride in the presence of nickel (II) chloride as catalyst. Other applied amine substrates were 5′-amino-5′-deoxythymidine and 5-aminouracil.*

Keywords Acyclic nucleosides; aminolysis; 5-substituted uracil derivatives; DMT-MM; amide bond formation

INTRODUCTION

Systems of amide-conjugated units of diversely functionalized acyclic nucleosides may be considered as potential medicines, for instance in combination anti-HIV therapy due to synergic effect, *e.g.* ddC-MKC analogue conjugated nucleoside (Figure 1, **I**).[1] Furthermore, 5′-acetamido-5′-deoxythymidine derivatives (**II**) exhibit significant activity against the P388 mouse leukaemia line.[2] 5′-Amino-5-fluoro-2′,5′-dideoxy-β-uridine derivatives (**III**)—with appropriate spacers containing the amide bonds—exhibit cytotoxicity of considerable decrease comparing to unsubstituted nucleosides.[3] Amide-linked heterodimer synthons consisting of acyclic nucleoside units and 5′-amino-5′-deoxythymidine are PNA/DNA chimeras (**IV**).[4] 6′-Amide-bond based aromatic and non-

Received 8 October 2008; accepted 10 December 2008.
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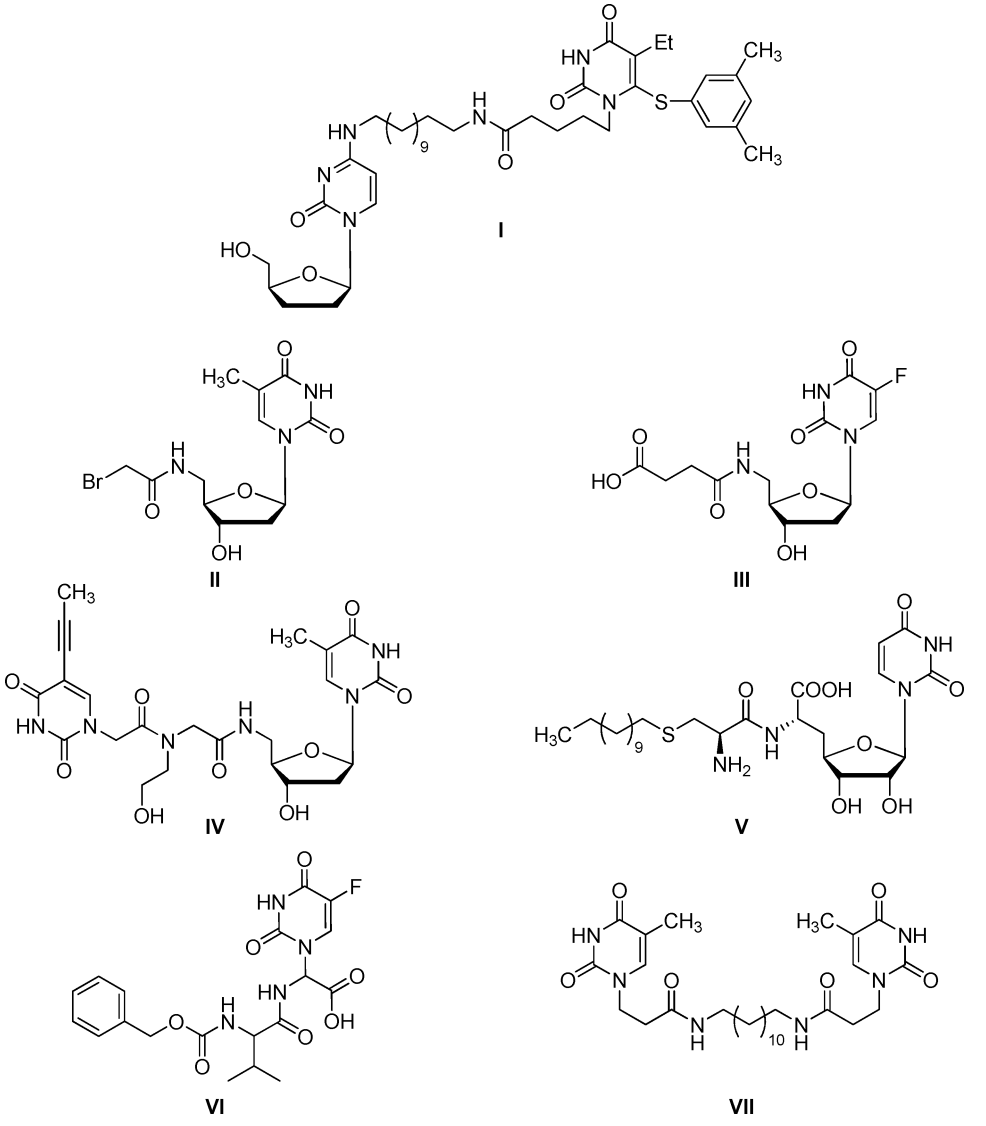


FIGURE 1 Various amide-conjugated analogues of nucleosides.

aromatic nikkomycin conjugates (**V**) greatly inhibit chitin synthases.[5] Polyamide-based minor-groove binders were designed as analogues of distamycin, netropsin, and the oligopeptide analogues.[6] Moreover, amide bonds are also exploited in the construction of diverse anticancer thermosensitive polymer-drug conjugates; a precursor bearing active moiety is represented by structure **VI**.[7] Separately from medicinal application of the title compounds, a very intriguing amide-conjugated quasi-nucleosides were reported (**VII**).[8] These structures, formally being 1,ω-bolaamphiphilic,

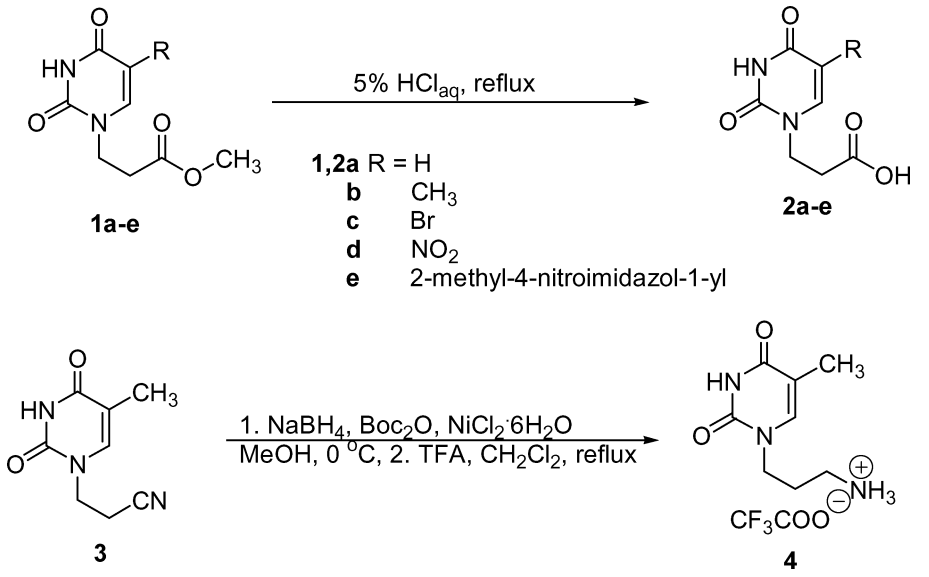
may form extraordinary topologies like supramolecular fibres (e.g., double-helical ropes), microtubes or microcrystals. The nature of the observed self-organization is based upon hydrogen bonds and π-π interactions, similarly to multiple interactions elucidating quaternary structure of nucleic acids, proteins and nucleic acid-protein complexes. Currently, a number of methods of amide bond formation in nucleoside derivatives is known and exploited. In a typical procedure, nucleoside containing amine group is treated with appropriate acyl halide or other acyl donors containing easily replaceable group under basic conditions.[9,10] Acylation using carboxylic acids,[11] esters,[12] or acyl chlorides are common.[13] The acylation reaction in the presence of magnesium chloride[14,15] or 9-borabicyclo[3.3.1]nonane (9-BBN) give products in excellent yields. [16] When acids are used as acylating agents the condensation reaction have to be supported in many cases by the presence of condensing agent, *e.g*. DCC.[17] The formed as a by-product 1,3-dicyclohexylurea is poorly soluble in most of organic solvents, but the removing of its traces may be troublesome. The formation of the amide bond in the reaction of appropriate carboxylic derivative with amine in the presence of 2-chloro-1-methylpyridinium iodide and triethylamine[18] or by treatment of pentachlorophenyl carboxylic esters with the corresponding amines[8] were also reported.

In this article, we report effortless and efficient method for novel conjugated nucleosides synthesis by coupling of two subunits bearing carboxylic and amino group respectively in the presence of DMT-MM as a condensing agent.

RESULTS AND DISCUSSIONS

The units containing carboxylic group (**2a–e**) were synthesized by acidic hydrolysis of Michael adducts of 5-substituted uracil derivatives to methyl acrylate (**1a–e**) (Scheme 1).[19] In turn, mainly utilized subunit carrying primary amine group, namely 1-(3′-aminopropyl)thymine trifluoroacetate **4** was synthesized by reduction of Michael adduct (**3**) of thymine to acrylonitrile with sodium borohydride, in the presence of nickel chloride (co-catalyst) and di-*tert*-butyl carbonate as a "trapping" agent in methanol.[20]

In preliminary experiments for the synthesis of amide-conjugated nucleosides the simple aminolysis approach was applied. Unfortunately, these experiments revealed several synthetic problems. First, aminolysis of methyl esters (instead of carboxylic acids) with primary aliphatic amines required significant (400% and higher) excess of nucleophile (methylamine and 2-aminoethanol). 5-Aminouracil appeared as entirely unreactive towards ester group under investigated conditions (Scheme 2). Moreover, prolonged time of the reaction course under basic conditions

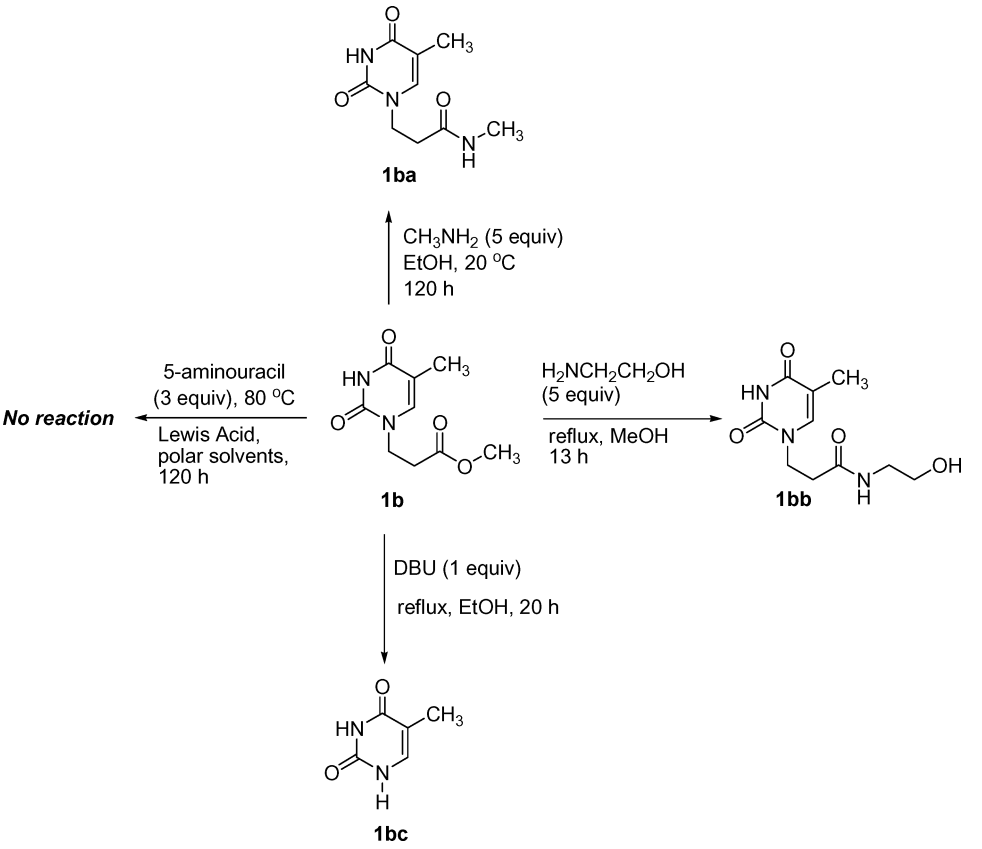


SCHEME 1 Synthesis of subunits for amide-conjugated nucleosides.

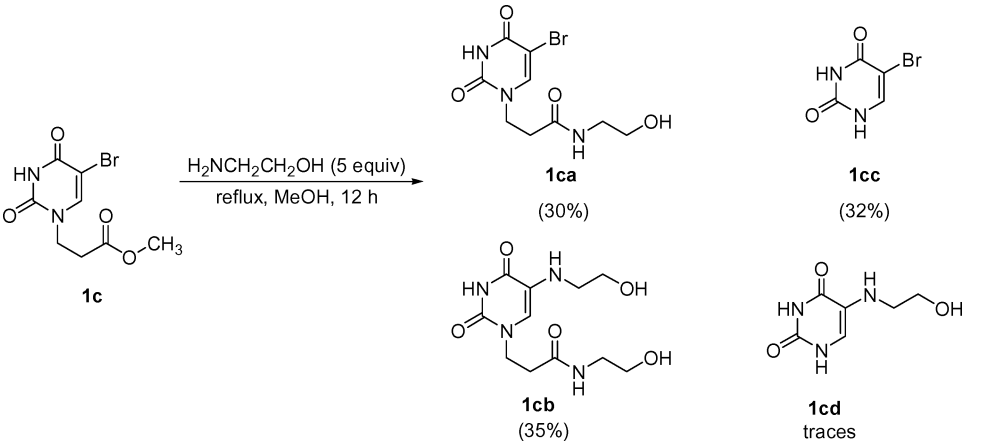
supported the formation of products of retro-Michael reaction. When **1b** was refluxed in the presence of DBU from post reaction mixture thymine **1bc** (Michael donor) was isolated. Furthermore, 5-bromo- and 5-nitrouracil derivatives under the above-mentioned conditions undergo undesired reactions. 5-Bromouracil derivative **1c** treated with *N*-centered nucleophiles under depicted conditions (Scheme 3) gave, apart from the desired 3-(5-bromo-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-*N*-(2-hydroxyethyl) propanamide **1ca**, 5-bromouracil **1cc** (formed via retro-Michael reaction) and product of subsequent substitution of bromine atom with 2-aminoethanol, namely *N*-(2-hydroxyethyl)-3-[5-(2-hydroxyethylamino)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]propanamide **1cb**. Our previous experiments also revealed that direct Michael-type addition of uracils to acrylic acid procured work-up problems in the purification of product and loss of selectivity. Thus, mainly due to the competitive polymerization reaction, an additional stage of hydrolysis had to be implicated.

As the consequence, this synthetic pathway had to be relinquished as completely ineffective in the case of the synthesis of intentional conjugated nucleosides.

Considering the optimal conditions of amide-coupling reaction led us to astonishingly convenient non-carbodiimide one-step condensing agent, namely 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride—DMT-MM.[21] Its versatility of usage owes to non-toxicity, non-irritation, working under mild conditions, capability of recycling, and practically total removal of products its dissolution from postreaction mixture:

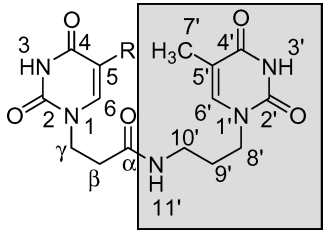


SCHEME 2 Aminolysis of 3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1-(2*H*)-yl) propanoate with selected *N*-nucleophiles.



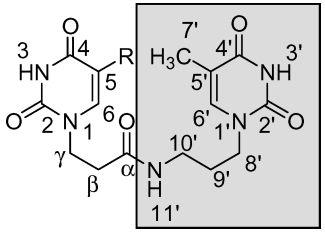
SCHEME 3 Aminolysis of 3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1-(2*H*)-yl)propanoate with 2-aminoethanol.

TABLE 1 ^{1}H NMR spectroscopic data of amide-conjugated nucleosides

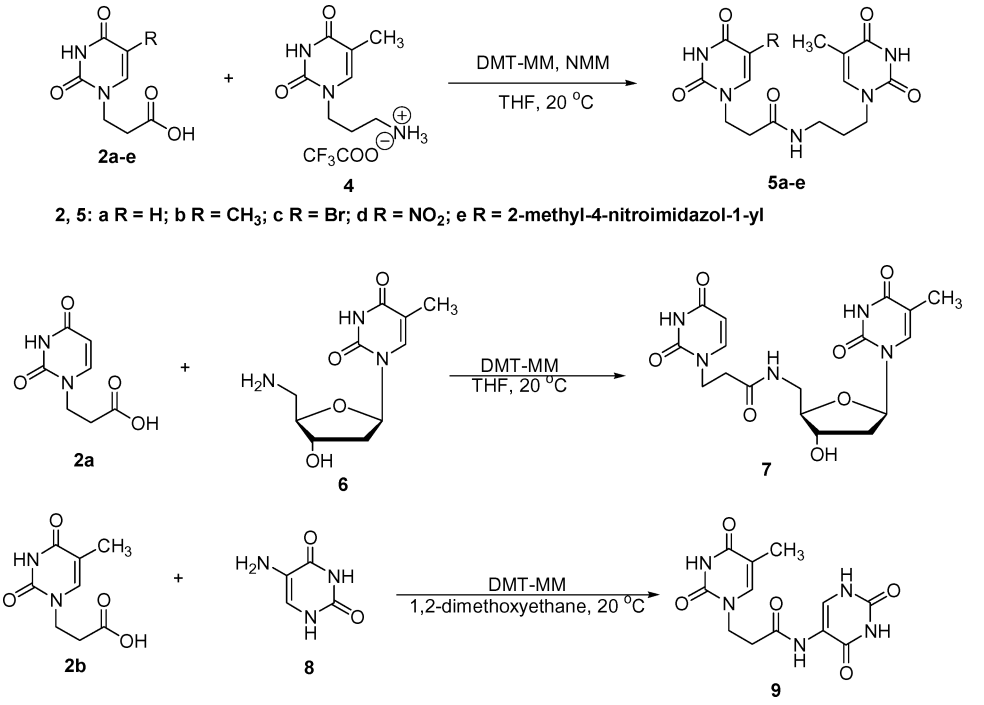


	H-3	H-6	CH_2 (γ)	CH_2 (β)	R	N-H (11′)	CH_2 (10′)	CH_2 (9′)	CH_2 (8′)	H-6′	CH_3 (7′)	H-3′
H (**5a**)	11.20 br s	7.53 d, 7.8 Hz	3.85 t, 6.3 Hz	2.44 t, 6.3 Hz	H-5 5.49 d, 7.8 Hz	8.00 br s	3.03 quartet, 6.0 Hz	1.67 quintet, 6.9 Hz	3.59 t, 6.9 Hz	7.48 s	1.75 s	11.20 br s
Me (**5b**)	11.22 br s	7.50 s	3.82 t, 6.6 Hz	2.43 t, 6.6 Hz	Me 1.75 s	8.02 t, 5.4 Hz	3.04 quartet, 6.3 Hz	1.69 quintet, 6.9 Hz	3.58 t, 6.9 Hz	7.40 s	1.71 s	11.22 br s
Br (**5c**)	11.76 br s	8.06 s	3.88 t, 6.3 Hz	2.46 t, 6.3 Hz	Br	8.04 br s	3.03 quartet, 6.3 Hz	1.67 quintet, 6.9 Hz	3.58 t, 6.9 Hz	7.49 s	1.74 s	11.21 br s
NO_2 (**5d**)	12.08 br s	9.18 s	4.07 t, 6.3 Hz	2.53 t, 6.3 Hz	NO_2	8.09 br s	3.03 quartet, 6.3 Hz	1.66 quintet, 6.9 Hz	3.58 t, 6.9 Hz	7.50 s	1.73 d, 0.9 Hz	11.21 br s
2-Methyl-4-nitroimidazol-1-yl (**5e**)	11.99 br s	8.31 s	3.94 t, 6.3 Hz	2.55 t, 6.3 Hz	imidazole H 8.26 s Me 2.25 s	8.09 t, 5.4 Hz	3.05 quartet, 6.0 Hz	1.69 quintet, 6.9 Hz	3.59 t, 6.9 Hz	7.51 s	1.74 s	11.22 br s

TABLE 2 ^{13}C NMR spectroscopic data of amide-conjugated nucleosides



	C-4	C-2	C-5	C-6	γ	β	α	10′	9′	8′	C-6′	C-5′	C-2′	C-4′	CH_3	Other
H (**5a**)	164.3	150.8	100.5	146.1	45.3	34.2	169.5	35.7	28.5	44.7	141.4	108.4	150.7	163.7	11.9	11.9 Me
Me (**5b**)	164.3	150.8	108.4	141.9	45.2	34.4	169.6	35.7	28.6	44.6	141.4	108.0	150.7	164.3	11.9	
Br (**5c**)	159.7	150.2	94.1	145.9	45.2	34.1	169.6	35.7	28.6	45.1	141.5	108.4	150.9	164.3	12.0	-
NO_2 (**5d**)	155.0	151.3	124.4	141.5	46.1	33.6	169.5	35.8	28.6	45.3	141.5	108.4	150.9	164.3	11.9	-
2-Methyl-4-nitroimidazol-1-yl (**5e**)	159.8	150.0	123.9	145.7	45.8	33.8	169.4	35.8	28.6	45.3	141.4	108.4	150.9	164.3	11.9	12.5 Me 109.9 C3 145.9 C2 146.5 C-NO_2



SCHEME 4 Synthesis of amide-conjugated nucleosides containing short linkage or a sugar constituent.

coupling by-product—4,6-dimethoxy-1*H*-1,3,5-triazin-2-one (DMT-OH) and product of DMT-MM decomposition 2,4-dimethoxy-6-morpholin-4-yl-1,3,5-triazine (DMTM). Hence, DMT-MM and its analogues are at present of high synthetic importance in the formation of both amide[21] (e.g., synthesis of peptides) and ester bonds.[22] The target compounds were synthesized via condensation of carboxylic and amine groups deriving from corresponding stricte- or quasi-nucleoside units (Scheme 4). Under typical applied conditions, the coupling reactions were performed at ambient temperature in THF or 1,2-dimethoxyethane with usage of 10% molar excess of amine substrate salt or pure amine and 10% molar excess of DMT-MM in relation to acidic substrate (Scheme 4). Total decay of limiting reagents was achieved in 1.5–24 hours. In a prevailing number of cases, products were precipitating during the course of the reactions and required only single crystallization. In the two cases (**5b**, **5e**), isolation on chromatographic column was applied. The products, conjugated nucleosides (**5a–5e**) were isolated in moderate to very good yields, except 5-nitrouracil derivatives. This fact may be colligated with the known susceptibility of the 5-nitrouracil ring to nucleophilic attack initiated by amino group, and in consequence, ring opening reactions.[23] To extend application of the explored condensation method, 5′-amino-5′-deoxythymidine **6** was applied as an amino substrate. The latter was synthesized in Mitsunobu reaction of thymidine with phthalimide, followed

by cleavage of phthalimide moiety under basic conditions.[24] The expected condensation product (**7**) was obtained in satisfactory yield. It should be mentioned that we did not observe products of competitive acylation neither on 3′-hydroxyl group nor on nitrogen N-3 of thymidine. On the other hand, the illustration of significant effectiveness of DMT-MM as the condensing agent was reaction between **2b** and 5-aminouracil **8**. Although all of the substrates were practically insoluble in most of commonly used solvents, product of coupling was isolated, after total time of reaction of 120 hours, in 52% yield. Spectroscopic data of the amide-conjugated nucleosides built from the above mentioned subunits are collected in Tables 1 (^{1}H NMR) and 2 (^{13}C NMR).

CONCLUSION

In summary, we have synthesized novel amide-conjugated nucleosides and their acyclic analogues with usage of convenient condensing agent DMT-MM. It was demonstrated that application of DMT-MM enables direct amide formation on various compounds bearing amine groups, *e.g.* in 5-aminouracil or 5′-amino-5′-deoxythymidine.

EXPERIMENTAL

General

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; δ values are in parts per million relative to tetramethylsilane as an internal standard. Elemental analyses were obtained using a Perkin-Elmer 240C (Poland) apparatus. All used reagents were purchased from Aldrich (Germany). TLC $60F_{254}$ plates and silica gel 60 (0.040–0.063 mm) were purchased from Merck (Poland). Melting points were measured at Betius apparatus and are uncorrected.

5-Substituted 3- (3,4-dihydro- 2,4-dioxopyrimidin-1 (2*H*)-yl) propanoic acids (2a–e). General procedure. Michael-type adduct of uracil to methyl acrylate (**1a–e**) (1 mmol) was refluxed in 5% HCl_{aq} (8 ml). During first 5–15 minutes of refluxing solid phase disappeared. During the next 15 minutes of boiling neo-crystalline material appeared. The mixture was then cooled to room temperature and kept for 16 hours in refrigerator. Afterwards crystals were filtered off, rinsed with ice water to neutral pH and dried on air.

3-(3,4-Dihydro-2,4-dioxopyrimidin-1(2*H*)-yl)propanoic acid (2a). Colorless needles; yield 62%; m.p. 176–179°C, lit.[25] 183–185°C; ^{1}H NMR: δ: 2.62 (t, 2H, CH_2, $J = 6.3$ Hz); 3.86 (t, 2H, $>NCH_2$, $J = 6.3$ Hz); 5.53 (d, 1H, H5, $J = 7.5$ Hz); 7.63 (d, 1H, H6, $J = 7.5$ Hz); 11.25 (br s, 1H, H3); 12.42 (br s, 1H, COOH); ^{13}C NMR: δ: 32.8; 44.22; 100.6; 146.2; 150.9; 163.8; 172.4.

3- (3,4- Dihydro-5- methyl-2,4- dioxopyrimidin-1 (2*H*)- yl) propanoic acid (2b). Colorless needles; yield 82%; m.p. 172–174°C, lit.[26] 174–175 °C; ^{1}H NMR: δ: 1.73 (s, 3H, Me); 2.59 (t, 2H, CH_2, $J = 6.9$ Hz); 3.81 (t, 2H, >NCH_2, $J = 6.9$ Hz); 7.49 (d, 1H, H6, $J = 0.9$ Hz); 11.23 (br s, 1H, H3); 12.41 (br s, 1H, COOH); ^{13}C NMR: δ: 12.0; 32.9; 44.0; 108.2; 141.9; 150.8; 164.4; 172.4.

3- (3,4-Dihydro- 5-bromo- 2,4-dioxopyrimidin-1 (2*H*) -yl) propanoic acid (2c). Colorless needles; yield 98%; m.p. 248–249°C, lit.[27] 250°C; ^{1}H NMR: δ: 2.63 (t, 2H, CH_2, $J = 6.9$ Hz); 3.87 (t, 2H, >NCH_2, $J = 6.9$ Hz); 8.17 (s, 1H, H6); 11.77 (br s, 1H, H3); 12.46 (br s, 1H, COOH); ^{13}C NMR: δ: 32.6; 44.4; 94.2; 145.9; 150.2; 159.7; 172.3.

3-(3,4-Dihydro-5-nitro-2,4-dioxopyrimidin-1(2*H*)-yl)propanoic acid (2d). Colorless needles; yield 96%; m.p. 247–249°C, lit.[28] 261–262 °C; ^{1}H NMR: δ: 2.69 (t, 2H, CH_2, $J = 6.6$ Hz); 4.04 (t, 2H, >NCH_2, $J = 6.6$ Hz); 9.25 (s, 1H, H6); 12.03 (br s, 1H, H3); 12.52 (br s, 1H, COOH); ^{13}C NMR: δ: 32.3; 45.5; 124.5; 149.3; 151.4; 155.1; 172.3.

3- (3,4-Dihydro- 5-(2-methyl- 4-nitroimidazol-1-yl)-2,4-dioxopyrimidin- 1 (2*H*) -yl)propanoic acid (2e). Light yellow needles; yield 65%; m.p. 240–241°C; ^{1}H NMR: δ: 2.27 (s, 3H, Me); 2.71 (t, 2H, CH_2, $J = 6.6$ Hz); 3.93 (t, 2H, >NCH_2, $J = 6.6$ Hz); 8.28 (s, 1H, *H*-imidazole); 8.36 (s, 1H, H6); 11.99 (br s, 1H, H3); 12.44 (br s, 1H, COOH); ^{13}C NMR: δ: 12.6; 32.4; 44.8; 110.0; 124.0; 145.8; 145.9; 146.6; 150.9; 159.8; 172.4. Analysis calcd. for $C_{11}H_{15}N_5O_6$ (309.24): C42.72, H3.59, N22.65. Found: C42.61. H3.53, N22.55. ESI MS: 2M+Na = 641.11; Calcd. 2M+Na = 641.47.

1-(3-Amino-propyl)-5-methyl-1*H*-pyrimidine-2,4-dione trifluoroacetate (4). A. Preparation of *t*-Butyl 3-(3,4-dihydro-5-methyl-2,4-dioxopyrimidin-1(2*H*)-yl)propylcarbamate (4-*Boc*-protected)

To a solution of uracil derivative **3** (10 mmol) in methanol (50 ml), cooled in an ice bath, nickel (II) chloride hydrate ($NiCl_2{\cdot}6H_2O$) (0.50 mmol) and di-*tert*-butyl carbonate (10 mmol) were added subsequently. After 10 minutes of vigorous stirring the suspension turned light green and $NaBH_4$ (70 mmol) was added in small portions while intensive stirring (0.5 hours). After addition of first portion, the mixture turned dark black and evolution of hydrogen appeared. The internal pressure was regulated by release of the stopper. After addition of $NaBH_4$, the ice bath was allowed to warm to ambient temperature (2.5 hours) and the reaction mixture turned transparent and light gray. TLC analysis (MeOH/$CHCl_3$ 1:9 vol./vol.) indicated total consumption of nitrile (**3**). The excess of $NaBH_4$ was removed by addition of ethyl acetate. The volatiles were removed under reduced pressure and product was isolated by column chromatography using a mixture MeOH/$CHCl_3$ (1:9 vol./vol.).

White crystals; 96%; m.p. 96–97 °C; ^{1}H NMR δ: 1.38 (s, 9H, $C(CH_3)_3$); 1.67 (t, 2H, $CH_2CH_2CH_2$, $J = 6.9$ Hz); 1.75 (d, 3H, CH_3 thymine, $J = 0.6$ Hz); 2.92 (q, 2H, CH_2NH, $J = 6$ Hz); 3.61 (t, 2H, >NCH_2, $J = 7.2$ Hz); 6.87 (br. t, 1H, N*H*-Boc, $J = 5.4$ Hz); 7.53 (d, 1H, *H*6, $J = 1.2$ Hz);

11.23 (br. s, 1H, N3-*H*). ^{13}C NMR δ: 11.96; 28.25; 28.90; 37.10; 45.25; 77.63; 108.41; 141.58; 150.89; 155.57; 164.32. MS *m/z* (relative intensity) 227 (17), 210 (18), 183 (25), 167 (32), 166 (M^+– *t*-BuOCONH, 100), 154 (83), 153 (58), 140 (67), 127 (24), 126 (60), 110 (51), 96 (36), 79 (25). Analysis calcd for $C_{13}H_{21}N_3O_4$ (283.33): C55.11, H7.47, N14.83. Found: C54.73, H7.20, N14.53.

B. Deprotection

Total amount of pure *N*-Boc (9.6 mmol) protected amine was refluxed in CH_2Cl_2 (15 ml) for 6 hours in the presence TFA (38.4 mmol). Solvent was removed under diminished pressure and colorless crystals of amine trifluoroacetate were dried in a vacuum desiccator, yield 98%. Colorless crystals; ^{1}H NMR: δ: 1.77 (s, 1H, Me); 1.89 (quin., 2H, $CH_2CH_2CH_2$, $J = 7.5$ Hz); 2.82 (tt, 2H, $CH_2NH_3^+$, $J = 6.6$ Hz, $J = 7.5$ Hz); 3.72 (t, 2H, $>NCH_2$, $J = 6.6$ Hz); 7.55 (s, 1H, H6); 7.89 (br s, 3H, NH_3^+); 11.30 (s, 1H, H3); ^{13}C NMR δ: 12.0; 26.9; 36.4; 44.6; 109.0; 116.0 (q, CF_3, $J_{C\text{-}F} = 292$ Hz); 141.3; 151.2; 158.7 (q, CF_3-CO, $J_{C\text{-}F} = 35$ Hz); 164.4.

Aminolysis of methyl 5-substituted 3-(3,4-dihydro-2,4-dioxopyrimidin-1(2*H*)-yl)propionates. Michael-type adducts of uracils to methyl acrylate (**1b**, **1c**) (1 mmol) were dissolved in methanol (or ethanol in the case of **1ba**) (8 ml). After dissolution, 2-aminoethanol (5 mmol) was added. The progress of reaction was monitored by TLC. For the precise conditions see Scheme 2. Products were isolated by column chromatography using 10% MeOH/$CHCl_3$ (v/v).

***N*-methyl-3- (5-methyl-2,4-dioxo -3,4-dihydropyrimidin- 1 (2*H*)-yl) propanamide (1ba).** White crystals, yield 74%; m.p. 172–173°C; ^{1}H NMR: δ: 1.73 (s, 3H, Me thymine); 2.42 (t, 2H, CH_2CO, $J = 6.6$ Hz); 2.56 (d, 3H, NH-*Me*, $J = 4.5$ Hz); 3.81 (t, 2H, $>CH_2$, $J = 6.6$ Hz); 7.41 (s, 1H, H6); 7.89 (d, 1H, CON*H*Me, $J = 4.5$ Hz); 11.21 (br s, 1H, H3); ^{13}C NMR δ: 12.0; 25.4; 34.2; 44.5; 108.0; 141.9; 150.7; 164.3; 169.9. Analysis calcd. for $C_9H_{13}N_3O_4$ (211.22): C51.18, H6.20, N19.89. Found: C50.95, H6.01, N19.65.

3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-*N*-(2-hydroxyethyl)-propanamide (1bb). White crystals, yield 91%; m.p. 187–189°C; ^{1}H NMR: δ: 1.73 (s, 3H, Me thymine); 2.44 (t, 2H, CH_2CONH, $J = 6.6$ Hz); 3.10 (q, 2H, CH_2OH, $J = 6.0$ Hz); 3.36 (q, 2H, $NHCH_2CH_2$, $J = 6.0$ Hz); 3.81 (t, 2H, $>NCH_2$, $J = 6.6$ Hz); 4.65 (t, 1H, OH, $J = 5.4$ Hz); 7.41 (s, 1H, H6); 7.99 (t, 1H, CON*H*, $J = 5.4$ Hz); 11.21 (br s, 1H, H3); ^{13}C NMR: δ: 11.9; 34.3; 41.6; 44.5; 59.8; 108.0; 141.9; 150.8; 164.3; 169.7. Analysis calcd. for $C_{10}H_{15}N_3O_4$ (241.25): C49.79, H6.27, N17.42. Found: C49.55, H6.07, N17.25.

3-(5-bromo-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-*N*-(2-hydroxyethyl)-propanamide (1ca). White crystals; yield 30%; m.p.194–195°C; ^{1}H NMR: δ: 2.47 (t, 2H, CH_2CO, $J = 6.6$ Hz); 3.10 (q, 2H, CH_2OH, $J = 6.0$ Hz); 3.36 (q, 2H, CH_2CH_2OH, $J = 6.0$ Hz); 3.88

(t, 2H, >NCH_2, J = 6.6 Hz); 4.66 (t, 1H, OH, J = 5.4 Hz); 8.00 (t, 1H, CONH, J = 5.4 Hz); 8.07 (s, 1H, H6); 11.75 (s, 1H, H3); ^{13}C NMR: δ: 34.0; 41.4; 45.1; 59.8; 94.0; 145.9; 150.2; 159.7; 169.6. Analysis calcd. for $C_9H_{12}BrN_3O_4$ (306.12): C35.31, H3.95, N13.73. Found: C34.96, H3.67, N13.45.

***N*-(2-hydroxyethyl)-3-[5-(2-hydroxyethylamino)-2,4-dioxo-3,4-dihydro (2*H*)pyrimidin-1-yl]propanamide (1cb).** White crystals; yield 35%; semi-solid compound; ^{1}H NMR: δ: 2.43 (t, 2H, CH_2CO, J = 6.9 Hz); 2.84 (br q, 2H, U-NHCH_2CH_2OH, J = 5.4 Hz); 3.10 (q, 2H, CH_2OH, J = 6.0 Hz); 3.36 (q, 2H, CONHCH_2, J = 6.0 Hz); 3.56 (q, 2H, U-NHCH_2, J = 5.4 Hz); 3.81 (t, 2H, >NCH_2, J = 6.9 Hz); 4.30 (br t, 1H, U-NHCH_2, J = 5.4 Hz); 4.67 (t, 1H, OH, J = 5.4 Hz); 4.78 (t, 1H, OH, J = 5.4 Hz); 6.56 (s, 1H, H6); 8.00 (t, 1H, CONHCH_2, J = 6.0 Hz); 11.34 (s, 1H, H3); ^{13}C NMR: δ: 34.5; 41.5; 44.4; 46.0; 58.9; 59.8; 117.0; 124.3; 148.5; 160.8; 169.7. Analysis calcd. for $C_{11}H_{18}N_4O_5$ (286.29): C46.15, H6.34, N19.57. Found: C46.06, H6.17, N19.25.

Conjugated Nucleosides: 3-(3,4-Dihydro-2,4-dioxopyrimidin-1 (2*H*)-yl)-*N*-(3-(3,4-dihydro-5-methyl-2,4-dioxopyrimidin-1(2*H*)-yl)propyl)propanamides (5a–e). General Procedure

To a suspension of 3-(3,4-dihydro-2,4-dioxopyrimidin-1(2*H*)-yl) propanoic acid **2** (1 mmol) in THF (5 ml) was added dropwise *N*-methylmorpholine (1 mmol), then 1-(3-aminopropyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione trifluoroacetate **4** (1.1 mmol) and after 10 minutes of stirring DMT-MM (1.1 mmol) were added. The progress of reaction was monitored by TLC analysis (20% vol. MeOH/$CHCl_3$). After 1.5–24 hours the white solid was filtered off and washed with cold methanol. The solvent from the filtrate was evaporated under diminished pressure and the residue was purified on column chromatography (20% vol. MeOH/$CHCl_3$). Fractions containing product after evaporation were combined with previously obtained solid and recrystallized from ethanol/water.

3-(3,4-Dihydro-2,4-dioxopyrimidin-1(2*H*)-yl)-*N*-(3-(3,4-dihydro-5-methyl-2,4-dioxopyrimidin-1(2*H*)-yl)propyl)propanamide (5a). White crystals; yield 85%; m.p. 223–225°C. Analalysis calcd. for $C_{15}H_{19}N_5O_5$ (349.34): C51.57, H5.48, N20.05. Found: C51.31, H5.18. N19.69.

3-(3,4-Dihydro-5-methyl-2,4-dioxopyrimidin-1(2*H*)-yl)-*N*-(3-(3,4-dihydro-5-methyl-2,4-dioxopyrimidin-1(2*H*)-yl)propyl)propanamide (5b). White crystals; yield 82%; m.p. 196–198°C. Analysis calcd. for $C_{16}H_{21}N_5O_5$ (363.37): C52.89, H5.83, N19.27. Found: C53.01, H5.80, N19.02.

3-(5-Bromo-3,4-dihydro-2,4-dioxopyrimidin-1(2*H*)-yl)-*N*-(3-(3,4-dihydro-5-methyl-2,4-dioxopyrimidin-1(2*H*)-yl)propyl)propanamide (5c). White crystals; yield 71%; m.p. 273–275°C. Analalysis calcd. for $C_{15}H_{18}BrN_5O_5$

(428.24): C42,07, H4.24, N16,35. Found: C41.67, H4.11, N15.98. ESI MS (m/z): M+Na = 450.05, 452.04, calcd. M+Na 451.23.

3-(3,4-Dihydro-5-nitro-2,4-dioxopyrimidin-1(2*H*)-yl)-*N*-(3-(3,4-dihydro-5-methyl-2,4-dioxopyrimidin-1(2*H*)-yl)propyl)propanamide (5d). Light yellow crystals; yield 30%; m.p. 240–241°C; Analysis calcd. for $C_{15}H_{18}N_6O_7$ (394.34): C45.69, H4.60, N21.31. Found: C45.52, H4.23, N21.22.

3-(3,4-Dihydro-5-(2-methyl-4-nitroimidazol-1-yl)-2,4-dioxopyrimidin-1(2*H*)-yl)-*N*-(3-(3,4-dihydro-5-methyl-2,4-dioxopyrimidin-1(2*H*)-yl)propyl)propanamide (5e). White crystals; yield 66%; Analysis calcd. for $C_{19}H_{22}N_8O_7$ (474.43): C48.10, H4.67, N23.62. Found: C48.22, H4.79, N23.29.

3-(2,4-Dioxo-3,4-dihydro-pyrimidin-1(2*H*)-yl)-*N*-[(2*R*,3*S*,5*R*)-3-hydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydro-pyrimidin-1(2*H*)-yl)-tetrahydro-furan-2-ylmethyl]-propanamide (7). To a suspension of 3-(3,4-dihydro-2,4-dioxopyrimidin-1(2*H*)-yl)propanoic acid 2a (1 mmol) in THF (5 ml) 5′-amino-5′-deoxythymidine 6 (1.1 mmol) and after 10 minutes of stirring DMT-MM (1.1 mmol) were added. The progress of reaction was monitored by TLC analysis (20% vol. MeOH/$CHCl_3$). After 24 hours the white solid was filtered off and washed with cold ethanol. The solvent from the filtrate was evaporated under diminished pressure and the residue was purified on column chromatography (20% vol. MeOH/$CHCl_3$). Fractions containing product after evaporation were combined with previously obtained solid and recrystallized from water. White crystals; yield 64%; m.p. 194–196 °C; ^{1}H NMR δ: 1.81 (s, 1H, Me); 1.91–2.16 (m, 2H, H′2); 2.49 (t, 2H, CH_2, J = 6.3 Hz); 3.18–3.41 (m, 2H, H′5); 3.71 (br s, 1H, H′4); 3.86 (t, 2H, >NCH_2, J = 6.3 Hz); 4.11 (br s, 1H, H′3); 5.30 (d, 1H, C′3-OH, J = 4.2 Hz); 5.47 (d, 1H, H5-uracil, 7.5 Hz); 6.13 (t, 1H, H′1, J = 6.9 Hz); 7.47 (s, 1H, H6-thymine); 7.52 (d, 1H, H6-uracil, 7.5 Hz); 8.18 (br s, 1H, CONH); 11.23 (br s, 1H, H3-thymine); 11.30 (br s, 1H, H3-uracil); ^{13}C NMR δ: 1.81 12.1; 34.1; 38.4; 41.0; 44.7; 71.2; 83.9; 84.9; 100.5; 109.7; 136.2; 146.1; 150.5; 150.8; 163.7; 169.9. Analysis calcd. for $C_{17}H_{21}N_5O_7$ (407.38): C50.12, H5.20, N17.19. Found: C50.30, H5.02, N6.92.

***N*-(2,4-Dioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl)-3-(5-methyl-2,4-dioxo-3,4-dihydro-pyrimidin-1(2*H*)-yl)-propionamide (9).** To a suspension of 3-(3,4-dihydro-5-methyl-2,4-dioxopyrimidin-1(2*H*)-yl)propanoic acid **2b** (0.5 mmol) in 1,2-dimethoxyethane (10 ml) was added 5-aminouracil **8** (0.75 mmol) and after 10 minutes of stirring DMT-MM (0.75 mmol). TLC analysis after 120 hours revealed only traces of the limiting substrate. Subsequently the solvent was removed under diminished pressure. Yellow residue was boiling in 10 minutes in 7 ml of water. Suspension was filtered off and precipitate collected from the filter was recrystallized from aqueous DMF. Yellowish crystals; yield 52%; m.p. 322–324 °C; ^{1}H NMR δ: 1.73 (s, 3H, Me); 2.71 (t, 2H, CH_2, J = 6.6 Hz); 3.84 (t, 2H, J = 6.6 Hz); 7.47 (s, 1H, H6-thymine); 8.01 (d, 1H, H6-uracil, J = 6.0 Hz); 9.31 (s, 1H, CONH);

10.67 (d, 1H, H1-uracil, $J = 5.1$ Hz); 11.24 (br s, 1H, H3-thymine); 11.44 (br s, 1H, H3-uracil); ^{13}C NMR δ: 12.0; 34.7; 44.3; 108.1; 113.0; 130.0; 141.8; 149.7; 150.8; 160.7; 164.3; 169.3. Analysis calcd. for $C_{12}H_{13}N_5O_5$ (307.26): C46.91, H 4.26, N22.79. Found: C47.28, H4.11, N22.36.

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